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Microbiological Transformations 40. Use of Fungal Epoxide Hydrolases for the Synthesis of Enantiopure Alkyl Epoxides

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Abstract: The enantioselective biohydrolyses of various substituted alkyl-epoxides using seven different fungi are described. These strains were used to achieve the preparation of these alkyl epoxides in high enantiomeric purity. A combined chemoenzymatic process is also described, allowing to enhance the overall yield of such an approach.

Synthesis of enantiopure compounds is nowadays an important topic because of the increasing demand for such molecules in the pharmaceutical, agrochemical or food flavour industry. In this context, intensive work has been devoted in recent years for the production of enantiopure epoxides and vicinal diols.[1-3] This can be achieved using for example the well known Sharpless methods for asymmetric epoxidation of allylic alcohols[4] or asymmetric dihydroxylation of alkenes,[5] or the Jacobsen-Katsuki method for the epoxidation of some particular (conjugated) olefins.[6] More recently, some salen cobalt or chromium catalysts have also been described by Jacobsen *et al.* to allow the opening of epoxides into enantiomerically enriched protected diols[7] or amino-alcohol precursors.[8] Besides these chemo-catalytic methods, various biocatalytic approaches -as for example alkene epoxidation by monooxygenases or bioreduction of α-halo-ketones into optically pure halohydrines[9]- have been shown to provide useful alternatives. In this context, one of the most promising way for the synthesis of such chirons is the use of cofactor-independent Epoxide Hydrolases (EHs). Indeed, these enzymes have been proven to catalyse the enantioselective hydrolysis of various racemic epoxides, thus allowing to recover, on one hand, the unreacted substrate in an optically pure form and, on the other hand, the corresponding diol which very often also shows good to excellent enantiomeric purity. However most of the enzymes studied were from mammalian origin and their use for preparative scale application was therefore severely hampered due to their low availability. Only recently, EH from microbial origin -*i.e.* from bacterial,[10] yeast[11] or fungal[12] sources- have been identified. These appear to be potentially much more interesting tools for asymmetric synthesis since these

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organisms can be easily cultured in large-scale. For example, we have shown that the fungi *Aspergillus niger* LCP 521 and *Beauveria bassiana*[13] ATCC 7159 were able to achieve -at a several gram-scale- the enantioselective hydrolysis of various types of epoxides derived from geraniol, limonene[12] or substituted styrene derivatives.[14,15]

In this context, and in order to broaden the applicability of fungal EHs, we explored the biohydrolysis of other diversely substituted epoxides -*i.e.* of the mono-, *gem*-, *trans*- and *cis*- disubstituted alkyl-substrates **1-4** (Scheme 1). This study was carried out with 7 different fungi having an EH activity: *A. niger*, *B. bassiana* and five new fungi selected from a screening[16] using 1,2-epoxyhexane and *trans*-2,3-epoxyhexane as substrates: *Aspergillus terreus* CBS 116-46, *Chaetomium globosum* LCP 679, *Cunninghamella elegans* LCP 1543, *Mortierella isabellina* ATCC 42613, and *Syncephalastrum racemosum* MUCL 28766. We described here the results we have obtained on an analytical scale, starting from the racemic alkyl-epoxides **1** to **4**, and the illustration of the possible use of these fungi for their preparation on an optically pure form.

RESULTS AND DISCUSSION

Biohydrolysis of the racemic epoxides 1 to 4:

The biohydrolyses were performed on an analytical scale by incubating the racemic substrates **1-4** (8mM) in 2 mL of buffered resting-cell suspension. The results obtained using the 7 studied strains are summarised in Table 1. For each of these reactions, we have determined -the initial rate, -the absolute configuration of the residual epoxide, -as well as the E value. This was aimed to allow the most accurate choice of the appropriate fungus for gram-scale preparation of these epoxides in enantiopure form.

Determination of the E value. This was achieved using equation 1 which involves the conversion rate c and the ee of the residual epoxide (ees).[17] At that stage, use of an equation involving the eep value is not valid, as stressed previously.[14] Therefore, for each reaction (performed with the seven fungi and the four substrates **1-4**), three to five sets of c and ees values were determined by periodical withdrawing of an aliquot and GC analysis. The c ratio was determined by quantification of the formed diol measured by direct injection of the aqueous phase in GC after addition of an internal standard. Also, in order to enhance accuracy, these E values were calculated by linear correlation between the numerator and the denominator of Equation 1, as described previously.[18] The values obtained are reported in Table 1.

