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 *c*. US $10 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 biocatalysts in their syntheses. This is mainly because, in their natural form, most of the enzymes are very sensitive catalysts that exert their activity mainly 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, 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, accord-

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ingly, 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, especially from 1999 on. 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

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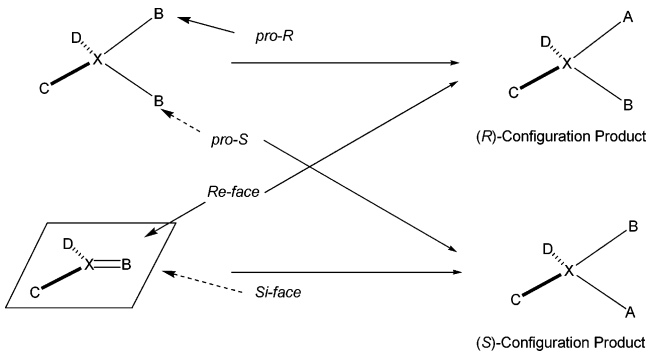
or can constitute a key step in a synthetic route, or aid to rationalize either the substrate specificity of an enzyme or the desymmetrization of a certain class of compounds. Accordingly, important parameters to which attention has been paid are enantioselectivity and the yield of the EED, which should 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,

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.

when it is arbitrarily assigned CIP priority[10] over the other enantiotopic group, the configuration of the

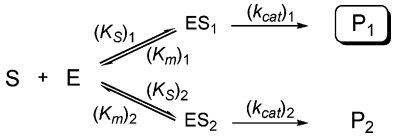
generated chiral center is assigned the stereodescriptor *R*. Alternatively, the other group is described as *pro-S*. Although this nomenclature is also applicable to the enantiotopic faces of a trigonal system, for this case, the *Re* and *Si* terminology is more often used. Thus, the stereoheterotropic face of a trigonal atom is 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

Scheme 2



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 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.

$$E = \frac{1 + ee_p}{1 - ee_p} \tag{4}$$

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] 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,[14] 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.[15] However, even if no information from the enzyme active site is available it is possible to improve its enantioselectivity by means of pseudo-controlled random mutagenesis performed over subsequent generations coming from a parent wild-type enzyme. Such an approach is termed directed evolution.[13,16]

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 ionic liquids is maybe the most striking advance in this field.[17] With respect to reactions carried out in conventional solvents, reactions in ionic liquids have different thermodynamic and kinetic behaviors, which often lead to improved process performance. Furthermore, ionic liquids 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.[18]

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.[19]

4. Hydrolyses and Transesterifications

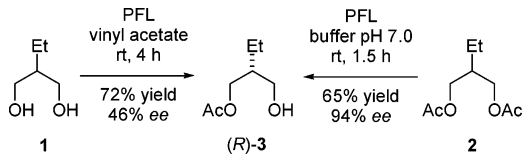
Hydrolases are one of the classes of enzymes most used in synthetic chemistry.[20] The lack of sensitive cofactors that would have to be recycled, and a large number of readily available enzymes possessing relaxed substrate specificities to choose from, are the main features that account for this preference. All 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 been recently 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.[21]

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[22] 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

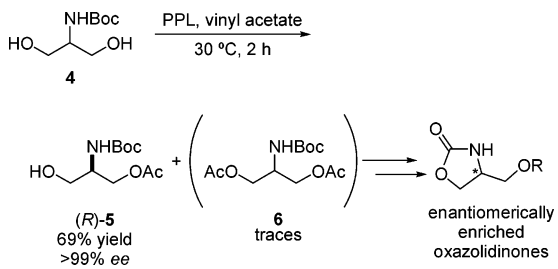
Scheme 3



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.

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[23] 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

Scheme 4

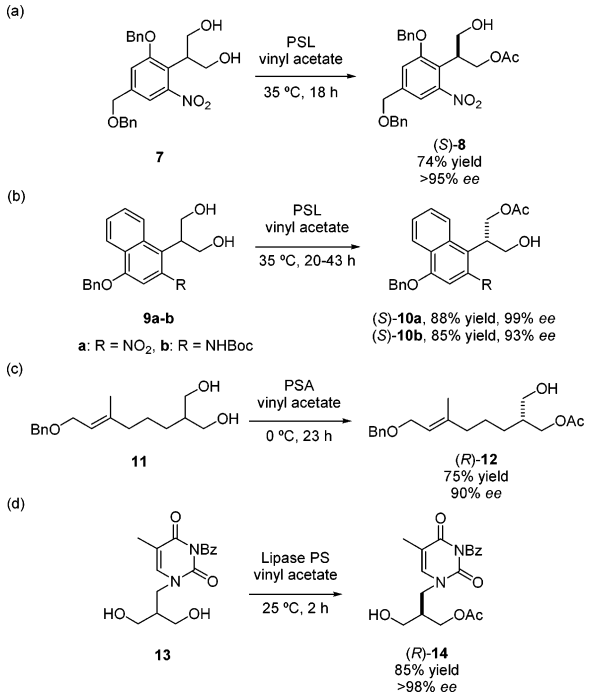


conditions (*R*)-(−)-3-*O*-acetyl-2-*N*-(*tert*-butoxycarbonyl)serinol [(*R*)-**5**] was obtained enantiopure and in high yield when PPL was used as catalyst in vinyl acetate. Depending on the reaction conditions, different amounts of the diacetate were obtained, which were presumably formed from the unwanted enan-

tiomer of the mono-acetylated product, thereby providing a self-correcting process.

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.[24] 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

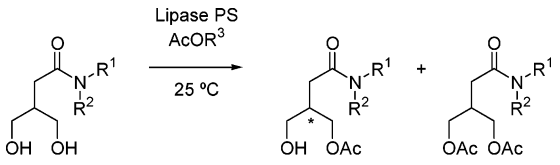
Scheme 5



corresponding monoacetate [(*S*)-**8**] was obtained in 74% yield and >95% *ee*. Using similar experimental conditions, Boger and co-workers[25] 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 hairpencils of male Danaus chrysippus (African Monarch),[26] and (*R*)-**14**, precursor of a novel β-amino acid[27] in high *ee*'s and yields.

Takabe et al.[28] 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 (**15a**−**d**), which, after optimization of the reaction conditions, afforded the corresponding monoacetates (**16a**−**d**) in high *ee*'s (Table 1). Unfortunately, the yields were somewhat low ,and significant amounts of the diacetates (**17a**−**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

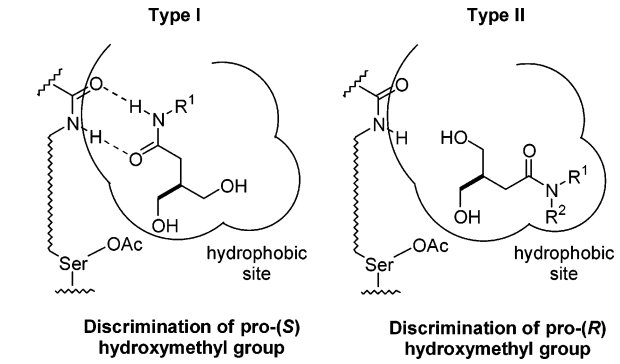
Table 1. EED of 2-Carbamoylmethyl-1,3-propanediols



					monoacetate		diacetate	
entry	substrate	R^1	R^2	R^3	yield (%)	*ee* (%)	config	yield (%)
1	**15a**	Me	H	Ph	26	>99	*S*	37
2	**15b**	Et	H	Ph	43	94	*S*	20
3	**15c**	Me	Me	Ph	77	96	*R*	trace
4	**15d**	Et	Et	vinyl	52	96	*R*	27

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

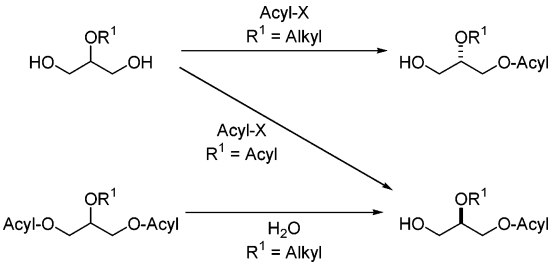
Chart 1



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 the preparation of different C_3 chiral building blocks. Lipase-catalyzed transesterifications[29] and hydrolyses[30] 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-

Scheme 6



2-*O*-benzylglycerol was incubated in phosphate buffer pH 7.[29c] 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*.[31]

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.[32] 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[33] 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 **18** (Scheme 7a) catalyzed by lipase AK. Subsequent protection of the resulting monoacetate with *tert*-butylchlorodimethylsilane afforded the *O*-protected monoalcohol (*S*)-**19** in good *ee* and high yield. Similarly, (*S*)-α-tocotrienol has been prepared in 19% overall yield[34] through a multistep synthesis that involves the CALB-catalyzed acetylation of the bicyclic triol **20** (Scheme 7b). The monoester (*S*)-**21** was obtained in 98% *ee* and 60% yield, which can be increased because the diester **22** (27%) can be easily recycled by nonenzymatic hydrolysis.

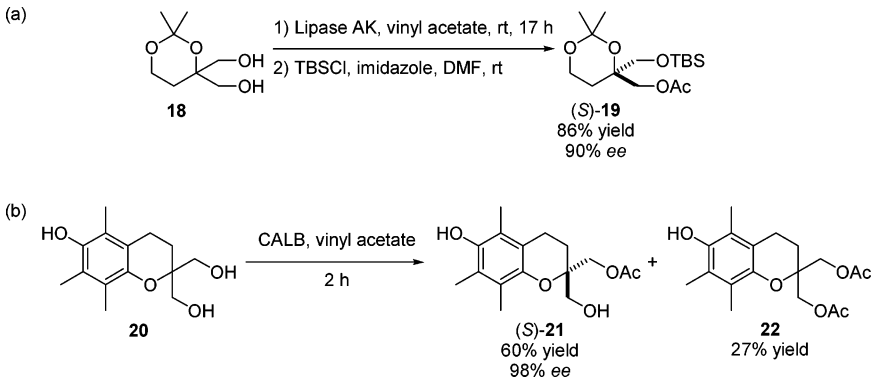
1-Aminocyclopropane-1-carboxylic acid and their derivatives are attractive synthetic targets because of their interesting properties either isolated or being part of the sequence of a peptide. Both enantiomers of 1-amino-2,2-difluorocyclopropane-1-carboxylic acid have been synthesized in a preparative 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 (**23**) and the corresponding diol (**25**) by means of a lipase PS-catalyzed hydrolysis and transesterification, respectively (Scheme 8).[35]

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 the monoester **27** was a key component (Scheme 9).[36] This compound was successfully prepared by means of a PLE-catalyzed hydrolysis of the diacetate **26**. The (*S*)-monoester was obtained as the major product in moderate *ee* and accompanied by different amounts of diol **28**, which depended on the reaction conditions. The authors have also made use of the so-obtained monoester (*S*)-**27** for the preparation of different α-substituted serine amino acid analogues.[37]

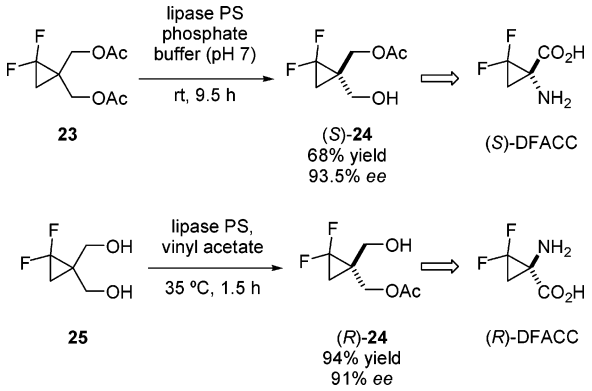
The EED of 2,2-disubstituted propane-1,3-diols using well-known acyl donors, i.e., vinyl and isopropenyl acetates, is a process that, although it has proven to be successful, usually suffers from low reactivity. Moreover, racemization of the products via acyl group migration occurs under different reaction 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.[38] Kita and co-workers have solved these problems by using different 1-ethoxyvinyl esters as acyl donors.[39] 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).[40]

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:[41] (1) It can be readily prepared from commercially available ethoxyacetylene and 2-furoic acid in a large scale in

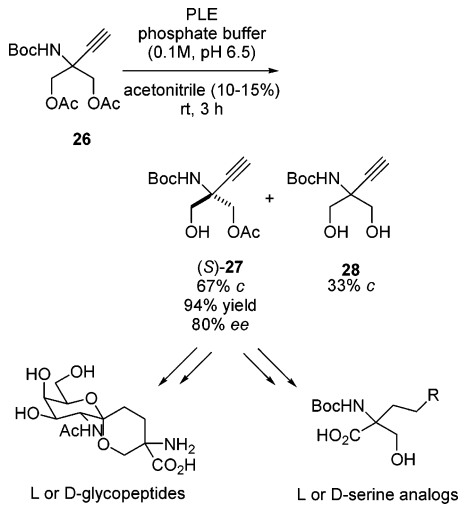
Scheme 7



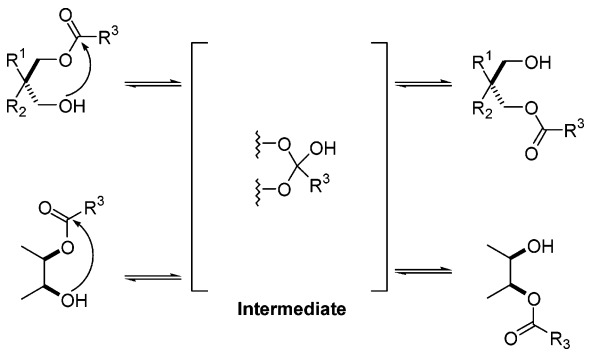
Scheme 8



Scheme 9



Scheme 10

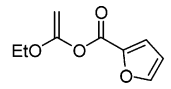


high yield and can be stored in a refrigerator for more than one year. (2) Conversely to other acyl donors, its reactions are usually complete within several hours to give the products in higher optical and chemical yields. (3) If necessary, the only side products, the diesters, can be recycled to the 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.

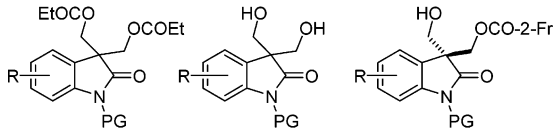
The desymmetrization of oxindoles bearing a quaternary stereogenic carbon is a very interesting process since its products can be found in different natural products and are also regarded as chiral building blocks in indole syntheses. In particular, the desymmetrization of prochiral 3,3-bis(hydroxymethyl)-1-methoxymethyl-2-oxindole (**30a**) 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).[42] After optimization of the biocatalyst and acyl donor, the hydrolysis of the dipropionate **29** 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 **30a**. 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).[43] 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 **30b**−**f** generally produced the corresponding (*R*)-monoalcohols [(*R*)-**31b**−**f**] with high optical and chemical yields (entries 4−8). On the contrary, the desymmetrization of the *N*-alkyl derivatives **30a**,**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 **30a**−**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*-**32** catalyzed by *Candida rugosa* lipase and using vinyl acetate as acylating agent.[44] The monoester (2*R*,3*R*,4*S*)-**33** 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 **34** by vinyl acetate (solvent and acyl donor) in the presence of porcine pancreas lipase (Scheme 11b).[45] This reaction afforded monoacetate (2*R*,3*R*,4*S*,5*R*,6*R*,7*S*,8*S*)-**35** in good yield and enantiopure, and the enzyme was highly regioselective for a primary alcohol end group, the two unproctected secondary alcohols being left untouched.

Table 2. EED of Oxindoles



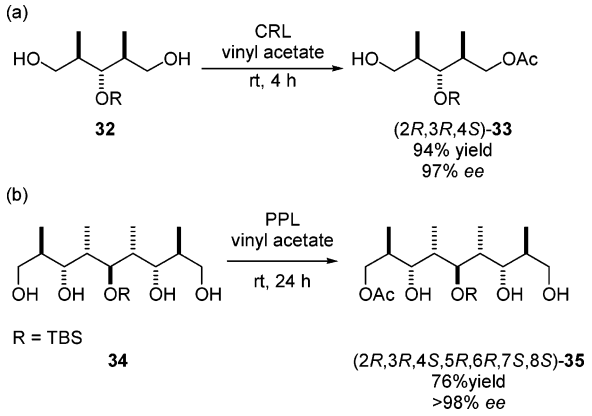
1-ethoxyvinyl 2-furoate



29 30a-h 31a-h

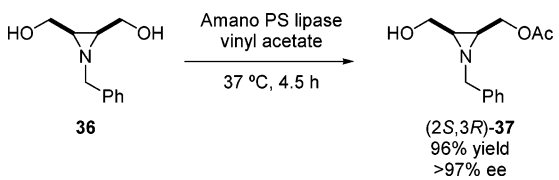
							monoalcohol	
entry	substrate	PG	R	reaction	*t* (h)	enzyme	yield (%)	*ee* (%)
1	**29**	MOM	H	hydrolysis	8	cholinesterase	20	70
2	**29**	MOM	H	hydrolysis	120	cholinesterase	38	95
3	**30a**	Me	H	transesterification	7	CRL	53	87
4	**30b**	BOC	H	transesterification	22	CRL	93	99
5	**30c**	BOC	5-OMe	transesterfication	3	CRL	77	98
6	**30d**	BOC	6-OMe	transesterfication	19	CRL	79	91
7	**30e**	Cbz	H	transesterification	58	CRL	71	98
8	**30f**	Ac	H	transesterification	48	CRL	90	97
9	**30g**	MOM	H	transesterification	64	CRL	34	86
10	**30h**	Bn	H	transesterification	144	CRL	59	68

Scheme 11



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[46] have carried out the Amano PS lipase-catalyzed desymmetrization of aziridine **36**, whose monoacetylated derivative, obtained in high yield and *ee* (Scheme 12), is related to a key inter-

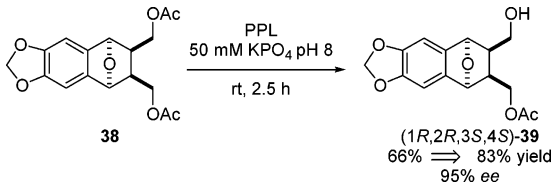
Scheme 12



mediate 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 **38** (Scheme 13),[47] the corre-

