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Review

Biocatalysis as an alternative for the production of chiral epoxides: A comparative review

Hui Lin[a,b], Jing-Yuan Liu[a,b], Hai-Bo Wang[a,b], Abeer Ahmed Qaed Ahmed[a,b], Zhong-Liu Wu[a,*]

[a] Chengdu Institute of Biology, Chinese Academy of Sciences, P.O. Box 416, 9 South Renmin Road, 4th Section, Chengdu, Sichuan 610041, China
[b] Graduate School of the Chinese Academy of Sciences, Beijing 100039, China

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ABSTRACT

Enantiopure epoxides are remarkably versatile intermediates for the synthesis of numerous biologically active targets, to which considerable efforts have been devoted either chemically or biologically during the past few decades. This review will emphasize the application of biocatalysis as an efficient alternative that complements conventional chemical reactions, with a special focus on the epoxidation reactions catalyzed with monooxygenases and chloroperoxidases and the hydrolytic kinetic resolution catalyzed with epoxide hydrolases. Their scopes and limitations will be elaborately discussed as compared with their chemical counterparts. These biocatalytic approaches have not only provided environmentally friendly alternatives, but also displayed advantages for certain types of enantiopure epoxides, and could serve as potential tools for synthetic chemists.

Contents

Abbreviations: AMO, alkene monooxygenase; CPO, chloroperoxidase; DIPT, Diisopropyl tartrate; EH, epoxide hydrolase; FAD, flavin adenine dinucleotide; HKR, hydrolytic kinetic resolution; NAD, Nicotinamide adenine dinucleotide; NADP, Nicotinamide adenine dinucleotide phosphate; P450, cytochrome P450; SMO, styrene monooxygenase; TBHP, *tert*-butyl hydroperoxide; XMO, xylene monooxygenase.

* Corresponding author. Tel.: +86 28 85238385; fax: +86 28 85238385.
E-mail address: wuzhl@cib.ac.cn (Z.-L. Wu).

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1. Introduction

Enantiopure epoxides are well recognized as extremely important building blocks in fine chemical industry, particularly for the synthesis of biologically active compounds and pharmaceuticals due to the increasing demand for single isomers under legislative pressure for safety issues [1–4]. The versatility of the epoxide is attributed to the oxirane function that can be opened by various nucleophiles or undergo elimination, reduction or rearrangements to a multitude of more elaborate intermediates with the retention or inversion of chirality [5–7].

Therefore, the development of efficient synthesis methods for enantiopure epoxides has been a fundamental research area in both organic synthesis and biocatalysis. There are two major approaches to chiral epoxide formation: the direct stereospecific epoxidation of alkenes and the resolution of racemates, such as the hydrolytic kinetic resolution (HKR) of racemic epoxides [8], both of which have been successfully devised by synthetic chemists. The most prominent catalytic reaction is the Sharpless epoxidation which allows the asymmetric epoxidation of prochiral allylic alcohols [9,10]. Other state of the art protocols, such as the Katsuki–Jacobsen epoxidation of unfunctionalized *cis*-substituted alkenes [11–13], and the Shi's epoxidation of *trans*-substituted alkenes[14], provide valuable complements to the Sharpless epoxidation. The Jacobsen HKR is one of the most powerful approaches for kinetic resolution of epoxides [8,15]. Even though the maximum theoretical yield of HKR is restricted to 50%, excellent enantiomeric excesses (ee) have been obtained with a diverse of terminal oxides.

Not as most of the above-mentioned chemical procedures which were catalyzed with transition-metal-based reagents, the biocatalytic procedures have shown remarkable advantages such as biodegradability, highly specific catalysts, and mild reaction conditions [16–18]. It is evident that almost all nature products that contain the oxirane function are non-racemic, which is caused by the chirality of the relative enzymes [19]. There are indeed a huge number of enzymes from microorganisms, plants and animals capable of catalyzing the asymmetric epoxidation or HKR reaction in a regio-, diastereo-, and enantio-selective fashion, such as various monooxygenases, chloroperoxidases (CPO), and epoxide hydrolases (EH). The biocatalytic processes typically yield epoxide products with excellent enantiomeric excesses. They are particularly valuable for those substrates that are poorly accepted by chemical procedures [20,21]. Collectively, the diverse enzymes that could generate enantiopure epoxides exhibit extremely broad substrate spectra. However, individually those enzymes commonly lack flexibility in the accommodation of diverse substrates, which becomes the limiting factor in the selection of the appropriate biocatalysts.

This review would serve as a brief guide of biocatalytic procedures that might be harnessed in organic synthesis to achieve chiral epoxides with high enantio-purity. Several reviews on the preparation of chiral epoxides have been published [3,7,8,22–26]. This review does not seek to provide a comprehensive list of all kinds of synthetic/enzymatic methods, but rather to emphasize the potential of biocatalysis in the preparation of chiral epoxides. Lipase catalyzed kinetic resolution [23] that does not react with the epoxide moiety is not discussed here. The main part of this review describes several predominant enzymatic approaches, including the epoxidation of alkenes by monooxygenase and CPO, and the HKR of racemic epoxides by EH, all of which can afford excellent enantioselectivity. The scope and limitation of those biocatalytic approaches will be discussed as compared with the corresponding classic chemical methods.

2. Monooxygenase as an alternative for the asymmetric epoxidation of alkenes

2.1. Introduction of monooxygenases

Monooxygenases introduce one oxygen atom from molecular oxygen into the substrate, with the other atom reduced to water [27]. The enzymes are involved in a wide variety of biological processes that include drug detoxification, biodegradation of aromatic compounds, biosynthesis of antibiotics and sidrophores, and many others [28–31]. In these reactions, the role of oxygen is not restricted to serving as electron acceptor but involves incorporation into the substrate. NAD(P)H is required as the ultimately reducing agent from which the electrons are delivered to the enzyme–substrate complex via a redox system. The most common cofactor of the monooxygenase complex appears to be either the heme-iron or FAD, while other cofactors, such as non-heme iron, tetrhydropteridine and copper ion, are also found in a wide variety of species [27,32,33].

It has been reported that monooxygenases can carry out an enormous amount of chemical reactions [34–37], among which enzymes that can transform alkenes into the corresponding oxides with excellent enantioselectivities, such as styrene monooxygenase (SMO), xylene monooxygenase (XMO) and alkene/alkane monooxygenase, will be described here in much detail. Other monooxygenases like cytochrome P450 enzymes (P450s) [38–40] and methane monooxygenases [41,42] are not covered because of their low to medium enantioselectivity in asymmetric epoxidation, which makes them impractical for synthetic chemists. For example, it has been reported that P450cam from *Pseudomonas putida* is capable of performing the transfer of *cis*-β-methylstyrene to the (*S*, *R*)-oxide with 78% ee [38]. The same limitation exists for methane monooxygenase, the basic function of which is to catalyze the transformation of methane into methanol, with 14–28% ee for the epoxidation of short-chain alkenes (C2–C4) [41].

2.1.1. Styrene monooxygenase

Styrene monooxygenase (SMO, EC 1.14.13.X) is an enzyme that transforms styrene into (*S*)-styrene oxide in the upper catabolic pathway of styrene degradation [43,44]. It belongs to flavoprotein monooxygenase family and contains a two-component flavoenzyme composed of a FAD-specific styrene epoxidase (StyA) and NADH-specific flavin reductase (StyB), encoded by *StyA* and *StyB* genes [45,46]. SMO is an attractive enzyme for the synthesis of epoxides, owning to its exquisite regio- and enantioselecties, mild reaction conditions, and the adoption of oxygen as an inexpensive nontoxic oxidant [47–50].

