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Enantioselective Bio-Hydrolysis of Various Racemic and *meso* Aromatic Epoxides Using the Recombinant Epoxide Hydrolase Kau2

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Abstract: Epoxide hydrolase Kau2 overexpressed in *Escherichia coli* RE3 has been tested with ten different racemic and *meso* α,β-disubstituted aromatic epoxides. Some of the tested substrates were bi-functional, and most of them are very useful building blocks in synthetic chemistry applications. As a general trend Kau2 proved to be an extremely enantioselective biocatalyst, the diol products and remaining epoxides of the bioconversions being obtained – with two exceptions – in nearly enantiomerically pure form. Furthermore, the reaction times were usually very short (around 1 h, except when stilbene oxides were used), and the use of organic co-solvents was well tolerated, enabling very high substrate concentrations (up to 75 g/L) to be reached. Even extremely sterically demanding epoxides such as *cis*- and *trans*-stilbene oxides were transformed on a reasonable time scale. All reactions were successfully conducted on a 1 g preparative scale, generating diol- and epoxide-based chiral synthons with very high enantiomeric excesses and isolated yields close to the theoretical maximum. Thus we have here demonstrated the usefulness and versatility of lyophilized *Escherichia coli* cells expressing Kau2 epoxide hydrolase as a highly enantioselective biocatalyst for accessing very valuable optically pure aromatic epoxides and diols through kinetic resolution of racemates or desymmetrization of *meso* epoxides.

Keywords: biotransformations; chiral resolution; enantioselectivity; epoxide hydrolase; kau2

Introduction

Optically enriched epoxides[1] and diols[2] are key synthons for the generation of chiral compounds of high synthetic value. Within this framework the epoxide hydrolase (EH) enzyme family has become a very popular source of biocatalysts during the last 20 years.[3] Indeed, EHs offer the advantage of being largely distributed within the various kingdoms of life,[4] functioning without added cofactors, and accepting a wide range of epoxide substrates. They are used in classical kinetic resolutions of racemates but can be used also in the desymmetrization of *meso*-epoxides or sometimes in enantioconvergent biotransformations. The classical limitation of kinetic resolution (i.e., a maximum theoretical yield of 50% for a pure enantiomer) can be overcome in the two latter cases, leading to a maximum theoretical yield of 100% for a pure diol product enantiomer. Furthermore, due to the development of genetic engineering techniques, it is now routine to search for biodiversity in specific biocatalysts, to overproduce them and to improve their performance in terms of specific activity, enantioselectivity or enantioconvergency by molecular evolution techniques.[5]

Recently, a new EH termed Kau2, whose gene was retrieved from environmental DNA of a microbial consortium, was successfully overexpressed in *E. coli* and shown to be of interest for kinetic resolution and enantioconvergent deracemization of *trans*- and *cis*-methylstyrene oxide, respectively.[6] To further explore the diversity of asymmetric epoxide and diol synthons generated through Kau2-catalyzed hydrolysis reactions, various aromatic racemic epoxides were tested, some of them being of high potential synthetic interest.[7] Indeed, some of the tested substrates were bifunctional, expanding thus their synthetic utility.

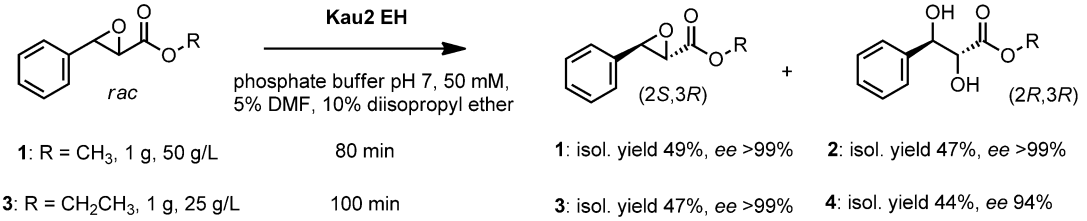
In this paper we describe the kinetic resolution of racemic methyl *trans*- and *cis*-3-phenylglycidate (**1**) and (**20**), ethyl *trans*-3-phenylglycidate (**3**), methyl *trans*-3-(4-methoxyphenyl)glycidate (**5**), *trans*- and *cis*-3-phenyloxirane-2-carbonitrile (**7**) and (**9**), *trans*-2-(bromomethyl)- and *trans*-2-(chloromethyl)-3-phenyloxirane (**11**) and (**13**), *trans*-stilbene oxide (**16**) as well as the desymmetrization of *cis*-stilbene oxide (**18**) using lyophilized cells of *E. coli* overexpressing the Kau2-EH as a biocatalyst. Compounds **1** and **3** are of synthetic interest since they can be used for the synthesis of the anticancer taxol side chain[8] or the nootropic drug (−)-Clausenamide.[9] Methyl 3-(4-methoxyphenyl)glycidate (**5**) in its (2*R*,3*S*)-stereochemical form is a very useful chiral synthon for the access to the calcium channel blocker Diltiazem, which acts as a potent vasodilator,[10] while each enantiomer of **5** as well as the corresponding diols can be used for the synthesis of all stereoisomers of isocytoxazone, the structural isomer of (−)-Cytoxazone, a cytokine modulator of microbial origin.[11] The chloro epoxide **13** has been used as a synthon to access the naturally occurring styryl lactones Leiocarpin C and Gonodiol.[7a] Furthermore, the diols **12** and **14** arising from ring-opening of bromo epoxide **11** and chloro epoxide **13** can easily be transformed under basic conditions (with retention of configuration) to get access to the terminal epoxide **15** which is useful for the synthesis of several natural and biologically active compounds.[7b,c] In almost all cases the biocatalytic transformation was of extremely high enantioselectivity, giving rise to an entire range of enantiomerically pure epoxides and diols. Furthermore, the tested reactions were successfully performed on a 1 g preparative scale, and the reaction times rarely exceeded 60–80 min. With these water-insoluble substrates an organic water-immiscible solvent was used without seriously affecting the enzyme activity. Another major advantage of such a biphasic biotransformation approach was that higher stabilities of the water-sensitive epoxidic substrates were achieved due to their reduced chemical hydrolysis in the organic phase.

Results and Discussion

Kinetic Resolution of Methyl and Ethyl *trans*-3-Phenylglycidate *rac*-1 and *rac*-3

Initially, *rac*-methyl *trans*-3-phenylglycidate **1** was tested as a substrate of the Kau2-EH (Scheme 1) in the 1 to 100 g/L concentration range in the presence of 5% DMF and 10% isooctane as a water-immiscible co-solvent. A ratio of 1 to 2 (mass of substrate to mass of lyophilized Kau2-expressing *E. coli* cells, m/m) was initially found convenient. For substrate concentrations lower than or equal to 10 g/L a nearly perfect kinetic resolution was observed, since both formed (2*R*,3*R*)-**2** and remaining (2*S*,3*R*)-**1** were obtained optically pure (*ee* >99%). Higher substrate concentrations of 25, 50 and 100 g/L resulted in *ee*s of 98% ($c=0.49$), 88% ($c=0.47$) and 69% ($c=0.41$) for the remaining epoxide, respectively, the *ee* of the formed diol being over 99% in all cases. Even when the reaction time was extended or new enzyme was added, the *ee* of the remaining epoxide could not be increased for these high substrate concentrations, suggesting that the reaction was blocked. In order to increase the substrate concentration in these kinetic resolutions, other water-immiscible solvents (10% v/v) than isooctane were tested by analyzing the *ee* of the remaining epoxide after one hour of reaction time at a substrate concentration of 50 g/L. While ethyl acetate was clearly the worst solvent (*ee* 79%), diisopropyl ether (*ee* 95%) proved to be better than isooctane (*ee* 87%), the *ee* of the residual epoxide remaining almost constant for extended reaction times (Figure 1).

Combining these results with the use of a more active Kau2-EH preparation of 1667 U/g (produced in a bioreactor) compared to 768 U/g (produced in flasks) enabled the substrate concentration to be increased to 50 g/L, ensuring ≥99% for both *ee*s of formed diol product and residual epoxide. Using these optimized conditions, the reaction was then conducted on a preparative scale (1 g) in 80 min, affording after extraction and purification the remaining epoxide (2*S*,3*R*)-**1** in 49% isolated yield (*ee* >99%) and the formed diol (2*R*,3*R*)-**2** in 47% isolated yield (*ee* >99%). These results compared particularly well with



Scheme 1. Preparative kinetic resolution of methyl and ethyl *trans*-3-phenylglycidate (*rac*-**1**) and (*rac*-**3**).

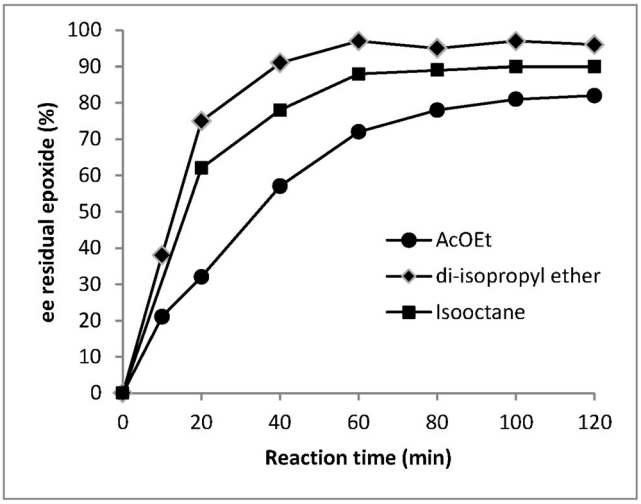


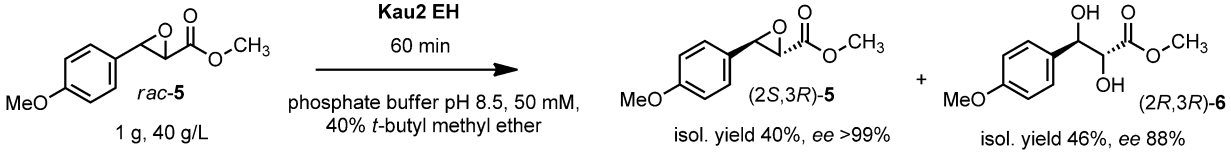
Figure 1. Comparison of the effects of the various used organic co-solvents on the final achievable *ee*s of the remaining substrate **1**.

a recent study[12] that used whole cells of *Galactomyces geotrichum* ZJUTZQ200, which enabled the access to (2*R*,3*S*)-**1** in 98.6% *ee* at 62.5% conversion from *rac*-**1**, resulting in an E value of 19 compared with an E value >200 in our case.

