CHEMICAL REVIEWS

REVIEW

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Update 1 of: Enantioselective Enzymatic Desymmetrizations in Organic Synthesis

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1. INTRODUCTION

During the recent past years, tremendous efforts have been made to establish enantioselective routes for the preparation of enantiomerically pure compounds due to their importance in the pharmaceutical, agricultural, and food industries. This is reflected in the fact that the sales of single-enantiomer small-molecule drugs has reached ca. US $159 billion in 2002.[1] Moreover, the FDA has become increasingly reluctant to permit the introduction of additional racemic drugs, as these therapies are by definition saddled with 50% of chemical ballast.[2] Enzymes are nowadays widely recognized among the most active and selective catalysts for the preparation of optically active compounds.[3] Some of the factors that account for this popularity are (1) They are chemo-, regio-, and stereoselective, and environmentally friendly. (2) Because of the mild conditions under which they operate, enzymatic reactions are affected to a lesser extent by side reactions (viz. isomerization, racemization, epimerization, and rearrangement of molecules) as compared to nonenzymatic processes. Nevertheless, organic chemists have been traditionally reluctant to employ enzymes in their syntheses. This is mainly because, in their natural form, most of the enzymes are sensitive catalysts that generally show their optimal activity in aqueous solution. Moreover, their handling requires some biochemistry knowledge. However, some recent advances carried out in the biocatalysis field have "approached" enzymes to organic synthesis: (a) they can operate in nonaqueous media accepting a broad range of substrates;[4] (b) immobilization techniques increase their stability and simplify their handling.[5] Thus, many enzymes can now be acquired and used as any other chemical.

Stereoselective biotransformations can be grouped into two main different classes: asymmetric synthesis and kinetic resolution of racemic mixtures (KR). Conceptually, they differ from each other in the fact that while asymmetric synthesis implies the formation of one or more chirality elements in a substrate, a KR is based on a transformation, which, subsequently, makes easier the separation of the two enantiomers of the racemic substrate. This fact involves a practical difference: in a kinetic resolution only half of the starting material is used. When only one enantiomer of a substrate is required, this fact constitutes a disadvantage of KRs and different approaches have been developed to overcome this limitation.[6] The one on which more attention has been recently paid is the dynamic KR[7] and consists of carrying out an in situ continuous racemization of the substrate, so that, theoretically,

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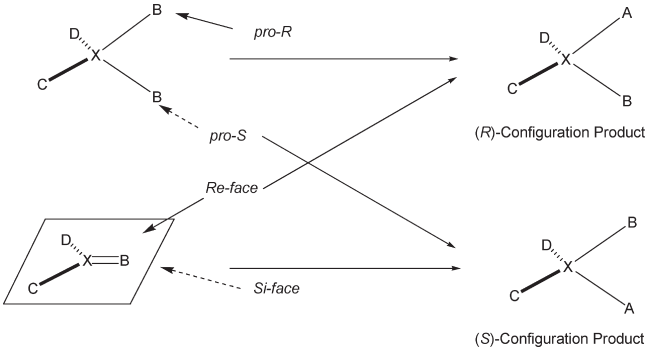
Scheme 1. Nomenclature of Enantiotopic Groups or Faces of Prochiral and *Meso* Compounds[a]



[a] X is a C atom or a heteroatom and A, B, C, and D are substituents with decreasing CIP priority.

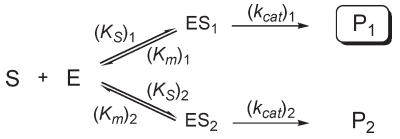
all of the racemic starting material can be used for transformation into one enantiomer. Nevertheless, many substrates employed in enzyme-catalyzed kinetic resolutions are not liable to undergo racemization.

The desymmetrization of symmetric compounds consists of a modification that eliminates one or more elements of symmetry of the substrate. If the symmetry elements that preclude chirality are eliminated, enantioselectivity can be achieved.[8] Enantioselective enzymatic desymmetrizations (EEDs) belong to the field of asymmetric synthesis and, accordingly, a maximum yield of 100% can be attained.[9] For this reason, they constitute a very interesting alternative to KRs for the preparation of optically active compounds, which is reflected in the increasing number of enzymatic desymmetrizations applied to synthesis published in the literature during the recent past years. This review deals with the developments made in the use of biocatalysts for the desymmetrization of *meso* and prochiral compounds. This is done in the format of a "Perennial Review" that updates the year 2005 version of this article (García-Urdiales, E.; Alfonso, I.; Gotor, V. *Chem. Rev.* **2005**, *105* (1), 313).[391] It is structured according to a synthetic rather than a biocatalytic point of view and, as a rule of thumb, only those examples useful from a synthetic point of view are included, i.e., EEDs that constitute or can constitute a key step in a synthetic route or aid to rationalize either the substrate specificity of an enzyme or the desymmetrization of a certain class of compounds. Accordingly, important parameters to which attention has been paid are enantioselectivity and the yield of the EED, which should be higher than 50% so that the desymmetrization implies a clear advantage over KRs. Nevertheless, exceptions can be made on the basis of novelty and difficulty of obtaining a compound by other means.

2. NOMENCLATURE, KINETICS, AND QUANTIFICATION

Meso and prochiral compounds have in common the presence of either two enantiotopic groups or a planar trigonal group with two enantiotopic faces (Scheme 1). An enantiotopic group is described as *pro-R* if, when it is arbitrarily assigned CIP priority[10] over the other enantiotopic group, the configuration of the generated chiral center is assigned the stereodescriptor *R*. Alternatively, the other group is described as *pro-S*. Although this nomenclature is also applicable to the enantiotopic faces of a trigonal system, for this case, the *Re* and *Si* terminology is more often used. Thus, the stereoheterotropic face of a trigonal atom is

Scheme 2



designated *Re* if the ligands of the trigonal atom appear in a clockwise sense in order of CIP priority when viewed from that side of the face. The opposite arrangement is termed *Si*.

During the EED of a *meso* or a prochiral compound, the enzymatic reaction takes place faster at one of the enantiotopic groups or faces of the substrate, thus affording the two enantiomers of the product in unequal amounts. This different rate of reaction arises from the different energy of the diastereomeric transition states between the enzyme and the substrate along the reaction coordinate ($\Delta\Delta G^{\ddagger}$), and which can be related to the enantioselectivity of the reaction by means of eq 1, where *E* is the parameter that quantifies the enantioselectivity displayed by the enzyme in the EED. Despite the different names used for this parameter, viz. "selectivity of the reaction" or "prochiral selectivity", we believe that there is no contradiction in using the term "enantiomeric ratio", with the same sense it is used in the enzymatic KR of racemic mixtures.

$$\Delta G^{\ddagger} = -RT \ln E \tag{1}$$

Many synthetically useful EEDs can be effectively described by Michaelis Menten kinetics,[11] in which no enzyme inhibition and no influence of product accumulation on reaction rate are prerequisites. According to this mechanism (Scheme 2), during an EED the *meso* or prochiral substrate (S) noncovalently binds to the active site of the enzyme to afford two diastereomeric Michaelis complexes (ES_1 and ES_2), which subsequently, undergo reaction to irreversibly yield the two enantiomers of the product (P_1 and P_2). Within this kinetic scheme, eq 2 describes the initial rates of formation of each enantiomer at infinitely low concentrations of substrate, where the rate constants are termed specificity constants and represent the pseudo-second-order rate constants of the whole transformation. Thereby, the enantiomeric ratio of an EED, i.e. the ratio of the rates of formation for each enantiomer of the product, corresponds to the ratio of the specificity constants (eq 3).

$$v_i = \left(\frac{k_{cat}}{K_m}\right)_i [E][S] \tag{2}$$

$$E = \frac{v_1}{v_2} = \frac{(k_{cat}/K_m)_1}{(k_{cat}/K_m)_2} \tag{3}$$

Unfortunately, eq 3 has no practical utility to experimentally determine enantiomeric ratios because in an EED it is not possible to measure the two Michaelis constants because there is only one substrate. Nevertheless, this equation can be transformed into an equivalent one in which *E* is related to the ee_p, a magnitude that can be easily measured experimentally (eq 4). In practice, ee_p is itself usually employed as the parameter of choice to quantify the enantioselectivity of an EED. This is because, conversely to KRs, the ratio of the rates of formation of both enantiomers does not change during the course of the desymmetrization. Thereby, ee_p remains constant throughout the whole transformation unless the reaction is reversible or the product of

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the reaction is not stable under the reaction conditions and it is further transformed. Thus, it is recommendable to keep in mind that if this were the case, ee_p could not be used to quantify enantioselectivity and, in addition to this, an optimal degree of conversion should be determined to maximize the optical purity of the product of the reaction.

$$E = \frac{1 + ee_p}{1 - ee_p} \quad (4)$$

3. ADVANCES IN ENANTIOSELECTIVITY ENGINEERING

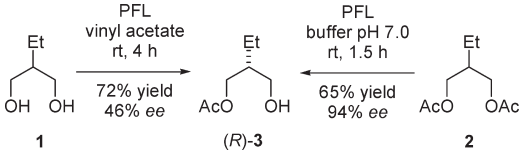
One of the most important goals in stereoselective biocatalysis is the development of methodologies that allow the optimization of the enantiomeric ratio of a given biotransformation. To achieve such a goal, it is usually desirable, in the first instance, to understand the factors that control the enzymatic enantioselectivity. With this regard, protein crystallography has proven to be a helpful tool by elucidating the three-dimensional structure of many enzymes.[12] This information itself, or in combination with protein modeling approaches, has permitted better understanding of the enantioselectivity displayed by many enzymes and, accordingly, allowed preparation of mutants with altered selectivity and even substrate specificity,[13] as well as the design of either small-molecule or biopolymer-based new catalysts inspired by enzymatic mechanisms or "artificial enzymes".[14] Nevertheless, in the absence of these structures, empirical qualitative models of the active sites of enzymes can be developed on the basis of the enantioselectivity displayed by an enzyme toward different substrates. Such an approach has led to renowned rules of thumb like Kazlauskas' and Prelog's rules,[15] which predict the major enantiomer obtained from lipase-catalyzed hydrolyses or (trans)esterifications of secondary alcohols and dehydrogenase-catalyzed reductions of prochiral ketones, respectively. Additionally, other similar but less generalizable models have been also developed for different enzymes.[16] However, even if no information from the enzyme active site is available, it is possible to improve its enantioselectivity by means of pseudocontrolled random mutagenesis performed over subsequent generations coming from a parent wild-type enzyme. Such an approach is termed directed evolution.[13,17]

Manipulation of the reaction conditions constitutes another alternative to optimize enantioselectivity. Appart from advances made by means of using traditional approaches such as the addition of cosolvents and additives, and the employment of biphasic systems in microbial reactions, the utilization of nonconventional solvents like ionic liquids,[18] supercritical carbon dioxide,[18d,f,19] and fluorous solvents[20] is maybe the most striking advance in this field. With respect to reactions carried out in conventional solvents, reactions in such media usually have different thermodynamic and kinetic behaviors, which often lead to improved process performance. Furthermore, they can also allow an enhanced stability of biocatalysts and an easy product recovery.

The enantioselectivity of microbial biotransformations can be usually diminished due to the presence of other enzymes of the microbial metabolism which either perform the same transformation with a lower enantioselectivity or further transform the major enantiomer of the product of the reaction. Different approaches have successfully overcome this limitation, thus effectively increasing the optical purity of the products of these reactions. With this regard, the addition of selective inhibitors, or the expression of the gene encoding the enzyme in a different microorganism, are examples of methods that have proven to be successful in solving this problem.[21]

Despite this progress, the search for new catalysts with improved or new selectivity profiles and the implementation of new catalysis assays in high-throughput format are still active areas of research.[22]

Scheme 3



4. HYDROLYSES AND TRANSESTERIFICATIONS

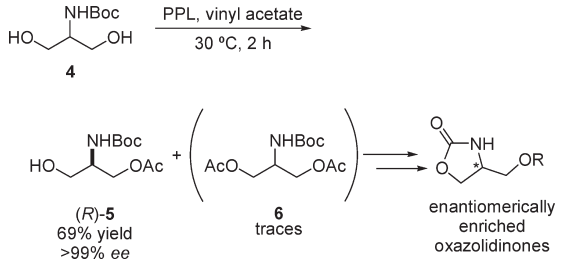
Hydrolases are one of the classes of enzymes most used in synthetic chemistry.[23] The lack of sensitive cofactors that would have to be recycled, and a large number of readily available enzymes possessing relaxed substrate specificities to choose from, are the main features that account for this preference. Many of them have in common the presence of a nucleophilic amino acid in the active site which attacks the group to be hydrolyzed, thus forming a covalent enzyme−substrate intermediate that is subsequently hydrolyzed by a water molecule. Moreover, their ability to accept different substrates apart from the natural ones, and their stability in nonaqueous media, have allowed the reversal of their natural reactions, thus widening the scope of transformations attainable with these catalysts. Furthermore, new reactivities of hydrolases have also been detected. For instance, the ability of their active site to stabilize negative charges has been used for the catalysis of Diels−Alder, Baeyer−Villiger, and Michael addition reactions by using the wild-type and mutants of different hydrolases.[24]

In this section, hydrolase-catalyzed EEDs of *meso* and prochiral alcohols, carboxylic acid esters and anhydrides, and nitriles are reviewed. The hydrolysis of *meso* epoxides generally displays low enantioselectivities. Moreover, these reactions have been traditionally connected to the enzymatic epoxidation of alkenes, thus allowing the stereoselective synthesis of *trans*-diols. Hence, the synthetically useful examples are described in a subsequent section.

4.1. Alcohols

Ester hydrolyses and transesterifications have been successfully used for the desymmetrization of different *meso* and prochiral alcohols. Traditionally two different approaches have been employed: (1) acylation of the free alcohol by means of a transesterification reaction, and (2) hydrolysis of an appropriate acyl derivative of the alcohol. If the enzyme maintains the enantiopreference in both processes, both approaches can be jointly used to produce, in high yields and *ee*'s, both enantiomers of the product. However, this is not always the case and, indeed, there are examples collected in the literature in which such a behavior is observed. For instance, Izquierdo and co-workers[25] have desymmetrized 2-ethylpropane-1,3-diol (**1**) and its di-*O*-acetate (**2**) through PFL-catalyzed transesterification and hydrolysis, respectively (Scheme 3). Both processes led to the monoacetate of (*R*)-configuration, the enzymatic enantioselectivity being higher for the hydrolytic transformation, from which (*R*)-**3** was isolated in high yield and *ee*. This chiral building block was further used for the preparation of a dioxaspiro compound that closely matches the skeleton of talaromycins.

Scheme 4

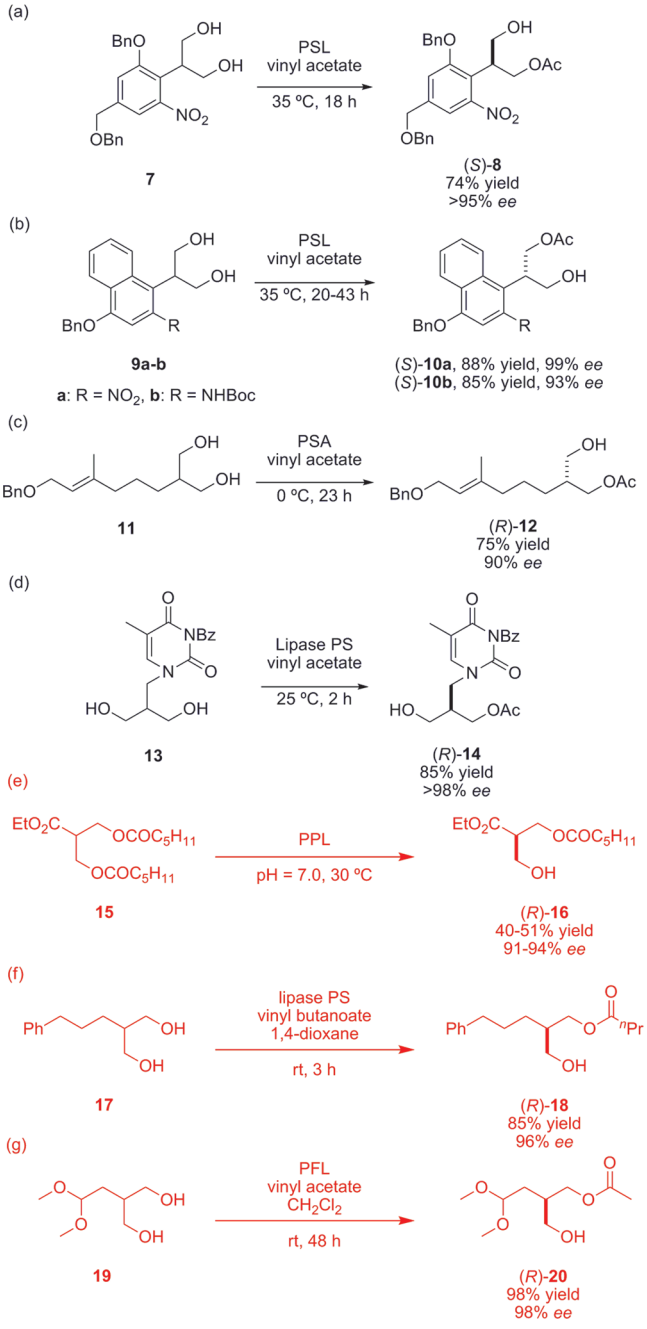


One of the family of substrates to which much attention has been paid is the one containing the propane-1,3-diol moiety, because its substructure is present or can easily lead to many molecules that play an important role in both medicinal chemistry and asymmetric synthesis. For instance, the introduction of enantiomerically pure 1,3-oxazolidin-2-ones (Evans chiral auxiliaries) can be considered a milestone in the history of asymmetric synthesis using covalently bound auxiliaries. Although they fulfill all the criteria required from a good auxiliary, their application can be limited by the availability of an appropriate enantiomerically pure amino acid or alcohol. Williams and co-workers[26] have successfully developed the synthesis of both enantiomers of the Evans auxiliary 4-hydroxymethyl-1,3-oxazolidin-2-one by means of a route that makes use of an enzymatic desymmetrization of *N*-BOC-protected serinol (**4**) (Scheme 4). After optimization of reaction conditions, (*R*)-(−)-3-*O*-acetyl-2-*N*-(*tert*-butoxycarbonyl)serinol [(*R*)-**5**] was obtained enantiopure and in high yield when PPL was used as catalyst in vinyl acetate. Depending on the reaction conditions, different amounts of the diacetate were obtained, which were presumably formed from the unwanted enantiomer of the monoacetylated product, thereby providing a self-correcting process. A more recent report described similar results using *N*-protected serinol derivatives to prepare enantiopure 2-hydroxymethyl aziridines.[27]

Enzymatic desymmetrizations of the propane-1,3-diol moiety have been also successfully employed for the synthesis of biologically active compounds. Accordingly, a formal enantioselective synthesis of the antitumor antibiotic (+)-FR900482 has been completed.[28] The key step was the desymmetrization of the propane-1,3-diol **7** by means of a PSL-catalyzed transesterification in vinyl acetate (Scheme 5a). The corresponding monoacetate [(*S*)-**8**] was obtained in 74% yield and >95% *ee*. Using similar experimental conditions, Boger and co-workers[29] have also developed effective asymmetric syntheses of key CBI precursors, a class of potent antitumor antibiotics, by means of enzymatic desymmetrizations of the prochiral diols **9a**−**b** (Scheme 5b). The corresponding (*S*)-monoacetates [(*S*)-**10a**−**b**] were isolated in excellent yields and *ee*'s. Additionally, the desymmetrization of propane-1,3-diols **11** and **13** by means of *Pseudomonas cepacia*- and lipase PS-catalyzed transesterifications (Scheme 5c,d) have allowed the syntheses of (*R*)-**12**, product isolated from the hair-pencils of male Danaus chrysippus (African Monarch),[30] and (*R*)-**14**, precursor of a novel *β*-amino acid[31] in high *ee*'s and yields.

Another interesting approach to simple enantioenriched 1,3-diols but using porcine pancreas lipase has been recently reported by Ishikawa and co-workers.[32] Thus, the PPL-catalyzed hydrolysis of symmetrical 2-(ethoxycarbonyl)propane-1,3-diyl dihexanoate (**15**) afforded the desymmetrized monohexanoate (*R*)-**16** in moderate yields and high *ee* values, even in a gram-scale

Scheme 5



reaction (Scheme 5e). Further synthetic manipulations of this simple chiral synthon afforded a 3-phenylpiperidine derivative as a model construction of the A−D ring system of lysergic acid. Moreover, the two isomers of the odorant Rosaphen have been recently prepared by using a chemoenzymatic methodology in which the key step is the lipase PS-catalyzed desymmetrization of the 2-substituted-propan-1,3-diol **17**.[33] After an optimization process that studied the effect of the solvent and the length of the acyl chain of the acyl donor on enantioselectivity, (*R*)-**18** was obtained in excellent yield and enantioselectivity (Scheme 5f). Similarly, diol **19** has been desymmetrized by PFL-catalyzed acetylation to yield the corresponding (*R*)-configured monoester (Scheme 5g), which was further used in the total synthesis of the alkaloid (+)-pilocarpine.[34]

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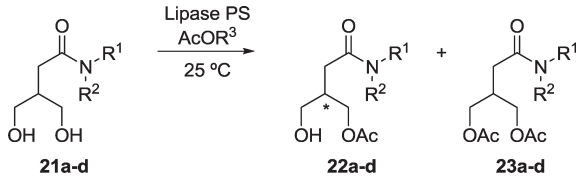
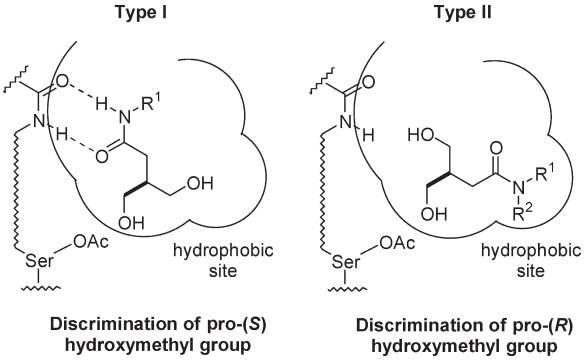
Table 1. EED of 2-Carbamoylmethyl-1,3-propanediols



					monoacetate		diacetate	
entry	substrate	R^1	R^2	R^3	yield (%)	*ee* (%)	config	yield (%)
1	**21a**	Me	H	Ph	26	>99	*S*	37
2	**21b**	Et	H	Ph	43	94	*S*	20
3	**21c**	Me	Me	Ph	77	96	*R*	trace
4	**21d**	Et	Et	vinyl	52	96	*R*	27

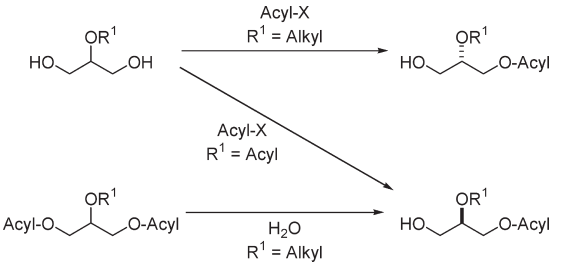
Chart 1



Takabe et al.[35] have published one of the few examples that rationalizes the desymmetrization of this class of compounds. They have investigated the lipase PS-catalyzed desymmetrization of different 2-carbamoylmethyl-1,3-propanediols (**21a**−**d**), which, after optimization of the reaction conditions, afforded the corresponding monoacetates (**22a**−**d**) in high *ee*'s (Table 1). Unfortunately, the yields were somewhat low, and significant amounts of the diacetates (**23a**−**d**) were usually obtained. On the other hand, they have found that the enantioselectivity shown by this lipase is highly dependent on the 2-carbamoyl substituent. Thus, the enantiopreference displayed toward the *N*-alkyl derivatives (entries 1 and 2) was the opposite to the one observed for the *N*,*N*-dialkyl 1,3-propanediols (entries 3 and 4). The authors have proposed a model that explains the enantioselectivity observed on the basis of hydrogen bonding between the enzyme and the substrates. In a plausible mechanism, shown in Chart 1, the *N*-monoalkylcarbamoyl group would establish a hydrogen bond with an amide group in the lipase; thus, the *pro*-*S* hydroxymethyl group would be stereoselectively acetylated (type I). On the other hand, in the reaction of *N*,*N*-dialkylcabamoyl-diols, the *N*,*N*-dialkylcarbamoyl group is more likely located at the hydrophobic site in the active-site model for the lipase (type II). Therefore, the *pro*-*R* hydroxymethyl group would be discriminated to give the corresponding (*R*)-monoacetates.

Glycerol and its derivatives comprise a very special class of prochiral propan-1,3-diols that are present almost everywhere in nature. Furthermore, they constitute a useful starting material for

Scheme 6



the preparation of different C_3 chiral building blocks. Lipase-catalyzed transesterifications[36] and hydrolyses[37] of different 2-*O*-alkylglycerol derivatives have been reported. The enantiopreference shown by lipases in these processes is *pro*-*S*, and it does not change for the hydrolyses of the corresponding diols, thus allowing the obtainment of both enantiomeric series (Scheme 6). Unfortunately, under the hydrolytic reaction conditions, the products of the reaction are usually unstable due to intramolecular acyl migration processes. Indeed, a racemization rate of *c* 2%/h has been observed when optically active (*S*)-1-*O*-acetyl- 2-*O*-benzylglycerol was incubated in phosphate buffer pH 7.[36c] The lipase-catalyzed transesterification of prochiral 2-*O*-acylglycerol derivatives has proven to be an effective solution for this problem because the products obtained are stable under the reaction conditions and the enantiopreference is inverted as compared to the transesterification of *O*-alkyl derivatives (Scheme 6). For instance, the PPL-catalyzed acetylation of 2-benzoyloxy-1,3-propanediol afforded the corresponding (*R*)-monoester in 63% yield and 96% *ee*.[38]

The synthesis of optically active compounds bearing a chiral quaternary center remains a challenge for synthetic organic chemists. Thereby, an increasing interest in the development of strategies for their preparation has been observed.[39] During the recent past years, the EED of prochiral 2,2-disubstituted propane-1,3-diols has permitted the stereoselective synthesis of different biologically active compounds bearing chiral quaternary centers. Thus, Fukuyama and co-workers[40] have recently reported the total synthesis of Leustroducsin, a potent colony-stimulating factor inducer isolated from the culture broth of *Streptomyces platensis* SANK 60191 by Sankyo's groups. One of the first steps of the synthesis is the desymmetrization of the glycerol derivative **24** (Scheme 7a) catalyzed by lipase AK. Subsequent protection of the resulting monoacetate with *tert*-butylchlorodimethylsilane afforded the *O*-protected monoalcohol (*S*)-**25** in good *ee* and high yield. Similarly, (*S*)-α-tocotrienol has been prepared in 19% overall yield[41] through a multistep synthesis that involves the CALB-catalyzed acetylation of the bicyclic triol **26** (Scheme 7b). The monoester (*S*)-**27** was obtained in 98% *ee* and 60% yield, which can be increased because the diester **28** (27%) can be easily recycled by nonenzymatic hydrolysis. A structurally related compound (2,2-bis-hydroxymethyl-3,4-dihydro-2*H*-naphthalen-1-one) has been recently studied using a very similar procedure.[42] However, in this case, the authors found the kinetic resolution of the corresponding racemic monosilylether much more efficient than the EED process of the free diol. On the other hand, the enzymatic desymmetrization of 2,2- and 2,5-disubstituted pyrroline-containing diols catalyzed by a lipoprotein lipase from *Pseudomonas* sp. has been reported (Scheme 7c−d) within synthetic studies on a route toward the polyhydroxylated pyrrolizidine

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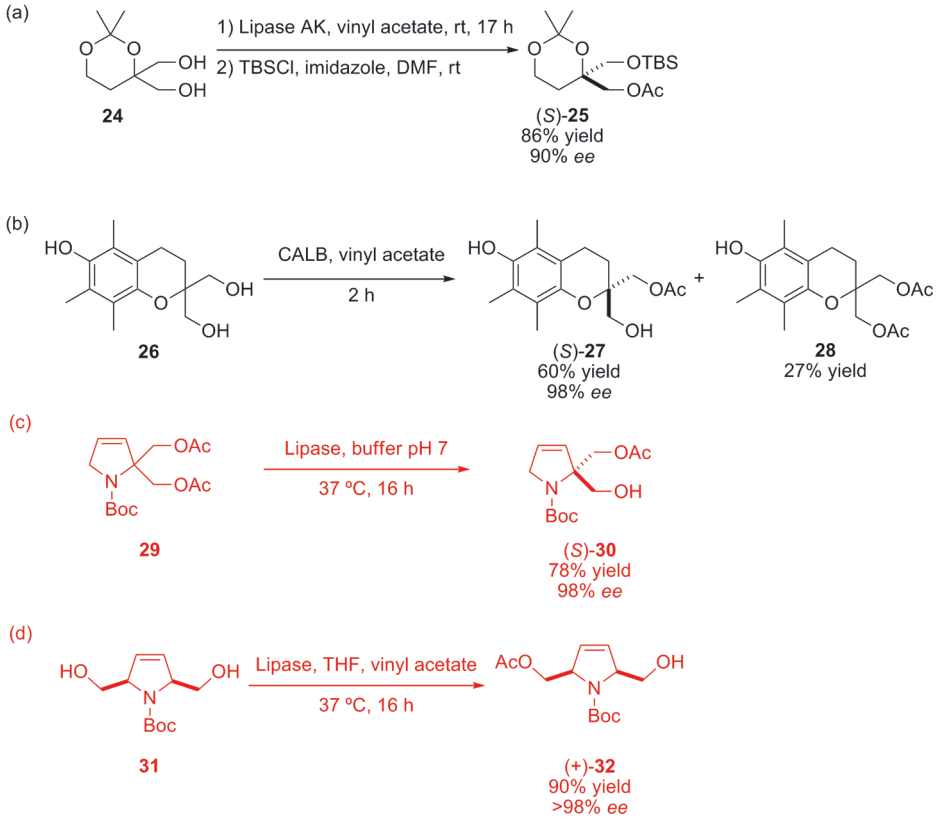
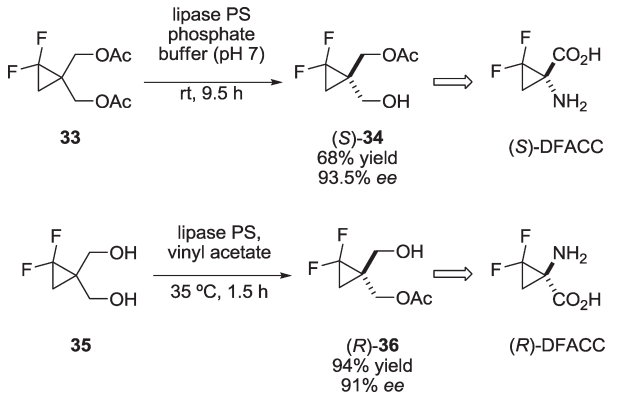
Scheme 7



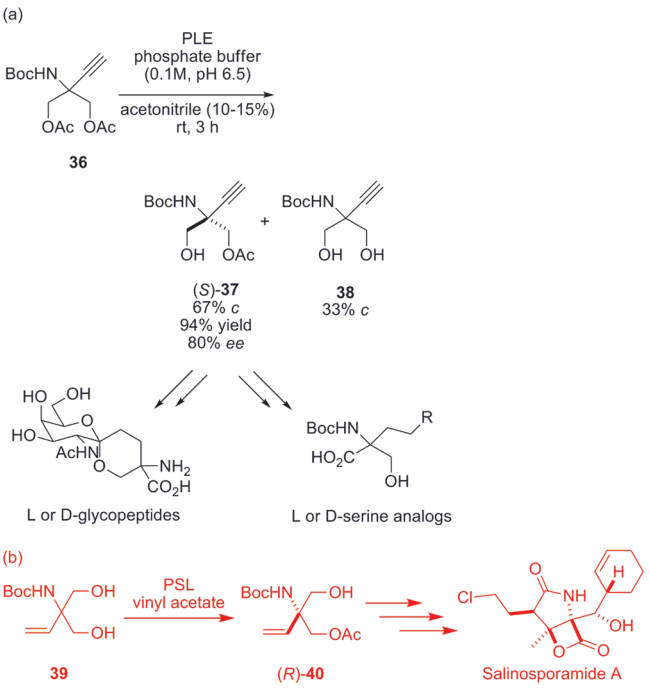
Scheme 8



natural products 1-epiaustraline and hyacinthacine A1.[43] In particular, the hydrolysis of diacetate **29** and diol **31** proceeded in excellent enantioselectivities and yields.

1-Aminocyclopropane-1-carboxylic acid and their derivatives are attractive synthetic targets because of their interesting properties either isolated or being part of the sequence of a peptide. Both enantiomers of 1-amino-2,2-difluorocyclopropane-1-carboxylic acid have been synthesized in a preparative scale in high yields and *ee*'s by means of analogue syntheses that make use of the desymmetrization of [1-(acetoxymethyl)-2,2-difluorocyclopropyl]methyl acetate (**33**) and the corresponding diol (**35**) by means of a lipase PS-catalyzed hydrolysis and transesterification, respectively (Scheme 8).[44]

Scheme 9



In an attempt to probe the mechanism by which glycosyltransferases recognize glycoproteins and assemble the core structures of *O*-linked oligosaccharides, two constrained glycopeptides were synthesized by means of a sequence in which

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the monoester **37** was a key component (Scheme 9a).[45] This compound was successfully prepared by means of a PLE-catalyzed hydrolysis of the diacetate **36**. The (*S*)-monoester was obtained as the major product in moderate *ee* and accompanied by different amounts of diol **38**, which depended on the reaction conditions. The authors have also made use of the so-obtained monoester (*S*)-**37** for the preparation of different α-substituted serine amino acid analogues.[46] A very similar diol (**39**) has been recently desymmetrized by using lipase from *Pseudomonas sp.* in $^{i}Pr_2O$-vinyl acetate (Scheme 9b).[47] The so-obtained optically active monoacetate [(*R*)-**40**] is the key synthetic intermediate for the total synthesis of Salinosporamide A, a potent 20S proteasome inhibitor from marine actinomycete *Salinospora tropicana*.

The EED of 2,2-disubstituted propane-1,3-diols using well-known acyl donors, i.e., vinyl and isopropenyl acetates, is a process that, although it has proven to be successful, usually suffers from low reactivity. Moreover, racemization of the products via acyl group migration occurs under different reaction conditions (Scheme 10), such as acidic or hydrogenolytic ones. Furthermore, this migration has also been observed for the products of the transesterification of other polyhydroxylated compounds such as different *meso* 1,2-diols.[48] Kita and co-workers have solved these problems by using different 1-ethoxyvinyl esters as acyl donors.[49] In particular, when the carbonyl carbon of the 1-ethoxyvinyl ester is linked to an aromatic moiety, acyl migrations are hampered since the formation of the corresponding ortho ester intermediate involves a greater loss in resonance energy as compared to the case of aliphatic acyl chains (Scheme 10).[50]

Screening of different 1-ethoxyvinyl esters for the EED of different 2,2-disubstituted propane-1,3-diols and *meso* 1,2-diols has revealed that 1-ethoxyvinyl 2-furoate is the acyl donor of choice for this type of compound because of the following reasons:[51] (1) It can be readily prepared from commercially available ethoxyacetylene and 2-furoic acid in a large scale in high yield and can be stored in a refrigerator for more than one year. (2) Conversely to other acyl donors, its reactions are usually complete within several hours to give the products in higher optical and chemical yields. (3) If necessary, the only side products, the diesters, can be recycled to the starting diols quantitatively. (4) Prolonging the reaction time increases the optical purity of the products through kinetic amplification. (5) As it has already been mentioned, products are sufficiently stable under acidic and/or oxidative conditions.

The desymmetrization of oxindoles bearing a quaternary stereogenic carbon is a very interesting process since its products can be found in different natural products and are also regarded

Scheme 10

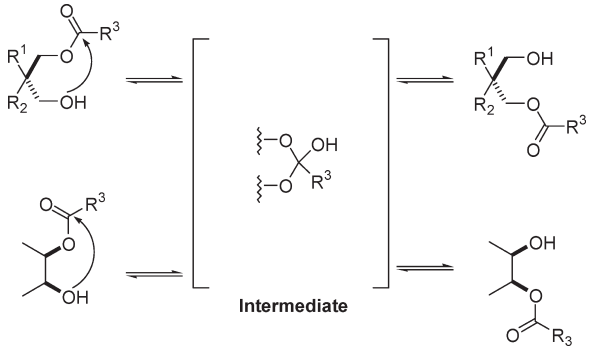
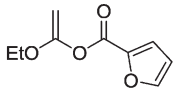
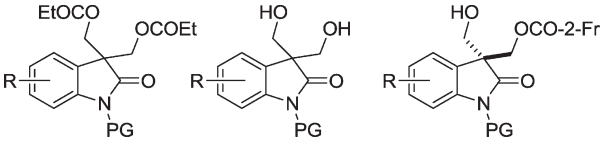


Table 2. EED of Oxindoles



1-ethoxyvinyl 2-furoate



41 **42a-h** **43a-h**

							monoalcohol	
entry	substrate	PG	R	reaction	*t* (h)	enzyme	yield (%)	*ee* (%)
1	**41**	MOM	H	hydrolysis	8	cholinesterase	20	70
2	**41**	MOM	H	hydrolysis	120	cholinesterase	38	95
3	**42a**	Me	H	transesterification	7	CRL	53	87
4	**42b**	BOC	H	transesterification	22	CRL	93	99
5	**42c**	BOC	5-OMe	transesterfication	3	CRL	77	98
6	**42d**	BOC	6-OMe	transesterfication	19	CRL	79	91
7	**42e**	Cbz	H	transesterification	58	CRL	71	98
8	**42f**	Ac	H	transesterification	48	CRL	90	97
9	**42g**	MOM	H	transesterification	64	CRL	34	86
10	**42h**	Bn	H	transesterification	144	CRL	59	68

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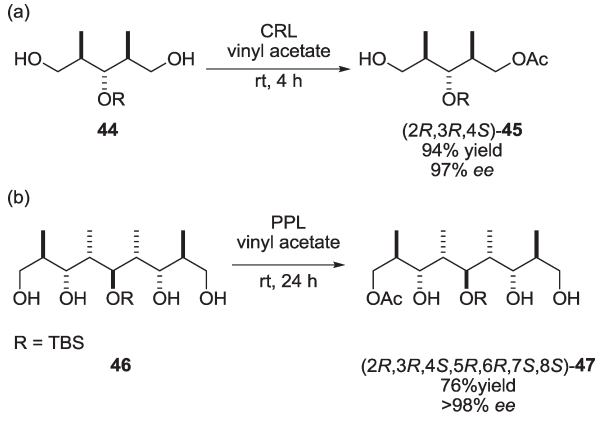
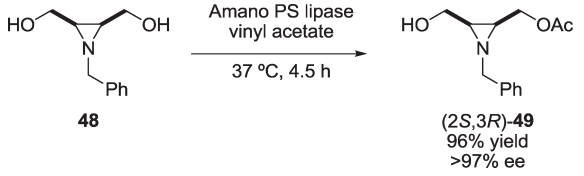
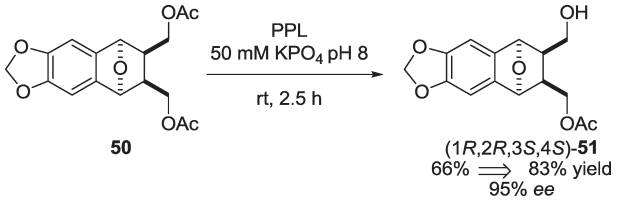
Scheme 11



Scheme 12



Scheme 13



as chiral building blocks in indole syntheses. In particular, the desymmetrization of prochiral 3,3-bis(hydroxymethyl)-1-methoxymethyl-2-oxindole (**42a**) by means of an enzymatic hydrolysis of the corresponding bis(acyloxymethyl) derivatives has been reported to afford the corresponding monoalcohols with optical activity (Table 2).[52] After optimization of the biocatalyst and acyl donor, the hydrolysis of the dipropionate **41** catalyzed by cholinesterase proved to be the most promising reaction. The fact that the *ee* of the (+)-monopropionate varied depending on the reaction time (entries 1 and 2), attributed to a subsequent enzymatic kinetic resolution, was used to optimize the desymmetrization process, the monoalcohol finally being obtained in 95% *ee* and 38% yield after 5 days of reaction (entry 2), together with significant amounts of the starting material (40%) and the diol (16%). Although the synthetic applicability of this process could have been improved by recycling these byproducts, the preparation of the starting material also constituted a disadvantage due to its somewhat low yield. This problem could be overcome by direct desymmetrization of the alcohol **42a**. Certainly, CRL (Meito OF) has proven to be an efficient catalyst in the transesterification of a closely related series of oxindoles by using the aforementioned 1-ethoxyvinyl 2-furoate as acyl donor (Table 2).[53] After optimization of the reaction conditions, it was found that the use of mixtures of $^{i}Pr_2O$−THF as solvent was crucial, the ratio 5:1 generally being the best choice. Results show that *N*-acyl derivatives **42b**−**f** generally produced the corresponding (*R*)-monoalcohols [(*R*)-**43b**−**f**] with high optical and chemical yields (entries 4−8). On the contrary, the desymmetrization of the *N*-alkyl derivatives **42a**,**g**−**h** resulted in lower optical and chemical yields (entries 1 and 9−10). Nevertheless, recrystallization was effective for obtaining the optically pure monoalcohols from those with unsatisfactory optical purities. Furthermore, the desymmetrization of the difuroates of **42a**−**h** opens up a route to the opposite enantiomeric series of these compounds although in lower yields (typically ≤50%).

Apart from prochiral derivatives of propane-1,3-diol and glycerol, other *meso* and prochiral alcohols have been desymmetrized with synthetic purposes. For instance, a secondary metabolite isolated from the skin of the anaspidean mollusk *Dolabrifera dolabrifera* was synthesized in five steps (58% overall yield) via the enzymatic desymmetrization of *meso*-**44** catalyzed by *Candida rugosa* lipase and using vinyl acetate as acylating agent.[54] The monoester (2*R*,3*R*,4*S*)-**45** was obtained in excellent yield and *ee* when intact molecular sieves was added to the medium to trap the byproduct acetaldehyde, which is essential to achieve high enantioselectivity (Scheme 11a). Additionally, the C(19)−C(27) fragment of rifamycin S has been successfully prepared by stereoselective acylation of the *meso* polyol **46** by vinyl acetate (solvent and acyl donor) in the presence of porcine pancreas lipase (Scheme 11b).[55] This reaction afforded monoacetate (2*R*,3*R*,4*S*,5*R*,6*R*,7*S*,8*S*)-**47** in good yield and enantiopure, and the enzyme was highly regioselective for a primary alcohol end group, the two unprotected secondary alcohols being left untouched. Finally, two enzymatic desymmetrizations of *meso*-diols related to **44** have been recently implemented in the total syntheses of complex biologically active compounds.[56]

Another important class of optically active alcohols obtained from EEDs are those ones usually derived from cyclic *meso* primary or secondary alcohols. Thus, *meso* diols including a three-membered ring constitute an interesting class of chiral building blocks for the synthesis of natural products. For instance, Prati and co-workers[57] have carried out the Amano PS lipase-catalyzed desymmetrization of aziridine **48**, whose monoacetylated derivative, obtained in high yield and *ee* (Scheme 12), is related to a key intermediate used in the total synthesis of the already mentioned antibiotic FR-900482. This reaction is part of the study of a chemoenzymatic route to prepare enantiomerically pure β-lactams from hydroxymethylaziridines via enzymatic KR or desymmetrization followed by carbonylative ring expansion.

Meso primary diols attached to larger ring systems than a cyclopropane have been also successfully desymmetrized. In this context, PPL has proven to be a suitable catalyst in the enantioselective hydrolysis of *meso* diacetate **50** (Scheme 13),[58] the corresponding monoaceteate (**51**) being obtained in high *ee*. Optimization of reaction conditions provided evidence that it was more convenient to terminate the reaction at about two-thirds conversion to minimize diol formation. Unreacted diacetate was then easily recycled and taking into account the recovered starting diacetate, this procedure provided for an 83% yield of desymmetrized tetracycle **51**, which was further used in the preparation of (−)-podophyllotoxin and its C_2-epimer, (−)-picropodophyllin.

The piperidine ring is a widespread structural fragment of biologically active compounds. In this sense, both enantiomers of

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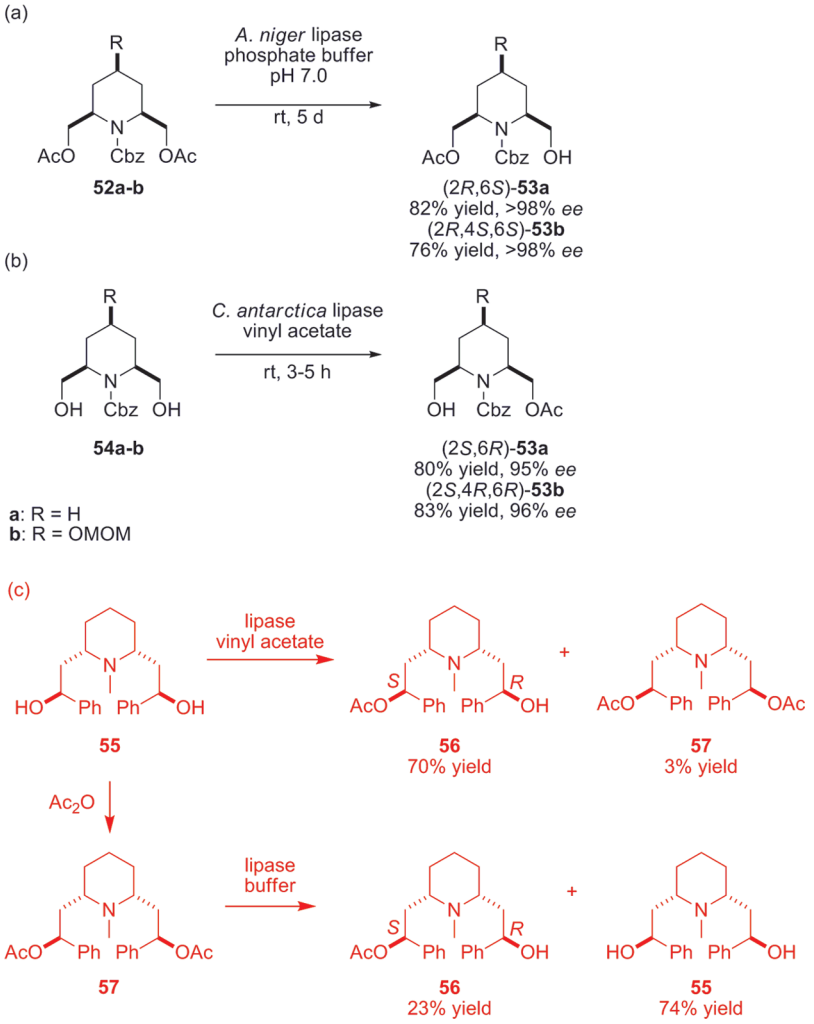
Scheme 14



different *cis*-2,6- and *cis,cis*-2,4,6-substituted piperidines have been obtained through desymmetrization strategies developed by Chênevert and co-workers. Namely, the *Aspergillus niger* lipase-catalyzed hydrolyses of diacetates **52a**−**b** afforded, at moderate rate, monoacetates **53a**−**b** enantiopure and in high yields (Scheme 14a).[59] The stereoselective acylation of the corresponding diols (**54a**−**b**) catalyzed by *Candida antarctica* lipase yielded, at a considerable faster rate, the opposite enantiomeric series, also in high yields and *ee*'s (Scheme 14b).[60] The usefulness of these so-obtained chiral building blocks was demonstrated by their use in the syntheses of different biologically active compounds.[60,61] Chênevert and Morin reported the chemoenzymatic synthesis of (−)-lobeline via enzymatic desymmetrization of lobelanidine, a piperidine-containing *meso* diol.[62] Similarly to the above-mentioned examples, two different methodologies were used: the acylation of lobelanidine (**55**) or the hydrolysis of its corresponding diacetate (**57**, Scheme 14c). Interestingly, this is an example where both processes are not complementary, since the monoacetate is also a susbrate for the enzyme. Therefore, in this case, the enzymatic acylation is more suitable to obtain the desired desymmetrized compound in good yield and excellent *ee* (≥98%).

The same research group also reported the enzymatic desymmetrization of various *meso*-*N*-Boc-2,5-*cis*-disubstituted pyrrolidines and pyrrolines derivatives (**58**, **60**, **62**, **64**) by ester hydrolysis or transesterification (Scheme 15a).[63] Several complementary approaches were assayed, and some of them rendered excellent results regarding yields and enantioselectivities. Besides, different substitution on the heterocyclic ring can be implemented. The clever combination of the monohydrolysis of the diacetate and the monoacylation of the free diol, in addition to a complete screening of biocatalysts and reaction conditions, have allowed them to give access to stereocomplementary desymmetrized compounds at will, in very good yields and moderate to good enantiomeric excesses. In this case, this is an example of the complementarity of both approaches (hydrolysis and acylation) for the accurate preparation of useful chiral compounds.

The synthesis of structurally related polyhydroxylated pyrrolidines can be also achieved using the biocatalytic desymmetrization of *meso*-diols (Scheme 15b).[64] Acylation of the protected diol **64** with vinyl acetate in the presence of Chirazyme L-2, c.-f., C2, lyo from *Candida antarctica* lipase-B, at room temperature led to the corresponding monoacetate in excellent yield and *ee*. The authors used this valuable intermediate for the stereoselctive synthesis of an iminocyclitol compound.

The desymmetrization of more elaborated acetylated derivatives of *meso* polyhydroxylated cyclohexanes by means of enzymatic hydrolyses has proven to be highly efficient. For instance,

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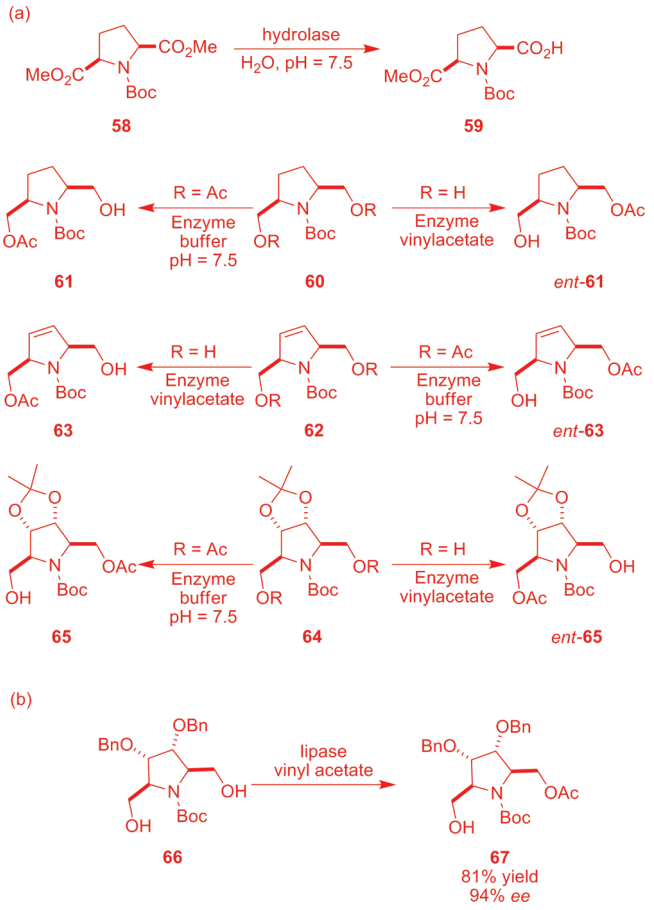
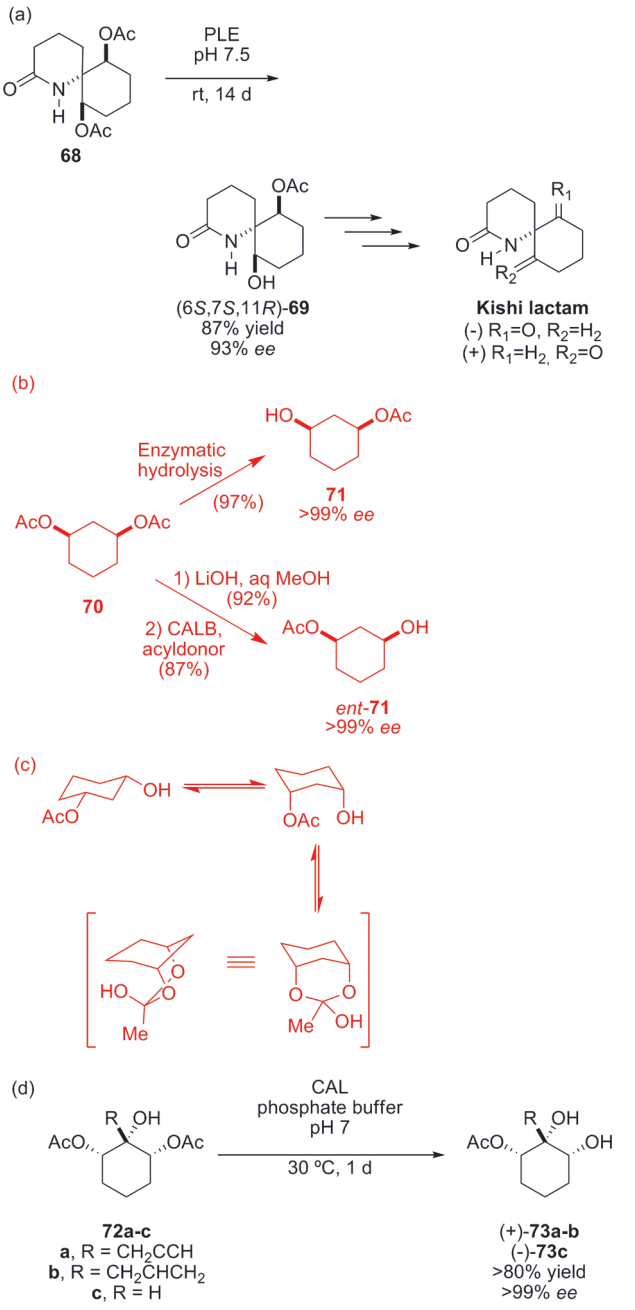
Scheme 15



Scheme 16



both antipodes of the Kishi lactam, the versatile intermediate for the synthesis of the perhydrohistrionicotoxin alkaloids, have been prepared by means of a synthetic route in which stereodivergence was introduced through a PLE-catalyzed hydrolysis of the *meso* lactam **68** (Scheme 16a).[65] This way, the corresponding monoacetate (6*S*,7*S*,11*R*)-**69** was obtained in high yield and *ee*. On the other hand, the simplest member of the *meso*-cyclohexane-1,3-diol family has been thoroughly studied by the group of Bäckvall.[66] Within this study, an efficient desymmetrization of *cis*-1,3-cyclohexanediol to (1*S*,3*R*)-3-(acetoxy)-1-cyclohexanol was performed via CALB-catalyzed transesterification, in high yield (up to 93%) and excellent enantioselectivity (*ee*'s up to >99.5%). Interestingly, also in this work, the metal- and enzyme-catalyzed dynamic transformations of *cis*/*trans*-1,3-cyclohexanediol using Lipase PS-C gave a high diastereoselectivity for *cis*-diacetate (*cis*/*trans* = 97:3). Then, the (1*R*,3*S*)-3-acetoxy-1-cyclohexanol was obtained from *cis*-diacetate by CALB-catalyzed hydrolysis in an excellent yield (97%) and selectivity (>99% *ee*). By deuterium labeling, it was shown that intramolecular acyl migration does not occur in the transformation of *cis*-monoacetate to the *cis*-diacetate. A reasonable explanation for this absence of migration has been also proposed by the authors. The *cis*-1,3-diol derivatives can possess two conformations, a diaxial and a diequatorial conformation, the latter being the most stable. For an intramolecular acyl transfer, the alcohol and acetate group must be close to one another, and this enforces the alcohol and acetate to be axial. In the intermediate for the intramolecular acetyl migration the oxygen substituents will be diaxial and, furthermore, the dioxane ring will have two carbons of the cyclohexane ring as axial carbons. For this reason, the energy of the acetyl migration intermediate is rather high for the cyclohexane system compared to the acyclic *syn*-1,3-diol system. Chênevert and co-workers also studied some structurally related molecules.[67] They recently reported the stereoselective acetylation of *meso*-2,2-dimethyl-1,3-cyclohexanediol by vinyl acetate in the presence of three lipases, which gave the (1*R*,3*S*)-monoester in high enantiomeric excess (*ee* ≥ 98%). The hydrolysis of the corresponding *meso*-diacetate in the presence of *Candida antarctica* lipase in phosphate buffer provided the opposite enantiomer. Finally, by following a similar approach, the diacetates of different *meso* 2-substituted cyclohexane-1,2,3-triols (**72a**−**c**) were

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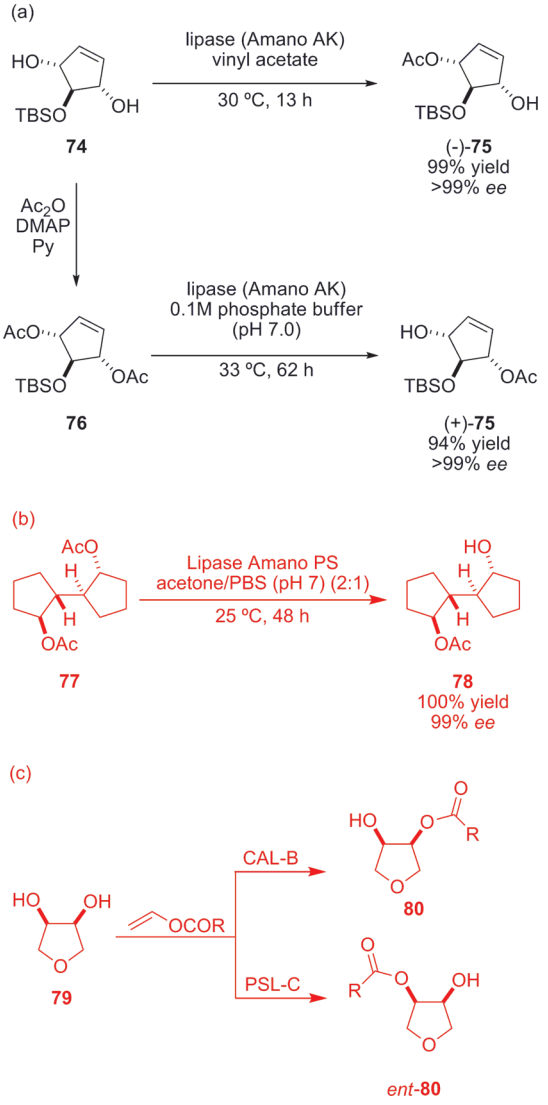
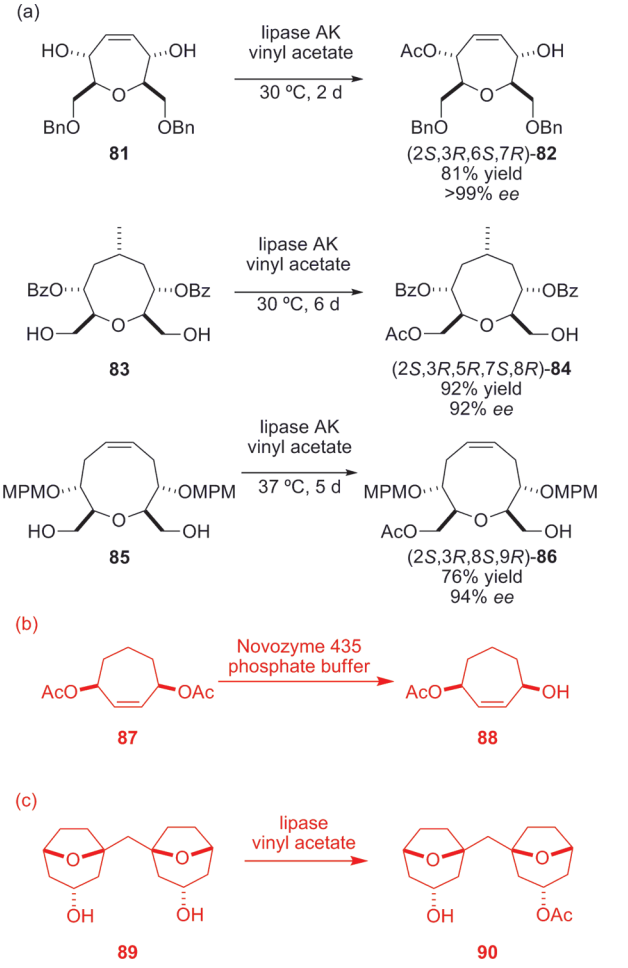
Scheme 17



Scheme 18



desymmetrized by means of CAL-catalyzed hydrolyses, from which the corresponding monoacetates were obtained enantiopure and in excellent yields (Scheme 16d).[68] Monoacetate (+)-**73a** was used as the starting material for the total synthesis of aquayamicin.