Extensive exploration for the SMOs from *Pseudomonas* sp. has been carried out, and the representative characteristics have been reported [46,51–53]. StyA, with a subunit size of 47 kDa, binds tightly to the reduced FAD, and StyB, with a molecular mass of 18 kDa, catalyzes NADH oxidation with two-electron reduction of FAD [46]. The crystal structure of StyA from *P. putida* S12 indicates that each subunit forms two distinct domains, in which the large cavity opening to the surface forms the FAD binding site and another cavity at the base of this pocket likely represents the styrene binding site [51,52].

In the epoxidation of styrene, molecular oxygen first reacts with StyA-FAD_{red} to yield an FAD C(4a)-peroxide intermediate, then styrene reacts with the peroxide intermediate and transforms into oxide [51]. In the StyB catalyzed reduction of FAD, the steady-state kinetics for StyB indicate a mechanism of sequential binding of NADH and flavin to StyB, and the presence of StyA has no interaction with the NADH oxidation activity [46].

2.1.2. Xylene monooxygenase

Xylene monooxygenase (XMO, EC 1.14.13.X), which catalyzes the insertion of an oxygen atom into a C–H bond with a histidine-rich active site, is a membrane-spanning, non-heme diiron enzyme [54]. The main function of XMO is to catalyze the hydroxylation of xylene and toluene, but it can as well be applied to the hydroxylation of alkanes and the conversion of styrene to (*S*)-styrene oxide [55,56].

XMO consists of two polypeptide subunits: the NADH acceptor reductase component, XylA, and the hydroxylase component, XylM [57]. The mechanism of the reaction for XMO is still obscure. It has been proposed that XylM hydroxylates alkanes by cleaving the C–H bond homolytically to generate a carbon-centered radical before the enzyme rebounds with Fe-OH to form an iron-coordinated alcohol [54]. However, the results could not eliminate the possibility of another mechanism involving an electrophilic Fe(III)OOH species that could insert OH^+ into a C–H bond [58].

2.1.3. Alkene monooxygenase

Alkene monooxygenase (AMO, EC 1.14.13.69), which belongs to the family of binuclear non-heme iron monooxygenases, catalyzes the oxidation of C2–C6 aliphatic alkenes to the corresponding *R*-enantiomer epoxides in high yield, a reaction catalyzed by very few oxygenase enzymes [59,60].

The AMOs from *Xanthobacter autotrophicus* Py2 and *Rhodococcus corallinus* (formerly *Nocardia corallina*) B-276 are the most thoroughly characterized. The AMO from *X. autotrophicus* Py2 is a four-component system comprised of an NADH oxidoreductase, a Rieske-type ferredoxin, an oxygenase and a small coupling protein [60]. However, the AMO from *R. corallinus* B-276 is simpler, consisting of a terminal oxygenase, an NADH-dependent reductase (37 kDa) and a regulatory component (14 kDa), encoded by the four-gene operon *amoABCD* [61]. The epoxygenase component comprises a large (53 kDa) and a small (35 kDa) subunit and contains a bridged diiron centre. It has been suggested that residues Ala91 and Ala185 control the configuration of the epoxide product by clamping the intermediate and/or the product of the reaction. The reductase component contains two prosthetic groups, an FAD centre and a [2Fe-2S] cluster that supply the energy required for the epoxidation reaction [62,63]. The AMO from *Mycobacterium* sp. is similar to the three-component system enzyme from *R. corallinus* B-276 [64].

2.1.4. Alkane monooxygenase

Alkane monooxygenase (ω-hydroxylase, EC 1.14.15.3) possesses important functions for the hydroxylation of fatty acids and alkanes, and the epoxidation of alkenes in the presence of a reduced pyridine nucleotide, a reductase, rubredoxin, and molecular oxygen [65].

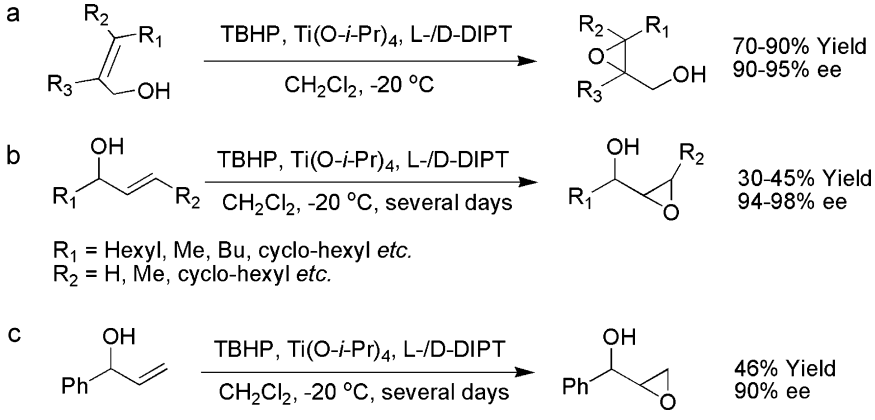
The alkane monooxygenase from *Pseudomonas oleovorans* has been purified by gel filtration, and characterized as a non-heme iron protein containing one iron atom and one cysteine residue per polypeptide chain [66,67]. Three protein components required for the hydrocarbon hydroxylation are rubredoxin (a non-heme iron protein containing no labile sulfide), a NADH-rubredoxin reductase and the ω-hydroxylase. During the hydroxylation process, the incorporated oxygen derived from O_2 does not need a reduced iron–sulfur protein as one donor. It has been proposed that the histidine motif in the enzyme plays a vital role as to coordinate the Fe ions comprising the diiron active site [68].

2.2. Asymmetric epoxidation of allylic alcohols

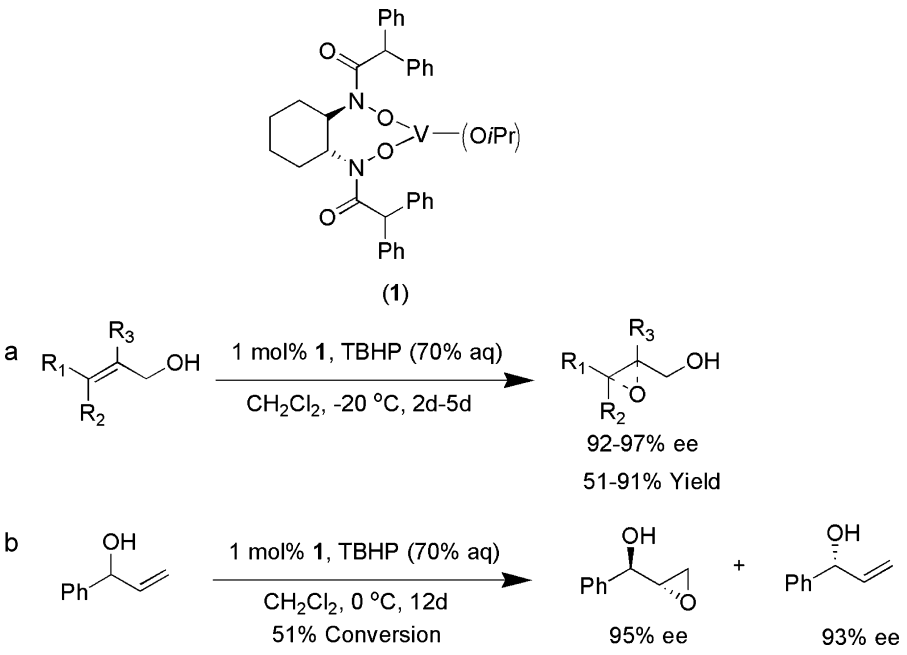
2.2.1. Classic methods for the asymmetric epoxidation of allylic alcohols

The predominant approach for the asymmetric epoxidation of allylic alcohols is through the titanium-catalyzed Sharpless epoxidation, which can usually achieve the enantioselective epoxidation of primary allylic alcohols catalyzed by L-(+)/D-(−)-diisopropyl tartrate and titanium tetraisopropoxide ($Ti(O\text{-}i\text{-}Pr)_4$) using *tert*-butyl hydroperoxide (TBHP) as the oxidant [10,22,69,70]. The absolute configuration of the resulting epoxide can be easily predicted using a rule developed by Sharpless, which correlates to the enantiomer of the tartrate used. Moreover, Sharpless asymmetric epoxidation is chemoselective, with only double bonds of allylic alcohols to be oxided (Scheme 1a).