Equation 1. $$E = \frac{\ln(1 - c)(1 - ees)}{\ln(1 - c)(1 + ees)}$$

Scheme 1. Epoxides 1-4. [19]

Table 1. Biohydrolysis of racemic epoxides 1-4.

Entry	Substrate	Fungus	Residual enantiomer[a]	E (Sd)[b]	Initial rate U/g[c]
1	(±)-**1**	*A. niger* LCP 521	(*S*)	3.7 (± 0.2)	4.2
2		*A. terreus* CBS 116-46	(*S*)	6.5 (± 0.2)	1.1
3		*B. bassiana* ATCC 7149	(*S*)	3.4 (± 0.1)	0.9
4		*C. globosum* LCP 679	(*S*)	1.7 (± 0.1)	2.1
5		*C. elegans* LCP 1543	(*S*)	2.4 (± 0.2)	1.0
6		*M. isabellina* ATCC 42613	(*S*)	31 (± 10)	3.5
7		*S. racemosum* MUCL 28766	(*S*)	3.5 (± 0.1)	0.5
8	(±)-**2**	*A. niger* LCP 521	(*S*)	9.0 (± 0.5)	6.3
9		*A. terreus* CBS 116-46	(*S*)	1.9 (± 0.1)	1.5
10		*B. bassiana* ATCC 7149	(*S*)	5.3 (± 0.7)	0.3
11		*C. globosum* LCP 679	(*R*)	8.4 (± 0.6)	0.3
12		*C. elegans* LCP 1543	(*R*)	1.3 (± 0.2)	0.7
13		*M. isabellina* ATCC 42613	ne[d]	ne[d]	3.5
14		*S. racemosum* MUCL 28766	(*R*)	2.7 (± 0.1)	2.8
15	(±)-**3**	*A. niger* LCP 521	(1*R*,2*R*)	5.9 (± 0.6)	2.7
16		*A. terreus* CBS 116-46	ne[d]	ne[d]	0.2
17		*B. bassiana* ATCC 7149	(1*S*,2*S*)	1.8 (± 0.1)	0.2
18		*C. globosum* LCP 679	(1*S*,2*S*)	6.5 (± 0.5)	1.2
19		*C. elegans* LCP 1543	(1*S*,2*S*)	3.2 (± 0.6)	0.3
20		*M. isabellina* ATCC 42613	(1*R*,2*R*)	18 (± 3)	0.7
21		*S. racemosum* MUCL 28766	(1*R*,2*R*)	4.7 (± 0.5)	0.3
22	(±)-**4**	*A. niger* LCP 521	(1*R*,2*S*)	6.3 (± 0.6)	1.5
23		*A. terreus* CBS 116-46	(1*R*,2*S*)	1.4 (± 0.1)	0.2
24		*B. bassiana* ATCC 7149	(1*R*,2*S*)	1.3 (± 0.1)	0.4
25		*C. globosum* LCP 679	(1*R*,2*S*)	6.9 (± 0.8)	1.1
26		*C. elegans* LCP 1543	(1*R*,2*S*)	2.5 (± 0.2)	0.8
27		*M. isabellina* ATCC 42613	(1*R*,2*S*)	1.3 (± 0.1)	1.3
28		*S. racemosum* MUCL 28766	(1*R*,2*S*)	3.7 (± 0.7)	0.8

(a) The absolute configuration was assigned *via* chiral GC analysis by comparison with authentic samples which absolute configuration have been determined on the basis of their optical rotation. *(b)* Standard deviation. *(c)* U/g = μmole of formed diol/min/gram of biomass (dry weight). *(d)* no enantioselectivity; E < 1.1.

It appears from the results obtained that:

(a) Depending on the strain used, either enantiomer of the *gem*-epoxide **2**, as well as of the *trans*-epoxide **3**, can be obtained. This is however not the case for the mono-substituted epoxide **1**, neither for the *cis*-substituted epoxide **4**.

(b) Although the E values obtained are only low (below 5) to moderate (between 6 to 31), an appropriate fungus (*i.e.* respectively *M. isabellina*, *A. niger*, *C. globosum*, *M. isabellina* and *C. globosum -entry 6, 8, 18, 20, 25*)[20] could be selected for achieving the preparation of optically pure (*S*)-**1**, (*S*)-**2**, (1*S*,2*S*) and (1*R*,2*R*)-**3** and (1*R*,2*S*)-**4**.