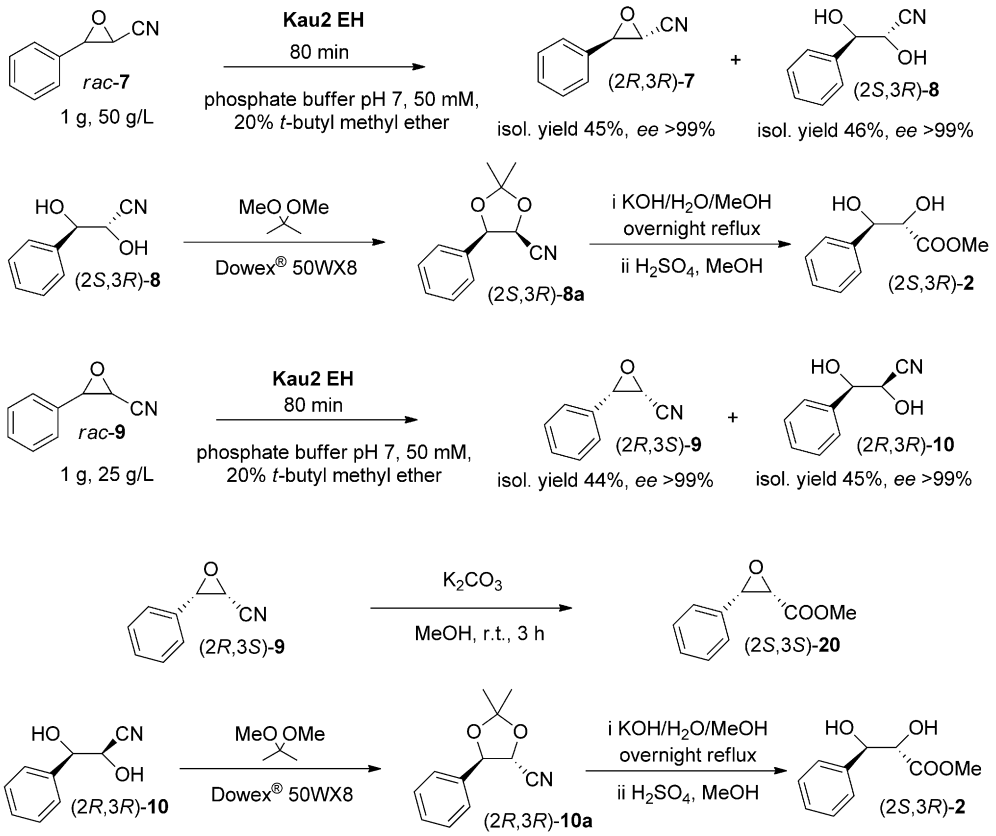
To obtain some additional information about the size of acceptable substrates of the Kau2-EH, compound **3** was also tested using the optimized reaction conditions defined for the transformation of the methyl ester **1** (Scheme 1). Essentially the same results were obtained for up to 10 g/L of substrate concentration, both *ee*s of remaining epoxide **3** and formed diol **4** reaching >99% at 50% conversion. At 25 g/L of substrate concentration, the *ee* of the epoxide reached >99% while the diol *ee* was around 96% during the entire experiment. At 50 g/L of substrate, the *ee* of **3** did not exceed 78% while the *ee* of the diol remained constant at 96% during the whole reaction. Thus at very high substrate concentrations the Kau2-EH was found to be less enantioselective towards the ethyl ester **3** compared to its methyl ester counterpart. Nevertheless, after 100 min of a preparative reaction which was conducted on a 1 g scale at 25 g/L, the remaining (2*S*,3*R*)-**3** and the formed (2*R*,3*R*)-**4** were isolated in 47 and 44% yields, and >99 and 94% *ee*, respectively. Interestingly, the Kau2-EH proved to be equally efficient[13] or compared favorably[12,14] with other EHs used in the kinetic resolution of *rac*-**3**. Essentially the same results as described above have been reported for a gelozyme preparation of the *Phaseolus radiatus* (Mung bean) EH acting on *rac*-**3**: the remaining epoxide (2*S*,3*R*)-**3** and the formed diol (2*R*,3*R*)-**4** were obtained in isolated yields of 45 and 40% with *ee* values of >99 and 94%, respectively.[13] Furthermore, the remaining epoxide (2*R*,3*S*)-**3** has been obtained either in 95% *ee* and 26% yield using whole cells of *Pseudomonas* sp. BZS21[14] or in more than 99% *ee* and 37% yield using whole cells of *Galactomyces geotrichum* ZJUTZQ200.[12] It should be noted that *rac*-methyl *cis*-3-phenylglycidate (**20**) was not a substrate of the Kau2-EH, in contrast to its *trans* counterpart **1**.

Kinetic Resolution of Methyl *trans*-3-(4-Methoxyphenyl)glycidate (*rac*-5)

We then tested the Kau2-EH with *rac*-**5** as a substrate (Scheme 2). Due to the presence of the *para*-methoxy substituent, **5** proved to be prone to chemical hydrolysis during the time course of the biocatalytic reaction. This was detrimental to both final yield in remaining epoxide as well as the *ee* of the formed diol. In order to minimize the chemical hydrolysis, the effects of the temperature (17 °C, 23 °C or 27 °C), the percentage (0%, 20% or 40%) of the non-miscible organic co-solvent methyl *tert*-butyl ether (MTBE), and the pH of the buffer phase (7.0, 7.5, 8.0, 8.5) were thoroughly tested. The best results were obtained at 17 °C with 40% MTBE at pH 8.5 (see the Supporting Information). Using these optimized reaction conditions, we tested substrate concentrations ranging from 1 to 40 g/L using a two-fold concentration of lyophilized biocatalyst (2 to 80 g/L). Generally, the reactions were quick with >99% *ee* for the epoxide reached in 1 h, except for low (1 g/L) and high substrate concentrations (40 g/L). The low mass transfer rate of the epoxide to the aqueous phase at 1 g/L likely limited the rate of the enzymatic reaction, whereas at 40 g/L of substrate the presence of organic solvent in conjunction with large cell quantities (80 g/L) generated a jelly-like phase that probably caused some inhibition of the bioconversion reaction. By slightly diminishing the substrate to catalyst ratio we were able to perform a preparative-scale reaction with 1 g (40 g/L)



Scheme 2. Preparative kinetic resolution of *rac*-methyl *trans*-3-(*4*-methoxyphenyl)glycidate (**5**).



Scheme 3. Kinetic resolution of *trans*- and *cis*-3-phenyloxirane-2-carbonitrile (*rac*-**7**) and (*rac*-**9**) and chemical transformations aimed at determining absolute configurations (see the Supporting Information).

of *rac*-**5** and 70 g/L of lyophilized biomass, affording in one hour remaining (2*S*,3*R*)-**5** in 40% isolated yield and >99% *ee*, and formed (2*R*,3*R*)-**6** in 46% isolated yield and 88% *ee*. Unfortunately and in line with the results obtained for glycidates **1** and **3**, residual (2*S*,3*R*)-**5** is not suitable for the synthesis of Diltiazem but could be used in the synthesis of one enantiomer of each *cis*- and *trans*-isocytoxazone.[11] When compared to previously reported kinetic resolutions of *rac*-**5**,[12,13] the Kau2 EH proved to be a better catalyst. Indeed, using whole cells of *Galactomyces geotrichum* ZJUTZQ200 an E value of only 3 was determined,[12] while using a gelozyme preparation of the *Phaseolus radiatus* (Mung bean) EH residual (2*S*,3*R*)-**5** was obtained in 45% yield and more than 99% *ee*, the formed diol-**6** being racemic in this case.

Kinetic Resolution of *trans*- and *cis*-3-Phenyloxirane-2-carbonitrile *rac*-7 and *rac*-9

Based on the excellent results obtained with the phenyl glycidate derivatives, we decided to test whether a cyano functionality could replace the previously used ester functionality. Due to the selected mode of synthesis, both *trans*-cyano (**7**) and *cis*-cyano-epoxides (**9**) were obtained and easily chromatographically separated, giving thus the possibility to test both substrates with the Kau2 EH. An *E. coli* biomass with a higher EH specific activity (2757 compared to 1667 U/g, see the Experimental Section) enabled the substrate/enzyme ratio to be reduced to 1/0.75 (m/m), and 20% MTBE were used to ensure a biphasic system. The *trans*-cyano derivative **7** was tested in the concentration range of 1–75 g/L and proved to be an excellent substrate (Scheme 3). Under these reaction conditions both residual (2*R*,3*R*)-**7** and formed (2*S*,3*R*)-**8** were obtained in >99% *ee* in 80 min for all tested concentrations, except for the 1 and 75 g/L concentrations. The slow mass transfer of the substrate to the aqueous phase at 1 g/L was probably the reason for the lower *ee* values; on the other hand, some unspecific inhibition at high substrate and biomass concentrations appeared to limit the *ee* of (2*R*,3*R*)-**7** to 75%. A preparative reaction run with 1 g of **7** at 50 g/L afforded after 80 min both residual (2*R*,3*R*)-**7** and formed (2*S*,3*R*)-**8** with *ee*s of >99% in 45 and 46% isolated yields, respectively (Scheme 3).