Several reports have demonstrated that the same system could be used to kinetically resolve racemic secondary allylic alcohols



Scheme 1. Sharpless epoxidation of allylic alcohols.



Scheme 2. Enantioselective epoxidation of allylic alcohols by vanadium and BHA complexes.

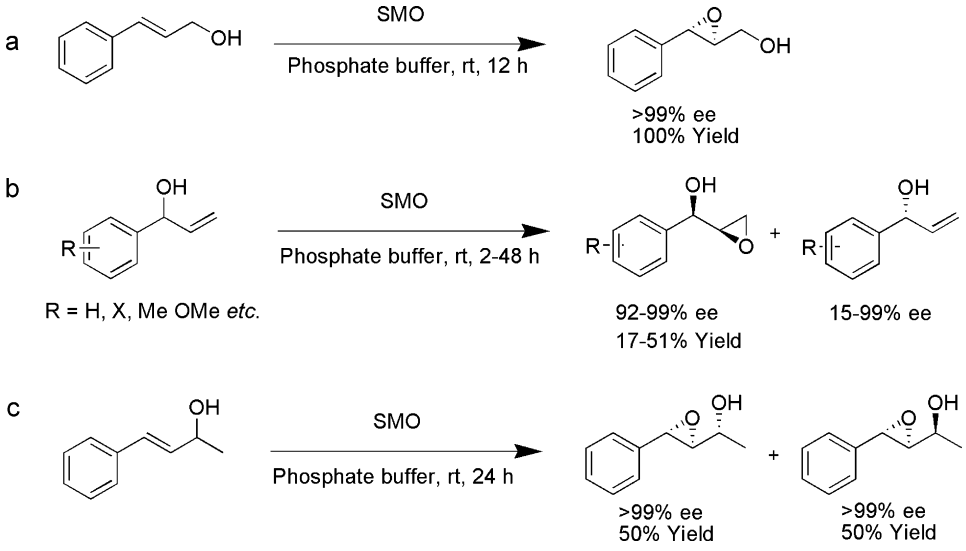
(Scheme 1b) [71–73]. Several aliphatic secondary allylic alcohols were transformed into the corresponding oxide with high enantio- (up to 98% ee) and diastereo-selecitvity (k_{fast}/k_{slow} up to 183) [71–75]. However, secondary phenyl allylic alcohols were not good substrates for this reaction. The kinetic resolution of 1-phenyl-2-propenol at −20 °C afforded the desired oxide with 90% ee in 46% yield after several days (Scheme 1c) [73].

An alternative method for the epoxidation of allylic alcohols is catalyzed with vanadium-complexes, which is also pioneered by Sharpless group [76]. Recently, Yamamoto et al. demonstrated that a complex of vanadium and C_2-symmetric bishydroxamic acid (**1**) in the presence of aqueous TBHP at −20 °C can provide epoxy alcohols with both good yields and high enantioselectivities (92–97% ee) (Scheme 2a) [77]. The reaction system was also applied to the kinetic resolution of 1-phenyl-2-propenol, which required 12 days at −20 °C, and the epoxy alcohol and the allylic alcohol were isolated with 95% and 93% ee, respectively (Scheme 2b) [77,78].

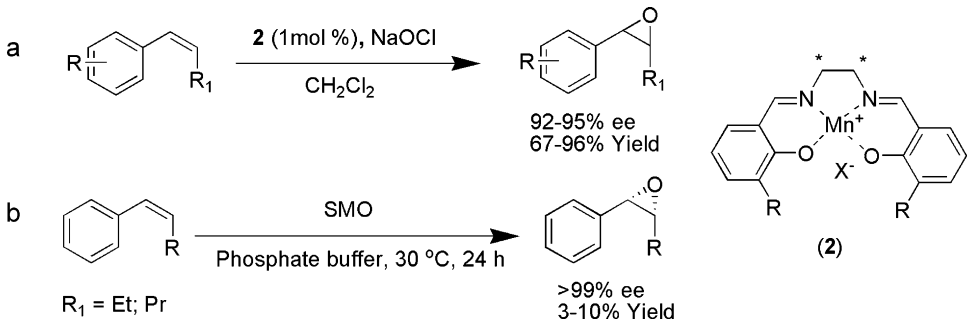
2.2.2. SMO-catalyzed asymmetric epoxidation of allylic alcohols

Primary allylic alcohols bearing a phenyl group could be a good substrate for SMO. For example, the epoxidation of (*Z*)-cinnamyl alcohol catalyzed with SMO from the genus of *Pseudomonas* yielded the corresponding (2*S*, 3*S*)-epoxide with >99% ee (Scheme 3a) [21,47], which is slightly better than that obtained from Sharpless epoxidation (98% ee) [79,80]. However, reports on the application of SMO to this group of substrates have been limited and yet to be explored.

A recent study demonstrated that SMO can also catalyze the kinetic resolution of secondary phenyl allylic alcohols [81]. The SMO from *Pseudomonas* sp. LQ26 shows excellent ability in the chiral recognition of a series of non-conjugated secondary allylic alcohols. For example, the kinetic resolution of racemic 1-phenylprop-2-enol produced (1*R*, 2*R*)-phenyl glycidol with >99% ee and 98% de, and (*R*)-alcohol was recovered with >99% ee at 50% conversion for 2 h (Scheme 3b) [81], which displayed advantage over previously established chemistry methods, such as the Sharp-



Scheme 3. SMO-catalyzed enantioselective epoxidation of allylic alcohols.



Scheme 4. Asymmetric epoxidation of *cis*-styrene derivatives.

less epoxidation and vanadium-based method, which required up to 12 days for the same substrate to reach 50% conversion, and yielded the epoxide with 90–93% ee (Schemes 1c and 2b) [73,77].