In this context, it is interesting to note that, in the course of studies conducted in parallel by the group of Faber *et al.*, it was observed that much better E values could be obtained for similar alkyl epoxides using EHs from bacterial origin.[10,21] However, on the other hand, more lipophylic epoxides (like styrene oxide derivatives for example) were poor substrates with these enzymes and, in fact, afford much higher E values with EHs from fungal origin.[14,15] Thus, a general trend seems to emerge that EHs from bacterial and fungal origin would be complementary tools depending on the type of substrate involved.

Preparative scale applications. These biohydrolyses were carried out on a preparative scale by incubating 2 g of racemic epoxide in 1 L of buffered resting-cell suspension of the appropriate strain, and the reaction was stopped when the ee of the residual epoxide was greater than 97%. The yields have been determined after extraction and purification of the products. From the results depicted in Table 2, it appears that this selection of strains indeed allowed the preparation of, at least, one enantiomer of each of the epoxides **1** to **4** in an optically pure form (ee > 97%), the corresponding (*R*) diols being formed with ees varying from 32 to 78%.

Table 2. Preparative-scale biohydrolysis of alkyl epoxides 1-4.

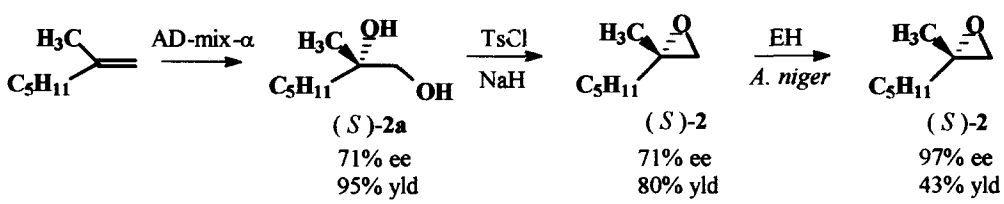
Substrate	Fungus	Cells[a] (*reaction time*)	epoxide Yield	epoxide ee (*Abs. Conf.*)	diol	diol Yield	diol ee (*Abs. Conf.*)
1	*M. isabellina*	35 (*6h*)	18%	97% (*S*)	**1a**	54%	35% (*R*)
2	*A. niger*	24 (*1h*)	22%	99% (*S*)	**2a**	62%	32% (*R*)
3	*C. globosum*	26 (*7.5h*)	12%	97% (1*S*,2*S*)	**3a**	60%	78% (1*R*,2*S*)
3	*M. isabellina*	49 (*4h*)	11%	98% (1*R*,2*R*)	**3a**	62%	59% (1*R*,2*S*)
4	*C. globosum*	30 (*12h*)	8%	97% (1*R*,2*S*)	**4a**	59%	58% (1*R*,2*R*)

(a) dry weight, expressed in grams.

Several reasons are to be taken into account in order to explain the moderate preparative yields obtained - i) because the reaction is a resolution, the maximal theoretical yield is 50% -ii) since the E value of these

reactions are only moderate, the maximal theoretical yield of enantiopure compound could not be higher than 21 to 41% depending on the specific case -iii) the recovery of the epoxide was difficult because of its adsorption on the cell walls of the fungi, a feature which was complicated by the fact that, because of the moderate specific activity of the biocatalysts, these reactions had to be carried out using high concentrations of cells (from 24 to 49 g dry weight per litre) for substrate concentrations of 2 g/L. It is however to be emphasised that this second step could be improved by using a crude enzymatic extract from *A. niger* which allows to lower the loss of product.[18] -iv) the volatility of these epoxides led to an important loss of product during the extraction/purification steps.

Further improvements. In spite of the low yields obtained, these results are quite interesting since they indicate that this approach allows the preparation of these alkyl substituted epoxides in enantiopure form, some of these being impossible to prepare in a simple way by other means. For instance, to the best of our knowledge, (*S*)-*gem*- **2** cannot be synthesised with a high ee using either the Sharpless or the Katsuki-Sharpless oxidation of the corresponding olefin. One of the major drawbacks of this biocatalytic approach is however the fact that such a resolution intrinsically involves a 50% yield limitation. In order to overcome this drawback, it was tempting to set up a combined chemo-enzymatic approach which could thus combine the advantages of both methods. The following example (scheme 2) illustrates how such a combination can be used to solve this problem. Thus, the Sharpless asymmetric dihydroxylation of 2-methyl-heptene using AD-mix-α,[22] followed by cyclisation of the diol thus formed, allowed to obtain the epoxide (*S*)-**2** with a 76% yield but an only moderate ee of 71%.[23] In order to prepare this product in enantiomerically pure form, it was thus necessary to get rid selectively of the (about) 15% of undesired (*R*) enantiomer present in the reaction product. This could be very easily achieved by incubation of this epoxide with an enzymatic extract of *A. niger*.[18] This thus afforded the (*S*)-**2** epoxide which showed an ee higher than 97% (analytical and preparative yields respectively 59% and 43%, overall preparative yield over the three steps 33%[24]).