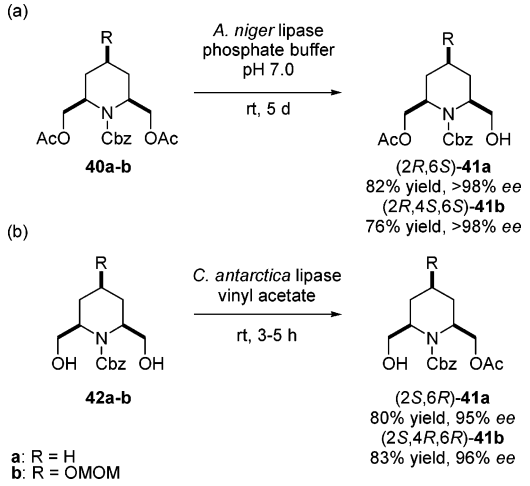
Scheme 13



sponding monoaceteate (**39**) 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 **39**, 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 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 **40a**−**b** afforded, at moderate rate, monoacetates **41a**−**b** enantiopure and in high yields (Scheme 14a).[48] The stereoselective acylation of the corresponding diols (**42a**−**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).[49] The usefulness of these so-obtained chiral building blocks was demonstrated

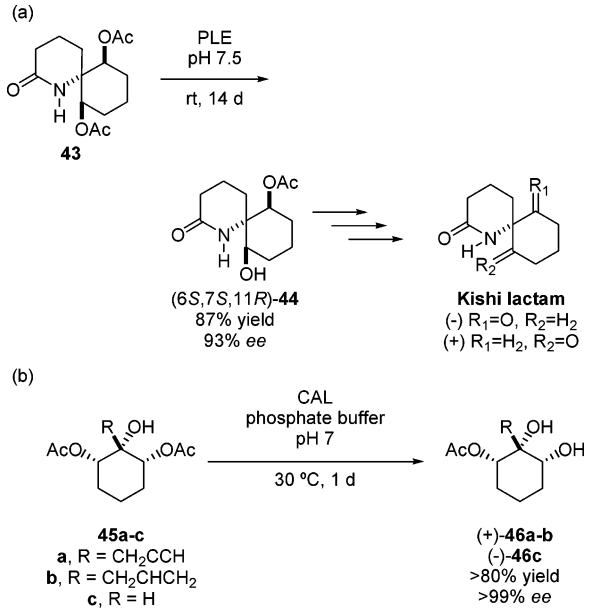
Scheme 14



by their use in the syntheses of different biologically active compounds.[49,50]

The desymmetrization of acetylated derivatives of *meso* polyhydroxylated cyclohexanes by means of enzymatic hydrolyses has proven to be highly efficient. For instance, 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 **43** (Scheme 15a).[51] This way, the corresponding monoacetate

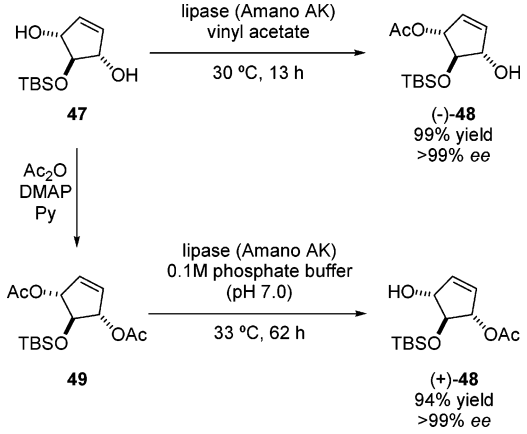
Scheme 15



(6*S*,7*S*,11*R*)-**44** was obtained in high yield and *ee*. Following a similar approach, the diacetates of different *meso* 2-substituted cyclohexane-1,2,3-triols (**45a**−**c**) were desymmetrized by means of CAL-catalyzed hydrolyses, from which the corresponding monoacetates were obtained enantiopure and in excellent yields (Scheme 15b).[52] Monoacetate (+)-**46a** 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 (**48**) were conveniently prepared via enzymatic desymmetrization of triol **47** and its derivative **49** (Scheme 16).[53] Lipase AK (Amano) efficiently catalyzed the

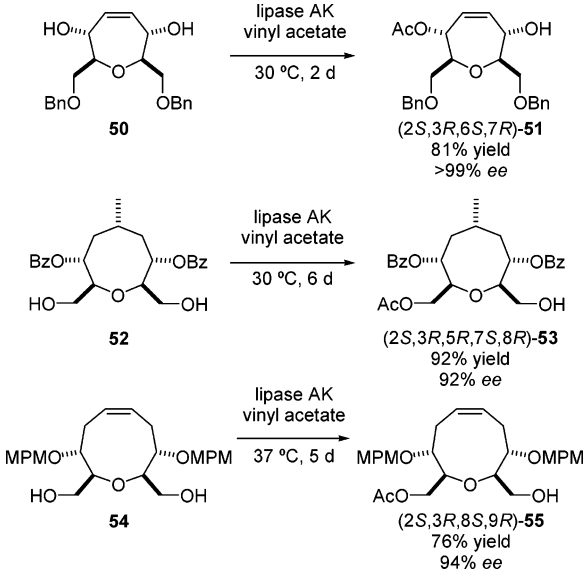
Scheme 16



transesterification of *meso* 5-*tert*-butyldimethylsilyloxy-2-cyclopentene-1,4-diol (**47**) as well as the hydrolysis of its diacetate (**49**). Both monoesters were obtained enantiopure and in very high yields.

This lipase 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.[54] The enantioselective transesterification with vinyl acetate of the *meso* alcohols **50**, **52**, and **54** (Scheme 17), obtained by a ring-expansion strategy,

Scheme 17

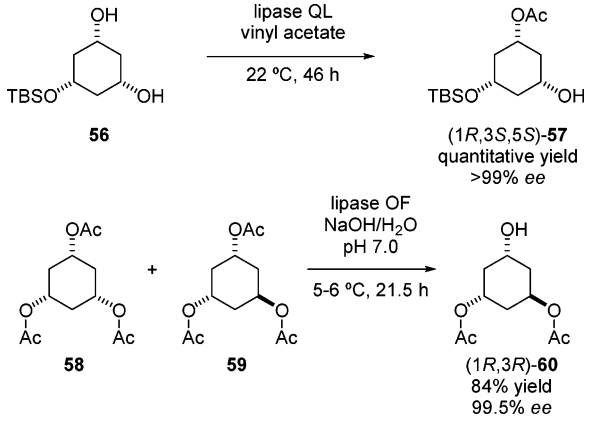


also afforded the corresponding monoacetates in high yields and *ee*'s.

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 18).[55] Lipase QL efficiently catalyzed

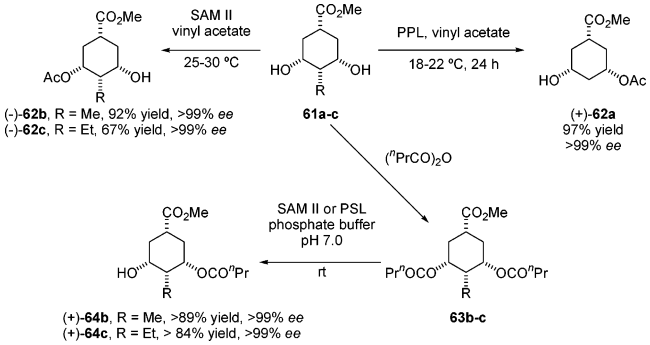
Scheme 18



the desymmetrization of the *meso* diol **56**, yielding the monoacetate (1*R*,3*S*,5*S*)-**57** quantitatively and enantiopure.[56] Additionally, Lipase OF catalyzed well the hydrolysis of only *trans*-(**59**) 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 (**61a**−**c**) was investigated (Scheme 19).[57] The results

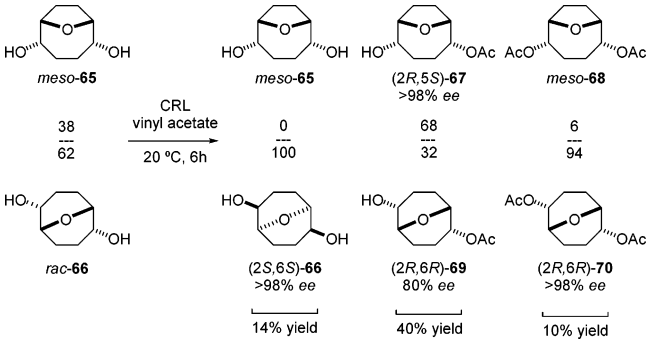
Scheme 19



obtained showed that preparative useful enantioselectivities were found for the PPL- and SAM II-catalyzed transesterifications of **61a** and **61b**−**c**, respectively, in which the two enzymes showed opposite enantiopreferences. The hydrolyses of the dibutyrates **63b**−**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.

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 20).[58] Namely, several li-

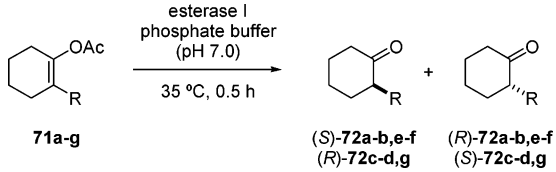
Scheme 20



pases and esterases were screened for the transesterification of the 38:62 mixture of *meso*-**65** and *rac*-**66**, obtained from the transannular *O*-heterocyclization of *cis*,*cis*-cycloocta-1,5-diene. When *C. rugosa* lipase, which proved to be the most efficient enzyme, was used as catalyst, a 67% of degree of conversion was reached after 6 h of reaction. That means that *meso*-**65** was transformed almost quantitatively, while the conversion of the racemate **66** stopped at 50% conversion. After separation of the enzyme, (−)-**66** was isolated enantiopure in 14% yield. Furthermore, (+)-**70** was also isolated enantiopure but was contaminated with 6% of *meso* **68**. Finally, a 68:32 mixture of enantiopure (+)-**67** and (+)-**69** (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*.[59] Several cyclohexanone enol acetates (**71a**−**g**) were subjected to enzymatic hydrolysis with these esterases to clarify the effect of various substituents at the β-position to the acetoxyl 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, and that only esterase I afforded, in some cases, synthetically useful results (Table 3). For this enzyme, the acetate **71a** was the best substrate (entry 1), (*S*)-**72a** being obtained enantiopure and in excellent yield (99%). Replacement of the methyl group by an ethyl or an *i*-propyl substituent (**71b**−**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 (**71d**−**g**, entries 4−7) an inversion of the enzymatic enantioselectivity was observed. The au-

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

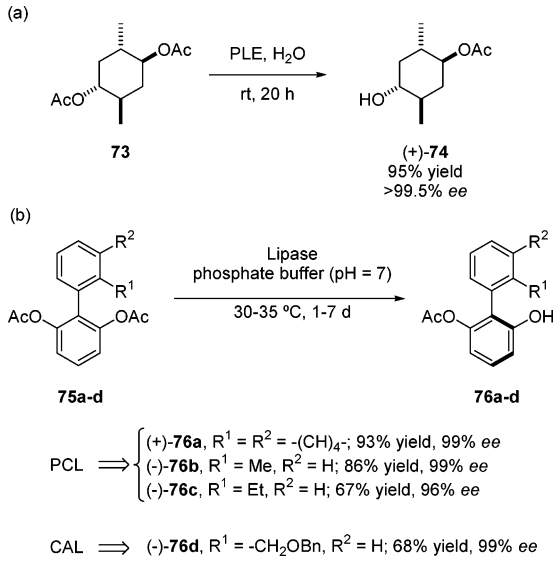


entry	substrate	R	product	*c* (%)	*ee* (%)
1	**71a**	Me	(*S*)-**72a**	>99	>99
2	**71b**	Et	(*S*)-**72b**	>99	14
3	**71c**	*i*-Pr	(*R*)-**72c**	10	17
4	**71d**	*t*-Bu	(*S*)-**72d**	21	5
5	**71e**	*n*-Pr	(*R*)-**72e**	>99	>99
6	**71f**	*n*-Pnt	(*R*)-**72f**	20	26
7	**71g**	Bn	(*S*)-**72g**	15	>99

thors 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.

Trauner and co-workers[60] have reported one of the few examples of the desymmetrization of molecules not possessing a symmetry plane (σ). Thus, the centrosymmetric *meso* 1,4-diacetoxy-2,5-dimethylcyclohexane (**73**), 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 21a). The monoester (+)-**74** was isolated

Scheme 21



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 **73** 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[61] have published the first application of an enzyme to desymmetrize axially chiral nonracemic biaryls (Scheme 21b). 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 **76a**−**d** in high *ee*'s and yields by desymmetrization of the corresponding σ-symmetric diacetates (**75a**−**d**) through lipase-catalyzed hydrolyses using lipases from *Candida antarctica* and *Pseudomonas fluorescens*.

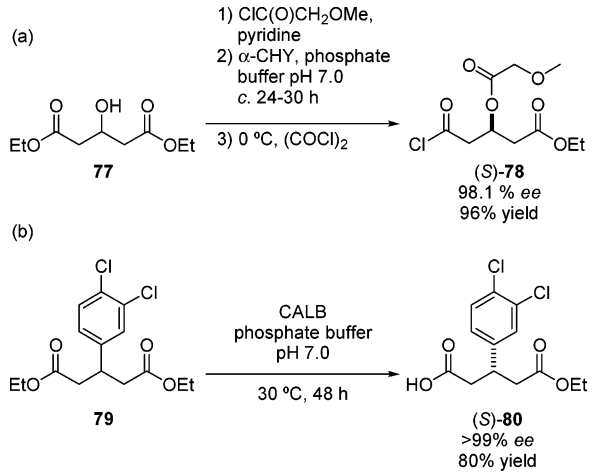
4.2. Carboxylic Acid Derivatives

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 during the recent past years.

For instance, 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.[62] 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 22a). Simi-

Scheme 22

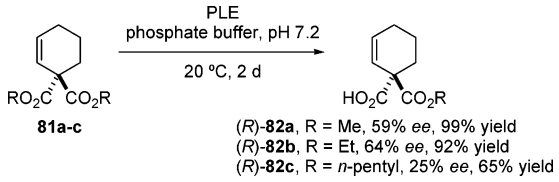


larly, the enzymatic desymmetrization of the prochiral diethyl 3-[3′,4′-dichlorophenyl]-glutarate (**79**), an intermediate in the synthesis of a series of neurokinin receptor antagonists, has been successfully developed and scaled up (Scheme 22b).[63] The hydrolysis of a similar prochiral glutarate diester had been studied, resulting in the product with the (*R*)-configuration.[64] 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 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*)-configura-

tion. In the pilot plant, the process produced 200 kg of enantiopure (*S*)-**80** 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.

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*.[65] Likewise, PLE-catalyzed hydrolysis of a series of dialkyl esters of 2-cyclohexene-1,1-dicarboxylate (**81a**−**c**) afforded the monoesters of (*R*)-configuration [(*R*)-**82a**−**c**] in high yields and moderate *ee*'s (Scheme 23).[66] The ethyl

Scheme 23

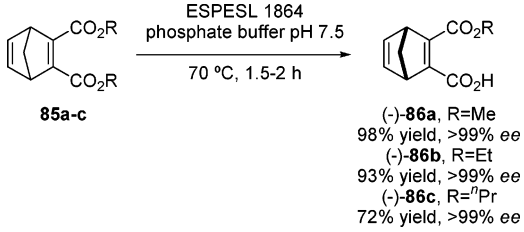


monoester (*R*)-**82b** was further employed in a synthetic route toward an α-substituted serine derivative.

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[67] has reported an attempt to extend this methodology to the desymmetrization of a series of *meso cis*-cyclohexane-1,3-dicarboxylic acid diesters (**83a**−**e**, Table 4), 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 (**83b**, entry 2) being the best substrate. This way both enantiomers of the monoester **84b** were prepared in high *ee* and overall yield (>80%).

Kashima et al. have reported the first example in which a termophilic esterase/lipase is used for the desymmetrization of a *meso* diester.[68] 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 24). In all cases examined, the monoesters

Scheme 24



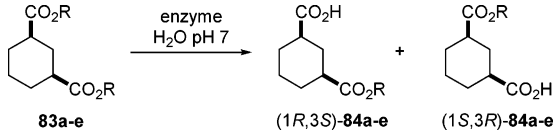
(−)-**86a**−**c** were obtained in high chemical yields and enantiomeric purities.

Recently, the first enzymatic desymmetrization of a prochiral phosphine oxide has been reported.[69] Namely, bis(methoxycarbonylmethyl)phenylphosphine oxide (**87**) 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 25a). The expected product, viz. the monoester of (*R*)-configuration [(*R*)-**88**], 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.[70] Additionally, both enantiomers of the related monoacetate **90** were obtained by means of enzymatic hydrolysis and transesterification reactions, respectively (Scheme 25b). Conversely to the case of dicarboxylate **87**, 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

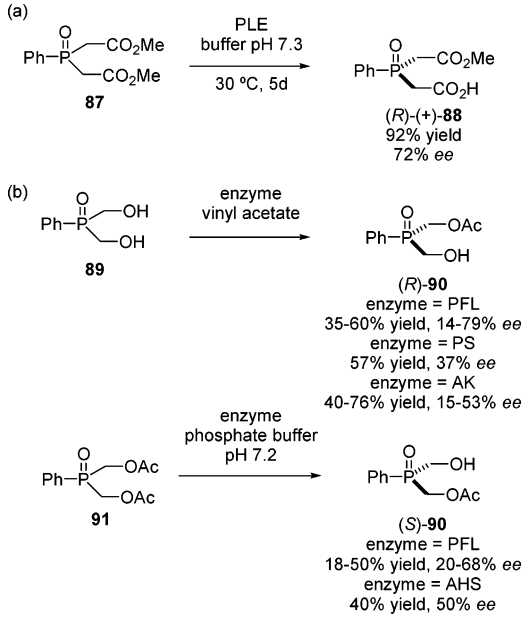
Although hydrolases are also able to catalyze, in a highly enantioselective fashion, the desymmetrization of other *meso* and prochiral carboxylic acid derivatives apart from esters, surprisingly, they have been used with little profusion. The employment of different synthetically equivalent reagents consti-

Table 4. 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	**83a**	Me	(1*R*,3*S*)-**84a**	82	(1*R*,3*S*)-**84a**	16	(1*S*,3*R*)-**84a**	62
2	**83b**	Et	(1*R*,3*S*)-**84b**	96	(1*R*,3*S*)-**84b**	34	(1*S*,3*R*)-**84b**	96
3	**83c**	*n*-Pr	(1*R*,3*S*)-**84c**	88	(1*R*,3*S*)-**84c**	32	(1*S*,3*R*)-**84c**	95
4	**83d**	*i*-Pr	(1*R*,3*S*)-**84d**	54	(1*R*,3*S*)-**84d**	32	(1*S*,3*R*)-**84d**	99
5	**83e**	*n*-Bu	(1*R*,3*S*)-**84e**	90	(1*R*,3*S*)-**84e**	47	(1*S*,3*R*)-**84e**	86

Scheme 25

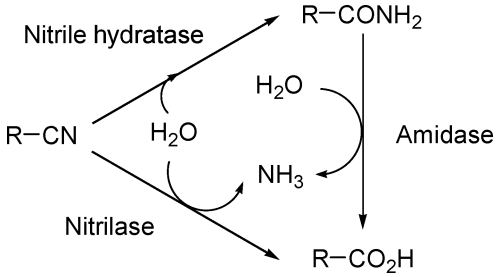


tutes a well-established methodology for the optimization of the enantioselectivity shown by an enzyme in the transformation of a particular class of compounds (substrate engineering). Thereby, we strongly believe that the application of acyl donors different from esters to biocatalysis and, more specifically, 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.[71] 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.[72] Ostaszewski et al.[73] have recently carried out the desymmetrization of 3-phenylglutaric anhydrides (**92a**−**c**) by means of enzymatic monoesterification (Table 5). The so-obtained optically active monoesters **93a**−**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 **92a** 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 **92a** > **92b** > **92c** independently of the catalyst used; meanwhile, for the enantioselectivity, although it also varied, no clearly defined trend was found.