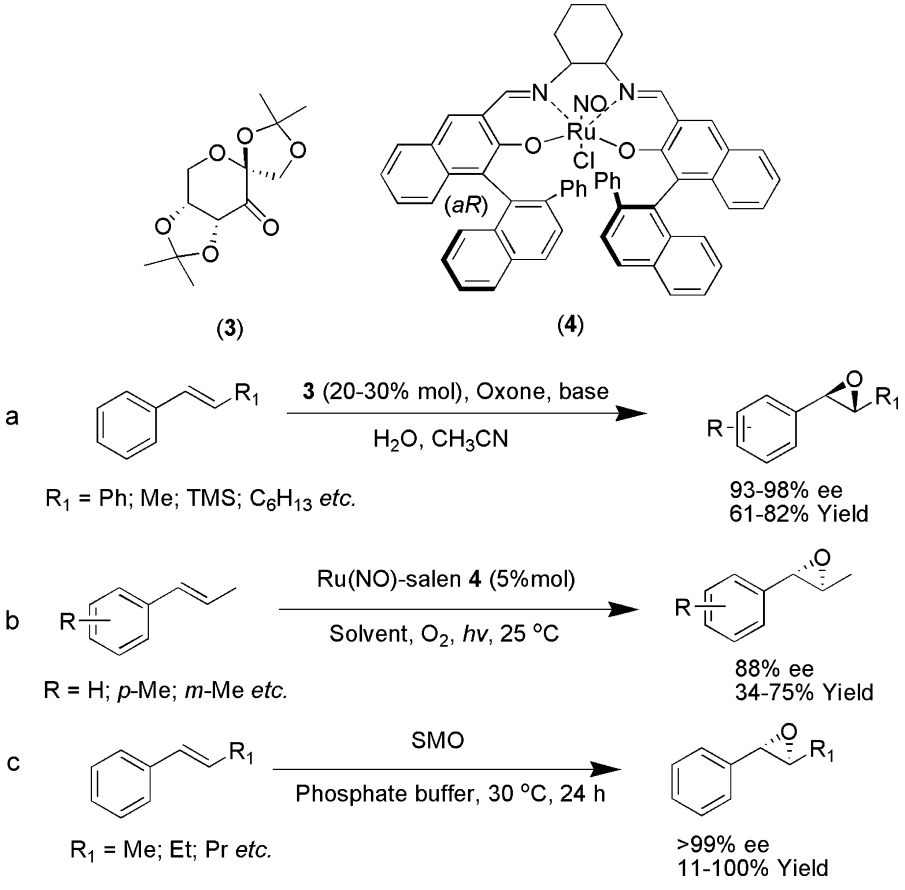
The epoxidation of conjugated secondary allylic alcohols, such as (*E*)-4-phenylbut-3-en-2-ol, can also be catalyzed by SMO, resulting in excellent enantioselectivity (>99% ee), albeit with low diastereoisomeric ratio (Scheme 3c) [21].

Although SMO can catalyze the epoxidation of aliphatic allylic alcohols, its enantio- and diastereo-selectivity are limited as compared with chemo-catalytic procedures [81]. Moreover, stereo-complementary SMO has not been reported yet. Currently, functionally characterized SMOs remain limited with the majority of them originated from the *Pseudomonas* species and sharing a high sequence similarity [48]. It awaits further exploration of the genetic diversity of SMOs to expand their applications.

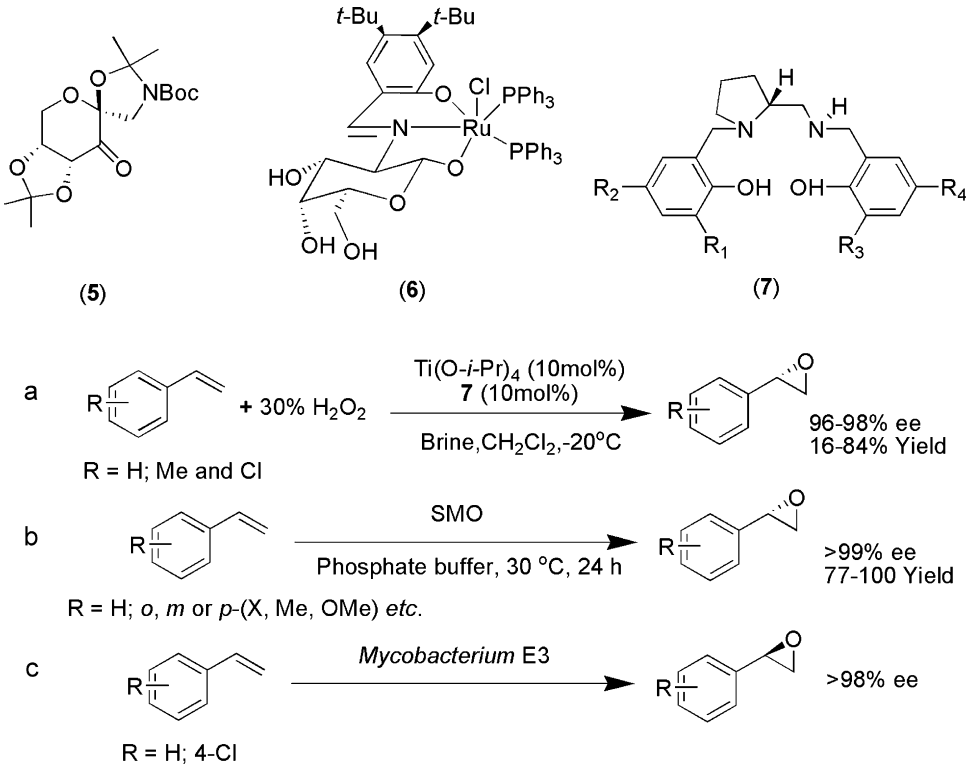
2.3. Asymmetric epoxidation of styrene and derivatives

2.3.1. Classic methods for the asymmetric epoxidation of styrene and derivatives

β-Substituented styrene derivatives can be *cis*- or *trans*- aromatic alkenes. The asymmetric epoxidation of *cis*-alkenes can be achieved using the Katsuki–Jacobsen epoxidation, in which chiral Mn(III)-salen (**2**) catalysts have been introduced with NaOCl/PhIO as an oxidant in CH_2Cl_2 at room temperature, giving good yields and higher selectivity (92–95% ee) (Scheme 4a) [11–13,82]. Either of the enantiomer can be produced by the change of the configuration of the catalyst. This method has been widely applied in the epoxidation of *cis*-alkenes and many salen derivatives have been introduced. However, *trans*-styrene derivatives are not good substrates for this reaction in terms of enantioselectivity (<88% ee) [11,12].



Scheme 5. Asymmetric epoxidation of *trans*-styrene derivatives.



Scheme 6. Asymmetric epoxidation of styrene derivatives.

The Shi epoxidation reaction has been successfully applied in the asymmetric epoxidation of *trans*-alkenes, which is catalyzed by the fructose-derived ketone (**3**, 20–30 mol%) and oxone in the mixture of water and acetonitrile, affording the corresponding (*R*, *R*)-oxides with 93–98% ee (Scheme 5a) [14,83,84]. Ketone **3** is commercially available, which facilitates the application of this method in many organic syntheses [85]. Another ligand for the epoxidation of *trans*-alkenes is a Ru(NO)-salen complex (**4**), which has been used by Katsuki and co-workers to catalyze the asymmetric epoxidation using oxygen as the oxidant in chlorobenzene. For *trans*-styrene derivatives, this method can afford the corresponding (*S*, *S*)-epoxides with medium to good enantioselectivity (76–92% ee) (Scheme 5b) [86].