In the enantioselective synthesis of the core system of the neocarzinostatin chromophore, both enantiomers of *trans*-4,5-dihydroxy-2-cyclopenten-1-one (**75**) were conveniently prepared via enzymatic desymmetrization of triol **74** and its derivative **76** (Scheme 17a).[69] Lipase AK (Amano) efficiently catalyzed the transesterification of *meso* 5-*tert*-butyldimethylsilyloxy-2-cyclopentene-1,4-diol (**74**) as well as the hydrolysis of its diacetate (**76**). Both monoesters were obtained enantiopure and in very high yields. Nicolaou and co-workers recently took advantage of the EED process of a more elaborate secondary cyclopentanol scaffold as the key step for the synthesis of vanussal B.[70] The corresponding synthon was prepared in high enantiomeric excess though a lipase-catalyzed monohydrolysis of the *meso* diacetate **77** (Scheme 17b). Moreover, in our group, we tested a *meso-cis*-diol derivative of tetrahydrofurane (**79**) as a substrate for the enzymatic acylation (Scheme 17c).[71] Two of the enzymes tested catalyzed the reaction: lipase PS-C and CALB. It is noteworthy that depending on the biocatalyst employed, an opposite stereochemical preference in the asymmetric acylation was observed. Lipase PS-C catalyzed the acylation of the OH group at the carbon having *R* configuration, while CALB catalyzed the acylation of the *S* stereocenter. Unfortunately, the lipase PS-C showed low enantioselectivity in these processes. After trying different solvents, acyl donors and even alkoxycarbonylation processes, we always obtained moderate enantioselectivities.

Lipase AK has also provided an expeditious entry to different enantiomeric cyclic polyols used as building blocks for the stereoselective synthesis of the D(E), I, and F rings of ciguatoxin, a polyether marine toxin.[72] The enantioselective transesterification with vinyl acetate of the *meso* alcohols **81**, **83**, and **85** (Scheme 18a), obtained by a ring-expansion strategy, also afforded the corresponding monoacetates in high yields and *ee*'s.

Recently, Saito et al. described the enantio-divergent synthesis of (*S*,*S*)- and (*R*,*R*)-2,6-diaminopimelic acid using a homochiral monoacetate (Scheme 18b) available from an enzymatic desymmetrization of the corresponding *meso*-diacetate **87** with CALB in phosphate buffer solution (81% yield, 99% *ee*).[73] On the other hand, the desymmetrization of the structurally related diol **89** was attempted in the presence of several commercially available lipases (Scheme 18c) using vinyl acetate as solvent.[74] Lipases from *Pseudomonas fluorescens*, *Pseudomonas cepacia*, *Aspergillus niger*, *Aspergillus orizae*, and *Rizopus orizae* were ineffective in

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catalyzing the corresponding transesterification. Lipase from *Candida rugosa* afforded a low conversion into the desired monoacetate and an enantiomeric excess of 76%. Finally, lipase from *Candida cylindracea* afforded the monoacetate with enantiomeric excesses ranging from 80% to 89%. The best results were obtained at 40 °C (10 h of reaction, 4000 U/mmol, 44% yield, 54% recovered starting diol).

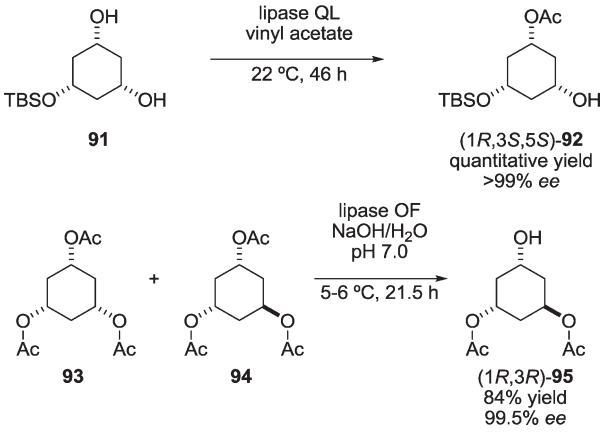
As it has already been mentioned, polyhydroxylated derivatives of six-membered rings constitute a particularly interesting class of compounds due to their role in carbohydrate and other natural products chemistry as well as their usefulness as chiral auxiliaries. Enzymes have again proven to be efficient catalysts in the selective transformation of polyhydroxylated cyclohexanes, which is still a challenging issue in asymmetric synthesis. EEDs of certain members of this class of compounds have been effectively used in vitamin D chemistry. Hilpert et al. have synthesized both enantiomers of useful building blocks to access 19-*nor* vitamin D derivatives by using highly selective enzymatic desymmetrizations of two *meso* 1,3,5-trihydroxy cyclohexane derivatives (Scheme 19).[75] Lipase QL efficiently catalyzed the desymmetrization of the *meso* diol **91**, yielding the monoacetate (1*R*,3*S*,5*S*)-**92** quantitatively and enantiopure.[76] Additionally, Lipase OF catalyzed well the hydrolysis of only *trans*-(**94**) from the *cis*/*trans* mixture of this triacetate. These routes have also provided access to the enantiopure (*S*)- and (*R*)-5-acetoxy-2-cyclohexenone, highly potential building blocks for natural product synthesis applying stereoselective conjugate organo cuprate addition reactions.

Likewise, within the context of a program directed toward the enantioselective total synthesis of natural products and analogues thereof containing a cyclohexane ring, the desymmetrization of *meso* all-*cis*-3,5-dihydroxy-1-(methoxycarbonyl)cyclohexane and the 4-methyl and ethyl substituted analogues (**96a**−**c**) was investigated (Scheme 20).[77] The results obtained showed that preparative useful enantioselectivities were found for the PPL- and SAM II-catalyzed transesterifications of **96a** and **96b**−**c**, respectively, in which the two enzymes showed opposite enantiopreferences. The hydrolyses of the dibutyrates **98b**−**c** using PSL or SAM II as catalysts afforded, also in preparative useful enantioselecitivities, the opposite enantiomeric series of the monoacetates obtained via enzymatic hydrolysis.

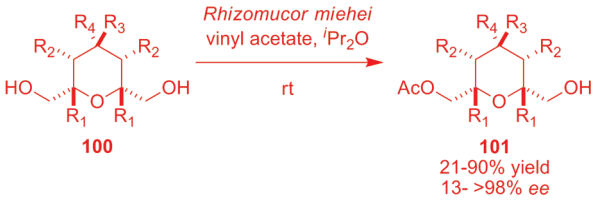
The efficient desymmetrization of highly substituted tetrahydropyranyl-2,6-methanols has been also accomplished by enzymatic acylation (Scheme 21).[78] The process was optimized for a large variety of compounds displaying different substitution in the six-membered ring (**100**). Both the yields and *ee* of the final monoacetate were very good except for those derivatives bearing bulky groups at the 3,5 positions (R_2 = Ph). Some of these enantioenriched building blocks could provide an easy access to both enantiomers of highly functionalized stereotetrads, and were used for the total synthesis of (+)-crocacin C.[79]

Hegemann et al. have recently published the first example of a lipase-catalyzed simultaneous separation of skeletal isomeric diols, with kinetic resolution of the one and desymmetrization of the other constitutional isomer (Scheme 22).[80] Namely, several lipases and esterases were screened for the transesterification of the 38:62 mixture of *meso*-**102** and *rac*-**103**, obtained from the transannular *O*-heterocyclization of *cis*,*cis*-cycloocta-1,5-diene. When *C. rugosa* lipase, which proved to be the most

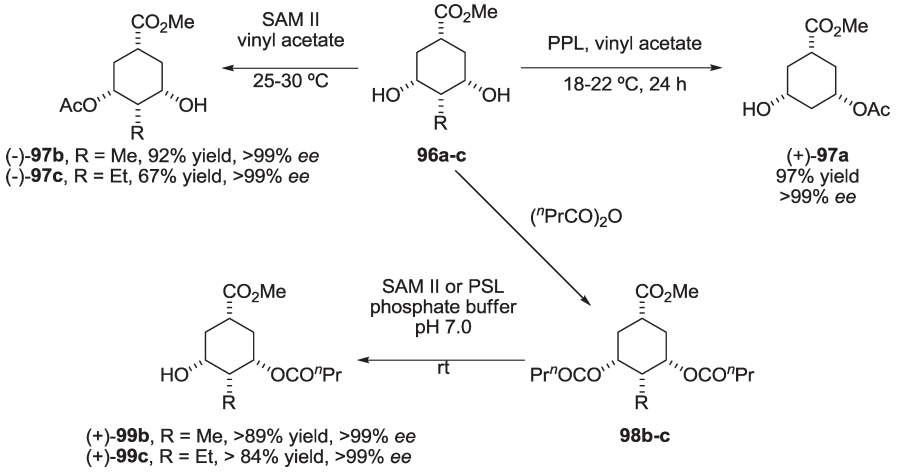
Scheme 19



Scheme 21



Scheme 20



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Scheme 22

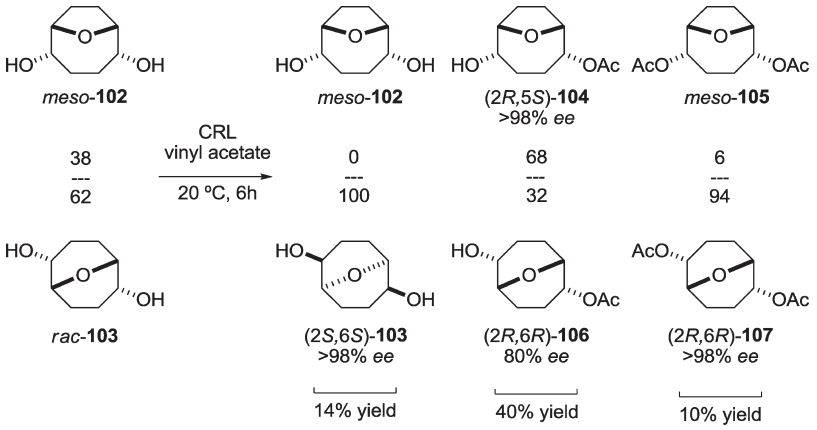
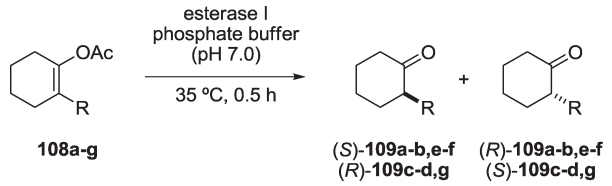


Table 3. EED of Prochiral α-Alkylated Cyclohexanone Enol Esters

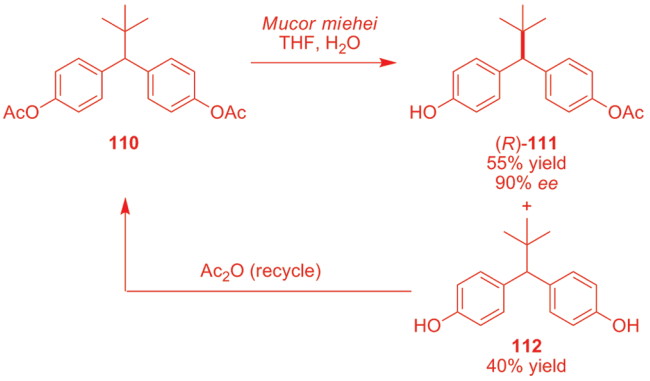


entry	substrate	R	product	*c* (%)	*ee* (%)
1	**108a**	Me	(*S*)-**109a**	>99	>99
2	**108b**	Et	(*S*)-**109b**	>99	14
3	**108c**	*i*-Pr	(*R*)-**109c**	10	17
4	**108d**	*t*-Bu	(*S*)-**109d**	21	5
5	**108e**	*n*-Pr	(*R*)-**109e**	>99	>99
6	**108f**	*n*-Pnt	(*R*)-**109f**	20	26
7	**108g**	Bn	(*S*)-**109g**	15	>99

efficient enzyme, was used as catalyst, a 67% of degree of conversion was reached after 6 h of reaction. That means that *meso*-**102** was transformed almost quantitatively, while the conversion of the racemate **103** stopped at 50% conversion. After separation of the enzyme, (−)-**103** was isolated enantiopure in 14% yield. Furthermore, (+)-**107** was also isolated enantiopure but was contaminated with 6% of *meso*-**105**. Finally, a 68:32 mixture of enantiopure (+)-**104** and (+)-**106** (80% *ee*) was the third fraction obtained from the crude reaction.

The enzymatic hydrolysis of prochiral α-substituted enol esters is an interesting process since the products of the reaction, the corresponding α-substituted enols, undergo enantioselective rearrangement in the active site of the enzyme to yield optically active α-substituted ketones. Hirata and co-workers have recently isolated two hydrolytic enzymes named esterases I and II from cultured plant cells of *Marchantia polymorpha*.[81] Several cyclohexanone enol acetates (**108a**−**g**) were subjected to enzymatic hydrolysis with these esterases to clarify the effect of various substituents at the *β*-position to the acetoxy group on the enantimeric ratio and the catalytic activity of the enzymes. Results showed that the enantioselectivities in the protonation of the enol intermediates were opposite between these enzymes

Scheme 23



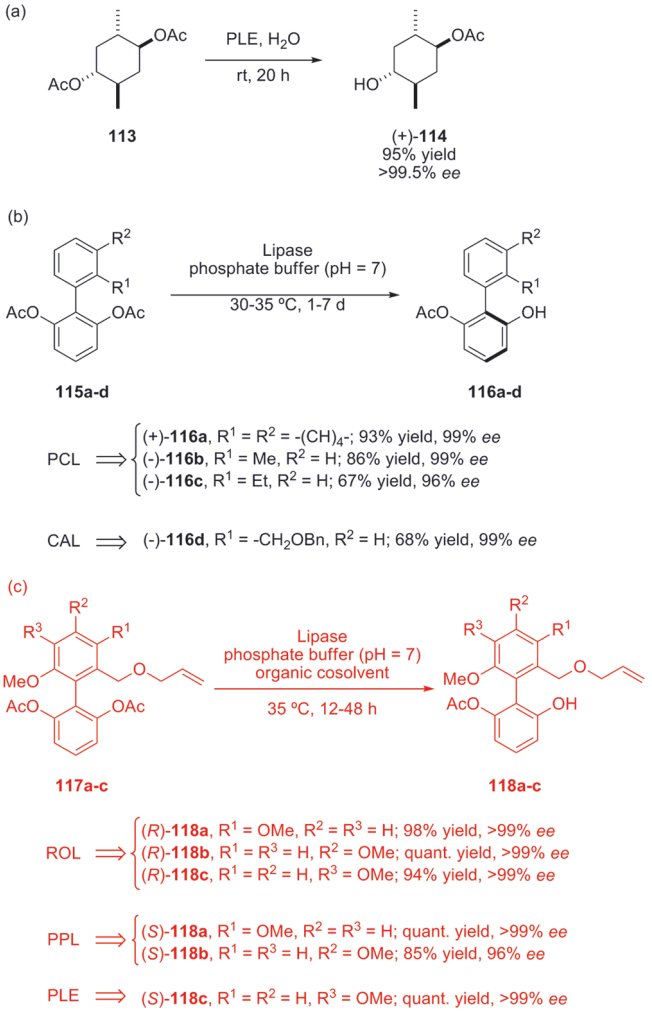
and that only esterase I afforded, in some cases, synthetically useful results (Table 3). For this enzyme, the acetate **108a** was the best substrate (entry 1), (*S*)-**109a** being obtained enantiopure and in excellent yield (99%). Replacement of the methyl group by an ethyl or an *i*-propyl substituent (**108b**−**c**, entries 2 and 3) led to the corresponding products with the same enantioselectivity but in lower *ee*'s. Moreover, when longer *β*-substituents were present in the substrate (**108d**−**g**, entries 4−7) an inversion of the enzymatic enantioselectivity was observed. The authors hypothesized that this enantioselectivity inversion could be attributed to the occurrence of a turnover of the substrate in the active site of the enzyme due to the steric hindrance offered by the α-substituents.

Miller and co-workers[82] described an interesting example of desymmetrization of a diacetate (**110**), where the reaction center is far away from the pro-stereogenic center (nearly 6 Å, Scheme 23). They used a biocatalytic hydrolysis to produce the monoacetate (**111**) in moderate yield and good enantiomeric excess. The side product in this case is the doubly hydrolyzed *meso* derivative **112**, which can be recycled by a simple chemical acylation. Interestingly, the authors also reported a synthetic, miniaturized enzyme mimic that catalyzes a comparable desymmetrization process, in this case by an acylation reaction. An exhaustive structural study gave some clues about the important noncovalent interactions responsible for the selectivity observed in the synthetic protein mimic.

Trauner and co-workers[83] have reported one of the few examples of the desymmetrization of molecules not possessing

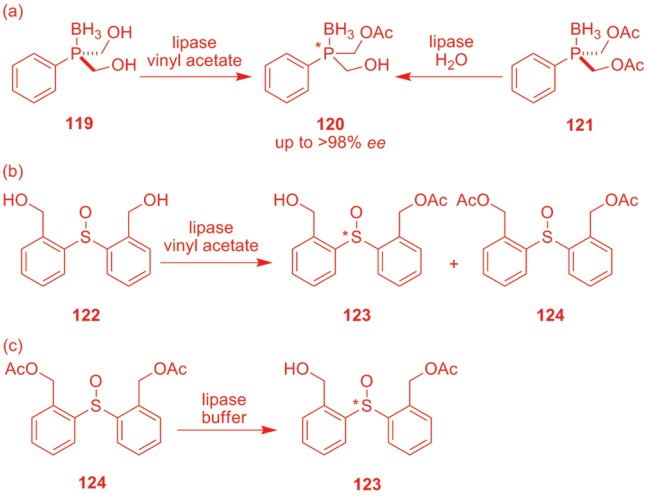
dx.doi.org/10.1021/cr100330u |*Chem. Rev.* 2011, 111, PR110–PR180

Scheme 24



a symmetry plane (σ). Thus, the centrosymmetric *meso* 1,4-diacetoxy-2,5-dimethylcyclohexane (**113**), which belongs to the molecular point group C_i (= S_2) and was obtained from *para*-xylene, was successfully hydrolyzed using PLE as catalyst (Scheme 24a). The monoester (+)-**114** was isolated enantiopure in excellent yield. Contrary to the well-known effect that the presence of organic cosolvents has on both the rate and selectivity of enzyme-catalyzed reactions, the addition of 10% DMSO only resulted in a moderate rate enhancement for the hydrolysis of **113** but no significant impact on the enantioselectivity. Similarly, only a few EEDs of compounds with a chirality axis or plane have been reported. As an exception, Matsumoto and co-workers[84] have published the first application of an enzyme to desymmetrize axially chiral nonracemic biaryls (Scheme 24b). This class of compounds is of current interest due to their importance as chiral ligands and auxiliaries in asymmetric synthesis. Furthermore, they also constitute a structural feature of many natural products. These authors have obtained biaryls **116a**−**d** in high *ee*'s and yields by desymmetrization of the corresponding σ-symmetric diacetates (**115a**−**d**) through lipase-catalyzed hydrolyses using lipases from *Candida antarctica* and *Pseudomonas fluorescens*. Moreover, these authors have recently applied this methodology to desymmetrize related biaryls but with full *ortho*-substitution (Scheme 24c).[85] For all

Scheme 25



substrates tested, the suitable choice of enzyme and reaction conditions allowed access to both enantiomeric series of the corresponding monoesters in excellent yields and enantiomeric purities.

Examples of EEDs for the preparation of chiral alcohols bearing the prochiral center in a heteroatom have been also described. For instance, the preparation of enantioenriched compounds with a chiral phosphorus has been efficiently carried out (Scheme 25a).[86] After screening different lipases, CALB was found to give excellent results in the desymmetrization of prochiral phosphine-boranes. Both enantiomers can be obtained in up to >98% optical purity via acetylation or hydrolysis, in processes that allow recycling of the substrates. The preparation of chiral sulfoxydes with an asymmetric sulfur center has been also carried out.[87] Two lipases, namely, CALB and lipoprotein lipase (LPL), proved particularly efficient to give 2-acetoxymethylphenyl 2-hydroxymethylphenyl sulfoxide in up to 98% yield and with up to 98% *ee* through acetylation reaction (Scheme 25b−c). On the basis of an X-ray analysis, the absolute configuration of the monoacetylated sulfoxyde was determined as (+)-(*R*). Interestingly, when assaying the hydrolysis of the corresponding diacetate as a complementary procedure, unexpected results were obtained. Thus, CALB again yielded the (+)-(*R*) enantiomer and LPL rendered different stereoselectivity depending on the organic cosolvent used. Anyway, the *ee*'s obtained for the hydrolysis in both cases were of limited synthetic utility.

4.2. Carboxylic Acid Derivatives

4.2.1. Esters. Hydrolases are also confirmed to be highly efficient in the desymmetrization of different *meso* and prochiral esters possessing the prochirality element in the acyl chain, compounds that can be easily prepared from inexpensive sources. This fact is reflected in the different examples collected in the literature.

A specially interesting family of sustrates for EED is that formed by α,α-disubstituted malonate diesters, since they bear a cuaternary prostereogenic center and they are synthetic precursors of non-natural amino acids. In two recent papers, Masterson[88] (Table 4, entries 1−3) and Back[89] (Table 4, entries 4−15) independently reported very good results in this field. In both cases, the monohydrolysis of the malonate diesters, catalyzed by PLE, rendered the corresponding monoacids with yields and *ee*'s

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Table 4. EED of α,α-Disubstituted Malonate Diesters

125 126

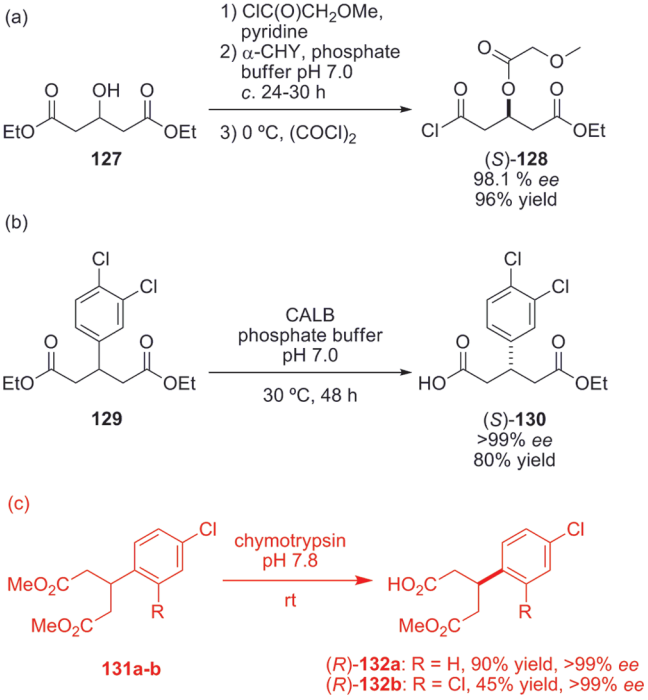
entry	substrate	R	R′	yield (%)	*ee* (%)
1[a]	**125a**	CH_2S-*t*Bu	Me	81	81
2[a]	**125b**	CH_2OBn	Me	69	70
3	**125c**	CH_2S-*t*Bu	Me	85	91
4	**125d**	$CH_3(CH_2)_5$	Me	85	67
5	**125e**	*i*-Pr	Me	82	42
6	**125f**	Me	$HC{\equiv}CCH_2$	85	63
7	**125g**	cyclohexyl (Cy)	Et	94	>98
8	**125h**	$CyCH_2$	Et	84	>98
9	**125i**	Ph	Me	90	>98
10	**125j**	$PhCH_2$	Et	98	52[b]
11	**125k**	$PhCH_2$	*n*-Pr	76	79[b]
12	**125l**	$PhCH_2CH_2$	Me	94	80[b]
13	**125m**	$PhCH_2CH_2$	Et	98	93[b]
14	**125n**	$PhCH_2CH_2$	*n*-Pr	89	94[b]
15	**125o**	$PhCH_2CH_2$	*n*-Bu	87	>98[b]

[a] Reaction carried out with the corresponding diethylester instead of the dimethylester. [b] Determined on a synthetic derivative.

ranging from moderate to very good (Table 4). Interestingly, the nature of the alcohol leaving group (EtOH or MeOH) slightly affected the enantioselectivity (compare entries 1 and 3 of Table 4). On the other hand, the nature of the substituents on the prochiral carbon seems to be the most important factor for the success of the EED. Although large steric differences between R and R′ are most likely beneficial for the final *ee*, some examples with longer chains in the "smaller" substituent showed very good results regarding both yields and stereoselectivity (entries 13−15). Both authors also reported the transformation of these valuable chiral synthons into their corresponding amino acid derivatives. Moreover, PLE-catalyzed desymmetrizations of related α,α-disubstituted malonate diesters have been recently implemented in the synthesis of polyketides[90] and silicon-containing unnatural amino acids.[91]

Öhrlein and co-workers have developed a multistep synthesis of Atorvastatin, a member of the statins family, which is a class of pharmaceuticals derived from the natural mevilonolacton.[92] This route proceeds through a versatile building block in the synthesis of statins, which is obtained in high yield and *ee* by desymmetrization of a derivative of diethyl glutarate using α-chymotrypsin as the biocatalyst of a hydrolysis reaction (Scheme 26a). Recently, large-scale direct desymmetrization of glutarate **127** has been accomplished by using lipase B from *Candida antactica*.[93] In less than an hour (*S*)-5-ethoxy-3-hydroxy-5-oxopentanoic acid was obtained in 95% yield and 90% *ee*. Similarly, the enzymatic desymmetrization of the prochiral diethyl 3-[3′,4′-dichlorophenyl]-glutarate (**129**), an intermediate in the synthesis of a series of neurokinin receptor antagonists, has been successfully developed and scaled up (Scheme 26b).[94] The hydrolysis of a similar prochiral glutarate diester had been studied, resulting in the product with the (*R*)-configuration.[95] In an attempt to find a catalyst with (*S*)-selectivity, the screening of about 200 commercial hydrolases was undertaken. Out of 11 candidates with *pro-S* selectivity, lipase B from *Candida antarctica* (Chirazyme L-2) was

Scheme 26



selected for further development. This hydrolysis, catalyzed by this lipase in either the free or the immobilized form, was carried out at 100 g/L of substrate and proceeded with an average conversion of 97% affording the monoacid of (*S*)- configuration. In the pilot plant, the process produced 200 kg of enantiopure (*S*)-**130** in three batches and in a high average isolated yield. The immobilized enzyme preparation was particularly effective, achieving over 70 000 enzyme turnovers per batch. Very similar diesters (**131a**−**b**) have been also recently prepared in excellent enantioselectivities and yields ranging from moderate to very good (45−90%) by an EED process (Scheme 26c).[96] The efficient monohydrolysis of the prochiral diester was catalyzed by chymotripsin, and the products [(*R*)-**132a**−**b**] were used for the synthesis of both enantiomers of two new inhibitors of the Botulinum neurotoxin serotype A protein.

PLE-catalyzed hydrolytic desymmetrizations of different *meso* and prochiral acyl donors have proven to be highly enantioselective. Thus, both paroxetine and its enantiomer have been synthesized through a chemoenzymatic approach in which chirality was introduced by means of a hydrolytic desymmetrization of dimethyl 3-(4′-fluorophenyl)glutarate. The corresponding (*S*)-monoester was thus obtained in 86% yield and 95% *ee*.[97] Likewise, PLE-catalyzed hydrolysis of a series of dialkyl esters of 2-cyclohexene-1,1-dicarboxylate (**133a**−**c**) afforded the monoesters of (*R*)-configuration [(*R*)-**134a**−**c**] in high yields and moderate *ee*'s (Scheme 27a).[98] The ethyl monoester (*R*)-**134b** was further employed in a synthetic route toward an α-substituted serine derivative.

A nice example of an industrial application of EEDs has been recently reported by Goswami et al. (Scheme 27b).[99] After trying different starting materials (diester or the corresponding cyclic anhydride), biocatalysts, and reaction conditions, they found an efficient process for the synthesis of the monoester, (1*S*,2*R*)-2-(methoxycarbonyl)cyclohex-4-ene-1-carboxylic acid **136** by *Candida antarctica* lipase (Novozym 435)-catalyzed desymmetrization

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of the corresponding diester, dimethyl-cyclohex-4-ene-*cis*-1,2-dicarboxylate. The process was optimized and scaled up to prepare a total of 3.15 kg of the 1*S*,2*R*-monoester from 3.42 kg of diester in two batches. The yield of the two batches ranged from 98.1 to 99.8% and the 1*S*,2*R*-monoester was enantiopure.

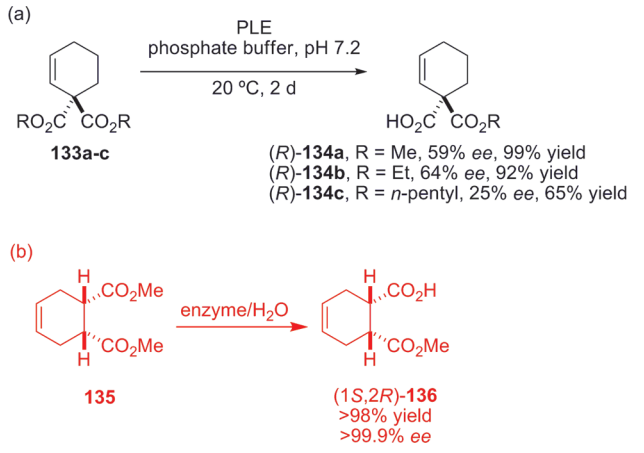
Additionally, pig liver esterase is known to be the enzyme of choice for the enantioselective hydrolysis of *meso cis*-1,2-cyclohexanedicarboxylic acid diesters. The so-obtained monoesters are useful chiral synthons for the preparation of both pharmaceuticals and natural products. Boaz[100] has reported an attempt to extend this methodology to the desymmetrization of a series of *meso cis*-cyclohexane-1,3-dicarboxylic acid diesters (**137a**−**e**, Table 5), prepared from the commercial cis/trans mixture of cyclohexane-1,3-dicarboxylic acids. Different enzymes were tested, and the analysis of the results showed, first, that contrary to the case of *cis*-cyclohexane-1,2-dicarboxylic acid diesters, PLE exhibited poor enantioselectivity in the EED of these 1,3-systems. Second, the lipase from *Pseudomonas cepacia* (PS-30) displayed opposite enantioselectivities to those of the lipase from *C. rugosa* (AY-30). Finally, the influence of the leaving group on both reaction rate and selectivity was high, the diethyl *cis*-cyclohexane-1,2-dicarboxylate (**137b**, entry 2) being the best substrate. This way both enantiomers of the monoester **138b** were prepared in high *ee* and overall yield (>80%).

Within the context of the enantioselective synthesis of the influenza neuraminidase inhibitor prodrug oseltamivir phosphate (Tamiflu) starting from cheap commercially available 2,6-dimethoxyphenol, a specific case of EED of *cis*-cyclohexane-1,3-dicarboxylic acid diesters has been reported (Scheme 28).[101] The PLE-catalyzed hydrolysis of the *meso*-diester **139** again afforded the (*S*)-monoacid **141** in high yield and *ee*. On the other hand, its enantiomer was similarly obtained via an EED of the same *meso*-diester with *Aspergillus oryzae* lipase, providing the (*R*)-monoacid **140** with even higher efficiency.

Kashima et al. have reported the first example in which a thermophilic esterase/lipase is used for the desymmetrization of a *meso* diester.[102] ESPESL 1864 proved to be superior to PLE and lipase AY from *C. rugosa* in the monohydrolyses of a series of *meso* dialkyl bicyclo[2.2.1]hept-2,5-diene-2,3-dicarboxylates (Scheme 29). In all cases examined, the monoesters (−)-**143a**−**c** were obtained in high chemical yields and enantiomeric purities.

Recently, the first enzymatic desymmetrization of a prochiral phosphine oxide has been reported.[103] Namely, bis(methoxycarbonylmethyl)phenylphosphine oxide (**144**) was subjected to hydrolysis in a phosphate buffer in the presence of several hydrolases, of which only pig liver esterase proved to be efficient (Scheme 30a). The expected product, viz. the monoester of (*R*)-configuration [(*R*)-**145**], was isolated after acidification and purification by column chromatography in 92% yield and 72% *ee*. The stereoselectivity showed by PLE is in accordance with the model established by Jones and co-workers.[104] Additionally, both enantiomers of the related monoacetate **147** were obtained by

Scheme 27



Scheme 28

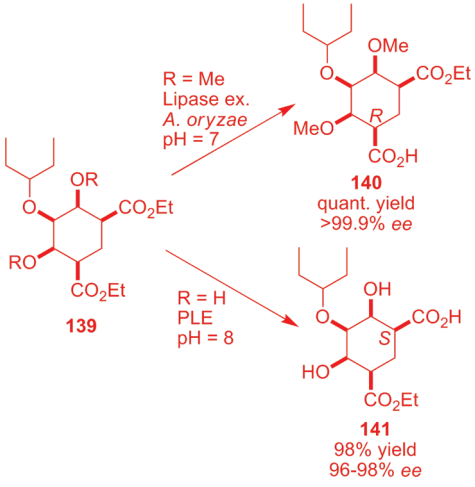
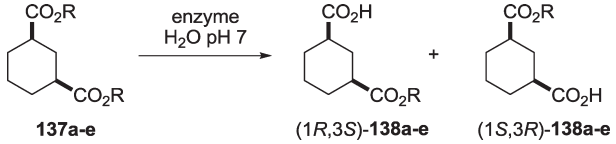


Table 5. EED of *meso cis*-Cyclohexane-1,3-dicarboxylic Acid Diesters



			PS-30		PLE		AY-30	
entry	diester	R	product	*ee* (%)	product	*ee* (%)	product	*ee* (%)
1	**137a**	Me	(1*R*,3*S*)-**138a**	82	(1*R*,3*S*)-**138a**	16	(1*S*,3*R*)-**138a**	62
2	**137b**	Et	(1*R*,3*S*)-**138b**	96	(1*R*,3*S*)-**138b**	34	(1*S*,3*R*)-**138b**	96
3	**137c**	*n*-Pr	(1*R*,3*S*)-**138c**	88	(1*R*,3*S*)-**138c**	32	(1*S*,3*R*)-**138c**	95
4	**137d**	*i*-Pr	(1*R*,3*S*)-**138d**	54	(1*R*,3*S*)-**138d**	32	(1*S*,3*R*)-**138d**	99
5	**137e**	*n*-Bu	(1*R*,3*S*)-**138e**	90	(1*R*,3*S*)-**138e**	47	(1*S*,3*R*)-**138e**	86

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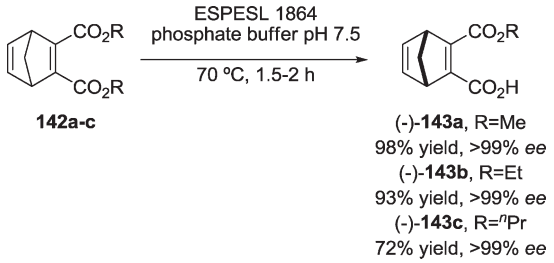
means of enzymatic hydrolysis and transesterification reactions, respectively (Scheme 30b). Conversely to the case of dicarboxylate **144**, several lipases proved to be suitable for these transformations and gave the aforementioned monoacetate in reasonable yields with moderate *ee*'s.

4.2.2. Anhydrides and Nitriles. Although hydrolases are also able to catalyze, in a highly enantioselective fashion, the desymmetrization of other *meso* and prochiral carboxylic acid derivatives apart from esters, surprisingly, they have been used with little profusion. The employment of different synthetically equivalent reagents constitutes a well-established methodology for the optimization of the enantioselectivity shown by an enzyme in the transformation of a particular class of compounds (substrate engineering). Thereby, we strongly believe that the application of acyl donors different from esters to biocatalysis and, more specifically, to EEDs, is going to be boosted soon.

The transesterification of *meso* and prochiral anhydrides can be efficiently catalyzed by hydrolases yielding the corresponding monoesters.[105] More especifically, for the desymmetrization of *meso* and prochiral glutaric anhydrides, it has been demonstrated that the appropriate choice of enzyme, protecting group, alcohol, and solvent allows access to either enantiomer of *O*-protected 3-hydroxy monoesters of glutaric acid.[106] Ostaszewski et al.[107] have carried out the desymmetrization of 3-phenylglutaric anhydrides (**149a**−**c**) by means of enzymatic monoesterification (Table 6). The so-obtained optically active monoesters **150a**−**c** were subsequently used for Ugi and Passerini multicomponent condensations, thus constituting a useful one-pot, two-step procedure for the preparation of optically active combinatorial libraries. Optimization of reaction conditions showed that ethereal solvents such as isopropyl and *tert*-butyl methyl ethers were the most suitable ones concerning both reaction rate and enantioselectivity. In addition to this, the utilization of different enzymes influenced the stereoselectivity and kinetics of the desymmetrizations. First, no nonimmobilized enzyme proved to be effective. Second, the *pro-S* selectivity observed for the majority of enzymes tested was reverted for the case of anhydride **149a** when Amano PS was used as catalyst. Third, a remarkable influence of the nature of substituents on the phenyl ring on both parameters was also evident. Additionally, reaction rates were observed to decrease in the series **149a** > **149b** > **149c** independently of the catalyst used; meanwhile, for the enantioselectivity, although it also varied, no clearly defined trend was found. In a more recent paper, the same authors deepen on the study of these substrates by performing the reaction by mainly assaying different reaction conditions.[108] They concluded that for 3-phenylglutaric anhydride **149a** and its chloro-substituted derivatives, Novozyme 435 showed the highest activity and enantioselectivity in *iso*-propyl ether, although the use of TBME was favorable in the case of the 4-methoxy-substituted anhydride (**149c**). Besides,

Scheme 29



Scheme 30

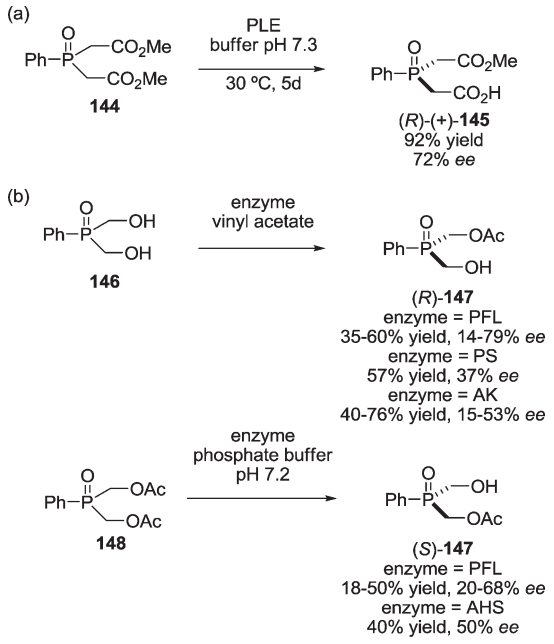
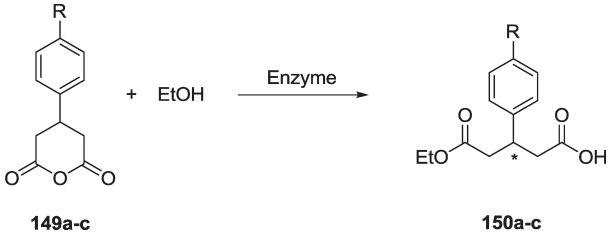


Table 6. EED of 3-Phenylglutaric Anhydrides



	150a (R = H)			**150b** (R = Cl)			**150c** (R = OMe)		
enzyme	major enantiomer	*ee* (%)	*t* (h)	major enantiomer	*ee* (%)	*t* (h)	major enantiomer	*ee* (%)	*t* (h)
Novozyme 435	*S*	78	61	*S*	67	120	*S*	67	528
Chirazyme L-2, C3	*S*	83	19	*S*	71	96	*S*	75	288
Amano PS	*R*	78	384						
Amano PS immobilized	*R*	78	19	*S*	17	96	*S*	99	168

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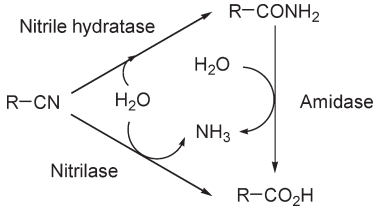
Scheme 31



the study on the effect of the enzyme amount demonstrated that the enantioselectivity of the reaction decreased significantly below a certain limit (135 mg of Novozym 435 per 1 mmol of anhydride). The reuse of the lipase was limited since each cycle was accompanied by a loss of the enzyme enantioselectivity and activity. Finally, they reported the first use of esters both as alkoxy donors and solvents for the EED of prochiral anhydrides.

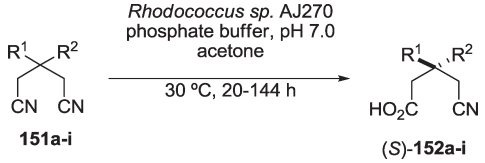
The enzyme-catalyzed hydrolysis of nitriles has been shown to proceed through two distinct pathways (Scheme 31).[109] Nitrilase enzymes convert a nitrile directly into the corresponding carboxylic acid and ammonia, whereas nitrile hydratases, metalloenzymes found in a number of bacteria, catalyze the hydration of the nitrile to the amide, which is then further transformed into the acid by the action of an amidase. In microorganisms containing a nitrile hydratase, usually also an amidase with a corresponding substrate specificity is found. In most cases, the amidase bears the highest enantioselectivity. As a rough rule, nitrilases are mostly active with aromatic nitriles; meanwhile, nitrile hydratases frequently present an assumed preference toward aliphatic nitrile substrates. So far, only a few examples of enantioselective nitrile hydratases leading to a product *ee* above 75% have been reported.[109]

Despite much attention given in recent years to the stereoselective biohydrolysis of nitriles, only a few examples of the EED of *meso* and prochiral nitriles have been reported. For instance, malonodinitriles bearing a chiral quaternary center at the 2 position have been effectively desymmetrized by using different *Rhodococcus rhodochrous* as catalysts. For instance, benzyl-[110] and butylmethylmalonodinitriles[111] have been transformed in excellent *ee*'s to the corresponding (*S*)-benzylmethyl malonoamide carboxylic acid and (*R*)-butylmethyl malonoamide carboxylic acid when *R. rhodochrous* IFO 15564 and ATCC 21197 were employed as catalysts, respectively. Enantioselectivity was attributed to the hydrolyses catalyzed by the corresponding amidases.

Rhodococcus sp. SP 361 cells from Novo Industri also catalyzed the desymmetrization of different *O*-protected 3-hydroxyglutarodinitriles affording the corresponding (*S*)-hydroxyglutaronitrile acids with moderate to good *ee*'s.[112] The enantioselectivity of the desymmetrization, confined to the nitrile hydratase, was higher when aryl protecting groups were employed. (*S*)-3-Hydroxyglutaronitrile monocarboxylic acid can be obtained enantiopure when *R. rhodochrous* IFO 15564 is employed. It catalyzed the hydrolysis of 3-benzoyloxyglutarodinitrile to the sole product (*S*)-cyanocarboxylic acid in an optically pure form without leaving any intermediate (*R*)-cyano amide.

The enantioselective hydrolysis of 3-alkyl- and 3-arylglutarodinitriles **151a**−**i** catalyzed by *Rhodococcus sp.* AJ270 cells, afforded the corresponding optically active cyanobutanoic acids (*S*)-**152a**−**i** with low to moderate enantiomeric excesses (Table 7).[113] Different additives were tested, and acetone, which presumably interacts with the biocatalyst, proved to dramatically enhance the enantioselectivity, giving, in some cases, enantiomeric

Table 7. EED of 3-Substituted Glutarodinitriles



151a-i (*S*)-**152a-i**

entry	compound	R^1	R^2	*t* (h)	yield (%)	*ee* (%)
1	**151a**	Ph	H	24	67	88
2	**151b**	4-F-C_6H_4	H	24	16	76
3	**151c**	4-Cl-C_6H_4	H	72	25	63
4	**151d**	4-Me-C_6H_4	H	24	25	95
5	**151e**	2-Me-C_6H_4	H	20	40	35
6	**151f**	4-MeO-C_6H_4	H	48	17	79
7	**151g**	Cy	H	20	60	83
8	**151h**	Bn	H	20	62	32
9	**151i**	Ph	Me	144		

excesses up to 95% (entry 4). It was additionally observed that dinitriles bearing electrodonating groups in the *para* position of the 3-phenyl ring seemed to lead to higher enantioselectivities (compare entries 4 and 6 with 2 and 3). Moreover, the presence of a chiral quaternary center in position 3 (**151i**, entry 9) causes loss of reactivity. Attempts to increase the yield of the monoacids (*S*)-**152a**−**i** by increasing the reaction time originated a decrease in the enantioselectivity. It was proposed that the enzymes of the microorganism responsible for the transformation are a nitrile hydratase and an amidase. The former would act as a regiospecific hydrating enzyme against the dinitrile and the latter would convert all the corresponding monocyanoamides into the acids rapid and completely. Moreover, the enantioselectivity of the overall hydrolysis would be derived from the action of the nitrile hydratase.

By means of the screening of genomic libraries coming from DNA directly extracted from environmental samples that have been collected from varying global habitats, DeSantis et al. have reported the discovery of over 200 new nitrilases, among which candidates able to enantioselectively desymmetrize 3-hydroxyglutarodinitrile (**153**) were found.[114] In particular, one of these (*R*)-specific nitrilases afforded (*R*)-**154** in excellent yield and *ee* (Scheme 32), a compound that previously could be only obtained with a highest 22% *ee*. However, attempts to develop a larger-scale process of this intermediate were plagued by lower *ee*'s due to high substrate concentrations. Nevertheless, application of the directed evolution technique Gene Site Saturation Mutagenesis permitted solving of this problem.[115] Thus, Ala190 and Phe191 were identified as enantioselective "hot spots". Particularly, the Ala190His mutant was the most selective and active biocatalyst, allowing complete conversion of **153** to (*R*)-**154** in 98% *ee* within 15 h at 2.25 M substrate concentration. On the other hand, these authors have also identified 22 nitrilases that afforded (*S*)-**154** with 90−98% *ee*.

Dinitriles bearing prochiral centers on atoms different from carbon have been also recently desymmetrized (Scheme 33). Thus, Kiełbasiński and Rutjes[116] have reported the successful transformation of bis(cyanomethyl) sulfoxide (**155**) into the corresponding optically active monoamide (**156**) and monoacid (**157**) with enantiomeric excesses ranging from low (10%) to very high (up to 99%) using a broad spectrum of nitrile hydrolyzing enzymes (Scheme 33a). Although the prochiral diacid (**158**) was

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also observed as a byproduct, among the five possible products of reaction, only the above-mentioned ones were isolated in representative amounts from the reaction mixture. The absolute configuration and *ee* of the final products largely depended on the enzyme used. The same group reported a similar study using prochiral bis(cyanomethyl)phenylphosphine oxide (**159**) as starting material, which yielded the corresponding optically active monoamide (**160**) and monoacid (**161**) with enantiomeric excesses ranging from low (15%) to very high (up to 99%, Scheme 33b).[117] In this case, these were the unique products observed. The analysis of the absolute configuration of both reaction products rendered some clues about the mechanism of their formation. The fact that identical absolute configurations are observed for both products in the same reaction clearly supports the concurrent formation of monoamide and monoacid over a sequential mechanism (Scheme 31). The cases in which the amide and the acid formed in the same reaction have opposite absolute configurations may suggest that the real stereorecognition is a result of a kinetic resolution at the stage of the hydrolysis of the initially formed racemic amide. Both situations were observed with different enzymes. However, in the latter case, the concurrent formation of both products cannot be excluded, with a bidirectional mechanism of action of nitrilases leading to both types of products. Taking into account the fact that amides are hardly accepted as substrates by nitrilases, the authors proposed the bidirectional mechanism as the most reasonable one.

5. AMINOLYSES AND AMMONOLYSES

In this section, we will focus on EEDs using primary amines and ammonia as nucleophiles, processes in which, in general, lipases are the enzymes of choice. Despite the importance of the amide bond in living systems, only a few examples of enantioselective amidation reactions by means of a desymmetrization process have been reported. Most of them have in common the utilization of glutaric acid diesters as substrates and lipase B from *Candida antarctica* as catalyst. In our research group, we have studied the desymmetrization of different dimethyl 3-substituted glutarates (**162a**−**g**) through enzymatic aminolyzes and ammonolyses (Table 8).[118] The reactions always stopped at the monoamide stage, and the enzyme showed a clear preference toward the *pro-R* ester groups, leading to the (*S*)-monoamides (*S*)-**164a**−**g**. Both chemical yield and stereoselectivity strongly depend on the substrate structure (compare for instance entries 1−3 with 13−15) and, to a lesser extent, on the nucleophile (**163a**−**c**, see for instance entries 1−3). As a general trend, those diesters bearing a heteroatom in R^1 (**162a**−**d**, entries 1−12) displayed the best yields and enantioselectivities. Conversely, diesters bearing the aliphatic methyl group (**162e**, entries 13−15) or the aromatic moieties Ph and *p*-F-Ph (**162f**−**g**, entries 16−21) led to longer reaction times and lower yields and *ee*'s. Nevertheless, higher *ee*'s can be obtained for these compounds when the reaction conditions are optimized (solvent and enzymatic preparation). It was hypothesized that the successful desymmetrization obtained with derivatives bearing a heteroatom at C3 was due to hydrogen bonding stabilization of these substrates in the enzyme active site. Additionally, some of

Scheme 32

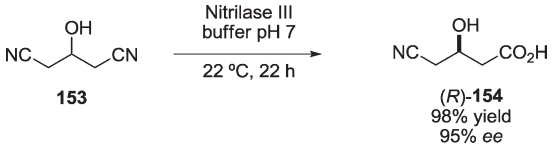
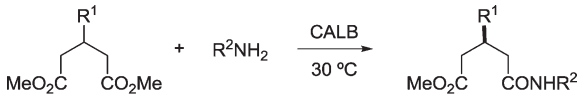


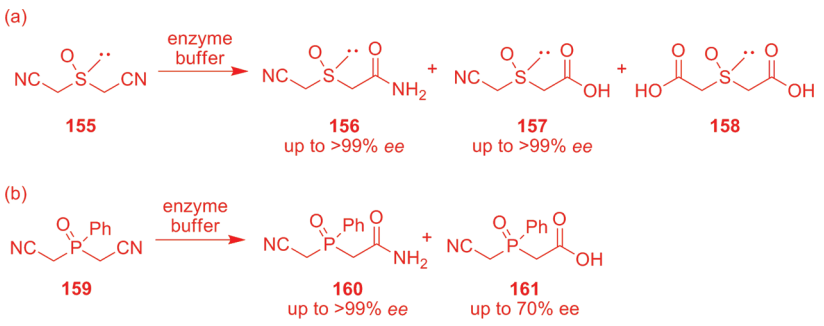
Table 8. EED of Dimethyl 3-Substituted Glutarates



162a-g **163a-c** (*S*)-**164a-g**

entry	diester (R^1)	amine (R^2)	*t* (h)	yield (%)	*ee* (%)
1	**162a** (OH)	**163a** (Bn)	9	98	>99
2		**163b** (Bu)		96	
3		**163c** (H)	5	98	
4	**162b** (NHBn)	**163a** (Bn)	48	92	>99
5		**163b** (Bu)		79	
6		**163c** (H)	72	85	
7	**162c** (OMe)	**163a** (Bn)	36	90	99
8		**163b** (Bu)		84	97
9		**163c** (H)	60	80	>99
10	**162d** (OAc)	**163a** (Bn)	36	40	>99
11		**163b** (Bu)	48	52	
12		**163c** (H)	72	69	
13	**162e** (Me)	**163a** (Bn)	72	67	76
14		**163b** (Bu)		60	71
15		**163c** (H)	96	63	72
16	**162f** (Ph)	**163a** (Bn)	240	12	88
17		**163b** (Bu)		17	92
18		**163c** (H)		12	
19	**162g** (*p*-F-Ph)	**163a** (Bn)	240	8	68
20		**163b** (Bu)		13	90
21		**163c** (H)		7	91

Scheme 33



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these monoamidoester derivatives have been used as starting materials for the efficient chemoenzymatic syntheses of biologically interesting compounds, such as the enantiopure γ-amino acids (*R*)-4-amino-3-hydroxybutanoic acid[119] and (*R*)-3,4-diaminobutanoic acid.[120]

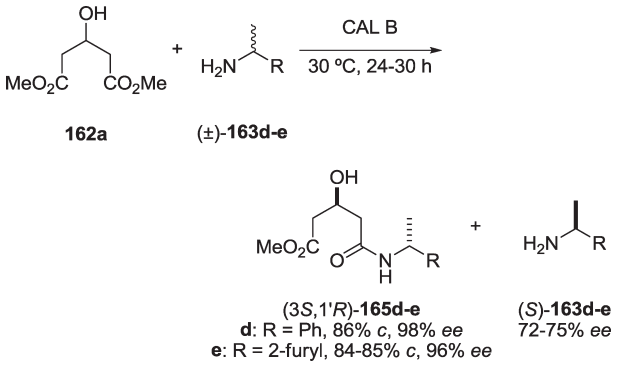
The synthetic potential of these desymmetrizations is even more interesting when it is combined with the concomitant resolution of the nucleophile. This way optically active compounds with more than one chiral center can be obtained one-pot. For instance, Sánchez et al.[121] have described an elegant desymmetrization-resolution scheme based on CAL B-catalyzed aminolysis reactions of dimethyl 3-hydroxyglutarate (**162a**) with different racemic amines [(±)-**163d**−**e**] (Scheme 34). Under suitable reaction conditions, the lipase was totally enantioselective toward the amines and highly enantioselective toward the diester, thus leading to the (3*S*,1′*R*)-monoamides **165d**−**e** with excellent yields, and enantiomeric and diastereomeric excesses.

Challis and co-workers have reported the enzymatic desymmetrization of citric acid (**166**) using spermidine (**167a**) as nucleophile (Scheme 35). Instead of a lipase, they employ as catalyst an ATP-dependent petrobactin biosynthetic enzyme from *Bacillus anthracis* (AsbA).[122] The enzyme belongs to the NIS peptide synthetase superfamily and it is highly selective: it stops at the monoamide stage and affords the corresponding (*S*)-amido acid in enantiopure form.[123] The enzyme is more specific for the acyl donor than for the nucleophile. Thus, among the many carboxylic acids tested, the enzyme only showed activity toward tricarballylic acid, an analogue of its natural substrate (citric acid) lacking the C-3 hydroxyl group. However, the condensation of several di- and triamine analogues of spermidine (**167b**−**e**) with citric acid also proceeded well.

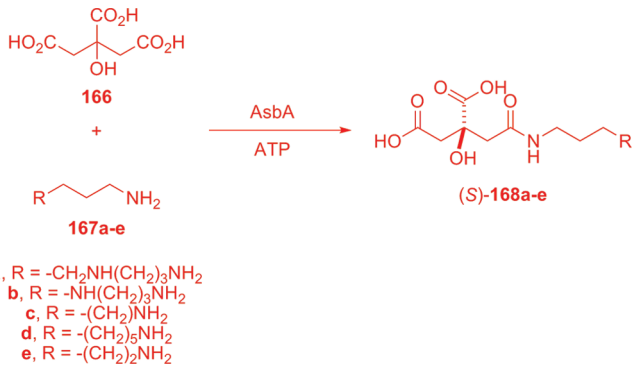
The novel NIS synthetase AcsD from *Pectobacterium chrysanthemi* has been also used for the desymmetrization of citric acid (**166**) with L-serine (**169**).[124] Similarly to AsbA from *Bacillus anthracis*, this enzyme is highly specific for citric acid (**166**) and ATP-dependent. However, the analysis of the reaction crude evidenced that, in this case, the product of the enzymatic desymmetrization is *O*-citryl-L-serine (**170**), which subsequently undergoes rearrangement to *N*-citryl-L-serine (**171**) under the reaction conditions via an intramolecular acyl migration (Scheme 36). Therefore, although the reaction is an enzymatic esterification, it can be regarded as a formal highly selective aminolysis in which the (*S*)-configured amido acid **171** is obtained enantiopure. Structural analysis of the crystallographic complexes formed by this enzyme with different substrates and substrate analogues, allowed the authors to propose a detailed mechanism of this reaction. First, the enzyme catalyzes the condensation of ATP and the *pro-R* carboxyl group of citrate to afford an adenylated intermediate of citric acid, and pyrophosphate. Enantioselectivity is achieved by the recognition of the central carboxylate of citrate by Thr301 and Arg305 and that of the hydroxyl group by Gln302. These interactions serve to position the *pro-R* carboxylate pointing toward the ATP site, which is consistent with a nucleophilic attack at the α-phosphate by citrate to make the enzyme-bound adenylate intermediate, which is finally decomposed by the attack of L-serine.

Similarly to the enzymatic amidation of glutarates, prochiral diamines can be also successfully desymmetrized. In particular, we have recently reported a general and straightforward methodology for the synthesis of optically active amino carbamates in high yields and enantiomeric excesses.[125] The key step is the lipase PS-catalyzed desymmetrization of prochiral 2-substituted

Scheme 34



Scheme 35



propane-1,3-diamines (**172a**−**t**, Table 9), which can be prepared from readily available starting materials. These compounds are valuable intermediates in organic synthesis since the selective cleavage of the allyloxycarbonyl moiety allows the functionalization at wish of both nitrogen atoms by means of a protection−deprotection strategy.[126] In all cases, the lipase shows a clear preference for the *pro-R* amino group, the enantioselectivity and reactivity being highly dependent on the nature of the 2-substituent bonded to the propan-1,3-diamine core. The computational study[127] of the two binding orientations of the parent compound of the series (**172a**, entry 1) shows that the enantioselectivity observed is due to the fact that the lowest energy conformation nicely fits the active site of lipase PS in a catalytically productive *pro-R* conformation, the unreacted amino group being optimally stabilized by means of hydrogen bonds. However, for the *pro-S* orientation to undergo catalysis, highly unfavorable contacts between the aromatic *ortho* carbons and the reactive amino group and the chiral carbon and the oxyanion have to occur. Furthermore, the exchange of the 2-phenyl group (entry 1) by a cyclohexyl one (**172o**, entry 15) evidence the key role played by aromaticity in the enantiorecognition mechanism of this type of substrate. This effect is further confirmed by the fact that the presence of 2-heteroaromatic groups (**172p**−**t**, entries 16−20) keep on rendering high enantioselectivities, although they decrease for the smallest heterocycles of the series(**172r**−**t**). The introduction of small *para* and *meta* substituents in the phenyl ring of **172a** (**172b**−**f**,**h**) slightly decreases the enantioselectivity (compare entry 1 with 2−6 and 8). This effect appears to be additive, as it is demonstrated by the

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Scheme 36

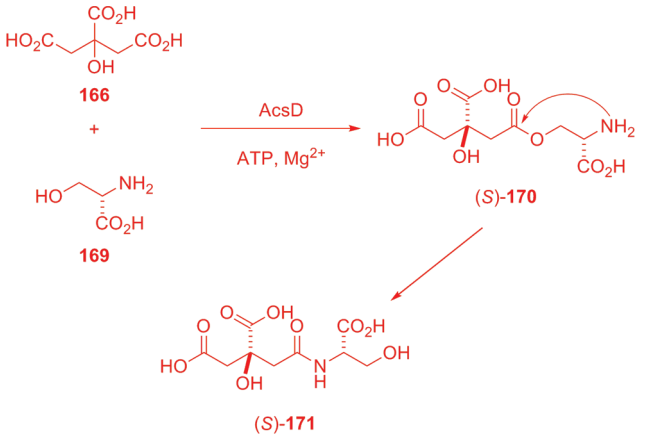
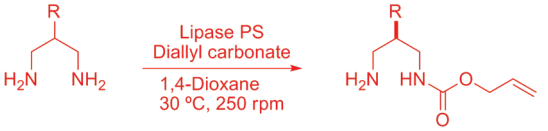


Table 9. EED of 2-Substituted-propan-1,3-diamines



entry	diamine	R	yield (%)	*ee* (%)
1	**172a**	phenyl	73	>99
2	**172b**	4-fluorophenyl	70	88
3	**172c**	4-methylphenyl	72	96
4	**172d**	4-methoxyphenyl	68	86
5	**172e**	4-trifluoromethylphenyl	65	91
6	**172f**	4-chlorophenyl	52	90
7	**172g**	4-biphenyl	62	64
8	**172h**	3-methoxyphenyl	62	91
9	**172i**	2-methoxyphenyl	39	60
10	**172j**	3,4-dimethoxyphenyl	65	79
11	**172k**	3,4,5-trimethoxyphenyl	64	70
12	**172l**	1-naphthyl	35	13
13	**172m**	2-naphthyl	61	87
14	**172n**	benzyl	70	69
15	**172o**	cyclohexyl	72	58
16	**172p**	pyridin-3-yl	58	89
17	**172q**	1*H*-indol-2-yl	47	88
18	**172r**	thien-2-yl	47	78
19	**172s**	furan-2-yl	48	71
20	**172t**	1*H*-pyrrol-2-y	53	76

results obtained with polysubstituted 2-phenyl rings (**172j,k,m**; entries 10,11,13). However, the *ortho* substitution almost abolishes the enzymatic selectivity and activity (**172i,l**; entries 9,12). This structure−enantioselectivity relationship can be explained on the basis of the available space in the active site of lipase PS for the fast-reacting orientation, as it is evidenced by the calculated relative solvent accessible surfaces of the atoms of the phenyl ring in the binding mode of *pro-R* **172a**. The case of the larger *para*-2-biphenyl substituent (**172g**, entry 7) constitutes, however, an exception, which has been attributed to deviations of the optimal torsion of a biphenyl group in the fast-reacting orientation of this diamine. Nevertheless, other factors like solvation effects cannot be discarded. Finally, the homologation of 2-aryl substituents by means of a methylene group (**172m**, entry 13) lowers the enantioselectivity by alleviating the conformational tension of the slow-reacting orientation of *pro-S* **172a**.

