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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-solvent 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; epoxide hydrolase; Kau2; enantioselectivity

15 Introduction

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

37 specific activity, enantioselectivity or
38 enantioconvergence by molecular evolution
39 techniques.^[5]

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

53 In this paper we describe the kinetic resolution of
54 racemic methyl *trans*- and *cis*-3-phenylglycidate-**1**
55 and-**20**, ethyl *trans*-3-phenylglycidate-**3**, methyl
56 *trans*-3-(4-methoxyphenyl)glycidate-**5**, *trans*- and
57 *cis*-3-phenyloxirane-2-carbonitrile-**7** and -**9**, *trans*-2-
58 (bromomethyl)- and *trans*-2-(chloromethyl)-3-
59 phenyloxirane-**11** and -**13**, *trans*-stilbene oxide-**16** as
60 well as the desymmetrization of *cis*-stilbene oxide-**18**

1 using lyophilized cells of *E. coli* overexpressing the
2 Kau2-EH as a biocatalyst. Compounds **1** and **3** are of
3 synthetic interest since they can be used for the
4 synthesis of the anticancer taxol side chain^[8] or the
5 nootropic drug (-)-Clausenamide.^[9] Methyl 3-(4-
6 methoxyphenyl)glycidate-**5** in its (2*R*,3*S*)
7 stereochemical form is a very useful chiral synthon
8 for the access to the calcium channel blocker
9 Diltiazem, which acts as a potent vasodilator^[10]
10 while each enantiomer of **5** as well as the
11 corresponding diols can be used for the synthesis of
12 all stereoisomers of isocytosazine, the structural
13 isomer of (-)-Cytosazine, acytokine modulator of
14 microbial origin.^[11] The chloro epoxide-**13** has been
15 used as a synthon to access the naturally occurring
16 styryl lactones Leiocarpin C and Gonodiol.^[7a]
17 Furthermore, the diols **12** and **14** arising from ring
18 opening of bromo epoxide-**11** and chloro epoxide-
19 **13** can easily be transformed under basic conditions
20 (with retention of configuration) to get access to the
21 terminal epoxide-**15** which is useful for the synthesis
22 of several natural and biologically active
23 compounds.^[7b,7c] In almost all cases the biocatalytic
24 transformation was of extremely high
25 enantioselectivity, giving rise to an entire range of
26 enantiomerically pure epoxides and diols.
27 Furthermore, the tested reactions were successfully
28 performed on a 1-g preparative scale, and the reaction
29 times rarely exceeded 60–80 minutes. With these
30 water-insoluble substrates an organic water-
31 immiscible solvent was used without seriously
32 affecting the enzyme activity. Another major
33 advantage of such a biphasic biotransformation
34 approach was that higher stabilities of the water-
35 sensitive epoxidic substrates were achieved due to
36 their reduced chemical hydrolysis in the organic
37 phase.

38 Results and Discussion

39 Kinetic resolution of methyl and ethyl *trans*-3- 40 phenylglycidate *rac*-**1** and *rac*-**3**.

41 Initially, *rac*-methyl *trans*-3-phenylglycidate-**1** was
42 tested as a substrate of the Kau2-EH (Scheme 1) in the
43 1 to 100 g/L concentration range in the presence of
44 5% DMF and 10% isooctane as a water-immiscible
45 co-solvent. A ratio of 1 to 2 (mass of substrate to
46 mass of lyophilized Kau2-expressing *E. coli* cells
47 m/m) was initially found convenient. For substrate
48 concentrations lower than or equal to 10 g/L a nearly
49 perfect kinetic resolution was observed, since both
50 formed (2*R*,3*R*)-**2** and remaining (2*S*,3*R*)-**1** were
51 obtained optically pure (ee>99%). Higher substrate
52 concentrations of 25, 50 and 100 g/L resulted in ees

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%), di-
isopropyl 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).

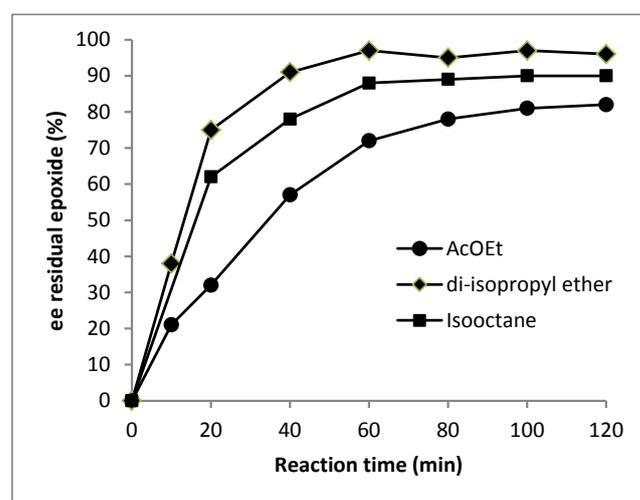


Figure 1. Comparison of the effects of the various used organic co-solvents on the final achievable ees of the remaining substrate **1**.

Combining these results with the use of a more active
Kau2-EH preparation of 1667 U/g (produced in
inabioreactor) compared to 768 U/g (produced in
flasks) enabled the substrate concentration to be
increased to 50 g/L, ensuring ≥99% for both ees of
formed diol product and residual epoxide. Using
these optimized conditions, the reaction was then
conducted on a preparative scale (1 g) in 80 minutes,
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 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.

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

5 To obtain some additional information about the size of acceptable substrates of the Kau2-EH, compound **5** 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 ees 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 10 minutes 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% yield, 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] Further, 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**.

6 **Kinetic resolution of methyl *trans*-3-(4-methoxyphenyl)glycidate *rac*-**5**.**

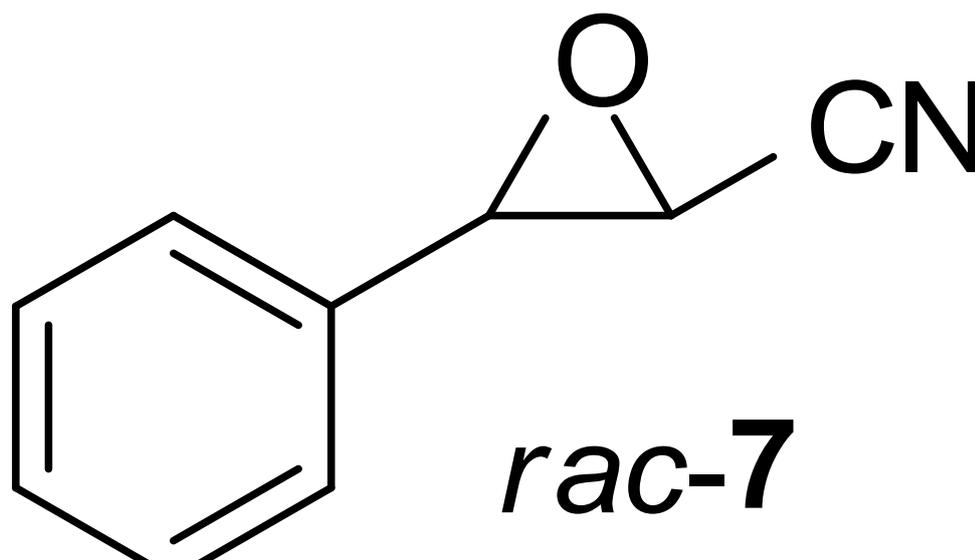
7 We then tested the Kau2-EH with *rac*-**5** as a substrate (Scheme 2). Due to the presence of the *mp*-methoxy substituent, **5** proved to be prone to chemical hydrolysis during the time course of the biocatalytic reaction. This was detrimental to both final yield in remaining epoxide as well as the 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 SI). Using these optimized reaction conditions, we tested substrate concentrations ranging from 1 to 40 g/L using a twofold 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) 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 glycidate **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*-isocytosaxone.^[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.

1
2
3 **Scheme 2.** Preparative kinetic resolution of *rac*-methyl *trans*-3-(4-methoxyphenyl)glycidate-5.

4
5 **Kinetic resolution of *trans*- and *cis*-3-phenyloxirane-2-carbonitrile *rac*-7 and *rac*-9.**

6
7
8 Based on the excellent results obtained with the
9 phenyl glycidate derivatives, we decided to test
10 whether a cyano functionality could replace the
11 previously used ester functionality. Due to the
12 selected mode of synthesis, both *trans*-cyano-7 and
13 *cis*-cyano-9 epoxides were obtained and easily
14 chromatographically separated, giving thus the
15 possibility to test both substrates with the Kau2-EH31
16 *AnE. coli* biomass with a higher EH specific activity
17 (2757 compared to 1667 U/g, see the Experimental
18 section) enabled the substrate/enzyme ratio to be
19 reduced to 1/0.75 (m/m), and 20% MTBE were used
20 to ensure a biphasic system. The *trans*-cyano

21 derivative 7 was tested in the concentration range of
22 1–75 g/L and proved to be an excellent substrate
23 (Scheme 3). Under these reaction conditions both
24 residual (*2R,3R*)-7 and formed (*2S,3R*)-8 were
25 obtained in > 99% ee in 80 minutes for all tested
26 concentrations, except for 1 and 75 g/L. The slow
27 mass transfer of the substrate to the aqueous phase at
28 1 g/L was probably the reason for the lower ee-
29 values; on the other hand, some unspecific inhibition
30 at high substrate and biomass concentrations appeared
31 to limit the ee of (*2R,3R*)-7 to 75%. A preparative
32 reaction run with 1 g of 7 at 50 g/L afforded after 80
33 minutes both residual (*2R,3R*)-7 and formed (*2S,3R*)-
34 8 with ees of > 99% in 45 and 46% isolated yield,
35 respectively (Scheme 3).