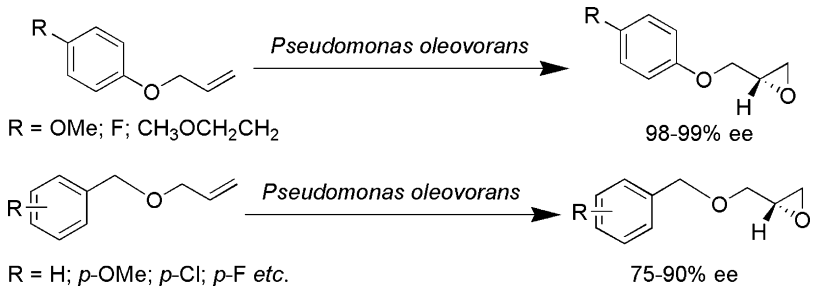
The direct epoxidation of terminal double bonds, such as in styrene, using Katsuki–Jacobsen epoxidation or Shi epoxidation usually suffers from insufficient enantioselectivities [11,84]. Using a modified carbohydrate-based chiral ketone (**5**, 15–30 mol%) designed for *cis*-olefins and terminal olefins with oxone (160 mol%) as an oxidant only resulted in terminal (*R*)-styrene epoxide derivatives with medium enantioselectivity (71–85% ee), the reactions were carried out with K_2CO_3 (4 equal) in dimethyl ether-dimethoxymethane (3:1, v/v) and buffer (0.2 M K_2CO_3–AcOH, pH 8.0) at −10 or 0 °C [87]. Better results were achieved using a mixed-ligand complexes of Ru(III) (**6**) containing tridentate chiral Schiff-base ligands derived from condensation of D-glucose amine and triphenylphosphine, which yielded styrene epoxide derivatives with 84–94% ee using TBHP as the oxidant in CH_2Cl_2 at room temperature [88]. Recently, a proline-derived C_1-symmetric salen ligand (**7**) with Ti(O-*i*-Pr)$_4$ catalyst exhibited improved enantioselcetivity in the epoxidation of terminal styrene derivatives. Several styrene derivatives were effectively converted to (*S*)-epoxides with 96–98% ee in CH_2Cl_2 at −20 °C (Scheme 6a) [89].

2.3.2. Monooxygenase-catalyzed asymmetric epoxidation of styrene and derivatives

The native substrate of SMO is styrene, which can undergo asymmetric epoxidation to enantiopure (*S*)-styrene oxide. Recombinant *E. coli* cells harboring the SMO genes from *Pseudomonas* sp. VLB120 [90,91], *P. fluorescens* ST [43,47], *P. putida* SN1 [92], *Pseudomonas* sp. LQ26 [21,48], *Rhodococcus opacus* 1CP [93] and from a metagenome library [94] have been cultivated and used in the epoxidation of the styrene (Scheme 6b). Most of the SMOs can yield (*S*)-styrene epoxide with excellent enantiomeric excess (>99% ee), which is difficult to achieve for terminal alkenes using chemo-catalyzed asymmetric epoxidation. SMO also accepts a series of styrene derivatives with substituents on the benzene ring, or at the α- or β-position of the carbon chain, or with changed aromatic core, resulting in high enantioselectivity in most cases (Schemes 4b, 5c and 6b). However, the enzymatic activity drops significantly for those bearing bulky substituent or electron withdrawing substituent [21,49,50,95,96].

A two-liquid phase system is routinely applied in SMO-catalyzed epoxidation, to partly release the effect of product/substrate inhibition, and has been successfully adopted in the pilot-scale preparation of enantiopure (*S*)-styrene oxide using the SMO from *Pseudomonas* sp. VLB120 expressed in recombinant *E. coli* [97,98]. The product concentration can reach up to 36.3 g L_{tot}^{-1}, and the bioprocess performs best in terms of production costs compared with three chemo-catalysis processes [97,99].

The strain of *P. putida* mt-2 harboring an XMO can also catalyze the epoxidation of styrene, providing (*S*)-styrene oxide with 93% ee [56,100,101]. The stereo-complementary reaction can be catalyzed by the strain of *Mycobacterium* sp. containing an AMO to yield (*R*)-styrene oxide with high enantioselectivity (98% ee) (Scheme 6c) [102]. However, both the XMO and AMO displayed limited substrate spectra. Besides styrene, only 3-chlorostyrene and



Scheme 7. Asymmetric epoxidation of alkenes catalyzed with *P. oleovorans*.

4-chlorostyrene could be transformed by XMO and AMO, respectively, with high enantioselectivity (>95% ee).

2.4. Monooxygenase-catalyzed asymmetric epoxidation of other substrates

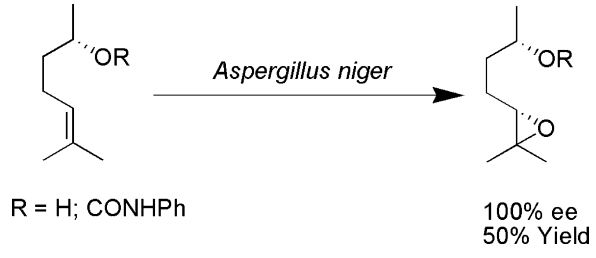
The alkane monooxygenase from *P. oleovorans* was at first reported to catalyze the asymmetric epoxidation of 1,7-octadiene with medium enantioselectivity, affording (*R*)-7,8-epoxy-1-octene with 80% ee [103]. Further investigation showed that allyl phenyl ethers and allyl benzyl ethers could be better substrates in terms of enantioselectivity, which yielded the corresponding (*S*)-epoxides with up to >98% ee (Scheme 7) [104]. This process has found industrial applications in the syntheses of the β-blockers Metoprolol and Atenolol [4].

In addition, a number of microorganisms with uncharacterized enzymes that might belong to the family of monooxygenase have been used in the epoxidation of alkenes and afforded the oxides with high enantioselectivities. For example, the strain of *Aerococcus* sp. M186 was reported to catalyze the transformation of propene and 1-butene into the (*R*)-oxides with 94% ee [105]. Furstoss and co-worker reported that a strain of the fungi *Aspergillus niger* could diastereoselectively transform (*S*)-sulcatol derivatives into a single enantiomer of (2*S*, 5*S*)-oxides (Scheme 8) [106].

3. Chloroperoxidase as an alternative for the asymmetric epoxidation of alkenes

3.1. Introduction of chloroperoxidase

The predominant chloroperoxidase (CPO, EC 1.11.1.10) investigated in biocatalysis is originated from the fungus *Caldariomyces fumago* as an exocellular enzyme with one ferriprotoporphyrine (IX) prosthetic group [107,108]. While the primary biological function of CPO is catalyzing the halogenation of organic compounds in the presence of halide ions and peroxides such as H_2O_2 [109,110], it can also catalyze halide-independent reactions, including asymmetric epoxidation, benzylic/allylic hydroxylation, sulfoxidation, oxidative halogenation, the oxidation of alcohols, aldehydes and amines [111,112]. The epoxidation reaction catalyzed by CPO is cofactor-independent, which makes the reaction easy to work with [111–113].



Scheme 8. Asymmetric epoxidation of (*S*)-sulcatols catalyzed with *A. niger*.

CPO exists in solutions at neutral pH as a monomer with a molecular weight of 42 kDa [108]. Its crystal structure shows that CPO folds into a unique structure with both a P450-like proximal pocket, in which one of the axial heme ligands derives from the sulfur atom of a cysteine residue, and a polar peroxidase-like distal pocket as the peroxide-binding site [114]. Despite intensive research over the decades, the mechanism of this enzyme is still controversial. Based on the known structural data, it is generally agreed that the halogenation is initiated with the oxidation of the resting high-spin Fe(III) enzyme by H_2O_2, followed by the reaction with a halide anion to give an Fe(III) hypohalite species [115]. Although the oxidation state of the Fe complex intermediate and the active site on which the halogenations occur is still obscure. Moreover, Ortiz de Montellano and co-workers have shown that epoxidation of CPO occurs via a ferryl oxygen transfer mechanism similar to the oxidation of styrene by P450 [116]. Despite the similarities, CPO can also catalyze a number of reactions not typical for P450 family of enzymes, that might be attributed to the more polar peroxidase-like heme environment as aforementioned [115].