6. REDOX REACTIONS

The redox transformation of different substrates is another biocatalytic process that has been widely used in organic synthesis.[128] The enzymes that catalyze these reactions are termed oxidoreductases, and one of their most characteristic features is that they require a cofactor to exert catalysis. In most of the cases, it is the nicotinamide adenine dinucleotide [NAD(H)$^+$] or its phosphorylated derivative [NADP(H)$^+$]. Other types of cofactors present in oxidoreductases are flavines (FMN or FAD) and pyrroloquinoline quinone (PQQ). All of them have in common that they are more or less unstable and that they are usually expensive. In addition to this, although oxidoreductases have shown a wide substrate specificity, they are highly specific for the cofactor This fact, together with the unstability of the biocatalyst under certain reaction conditions, are usually the major bottlenecks of the application of oxidoreductases in industrial processes.[129] This explains why the modification of the oxidoreductase cofactor specificity,[130] together with the development of efficient cofactor recycling systems,[129,131] are probably the most active fields of research within this area of the biocatalysis.

There is an enormous amount of examples of EEDs by means of redox processes using either whole cells[132] or isolated enzymes[133] from different sources. In this section, we try to summarize the most outstanding examples corresponding to desymmetrizations of *meso* and prochiral ketones, alkenes, diols, carbon−hydrogen bonds, arenes, and sulfides, processes in which high enantioselectivities can be achieved.

6.1. Reduction of Ketones

Dehydrogenases and reductases are enzymes that catalyze the reversible reduction of carbonyl groups to alcohols. The natural substrates of the enzymes are alcohols such as ethanol, lactate or glycerol, and the corresponding carbonyl compounds; however, unnatural ketones can also be enantioselectively reduced. To exhibit catalytic activities, the enzymes require a cofactor such as NADH or NADPH, from which a hydride is transferred to the substrate carbonyl carbon. The oxidation of a hydrogen source (cosubstrate), usually by the same enzyme (substrate-coupled approach) or a different one (enzyme-coupled approach), is combined with the reduction of the oxidized cofactor and thereby is responsible for its recycling. In this sense, alcohols such as ethanol and 2-propanol and glucose, or formic acid and dihydrogen, among others, can be used as cosubstrates. During the recent past years, many advances have been done in the field of cofactor recycling,[129,134] thus allowing the utilization of biocatalysts which consume cheaper and environmentally friendly sources of energy. Although to carry out the enzymatic reduction of carbonyl compounds, whole-cell catalysts have traditionally been preferred because the cofactor is recycled by the cell metabolic machinery by simply adding the appropriate nutrient to the reaction medium, the enantioselectivity and the yield of these processes can be seriously affected by the presence of competing enzymes, as well as (co)substrate/(co)product

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permeability or toxicity problems can occur. This is why, in the recent past years, isolated oxidoreductases have been by far mostly used.

Baker's yeast (*Saccharomyces cerevisiae*) has been the most popular whole-cell biocatalyst for the desymmetrization of prochiral ketones.[135] Reductions catalyzed by this microorganism tolerate a large diversity of carbonyl substrates and, in general, follow Prelog's rule. This broad substrate acceptance is due to the presence of a number of reductase enzymes, all of which have been cloned. Although some of these reductases possess overlapping substrate specificities with opposite enantioselectivities, which often diminish the optical purities of the alcohol products, many approaches have been developed to selectively modify their activity. Thus, variations in substrate structure and concentration, the use of organic solvents in place of water, heat treatment, lyophilization and immobilization of the cells, the addition of exogenous modifiers, genetic manipulation, and the employment of isolated or overexpressed ketoreductases have allowed the optimization of Baker's yeast and other microorganisms enantioselectivity and opened a route to obtain both enantiomeric series of secondary alcohols.[136] Nevertheless, these goals also can be achieved by employing other biocatalysts. As it can be seen from the following examples, this option has been indeed used with profusion.

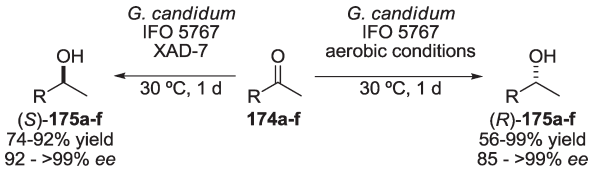
Nowadays, the biocatalytic reduction of simple prochiral alkyl aryl ketones is a well-established methodology. As a result, in most of the cases a proper biocatalyst that catalyzes the formation of the corresponding optically active secondary alcohols in excellent *ee*'s can be found. Conversely, the EED of small dialkyl ketones still remains a major challenge in organic chemistry. This is because microorganisms that effectively discriminate groups which are different by one or more methylene units are rare. With this regard, dried cells of the dimorphic fungus *Geotrichum candidum* IFO 4597 (APG4) constitute an exception. Thus, Matsuda et al. have demonstrated that when this microorganism was employed in the presence of $NAD(P)^+$ and 2-propanol, it not only effectively reduced different methyl aryl ketones[137] but also simple aliphatic ones (e.g., 2-pentanone, 2-butanone, and 3-hexanone). This way the corresponding (*S*)-alcohols were obtained with excellent enantioselectivities (94 to >99% *ee*) and in moderate to excellent yields (35−97%).[138]

Another way of improving the low enantioselectivity displayed by *G. candidum* in the preparation of (*R*)- or (*S*)- arylethanols is its immobilization on a water-absorbing polymer (BL-100). This way, and in the presence of additives such as hexan-2-ol, a series of enantiomerically pure (*S*)-1-arylethanols were prepared in high yields.[139] As a general trend, it was observed that the reduction of *ortho*-substituted acetophenones gave relatively better yields than that of *para*-substituted acetophenones, probably because the reverse reaction, oxidation, does not proceed for the *ortho*-substituted substrates. Moreover, a methyl group but not an ethyl or a propyl group is suitable as the smaller group adjacent to the keto group. On the other hand, the addition of another hydrophobic polymer has also permitted maximizing of the enantioselectivity of *G. candidum* in the reduction of a series of ketones to afford the corresponding (*S*)-alcohols.[140] In this case, this effect is because the hydrophobic polymer reduces the substrate and the product concentrations in the aqueous phase, since the substrate locates mainly in the polymer surface rather than in the aqueous phase. As the enzyme with the smallest K_m value reacts preferentially at low substrate concentrations, the (*S*)-enzyme, which is thought to have the smallest K_m value, would predominantly contribute to the reduction. Therefore, the (*S*)-alcohol is obtained preferentially. Namely, when Amberlite XAD-7 was employed in the reduction of different aliphatic (alkyl methyl and alkyl ethyl) and aromatic ketones with this microorganism, the *ee*'s and/or the yields were dramatically increased. However, for some candidates for which low enantioselectivities had been observed, the increase was not sufficient to apply the desymmetrizations with synthetic purposes. This fact was attributed to the preferential oxidation of the (*S*)-alcohols over the (*R*)-ones, thus lowering the obtained *ee*'s. To inhibit oxidation, the reductions of aromatic ketones were carried out in an argon atmosphere. This way, enantioselectivities were again increased regardless of the presence of XAD-7 in anaerobic conditions.

G. candidum in conjunction with Amberlite XAD-7 has been also successfully used for the preparation of both enantiomers of different secondary alcohols (**175a**−**f**) using ketones **174a**−**f** as the starting materials (Scheme 37).[141] *G. candidum* IFO 5767 afforded the corresponding (*S*)-alcohols in an excellent *ee* when Amberlite XAD-7 was added to the reaction system; meanwhile, when the reaction was conducted under aerobic conditions, the (*R*)-alcohols were isolated in an excellent *ee*. Again, it was hypothesized that aerobic conditions would activate the oxidation from the (*S*)-alcohol to the corresponding ketone, which is thought to be, in contrast to the reduction to the (*R*)-alcohol, a reversible process.

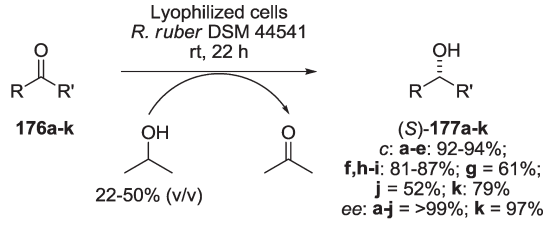
Large-scale application of the methods for the enantioselective hydrogen transfer based on alcohol dehydrogenases (ADHs) usually means high concentrations of the substrate of the reaction. Additionally, high concentrations of the cosubstrate are also required to drive the reaction to completion. In general, under these conditions, instability of the biocatalyst is observed, which impedes its application to synthesis. Nevertheless, as it is going to be shown in the following sections, some exceptions can be found in the literature. For instance, Kroutil and co-workers[142] have presented a highly enantioselective *sec*-alcohol dehydrogenase from *Rhodococcus ruber* DSM 44541, which is exceptionally stable toward organic solvents and which is able to enantioselectively catalyze the reduction of a broad spectrum of prochiral ketones **176a**−**k**, giving (*S*)-configured alcohols (*S*)-**177a**−**k** in high yields and excellent *ee*'s using 2-propanol as a hydrogen donor at 22% v/v (Scheme 38).[143] As an exception, the bicyclic ketones 1-indanone and 1-tetralone could not be reduced by employing this method. Although a single enzyme named ADH-"A" catalyzes both the reduction of the ketones and the oxidation of the cosubstrate, it was demonstrated that further ADHs compete for the substrates. Thus, the employment of lyophilized cells of *E. coli* containing the overexpressed ADH led to impressive enhancements of enantioselectivity for most of the ketones poorly desymmetrized by the wild type cells.[144] Both lyophilized cells of *E. coli* and recombinant ADH-"A" operate well a wide range of organic solvents up to 50% v/v, which emphasizes the robustness of this enzyme. However, higher concentrations of organic solvents are only tolerated by the cells, which can work under microaqueous conditions in hydrophobic organic solvents. Thus, a nice correlation between the logP of the solvent and the catalytic activity is observed.[145] The short-chain ADH from *Paracoccus pantotrophus* DSM 11072 has been also found to behave in a similar way. However, in this case, DMSO, which exhibits the lowest logP value of all the solvents investigated, was not only tolerated but led to a higher conversion and relative activity than the reaction carried out in aqueous buffer.[146]

Scheme 37



R: **a** = Ph; **b** = *p*-Br-Ph; **c** = $(CH_2)_2$Ph; **d** = CH_2OPh; **e** = 2-Py; **f** = 3-Py;

Scheme 38

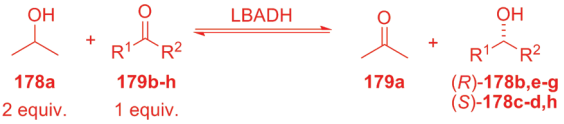


R: **a-j** = Me; **k** = Et
R': **a** = n-C_8H_{17}; **b** = n-C_7H_{15}; **c** = n-C_6H_{13}; **d** = n-C_5H_{11};
e = $(CH_3)_2C{=}CH(CH_2)_2$; **f** = Ph; **g** = *p*-$MeOC_6H_4$; **h** = *c*-C_6H_{11};
i = 2-Nf; **j** = (*E*)-Ph-HC=CH; **k** = n-C_5H_{11};

Gröger and co-workers have reported on the use of "designer cells" to carry out enantioselective ketone reductions in aqueous media at substrate concentrations >100 $g \cdot L^{-1}$ and without the need for any external cofactor.[147] In particular, the *E. coli* strain DSM14459 was used as host organism where an *R*-specific alcohol dehydrogenase from *Lactobacillus kefir* and a glucose dehydrogenase from *Thermoplasma acidophilum* were overexpressed. The analogous *S*-selective cells were similarly prepared by choosing an ADH from *Rhodococcus erythropolis* and the recombinant glucose dehydrogenase from *Bacillus subtilis*. Both whole-cell biocatalysts were tested against a diverse subset of ketones affording both *R*- and *S*-configured alcohols with conversions typically >90% and enantioselectivities of >99% in most cases. On the other hand, directed evolution can be also employed to improve the tolerance of oxidoreductases to high concentrations of organic solvents. Thus, phenylacetaldehyde reductase from *Rhodococcus sp.* has been engineered by a method that consists in the screening of a mutant library followed by the manual combination of the advantageous mutations, which led to an engineered enzyme that tolerated concentrations of 2-propanol higher than 20% v/v without affecting the enantioselectivity measured for a model substrate.[148]

The biocatalyzed reduction of haloketones proceeds quasi-irreversibly when coupled with (almost) stoichiometric amounts of different secondary alcohols, thus offering an alternative method to improve the stability of the enzyme by minimizing the concentration of cosubstrate.[149] Although ADHs nicely reduce α-chloroketones into the corresponding 1-chloro-2-alcohols, the oxidation of halohydrins like 1-chloro-2-octanol and 2-chloro-1-phenylethanol by a library of more than 60 commercial ADHs employing acetone as hydrogen acceptor could not be detected.[150] Analysis of the degrees of conversion shown by a selected set of ketones (Table 10) showed that electron-withdrawing groups on either side of the ketone increase the degree of conversion (**179c**−**e**,**g**−**h**; entries 2−4,6−7) with respect to the

Table 10. Substrate-Coupled EED of Alkyl Aryl Ketones Catalyzed LBADH



entry	ketone	alcohol	R^1	R^2	conversion (%)
1	**179b**	(*R*)-**178b**	Ph	Me	49
2	**179c**	(*S*)-**178c**	Ph	CH_2Cl	>99
3	**179d**	(*S*)-**178d**	Ph	CH_2N_3	96
4	**179e**	(*R*)-**178e**	*p*-O_2N-Ph	Me	84
5	**179f**	(*R*)-**178f**	*p*-MeO-Ph	Me	22
6	**179g**	(*R*)-**178g**	$MeOCH_2$	Me	89
7	**179h**	(*S*)-**178h**	MeO_2CCH_2	CH_2Cl	>99

reference substrate (**179b**, entry 1).[151] Moreover, the opposite effect is also observed (**179f**, entry 5). The employment of metal catalysts and the docking of ketones **179b**−**c** in the active site of LBADH revealed that the catalyst does not play any role on the degrees of conversion attained. Kinetic measurements and IR absorption bands of the carbonyl stretching band suggest, contrary to the general belief, that the presence of electron-withdrawing groups does not increase the electrophilicity of the corresponding carbonyl carbons but the double bond character of the ketones. Finally, ab initio calculations of the corresponding pairs alcohol−ketone discarded the blockade of the reaction by means of intramolecular hydrogen bonds and indicated that the conversions listed in Table 10 are mainly due to the thermodynamics of the reaction. This fact was proven by the oxidation of halohydrin **178c** with 1-chloropropan-2-one, a ketone with a suitable redox potential.

Another common approach to increase the enzymatic enantioselectivity of a microbial transformation is the purification of the enzyme responsible for the transformation to minimize the impact of the side reactions due to other enzymes present in the microorganism. This was the case of a carbonyl reductase purified from Baker's yeast by Ema et al.[152] This enzyme showed high enantioselectivity and broad substrate specificity in the reduction of different prochiral ketones. In most of the cases, the enantioselectivities were higher than the ones measured for the corresponding whole-cell reductions. Namely, 13 out of the so-obtained 20 alcohols had an enantiomeric purity of >98% *ee*; meanwhile, for the whole-cell transformations such *ee* values were only detected for 2 out of 20 substrates. Furthermore, the isolated yields for some ester-containing alcohols were higher in the enzymatic reductions than in the corresponding whole-cell ones. Concerning the substrate specificity of this isolated enzyme, the results obtained suggested the existence of a binding pocket with a marked preference toward ester and hydrophobic groups, the former being accommodated with a higher affinity than the latter. Thus, the enantiopreference observed for the enzyme (Prelog vs anti-Prelog alcohols) could be explained by means of this hypothesis. Moreover, the more unbalanced the putative binding abilities of the two substitutents flanking the carbonyl group were, the higher would be the enzymatic enantioselectivity.

Understanding the enzymatic enantioselectivity in terms of protein−substrate interactions is a useful approach that usually allows the identification of key interacting amino acids and, hence, lets know how substrates and/or enzymes can be

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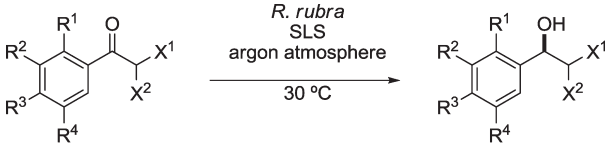
Table 11. EED of ω-Bromoacetophenone Derivatives



180a-h (*R*)-**181a-f**

entry	compound	R^1	R^2	R^3	R^4	X^1	X^2	*t* (h)	yield (%)	*ee* (%)
1	**180a**	H	H	H	H	H	Br	30	90	95
2	**180b**	H	H	H	Cl	H	Br	48	77	95
3	**180c**	H	H	$PhCH_2O$	H	H	Br	48	81	94
4	**180d**	H	H	$PhCH_2O$	CH_2OH	H	Br	48	79	95
5	**180e**	H	H	$PhCH_2O$	CH_2OH	Br	Br	40	82	92
6	**180f**	H	H	$-OCH_2OCH_2-$		H	Br	72	69	89
7	**180g**	OH	Br	H	CH_3	H	H	140		
8	**180h**	OH	H	H	CH_3	H	Br	140		

engineered in order to optimize their enzymatic transformations. With this regard, docking studies suggested a key role of Gln245 in the unusually low enantioselectivity shown by a carbonyl reductase from *Sporobolomyces salmonicolor* in the reduction of *para*-substituted acetophenones.[153] Saturation mutagenesis of this residue confirmed this hypothesis and allowed the identification of three mutants (Q245H, Q245P, and Q245L) with reversed enantiopreference and which allow the EED of this type of ketone in very high *ee*'s. This enantioselectivity behavior was rationalized in terms of "new" hydrophobic interactions between the mutant residues and the substrates.

The SADH from *Thermoanaerobacter ethanolicus* shows high enantioselectivity in the EED of ethynyl ketones and ketoesters.[181] This fact has been explained on the basis of the accepted enantiorecognition mechanism, which consists in two hydrophobic sites of different size in the active site of the enzyme that would be occupied by the two alkyl groups of these ketones.[154] On the basis of this information, it was envisioned that the W110A mutation would allow the large pocket also to bind phenyl ring-containing ketones. The results obtained did confirm this hypothesis since the mutant enzyme was able to reduce a series of such ketones related to the 2-butanone and acetone cores, thus yielding the corresponding Prelog alcohols in high yields and *ee*'s.[155] Moreover, immobilization of this mutant in a xerogel-encapsulated form confers it with a much higher tolerance to organic solvents.[156] Finally, and by following again a size rationale, the I86A mutant also allowed the acceptance of acetophenone derivatives with an inversion of the stereopreference of the enzyme, the anti-Prelog alcohols being now obtained in excellent optical purities.[157]

As it has already been mentioned, the screening for a suitable biocatalyst continues to be a major strategy when a given ketone or family of ketones has to be desymmetrized. In this sense, within the frame of a program to find a new enantioselective route to 2-substituted amino-1-arylethanols, several ω-bromoacetophenone derivatives **180a**−**h** were subjected to reduction to yield the (*R*)-2-bromo-(1-phenyl/substituted phenyl) ethanol derivatives (*R*)-**181a**−**f** (Table 11).[158] Among the biocatalysts tested, *Rhodotorula rubra* turned out to be the microorganism of choice. Analysis of different reaction conditions revealed that yields and *ee*'s were maximized when sodium lauryl sulfate was employed as surfactant under argon atmosphere. This way, bioreduction of phenacyl bromide (**180a**, entry 1) was improved to yield alcohol (*R*)-**181a** in excellent *ee* and yield. Similar results were obtained for the rest of substrates tested (entries 2−6) with the exception of **180g**−**h** (entries 7−8), for which the reaction did not proceed. This effect was attributed to the presence of free phenolic groups in these substrates. Bromoalcohols (*R*)-**181c**−**d** were subsequently employed to synthesize the active components of denopamine and salmeterol, respectively.[159]

The development of rapid screening and selection methodologies is essential to avoid time-consuming searches for a suitable biocatalyst. Homann et al. have recently achieved this goal by assembling a "targeted" library of microbes containing about 300 cultures representing 55 genera of bacteria, yeast, and filamentous fungi and testing it against different commercially available UV active alkyl aryl ketones for the ability to generate chiral secondary alcohols by enantioselective reduction (Chart 2).[160] The screening of this library, arrayed in multiwell plates, rapidly yielded a set of 60 microbes that reduced a series of 24 ketones, providing both the (*R*)- and (*S*)-enantiomers of the corresponding alcohols in 92−99% *ee* with yields up to 95% at 1−4 g/L. It is estimated that the described plate format afforded a 5−10-fold reduction in the average screen time compared to a conventional flask-based screening protocol. To demonstrate the utility of the targeted library approach, it was used to identify cultures capable of selective reduction of several target ketones, whose optically active alcohol derivatives were used as chiral intermediates in the preparation of three antiviral CCR5 antagonists (**182a**−**c**), an antidepressant NK1 receptor antagonist (**182d**), and an antimuscarinic M2 receptor antagonist (**182e**). This way, the bioconversions of all five ketone intermediates were identified within just a few days. Furthermore, by altering the time of substrate addition or by using a high concentration of resting cells, further improvements in reaction productivity were achieved. Li et al. have also reported on a practical approach to quickly discover new biocatalysts.[161] It consists of a preliminary selection of microorganisms that possibly contain a desired ADH, based on their degradation ability. The experiments are carried out in 96 deep wells of a microtiter plate at a 2 mM concentration of substrate using 0.55 mL of buffer. Then, a rapid screen of the enantioselectivities of these strains by means of GC

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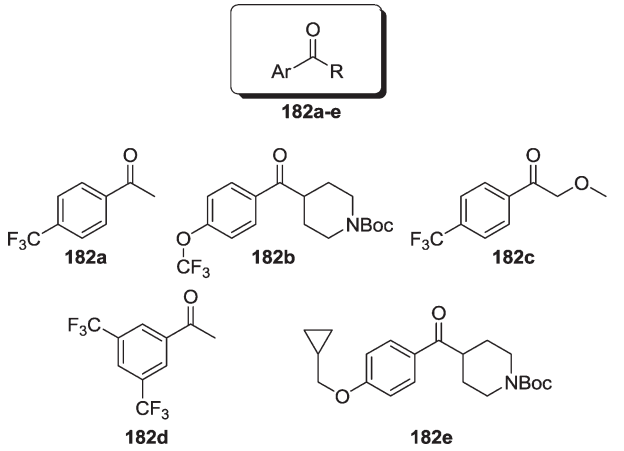
Chart 2

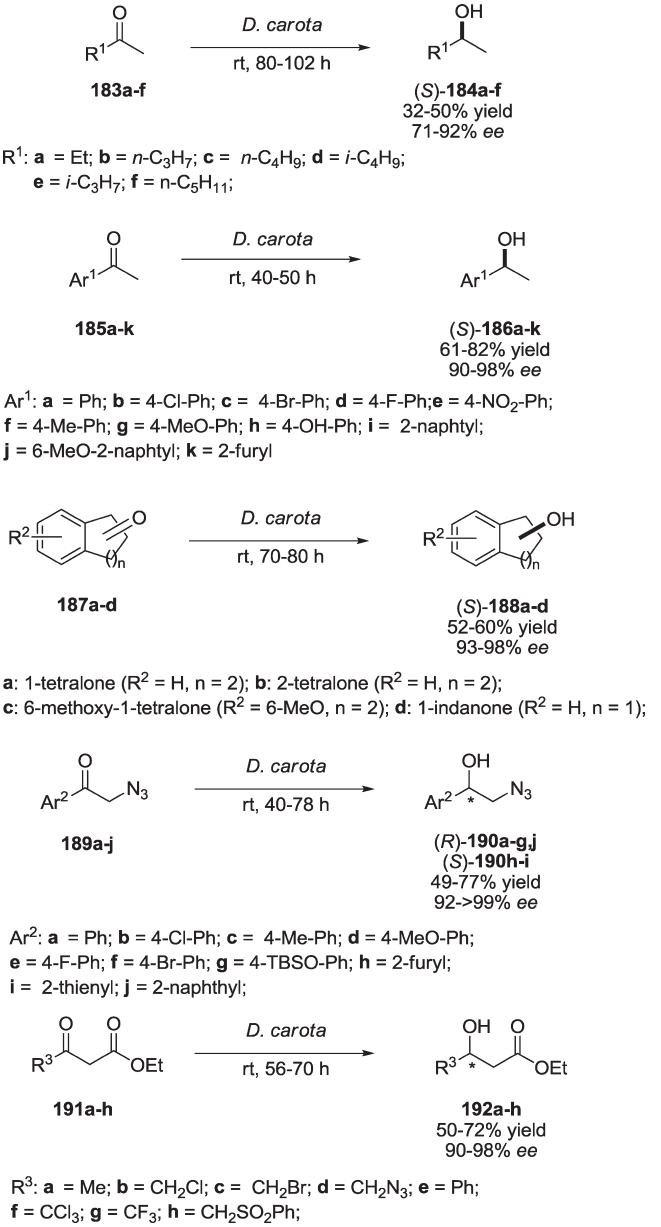


with an autosampler follows. The analysis is fast (12 min approx) and very sensitive, as it allows for product *ee* determinations in samples with only 2% conversion. The authors successfully applied this new methodology to the detection of suitable whole-cell catalysts for difficult-to-reduce β-ketoesters. In both cases, suitable catalysts were found and the preparative syntheses of both enantiomers of each of the respective alcohols were carried out in >80% yield and >87% *ee*.

The employment of whole plant cells in biotransformations is highly desirable because they can operate under extremely mild and evironmentally benign conditions. Unfortunately, their use has been impeded due to their usually slow rate of growth. However, some successful applications can be found in the literature. Recently, Baldasarre et al. have first reported the use of whole cells of carrot root (*Daucus carota*) for the enantioselective reduction of prochiral ketones.[162] Later, Yadav and coworkers systematically studied the bioreduction of aliphatic (**183a**−**f**), aromatic (**185a**−**k**), cyclic (**187a**−**d**), and azido ketones (**189a**−**j**) as well as of β-ketoesters (**191a**−**h**) using *D. carota* root cells (Scheme 39).[163] The results obtained showed that these cells efficiently catalyzed the desymmetrization of the ketones tested, with the exception of aliphatic ketones, for which somewhat lower *ee*'s and yields and higher reaction times were measured. In general, it was also observed that electron-withdrawing substituents attached to the carbonyl carbon had a positive effect on the reaction rate. The opposite was also true for electron-donating substituents. In particular, reduction of 2-azido-1-aryl ketones **189a**−**j** turned out to be a more convenient process than when Baker's yeast was used as catalyst. The azido alcohols **190d**−**g** were further used for the preparation of (*R*)-(−)-denopamine, (*R*)-(−)-tembamide, and (*R*)-(−)-aegeline.[164]

Apart from relatively simple dialkyl or alkyl aryl ketones, other more highly functionalized ketones also can be successfully desymmetrized by means of enzyme-catalyzed reductions in a chemoselective fashion. Thus, an ADH from *Pyrococcus furiosus* was shown to be an efficient catalyst in the EED of a wide series of α-chloroacetophenones, yielding the corresponding (*R*)-configured halohydrins in excellent *ee*'s also in a preparative scale.[165] On the other hand, the fungus *Rhizopus arrhizus* has been found an excellent catalyst for the reduction of acetophenones bearing halogen substituents on the aromatic ring (Table 12).[166] As a general feature, the presence of the electron-withdrawing

Scheme 39



halogens increases the reactivity of these ketones (compare the yields of acetophenone (entry 1, **193a**) and derivatives **193b**−**j** (entries 2−10)). Concerning enantioselectivity, the presence of a *m*-halogen substituent (**193c**,**f**,**i**), drastically reduces it (compare entry 1 with 3, 6 and 9). Conversely, the presence of *o*-halogens (**193b**,**e**,**h**) leads to excellent *ee* values (compare entry 1 with 2, 5, and 8). Finally, the *p*-substitution (**193d**,**g**,**j**) seems to have a much smaller effect on enantioselectivity (compare entry 1 with 4, 7, and 10). With regard to the effect of the halogen, the enantioselectivity increases with its size (F < Cl < Br; compare entries 2−4 with 5−7 and 8−10). Therefore, the effect of this type of substituent on the enantioselectivity seems to be rather steric than electronic. This also explains the observed "*ortho* effect", since such a substitution pattern leads to more hindered ketones. The inclusion of more halogen substituents had a detrimental effect on the yield of the reaction, although the reaction proceeded with excellent enantioselectivity (**193k**,**l**,

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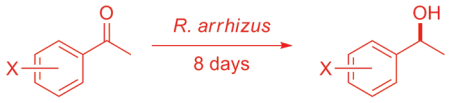
Table 12. EED of Halogenated Acetophenones Catalyzed by *Rhizopus arrhizus*



193a-l (*S*)-194a-l

entry	ketone	X	R^1	conversion (%)	*ee* (%)
1	**193a**	H	Me	63	75
2	**193b**	2-F	Me	73	96
3	**193c**	3-F	Me	66	40
4	**193d**	4-F	Me	48	72
5	**193e**	2-Cl	Me	77	97
6	**193f**	3-Cl	Me	78	48
7	**193g**	4-Cl	Me	80	85
8	**193h**	2-Br	Me	73	>99
9	**193i**	3-Br	Me	71	60
10	**193j**	4-Br	Me	72	88
11	**193k**	2,4-dichloro	Me	32	94
12	**193l**	2,3,4-trichloro	Me	15	92

entries 11−12). The study was also extended to the corresponding halogenated propiophenones. Although the results show a similar trend as with acetophenones, the extra methylene group causes a general drop in the yield. However, the enantioselectivity increases.

Halohydrins bearing halogen substituents in the aromatic ring are interesting chiral synthons in the preparation of many pharmaceuticals. Thus, the synthesis of a leading candidate compound in an anticancer program required (*S*)-2-chloro-1-(3-chlorophenyl)- ethanol as an intermediate.[167] Screening of 119 microbial cultures for *S*-enantioselectivity afforded *Hansenula polymorpha* ATCC 58401 (73.8% *ee*) as a potential hit. From it, a ketoreductase was purified, cloned, and overexpressed in *E. coli* together with a glucose 6-phosphate dehydrogenase from *Saccharomyces cerevisiae* to allow NADP(H) recycling. The so-prepared catalyst was used to prepare the target alcohol and two other related ones [(*S*)-2-chloro-1-(3-chloro-4-fluorophenyl)-ethanol and (*S*)-2-chloro-1-(3-chloro-4-methoxyphenyl)-ethanol] in high yields and enantiopure form. A similar ketone, 3,4-dichlorophenacyl chloride (**195**) was reduced with growing cells from *G. candidum* (CBS 233.76) in the presence of a hydrophobic adsorbing resin at 4 g/L to yield the corresponding alcohol (*S*)-**196** in excellent *ee* and yield (Scheme 40).[168] This compound was further transformed into (*R*)-3,4-dichlorophenylbutanolide, intermediate in the synthesis of (+)-*cis*-(1*S*,4*S*)-sertraline. On the other hand, employment of growing cultures of *Rhodotorula mucillaginosa* (CBS 2378) afforded the opposite enantiomer of the aforementioned alcohol (*R*)-**196** in excellent yield and *ee* as well.

Trifluoromethyl substituents are important to medicinal chemistry as a useful tool to prevent degradation of the active compounds where they are present by human metabolism. Thus, the obtaining of perfluorinated optically active alcohols is an active field of research in the pharmaceutical industry. With this regard, perfluorinated prochiral ketones (**197**−**200**, Chart 3) were desymmetrized by using isolated ADHs from different sources (*Lactobacillus brevis*, *Thermoanaerobacter* species and

Scheme 40

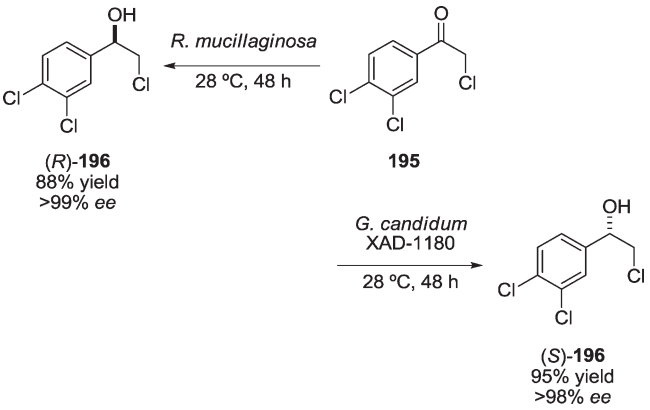
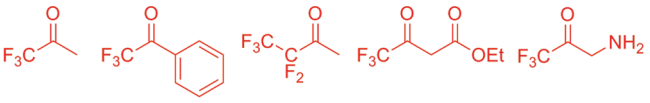


Chart 3



197 198 199 200 201

two from *Rhodococcus species*).[169] The results revealed, in general, good activities but for ADH RS2. Comparison of the activities with the corresponding nonfluorinated ketones shows that replacement of a CH_3 by a CF_3 group leads to significantly decreased activity, thus indicating the larger steric demand of the trifluoromethyl group. However, higher activities were found for 3,3,4,4,4-pentafluorobutanone (**199**) as compared to 1,1,1-trifluoroacetone (**197**) and 2-butanone, which indicates a higher reactivity of the carbonyl group of this ketone. Concerning enantioselectivity, both antipodes of the corresponding alcohols were obtained in all cases in *ee* values ranging from 93% to >99% by a sensible selection of enzyme and cofactor recycling system. Although in a few cases the degrees of conversion attained were not that satisfactory (<50%), it is worthy of mention that this methodology allowed the preparation of both enantiomers of the highly symmetric alcohol 1,1,1-trifluoropropan-2-ol in a 100 g scale. On the other hand, the prochiral ketone **182d** (Chart 2), which bears two trifluoromethyl groups in the aromatic ring of the acetopheone core, has been efficiently desymmetrized by an isolated alcohol dehydrogenase from *Rhodococcus erythropolis*.[170] This enzyme afforded, in almost quantitative conversion, the corresponding enantiopure (*S*)-alcohol, key compound in the preparation of different NK1 antagonists under clinical evaluation at the time of publishing. Process optimization allowed the pilot scale preparation of this alcohol using high substrate concentration (390 mM) and achieving excellent isolation yields (>90%) and effective space time yield (100−110 g/L d). In this same sense, α,α-difluorinated-1,3-dicarbonylic compounds have been desymmetrized in excellent conversions and *ee*'s into the corresponding (*S*)-configured monoalcohols by employing a carbonyl reductase from *Saccharomyces cerevisiae* overexpressed in *E. coli*.[171] Moreover, the opposite enantiomeric series, which could be also accessed by using enzyme E039, served as starting material for the preparation of an α,α-difluorinated derivative of (*R*)-β-aminobutyric acid in three steps. Finally, the small but

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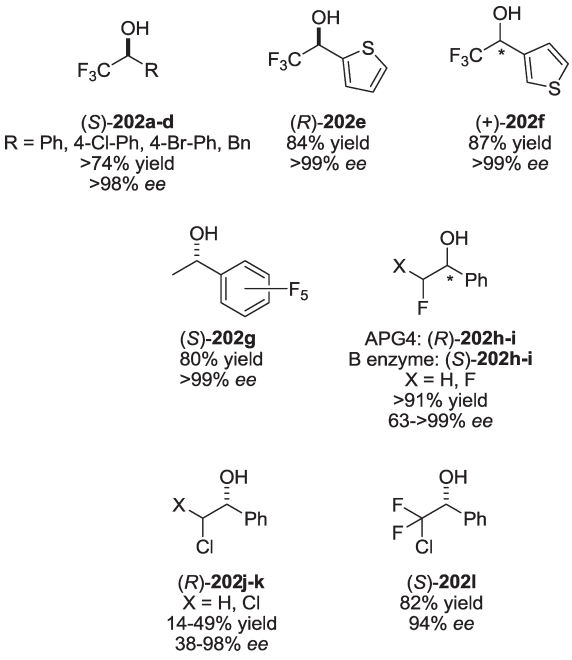
Chart 4

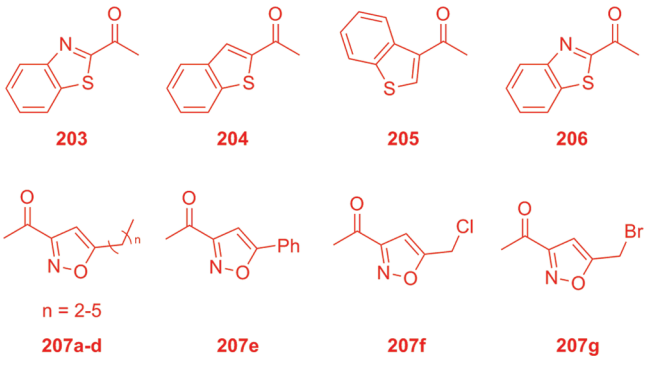


versatile β-amino trifluoromethylketone **201** (Chart 3) has been successfully desymmetrized by using the commercially available reductase KRED102. Thus, the corresponding (*R*)-alcohol was obtained in >99% *ee* and 95% yield.[172]

Reduction of different trifluoromethyl ketones using *G. candidum* IFO 4597 (APG4) as catalyst afforded the corresponding (*S*)-alcohols in excellent *ee*'s and yields but with opposite configuration to that observed in the reduction of the analogous methyl ketones.[137] Investigation of this behavior led to the isolation of two enzymes with opposite enantioselectivities: one of them (the "A enzyme") catalyzed the reduction of methyl ketones, and the other (the "B enzyme") catalyzed reduction of trifluoromethyl ketones. When whole cells of this microorganism are used, dihalomethyl ketones constitute the saddle point in the inversion of the enantioselectivity as a function of the number of halogen atoms attached to the methyl group. Next, both APG4 and the isolated "B enzyme" were applied to the reduction of different fluorinated ketones on a preparative scale, which afforded the corresponding optically active alcohols (**202a**−**l**) in excellent *ee*'s (Chart 4).

Heteroaryl containing synthons are very useful in organic synthesis for a wide variety of reasons. Accordingly, much attention has been paid to the preparation of optically active heteroaryl secondary alcohols. For instance, Irimie and co-workers have recently set up a chemoenzymatic methodology for the preparation of both enantiomers of a small series of secondary alcohols that contain a benzofusionated 5-membered aromatic heterocycle (**203**−**206**, Chart 5).[173] Nonfermenting Baker's yeast catalyzes the formation of the (*S*)-alcohols at a preparative scale in *ee* > 98% and yields >90%. Subsequently, a Mitsunobu reaction affords the (*R*)-alcohols without racemization and in yields >80%. On the other hand, plant cultured cells of *Caragana chamlagu* efficiently catalyze the EED of a series of 3-acetyl isoxazole derivatives (**207a**−**g**, Chart 5).[174] The corresponding (*S*)-alcohols were obtained in high *ee* values (>90%) and yields (>70%, with the exception of **207g**, 41%) after 12−24 h of reaction.

Chart 5



Chiral 4-(dimethylamino)pyridine (DMAP) derivatives are interesting compounds due to their importance as (scaffolds for) chiral ligands to be used in asymmetric synthesis. We have recently reported the preparation of a series of 3-substituted DMAP derivatives containing a secondary alcohol function (**208a**−**e**) by means of Baker's yeast mediated reduction of the corresponding ketones (Scheme 41a).[175] The resulting (*S*)-alcohols [(*S*)-**209a**−**e**] were obtained in high yields and excellent *ee*'s. They were subsequently transformed into the corresponding DMAP derivatives, which after suitable protection of the hydroxyl group, were tested as catalysts in different enantioselective chemical reactions like the kinetic resolution of 1-phenylethanol with chloroformiates and $ZnCl_2$, the addition of $ZnEt_2$ to benzaldehyde, and the Steglich rearrangement of an enol carbonate. In all cases, the reactions occurred with enantioselectivity. Baker's yeast has been also shown to be a useful catalyst in the preparation of alcohol (*S*)-**211** using ketone **210** as starting material (Scheme 41b).[176] Subsequent transformation of the hydroxyl group into amine yielded the pincer ligand (*S*)-**212** in enantiopure form. This type of compound complexed with Os and Ru are useful catalysts in the enantioselective transfer hydrogenation of prochiral ketones.

A series of *o*-, *m*-, and *p*-substituted α-hydroxyphenylethanones (**213a**−**h**) have been reduced to the corresponding 1,2-diols using *Geotrichum* sp. cells as catalyst (Table 13).[177] The position of the substituent had remarkable effects on the enantioselectivity displayed by this microorganism. Thus, *o*-substituted ketones gave generally (*R*)-1,2-diols in moderate *ee*'s (**213b**,**e**; entries 2 and 5), whereas (*S*)-1,2-diols were obtained by reduction of unsubstituted or *m*- and *p*-substituted phenacyl alcohols (**213a**,**c**−**d**,**f**−**h**) in good to excellent *ee*'s (entries 1, 3−4, and 6−8). From the reduction of **213h**, it can be hypothesized that the steric bulk of the substituents also affects the rate of the bioreduction (compare entries 1−7 with entry 8). Additionally, the analogous desymmetrization of the corresponding α-acetoxyphenylethanones turned out to be a less enantioselective and also more complex transformation due to the concomitant biohydrolysis of the ester moiety present in these substrates.

A concise highly chemo- and enantioselective preparation of (*S*)-2-ethyl-1-phenylprop-2-en-1-ol [(*S*)-**216**] via extractive biocatalysis by the yeast *Pichia stipitis* CCT 2617-mediated reduction of 2-ethyl-1-phenylprop-2-en-1-one (**215**) adsorbed on Amberlite XAD-7 has been reported (Scheme 42).[178] The employment of the polymer eliminated the limitations observed

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Scheme 41

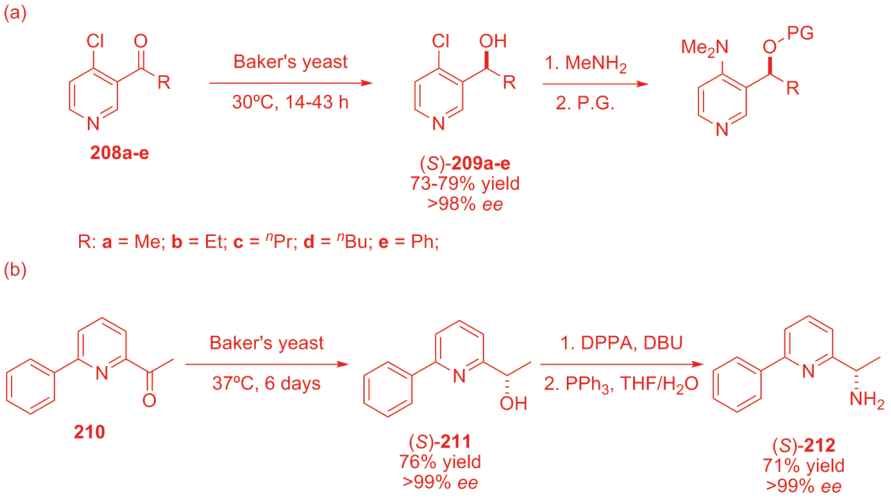
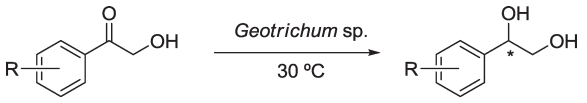


Table 13. EED of Substituted α-Hydroxyphenylethanones



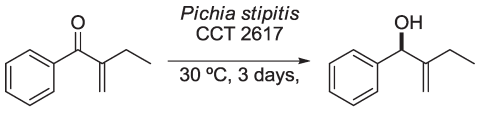
entry	substrate	R	*t* (h)	yield (%)	*ee* (%)	configuration
1	**213a**	H	16	62	91	*S*
2	**213b**	*o*-Cl	15	77	60	*R*
3	**213c**	*m*-Cl	12	93	77	*S*
4	**213d**	*p*-Cl	12	91	96	*S*
5	**213e**	*o*-NO_2	15	37	47	*R*
6	**213f**	*m*-NO_2	12	84	98.5	*S*
7	**213g**	*p*-NO_2	10	95	100[a]	*S*
8	**213h**	*p*-CH_3SO_2NH	48	67	99.5	*S*

[a] Theoretical value.

in the degree of conversion and yield when the reaction was carried out conventionally.

Similarly, propargylic alcohols have been prepared through chemoselective EED of acetylenic ketones. For instance, the oxidoreductases *Lactobacillus brevis* alcohol dehydrogenase (LBADH), easily available in the form of a crude cell extract from a recombinant *Escherichia coli* strain (recLBADH), and *Candida parapsilosis* carbonyl reductase (CPCR) are suitable catalysts for the reduction of a broad variety of ketones of this type (**217a**−**r**), affording the corresponding propargylic alcohols (**218a**−**r**) in good yields and excellent *ee*'s (Table 14).[179] More specifically, recLBADH affords the alcohols of (*R*)-configuration; meanwhile, CPCR presents a preference toward the (*S*)-configuration. When the propargylic ketones contained an aromatic moiety conjugated to the α,β-unsaturated ketone fragment, both (*R*)- and (*S*)-**218a**−**i** were obtained enantiopure, regardless of the nature of the aromatic system (entries 1−9). The synthesis of both enantiomers of (*R*)- and (*S*)-3-butyn-2-ol

Scheme 42



was also attempted. Its preparation has remained as a great challenge in organic synthesis due to the similar steric demands of the methyl and ethynyl residues which flank the ketone moiety. In fact, the bioreduction of this substrate (entry 10) afforded the corresponding alcohols with moderate *ee*'s. Nevertheless, protection of the acetylenic moiety with silyl groups proved to be a successful approach, and when the phenyldimethylsilyl moiety was employed, both alchols were obtained enantiopure. This way, it was demonstrated that a bulky substituent at the alkyne moiety results in a higher selectivity in the reduction, which is more pronounced for the case of recLBADH (compare entries 10−14 for both enzymes). It was also studied that by changing the steric demand of the substituents the *ee* values can be adjusted and even the configurations of the products can be altered (entries 15−18). It is again evidenced that recLBADH is more sensitive to changes in the enantioselectivity due to modification of the substituents than CPCR.

These authors have also succeeded in the enzymatic reduction of the α-halo propargylic ketones **217s**−**v** (Chart 6).[180] Namely, horse liver ADH (HLADH) and *Thermoanaeroium brockii* ADH (TBADH) afforded the corresponding enantiopure (*R*)-α,β-alkynyl α-chloro or α-bromohydrins; meanwhile, recLBADH allowed the preparation of the opposite enantiomeric series in >98.5% *ee*. For the case of ketone **217a**, the enzymatic reductions were carried out on a preparative scale and the products were isolated in >95% yield. The so-obtained halohydrins were subsequently transformed into the corresponding propargylic epoxides in good yield and without racemization.

Secondary alcohol dehydrogenase (SADH) from *Thermoanaerobacter ethanolicus*, an NADP-dependent, thermostable

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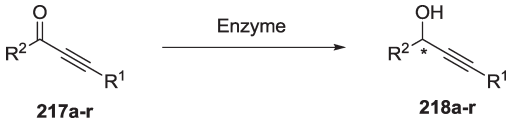
Table 14. EED of Acetylenic Ketones



217a-r **218a-r**

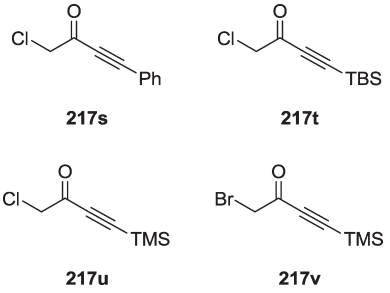
	alcohol			recLBADH			CPCR		
entry	**218**	R^1	R^2	*c* (%)	*ee* (%)	config	*c* (%)	*ee* (%)	config
1	**a**	Ph	Me	>99	>99	*R*	80	>99	*S*
2	**b**	4-MeO-Ph	Me	70	>99	*R*	30	>99	*S*
3	**c**	4-F-Ph	Me	100[a]	>99	*R*	60	>99	*S*
4	**d**	4-Cl-Ph	Me	85	>99	*R*	100[a]	>99	*S*
5	**e**	4-Br-Ph	Me	85	>99	*R*	20	>99	*S*
6	**f**	3-Br-Ph	Me	75	>99	*R*	55	>99	*S*
7	**g**	2-Br-Ph	Me	70	>99	*R*	20	>99	*S*
8	**h**	2-Py	Me	100[a]	>99	*R*	40	>99	*S*
9	**i**	(3-Me)-2-thienyl	Me	100[a]	>99	*R*	60	>99	*S*
10	**j**	H	Me	100[a]	60	*R*	100[a]	49	*S*
11	**k**	$SiMe_3$	Me	100[a]	>99	*R*	100[a]	57	*S*
12	**l**	$SiEt_3$	Me	90	>99	*R*	65	91.5	*S*
13	**m**	$SiMe_2{}^tBu$	Me	40	>99	*R*	40	98.5	*S*
14	**n**	$SiMe_2Ph$	Me	60	>99	*R*	80	>99	*S*
15	**o**	H	Me	100[a]	60	*R*	100[a]	49	*S*
16	**p**	H	Et	90	34	*S*	90	67	*S*
17	**q**	H	^{n}Pr	100[a]	>99	*S*	65	76	*S*
18	**r**	H	^{n}Pn	100[a]	>99	*S*	<5	62	*R*

[a] Theoretical value.

oxidoreductase, is also able to afford propargylic alcohols by catalyzing the chemo- and enantioselective reduction of ethynyl ketones **217o−q** and **219a−g**, and ethynylketoesters **220a−f** (Table 15).[181] These substrates require higher concentrations of SADH than unconjugated ketones because they decompose slowly under the reaction conditions, and, more significantly, they cause irreversible inactivation of the enzyme. In general, ethynyl ketones are reduced with moderate enantioselectivity with the exception of ketone **219a** (entry 3). Furthermore, ethynyl ketones bearing a small (up to ^{n}Pr) alkyl substituent (**217o−q**, **219a−b**) are reduced to (*S*)-alcohols (entries 1−5), meanwhile larger ones (**219c−g**) give the (*R*)-alcohols (entries 6−10). Conversely, ethynylketoesters **220a−f** are converted to (*R*)-ethynylhydroxyesters of excellent optical purity (entries 11−16). This fact suggests that the ester group plays a significant role in substrate binding to the active site. The enantioselectivity of the reaction depends on the number of methylenes between the carbonyl and the ester moieties as well as on the size of the latter. Thus, isopropyl ethynylketoesters (**220c,e**) give higher chemical yields and higher enantioselectivities of ethynylhydroxyesters than methyl or ethyl ethynylketoesters (**220a−b,d,f**) (compare for instance entries 11 and 12 with 13). The so-obtained optically pure ethynylhydroxyesters may serve as useful chiral building blocks for asymmetric synthesis.

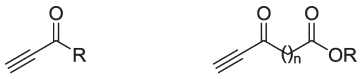
The reductive EED of different 2-azido-1-aryl ketones (**189a−f,i−j**) catalyzed by fermented Baker's yeast has been

Chart 6



217s **217t** **217u** **217v**

Table 15. EED of Ethynyl Ketones and Ketoesters



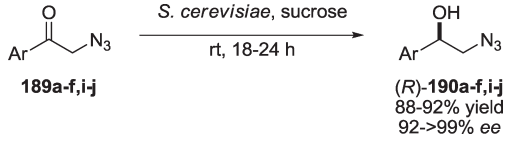
217o-q 219a-g **220a-f**

entry	compd	R	*n*	abs conf	*t* (h)	yield (%)	*ee* (%)
1	**217o**	Me		*S*	0.5	30	60
2	**217p**	Et		*S*	1	32	80
3	**219a**	^{i}Pr		*S*	2	50	>98
4	**219b**	^{t}Bu		*S*	24		85
5	**217q**	^{n}Pr		*S*	1	28	51
6	**219c**	^{i}Bu		*R*	1	20	50
7	**219d**	^{neo}Pn		*R*	7		66
8	**219e**	^{n}Bu		*R*	4	32	42
9	**219f**	^{s}Pn					
10	**219g**	^{i}Pn		*R*	5	55	80
11	**220a**	Me	2	*R*	2.5	35	82
12	**220b**	Et	2	*R*	1.5	51	90
13	**220c**	^{i}Pr	2	*R*	1.0	88	>98
14	**220d**	Et	3	*R*	1.5	76	97
15	**220e**	^{i}Pr	3	*R*	1.5	76	>98
16	**220f**	Me	4	*R*	2.5	68	>98

reported by Yadav and co-workers (Scheme 43).[182] The yeast cells were treated with allyl alcohol to inhibit the (*S*)-oxidoreductase. Thus, the corresponding (*R*)-azido alcohols (*R*)-**190a−f,i,j** were obtained in high yields and enantioselectivities. These compounds are interesting building blocks for the preparation of biologically active molecules because they serve as precursors of a wide variety of synthons like aziridines and β-amino alcohols, substructures present in compounds such as β-adrenergic blockers. For instance, (*R*)-(−)-epinephrine, (*R*)-(−)-norepinephrine, and (*R*)-(−)-isoproterenol can be prepared from (*R*)- 2-azido-1-(1,3-benzodioxol-5-yl)-ethan-1-ol. This alcohol can be obtained enantiopure and in quatitative yield by the reduction of the corresponding azidoketone catalyzed by *Rhodotorula glutinis* CCT 2182.[183] The same authors have further explored the potential of this biocatalyst in the reduction of this type of compounds by using a broader series of 4′-substituted α-azidoacetophenones (**189a−d,f**) as substrates.[184] Again, in all cases, the (*R*)-configured alcohols **190a−d,f** were

dx.doi.org/10.1021/cr100330u |*Chem. Rev.* 2011, 111, PR110–PR180

Scheme 43



obtained in 88−98% yield and enantiopure. Moreover, *Geotrichum candidum* can be used as a complementary catalyst: the alcohols of (*S*)-configuration were now isolated in 93−99% yield and enantiopure. The only exception was (*S*)-**190a**, for which an *ee* value of 40% was measured.

β-Azidoalcohols have recently become even more appealing since the azide group can be used with alkynes in click chemistry approaches to afford the corresponding triazoles. With this goal in mind, both enantiomers this type of compound have been prepared by using recombinant enzymes and a wide series of α-azidoacetophenone derivatives as substrates.[185] Namely, the carbonyl reductase from *Candida magnoliae* afforded the (*S*)-series. After 24 h of reaction, the corresponding alcohols were obtained in high yields (>80%) and enantiopure regardless the substituents. In turn, by an ADH from *Saccharomyces cerevisiae* (Ymr226c) allowed the access to the (*R*)-series. However, in this case, the substituents did have a pronounced effect on the enzymatic activity. Thus, for unsubstituted and *para*-halo-substituted (*R*)-azidoacetophenones, the reduction was completed in about 1 day, while the reaction was much slower for the substrate with 4-methyl, 3- or 4-nitro, and 3- or 4-methoxy groups on the phenyl ring. No reduction was observed for (*R*)-azido-4′-cyanoacetophenone. In any case, if the reaction proceeded, the (*R*)-alcohol was always obtained enantiopure, with the sole exception of (*R*)-azido-*p*-nitroacetophenone (63 *ee*). Finally, (*S*)-2-Azido-1-(*p*-chlorophenyl)ethanol reacted with phenylacetylene or propargyl alcohol under the action of a Cu(I) catalyst (Huisgen's cycloaddition) to yield (*S*)-1-(4-chlorophenyl)-2-(4-phenyl-1*H*-1,2,3-triazol-1-yl)ethanol and (*S*)-1-(4-chlorophenyl)-2-(4-(hydroxymethyl)-1*H*-1,2,3-triazol-1-yl)ethanol in 90% and 83% yield, respectively.