37
38
39 **Scheme 3.** Kinetic resolution of *trans*- and *cis*-3-phenyloxirane-2-carbonitrile *rac*-7 and *rac*-9 and chemical
40 transformations aimed at determining absolute configurations (see ES).

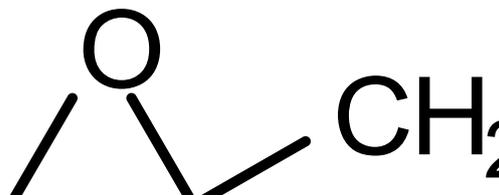
41
42 The Kau2-EH was previously reported to
43 enantioconvergently transform *cis*- β -methylstyrene
44 oxide into the corresponding (*1R,2R*)-diol in 97%
45 isolated yield and 98.3% ee.^[6] As methyl *cis*-3-
46 phenylglycidate-20 was not a substrate of the Kau2-
47 EH, we were pleased to note that closely related *cis*-3-

1 phenyloxirane-2-carbonitrilerac-9 underwent 23
 2 kinetic resolution using this enzyme. The optima24
 3 substrate/enzyme ratio was 1/1 (m/m), and 20%25
 4 MTBE were used to ensure the presence of a biphasi26
 5 system. Once again the kinetic resolution was almos27
 6 perfect for substrate concentrations ranging from 1 t28
 7 25 g/L, both residual epoxide and formed diol being29
 8 recovered essentially optically pure after 1 h. At 530
 9 g/L of substrate concentration the reaction stoppe31
 10 after the ee of the remaining epoxide reached 93%32
 11 the obtained diol being nearly optically pure. A33
 12 preparative reaction was then conducted on a 1-g34
 13 scale at 25 g/L of rac-9, affording after 80 minute35
 14 and silica gel purification residual (2R,3S)-9 an36
 15 formed (2R,3R)-10 in nearly enantiopure form37
 16 (ee>99%), and 44 and 45% isolated yield38
 17 respectively. The results described here for the Kau239
 18 catalyzed biohydrolysis of both rac-7 and rac40
 19 9 compared particularly well with those described in41
 20 the literature using whole cells of *Mortierella*42
 21 *isabellina* DSM 1414 in an attempt to kinetically43
 22 resolve these epoxides.^[15] Indeed, for both rac-7 and44

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 ees exceeding 99% after 1 h of reaction time.



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 ES).

46
 47
 48
 49
 50
 51 When run on a preparative scale (1 g of rac-11, 755
 52 g/L) in the presence of 0.8 g of biocatalyst (60 g/L)66
 53 the reaction afforded optically pure (2*S*,3*R*)-11 an67
 54 (2*R*,3*R*)-12 in 44% and 48% isolated yields68
 55 respectively (Scheme 4). A preparative-scale reaction69
 56 with chloro-epoxide-13 (1 g of 13, 50 g/L; 0.5 g of70
 57 biocatalyst (2011 U/g)), afforded optically pur71
 58 (2*S*,3*R*)-13 (43% isolated yield) and (2*R*,3*R*)-1472
 59 (44.5% isolated yield) (Scheme 4). A(2*S*,3*R*) absolut73
 60 configuration was assigned to remaining bromo74
 61 epoxide-11 by analogy with the stereochemistry dat75
 62 obtained for chloro-epoxide-13, assuming the sam76
 63 stereochemistry of epoxide opening during the Kau277
 64 mediated hydrolysis reaction for both compounds78

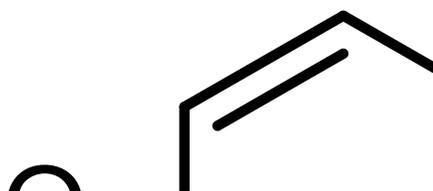
Such an equivalent behavior was supported by the fact that formed bromo-diol-12 and chloro-diol-14 possessed the same absolute configuration (Scheme 4 and ES).

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

1 substrate than the above-mentioned epoxidid
 2 compounds. Indeed, the remaining epoxide wa
 3 obtained in enantiomerically pure form only after
 4 48 h of reaction time, compared to approximat
 5 for the above-mentioned substrates. With th
 6 exception of 1 g/L of substrate concentration, wh
 7 resulted in an ee of 93% for the remaining epoxid
 8 after 105 h, higher concentrations (5, 10 and 25 g/L)
 9 enabled enantiomerically pure epoxide to be obtain
 10 after 24h (Scheme 5). At the highest substrat
 11 concentration of 50 g/L an enantiomerically pure

remaining epoxide was obtained but only after 48h.
 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.



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

23
 24
 25
 26 The reaction portfolio was then extended to th
 27 desymmetrization of the *meso*-epoxide *cis*-stilben
 28 oxide-**18** (Scheme 5). The *cis*-epoxide exhibit
 29 quantitative transformation in the range from 1 to 25
 30 g/L (the biomass concentration being double that of
 31 the substrate, m/m), the formed (1*R*,2*R*)-**19** being in
 32 all cases almost enantiomerically pure (ee>99%) with
 33 more than 90% analytical yield in 24 h. At 50 g/L the
 34 yield of formed **19** dropped considerably to 20% after
 35 24h of reaction time. Thus a preparative reaction was
 36 run on a 1-g scale at 25 g/L of **18**. After 30 h of
 37 reaction time the epoxide was totally consumed and
 38 the formed (1*R*,2*R*)-diol-**19** was recovered in 99% ee
 39 and 84.5% isolated yield. This result is comparable to
 40 the data of a previous report dealing with the
 41 desymmetrization of *meso*-epoxides using various
 42 EHs obtained from a metagenomic library.^[16] Using
 43 cell extracts of 4 different EHs (BD 8877, BD 8676,
 44 BD 9300, BD 9883) (1*R*,2*R*)-diol-**19** was obtained
 45 with ees ranging from 96 to 99.5%, while its antipode
 46 (1*S*,2*S*)-diol-**19** was obtained in 99% ee using the BD
 47 9196 EH.^[16]
 48 We can conclude from the above-mentioned results
 49 that the Kau2-EH is a particularly useful enzyme for
 50 the kinetic resolution/desymmetrization of 1,2-
 51 disubstituted epoxides which contain at least one
 52 phenyl ring substituent. Indeed, exceptionally high
 53 stereoselectivity was demonstrated with this enzyme,
 54 leading in all but two cases to ees of at least 99% for

both remaining epoxide and formed diol, which
 represents a nearly perfect kinetic resolution (Table
 1).

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)	<i>meso</i> (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.

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 enzymetolerated well the presence of water-immiscible organic solvents such as di-isopropyl ether and MTBE, alleviating or diminishing the

1 chemical hydrolysis of the epoxide as well as
 2 substrate and product inhibition. Further, the use of
 3 organic co-solvents enabled the dissolution of very
 4 high quantities of substrate and product. The
 5 biocatalyst was also tolerant to pH changes in the
 6 alkaline region (pH 7.0 to pH 8.5), offering the
 7 possibility to further minimize the spontaneous
 8 chemical hydrolysis of fairly unstable epoxides.
 9 Some of the tested substrates are potentially very
 10 valuable chiral synthons for the synthesis of
 11 numerous biologically active target molecules. When
 12 compared to a recent report about the kinetic
 13 resolution of glycidates **1**, **3** and **5** with whole cells of
 14 *Galactomyces geotrichum*, the Kau2-EH expressed in
 15 *E. coli* proved to be an outstanding biocatalyst for
 16 getting access to optically pure epoxides.^[12] The
 17 residual epoxides ((*2S,3R*)-**1** and (*2S,3R*)-**3**) are
 18 suitable as chiral synthons for accessing the Taxol
 19 side chain^[17a,b] as well as for being used in the
 20 synthesis of the nootropic drug (-)-
 21 Clausenamide.^[9] Unfortunately, the residual epoxide
 22 (*2S,3R*)-**5** did not bear the correct configuration to be
 23 used as a chiral synthon for accessing Diltiazem. We
 24 have shown that bi-functional epoxides can be used
 25 as substrates of the Kau2-EH with virtually no
 26 noticeable influence of different functionalities such
 27 as nitrile, methyl or ethyl ester and bromine and
 28 chlorine on their reactivity. In the latter cases, new
 29 terminal epoxides were easily accessible without any
 30 loss in enantiopurity from the enzymatically formed
 31 diol by simply using basic reaction conditions. Even
 32 very sterically demanding substrates such as *trans*-
 33 and *cis*-stilbene oxides were also transformed by the
 34 enzyme; however, lower reaction rates were observed.
 35 Even these demanding substrates reacted to
 36 completion on a preparative scale within one day
 37 using the Kau2-EH. In order to compare the
 38 efficiencies of the Kau2-EH in the kinetic resolutions
 39 of the various substrates described above, we present
 40 in Figure 2 the results of all the tested substrates at the
 41 same concentration of 25 g/L under the various
 42 reaction conditions described in the Experimental
 43 section. This comparison is based on the number of
 44 μmoles of remaining epoxide at the end of the
 45 resolution process (epoxide ee > 99%) divided by the
 46 reaction time and the number of enzymatic units used
 47 in the reaction. That means that a twofold higher
 48 efficiency leads to an approximately twofold quicker
 49 resolution process (ee of the remaining epoxide
 50 >99%) based on the same amount of enzyme
 51 used. We can conclude: (1) the efficiency appears to
 52 be inversely proportional to the size of the substituent
 53 (CH₂Br > CH₂Cl > CN > COOMe > COOEt > Phe), and (2)
 54 enzyme catalyzed resolution of *trans*-configured
 55 substrates exhibited higher efficiencies than for their
 56 *cis*-counterparts.
 57