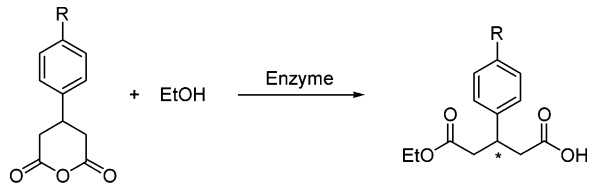
The enzyme-catalyzed hydrolysis of nitriles has been shown to proceed through two distinct pathways (Scheme 26).[74] Nitrilase enzymes convert a nitrile

Scheme 26



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.[74]

Table 5. EED of 3-Phenylglutaric Anhydrides



92a-c 93a-c

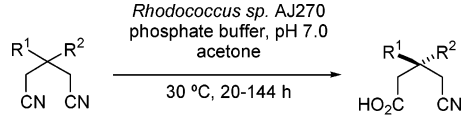
	93a (R = H)			**93b** (R = Cl)			**93c** (R = OMe)		
enzyme	major enantiomer	*ee* (%)	*t* (h)	major enantiomer	*ee* (%)	*t* (h)	major enantiomer	*ee* (%)	*t* (h)
novozyme 435	*S*	78	61	*S*	67	120	*S*	67	528
chirazyme L-2, C3	*S*	83	19	*S*	71	96	*S*	75	288
Amano PS	*R*	78	384						
Amano PS immobilized	*R*	78	19	*S*	17	96	*S*	99	168

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-[75] and butylmethylmalonodinitriles[76] 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.[77] 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 **94a**−**i** catalyzed by *Rhodococcus sp.* AJ270 cells, afforded the corresponding optically active cyanobutanoic acids (*S*)-**95a**−**i** with low to moderate enantiomeric excesses (Table 6).[78]

Table 6. EED of 3-Substituted Glutarodinitriles



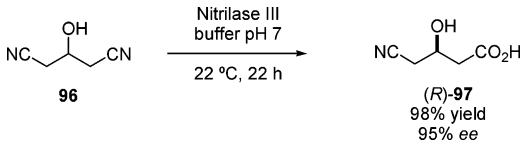
94a-i (*S*)-95a-i

entry	compound	R^1	R^2	*t* (h)	yield (%)	*ee* (%)
1	**94a**	Ph	H	24	67	88
2	**94b**	4-F-C_6H_4	H	24	16	76
3	**94c**	4-Cl-C_6H_4	H	72	25	63
4	**94d**	4-Me-C_6H_4	H	24	25	95
5	**94e**	2-Me-C_6H_4	H	20	40	35
6	**94f**	4-MeO-C_6H_4	H	48	17	79
7	**94g**	Cy	H	20	60	83
8	**94h**	Bn	H	20	62	32
9	**94i**	Ph	Me	144		

Different additives were tested and acetone, which presumably interacts with the biocatalyst, proved to dramatically enhance the enantioselectivity, giving, in some cases, enantiomeric 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 (**94i**, entry 9) causes loss of reactivity. Attempts to increase the yield of the monoacids (*S*)-**95a**−**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 (**96**) were found.[79] In particular, one of these (*R*)-specific nitrilases afforded (*R*)-**97** in excellent yield and *ee* (Scheme 27), a

Scheme 27



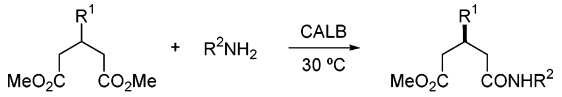
96 (*R*)-97
98% yield
95% *ee*

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 novel directed evolution technique Gene Site Saturation Mutagenesis permitted solving of this problem.[80] 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 **96** to (*R*)-**97** 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*)-**97** with 90−98% *ee*.

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. All 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 recently studied the desymmetrization of different dimethyl 3-substituted glutarates (**98a**−**g**) through enzymatic aminolyses and ammonolyses (Table 7).[81] 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*)-**100a**−**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 (**99a**−**c**, see for instance entries 1−3). As a general trend, those diesters

Table 7. EED of Dimehyl 3-Substituted Glutarates



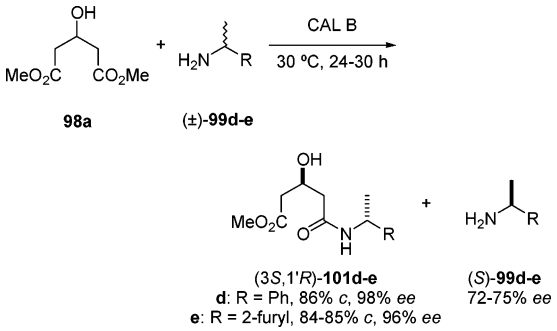
98a-g **99a-c** (*S*)-**100a-g**

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

bearing a heteroatom in R^1 (**98a**−**d**, entries 1−12) displayed the best yields and enantioselectivities. Conversely, diesters bearing the aliphatic methyl group (**98e**, entries 13−15) or the aromatic moieties Ph and *p*-F−Ph (**98f**−**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 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[82] and (*R*)-3,4-diaminobutanoic acid.[83]

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.[84] have described an elegant desymmetrization-resolution scheme based on CAL B-catalyzed aminolysis reactions of dimethyl 3-hydroxyglutarate (**98a**) with different racemic amines [(±)-**99d**−**e**] (Scheme 28). 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 **101d**−**e** with excellent yields, and enantiomeric and diastereomeric excesses.

Scheme 28



98a (±)-**99d-e**

(3*S*,1′*R*)-**101d-e**
d: R = Ph, 86% *c*, 98% *ee*
e: R = 2-furyl, 84-85% *c*, 96% *ee*

(*S*)-**99d-e**
72-75% *ee*

6. Redox Reactions

The redox transformation of different substrates is another biocatalytic process that has been widely used in organic synthesis. 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, making its substitution by synthetic ones not a promising alternative. These facts are still responsible for hampering the application of isolated oxidoreductases in industrial processes.

There is an enormous amount of examples of EEDs by means of redox processes using cells 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 activites, the enzymes require a cofactor such as NADH or NADPH, from which a hydride is transferred to the substrate carbonyl carbon, and a hydrogen source. The enzymatic oxidation of the latter (cosubstrate) is coupled 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,[85] thus allowing the utilization of whole-cell catalysts which consume cheaper and environmentally friendly sources of energy. Although it is true that these systems also facilitate the utilization of isolated enzymes, as only catalytic amounts of the cofactor are needed, whole-cell catalysts continue to be mostly used.

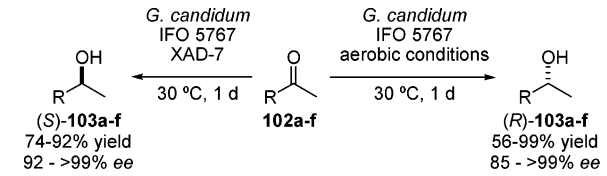
Baker's yeast (*Saccharomyces cerevisiae*) has been the most popular whole-cell biocatalyst for the desymmetrization of prochiral ketones.[86] Reductions catalyzed by this microorganism tolerate a large diversity of carbonyl substrates and, in general, follow Prelog's rule. This broad substrate acceptance 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, and genetic manipulation have allowed the optimization of Baker's yeast and other microorganisms enantioselectivity and opened a route to obtain both enantiomeric series of secondary alcohols.[87] 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[88] 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%).[89]

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.[90] 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.[91] 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 (**103a**−**f**) using ketones **102a**−**f** as the starting materials (Scheme 29).[92] *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.

Scheme 29

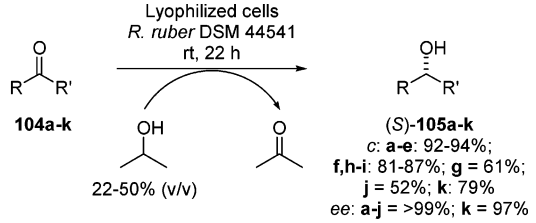


R: **a** = Ph; **b** = *p*-Br-Ph; **c** = $(CH_2)_2$Ph; **d** = CH_2OPh; **e** = 2-Py; **f** = 3-Py;

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[93] have recently 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 **104a**−**k** giving (*S*)-configured alcohols (*S*)-**105a**−**k** in high yields and excellent *ee*'s using 2-propanol as a hydrogen donor at 22% v/v (Scheme 30).[94] As an exception, the bicyclic ketones 1-indanone

Scheme 30



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};

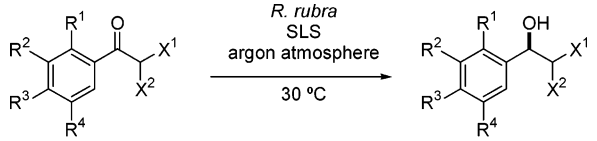
and 1-tetralone could not be reduced employing this method. Furthermore, this microbial redox system is able to produce the opposite enantiomeric series of these alcohols through oxidative KR of the corresponding racemic alcohols. Comparison of the structure activity/enantioselectivity relationships for the alcohols and their ketone counterparts revealed different trends for both processes.

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.[95] 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.

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 **106a**−**h** were subjected to reduction to yield the (*R*)-2-bromo-(1-phenyl/substituted phenyl) ethanol derivatives (*R*)-**107a**−**f** (Table 8).[96] 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 (**106a**, entry 1) was improved to yield alcohol (*R*)-**107a** in excellent *ee* and yield. Similar results were obtained for the rest of substrates tested (entries 2−6) with the exception of **106g**−**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*)-**107c**−**d** were subsequently employed to synthesize the active components of denopamine and salmeterol, respectively.[97]

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).[98] 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*)-

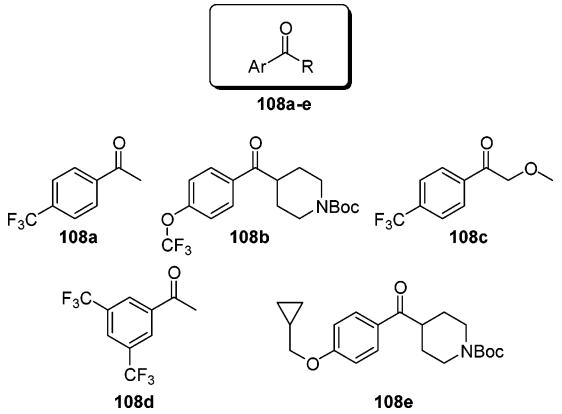
Table 8. EED of ω-Bromoacetophenone Derivatives



106a-h (*R*)-**107a-f**

entry	compound	R^1	R^2	R^3	R^4	X^1	X^2	*t* (h)	yield (%)	*ee* (%)
1	**106a**	H	H	H	H	H	Br	30	90	95
2	**106b**	H	H	H	Cl	H	Br	48	77	95
3	**106c**	H	H	$PhCH_2O$	H	H	Br	48	81	94
4	**106d**	H	H	$PhCH_2O$	CH_2OH	H	Br	48	79	95
5	**106e**	H	H	$PhCH_2O$	CH_2OH	Br	Br	40	82	92
6	**106f**	H	H	-OCH_2OCH_2-		H	Br	72	69	89
7	**106g**	OH	Br	H	CH_3	H	H	140		
8	**106h**	OH	H	H	CH_3	H	Br	140		

Chart 2

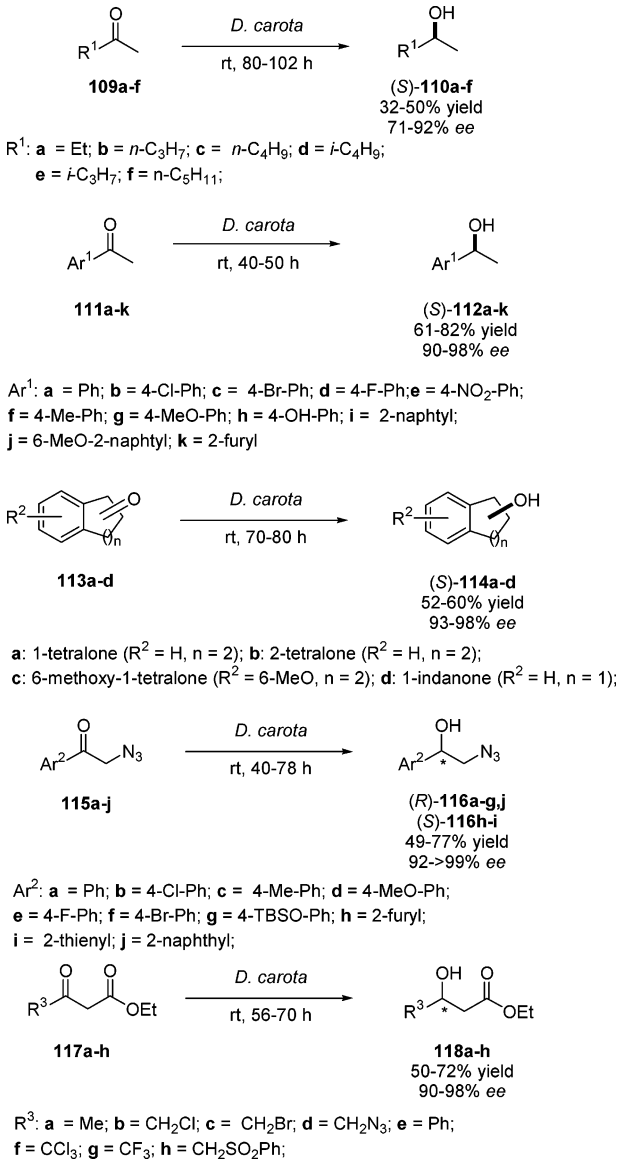


enantiomers of the corresponding alcohols in 92−99% *ee* with yields up to 95% at 1−4 g/L. It is estimated that the described plate format afforded a 5−10-fold reduction in the average screen time compared to a conventional flask-based screening protocol. To demonstrate the utility of the targeted library 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 (**108a**−**c**), an antidepressant NK1 receptor antagonist (**108d**) and an antimuscarinic M2 receptor antagonist (**108e**). 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.

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.[99] Later, Yadav and co-workers systematically studied the bioreduction of aliphatic (**109a**−**f**), aromatic (**111a**−**k**), cyclic (**113a**−**d**), and azido ketones (**115a**−**j**) as well as of β-ketoesters (**117a**−**h**) using *D. carota* root cells (Scheme 31).[100] 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 **115a**−**j** turned out to be a more convenient process than when Baker's yeast was used as catalyst. The azido alcohols **116d**−**g** were further used for the preparation of (*R*)−(−)-denopamine, (*R*)−(−)-tembamide, and (*R*)−(−)-aegeline.[101]

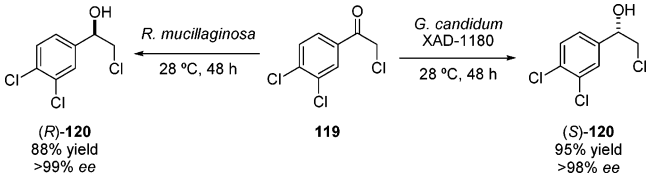
Apart from relatively simple dialkyl or alkyl aryl ketones, other more highly functionalized ketones

Scheme 31



also can be successfully desymmetrized by means of enzyme-catalyzed reductions in a chemoselective fashion. Thus, 3,4-dichlorophenacyl chloride (**119**) 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*)-**120** in excellent *ee* and yield (Scheme 32).[102] This compound was further transformed into

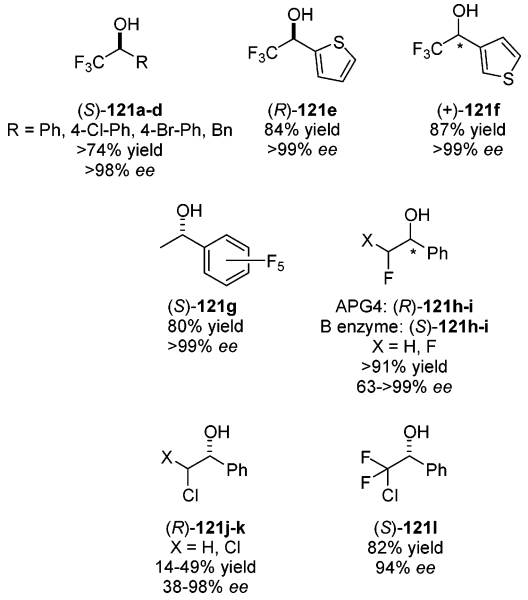
Scheme 32



(*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*)-**120** in excellent yield and *ee* as well.

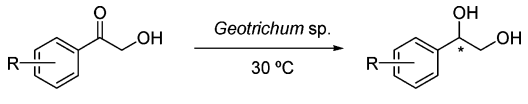
Reduction of different trifluoromethyl ketones using *G. candidum* IFO 4597 (APG4) as catalyst afforded the corresponding (*S*)-alcohols in excellent *ee*'s and yields but with opposite configuration to that observed in the reduction of the analogous methyl ketones.[88] Investigation of this behavior 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 (**121a**−**l**) in excellent *ee*'s (Chart 3).

Chart 3



A series of *o*-, *m*-, and *p*-substituted α-hydroxyphenylethanones (**122a**−**h**) have been reduced to the corresponding 1,2-diols using *Geotrichum* sp. cells as catalyst (Table 9).[103] 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 (**122b**,**e**; entries 2 and 5), whereas (*S*)-1,2-diols were obtained by reduction of unsubstituted or *m*- and *p*-substituted phenacyl alcohols (**122a**,**c**−**d**,**f**−**h**) in good to excellent *ee*'s (entries 1, 3−4, and 6−8). From the reduction of **122h**, 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.