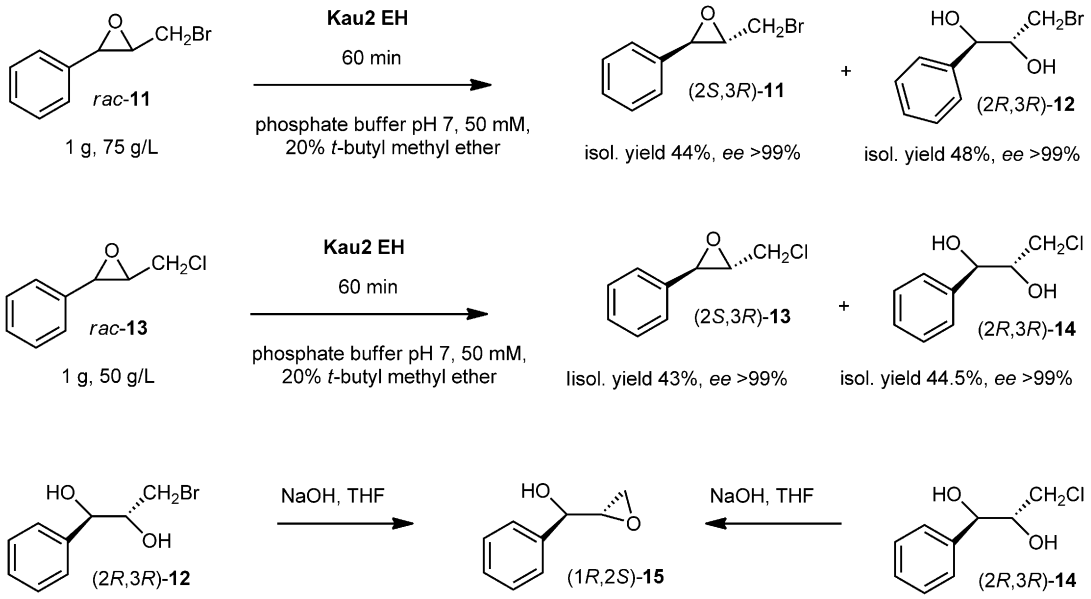
The Kau2 EH was previously reported to enantioconvergently transform *cis*-β-methylstyrene oxide into the corresponding (1*R*,2*R*)-diol in 97% isolated yield and 98.3% *ee*.[6] As methyl *cis*-3-phenylglycidate (**20**)

was not a substrate of the Kau2 EH, we were pleased to note that closely related *cis*-3-phenyloxirane-2-carbonitrile (*rac*-**9**) underwent a kinetic resolution using this enzyme. The optimal substrate/enzyme ratio was 1/1 (m/m), and 20% MTBE were used to ensure the presence of a biphasic system. Once again the kinetic resolution was almost perfect for substrate concentrations ranging from 1 to 25 g/L, both residual epoxide and formed diol being recovered essentially optically pure after 1 h. At 50 g/L of substrate concentration the reaction stopped after the *ee* of the remaining epoxide reached 93%, the obtained diol being nearly optically pure. A preparative reaction was then conducted on a 1 g scale at 25 g/L of *rac*-**9**, affording after 80 min and silica gel purification residual (2*R*,3*S*)-**9** and formed (2*R*,3*R*)-**10** in nearly enantiopure form (ee >99%), and 44 and 45% isolated yields, respectively. The results described here for the Kau2-catalyzed biohydrolysis of both *rac*-**7** and *rac*-**9** compared particularly well with those described in the literature using whole cells of *Mortierella isabellina* DSM 1414 in an attempt to kinetically resolve these epoxides.[15] Indeed, for both *rac*-**7** and of *rac*-**9** an E value of only 1 was determined in this case.

Kinetic Resolution of *trans*-2-(Bromomethyl)- and *trans*-2-(Chloromethyl)-3-phenyloxirane (*rac*-11) and (*rac*-13)

To further extend the substrate range of the Kau2 EH, we turned our attention to the very interesting compounds *trans*-2-(bromomethyl)-3-phenyloxirane (*rac*-**11**) and *trans*-2-(chloromethyl)-3-phenyloxirane (*rac*-**13**). It is worthwhile to note that when tested at a concentration of 25 g/L, both *rac*-**11** and *rac*-**13** behaved similarly in Kau2-mediated hydrolysis reactions, *rac*-**11** being transformed slightly quicker than *rac*-**13**. Using a substrate/enzyme ratio of 2.5/1 (m/m) at 1–25 g/L of *rac*-**11**, a 2/1 ratio at 50 g/L of *rac*-**11** and a 1/1 ratio at 75 g/L of *rac*-**11** in the presence of 20% MTBE, a nearly perfect kinetic resolution was observed in each case, affording (2*S*,3*R*)-**11** and (2*R*,3*R*)-**12** with *ee*s exceeding 99% after 1 h of reaction time.

When run on a preparative scale (1 g of *rac*-**11**, 75 g/L) in the presence of 0.8 g of biocatalyst (60 g/L), the reaction afforded optically pure (2*S*,3*R*)-**11** and (2*R*,3*R*)-**12** in 44% and 48% isolated yields, respectively (Scheme 4). A preparative-scale reaction with chloro-epoxide-**13** [1 g of **13**, 50 g/L; 0.5 g of biocatalyst (2011 U/g)], afforded optically pure (2*S*,3*R*)-**13** (43% isolated yield) and (2*R*,3*R*)-**14** (44.5% isolated yield) (Scheme 4). A (2*S*,3*R*) absolute configuration was assigned to remaining bromo-epoxide (**11**) by analogy with the stereochemistry data obtained for chloro-epoxide (**13**), assuming the same stereochemistry of epoxide opening during the Kau2-mediated hydrolysis reaction for both compounds. Such an equivalent behavior was supported by the fact that formed bromo-diol (**12**) and chloro-diol (**14**) possessed the same absolute configuration (Scheme 4 and the Supporting Information).



Scheme 4. Kinetic resolution of *trans*-2-(bromomethyl)- and *trans*-2-(chloromethyl)-3-phenyloxirane (*rac*-**11**) and (*rac*-**13**) and chemical transformations aimed at determining absolute configurations of diols (see the Supporting Information).

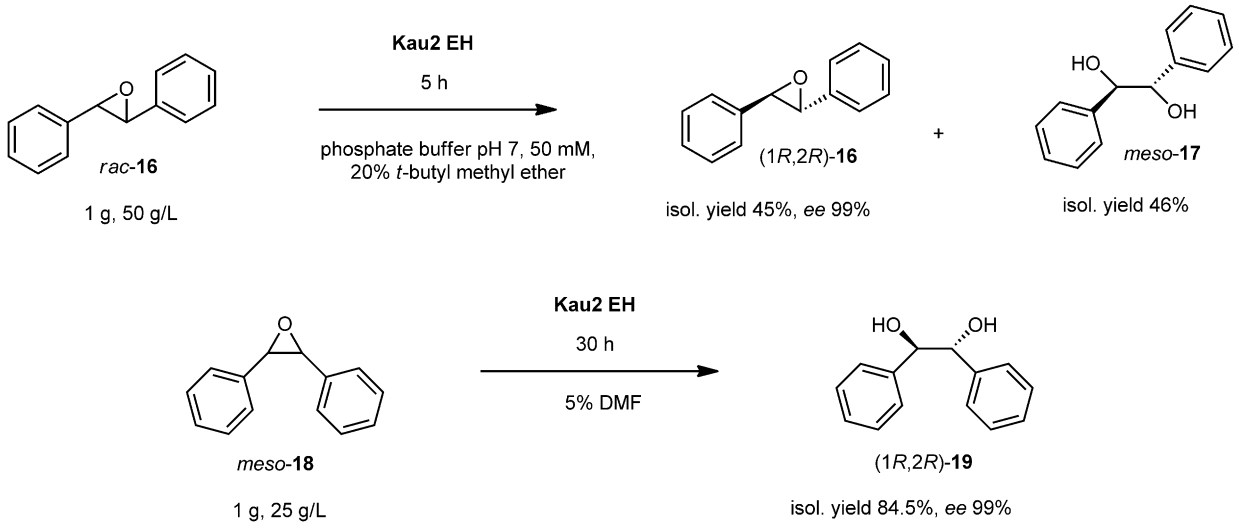
Kinetic Resolution of *trans*-Stilbene Oxide (16) and Desymmetrization of *cis*-Stilbene Oxide (18)

In an attempt to further explore the substrate scope of the Kau2 EH, sterically more demanding epoxides such as *trans*-stilbene and *cis*-stilbene oxides were tested. Although these epoxides are not bi-functional, they are of great interest as scouting molecules of the active site of Kau2 EH. As anticipated, the sterically challenging *trans*-stilbene oxide (**16**) was a poorer substrate than the above-mentioned epoxidic compounds. Indeed, the remaining epoxide was obtained in enantiomerically pure form only after 24–48 h of reaction time, compared to approximately 1 h for the above-mentioned substrates. With the exception of 1 g/L of substrate concentration, which resulted in an *ee* of 93% for the remaining epoxide after 105 h, higher concentrations (5, 10 and 25 g/L) enabled enantiomerically pure epoxide to be obtained after 24 h (Scheme 5). At the highest substrate concentration of 50 g/L an enantiomerically pure remaining epoxide was obtained but only after 48 h. In all cases 20% MTBE were used as an immiscible organic phase with the biocatalyst concentration being twice as high as that of the substrate (m/m). The reaction products of the *trans*-stilbene oxide transformations comprised *meso*-diol **17** and residual (1*R*,2*R*)-**16**. When run on a preparative scale with 1 g of *rac*-**16** at 50 g/L for 5 h, the reaction afforded residual (1*R*,2*R*)-**16** with an *ee* of >99% in 45% isolated yield and *meso*-diol **17** in 46% isolated yield.

The reaction portfolio was then extended to the desymmetrization of the *meso*-epoxide *cis*-stilbene oxide (**18**) (Scheme 5). The *cis*-epoxide exhibited quantitative transformation in the range from 1 to 25 g/L (the biomass concentration being double that of the substrate, m/m), the formed (1*R*,2*R*)-**19** being in all cases almost enantiomerically pure (*ee* >99%) with more than 90% analytical yield in 24 h. At 50 g/L the yield of formed **19** dropped considerably to 20% after 24 h of reaction time. Thus a preparative reaction was run on a 1 g scale at 25 g/L of **18**. After 30 h of reaction time the epoxide was totally consumed and the formed (1*R*,2*R*)-diol-**19** was recovered in 99% *ee* and 84.5% isolated yields. This result is comparable to the data of a previous report dealing with the desymmetrization of *meso*-epoxides using various EHs obtained from a metagenomic library.[16] Using cell extracts of 4 different EHs (BD 8877, BD 8676, BD 9300, BD 9883) (1*R*,2*R*)-diol (**19**) was obtained with *ee*s ranging from 96 to 99.5%, while its antipode (1*S*,2*S*)-diol (**19**) was obtained in 99% *ee* using the BD 9196 EH.[16]

We can conclude from the above-mentioned results that the Kau2 EH is a particularly useful enzyme for the kinetic resolution/desymmetrization of 1,2-disubstituted epoxides which contain at least one phenyl ring substituent. Indeed, exceptionally high stereoselectivity was demonstrated with this enzyme, leading in all but two cases to *ee*s of at least 99% for both remaining epoxide and formed diol, which represents a nearly perfect kinetic resolution (Table 1).