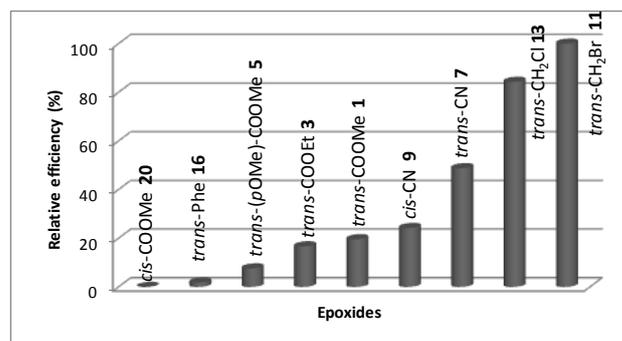
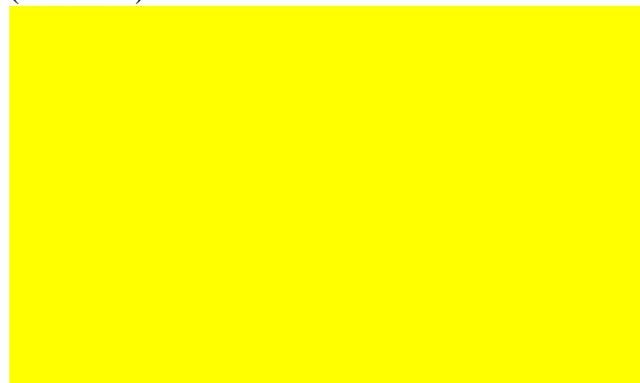


Figure 2. Comparison of the relative Kau2-EH efficiencies (vertical bars) for the kinetic resolutions of the various epoxidic substrates at the same concentration of 25 g/L, but under reaction conditions adapted for each substrate (see experimental part).

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).



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

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

1 (*rac*-**9**) led to the loss of the high enantioconvergence⁵⁵
2 found previously⁶¹ in favour of a perfect⁵⁶
3 enantioselectivity. It should be noted in that case⁵⁷
4 that, in contrast to its *trans*-counterpart, the reactive⁵⁸
5 enantiomer corresponded to the one bearing the⁵⁹
6 benzylic carbon atom of *R*-absolute configuration, the⁶⁰
7 enzymatic attack occurring selectively at the β⁶¹
8 carbon atom bearing the cyano group.

9 Conclusion

10 In conclusion, we have shown that the Kau2-EH is a⁶²
11 outstanding catalyst, which enables an easy, robust⁶³
12 and efficient access to numerous useful optically pure⁶⁴
13 diols and epoxides that can be used for example as⁶⁵
14 chiral building blocks in the synthesis of various⁶⁶
15 biologically active chemicals.

16 Experimental Section

17 General Remarks

18
19 All reagents were used as received from Sigma-Aldrich⁶⁷
20 Acros or Fluka. Dimethylformamide (DMF)⁶⁸
21 tetrahydrofuran (THF), dichloromethane, methanol, di⁶⁹
22 isopropyl ether, *t*-butyl methyl ether (MTBE) and *iso*⁷⁰
23 octane were of analytical grade. Acetonitrile, hexane and⁷¹
24 isopropanol were of HPLC grade. The epoxides *rac*⁷²
25 methyl *trans*-3-(4-methoxyphenyl)glycidate-**5**, *rac-trans*⁷³
26 stilbene oxide-**16** and *meso-cis*-stilbene oxide-**18** were from⁷⁴
27 Sigma-Aldrich. All other reagents were from Sigma⁷⁵
28 Aldrich, ACROS or Fluka. ¹H and ¹³C NMR spectra were⁷⁶
29 recorded on a Bruker Avance 300 Ultrashield NMR⁷⁷
30 spectrometer. Chemical shifts are given in ppm relative to⁷⁸
31 residual peaks of chloroform-*d*₃ or acetone-*d*₆. The⁷⁹
32 coupling constants are given in Hertz (Hz). The⁸⁰
33 abbreviations br, s, d, t and m correspond to a broad signal⁸¹
34 a singlet, a doublet, a triplet and a multiplet, respectively.

36 Biocatalyst production and EH activity.

37
38 **Buffer and culture media:** Buffers A and B were⁸²
39 composed of 50 mM Na-phosphate, adjusted to pH 7.0 and⁸³
40 8.5, respectively. Luria Bertani (LB) medium⁸⁴
41 contained (per litre) 10 g of peptone, 5 g of yeast extract⁸⁵
42 and 5 g of NaCl and was adjusted to pH 7.5. For solid⁸⁶
43 media 15 g/L of agar were added. The minimal growth⁸⁷
44 medium was composed of (per litre) sucrose 10 g,⁸⁸
45 KH₂PO₄ 13.6 g, (NH₄)₂SO₄ 4 g, NaOH 3 g, MgSO₄·7H₂O 2 g,⁸⁹
46 CaCl₂·0.377 g, FeSO₄·7H₂O 0.01 g and adjusted to pH 7.4.⁹⁰

47 **Engineering *E. coli* RE3 expressing Kau2-EH.** The⁹¹
48 construction of the IPTG-inducible Kau2-EH-encoding⁹²
49 plasmid was described elsewhere.⁶¹ Transformation of *E. coli*⁹³
50 RE3 with this DNA construct resulted in ampicillin⁹⁴
51 resistant Kau2-overproducers that were able to grow in⁹⁵
52 minimal medium with sucrose as the sole carbon source.¹⁸¹

53 **Kau2-EH production.** Frozen glycerol stock suspension⁹⁶
54 of *E. coli* RE3 cells expressing Kau2-EH were spread on⁹⁷

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, pO₂ was maintained
above 20% (air flow 1 VVM), and the stirring speed was
set at 300 rpm. After 8–9 hours 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 (BioBundle bioreactor
from Applikon Biotechnology, 10 L).

Determination of Kau2-EH activity. The EH activity
was determined using (*S*)-*p*-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 minutes, the supernatant (200
μL) was then filtered (0.45 μm) and analyzed by HPLC
(Agilent 1100 Series) on a NUCLEOSIL[®] C₁₈ 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.*¹⁹¹

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-T²⁰¹ were added, and the mixture
was stirred at room temperature for 30 minutes. The
formed bromohydrine was cyclized by adding 5.2 g of
K₂CO₃, leaving the reaction under stirring at room
temperature overnight. The reaction was stopped by
addition of 1.0 g of Na₂S₂O₃. The mixture was poured into
a separatory funnel, extracted 3 times with 50 mL of
diethyl ether, the organic phases were collected, dried over
MgSO₄, 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 0.81 g of *rac*-methyl
trans-3-phenylglycidate-**1** as a colourless liquid (82%
yield). ¹H NMR (CDCl₃, 300 MHz): δ (ppm) = 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). ¹³C NMR (CDCl₃, 75 MHz): δ (ppm) = 168.6,
134.9, 129.0, 128.7, 125.8, 58.0, 56.6, 52.6.

Synthesis of ethyl trans-3-phenylglycidate rac-3.¹⁹¹ In a
100-mL round bottom flask were weighed 0.979 g (5.5

1 mmol) of *trans*-ethyl cinnamate, followed by the addition
2 of 20 mL of acetonitrile and 5 mL of distilled water. The
3 3.949 g of dibromoamine-T were added, and the mixture
4 was stirred at room temperature for 30 minutes. The next
5 preparatory steps were as described above for the synthesis
6 of **1**. Flash chromatography (as described above) afforded
7 0.82 g of *rac*-ethyl *trans*-3-phenylglycidate-**3** as a
8 colourless liquid (78% yield). ¹H NMR (CDCl₃, 300
9 MHz): δ(ppm) = 7.2-7.3 (brm, 5H), 4.28 (q, *J* = 7.2 Hz, 1H),
10 4.27 (q, *J* = 7.2 Hz, 1H), 4.1 (d, *J* = 1.7 Hz, 1H), 3.5 (d,
11 *J* = 1.7 Hz, 1H), 1.3 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (CDCl₃,
12 75 MHz): δ(ppm) = 168.2, 135.0, 129.0, 128.6, 125.8, 61.8, 57.9,
13 56.7, 14.1.