Table 9. EED of Substituted α-Hydroxyphenylethanones



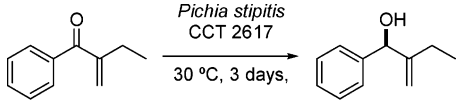
122a-h **123a-h**

entry	substrate	R	*t* (h)	yield (%)	*ee* (%)	configuration
1	**122a**	H	16	62	91	*S*
2	**122b**	*o*-Cl	15	77	60	*R*
3	**122c**	*m*-Cl	12	93	77	*S*
4	**122d**	*p*-Cl	12	91	96	*S*
5	**122e**	*o*-NO_2	15	37	47	*R*
6	**122f**	*m*-NO_2	12	84	98.5	*S*
7	**122g**	*p*-NO_2	10	95	100[a]	*S*
8	**122h**	*p*-CH_3SO_2NH	48	67	99.5	*S*

[a] Theoretical value.

A concise highly chemo- and enantioselective preparation of (*S*)-2-ethyl-1-phenylprop-2-en-1-ol [(*S*)-**125**] via extractive biocatalysis by the yeast *Pichia stipitis* CCT 2617-mediated reduction of 2-ethyl-1-phenylprop-2-en-1-one (**124**) adsorbed on Amberlite XAD-7 has been reported (Scheme 33).[104] The employment

Scheme 33

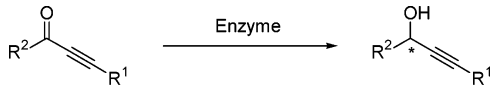


124 (*S*)-**125**
65% yield
>99% *ee*

of the polymer eliminated the limitations observed 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 (**126a**−**r**), affording the corresponding propargylic alcohols (**127a**−**r**) in good yields and excellent *ee*'s (Table 10).[105] 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*)-**127a**−**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 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

Table 10. EED of Acetylenic Ketones



126a-r 127a-r

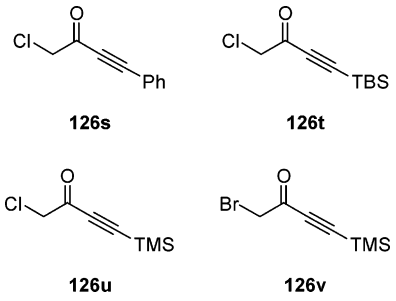
	alcohol			recLBADH			CPCR		
entry	**127**	R^1	R^2	*c* (%)	*ee* (%)	config	*c* (%)	*ee* (%)	config
1	**a**	Ph	Me	>99	>99	*R*	80	>99	*S*
2	**b**	4-MeO-Ph	Me	70	>99	*R*	30	>99	*S*
3	**c**	4-F-Ph	Me	100[a]	>99	*R*	60	>99	*S*
4	**d**	4-Cl-Ph	Me	85	>99	*R*	100[a]	>99	*S*
5	**e**	4-Br-Ph	Me	85	>99	*R*	20	>99	*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.

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 **126s**−**v** (Chart 4).[106] Namely, horse liver ADH

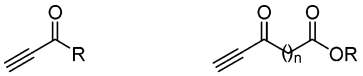
Chart 4



(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 **126a**, 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 oxidoreductase, is also able to afford propargylic alcohols by catalyzing the chemo- and enantioselective reduction of ethynyl ketones **126o**−**q** and **128a**−**g**, and ethynylketoesters **129a**−**f** (Table 11).[107] 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 **128a** (entry 3). Furthermore, ethynyl ketones bearing a small (up to ^{n}Pr) alkyl substituent (**126o**−**q**, **128a**−**b**) are reduced to (*S*)-alcohols (entries 1−5), meanwhile larger ones (**128c**−**g**) give the (*R*)-alcohols (entries 6−10). Conversely, ethynylketoesters **129a**−**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 (**129c**,**e**) give higher chemical yields and higher enantioselectivities of ethynylhydroxyesters than methyl or ethyl ethynylketoesters (**129a**−**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.

Table 11. EED of Ethynyl Ketones and Ketoesters

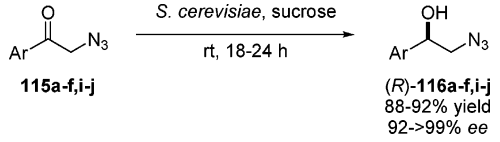


126o-q 128a-g 129a-f

entry	compound	R	*n*	abs conf	*t* (h)	yield (%)	*ee* (%)
1	**126o**	Me		*S*	0.5	30	60
2	**126p**	Et		*S*	1	32	80
3	**128a**	^{i}Pr		*S*	2	50	>98
4	**128b**	^{t}Bu		*S*	24		85
5	**126q**	^{n}Pr		*S*	1	28	51
6	**128c**	^{i}Bu		*R*	1	20	50
7	**128d**	^{neo}Pn		*R*	7		66
8	**128e**	^{n}Bu		*R*	4	32	42
9	**128f**	^{s}Pn					
10	**128g**	^{i}Pn		*R*	5	55	80
11	**129a**	Me	2	*R*	2.5	35	82
12	**129b**	Et	2	*R*	1.5	51	90
13	**129c**	^{i}Pr	2	*R*	1.0	88	>98
14	**129d**	Et	3	*R*	1.5	76	97
15	**129e**	^{i}Pr	3	*R*	1.5	76	>98
16	**129f**	Me	4	*R*	2.5	68	>98

The reductive EED of different 2-azido-1-aryl ketones (**115a**−**f**,**i**−**j**) catalyzed by fermented Baker's yeast has been reported by Yadav and co-workers (Scheme 34).[108] The yeast cells were treated with allyl alcohol to inhibit the (*S*)-oxidoreductase. Thus, the corresponding (*R*)-azido alcohols (*R*)-**116a**−**f**,**i**,**j** were obtained in high yields and enantioselectivities. These compounds are interesting building blocks for

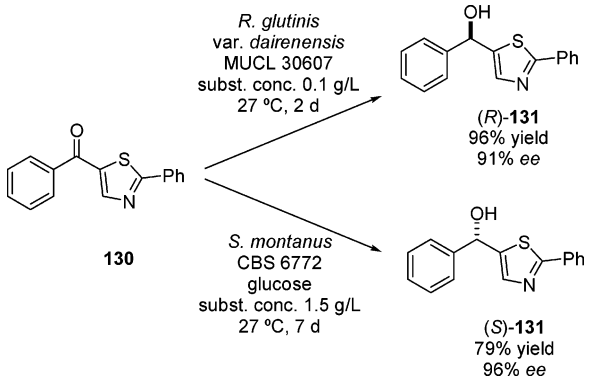
Scheme 34



the preparation of biologically active molecules such as β-adrenergic blockers.

Bulky ketones such as bisaryl ketones can be also 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*)-**131**] by enantioselective bioreduction of 1-phenyl-1-(2-phenylthiazol-5-yl)-methanone with whole microbial cells (Scheme 35).[109] A short screening of 38 microbial

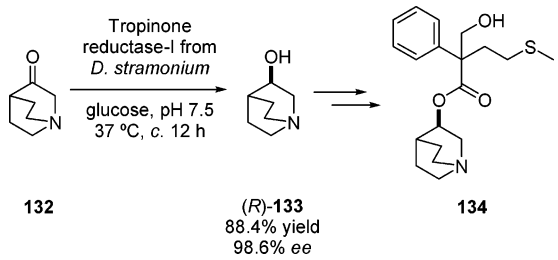
Scheme 35



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.

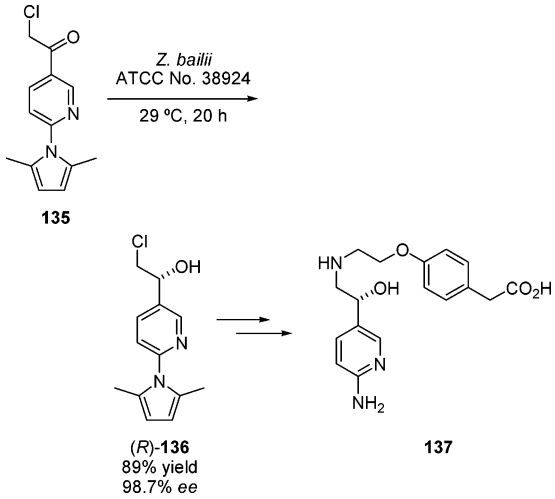
Prochiral amino ketones are attractive substrates for the preparation of pharmaceuticals. The corresponding amino alcohols can be obtained in high yield and *ee* by means of reductive EEDs. In this sense, the enantioselective reduction of different ketones using tropinone reductase-I derived from plants such as *Datura stramonium* and *Hyoscyamus niger* can produce optically active secondary alcohols with high enantioselectivity. Namely, Yamamoto et al.[110] 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 **132** giving (*R*)-3-quinuclidinol [(*R*)-**133**] in excellent yield and *ee* (Scheme 36). This compound has been used as an intermediate to produce a variety of physiologically or pharmacologically active agents such as squalene synthase inhibitor **134**.

Scheme 36



Similarly, a method for the microbial stereoselective reduction of 2-chloro-1-[6-(2,5-dimethylpyrrol-1-yl)-pyridin-3-yl]ethanone (**135**) has been reported by Burns et al.,[111] resulting in the enantioselective formation of the (*R*)-enantiomer of the chlorohydrin, (*R*)-**136**, which can be used in the synthesis of β-adrenergic receptor agonists, such as compound **137**. Initial screening of a number of microbiological catalysts identified cultures producing the desired alcohol with 62 to 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 **135** (2 g/L) were reacted, an 89% yield of (*R*)-**136** was isolated in excellent *ee* (Scheme 37).

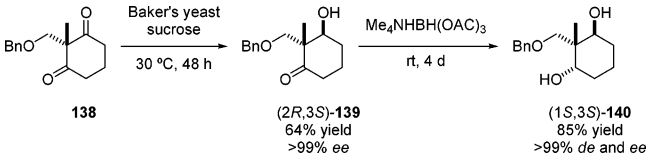
Scheme 37



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 (**138**).[112] The (3*S*)-hydroxyketone (2*R*,3*S*)-**139** was obtained enantiopure and in 64% yield, together with 15% of the starting material (Scheme 38). After chromatographic separation, the remaining ketone functionality in **139** was further reduced in a highly diastereoselective fashion to yield the corresponding *trans*-1,3-diol (1*S*,3*S*)-**140** in >99% *de* and *ee* and in 85% yield.

Sugai and co-workers have investigated the substrate specificity of the reduction of different trike-

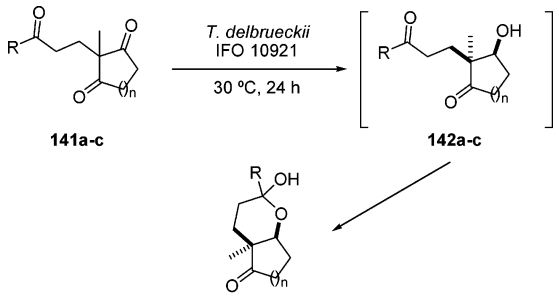
Scheme 38



138 (2*R*,3*S*)-**139** 64% yield >99% *ee* (1*S*,3*S*)-**140** 85% yield >99% *de* and *ee*

tones with the yeast strain *Torulaspora delbrueckii* IFO10921.[113] They have carried out the enantioselective reduction of the prochiral triketones **141a**−**c** shown in Scheme 39. In all cases, the *pro-R* carbonyl

Scheme 39



141a-c **142a-c**

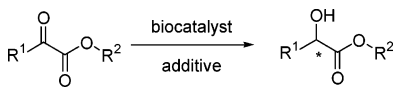
(1*S*,3*S*,6*S*)-**143a**, R = Me, n = 2, 60% yield, >99.9% *ee*
(1*S*,6*S*)-**143b**, R = Et, n = 2, 56% yield, 98.7 *ee*
(1*S*,6*S*)-**143c**, R = Me, n = 1, 63% yield, >99.9% *ee*

was preferentially reduced, yielding intermediates **142a**−**c** which in the reaction medium underwent cyclization to afford the bicyclic acetals (1*S*,6*S*)-**143a**−**c** in high yields and excellent *ee*'s. In contrast to **141a**−**b**, two byproducts were also isolated for the case of triketone **141c**. The first was the open-chain form of hydroxyketone **142c** (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. Furthermore, reduction of other triketones afforded interesting structure−activity relationships: (1) introduction of a 5,5-dimethyl group on the ring of **141a** 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.[114] Both the yield and the enantioselectivities of these reductions were maximized when methyl esters **144a**−**c** were used, the (*R*)-alcohols **145a**−**c** being isolated in all instances (Table 12, 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 appart 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 12).[115] Among all the results obtained, it is worth mentioning that at 37 °C and in the absence of additives ethyl 3-methyl-2-oxobutanoate (**144f**) and ethyl benzoylformate (**144g**) 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 **144f**−**g** in the presence of glutamic acid gave the hydroxyesters of opposite configuration [(*S*)-**145f**−**g**] in excellent *ee*'s (entries 8 and 9). Additionally, ethyl 2-oxobutanoate (**144d**) and ethyl 2-oxopentanoate (**144e**) were also reduced to the corresponding (*S*)-alcohols [(*S*)-**145d**−**e**] in >99% *ee* in the presence of an amino acid such as asparagine or aspartic acid (entries 4 and 5).

Table 12. EED of α-Ketoesters

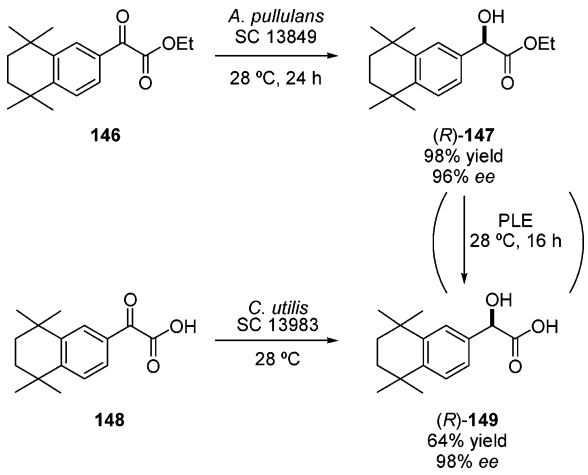


144a-g **145a-g**

entry	substrate	R^1	R^2	product	biocatalyst	additive	*T* (°C)	*t* (h)	yield (%)	*ee* (%)
1	**144a**	Me	Me	(*R*)-**145a**	B.Y.		30	24	36	92
2	**144b**	Ph	Me	(*R*)-**145b**	B.Y.		30	24	91	98
3	**144c**	MeO_2CCH_2	Me	(*R*)-**145b**	B.Y.		30	24	64	>98
4	**144d**	Et	Et	(*S*)-**145d**	S.T.	Asp/Asn	37	30		>99
5	**144e**	*n*-Pr	Et	(*S*)-**145e**	S.T.	Asp/Asn	37	30		>99
6	**144f**	*i*-Pr	Et	(*R*)-**145f**	S.T.		37	20	57	>99
7	**144f**	Ph	Et	(*R*)-**145g**	S.T.		37	20	65	>99
8	**144g**	*i*-Pr	Et	(*S*)-**145f**	S.T.	Glu	37	30		>99
9	**144g**	Ph	Et	(*S*)-**145g**	S.T.	Glu	37	30		>99

Similarly, *Aureobasidium pullulans* SC 13849 catalyzed the enantioselective reduction of the ethyl α-keto ester **146** to afford the chiral hydroxyester (*R*)-**147**. This compound and its related optically active hydroxy acid (*R*)-**149** are intermediates in the synthesis of a retinoic acid receptor gamma-specific agonist (Scheme 40).[116] PLE-catalyzed hydrolysis of

Scheme 40

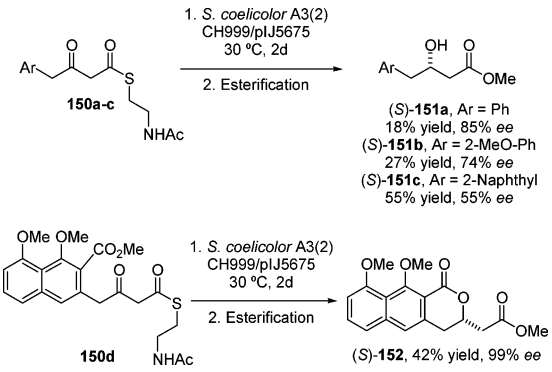


alcohol (*R*)-**147**, obtained in excellent yield and *ee*, afforded the desired hydroxy acid (*R*)-**149**. Alternatively, this compound can be also directly obtained by reduction of the corresponding α-keto acid **148**. Among the microorganisms screened, *Candida maltosa* SC 16112 and two strains of *Candida utilis* (SC 13983, SC 13984) gave reaction yields of >53% in >96% *ee*'s.

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. Recently, 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.[117] 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 **150a**−**d** shown in Scheme 41. 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*)-**151a**−**c** were isolated in good to excellent *ee*'s and moderate yields. Additionally, lactone (*S*)-**152** was obtained as the sole product of the reduction of **150d**. It was formed as essentially a single enantiomer by cyclization of the initially formed β-hydroxy acid (*S*)-**151d**.

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.