Furthermore, the Kau2 EH-catalyzed hydrolysis was conducted at very high substrate concentrations (from 25 to 75 g/L) and was generally complete within 1 h, thus closely approaching industrial needs. The enzyme tolerated well the presence of water-immiscible organic solvents such as di-isopropyl ether and MTBE, alleviating or diminishing the chemical hydrolysis of the epoxide as well as substrate and product inhibition. Furthermore, the use of organic co-solvents enabled the dissolution of very high quantities of substrate and product. The biocatalyst was also tolerant to pH changes in the alkaline region (pH 7.0 to pH 8.5), offering the possibility to further



Scheme 5. Kinetic resolution of *trans*-stilbene oxide (**16**) and desymmetrization of *cis*-stilbene oxide (**18**).

Table 1. Comparison of the preparative scale reaction of the various tested substrates (1 g/L) using the Kau2 EH.

Substrate	ee_S [%] (isolated yield [%])	ee_P [%] (isolated yield [%])	E
1	>99 (49)	>99 (47)	>200
3	>99 (47)	94 (44)	>200
5	>99 (40)	88 (46)	>100[a]
7	>99 (45)	>99 (46)	>200
9	>99 (44)	>99 (45)	>200
11	>99 (44)	>99 (48)	>200
13	>99 (43)	>99 (44.5)	>200
16	99 (45)	meso (46)	>200
18	–	99 (84.5)	199
20	no reaction		

[a] The E value is probably lower due to spontaneous chemical hydrolysis of the substrate.

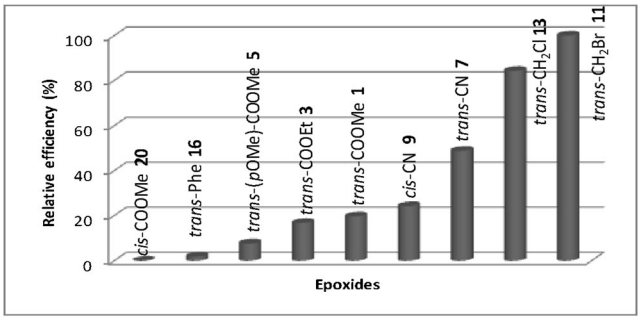
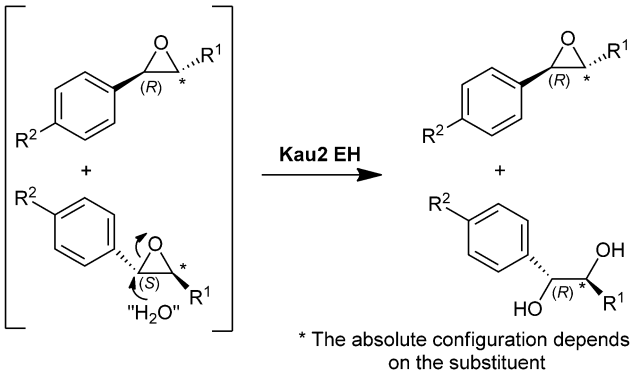


Figure 2. Comparison of the relative Kau2 EH efficiencies (vertical bars) for the kinetic resolutions of the various epoxidic substrates at the same concentration of 25 g/L, but under reaction conditions adapted for each substrate (see the Experimental Section).

minimize the spontaneous chemical hydrolysis of fairly unstable epoxides. Some of the tested substrates are potentially very valuable chiral synthons for the synthesis of numerous biologically active target molecules. When compared to a recent report about the kinetic resolution of glycidates **1**, **3** and **5** with whole cells of *Galactomyces geotrichum*, the Kau2 EH expressed in *E. coli* proved to be an outstanding biocatalyst for getting access to optically pure epoxides.[12] The residual epoxides [(2*S*,3*R*)-**1** and (2*S*,3*R*)-**3**] are suitable as chiral synthons for accessing the taxol side chain[17a,b] as well as for being used in the synthesis of the nootropic drug (−)-Clausenamide.[9] Unfortunately, the residual epoxide (2*S*,3*R*)-**5** did not bear the correct configuration to be used as a chiral synthon for accessing Diltiazem. We have shown that bi-functional epoxides can be used as substrates of the Kau2 EH with virtually no noticeable influence of different functionalities such as nitrile, methyl or ethyl ester and bromine and chlorine on the reactivity. In the latter cases, new terminal epoxides were easily accessible without any loss in enantiopurity from the enzymatically formed diol by simply using basic reaction conditions. Even very sterically demanding substrates such as *trans*- and *cis*-stilbene oxides were also transformed by the enzyme; however, lower reaction rates were observed. Even these demanding substrates reacted to completion on a preparative scale within one day using the Kau2 EH. In order to compare the efficiencies of the Kau2-EH in the kinetic resolutions of the various substrates described above, we present in Figure 2 the results of all the tested substrates at the same concentration of 25 g/L *under the various reaction conditions* described in the Experimental Section. This comparison is based on the number of μmoles of remaining epoxide at the end of the resolution process (epoxide *ee* >99%) divided by the reaction time and the number of enzymatic units used in the reaction. That means that a two-fold higher efficiency leads to an approximately two-fold quicker resolution process (*ee* of the remaining epoxide >99%) based on the same amount of enzyme used. We can conclude: (i) the efficiency appears to be inversely proportional to the size of the substituent ($CH_2Br \approx CH_2Cl >$ CN > COOMe > COOEt > Ph), and (ii) enzyme-catalyzed resolution of *trans*-configured substrates exhibited higher efficiencies than for their *cis*-counterparts.

It clearly appears that a β-substitution at the oxirane ring (with respect to the phenyl group) plays a critical role on the outcome of the biohydrolysis. Indeed, the presence of a substituent alkyl, phenyl, ester, cyano, chloro or bromo) located at the β-carbon atom seems to preclude one enantiomer of the various tested racemates from reacting with the Kau2 EH, leading to the observed very high enantioselectivities found in almost all cases. More precisely, with *trans*-configured epoxides the reactive enantiomer always corresponded to the one bearing the benzylic carbon atom of *S*-absolute configuration, the enzymatic attack occurring selectively at this carbon atom (Scheme 6).

In the case of *cis*-substitution the Kau2 EH proved to be totally inactive with *cis*-methyl phenylglycidate (**20**). This is astonishing in view of the considerable enzymatic activities determined with structurally related compounds such as *cis*-methyl styrene oxide,[6] *rac*-**9**, and *meso*-**18**. For the time being, no explanation of this fact can be given. We can only speculate that the active site of the Kau2 EH is not compatible with the methyl ester group of **20**, in contrast to the less polar methyl, cyano and phenyl groups. Another intriguing result was that the replacement of a methyl group by a cyano group (*rac*-**9**) led to the loss of the high enantioconvergency found previously[6] in favor of a perfect enantioselectivity. It should be noted in that case that, in contrast to its *trans*-counterpart, the reactive enantiomer corresponded to the one bearing



For R^2 = H, R^1 = Me, COOMe, COOEt, CN, CH_2Cl, CH_2Br or Ph

For R^2 = OMe, R^1 = COOMe

Scheme 6. Kau2 EH attack at the benzylic carbon atom of *trans*-configured 1,2-disubstituted aromatic epoxides.

the benzylic carbon atom of *R*-absolute configuration, the enzymatic attack occurring selectively at the β-carbon atom bearing the cyano group.

Conclusions

In conclusion, we have shown that the Kau2 EH is an outstanding catalyst, which enables an easy, robust and efficient access to numerous useful optically pure diols and epoxides that can be used, for example, as chiral building blocks in the synthesis of various biologically active chemicals.

Experimental Section

General Remarks

All reagents were used as received from Sigma–Aldrich, Acros or Fluka. Dimethylformamide (DMF), tetrahydrofuran (THF), dichloromethane, methanol, diisopropyl ether, *tert*-butyl methyl ether (MTBE) and isooctane were of analytical grade. Acetonitrile, hexane and isopropyl alcohol were of HPLC grade. The epoxides *rac*-methyl *trans*-3-(4-methoxyphenyl)glycidate (**5**), *rac-trans*-stilbene oxide (**16**) and *meso-cis*-stilbene oxide (**18**) were from Sigma–Aldrich. All other reagents were from Sigma–Aldrich, ACROS or Fluka. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 Ultrashield NMR spectrometer. Chemical shifts are given in ppm relative to residual peaks of chloroform-d_3 or acetone-d_6. The coupling constants *J* are given in Hertz (Hz). The abbreviations br, s, d, t and m correspond to a broad signal, a singlet, a doublet, a triplet and a multiplet, respectively.

Biocatalyst Production and EH Activity

Buffer and culture media: Buffers A and B were composed of 50 mM Na-phosphate, adjusted to pH 7.0 and 8.5, respectively. Luria Bertani (LB) medium contained (per litre) 10 g of peptone, 5 g of yeast extract and 5 g of NaCl and was adjusted to pH 7.5. For solid media 15 g/L of agar were added. The minimal growth medium was composed of (per litre) sucrose 10 g, KH_2PO_4 13.6 g, $(NH_4)_2SO_4$ 4 g, NaOH 3 g, $MgSO_4 \cdot 7H_2O$ 2 g, $CaCl_2$ 0.377 g, $FeSO_4 \cdot 7H_2O$ 0.01 g and adjusted to pH 7.4.

Engineering E. coli RE3 expressing Kau2 EH: The construction of the IPTG-inducible Kau2 EH-encoding plasmid was described elsewhere.[6] Transformation of *E. coli* RE3 with this DNA construct resulted in ampicillin-resistant Kau2-overproducers that were able to grow in minimal medium with sucrose as the sole carbon source.[18]

Kau2 EH production: Frozen glycerol stock suspensions of *E. coli* RE3 cells expressing Kau2 EH were spread on Petri dishes filled with LB agar containing 100 μg/mL of ampicillin and grown for 24 h at 28 °C. One colony was used to inoculate an Erlenmeyer flask (250 mL) filled with 30 mL of pre-culture minimal medium or with 30 mL of LB medium, both containing 100 μg/mL of ampicillin. The pre-culture was grown overnight at 31 °C. The bioreactor was filled with 3 L of minimal medium (adjusted to pH 7.4) and was inoculated directly using the entire pre-culture. The cultivation temperature was 28 °C, pO_2 was maintained above 20% (air flow 1 VVM), and the stirring speed was set at 300 rpm. After 8–9 h of cultivation, the temperature was reduced from 28 °C to 25 °C, and subsequently IPTG (1 M in distilled water) was added to a final concentration of 0.45 mM. After 15 h of cultivation at 25 °C, the cells were harvested by centrifugation and then lyophilized. Two cell samples were obtained with specific activities of 1667 U/g and 2757 U/g (Bio-Bundle bioreactor from Applikon Biotechnology, 10 L).