14 **Synthesis of *trans*- and *cis*-3-phenyloxirane-2-
15 carbonitrile *rac*-**7** and *rac*-**9**.**^[21]To a solution of
16 benzaldehyde (380 mg, 3.0 mmol), chloroacetonitrile (457
17 mg, 6.0 mmol) and tetrahexylammonium bromide (154 mg,
18 0.36 mmol) in THF (15.0 mL) was added KOH (400 mg,
19 7.2 mmol) at room temperature. After 22 h under stirring
20 NaBH₄ (200 mg) was added to the mixture to remove the
21 remaining benzaldehyde. After 5 minutes, the reaction was
22 quenched with water, and the mixture was extracted 3
23 times with 15 mL of ethyl acetate. The combined organic
24 layers were washed with brine and water, dried over
25 MgSO₄, filtrated and finally concentrated under vacuum.
26 The crude mixture was purified by flash chromatography
27 on silica gel (230-400 mesh) using a 9/1 mixture of
28 pentane and diethyl ether as an eluent, affording 0.09 g
29 *trans*-**7** as a colourless liquid (22.5% yield) and 0.16 g
30 *cis*-**9** as a white solid (40% yield, m. p. = 54–55°C). **7**: ¹H
31 NMR (CDCl₃, 300 MHz): δ(ppm) = 7.4-7.5 (brm, 3H), 7.29
32 7.3 (brm, 2H), 4.3 (d, *J* = 1.8 Hz, 1H), 3.4 (d, *J* = 1.8
33 Hz, 1H). ¹³C NMR (CDCl₃, 75 MHz): δ(ppm) = 132.8,
34 129.8, 129.0, 125.8, 116.2, 58.5, 44.7; **9**: ¹H NMR (CDCl₃,
35 300 MHz): δ(ppm) = 7.4-7.5 (m, 5 H), 4.3 (d, *J* = 3.7
36 Hz, 1H), 3.8 (d, *J* = 3.7 Hz, 1H). ¹³C NMR (CDCl₃,
37 75 MHz): δ(ppm) = 131.4, 129.7, 128.7, 126.3, 115.0, 57.9,
38 45.1.

39 **Synthesis of *trans*-2-(bromomethyl)- and *trans*-
40 (chloromethyl)-3-phenyloxirane *rac*-**11** and *rac*-**13**.**To a
41 solution of 70% *m*-chloroperoxybenzoic acid (*m*CPBA,
42 8.5 mmol) in 20 mL of CH₂Cl₂ was added a 30 mL
43 cinnamyl bromide (571 mg, 2.9 mmol) solution in CH₂Cl₂.
44 Then 20 mL of 0.5 M NaHCO₃ were added, and the
45 heterogeneous mixture was stirred at 25°C for 17 h. The
46 resulting mixture was washed 3 times with 10% sodium
47 bicarbonate, and the combined aqueous phases were
48 extracted 3 times with 30 mL of diethyl ether. The
49 combined organic layers were finally washed with brine
50 then water, dried over MgSO₄, filtrated and then
51 concentrated under vacuum. The crude product was first
52 purified by flash chromatography on silica gel (230-400
53 mesh) using a 9/1 mixture of pentane and diethyl ether as
54 eluent and then by bulb-to-bulb distillation at 200°C under
55 vacuum (0.4 mbar), affording 0.302 g of *trans*-
56 (bromomethyl)-3-phenyloxirane *rac*-**11** as a slightly yellow
57 liquid in 49% yield. ¹H NMR (CDCl₃, 300 MHz): δ (ppm)
58 = 7.3-7.4 (brm, 5H), 3.7 (d, *J* = 1.8 Hz, 1H), 3.4 (d, *J* = 5.2
59 Hz, 2H), 3.2 (dt, *J* = 1.8 Hz, *J* = 5.8 Hz, 1H). ¹³C NMR
60 (CDCl₃, 75 MHz): δ (ppm) = 136.0, 128.6, 128.6, 125.7,
61 61.0, 60.3, 31.9. A similar procedure starting from
62 cinnamyl chloride afforded 0.275 g of *trans*-

(chloromethyl)-3-phenyloxirane *rac*-**13** as a colourless
liquid (yield 52.1%). ¹H NMR (CDCl₃, 300 MHz): δ (ppm)
= 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); ¹³C
NMR (CDCl₃, 75 MHz): δ (ppm) = 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 hours and then acidified with diluted HCl for 2 hours at
4°C. The reaction solution was then extracted 3 times by
ethyl acetate (30 mL), the combine organic layers were
washed with brine then water, dried over MgSO₄, 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 0.287 g of *rac*-methyl *cis*-3-
phenylglycidate-**20** as a colourless liquid in 80% yield. ¹H
NMR (CDCl₃, 300 MHz): δ (ppm) = 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).
¹³C NMR (CDCl₃, 75 MHz): δ (ppm) = 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 H₂SO₄ (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
hydrolysis of *rac*-**3**, **-5**, **-7**, **-9**, **-11**, **-13** and **-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 (1*R*,2*S*)-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 hours. The mixture was
poured into a separatory funnel, extracted 3 times with 15
mL of ethyl acetate, the organic phases were collected,
dried over MgSO₄, filtered and evaporated under vacuum.
The product was purified by flash chromatography on
silica gel (230-400 mesh) using a 7/3 mixture of pentane
and diethyl ether as eluent, affording 57 mg of (1*R*,2*S*)-1-
phenylglycidol-**15** as a colourless liquid (59% yield).
(2*R*,3*S*)-**15**: ¹H NMR (CDCl₃, 300 MHz): δ (ppm) = 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). ¹³C NMR (CDCl₃,
75 MHz): δ (ppm) = 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 (2*S*,3*R*)-2 from cyano-diols (2*S*,3*R*)-8** and
(2*R*,3*R*)-**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

1 h, and then it was filtered and evaporated under reduced
2 pressure. The residue was dissolved in a mixture of
3 methanol and water (2:1), and KOH (0.59 g) was added to
4 the solution. Then the reaction solution was refluxed
5 overnight at 75°C. The reaction was stopped by adding
6 HCl (20%) and adjusted to pH 2. The mixture was
7 extracted 3 times with 40 mL diethyl ether and then dried
8 over MgSO₄. The diethyl ether was removed by rotary
9 evaporation, the crude product was dissolved in methanol
10 and a few drops of H₂SO₄ (98%) were added to the solution.
11 The mixture was stirred at room temperature for 1.5 h, and it
12 was quenched with a saturated aqueous solution of sodium
13 bicarbonate (20 mL). The mixture was extracted 3 times
14 with 40 mL diethyl ether, dried over MgSO₄ and
15 evaporated under vacuum. The product was purified by
16 flash chromatography on silica gel (230-400 mesh) using
17 an 50/50 mixture of pentane and diethyl ether as eluent
18 affording 85 mg of (2*S*,3*R*)-**2**. The same protocol was used
19 for (2*R*,3*R*)-**10**, which was obtained from bioconversion of
20 *rac*-**9**, leading to the same (2*S*,3*R*)-**2**.

22 **Kau2-EH enantioselective hydrolysis of epoxide 23 substrates.**

25 **Bioconversion of methyl trans-3-phenylglycidate *rac*-1.**

26 *Analytical scale.* A specific volume of a stock solution of
27 2.8 M of *rac*-**1** (final concentration 1–100 g/L) in DMF
28 (final concentration of DMF adjusted to 5%) and
29 lyophilized biomass with an EH activity of 1667 U/g (final
30 concentration 2–200 g/L) were added to 0.4 mL of buffer
31 containing 50 µL of di-isopropyl ether in a 10 mL round
32 bottom flask (final reaction volume 0.5 mL). The
33 enzymatic reactions were incubated at 27°C under
34 magnetic stirring (1200 rpm). Samples, which were
35 withdrawn at regular intervals, were extracted with ethyl
36 acetate and analyzed by chiral GC on a Lipodex-G column
37 (0.25 µm, 25 m × 0.25 mm, Macherey-Nagel) for enantiomer
38 analysis of both residual substrate and formed diol, using
39 bromo-acetophenone being used as an internal standard.
40 The flow rate of the carrier gas (H₂) was set at 2.0
41 mL·min⁻¹. The following oven temperature program
42 enabled separation of the enantiomers: 110°C for 10
43 minutes, followed by an increase to 140°C using a rate
44 10°C/min. The retention times were as follows. (2*R*,3*S*)-**1**:
45 1:17.5 min, (2*S*,3*R*)-**1**:17.9 min, (2*R*,3*R*)-**2**:38.1
46 min, (2*S*,3*S*)-**2**:38.6 min, and for the 2 *syn*-diols-**2**: 39.1 and
47 40.1 min.