The EED of bulky−bulky ketones is a challenging process because both substituents usually show a high symmetry concerning steric demand. Hence, the search for suitable catalysts in this type of transformation is always of interest. For example, Kroutil and co-workers have recently identified that a recombinant short chain ADH from *Ralstonia sp.* DSM 6428 overexpressed in *E. coli* is an excellent catalyst in the reduction of the bulky−bulky aryl alkyl ketones **221c**−**e** shown in Table 16 and the α-ketoester **256b** shown in Table 18.[186] Similarly, Hua and co-workers have studied the substrate specificity shown by a purified carbonyl reductase from *Sporobolomyces salmonicolor* toward aliphatic and aromatic ketones as well as α- and β-ketoesters.[187] Interestingly, the enzyme shows the highest activity for α-ketoesters and, in general, very high *ee* values are obtained for ketones bearing bulky substituents. Analysis of the structure and enantioselectivity of a carefully selected subset of aryl alkyl ketones (Table 16) afforded the following relationships: (1) The reduction of acetophenone (**221a**, entry 1) and propiophenone (**221b**, entry 2) produced the (*R*)-enantiomers of the corresponding alcohols in low *ee*. (2) A further increase of the length of the aliphatic substituent causes a reversal of enantioselectivity, and the (*S*)-alcohols are then obtained as major products. (3) The size increase from *n*-propyl (**221c**, entry 3) or

Table 16. EED of Aryl Alkyl Ketones Catalyzed by Carbonyl Reductase from *Sporobolomyces salmonicolor*

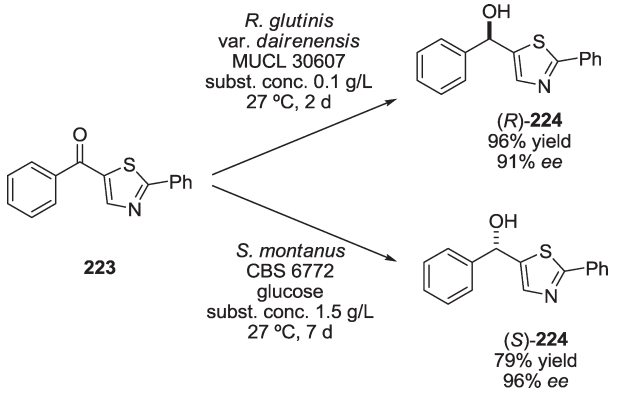
entry	ketone	Ar	R	*ee* (%)	absolute configuration
1	**221a**	Ph	Me	42	*R*
2	**221b**	Ph	Et	28	*R*
3	**221c**	Ph	^{n}Pr	88	*S*
4	**221d**	Ph	^{n}Bu	87	*S*
5	**221e**	Ph	^{n}Pn	34	*S*
6	**221f**	Ph	^{n}Hex	27	*S*
7	**221g**	Ph	^{i}Pr	98	*R*
8	**221h**	Ph	^{t}Bu	98	*R*
9	**221i**	Ph	*cyclo*-Pr	96	*R*
10	**221j**	4-F-C_6H_4	*cyclo*-Pr	98	*R*
11	**221k**	4-Cl-C_6H_4	*cyclo*-Pr	98	*R*

n-butyl (**221d**, entry 4) to C5 and C6 chains (entries 5−6) decreases the (*S*)-enantioselectivity. (4) Interestingly, when the alkyl group becomes branched (**221g**−**i**, entries 7−8) the (*R*)-enantioselectivity comes back and in very high *ee* values.[188] The corresponding favored binding modes could be predicted by means of an automated docking protocol which makes use of the interaction energy of the substrates in the active site of the enzyme as scoring function.[189]

Bisaryl ketones are an example of bulky−bulky ketones, the reduction of which affords optically active diarylmethanols, important chiral synthons. This type of ketone can be successfully desymmetrized by means of microbe-catalyzed reductions. For instance, Roy et al. have investigated the preparation of (*R*)- and (*S*)-1-phenyl-1-(2-phenylthiazol-5-yl)-methanol [(*R*)- and (*S*)-**224**] by enantioselective bioreduction of 1-phenyl-1-(2-phenylthiazol-5-yl)-methanone with whole microbial cells (Scheme 44).[190] A short screening of 38 microbial strains allowed the selection of two suitable yeast strains fulfilling enantiocomplementarity. Gram-scale preparations of the (*S*)- and (*R*)-alcohols were achieved in good to high optical purity and high yield using, respectively, *Saccharomyces montanus* CBS 6772 and *Rhodotorula glutinis* var. *dairenensis* MUCL 30607. Commercially available purified ketoreductases have been also used for the biocatalytic reduction of a wide series of bisaryl ketones (Chart 7) to yield both enantiomeric series of the corresponding alcohols in high yields and enantioselectivities.[191] In general, monosubstituted ketones in the *ortho* position (**225a**−**d**) afforded better enantioselectivities than the corresponding *metha* (**225e**−**f**,**h**) and *para* (**225j**−**l**) counterparts. However, and contrary to results obtained with most chemical catalysts, *ortho*-substitution is not necessary to obtain good enantioselectivities as long as electron-withdrawing substituents are present (**225i**,**n**). Finally, some disubstituted bisaryl ketones (**225t**−**u**) as well as several benzoylpyridines (**225q**−**s**) were also efficiently desymmetrized by this means. The above-mentioned purified carbonyl reductase from *Sporobolomyces salmonicolor* enzyme was also tested in the EED of diaryl ketones with high electronic symmetry.[192] As suspected, the SSCR showed a high

dx.doi.org/10.1021/cr100330u |*Chem. Rev.* 2011, 111, PR110–PR180

Scheme 44

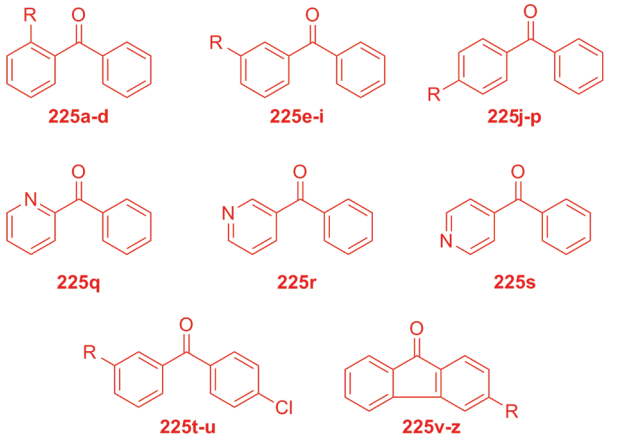


activity when THF or ^{i}PrOH were used as cosolvents. In the majority of cases the enzyme afforded the alcohols of (*R*)-configuration, the enantioselectivity of these processes ranging from moderate to excellent depending on the structure of the ketone used. Conversely to the above-mentioned purified carbonyl reductases, SSCR was more enantioselective toward the reduction of diaryl ketones with a *para*-substituent on one of the phenyl groups (**225j,m,o**−**p**) than those with an *ortho*-(**225a,d**) or *meta*-substituent (**225g,i**). Moreover, diaryl ketones with a pyridine ring (**225q**−**r**) were reduced, yielding the corresponding alcohols in enantiopure form. Finally, as in the case of substituted acetophenones,[153] the mutant Q245P reduced these substrates although with the opposite enantioselectivity in most of the cases. Thus, the corresponding (*S*)-alcohols were obtained in *ee* values ranging from moderate to excellent depending on the substrate structure.

Grape cells have been also successfully used for the anti-Prelog EED of various 2-substituted fluorenones (**225v**−**z**, Chart 7).[193] The high enantioselectivities (>92% *ee*) achieved with the halogenated substrates **225v**−**y** and the moderate *ee* observed for the 2-methyl-fluorenone (**225z**) support the hypothesis that the enzymatic enantioselectivity is mainly driven by electronic effects in these highly symmetric substrates. Moreover, the degrees of conversion are dependent on the solubility of the substrates. Thus, the presence of small amounts (1%) of the Triton X-100 surfactant is required to achieve conversion rates >91%.

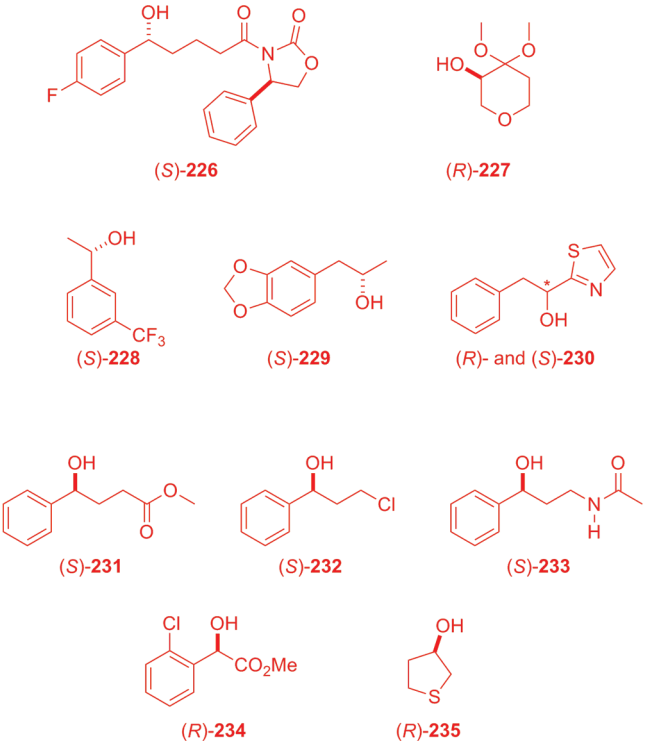
The biocatalytic reduction of prochiral ketones has been used with profusion as the key step in the preparation of many optically active pharmaceuticals. For instance, the synthesis of ezetimibe, a selective acyl-coenzyme A cholesterol acyltransferase inhibitor used in hypercholesterolemia, requires enantiopure alcohol (*S*)-**226** (Chart 8) as a crucial intermediate.[194] The most enantioselective synthesis of this chiral synthon reported so far consists in the EED of the corresponding ketone by using the microbial catalyst *Schizosaccharomyces octosporus* ATCC 2479. The screening of a library of cultures obtained from soil has recently allowed to identify *Burkholderia cenocepacea* as a more efficient catalyst: the yield of the reduction of (*S*)-**226** increased from 33% to 54%, and the corresponding alcohol was obtained in enantiopure form. A similar approach was undertaken to prepare (*R*)-4,4-dimethoxytetrahydro-2*H*-pyran-3-ol [(*R*)-**227**], a key intermediate in the synthesis of a chemokine receptor inhibitor.[195] Thus, previous approaches to selectively synthesize this alcohol involved the use of potassium osmate, and the

Chart 7



a,**e**,**j**,**z**: R = Me; **b**,**f**,**k**: R = OH; **c**,**g**,**l**: R = NH_2; **d**,**h**,**m**,**w**: R = Cl; **i**,**n**: R = NO_2; **o**: R = OMe; **p**,**v**: R = F; **t**: R = CN; **u**: R = CO_2Me; **x**: R = Br; **y**: R = I;

Chart 8



desired product was only obtained in 92% *ee*. The screening of commercially available ketoreductases revealed that KRED101 reduced the corresponding ketone to enantiopure (*R*)-**227** in 96−98% yield. The optimized process was used at pilot plant scale to produce 80 kg of the target alcohol. Berkowitz and coworkers have recently screened a library of isolated ketoreductases, which has allowed them to carry out the first viable EED catalyzed by this type of enzyme of an array of ketones, the reduction of which leads to optically active alcohols **228**−**233**

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(Chart 8), chiral synthons in the preparation of a wide variety of pharmaceuticals.[196]

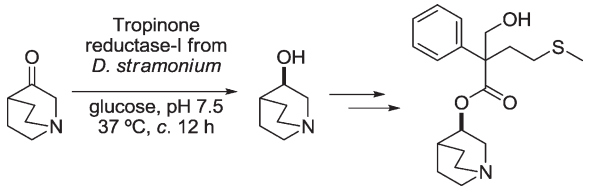
E. coli cells overexpressing a versatile carbonyl reductase[136d] were used for the production of methyl (*R*)-*o*-chloromandelate [(*R*)-**234**, Chart 8], intermediate in the synthesis of clopidogrel, a platelet aggregation inhibitor.[197] The optimized EED of the corresponding prochiral ketone afforded this alcohol enantiopure and in yields >80%, thus considerably improving the existing syntheses, which, at best, afforded the ethyl ester analogue in 76% *ee*. Finally, the directed evolution of a ketoreductase has allowed to increase the enantioselectivity of the challenging reduction of the highly symmetric tetrahydrothiophene-3-one to (*R*)-tetrahydrothiophene-3-ol [(*R*)-**235**, Chart 8] from 63 to >99% *ee*.[198] This alcohol is a key component of sulopenem, a potent antibacterial, and the EED has been successfully scaled to 100 kg, thus obviating the need for the hazardous reactions, reagents, and intermediates present in the alternative chiral pool route in which this chiral alcohol is obtained in five steps from L-aspartic acid.

Prochiral amino ketones are attractive substrates for the preparation of pharmaceuticals. The corresponding amino alcohols can be obtained in high yield and *ee* by means of reductive EEDs. In this sense, the enantioselective reduction of different ketones using tropinone reductase-I derived from plants such as *Datura stramonium* and *Hyoscyamus niger* can produce optically active secondary alcohols with high enantioselectivity. Namely, Yamamoto et al.[199] have isolated the gene for tropinone reductase from *D. stramonium* or *H. niger* and incorporated it into a plasmid vector containing a gene for glucose dehydrogenase derived from *Bacillus subtilis* or from *Thermoplasma acidophilum*. The resulting plasmid was cloned into *E. coli*, and the resulting transformants were employed to catalyze the enantioselective reduction. Thus, cells of *E. coli* transformed with pSG-DSR1 were added to a reaction solution containing amino ketone **236** giving (*R*)-3-quinuclidinol [(*R*)-**237**] in excellent yield and *ee* (Scheme 45). This compound has been used as an intermediate to produce a variety of physiologically or pharmacologically active agents such as squalene synthase inhibitor **238**.

Similarly, a method for the microbial stereoselective reduction of 2-chloro-1-[6-(2,5-dimethylpyrrol-1-yl)-pyridin-3-yl]ethanone (**239**) has been reported by Burns et al.,[200] resulting in the enantioselective formation of the (*R*)-enantiomer of the chlorohydrin, (*R*)-**240**, which can be used in the synthesis of β-adrenergic receptor agonists, such as compound **241**. Initial screening of a number of microbiological catalysts identified cultures producing the desired alcohol with 62−91% *ee* at 20−to 49% conversion. *Zygosaccharomyces bailii* ATCC No. 38924 was the most enantioselective biocatalyst for this application. When 8.0 L of a culture of *Z. bailii* and ethanone **239** (2 g/L) were reacted, an 89% yield of (*R*)-**240** was isolated in excellent *ee* (Scheme 46a).

Other more complex amino ketones have been also desymmetrized for the preparation of different pharmaceutical. Thus, *Lactobacillus kefiri* DSM 20587 cells immobilized in calcium alginate and carrageenan have been used as catalysts for the EED of denbufylline (**242**), a cAMP-phosphodiesterase inhibitor, into its most important pharmacologically active metabolite (*R*)-**243**.[201] After process optimization, the target alcohol was obtained in excellent yield and *ee* (Scheme 46b). On the other hand, the preparation of allylic alcohol (*R*)-**245** is required for the asymmetric synthesis of an integrin $\alpha_{v}\beta_{3}$ receptor antagonist. Whole cells of *Candida chilensis* are able to reduce the corresponding prochiral ketone to the desired alcohol in 95% *ee* (Scheme 46c).[202] Further optimization of the reaction allowed the setup of a reproducible and scaleable process at pilot scale in which the overall yield was improved from an initial value of 10% up to more than 80%.

Scheme 45



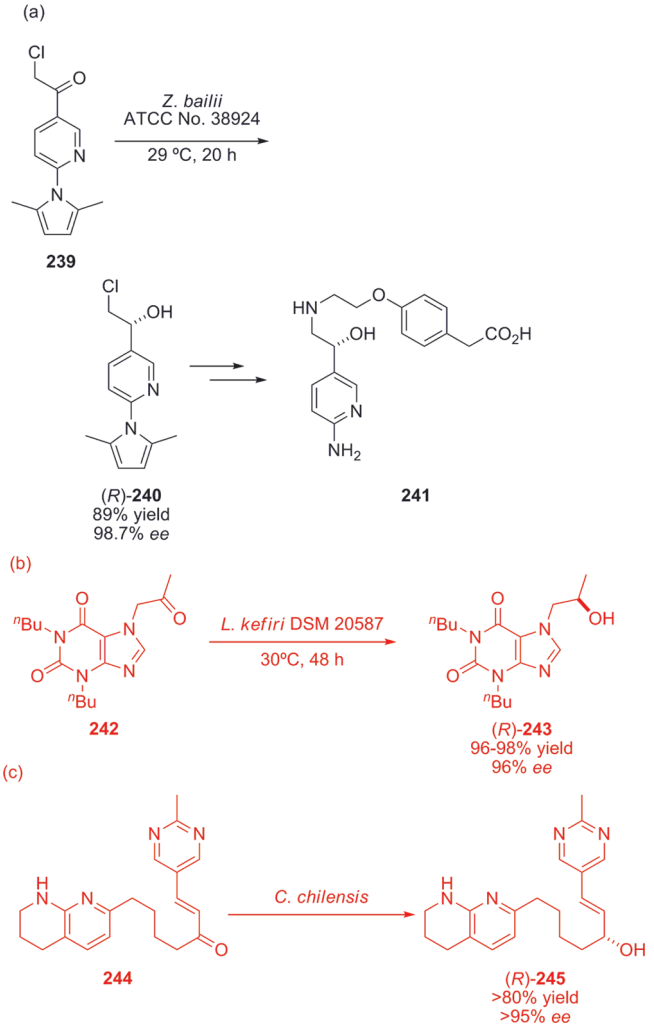
236 (*R*)-**237** 88.4% yield 98.6% *ee* **238**

The importance of the EED of prochiral polyketones which would produce a new quaternary chiral center constitutes a challenging transformation difficult to achieve by conventional methods. Moreover, the highly functionalized chiral intermediates obtained from this type of process are interesting targets in asymmetric synthesis. In this regard, synthetic studies on some terpenoids have led Iwamoto and co-workers to the desymmetrization of 2-benzyloxymethyl-2-methylcyclohexane-1,3-dione (**246a**).[203] The (3*S*)-hydroxyketone (2*R*,3*S*)-**247a** was obtained enantiopure and in 64% yield, together with 15% of the starting material (Table 17, entry 1). After chromatographic separation, the remaining ketone functionality in **247a** was further reduced in a highly diastereoselective fashion to yield the corresponding *trans*-1,3-diol (1*S*,3*S*)-**249a** in >99% *de* and *ee* and in 85% yield. Further studies on the desymmetrization of this substrate allowed the optimization of the yield of this EED (91%) by adding yeast extract to the reaction medium.[204] Moreover, extension of this methodology to the related 5-, 7- and 8-membered ring diketones **246b**−**e** afforded the following structure-selectivity relationships: (1) Desymmetrization of the cyclopentane analogue (**246b**, entry 2) proceeded with opposite and lower diastereoselectivity, although each of the diastereomers (**247b** and **248b**) were isolated in enantiopure form. (2) Expansion of the diketone ring size by one unit (**246c**−**d**) maintains the regioselectivity observed for the parent substrate of this series (**246a**, entry 1) as well as the enantioselectivity. Thus, regardless the protecting group used the monoketones **247c**−**d** were obtained enantio- and diastereopure and in very high yields (entries 3−4). Although in this case the borane hydride used so far failed to further reduce the remaining keto group, the employment of the CBS catalyst allowed the obtaining of polyol **249d** by using the pivaloyl-protected monoketone **247d** as substrate. 3) Finally, the results obtained with the pivaloyl protected cyclooctane dione **246e** (entry 5) show again a reversal of the diastereoselectivity of this reaction as compared to the cyclohexanedione **246a** (entry 1). This hampers somehow the rationalization of the regioselectivity of the EED of this type of substrate in terms of the ring size of the substrates, as it is further demonstrated by the concentration-dependence of the regioselectivity shown by this catalyst in the reduction of some related cyclopentan- and cyclohexandiones.[205]

The structurally related cyclopentan-1,3-dione **250** has been desymmetrized by using cultured cells of higher plants, the

dx.doi.org/10.1021/cr100330u |*Chem. Rev.* 2011, 111, PR110–PR180

Scheme 46



suitable election of which allows access to the two diastereomers of **251** shown in Scheme 47.[206] Although the enantioselectivities were excellent in both cases, the *de* value of (2*R*,3*S*)-**251** was moderate (58%). This problem has been overcome by using different strains of *Geotrichum candidum* under anaerobic and aerobic conditions.[207] After optimization of the reaction conditions, both isomeric hidroxycyclopentanones were obtained diastereo- and enantiopure and in very high yields (Scheme 47).

Sugai and co-workers have investigated the substrate specificity of the reduction of different triketones with the yeast strain *Torulaspora delbrueckii* IFO10921.[208] They have carried out the enantioselective reduction of the prochiral triketones **252a**−**d** shown in Scheme 48. In all cases, the *pro-R* carbonyl was preferentially reduced, yielding intermediates **253a**−**d**, which in the reaction medium underwent cyclization to afford the bicyclic acetals (1*S*,6*S*)-**254a**−**d** in high yields and excellent *ee*'s. In contrast to **252a**−**b**, two byproducts were also isolated for the case of triketone **252c**−**d**. For the case of the five-membered substrate, the first was the open-chain form of hydroxyketone **253c** (7%) whose relationship between the hydroxy group and the methylene side chain was *trans*. The other side product (14%) was also a bicycle probably due to the nonenzymatic intramolecular aldol reaction. For the case of **253d**, the introduction of a sterically bulky isopropyl group greatly affected the stereoselectivity of the reaction in comparison to the methyl and ethyl groups of **253a**−**b**. Thus, a mixture of diastereomers is obtained due to the reduction of pro-(*S*) carbonyl group, is now obtained. Further attempts to increase the selectivity of the EED of this key cyclohexanedione by using other microorganisms slightly increased the ratio of the target diastereomer, but chromatographic separation could not be avoided.[208c] On the other hand, reduction of other triketones afforded interesting structure−activity relationships: (1) introduction of a 5,5-dimethyl group on the ring of **252a** and (2) the one methylene group subtraction between the quaternary center and the side chain carbonyl group of this same substrate only resulted in very low activity of enzymes. Finally, the reduction worked also well with use of an air-dried, long-term preservable cell preparation, thus greatly increasing the applicability of this yeast-mediated reduction in synthetic organic chemistry.

The reductive EED of prochiral α-ketoesters is an interesting transformation in organic chemistry due to the importance of the resulting optically active α-hydroxy acids as chiral building blocks. Hence, several routes to these compounds have been devised. For instance, Baker's yeast has been used to reduce a series of alkyl esters derived from pyruvate and benzoylformate.[209] Both the yield and the enantioselectivities of these reductions were maximized when methyl esters **256a**−**c** were used, the (*R*)-alcohols **257a**−**c** being isolated in all instances (Table 18, entries 1−3). It is worth mentioning that yeast-mediated ester hydrolysis was a significant side reaction for related substrates derived from long-chain alcohols. Moreover, in certain cases, to increase the enantioselectivity, an approach based on the employment of known inhibitors of yeast reductases was adopted. Thus, in the case of ethyl benzoylformate, the addition of methyl vinyl ketone increased slightly both the enantioselectivity and the yield of the reduction, but the simultaneous presence of methyl vinyl ketone and ethyl chloroacetate was toxic to the yeast cells, and only starting material was recovered.

Other microorganisms apart from the renowned Baker's yeast have proven to be valuable biocatalysts for these transformations. For instance, Ishihara and co-workers have studied the enantioselectivity of the reduction of different α-keto esters catalyzed by a thermophilic actinomycete, *Streptomyces thermocyaneviolaceus* IFO 14271 (Table 18).[210] Among all the results obtained, it is worth mentioning that at 37 °C and in the absence of additives ethyl 3-methyl-2-oxobutanoate (**256f**) and ethyl benzoylformate (**256g**) were reduced to the corresponding (*R*)-alcohols in good yields and excellent enantioselectivities (entries 6 and 7), while the rest of the substrates tested afforded the (*S*)-alcohols in moderate to low *ee*'s and yields. The presence of different additives greatly modified the enantioselectivity displayed by this microorganism. For instance, the reduction of **256f**−**g** in the presence of glutamic acid gave the hydroxyesters of opposite configuration [(*S*)-**257f**−**g**] in excellent *ee*'s (entries 8 and 9). Additionally, ethyl 2-oxobutanoate (**256d**) and ethyl 2-oxopentanoate (**256e**) were also reduced to the corresponding (*S*)-alcohols [(*S*)-**257d**−**e**] in >99% *ee* in the presence of an amino acid such as asparagine or aspartic acid (entries 4 and 5).

Similarly, *Aureobasidium pullulans* SC 13849 catalyzed the enantioselective reduction of the ethyl α-keto ester **258** to afford

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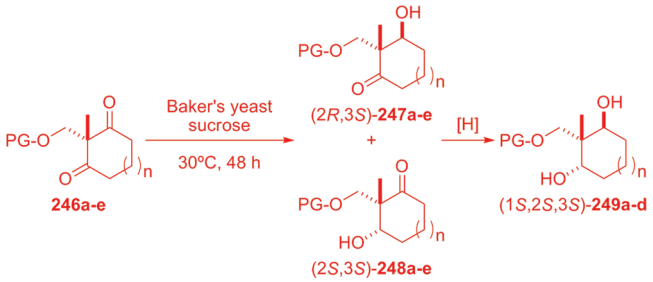
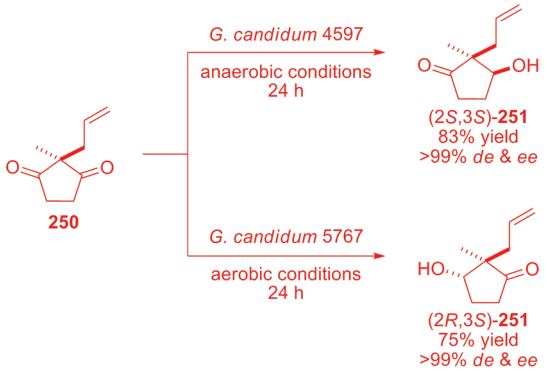
Table 17. EED of 2,2-Disubstituted-cyclohexan-1,3-diones Catalyzed by Baker's Yeast



				247		248		249		
entry	diketone	*n*	PG	yield (%)	*ee* (%)	yield (%)	*ee* (%)	yield (%)	*ee* (%)	*de* (%)
1	**246a**	1	Bn	64 (91[a])	>99	nd		85[b]	>99	>99
2	**246b**	0	Bn	6[a]	>99	73[a]	>99	93[b]	>99	>99
3	**246c**	2	Bn	91	>99	nd				
4	**246d**	2	pivaloyl	95	>99	nd		86[c]	>99	>99
5	**246e**	3	pivaloyl	n.d.		93[a]	>99			

[a] Reaction carried out with yeast extract. [b] Reduction conditions: $Me_4NBH(OAc)_3$, 4 days, rt. [c] Reduction conditions: $BH_3 \cdot SMe_2$, (*R*)-CBS catalyst, CH_2Cl_2, 10 h, 30 °C.

Scheme 47



the chiral hydroxyester (*R*)-**259**. This compound and its related optically active hydroxy acid (*R*)-**261** are intermediates in the synthesis of a retinoic acid receptor gamma-specific agonist (Scheme 49).[211] PLE-catalyzed hydrolysis of alcohol (*R*)-**259**, obtained in excellent yield and *ee*, afforded the desired hydroxy acid (*R*)-**261**. Alternatively, this compound can be also directly obtained by reduction of the corresponding α-keto acid **260**. Among the microorganisms screened, *Candida maltosa* SC 16112 and two strains of *Candida utiliz* (SC 13983, SC 13984) gave reaction yields of >53% in >96% *ee*'s.

Recombinant dehydrogenases have been also used in the EED of this type of substrate. For example, Hua and co-workers have purified a thermostable 7α-hydroxysteroid dehydrogenase from *Bacteroides fragilis* ATCC 25285.[212] Although the enzyme shows almost no activity toward acetophenone derivatives, it is very active in the reduction of a wide series of α-ketoesters, which are more sterically demanding. This behavior has been attributed to a spacious active site designed to host the bulky steroidal native substrate. The substituents at the phenyl ring of aromatic α-ketoesters greatly affected the activity of the enzyme, but their effects on the enantioselectivity (typically >98%) were minimal. For the case of other α-ketoesters, the enantioselectivities were excellent as long as the alkyl substituent was sterically demanding (cyclohexyl, *iso*-propyl or *tert*-butyl).

The enantioselective reduction of β-ketoesters is a well-established transformation that has been studied with more profusion than the reductive EED of α-ketoesters. The excellent yields and *ee*'s of the former can account for this situation. Anson et al. have established the feasibility of novel bioreductions of β-ketoesters based on ketide synthase-deficient strains of *Streptomyces coelicolor* A3(2) (CH999), a soil bacterium that produces the dimeric benzoisochromanequinone antibiotic actinorhodin.[213] This was carried out with the express aim of feeding unnatural β-ketoester substrates so that the action of the *act*VIORF1 reductase present in this bacterium would yield, enantioselectively, β-hydroxyesters. Namely, the authors tested the so-created microbe with the *N*-acetylcysteamine β-ketothioesters **262a**−**d** shown in Scheme 50. Thus, their incubation with mycelium of CH999/pIJ5675 afforded the corresponding hydroxyacids, which were conventionally esterified to aid purification. This way, the methyl esters (*S*)-**263a**−**c** were isolated in good to excellent *ee*'s and moderate yields. Additionally, lactone (*S*)-**264** was obtained as the sole product of the reduction of **262d**. It was formed as essentially a single enantiomer by cyclization of the initially formed β-hydroxy acid (*S*)-**263d**. On the other hand, a toolbox consisting of more than 30 recombinant ketoreductases obtained from genome mining and protein engineering has been evaluated against a series of diverst β-ketoesters.[214] The results obtained show that, although both enantiomeric series of the corresponding β-hydroxyesters can be generally accessed in high enantioselectivity by using a proper enzyme, the selectivity and activity of these ketoreductases is greatly affected by the structure

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Scheme 48

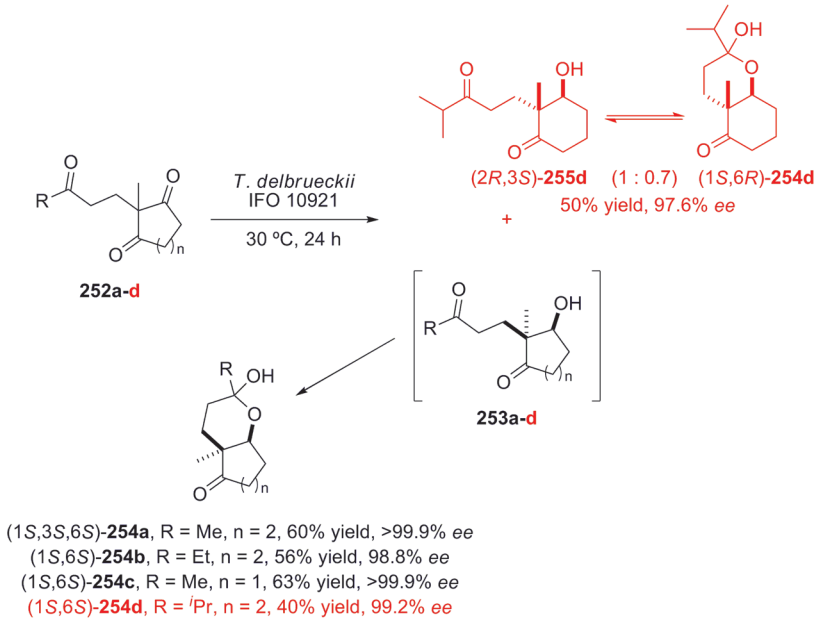
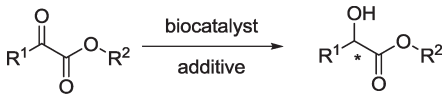


Table 18. EED of α-Ketoesters



entry	substrate	R^1	R^2	product	biocatalyst	additive	T (°C)	t (h)	yield (%)	*ee* (%)
1	**256a**	Me	Me	(*R*)-**257a**	BY		30	24	36	92
2	**256b**	Ph	Me	(*R*)-**257b**	BY		30	24	91	98
3	**256c**	MeO_2CCH_2	Me	(*R*)-**257b**	BY		30	24	64	>98
4	**256d**	Et	Et	(*S*)-**257d**	ST	Asp/Asn	37	30		>99
5	**256e**	*n*-Pr	Et	(*S*)-**257e**	ST	Asp/Asn	37	30		>99
6	**256f**	*i*-Pr	Et	(*R*)-**257f**	ST		37	20	57	>99
7	**256f**	Ph	Et	(*R*)-**257g**	ST		37	20	65	>99
8	**256g**	*i*-Pr	Et	(*S*)-**257f**	ST	Glu	37	30		>99
9	**256g**	Ph	Et	(*S*)-**257g**	ST	Glu	37	30		>99

of the substrates. Thus, the enzymes could be grouped in three major classes according to their response to the structure of the β-substituent.

Recently, Müller and co-workers have elegantly extended the scope of the biocatalytic reductive desymmetrization of β-ketoesters with the study of the transformation of the more challenging β,δ-diketoesters to optically active δ-hydroxy-β-ketoesters. In particular, these authors have proven that alcohol dehydrogenases (ADHs) are adequate catalysts for the regio- and enantioselective reduction of these compounds by measuring the activity of several candidates from different sources in the reduction of a set of 4,6-disubstituted β,δ-diketohexanoates. From this screening, it was found that ADH from *Lactobacillus brevis*, available in the form of a crude cell extract from a recombinant *E. coli* (recLBADH), was able to accept a broad range of β,δ-diketo esters.[215,216] Additionally, it was observed that substitution at C-6 played a key role in the activity of the enzyme. Thus, chain elongation at this position decreased the activity and the presence of 6-alkoxy substituents abolished it. Conversely, the enzyme turned out ot be less sensitive to variations of the alcoholic part of the ester.

In particular, diketoesters **265a**−**c** were subjected to preparative recLBADH-catalyzed bioreduction (Scheme 51). It selectively occurs at the δ-keto group, thus affording the corresponding optically active δ-hydroxy-β-keto esters **266a**−**c**. These compounds are versatile intermediates in organic synthesis due to their high functionalization. Thus, its cyclization to afford the corresponding δ-lactones (**268a**−**c**) occurs with good yields. In particular, the lactone (*R*)-**268c** has been described as a natural fragrance.[217] On the other hand, different

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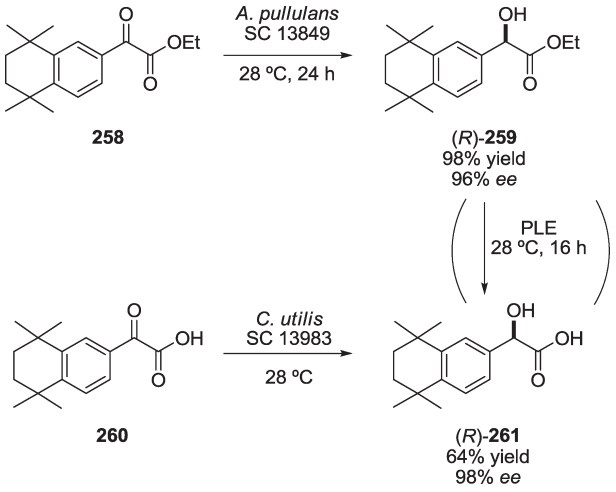
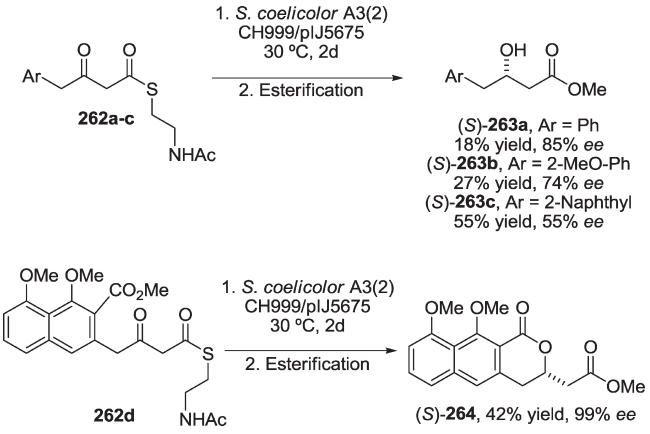
transformations of lactone (*S*)-**268a** have been employed in the syntheses of (−)-callystatin A and (−)-20-*epi*-callystatin A via the aldehyde **269**,[218,219] and (*S*)-argentilactone and (*S*)-goniothalamin.[220] Furthermore, transformation of (*S*)-**266a** into lactone **270** constitutes a key step in the synthesis of (*R*)-*semi*-vioxanthin.[221] Finally, the β,δ-dihydroxy esters *syn*-(3*R*,5*S*)- and *anti*-(3*S*,5*S*)-**267a** were prepared from (*S*)-**266a**. These compounds are valuable precursors to prepare synthetic statins of the mevinic acid type, like atorvastatin and rosuvastatin, important lipid regulating drugs for the treatment of diseases related to hyperlipidaemia. It is worth mentioning that (*R*)-**266a** has also been prepared with synthetic purposes on a 100 g scale[222] and through bioreduction in a biphasic system using Baker's yeast as catalyst (50% yield, 90−94% *ee*).[216]

Optically active hydroxyketones are widespread structural features of many biologically active molecules and have been also used with profusion as chiral building blocks since they can be easily transformed into other highly functionalized compounds such as diols, diamines, amino alcohols, and epoxides among others. Many efforts have been recently devoted to the search for selective catalysts and reaction conditions that avoid the overreduction of this type of substrate to the corresponding diols, which considerably lowers the yield of these processes. Thus, the preparation of (5*R*)- and (5*S*)-hydroxy-2-hexanone, essential intermediate for the synthesis of biodegradable polymers, pharmaceuticals, and aroma compounds, has been achieved by using 2,5-hexanedione (**273a**, Chart 9) as starting material and whole cells of *Lactobacillus kefiri* and *Saccharomyces cerevisiae*, respectively, as catalysts.[223] Subsequent process optimization protocols have allowed the obtaining of both enantiomers of this hydroxyketone in excellent enantioselectivities and yields. Diketones **271a,c,d**, **272b**, and **273a** (Chart 9) have been also regio- and enantioselectively reduced by using the lyophilized cells of *Rhodococcus ruber* DSM 44541 containing the alcohol dehydrogenase ADH-"A".[142,224] A strong preference for the (*S*)-configured alcohols and keto groups bearing not too large small substituents was observed. Hence, in a ketone like 2,4-octanedione, only the 2-keto group is reduced since the ketone in position 4 bears two substituents made of four heavy atoms each. Thus, enantiopure (*S*)-2-hydroxy-4-octanone is obtained in excellent yield (90%). But for diketones not fulfilling this requirement, mixtures of the corresponding hydroxyketone

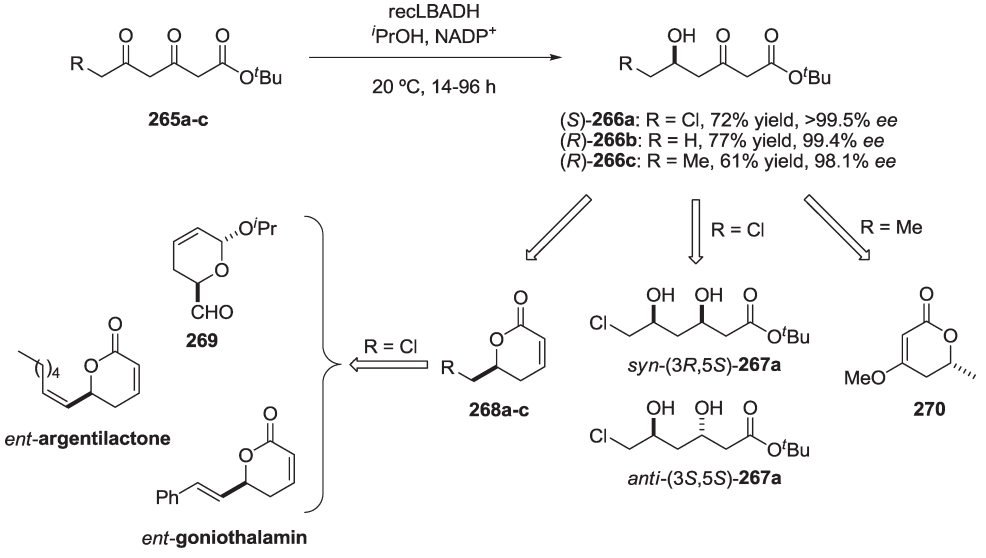
Scheme 49



Scheme 50



Scheme 51



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and diol are observed due to the reduction of both keto groups by means of a process consisting of an EED and a kinetic resolution.

A similar behavior was observed in the reduction of the β- and γ-diketones **272a,c** and **273b** shown in Chart 9 by three purified ketoreductases.[225] Namely, alcohol dehydrogenase from *Lactobacillus brevis* afforded the corresponding (2*R*)-hydroxyketones in enantiopure form and, usually, in high degrees of conversion. Conversely, the ADHs from *Rhodococcus ruber*[142] and *Thermoanaerobacter* sp. (ADH-T) afforded the opposite enantiomeric series. Again, the fact that the reductions stop at the hydroxyketone stage can be explained on the basis of the size of the binding pockets that host each substituents of each keto group. Thus, the large substituents of the 4- and 5-keto groups of **272a,c** and **273b** are too large to fit in the active site of these enzymes and, hence, their reduction is not detected. However, the 1,2-diketones **271a**−**c** are less sterically demanding and in these cases the corresponding diols are detected. Both the ratio diol:hydroxyketone and the absolute configuration of the isomers obtained are explained in terms of substrate binding to a site made of two sites of different size.

A set of 34 commercially available ketoreductases have been screened for the EED of the symmetric 3-substituted 2,4-diones **274a**−**j** (Table 19).[226] The results obtained show that most enzymes give the keto alcohol as the only product, and only a couple of them are able to further reduce it upon addition of an excess of enzyme at longer reaction times. On the other hand, the majority of them also follow the Prelog's rule and, conversely to the case of the chiral center of the α-substituent, in very high enantioselectivity. As a general feature, the employment of KRED102 affords the *syn* enantiomers (3*R*,4*S*)-**275a**−**g** in excellent yields and enantioselectivities (entries 1−2,4−6,8−9). Similarly, among the few KREDs that show *anti* selectivity, KRED118, 119, and 120 are able to reduce ketones **274b,e,h** in high enantioselectivities (entries 3, 7 and 10). This also applies to the case of the α,α-disubstituted ketones **274i,j** (entries 11 and 13). However, to achieve the *syn* selectivity in ketones bearing a quaternary center, KRED 101 and 112 have to be used (entry 12).

Although the reductive preparation of both enantiomers of benzoin (**277a**) in high *ee*'s by using whole-cell catalysts has been explored in profusion,[227] derivatives of this hydroxyketone have traditionally received much less attention. As an exception, a collection of 21 different microorganisms was recently screened for the EED of several benzils to optically active benzoins (Scheme 52).[228] Whole cells of *Fusarium roseum* OUT4019 were found to be an excellent catalyst and afforded the corresponding (*S*)-configured benzoins in >90% *ee*. The only exception was the 2-methoxybenzil ketone **276b**, for which *Aspergillus oryzae* cells turned out to be an excellent catalyst (91% *ee*).

Chart 9

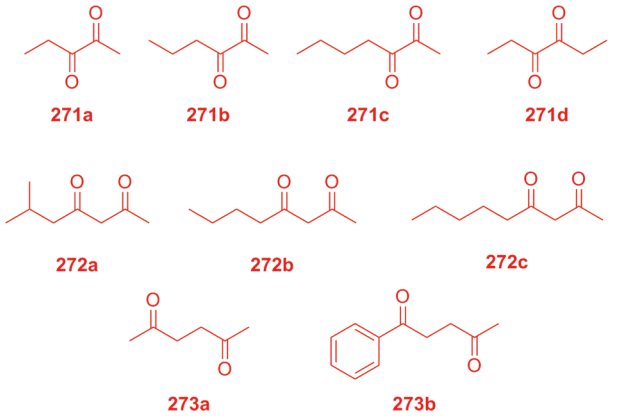
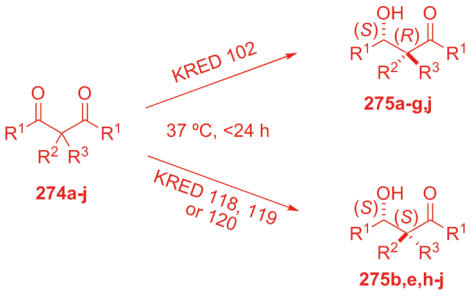


Table 19. EED of 1,3-Diketones



entry	substrate	R^1	R^2	R^3	product	biocatalyst	yield (%)	*ee* (%)
1	**274a**	Me	Me	H	(3*R*,4*S*)-**275a**	KRED 102	100	>99
2	**274b**	Me	Et	H	(3*R*,4*S*)-**275b**	KRED 102	102	>99
3	**274b**	Me	Et	H	(3*S*,4*S*)-**275b**	KRED 118	100	>99
4	**274c**	Me	^{n}Pr	H	(3*R*,4*S*)-**275c**	KRED 102	100	>99
5	**274d**	Me	^{t}Bu	H	(3*R*,4*S*)-**275d**	KRED 102	94	>99
6	**274e**	Me	allyl	H	(3*R*,4*S*)-**275e**	KRED 102	100	>99
7	**274e**	Me	allyl	H	(3*S*,4*S*)-**275e**	KRED 120	100	>99
8	**274f**	Me	3-butenyl	H	(3*R*,4*S*)-**275f**	KRED 102	100	>99
9	**274g**	Me	2-buten-3-methyl	H	(3*R*,4*S*)-**275g**	KRED 102	100	>99
10	**274h**	Et	Me	H	(4*S*,5*S*)-**275h**	KRED 119	100	>99
11	**274i**	Me	Et	Me	(3*S*,4*S*)-**275i**	KRED 118 and 119	100	>99
12	**274j**	Me	allyl	Me	(3*R*,4*S*)-**275j**	KRED 101 and 112	100	96
13	**274j**	Me	allyl	Me	(3*S*,4*S*)-**275j**	KRED 118 and 119	100	>99

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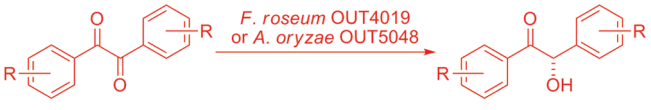
Ketoreductases can chemo-and enantioselectively differentiate between methyl and trifluoromethyl ketones within the same molecule as it has been demonstrated for several substituted trifluoroacetyl acetophenones (Scheme 53).[229] Thus, a library of 72 commercially available ketoreductases was screened for its ability to distinguish the different steric and electronic properties of both types of carbonyl groups. Although the *o*-diketone was isolated as a cyclized dihydrate and found to be inert, the results obtained with the *p*-diketone **278b** are excellent. Thus, the selective reduction of the more reactive trifluoromethyl ketone can be accomplished by numerous enzymes to yield exclusively both the (*S*)- (KRED 112 and A1i) and the (*R*)-enantiomers (KRED 129, 131, A1n, and A1x) of the corresponding trifluoromethyl hydroxyketone **279b** in enantiopure form. The identification of catalysts that chemoselectively reduce the less reactive methyl ketone was more difficult. Thus, only (*S*)-**280b** could be prepared in high yield and *ee* by using an ADH from *Candida parapsilosis*. Overall, the observed chemoselectivity under the conditions assayed for diketone **278b** was lower for the case of the *m*-diketone **278a**. Thus, although the (*S*)- and (*R*)-enantiomers of the *m*-trifluoromethyl hydroxyketone **279a** can be accessed in enantiopure form by using KRED 112 and KRED A1i, and ADH-RE, respectively, significant amounts of the corresponding diol (11−18%) cannot be avoided. Moreover, only ADH−CP afforded the (*S*)-enantiomer of *m*-methyl hydroxyketone **280a** as major product but in 90% *ee*.

In our research group, we have worked on the enantioselective preparation of optically active β-hydroxynitriles using β-keto nitriles as starting material. Preliminary studies afforded evidence that β-keto nitriles behave differently from β-ketoesters under similar reaction conditions. Thus, during the screening for a suitable strain to enantioselectively reduce a ethanolic solution of benzoyl acetonitrile (**281a**), we found out that growing cells of the fungus *Curvularia lunata* CECT 2130 produced an almost equimolar mixture of 3-hydroxy-3-phenylpropanenitrile and a compound in which an ethyl group had been incorporated to the α-position of the nitrile (**283aa**).[230] Further investigations highlighted that the ethyl moiety present in the unexpected product came from (1) enzymatic oxidation of the cosolvent ethanol followed by (2) nonenzymatic aldol condensation and (3) enzymatic reduction of the C=C double bond. Moreover, a dramatic influence of the growing state of the microorganism on the ratio of nonalkylated and alkylated reduced products was also observed and associated with the metabolism of the microorganism. Thus, when 4-day-old growing cells were employed, **283aa** was obtained in 96% *de* and 98% *ee* (Table 20, entry 1). Other alcohols and β-keto nitriles were employed to study the substrate specificity of this interesting biotransformation.[231] First, it was observed that MeOH inhibited the reaction, but other short chain alcohols behaved as ethanol (**282b**−**d**, entries 2−4), the corresponding alkyl groups being incorporated in the α-position (**283ab**−**ad**). It can be seen that the longer the alkyl chain, the lower the yield of the corresponding α-alkyl-β-hydroxy nitrile, but no clear trend was observed for both *ee* and *de*. Second, when the ethanolic solutions of other β-keto nitriles were employed (**281b**−**f**, entries 5−9) lower enantioselectivities and diastereoselectivities were observed, in general, as compared to benzoyl acetonitrile (**281a**, entry 1). Nevertheless, its alkylation-reduction resulted in highly diastereo- and enantioselective reactions leading to α-alkyl-β-hydroxy nitriles (2*R*,1′*R*)-**283ba**−**fa**, which, together with (2*R*,1′*R*)-**283aa**−**ad**, were easily reduced to the corresponding γ-amino alcohols. It is of note that the employment of aliphatic β-keto nitriles gave disappointing results concerning both yields and optical purities.

As it has already been pointed out, the use of methanol as cosolvent allowed, in turn, the chemoselective reduction of the aforementioned aromatic β-keto nitriles **281a**,**f**−**i** by the fungus *Curvularia lunata* CECT 2130 to yield the corresponding (*S*)-β-hydroxy nitriles (*S*)-**284a**,**f**−**i** in a highly enantioselective way (Scheme 54).[232] The role of methanol still is not fully understood. Either (a) MeOH is able to substitute EtOH in the cofactor regenerating process, or (b) MeOH inhibits bioreduction of the double bond of the intermediate unsaturated ketone by the enoate reductase. This fungal strain is therefore a versatile biocatalyst, and depending on the experimental conditions, it is possible to selectively obtain the alkylated and nonalkylated reduced products.

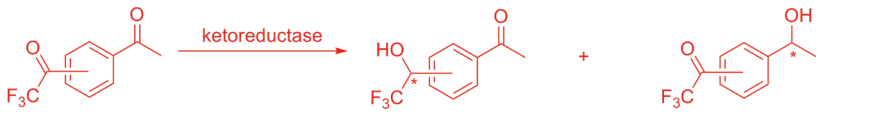
Taking into account that the best stereochemical result was obtained in the case of benzoyl acetonitrile (**281a**), a series of derivatives bearing different substituents on the phenyl moiety were also tested (Table 21). All the β-hydroxy nitriles were obtained in high to very high *ee*'s with moderate yields. Some general tendencies in the enantioselectivity of this biotransformation were observed: (a) a substituent in the *para* position (entries 4, 7, and 9) decreases the *ee*, whereas a substituent in the *ortho* (entry 2) or *meta* (entries 3, 6, and 8) positions does not result in significant changes in enantioselectivity, and sometimes results in even higher values than those obtained with **281a**; (b) concerning substitution at the *para* position, a methyl (entry 7) or a methoxy (entry 9) group results in a much higher *ee* value than a chlorine atom (entry 4); (c) the nature of the substituent

Scheme 52



R = **a**: H; **b**: *o*-MeO; **c**: *m*-MeO; **d**: *p*-MeO; **e**: *o*-F; **f**: *m*-F; **g**: *p*-F;

Scheme 53



a: *m*-trifluoromethylketone;
b: *p*-trifluoromethylketone;

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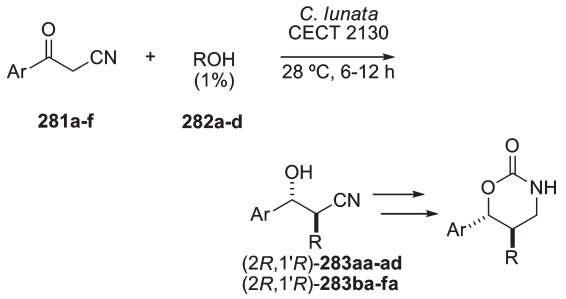
Table 20. *C. lunata*-Catalyzed Alkylation-Reduction of *β*-Keto Nitriles



entry	substrate	Ar	alcohol	R	product	yield (%)	*de* (%)	*ee* (%)
1	**281a**	Ph	**282a**	Et	**283aa**	69	96	98
2	**281a**	Ph	**282b**	^{n}Pr	**283ab**	38	98	98
3	**281a**	Ph	**282c**	^{n}Bu	**283ac**	13	87	86
4	**281a**	Ph	**282d**	^{t}Bu	**283ad**	14	94	97
5	**281b**	3-Me-Ph	**282a**	Et	**283ba**	42	78	70
6	**281c**	4-Me-Ph	**282a**	Et	**283ca**	64	92	83
7	**281d**	4-MeO-Ph	**282a**	Et	**283da**	58	88	75
8	**281e**	2-thienyl	**282a**	Et	**283ea**	63	94	93
9	**281f**	2-furyl	**282a**	Et	**283fa**	59	72	87

when it is placed on a *meta* position (entries 3, 6, and 8) is not so influential; (d) a double substitution in *meta* and *para* (entry 5) leads again to a high *ee* value, much closer to that obtained with **281k** than that with **281l** (entries 3 and 4, respectively).

A wide series of *β*-ketosulfones and *β*-ketosulfides have been reduced to the corresponding optically active sulfur-containing alcohols by using Baker's yeast as catalyst in a mixture of 10 mL of diisopropyl ether and 0.7 mL of water as solvent.[233] As a general feature, methyl ketones were always reduced in very high enantioselectivities (>96%), which gradually decreased with an increase of the size of the small substituent. On the other hand, the nature of the substituents at the phenyl ring bonded to the sulfur atom did have little effect on the enantioselectivity of the reaction. However, isolated yields (47−99%) were more sensitive to this type of structural variation. On the other hand, the enantioselective reduction of α-thiocyanatoketones yields optically active *β*-hydroxythiocyanates, which serve as substrates for the preparation of optically active thiiranes. For example, substrates **285a**−**e** have been desymmetrized with Baker's yeast in organic solvent to furnish the corresponding alcohols which, after isolation, were further transformed into the (*R*)-configured thiiranes **287a**−**e** in excellent yields and enantioselectivities (Scheme 55).[234] However, the EED of the analogous 1-arylsulphanyl-3-thiocyanatopropan-2-ones proceeded in low enantioselectivities.

Cascade or domino reactions involve two or more sequential transformations without isolation of intermediates using one or more catalysts. Although the tuning of the reaction conditions in order to ensure the compatibility of all catalysts and avoid undesired side reactions can be a time-consuming process, the well-known advantages of one-pot processes together with their inherent elegance are the main reasons why cascade reactions are gaining significant attention. In the recent past years, α-haloketones have been intensively used as substrates in chemoenzymatic cascade reactions. The first step makes use of an ADH to

Scheme 54

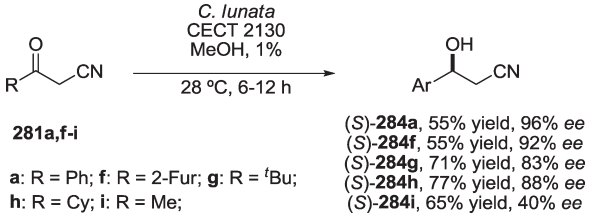
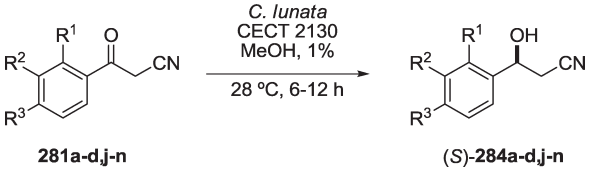


Table 21. *C. lunata*-Catalyzed Reduction of *β*-Keto Nitriles



entry	substrate	R^1	R^2	R^3	yield (%)	*ee* (%)
1	**281a**	H	H	H	55	96
2	**281j**	Cl	H	H	42	98
3	**281k**	H	Cl	H	57	97
4	**281l**	H	H	Cl	47	50
5	**281m**	H	Cl	Cl	43	92
6	**281b**	H	Me	H	59	94
7	**281c**	H	H	Me	54	82
8	**281n**	H	CF_3	H	41	98
9	**281d**	H	H	MeO	42	83

enantioselectively desymmetrize the prochiral substrate, thus yielding an optically active halohydrin, which either spontaneously or by means of a catalyst undergoes an S_N2 ring closure reaction to afford the corresponding epoxide without any racemization. Furthermore, the epoxides can be also in situ further transformed, thus extending the length of the cascade reaction. For example, Kroutil and co-workers have employed the haloketones **288a**−**e** as substrates in different cascade reactions (Scheme 56a).[235] Enzymes of opposite enantioselectivities were first employed to obtain both enantiomeric series of the target products. In particular, the ADH-"A" from *Rhodococcus ruber* DSM 44541, and ADHs from *Pseudomonas fluorescens* DSM 50106 and *Lactobacillus brevis* were used as Prelog and anti-Prelog enzymes, respectively. Next, the epoxidation of the halohydrin was accomplished in different fashions. Thus, if the EED of the α-haloketone is carried out at pH ∼ 13, the resulting halohydrin spontaneously cyclizes to the epoxide in a one-pot one-step fashion.[235a] Unfortunately, only the whole-cell preparate of *Rhodococcus ruber* could tolerate these pH conditions and, therefore, a one-pot two-step protocol in which the pH is adjusted once the enzymatic EED is finished had to be used to obtain the epoxides **290a**−**e**. Alternatively, an epoxidation step catalyzed by a nonselective halohydrin dehalogenase was also successfully used.[235b] However, the latter transformation is an equilibrium and approaches to efficiently displace it in the desired direction are required to obtain high conversions. In this regard, a subsequent ring-opening reaction has been coupled.[235c] Thus, azide and cyanide were successfully used as nucleophiles in a process also catalyzed by the halohydrine

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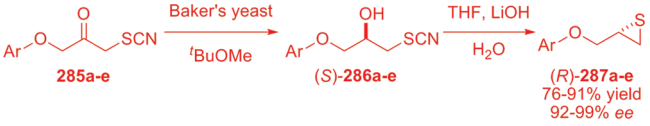
Scheme 55



a: Ar = Ph; **b**: Ar = *p*-Cl-C_6H_4; **c**: Ar = *p*-Me-Ph; **d**: Ar = *o*-Cl-Ph; **e**: Ar = *o*-Me-Ph;

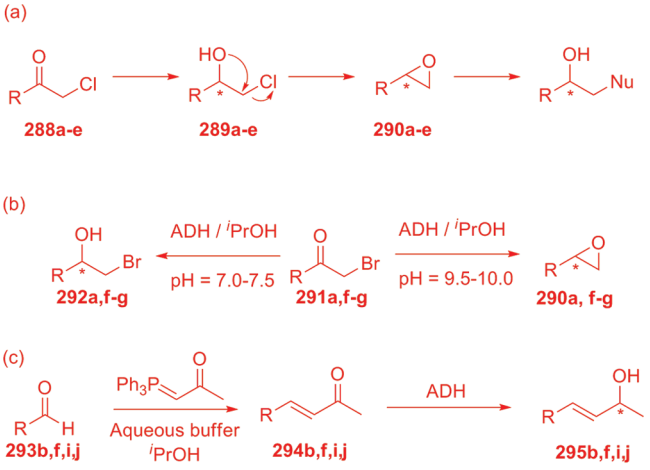
dehalogenase and yielding enantiopure β-hydroxy azides and cyanides in a three-step, two-enzyme one-pot fashion. Alternatively, the addition of hydroxide-loaded anion exchangers also proved helpful as they remove the chloride ions liberated during epoxide formation.[235d]

The employment of α-bromo instead of α-chloroketones would lead to optically active bromoydrines, in which the better leaving group bromine should allow the epoxidation to occur at lower pH values, more compatible with the stability of the ADHs employed in these cascade processes (Scheme 56a). This was demonstrated by Gröger and co-workers, who have employed a designer cell overexpressing an (*R*)-ADH to produce the epoxide (*S*)-**290a**,[236] compound, which was difficult to obtain in high enantioselectivity and yield.[235] The results obtained indeed showed that by setting the pH of a solution of (*S*)-1-bromo-2-octanol, obtained enantiopure and in very high yield, to a value of 11, is enough to obtain the corresponding epoxide in 2 h. However, when the reaction is carried out one-pot, the overall yield is very low and, finally, the optimized the process has to be carried out in a stepwise fashion. Additionally, we have recently shown that although bromohydrines do undergo spontaneous epoxidation at much lower pH values than chlorohydrines, they also decompose much faster to yield, via a S_N2 mechanism, the corresponding hydroxyketones in aqueous conditions.[237] This side reaction can be suppressed by addition of an organic cosolvent and the proper adjustment of pH to either 7.0−7.5 or 9.5−10.0 allows the obtaining of the corresponding either bromohydrins or epoxides, respectively (Scheme 56b). These conditions are compatible with the stability of the two isolated ADHs employed as catalysts, thus offering a route to both enantiomeric series of these types of compounds in a one-pot fashion. Finally, the Staudinger reduction of the isolated azido derivatives **290g** and **292g** allowed the preparation the cyclic aminoalcohols (*R*)-prolinol and (*S*)-piperidin-3-ol enantiopure and in high overall yields.