Determination of Kau2 EH activity: The EH activity was determined using (*S*)-*para*-chlorostyrene oxide as a substrate. Fifteen μL of a 400 mM stock solution in acetonitrile (final concentration 3.0 mM) and lyophilized cells expressing the Kau2-EH (0.1 g/L) were added to 2 mL of buffer A in a 10-mL round-bottom flask. The enzymatic reaction was incubated at 27 °C under stirring (800 rpm). Samples of 200 μL were withdrawn at regular intervals, diluted with 200 μL of acetonitrile and centrifuged at 12000 *g* for 3 min, the supernatant (200 μL) was then filtered (0.45 μm) and analyzed by HPLC (Agilent 1100 Series) on a NUCLEOSIL® C_{18} column (250 × 4.60 mm, Macherey–Nagel) in isocratic mode (55% acetonitrile in distilled water, flow rate of 0.7 mL/min). Residual epoxide and formed diol eluted at 15.5 and 5.5 min, respectively (detection at 220 nm), they were quantified using hydrobenzoin as a standard with a retention time of 10.1 min.

Chemical Synthesis

Synthesis of methyl trans-3-phenylglycidate (rac-1):[19] In a 100-mL round-bottom flask were weighed 0.896 g (5.5 mmol) of *trans*-methyl cinnamate, followed by the addition of 20 mL of acetonitrile and 5 mL distilled water. Then 1.87 g of dibromoamine-T[20] were added, and the mixture was stirred at room temperature for 30 min. The formed bromohydrin was cyclized by adding 5.2 g of K_2CO_3, leaving the reaction under stirring at room temperature overnight. The reaction was stopped by addition of 1.0 g of $Na_2S_2O_3$. The mixture was poured into a separatory funnel, extracted

3 times with 50 mL of diethyl ether, the organic phases were collected, dried over $MgSO_4$, filtered and evaporated under vacuum. The product was purified by flash chromatography on silica gel (230–400 mesh) using an 8/2 mixture of pentane and diethyl ether as eluent, affording *rac*-methyl *trans*-3-phenylglycidate (**1**) as a colorless liquid; yield: 0.81 g (82%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.2–7.3 (brm, 5H), 4.1 (d, J = 1.5 Hz, 1H), 3.8 (s, 3H), 3.5 (d, J = 1.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 168.6, 134.9, 129.0, 128.7, 125.8, 58.0, 56.6, 52.6.

Synthesis of ethyl trans-3-phenylglycidate (rac-3):[19] In a 100-mL round-bottom flask were weighed 0.979 g (5.5 mmol) of *trans*-ethyl cinnamate, followed by the addition of 20 mL of acetonitrile and 5 mL of distilled water. Then 3.949 g of dibromoamine-T were added, and the mixture was stirred at room temperature for 30 min. The next preparatory steps were as described above for the synthesis of **1**. Flash chromatography (as described above) afforded *rac*-ethyl *trans*-3-phenylglycidate (**3**) as a colorless liquid; yield: 0.82 g (78%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.2–7.3 (brm, 5H), 4.28 (q, J = 7.2 Hz, 1H), 4.27 (q, J = 7.2 Hz, 1H), 4.1 (d, J = 1.7 Hz, 1H), 3.5 (d, J = 1.7 Hz, 1H), 1.3 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 168.2, 135.0, 129.0, 128.6, 125.8, 61.8, 57.9, 56.7, 14.1.

Synthesis of trans- and cis-3-phenyloxirane-2-carbonitrile (rac-7) and (rac-9):[21] To a solution of benzaldehyde (380 mg, 3.0 mmol), chloroacetonitrile (450 mg, 6.0 mmol) and tetrahexylammonium bromide (154 mg, 0.36 mmol) in THF (15.0 mL) was added KOH (400 mg, 7.2 mmol) at room temperature. After 22 h under stirring, $NaBH_4$ (200 mg) was added to the mixture to remove the remaining benzaldehyde. After 5 min, the reaction was quenched with water, and the mixture was extracted 3 times with 15 mL of ethyl acetate. The combined organic layers were washed with brine and water, dried over $MgSO_4$, filtrated and finally concentrated under vacuum. The crude mixture was purified by flash chromatography on silica gel (230–400 mesh) using a 9/1 mixture of pentane and diethyl ether as an eluent, affording *trans*-**7** as a colorless liquid (yield: 0.09 g, 22.5%) and *cis*-**9** as a white solid (yield: 0.16 g, 40% yield, mp 54–55 °C). **7**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.4–7.5 (brm, 3H), 7.2–7.3 (brm, 2H), 4.3 (d, J = 1.8 Hz, 1H), 3.4 (d, J = 1.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 132.8, 129.8, 129.0, 125.8, 116.2, 58.5, 44.7. **9**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.4–7.5 (m, 5H), 4.3 (d, J = 3.7 Hz, 1H), 3.8 (d, J = 3.7 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 131.4, 129.7, 128.7, 126.3, 115.0, 57.7, 45.1.

Synthesis of trans-2-(bromomethyl)- and trans-2-(chloromethyl)-3-phenyloxirane (rac-11) and (rac-13): To a solution of 70% *meta*-chloroperoxybenzoic acid (*m*CPBA) (8.5 mmol) in 20 mL of CH_2Cl_2 was added 30 mL of a cinnamyl bromide (571 mg, 2.9 mmol) solution in CH_2Cl_2. Then 20 mL of 0.5 M $NaHCO_3$ were added, and the heterogeneous mixture was stirred at 25 °C for 17 h. The resulting mixture was washed 3 times with 10% sodium bicarbonate, and the combined aqueous phases were extracted 3 times with 30 mL of diethyl ether. The combined organic layers were finally washed with brine then water, dried over $MgSO_4$, filtrated and then concentrated under vacuum. The crude product was first purified by flash chromatography on silica gel (230–400 mesh) using a 9/1 mixture of pentane and diethyl ether as eluent and then by bulb-to-bulb distillation at 200 °C under vacuum (0.4 mbar), affording *trans*-2-(bromomethyl)-3-phenyloxirane *rac*-**11** as a slightly yellow liquid; yield: 0.302 g (49%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.3–7.4 (brm, 5H), 3.7 (d, J = 1.8 Hz, 1H), 3.4 (d, J = 5.8 Hz, 2H), 3.2 (dt, J = 1.8 Hz, J = 5.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 136.0, 128.6, 128.6, 125.7, 61.0, 60.3, 31.9.

A similar procedure starting from cinnamyl chloride afforded *trans*-2-(chloromethyl)-3-phenyloxirane *rac*-**13** as a colourless liquid; yield: 0.275 g (52.1%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.2–7.4 (brm, 5H), 3.8 (d, J = 1.8 Hz, 1H), 3.72 (dd, J = 5.0 Hz, J = 11.7 Hz, 1H), 3.66 (dd, J = 5.7 Hz, J = 11.7 Hz, 1H), 3.3 (ddd, J = 1.9 Hz, J = 5.0 Hz, J = 5.7 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 135.9, 128.6, 128.6, 125.7, 61.0, 58.6, 44.4.

Synthesis of methyl cis-3-phenylglycidate (rac-20):[22] A mixture of *cis*-3-phenyloxirane-2-carbonitrile-**9** (0.302 g, 2 mmol), dry potassium carbonate (0.289 g, 2.0 mmol) and dry methanol (10 mL) was stirred at room temperature for 3 h and then acidified with dilute HCl for 2 h at 4 °C. The reaction solution was then extracted 3 times by ethyl acetate (30 mL), the combined organic layers were washed with brine then water, dried over $MgSO_4$, filtrated and finally concentrated under vacuum. The crude product was purified by flash chromatography on silica gel (230–400 mesh) using a 7/3 mixture of pentane and diethyl ether as an eluent, affording*rac*-methyl *cis*-3-phenylglycidate-**20** as a colorless liquid; yield: 0.287 g (80%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.3–7.4 (brm, 5H), 4.3 (d, J = 4.5 Hz, 1H), 3.8 (d, J = 4.5 Hz, 1H), 3.6 (brs, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 166.3, 133.0, 128.4, 128.3, 126.3, 58.3, 56.0, 53.3.

Chemical hydrolysis of methyl trans-3-phenylglycidate (rac-1): For access to racemic forms of the various diol products, acidic opening of the corresponding epoxides was carried out as exemplified for *rac*-**1**. In a 100-mL round bottomed flask were added 100 mg of *rac*-**1**, 20 mL of water and two drops of H_2SO_4 (98%). The reactions were performed overnight at room temperature under magnetic stirring (800 rpm). The reaction mixture was then extracted with 20 mL of ethyl acetate, and the formed *rac*-diols analyzed by chiral GC or HPLC. The chemical hydrolyses of *rac*-**3**, *rac*-**5**, *rac*-**7**, *rac*.**9**, *rac*-**11**, *rac*-**13** and *rac*-**16** were carried out using the same protocol leading to diastereoisomeric mixtures of corresponding diols **4**, **6**, **8**, **10**, **12**, **14** and **17**.

Synthesis of (1R,2S)-1-phenylglycidol (15): In a 25-mL round-bottom flask were weighed 123.5 mg (7.0 mmol) of (2*R*,3*R*)-**12**, followed by the addition of 3 mL of THF. Then 56 mg (14.0 mmol) of NaOH were added, and the mixture was stirred at 0 °C for 2 h. The mixture was poured into a separatory funnel, extracted 3 times with 15 mL of ethyl acetate, the organic phases were collected, dried over $MgSO_4$, filtered and evaporated under vacuum. The product was purified by flash chromatography on silica gel (230–400 mesh) using an 7/3 mixture of pentane and diethyl ether as eluent, affording (1*R*,2*S*)-1-phenylglycidol (**15**) as a colorless liquid; yield: 57 mg (59%). (2*R*,3*S*)-**15**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.3–7.4 (brm, 5H), 5.0 (d, J = 2.7 Hz, 1H), 3.3 (dt, J = 2.9 Hz, J = 3.9 Hz,1H), 3.0 (dd, J = 2.8 Hz, J = 4.9 Hz, 1H), 2.8 (dd, J = 4.1 Hz, J = 4.9 Hz, 1H), 2.3 (brs, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 139.5, 128.6, 128.3, 126.4, 70.8, 55.1, 43.6.