48 *Preparative scale.* One g of *rac*-**1** dissolved in 1 mL of
49 DMF was added to 17 mL of buffer A and 2 mL of di-
50 isopropyl ether in a 100-mL round bottomed flask. The
51 biocatalytic reaction was initiated at 27°C by the addition
52 of 1.5 g of lyophilized biomass with an EH activity of
53 1667 U/g under magnetic stirring (1200 rpm). After 60 min,
54 the reaction mixture was extracted 3 times with 40 mL
55 ethyl acetate. The combined organic phases were dried
56 over MgSO₄, filtered and then evaporated under reduced
57 pressure. The products were purified by flash
58 chromatography (pentane/diethyl ether, 7/3), affording
59 0.49 g of (2*S*,3*R*)-**1** (ee>99%, yield 49%) as a colourless
60 liquid and 0.52 g of (2*R*,3*R*)-**2** (ee>99%, yield 47%) as a
61 white solid (m. p. = 77–78°C). Absolute configurations
62 were determined by comparison of the optical rotations

found in the literature for (2*R*,3*R*)-**2** ($[\alpha]_D^{22} = -41.3$ (c
0.48, CHCl₃)^[23] and (2*S*,3*R*)-**1** ($[\alpha]_D^{22} = +171$ (c 1.0,
CHCl₃)^[9] with the ones experimentally determined in this
work ($[\alpha]_D^{22} = -44$ (c 0.5, CHCl₃) and $[\alpha]_D^{15} = +171.9$ (c
1.0, CHCl₃), respectively). (2*R*,3*R*)-**2**: ¹³C NMR (CDCl₃, 75
MHz): δ (ppm) = 172.4, 138.5, 128.3, 128.2, 126.4, 75.0,
74.8, 52.4. ¹H NMR (CDCl₃, 300 MHz): δ (ppm) = 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 (H₂) was set at 2.67
mL/min. The column temperature was set at 150°C and
kept at this temperature for 20 minutes 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/isopropanol (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 di-isopropyl 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 MgSO₄, filtered and then
evaporated under reduced pressure. The products were
purified by flash chromatography (pentane/diethyl ether,
7/3), affording 0.47 g of (2*S*,3*R*)-**3** as a colourless liquid
(ee>99%, yield 47%) and 0.49 g of (2*R*,3*R*)-**4** as a
colourless liquid (ee 94%, yield 44%), $[\alpha]_D^{25} = -37.5$ (c
1.0, CHCl₃). 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, CHCl₃)^[24] with the
one experimentally determined in this work ($[\alpha]_D^{25} = +$
154.7 (c 1.0, CHCl₃)). 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 (CDCl₃, 300 MHz):
δ (ppm) = 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). ¹³C NMR (CDCl₃, 75 MHz): δ (ppm) = 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.*

1 *Analytical scale.* A specific volume of a stock solution of 63
2 100 g/L of *rac-5* (final concentration 1-50 g/L) in MTBE 64
3 (final concentration of MTBE 40%) and lyophilized 65
4 biomass with an EH activity of 1667 U/g (final 66
5 concentration 2-100 g/L) were added to 0.3 mL of buffer 67
6 in a 10 mL round bottom flask (final reaction volume 0.68
7 mL). The enzymatic reactions were incubated at 17°C 69
8 under magnetic stirring (800 rpm). Regularly withdrawn 70
9 aliquots were saturated with NaCl and extracted with ethyl 71
10 acetate (200 µL). Then 100 µL of the organic phase were 72
11 evaporated under reduced pressure, and the residues were 73
12 dissolved in 100 µL of isopropyl alcohol. Then 20 µL of 74
13 this solution were analyzed by HPLC to determine the ee 75
14 of both remaining epoxide and formed diol. A Lu 76
15 Cellulose-4 chiral column (250×4.60 mm, Phenomenex 77
16 was used in the isocratic mode with an 8/2 mixture of 78
17 hexane/isopropanol at a flow rate of 1.2 mL/min. UV 79
18 detection was set at 230 nm. The retention times were as 80
19 follows: (2*S*,3*R*)-**5**: 8.4 min, (2*R*,3*S*)-**5**: 8.8 min, (2*S*,3*S*)- 81
20 17.7 min, (2*R*,3*R*)-**6**: 19.8 min, and for the 2 *syn*-diols- 82
21 21.7 and 27.4 min. 83

22 *Preparative scale.* In a 100 mL round bottomed flask was 84
23 added 1.0 g of *rac-5* dissolved in 10 mL of MTBE and 1 85
24 mL of buffer B. The biocatalytic reaction was initiated by 86
25 addition of 1.75 g of lyophilized biomass with an EH 87
26 activity of 1667 U/g, and the reaction mixture was kept at 88
27 17°C under magnetic stirring (800 rpm). After 60 min, the 89
28 reaction mixture was extracted 3 times with 40 mL ethyl 90
29 acetate. The combined organic phases were dried over 91
30 MgSO₄, filtered and then evaporated under reduced 92
31 pressure. The products were purified by flash 93
32 chromatography (pentane/diethyl ether, 7/3), affording 94
33 0.40 g of (2*S*,3*R*)-**5** as a colourless liquid (ee >99%, 40% 95
34 yield) and 0.51 g of (2*R*,3*R*)-**6** as a white solid (ee 88% 96
35 46 % yield), m. p. = 105-106 °C. The absolute 97
36 configurations were determined by comparison of the 98
37 optical rotation values found in the literature for (2*R*,3*S*)- 99
38 ([α]_D²⁴ = -205 (c 1.0, MeOH))^[25] and (2*R*,3*R*)-**6** ([α]_D¹⁸ = 100
39 - 43.9 (c 1.0, CHCl₃))^[23] with the ones experimentally 101
40 determined in this work ([α]_D²⁴ = +190.7 (c 1.0, MeOH) 102
41 and ([α]_D¹⁸ = -39.2 (c 1.0, CHCl₃), respectively). (2*R*,3*R*)- 103
42 **6**: ¹H NMR (CDCl₃, 300 MHz): δ (ppm) = 7.2-7.3 (brm, 2H) 104
43 6.8-6.9 (brm, 2H), 5.0 (d, *J* = 4.3 Hz, 1H), 4.5 (d, *J* = 4.3 105
44 Hz, 1H), 3.8 (s, 3H), 3.7 (s, 3H), 3.0 (brs, 2H). ¹³C NMR 106
45 (CDCl₃, 75 MHz): δ (ppm) = 172.5, 159.5, 130.1, 127.1, 107
46 113.7, 74.8, 74.6, 55.2, 52.5. 108

47 109 48 **Bioconversion of trans-3-phenyloxirane-2-carbonitrile** 110 49 *rac-7*. 111

50 112
51 *Analytical scale.* A specific volume of a stock solution of 113
52 200 g/L of *rac-7* (final concentration 1-100 g/L) in MTBE 114
53 (final concentration of MTBE 20%) and lyophilized 115
54 biomass with an EH activity of 2757 U/g (final 116
55 concentration 0.75-75 g/L) were added to 0.8 mL of buffer 117
56 A in a 10 mL round bottom flask (final volume of reaction 118
57 1 mL). The enzymatic reactions were incubated at 27°C 119
58 under magnetic stirring (800 rpm). Regularly withdrawn 120
59 aliquots were saturated with NaCl and extracted with ethyl 121
60 acetate (200 µL). Then 100 µL of the organic phase were 122
61 evaporated under reduced pressure, and the residues were 123
62 dissolved in 100 µL of isopropyl alcohol. Then 20 µL of 124

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/isopropanol at a flow
rate of 1.2 mL/min. 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 MgSO₄, filtered and then evaporated under
reduced pressure. The products were purified by flash
chromatography (pentane/diethyl ether, 7/3), affording
0.45 g of (2*R*,3*R*)-**7** as a slightly yellow liquid (ee 99%,
45 % yield) and 0.52 g of (2*S*,3*R*)-**8** as a white solid (ee
99%, 46% yield). [α]_D²² = -44.3 (c 1.0, EtOH), m. p. =
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, [α]_D²⁵ = +
24.3 (c 0.86, EtOH))^[15] with the one experimentally
determined in this work ([α]_D²⁰ = +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 (¹H NMR, see
Supplementary material), 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** ([α]_D²⁰ = -12.7 (c 0.71,
CH₂Cl₂)) was compared with the one found in the literature
for (2*S*,3*R*)-*syn*-methyl-ester-diol-**2** ([α]_D²⁰ = -16.2 (c 0.69,
CH₂Cl₂))^[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**: ¹H NMR (Acetone-d₆, 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).
¹³C NMR (Acetone-d₆, 75 MHz): δ = 139.9, 128.1, 127.9,
126.7, 118.8, 74.4, 67.1.