Scheme 2. Chemoenzymatic synthesis of enantiopure (*S*)-2.

CONCLUSION

In the course of this study, we have achieved the enantioselective hydrolysis of various substituted alkyl-epoxides using seven fungi equipped with an EH activity. These strains were shown to allow the preparation of these alkyl substituted epoxides, which were thus obtained in high enantiomeric purity but with only moderate yields. However, in order to improve the yield of these preparations, a combined chemoenzymatic strategy could be devised.

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EXPERIMENTAL SECTION

General. The strains used in this work were purchased at the ATCC, CBS, LCP and MUCL collections. ^1H NMR spectra were recorded in $CDCl_3$ solution on a Brüker AC 250 apparatus. Optical rotation values were measured on a Perkin-Elmer 241C polarimeter at 589 nm or 578 nm. Vapor phase chromatography (GC) analyses were performed using, for quantitative determination, a capillary column OPTIMA 1701 (0.32 mm, 30 m, Macherey-Nagel), and for ee determination, one of the four following "chiral" columns (0.22 mm, 25 m, Macherey-Nagel) *i.e.*: Heptakis (6-O-methyl-2,3-di-O-pentyl)-β-cyclodextrin (col. I), Lipodex E [Octakis (3-O-butyryl-2,6-di-O-pentyl)-γ-cyclodextrin] in OV 1701 (1/1) (col. II), Lipodex E [Octakis (3-O-butyryl-2,6-di-O-pentyl)-γ-cyclodextrin] (col. II*bis*), Octakis (6-O-methyl-2,3-di-O-pentyl)-γ-cyclodextrin. In OV 1701 (col. III).

Synthesis of substrates 1 to 4.

(±)-hexyl-oxirane **1** was purchased from Fluka.

(±)-gem-1-pentyl-1-methyl-oxirane **2.** To a stirred solution of 8 g (36 mmoles) of trimethylsulfonium iodide in 50 mL of DMSO, placed under argon, was added 1.4 g (36 mmoles) of sodium hydride (60% mineral oil dispersion).[25] After stirring for 30 min, a solution of 3.4 g (30 mmoles) of 2-heptanone in 10 mL of DMSO was added and the reaction mixture was stirred at room temperature overnight. After cooling and addition of water, the mixture was extracted with ether (3 times). The combined extracts were washed with brine, dried over $MgSO_4$ and evaporated to give crude product. Purification by flash chromatography (pentane/ether 96/4) yielded 2.9 g (77% yield) of **2** as a colorless liquid. ^1H NMR[26] δ: 0.89 (t, 3H, J = 6.8 Hz, ω-CH_3); 1.30 (s, 3H, CH_3); 1.30-1.66 (m, 8H, $(CH_2)_4$); 2.57 and 2.61 (d, 1H, J_{gem}= 4.9 Hz, CH_2-O).

(±)-trans-1-pentyl-2-methyl-oxirane **3**. To a stirred solution of 4 g (35.7 mmoles) of *trans*-2-octene in 10 mL of CH_2Cl_2, cooled in an ice bath, was added 7.5 g of *m*-chloroperoxybenzoic acid (70%, 1.1 equ.) dissolved in 100 mL of CH_2Cl_2. After removing the ice bath and stirring 3 h, the reaction mixture was washed with a saturated solution of $NaHSO_3$, aqu. NaOH 10% and brine, and dried over $MgSO_4$. Purification by flash chromatography (pentane/ether 96/4) yielded 4 g (87% yield) of **3** as a colorless liquid. ^1H NMR[27] δ: 0.89 (t, 3H, J = 6.8 Hz, ω-CH_3); 1.29 (d, 3H, J = 5.2 Hz, CH_3); 1.32-1.55 (m, 8H, $(CH_2)_4$); 2.62 (td, 1H, J_{1-2} = 2.1 Hz, J = 5.2 Hz, H_1); 2.74 (qd, 1H, J_{1-2} = 2.2 Hz, J = 5.2 Hz, H_2).