Gröger et al. have tested the performance of a Wittig reaction designed for pure water in mixtures of aqueous buffer/2-propanol as solvent. Such media are suitable for the catalytic activity of ADHs and would allow the one-pot two-step preparation of the corresponding optically active alcohols starting from aldehydes and phosphorus ylides without the need to isolate the intermediate α,β-unsaturated ketones (Scheme 56c). In particular, the new media did have little effect on the performance of the Wittig reaction. Moreover, the presence of the ylide at the concentrations required only slightly decreased the ADH activity, which allowed the preparation of allylic alcohols (*S*)-**295b,f,i,j** and (*R*)-**295j** enantiopure and in degrees of conversion ranging from moderate to excellent (30−90%).[238]

Ketones can be also reduced to primary amines by means of a reductive amination reaction. The biocatalytic version of such process can be catalyzed by α-amino acid transaminases, which use 2-oxocarboxylic acids as substrates.[239] These enzymes require an amino donor (i.e., an α-amino acid like L-alanine), the relative amount of which will determine the final equilibrium

Scheme 56



a: R = *n*-C_6H_{13}; **b**: R = Ph; **c**: R = Bn; **d**: R = $CH_2CO_2{}^iPr$; **e**: R = CH_2OPh; **f**: R = *p*-NO_2-Ph; **g**: R = $N_3(CH_2)_3$; **i**: R = *o*-MeO-Ph; **j**: R = *p*-Me-Ph;

concentrations of both the target product and the amino donor. For the preparation of primary amines not adjacent to carboxylic acid groups, ω-transaminases have to employed.[239b] For example, the preparation of amines **296**−**299** (Chart 10) in enantiopure form has been recently accomplished by means of optimized processes that make use of ω-transaminases present in the cells of *Arthrobacter* sp. KNK168 and *Pseudomonas* sp. KNK425.[240] Moreover, the synthesis of the antidiabetic amine sigtagliptin [(*R*)-**304**] has been recently accomplished by means of an elegant optimization process that consisted in an initial substrate walking, modeling, and mutational approach that created a transaminase with marginal activity for the corresponding ketone, starting from a transaminase only active toward methyl ketones and small cyclic ones.[241] Further engineering of this variant in a directed evolution fashion yielded the final mutant transaminase, which contained 27 mutations with respect to the wild-type enzyme and converted 200 g/L of prositagliptin ketone to sitagliptin of >99.95% *ee* by using 6 g/L enzyme in 50% DMSO with a 92% assay yield at the end of reaction. Moreover, the so-obtained catalyst was also very efficient in the enantioselective preparation of various trifluoromethyl-substituted amines as well as phenylethylamines with electronrich substituents, which cannot be generated via traditional reductive amination, and chiral pyrrolidines.

In this type of process, however, the equilibrium of the reaction is even more displaced toward the side of the substrate ketone as compared to 2-oxocarboxylic acids, which means that only small amounts of the target amine are formed. Therefore, different strategies have been developed for the displacement of the equilibrium. For instance, Bornscheuer and co-workers have recently observed that coupling a ω-transaminase with a pyruvate decarboxylase efficiently displaces this equilibrium by production of highly volatile CO_2 from the lactate formed from the amino donor (L-alanine). Thus, amines **300**−**303** have been efficiently synthesized (Chart 10).[242]

On the other hand, Kroutil and co-workers have envisaged a cascade reaction that consists of (1) a ω-transaminase to transfer an amino group from an amino acid like L-alanine to a substrate ketone, (2) an amino acid dehydrogenase which recycles the amino donor from the so-formed pyruvate at the expense of ammonium and NADH, and (3) an ADH like formate dehydrogenase to finally recycle the oxidized cofactor by oxidizing

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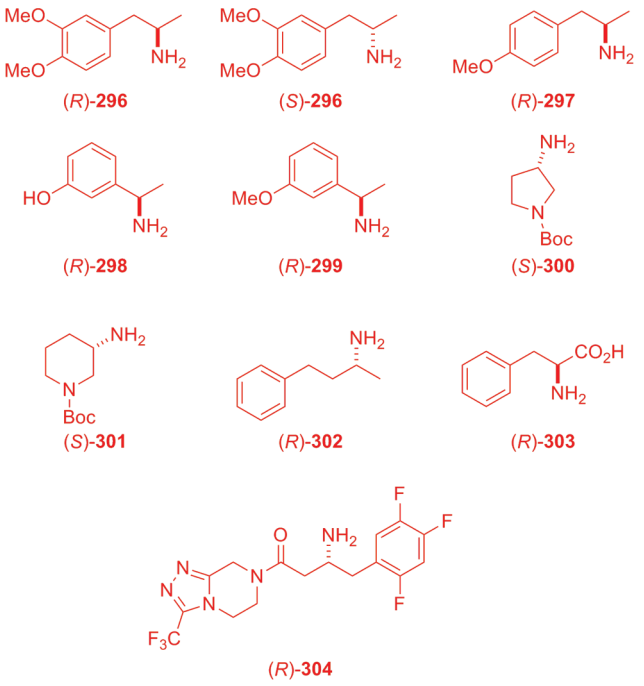
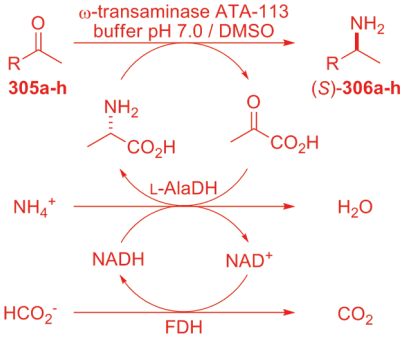
Chart 10



formate to carbon dioxide, thus ensuring the displacement of the equilibrium in the desired direction (Table 22).[243] Application of such a system to a wide series of dialkyl and aryl alkyl ketones (**305a**−**h**) afforded the corresponding primary amines (*S*)-**306a**−**h** in high conversion and excellent enantioselectivity in most of the cases. To do so, from the 19 commercially available ω-transaminases tested, ATA-113 was selected as the enzyme of choice. Additionally, the process optimization also required the addition of DMSO (15% v/v) as cosolvent and a concentration of the amine donor (L-alanine) of 250 mM. Moreover, the employment of ATA-117 allowed the obtainment of (*R*)-**306a** in >99% *ee*. Both D- and L-alanine can be used as donor without affecting the enantioselectivity of the reductive amination. The only difference is observed in the reaction rate, which was 20 times slower with L-alanine.

Table 22. Reductive Amination of Simple Ketones Catalyzed by ω-Transaminase ATA-113



entry	substrate	R	*c* (%)	*ee* (%)
1	**305a**	^{n}Pr	92	93
2	**305b**	Et	>99	>99
3	**305c**	^{n}Hex	89	>9
4	**305d**	Ph	6	>99
5	**305e**	2-Ph-$(CH_2)_2$	>99	34
6	**305f**	*p*-MeO-Ph−CH_2	>99	>99
7	**305g**	PhO−CH_2	50	86
8	**305h**	EtO_2C−CH_2	>99	94

6.2. Reduction of Carbon−Carbon Double Bonds

As it has already been pointed out, similarly to carbon−oxygen double bonds, carbon−carbon double bonds of prochiral alkenes can be enantioselectively reduced to obtain the corresponding optically active saturated compounds. Although isolated carbon−carbon double bonds can be successfully reduced through this approach,[244] the bioreduction of alkenes conjugated with a carbonyl group has been more studied. These processes are typically catalyzed by enoate reductases (ERs), enzymes which belong to the old yellow enzyme (OYE) family. These flavin-dependent oxidoreductases catalyze the asymmetric reduction of electronically activated carbon−carbon double bonds with at least one electron-withdrawing substituent such as a carbonyl- or nitro-group. In the catalytic mechanism of enoate reductases, a hydride derived from the flavin co-factor is transferred enantioselectively onto the C_β of the alkene, while a catalytic conserved Tyr adds a proton, which is ultimately derived from the solvent, onto the C_α from the opposite side.[245] This mechanism, which resembles that of the Michael addition, is consequently also strictly *trans*-specific. Another consequence of this mechanism is that carbon−oxygen and nonactivated carbon−carbon double bonds are completely unreactive. The catalytic cycle of enoate reductases requires the reduction of the oxidized flavin at the expense of NAD(P)H, which is ultimately derived from an external H-donor via another redox-reaction. Due to the complexity of cofactor recycling, the vast majority of biotransformations using enoate reductases have been performed using whole cells. Thus, the EED of α,β-unsaturated aldehydes, ketones, and carboxylic acid derivatives by chemoselective reduction of the olefinic bond, leaving the carbonyl group untouched, can be achieved. For instance, the bioreduction of methyleneketones **307a**−**c** was carried out with *Rhizopus arrhizus*, *P. fluorescens*, and immobilized *S. cerevisiae* (Scheme 57).[246] The best results were obtained with *P. fluorescens*, which afforded the corresponding optically active saturated ketones (*R*)-**308a** and (*S*)-**308b**−**c** in excellent *ee*'s and yields.

The application of oxygen-stable ERs to synthesis is hampered, on the one hand, by their limited substrate space, which has not been fully explored. On the other hand, the yields of these processes tend to be low due to side reactions like the reduction/oxidation of the carbonyl group. The absence of competing oxidoreductases is more difficult to attain than in the case of the ADH-catalyzed reduction of keto groups because these enzymes are usually required to recycle NAD(P)H, which is used to reduce the flavin cofactor. However, the development of nicotinamide-independent recycling systems has been an active and successful field of research. For example, the enantioselective reduction of ketoisophorone catalyzed by the OYE homologue from *Bacillus subtilis* YqjM was carried out in absolute chemoselectivity by using a light-driven regeneration system which profits from the photoexcitability of flavins.[247] It was demonstrated that such a regeneration system does not affect the enantioselectivity of this process, which was however poor due to the presence of competing enoate reductases. Moreover, Faber and co-workers have recently presented another nicotinamide-independent enantioselective reduction of the carbon−carbon double bond of several activated

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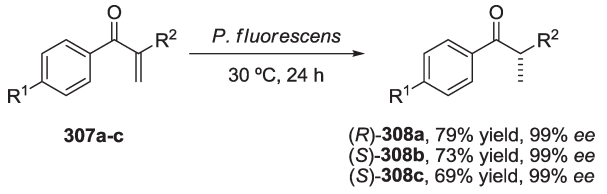
alkenes in a substrate-coupled fashion.[248] β-Substituted cyclohexenones, which were not reduced with ERs, serve as H-donors and are oxidized to the corresponding phenols, while the substrates selected are reduced. Thus, the flavin cofactor is recycled without the need of NAD(P)H or an ADH.

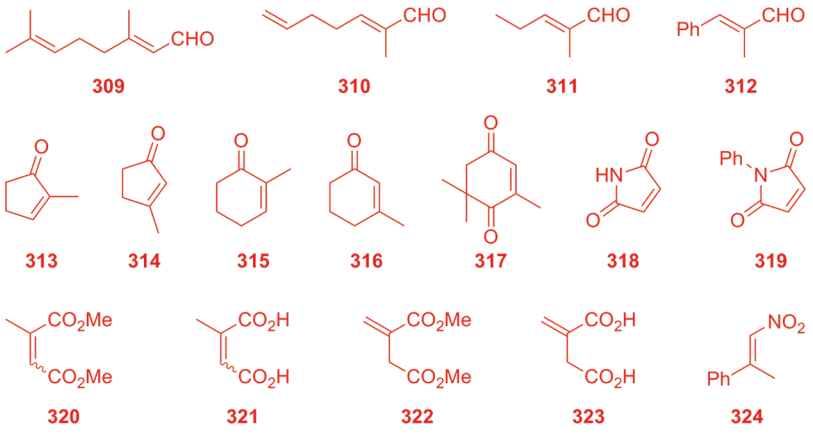
Concerning the substrate spectra of ERs, recent progress has been also recently made. On the one hand, the enantioselectivity of an ER can be successfully modulated by means of the preparation of mutant enzymes.[249] On the other hand, the structural diverse subset of alkenes shown in Chart 11 have been systematically used to probe the substrate space of the following overexpressed or purified ERs:[250] XenA from *Pseudomonas putida*, KYE1 from *Kluyveromyces lactis*, Yers-ER from *Yersinia bercovieri*, 12-oxophytodienoate reductase isoenzymes OPR-1 and OPR-3 from *Lycopersicon esculentum*, YqjM from *Bacillus subtilis*, NCR from *Zymomonas mobiliz*, OYE-1 from *Saccharomyces carlsbergensis*, OYE-2 and OYE 3 from *Saccharomyces cerevisiae*, pentaerythritol tetranitrate reductase, morphinone reductase, *N*-ethylmaleimide reductase, and estrogen-binding protein. In general, many of them can be obtained in very high chemo- and enantioselectivities, the latter being highly dependent on the substrate and enzyme used. Thus, the position occupied by the substituent of the alkene (α or β) or the configuration of the double bond (*cis* or *trans*) can reverse the enantiomeric ratio of these EEDs.[250c] Moreover, enantiocomplementary enzymes were also found. Thus, for nitroalkene **324**, OPR-1 and OPR-3 from *Lycopersicon esculentum* afforded opposite enantioselectivities,[250b] a fact explained on the basis of geometrical differences in the active sites of these homologues due to nonconserved loops.[250d] Similarly, morphinone reductase and estrogen-binding protein often a complementary enantiopreference in comparison to that of previously investigated OYEs.[250f]

Scheme 57



Citral (**309**, Chart 11) is an antimicrobial terpene widely used in the flavor and fragrance industry. It consists of a mixture of *cis* and *trans* isomers referred to as geranial and neral, respectively. The enantioselective reduction of the conjugated carbon−carbon double bond of this relatively inexpensive commodity produces the much more expensive citronellal. The preparation of this compound is highly challenging due to the presence of several double bonds, which requires the corresponding process not only to be highly enantioselective but also chemo- and regioselective. In this regard, Rosche and co-workers have screened 46 yeast and bacterial strains for enantioselective reduction of the carbon−carbon double bond of citral conjugated with the aldehyde group.[251] The results obtained show that the employment of a two-phase medium affords higher hit rates than aqueous media, probably due to higher effective concentrations of the substrate, better permeabilization of the cells, and decreased ADH activity. In particular, toluene and methyl *tert*-butyl ether were the solvents of choice. Concerning selectivity, both the amounts of undesired alcohol products, which lower the yields of these processes, and the *ee* values of citronellal were dependent on the microorganism used. As a general feature, higher citronellal concentrations were obtained by using the prokaryotic strains *Zymomonas mobiliz* and *Citrobacter freundii*, although only the former showed very high enantioselectivity in the production of the (*S*)-enantiomer (>99%). In contrast, the eukaryotic strains showed opposite enantioselectivity with *ee* values of >96% (*Candida rugosa*, *Saccharomyces bayanus*, and *Saccharomyces cerevisae*). Overexpression of OYEs from *Saccharomyces cerevisiae*, *Saccharomyces pastorianus*, and *Zymomonas mobiliz* in *Escherichia coli* strains leads to improved enoate reductase activities.[252] Thus, citral (**309**) and the other α,β-usaturated carbonyl compounds tested (Chart 11; **311**, **312**, **315**, and **316**) were reduced in all cases to the corresponding saturated carbonylic derivatives with no detection of the corresponding alcohol byproduct. However, the enantioselectivities differed with the catalyst and substrate employed. In particular, the following factors were identified to influence the enantioselectivity of these EEDs: (i) the position of the methyl group in α- or β-position (OYE 1 reduced α-methyl 2-cyclohexenone (**315**) to the (*R*)-enantiomer and β-methyl 2-cyclohexenone (**316**) to the (*S*)-enantiomer.[253] (ii) The *E*- or *Z*-configuration of the double-bond as illustrated by different enantio-specificities observed with *E*- and *Z*-citral (**309**). (iii) The substituent of the β-position (opposite enantiomers are obtained from *E*-2-methyl-pentenal (**311**) and

Chart 11



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E-3-phenyl-2-methyl-propenal (**312**) which differ only with regards to an ethyl versus phenyl group).

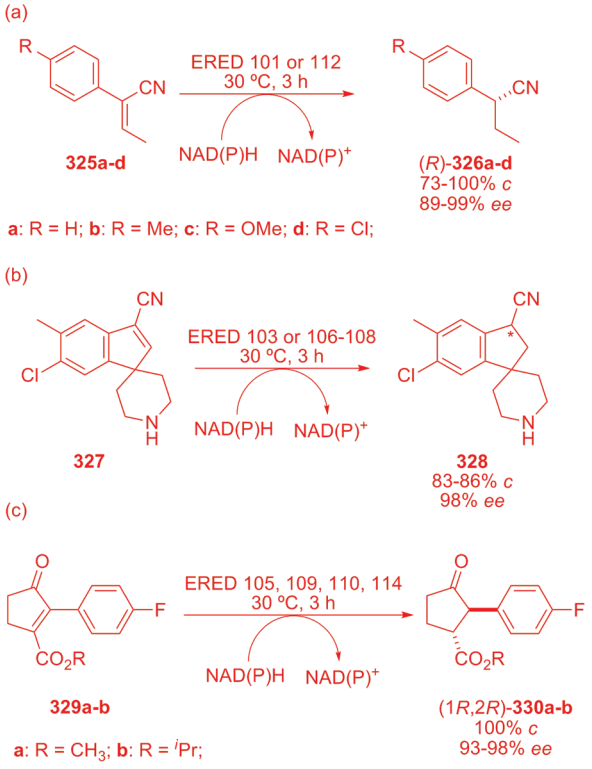
The enantioselective reduction of the carbon−carbon double bond of conjugated nitriles remains a challenge due to their inherent low reactivity. In this regard, Kosjek and co-workers have shown that commercial isolated ERs can successfully desymmetrize a set of α,β-unsaturated nitriles (**325a−d**) to afford the corresponding (*R*)-configured saturated nitriles in high yields and optical purities (Scheme 58a).[254] Similarly, the more complex pharmaceutical building block **327** can be also desymmetrized in high yield and excellent optical purity by 4 out of the 14 screened enzymes (Scheme 58b). On the other hand, the same authors have also tested the performance of these enzymes on the 2,3-disubstituted cyclopentenones **329a−b** (Scheme 58c). This type of substrate is very interesting since the reduction of its carbon−carbon double bond affords ketones with two chiral centers. The results obtained show that for both substrates, the *trans*-isomers are the major one (>92%), which are obtained in quantitative conversions and excellent enantioselectivities. Finally, the applicability of these biotransformations was demonstrated on a 200 mg scale for nitrile **325a** and ketone **329a**. ERED 112 and 114 were selected as catalysts, respectively, and an in situ nicotinamide cofactor recycling system based on a sodium phosphite/phosphite dehydrogenase was employed to yield the corresponding saturated compounds in >70% yield and with the same optical purity observed in the screening process.

The concurrent reduction of the carbonyl group of unsaturated aldehydes can be beneficial depending if optically active primary alcohols, compounds difficult to achieve by means of enzymatic KRs, are targeted. Thus, Fuganti et al.[255] have converted the unsaturated aldehydes **331a−c** into the saturated alcohols (*S*)-**332a−c** by using fermenting cells of Baker's yeast as catalyst (Scheme 59). The microbial saturation of these substrates adsorbed on a nonpolar resin proceeds in good yields and excellent enantioselectivities. The usefulness of enantiopure (*S*)-**332a−c** as chiral building blocks for the synthesis of bisabolane sesquiterpenes was shown in the preparation of (*S*)-(+)-curcuphenol, (*S*)-(+)-xanthorrhizol, (*S*)-(−)-curcuquinone, and (*S*)-(+)-curcuhydroquinone.

The Baker's yeast-catalyzed reduction of acetals **333a−d** afforded the corresponding optically active alcohols in high yields and in *ee*'s ranging from 90 to 98% (Scheme 60).[256] These derivatives are much more efficiently hydrogenated than the related allylic alcohols (14 days) or the aldehydes (>4 days), which although are believed to be the real substrates of this transformation, presumably inhibit the enzymatic system.[257]

Serra et al. have carried out a study on the chemo- and enantioselectivity of the Baker's yeast-mediated reduction of sulfur-functionalized methacroleins to obtain the corresponding sulfur-functionalized allylic alcohols, suitable precursors for the preparation of the bifunctional methyl branched C4 chiral synthons (Scheme 61).[258] They have found that the stereochemical behavior of the double bond biohydrogenation depends on the position of the double bond and also on the oxidation state of sulfur. Thus, the reduction of aldehydes **335a−b** afforded the corresponding saturated alcohols of opposite configuration in good *ee*'s but somewhat low yields. This fact was due both to low degrees of conversion and to incomplete reduction of the starting material, the allylic alcohols thus being obtained in significant yields. Nevertheless, recycling of the allylic alcohols by means of an oxidation process would allow optimization of this process. On the other hand, reduction of aldehyde **338** afforded

Scheme 58



the corresponding saturated alcohol (*S*)-**337a** with moderate *ee*, which decreased with increasing reaction time. The optically active saturated acohols containing the sulfide moiety [(*R*)- and (*S*)-**337a**] were easily transformed into the corresponding sulfones, thus giving access to both enantiomers of the sulfur-functionalized alcohol **337b** in high *ee*'s.

6.3. Oxidation of Alcohols

Dehydrogenases are not only able to catalyze the enantioselective reduction of prochiral ketones but can also effectively desymmetrize *meso* and prochiral diols through enantioselective oxidation. Optically active hydroxyketones and hydroxycarboxylic acids, or related derivatives, are thus obtained as products. In fact, some examples can be found in the literature. For instance, (*R*)-3-hydroxy-2-methyl propionic acid [(*R*)-**341**], key intermediate of the synthesis of Captopril, has been prepared by oxidative EED of prochiral 2-methyl-1,3-propanediol (**340**).[259] After screening of different acetic acid bacteria, *Acetobacter pasteurianus* DSM 8937 afforded the best results. Optimization of reaction conditions provided evidence for the importance of the type of carbon source used and allowed the production of (*R*)-**341** in 97% *ee* (Scheme 62).

Romano et al. have investigated the catalytic and enantiodiscriminative capabilities of acetic acid bacteria belonging to the genuses *Acetobacter* and *Gluconobacter* in the oxidation of different primary alcohols and chiral and prochiral diols.[260] In particular, they have carried out the EED of *meso*-2,3-butandiol (**342**), finding out that the biotransformation proceeded without diacetyl formation. Moreover, with the sole exception of *G. cerinus* DSM 9534, a general preference for the oxidation of the hydroxyl in the (*R*)-configuration was also observed. Different strains afforded (*S*)-hydroxyketone (*S*)-**343** in excellent *ee*'s, being *G. asaii* MIM 1000/9 the best one. After 1 h, this

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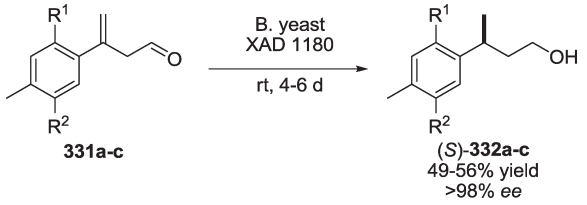
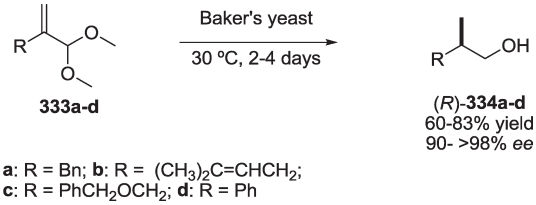
Scheme 59



Scheme 60



microorganism yielded (*S*)-**343** in complete conversion and >97% *ee* (Scheme 63a). Conversely, the recently reported lyophilized cells of *Rhodococcus ruber* DSM 44541 containing alcohol dehydrogenase ADH-"A"[142] show a marked preference for the oxidation of (*S*)-configured alcohols, the enantioselectivity being excellent for relatively large substrates.[224] For instance, *meso* diol **344** is converted to the corresponding hydroxyketone in >99% *ee* and 62% yield (Scheme 63b). On the other hand, *meso* diol **346** was transformed into the synthetically interesting lactone (+)-**347** by means of horse liver alcohol dehydrogenase-catalyzed oxidation (Scheme 63c).[261] This way, the title lactone was obtained in good yield and excellent *ee*. Moreover, the employment of different catalysts (cells of *Geotrichum candidum* 5767 under aerobic conditions or of *Mucor heimalis* 6095) allows the obtaining of either (2*R*,3*R*)-**251** or (2*S*,3*R*)-**251** by oxidation of *meso* 2-methyl-2-(2-propen-1-yl)-cyclopentan-1,3-diol in excellent yields and enantioselectivities.[207] These biotransformations, together with the reductive EEDs of the corresponding diketone shown in Scheme 47, allow access to the four possible diastereomers of the hydroxyketone **251**.

Finally, the desymmetrization of *meso* pyrrolidines by means of the oxidation of an isosteric amine group has been recently reported.[262] The enzyme employed a monoamine oxidase from *Aspergillus niger* (MAO-N) and proved to be a very efficient catalyst in the EED of pyrrolidines **348a**−**f** (Scheme 64). Moreover, the feasibility of the subsequent addition of a nucleophile to render 3,4-disubstituted proline derivatives was demonstrated by the study of the addition of HCN under different conditions, which also included cascade reactions. The corresponding proline analogues were thus obtained in diastereomeric ratios that strongly depended on the reaction conditions.

6.4. Monohydroxylation of Methylene Carbons

Optically active secondary alcohols can be obtained from prochiral alkanes by means of enantioselective hydroxylase-catalyzed desymmetrizations, transformations that still remain a challenge by other means. The vast majority of these transformations are catalyzed by cytochrome P450-dependent monooxygenases. Their membrane-bound nature, together with their functional dependence on cofactors and their related electron transport proteins, has ensured that whole-cells catalysts are the preferred ones.

Most of the examples of oxyfunctionalization of unactivated C−H bonds catalyzed by microorganisms make use of natural products as starting material.[263] Simple alkanes, which can be regarded as more "difficult" substrates, usually have been hydroxylated by the employment of methanogenous microorganisms. However, this type of catalyst shows the highest efficiency with methane as substrate, while for substrates with increased chain length, the yields are significantly reduced. Yet, Adam et al. have recently demonstrated that *Bacillus megaterium* hydroxylates a variety of simple *n*-alkanes (Chart 12, **190a**−**d**) to afford, in some cases, enantioenriched alcohols in up to 99% *ee*.[264] Although the synthetic utility of these biotransformations is limited by the fact that mixtures of regioisomers in low yields are usually obtained, the authors extract some interesting conclusions: (1) the time of substrate addition plays a crucial role with regard to the product formation, (2) the observed *ee* values were ascribed to the direct hydroxylation of the *n*-alkanes by the microorganism, (3) no primary alcohols were detected, and (4) only small enantioselectivities were obtained when the substituents that flank the hydroxylated position were similar in size.

The same strain was also able to perform the chemo- and enantioselective hydroxylation of the unfunctionalized arylalkanes **350e**−**l** (Chart 12).[265] In general, and conversely to the previous oxidation of alkanes, lower enantioselectivities were achieved. However, the α-oxidation of **350j** (91% *ee*) and the *β*-, *γ*-, and *δ*-oxidations of **350l** (88, 86, and >99% *ee*, respectively) constituted remarkable exceptions. Furthermore, the (*R*)-enantiomers were preferably formed with the exceptions of the *β*- an *γ*-oxidations of **350l**. This substrate was also the only that showed measurable overoxidation of the resulting alcohols to the corresponding ketones. Surprisingly, nonterminal homobenzylic positions were preferred to the benzylic ones, substrate **350f** being the only exception. The low yields obtained in this biotransformations can be effectively improved by immobilization of the *B. megaterium* cells in alginate gel.[265b]

The hydroxylation of nonactivated C−H bonds has also been applied with synthetic purposes. Thus, a process has been developed for the preparation on a 50 mL scale of (*S*)-4-hydroxy-2-pyrrolidinones, useful intermediates for the preparation of antibiotics, in which an oxygen atom is inserted regio- and stereoselectively into the corresponding nonhydroxylated 2-pyrrolidinones by resting cells of *Sphingomonas* sp. HXN-200 (Scheme 65a).[266] Namely, hydroxylation of *N*-benzyl-pyrrolidin-2-one (**351a**) gave a 75% conversion of enantiopure (*S*)-*N*-benzyl-4-hydroxypyrrolidin-2-one [(*S*)-**352a**]. The presence of 2% glucose, which contributed to cofactor regeneration, was mandatory. Although the enantioselectivity for hydroxylation of the *N*-BOC compound **351b** was lower, the *ee* was increased by recrystallization to afford enantiopure (*S*)-**352b**. Moreover, this strain has also proven to hydroxylate other related prochiral heterocycles although in lower enantioselectivities.[267] Recently, substrate and protein engineering have been combined to improve the synthetic outcome of the biohydroxylation of carboxylic acid **353** (Scheme 65b).[268] Thus, this substrate was first transformed into 2-cyclopentylbenzoxazole (**354**), which was amenable to biohydroxylation with a library of mutants of a medium chain fatty acid hydroxylase from *Bacillus megaterium* (cytochrome P450 BM-3), both in whole cell and purified format. Depending on the mutant used, both enantiomers of the hydroxylated derivative in position 3 of the cyclopentane ring were

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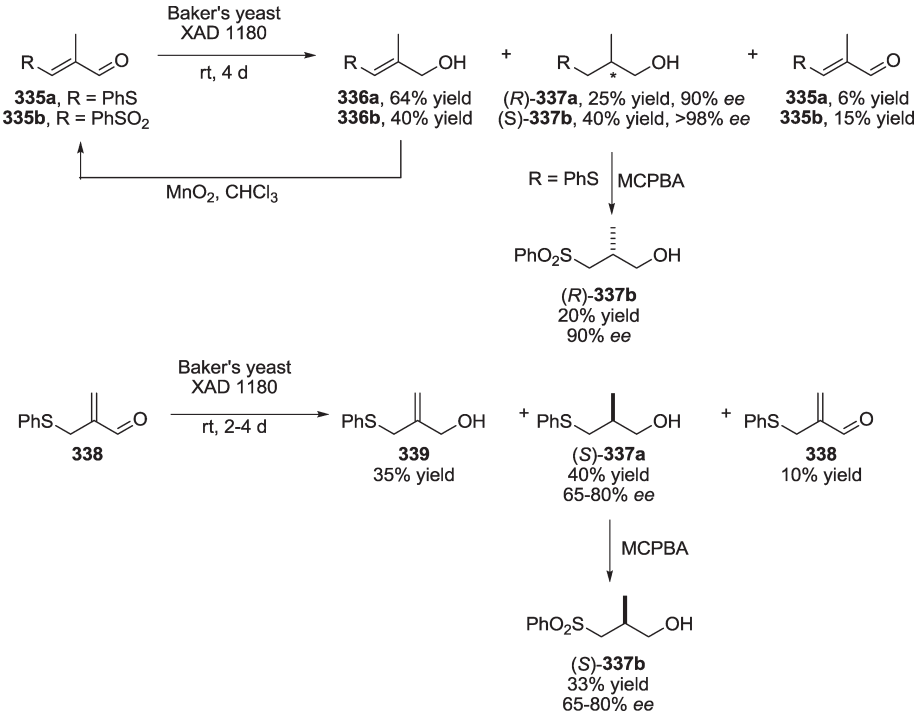
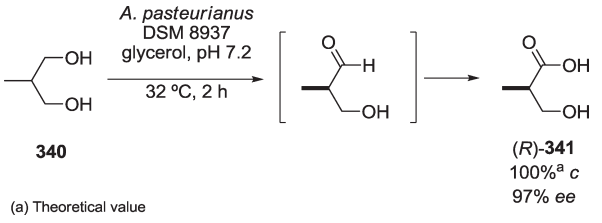
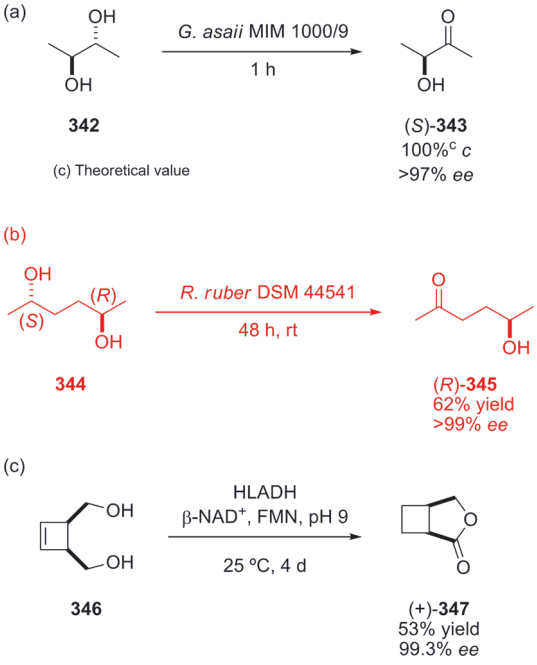
Scheme 61



Scheme 62



Scheme 63



obtained in high enantio- and diastereoselectivities, although yields still have to be optimized in order to attain synthetic usefulness. Subsequently, protecting group removal provided end product.

Peroxidases are readily available enzymes that do have the potential to be useful catalysts.[269] The reason is that they do not require additional cofactors, and hence, they can be used isolated. In this regard, Hu and Hager have published one of the very few reports concerning the enantioselective desymmetrization of acetylenes to afford optically active propargylic alcohols.[270] In particular, they report, for the first time, the chloroperoxidase (CPO)-catalyzed hydroxylation of alkynes **356a**−**k** to yield the corresponding (*R*)-alcohols (Table 23). This enzyme is a heme-iron-containing peroxidase, which uses hydrogen peroxide as oxidant. Results obtained showed that, in most cases, the yields could be increased by the addition of more enzyme and oxidant. However, they focused on the study of the enantioselectivity displayed by this enzyme. When the smallest alkyne (**356a**, entry 1) served as substrate, both the *ee* and yield were very moderate, but both parameters dramatically increased for an acetylene having an additional carbon atom (**356b**, entry 2). Moreover, CPO proved to be progressively less enantioselective toward alkynes possessing additional carbons (**356c**−**d**, entries 3 and 4) with the exception of phenyl acetylene (**356e**, entry 5), which was hydroxylated with good enantioselectivity, although the yield is somewhat low. Surprisingly, when an electron-withdrawing group is attached to the methyl group remote from the prochiral propargylic carbon (**356f**−**g**, entries 6 and 7), the enantioselectivities and yields showed remarkable enhancements. When these functional groups were more remote from the triple bond, the *ee* still remained high for **356i** (entry 9), but there was a

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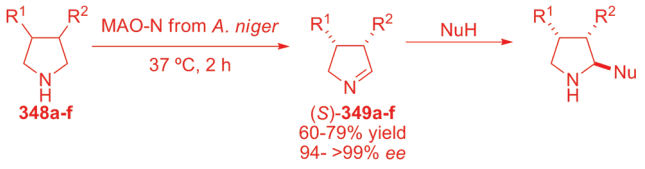
significant decrease for **356h** (entry 8). However, in both cases, the yields were significantly lower. Furthermore, the presence of an ethyl group adjacent to the prochiral propargylic carbon promoted high stereoselectivity and a dramatic decrease of the yield. These results suggest that the active site of CPO is very sterically constrained.

Other reagents such as *tert*-butyl hydroperoxide can be used as oxidants, but, in all cases, lower enantioselectivities were obtained. This effect was attributed to the higher concentrations of this oxidant as compared to H_2O_2, a fact that promoted further oxidation of the propargylic alcohols to α,β-ynones. Moreover, keeping very low concentrations of the oxidant in the reaction medium was also critical to protect the enzyme from inactivation.

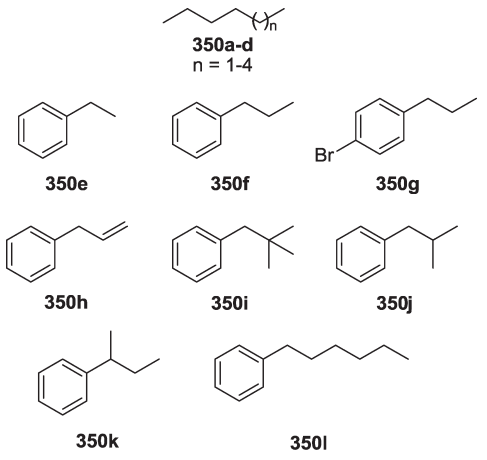
The germinating peas α-oxidase-catalyzed α-oxidation of different fatty acids (**358a**−**n**) led to intermediary α-hydroxyperoxy acids which preferentially decarboxylated to the corresponding aldehydes (**360a**−**n**) in competition with reduction to the (*R*)-2-hydroxy acids **359a**−**n** (Scheme 66).[271] A major breakthrough for preparative-scale applications was the use of tin(II) chloride as an in situ reducing agent. Thereby, the undesirable competitive decarboxylation of the 2-hydroperoxy acids to the aldehydes was circumvented and a biocatalytic α-hydroxylation of the carboxylic acids has been made available for the direct synthesis of enantiopure 2-hydroxyacids in excellent yields. Moreover, these results demonstrate that the α-oxidase system selectively autoxidizes the C−H^R bond of the prochiral α-methylenic group in carboxylic acids.

Scheme 64



a: $R^1 + R^2 = -CH_2-$, **b**: $R^1 + R^2$ = 1,3-cyclopentyl,
c: $R^1 + R^2$ = 1,3-cyclopent-2-enyl
d: $R^1 = R^2$ = H, **e**: $R^1 + R^2 = -CH=CH-$, **f**: $R^1 + R^2 = -CH_2-CH_2-$

Chart 12



6.5. Oxidation of Sulfides

Peroxidases can also efficiently catalyze the enantioselective sulfoxidation of a variety of sulfides. Despite their enormous potential, their application is hampered by their low operational stability. In this regard, during the recent past years many advances have been made to improve their performance,[272] especially by means of medium engineering,[273] immobilization techniques,[274] or the development of bienzymatic systems that progressively in situ generate H_2O_2 at the expense of air and a carbon source.[275] Nevertheless, other types of biocatalysts such as a vanadate containing phytase (an acid phosphorylase),[276] monooxygenases,[277] dioxygenases,[278] and catalytic antibodies[279] have also demonstrated their ability to catalyze the EED of sulfides.

In parallel with these advances, several authors have also studied the substrate specificity of new promising strains able to catalyze this transformation. For instance, Adam et al. have recently described the enantioselective sulfoxidation of a series of organic sulfides (**361a**−**h**) catalyzed by *Pseudomonas frederiksbergensis* sp. Nov. (Table 24).[280] It was first observed that both

Scheme 65

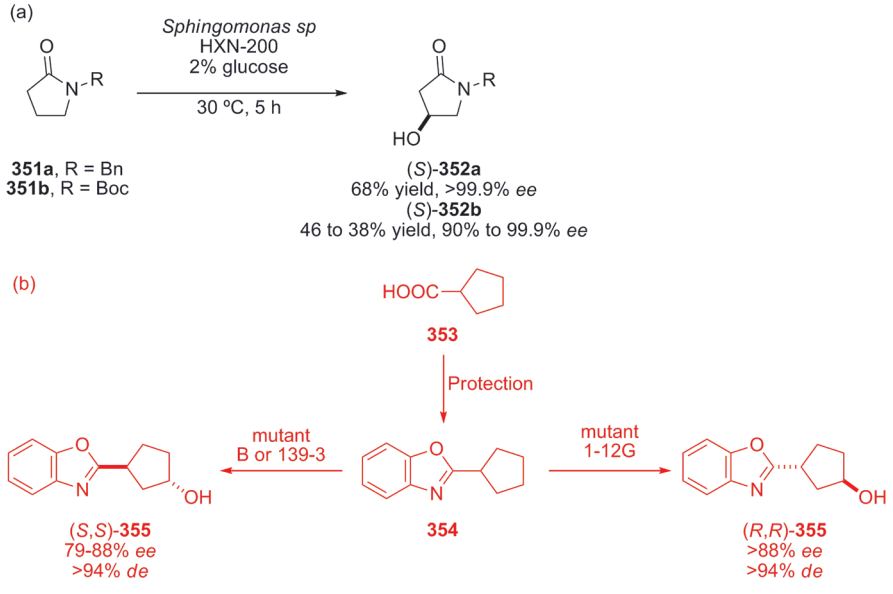


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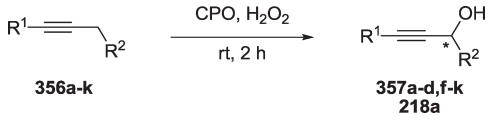
Table 23. EED of Propargylic Methylenes

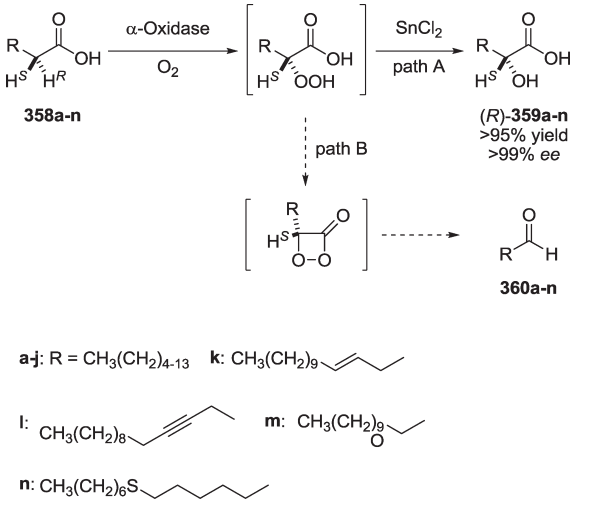


entry	alkyne	R^1	R^2	yield (%)	*ee* (%)	config
1	**356a**	Me	Me	7	57	*R*
2	**356b**	Et	Me	26	91	*R*
3	**356c**	Pr	Me	30	87	*R*
4	**356d**	Bu	Me	8	78	*R*
5	**356e**	Ph	Me	15	86	*R*
6	**356f**	CH_2OAc	Me	52	95	*R*
7	**356g**	CH_2Br	Me	65	94	*R*
8	**356h**	$(CH_2)_2OAc$	Me	26	83	*R*
9	**356i**	$(CH_2)_2Br$	Me	25	94	*R*
10	**356j**	CH_2OAc	Et	8	87	n.d.
11	**356k**	$COCH_3$	Me	n.r.	n.r.	n.r.

the reaction time and the pH influenced the yield and the enantioselectivity in which (*S*)-**362a**, the model compound, was obtained. Then the rest of sulfides (**361b**−**h**) were subjected to oxidation with this microorganism and the corresponding (*S*)-sulfoxides (*S*)-**362b**−**h** were obtained in nonoptimized yields ranging from 4 to 48% and in good to excellent enantioselectivities. In particular, **361a** was sulfoxidized in 91% *ee* (entry 1). In most of the cases, substitution in the *para* position yielded enantiopure sulfoxides regardless the nature of the substituent (**361b**−**d**, entries 2−4). To assess the effect of the alkyl side chain on the enantioselectivity, the *n*-propyl derivative **361e** was tested together with **361f** (entries 5 and 6) and compared to the results obtained for the methyl phenyl sulfide (**361a**, entry 1). As it can be seen from the table, these results indicate a decrease in both the reaction rate and the enantioselectivity due to the increase of the size of the alkyl substituent, an effect that is more pronounced for the case of sulfide **361f**. Moreover, intercalation of a methylene unit between the sulfur atom and the aryl substituent has a deleterious effect on both enantioselectivity and reactivity (**361g**, entry 7). It is also worth mentioning that dialkyl sulfides, such as **361h**, are sulfoxidized in good enantioselectivities as well (entry 8).

Porto et al. have prepared sulfoxides (*S*)-**364a**−**f** by using *Aspergillus terreus* CCT 3320 cells, which oxidized the precursor sulfides **363a**−**f** (Scheme 67).[281] These biotransformations led, in most cases, to *ee*'s better than 95%. Additionally, variation of the *ee* values depending on the reaction time was observed. This fact was rationalized as a double step process consisting of an initial desymmetrization followed by KR of the resulting optically active sulfoxides to yield the corresponding sulfones. The enantiotopic differentiation of the first step would be moderate but enhanced in a highly enantioselective fashion by the subsequent KR. The efficiency of the latter was confirmed by using a racemic mixture of (±)-**364a**, the corresponding sulfone and sulfoxide obtained being enantiopure. In an attempt to improve the biocatalytic process, the cells were immobilized on two supports, chrysotile and cellulose/TiO_2. Although the immobilized cells showed a similar biocatalytic behavior, they allowed a faster separation from the reaction media and an easier reuse of the biocatalyst. This behavior was attributed to the fact that the

Scheme 66



immobilized cells are intertwined with the fibers of both supports, which was corroborated by means of scanning electron microscopy micrographs. Besides, supported cells stored for at least 3 months showed no loss of activity.

The efficient sulfide-monooxygenase producing strain ECU0066 has been recently isolated and identified as *Rhodococcus* sp.[282] Resting cells of this biocatalyst could transform phenylmethyl sulfide (**365a**) into (*S*)-phenylmethyl sulfoxide (**366a**) in enantiopure form and, as in the above-mentioned example, the yield and *ee* values are due to a sequential process consisting of an EED and a subsequent KR. Although the employment of recombinant protein, that suppresses the KR that overoxidates the product sulfoxide to the corresponding sulfone, did increase the final yield, the enantioselectivity decreased considerably (86.5% *ee*).[283] However, some other sulfides (**365i,j,n**) could be prepared in high yields (>60%) and enantioselectivities (>91.7%) by using the whole-cell catalyst.

Washed-cell preparations of recombinant *E. coli* JM109-(pDTG141), engineered to express the naphthalene dioxygenase (NDO) gene from *Pseudomonas* sp. NCIB 9816-4, have been used to biooxidize a range of aryl alkyl- (**365a**−**t**) and dialkyl- (**365u**−**y**) sulfides (Table 25) to the corresponding optically active sulfoxides to determine its substrate specificity.[278] Despite the predilection of this enzyme to form *cis*-dihydrodiols with various aromatic hydrocarbons and the proposed requirement for at least one aromatic ring for substrate binding by NDO, the aromatic ring of the aryl alkyl sulfides tested is not a site for enzymatic oxidative attack, nor can it be a prerequisite for heteroatom biooxidation.

NDO-mediated sulfoxidation of the sulfides tested resulted in predominantly *pro-S* attack. Notable exceptions to this trend were the oxidation of aryl alkyl sulfides possessing either *n*- or *i*-alkyl groups with ≥3 carbon atoms (**365c**−**e**, entries 3−5), for which the (*R*)-selectivity was favored. The same behavior was observed for those dialkyl sulfides in which the largest alkyl substituent contained ≥7 carbon atoms (**365w**−**y**, entries 23−25). Nevertheless, in this case negligible sulfoxidation was detected, although significant substrate disappearance linked to the appearance of an uncharacterized product was observed.

In the desymmetrization of aryl alkyl sulfides, the addition of some electron-donating or electron-withdrawing groups to the

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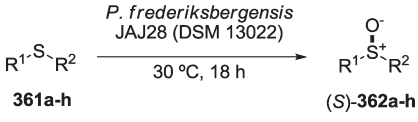
Table 24. *P. frederiksbergensis*-Catalyzed Desymmetrization of Sulfides



361a-h (*S*)-**362a-h**

entry	substrate	R^1	R^2	*c* (%)	*ee* (%)
1	**361a**	Ph	Me	87	91
2	**361b**	4-Me-Ph	Me	100[a]	>99
3	**361c**	4-MeO-Ph	Me	98	>99
4	**361d**	4-Cl-Ph	Me	86	96
5	**361e**	Ph	^{n}Pr	27	81
6	**361f**	Ph	^{i}Pr	<5	
7	**361g**	Bn	Me	<5	
8	**361h**	Cy	Me	98	70

[a] Theoretical value.

phenyl ring can decrease the yield and/or the stereoselectivity of their NDO-catalyzed desymmetrization. In particular, electron influences on the phenyl ring due to *para*-substitution (**365g**−**n**) do not appear to have a significant effect on the stereochemichal outcome of the NDO-catalyzed sulfoxidation (compare entries 1 and 2 with entries 7−14). Conversely, this was not the case when the aryl moiety was *meta*- or *ortho*-substituted (**365o**−**p**, entries 15−16). Actually, it was observed that the closer the substitutent approached the sulfur atom of the substrate the lower the *ee* value of the resultant sulfoxide was (*para* > *meta* > *ortho*; entries 10 and 15−16). These results suggest that nonsusbstituted and, at least, *para*-substituted methyl phenyl sulfides must occupy a similar orientation within the active site of NDO.

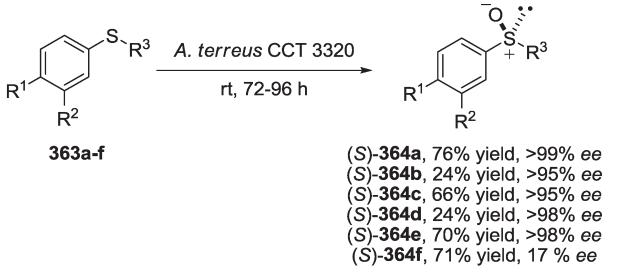
Benzyl alkyl sulfides (**365q**−**r**) were also shown to be suitable substrates for NDO. However, both the yield and the selectivity were dramatically reduced compared to the biotransformation of phenyl alkyl sulfides (compare entries 1 and 2 with entries 17 and 18). Moreover, increase of the distance between the aryl ring and the heteroatom by introducing additional methylene groups (**365s**−**t**) further continued this trend of decreasing the yield of sulfoxide formed (entries 19 and 20).

Finally, *n*-hexyl methyl and cyclohexyl methyl sulfides (**365u**−**v**) were shown to be the only suitable dialkyl substrates for NDO (compare entries 21 and 22 with entries 23−25). It is of note that bicyclic sulfides such as thiochroman and thiochroman-4-one are poor substrates giving negligible yields of sulfoxide with low *ee* values.

Some more elaborate sulfides have been also successfully desymmetrized by transformation into sulfoxides. Thus, the biotransformation of benzhydrylsulfanyl acetic acid **367** was tested using several fungal strains.[284] This compound can be used for the synthesis of (±)-modafinil, a psychostimulant agent. *Beauveria bassiana* (ATCC-7159) biotransformed benzhydrylsulfanyl acetic acid into (*S*)-sulfinyl carboxylic acid **368** in excellent yield and enantioselectivity (99%) (Scheme 68).

Baeyer−Villiger monooxygenases are also highly efficient catalysts in the oxidative EED of sulfides. Thus, the substrate spectrum of recombinant 4-hydroxyacetophenone monooxygenase (HAPMO) from *Pseudomonas fluorescens* has been recently studied by using a wide series of aryl alkyl sulfides as substrates.[285] The results obtained show that, in general, alkyl

Scheme 67



363a-f

(*S*)-**364a**, 76% yield, >99% *ee*
(*S*)-**364b**, 24% yield, >95% *ee*
(*S*)-**364c**, 66% yield, >95% *ee*
(*S*)-**364d**, 24% yield, >98% *ee*
(*S*)-**364e**, 70% yield, >98% *ee*
(*S*)-**364f**, 71% yield, 17 % *ee*

a: $R^1 = R^2 = H$, $R_3 = Et$; **b**: $R^1 = R^2 = H$, $R^3 = Me$;
c: $R^1 = Me$, $R^2 = H$, $R^3 = Et$; **d**: $R^1 = R^3 = Me$, $R^2 = H$;
e: $R^1 = H$, $R^2 = Cl$, $R^3 = Et$; **f**: $R^1 = H$, $R^2 = Cl$, $R^3 = Me$;

phenyl sulfides bearing an alkyl substituent up to three non-hydrogen atoms are the best substrates for the enzyme, which yields the (*S*)-sulfoxides in high enantioselectivities (>95% *ee*) and conversions (>63%). For larger alkyl substituents, the enantioselectivity considerably drops, as well as for benzyl sulfides, which are, however, good substrates for phenylacetone monooxygenase (PAMO) from *Thermobifida fusca*.[286] Concerning the effect of *para*-substitution of the aryl ring on the enzyme, higher selectivities were observed for electron-donating groups, while strong withdrawing ones had a negative effect on selectivity and efficiency. The atom efficiency of the BVMO-catalyzed oxidative EED of sulfides can be considerably enhanced by coupling it with the ADH-catalyzed KR of racemic secondary alcohols.[287] These two productive parallel processes are connected by the mutual recycling of the NADP(H) cofactor and, hence, they have been termed parallel interconnected kinetic asymmetric transformations. The results obtained show a similar enzymatic performance as compared to the corresponding simple processes.

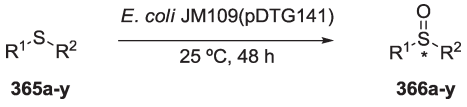
6.6. *cis*-Dihydroxylation of Carbon−Carbon Double Bonds

Dioxygenases found in prokaryotic microorganisms possess the remarkable ability to desymmetrize aromatic and conjugated substrates through oxidation of a carbon−carbon double bond to produce optically active vicinal *cis*-diols. The mostly studied enzymes that catalyze these biotransformations are the following three aromatic dioxygenases: toluene dioxygenase (TDO), naphthalene dioxygenase (NDO), and biphenyl dioxygenase (BPDO). They generally contain three components.[288] For example, NDO comprises an iron−sulfur flavoprotein reductase, an iron−sulfur ferredoxin that transfers electrons from NADP(H), and a catalytic oxygenase component with a mononuclear iron site. Although the dioxygenation reaction is believed to occur at the mononuclear iron site, the precise mechanism of dioxygenation remains unknown.[289]

The *cis*-dihydroxylation reaction is normally part of the pathway for the metabolism of aromatic substrates by such microorganisms and can only be exploited for the production of *cis*-diols when the diol dehydrogenase enzyme responsible for the oxidation of the dihydroarene *cis*-diol to a catechol is suppressed or absent. This scenario has been mostly realized by the employment of either a mutant version of the parent microorganism, or a host microorganism in which the dioxygenase enzyme is expressed in the absence of the enzymes for the remainder of the metabolic pathway. This way, the regio- and enantioselectivity

dx.doi.org/10.1021/cr100330u |*Chem. Rev.* 2011, 111, PR110–PR180

Table 25. NDO-Catalyzed Desymmetrization of Sulfides



365a-y 366a-y

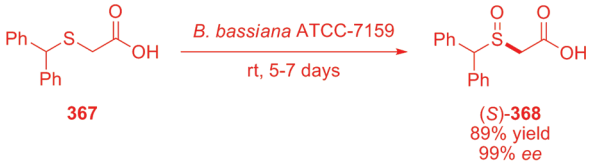
entry	sulfide	R^1	R^2	*c* (%)	*ee* (%)	config
1	**365a**	Ph	Me	98	98	*S*
2	**365b**	Ph	Et	87	86	*S*
3	**365c**	Ph	^{n}Pr	58	76	*R*
4	**365d**	Ph	^{i}Pr	69	74	*R*
5	**365e**	Ph	^{n}Bu	25	97	*R*
6[a]	**365f**	Ph	CH=CH_2	98	93	unknown
7	**365g**	4-NO_2-Ph	Me	5	>98	*S*
8	**365h**	4-CN-Ph	Me	13	>98	*S*
9	**365i**	4-F-Ph	Me	97	98	*S*
10	**365j**	4-Cl-Ph	Me	10	90	*S*
11	**365k**	4-Cl-Ph	Et	31	>98	unknown
12	**365l**	4-Br-Ph	Me	25	90	*S*
13	**365m**	4-MeO-Ph	Me	25	92	*S*
14	**365n**	4-Me-Ph	Me	96	>98	*S*
15[a]	**365o**	3-Cl-Ph	Me	21	75	*S*
16	**365p**	2-Cl-Ph	Me	12	31	unknown
17	**365q**	Bn	Me	20	<1	*rac*
18	**365r**	Bn	Et	11	5	*S*
19	**365s**	Ph-$(CH_2)_2$–	Me	10	54	unknown
20	**365t**	Ph-$(CH_2)_3$–	Me			
21	**365u**	Me	^{n}Hex	70	74	*S*
22	**365v**	Me	Cy	70	85	*S*
23	**365w**	Me	^{n}Hep	<5	3	*R*
24	**365x**	Me	^{n}Oct	<5	4	*R*
25	**365y**	Me	^{n}Non	<5	5	*R*

[a] Traces of the corresponding sulfone were found.

displayed by these dioxygenases in the *cis*-dihydroxylation of many monocyclic, fused, and linked aromatic and heteroaromatic systems as well as conjugated dienes, have been studied and quantified, thus mapping the substrate specificity of these enzymes.[290] It is of note that because dioxygenases are also able to catalyze the monooxidation of sulfides and activated carbons, such as benzylic ones, when these functionalities were present in a substrate, tandem oxidation metabolites with more than two chiral centers were obtained in a highly enantioselective fashion.[290]

Although in many cases yields have to be improved to allow a full implementation of enzymatic *cis*-dihydroxylations to organic synthesis, these desymmetrizations possess an enormous synthetic potential because of the following reasons: (1) The products obtained from these biotransformations are extremely interesting chiral synthons.[291] (2) There is an ever greater knowledge of substrate's structure- and biocatalyst-regioselectivity relationships, which make possible that, in many cases, regioselectivity can be predicted and even controlled by means of the employment of different enzymes or the introduction of certain substituents on the substrates.[290] (3) So far, with very few exceptions, the products are obtained with absolute enantiomeric purity. (4) Different synthetic and biocatalytic strategies that allow enantiodivergence have been developed, which confers a

Scheme 68



367 (*S*)-**368** 89% yield 99% *ee*

high versatility on *cis*-dihydroxylations.[292] It is worth mentioning that X-ray crystallographic studies of NDO[289,293] have provided a valuable structural model for dioxygenases which has been translated into the preparation of mutants of this enzyme with modified regio- and enantioselectivity.[294] Indeed, the employment of the NDO mutant F352 V in combination with NDO_{wt}, TDO, and carbazole dioxygenase has provided four of the *cis*-dihydrodiol isomers from biphenyl.[294a]

The potential of EEDs based on *cis*-dihydroxylations has been reflected in their employment in chemoenzymatic syntheses of different biologically active compounds. Thus, Banwell and co-workers have developed routes leading to (−)-gabosine A[295] and 6C-methyl-D-mannoses[296] using as starting materials the enantiopure *cis*-diols coming from the TDO-catalyzed dihydroxylation of iodobenzene and toluene, respectively. Likewise, Fessner and co-workers have reported the synthesis of a polycyclic lactone as a valuable pancratistatin analogue.[297] One of the key steps was the enzymatic dihydroxylation of dioxole **369** using recombinant *E. coli* cells expressing a naphthalene dioxygenase from *Pseudomonas putida* G7 (Scheme 69). The corresponding polycyclic diol (5*R*,6*S*)-**370** was obtained in good yield and *ee* after 1 day of reaction. More recently, dioxygenase-catalyzed *cis*-dihydroxylation of aromatic compounds has been used as first step in the preparation of a wide variety of optically active highly functionalized cyclohexanes[298] and tetrahydrofurans,[299] different sugars and carbasugars,[300] the antiviral oseltamivir[301] and other biologically active polyhydroxylated cyclic compounds,[302] and different chiral ligands.[303] Moreover, *cis*-1,2-diols can be used as starting material for the preparation of enantiopure *trans*-1,2-diols by means of chemoenzymatic routes consisting of up to seven steps.[304]

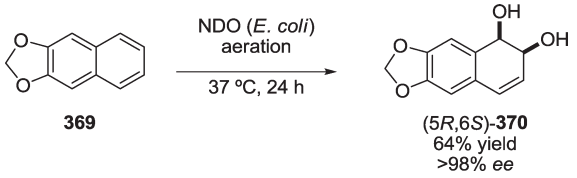
6.7. *trans*-Dihydroxylation of Carbon−Carbon Double Bonds

The epoxidation of alkenes, when followed by hydrolysis of the resulting epoxides, constitutes a sequence that leads to *trans*-diols. When enzymes are used to catalyze either one or both of these two steps, optically active *trans*-diols can be obtained. More specifically, the EED of prochiral alkenes affords optically active epoxides whose *ee* depends on the enantioselectivity of the desymmetrization. In case it were not satisfactory, its optimization is always possible by carrying out the subsequent hydrolytic KR. On the other hand, certain symmetric alkenes give *meso*-epoxides. Thereby, in this case enantioselectivity is solely achieved during the hydrolysis of the epoxide.