51 112 52 **Bioconversion of cis-3-phenyloxirane-2-carbonitrile** 113 53 *rac-9*. 114

54 115
55 *Analytical scale.* A specific volume of a stock solution of 116
56 100 g/L of *rac-9* (final concentration 1-50 g/L) in MTBE 117
57 (final concentration of MTBE 20%) and lyophilized 118
58 biomass with an EH activity of 2757 U/g (final 119
59 concentration 1-50 g/L) were added to 0.8 mL of buffer A 120
60 in a 10 mL round bottom flask (final volume of reaction: 1 121
61 mL). Enzymatic reactions were incubated at 27°C under 122
62 magnetic stirring (800 rpm). Regularly withdrawn aliquots 123
were saturated with NaCl and extracted with ethyl acetate
(200 µL). Then 100 µL of the organic phase were

1 evaporated under reduced pressure, and the residues were
2 dissolved in 100 μ L of isopropyl alcohol. Then 20 μ L of
3 this solution were analyzed by HPLC to determine the ee
4 of both remaining epoxide and formed diol. A Chiralcel
5 OD-H column (250 \times 4.60 mm) was used in isocratic mode
6 with a 92.5/7.5 mixture of hexane/isopropanol at a flow
7 rate of 1.2 mL/min. UV detection was set at 215 nm. The
8 retention times were as follows: (2*S*,3*R*)-**9**: 11.2 min
9 (2*R*,3*S*)-**9**: 12.0 min, (2*R*,3*R*)-**10**: 20.6 min, (2*S*,3*S*)-**10**:
10 23.9 min.

11 **Preparative scale.** In a 100 mL round bottomed flask was
12 added 1.0 g of *rac*-**9** dissolved in 8 mL of MTBE and 3.74
13 mL of buffer A. The biocatalytic reaction was initiated by
14 addition of 1 g of lyophilized biomass with an EH activity
15 of 2757 U/g. The reaction was performed at 27 $^{\circ}$ C under
16 magnetic stirring (800 rpm). After 60 min, the reaction
17 mixture was extracted 3 times with 40 mL ethyl acetate
18 The combined organic phases were dried over MgSO₄,
19 filtered and then evaporated under reduced pressure. The
20 products were purified by flash chromatography
21 (pentane/diethyl ether, 7/3), affording 0.44 g of (2*R*,3*S*)-**9a**
22 a white solid (ee > 99%, 44% yield), $[\alpha]_D^{25} = +109.8$ (c
23 0.86, EtOH), m. p. = 54–55 $^{\circ}$ C and 0.51 g of (2*R*,3*R*)-**10a**
24 slightly yellow liquid (ee > 99%, 45.2% yield), $[\alpha]_D^{25} =$
25 32.1 (c 1.0, EtOH). The absolute configuration of residual
26 epoxide **9** was determined after transformation of **9** to its
27 *cis*-methyl-ester counterpart (2*S*,3*S*)-**20** (Scheme 3).^[27] The
28 absolute configuration of (2*S*,3*S*)-**20** was determined by
29 comparison of the optical rotation value found in the
30 literature for (2*R*,3*R*)-**20** ($[\alpha]_D^{25} = +10.8$ (c 1.0392
31 CH₂Cl₂)^[22] with the one determined experimentally in this
32 work ($[\alpha]_D^{20} = -9.1$ (c 0.98, CH₂Cl₂)). In order to
33 determine the absolute configuration of the formed diol-**10**
34 the same protocol was used as for diol-**8** (Scheme 3).^[26]
35 The optical rotation of the formed diol-**2** ($[\alpha]_D^{20} = -14.7$ (c
36 0.73, CH₂Cl₂)) was compared to the one found in the
37 literature for (2*S*,3*R*)-*syn*-methyl-ester-diols ($[\alpha]_D^{20} =$
38 16.2 (c 0.69, CH₂Cl₂)^[26], establishing the same absolute
39 configuration for the obtained methyl-ester-diols-**10**
40 the (2*R*,3*R*) configuration for *trans*-acetone-**10a** and the
41 the same (2*R*,3*R*) configuration for the biocatalytically
42 formed cyano-diols-**10** (Scheme 3). (2*R*,3*R*)-**10**: ¹H NMR
43 (Acetone-*d*₆, 300 MHz): $\delta = 7.5$ – 7.6 (brm, 2H), 7.4 – 7.0
44 (brm, 3H), 5.7 (brs, 1H), 5.2 (brs, 1H), 4.9 (d, $J = 5.8$ Hz,
45 1H), 4.7 (d, $J = 5.8$ Hz, 1H). ¹³C NMR (Acetone-*d*₆,
46 MHz): $\delta = 139.5$, 128.2, 128.0, 127.3, 118.8, 74.4, 66.4.

48 **Bioconversion of 2-(bromomethyl)-3-phenyloxirane** 49 *rac*-**11**

50
51 **Analytical scale.** A specific volume of a stock solution of
52 100 g/L of *rac*-**11** (final concentration 1–100 g/L) in MTBE
53 (final concentration of MTBE 20%) and lyophilized
54 biomass with an EH activity of 2757 U/g (varying
55 concentrations: see text) were added to 0.8 mL of buffer
56 in a 10 mL round bottom flask (final volume of reaction
57 mL). Enzymatic reactions were incubated at 27 $^{\circ}$ C under
58 magnetic stirring (800 rpm). Regularly withdrawn aliquots
59 were saturated with NaCl and extracted with ethyl acetate
60 (200 μ L). Then 100 μ L of the organic phase were
61 evaporated under reduced pressure and the residues were
62 dissolved in 100 μ L of isopropyl alcohol. Then 20 μ L 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 \times 4.60
mm, Phenomenex) was used in isocratic mode with a
92.5/7.5-mixture of hexane/isopropanol 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 $^{\circ}$ 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 MgSO₄, filtered and then evaporated under reduced
pressure. The products were purified by flash
chromatography (pentane/diethyl ether, 7/3), affording
0.44 g of (2*S*,3*R*)-**11a** as a colourless liquid (ee 99%, 44%
yield), $[\alpha]_D^{24} = +11.3$ (c 1, CHCl₃) and 0.53 g of (2*R*,3*R*)-
12a as a colourless liquid (ee 99%, 48% yield), $[\alpha]_D^{24} =$
8.5 (c 1, CHCl₃). Diol **12** 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,
CHCl₃)^[24] with the one experimentally obtained in this
work for epoxy alcohol-**15** arising from bromo-diols ($[\alpha]_D^{25}$
= -104 (c 2.74, CHCl₃)). (2*R*,3*R*)-**12**: ¹H NMR (Acetone-*d*₆,
300 MHz): $\delta = 7.3$ – 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). ¹³C NMR (CDCl₃, 75 MHz): δ
(ppm) = 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 $^{\circ}$ 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 MgSO₄,
filtered and then evaporated under reduced pressure. The
products were purified by flash chromatography
(pentane/diethyl ether, 7/3), affording 0.43 g of (2*S*,3*R*)-
13a as a colourless liquid (ee > 99%, 43% yield) and 0.49 g
of (2*R*,3*R*)-**14a** as a slightly yellow liquid (ee > 99%, 44.5%
yield), $[\alpha]_D^{24} = -4.6$ (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, CHCl₃)^[29] with the one
experimentally determined in this work ($[\alpha]_D^{20} = +20$ (c
0.6, CHCl₃)). 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*)-

1 **15** ($[\alpha]_{\text{D}}^{25} = -100.2$ (c 2.31, CHCl_3)^[30] with the one
2 experimentally obtained in this work for epoxy alcohol-**15**
3 arising from chloro-diol ($[\alpha]_{\text{D}}^{25} = -100$ (c 2.74, CHCl_3)
4 (*2R,3R*)-**14**. ¹H NMR (CDCl_3 , 300 MHz): δ (ppm) = 7.3
5 7.4 (brn, 5H), 4.8 (d, $J = 5.0$ Hz, 1H), 3.9 (m, 1H), 3.5 (m
6 2H), 2.9-3.2 (brs, 2H). ¹³C NMR (CDCl_3 , 75 MHz):
7 (ppm) = 139.6, 128.6, 128.3, 126.6, 74.9, 74.6, 46.1.

8 **Bioconversion of trans-stilbene oxide rac-16.**

10 **Analytical scale.** In all experiments 100 mg of *rac*-**16** were
11 dissolved in MTBE (from 0.4 to 20 mL) and buffer A was
12 added to make up a final MTBE concentration of 20% and
13 a final total concentration of **16** in the range of 1–50 g·L⁻¹.
14 The reaction was initiated by the addition of 200 mg of
15 lyophilized biomass with an EH activity of 2757 U/g. The
16 biotransformations were performed at 27°C under stirring
17 (1200 rpm). Regularly withdrawn aliquots were saturated
18 with NaCl and extracted with ethyl acetate (200 μL). Then
19 100 μL of the organic phase were evaporated under
20 reduced pressure, and the residues were dissolved in 100
21 μL of isopropyl alcohol. Then 20 μL of this solution were
22 analyzed by HPLC to determine the ees of both remaining
23 epoxide and formed diol. A Lux Cellulose-4 chiral column
24 (250×4.60 mm, Phenomenex) was used in isocratic mode
25 with a 9/1-mixture of hexane/isopropanol at a flow rate of
26 1 mL/min. UV detection was set at 215 nm. The retention
27 times were as follows: (*1R,2R*)-**16**: 4.9 min, (*1S,2S*)-**16**: 7.8
28 min, *meso*-diol-**17**: 17.3 min.