Scheme 41



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.[118,119] 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 **153a**−**c** were subjected to preparative recLBADH-catalyzed bioreduction (Scheme 42). It selectively occurs at the δ-keto group, thus affording the corresponding optically active δ-hydroxy-β-keto esters **154a**−**c**. These compounds are versatile intermediates in organic synthesis due to their high functionalization. Thus, its cyclization to afford the corresponding δ-lactones (**156a**−**c**) occurs with good yields. In particular, the lactone (*R*)-**156c** has been described as a natural fragrance.[120] On the other hand, different transformations of lactone (*S*)-**156a** have been employed in the syntheses of (−)-callystatin A and (−)-20-*epi*-callystatin A via the aldehyde **157**,[121,122] and (*S*)-argentilactone and (*S*)-goniothalamin.[123] Furthermore, transformation of (*S*)-**154a** into lactone **158** constitutes a key step in the synthesis of (*R*)-*semi*-vioxanthin.[124] Finally, the β,δ-dihydroxy esters *syn*-(3*R*,5*S*)- and *anti*-(3*S*,5*S*)-**155a** were prepared from (*S*)-**154a**. It is worth mentioning that (*R*)-**154a** has also been prepared with synthetic purposes through bioreduction in a biphasic system using Baker's yeast as catalyst (50% yield, 90−94% *ee*).[119]

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 (**159a**), we found out that growing cells of the fungus *Curvularia lunata* CECT 2130 produced an almost

Scheme 42

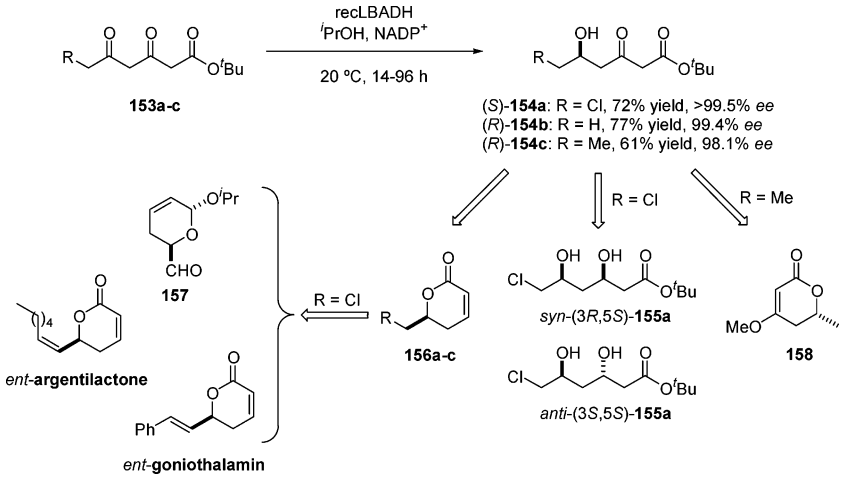
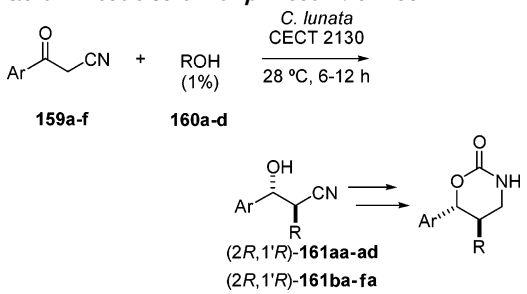


Table 13. *C. lunata*-Catalyzed Alkylation−Reduction of β-Keto Nitriles

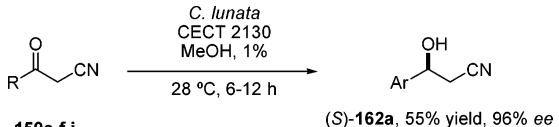


entry	substrate	Ar	alcohol	R	product	yield (%)	*de* (%)	*ee* (%)
1	**159a**	Ph	**160a**	Et	**161aa**	69	96	98
2	**159a**	Ph	**160b**	^{n}Pr	**161ab**	38	98	98
3	**159a**	Ph	**160c**	^{n}Bu	**161ac**	13	87	86
4	**159a**	Ph	**160d**	^{t}Bu	**161ad**	14	94	97
5	**159b**	3-Me-Ph	**160a**	Et	**161ba**	42	78	70
6	**159c**	4-Me-Ph	**160a**	Et	**161ca**	64	92	83
7	**159d**	4-MeO-Ph	**160a**	Et	**161da**	58	88	75
8	**159e**	2-thienyl	**160a**	Et	**161ea**	63	94	93
9	**159f**	2-furyl	**160a**	Et	**161fa**	59	72	87

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 (**161aa**).[125] 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 **161aa** was obtained in 96% *de* and 98% *ee* (Table 13, entry 1). Other alcohols and β-keto nitriles were employed to study the substrate specificity of this interesting biotransformation.[126] First, it was observed that MeOH inhibited the reaction, but other short chain alcohols behaved as ethanol (**160b**−**d**, entries 2−4), the corresponding alkyl groups being incorporated in the α-position (**161ab-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 (**159b**−**f**, entries 5−9) lower enantioselectivities and diastereoselectivities were observed, in general, as compared to benzoyl acetonitrile (**159a**, entry 1). Nevertheless, its alkylation−reduction resulted in highly diastereo- and enantioselective reactions leading to α-alkyl-β-hydroxy nitriles (2*R*,1′*R*)-**161ba-fa**, which, together with (2*R*,1′*R*)-**161aa-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.

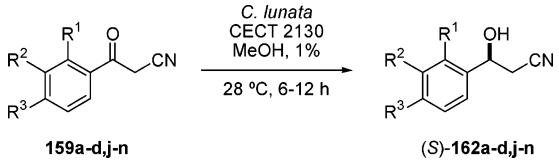
As it has already been pointed out, the use of methanol as cosolvent allowed, in turn, the chemoselective reduction of the aforementioned aromatic β-keto nitriles **159a**,**f**−**i** by the fungus *Curvularia lunata* CECT 2130 to yield the corresponding (*S*)-β-hydroxy nitriles (*S*)-**162a**,**f**−**i** in a highly enantioselective way (Scheme 43).[127] 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.

Scheme 43



Taking into account that the best stereochemical result was obtained in the case of benzoyl acetonitrile (**159a**), a series of derivatives bearing different substituents on the phenyl moiety were also tested (Table 14). All the β-hydroxy nitriles were obtained

Table 14. *C. lunata*-Catalyzed Reduction of β-Keto Nitriles



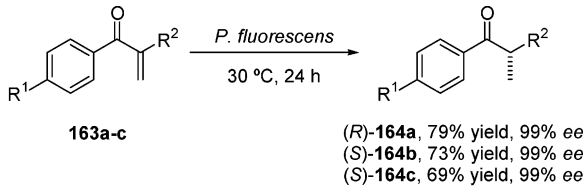
entry	substrate	R^1	R^2	R^3	yield (%)	*ee* (%)
1	**159a**	H	H	H	55	96
2	**159j**	Cl	H	H	42	98
3	**159k**	H	Cl	H	57	97
4	**159l**	H	H	Cl	47	50
5	**159m**	H	Cl	Cl	43	92
6	**159b**	H	Me	H	59	94
7	**159c**	H	H	Me	54	82
8	**159n**	H	CF_3	H	41	98
9	**159d**	H	H	MeO	42	83

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 **159a**; (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 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 **159k** than that with **159l** (entries 3 and 4, respectively).

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,[128] the bioreduction of alkenes conjugated with a carbonyl group has been more studied. Thus, α,β-unsaturated aldehydes, ketones, and carboxylic acid derivatives are typically used as starting materials for this transformation. Depending on the biocatalyst used and on the reaction conditions the chemoselective reduction of the olefinic bond, leaving the carbonyl group untouched, can be achieved. For instance, the bioreduction of methyleneketones **163a**−**c** was carried out with *Rhizopus arrhizus*, *P. fluorescens*, and immobilized *S. cerevisiae* (Scheme 44).[129] The best results were obtained with

Scheme 44

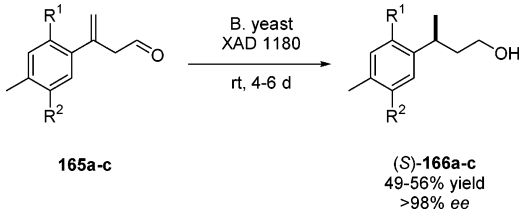


a: R^1 = H, R^2 = Et; **b**: R^1 = H, R^2 = CH_2CO_2Me;
c: R^1 = OMe, R^2 = CH_2CO_2Me;

P. fluorescens, which afforded the corresponding optically active saturated ketones (*R*)-**164a** and (*S*)-**164b**−**c** in excellent *ee*'s and yields.

Concurrent reduction of the carbonyl group of unsaturated aldehydes permits the obtainment of optically active primary alcohols, compounds that are difficult to achieve by means of enzymatic KRs. Fuganti et al.[130] have converted the unsaturated aldehydes **165a**−**c** into the saturated alcohols (*S*)-**166a**−**c** by using fermenting cells of Baker's yeast as catalyst (Scheme 45). The microbial saturation of

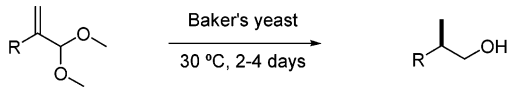
Scheme 45



these substrates adsorbed on a nonpolar resin proceeds in good yields and excellent enantioselectivities. The usefulness of enantiopure (*S*)-**166a**−**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 **167a**−**d** afforded the corresponding optically active alcohols in high yields and in *ee*'s ranging from 90 to 98% (Scheme 46).[131] These derivatives are much more

Scheme 46



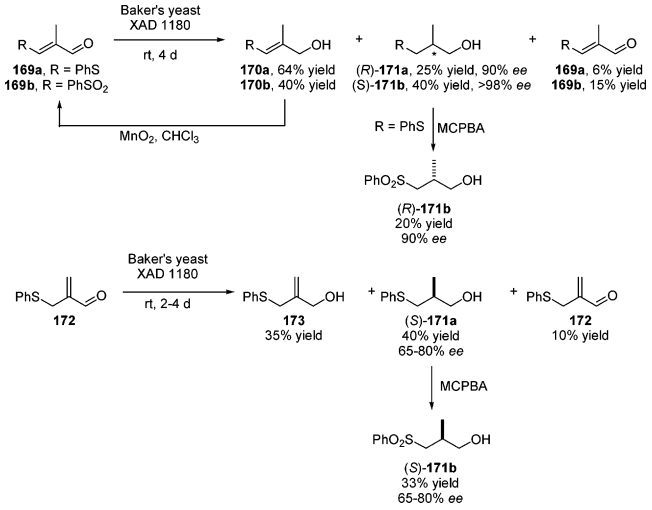
a: R = Bn; **b**: R = $(CH_3)_2C{=}CHCH_2$;
c: R = $PhCH_2OCH_2$; **d**: R = Ph

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.[132]

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 47).[133] 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 **169a**−**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 **172** afforded the corresponding saturated alcohol (*S*)-**171a** with moderate *ee*, which decreased with increasing reaction time. The optically active saturated acohols containing the sulfide moiety [(*R*)- and (*S*)-**171a**] were easily transformed into the corresponding sulfones, thus giving access to both enantiomers of the sulfur-functionalized alcohol **171b** in high *ee*'s.

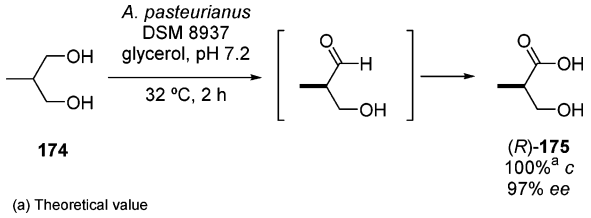
Scheme 47



6.3. Oxidation of Alcohols

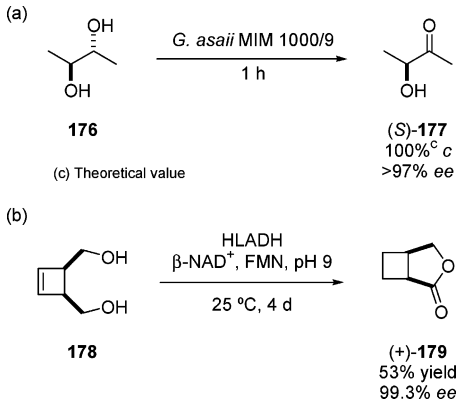
Dehydrogenases are not only able to catalyze the enantioselective reduction of prochiral ketones but can also effectively desymmetrize *meso* and prochiral diols through enantioselective oxidation. Optically active hydroxyketones and hydroxycarboxylic acids, or related derivatives, are thus obtained as products. In fact, some recent examples can be found in the literature. For instance, (*R*)-3-hydroxy-2-methyl propionic acid [(*R*)-**175**], key intermediate of the synthesis of Captopril, has been prepared by oxidative EED of prochiral 2-methyl-1,3-propanediol (**174**).[134] 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*)-**175** in 97% *ee* (Scheme 48).

Scheme 48



Romano et al. have investigated the catalytic and enantiodiscriminative capabilities of acetic acid bacteria belonging to the genus *Acetobacter* and *Gluconobacter* in the oxidation of different primary alcohols, and chiral and prochiral diols.[135] In particular, they have carried out the EED of *meso*-2,3-butandiol (**176**), 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*)-**177** in excellent *ee*'s, being *G. asaii* MIM 1000/9 the best one. After 1 h this microorganism yielded (*S*)-**177** in complete conversion and >97% *ee* (Scheme 49a).

Scheme 49



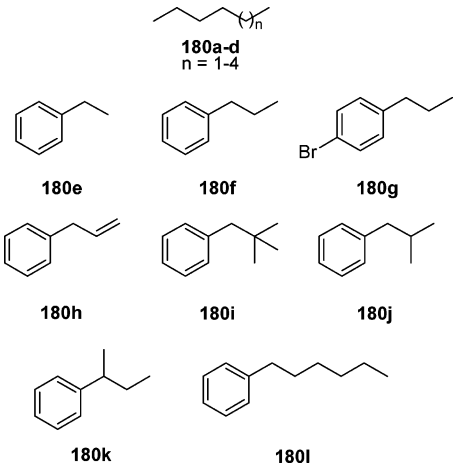
Similarly, *meso* diol **178** was transformed into the synthetically interesting lactone (+)-**179** by means of horse liver alcohol dehydrogenase-catalyzed oxidation (Scheme 49b).[136] This way, the title lactone was obtained in good yield and excellent *ee*.

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.[137] 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 5,

Chart 5

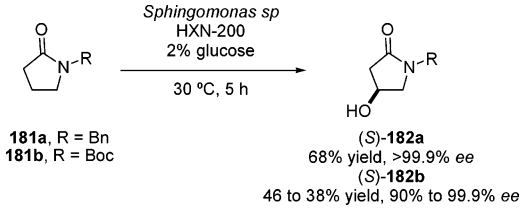


180a−**d**) to afford, in some cases, enantioenriched alcohols in up to 99% *ee*.[138] Although the synthetic utility of these biotransformations is limited by the fact that mixtures of regioisomers in low yields are usually obtained, the authors extract some interesting conclusions: (1) the time of 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 **180e**−**l** (Chart 5).[139] In general, and conversely to the previous oxidation of alkanes, lower enantioselectivities were achieved. However, the α-oxidation of **180j** (91% *ee*) and the *β*-, *γ*-, and *δ*-oxidations of **180l** (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 **180l**. 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 **180f** 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.[139b]

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 non-hydroxylated 2-pyrrolidinones by resting cells of *Sphingomonas* sp. HXN-200 (Scheme 50).[140] Namely, hydroxylation of

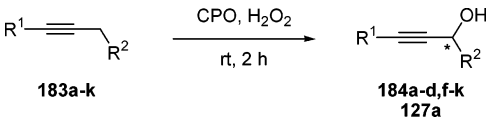
Scheme 50



N-benzyl-pyrrolidin-2-one (**181a**) gave a 75% conversion of enantiopure (*S*)-*N*-benzyl-4-hydroxypyrrolidin-2-one [(*S*)-**182a**]. The presence of 2% glucose, which contributed to cofactor regeneration, was mandatory. Although the enantioselectivity for hydroxylation of the *N*-BOC compound **181b** was lower, the *ee* was increased by recrystallization to afford enantiopure (*S*)-**182b**. Moreover, this strain has also proven to hydroxylate other related prochiral heterocycles although in lower enantioselectivities.[141]

Peroxidases are readily available enzymes that do have the potential to be useful catalysts.[142] 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.[143] In particular, they report, for the first time, the chloroperoxidase (CPO)-catalyzed hydroxylation of alkynes **183a**−**k** to yield the corresponding (*R*)-alcohols (Table 15). This enzyme is a

Table 15. EED of Propargylic Methylenes



entry	alkyne	R^1	R^2	yield (%)	*ee* (%)	config
1	**183a**	Me	Me	7	57	*R*
2	**183b**	Et	Me	26	91	*R*
3	**183c**	Pr	Me	30	87	*R*
4	**183d**	Bu	Me	8	78	*R*
5	**183e**	Ph	Me	15	86	*R*
6	**183f**	CH_2OAc	Me	52	95	*R*
7	**183g**	CH_2Br	Me	65	94	*R*
8	**183h**	$(CH_2)_2OAc$	Me	26	83	*R*
9	**183i**	$(CH_2)_2Br$	Me	25	94	*R*
10	**183j**	CH_2OAc	Et	8	87	n.d.
11	**183k**	$COCH_3$	Me	n.r.	n.r.	n.r.

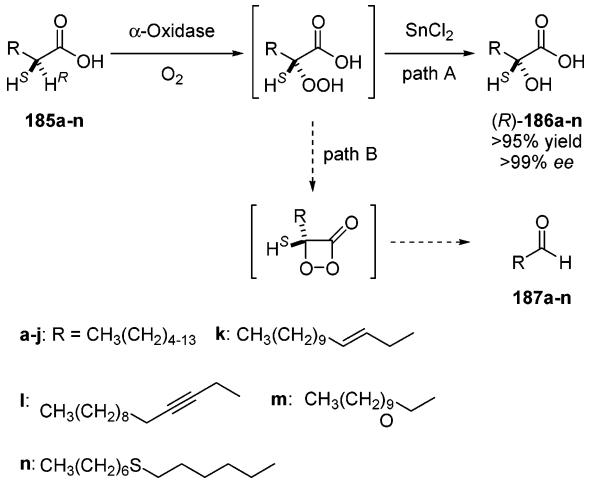
heme-iron-containing peroxidase, which uses hydrogen peroxide as oxidant. Results obtained showed that, in most cases, the yields could be increased by the addition of more enzyme and oxidant. However, they focused on the study of the enantioselectivity displayed by this enzyme. When the smallest alkyne (**183a**, 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 (**183b**, entry 2). Moreover, CPO proved to be progressively less enantioselective toward alkynes possessing additional carbons (**183c**−**d**, entries 3 and 4) with the exception of phenyl acetylene (**183e**, 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 (**183f**−**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 **183i** (entry 9), but there was a significant decrease for **183h** (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, 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 (**185a**−**n**) led to intermediary α-hydroxyperoxy acids which preferentially decarboxylated to the corresponding aldehydes (**187a**−**n**) in competition with reduction to the (*R*)-2-hydroxy acids **186a**−**n** (Scheme 51).[144] A major

Scheme 51



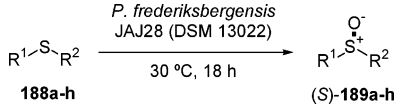
breakthrough for preparative-scale applications was the use of tin(II) chloride as an in situ reducing agent. Thereby, the undesirable competitive decarboxylation of the 2-hydroperoxy acids to the aldehydes was circumvented and a biocatalytic α-hydroxylation of the carboxylic acids has been made available 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.