The same protocol was used for (2*R*,3*R*)-**14** leading to the same (1*R*,2*S*)-1-phenylglycidol (**15**).

Synthesis of (2S,3R)-2 from cyano-diols (2S,3R)-8 and (2R,3R)-10: (2*S*,3*R*)-**8** from the bioconversion of *rac*-**7** (400 mg, 2.45 mmol) was dissolved in acetone dimethyl acetal (40 mL), and then Dowex® 50WX8 (4 g) was added to the solution. The reaction was monitored by TLC for 1.5 h, and then the mixture was filtered and the filtrate evaporated under reduced pressure. The residue was dissolved in a mixture of methanol and water (2:1), and KOH (0.59 g) was added to the solution. Then the reaction solution was refluxed overnight at 75 °C. The reaction was stopped by adding HCl (20%) and adjusted to pH 2. The mixture was extracted 3 times with 40 mL diethyl ether and then dried over $MgSO_4$. The diethyl ether was removed by rotary evaporation, the crude product was dissolved in methanol, and a few drops of H_2SO_4 (98%) were added to the solution. The mixture was stirred at room temperature for 1.5 h, and was quenched with a saturated aqueous solution of sodium bicarbonate (20 mL). The mixture was extracted 3 times with 40 mL diethyl ether, dried over $MgSO_4$ and evaporated under vacuum. The product was purified by flash chromatography on silica gel (230–400 mesh) using an 50/50 mixture of pentane and diethyl ether as eluent, affording (2*S*,3*R*)-**2**; yield: 85 mg. The same protocol was used for (2*R*,3*R*)-**10**, which was obtained from bioconversion of *rac*-**9**, leading to the same (2*S*,3*R*)-**2**.

Kau2 EH Enantioselective Hydrolysis of Epoxide Substrates

Bioconversion of methyl trans-3-phenylglycidate (rac-1): *Analytical scale* – A specific volume of a stock solution of 2.8 M of *rac*-**1** (final concentration 1–100 g/L) in DMF (final concentration of DMF adjusted to 5%) and lyophilized biomass with an EH activity of 1667 U/g (final concentration 2–200 g/L) were added to 0.4 mL of buffer A containing 50 μL of diisopropyl ether in a 10-mL round bottom flask (final reaction volume 0.5 mL). The enzymatic reactions were incubated at 27 °C under magnetic stirring (1200 rpm). Samples, which were withdrawn at regular intervals, were extracted with ethyl acetate and analyzed by chiral GC on a Lipodex-G column (0.25 μm, 25 m × 0.25 mm, Macherey–Nagel) for *ee* analysis of both residual substrate and formed diol, 4-bromoacetophenone being used as an internal standard. The flow rate of the carrier gas (H_2) was set at 2.96 mL/min. The following oven temperature program enabled separation of the enantiomers: 110 °C for 20 min, followed by an increase to 140 °C using a rate of 10 °C min^{-1}. The retention times were as follows. (2*R*,3*S*)-**1**: 17.5 min, (2*S*,3*R*)-**1**: 17.9 min, (2*R*,3*R*)-**2**: 38.2 min, (2*S*,3*S*)-**2**: 38.6 min, and for the 2 *syn*-diols-**2**: 39.1 and 40.1 min.

Preparative scale – One g of *rac*-**1** dissolved in 1 mL of DMF was added to 17 mL of buffer A and 2 mL of diisopropyl ether in a 100-mL round-bottomed flask. The biocatalytic reaction was initiated at 27 °C by the addition of 1.5 g of lyophilized biomass with an EH activity of 1667 U/g under magnetic stirring (1200 rpm). After 60 min, the reaction mixture was extracted 3 times with 40 mL ethyl acetate. The combined organic phases were dried over $MgSO_4$, filtered and then evaporated under reduced pressure. The products were purified by flash chromatography (pentane/diethyl ether, 7/3), affording(2*S*,3*R*)-**1** as a colorless liquid (yield: 0.49 g, 49%; *ee* >99%) and (2*R*,3*R*)-**2** as a white solid (mp 77–78 °C) (yield: 0.52 g. 47%; *ee* >99%). Absolute configurations were determined by comparison of the optical rotations found in the literature for (2*R*,3*R*)-**2** {$[\alpha]_D^{20}$: −41.3 (*c* 0.48, $CHCl_3$)}[23] and (2*S*,3*R*)-**1** {$[\alpha]_D^{22}$: +171 (*c* 1.0, $CHCl_3$)}[9] with the ones experimentally determined in this work {$[\alpha]_D^{22}$: −44 (*c* 0.5, $CHCl_3$) and $[\alpha]_D^{15}$: +171.9 (*c* 1.0, $CHCl_3$), respectively}. (2*R*,3*R*)-**2**: ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 172.4, 138.5, 128.3, 128.2, 126.4, 75.0, 74.8, 52.4; ^1H NMR ($CDCl_3$, 300 MHz): δ = 7.2–7.3 (brm, 5H), 4.9 (brdd, 1H), 4.4 (brdd, *J* = 4.4 Hz, *J* = 5.9 Hz, 1H), 3.6 (s, 3H), 2.9 (2brs, 2H).

Bioconversion of ethyl trans-3-phenylglycidate (rac-3): *Analytical scale* – The same protocol as described above was used. The samples were analyzed by chiral GC with a Cyclosil B column (60 m, 0.25 μm × 0.25 mm, Agilent, USA), the flow rate of the carrier gas (H_2) was set at 2.67 mL/min. The column temperature was set at 150 °C and kept at this temperature for 20 min and then increased to 180 °C at a rate of 10 °C/min. The retention times were as follows. (2*R*,3*S*)-**3**: 22.7 min, and (2*S*,3*R*)-**3**: 23.0 min. The formed diols were analyzed at 180 °C without derivatization, the retention times were as follows: (2*R*,3*R*)-**4**, 17.7 min; (2*S*,3*S*)-**4**, 18.0 min; and for the 2 *syn*-diols-**4** 18.5 and 18.8 min. A Chiralpak AD-H column (250 × 4.60 mm) in isocratic mode was used for the determination of the absolute configuration of (2*R*,3*R*)-**4** using a mixture of hexane/isopropyl alcohol (85/15) at a flow rate of 0.7 mL/min. UV detection was set at 230 nm. The retention times were as follows. (2*S*,3*S*)-**4**: 15.9 min, (2*R*,3*R*)-**4**: 16.7 min, and for the 2 *syn*-diols-**4**: 18.8 and 20.6 min.

Preparative scale – In a 100-mL round-bottomed flask was added 1.0 g of *rac*-**3** dissolved in 2 mL of DMF, 34 mL of buffer A and 4 mL of diisopropyl ether. The biocatalytic reaction was initiated by the addition of 2.0 g of lyophilized biomass with an EH activity of 1667 U/g. The reaction was performed at 27 °C under magnetic stirring (1200 rpm). After 60 min, the reaction mixture was extracted 3 times with 40 mL ethyl acetate. The combined organic phases were dried over $MgSO_4$, filtered and then evaporated under reduced pressure. The products were purified by flash chromatography (pentane/diethyl ether, 7/3), affording (2*S*,3*R*)-**3** as a colourless liquid (yield: 0.47 g, 47%; *ee* >99%) and (2*R*,3*R*)-**4** as a colorless liquid (yield: 0.49 g, 44%; *ee* 94%); $[\alpha]_D^{25}$: −37.5 (*c* 1.0, $CHCl_3$). The absolute configuration of (2*S*,3*R*)-**3** was determined by comparison of the optical rotation found in the literature {$[\alpha]_D^{20}$: + 152 (*c* 1.0, $CHCl_3$)}[24] with the one experimentally determined in this work {$[\alpha]_D^{25}$: +154.7 (*c* 1.0, $CHCl_3$)}. The absolute configuration of (2*R*,3*R*)-**4** was deduced from the published elution order of (2*S*,3*S*)-**4** and (2*R*,3*R*)-**4** on a Chiralpak AD-H column.[13] (2*R*,3*R*)-**4**: ^1H NMR ($CDCl_3$, 300 MHz): δ = 7.2–7.3 (brm, 5H), 4.9 (brd, *J* = 4.0 Hz, 1H), 4.4 (brs, 1H), 4.1 (q, *J* = 7.2 Hz, 2H), 3.0 (brs, 2H), 1.1 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 171.9, 138.6, 128.3, 128.2, 126.4, 75.0, 74.7, 61.9, 14.0.

Bioconversion of methyl trans-3-(4-methoxyphenyl)glycidate (rac-5): *Analytical scale* – A specific volume of a stock solution of 100 g/L of *rac*-**5** (final concentration 1–50 g/L) in MTBE (final concentration of MTBE 40%) and lyophilized biomass with an EH activity of 1667 U/g (final concentration 2–100 g/L) were added to 0.3 mL of buffer B in a 10-mL round-bottom flask (final reaction volume 0.5 mL). The en-

zymatic reactions were incubated at 17 °C under magnetic stirring (800 rpm). Regularly withdrawn aliquots were saturated with NaCl and extracted with ethyl acetate (200 μL). Then 100 μL of the organic phase were evaporated under reduced pressure, and the residues were dissolved in 100 μL of isopropyl alcohol. Then 20 μL of this solution were analyzed by HPLC to determine the *ee*s of both remaining epoxide and formed diol. A Lux Cellulose-4 chiral column (250 × 4.60 mm, Phenomenex) was used in the isocratic mode with an 8/2 mixture of hexane/isopropanol at a flow rate of 1.2 mL/min. UV detection was set at 230 nm. The retention times were as follows: (2*S*,3*R*)-**5**: 8.4 min, (2*R*,3*S*)-**5**: 8.8 min, (2*S*,3*S*)-**6**: 17.7 min, (2*R*,3*R*)-**6**: 19.8 min, and for the 2 *syn*-diols-**6**: 21.7 and 27.4 min.