30 **Preparative scale.** In a 100 mL round bottomed flask was
31 added 1.0 g of *rac*-**16** dissolved in 4 mL of MTBE and 16
32 mL of buffer A. The biocatalytic reaction was initiated by
33 addition of 2 g of lyophilized biomass with an EH activity
34 of 2757 U/g. The reaction was performed at 27°C under
35 magnetic stirring (800 rpm). After 5 h, the reaction mixture
36 was extracted 3 times with 40 mL ethyl acetate. The
37 combined organic phases were dried over MgSO_4 , filtered
38 and then evaporated under reduced pressure. The products
39 were purified by flash chromatography (pentane/diethyl
40 ether, 1/1), affording 0.45 g of a slightly yellow solid
41 (*1R,2R*)-**16** (ee% 99%, 45% yield), m. p. = 65–66°C and
42 0.51 g of *meso*-**17** as a white solid (46 % yield), m. p. =
43 122–124°C. The absolute configuration of (*1R,2R*)-**16** was
44 determined by comparison of the optical rotation value
45 found in the literature for (*1R,2R*)-**16** ($[\alpha]_{\text{D}}^{20} = +357$ (c
46 0.59, benzene)^[31] and the one experimentally determined
47 in this work ($[\alpha]_{\text{D}}^{20} = +348$ (c 0.59, benzene)). *meso*-**17**
48 ¹H NMR (Acetone- d_6 , 300 MHz): δ (ppm) = 7.2-7.3 (brn,
49 10H), 4.9 (brs, 2H), 4.3 (s, 2H). ¹³C NMR (Acetone- d_6 ,
50 MHz) δ (ppm) = 142.0, 127.4, 127.3, 126.9, 77.7.

52 **Bioconversion of meso-cis-stilbene oxide-18.**

54 **Analytical scale.** A specific volume of a stock solution of
55 100 g/L of *meso*-**18** (final concentration 1–50 g/L) in DMF
56 (final concentration of DMF 5%) and lyophilized biomass
57 with an EH activity of 2757 U/g (final concentration 2–100
58 g/L) were added to 1.9 mL of buffer A in a 25 mL round
59 bottom flask (final reaction volume: 2 mL). The enzymatic
60 reactions were performed at 27°C under magnetic stirring
61 (1200 rpm). The next preparatory steps were performed as
62 described for the analytical-scale bioconversion of

compound **16**. The retention times were as follows: *meso*-
18: 5.6 min, (*1S,2S*)-**19**: 23.9 min, and (*1R,2R*)-**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 0.92 g of (*1R,2R*)-
19 as a slightly yellow solid (ee% 99%, 84.5% yield), m. p.
= 136–138°C. The absolute configuration was determined
by comparison of the optical rotation value found in the
literature for (*1R,2R*)-**19**, ($[\alpha]_{\text{D}}^{28} = +92.7$ (c 1, EtOH))^[32]
with the one experimentally determined in this work
($[\alpha]_{\text{D}}^{28} = +93.8$ (c 1, EtOH)). (*1R,2R*)-**19**: ¹H NMR
(Acetone- d_6 , 300 MHz): δ (ppm) = 6.9-7.1 (brn, 10H), 4.52
(brs, 2H), 4.47 (brs, 2H). ¹³C NMR (Acetone- d_6 , 75 MHz):
 δ (ppm) = 141.7, 127.5, 127.2, 127.1, 78.9.

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References

- [1] P. Kumar, V. Naidu, P. Gupta, *Tetrahedron* **2007**, *63*, 2745–2785.
- [2] Y. Chen, C. Chen, X. Wu, *Chem. Soc. Rev.* **2012**, *41*, 1742–1753.
- [3] a) A. Archelas, R. Furstoss, *Curr. Opin. Chem. Biol.* **2001**, *5*, 112–119; b) A. Steinreiber, K. Faber, *Curr. Opin. Biotechnol.* **2001**, *12*, 552–558; c) E.J. de Vries, D.B. Janssen, *Curr. Opin. Biotechnol.* **2003**, *14*, 414–420; d) E.Y. Lee, *Biotechnol. Lett.* **2008**, *30*, 1509–1514; e) W.J. Choi, *Appl. Microbiol. Biotechnol.* **2009**, *84*, 239–247; f) M. Kotik, A. Archelas, R. Wohlgenuth, *Curr. Org. Chem.* **2012**, *16*, 451–482.
- [4] B. van Loo, J. Kingma, M. Arand, M.G. Wubbolts, D. B. Janssen, *Appl. Env. Microbiol.* **2006**, *72*, 2905–2917.
- [5] a) M. Arand, F. Müller, A. Mecky, W. Hinz, P. Urban, D. Pompon, R. Kellner, F. Oesch, *Biochem. J.* **1999**, *337*, 37–43; b) R. Rink, J.H. Lutje Spelberg, R.J. Pieters, J. Kingma, M. Nardini, R.M. Kellogg, B.W. Dijkstra, D.B. Janssen, *J. Am. Chem. Soc.* **1999**, *121*, 7417–7418; c) B. van Loo, J. Kingma, G. Heyman, A. Wittenaar, J.H. Lutje Spelberg, T. Sonke, D.B. Janssen, *Enzyme Microb. Technol.* **2009**, *44*, 145–153; d) M.T. Reetz, L.-W. Wang, M. Bocola, *Angew. Chem. Int. Ed.* **2006**, *45*, 1236–1241; e) M.T. Reetz, D. Kahakeaw, R. Lohmer, *ChemBioChem* **2008**, *9*, 1797–1804; f) M.T. Reetz, H.