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,[145] especially by means of medium engineering,[146] immobilization techniques,[147] or the development of bienzymatic systems that progressively in situ generate H_2O_2 at the expense of air and a carbon source.[148] Nevertheless, other types of biocatalysts such as a vanadate containing phytase (an acid phosphorylase),[149] cyclohexanone monooxygenase,[150] naphthalene dioxygenase,[151] and catalytic antibodies[152] 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 (**188a**−**h**) catalyzed by *Pseudomonas frederiksbergensis* sp. Nov. (Table 16).[153] It was

Table 16. *P. frederiksbergensis*-Catalyzed Desymmetrization of Sulfides



entry	substrate	R^1	R^2	*c* (%)	*ee* (%)
1	**188a**	Ph	Me	87	91
2	**188b**	4-Me-Ph	Me	100[a]	>99
3	**188c**	4-MeO-Ph	Me	98	>99
4	**188d**	4-Cl-Ph	Me	86	96
5	**188e**	Ph	^{n}Pr	27	81
6	**188f**	Ph	^{i}Pr	<5	
7	**188g**	Bn	Me	<5	
8	**188h**	Cy	Me	98	70

[a] Theoretical value.

first observed that both the reaction time and the pH influenced the yield and the enantioselectivity in which (*S*)-**189a**, the model compound, was obtained. Then the rest of sulfides (**188b**−**h**) were subjected to oxidation with this microorganism and the corresponding (*S*)-sulfoxides (*S*)-**189b**−**h** were obtained in nonoptimized yields ranging from 4 to 48% and in good to excellent enantioselectivities. In particular, **188a** 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 (**188b**−**d**, entries 2−4). To assess the effect of the alkyl side chain on the enantioselectivity, the *n*-propyl derivative **188e** was tested together with **188f** (entries 5 and 6) and compared to the results obtained for the methyl phenyl sulfide (**188a**, 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 **188f**. Moreover, intercalation of a methylene unit between the sulfur atom and the aryl substituent has a deleterious effect on both enantioselectivity and reactivity (**188g**, entry 7). It is also worth mentioning that dialkyl sulfides, such as **188h**, are sulfoxidized in good enantioselectivities as well (entry 8).

Porto et al. have prepared sulfoxides (*S*)-**191a**−**f** by using *Aspergillus terreus* CCT 3320 cells, which oxidized the precursor sulfides **190a**−**f** (Scheme 52).[154] These biotransformations led, in most cases,

Scheme 52

190a-f

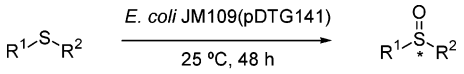
(*S*)-**191a**, 76% yield, >99% *ee*
(*S*)-**191b**, 24% yield, >95% *ee*
(*S*)-**191c**, 66% yield, >95% *ee*
(*S*)-**191d**, 24% yield, >98% *ee*
(*S*)-**191e**, 70% yield, >98% *ee*
(*S*)-**191f**, 71% yield, 17 % *ee*

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**: $R^1 = H$, $R^2 = Cl$, $R^3 = Me$;

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 (±)-**191a**, 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 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.

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- (**192a**−**t**) and dialkyl- (**192u**−**y**) sulfides (Table 17) to the corresponding optically active sulfoxides to determine its substrate specificity.[151] 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.

Table 17. NDO-Catalyzed Desymmetrization of Sulfides



192a-y **193a-y**

entry	sulfide	R^1	R^2	*c* (%)	*ee* (%)	config
1	**192a**	Ph	Me	98	98	*S*
2	**192b**	Ph	Et	87	86	*S*
3	**192c**	Ph	^{n}Pr	58	76	*R*
4	**192d**	Ph	^{i}Pr	69	74	*R*
5	**192e**	Ph	^{n}Bu	25	97	*R*
6[a]	**192f**	Ph	CH=CH_2	98	93	unknown
7	**192g**	4-NO_2-Ph	Me	5	>98	*S*
8	**192h**	4-CN-Ph	Me	13	>98	*S*
9	**192i**	4-F-Ph	Me	97	98	*S*
10	**192j**	4-Cl-Ph	Me	10	90	*S*
11	**192k**	4-Cl-Ph	Et	31	>98	unknown
12	**192l**	4-Br-Ph	Me	25	90	*S*
13	**192m**	4-MeO-Ph	Me	25	92	*S*
14	**192n**	4-Me-Ph	Me	96	>98	*S*
15[a]	**192o**	3-Cl-Ph	Me	21	75	*S*
16	**192p**	2-Cl-Ph	Me	12	31	unknown
17	**192q**	Bn	Me	20	<1	*rac*
18	**192r**	Bn	Et	11	5	*S*
19	**192s**	Ph-$(CH_2)_2$-	Me	10	54	unknown
20	**192t**	Ph-$(CH_2)_3$-	Me			
21	**192u**	Me	^{n}Hex	70	74	*S*
22	**192v**	Me	Cy	70	85	*S*
23	**192w**	Me	^{n}Hep	<5	3	*R*
24	**192x**	Me	^{n}Oct	<5	4	*R*
25	**192y**	Me	^{n}Non	<5	5	*R*

[a] Traces of the corresponding sulfone were found.

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 (**192c**−**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 (**192w**−**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 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 (**192g**−**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 (**192o**−**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 (**192q**−**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 (**192s**−**t**) further continued this trend of decreasing the yield of sulfoxide formed (entries 19 and 20).

Finally, *n*-hexyl methyl and cyclohexyl methyl sulfides (**192u**−**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.

6.6. *Cis*-Dihydroxylation of Carbon–Carbon Double Bonds

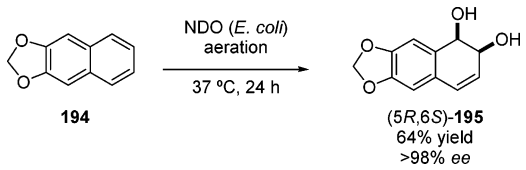
Dioxygenases found in prokaryotic microorganisms possess the remarkable ability to desymmetrize aromatic and conjugated substrates through oxidation of a carbon−carbon double bond to produce optically active vicinal *cis*-diols. The mostly studied enzymes that catalyze these biotransformations are the following three aromatic dioxygenases: toluene dioxygenase (TDO), naphthalene dioxygenase (NDO), and biphenyl dioxygenase (BPDO). They generally contain three components.[155] 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.[156]

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, Boyd and co-workers have recently studied and quantified the regio- and enantioselectivity displayed by these dioxygenases in the *cis*-dihydroxylation of many monocyclic, fused, and linked aromatic and heteroaromatic systems as well as conjugated dienes,[157] thus mapping the substrate specificity of these enzymes. 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.[157]

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.[158] (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.[157] (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 high versatility on *cis*-dihydroxylations.[159] It is worth mentioning that X-ray crystallographic studies of NDO[156,160] 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.[161] Indeed, the employment of the NDO mutant F352V in combination with NDO_{wt}, TDO, and carbazole dioxygenase has provided four of the *cis*-dihydrodiol isomers from biphenyl.[161a]

Recently, this potential of EEDs based on *cis*-dihydroxylations has been reflected in the syntheses of different biologically active compounds. Thus, Banwell and co-workers have developed routes leading to (−)-gabosine A[162] and 6*C*-methyl-D-mannoses[163] 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 recently reported the synthesis of a policyclic lactone as a valuable pancratistatin analogue.[164] One of the key steps was the enzymatic dihydroxylation of dioxole **194** using recombinant *E. coli* cells expressing a naphthalene dioxygenase from *Pseudomonas putida* G7 (Scheme 53). The corresponding policyclic diol (5*R*,6*S*)-**195** was obtained in good yield and *ee* after 1 day of reaction.

Scheme 53

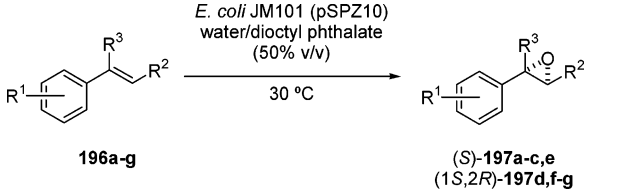


6.7. *Trans*-Dihydroxylation of Carbon–Carbon Double Bonds

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 non-heme iron complex, or in flavin monooxygenases as 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. 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 **196a**−**g** allowed, after recovery of the reaction products via distillation from the organic phase, the preparation of the corresponding optically active oxiranes (**197a**−**g**) in good to excellent yields (Table 18).[165]

Table 18. EED of Styrenes

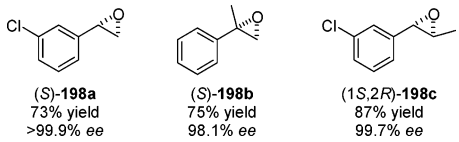


entry	substrate	R^1	R^2	R^3	t (h)	yield (%)	*ee* (%)
1	**196a**	H	H	H	17	76.3	99.5
2	**196b**	*p*-Me	H	H	17	46.5	99.9
3	**196c**	H	H	Me	23.5	74.8	96.7
4	**196d**	H	Me	H	20	87.2	99.8
5	**196e**	*m*-Cl	H	H	17.5	87.3	99.4
6	**196f**	*o*-CH_2−CH_2-		H	45.5	53.0	98.5
7	**196g**	*o*-CH_2-		H	19	47.9	98.0

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.[166] 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.[161] 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.[167] Namely, they have achieved the gram-scale production of epoxides **198a**−**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 6).

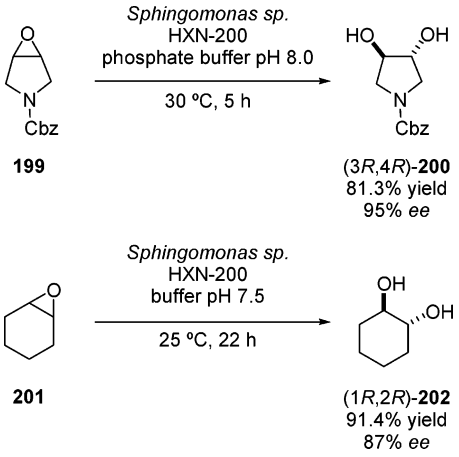
Chart 6



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.[168] 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.[19e,168,169] 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.[170] Likewise, Chang et al. have lately reported

an example of enantioselective hydrolysis of *meso*-epoxides with a bacterial epoxide hydrolase.[171] Namely, they have studied and carried out the preparative hydrolyses of epoxides **199** and **201** under the conditions shown in Scheme 54. In both

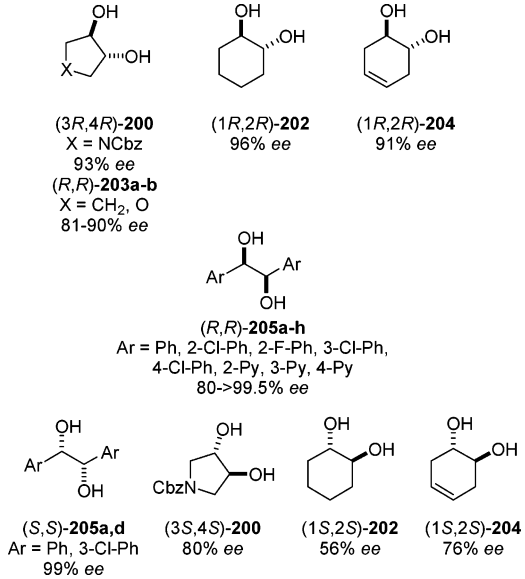
Scheme 54



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*)-**200** through a sequence that implied the previous action of a monooxygenase.[172]

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 were capable of selectively hydrolyzing a wide range of cyclic and aryl *meso*-epoxides.[173] From these processes, the corresponding chiral (*R*,*R*)-diols were obtained in high *ee*'s (Chart 7). To confirm the synthetic utility of these enzymes, some hydrolyses were carried out at a gram scale and

Chart 7

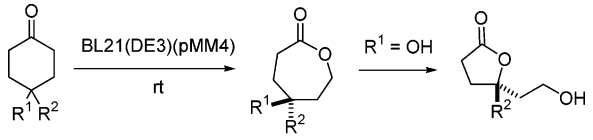


the corresponding diols were obtained in high yields. Moreover, they have also found the first epoxide hydrolases providing access to complementary (*S*,*S*)-diols (Chart 7).

6.8. Baeyer–Villiger Oxidations

Baeyer–Villiger monooxygenases (BVMO) efficiently catalyze the nucleophilic and electrophilic oxygenation of different functional groups. In particular, the enzymatic Baeyer–Villiger oxidation of ketones offers an environmentally benign entry to chiral lactones, important building blocks in synthetic chemistry.[174] 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.[175] 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[176] 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 co-workers 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 (**206a**–**k**; Table 19).[177] The efficient

Table 19. EED of 4-Mono- and 4,4-Disubstituted Cyclohexanones



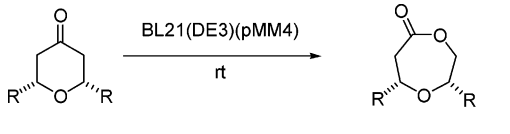
entry	substrate	R^1	R^2	yield (%)	*ee* (%)	config
1	**206a**	Me	H	61	≥98	(*S*)-(−)
2	**206b**	Me	Me	61		
3	**206c**	Et	H	91	97	(*S*)-(−)
4	**206d**	Et	Me	91	75	
5	**206e**	Et	Et	60		
6	**206f**	*cyclo*-CH_2CH_2		74		
7	**206g**	OH	H	61	9.1	(−)
8	**206h**	Et	OH	54	94	(−)
9	**206i**	OMe	H	84	78	(*S*)-(+)
10	**206j**	Br	H	63	97	(*S*)-(−)
11	**206k**	I	H	60	97	(*S*)-(−)

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 **207d**,**i** and **208g** 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 **206g** (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 **207b**,**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 **206d** was transformed (entry 4; 75% *ee*) as compared to ketones **206a**,**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 **206g**−**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.[178] 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 **209a**−**e** was oxidized to the corresponding lactones (**210a**−**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 55).[179] 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 (**209a**) and ethyl (**209b**), were oxidized in excellent yields, significantly decreased conversions or no reaction at all were measured for ketones bearing larger substituents (**209c**−**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.

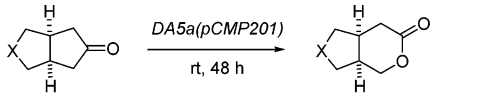
Scheme 55



Cyclopentanone monooxygenase (CPMO) 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, recent results show that 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.[180] 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 the prochiral bicyclo[4.3.0]-ketones **211a**−**b** have been compared (Scheme 56).[181]

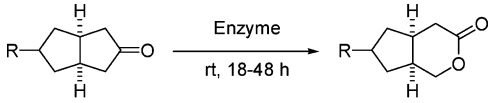
Scheme 56



Results showed that 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 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 **213a**−**e** (Table 20).[182] The results obtained confirmed, first, that some substrates were transformed by both enzymes in an enantiodivergent fashion (**213a,d**−**e**, entries 1 and 2, 7−10). Second, for the case of the

Table 20. EED of Pentalenones

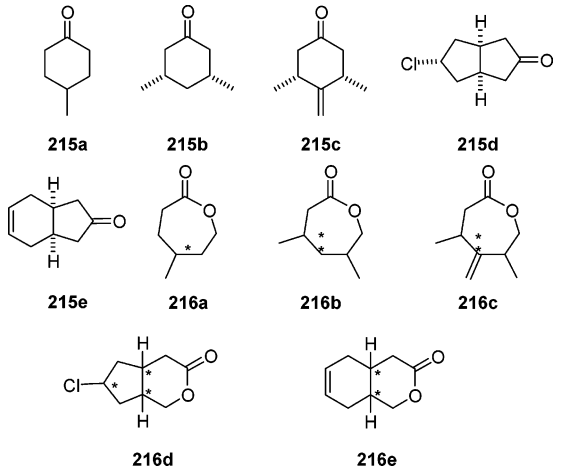


entry	substrate	R	enzyme	yield (%)	*ee* (%)	config
1	**213a**	*endo* MeO	CHMO	24	9	(−)
2	**213a**	*endo* MeO	CPMO	81	34	(+)
3	**213b**	*exo* MeO	CHMO	40	96	(−)
4	**213b**	*exo* MeO	CPMO	75	11	(−)
5	**213c**	*endo* Cl	CHMO	75	80	(−)
6	**213c**	*endo* Cl	CPMO	79	60	(−)
7	**213d**	*exo* Cl	CHMO	78	>99	(−)
8	**213d**	*exo* Cl	CPMO	92	>99	(+)
9	**213e**	$=CH_2$	CHMO	43	61	(−)
10	**213e**	$=CH_2$	CPMO	85	41	(+)

CHMO biotransformations, the *exo* geometry present in the bicycloketones **213b**,**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 **213a**,**c** (entries 1 and 5) and for the sp^2-hybridized ketone **213e** (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 **213d**.

In the last 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 (**215a**−**e**) with recombinant *E. coli* cells expressing two new monooxygenases from *Brevibacterium* ($CHMO_{BreviI}$ and $CHMO_{BreviII}$) were investigated (Table 21).[183] With the

Table 21. $CHMO_{Brevi}$-Catalyzed Desymmetrization of *meso* and Prochiral Ketones

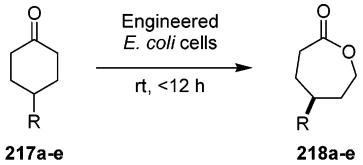


entry	substrate	product	enzyme	yield (%)	*ee* (%)	config
1	**215a**	**216a**	$CHMO_{BreviI}$	65	>99	(−)-*S*
2			$CHMO_{BreviII}$	60	44	(+)-*R*
3	**215b**	**216b**	$CHMO_{BreviI}$	61	97	(−)-4*S*,6*R*
4			$CHMO_{BreviII}$	56	99	(+)−4*R*,6*S*
5	**215c**	**216c**	$CHMO_{BreviI}$	70	>99	(+)
6			$CHMO_{BreviII}$	44	>99	(−)
7	**215d**	**216d**	$CHMO_{BreviI}$	55	>99	(−)
8			$CHMO_{BreviII}$	59	>99	(+)
9	**215e**	**216e**	$CHMO_{BreviI}$	10	71	n.d.
10			$CHMO_{BreviII}$	92	94	(+)

exception of the $CHMO_{BreviI}$-catalyzed oxidation of **215e** (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 **215c**, its oxidation occurred chemoselectively without concomitant epoxidation of the double bond (entries 5 and 6).

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.[184] 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 57) and with the exception of substrates

Scheme 57



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

217c,**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 **217c** with very high (*S*)-enantioselectivity, and the *Brevibacterium* sp. ChnB1 and *Rhodococcus* SC1 enzymes also showed complete stereoselectivity in the oxidation of **217e**.

7. Carbon–Carbon Bond Formation

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.

7.1. Aldol Reactions

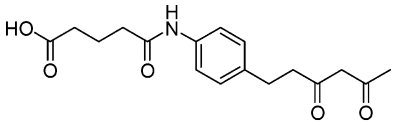
The aldol reaction is one of the most powerful methods of forming carbon−carbon bonds. There are two types of enzymatic catalysts that effect the aldol addition: aldolases and catalytic antibodies. Aldolases are a specific group of lyases that typically catalyze the stereoselective addition of a ketone 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.[185] These features could account for the fact that, to the best of our knowledge, no synthetically useful aldolase-catalyzed EED has been published during the recent past years.