Enzymatic epoxidation of olefins can be performed by monooxygenases and peroxidases. Although peroxidases would be a priori more attractive catalysts, since they are independent of cofactors, some limitations concerning operational stability have made monooxygenases the traditionally preferred biocatalysts for this transformation. They use molecular oxygen as oxidant, which is activated only in the active site of the enzyme at a heme or nonheme iron complex, or in flavin monooxygenases as

dx.doi.org/10.1021/cr100330u |*Chem. Rev.* 2011, 111, PR110–PR180

Scheme 69



peroxoflavin. Reducing equivalents needed for the reductive activation of dioxygen are mostly supplied from reduced nicotinamide cofactors. Hence, cofactor regeneration is a major issue if isolated enzymes have to be employed and, thereby, whole-cell biocatalysts have been mainly used.[305] Again, problems related to low concentrations of enzyme can be alleviated by employing overexpressed monooxygenases in recombinant cells. For instance, the use of recombinant *E. coli* growing cells containing overexpressed styrene monooxygenase in a suitable organic/aqueous reaction medium for the epoxidation of styrenes **371a**−**g** allowed, after recovery of the reaction products via distillation from the organic phase, the preparation of the corresponding optically active oxiranes (**372a**−**g**) in good to excellent yields (Table 26).[306]

Because of the inhibition effect that certain substrates have on *E. coli*, a cell-free biotransformation system based on styrene monooxygenase would give access to an even broader range of chiral oxiranes. In fact, Schmid and co-workers have recently reported that the organometallic complex $[Cp^*Rh(bpy)(H_2O)]^{2+}$ catalyzes the transhydrogenation reaction between formate and isoalloxazine-based cofactors such as FAD and FMN.[307] Coupling this $FADH_2$ regeneration reaction to the $FADH_2$-dependent styrene monooxygenase (StyA) resulted in a cell-free chemoenzymatic epoxidation reaction in which the organometallic compound substitutes for the native reductase (StyB), responsible for $FAD(H_2)$ regeneration using NADH as cofactor, the nicotinamide coenzyme (NAD), and an artificial NADH regeneration system such as formate dehydrogenase. Optimization of the reaction conditions revealed the need for high biocatalyst concentrations relative to regeneration catalyst and FAD concentrations. This way, the electron transfer yield was maximized although it was highly dependent on the substrate used. Additionally, it was also observed that a correct amount of FAD present in the reaction medium was crucial for high efficiency. Various styrene derivatives were tested as substrates for this system. Apart from trace amounts of diols, only epoxides were detected as products formed from the vinyl aromatic substrates. With the exception of *p*-bromostyrene oxide (*ee* = 95.6%), the *ee* values of the products were generally >98% and thereby comparable to the *ee* values obtained in the in vivo reactions.[294] In contrast to whole-cell reactions, all substrates tested excluding *m*-chlorostyrene were converted at higher initial rates than styrene. This is most likely due to the lack of the transport limitations across microbial membranes and other influences of complex whole-cell systems. Two groups of compounds, from the vinyl aromatic substrates employed, were not converted by the enzymatic system: (1) Bulky substrates such as *p*-*tert*-butylstyrene, *cis*- and *trans*-stilbene, or vinyl ferrocene. (2) Inhibitors of the formate driven formation of the catalytically active hydrorhodium complex such as *p*-aminostyrene, indole, and different pyridines.

These authors have also recently published an example of the preparation and synthetic application of a bacterial monooxygenase as a reagent for asymmetric cell-free epoxidation.[308] Namely, they have achieved the gram-scale production of epoxides **373a**−**c** in excellent *ee*'s and yields by epoxidation of the corresponding styrenes catalyzed by a lyophilized preparation of the soluble flavin- and NADH-dependent styrene monooxygenase (StyAB) from *Pseudomonas* sp. VLB120. NADH regeneration was achieved by the formate/formate dehydrogenase (FDH) system of *Pseudomonas* sp. 101 (Chart 13).

The enantioselective epoxidation of simple linear terminal alkenes is a challenging transformation because of low enantioselectivities. In this regard, Arnold and co-workers have employed directed-evolution to overcome this limitation.[309] In particular, they used a previous mutant of the fatty acid hydroxylase cytochrome P450 BM-3 from *Bacillus megaterium* as starting point. Saturation mutagenesis of 11 amino acids selected by a structure-based criterion led to eight variants from five libraries with improved activity and enantioselectivity in the epoxidation of 1-hexene. Recombination of these mutants afforded a new library from which two variants (SH-44 and RH-47) were selected. They show opposite enantioselectivities over a series of simple linear terminal alkenes and, although the maximum enantioselectivity was 83% *ee*, no catalyst (chemical or enzymatic) is known to epoxidize such substrates with more than 50% *ee*. Finally, the synthetic applicability of the new biocatalyst was demonstrated by its usage in a cell-free format by using the ADH from *Thermoanaerobium brockii* to regenerate the nicotinamide cofactor.

Epoxide hydrolases isolated from a wide range of sources have been extensively used for the KR of racemic epoxides, a process that is stereochemically more complex than the KR of other substrates such as alcohols or carboxylic acids and their derivatives. The reason is that not only enantioselectivity influences the stereochemical outcome of the reaction but also regioselectivity does.[310] Although the hydrolysis of *meso*-epoxides does not suffer from such complexity, the enantioselectivity achieved in these processes is usually low, especially for microbial epoxide hydrolases.[22e,310,311] Consequently, new enzymes of this class keep on being searched for.[312] Nevertheless, in some cases, high *ee*'s can be achieved. For instance, Weijers and de Bont have investigated the substrate specificity and enantioselectivity of the recently discovered yeast epoxide hydrolase from *Rhodotorula glutinis*. Some *meso*-epoxides were included in the set of substrates tested, which, with the exception of *meso*-1,2-epoxycyclooctane, were hydrolyzed in excellent *ee*'s and yields.[313] Likewise, Chang et al. have lately reported an example of enantioselective hydrolysis of *meso*-epoxides with a bacterial epoxide hydrolase.[314] Namely, they have studied and carried out the preparative hydrolyses of epoxides **374** and **376** under the conditions shown in Scheme 70. In both cases, the corresponding *trans*-diols were isolated in high yields and *ee*'s. Additionally, when *N*-Cbz 3-pyrroline was used as substrate, this bacterial strain afforded the same product (3*R*,4*R*)-**375** through a sequence that implied the previous action of a monooxygenase.[315]

Since no single enzyme is likely to function optimally on all types of epoxides, a general viable solution for the EED of *meso*-epoxides can be developed using a diverse set of microbial epoxide hydrolases. In fact, Zhao et al. have recently demonstrated the utility of this approach by creating a library of epoxide hydrolases discovered from nature, from which some enzymes

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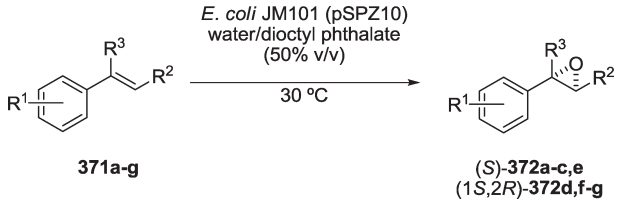
Table 26. EED of Styrenes



entry	substrate	R^1	R^2	R^3	t (h)	yield (%)	*ee* (%)
1	**371a**	H	H	H	17	76.3	99.5
2	**371b**	*p*-Me	H	H	17	46.5	99.9
3	**371c**	H	H	Me	23.5	74.8	96.7
4	**371d**	H	Me	H	20	87.2	99.8
5	**371e**	*m*-Cl	H	H	17.5	87.3	99.4
6	**371f**	*o*-CH_2−CH_2−		H	45.5	53.0	98.5
7	**371g**	*o*-CH_2−		H	19	47.9	98.0

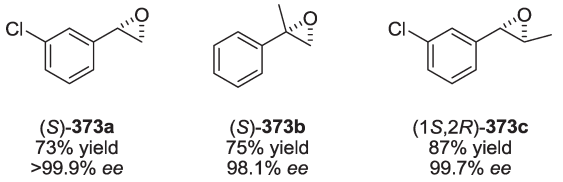
were capable of selectively hydrolyzing a wide range of cyclic and aryl *meso*-epoxides.[316] From these processes, the corresponding chiral (*R*,*R*)-diols were obtained in high *ee*'s (Chart 14). To confirm the synthetic utility of these enzymes, some hydrolyses were carried out at a gram scale and the corresponding diols were obtained in high yields. Moreover, they have also found the first epoxide hydrolases providing access to complementary (*S*,*S*)-diols (Chart 14).

The enzymatic hydrolysis of methylene interrupted *meso*-bis-epoxide **381** catalyzed by whole resting cells of *Rhodococcus* sp. CBS 717.73 yielded the tetrahydrofuran derivative **382b** in 94% *ee* and 89% *de* at 85% conversion (path B, Scheme 71).[317] A mechanistic proposal based on a cascade initiated by hydrolytic opening of an epoxy moiety catalyzed by epoxide hydrolases to yield an intermediate epoxydiol, which would undergo a ring-closure to furnish the cyclization product, was put forward. This hypothesis was tested by using a range of cloned epoxide hydrolases of different origins.[318] Depending on the enzyme, this substrate (**381**) was transformed into three (**382a**, **382b**, and *ent*-**382a**) out of the four possible stereoisomeric tetrahydrofuran derivatives by following the cascade pathways A, B, and D, respectively (Scheme 71) and with varying enantioselectivities. Analysis of the stereochemical courses of the cascades by molecular modeling revealed that the hydrolytic initiation of the cascade was solely determined by the stereopreferences of the enzymes, whereas the spontaneous cyclizations of the intermediate epoxy-diols were governed by Baldwin's rules. Hence, no cyclase is involved in the mechanism of formation of this type of product.

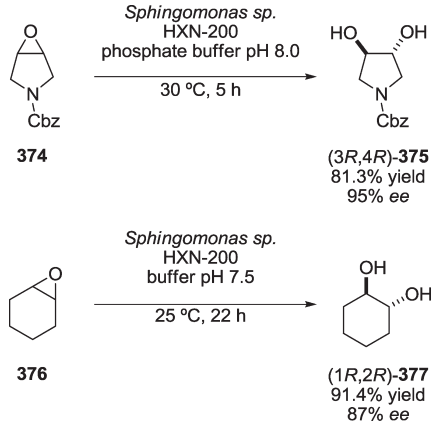
6.8. Baeyer−Villiger Oxidations

Baeyer−Villiger monooxygenases (BVMO) efficiently catalyze the nucleophilic and electrophilic oxygenation of different functional groups.[319] In particular, the enzymatic Baeyer−Villiger oxidation of ketones offers an environmentally benign entry to chiral lactones, important building blocks in synthetic chemistry.[320] In this sense, when *meso* or prochiral ketones are used as substrates, the BVMO-catalyzed desymmetrization usually occurs with exquisite enantioselectivity. Although many bacterial species carry monooxygenases capable of performing Baeyer−Villiger oxidations, cyclohexanone monooxygenase (CHMO) from *Acinetobacter calcoaceticus* NCIMB 9871 has been the most extensively investigated.[321] Its broad substrate acceptability and high enantioselectivity can account for this popularity, but the problems associated with cell growth, overmetabolism, enzyme isolation, and recycling of the cofactor have been major obstacles in its use in organic synthesis. Nevertheless, the construction of Baker's yeast and *E. coli* strains overexpressing CHMO[322] has permitted researchers to overcome these problems, and, indeed, many examples of Baeyer−Villiger desymmetrizations using overexpressed CHMO can be found in the recent literature. For instance, Mihovilovic and coworkers have employed whole cells of an *E. coli* strain that overexpresses *Acinetobacter* sp. NCIB 9871 CHMO for the Baeyer−Villiger oxidation of a variety of 4-mono- and 4,4-disubstituted cyclohexanones (**383a**−**k**; Table 27).[323] The efficient production of CHMO in the *E. coli* expression system allowed these oxidations to reach completion in approximately half the time required for the engineered Baker's yeast strain that expresses the same enzyme. All lactones except **384d**,**i** and **385g** were obtained in synthetically useful yields and very high *ee*'s. Moreover, the results provide a means to explore the enantiodiscriminative requirements of this enzyme. On one hand, the unusual low enantioselectivity obtained for lactone **385g** (entry 7) may signal unique interactions with the enzyme active site made possible by the hydroxyl substituent. On the other hand, it has been postulated that the enantioselectivity of this enzyme arises from a combination of modest intrinsic chiral discrimination coupled with conformational preferences of bound substrates. In this particular case, although the high yields in which lactones **384b**,**e** were obtained (entries 2 and 5) proved that products derived from a Criegee-like intermediate possessing an axial substituent at the 4-position are not prohibited, the lower enantioselectivity with which ketone **383d** was transformed

Chart 13



Scheme 70



dx.doi.org/10.1021/cr100330u |*Chem. Rev.* 2011, 111, PR110–PR180

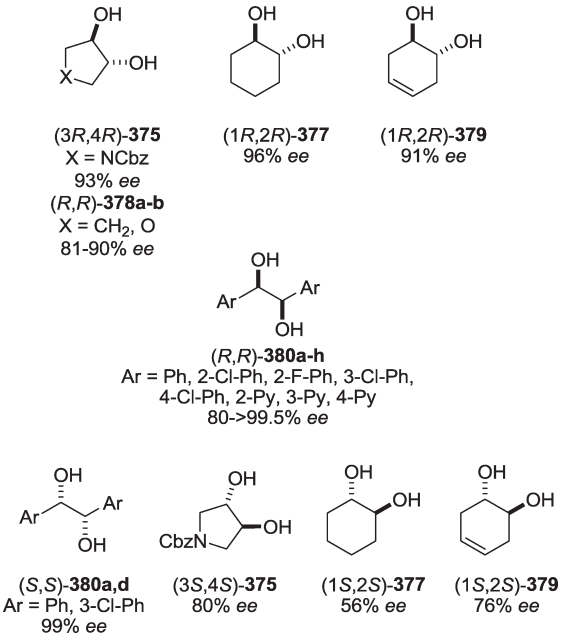
(entry 4; 75% *ee*) as compared to ketones **383a,c** (entries 1 and 3 ≥98 and 97% *ee*, respectively) was accordingly attributed to the lower conformational bias between the corresponding Criegee-like intermediates. Unfortunately, this analysis is more difficult to apply to the oxidations of ketones containing polar substituents such as **383g−k** since their conformational behavior is more complex and influenced by the presence of the carbonyl group dipole and additional protein−substrate interactions such as hydrogen bonds.

Cells of *A. calcoaceticus* NCIMB 9871 also have been proven to catalyze the desymmetrization of heterocyclic ketones without side reactions of oxidation-sensitive functionalities such as sulfur or nitrogen.[324] Unfortunately, for ketones with high polarity somewhat low yields were observed and attributed to membrane penetration problems. This problem was overcome by employing the aforementioned *E. coli* strain. Namely, a series of *cis*-2,6-dialkylperhydropyrans **386a−e** was oxidized to the corresponding lactones (**387a−e**). The substituents were chosen with the aim of also investigating the spatial requirements of the CHMO active site for this type of substrates (Scheme 72a).[325] Results showed that lactones were obtained in excellent *ee*'s, the size of the R group having a substantial influence on the conversion of the substrates. While ketones containing small straight chains, such as methyl (**386a**) and ethyl (**386b**), were oxidized in excellent yields, significantly decreased conversions, or no reaction at all were measured for ketones bearing larger substituents (**386c−e**). Hence, a straight chain with three carbons seems to represent the borderline with respect to steric demands of the substrate in the active site. On the other hand, cyclopentanone monooxygenase (CPMO) from *Comamonas* sp. NCIMB 9872, known for accepting sterically demanding substrates, was used to desymmetrize the oxygen-containing heterobicyclic ketone **388** (Scheme 72b).[326] Optimization of the reaction conditions afforded the corresponding lactone in 95% *ee* and 70% isolated yield and was subsequently used as starting material for the preparation of various natural products containing a tetrahydrofuran structural motif. Later on, the same authors reported that, similarly to this CPMO, overexpressed CHMO from *Xanthobacter* sp. ZL5 is also able to perform the Baeyer−Villiger desymmetrization of sterically demanding ketones with excellent enantioselectivities while remaining as active and selective toward other cyclic ketones as the CHMO from *Acinetobacter* sp. NCIB 9871.[327]

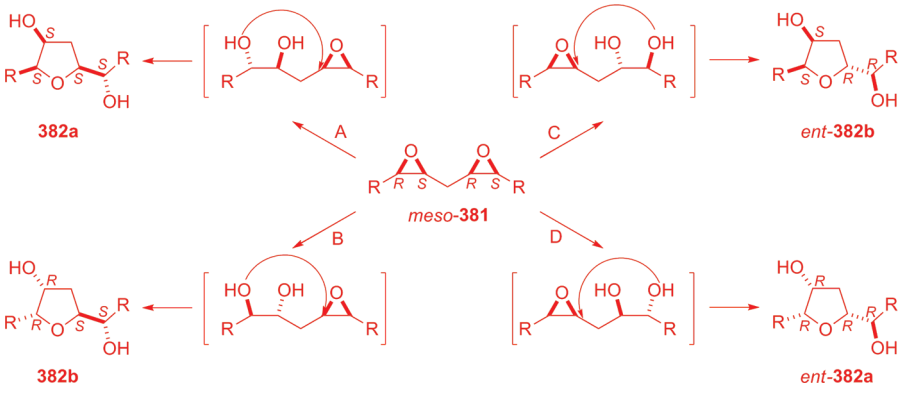
Cyclopentanone monooxygenase from *Comamonas* (previously *Pseudomonas*) sp. NCIMB 9872 has been relatively little used in enzymatic Baeyer−Villiger oxidations because of the general belief that it was less enantioselective than the renowned CHMO. However, as it has been already been shown in Scheme 72b, this is not completely true, thus reopening the interest in this enzyme. In this sense, Wang et al. have demonstrated that, conversely to CHMO, the CPMO-catalyzed oxidation of different 4-hydroxycyclohexanone derivatives turned out to be a more promising concerning yield and, to a lesser extent, enantioselectivity.[328] Moreover, CPMO readily accepts cyclohexanones with large substituents in the 4-position, improving the enantioselectivity with an increasing size of this group. In the case of 4-halocyclohexanones, an opposite enantioselectivity to that displayed by CHMO was observed.

The performances of recombinant *E. coli* overexpressing *Comamonas* sp. NCIMB 9872 CPMO and *Acinetobacter* sp. NCIMB 9871 CHMO in the whole-cell biotransformation of prochiral 4-substituted-3,5-dimethylcyclohexanones **390a−g** and bicyclo[4.3.0]ketones **392a−b** have been compared (Scheme 73).[329b] Whenever a hydroxyl group was present in the 4-position, the lactones rearranged spontaneously to yield the corresponding γ-butyrolactones. However, CPMO-expressing cells were only successful in two cases (**390e** and **390g**, >58% yield,

Chart 14

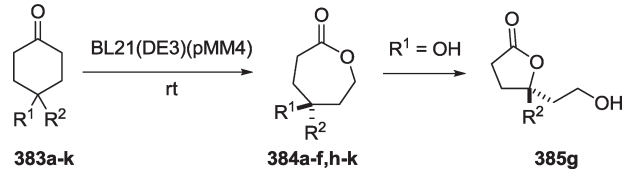


Scheme 71



dx.doi.org/10.1021/cr100330u |Chem. Rev. 2011, 111, PR110–PR180

Table 27. EED of 4-Mono- and 4,4-Disubstituted Cyclohexanones



383a-k **384a-f,h-k** **385g**

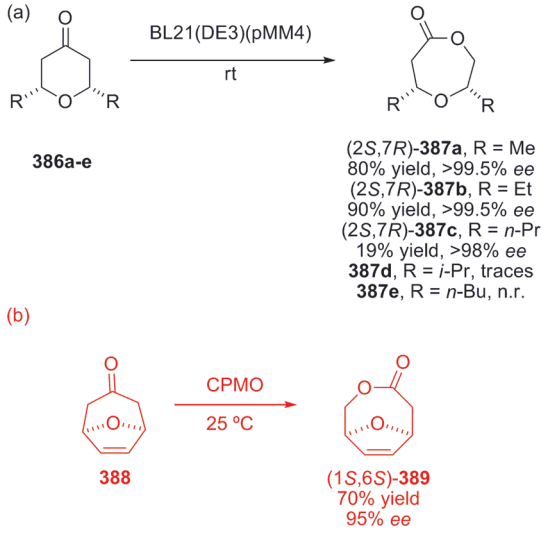
entry	substrate	R^1	R^2	yield (%)	*ee* (%)	config
1	**383a**	Me	H	61	≥98	(*S*)-(−)
2	**383b**	Me	Me	61		
3	**383c**	Et	H	91	97	(*S*)-(−)
4	**383d**	Et	Me	91	75	
5	**383e**	Et	Et	60		
6	**383f**		*cyclo*-CH_2CH_2			74
7	**383g**	OH	H	61	9.1	(−)
8	**383h**	Et	OH	54	94	(−)
9	**383i**	OMe	H	84	78	(*S*)-(+)
10	**383j**	Br	H	63	97	(*S*)-(−)
11	**383k**	I	H	60	97	(*S*)-(−)

>91% *ee*). However, these enzymes showed an opposite behavior for the case of the bicyclic ketones (Scheme 73b).[329a] Thus, while CHMO afforded the corresponding lactones in almost racemic mixtures, the CPMO-expression system furnished these compounds in excellent yields and enantioselectivities. Additionally, it was of interest that, in all cases, CPMO and CHMO promoted the formation of the opposite enantiomeric series.

Later, these authors extended this comparison to the Baeyer−Villiger oxidation of the pentalenones **394a−e** (Table 28).[330] The results obtained confirmed, first, that some substrates were transformed by both enzymes in an enantiodivergent fashion (**394a,d−e**, entries 1 and 2, 7−10). Second, for the case of the CHMO biotransformations, the *exo* geometry present in the bicycloketones **394b,d**, (entries 3 and 7) seemed to be a prerequisite for high optical purity of product lactones. This hypothesis was supported by the results obtained for the *endo* substrates **394a,c** (entries 1 and 5) and for the sp^2-hybridized ketone **394e** (entry 9), which gave enantioselectivities approximately between those of the *endo* and *exo* substrates. Additionally, the presence of a less-polar functional group (Cl < MeO) was favorable to obtain both high yield and enantioselectivity (compare entries 3 and 7). On the other hand, the biotransformations with CPMO-producing cells did not display such a significant trend, but, as in the case of CHMO, the best result was obtained for substrate **394d**.

In the past few years, different MOs have been characterized and cloned in *E. coli*, thereby enriching the diversity of the BVMOs available. In this sense, the microbial Baeyer−Villiger oxidation of representative *meso* and prochiral ketones (**396a−e**) with recombinant *E. coli* cells expressing two new monooxygenases from *Brevibacterium* ($CHMO_{BreviI}$ and $CHMO_{BreviII}$) were investigated (Table 29).[331] With the exception of the $CHMO_{BreviI}$-catalyzed oxidation of **396e** (entry 9), all lactones were obtained in synthetically useful yields. Concerning enantioselectivities, both CHMOs showed an enantiodivergent behavior, *ee* values being excellent in most cases. It is worth mentioning that for the case of ketone **396c**, its oxidation occurred chemoselectively without concomitant epoxidation of the double bond (entries 5 and 6).

Scheme 72



Likewise, Kyte et al. have recently cloned the genes encoding eight BVMOs from bacteria inhabiting a wastewater treatment plant (*Brevibacterium* sp. ChnB1, *Brevibacterium* sp. ChnB2, *Acidovorax* CHX, *Acinetobacter* SE19, *Arthrobacter* BP2, *Rhodococcus* ph1, *Rhodococcus* phi2, and *Rhodococcus* SC1) and carried out a systematic investigation of their substrate specificity.[332] The results were compared with the ones obtained for the well-known CHMO from *Acinetobacter* sp. NCIB 9871. For the particular case of the 4-alkyl-substituted cyclohexanones tested (Scheme 74) and with the exception of substrates **398c,e**, CHMO showed good (*S*)-selectivity (≥98% *ee*). The newly cloned enzymes also afforded (*S*)-selectivity and in the few cases in which (*R*)-lactones were preferentially formed, the enantiomeric purities were not synthetically useful (<60%). Additionally, this collection of enzymes did provide two (*S*)-ε-caprolactones which were not produced effectively by the model *Acinetobacter* enzyme. More specifically, CHMOs from *Brevibacterium* sp. (ChnB1), *Acidovorax* CHX, and *Rhodococcus* SC1 all oxidized **398c** with very high (*S*)-enantioselectivity, and the *Brevibacterium* sp. ChnB1 and *Rhodococcus* SC1 enzymes also showed complete stereoselectivity in the oxidation of **398e**. More recently, a directed-evolution approach intended to induce allostery in phenylacetone monooxygenase from *Thermobifida fusca* led to the discovery of a double mutant (Gln93Asn/Pro94Asp) which catalyzed the EED of prochiral cyclohexanones **398a−b,e** in excellent enantioselectivities (>97% *ee*).[333] More interestingly, the (*R*)-configuration of the lactone products is opposite to that obtained by using CHMO.

A clustering of BVMOs into two main groups on the basis of protein sequence and enantiopreference has been proposed.[334] The first group contains the CHMO-type enzymes: cyclohexanone monooxygenases from *A. calcoaceticus* 9871 ($CHMO_{Acineto}$), from *Brevibacterium* DPR 14 ($CHMO_{Brevi1}$), from *Rhodococcus* DPR 455 ($CHMO_{Rhodo1}$) and *Rhodococcus* DPR 460 ($CHMO_{Rhodo2}$), from *Arthrobacter* DPR 453 ($CHMO_{Arthro}$), and from *Brachymonas* DPR 192 ($CHMO_{Brachy}$). The second group consists of CPMO-type enzymes: cyclopentanone monooxygenase from *Comamonas* NCIMB 9872 ($CPMO_{Coma}$) and a second CHMO from *Brevibacterium* DPR 399 ($CHMO_{Brevi2}$).

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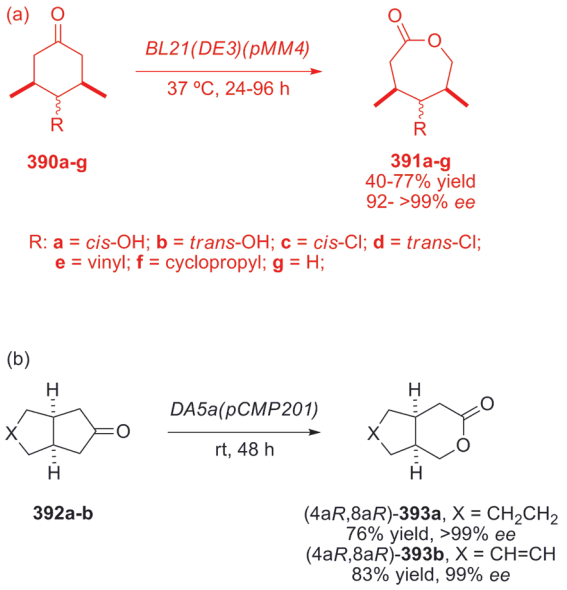
Scheme 73

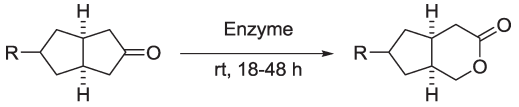


When prochiral ketones **394c**−**d**, **396b**−**c**, **396e**, **398a** and **400c**, **401**−**403** (Chart 15) were desymmetrized by these BVMOs overexpressed in *Escherichia coli* cells, each group generally afforded opposite enantiopreferences in moderate to excellent enantioselectivities.

This library of enzymes has been used with profusion in the Baeyer−Villiger oxidation of a wide range of prochiral ketones. Thus, the oxidative EED of 2-substituted cyclobutanones results in optically active butyrolactones, which are interesting chiral synthons in the preparation of different biologically active compounds such as analgesics, GABA receptor inhibitors, β-amino acids, and structurally related lignans. After initial screening, the best substrates were selected to carry out the biotransformations at a preparative scale (Scheme 75).[335] Eight (**400a**−**h**) out of the 10 ketones tested (R = ^{t}Bu and $-CH_2OCH_2Ph$) could be desymmetrized in >50% yield and >90% *ee* by using the proper enzyme. On the other hand, enantioselective Baeyer−Villiger oxidations have been employed as the enantiotopos-differentiating reaction in a synthetic route designed to prepare optically active bicycle[4.2.0]octanes.[336] In particular, for the example shown in Scheme 76, both antipodes of the corresponding lactone can be accessed by the choice of a suitable enzyme. Thus, using monooxygenase $CHMO_{Brevi1}$, *ent*-**406f** (96% *ee*) was predominantly obtained, while $CHMO_{Brevi2}$ and $CPMO_{Coma}$ gave the enantiomeric lactone **406f**. So far, monooxygenases producing predominantly enantiomer **406f** gave lower conversions and selectivities than monooxygenases with a preference for enantiomer *ent*-**406f**.

This library of enzymes has been also screened for activity in the Baeyer−Villiger oxidation of various bridged cycloketones.[337] From these processes, polycyclic lactones with four to six newly generated chiral centers can be obtained. The results of the screening indicated that only saturated ketones **405a**−**f** were converted into the corresponding lactones, the best conditions being tested at a preparative scale (Table 30).[337b] As can be seen from the table, the enzyme library allowed, in several cases, access to antipodal lactone products (**405a**,**b**,**d**; entries 1−4,6−7). In all cases, the isolated yields ranged between 49 and 78% and the enantioselectivities were excellent with the exception of the EED

Table 28. EED of Pentalenones



394a-e 395a-e

entry	substrate	R	enzyme	yield (%)	*ee* (%)	config
1	**394a**	*endo* MeO	CHMO	24	9	(−)
2	**394a**	*endo* MeO	CPMO	81	34	(+)
3	**394b**	*exo* MeO	CHMO	40	96	(−)
4	**394b**	*exo* MeO	CPMO	75	11	(−)
5	**394c**	*endo* Cl	CHMO	75	80	(−)
6	**394c**	*endo* Cl	CPMO	79	60	(−)
7	**394d**	*exo* Cl	CHMO	78	>99	(−)
8	**394d**	*exo* Cl	CPMO	92	>99	(+)
9	**394e**	$=CH_2$	CHMO	43	61	(−)
10	**394e**	$=CH_2$	CPMO	85	41	(+)

of ketone **405d** catalyzed by $CPMO_{Coma}$ (entry 7). These results further support the proposed classification into two groups of cycloketone converting BVMOs.

7. CARBON−CARBON BOND FORMATION AND BREAKING

The construction of carbon−carbon bonds starting from achiral substrate(s) and with complete control of the stereochemical course of the reaction is of utmost importance for organic synthesis. These transformations are facilitated, in nature, by lyases. There are three main types of carbon−carbon forming EEDs with general applicability to synthesis: the aldol and acyloinic reactions and the hydrocyanantion of carbonyl compounds. Besides, very recent EEDs involving the reverse reaction (i.e., the breaking of a C−C bond) will be discussed in the last section.

7.1. Aldol Reactions

The aldol reaction is one of the most powerful methods of forming carbon−carbon bonds. There are two types of enzymatic catalysts that effect the aldol addition: aldolases and catalytic antibodies. Aldolases are a specific group of lyases that typically catalyze the stereoselective addition of a ketone donor to an aldehyde acceptor. Two distinct types of aldolases have been identified and classified according to their mechanism. Type I aldolases make use of a unique chemically reactive lysine residue to form a Schiff base as an intermediate, while Type II aldolases contain a Zn^{2+} cofactor in the active site which is believed to activate the carbonyl donor by direct coordination with it. These enzymes generally tolerate a somewhat broad range of acceptor substrates but have stringent requirements for donor substrates. In addition to this, as in aldolase-catalyzed aldol reactions usually chiral aldehydes are employed as acceptors, only a small group of carbonyl compounds can be efficiently desymmetrized by the use of these enzymes.[338] These features have made that the use of aldolases for EED processes was originally very limited. This situation has recently changed after the discovery of new aldolase activities. Thus, an L-*allo*-threonine aldolase from *Aeromonas jandaei* and a D-threonine aldolase from *Pseudomonas* sp. can also accept, apart from glycine, D-alanine, D-serine, and D-cisteine as donors.[339] Aromatic aldehydes bearing

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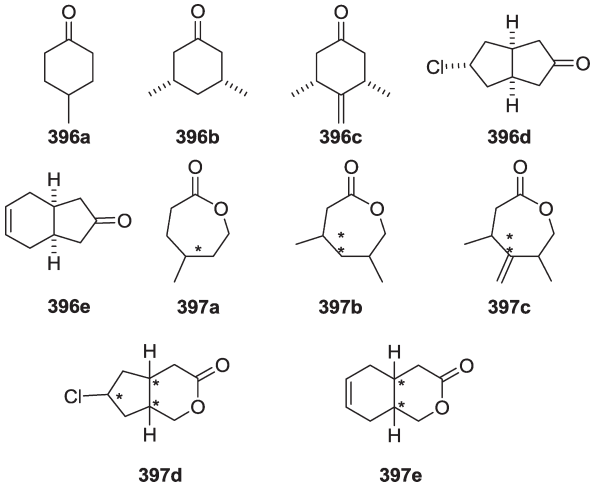
Table 29. $CHMO_{Brevi}$-Catalyzed Desymmetrization of *meso* and Prochiral Ketones

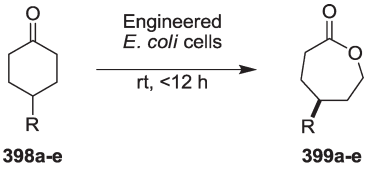


entry	substrate	product	enzyme	yield (%)	*ee* (%)	config
1	**396a**	**397a**	$CHMO_{BreviI}$	65	>99	(−)-*S*
2			$CHMO_{BreviII}$	60	44	(+)-*R*
3	**396b**	**397b**	$CHMO_{BreviI}$	61	97	(−)-4*S*,6*R*
4			$CHMO_{BreviII}$	56	99	(+)-4*R*,6*S*
5	**396c**	**397c**	$CHMO_{BreviI}$	70	>99	(+)
6			$CHMO_{BreviII}$	44	>99	(−)
7	**396d**	**397d**	$CHMO_{BreviI}$	55	>99	(−)
8			$CHMO_{BreviII}$	59	>99	(+)
9	**396e**	**397e**	$CHMO_{BreviI}$	10	71	nd
10			$CHMO_{BreviII}$	92	94	(+)

electron-withdrawing groups have been shown to be the best acceptors, thus rendering the corresponding aldol aducts in very high enantioselectivity although in a moderate diastereoselectivity. On the other hand, D-fructose-6-phosphate aldolase (FSA), a Type I aldolase showing very good activity with a broad variety of both donor and acceptor substrates (Table 31), has been also recently discovered.[340] Thus, the acceptor can be a nonchiral aldehyde (**407a**−**e**) and the donor does not need the presence of phosphate, being dihydroxyacetone (**408a**, entries 1, 2, 4 and 7) or even hydroxyacetone (**408b**, entries 3, 5, 6 and 8) very good substrates for FSA. The reaction proceeds with good conversion and isolated yields, considering the reversibility of the process and the problems associated with the isolation and purification of the compounds. Apart from the case of using **407d** as the acceptor and **408b** as the donor (entry 6), the stereoselectivity was complete toward the *threo* (3*S*,4*R*) aldol adduct. This reaction opens the way toward the efficient chemoenzymatic synthesis of chiral polyhydroxylated compounds such as sugar derivatives or iminocyclitols.

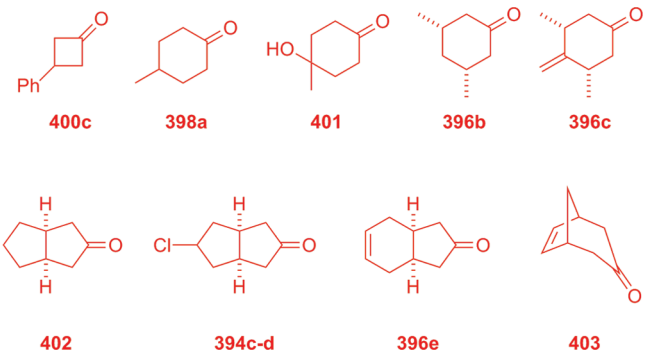
A further expansion of the synthetic applications of FSA has been recently reported,[341] when Clapés and co-workers discovered that glycoaldehyde (**407d**) can act as both acceptor and donor in a self-aldol reaction process, leading to an efficient and very simple synthesis of D-threose (**410a**, Scheme 77). By a careful study of the kinetic parameters, the authors were able to control the self-aldol/cross-aldol reaction processes, allowing the use of **407d** as the donor with a broad variety of aldehyde acceptors (Scheme 77). The aldol adducts (**410b**−**d**) bear an

Scheme 74

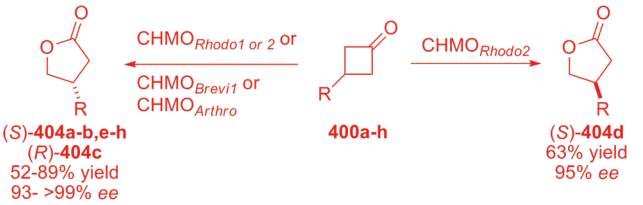


a: R = Me; **b**: R = Et; **c**: R = n-Pr;
d: R = i-Pr; **e**: R = n-Bu;

Chart 15



Scheme 75



R: **a** = *n*-Bu; **b** = Bn; **c** = Ph; **d** = *p*-Cl-Ph; **e** = *m*-MeO-Bn;
f = *p*-MeO-Bn; **g** = piperonyl; **h** = 3,4,5-$(MeO)_3$-Bn;

aldehyde group which can be susceptible of further chemoenzymatic transformations. In all the cases, the newly formed chiral centers also followed the same stereoselectivity previously observed. Some further mutations on the FSA active site has allowed to get a biocatalyst with an improved selectivity toward dihydroxyacetone (**408a**) for the synthesis of polyhydroxylated compounds, some of them through true EED processes.[342]

By the design of appropriate antigens, specific functional groups can be induced into the binding site of an antibody to perform general acid/base catalysis, nucleophilic/electrophilic catalysis, and catalysis by strain or proximity effects. Unlike the natural enzymes, aldolase catalytic antibodies have the ability to accept a more diverse range of substrates. This flexibility in their substrate specificity has converted aldolase antibodies into the most recently used biocatalysts to perform aldol-type EEDs.

The commercially available ab38C2 (Aldrich) is one of the most used aldolase antibodies.[343] It was obtained by using hapten **411** (Chart 16), in which the β-diketone functionality should act as a reactive immunogen to trap a chemically reactive lysine

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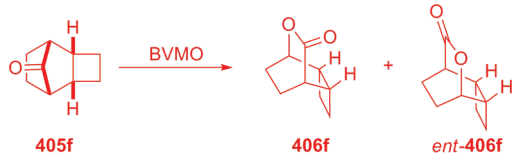
Scheme 76



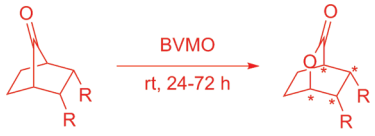
residue in the active site of an antibody. Covalent trapping was facilitated by intramolecular hydrogen bonding that stabilizes an enaminone in the active site of the antibody. The chemical mechanism leading up to the stabilized enaminone should match that of Type I aldolases over this portion of the reaction coordinate. This way, the aforementioned commercially available ab38C2 (Aldrich) together with ab33F12 were obtained.[344]

In an effort to increase the repertoire of catalysts for the enantioselective enzymatic reversible aldol reaction, Barbas III and co-workers have also designed hapten **412** (Chart 17). As compared to hapten **411**, it additionally includes the sulfone moiety, responsible for the structural analogy with the rate-determining transition state of the aldol reaction.[345] After immunization of mice with this hapten, 17 monoclonal antibodies were prepared and purified, from which two (93F3 and 84G3) were characterized in detail and compared to antibodies 38C2 and 33F12. Namely, the enantioselectivity displayed by these antibodies in the formation of the aldol adducts **413a**−**e** is substrate dependent. Aldols (*R*)-**413a,c**−**d** are provided in essentially enantiomerically pure form with either catalyst, while a moderate enantioselectivity is obtained in the synthesis of (*S*)-**413b** (69% *ee* with ab93F3 or 54% *ee* with ab84G3). These values are similar to those obtained with the well-known commercial ab38C2; however, the enantioselectivity is reversed. The diastereoselectivity displayed by ab93F3 was also studied by means of the preparation of the aldol adduct **413e**. The syn isomer is the preferred one (90% *de* and *ee*), thus evidencing again a selectivity opposite to that obtained with ab38C2.

Direct aldol reactions that involve unsymmetrical ketones constitute a major challenge since simultaneous control of the regio-, diastereo-, and enantioselectivity of the reaction has to be achieved. In this sense, the aforementioned aldolase antibodies have proven to be effective catalysts. Namely, Maggiotti et al.[346] have studied the regioselectivity displayed by antibodies 38C2, 33F12, 84G3, and 93F3 in the aldol reaction of various unsymmetrical methyl ketones with *para*-nitrobenzaldehyde. It was found that the sense and level of regioselectivity for the reactions catalyzed by antibodies 38C2 and 33F12 were highly dependent on the structure of the substrates. In general, ab38C2, like the noncatalyzed reaction, favored the formation of the branched regioisomers resulting from a reaction on the more substituted carbon. In contrast, antibodies 84G3 and 93F3 catalyzed the exclusive formation of the linear regioisomer independent of the structure of the reactants examined. Analysis of the enantioselectivity of the ab84G3-catalyzed direct aldol reaction of ketones **414a**−**h** with *para*-nitrobenzaldehyde was carried out (Scheme 78). In all cases, the linear aldol adducts of (*R*)-configuration were obtained in excellent *ee*'s, the opposite enantiomeric series of these compounds being accessible via ab84G3-catalyzed retro-aldol KR of (±)-**415a**−**h**.

Griengl and co-workers have recently expanded the repertoire of biocatalytic aldol reactions by demonstrating that hydroxynitrile lyase from *Hevea brasiliensis* (*Hb*HNL) can accept nitrocompounds as donors instead of cyanide. It thus constitutes the first report of a biocatalytic enantioselective nitroaldol (or Henry) reaction.[347] The employment of several substrates afforded the following conclusions: (1) The enzyme is less active than in the hydrocyanation of the corresponding aldehydes. (2) *Hb*HNL can accept a wide range of aldehydes although its nucleophile promiscuity is much smaller (only nitromethane and nitroethane were tested). (3) With the exception of the reaction with benzaldehyde, the rest of desymmetrizations tested usually show low yields, although the addition products generally show very high *ee* values.

Table 30. Baeyer−Villiger Desymmetrization of Bridged Cycloketones



entry	substrate	R (*endo*)	enzyme	yield (%)	*ee* (%)	config[a]
1	**405a**	$-(CH_2)_3-$	$CHMO_{Rhodo2}$	63	99	(−)
2	**405a**	$-(CH_2)_3-$	$CHMO_{Brevi2}$	67	92	(+)
3	**405b**	$-(CH_2)_4-$	$CHMO_{Rhodo1}$	58	99	(−)
4	**405b**	$-(CH_2)_4-$	$CHMO_{Brevi2}$	78	94	(+)
5	**405c**	1,3-cyclopentyl	$CHMO_{Acineto}$	51	98	(−)
6	**405d**	CO_2Me	$CHMO_{Acineto}$	53	92	(−)
7	**405d**	CO_2Me	$CPMO_{Coma}$	49	71	(+)
8	**405e**	$-CH_2-O-CH_2-$	$CHMO_{Brevi1}$	72	96	(−)
9	**405f**	$-(CH_2)_2-$	$CHMO_{Rhodo2}$	60	na	na

[a] α_D^{20} ($CHCl_3$)

7.2. Acyloinic Reactions

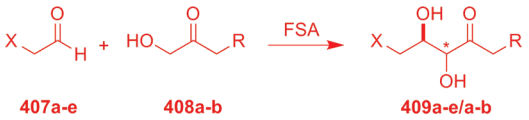
The acyloinic condensation is another method of forming carbon−carbon bonds. The enzymatic reaction is catalyzed by the group of the thiamin diphosphate (ThDP)-dependent enzymes[348] through a mechanism in which, usually, the decarboxylation of a ketoacid is promoted in the presence of the ThDP cofactor. The so-formed umpolung acyl anion (donor substrate) is then susceptible to undergo addition to aldehydes, thus leading to optically active 2-hydroxyketones. The synthetic applications of these enzymatic transformations was increased when it was found that simple aldehydes could act as suitable substrates for these enzymes, thus avoiding the use of expensive ketoacids.[349]

Pyruvate decarboxylase (PDC) is one of the first enzymes that was used for synthetic purposes.[350] Although it is active toward a variety of aldehydes, it has been found that acetaldehyde is the preferred donor substrate. For instance, the PDC-catalyzed formation of (*R*)-1-hydroxy-1-phenyl-2-propanone (*R*)-**417a**/**h**, synthetic precursor of (−)-ephedrine, using acetaldehyde and benzaldehyde as starting materials, has been in the focus of research of many working groups with the emphasis on optimization of the yeast strains for biotransformation.[350,351]

Benzoylformate decarboxylase (BFD) is able to bind a broader range of aromatic aldehydes to ThDP prior to ligation to the acceptor aldehyde.[352] This way, different aromatic β-hydroxyketones can be prepared on a preparative scale in moderate to high yields and good *ee*'s (Table 32). Interestingly, the absolute configuration of the final compound depends on the nature of

dx.doi.org/10.1021/cr100330u |*Chem. Rev.* 2011, 111, PR110–PR180

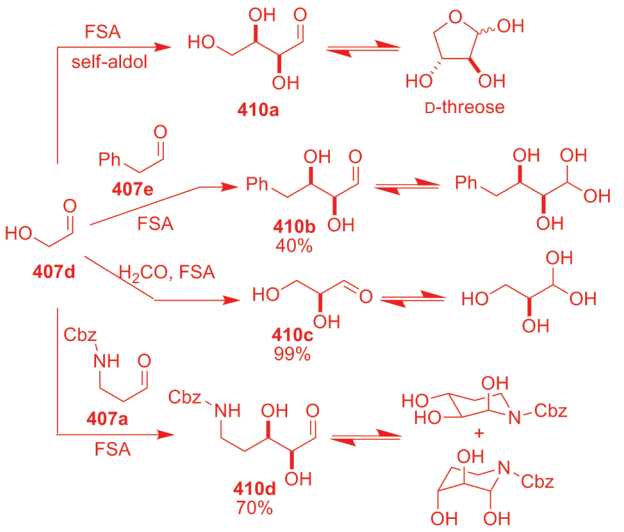
Table 31. FSA-Catalyzed Aldol Desymmetrization Reactions



entry	aldehyde	X	ketone	R	major product	conversion (%)	isolated yield (%)
1	**407a**	CH_2NHCbz	**408a**	OH	*threo*	79−82	69
2	**407b**	NHCbz	**408a**	OH	*threo*	60	40
3	**407b**	NHCbz	**408b**	H	*threo*	96	75
4	**407c**	OBn	**408a**	OH	*threo*	35	28
5	**407c**	OBn	**408b**	H	*threo*	95	71
6	**407d**	OH	**408b**	H	*erythro*	∼100	51[a]
7	**407e**	Ph	**408a**	OH	*threo*	77	46
8	**407e**	Ph	**408b**	H	*threo*	70	48

[a] Isolated as a mixture of the open chain and cyclic hemiacetalic forms of the *threo* (major, 3*S*,4*R*) and the *erythro* (minor, 3*R*,4*R*) isomers.

Scheme 77

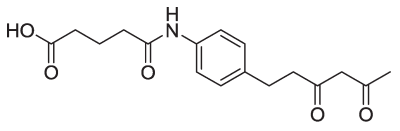


the acceptor substrate, being *S* for acetaldehyde (**416h**, entries 1−5) and *R* for aromatic aldehydes (**416a**,**f**−**g**, entries 6−8). Besides, for highly hydrophobic substrates, DMSO can be used as cosolvent with no loss of BFD ligase activity.[353]

Benzaldehyde lyase (BAL) is perhaps the most versatile enzyme concerning the acceptance of aromatic donors. Demir et al.[354] have shown that this enzyme is able to activate and dimerize a broad range of heteroaromatic, and *ortho*-, *meta*-, and *para*-substituted aromatic aldehydes, which bear groups with both electron-releasing and electron-withdrawing properties (Scheme 79a). In all cases, both the yields and the *ee*'s are synthetically useful. The preference of BAL for aromatic aldehydes as donor substrates has been also exploited for their cross coupling with acetaldehyde (**416h**). This way, the corresponding (*R*)-2-hydroxypropiophenones (*R*)-**417** have been obtained in high yields and optical purities (Scheme 79b).

The synthetic value of this kind of C−C forming reaction has been reported by Müller et al.[355] In an unprecedented paper, these authors combine the preferences of BAL and the BFD mutant H281A with the donor−acceptor concept to prepare a wide range of mixed benzoins with very high conversions,

Chart 16



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selectivities, and *ee*'s. After that, further developments in these reactions have been published, expanding the scope of the synthetic applications of this type of biotransformations.[356]

A special case of acyloinic-type reaction has been recently studied by Hailes and co-workers, by using wild-type as well as mutant transketolase (TK) enzymes. Although the process involves the decarboxylation of a donor β-hydroxypyruvate and the formal addition to the acyl anion to an aldehyde acceptor (Scheme 80), we have classified this reaction within this subsection for two main reasons. First of all, the final product structurally resembles those obtained in acyloinic condensations. On the other hand, TK are also ThDP-dependent anzymes, requiring the addition of this cofactor and the presence of Mg^{2+} for its suitable activity. In vivo, TK catalyzes the reversible transfer of a ketol unit to D-ribose-5-phosphate or D-eritrose-4-phosphate,[357] but the reaction is made reversible by using hydroxypyruvate as the donor. This fact has been exploited for synthetic applications in EEDs, since the enzyme showed some degree of stereoselectivity toward nonchiral aldehydes,[358] including nonhydroxylated aldehydes.[359] Different mutants have been prepared which showed increased activity toward non-natural acceptors and even reversing of the stereoselectivity,[360] which is 3*S* in the WT enzyme. In this way, a broad family of enantioenriched α,α′-dihydroxyketones can be easily prepared in good yields and moderate to excellent *ee*'s.

7.3. Hydrocyanation of Carbonyl Compounds

Oxynitrilases or hydroxynitrile lyases (HNL) catalyze the reversible addition of hydrogen cyanide to the carbonyl group of aldehydes and ketones. Under appropriate conditions, they allow the desymmetrization of prochiral aldehydes and ketones to furnish enantiomerically pure or enriched cyanohydrins. Pure and in situ generated HCN can be successfully used as donor for

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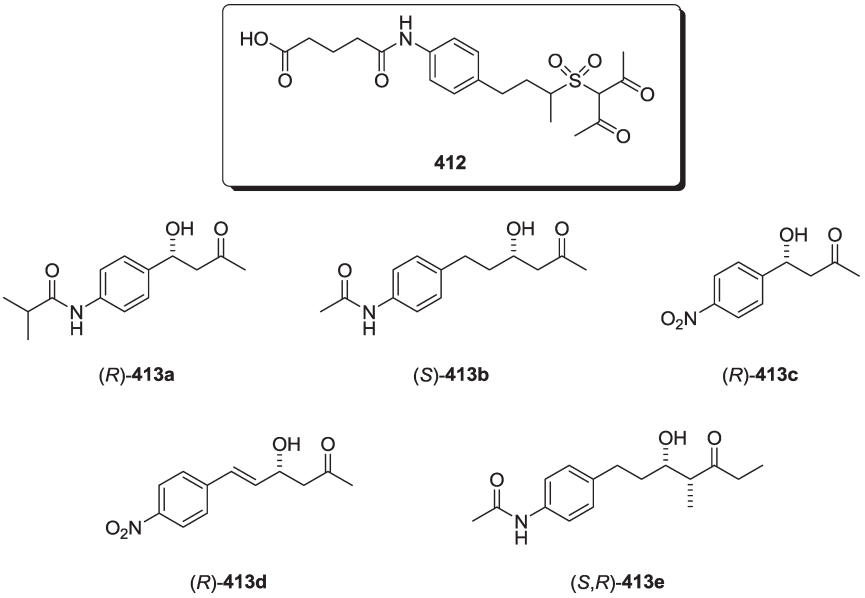
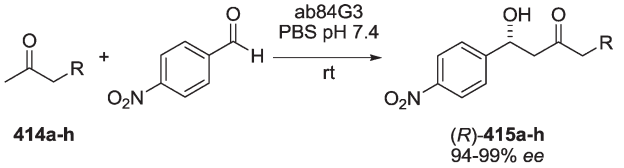
Chart 17



Scheme 78



R: **a** = Et; **b** = *n*-Pr; **c** = OMe; d = SMe; e = F; **f**: CH_2OH; **g** = CH_2OCH_3; **h** = CH_2SCH_3

this addition. Nevertheless, its toxicity makes the transhydrocyanation of ketone cyanohydrins a safer alternative.[361]

Oxynitrilases from different sources have been isolated and purified, and they can be divided into two classes, discernible by the presence or absence of FAD as a cofactor. In fact, only the oxynitrilases from the plant family *Rosaceae* contain this cofactor, a fact that has been explained in terms of evolutionary grounds; it is not involved in catalysis but would appear to exert a structure-stabilizing effect. Although a Ser-Asp-His catalytic triad has been identified for certain oxynitrilases, the catalytic mechanism is still a matter of dispute.[362] Thus, the formation of a hemiacetal/ketal intermediate, and the activation of the carbonyl oxygen by the catalytic Ser and a Thr residue, followed by attack of hydrogen cyanide, which would be deprotonated by the catalytic His, are the two most likely hypotheses.

Oxynitrilases can accept a wide range of substrates and both (*R*)- and (*S*)-cyanohydrins can be in principle prepared by using (*R*)- and (*S*)-oxynitrilases.[361] One restriction to their general use in synthesis is the limited quantities in which they can be obtained. That is why the (*R*)-oxynitrilase from *Prunus amygdalus* (almonds) is the most heavily researched of all the (*R*)-selective enzymes, as it can be easily obtained in large quantities by extraction from almonds. Nevertheless, the overexpression of oxynitrilases in host microorganisms has proven to be successful in providing sufficient quantites of these enzymes for their large-scale application, particularly for the case of (*S*)-oxynitrilases.[361a]

Despite recent advances in catalyst engineering that facilitate the application of enzymatic hydrocyanations on an industrial scale,[363] undoubtedly, the implementation of the enzyme-catalyzed cyanohydrin reaction as a method of great synthetic importance has been principally possible due to the suppression of the nonenzymatic reaction, which lowers the yield and optical purity of the resulting products.[361b,d,364] This drawback can be overcome by appropriate control of the pH of the reaction medium when the reaction is carried out in an aqueous environment or in a biphasic system. Nevertheless, the enantiomeric purity of the enzymatic products can be further compromised by their racemization in the aqueous buffer during the course of the reaction. Conversely, the employment of oxynitrilases in organic solvents not miscible with water constitutes an elegant solution for these problems. In this regard, since the pioneering contribution of Effenberger and co-workers,[365] many advances have been done in the field. Lin et al. have found that when dried almond powder is used in organic solvents, no hydrocyanation product is detected even if the reaction is carried out in water-saturated *iso*-propyl ether. However, when almond meal with a 9% water content (microaqueous conditions) is employed as catalyst, the reaction can be carried out in an organic solvent with a satisfactory activity over the temperature range of 4 to 30 °C.[366] These authors have tested the utility of these conditions by preparing a series of (*R*)-cyanohydrins in high yields and enantioselectivities starting from a series of aliphatic and aromatic aldehydes and ketones using the (*R*)-oxynitrilases from almond, peach, and loquat as catalysts.[367] Results showed that the microaqueous reaction system was superior to the conventionally used water-organic biphasic reaction system, even at 30 °C. Additionally, the accessible substrates by the peach enzyme were very similar to those by the almond enzyme. However, the substrate scope of the loquat enzyme, unlike the almond or peach enzymes, was restricted to aromatic and heteroaromatic aldehydes.

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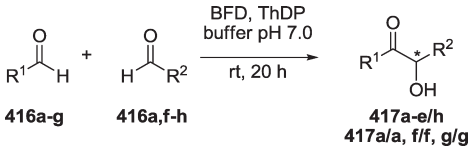
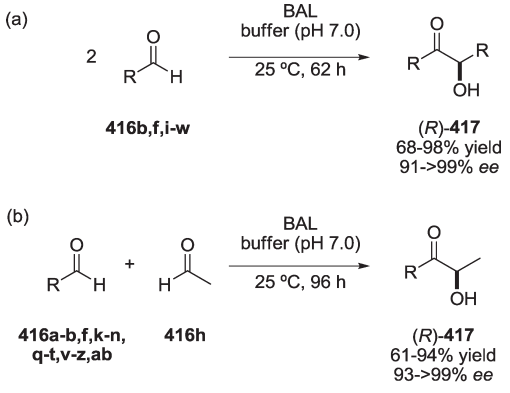
Table 32. EED of Aldehydes Catalyzed by BFD



entry	substrates	product	R^1	R^2	yield (%)	*ee* (%)
1	**416a/416h**	(*S*)-**417a/h**	Ph	Me	90	92
2	**416b/416h**	(*S*)-**417b/h**	3-MeO-C_6H_4	Me	97	96
3	**416c/416h**	(*S*)-**417c/h**	3-*i*-PrO-C_6H_4	Me	91	>99
4	**416d/416h**	(*S*)-**417d/h**	3,5-di-MeO-C_6H_4	Me	40	97
5	**416e/416h**	(*S*)-**417e/h**	2-naphthyl	Me	32	88
6	**416a/416a**	(*R*)-**417a/a**	Ph	Ph	70	>99
7	**416f/416f**	(*R*)-**417f/f**	2-F-C_6H_4	2-F-C_6H_4	68	>99
8	**416g/416g**	(*R*)-**417g/g**	4-Me-C_6H_4	4-Me-C_6H_4	69	>99

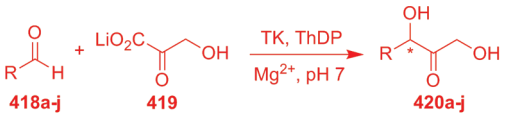
Scheme 79



R = **a**: Ph; **b**: 3-MeO-C_6H_4; **f**: 2-F-C_6H_4; **i**: 2-Cl-C_6H_4; **j**: 2-Br-C_6H_4; **k**: 2-MeO-C_6H_4; **l**: 3-F-C_6H_4; **m**: 3-Cl-C_6H_4; **n**: 3-Br-C_6H_4; **o**: 3-OH-C_6H_4; **p**: 4-F-C_6H_4; **q**: 4-Cl-C_6H_4; **r**: 4-Br-C_6H_4; **s**: 4-MeO-C_6H_4; **t**: 2,4-F_2-C_6H_3; **u**: 2-naphthalenyl; **v**: 2-furanyl; **w**: 2-thienyl; **x**: 3,5-F_2-C_6H_3; **y**: 3-OH-4-MeO-C_6H_3; **z**: 3-MeO-4-OH-C_6H_3; **ab**: 3,4,5-$(MeO)_3$-C_6H_2;

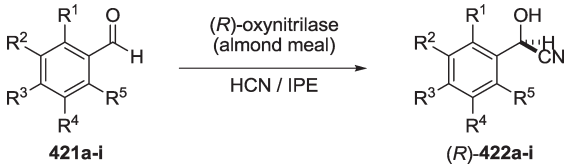
Subsequently, these authors have exploited the usefulness of the microaqueous conditions for the desymmetrization of different aromatic aldehydes. Namely, they have published the first enantioselective synthesis of (*R*)-fluorinated mandelonitriles using the aforementioned preparation of almond meal as catalyst (Table 33).[368] As it can be seen from the results obtained, the influence of the fluoro-substitution on *ee* varies with both the number and position of the fluorine atoms attached to the phenyl ring. Thus, as compared to the reaction of benzaldehyde, which was transformed into enantiopure mandelonitrile, fluoro-substitution decreases the enantioselectivity of the reaction in the series monosubstituted (**421a**−**b**; entries 1 and 2) < disubstituted (**421c**−**e**; entries 3−5) < tri- and pentasubstituted (**421f**−**g**; entries 6 and 7). For the latter compounds as well as for **421h** (entry 8), no enantioselectivity was observed. The position of the fluorine atoms also played a role on the stereoselectivity of the reaction. Thus, *ortho*-substitution (**421b,d**−**e**; entries 2, and 4−5) caused more significant reduction in the enantioselectivity

Scheme 80



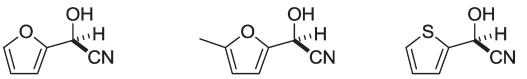
R = **a**: CH_2OH; **b**:C_2H_5; **c**: C_3H_7; **d**: C_4H_9; **e**: C_5H_{11}; **f**: C_6H_{13}; **g**: C_7H_{15}; **h**: cyclopropyl; **i**: cyclopentyl; **j**: cyclohexyl;

Table 33. EED of Benzaldehyde Derivatives



entry	substrate	R^1	R^2	R^3	R^4	R^5	*t* (h)/*T* (°C)	yield (%)	*ee* (%)
1	**421a**	H	H	F	H	H	24/20	90	94.2
2	**421b**	H	H	H	H	F	24/20	96	84
3	**421c**	H	F	F	H	H	24/28	71	84.3
4	**421d**	F	F	H	H	H	24/20	92	46.1
5	**421e**	F	H	H	H	F	24/12	70	41.0
6	**421f**	H	F	F	F	H	24/12	37	0
7	**421g**	F	F	F	F	F	48/12	90	0
8	**421h**	H	H	CF_3	H	H	24/12	67	0

Chart 18

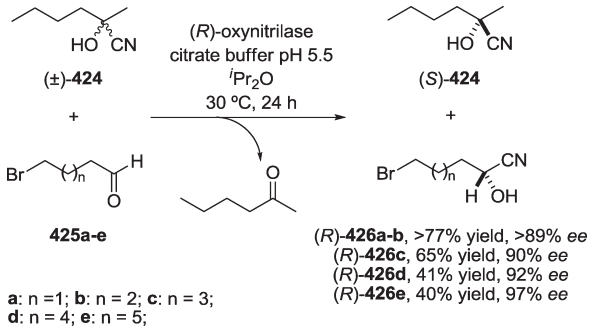


of the reaction than substitution at other positions (**421a,c**; entries 1 and 3). These effects were attributed to the strongly electronegative character of the fluorine atoms, which depending on their number and position, facilitate, to a different extent, the racemization of the products formed.