- 1 Zheng, *ChemBioChem***2011**, 12, 1529–1535; g) B. van Loon, *J. Am. Chem. Soc.* **2004**, 126, 11156–11157.
- 2 J.H. L. Spelberg, J. Kingma, T. Sonke, M.G. Wubbolts, *J. Am. Chem. Soc.* **2004**, 126, 11156–11157.
- 3 D.B. Janssen, *Chem. Biol.* **2004**, 11, 981-990; h) M. Kotik, *J. Mol. Catal. B: Enzym.* **2010**, 65, 41–48.
- 4 A. Archelas, V. Faměrova, P. Oubrechtova, V. Křen, *Biotechnol.* **2011**, 156, 1–10; i) H. Zheng, M.T. Reetz, *Am. Chem. Soc.* **2010**, 132, 15744–15751; j) M. Kotik, Zhao, G. Iacazio, A. Archelas, *J. Mol. Catal. B: Enzym.* **2013**, 91, 44–51.
- 5 *Biotechnol.* **2011**, 156, 1–10; i) H. Zheng, M.T. Reetz, *Am. Chem. Soc.* **2010**, 132, 15744–15751; j) M. Kotik, Zhao, G. Iacazio, A. Archelas, *J. Mol. Catal. B: Enzym.* **2013**, 91, 44–51.
- 6 *Am. Chem. Soc.* **2010**, 132, 15744–15751; j) M. Kotik, Zhao, G. Iacazio, A. Archelas, *J. Mol. Catal. B: Enzym.* **2013**, 91, 44–51.
- 7 Zhao, G. Iacazio, A. Archelas, *J. Mol. Catal. B: Enzym.* **2013**, 91, 44–51.
- 8 *Enzym.* **2013**, 91, 44–51.
- 9 [6] M. Kotik, V. Štěpánek, M. Grulich, P. Kyslík, Archelas, *J. Mol. Catal. B: Enzym.* **2010**, 65, 41–48.
- 10 Archelas, *J. Mol. Catal. B: Enzym.* **2010**, 65, 41–48.
- 11 [7] a) J.S. Yadav, K. Premalatha, S.J. Harshavardhan, B. Subba Reddy, *Tetrahedron Lett.* **2008**, 49, 6765–6767; b) Palin, D.R. Barn, J.K. Clark, J.E. Cottney, P.M. Cowley, M. Crockatt, L. Evans, H. Feilden, R.R. Goodwin, Griekspoor, S.J.A. Grove, A.K. Houghton, P.S. Jones, R. Morphy, A.R.C. Smith, H. Sundaram, D. Vrolijk, M. Weston, G. Wishart, P. Wren, *Bioorg. Med. Chem. Lett.* **2005**, 15, 589-593; c) P. Kumar, A. Dubey, Harbindu, *Org. Biomol. Chem.* **2012**, 10, 6987-6994.
- 12 Subba Reddy, *Tetrahedron Lett.* **2008**, 49, 6765–6767; b) Palin, D.R. Barn, J.K. Clark, J.E. Cottney, P.M. Cowley, M. Crockatt, L. Evans, H. Feilden, R.R. Goodwin, Griekspoor, S.J.A. Grove, A.K. Houghton, P.S. Jones, R. Morphy, A.R.C. Smith, H. Sundaram, D. Vrolijk, M. Weston, G. Wishart, P. Wren, *Bioorg. Med. Chem. Lett.* **2005**, 15, 589-593; c) P. Kumar, A. Dubey, Harbindu, *Org. Biomol. Chem.* **2012**, 10, 6987-6994.
- 13 Palin, D.R. Barn, J.K. Clark, J.E. Cottney, P.M. Cowley, M. Crockatt, L. Evans, H. Feilden, R.R. Goodwin, Griekspoor, S.J.A. Grove, A.K. Houghton, P.S. Jones, R. Morphy, A.R.C. Smith, H. Sundaram, D. Vrolijk, M. Weston, G. Wishart, P. Wren, *Bioorg. Med. Chem. Lett.* **2005**, 15, 589-593; c) P. Kumar, A. Dubey, Harbindu, *Org. Biomol. Chem.* **2012**, 10, 6987-6994.
- 14 M. Crockatt, L. Evans, H. Feilden, R.R. Goodwin, Griekspoor, S.J.A. Grove, A.K. Houghton, P.S. Jones, R. Morphy, A.R.C. Smith, H. Sundaram, D. Vrolijk, M. Weston, G. Wishart, P. Wren, *Bioorg. Med. Chem. Lett.* **2005**, 15, 589-593; c) P. Kumar, A. Dubey, Harbindu, *Org. Biomol. Chem.* **2012**, 10, 6987-6994.
- 15 Griekspoor, S.J.A. Grove, A.K. Houghton, P.S. Jones, R. Morphy, A.R.C. Smith, H. Sundaram, D. Vrolijk, M. Weston, G. Wishart, P. Wren, *Bioorg. Med. Chem. Lett.* **2005**, 15, 589-593; c) P. Kumar, A. Dubey, Harbindu, *Org. Biomol. Chem.* **2012**, 10, 6987-6994.
- 16 Morphy, A.R.C. Smith, H. Sundaram, D. Vrolijk, M. Weston, G. Wishart, P. Wren, *Bioorg. Med. Chem. Lett.* **2005**, 15, 589-593; c) P. Kumar, A. Dubey, Harbindu, *Org. Biomol. Chem.* **2012**, 10, 6987-6994.
- 17 Weston, G. Wishart, P. Wren, *Bioorg. Med. Chem. Lett.* **2005**, 15, 589-593; c) P. Kumar, A. Dubey, Harbindu, *Org. Biomol. Chem.* **2012**, 10, 6987-6994.
- 18 *Lett.* **2005**, 15, 589-593; c) P. Kumar, A. Dubey, Harbindu, *Org. Biomol. Chem.* **2012**, 10, 6987-6994.
- 19 Harbindu, *Org. Biomol. Chem.* **2012**, 10, 6987-6994.
- 20 [8] H. Hamamoto, V.A. Mamedov, M. Kitamoto, Hayashib, S. Tsuboi, *Tetrahedron: Asymmetry* **2000**, 11, 4485–4497.
- 21 Hayashib, S. Tsuboi, *Tetrahedron: Asymmetry* **2000**, 11, 4485–4497.
- 22 4485–4497.
- 23 [9] G.J. Zheng, Q. Yuan, L. Yang, X. Zhang, J.J. Wang, W.R. Sun, *J. Mol. Catal. B: Enzym.* **2006**, 43, 133-136.
- 24 W.R. Sun, *J. Mol. Catal. B: Enzym.* **2006**, 43, 133-136.
- 25 [10] A. Gentile, C. Giordano, C. Fuganti, L. Ghirotto, Servi, *J. Org. Chem.* **1992**, 57, 6635-6637.
- 26 A. Gentile, C. Giordano, C. Fuganti, L. Ghirotto, Servi, *J. Org. Chem.* **1992**, 57, 6635-6637.
- 27 [11] Z. Hameršák, D. Šepac, D. Žiher, V. Šunjić, *Synthesis* **2003**, 3, 375-382.
- 28 Z. Hameršák, D. Šepac, D. Žiher, V. Šunjić, *Synthesis* **2003**, 3, 375-382.
- 29 [12] C. Wei, J. Ling, H. Shen, Q. Zhu, *Molecules* **2014**, 19, 8067-8079.
- 30 C. Wei, J. Ling, H. Shen, Q. Zhu, *Molecules* **2014**, 19, 8067-8079.
- 31 [13] A. V. Devi, C. Lahari, L. Swarnalatha, N. Fadnavis, *Tetrahedron: Asymmetry* **2008**, 19, 1139–1144.
- 32 A. V. Devi, C. Lahari, L. Swarnalatha, N. Fadnavis, *Tetrahedron: Asymmetry* **2008**, 19, 1139–1144.
- 33 [14] C. Li, Q. Liu, X. Song, D. Di, A. Ji, Y. Qu, *Biotechnol. Lett.* **2003**, 25, 2113-2116.
- 34 C. Li, Q. Liu, X. Song, D. Di, A. Ji, Y. Qu, *Biotechnol. Lett.* **2003**, 25, 2113-2116.
- 35 [15] M. Zagozda, J. Plenkiewicz, *Tetrahedron: Asymmetry* **2008**, 19, 1455-1460.
- 36 M. Zagozda, J. Plenkiewicz, *Tetrahedron: Asymmetry* **2008**, 19, 1455-1460.
- 37 [16] L. Zhao, B. Han, Z. Huang, M. Miller, H. Huang, D. Malashock, Z. Zhu, A. Milan, D. E. Robertson, D. Weiner, M. J. Burk, *J. Am. Chem. Soc.* **2004**, 126, 13198-13199.
- 38 L. Zhao, B. Han, Z. Huang, M. Miller, H. Huang, D. Malashock, Z. Zhu, A. Milan, D. E. Robertson, D. Weiner, M. J. Burk, *J. Am. Chem. Soc.* **2004**, 126, 13198-13199.
- Weiner, M. J. Burk, *J. Am. Chem. Soc.* **2004**, 126, 11156–11157.
- [17] a) D.-M. Gou, Y.-C. Liu, C.-S. Chen, *J. Org. Chem.* **1993**, 58, 1287-1289; b) A. A. Afon'kin, L. M. Kostrikin, A. E. Shumeiko, A. F. Popov, A. A. Matveev, V. N. Matvienko, A. F. Zadbudkin, *Russian. Chem. Bull.* **2012**, 61, 2149-2162.
- [18] M. Grulich, J. Marsalek, P. Kyslík, V. Štěpánek, M. Kotik, *Process Biochem.* **2011**, 46, 526-532.
- [19] I. Saikia, B. Kashyap, P. Phukan, *Synth. Comm.* **2010**, 40, 2647-2652.
- [20] C. Nair, P. Indrasenan, *Talanta* **1976**, 23, 239-241.
- [21] L. Mhamdi, H. Bohli, Y. Moussaoui, R. ben Salem, *Intern. J. Org. Chem.* **2011**, 1, 119-124.
- [22] J. Wang, F. D. Rochon, Y. Yang, L. Hua, M. M. Kayser, *Tetrahedron: Asymmetry* **2007**, 18, 1115-1123.
- [23] B.R. Matthews, W.R. Jackson, H.A. Jacobs, K.G. Watson, *Aust. J. Chem.* **1990**, 43, 1195-1214.
- [24] O. Cabon, D. Buisson, M. Larcheveque, R. Azerad, *Tetrahedron: Asymmetry* **1995**, 6, 2211-2218.
- [25] R. Imashiro, M. Seki, *J. Org. Chem.* **2004**, 69, 4216-4226.
- [26] F. Effenberger, M. Hopf, T. Ziegler, J. Hudelmayer, *Chem. Ber.* **1991**, 124, 1651-1659.
- [27] J. Svoboda, Z. Kocfeldová, J. Paleček, *Collect. Czech. Chem. Commun.* **1998**, 53, 822-832.
- [28] N.K. Jobson, A.R. Crawford, D. Dewar, S.L. Pimlott, A. Sutherland, *Bioorg. Med. Chem. Lett.* **2009**, 19, 4996-4998.
- [29] Z.-X. Wang, Y. Shi, *J. Org. Chem.* **1997**, 62, 8622-8623.
- [30] J.M. Palazon, B. Anorbe, V.S. Martin, *Tetrahedron Lett.* **1986**, 27, 4987-4990.
- [31] G. Berti, F. Bottari, P.L. Ferrarini, B. Macchia, *J. Org. Chem.* **1965**, 30, 4091-4096.
- [32] N. Takenaka, G. Xia, H. Yamamoto, *J. Am. Chem. Soc.* **2004**, 126, 13198-13199.

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

Adv. Synth. Catal. **Year**, *Volume*, Page – Page

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