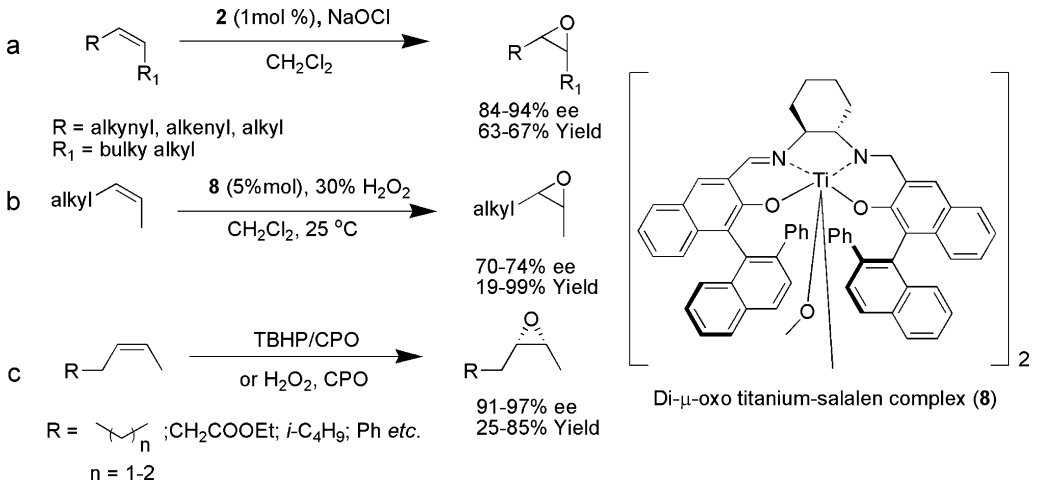
3.2. Asymmetric epoxidation of aliphatic cis-alkenes

3.2.1. Classic methods for the asymmetric epoxidation of aliphatic cis-alkenes

Aliphatic *cis*-alkenes can be epoxidized using the Katsuki–Jacobsen epoxidation as described for aromatic *cis*-alkenes with good yield and high enantioselectivity (84–94% ee) (Scheme 9a) [13,117]. Recently, a di-μ-oxo titanium-salen complex (di-μ-oxo Ti-salalen, **8**) has been developed to catalyze the formation of oxides from aliphatic *cis*-alkenes with aqueous hydrogen peroxide as the oxidant. Although it can be applied to a wide range of non-activated *cis*-alkenes, only medium enantioselectivity is obtained (70–74% ee) (Scheme 9b) [118].

3.2.2. CPO-catalyzed asymmetric epoxidation of aliphatic cis-alkenes

In general, the substrate spectrum of CPO is relatively narrow, but it shows excellent performance for some *cis*-alkenes. The enzyme can catalyze the asymmetric epoxidation of *cis*-disubstituted alkenes bearing alkyl groups with good enantioselectivity, and the best result has been obtained with *cis*-1-methyl substituted alkenes, yielding the corresponding (*R*, *S*)-oxides with up to 97% ee (Scheme 9c) [113,119–121]. Functionalized *cis*-2-alkenes, such as unsaturated carboxylic ester, and alkenes with a terminal bromine atom, have also been introduced into the asymmetric epoxidation by CPO with excellent enantioselectivities (91–97% ee), whereas *cis*-2-buten-l-ol was found to oxidized to the corresponding unsaturated aldehyde instead of epoxide



Scheme 9. Asymmetric epoxidation of aliphatic *cis*-alkenes.

[122] and *trans*-alkenes are very unreactive substrates for the CPO.

3.3. Asymmetric epoxidation of terminal aliphatic alkenes

3.3.1. Classic methods for the asymmetric epoxidation of terminal aliphatic alkenes

The asymmetric epoxidation of terminal aliphatic alkenes is rarely performed in the area of chemo-catalysis. A platinum catalyst reported by Strukul and co-workers has been applied to the epoxidization of several terminal olefins with medium to good enantioselective (58–83% ee) with H_2O_2 as the terminal oxidant in CH_2Cl_2 [123]. Recently, the di-μ-oxo Ti-salalen complex (**8**) has been used for the epoxidation of some terminal aliphatic alkenes in CH_2Cl_2 with aqueous hydrogen peroxide as the oxidant, affording chiral oxides with good enantioselectivity (79–97% ee) (Scheme 10a) [118].

3.3.2. CPO- and AMO-catalyzed asymmetric epoxidation of aliphatic terminal alkenes

1,1-Disubstituted terminal alkenes with one substituent being methyl can serve as good substrates for CPO to yield (*R*)-epoxides with excellent enantioselectivities (89–95% ee) using TBHP or H_2O_2 as the terminal oxidant with high turnover numbers ranging from 440 to 4200 (mol of epoxide/mol of CPO) (Scheme 10b) [121,124–127]. The enantioselectivity is largely depends on the length of the carbon chain of the substrate [126].

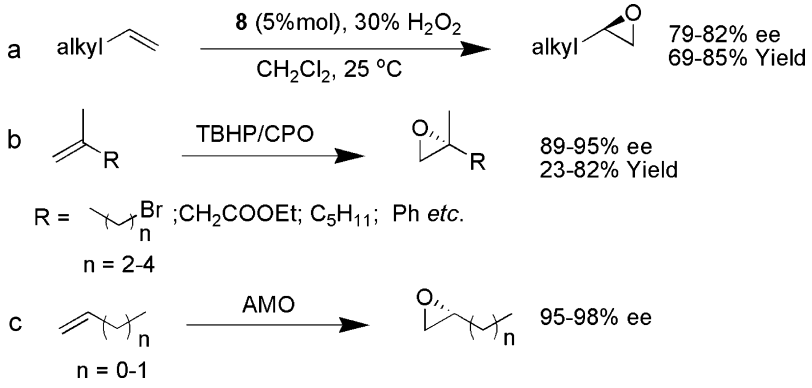
Another useful biocatalytic approach for terminal aliphatic alkenes is using the whole-cell of *R. corallinus* B-276 harboring an AMO, which can catalyze the asymmetric epoxidation of propene and 1-butene to synthesize the corresponding (*R*)-oxides with 95–98% ee (Scheme 10c) [59,128,129].

4. Epoxide hydrolase as an alternative for the kinetic resolution of epoxides

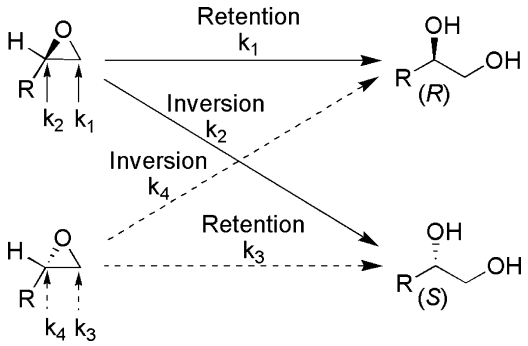
4.1. Introduction of epoxide hydrolase

Epoxide hydrolase (EH, EC 3.3.2.3) is so ubiquitous that it has been found in all types of living organisms, from microorganisms to plants and animals [130,131]. Functioned as catalyzing the kinetic resolution and the enantioconvergent hydrolysis of racemic epoxides into the corresponding diols, the enzyme has been intensively explored in the past decades, and a number of excellent reviews focusing on its application in organic synthesis have been published [24,131–133]. The cofactor-independent EH catalysis has several advantages, such as the broad substrate specificities with high regio- and enantioselectivity and activity in organic solvents, which leads to many successful applications [132].