Preparative scale – In a 100-mL round-bottomed flask was added 1.0 g of *rac*-**5** dissolved in 10 mL of MTBE and 15 mL of buffer B. The biocatalytic reaction was initiated by addition of 1.75 g of lyophilized biomass with an EH activity of 1667 U/g, and the reaction mixture was kept at 17 °C under magnetic stirring (800 rpm). After 60 min, the reaction mixture was extracted 3 times with 40 mL ethyl acetate. The combined organic phases were dried over $MgSO_4$, filtered and then evaporated under reduced pressure. The products were purified by flash chromatography (pentane/diethyl ether, 7/3), affording (2*S*,3*R*)-**5** as a colorless liquid (yield: 0.40 g, 40%; *ee* >99%) and (2*R*,3*R*)-**6** as a white solid (yield: 0.51 g, 46%; *ee* 88%), mp 105–106 °C. The absolute configurations were determined by comparison of the optical rotation values found in the literature for (2*R*,3*S*)-**5** {$[\alpha]_D^{24}$: −205 (*c* 1.0, MeOH)}[25] and (2*R*,3*R*)-**6** {$[\alpha]_D^{18}$: −43.9 (*c* 1.0, $CHCl_3$)}[23] with the ones experimentally determined in this work {<$[\alpha]_D^{24}$: +190.7 (*c* 1.0, MeOH) and $[\alpha]_D^{18}$: −39.2 (*c* 1.0, $CHCl_3$), respectively}. (2*R*,3*R*)-**6**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.2–7.3 (brm, 2H), 6.8–6.9 (brm, 2H), 5.0 (d, *J* = 4.3 Hz, 1H), 4.5 (d, *J* = 4.3 Hz, 1H), 3.8 (s, 3H), 3.7 (s, 3H), 3.0 (brs, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 172.5, 159.5, 130.1, 127.7, 113.7, 74.8, 74.6, 55.2, 52.5.

Bioconversion of trans-3-phenyloxirane-2-carbonitrile (rac-7): *Analytical scale* – A specific volume of a stock solution of 200 g/L of *rac*-**7** (final concentration 1–100 g/L) in MTBE (final concentration of MTBE 20%) and lyophilized biomass with an EH activity of 2757 U/g (final concentration 0.75–75 g/L) were added to 0.8 mL of buffer A in a 10-mL round-bottom flask (final volume of reaction 1 mL). The enzymatic reactions were incubated at 27 °C under magnetic stirring (800 rpm). Regularly withdrawn aliquots were saturated with NaCl and extracted with ethyl acetate (200 μL). Then 100 μL of the organic phase were evaporated under reduced pressure, and the residues were dissolved in 100 μL of isopropyl alcohol. Then 20 μL of this solution were analyzed by HPLC for *ee* determination of both remaining epoxide and formed diol. A Chiralcel OD-H column (250 × 4.60 mm) was used in isocratic mode with a 92.5/7.5 mixture of hexane/isopropyl alcohol at a flow rate of 1.2 mL min^{-1}. UV detection was set at 215 nm. The retention times were as follows: (2*R*,3*R*)-**7**: 16.8 min, (2*S*,3*S*)-**7**: 19.4 min, (2*S*,3*R*)-**8**: 21.6 min, (2*R*,3*S*)-**8**: 25.5 min.

Preparative scale – In a 100-mL round-bottomed flask was added 1.0 g of *rac*-**7** dissolved in 4 mL of MTBE and 16 mL of buffer A. The biocatalytic reaction was initiated by addition of 0.75 g of lyophilized biomass with an EH activity of 2757 U/g, and the reaction mixture was maintained at 27 °C under magnetic stirring (800 rpm). After 60 min, the reaction mixture was extracted 3 times with 40 mL ethyl acetate. The combined organic phases were dried over $MgSO_4$, filtered and then evaporated under reduced pressure. The products were purified by flash chromatography (pentane/diethyl ether, 7/3), affording (2*R*,3*R*)-**7** as a slightly yellow liquid (yield: 0.45 g, 45%; *ee* 99%) and (2*S*,3*R*)-**8** as a white solid (yield: 0.52 g, 46%; *ee* 99%). $[\alpha]_D^{22}$: −44.3 (*c* 1.0, EtOH), mp 83–84 °C. The absolute configuration of (2*R*,3*R*)-**7** was determined by comparison of the optical rotation value found in the literature for (2*R*,3*R*)-**7** {29% *ee*, $[\alpha]_D^{25}$: +24.3 (*c* 0.86, EtOH)}[15] with the one experimentally determined in this work {$[\alpha]_D^{20}$: +150.8 (*c* 1.1, EtOH)}. The absolute configuration of the formed diol **8** was determined by its transformation to the acetonide **8a**, which proved to be of *cis*-configuration (^{1}H NMR, see the Supporting Information), and subsequently to the corresponding *syn*-methyl ester diol **2** according to a previously described method (Scheme 3).[26] It should be noted that during the overnight reflux an epimerization of *cis*-acetonide **8a** occurred at carbon-2. Such an epimerization did not occur when starting from the corresponding *trans*-acetonide (*vide infra*).[26] The optical rotation of the formed diol **2** {$[\alpha]_D^{20}$: −12.7 (*c* 0.71, CH_2Cl_2)} was compared with the one found in the literature for (2*S*,3*R*)-*syn*-methyl-ester-diol **2** {$[\alpha]_D^{20}$: −16.2 (*c* 0.69, CH_2Cl_2)},[26] establishing the absolute configuration of the obtained methyl-ester-diol **2** and consequently the (2*S*,3*R*) configuration of bio-catalytically formed cyano-diol **8**. (2*S*,3*R*)-**8**: ^{1}H NMR (acetone-d_6, 300 MHz): δ = 7.5 (brm, 2H), 7.3–7.4 (brm, 3H), 5.7 (d, *J* = 6.8 Hz, 1H), 5.3 (d, *J* = 4.3 Hz, 1H), 4.9 (t, *J* = 4.3 Hz, 1H), 4.6 (d, *J* = 5.7 Hz, 1H); ^{13}C NMR (acetone-d_6, 75 MHz): δ = 139.9, 128.1, 127.9, 126.7, 118.8, 74.4, 67.1.

Bioconversion of cis-3-phenyloxirane-2-carbonitrile (rac-9): *Analytical scale* – A specific volume of a stock solution of 100 g/L of *rac*-**9** (final concentration 1–50 g/L) in MTBE (final concentration of MTBE 20%) and lyophilized biomass with an EH activity of 2757 U/g (final concentration 1–50 g/L) were added to 0.8 mL of buffer A in a 10-mL round-bottom flask (final volume of reaction: 1 mL). Enzymatic reactions were incubated at 27 °C under magnetic stirring (800 rpm). Regularly withdrawn aliquots were saturated with NaCl and extracted with ethyl acetate (200 μL). Then 100 μL of the organic phase were evaporated under reduced pressure, and the residues were dissolved in 100 μL of isopropyl alcohol. Then 20 μL of this solution were analyzed by HPLC to determine the *ee*s of both remaining epoxide and formed diol. A Chiralcel OD-H column (250 × 4.60 mm) was used in isocratic mode with a 92.5/7.5 mixture of hexane/isopropyl alcohol at a flow rate of 1.2 mL/min. UV detection was set at 215 nm. The retention times were as follows: (2*S*,3*R*)-**9**: 11.2 min, (2*R*,3*S*)-**9**: 12.0 min, (2*R*,3*R*)-**10**: 20.6 min, (2*S*,3*S*)-**10**: 23.9 min.

Preparative scale – In a 100-mL round-bottomed flask was added 1.0 g of *rac*-**9** dissolved in 8 mL of MTBE and 32 mL

of buffer A. The biocatalytic reaction was initiated by addition of 1 g of lyophilized biomass with an EH activity of 2757 U/g. The reaction was performed at 27 °C under magnetic stirring (800 rpm). After 60 min, the reaction mixture was extracted 3 times with 40 mL ethyl acetate. The combined organic phases were dried over $MgSO_4$, filtered and then evaporated under reduced pressure. The products were purified by flash chromatography (pentane/diethyl ether, 7/3), affording (2*R*,3*S*)-**9** as a white solid (yield: 0.44 g, 44%; *ee* >99%), $[\alpha]_D^{25}$: +109.8 (*c* 0.86, EtOH), mp 54–55 °C and (2*R*,3*R*)-**10** as a slightly yellow liquid (yield: 0.51 g, 45.2%; *ee* >99%), $[\alpha]_D^{25}$: −32.1 (*c* 1.0, EtOH). The absolute configuration of residual epoxide **9** was determined after transformation of **9** to its *cis*-methyl-ester counterpart (2*S*,3*S*)-**20** (Scheme 3).[27] The absolute configuration of (2*S*,3*S*)-**20** was determined by comparison of the optical rotation value found in the literature for (2*R*,3*R*)-**20** { $[\alpha]_D^{25}$: +10.8 (*c* 1.03, CH_2Cl_2)}[22] with the one determined experimentally in this work { $[\alpha]_D^{20}$: −9.1 (*c* 0.98, CH_2Cl_2)}. In order to determine the absolute configuration of the formed diol **10**, the same protocol was used as for diol **8** (Scheme 3).[26] The optical rotation of the formed diol **2** { $[\alpha]_D^{20}$: −14.7 (*c* 0.73, CH_2Cl_2)} was compared to the one found in the literature for (2*S*,3*R*)-*syn*-methyl-ester-diol **2** { $[\alpha]_D^{20}$: −16.2 (*c* 0.69, CH_2Cl_2)},[26] establishing the same absolute configuration for the obtained methyl-ester-diol **2**, the (2*R*,3*R*) configuration for *trans*-acetonide **10a** and thus the same (2*R*,3*R*) configuration for the biocatalytically formed cyano-diol **10** (Scheme 3). (2*R*,3*R*)-**10**: ^{1}H NMR (acetone-d_6, 300 MHz): δ = 7.5–7.6 (brm, 2H), 7.4–7.3 (brm, 3H), 5.7 (brs, 1H), 5.2 (brs, 1H), 4.9 (d, *J* = 5.8 Hz, 1H), 4.7 (d, *J* = 5.8 Hz, 1H); ^{13}C NMR (acetone-d_6, 75 MHz): δ = 139.5, 128.2, 128.0, 127.3, 118.8, 74.4, 66.4.