The almond meal-catalyzed hydrocyanation under microaqueous conditions of a wide range of prochiral heteroaryl carboxaldehydes has been studied due to the special importance of optically active heteroaryl cyanohydrins in synthesis.[369] The results obtained showed that, as a general trend, the microaqueous conditions did afford similar results to those obtained under standard ones for the aldehydes leading to **423a**−**c** (Chart 18). Additionally, the following structure−activity relationships could be outlined (data not shown): (1) *N*-Containing heteroaryl carboxaldehydes turned out to be not satisfactory substrates from an enantioselective point of view. (2) As a general trend, the introduction of substituents led to yields and *ee*'s that were no better than the corresponding values obtained for the parent compounds. (3) Electron-donating substituents such as the methyl group reduced the reactivity of the aldehydes, thereby

dx.doi.org/10.1021/cr100330u |*Chem. Rev.* 2011, 111, PR110–PR180

Scheme 81

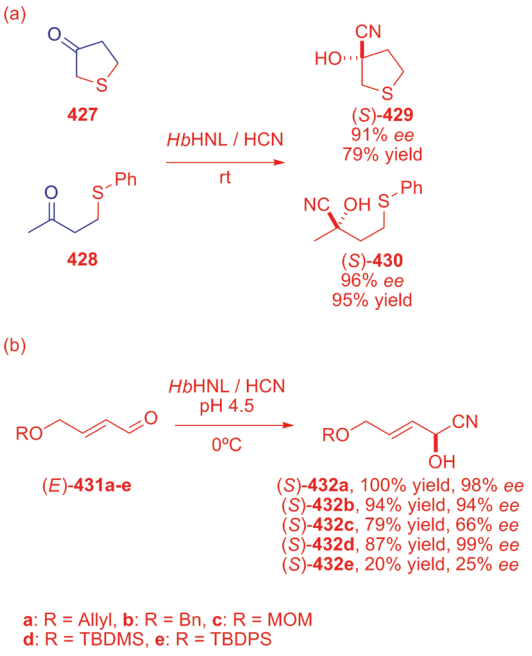


affording lower yields. (4) The presence of strong electron-withdrawing substituents such as the nitro group, activated the aldehydes so much that a black tar was formed without any desired products detected. (5) Heteroaryl carboxaldehydes substituted with moderate electron-withdrawing substituents such as halogen and cyano groups usually gave modest yields and *ee*'s. In addition to this, HNL from *Hevea brasiliensis* has also proven to be an excellent catalyst for the desymmetrizations of aromatic aldehydes. Thus, the corresponding cyanhydrines derived from formyl- and 1,1′-diformyl- ferrocenes have been obtained in excellent yields and enantioselectivities.[370] Moreover, this enzyme also allowed the production of (*R*)-**423a** on kilogram scale in excellent enantioselectivity and yield. The subsequent reduction of the nitrile group afforded the corresponding β-amino alcohol thus allowing the efficient laboratory production of this compound.[371]

In our research group, we have extended the optimized procedure for the almond meal (*R*)-oxynitrilase-catalyzed transcyanation of the ω-bromoaldehydes **425a,b**[372] to compounds **425c**−**e** (Scheme 81).[373] (±)-2-Methyl-2-hydroxyhexanenitrile [(±)-**424**] was used as the hydrogen source, cyanohydrins (*R*)-**426c**−**e** being obtained in good *ee*'s. However, a decrease in the yield associated with an increase in the length of the alkyl chain between the bromine and the aldehyde was detected. Additionally, we have demonstrated the synthetic versatility of the so-obtained (*R*)-cyanohydrines by using them to prepare different biologically active compounds such as (*S*)-pipecolic acid and 2-substituted piperidine alkaloids,[374] optically active azacycloalkan-3-ols,[373] and 2,3-disubstituted piperidines.[375]

Substrate engineering has been efficiently used as strategy to optimize the enantiomeric ratio of some hydrocyanation reactions that initially gave unsatisfactory enantioselectivities. For example, *Hevea brasiliensis*, HNL, is known not to be able to successfully differentiate between similar small-sized groups such as the ethyl and methyl group in 2-butanone. However, the employment of equivalent *thio-disguised* substrates **427** and **428** raised the initial *ee* value from 54% to >90% (Scheme 82a). Subsequent hydrolysis and deprotection of the corresponding products afforded the target (*S*)-2-hydroxy-2-methylbutyric acid in enantiopure form.[376] Similarly, the EED of the (*E*)-4-alkoxy-2-butenal has been optimized by using different hydroxylic protecting groups.[377] Thus, as it can be seen in Scheme 82b, the enantioselectivity of the hydrocyanation catalyzed by *Hb*HNL strongly depended on the nature of the protecting group used: while MOM and TBDPS afforded the worst results, the employment of TBDMS as protecting group afforded the corresponding (*S*)-configured cyanhydrine in enantiopure form and very high yield. Although for the case of the (*Z*)-configured butenals the

Scheme 82



enzymatic enantioselectivity drops considerably, the allyl protecting group still afforded an enantioselectivity high enough to allow the employment of the corresponding cyanhydrine for the de novo synthesis of unnatural pentoses. Alternatively, protein engineering techniques like rational mutation and directed evolution, have been also successfully used to improve the enantioselectivity displayed by the *Prunus amigdalus* HNL isoenzyme 5 toward non-natural aromatic substrates[378] and hydroxypivaladehyde,[379] respectively. In both cases, the initial optical purities (89% *ee* approx) were finally raised to values >96% *ee*.

More recently, some new HNLs have been discovered, which has expanded their utility in EED processes. For instance, Asano and co-workers have isolated the oxynitrilase from *Prunus mume* (the seed of the Japanese apricot), which has shown to be very efficient for the reaction with a large variety of aromatic[380] and saturated and unsaturated aliphatic[381] aldehydes. On the other hand, Han et al. described the activity of an oxynitrilase from the defatted seed meal of common vetch (*Vicia sativa* L.) toward aromatic and heteroaromatic aldehydes.[382] A few years later, Nanda reported a new hydroxynitrile lyase from *Prunus armeniaca* (the white apricot, shakarpara cultivar), which is able to catalyze the biotransformations from polycyclic aromatic aldehydes and aromatic aldehydes with bulky substitution.[383] In all these works, the final cyanohydrins were of *R* configuration, although the *ee* of the final product depended on the biocatalyst and the substrate structure.

7.4. Breaking of C−C Bonds

Although enzymatic reactions involving the cleavage of a carbon−carbon bond are rare, recent examples using this kind of transformation have been reported in the field of EEDs of prochiral compounds. One interesting example is the reaction catalyzed by 6-oxocamphor hydrolase (OCH) from *Rhodococcus* sp. NCIMB 9784, which naturally promotes the desymmetrization of 6-oxocamphor to α-campholinic acid in moderate stereoselectivity.[384] Very remarkably, this enzyme showed a

dx.doi.org/10.1021/cr100330u |*Chem. Rev.* 2011, 111, PR110–PR180

Scheme 83

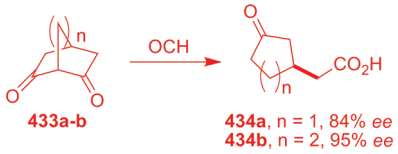
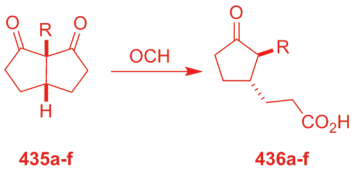


Table 34. OCH-Catalyzed EED of Bicyclic Diketones



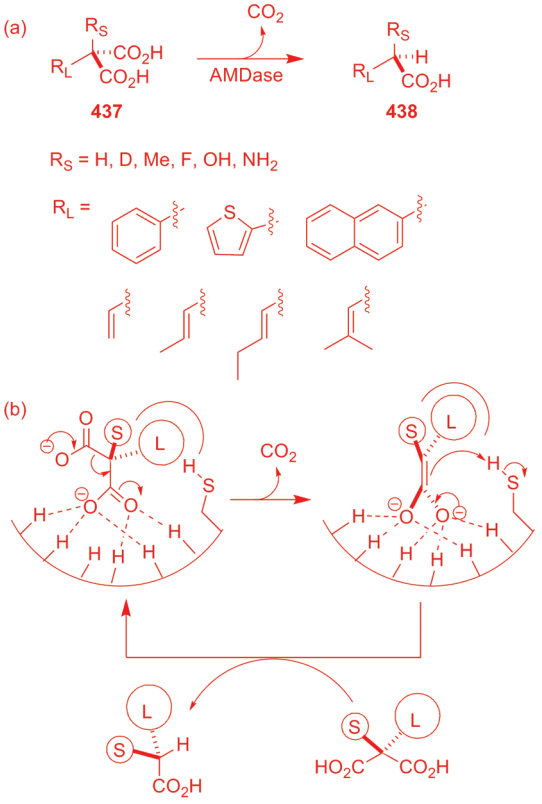
entry	substrate	R	*de* (%)	*ee* (%)
1	**435a**	H		
2	**435b**	Me	82	>95
3	**435c**	Et	81	>95
4	**435d**	allyl	86	>95
5	**435e**	prop-2-ynyl	78	91
6	**435f**	pent-2-ynyl	75	75

better stereodifferentiation of other structurally related bicyclic prochiral ketones (Scheme 83).[385] The somehow lower *ee* of the five-membered ring ketoacid was attributed to a background nonbiocatalytic hydrolysis.

In a further improvement of this process, Grogan and coworkers overexpressed OCH in *Escherichia coli* and used the enzyme to catalyze the EED of different bicyclo[2.2.2]octane-2,6-dione derivatives (Table 34).[386] Although the simplest compound of this series was very slowly tranformed (**435a**, entry 1), the ones with an alkyl or allyl substituent at the carbon between the two carbonyl groups (**435b**−**d**) rendered very good diastereoselectivities and excellent *ee*'s (entries 2−4). Those bearing more rigid and longer groups (**435e**−**f**) showed slightly lower stereoselectivities (entries 5 and 6). This EED opens the way to the chemoenzymatic synthesis of chiral cyclopentanone-based natural products in an easy, efficient, and stereocontrolled fashion.

The EED by stereoselective decarboxylation of prochiral α-disubstituted malonic acids is an attractive route to produce homochiral α-substituted carboxylic acids. Regarding that, the arylmalonate decarboxylase (AMDase) isolated from *Bordetella bronchiseptica*[387] has shown to be highly effective in the decarboxylation of α-arylmalonates (**437**) to give enantiomerically pure α-arylcarboxylic acids (**438**, Scheme 84a).[388] This is a very interesting enzyme with a high potential for synthetic applications since it is very robust and does not require cofactor. One of the main disadvantages for the synthetic applications of AMDase has been the need of an aromatic group attached to the prochiral center of the malonic acid. After different structural studies,[389] a proposal for the mechanism of action has come out (Scheme 84b). The active site of the AMDase present a

Scheme 84



"dioxyanion hole" (formed by six H-bonds) which would stabilize the large anionic charge density of a putative enediolate intermediate, produced after the decarboxylation of the prochiral malonate dianion. This enediolate would undergo stereoselective protonation at the *Si* face from a cysteinyl thiol, rendering the final carboxylic acid with *R* configuration. The understanding of the mechanism allowed Leys and Mickelfield to prepare new-AMDase mutants by structure guided directed evolution, which showed enhanced catalytic efficiency with a range of substrates, namely alkenylmalonate derivatives.[390]

8. SUMMARY AND OUTLOOK

Enantioselective enzymatic desymmetrizations of *meso* and prochiral substrates have proven to be a powerful methodology that allows the preparation of a wide range of optically active building blocks in a highly enantioselective fashion and in high yields. In many cases, enantiodivergence can be achieved by means of the employment of different enzymes, substrate engineering, or combination with other approaches such as KRs or metal catalysis. More recently, the feasibility to scale up these transformations and to mutate enzymes, not only to alter their activity or selectivity, but to additionally meet the needs of industrial processes, have been also shown. These facts make EEDs an attractive tool of asymmetric synthesis and convert them into an active field of research. We strongly believe that the discovery and characterization of new biotransformations, together with genetic engineering techniques, are going to play a major role in the future of biocatalysis but without detriment to

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other more traditional approaches such as the screening for new biocatalysts.

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BIOGRAPHIES

Eduardo García-Urdiales graduated in chemistry in 1996 at the University of Oviedo. In 2001 he completed his Ph.D. under the guidance of Prof. Gotor. During his doctoral studies, he also spent a short stay at the group of Prof. Karl Hult (KTH, Stockholm). In 2001, he moved to Solvay Pharmaceuticals GmbH in Hannover, Germany, as a postdoctoral fellow in the Computer Aided Drug Design unit, where he stayed for two years. After that, he moved back to Oviedo, and in 2005 he joined the Structural and Computational Biology Unit of the EMBL-Heidelberg as a postdoctoral fellow. He is currently working as a research associate at the University of Oviedo. His research interests are focused on the study of protein−ligand interactions and the rational design of small-molecule modulators of the protein activity.

Ignacio Alfonso was born in 1972 and graduated in chemistry in 1995 at the University of Oviedo. He completed his PhD Thesis (1996−2000) under the guidance of Prof. Gotor and a postdoctoral stay (2000−2002) at The Scripps Research Institute with Prof. Ghadiri. After two different stays as research associate at the University of Oviedo (2002−2004) and the University Jaume I in Castellón (2004−2007, Ramón y Cajal contract), he obtained a permanent position as a Tenured Scientist in the Spanish Council for Scientific Research (CSIC), where he started his independent research career. He is currently the group leader of the Supramolecular Chemistry group at the Institute of Advanced Chemistry of Catalonia (IQAC). His research interests are supramolecular and bioorganic chemistry, molecular recognition, self-assembling, and constitutional dynamic chemistry.

Vicente Gotor received his Ph.D. from the University of Zaragoza in 1974. After leaving Zaragoza, Dr. Gotor carried out two years of postdoctoral studies at Max Planck Institut für Kohlenforschung (Mülhein/Ruhr, Germany) in the area of organometallic chemistry. He joined the Chemistry Faculty at the University of Oviedo as Assistant Professor in 1977; he assumed his current position as Professor of Organic Chemistry at the same institution in 1982. His research fields include the areas of Heterocyclic and Bioorganic Chemistry. Specific areas of his research interest are enzymatic amidation reactions with hydrolases, enzymatic chemoselective transformations on natural products, biotransformations with oxynitrilases and oxidoreductases, and chiral recognition with azamacrocycles. He was Vice-chancellor of Research of Oviedo University for four years until June 2000, Head of Department of Organic and Inorganic Chemistry for five years (June 2003 until May 2008). At present, he is Rector of Oviedo University and he has published more than 300 papers, 11 patents, and supervised 51 doctoral theses.

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ABBREVIATIONS

ab	antibody
ADH	alcohol dehydrogenase
AHS	lipase AHS (Amano)
BAL	benzaldehyde lyase
BFD	benzoylformate decarboxylase
BOC	*t*-butoxycarbonyl
BPDO	biphenyl dioxygenase
BVMO	Baeyer−Villiger monooxygenase
c	(degree of) conversion
CAL(B)	lipase (B) from *Candida antarctica*
Cbz	benzyloxycarbonyl

Chirazyme C-3	commercially available preparation of CALB (Roche)
Chirazyme L-2	commerically available preparation of CALB (Roche)
CHMO	cyclohexanone monooxygenase
α-CHY	α-chymotripsin
CIP	Cahn−Ingold−Prelog
CPCR	*Candida parapsilosis* carbonyl reductase
CPMO	cyclopentanone monooxygenase
CPO	chloroperoxidase
CRL	lipase from *Candida rugosa*
Cy	cyclohexyl
de	diastereomeric excess
DFACC	2,2-difluoro-1-aminocyclopropane-1-carboxylic acid
DMAP	4-(dimethylamino)pyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
E	enantiomeric ratio
ee	enantiomeric excess
EED	enantioselective enzymatic desymmetrization
ER	enoate reductase
ESPESL 1864	commercially available termophilic enzyme (Diversa Corp.)
FDA	U.S. Food and Drug Administration
$FAD(H_2)$	flavine-adenine dinucleotide
FMN(H)	flavine mononucleotide
FSA	D-fructose-6-phosphate aldolase
HAPMO	4-hydroxyacetophenone monooxygenase
HLADH	horse liver alcohold dehydrogenase
HNL	hydroxynitrile lyase
IPE	*i*-propyl ether
KR	kinetic resolution
LBADH	*Lactobacillus brevis* alcohol dehydrogenase
Lipase AK	commercially available preparation of PFL (Amano Pharmaceutical Co.)
Lipase OF	commercially available preparation of CRL (Meito Sangyo Co.)
Lipase PS	commercially available preparation of lipase from *Burkholderia cepacia* (Amano Pharmaceutical Co.)
Lipase QL	commercially available preparation of lipase from *Alcaligenes* sp. (Meito Sangyo Co.)
LPL	lipoprotein lipase
MPMO	*p*-methoxybenzyloxy
MOM	methoxymethyl
NAD(H)	nicotinamide adenine dinucleotide
NADP(H)	nicotinamide adenine dinucleotide phosphate
NDO	naphthalene dioxygenase
NIS	NRPS-independent siderophore
NK	neurokinin
Novozyme 435	commercially available immobilized CALB (Novo Nordisk)
NRPS	nonribosomal peptide synthetase
OCH	6-oxocamphor hydrolase
OYE	old yellow enzyme
PAMO	phenylacetone monooxygenase
PBS	phosphate buffered saline
PDC	pyruvate decarboxylase
PFL	lipase from *Pseudomonas fluorescens*
PG	protective group
PLE	pig liver esterase
PPL	porcine pancreas lipase
PQQ	pyrroloquinoline quinone
PSA	preparation of *Pseudomonas cepacia* lipase
PSL	*Pseudomonas* sp. lipase
recLBADH	recombinant *Escherichia coli* strain expressing LBADH
SADH	secondary alcohol dehydrogenase
SAM II	commercially available preparation of *Pseudomonas* lipase (Fluka)
SLS	sodium lauryl sulfate
Sty	styrene monooxygenase
TBADH	*Termoanaerobium brockii* ADH
TBSCl	*t*-butylchlorodimethylsilane
TBSO	*t*-butyldimethylsilyloxy
TDO	toluene dioxygenase
ThDP	thiamin diphosphate
THF	tetrahydrofuran
TK	transketolase

REFERENCES

(1) Rouhi, A. M. *Chem. Eng. News* **2003**, *81* (18), 45.

(2) (a) Crosby, J. *Chirality in Industry II, Development in the Commercial Manufacture and Applications of Optically Active Compounds*; Wiley & Sons: Chichester, 1997; Chapter 1. (b) Blumenstein, J. J. *Chiral Drugs: Regulatory Aspects*; Wiley & Sons: Chichester, 1997; Chapter 2.

(3) (a) Bommarius, A. S.; Riebel, B. R. *Biocatalysis*; Wiley-VCH: Weinheim, 2004. (b) Patel, R. P. *Stereoselective Biocatalysis*; Marcel Dekker: New York, 2000. (c) Faber, K. *Biotransformations in Organic Chemistry*; Springer-Verlag: Heidelberg, 2004. (d) Drauz, K.; Waldmann, H. *Enzyme Catalysis in Organic Synthesis: A Comprehensive Handbook*; Wiley-VCH: Weinheim, 2002. (e) García-Urdiales, E.; Alfonso, I.; Gotor, V. *Asymmetric Organic Synthesis with Enzymes*; Wiley-VCH: Weinheim, 2008.(f) Patel, R. N. *Biocatalysis in the Pharmaceutical and Biotechnology Industries*; CRC Press: Boca Raton, FL, 2007. (g) Fessner, W.-D.; Anthonsen, T. *Modern Biocatalysis*; Wiley-VCH: Weinheim, 2009.

(4) (a) Klibanov, A. M. *Nature* **2001**, *409*, 241. (b) Carrea, G.; Riva, S. *Angew. Chem., Int. Ed.* **2000**, *39*, 2226.

(5) (a) Cao, L.; van Langen, L.; Sheldon, R. A. *Curr. Opin. Biotechnol.* **2003**, *14*, 387. (b) Sheldon, R. A. *Adv. Synth. Catal.* **2007**, *349*, 1289. (c) Hanefeld, U.; Gardossi, L.; Magner, E. *Chem. Soc. Rev.* **2009**, *38*, 453.

(6) See for instance: Faber, K. *Chem.—Eur. J.* **2001**, *7*, 5004and references therein.

(7) (a) Pàmies, O.; Bäckvall, J.-E. *Chem. Rev.* **2003**, *103*, 3247. (b) Pellicer, H. *Tetrahedron* **2003**, *59*, 8291.(c) Martín-Matute, B.; Bäckvall, J.-E. *Curr. Opin. Chem. Biol.* **2007**, *11*, 226.

(8) Willis, M. C. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1765.

(9) Schoffers, E.; Golebiowski, A.; Johnson, C. R. *Tetrahedron* **1996**, *52*, 3769.

(10) (a) Cahn, R. S.; Ingold, C. K.; Prelog, V. *Angew. Chem.,Int. Ed.* **1966**, *5*, 385. (b) Prelog, V.; Helmchen, G. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 567.

(11) Fehrst, A. *Structure and Mechanism in Protein Science. Guide to Enzyme Catalysis and Protein Folding*; W. H. Freeman and Co.: New York, 1999.

(12) To access the repository for the processing and distribution of 3-D biological macromolecular structure data, visit http:// www.rcsb.org/pdb.

(13) For a recent review see: Hult, K.; Berglund, P. *Curr. Opin. Biotechnol.* **2003**, *14*, 395.

(14) (a) Letondor, C.; Humbert, N.; Ward, T. R. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 4683. (b) Letondor, C.; Pordea, A.; Humbert, N.; Ivanova, A.; Mazurek, S.; Novic, M.; Ward, T. R. *J. Am. Chem. Soc.* **2006**, *128*, 8320. (c) Creus, M.; Pordea, A.; Rossel, T.; Sardo, A.; Letondor, C.;

Ivanova, A.; LeTrong, I.; Stenkamp, R. E.; Ward, T. R. *Angew. Chem., Int. Ed.* **2008**, *47*, 1400. (d) Bjerre, J.; Rousseau, C.; Marinescu, L.; Bols, M. *Appl. Microbiol. Biotechnol.* **2008**, *81*, 1. (e) Pordea, A.; Creus, M.; Panek, J.; Duboc, C.; Mathis, D.; Novic, M.; Ward, T. R. *J. Am. Chem. Soc.* **2008**, *130*, 8085.(f) Nanda, V.; Koder, R. L. *Nature Chem.* **2010**, *2*, 15.

(15) (a) Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656. (b) Prelog, V. *Pure Appl. Chem.* **1964**, *9*, 119.

(16) Ottolina, G.; Carrea, G. *Can. J. Chem.* **2002**, *80*, 559.

(17) (a) Valetti, F.; Gilardi, G. *Nature Prod. Rep.* **2004**, *21*, 490. (b) Arnold, F. H. *Acc. Chem. Res.* **1998**, *31*, 125. (c) Reetz, M. T. *J. Org. Chem.* **2009**, *74*, 5767.(d) Turner, N. J. *Nature Chem. Biol.* **2009**, *5*, 567. (e) Tracewell, C. A.; Arnold, F. H. *Curr. Opin. Chem. Biol.* **2009**, *13*, 3. (f) Hudlicky, T.; Reed, J. W. *Chem. Soc. Rev.* **2009**, *38*, 3117.(g) Lafaquière, V.; Barbe, S.; Puech-Guenot, S.; Guieysse, D.; Cortés, J.; Monsan, P.; Siméon, T.; André, I.; Remaud-Siméon, M. *ChemBioChem* **2009**, *10*, 2760.(h) Reetz, M. T.; Soni, P.; Acevedo, J. P.; Sanchis, J. *Angew. Chem., Int. Ed.* **2009**, *48*, 8268.(i) Reetz, M. T.; Prasad, S.; Carballeira, J. D.; Gumulya, Y.; Bocola, M. *J. Am. Chem. Soc.* **2010**, *132*, 9144.(j) Engström, K.; Nyhlén, J.; Sandström, A. G.; Bäckvall, J.-E. *J. Am. Chem. Soc.* **2010**, *132*, 7038.

(18) (a) Baudequin, C.; Baudoux, J.; Levillain, J.; Cahard, D.; Gaumont, A.-C.; Plaquevent, J.-C. *Tetrahedron: Asymmetry* **2003**, *14*, 3081. (b) van Rantwijk, F.; Sheldon, R. A. *Chem. Rev.* **2007**, *107*, 2757. (c) Yang, Z. *J. Biotechnol.* **2009**, *144*, 12. (d) Habulin, M.; Primozic, M.; Knez, Z. *Modern Biocatalysis: Stereoselective and Environmentally Friendly Reactions*; Wiley-VCH: Weinheim, 2009; p 109. (e) Gorke, J.; Srienc, F.; Kazlauskas, R. *Biotechnol. Bioprocess Eng.* **2010**, *15*, 40.(f) Fan, Y.; Qian, J. *J. Mol. Catal. B: Enzym* **2010**, *66*, 1.(g) Abe, Y.; Yoshiyama, K.; Yagi, Y.; Hayase, S.; Kawatsura, M.; Itoh, T. *Green Chem.* **2010**, *12*, 1976.(h) Filice, M.; Guisan, J. M.; Palomo, J. M. *Green Chem.* **2010**, *12*, 1365.(i) Yang, Z.; Zhang, K.-P.; Huang, Y.; Wang, Z. *J. Mol. Catal. B: Enzym.* **2010**, *63*, 23.

(19) (a) Matsuda, T.; Marukado, R.; Mukouyama, M.; Harada, T.; Nakamura, K. *Tetrahedron: Asymmetry* **2008**, *19*, 2272.(b) Harada, T.; Kubota, Y.; Kamitanaka, T.; Nakamura, K.; Matsuda, T. *Tetrahedron Lett.* **2009**, *50*, 4934.

(20) Shipovskov, S. *Biotechnol. Prog.* **2008**, *24*, 1262.

(21) (a) Stewart, J. D. *Curr. Opin. Biotechnol.* **2000**, *11*, 363. (b) Nakamura, K.; Yamanaka, R.; Matsuda, T.; Harada, T. *Tetrahedron: Asymmetry* **2003**, *14*, 2659.

(22) (a) Taran, F.; Gauchet, C.; Mohar, B.; Meunier, S.; Valleix, A.; Renard, P. Y.; Créminon, C.; Grassi, J.; Wagner, A.; Mioskowski, C. *Angew. Chem., Int. Ed.* **2002**, *41*, 124. (b) Schrader, W.; Eipper, A.; Pugh, D. J.; Reetz, M. T. *Can. J. Chem.* **2002**, *80*, 626. (c) Wahler, D.; Reymond, J.-L. *Curr. Opin. Chem. Biol.* **2001**, *5*, 152. (d) Cagnon, J. R.; Marsaioli, A. J.; Riatto, V. B.; Pilli, R. A.; G. P.; Eguchi, S. Y. *Chemosphere* **1999**, *38*, 2243. (e) Cagnon, J. R.; Marsaioli, A. J.; Riatto, V. B.; Pilli, R. A.; Manfio, G. P.; Eguchi, S. Y. *Chemosphere* **1999**, *38*, 2237. (f) Reymond, J.-L. *Enzyme Assays: High-Throughput Screening, Genetic Selection and Fingerprinting*; Wiley-VCH: Weinheim, 2005.(g) Reymond, J.-L.; Fluxà, V. S.; Maillard, N. *Chem. Commun.* **2009**, 34.

(23) Bornscheuer, U. T.; Kazlauskas, R. J. *Hydrolases in Organic Synthesis*; Verlag Chemie: Weinheim, 1999.

(24) (a) Branneby, C.; Carlqvist, P.; Magnusson, A.; Hult, K.; Brinck, T.; Berglund, P. *J. Am. Chem. Soc.* **2003**, *125*, 874. (b) Carlqvist, P.; Eklund, R.; Hult, K.; Brinck, T. *J. Mol. Model.* **2003**, *9*, 164. (c) Torre, O.; Alfonso, I.; Gotor, V. *Chem. Commun.* **2004**, 1724. (d) Cai, Y.; Yao, S.-P.; Wu, Q.; Lin, X.-F. *Biotechnol. Lett.* **2004**, *26*, 525. (e) Cai, Y.; Sun, X.-F.; Wang, N.; Lin, X.-F. *Synthesis* **2004**, 671. (f) Yao, S.-P.; Lu, D.-S.; Wu, Q.; Cai, Y.; Xua, S.-H.; Lin, X.-F. *Chem. Commun.* **2004**, 2006. (g) Sun, X.-F.; Wang, N.; Lin, X.-F. *Synthesis* **2004**, 671. (h) Branneby, C.; Carlqvist, P.; Hult, K.; Brinck, T.; Berglund, P. *J. Mol. Catal. B: Enzym.* **2004**, *31*, 123. (i) Carlqvist, P.; Svedendahl, M.; Branneby, C.; Hult, K.; Brinck, T.; Berglund, P. *ChemBioChem* **2005**, *6*, 331. (j) Li, C.; Feng, X.-W.; Wang, N.; Zhou, Y.-J.; Yu, X.-Q. *Green Chem.* **2008**, *10*, 616. (k) Priego, J.; Ortiz-Nava, C.; Carrillo-Morales, M.; Lopez-Munguia, A.; Escalante, J.; Castillo, E. *Tetrahedron* **2009**, *65*, 536.(l) de Souza, R. O. M. A.; Matos, L. M. C.; Gonçalves, K. M.; Costa, I. C. R.; Babics, I.; Leite, S. G. F.; Oestreicher, E. G.; Antunes, O. A. C. *Tetrahedron Lett.* **2009**, *50*, 2017.(m) Feng, X.-W.; Li, C.; Wang, N.; Li, K.; Zhang, W.-W.; Wang, Z.; Yu, X.-Q. *Green Chem.* **2009**, *11*, 1933.

(25) Izquierdo, I.; Plaza, M. T.; Rodríguez, M.; Tamayo, J. *Tetrahedron: Asymmetry* **1999**, *10*, 449.

(26) Neri, C.; Williams, J. M. J. *Adv. Synth. Catal.* **2003**, *345*, 835.

(27) Choi, J. Y.; Borch, R. F. *Org. Lett.* **2007**, *9*, 215.

(28) Fellows, I. M.; Kaelin, D. E.; Martin, S. F. *J. Am. Chem. Soc.* **2000**, *122*, 10781.

(29) Kastrinsky, D. B.; Boger, D. L. *J. Org. Chem.* **2004**, *69*, 2284.

(30) Takabe, K.; Mase, N.; Hashimoto, H.; Tsuchiya, A.; Ohbayasi, T.; Yoda, H. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1967.

(31) Yokomatsu, T.; Takada, K.; Yasumoto, A.; Yuasa, Y.; Shibuya, S. *Heterocycles* **2002**, *56*, 545.

(32) Miyata, S.; Kumamoto, T.; Ishikawa, T. *Helv. Chim. Acta* **2007**, *90*, 1420.

(33) Kawasaki, M.; Toyooka, N.; Saka, T.; Goto, M.; Matsuya, Y.; Kometani, T. *J. Mol. Catal. B: Enzym.* **2010**, *67*, 135.

(34) Davies, S. G.; Roberts, P. M.; Stephenson, P. T.; Storr, H. R.; Thomson, J. E. *Tetrahedron* **2009**, *65*, 8283.

(35) Takabe, K.; Iida, Y.; Hiyoshi, H.; Ono, M.; Hirose, Y.; Fukui, Y.; Yoda, H.; Mase, N. *Tetrahedron: Asymmetry* **2000**, *11*, 4825.

(36) (a) Murata, M.; Terao, Y.; Achiwa, K.; Nishio, T.; Seto, K. *Chem. Pharm. Bull.* **1989**, *10*, 2670. (b) Terao, Y.; Murata, M.; Achiwa, K.; Nishio, T.; Akamtsu, M.; Kamimura, M. *Tetrahedron Lett.* **1988**, *29*, 5173. (c) Wang, Y. F.; Lalonde, J. J.; Momongan, M.; Bergbreiter, D.; Wong, C. H. *J. Am. Chem. Soc.* **1988**, *110*, 7200.

(37) (a) Breitgoff, D.; Laumen, K.; Schneider, M. *J. Chem. Soc., Chem. Commun.* **1988**, *110*, 7200. (b) Wirz, B.; Schmid, R.; Foricher, J. *Tetrahedron: Asymmetry* **1992**, *3*, 137. (c) Suemune, H.; Mizuhara, Y.; Akita, H.; Sakai, K. *Chem. Pharm. Bull.* **1986**, *34*, 3440. (d) Kerschner, V.; Kreiser, W. *Tetrahedron Lett.* **1987**, *28*, 531.

(38) Bódai, V.; Novák, L.; Poppe, L. *Synlett* **1999**, 759.

(39) (a) Ramon, D. J.; Yus, M. *Curr. Org. Chem.* **2004**, *8*, 149. (b) Corey, E. J.; Guzman-Perez, A. *Angew. Chem., Int. Ed.* **1998**, *37*, 388.

(40) Shimada, K.; Kauragi, Y.; Fukuyama, T. *J. Am. Chem. Soc.* **2003**, *125*, 4048.

(41) Chênevert, R.; Courchesne, G. *Tetrahedron Lett.* **2002**, *43*, 7971.

(42) Mahapatra, T.; Das, T.; Nanda, Samik. *Tetrahedron: Asymmetry* **2008**, *19*, 2497.

(43) Donohoe, T. J.; Rigby, C. L.; Thomas, R. E.; Nieuwenhuys, W. F.; Bhatti, F. L.; Cowley, A. R.; Bhalay, G.; Linney, I. D. *J. Org. Chem.* **2006**, *71*, 6298.

(44) Kirihara, M.; Kawasaki, M.; Takuwa, T.; Kakuda, H.; Wakikawa, T.; Takeuchi, Y.; Kirk, K. L. *Tetrahedron: Asymmetry* **2003**, *14*, 1753.

(45) Lane, J. W.; Halcomb, R. L. *J. Org. Chem.* **2003**, *68*, 1348.

(46) Lane, J. W.; Halcomb, R. L. *Org. Lett.* **2003**, *5*, 4017.

(47) Fukuda, T.; Sugiyama, K.; Arima, S.; Harigaya, Y.; Nagamitsu, T.; Ōmura, S. *Org. Lett.* **2008**, *10*, 4239.

(48) (a) Fadel, A.; Arzel, P. *Tetrahedron: Asymmetry* **1997**, *8*, 283. (b) Kirihara, M.; Takuwa, T.; Kawasaki, M.; Kakuda, H.; Hirokami, S.; Takahata, H. *Chem. Lett.* **1999**, 405. (c) Ihara, M.; Suzuki, M.; Fukumoto, K.; Kabuto, C. *J. Am. Chem. Soc.* **1990**, *112*, 1164. (d) Toyooka, N.; Nishino, A.; Momose, T. *Tetrahedron Lett.* **1993**, *34*, 4539. (e) Suemune, H.; Harabe, T.; Xie, Z.-F.; Sakai, K. *Chem. Pharm. Bull.* **1988**, *36*, 4337.

(49) (a) Kita, Y.; Takebe, Y.; Murata, K.; Naka, T.; Akai, S. *Tetrahedron Lett.* **1996**, *37*, 7369. (b) Kita, Y.; Naka, T.; Imanishi, M.; Akai, S.; Takebe, Y.; Matsugi, M. *Chem. Commun* **1998**, 1183. (c) Kita, Y.; Takebe, Y.; Murata, K.; Naka, T.; Akai, S. *J. Org. Chem.* **2000**, *65*, 83.

(50) For the case of 1-ethoxyvinyl benzoate, see: (a) Akai, S.; Naka, T.; Takebe, Y.; Kita, Y. *Tetrahedron Lett.* **1997**, *38*, 4243. (b) Akai, S.; Naka, T.; Takebe, Y.; Kita, Y. *Chem. Pharm. Bull.* **2000**, *48*, 1519.

(51) (a) Akai, S.; Naka, T.; Fujita, T.; Takebe, Y.; Kita, Y. *Chem. Commun.* **2000**, 1461. (b) Akai, S.; Naka, T.; Fujita, T.; Takebe, Y.; Tsujino, T.; Kita, Y. *J. Org. Chem.* **2002**, *67*, 411.(c) Akai, S.; Tsujino, T.; Fukuda, N.; Iio, K.; Takeda, Y.; Kawaguchi, K.-i.; Naka, T.; Higuchi, K.; Akiyama, E.; Fujioka, H.; Kita, Y. *Chem.—Eur. J.* **2005**, *11*, 6286.
(52) Nakazawa, K.; Hayashi, M.; Tanaka, M.; Aso, M.; Suemune, H. *Tetrahedron: Asymmetry* **2001**, *12*, 897.
(53) (a) Akai, S.; Tsujino, T.; Naka, T.; Tanimoto, K.; Kita, Y. *Tetrahedron Lett.* **2001**, *42*, 7315. (b) Akai, S.; Tsujino, T.; Akiyama, E.; Tanimoto, K.; Naka, T.; Kita, Y. *J. Org. Chem.* **2004**, *69*, 2478.
(54) Chênevert, R.; Courchesne, G.; Caron, D. *Tetrahedron: Asymmetry* **2003**, *14*, 2567.
(55) Chênevert, R.; Rose, Y. S. *J. Org. Chem.* **2000**, *65*, 1707.
(56) (a) Schmidt, Y.; Lehr, K.; Breuninger, U.; Brand, G.; Reiss, T.; Breit, B. *J. Org. Chem.* **2010**, *75*, 4424. (b) Hoffman, T. J.; Kolleth, A.; Rigby, J. H.; Arseniyadis, S.; Cossy, J. *Org. Lett.* **2010**, *12*, 3348.
(57) Davoli, P.; Caselli, E.; Bucciarelli, M.; Forni, A.; Torre, G.; Prati, F. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1948.
(58) Berkowitz, D. B.; Choi, S.; Maeng, J. H. *J. Org. Chem.* **2000**, *65*, 847.
(59) Chênevert, R.; Dickmann, M. *J. Org. Chem.* **1996**, *61*, 3332.
(60) Chênevert, R.; Ziarani, G. M.; Morin, M. P.; Dasser, M. *Tetrahedron: Asymmetry* **1999**, *10*, 3117.
(61) (a) Chênevert, R.; Morin, M.-P. *J. Org. Chem.* **1999**, *64*, 3178. (b) Chênevert, R.; Ziarani, G. M.; Dasser, M. *Heterocycles* **1999**, *51*, 593.
(62) Chênevert, R.; Morin, P. *Bioorg. Med. Chem.* **2009**, *17*, 1837.
(63) Chênevert, R.; Jacques, F.; Giguère, P.; Dasser, M. *Tetrahedron: Asymmetry* **2008**, *19*, 1333.
(64) Izquierdo, I.; Plaza, M. T.; Tamayo, J. A.; Franco, F.; Sánchez-Cantalejo, F. *Tetrahedron* **2008**, *64*, 4993.
(65) Luzzio, F. A.; Fitch, R. W. *J. Org. Chem.* **1999**, *64*, 5485.
(66) Fransson, A.-B. L.; Xu, Y.; Leijondahl, K.; Bäckvall, J.-E. *J. Org. Chem.* **2006**, *71*, 6309.
(67) Chênevert, R.; Lévesque, C.; Morin, P. *J. Org. Chem.* **2008**, *73*, 9501.
(68) Matsumoto, T.; Konegawa, T.; Yamaguchi, H.; Nakamura, T.; Sugai, T.; Suzuki, K. *Synlett* **2001**, 1650.
(69) Toyama, K.; Iguchi, S.; Sakazaki, H.; Oishi, T.; Hirama, M. *Bull. Chem. Soc. Jpn.* **2001**, *74*, 997.
(70) Nicolaou, K. C.; Zhang, H.; Ortiz, A.; Dagneau, P. *Angew. Chem., Int. Ed.* **2008**, *47*, 8605.
(71) Recuero, V.; Brieva, R.; Gotor, V. *Tetrahedron: Asymmetry* **2008**, *19*, 1684.
(72) Oishi, T.; Maruyama, M.; Shoji, M.; Maeda, K.; Kumahara, N.; Tanaka, S.-i.; Hirama, M. *Tetrahedron* **1999**, *55*, 7471.
(73) Saito, Y.; Shinkai, T.; Yoshimura, Y.; Takahata, H. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5894.
(74) Coste, G.; Gerber-Lemaire, S. *Tetrahedron: Asymmetry* **2005**, *16*, 2277.
(75) Hilpert, H.; Wirz, B. *Tetrahedron* **2001**, *57*, 681.
(76) Wirz, B.; Iding, H.; Hilpert, H. *Tetrahedron: Asymmetry* **2000**, *11*, 4171.
(77) Zhao, Y.; Wu, Y.; De Clerq, P.; Vandewalle, M.; Maillos, P.; Pascal, J.-C. *Tetrahedron: Asymmetry* **2000**, *11*, 3887.
(78) Candy, M.; Audran, G.; Bienaymé, H.; Bressy, C.; Pons, J.-M. *Org. Lett.* **2009**, *11*, 4950.
(79) Candy, M.; Audran, G.; Bienaymé, H.; Bressy, C.; Pons, J.-M. *J. Org. Chem.* **2010**, *75*, 1354.
(80) Hegemann, K.; Schimanski, H.; Höweler, U.; Haufe, G. *Tetrahedron Lett.* **2003**, *44*, 2225.
(81) Hirata, T.; Shimoda, K.; Kawano, T. *Tetrahedron: Asymmetry* **2000**, *11*, 1063.
(82) Lewis, C. A.; Gustafson, J. L.; Chiu, A.; Balsells, J.; Pollard, D.; Murry, J.; Reamer, R. A.; Hansen, K. B.; Miller, S. J. *J. Am. Chem. Soc.* **2008**, *130*, 16358.
(83) Böhm, C.; Austin, W. F.; Trauner, D. *Tetrahedron: Asymmetry* **2003**, *14*, 71.
(84) Matsumoto, T.; Konegawa, T.; Nakamura, T.; Suzuki, K. *Synlett* **2002**, 122.
(85) Okuyama, K.; Shingubara, K.; Tsujiyama, S.-i.; Suzuki, K.; Matsumoto, T. *Synlett* **2009**, 941.
(86) Wiktelius, D.; Johansson, M. J.; Luthman, K.; Kann, N. *Org. Lett.* **2005**, *7*, 4991.
(87) Rachwalski, M.; Kwiatkowska, M.; Drabowicz, J.; Kłos, M.; Wieczorek, W. M.; Szyrej, M.; Sieroń, L.; Kiełbasiński, P. *Tetrahedron: Asymmetry* **2008**, *19*, 2096.
(88) Masterson, D. S.; Kinkini, R.; Rosado, D. A.; Fouche, M. *J. Pept. Sci.* **2008**, *14*, 1151.
(89) Iosub, V.; Haberl, A. R.; Leung, J.; Tang, M.; Vembaiyan, K.; Parvez, M.; Back, T. G. *J. Org. Chem.* **2010**, *75*, 1612.
(90) Prantz, K.; Mulzer, J. *Chem.—Eur. J.* **2010**, *16*, 485.
(91) Falgner, S.; Burschka, C.; Wagner, S.; Böhm, A.; Daiss, J. O.; Tacke, R. *Organometallics* **2009**, *28*, 6059.
(92) Öhrlein, R.; Baisch, G. *Adv. Synth. Catal.* **2003**, *345*, 713.
(93) Schönherr, H.; Mollitor, J.; Schneider, C. *Eur. J. Org. Chem.* **2010**, 3908.
(94) Homann, M. J.; Vail, R.; Morgan, B.; Sabesan, V.; Levy, C.; Dodds, D. R.; Zaks, A. *Adv. Synth. Catal.* **2001**, *343*, 744and references therein.
(95) See refs 10−16 from ref 63.
(96) Stowe, G. N.; Šilhár, P.; Hixon, M. S.; Silvaggi, N. R.; Allen, K. N.; Moe, S. T.; Jacobson, A. R.; Barbieri, J. T.; Janda, K. D. *Org. Lett.* **2010**, *12*, 756.
(97) Yu, M. S.; Lantos, I.; Peng, Z.-Q.; Yu, J.; Cacchio, T. *Tetrahedron Lett.* **2000**, *41*, 5647.
(98) Honda, T.; Koizumi, T.; Komatsuzaki, Y.; Yamashita, R.; Kanai, K.; Nagase, H. *Tetrahedron: Asymmetry* **1999**, *10*, 2703.
(99) Goswami, A.; Kissick, T. P. *Org. Process Res. Dev.* **2009**, *13*, 483.
(100) Boaz, N. W. *Tetrahedron: Asymmetry* **1999**, *10*, 813 and references therein.
(101) Zutter, U.; Iding, H.; Spurr, P.; Wirz, B. *J. Org. Chem.* **2008**, *73*, 4895.
(102) Kashima, Y.; Liu, J.; Takenami, S.; Niwayama, S. *Tetrahedron: Asymmetry* **2002**, *13*, 953.
(103) Kiełbasiński, P.; Żurawiński, R.; Albrycht, M.; Mikołajczyk, M. *Tetrahedron: Asymmetry* **2003**, *14*, 3379.
(104) (a) Toone, E. J.; Werth, M. J.; Jones, J. B. *J. Am. Chem. Soc.* **1990**, *112*, 4946. (b) Provencher, L.; Wynn, H.; Jones, J. B.; Krawczyk, A. R. *Tetrahedron: Asymmetry* **1993**, *4*, 2025. (c) Provencher, L.; Jones, J. B. *J. Org. Chem.* **1994**, *59*, 2729.
(105) (a) See ref 20, Chapter 5.2.6. (b) Ozegowski, R.; Kunath, A.; Schick, H. *Tetrahedron: Asymmetry* **1993**, *4*, 695.
(106) Ozegowski, R.; Kunath, A.; Schick, H. *Liebigs Ann. Chem.* **1993**, 805.
(107) Ostaszewski, R.; Portlock, D. E.; Fryszkowska, A.; Jeziorska, K. *Pure Appl. Chem.* **2003**, *75*, 413.
(108) Fryszkowska, A.; Komar, M.; Koszelewski, D.; Ostaszewski, R. *Tetrahedron: Asymmetry* **2006**, *17*, 961.
(109) See ref 3b, Chapter 17.
(110) Ohta, H. *Chimia* **1996**, *50*, 434.
(111) Yokoyama, M.; Sugai, T.; Ohta, H. *Tetrahedron: Asymmetry* **1993**, *4*, 1081.
(112) (a) Crosby, J. A.; Parratt, J. S.; Turner, N. J. *Tetrahedron: Asymmetry* **1992**, *12*, 1547. (b) Beard, T.; Cohen, M. A.; Parratt, J. S.; Turner, N. J.; Crosby, J.; Moillet, J. *Tetrahedron: Asymmetry* **1993**, *4*, 1085.
(113) Wang, M.-X.; Liu, C.-S.; Li, J.-S. *Tetrahedron: Asymmetry* **2001**, *12*, 3367.
(114) DeSantis, G.; Zhu, Z.; Greenberg, W. A.; Wong, K.; Chaplin, J.; Hanson, S. R.; Farwell, B.; Nicholson, L. W.; Rand, C. L.; Weiner, D. P.; Robertson, D. E.; Burk, M. J. *J. Am. Chem. Soc.* **2002**, *124*, 9024.
(115) DeSantis, G.; Wong, K.; Farwell, B.; Chatman, K.; Zhu, Z.; Tomlinson, G.; Huang, H.; Tan, X.; Bibbs, L.; Chen, P.; Kretz, K.; Burk, M. J. *J. Am. Chem. Soc.* **2003**, *125*, 11476.
(116) Kiełbasiński, P.; Rachwalski, M.; Mikołajczyk, M.; Szyrej, M.; Wieczorek, M. W.; Wijtmans, R.; Rutjes, F. P. J. T. *Adv. Synth. Catal.* **2007**, *349*, 1387.

(117) Kiełbasiński, P.; Rachwalski, M.; Kwiatkowska, M.; Mikołajczyk, M.; Szyrej, M.; Wieczorek, M. W.; Szyrej, M.; Sieroń, L.; Rutjes, F. P. J. T. *Tetrahedron: Asymmetry* **2007**, *18*, 2108.
(118) López-García, M.; Alfonso, I.; Gotor, V. *Tetrahedron: Asymmetry* **2003**, *14*, 603.
(119) Puertas, S.; Rebolledo, F.; Gotor, V. *J. Org. Chem.* **1996**, *61*, 6024.
(120) López-García, M.; Alfonso, I.; Gotor, V. *J. Org. Chem.* **2003**, *68*, 648.
(121) Sánchez, V. M.; Rebolledo, F.; Gotor, V. *J. Org. Chem.* **1999**, *64*, 1464.
(122) Oves-Costales, D.; Kadi, N.; Foff, M. J.; Song, L.; Wilson, K. S.; Challis, G. L. *J. Am. Chem. Soc.* **2007**, *129*, 8416.
(123) Oves-Costales, D.; Song, L.; Challis, G. L. *Chem. Commun.* **2009**, 1389.
(124) Schmelz, S.; Kadi, N.; McMahon, S. A.; Song, L.; Oves-Costales, D.; Oke, M.; Liu, H.; Johnson, K. A.; Carter, L. G.; Botting, C. H.; White, M. F.; Challis, G. L.; Naismith, J. H. *Nature Chem. Biol.* **2009**, *5*, 174.
(125) (a) Busto, E.; Gotor-Fernández, V.; Montejo-Bernardo, J.; García-Granda, S.; Gotor, V. *Org. Lett.* **2007**, *9*, 4203. (b) Ríos-Lombardía, N.; Busto, E.; García-Urdiales, E.; Gotor-Fernández, V.; Gotor, V. *J. Org. Chem.* **2009**, *74*, 2571.(c) Ríos-Lombardía, N.; Busto, E.; Gotor-Fernández, V.; Gotor, V. *Eur. J. Org. Chem.* **2010**, 484.
(126) Busto, E.; Gotor-Fernández, V.; Montejo-Bernardo, J.; García-Granda, S.; Gotor, V. *Tetrahedron* **2009**, *65*, 8393.
(127) García-Urdiales, E.; Busto, E.; Ríos-Lombardía, N.; Gotor-Fernández, V.; Gotor, V. *ChemBioChem* **2009**, *10*, 2875.
(128) For a recent review on the topic, see: Matsuda, T.; Yamanaka, R.; Nakamura, K. *Tetrahedron: Asymmetry* **2009**, *20*, 513.
(129) (a) Canivet, J.; Suss-Fink, G.; Stepnicka, P. *Eur. J. Inorg. Chem.* **2007**, *30*, 4736. (b) De Wildeman, S. M. A.; Sonke, T.; Schoemaker, H. E.; May, O. *Acc. Chem. Res.* **2007**, *40*, 1260. (c) Moore, J. C.; Pollard, D. J.; Kosjek, B.; Devine, P. N. *Acc. Chem. Res.* **2007**, *40*, 1412. (d) Huisman, G. W.; Liang, J.; Krebber, A. *Curr. Opin. Chem. Biol.* **2010**, *14*, 122.
(130) (a) Morikawa, S.; Nakai, T.; Yasohara, Y.; Nanba, H.; Kizaki, N.; Hasegawa, J. *Biosci., Biotechnol., Biochem.* **2005**, *69*, 544. (b) Schlieben, N. H.; Niefind, K.; Müller, J.; Riebel, B.; Hummel, W.; Schomburg, D. *J. Mol. Biol.* **2005**, *349*, 801.(c) Yang, W.; Xu, J.-H.; Pan, J.; Xu, Y.; Wang, Z.-L. *Biochem. Eng. J.* **2008**, *42*, 1.(d) Zhang, R.; Xu, Y.; Sun, Y.; Zhang, W.; Xiao, R. *Appl. Environ. Microbiol.* **2009**, *75*, 2176. (e) Machielsen, R.; Looger, L. L.; Raedts, J.; Dijkhuizen, S.; Hummel, W.; Hennemann, H.-G.; Daussmann, T.; van der Oost, J. *Eng. Life Sci.* **2009**, *9*, 38.
(131) (a) Zhang, J.; Witholt, B.; Li, Z. *Chem. Commun.* **2006**, *4*, 398. (b) Zhang, J.; Witholt, B.; Li, Z. *Adv. Synth. Catal.* **2006**, *348*, 429.
(132) Goldberg, K.; Schroer, K.; Luetz, S.; Liese, A. *Appl. Microbiol. Biotechnol.* **2007**, *76*, 249.
(133) Goldberg, K.; Schroer, K.; Luetz, S.; Liese, A. *Appl. Microbiol. Biotechnol.* **2007**, *76*, 237.
(134) (a) van der Donk, W. A.; Zhao, H. *Curr. Opin. Biotechnol.* **2003**, *14*, 421. (b) Mertens, R.; Greiner, L.; van den Ban, E. C. D.; Haaker, H. B. C. M.; Liese, A. *J. Mol. Catal. B: Enzym.* **2003**, *24*−*25*, 39. (c) Wagenknecht, P. S.; Penney, J. M.; Hembre, R. T. *Organometallics* **2003**, *22*, 1180. (d) Nakamura, K.; Yamanaka, R. *Chem. Commun* **2002**, 1782. (e) Endo, T.; Koizumi, S. *Adv. Synth. Catal.* **2001**, *343*, 521. (f) Nakamura, K.; Yamanaka, R.; Tohi, K.; Hamada, H. *Tetrahedron Lett.* **2000**, *41*, 6799.
(135) (a) Csuk, R.; Glanzer, B. I. *Chem. Rev.* **1991**, *91*, 49. (b) Stewart, J. D. *Curr. Opin. Drug Discovery Dev.* **1998**, *1*, 278.
(136) (a) Nakamura, K.; Yamanaka, R.; Matsuda, T.; Harada, T. *Tetrahedron: Asymmetry* **2003**, *14*, 2659.(b) Kaluzna, I. A.; Matsuda, T.; Sewell, A. K.; Stewart, J. D. *J. Am. Chem. Soc.* **2004**, *126*, 12827.(c) Ema, T.; Yagasaki, H.; Okita, N.; Nishikawa, K.; Korenaga, T.; Sakai, T. *Tetrahedron: Asymmetry* **2005**, *16*, 1075.(d) Ema, T.; Yagasaki, H.; Okita, N.; Takeda, M.; Sakai, T. *Tetrahedron* **2006**, *62*, 6143.(e) Yang, Y.; Zhu, D.; Piegat, T. J.; Hua, L. *Tetrahedron: Asymmetry* **2007**, *18*, 1799.
(137) Matsuda, T.; Harada, T.; Nakajima, N.; Itoh, T.; Nakamura, K. *J. Org. Chem.* **2000**, *65*, 157.
(138) Matsuda, T.; Nakajima, Y.; Harada, T.; Nakamura, K. *Tetrahedron: Asymmetry* **2002**, *13*, 971.
(139) Nakamura, K.; Inoue, Y.; Matsuda, T.; Misawa, I. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2397.
(140) Nakamura, K.; Fuiji, M.; Ida, Y. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3205.
(141) Nakamura, K.; Takenaka, K.; Fujii, M.; Ida, Y. *Tetrahedron Lett.* **2002**, *43*, 3629.
(142) Stampfer, W.; Kosjek, B.; Faber, K.; Kroutil, W. *J. Org. Chem.* **2003**, *68*, 402.
(143) Stampfer, W.; Kosjek, B.; Moitzi, C.; Kroutil, W.; Faber, K. *Angew. Chem., Int. Ed.* **2002**, *41*, 1014.
(144) Edegger, K.; Gruber, C. C.; Poessl, T. M.; Wallner, S. R.; Lavandera, I.; Faber, K.; Niehaus, F.; Eck, J.; Oehrlein, R.; Hafnerd, A.; Kroutil, W. *Chem. Commun.* **2006**, 2402.
(145) de Gonzalo, G.; Lavandera, I.; Faber, K.; Kroutil, W. *Org. Lett.* **2007**, *9*, 2163.
(146) Lavandera, I.; Kern, A.; Schaffenberger, M.; Gross, J.; Glieder, A.; de Wildeman, S.; Kroutil., W. *ChemSusChem* **2008**, *1*, 431.
(147) Gröger, H.; Chamouleau, F.; Orologas, N.; Rollmann, C.; Drauz, K.; Hummel, W.; Weckbecker, A.; May, O. *Angew. Chem., Int. Ed.* **2006**, *45*, 5677.
(148) Makino, Y.; Inoue, K.; Dairi, T.; Itoh, N. *Appl. Environ. Microbiol.* **2005**, *71*, 4713.
(149) Bisogno, F. R.; Lavandera, I.; Kroutil, W.; Gotor, V. *J. Org. Chem.* **2009**, *74*, 1730.
(150) Lavandera, I.; Kern, A.; Resch, V.; Ferreira-Silva, B.; Glieder, A.; Fabian, W. M. F.; de Wildeman, S.; Kroutil, W. *Org. Lett.* **2008**, *10*, 2155.
(151) Bisogno, F. R.; García-Urdiales, E.; Valdés, H.; Lavandera, I.; Kroutil, W.; Suárez, D.; Gotor, V. *Chem.—Eur. J.* **2010**, *16*, 11012.
(152) (a) Ema, T.; Sugiyama, Y.; Fukumoto, M.; Moriya, H.; Cui, J.-N.; Sakai, T.; Utaka, M. *J. Org. Chem.* **1998**, *63*, 4996. (b) Ema, T.; Moriya, H.; Kofukuda, T.; Ishida, T.; Maehara, K.; Utaka, M.; Sakai, T. *J. Org. Chem.* **2001**, *66*, 8682.
(153) Zhu, D.; Yang, Y.; Majkowicz, S.; Pan, T, H.-Y.; Kantardjieff, K.; Hua, L. *Org. Lett.* **2008**, *10*, 525.
(154) Keinan, E.; Hafeli, E. K.; Seth, K. K.; Lamed, R. *J. Am. Chem. Soc.* **1986**, *108*, 162.
(155) Musa, M. M.; Ziegelmann-Fjeld, K. I.; Vieille, C.; Zeikus, J. G.; Phillips, R. S. *J. Org. Chem.* **2007**, *72*, 30.
(156) Musa, M. M.; Ziegelmann-Fjeld, K. I.; Vieille, C.; Zeikus, J. G.; Phillips, R. S. *Angew. Chem., Int. Ed.* **2007**, *46*, 3091.
(157) Musa, M. M.; Lott, N.; Laivenieks, M.; Watanabe, L.; Vieille, C.; Phillips, R. S. *ChemCatChem* **2009**, *1*, 89.
(158) Goswami, A.; Bezbaruah, R. L.; Goswami, J.; Borthakur, N.; Dey, D.; Hazarika, A. K. *Tetrahedron: Asymmetry* **2000**, *11*, 3701.
(159) Goswami, J.; Bezbaruah, R. L.; Goswami, A.; Borthakur, N. *Tetrahedron: Asymmetry* **2001**, *12*, 3343.
(160) Homann, M. J.; Vail, R. B.; Previte, E.; Tamarez, M.; Morgan, B.; Dodds, D. R.; Zaks, A. *Tetrahedron* **2004**, *60*, 789.
(161) Zhang, J.; Duetz, W. A.; Witholt, B.; Li, Z. *Chem. Commun.* **2004**, 2120.
(162) Baldasarre, F.; Bertoni, G.; Chiappe, C.; Marioni, F. *J. Mol. Catal. B: Enzym.* **2000**, *11*, 55–58.
(163) Yadav, J. S.; Nanda, S.; Reddy, P. T.; Rao, A. B. *J. Org. Chem.* **2002**, *67*, 3900.
(164) Yadav, J. S.; Reddy, P. T.; Nanda, S.; Rao, A. B. *Tetrahedron: Asymmetry* **2001**, *12*, 3381.
(165) Zhu, D.; Hyatt, B. A.; Hua, L. *J. Mol. Catal. B: Enzym.* **2009**, *56*, 272.
(166) Salvi, N. A.; Chattopadhyay, S. *Tetrahedron: Asymmetry* **2008**, *19*, 1992.
(167) Hanson, R. L.; Goldberg, S.; Goswami, A.; Tully, T. P.; Patel, R. N. *Adv. Synth. Catal.* **2005**, *347*, 1073.