The first determined structure of a microbial EH was from *Agrobacterium radiobacter* AD1, which shows a two-domain structure that consists of a core domain and a lid domain [134]. Then, the X-ray structure of the EH from *A. niger* has been reported, which consists of two 44 kDa subunits, and each subunit involves a core α/β domain, a lid domain and a long N-terminal meander. The α/β-hydrolase fold contains the catalytic triad Asp192, Asp348 and His374. The lid is composed of six α-helices which covers the catalytic site [135].



Scheme 10. Asymmetric epoxidation of aliphatic terminal alkenes.



Scheme 11. EH-catalyzed enantioselective ring opening of racemic epoxides.

The reaction mechanism of EH involves two steps. Two tyrosine residues in the lid domain form hydrogen bonds with epoxide ring, facilitating the nucleophilic attack of an aspartic residue in the active site [136]. In the following step, the intermediate is hydrolyzed by a water molecule with the help of a charge relay system composed of histidine and glutamic acid or aspartic acid and then released the diols. The second step is the rate-limiting step because of the higher activation energy [137].

Since the nucleophile can attack both carbon atoms of the epoxide ring, the reaction would proceed in four possible pathways (k_1–k_4, Scheme 11) [24,131]. The absolute configuration of the product is a result of both the enantioselectivity and the regioselectivity of the reaction, which depends on the enzyme origin and the nature of the substituent on the epoxide ring. Therefore, enantioconvergent processes are possible for EH-catalyzed reactions. In case of mono-substituted epoxides, it is common that the enantioconvergent reactions proceed via both the k_1 and k_4 pathways, leading to the (*R*)-diol in 100% theoretical yield [24,132,138].

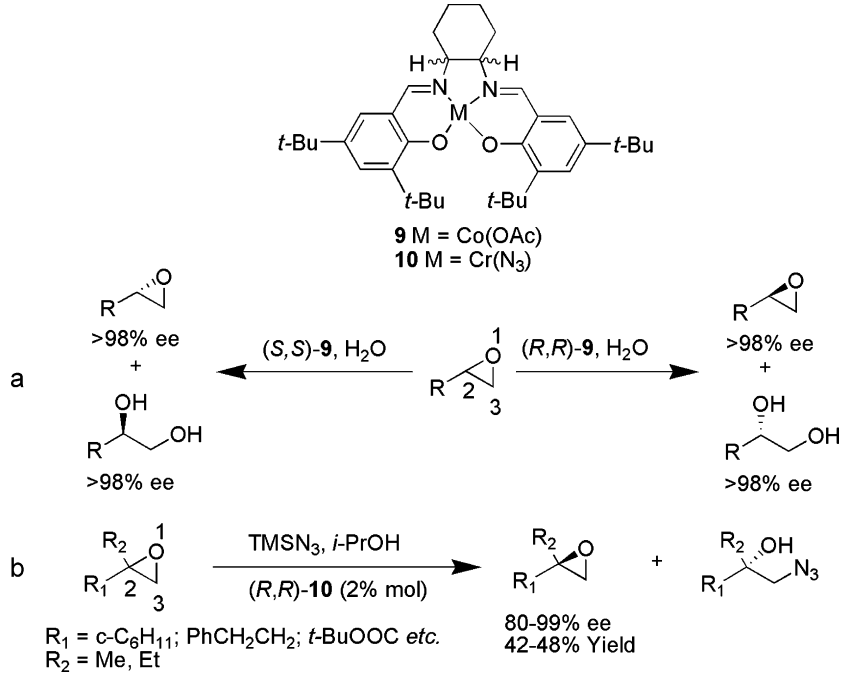
4.2. Jacobson's HKR for kinetic resolution of epoxides

Terminal epoxides in enantiomerically pure form are less accessible than other epoxides. The hydrolytic kinetic resolution (HKR) developed by Jacobsen's group has therefore proven to be one of the most powerful approaches to synthesize both terminal epoxides and their corresponding diols in highly enantiopure form [8,15].The technology is based on the (salen)Co(III)(OAc) (**9**) catalysts. A racemic terminal epoxide is combined with approximately half an equivalent of water in the presence 0.2–2 mol% of a chiral Co(salen) catalyst proceeded at room temperature to afford a mixture of unreacted epoxide and 1,2-diol in almost equal amounts (Scheme 12a) [15,139]. Either enantiomer of the oxides can be obtained using different enantiomers of the (salen)Co(III)(OAc) complex. A broad spectrum of racemic terminal epoxides bearing a wide range of functional groups can afford the corresponding chiral epoxides with >99% ee through this process [139]. The application of oligomeric Co(salen) catalyst system can further increase the reactivity to up to 50-fold per Co(salen) unit basis [140–144]. It is worth noting that both enantiomers of the catalyst **9** can be obtained from commercial sources or easily synthesized from (salen)Co(II), which enhances the advantage of this approach.

Although 2,2-disubstituted epoxides are unreactive under Jacobson's HKR conditions with catalyst **9** [139], a Cr catalyst with the same salen ligand (**10**) works for the resolution of racemic 2,2-disubstituted epoxides in the presence of HN_3, providing the desired enantio-enriched epoxides with 80–99% ee (Scheme 12b) [145,146].

4.3. EH-catalyzed kinetic resolution of epoxides

An attractive alternative to the Jacobson's HKR reaction is the use of EHs, which are readily available from a wide range of microorganism sources in sufficient quantities, and the large enzyme reservoir provides feasible tools to meet the potential requirements of synthetic chemists [24,147]. Currently, the kinetic resolution of racemic epoxides by EH has been well developed, and excellent enantioselectivity has been achieved for a variety of substrates (Fig. 1, and Tables 1 and 2). Several preparative-scale EH-catalyzed hydrolytic kinetic resolutions have been reported for the synthesis of enantiopure intermediates such as Eliprodil and (*S*)-Ibuprofen [148,149].



Scheme 12. Jacobsen's hydrolytic kinetic resolution reaction.

Table 1
EH-catalyzed hydrolysis of terminal epoxides.

Entry	Substrate	Enzyme source	Epoxide (ee %, config.)	Epoxide (yield %)	Reference
1	**11**, **12**	*R. glutinis*; *A. niger*; *Rhodotorula* sp.; *Rhodosporidium* sp.; *Trichosporon* sp.; *Mortierella isabellina*; *Chryseomonas luteola*; *Sphingomonas* sp.; *Mugil cephalus*	Up to >99 (*S*)	15–52	[150–163]
2	**11**, **12**	*Beauveria sulfurescens*; *Beauveria* densa; *Caulobacter crescentus*	Up to >99 (*R*)	17–34	[23,164–167]
3	**13**	*A. niger*; *A. radiobacter*	>99 (*S*)	6–43	[20,177–179]
4	Glycidyl ether derivatives	*Bacillus megaterium*; *Bacillus alcalophilus* MTCC10234;	>99 (*S*)	24–44	[168–171,175]
5	Glycidyl ether derivatives	*A. radiobacter Trichosporon loubierii* ECU1040 *Bacillus megaterium* ECU1001	99 (*R*)	28–41	[172–175]

Table 2
EH-catalyzed hydrolysis of disubstituted epoxides **14**–**17**.