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.[186] It was obtained by using hapten **219** (Chart 8), in which the

Chart 8

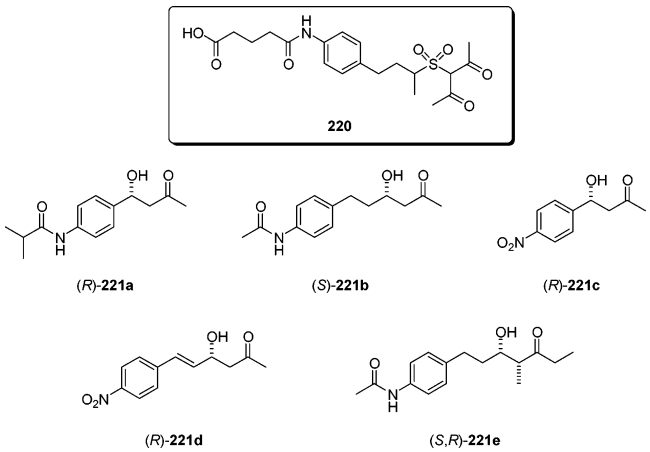


219

β-diketone functionality should act as a reactive immunogen to trap a chemically reactive lysine 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.[187]

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 **220** (Chart 9). As compared to hapten

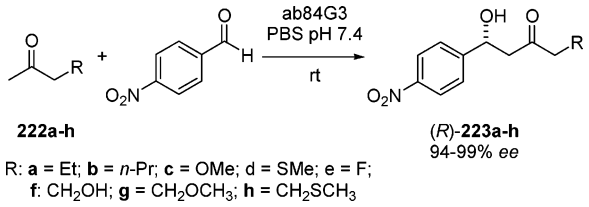
Chart 9



219, it additionally includes the sulfone moiety, responsible for the structural analogy with the rate-determining transition state of the aldol reaction.[188] 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 **221a**−**e** is substrate dependent. Aldols (*R*)-**221a**,**c**−**d** are provided in essentially enantiomerically pure form with either catalyst, while a moderate enantioselectivity is obtained in the synthesis of (*S*)-**221b** (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 **221e**. 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.[189] 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 **222a**−**h** with *para*-nitrobenzaldehyde was carried out (Scheme 58). In all cases, the linear aldol adducts

Scheme 58

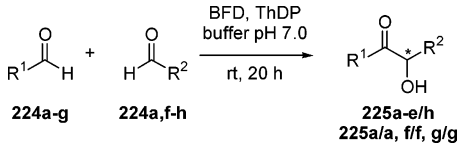


of (*R*)-configuration were obtained in excellent *ee*'s, the opposite enantiomeric series of these compounds being accessible via ab84G3-catalyzed retro-aldol KR of (±)-**223a**−**h**.

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[190] through a mechanism in which, usually, the decarboxylation of a ketoacid is promoted in the presence of the ThDP

Table 22. EED of Aldehydes Catalyzed by BFD



entry	substrates	product	R^1	R^2	yield (%)	*ee* (%)
1	**224a/224h**	(*S*)-**225a/h**	Ph	Me	90	92
2	**224b/224h**	(*S*)-**225b/h**	3-MeO−C_6H_4	Me	97	96
3	**224c/224h**	(*S*)-**225c/h**	3-*i*-PrO−C_6H_4	Me	91	>99
4	**224d/224h**	(*S*)-**225d/h**	3,5-di-MeO−C_6H_4	Me	40	97
5	**224e/224h**	(*S*)-**225e/h**	2-naphthyl	Me	32	88
6	**224a/224a**	(*R*)-**225a/a**	Ph	Ph	70	>99
7	**224f/224f**	(*R*)-**225f/f**	2-F-C_6H_4	2-F-C_6H_4	68	>99
8	**224g/224g**	(*R*)-**225g/g**	4-Me-C_6H_4	4-Me-C_6H_4	69	>99

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.[191]

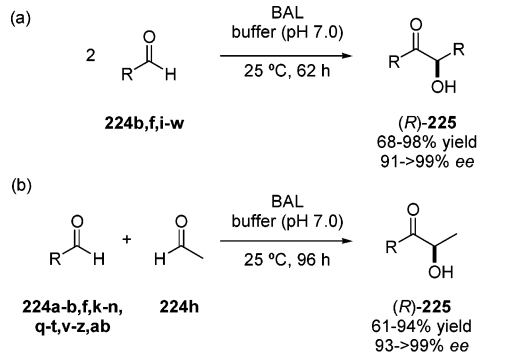
Pyruvate decarboxylase (PDC) is one of the first enzymes that was used for synthetic purposes.[192] Although it is active toward a variety of aldehydes, it has been found that acetaldehyde is the preferred donor substrate. For instance, the PDC-catalyzed formation of (*R*)-1-hydroxy-1-phenyl-2-propanone (*R*)-**225a/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.[192,193]

Benzoylformate decarboxylase (BFD) is able to bind a broader range of aromatic aldehydes to ThDP prior to ligation to the acceptor aldehyde.[194] This way, different aromatic β-hydroxyketones can be prepared on a preparative scale in moderate to high yields and good *ee*'s (Table 22). Interestingly, the absolute configuration of the final compound depends on the nature of the acceptor substrate, being *S* for acetaldehyde (**224h**, entries 1−5) and *R* for aromatic aldehydes (**224a**,**f**−**g**, entries 6−8). Besides, for highly hydrophobic substrates, DMSO can be used as cosolvent with no loss of BFD ligase activity.[195]

Benzaldehyde lyase (BAL) is perhaps the most versatile enzyme concerning the acceptance of aromatic donors. Demir et al.[196] 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 59a). 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 (**224h**). This way, the corresponding (*R*)-2-hydroxypropiophenones (*R*)-**225** have been obtained in high yields and optical purities (Scheme 59b).

Finally, the synthetic value of this kind of C−C forming reaction has been recently reported by

Scheme 59



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;

Müller et al.[197] 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, selectivities, and *ee*'s.

7.3. Hydrocyanation of Carbonyl Compounds

Oxynitrilases (hydroxynitrile lyases) 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 this addition. Nevertheless, its toxicity makes the transhydrocyanation of ketone cyanohydrins a safer alternative.[198]

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.[199] Thus, the formation of a hemiacetal/ketal intermediate, and the

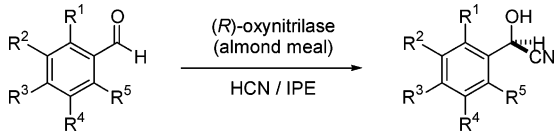
activation of the carbonyl oxygen by the catalytic Ser and a Thr residue, followed by attack of hydrogen cyanide, which would be deprotonated by the catalytic His, are the two most likely hypotheses.

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.[198] 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.[198a]

Despite recent advances in catalyst engineering that facilitate the application of enzymatic hydrocyanations on an industrial scale,[200] undoubtedly, the implementation of the enzyme-catalyzed cyanohydrin reaction as a method of great synthetic importance has been principally possible due to the supression of the nonenzymatic reaction, which lowers the yield and optical purity of the resulting products.[198b,d,201] 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,[202] 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.[203] 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.[204] 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.

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 23).[205] As it can be seen from the

Table 23. EED of Benzaldehyde Derivatives



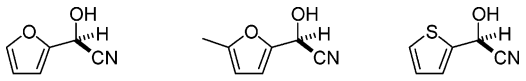
226a-i (*R*)-**227a-i**

entry	substrate	R^1	R^2	R^3	R^4	R^5	*t* (h)/*T* (°C)	yield (%)	*ee* (%)
1	**226a**	H	H	F	H	H	24/20	90	94.2
2	**226b**	H	H	H	H	F	24/20	96	84
3	**226c**	H	F	F	H	H	24/28	71	84.3
4	**226d**	F	F	H	H	H	24/20	92	46.1
5	**226e**	F	H	H	H	F	24/12	70	41.0
6	**226f**	H	F	F	F	H	24/12	37	0
7	**226g**	F	F	F	F	F	48/12	90	0
8	**226h**	H	H	CF_3	H	H	24/12	67	0

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 (**226a**−**b**; entries 1 and 2) < disubstituted (**226c**−**e**; entries 3−5) < tri- and pentasubstituted (**226f**−**g**; entries 6 and 7). For the latter compounds as well as for **226h** (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 (**226b**,**d**−**e**; entries 2, and 4−5) caused more significant reduction in the enantioselectivity of the reaction than substitution at other positions (**226a**,**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.

These authors have also studied the almond meal-catalyzed hydrocyanation under microaqueous conditions of a wide range of prochiral heteroaryl carboxaldehydes due to the special importance of optically active heteroaryl cyanohydrins in synthesis.[206] 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 **228a**−**c** (Chart 10). Additionally, the

Chart 10



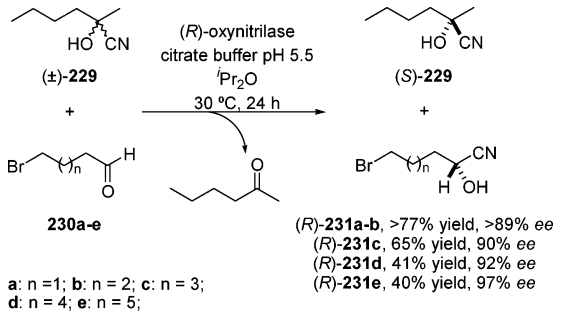
(*S*)-**228a** 100% yield 99% *ee*
(*S*)-**228b** 60% yield 97% *ee*
(*S*)-**228c** 70% yield 99% *ee*

following structure−activity relationships could be outlined (data not shown): (1) *N*-Containing heteroaryl carboxaldehydes turned out to be not satis-

factory 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 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 our research group, we have extended the optimized procedure for the almond meal (*R*)-oxynitrilase-catalyzed transcyanation of the ω-bromoaldehydes **230a**,**b**[207] to compounds **230c**−**e** (Scheme 60).[208] (±)-2-Methyl-2-hydroxyhexanenitrile [(±)-**229**]

Scheme 60



was used as the hydrogen source, cyanohydrins (*R*)-**231c**−**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,[209] optically active azacycloalkan-3-ols,[208] and 2,3-disubstituted piperidines.[210]

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. Moreover, 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. These facts make EEDs an attractive tool of asymmetric synthesis and convert them into an active field of research. We strongly believe that genetic engineering techniques (e.g., improvement of the enzymatic specificity by mutation or overexpression of an enzyme in a well-known and easy-to-handle host microorganism) are going to continue to play a major role in the future research of enzymatic biocatalysis but without detriment to other more traditional approaches such as medium engineering and the screening for new biocatalysts.

9. 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
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
E	enantiomeric ratio
ee	enantiomeric excess
EED	enantioselective enzymatic desymmetrization
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
HLADH	horse liver alcohold dehydrogenase
IPE	*i*-propyl ether
KR	kinetic resolution
LABDH	*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.)
MPMO	*p*-methoxybenzyloxy
MOM	methoxymethyl
NAD(H)	nicotinamide adenine dinucleotide
NADP(H)	nicotinamide adenine dinucleotide phosphate
NDO	naphthalene dioxygenase
NK	neurokinin
Novozyme 435	commercially available immobilized CALB (Novo Nordisk)
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

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11. References

(1) Rouhi, A. M. *Chem. Eng. News* **2003**, *81*, 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.
(4) (a) Klibanov, A. M. *Nature* **2001**, *409*, 241. (b) Carrea, G.; Riva, S. *Angew. Chem., Int. Ed.* **2000**, *39*, 2226.
(5) Cao, L.; van Langen, L.; Sheldon, R. A. *Curr. Opin. Biotechnol.* **2003**, *14*, 387.
(6) See for instance: Faber, K. *Chem. Eur. J.* **2001**, *7*, 5005, and references therein.
(7) (a) Pàmies, O.; Bäckvall, J.-E. *Chem. Rev.* **2003**, *103*, 3247. (b) Pellicer, H. *Tetrahedron* **2003**, *59*, 8291.
(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) 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.
(15) Ottolina, G.; Carrea, G. *Can. J. Chem.* **2002**, *80*, 559.
(16) (a) Valetti, F.; Gilardi, G. *Nat. Prod. Rep.* **2004**, *21*, 490. (b) Arnold, F. H. *Acc. Chem. Res.* **1998**, *31*, 125.
(17) Baudequin, C.; Baudoux, J.; Levillain, J.; Cahard, D.; Gaumont, A.-C.; Plaquevent, J.-C. *Tetrahedron: Asymmetry* **2003**, *14*, 3081.
(18) (a) Stewart, J. D. *Curr. Opin. Biotechnol.* **2000**, *11*, 363. (b) Nakamura, K.; Yamanaka, R.; Matsuda, T.; Harada, T. *Tetrahedron: Asymmetry* **2003**, *14*, 2659.
(19) (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.
(20) Bornscheuer, U. T.; Kazlauskas, R. J. *Hydrolases in Organic Synthesis*; Verlag Chemie: Weinheim, 1999.
(21) (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**, in press. (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.
(22) Izquierdo, I.; Plaza, M. T.; Rodríguez, M.; Tamayo, J. *Tetrahedron: Asymmetry* **1999**, *10*, 449.
(23) Neri, C.; Williams, J. M. J. *Adv. Synth. Catal.* **2003**, *345*, 835.
(24) Fellows, I. M.; Kaelin, D. E.; Martin, S. F. *J. Am. Chem. Soc.* **2000**, *122*, 10781.
(25) Kastrinsky, D. B.; Boger, D. L. *J. Org. Chem.* **2004**, *69*, 2284.
(26) Takabe, K.; Mase, N.; Hashimoto, H.; Tsuchiya, A.; Ohbayasi, T.; Yoda, H. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1967.
(27) Yokomatsu, T.; Takada, K.; Yasumoto, A.; Yuasa, Y.; Shibuya, S. *Heterocycles* **2002**, *56*, 545.
(28) Takabe, K.; Iida, Y.; Hiyoshi, H.; Ono, M.; Hirose, Y.; Fukui, Y.; Yoda, H.; Mase, N. *Tetrahedron: Asymmetry* **2000**, *11*, 4825.
(29) (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.
(30) (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.
(31) Bódai, V.; Novák, L.; Poppe, L. *Synlett* **1999**, 759.
(32) (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.
(33) Shimada, K.; Kauragi, Y.; Fukuyama, T. *J. Am. Chem. Soc.* **2003**, *125*, 4048.
(34) Chênevert, R.; Courchesne, G. *Tetrahedron Lett.* **2002**, *43*, 7971.
(35) Kirihara, M.; Kawasaki, M.; Takuwa, T.; Kakuda, H.; Wakikawa, T.; Takeuchi, Y.; Kirk, K. L. *Tetrahedron: Asymmetry* **2003**, *14*, 1753.
(36) Lane, J. W.; Halcomb, R. L. *J. Org. Chem.* **2003**, *68*, 1348.
(37) Lane, J. W.; Halcomb, R. L. *Org. Lett.* **2003**, *5*, 4017.
(38) (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.
(39) (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.
(40) 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.
(41) (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.
(42) Nakazawa, K.; Hayashi, M.; Tanaka, M.; Aso, M.; Suemune, H. *Tetrahedron: Asymmetry* **2001**, *12*, 897.
(43) (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.
(44) Chênevert, R.; Courchesne, G.; Caron, D. *Tetrahedron: Asymmetry* **2003**, *14*, 2567.
(45) Chênevert, R.; Rose, Y. S. *J. Org. Chem.* **2000**, *65*, 1707.
(46) Davoli, P.; Caselli, E.; Bucciarelli, M.; Forni, A.; Torre, G.; Prati, F. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1948.
(47) Berkowitz, D. B.; Choi, S.; Maeng, J. H. *J. Org. Chem.* **2000**, *65*, 847.
(48) Chênevert, R.; Dickmann, M. *J. Org. Chem.* **1996**, *61*, 3332.
(49) Chênevert, R.; Ziarani, G. M.; Morin, M. P.; Dasser, M. *Tetrahedron: Asymmetry* **1999**, *10*, 3117.
(50) (a) Chênevert, R.; Morin, M.-P. *J. Org. Chem.* **1999**, *64*, 3178. (b) Chênevert, R.; Ziarani, G. M.; Dasser, M. *Heterocycles* **1999**, *51*, 593.
(51) Luzzio, F. A.; Fitch, R. W. *J. Org. Chem.* **1999**, *64*, 5485.
(52) Matsumoto, T.; Konegawa, T.; Yamaguchi, H.; Nakamura, T.; Sugai, T.; Suzuki, K. *Synlett* **2001**, 1650.