Bioconversion of 2-(bromomethyl)-3-phenyloxirane (rac-11): *Analytical scale* – A specific volume of a stock solution of 100 g/L of *rac*-**11** (final concentration 1–100 g/L) in MTBE (final concentration of MTBE 20%) and lyophilized biomass with an EH activity of 2757 U/g (varying concentrations: see text) were added to 0.8 mL of buffer A in a 10-mL round-bottom flask (final volume of reaction 1 mL). Enzymatic reactions were incubated at 27 °C under magnetic stirring (800 rpm). Regularly withdrawn aliquots were saturated with NaCl and extracted with ethyl acetate (200 µL). Then 100 µL of the organic phase were evaporated under reduced pressure and the residues were dissolved in 100 µL of isopropyl alcohol. Then 20 µL of this solution were analyzed by HPLC to determine the enantiomeric excesses of both remaining epoxide and formed diol. A Lux Cellulose-4 chiral column (250 × 4.60 mm, Phenomenex) was used in isocratic mode with a 92.5/7.5 mixture of hexane/isopropyl alcohol at a flow rate of 1 mL/min. UV detection was set at 215 nm. The retention times were as follows: (2*S*,3*R*)-**11**: 8.5 min, (2*R*,3*S*)-**11**: 10.2 min, (2*R*,3*R*)-**12**: 20.6 min, (2*S*,3*S*)-**12**: 22.6 min, and for the 2 *syn*-diols: 25.6 and 26.7 min.

Preparative scale – In a 100-mL round-bottomed flask was added 1.0 g of *rac*-**11** dissolved in 2.6 mL of MTBE and 10.7 mL of buffer A. The biocatalytic reaction was initiated by addition of 0.8 g of lyophilized biomass with an EH activity of 2757 U/g. The reaction mixture was kept at 27 °C under magnetic stirring (800 rpm). After 60 min, the reaction mixture was extracted 3 times with 40 mL ethyl acetate. The combined organic phases were dried over $MgSO_4$, filtered and then evaporated under reduced pressure. The products were purified by flash chromatography (pentane/diethyl ether, 7/3), affording (2*S*,3*R*)-**11** as a colourless liquid (yield: 0.44 g, 44%; *ee* 99%), $[\alpha]_D^{24}$: +11.3 (*c* 1, $CHCl_3$) and (2*R*,3*R*)-**12** as a colourless liquid (yield: 0.53 g, 48%; *ee* 99%), $[\alpha]_D^{20}$: M8.5 (*c* 1, $CHCl_3$). Diol **12** was cyclized under basic conditions to the corresponding 1-phenylglycidol **15**,[28] whose absolute configuration was established as (1*R*,2*S*) by comparison of the optical rotation data found in the literature for (1*R*,2*S*)-**15** {$[\alpha]_D^{25}$: −100.2 (*c* 2.31, $CHCl_3$)}[24] with the one experimentally obtained in this work for epoxy alcohol **15** arising from bromo-diol {$[\alpha]_D^{25}$: −104 (*c* 2.74, $CHCl_3$). (2*R*,3*R*)-**12**: ^{1}H NMR (acetone-d_6, 300 MHz): δ = 7.3–7.4 (brm, 5H), 4.8 (d, *J* = 5.4 Hz, 1H), 4.0 (ddd, *J* = 3.5 Hz, *J* = 5.3 Hz, *J* = 7.8 Hz 1H), 3.5 (dd, *J* = 7.7 Hz, *J* = 10.6 Hz, 1H), 3.4 (dd, *J* = 3.4 Hz, *J* = 10.7 Hz, 1H), 2.3–3.1 (brs, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 139.6, 128.7, 128.3, 126.6, 75.0, 74.7, 35.8.

Bioconversion of 2-(chloromethyl)-3-phenyloxirane (rac-13): *Preparative scale* – In a 100-mL round-bottomed flask was added 1.0 g of *rac*-**13** dissolved in 4 mL of MTBE and 16 mL of buffer A. The biocatalytic reaction was initiated by addition of 0.5 g of lyophilized biomass with an EH activity of 2757 U/g. The reaction was performed at 27 °C under magnetic stirring (800 rpm). After 40 min, the reaction mixture was extracted 3 times with 40 mL ethyl acetate. The combined organic phases were dried over $MgSO_4$, filtered and then evaporated under reduced pressure. The products were purified by flash chromatography (pentane/diethyl ether, 7/3), affording of (2*S*,3*R*)-**13** as a colourless liquid (yield: 0.43 g, 43%; *ee* >99%) and (2*R*,3*R*)-**14** as a slightly yellow liquid (yield: 0.49 g, 44.5%; *ee* >99%), $[\alpha]_D^{20}$: −4.6 (*c* 1, EtOH). The absolute configuration of (2*S*,3*R*)-**13** was determined by comparison of the optical rotation value found in the literature for (2*S*,3*R*)-**13** {$[\alpha]_D^{25}$: +21.4 (*c* 0.6, $CHCl_3$)}[29] with the one experimentally determined in this work {$[\alpha]_D^{20}$: +20 (*c* 0.6, $CHCl_3$)}. Diol **14** was cyclized in basic conditions to the corresponding 1-phenylglycidol **15**,[28] whose absolute configuration was established as (1*R*,2*S*) by comparison of the optical rotation data found in the literature for (1*R*,2*S*)-**15** {$[\alpha]_D^{25}$: −100.2 (*c* 2.31, $CHCl_3$)}+[30] with the one experimentally obtained in this work for epoxy alcohol **15** arising from chloro-diol {$[\alpha]_D^{25}$: −100 (*c* 2.74, $CHCl_3$)}. (2*R*,3*R*)-**14**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.3–7.4 (brm, 5H), 4.8 (d, *J* = 5.0 Hz, 1H), 3.9 (m, 1H), 3.5 (m, 2H), 2.9–3.2 (brs, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 139.6, 128.6, 128.3, 126.6, 74.9, 74.6, 46.1.

Bioconversion of trans-stilbene oxide (rac-16): *Analytical scale* – In all experiments 100 mg of *rac*-**16** were dissolved in MTBE (from 0.4 to 20 mL) and buffer A was added to make up a final MTBE concentration of 20% and a final total concentration of **16** in the range of 1–50 g/L. The reaction was initiated by the addition of 200 mg of lyophilized biomass with an EH activity of 2757 U/g. The biotransformations were performed at 27 °C under stirring (1200 rpm). Regularly withdrawn aliquots were saturated with NaCl and extracted with ethyl acetate (200 µL). Then 100 µL of the organic phase were evaporated under reduced pressure, and the residues were dissolved in 100 µL of isopropyl alcohol. Then 20 µL of this solution were analyzed by HPLC to determine the *ee*s of both remaining epoxide and formed diol. A Lux Cellulose-4 chiral column (250 × 4.60 mm, Phenomenex) was used in isocratic mode with a 9/1 mixture of hexane/

isopropyl alcohol at a flow rate of 1 mL/min. UV detection was set at 215 nm. The retention times were as follows: (1*R*,2*R*)-**16**: 4.9 min, (1*S*,2*S*)-**16**: 7.9 min, *meso*-diol-**17**: 17.3 min.

Preparative scale – In a 100-mL round-bottomed flask was added 1.0 g of *rac*-**16** dissolved in 4 mL of MTBE and 16 mL of buffer A. The biocatalytic reaction was initiated by addition of 2 g of lyophilized biomass with an EH activity of 2757 U/g. The reaction was performed at 27 °C under magnetic stirring (800 rpm). After 5 h, the reaction mixture was extracted 3 times with 40 mL ethyl acetate. The combined organic phases were dried over $MgSO_4$, filtered and then evaporated under reduced pressure. The products were purified by flash chromatography (pentane/diethyl ether, 1/1), affording (1*R*,2*R*)-**16** as a slightly yellow solid (yield: 0.45 g, 45%; *ee* 99%), mp 65–66 °C and *meso*-**17** as a white solid (yield: 0.51 g, 46%), mp 122–124 °C. The absolute configuration of (1*R*,2*R*)-**16** was determined by comparison of the optical rotation value found in the literature for (1*R*,2*R*)-**16** {$[\alpha]_D^{20}$: +357 (*c* 0.59, benzene)}[31] and the one experimentally determined in this work {$[\alpha]_D^{20}$: +348 (*c* 0.59, benzene)}. *meso*-**17**: ^{1}H NMR (acetone-d_6, 300 MHz): δ = 7.2–7.3 (brm, 10H), 4.9 (brs, 2H), 4.3 (s, 2H); ^{13}C NMR (acetone-d_6, 75 MHz): δ = 142.0, 127.4, 127.3, 126.9, 77.7.

Bioconversion of meso-cis-stilbene oxide (18): *Analytical scale* – A specific volume of a stock solution of 100 g/L of *meso*-**18** (final concentration 1–50 g/L) in DMF (final concentration of DMF 5%) and lyophilized biomass with an EH activity of 2757 U/g (final concentration 2–100 g/L) were added to 1.9 mL of buffer A in a 25-mL round-bottom flask (final reaction volume: 2 mL). The enzymatic reactions were performed at 27 °C under magnetic stirring (1200 rpm). The next preparatory steps were performed as described for the analytical scale bioconversion of compound **16**. The retention times were as follows: *meso*-**18**: 5.6 min, (1*S*,2*S*)-**19**: 23.9 min, and (1*R*,2*R*)-**19**: 26.9 min.

Preparative scale – In a 100-mL round-bottomed flask was added 1.0 g of *rac*-**18** dissolved in 2 mL of DMF and 38 mL of buffer A. The biocatalytic reaction was initiated by the addition of 2 g of lyophilized biomass with an EH activity of 2757 U/g. The reaction was performed at 27 °C under magnetic stirring (800 rpm). After 30 h, the reaction mixture was extracted 3 times with 40 mL ethyl acetate. The combined organic phases were dried over $MgSO_4$, filtered and then evaporated under reduced pressure. The products were purified by flash chromatography (pentane/diethyl ether, 1/1), affording (1*R*,2*R*)-**19** as a slightly yellow solid (yield: 0.92 g, 84.5%; *ee* 99%), mp 136–138 °C. The absolute configuration was determined by comparison of the optical rotation value found in the literature for (1*R*,2*R*)-**19**, {$[\alpha]_D^{28}$: +92.7 (*c* 1, EtOH)}[32] with the one experimentally determined in this work {$[\alpha]_D^{28}$: +93.8 (*c* 1, EtOH)}. (1*R*,2*R*)-**19:** ^{1}H NMR (acetone-d_6, 300 MHz): δ = 6.9–7.1 (brm, 10H), 4.52 (brs, 2H), 4.47 (brs, 2H); ^{13}C NMR (acetone-d_6, 75 MHz): δ = 141.7, 127.5, 127.2, 127.1, 78.9.

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