(168) Barbieri, C.; Caruso, E.; D'Arrigo, P.; Fantoni, G. P.; Servi, S. *Tetrahedron: Asymmetry* **1999**, No. 10, 3931.
(169) Rosen, T. C.; Feldmann, R.; Dünkelmann, P.; Daussmann, T. *Tetrahedron Lett.* **2006**, *47*, 4803.
(170) Pollard, D.; Truppo, M.; Pollard, J.; Chen, C.-y.; Moore, J. *Tetrahedron: Asymmetry* **2006**, *17*, 554.
(171) Ema, T.; Kadoya, T.; Akihara, K.; Sakai, T. *J. Mol. Catal. B: Enzym.* **2010**, *66*, 198.
(172) Wei, L.; Makowski, T.; Martinez, C.; Ghosh, A. *Tetrahedron: Asymmetry* **2008**, *19*, 2648.
(173) Toşa, M. I.; Podea, P. V.; Paizs, C.; Irimie, F. D. *Tetrahedron: Asymmetry* **2008**, *19*, 2068.
(174) Itoh, K.-i.; Sakamaki, H.; Nakamura, K.; Horiuchi, C. A. *Tetrahedron: Asymmetry* **2005**, *16*, 1403.
(175) (a) Busto, E.; Gotor-Fernández, V.; Gotor, V. *Tetrahedron: Asymmetry* **2006**, *17*, 1007.(b) Busto, E.; Gotor-Fernández, V.; Gotor, V. *Adv. Synth. Catal.* **2006**, *348*, 2626.
(176) Felluga, F.; Baratta, W.; Fanfoni, L.; Pitacco, G.; Rigo, P.; Benedetti, F. *J. Org. Chem.* **2009**, *74*, 3547.
(177) Wei, Z.-L.; Lin, G.-Q.; Li, Z.-Y. *Bioorg. Med. Chem.* **2000**, *8*, 1129.
(178) Conceição, G. J. A.; Moran, P. J. S.; Rodrigues, J. A. R. *Tetrahedron: Asymmetry* **2003**, *14*, 43.
(179) Schubert, T.; Hummel, W.; Kula, M.-R.; Müller, M. *Eur. J. Org. Chem.* **2001**, 4181.
(180) Schubert, T.; Hummel, W.; Müller, M. *Angew. Chem., Int. Ed.* **2002**, *41*, 634.
(181) Heiss, C.; Phillips, R. S. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2821.
(182) Yadav, J. S.; Reddy, P. T.; Nanda, S.; Rao, A. B. *Tetrahedron: Asymmetry* **2001**, *12*, 63.
(183) Antunes, H.; Fardelone, L. C.; Rodrigues, J. A. R.; Moran, P. J. S. *Tetrahedron: Asymmetry* **2004**, *15*, 2615.
(184) Fardelone, L. C.; Rodrigues, J. A. R.; Moran, P. J. S. *J. Mol. Catal. B: Enzym.* **2006**, *39*, 9.
(185) Ankati, H.; Yang, Y.; Zhu, D.; Biehl, E. R.; Hua, L. *J. Org. Chem.* **2008**, *73*, 6433.
(186) Lavandera, I.; Kern, A.; Ferreira-Silva, B.; Glieder, A.; de Wildeman, S.; Kroutil, W. *J. Org. Chem.* **2008**, *73*, 6003.
(187) Zhu, D.; Yang, Y.; Buynak, J. D.; Hua, L. *Org. Biomol. Chem.* **2006**, *4*, 2690.
(188) Zhu, D.; Hua, L. *J. Org. Chem.* **2006**, *71*, 9484.
(189) Cundari, T. R.; Dinescu, A.; Zhu, D.; Hua, L. *J. Mol. Model.* **2007**, *13*, 685.
(190) Roy, S.; Alexandre, V.; Neuwels, M.; Le Texier, L. *Adv. Synth. Catal.* **2001**, *343*, 738.
(191) Truppo, M. D.; Pollard, D.; Devine, P. *Org. Lett.* **2007**, *9*, 335.
(192) Li, H.; Zhu, D.; Hua, L.; Biehl, E. R. *Adv. Synth. Catal.* **2009**, *351*, 583.
(193) Xie, B.; Yang, J.; Yang, Q.; Yuan, W. *J. Mol. Catal. B: Enzym* **2009**, *61*, 284.
(194) Singh, A.; Basit, A.; Banerjee, U. C. *J. Ind. Microbiol. Biotechnol.* **2009**, *36*, 1369.
(195) Kosjek, B.; Nti-Gyabaah, J.; Telari, K.; Dunne, L.; Moore, J. C. *Org. Process Res. Dev.* **2008**, *12*, 584.
(196) Broussy, S.; Cheloha, R. W.; Berkowitz, D. B. *Org. Lett.* **2009**, *11*, 305.
(197) (a) Ema, T.; Okita, N.; Ide, S.; Sakai, T. *Org. Biomol. Chem.* **2007**, *5*, 1175.(b) Ema, T.; Ide, S.; Okita, N.; Sakai, T. *Adv. Synth. Catal.* **2008**, *350*, 2039.
(198) Liang, J.; Mundorff, E.; Voladri, R.; Jenne, S.; Gilson, L.; Conway, A.; Krebber, A.; Wong, J.; Huisman, G.; Truesdell, S.; Lalonde, J. *Org. Process Res. Dev.* **2010**, *14*, 188.
(199) Yamamoto, H.; Ueda, M.; Ritsuzui, P.; Hamatani, T. E. U.S. Patent 1,318,200, 2003; *Chem. Abstr.* **2003**, *139*, 35200.
(200) Burns, M. P.; Wong, J. W. U.S. Patent 6,451,587, 2002; *Chem. Abstr.* **2002**, *137*, 246600h.
(201) Pękala, E.; Burbiel, J. C.; Müller, C. E. *Chirality* **2009**, *21*, 713.
(202) Pollard, D. J.; Telari, K.; Lane, J.; Humphrey, G.; McWilliams, C.; Nidositko, S.; Salmon, P.; Moore, J. *Biotechnol. Bioeng.* **2006**, *93*, 674and references therein.
(203) Iwamoto, M.; Kawada, H.; Tanaka, T.; Nakada, M. *Tetrahedron Lett.* **2003**, *44*, 7239.
(204) Watanabe, H.; Iwamoto, M.; Nakada, M. *J. Org. Chem.* **2005**, *70*, 4652.
(205) Kato, T.; Mizumoto, S.; Fudesaka, M.; Nakashima, Y.; Kajimoto, T.; Node, M. *Synlett* **2006**, 2176.
(206) Shimoda, K.; Kubota, N.; Hamada, H.; Hamada, H. *Tetrahedron Lett.* **2006**, *47*, 1541.
(207) Fujii, M.; Takeuchi, M.; Akita, H.; Nakamura, K. *Tetrahedron Lett.* **2009**, *50*, 4941.
(208) (a) Fuhshuku, K.-i.; Funa, N.; Akeboshi, T.; Ohta, H.; Hosomi, H.; Ohba, S.; Sugai, T. *J. Org. Chem.* **2000**, *65*, 129. (b) Fuhshuku, K.-i.; Tomita, M.; Sugai, T. *Adv. Synth. Catal.* **2003**, *345*, 766. (c) Fujieda, S.; Tomita, M.; Fuhshuku, K.-i.; Ohba, S.; Nishiyama, S.; Sugai, T. *Adv. Synth. Catal.* **2005**, *347*, 1099.
(209) Kayser, M. M.; Mihovilovic, M. D.; Kearns, J.; Feicht, A.; Stewart, J. D. *J. Org. Chem.* **1999**, *64*, 6603.
(210) Ishihara, K.; Yamaguchi, H.; Hamada, H.; Nakajiman, N.; Nakamura, K. *J. Mol. Catal. B: Enzymol.* **2000**, *10*, 429.
(211) Patel, R. N.; Chu, L.; Chidambaram, R.; Zhu, J.; Kant, J. *Tetrahedron: Asymmetry* **2002**, *13*, 349.
(212) Zhu, D.; Stearns, J. E.; Ramirez, M.; Hua, L. *Tetrahedron* **2006**, *62*, 4535.
(213) Anson, C. E.; Bibb, M. J.; Booker-Milburn, K. I.; Clissold, C.; Haley, P. J.; Hopwood, D. A.; Ichinose, K.; Revill, W. P.; Stephenson, G. R.; Surti, C. M. *Angew. Chem., Int. Ed.* **2000**, *39*, 224.
(214) Zhu, D.; Mukherjee, C.; Rozzell, J. D.; Kambourakis, S.; Hua, L. *Tetrahedron* **2006**, *62*, 901.
(215) Wolberg, M.; Hummel, W.; Wandrey, C.; Müller, M. *Angew. Chem., Int. Ed.* **2000**, *39*, 4306.
(216) Wolberg, M.; Hummel, W.; Müller, M. *Chem.—Eur. J.* **2001**, *7*, 4562.
(217) Wolberg, M.; Ji, A.; Hummel, W.; Müller, M. *Synthesis* **2001**, 937.
(218) Enders, D.; Vicario, J. L.; Job, A.; Wolberg, M.; Müller, M. *Chem.—Eur. J.* **2002**, *8*, 4272.
(219) Vicario, J. L.; Job, A.; Wolberg, M.; Müller, M.; Enders, D. *Org. Lett.* **2002**, *4*, 1023.
(220) Job, A.; Wolberg, M.; Müller, M.; Enders, D. *Synthesis* **2001**, 1796.
(221) Drochner, D.; Müller, M. *Eur. J. Org. Chem.* **2001**, 211.
(222) Wolberg, M.; Filho, M. V.; Bode, S.; Geilenkirchen, P.; Feldmann, R.; Liese, A.; Hummel, W.; Müller, M. *Bioprocess Biosyst. Eng.* **2008**, *31*, 183.
(223) (a) Tan, A. W. I.; Fischbach, M.; Huebner, H.; Buchholz, R.; Hummel, W.; Daussmann, T.; Wandrey, C.; Liese, A. *Appl. Microbiol. Biotechnol.* **2006**, *71*, 289.(b) Katzberg, M.; Wechler, K.; Müller, M.; Dünkelmann, P.; Stohrer, J.; Hummel, W.; Bertau, M. *Org. Biomol. Chem.* **2009**, *7*, 304.
(224) Edegger, K.; Stampfer, W.; Seisser, B.; Faber, K.; Mayer, S. F.; Oehrlein, R.; Hafner, A.; Kroutil, W. *Eur. J. Org. Chem.* **2006**, 1904.
(225) Kurina-Sanz, M.; Bisogno, F. R.; Lavandera, I.; Orden, A. A.; Gotor, V. *Adv. Synth. Catal.* **2009**, *351*, 1842.
(226) (a) Kalaitzakis, D.; Rozzell, J. D.; Kambourakis, S.; Smonou, I. *Org. Lett.* **2005**, *7*, 4799. (b) Kalaitzakis, D.; Rozzell, J. D.; Kambourakis, S.; Smonou, I. *Eur. J. Org. Chem.* **2006**, 2309. (c) Kalaitzakis, D.; Rozzell, J. D.; Smonou, I.; Kambourakis, S. *Adv. Synth. Catal.* **2006**, *348*, 1958.
(227) (a) Imuta, M.; Ziffer, H. *J. Org. Chem.* **1978**, *43*, 3319. (b) Ohta, H.; Konishi, J.; Kato, Y.; Tsuchihashi, G. *Agric. Biol. Chem.* **1987**, *51*, 2421.(c) Maruyama, R.; Nishizawa, M.; Itoi, Y.; Ito, S.; Inoue, M. *J. Biotechnol.* **2002**, *94*, 157.(d) Demir, A. S.; Hamamcı, H.; Ayhan, P.; Duygu, A. N.; Iğdir, A. Ç.; Çapanoğlu, D. *Tetrahedron: Asymmetry* **2004**, *15*, 2579.
(228) Demir, A. S.; Ayhan, P.; Demirtaş, U.; Erkılıç, U. *J. Mol. Catal. B: Enzym.* **2008**, *55*, 164.

(229) Grau, B. T.; Devine, P. N.; DiMichele, L. N.; Kosjek, B. *Org. Lett.* **2007**, *9*, 4951.
(230) Gotor, V.; Dehli, J. R.; Rebolledo, F. *J. Chem. Soc., Perkin Trans. 1* **2000**, 307.
(231) Dehli, J. R.; Gotor, V. *Tetrahedron: Asymmetry* **2001**, *12*, 1485.
(232) Dehli, J. R.; Gotor, V. *Tetrahedron: Asymmetry* **2000**, *11*, 3693.
(233) Chen, Q.; Wang, K.; Yuan, C. *New J. Chem.* **2009**, *33*, 972.
(234) Łukowska, E.; Plenkiewicz, J. *Tetrahedron: Asymmetry* **2007**, *18*, 1202.
(235) (a) Poessl, T. M.; Kosjek, B.; Ellmer, U.; Gruber, C. C.; Edegger, K.; Faber, K.; Hildebrandt, P.; Bornscheuer, U. T.; Kroutil, W. *Adv. Synth. Catal.* **2005**, *347*, 1827. (b) Seisser, B.; Lavandera, I.; Faber, K.; Spelberg, J. H. L.; Kroutil, W. *Adv. Synth. Catal.* **2007**, *349*, 1399. (c) Schrittwieser, J. H.; Lavandera, I.; Seisser, B.; Mautner, B.; Kroutil, W. *Eur. J. Org. Chem.* **2009**, 2293.(d) Schrittwieser, J. H.; Lavandera, I.; Seisser, B.; Mautner, B.; Spelberg, J. H. L.; Kroutil, W. *Tetrahedron: Asymmetry* **2009**, *20*, 483.
(236) Berkessel, A.; Rollmann, C.; Chamouleau, F.; Labs, S.; May, O.; Gröger, H. *Adv. Synth. Catal.* **2007**, *349*, 2697.
(237) Bisogno, F. R.; Cuetos, A.; Orden, A. A.; Kurina-Sanz, M.; Lavandera, I.; Gotor, V. *Adv. Synth. Catal.* **2010**, *352*, 1657.
(238) Krausser, M.; Hummel, W.; Gröger, H. *Eur. J. Org. Chem.* **2007**, 5175.
(239) (a) Breuer, M.; Ditrich, K.; Habicher, T.; Hauer, B.; Kesseler, M.; Stürmer, R.; Zelinski, T. *Angew. Chem., Int. Ed.* **2004**, *43*, 788. (b) Hwang, B.-Y.; Chob, B.-K.; Yun, H.; Koteshwar, K.; Kim, B.-G. *J. Mol. Catal. B: Enzym.* **2005**, *37*, 47.
(240) (a) Iwasaki, A.; Yamada, Y.; Ikenaka, Y.; Hasegawa, J. *Biotechnol. Lett.* **2003**, *25*, 1843.(b) Iwasaki, A.; Yamada, Y.; Kizaki, N.; Ikenaka, Y.; Hasegawa, J. *Appl. Microbiol. Biotechnol.* **2006**, *69*, 499.
(241) Savile, C. K.; Janey, J. M.; Mundorff, E. C.; Moore, J. C.; Tam, S.; Jarvis, W. R.; Colbeck, J. C.; Krebber, A.; Fleitz, F. J.; Brands, J.; Devine, P. N.; Huisman, G. W.; Hughes, G. J. *Science* **2010**, *329*, 305.
(242) Höhne, M.; Kühl, S.; Robins, K.; Bornscheuer, U. T. *ChemBioChem* **2008**, *9*, 363.
(243) Koszelewski, D.; Lavandera, I.; Clay, D.; Guebitz, G. M.; Rozzell, D.; Kroutil, W. *Angew. Chem., Int. Ed.* **2008**, *47*, 9337.
(244) For a recent reference, see: Fuganti, C.; Serra, S.; Dulio, A. *J. Chem. Soc., Perkin Trans. 1* **1999**, 279and references therein.
(245) Kohli, R. M.; Massey, V. *J. Biol. Chem.* **1998**, *273*, 32763.
(246) de Mancilha, M.; De Conti, R.; Moran, P. J. S.; Rodrigues, J. A. R. *ARKIVOC* **2001**, 85.
(247) Grau, M. M.; van der Toorn, J. C.; Otten, L. G.; Macheroux, P.; Taglieber, A.; Zilly, F. E.; Arends, I. W. C. E.; Hollmann, F. *Adv. Synth. Catal.* **2009**, *351*, 3279.
(248) Stueckler, C.; Reiter, T. C.; Baudendistel, N.; Faber, K. *Tetrahedron* **2010**, *66*, 663.
(249) (a) Padhi, S. K.; Bougioukou, D. J.; Stewart, J. D. *J. Am. Chem. Soc.* **2009**, *131*, 2371.(b) Bougioukou, D. J.; Kille, S.; Taglieber, A.; Reetz, M. T. *Adv. Synth. Catal.* **2009**, *351*, 3287.
(250) (a) Chaparro-Riggers, J. F.; Rogers, T. A.; Vazquez-Figueroa, E.; Polizzi, K. M.; Bommarius, A. S. *Adv. Synth. Catal.* **2007**, *349*, 1521. (b) Hall, M.; Stueckler, C.; Kroutil, W.; Macheroux, P.; Faber, K. *Angew. Chem., Int. Ed.* **2007**, *46*, 3934. (c) Stueckler, C.; Hall, M.; Ehammer, H.; Pointner, E.; Kroutil, W.; Macheroux, P.; Faber, K. *Org. Lett.* **2007**, *9*, 5409. (d) Hall, M.; Stueckler, C.; Ehammer, H.; Pointner, E.; Oberdorfer, G.; Gruber, K.; Hauer, B.; Stuermer, R.; Kroutil, W.; Macheroux, P.; Faber, K. *Adv. Synth. Catal.* **2008**, *350*, 411. (e) Hall, M.; Stueckler, C.; Hauer, B.; Stuermer, R.; Friedrich, T.; Breuer, M.; Kroutil, W.; Faber, K. *Eur. J. Org. Chem.* **2008**, 1511. (f) Mueller, N. J.; Stueckler, C.; Hauer, B.; Baudendistel, N.; Housden, H.; Bruce, N. C.; Faber, K. *Adv. Synth. Catal.* **2010**, *352*, 387.
(251) Müller, A.; Hauer, B.; Rosche, B. *J. Mol. Catal. B: Enzym.* **2006**, *38*, 126.
(252) Müller, A.; Hauer, B.; Rosche, B. *Biotechnol. Bioeng.* **2007**, *98*, 22.
(253) Swiderska, M. A.; Stewart, J. D. *J. Mol. Catal. B: Enzym.* **2006**, *42*, 52.
(254) Kosjek, B.; Fleitz, F. J.; Dormer, P. G.; Kuethe, J. T.; Devine, P. N. *Tetrahedron: Asymmetry* **2008**, *19*, 1403.
(255) Fuganti, C.; Serra, S. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3758.
(256) Ferraboschi, P.; Reza-Elahi, S.; Verza, E.; Santaniello, E. *Tetrahedron: Asymmetry* **1999**, *10*, 2639.
(257) (a) Ferraboschi, P.; Casati, S.; Sataniello, E. *Tetrahedron: Asymmetry* **1994**, *5*, 19. (b) Ferraboschi, P.; Reza-Elahi, S.; Verza, E.; Meroni Rivolta, F.; Santaniello, E. *Synlett* **1996**, 1176.
(258) Serra, S.; Fuganti, C. *Tetrahedron: Asymmetry* **2001**, *12*, 2191.
(259) Molinari, F.; Gandolfi, R.; Villa, R.; Urban, E.; Kiener, A. *Tetrahedron: Asymmetry* **2003**, *14*, 2041.
(260) Romano, A.; Gandolfi, R.; Nitti, P.; Rollini, M.; Mollinari, F. *J. Mol. Catal. B: Enzymol.* **2002**, *17*, 235.
(261) Gourdel-Martin, M.-E.; Comoy, C.; Huet, F. *Tetrahedron: Asymmetry* **1999**, *10*, 403.
(262) Köhler, V.; Bailey, K. R.; Znabet, A.; Raftery, J.; Helliwell, M.; Turner, N. J. *Angew. Chem., Int. Ed.* **2010**, *49*, 2182.
(263) (a) Holland, H. L. *Biotechnology*; Wiley-VCH: Weinheim, 1998; Vol. 8a. (b) Holland, H. L. *Curr. Opin. Biol.* **1999**, *3*, 22.
(264) Adam, W.; Lukacs, Z.; Saha-Möller, C. R.; Weckerle, B.; Schreier, P. *Eur. J. Org. Chem.* **2000**, 2923.
(265) (a) Adam, W.; Likacs, Z.; Harmsen, D.; Saha-Möller, C. R.; Schreier, P. *J. Org. Chem.* **2000**, *65*, 878. (b) Adam, W.; Lukacs, Z.; Kahle, C.; Saha-Möller, C. R.; Schreier, P. *J. Mol. Catal. B: Enzymol.* **2001**, *11*, 377.
(266) Chang, D.; Wiltholt, B.; Li, Z. *Org. Lett.* **2000**, *2*, 3949.
(267) (a) Li, Z.; Feiten, H.-J.; van Beilen, J. B.; Duetz, W.; Witholt, B. *Tetrahedron: Asymmetry* **1999**, *10*, 1323. (b) Li, Z.; Feiten, H.-J.; Chang, D.; Duetz, W. A.; van Beilen, J. B.; Witholt, B. *J. Org. Chem.* **2001**, *66*, 8424. (c) Chang, D.; Feiten, H.-J.; Witholt, B.; Li, Z. *Tetrahedron: Asymmetry* **2002**, *13*, 2141.
(268) Münzer, D. F.; Meinhold, P.; Peters, M. W.; Feichtenhofer, S.; Griengl, H.; Arnold, F. H.; Gliedeerc, A.; de Raadt, A. *Chem. Commun.* **2005**, 2597.
(269) Littlechild, J. *Curr. Opin. Chem. Biol.* **1999**, *3*, 28.
(270) Hu, S.; Hager, L. P. *J. Am. Chem. Soc.* **1999**, *121*, 872.
(271) Adam, W.; Boland, W.; Hartmann-Schreier, J.; Humpf, H.-U.; Lazarus, M.; Saffert, A.; Saha-Möller, C. R.; Schreier, P. *J. Am. Chem. Soc.* **1998**, *120*, 11044.
(272) For a recent review, see: van de Velde, F.; van Rantwijk, F.; Sheldon, R. A. *Trends Biotechnol.* **2001**, *19*, 73.
(273) (a) Dai, L.; Klibanov, A. M. *Biotechnol. Bioeng.* **2000**, *70*, 353. (b) Pasta, P.; Carrea, G.; Monzani, E.; Gaggero, N.; Colonna, S. *Biotechnol. Bioeng.* **1999**, *62*, 489.
(274) (a) Kadnikova, E. N.; Kostić, N. M. *J. Non-Cryst. Solids* **2001**, *283*, 63. (b) Kadnikova, E. N.; Kostić, N. M. *J. Org. Chem.* **2003**, *68*, 2600.
(275) (a) Okrasa, K.; Guibé-Jampel, E.; Therisod, M. *J. Chem. Soc., Perkin Trans. 1* **2000**, 1077. (b) van de Velde, F.; Lourenço, N. D.; Bakker, M.; van Rantwijk, F.; Sheldon, R. A. *Biotechnol. Bioeng.* **2000**, *69*, 286. (c) Okrasa, K.; Falcimaigne, A.; Guibé-Jampel, E.; Therisod, M. *Tetrahedron: Asymmetry* **2002**, *13*, 519.
(276) (a) van de Velde, F.; Arends, I. W. C. E.; Sheldon, R. A. *J. Inorg. Biochem.* **2000**, *80*, 81. (b) van de Velde, F.; Könemann, L.; van Rantwijk, F.; Sheldon, R. A. *Biotechnol. Bioeng.* **2000**, *67*, 87.
(277) (a) Chen, G.; Kayser, M. M.; Mihovilovic, M. D.; Mrstik, M. E.; Martinez, C. A.; Stewart, J. D. *New J. Chem.* **1999**, *23*, 827. (b) Feingersch, R.; Shainsky, J.; Wood, T. K.; Fishman, A. *Appl. Environ. Microbiol.* **2008**, *74*, 1555.
(278) Kerridge, A.; Willetts, A.; Holland, H. *J. Mol. Catal. B: Enzymol.* **1999**, *6*, 59.
(279) Nimri, S.; Keinan, E. *J. Am. Chem. Soc.* **1999**, *121*, 8978.
(280) Adam, W.; Heckel, F.; Saha-Möller, C. R.; Taupp, M.; Screier, P. *Tetrahedron: Asymmetry* **2004**, *15*, 983.
(281) Porto, A. L. M.; Cassiola, F.; Dias, S. L. P.; Joekes, I.; Gushikem, Y.; Rodrigues, J. A. R.; Moran, P. J. S.; Manfio, G. P.; Marsaioli, A. J. *J. Mol. Catal. B: Enzymol.* **2002**, *19*−*20*, 327.

(282) Li, A.-T.; Zhang, J.-D.; Xu, J.-H.; Lu, W.-Y.; Lin, G.-L. *Appl. Environ. Microbiol.* **2009**, *75*, 551.
(283) Zhang, J.-D.; Li, A.-T.; Yang, Y.; Xu, J.-H. *Appl. Microbiol. Biotechnol.* **2010**, *85*, 615.
(284) Olivo, H. F.; Osorio-Lozada, A.; Peeples, T. L. *Tetrahedron: Asymmetry* **2005**, *16*, 3507.
(285) de Gonzalo, G.; Torres Pazmiño, D. E.; Ottolina, G.; Fraaije, M. W.; Carrea, G. *Tetrahedron: Asymmetry* **2006**, *17*, 130.
(286) de Gonzalo, G.; Torres Pazmiño, D. E.; Ottolina, G.; Fraaije, M. W.; Carrea, G. *Tetrahedron: Asymmetry* **2005**, *16*, 3077.
(287) (a) Rioz-Martínez, A.; Bisogno, F. R.; Rodríguez, C.; de Gonzalo, G.; Lavandera, I.; Torres Pazmiño, D. E.; Fraaije, M. W.; Gotor, V. *Org. Biomol. Chem.* **2010**, *8*, 1431.(b) Bisogno, F. R.; Rioz-Martínez, A.; Rodríguez, C.; Lavandera, I.; de Gonzalo, G.; Torres Pazmiño, D. E.; Fraaije, M. W.; Gotor, V. *ChemCatChem* **2010**, *2*, 946.
(288) Solomon, E. I.; Brunold, T. C.; Davis, M. I.; Kemsley, J. N.; Lee, S.-K.; Lehnert, N.; Neese, F.; Skulan, A. J.; Yang, Y.-S.; Zhou, J. *Chem. Rev.* **2000**, *100*, 235.
(289) Carredano, E.; Karlsson, A.; Kauppi, B.; Choudhury, D.; Parales, R. E.; Parales, J. V.; Lee, K.; Gibson, D. T.; Eklund, H.; Ramaswamy, S. *J. Mol. Biol.* **2000**, *182*, 5495.
(290) (a) Boyd, D. R.; Sharma, N. D.; Ljubez, V.; Byrne, B. E.; Shepherd, S. D.; Allen, C. C. R.; Kulakov, L. A.; Larkin, M. J.; Dalton, H. *Chem. Commun.* **2002**, 1914. (b) Boyd, D. R.; Sharma, N. D.; Haughey, S. A.; Malone, J. F.; King, A. W. T.; McMurray, B. T.; Alves-Areias, A.; Allen, C. C. R.; Holt, R.; Dalton, H. *J. Chem. Soc., Perkin Trans. 1* **2001**, 3288. (c) Boyd, D. R.; Sharma, N. D.; Allen, C. C. R. *Curr. Opin. Biotechnol.* **2001**, *12*, 564and references therein. (d) Boyd, D. R.; Sharma, N. D.; Bowers, N. I.; Boyle, R.; Harrison, J. S.; Lee, K.; Bugg, T. D. H.; Gibson, D. T. *Org. Biomol. Chem.* **2003**, *1*, 1298. (e) Boyd, D. R.; Sharma, N. D.; Bowers, N. I.; Brannigan, I. N.; Groocock, M. R.; Malone, J. F.; McConville, G.; Allen, C. C. R. *Adv. Synth. Catal.* **2005**, *347*, 1081. (f) Boyd, D. R.; Sharma, N. D.; O'Dowd, C. R.; Carroll, J. G.; Lokea, P. L.; Allen, C. C. R. *Chem. Commun.* **2005**, 3989.(g) Finn, K. J.; Pavlyuk, O.; Hudlicky, T. *Collect. Czech. Chem. Commun.* **2005**, *70*, 1709. (h) Finn, K. J.; Rochon, L.; Hudlicky, T. *Tetrahedron: Asymmetry* **2005**, *16*, 3606. (i) Boyd, D. R.; Sharma, N. D.; Belhocine, T.; Malone, J. F.; McGregor, S.; Allen, C. C. R. *Chem. Commun.* **2006**, 4934. (j) Finn, K. J.; Collins, J.; Hudlicky, T. *Tetrahedron* **2006**, *62*, 7471. (k) Boyd, D. R.; Sharma, N. D.; Coen, G. P.; Gray, P. J.; Malone, J. F.; Gawronski, J. *Chem.—Eur. J.* **2007**, *13*, 5804.(l) Boyd, D. R.; Sharma, N. D.; Ljubez, V.; Malone, J. F.; Allen, C. C. R. *J. Chem. Technol. Biotechnol.* **2007**, *82*, 1072. (m) Kwit, M.; Gawronski, J.; Boyd, D. R.; Sharma, N. D.; Kaik, M.; O'Ferrall, R. A. M.; Kudavalli, J. S. *Chem.—Eur. J.* **2008**, *14*, 11500. (n) Boyd, C. R.; Sharma, N. D.; Coen, G. P.; Hempenstall, F.; Ljubez, V.; Malone, J. F.; Allen, C. C. R.; Hamilton, J. T. G. *Org. Biomol. Chem.* **2008**, *6*, 3957.(o) Boyd, D. R.; Sharma, N. D.; Malone, J. F.; Allen, C. C. R. *Chem. Commun.* **2009**, 3633.
(291) (a) Hudlicky, T.; Gonzalez, D.; Gibson, D. T. *Aldrichimica Acta* **1999**, *32*, 35.(b) Hudlicky, T.; Reed, J. W. *Synlett* **2009**, 685.
(292) See, for instance, ref 152, section 4.
(293) Kauppi, B.; Lee, K.; Carredano, E.; Parales, R. E.; Gibson, D. T.; Eklund, H.; Ramaswamy, S. *Structure* **1998**, *6*, 571.
(294) (a) Parales, R. E.; Resnick, S. M.; Yu, C.-L.; Boyd, D. R.; Sharma, N. D.; Gibson, D. T. *J. Bacteriol.* **2000**, *182*, 5495. (b) Parales, R. E.; Lee, K.; Resnick, S. M.; Jiang, H.; Lessner, D. J.; Gibson, D. T. *J. Bacteriol.* **2000**, *182*, 1641. (c) Parales, R. E.; Parales, J. V.; Gibson, D. T. *J. Bacteriol.* **1999**, *181*, 1831.
(295) Banwell, M. G.; Bray, A. M.; Wong, D. J. *New J. Chem.* **2001**, *25*, 1351.
(296) Banwell, M. G.; Bray, A. M.; Edwards, A. J.; Wong, D. J. *New J. Chem.* **2001**, *25*, 3.
(297) Phung, A. N.; Zannetti, M. T.; Whited, G.; Fessner, W.-D. *Angew. Chem., Int. Ed.* **2003**, *42*, 4821.
(298) (a) Fonseca, G.; Seoane, G. A. *Tetrahedron: Asymmetry* **2005**, *16*, 1393. (b) Carrera, I.; Brovetto, M. C.; Seoane, G. *Tetrahedron: Lett.* **2006**, *47*, 7849. (c) Bellomo, A.; Gonzalez, D. *Tetrahedron: Asymmetry* **2006**, *17*, 474. (d) Bellomo, A.; Gonzalez, D. *Tetrahedron Lett.* **2007**, *48*, 3047. (e) Carrera, I.; Brovetto, M. C.; Seoane, G. *Tetrahedron* **2007**, *63*, 4095. (f) Bellomo, A.; Giacomini, C.; Brena, B.; Seoane, G.; Gonzalez, D. *Synth. Commun.* **2007**, *37*, 3509. (g) Bellomo, A.; Gonzalez, D.; Stefani, H. A. *J. Organomet. Chem.* **2008**, *693*, 1136. (h) Bellomo, A.; Camarano, S.; Rossini, C.; Gonzalez, D. *Carbohydr. Res.* **2009**, *344*, 44. (i) Bellomo, A.; Weber, M.; Gonzalez, D.; Stefani, H. A. *Catal. Commun.* **2009**, *10*, 1647. (j) Bellomo, A.; Bertucci, A.; Stefani, H.; Vázquez, A.; Gonzalez, D. *Tetrahedron: Asymmetry* **2009**, *20*, 2673. (k) Boyd, D. R.; Sharma, N. D.; Berberian, M. V.; Dunne, K. S.; Hardacre, C.; Kaik, M.; Kelly, B.; Malone, J. F.; McGregor, S. T.; Stevenson, P. J. *Adv. Synth. Catal.* **2010**, *352*, 855.
(299) Brovetto, M.; Seoane, G. *J. Org. Chem.* **2008**, *73*, 5776.
(300) (a) Bellomo, A.; Bonilla, J. B.; López-Prados, J.; Martín-Lomas, M.; Gonzalez, D. *Tetrahedron: Asymmetry* **2009**, *20*, 2061. (b) Ramos, J. C.; Bracco, P.; Mazzini, M.; Fernández, J. R.; Gamenara, D.; Seoane, G. A. *Tetrahedron: Asymmetry* **2010**, *21*, 969. (c) Boyd, D. R.; Sharma, N. D.; Bowers, N. I.; Coen, G. B.; Malone, J. F.; O'Dowd, C. R.; Stevenson, P. J.; Allen, C. C. R. *Org. Biomol. Chem.* **2010**, *8*, 1415.
(301) (a) Matveenko, M.; Willis, A. C.; Banwell, M. G. *Tetrahedron Lett.* **2008**, *49*, 7018. (b) Shie, J.-J.; Fang, J.-M.; Wong, C.-H. *Angew. Chem., Int. Ed.* **2008**, *47*, 5788. (c) Sullivan, B.; Carrera, I.; Drouin, M.; Hudlicky, T. *Angew. Chem., Int. Ed.* **2009**, *48*, 4229. (d) Werner, L.; Machara, A.; Hudlicky, T. *Adv. Synth. Catal.* **2010**, *352*, 195.
(302) (a) Moser, M.; Sun, X.; Hudlicky, T. *Org. Lett.* **2005**, *7*, 5669. (b) Hudlicky, T.; Rinner, U.; Finn, K. J.; Ghiviriga, I. *J. Org. Chem.* **2005**, *70*, 3940. (c) Hudlicky, T.; Moser, M.; Banfield, S. C.; Rinner, U.; Chapuis, J.-C.; Pettit, G. R. *Can. J. Chem.* **2006**, *84*, 1313. (d) Omori, A. T.; Finn, K. J.; Leisch, H.; Carroll, R. J.; Hudlicky, T. *Synlett* **2007**, 2859. (e) Zezula, J.; Rice, K. C.; Hudlicky, T. *Synlett* **2007**, 2863. (f) Collins, J.; Drouin, M.; Sun, X.; Rinner, U.; Hudlicky, T. *Org. Lett.* **2008**, *10*, 361. (g) Sullivan, B.; Hudlicky, T. *Tetrahedron Lett.* **2008**, *49*, 5211. (h) Gilmet, J.; Sullivan, B.; Hudlicky, T. *Tetrahedron* **2009**, *65*, 212. (i) Leisch, H.; Omori, A. T.; Finn, K. J.; Gilmet, J.; Bissett, T.; Ilceski, D.; Hudlicky, T. *Tetrahedron* **2009**, *65*, 9862. (j) Fabris, F.; Collins, J.; Sullivan, B.; Leisch, H.; Hudlicky, T. *Org. Biomol. Chem.* **2009**, *7*, 2619. (k) Collins, J.; Rinner, U.; Moser, M.; Hudlicky, T.; Ghiviriga, I.; Romero, A. E.; Kornienko, A.; Ma, D.; Griffin, C.; Pandey, S. *J. Org. Chem.* **2010**, *75*, 3069. (l) Werner, L.; Hudlicky, J. R.; Wernerova, M.; Hudlicky, T. *Tetrahedron* **2010**, *66*, 3761.
(303) (a) Boyd, D. R.; Sharma, N. D.; Sbircea, L.; Murphy, D.; Belhocine, T.; Malone, J. F.; James, S. L.; Allen, C. C. R.; Hamilton, J. T. G. *Chem. Commun.* **2008**, 5535.(b) Sbircea, L.; Sharma, N. D.; Clegg, W.; Harrington, R. W.; Horton, P. N.; Hursthouse, M. B.; Apperley, D. C.; Boyd, D. R.; James, S. L. *Chem. Commun.* **2008**, 5538. (c) Boyd, D. R.; Sharma, N. D.; Sbircea, L.; Murphy, D.; Malone, J. F.; James, S. L.; Allen, C. C. R.; Hamilton, J. T. G. *Org. Biomol. Chem.* **2010**, *8*, 1081.
(304) (a) Boyd, D. R.; Sharma, N. D.; Llamas, N. M.; O'Dowd, C. R.; Allen, C. C. R. *Org. Biomol. Chem.* **2006**, *4*, 2208. (b) Boyd, D. R.; Sharma, N. D.; Llamas, N. M.; Coen, G. P.; McGeehin, P. K. M.; Allen, C. C. R. *Org. Biomol. Chem.* **2007**, *5*, 514.
(305) For recent examples on microbial asymmetric epoxidations seeHwang, S.; Choi, C. Y.; Lee, E. Y. *J. Ind. Eng. Chem.* **2010**, *16*, 1.
(306) Schmid, A.; Hofstetter, K.; Feiten, H.-J.; Hollmann, F.; Witholt, B. *Adv. Synth. Catal.* **2001**, *343*, 732.
(307) Hollmann, F.; Lin, P.-C.; Witholt, B.; Schmid, A. *J. Am. Chem. Soc.* **2003**, *125*, 8209.
(308) Hofstetter, K.; Lutz, J.; Lang, I.; Witholt, B.; Schmid, A. *Angew. Chem., Int. Ed.* **2004**, *43*, 2163.
(309) Kubo, T.; Peters, M. W.; Meinhold, P.; Arnold, F. H. *Chem.—Eur. J.* **2006**, *12*, 1216.
(310) See, for instance: Steinreiber, A.; Faber, K. *Curr. Opin. Biotechnol.* **2001**, *12*, 552.
(311) Chiappe, C.; De Rubertis, A.; Marioni, F.; Simonetti, A. *J. Mol. Catal. B: Enzym.* **2000**, *10*, 539.
(312) van Loo, B.; Kingma, J.; Arand, M.; Wubbolts, M. G.; Janssen, D. B. *Appl. Environ. Microbiol.* **2006**, *72*, 2905.

(313) Weijers, C. A. G. M.; de Bont, J. A. M. *J. Mol. Catal. B: Enzym.* **1999**, *6*, 199.
(314) Chang, D.; Wang, Z.; Heringa, M. F.; Wirthner, R.; Witholt, B.; Li, Z. *Chem. Commun.* **2003**, 960.
(315) Chang, D.; Heringa, M. F.; Witholt, B.; Li, Z. *J. Org. Chem.* **2003**, *68*, 8599.
(316) Zhao, L.; Han, B.; Huang, Z.; Miller, M.; Huang, H.; Malashock, D. S.; Zhu, Z.; Milan, A.; Robertson, D. E.; Weiner, D. P.; Burk, M. J. *J. Am. Chem. Soc.* **2004**, *126*, 11156.
(317) Glueck, S. M.; Fabian, W. M. F.; Faber, K.; Mayer, S. F. *Chem.—Eur. J.* **2004**, *10*, 3467.
(318) Ueberbacher, B. T.; Oberdorfer, G.; Gruber, K.; Faber, K. *ChemBioChem* **2009**, *10*, 1697.
(319) de Gonzalo, G.; Mihovilovic, M. D.; Fraaije, M. W. *ChemBioChem* **2010**, *11*, 2208.
(320) For recent reviews covering the topic see (a) Alphand, V.; Carrea, G.; Wohlgemuth, R.; Furstoss, R.; Woodley, J. M. *Trends Biotechnol.* **2003**, *21*, 318. (b) Kamerbeek, N. M.; Janssen, D. B.; van Berkel, W. J. H.; Fraaije, M. W. *Adv. Synth. Catal.* **2003**, *345*, 667. (c) Mihovilovic, M. D.; Müller, B.; Stanetty, P. *Eur. J. Org. Chem.* **2002**, 3711.
(321) Stewart, J. D. *Curr. Org. Chem.* **1998**, *2*, 211.
(322) (a) Kayser, M. M.; Chen, G.; Stewart, J. D. *Synlett* **1999**, 153. (b) Kayser, M. M.; Chen, G.; Stewart, J. D. *J. Org. Chem.* **1998**, *63*, 7103. (c) Stewart, J. D.; Reed, K. W.; Martínez, J.; Zhu, J.; Chen, G.; Kayser, M. M. *J. Am. Chem. Soc.* **1998**, *120*, 3541.
(323) Mihovilovic, M. D.; Chen, G.; Wang, S.; Kyte, B.; Rochon, F.; Kayser, M. M.; Stewart, J. D. *J. Org. Chem.* **2001**, *66*, 733.
(324) Mihovilovic, M. D.; Müller, B.; Kayser, M. M.; Stewart, J. D.; Fröhlich, J.; Stanetty, P.; Spreitzer, H. *J. Mol. Catal. B: Enzym.* **2001**, *11*, 349.
(325) Mihovilovic, M. D.; Rudroff, F.; Kandioller, W.; Grötzl, B.; Stanetty, P.; Spreitzer, H. *Synlett* **2003**, 1973.
(326) Mihovilovic, M. D.; Bianchi, D. A.; Rudroff, F. *Chem. Commun.* **2006**, 3214.
(327) Rial, D. V.; Bianchi, D. A.; Kapitanova, P.; Lengar, A.; van Beilen, J. B.; Mihovilovic, M. D. *Eur. J. Org. Chem.* **2008**, 1203.
(328) Wang, S.; Kayser, M. M.; Iwaki, H.; Lau, P. C. K. *J. Mol. Catal. B: Enzym.* **2003**, *22*, 211.
(329) (a) Mihovilovic, M. D.; Müller, B.; Kayser, M. M.; Stanetty, P. *Synlett* **2002**, 700.(b) Mihovilovic, M. D.; Rudroff, F.; Grötzl, B.; Stanetty, P. *Eur. J. Org. Chem.* **2005**, 809.
(330) Mihovilovic, M. D.; Müller, B.; Schulze, A.; Stanetty, P.; Kayser, M. M. *Eur. J. Org. Chem.* **2003**, 2243.
(331) Mihovilovic, M. D.; Rudroff, F.; Müller, B.; Stanetty, P. *Bioorg. Med. Chem. Lett.* **2003**, 1479.
(332) Kyte, B. G.; Rouvière, P.; Cheng, Q.; Stewart, J. D. *J. Org. Chem.* **2004**, *69*, 12.
(333) Wu, S.; Acevedo, J. P.; Reetz, M. T. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 2775.
(334) Mihovilovic, M. D.; Rudroff, F.; Grötzl, B.; Kapitan, P.; Snajdrova, R.; Rydz, J.; Mach, R. *Angew. Chem., Int. Ed.* **2005**, *44*, 3609.
(335) Rudroff, F.; Rydz, J.; Ogink, F. H.; Fink, M.; Mihovilovic, M. D. *Adv. Synth. Catal.* **2007**, *349*, 1436.
(336) Braun, I.; Rudroff, F.; Mihovilovic, M. D.; Bach, T. *Angew. Chem., Int. Ed.* **2006**, *45*, 5541.
(337) (a) Mihovilovic, M. D.; Snajdrova, R.; Winninger, A.; Rudroff, F. *Synlett* **2005**, 2751. (b) Snajdrova, R.; Braun, I.; Bach, T.; Mereiter, K.; Mihovilovic, M. D. *J. Org. Chem.* **2007**, *72*, 9597.
(338) See, for instance: (a) Machajewski, T. D.; Wong, C.-H. *Angew. Chem., Int. Ed.* **2000**, *39*, 1352. (b) Ref 3a, Chapter 9. (c) Steinreiber, J.; Fesko, K.; Reisinger, C.; Schürmann, M.; van Assema, F.; Wolberg, M.; Mink, D.; Griengl, H. *Tetrahedron* **2007**, *63*, 918. (d) Steinreiber, J.; Fesko, K.; Mayer, C.; Reisinger, C.; Schürmann, M.; Griengl, H. *Tetrahedron* **2007**, *63*, 8088.
(339) Fesko, K.; Uhl, M.; Steinreiber, J.; Gruber, K.; Griengl, H. *Angew. Chem., Int. Ed.* **2010**, *49*, 121.
(340) (a) Castillo, J. A.; Calveras, J.; Casas, J.; Mitjans, M.; Vinardell, M. P.; Parella, T.; Inoue, T.; Sprenger, G. A.; Joglar, J.; Clapés, P. *Org. Lett.* **2006**, *8*, 6067. (b) Concia, A. L.; Lozano, C.; Castillo, J. A.; Parella, T.; Joglar, J.; Clapés, P. *Chem.—Eur. J.* **2009**, *15*, 3808.
(341) Garrabou, X.; Castillo, J. A.; Guérard-Hélaine, C.; Parella, T.; Joglar, J.; Lemaire, M.; Clapés, P. *Angew. Chem., Int. Ed.* **2009**, *48*, 5521.
(342) Castillo, J. A.; Guérard-Hélaine, C.; Gutiérrez, M.; Garrabou, X.; Sancelme, M.; Schürmann, M.; Inoue, T.; Hélaine, V.; Charmantray, F.; Gefflaut, T.; Hecquet, L.; Joglar, J.; Clapés, P.; Sprenger, G. A.; Lemaire, M. *Adv. Synth. Catal.* **2010**, *352*, 1039.
(343) See, for instance: (a) Shabat, D.; List, B.; Lerner, R. A.; Barbas, C. F., III. *Tetrahedron Lett.* **1999**, *40*, 1437. (b) List, B.; Lerner, R. A.; Barbas, C. F., III. *Org. Lett.* **1999**, *1*, 59.
(344) (a) Wagner, J.; Lerner, R. A.; Barbas, C. F., III. *Science* **1995**, *270*, 1797. (b) Barbas, C. F., III; Heine, A.; Zhong, G.; Hoffmann, T.; Gramatikova, S.; Björnestedt, R.; List, B.; Anderson, J.; Stura, E. A.; Wilson, I. A.; Lerner, R. A. *Science* **1997**, *278*, 2085.
(345) Zhong, G.; Lerner, R. A.; Barbas, C. F., III. *Angew. Chem., Int. Ed.* **1999**, *38*, 3738.
(346) (a) Maggiotti, V.; Resmini, M.; Gouverneur, V. *Angew. Chem., Int. Ed.* **2002**, *41*, 1012. (b) Maggiotti, V.; Bahmanyar, S.; Reiter, M.; Resmini, M.; Houk, K. N.; Gouverneur, V. *Tetrahedron* **2004**, *60*, 619.
(347) (a) Purkarthofer, T.; Gruber, K.; Gruber-Khadjawi, M.; Waich, K.; Skranc, W.; Mink, D.; Griengl, H. *Angew. Chem., Int. Ed.* **2006**, *45*, 3454.(b) Gruber-Khadjawi, M.; Purkarthofer, T.; Skranc, W.; Griengl, H. *Adv. Synth. Catal.* **2007**, *349*, 1445.
(348) For an recent review on ThDP-dependent enzymes, see: Pohl, M.; Lingen, B.; Müller, M. *Chem.—Eur. J.* **2002**, *8*, 5288.
(349) Iding, H. Ph.D. Thesis, University of Düsseldorf, 1998.
(350) For a review, see: Iding, H.; Siegert, P.; Mesch, K.; Pohl, M. *Biochim. Biophys. Acta* **1998**, *1385*, 307and references therein.
(351) For a review, see: Oliver, A. L.; Anderson, B. N.; Roddick, F. A. *Adv. Microb. Physiol.* **1999**, *41*, 1.
(352) Iding, H.; Dünnwald, T.; Griner, L.; Liese, A.; Müller, M.; Siegert, P.; Grötzinger, J.; Demir, A. S.; Pohl, M. *Chem.—Eur. J.* **2000**, *6*, 1483.
(353) Dünnwald, T.; Demir, A. S.; Siegert, P.; Pohl, M.; Müller, M. *Eur. J. Org. Chem.* **2000**, 2161.
(354) Demir, A. S.; Sesenoglu, Ö.; Eren, E.; Hosrik, B.; Pohl, M.; Janzen, E.; Kolter, D.; Feldmann, R.; Dünkelmann, P.; Müller, M. *Adv. Synth. Catal.* **2002**, *344*, 96.
(355) Dünkelmann, P.; Kolter, D.; Nitsche, A.; Demir, A. S.; Siegert, P.; Lingen, B.; Pohl, M.; Müller, M. *J. Am. Chem. Soc.* **2002**, *124*, 12084.
(356) (a) Müller, M.; Gocke, D.; Pohl, M. *FEBS J.* **2009**, *276*, 2894. (b) Cosp, A.; Dresen, C.; Pohl, M.; Walter, L.; Röhr, C.; Müller, M. R. *Adv. Synth. Catal.* **2008**, *350*, 759. (c) Domínguez de María, P.; Stillger, T.; Pohl, M.; Kiesel, M.; Liese, A.; Gröger, H.; Trauthwein, H. *Adv. Synth. Catal.* **2008**, *350*, 165. (d) Domínguez de María, P.; Pohl, M.; Gocke, D.; Gröger, H.; Trauthwein, H.; Stillger, T.; Walter, L.; Müller, M. *Eur. J. Org. Chem.* **2007**, 2940.
(357) Racker, E. In *The Enzymes*; Boyer, P. D.; Lardy, H.; Myrzback, K., Ed.; Academic Press: New York, 1961, Vol. 5, p 397.
(358) (a) Hibbert, E. G.; Senussi, T.; Costelloe, S. J.; Lei, W.; Smith, M. E. B.; Ward, J. M.; Hailes, H. C.; Dalby, P. A. *J. Biotechnol.* **2007**, *131*, 425. (b) Ingram, C. U.; Bommer, M.; Smith, M. E. B.; Dalby, P. A.; Ward, J. M.; Hailes, H. C.; Lye, G. J. *Biotechnol. Bioeng.* **2007**, *96*, 559.
(359) Hibbert, E. G.; Senussi, T.; Smith, M. E. B.; Costelloe, S. J.; Ward, J. M.; Hailes, H. C.; Dalby, P. A. *J. Biotechnol.* **2008**, *134*, 240.
(360) (a) Smith, M. E. B.; Hibbert, E. G.; Jones, A. B.; Dalby, P. A.; Hailes, H. C. *Adv. Synth. Catal.* **2008**, *350*, 2631. (b) Cázares, A.; Galman, J. L.; Crago, L. G.; Smith, M. E. B.; Strafford, J.; Ríos-Solís, L.; Lye, G. J.; Dalby, P. A.; Hailes, H. C. *Org. Biomol. Chem.* **2010**, *8*, 1301.
(361) For general reviews on the topic, see: (a) North, M. *Tetrahedron: Asymmetry* **2003**, *14*, 147. (b) Gröger, H. *Adv. Synth. Catal.* **2001**, *343*, 547. (c) Effenberger, F.; Förster, S.; Wajant, H. *Curr. Opin. Biotechnol.* **2000**, *11*, 532.(d) Ref 3b, Chapters 11−12. (e) Gregory, R. J. H. *Chem. Rev.* **1999**, *99*, 3649. (f) Effenberger, F. *Chimia* **1999**, *53*, 3.
(362) (a) Trummler, K.; Roos, J.; Schwaneberg, U.; Effenberger, F.; Förster, S.; Pfizenmaier, W. W. *Plant Sci.* **1998**, *139*, 19. (b) Gruber, K.; Gugganig, M.; Wagner, U. G.; Kratky, C. *Biol. Chem.* **1999**, *380*, 993.

(c) Zuegg, J.; Gruber, K.; Gugganig, M.; Wagner, U. G.; Kratky, C. *Protein Sci.* **1999**, *8*, 1990. (d) Johnson, D. V.; Griengl, H. *Adv. Biochem. Eng. Biotechnol.* **1999**, *63*, 32. (e) Schmidt, M.; Griengl, H. *Top. Curr. Chem.* **1999**, *200*, 194.

(363) See, for instance: Gröger, H.; Capan, E.; Barthuber, A.; Vorlop, K.-D. *Org. Lett.* **2001**, *3*, 1969and references therein.

(364) Johnson, D. V.; Zabelinskaja-Mackova, A. A.; Griengl, H. *Curr. Opin. Chem. Biol.* **2000**, *4*, 103and references therein.

(365) Effenberger, F.; Ziegler, T.; Förster, S. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 458.

(366) Han, S.; Lin, G.; Li, Z. *Tetrahedron: Asymmetry* **1998**, *9*, 1835.

(367) Lin, G.; Han, S.; Li, Z. *Tetrahedron* **1999**, *55*, 3531.

(368) Han, S.; Chen, P.; Lin, G.; Huang, H.; Li, Z. *Tetrahedron: Asymmetry* **2001**, *12*, 843.

(369) Chen, P.; Han, S.; Lin, G.; Huang, H.; Li, Z. *Tetrahedron: Asymmetry* **2001**, *12*, 3273.

(370) Fröhlich, R. F. G.; Zabelinskaja-Mackova, A. A.; Fechter, M. H.; Griengl, H. *Tetrahedron: Asymmetry* **2003**, *14*, 355.

(371) Purkarthofer, T.; Pabst, T.; van den Broek, C.; Griengl, H.; Maurer, O.; Skranc, W. *Org. Process Res. Dev.* **2006**, *10*, 618.

(372) Menéndez, E.; Brieva, R.; Rebolledo, F.; Gotor, V. *Chem. Commun.* **1995**, 989.

(373) Monterde, M. I.; Nazabadioko, S.; Rebolledo, F.; Brieva, R.; Gotor, V. *Tetrahedron: Asymmetry* **1999**, *10*, 3449.

(374) Nazabadioko, S.; Pérez, R. J.; Brieva, R.; Gotor, V. *Tetrahedron: Asymmetry* **1998**, *9*, 1597.

(375) Monterde, M. I.; Brieva, R.; Gotor, V. *Tetrahedron: Asymmetry* **2001**, *12*, 525.

(376) Fechter, M. H.; Gruber, K.; Avi, M.; Skranc, W.; Schuster, C.; Pöchlauer, P.; Klepp, K. O.; Griengl, H. *Chem.—Eur. J.* **2007**, *13*, 3369.

(377) Avi, M.; Gaisberger, R.; Feichtenhofer, S.; Griengl, H. *Tetrahedron* **2009**, *65*, 5418.

(378) Weis, R.; Gaisberger, R.; Skranc, W.; Gruber, K.; Glieder, A. *Angew. Chem., Int. Ed.* **2005**, *44*, 4700.

(379) Pscheidt, B.; Liu, Z.; Gaisberger, R.; Avi, M.; Skranc, W.; Gruber, K.; Griengl, H.; Glieder, A. *Adv. Synth. Catal.* **2008**, *350*, 1943.

(380) Nanda, S.; Kato, Y.; Asano, Y. *Tetrahedron* **2005**, *61*, 10908.

(381) Nanda, S.; Kato, Y.; Asano, Y. *Tetrahedron: Asymmetry* **2006**, *17*, 735.

(382) Han, S.-Q.; Ouyang, P.-K.; Wei, P.; Hu, Y. *Biotechnol. Lett.* **2006**, *28*, 1909.

(383) Bhunya, R.; Mahapatra, T.; Nanda, S. *Tetrahedron: Asymmetry* **2009**, *20*, 1526.

(384) Chapman, P. J.; Meerman, G.; Gunsalus, I. C.; Srinivasan, R.; Rinehart, K. *J. Am. Chem. Soc.* **1966**, *88*, 618.

(385) Grogan, G.; Graf, J.; Jones, A.; Parsons, S.; Turner, N. J.; Flitsch, S. L. *Angew. Chem., Int. Ed.* **2001**, *40*, 1111.

(386) Hill, C. L.; Verma, C. S.; Grogan, G. *Adv. Synth. Catal.* **2007**, *349*, 916.

(387) Miyamoto, K.; Ohta, H. *J. Am. Chem. Soc.* **1990**, *112*, 4077.

(388) (a) Matoishi, K.; Hanzawa, S.; Kakidani, H.; Suzuki, M.; Sugai, T.; Ohya, H. *Chem. Commun.* **2000**, 1519. (b) Matoishi, K.; Ueda, M.; Miyamoto, K.; Ohta, H. *J. Mol. Catal. B: Enzym.* **2004**, *27*, 161. (c) Ijima, Y.; Matoishi, K.; Terao, Y.; Doi, N.; Yanagawa, H.; Ohta, H. *Chem. Commun.* **2005**, 877.(d) Terao, Y.; Ijima, Y.; Miyamoto, K.; Ohta, H. *J. Mol. Catal. B: Enzym.* **2007**, *45*, 15.

(389) (a) Okrasa, K.; Levy, C.; Hauer, B.; Baudendistel, N.; Leys, D.; Micklefield, J. *Chem.—Eur. J.* **2008**, *14*, 6609. (b) Kuettner, E. B.; Keim, A.; Kircher, M.; Rosmus, S.; Sträter, N. *J. Mol. Biol.* **2008**, *377*, 386.

(390) Okrasa, K.; Levy, C.; Wilding, M.; Goodall, M.; Baudendistel, N.; Hauer, B.; Leys, D.; Micklefield, J. *Angew. Chem., Int. Ed.* **2009**, *48*, 7691.

(391) García-Urdiales, E.; Alfonso, I.; Gotor, V. *Chem. Rev.* **2005**, *105* (1), 313.