Entry	Substrate	Enzyme source	Epoxide(ee%, config.)	Epoxide(yield %)	Reference
1	**14**	*Nocardia* sp.; *Rhodococcus* sp.	Up to 99 (*R*)	21–50	[176,180–183]
2	**14**, **15**	*A. niger*; *Methylobacterium* sp. *Mycoplana rubra* SM 73	Up to 99 (*S*)	10–47	[149,161,183–185]
4	**16**	*Chaetomium globosum*; *R. glutinis*; *Pseudomonas* sp.	>95 (*R*, *S*)	8–48	[158,161,186]
5	**17**	*Beauveria bassiana*; *R. glutinis*; *M. isabellina*	>98 (*R*, *R*)	11–48	[158,161,185]
6	**17**	*C. globosum*	97 (*S*, *S*)	12	[161]

In general, most enantiopure epoxides that acquired from the Jacobsen HKR reaction can be achieved through EH-catalyzed kinetic resolution, and cases of enantio-complementary are not rare owing to the great diversity of this group of enzymes (Table 1, entries 1–2, 4–5) [23,150–175]. At the same time, some types of epoxides that are not preferred by the Jacobsen HKR, such as pyridyloxirane and 2,2-disubstituted and 2,3-disubstituted epoxides, could be well accepted by EHs [20,158,161,176]. For example, a kinetic resolution of pyridyl oxiranes (**13**) were catalyzed by the whole cells of *A. niger* or *A. radiobacter*, preparing the (*S*)-epoxides as a single enantiomer with 27% yield at a substrate concentration of as high as 10 g L^{-1} (Table 1, entry 3) [20,177–179], while the Jacobsen's HKR reaction yielded products with <5% ee for the same set of substrates [20].

EH-catalyzed resolution of 2,2-disubstituted and 2,3-disubstituted epoxides, unfavorable substrates for the Jacobsen's HKR, have been reported to have an excellent enantioselectivity (Table 2, entries 1–6). Most of the bacterial EHs showed a preference for the (*S*)-2,2-disubstituted enantiomer, providing the (*R*)-epoxides with over 99% ee [176,180–183]. EHs with the opposite enantioselectivity to yield (*S*)-epoxides are also available (Table 2, entry 2) [149,161,183–185], an illustration of which is the synthesis of (*S*)-*p*-isobutyl-α-methyl styrene oxide (96% ee) that serves as an intermediate for (*S*)-Ibuprofen catalyzed with a strain of *A. niger* [149]. In the EH-catalyzed resolution of *trans*-2,3-disubstituted epoxides (**17**), mixed enantio-preference is common, which can provide enantio-complementary chiral epoxides simply by the choice of the appropriate enzymes or EH-containing strains (Table 2, entries 5–6) [158,161,185]. For *cis*-2,3-disubstituted epoxides (**16**), several EHs from different sources can provide the enantio-enriched (*R*, *S*)-epoxides (Table 2, entry 4) [158,161,186], while the opposite enantio-preference with high selectivity is rare. Only in one report, the (9*S*, 10*R*)-enantiomer of 8-(3-(5-methylhexyl)oxiran-2-yl)octanoic acid was prepared with >90% ee and 50% conversion using the EH from *Pseudomonas* NRRL B-2994 [187].

In addition, one trisubstituted racemic epoxide, 1-methyl-1,2-epoxy-cyclohexane, has also been transformed to the corresponding (1*S*, 2*S*)-diol, leaving the (1*S*, 2*R*)-epoxide unchanged with >99% ee and 30% yield with the whole cell of *Corynebacterium* C12 containing EH [188]. This type of substrate has not been reported for the Jacobsen's HKR.

Up until now, the whole cells of native strains containing EH activity are used more frequently. However, genes encoding various types of EHs have been cloned and expressed in heterologous hosts and these recombinant EHs have also been applied, which opens up more opportunities to control the product quality and purity [152,175], as well as to manipulate the enzymes at genetic level to improve their properties [189–191]. A recent report on a recombinant EH from *Rhodutorula glutinis* expressed in *S. cerevisiae*

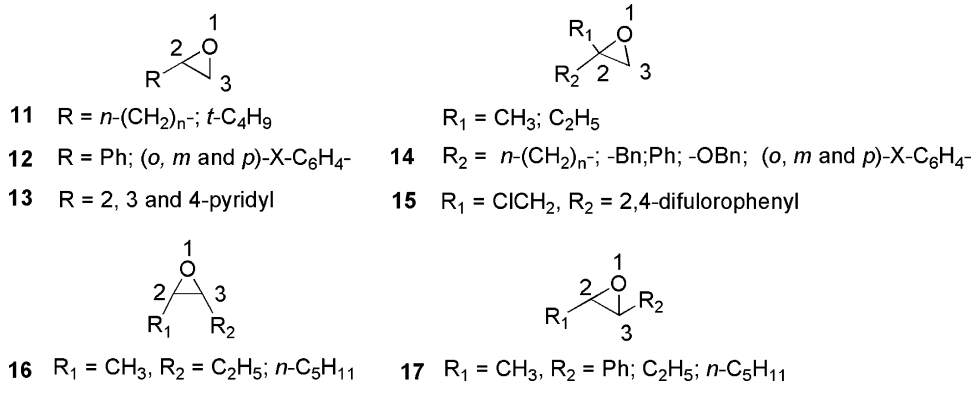


Fig. 1. Various substrates for EH-catalyzed hydrolytic kinetic resolution.

and *E. coli* showed that the reaction system could tolerate a styrene oxide concentration of 40 mM, resulting in (*S*)-styrene oxide with an enantiomeric excess higher than 99% and 21.3% yield [153,154].

Moreover, other forms of the catalysts, such as enzyme extract, lyophilized cells, and immobilization enzymes [152,192], have all been applied in the EH-catalyzed hydrolytic resolution, which facilitates the storage and application of these enzymes, and may encourage a broader spectrum of researches into this area.

5. Conclusions

Although classic chemo-catalysis for the enantioselective synthesis of chiral epoxides is extensively developed, biocatalytic approaches remain extremely attractive for several reasons. Firstly, they are typically highly stereo-selective and regio-selective, and can provide substrate spectrum complementary to chemo-catalysis for certain types of molecules. Secondly, they can be routinely carried out at ambient temperature under atmospheric pressure to achieve the best performance. Thirdly, the level of pollution caused could be much less as compared to chemo-catalysis for a lot of processes.

Common challenges for biocatalytic approaches do exist, such as limited enzyme availability and substrate spectrum, poor stability and sometimes low catalytic efficiency of the catalysts. In addition, biological catalysts with complementary stereoselectivity are not easily obtained as compared with metal catalysts which can be achieved simply by controlling the configuration of the ligands. Nonetheless, successes achieved at protein heterologous expression and *in vitro* engineering during the last decades have opened up unlimited access to a variety of enzymes with enhanced thermo-stability, pH-stability, organic solvent tolerance, extended substrate spectrum and high enantioselectivity, which is expected to tremendously promote the application of enzymes in organic synthesis, in particular, for chiral compounds.

Currently, several enzymes mentioned in this review are commercially available, such as the CPO from *C. fumago* as crude suspension in phosphate buffer (Sigma) and the EHs from *A. niger* and *Rhodococcus rhodochrous* as lyophilized powder (Sigma). Many others with known protein sequence, such as a variety of SMOs, AMOs and EHs, have been functionally expressed in commercially available microbial expression systems. It can be anticipated that with the increased variety and availability of biocatalysts, organic chemists would have abundant choices to obtain enantiopure epoxides through the biocatalytic approach in the near future.

Acknowledgements

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