(53) Toyama, K.; Iguchi, S.; Sakazaki, H.; Oishi, T.; Hirama, M. *Bull. Chem. Soc. Jpn.* **2001**, *74*, 997.
(54) Oishi, T.; Maruyama, M.; Shoji, M.; Maeda, K.; Kumahara, N.; Tanaka, S.-i.; Hirama, M. *Tetrahedron* **1999**, *55*, 7471.
(55) Hilpert, H.; Wirz, B. *Tetrahedron* **2001**, *57*, 681.
(56) Wirz, B.; Iding, H.; Hilpert, H. *Tetrahedron: Asymmetry* **2000**, *11*, 4171.
(57) Zhao, Y.; Wu, Y.; De Clerq, P.; Vandewalle, M.; Maillos, P.; Pascal, J.-C. *Tetrahedron: Asymmetry* **2000**, *11*, 3887.
(58) Hegemann, K.; Schimanski, H.; Höweler, U.; Haufe, G. *Tetrahedron Lett.* **2003**, *44*, 2225.
(59) Hirata, T.; Shimoda, K.; Kawano, T. *Tetrahedron: Asymmetry* **2000**, *11*, 1063.
(60) Böhm, C.; Austin, W. F.; Trauner, D. *Tetrahedron: Asymmetry* **2003**, *14*, 71.
(61) Matsumoto, T.; Konegawa, T.; Nakamura, T.; Suzuki, K. *Synlett* **2002**, 122.
(62) Öhrlein, R.; Baisch, G. *Adv. Synth. Catal.* **2003**, *345*, 713.
(63) Homann, M. J.; Vail, R.; Morgan, B.; Sabesan, V.; Levy, C.; Dodds, D. R.; Zaks, A. *Adv. Synth. Catal.* **2001**, *343*, 744, and references therein.
(64) See refs 10−16 from ref 63.
(65) Yu, M. S.; Lantos, I.; Peng, Z.-Q.; Yu, J.; Cacchio, T. *Tetrahedron Lett.* **2000**, *41*, 5647.
(66) Honda, T.; Koizumi, T.; Komatsuzaki, Y.; Yamashita, R.; Kanai, K.; Nagase, H. *Tetrahedron: Asymmetry* **1999**, *10*, 2703.
(67) Boaz, N. W. *Tetrahedron: Asymmetry* **1999**, *10*, 813, and references therein.
(68) Kashima, Y.; Liu, J.; Takenami, S.; Niwayama, S. *Tetrahedron: Asymmetry* **2002**, *13*, 953.
(69) Kiełbasiński, P.; Żurawiński, R.; Albrycht, M.; Mikołajczyk, M. *Tetrahedron: Asymmetry* **2003**, *14*, 3379.
(70) (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.
(71) (a) See ref 20, Chapter 5.2.6. (b) Ozegowski, R.; Kunath, A.; Schick, H. *Tetrahedron: Asymmetry* **1993**, *4*, 695.
(72) Ozegowski, R.; Kunath, A.; Schick, H. *Liebigs Ann. Chem.* **1993**, 805.
(73) Ostaszewski, R.; Portlock, D. E.; Fryszkowska, A.; Jeziorska, K. *Pure Appl. Chem.* **2003**, *75*, 413.
(74) See ref 3b, Chapter 17.
(75) Ohta, H. *Chimia* **1996**, *50*, 434.
(76) Yokoyama, M.; Sugai, T.; Ohta, H. *Tetrahedron: Asymmetry* **1993**, *4*, 1081.
(77) (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.
(78) Wang, M.-X.; Liu, C.-S.; Li, J.-S. *Tetrahedron: Asymmetry* **2001**, *12*, 3367.
(79) 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.
(80) 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.
(81) López-García, M.; Alfonso, I.; Gotor, V. *Tetrahedron: Asymmetry*, **2003**, *14*, 603.
(82) Puertas, S.; Rebolledo, F.; Gotor, V. *J. Org. Chem.* **1996**, *61*, 6024.
(83) López-García, M.; Alfonso, I.; Gotor, V. *J. Org. Chem.* **2003**, *68*, 648.
(84) Sánchez, V. M.; Rebolledo, F.; Gotor, V. *J. Org. Chem.* **1999**, *64*, 1464.
(85) (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: Enzymol.* **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.
(86) (a) Csuk, R.; Glanzer, B. I. *Chem. Rev.* **1991**, *91*, 49. (b) Stewart, J. D. *Curr. Opin. Drug Discov. Dev.* **1998**, *1*, 278.
(87) For a recent review on the topic see Nakamura, K.; Yamanaka, R.; Matsuda, T.; Harada, T. *Tetrahedron: Asymmetry* **2003**, *14*, 2659.
(88) Matsuda, T.; Harada, T.; Nakajima, N.; Itoh, T.; Nakamura, K. *J. Org. Chem.* **2000**, *65*, 157.
(89) Matsuda, T.; Nakajima, Y.; Harada, T.; Nakamura, K. *Tetrahedron: Asymmetry* **2002**, *13*, 971.
(90) Nakamura, K.; Inoue, Y.; Matsuda, T.; Misawa, I. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2397.
(91) Nakamura, K.; Fuiji, M.; Ida, Y. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3205.
(92) Nakamura, K.; Takenaka, K.; Fujii, M.; Ida, Y. *Tetrahedron Lett.* **2002**, *43*, 3629.
(93) Stampfer, W.; Kosjek, B.; Faber, K.; Kroutil, W. *J. Org. Chem.* **2003**, *68*, 402.
(94) Stampfer, W.; Kosjek, B.; Moitzi, C.; Kroutil, W.; Faber, K. *Angew. Chem., Int. Ed.* **2002**, *41*, 1014.
(95) (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.
(96) Goswami, A.; Bezbaruah, R. L.; Goswami, J.; Borthakur, N.; Dey, D.; Hazarika, A. K. *Tetrahedron: Asymmetry* **2000**, *11*, 3701.
(97) Goswami, J.; Bezbaruah, R. L.; Goswami, A.; Borthakur, N. *Tetrahedron: Asymmetry* **2001**, *12*, 3343.
(98) Homann, M. J.; Vail, R. B.; Previte, E.; Tamarez, M.; Morgan, B.; Dodds, D. R.; Zaks, A. *Tetrahedron* **2004**, *60*, 789.
(99) Baldasarre, F.; Bertoni, G.; Chiappe, C.; Marioni, F. *J. Mol. Catal. B: Enzymol.* **2000**, *11*, 55−58.
(100) Yadav, J. S.; Nanda, S.; Reddy, P. T.; Rao, A. B. *J. Org. Chem.* **2002**, *67*, 3900.
(101) Yadav, J. S.; Reddy, P. T.; Nanda, S. Rao, A. B. *Tetrahedron: Asymmetry* **2001**, *12*, 3381.
(102) Barbieri, C.; Caruso, E.; D'Arrigo, P.; Fantoni, G. P.; Servi, S. *Tetrahedron: Asymmetry* **1999**, *10*, 3931.
(103) Wei, Z.-L.; Lin, G.-Q.; Li, Z.-Y. *Bioorg. Med. Chem.* **2000**, *8*, 1129.
(104) Conceição, G. J. A.; Moran, P. J. S.; Rodrigues, J. A. R. *Tetrahedron: Asymmetry* **2003**, *14*, 43.
(105) Schubert, T.; Hummel, W.; Kula, M.-R.; Müller, M. *Eur. J. Org. Chem.* **2001**, 4181.
(106) Schubert, T.; Hummel, W.; Müller, M. *Angew. Chem., Int. Ed.* **2002**, *41*, 634.
(107) Heiss, C.; Phillips, R. S. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2821.
(108) Yadav, J. S.; Reddy, P. T.; Nanda, S.; Rao, A. B. *Tetrahedron: Asymmetry* **2001**, *12*, 63.
(109) Roy, S.; Alexandre, V.; Neuwels, M.; Le Texier, L. *Adv. Synth. Catal.* **2001**, *343*, 738.
(110) Yamamoto, H.; Ueda, M.; Ritsuzui, P.; Hamatani, T. E. Patent 1,318,200, 2003; *Chem. Abstr.* **2003**, *139*, 35200.
(111) Burns, M. P.; Wong, J. W. U.S. Patent 6,451,587, 2002; *Chem. Abstr.* **2002**, *137*, 246600h.
(112) Iwamoto, M.; Kawada, H.; Tanaka, T.; Nakada, M. *Tetrahedron Lett.* **2003**, *44*, 7239.
(113) (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.
(114) Kayser, M. M.; Mihovilovic, M. D.; Kearns, J.; Feicht, A.; Stewart, J. D. *J. Org. Chem.* **1999**, *64*, 6603.
(115) Ishihara, K.; Yamaguchi, H.; Hamada, H.; Nakajiman, N.; Nakamura, K. *J. Mol. Catal. B: Enzymol.* **2000**, *10*, 429.
(116) Patel, R. N.; Chu, L.; Chidambaram, R.; Zhu, J.; Kant, J. *Tetrahedron: Asymmetry* **2002**, *13*, 349.
(117) 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.
(118) Wolberg, M.; Hummel, W.; Wandrey, C.; Müller, M. *Angew. Chem., Int. Ed.* **2000**, *39*, 4306.
(119) Wolberg, M.; Hummel, W.; Müller, M. *Chem. Eur. J.* **2001**, *7*, 4562.
(120) Wolberg, M.; Ji, A.; Hummel, W.; Müller, M. *Synthesis* **2001**, 937.
(121) Enders, D.; Vicario, J. L.; Job, A.; Wolberg, M.; Müller, M. *Chem. Eur. J.* **2002**, *8*, 4272.
(122) Vicario, J. L.; Job, A.; Wolberg, M.; Müller, M.; Enders, D. *Org. Lett.* **2002**, *4*, 1023.
(123) Job, A.; Wolberg, M.; Müller, M.; Enders, D. *Synthesis* **2001**, 1796.
(124) Drochner, D.; Müller, M. *Eur. J. Org. Chem.* **2001**, 211.
(125) Gotor, V.; Dehli, J. R.; Rebolledo, F. *J. Chem. Soc., Perkin Trans. 1* **2000**, 307.
(126) Dehli, J. R.; Gotor, V. *Tetrahedron: Asymmetry* **2001**, *12*, 1485.
(127) Dehli, J. R.; Gotor, V. *Tetrahedron: Asymmetry* **2000**, *11*, 3693.
(128) For a recent reference see Fuganti, C.; Serra, S.; Dulio, A. *J. Chem. Soc., Perkin Trans. 1* **1999**, 279, and references therein.
(129) de Mancilha, M.; De Conti, R.; Moran, P. J. S.; Rodrigues, J. A. R. *Arkivoc* **2001**, 85.
(130) Fuganti, C.; Serra, S. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3758.
(131) Ferraboschi, P.; Reza-Elahi, S.; Verza, E.; Santaniello, E. *Tetrahedron: Asymmetry* **1999**, *10*, 2639.
(132) (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.
(133) Serra, S.; Fuganti, C. *Tetrahedron: Asymmetry* **2001**, *12*, 2191.
(134) Molinari, F.; Gandolfi, R.; Villa, R.; Urban, E.; Kiener, A. *Tetrahedron: Asymmetry* **2003**, *14*, 2041.
(135) Romano, A.; Gandolfi, R.; Nitti, P.; Rollini, M.; Mollinari, F. *J. Mol. Catal. B: Enzymol.* **2002**, *17*, 235.

(136) Gourdel-Martin, M.-E.; Comoy, C.; Huet, F. *Tetrahedron: Asymmetry* **1999**, *10*, 403.
(137) (a) Holland, H. L. *Biotechnology*; Wiley-VCH: Weinheim, 1998; Vol. 8a. (b) Holland, H. L. *Curr. Opin. Biol.* **1999**, *3*, 22.
(138) Adam, W.; Lukacs, Z.; Saha-Möller, C. R.; Weckerle, B.; Schreier, P. *Eur. J. Org. Chem.* **2000**, 2923.
(139) (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.
(140) Chang, D.; Wiltholt, B. Li, Z. *Org. Lett.* **2000**, *2*, 3949.
(141) (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.
(142) Littlechild, J. *Curr. Opin. Chem. Biol.* **1999**, *3*, 28.
(143) Hu, S.; Hager, L. P.; *J. Am. Chem. Soc.* **1999**, *121*, 872.
(144) 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.
(145) For a recent review see: van de Velde, F.; van Rantwijk, F.; Sheldon, R. A. *Trends Biotechnol.* **2001**, *19*, 73.
(146) (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.
(147) (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.
(148) (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.
(149) (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.
(150) Chen, G.; Kayser, M. M.; Mihovilovic, M. D.; Mrstik, M. E.; Martinez, C. A.; Stewart, J. D. *New J. Chem.* **1999**, *23*, 827.
(151) Kerridge, A.; Willetts, A.; Holland, H. *J. Mol. Catal. B: Enzymol.* **1999**, *6*, 59.
(152) Nimri, S.; Keinan, E. *J. Am. Chem. Soc.* **1999**, *121*, 8978.
(153) Adam, W.; Heckel, F.; Saha-Möller, C. R.; Taupp, M.; Screier, P. *Tetrahedron: Asymmetry* **2004**, *15*, 983.
(154) 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.
(155) 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**, *199*, 235.
(156) 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.
(157) (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*, 564, and references therein.
(158) Hudlicky, T.; Gonzalez, D.; Gibson, D. T. *Aldrichimica Acta* **1999**, *32*, 35.
(159) See for instance ref 152, section 4.
(160) Kauppi, B.; Lee, K.; Carredano, E.; Parales, R. E.; Gibson, D. T.; Eklund, H.; Ramaswamy, S. *Structure* **1998**, *6*, 571.
(161) (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.
(162) Banwell, M. G.; Bray, A. M.; Wong, D. J. *New J. Chem.* **2001**, *25*, 1351.
(163) Banwell, M. G.; Bray, A. M.; Edwards, A. J.; Wong, D. J. *New J. Chem.* **2001**, *25*, 3.
(164) Phung, A. N.; Zannetti, M. T.; Whited, G.; Fessner, W.-D. *Angew. Chem., Int. Ed.* **2003**, *42*, 4821.
(165) Schmid, A.; Hofstetter, K.; Feiten, H.-J.; Hollmann, F.; Witholt, B. *Adv. Synth. Catal.* **2001**, *343*, 732.
(166) Hollmann, F.; Lin, P.-C.; Witholt, B.; Schmid, A. *J. Am. Chem. Soc.* **2003**, *125*, 8209.
(167) Hofstetter, K.; Lutz, J.; Lang, I.; Witholt, B.; Schmid, A. *Angew. Chem., Int. Ed.* **2004**, *43*, 2163.
(168) See for instance Steinreiber, A.; Faber, K. *Curr. Opin. Biotechnol.* **2001**, *12*, 552.
(169) Chiappe, C.; De Rubertis, A.; Marioni, F.; Simonetti, A. *J. Mol. Catal. B: Enzymol.* **2000**, *10*, 539.
(170) Weijers, C. A. G. M.; de Bont, J. A. M. *J. Mol. Catal. B: Enzymol.* **1999**, *6*, 199.
(171) Chang, D.; Wang, Z.; Heringa, M. F.; Wirthner, R.; Witholt, B.; Li, Z. *Chem. Commun.* **2003**, 960.
(172) Chang, D.; Heringa, M. F.; Witholt, B.; Li, Z. *J. Org. Chem.* **2003**, *68*, 8599.
(173) 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.
(174) 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.
(175) Stewart, J. D. *Curr. Org. Chem.* **1998**, *2*, 211.
(176) (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.
(177) Mihovilovic, M. D.; Chen, G.; Wang, S.; Kyte, B.; Rochon, F.; Kayser, M. M.; Stewart, J. D. *J. Org. Chem.* **2001**, *66*, 733.
(178) Mihovilovic, M. D.; Müller, B.; Kayser, M. M.; Stewart, J. D.; Fröhlich, J.; Stanetty, P.; Spreitzer, H. *J. Mol. Catal. B: Enzymol.* **2001**, *11*, 349.
(179) Mihovilovic, M. D.; Rudroff, F.; Kandioller, W.; Grötzl, B.; Stanetty, P.; Spreitzer, H. *Synlett* **2003**, 1973.
(180) Wang, S.; Kayser, M. M.; Iwaki, H.; Lau, P. C. K. *J. Mol. Catal. B: Enzymol.* **2003**, *22*, 211.
(181) Mihovilovic, M. D.; Müller, B.; Kayser, M. M.; Stanetty, P. *Synlett* **2002**, 700.
(182) Mihovilovic, M. D.; Müller, B.; Schulze, A.; Stanetty, P.; Kayser, M. M. *Eur. J. Org. Chem.* **2003**, 2243.
(183) Mihovilovic, M. D.; Rudroff, F.; Müller, B.; Stanetty, P. *Bioorg. Med. Chem. Lett.* **2003**, 1479.
(184) Kyte, B. G.; Rouvière, P.; Cheng, Q.; Stewart, J. D. *J. Org. Chem.* **2004**, *69*, 12.
(185) See for instance (a) Machajewski, T. D.; Wong, C.-H. *Angew. Chem., Int. Ed.* **2000**, *39*, 1352. (b) Ref 3a, Chapter 9.
(186) 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.
(187) (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.
(188) Zhong, G.; Lerner, R. A.; Barbas, C. F., III. *Angew. Chem., Int. Ed.* **1999**, *38*, 3738.
(189) (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.
(190) For an recent review on ThDP-dependent enzymes see Pohl, M.; Lingen, B.; Müller, M. *Chem. Eur. J.* **2002**, *8*, 5288.
(191) Iding, H. Ph.D. Thesis, University of Düsseldorf, 1998.
(192) For a review see Iding, H.; Siegert, P.; Mesch, K.; Pohl, M. *Biochim. Biophys. Acta* **1998**, *1385*, 307, and references therein.
(193) For a review see Oliver, A. L.; Anderson, B. N.; Roddick, F. A. *Adv. Microb. Physiol.* **1999**, *41*, 1.
(194) 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.
(195) Dünnwald, T.; Demir, A. S.; Siegert, P.; Pohl, M.; Müller, M. *Eur. J. Org. Chem.* **2000**, 2161.
(196) 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.
(197) Dünkelmann, P.; Kolter, D.; Nitsche, A.; Demir, A. S.; Siegert, P.; Lingen, B.; Pohl, M.; Müller, M.; *J. Am. Chem. Soc.* **2002**, *124*, 12084.
(198) For general reviews on the topic see (a) North, M. *Tetrahedron: Asymmetry* **2003**, *14*, 147. (b) Gröger, H. *Adv. Synth. Catal.* **2001**, *343*, 547. (d) ref 3b, Chapters 11−12. (c) Effenberger, F.; Förster, S.; Wajant, H. *Curr. Opin. Biotechnol.* **2000**, *11*, 532. (e) Gregory, R. J. H. *Chem. Rev.* **1999**, *99*, 3649. (f) Effenberger, F. *Chimia* **1999**, *53*, 3.
(199) (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.
(200) See for instance Gröger, H.; Capan, E.; Barthuber, A.; Vorlop, K.-D. *Org. Lett.* **2001**, *3*, 1969, and references therein.
(201) Johnson, D. V.; Zabelinskaja-Mackova, A. A.; Griengl, H. *Curr. Opin. Chem. Biol.* **2000**, *4*, 103, and references therein.
(202) Effenberger, F.; Ziegler, T.; Förster, S. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 458.

(203) Han, S.; Lin, G.; Li, Z. *Tetrahedron: Asymmetry* **1998**, *9*, 1835.
(204) Lin, G.; Han, S.; Li, Z. *Tetrahedron* **1999**, *55*, 3531.
(205) Han, S.; Chen, P.; Lin, G.; Huang, H.; Li, Z. *Tetrahedron: Asymmetry* **2001**, *12*, 843.
(206) Chen, P.; Han, S.; Lin, G.; Huang, H.; Li, Z. *Tetrahedron: Asymmetry* **2001**, *12*, 3273.
(207) Menéndez, E.; Brieva, R.; Rebolledo, F.; Gotor, V. *Chem. Commun.* **1995**, 989.
(208) Monterde, M. I.; Nazabadioko, S.; Rebolledo, F.; Brieva, R.; Gotor, V. *Tetrahedron: Asymmetry* **1999**, *10*, 3449.
(209) Nazabadioko, S.; Pérez, R. J.; Brieva, R.; Gotor, V. *Tetrahedron: Asymmetry* **1998**, *9*, 1597.
(210) Monterde, M. I.; Brieva, R.; Gotor, V. *Tetrahedron: Asymmetry* **2001**, *12*, 525.

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