(±)-cis-1-pentyl-2-methyl-oxirane **4**. Racemic **4** was obtained by epoxidation of the corresponding *cis*-olefin formed by catalytic hydrogenation of 2-octyne.[28] To 450 mg of Lindlar catalyst in 60 mL of pentane, placed under hydrogen at room temperature, was added dropwise 4 g (36 mmoles) of 2-octyne, and the mixture was

stirred. After 2 h of reaction, no more hydrogen was consumed. The solids were then filtered on celite, and the pentane was partially evaporated. The crude was dissolved in 10 mL of CH_2Cl_2, and the epoxide **4** was obtained using the same procedure as that described for **3** (yield 96%). ^1H NMR[27] δ: 0.89 (t, 3H, J = 6.8 Hz, ω-CH_3); 1.29 (d, 3H, J = 5.2 Hz, CH_3); 1.32-1.55 (m, 8H, $(CH_2)_4$); 2.62 (td, 1H, $J_{1\text{-}2}$ = 2.1 Hz, J = 5.2 Hz, H_1); 2.74 (qd, 1H, $J_{1\text{-}2}$ = 2.2 Hz, J = 5.2 Hz, H_2). Using these experimental conditions, a small proportion (3%) of *trans*-1-pentyl-2-methyl-oxirane was also formed.

Synthesis of reference material for the diols 1a-4a. *(±)-hexyl-ethanediol* **1a** was purchased from Fluka. The *(±)-1-pentyl-1-methyl-ethanediol* **2a**, *(±)-erythro-1-pentyl-2-methyl-ethanediol* **3a**, and *(±)-threo-1-pentyl-2-methyl-ethanediol* **4a**, were prepared by hydrolysis of the corresponding epoxides as follows. To 10 mg epoxide dissolved in 1 mL of a mixture THF/water (5/1), two drops of concentrated sulfuric acid were added. After stirring overnight, the solution was neutralized by addition of saturated $NaHCO_3$ solution, and extracted with ether. Evaporation of the washed (saturated NaCl solution) organic phase gave the crude racemic diol which was further analyzed by GC on a chiral stationary phase. These ee determinations were performed after derivatisation in acetonide for the diols **1a-3a**, and after cyclisation in the corresponding *trans*-epoxide **3** for the diol **4a**.

General procedure for the derivatisation of the diols into the corresponding acetonide. As described previously,[14] the acetonide derivative was obtained by reaction of the diol with 2,2-dimethoxypropane in presence of a catalytic amount of APTS.

Procedure for the cyclisation of the diol 4a into the corresponding epoxide 3 according to Golding et al[29] The crude diol **4a** obtained after extraction of the reaction mixture with ether and evaporation of the solvent was placed under nitrogen and 2 drops of HBr in glacial acetic acid (33%) were added. After stirring for 1 h, the mixture was neutralized by addition of water and solid Na_2CO_3, and extracted with ether (2 times). The combined organic layers were evaporated and dried under vacuum. After stirring 30 min with 50 µL of KOH in MeOH (1N), the mixture was washed with brine and extracted with ether to give the epoxide **3**.

General procedure for the growth of fungi. The fungal strains were maintained on agar slants and grown on a medium consisting of 20 g of Corn Steep Liquor (Roquette SA), and 10 g of glucose for 1 L of tap water (for the culture of *Chaetomium globosum*, 10 g/L of KH_2PO_4 and 5 g/L of K_2HPO_4 were added to obtain a pH 6). *For small scale biotransformations* (analytical scale), approximately 0.5 cm^2 of fungal mycelium and/or spores was used to inoculate 100 mL of medium in a baffled 500 mL Erlenmeyer flask. After 3 days of culture at 27°C, the biomass was filtered off and washed with water. *For larger scale biotransformations* (preparative scale), the fungi were grown in 2 or 7 L fermentors containing respectively 1.4 or 5 L of medium in which 0.2 g/L of Pluronic PE 8100 (BASF) and 50 µL/L of antifoam silicone 426R (Prolabo) were added (to prevent overflowing during the growth). The medium was maintained at 27°C, stirred at 500-700 rpm and aerated at 0.1-0.3 VVM. The methods of inoculation were different in function of the fungus, *i.e.* -i) for *Aspergillus niger*: the method was identical to the one employed for the inoculation of the Erlenmeyer flask; -ii) for *Beauveria bassiana* and *Mortierella isabellina*: after 2 days of growth in Erlenmeyer flask, 10 mL of the broth was used to inoculate the fermentors; and -iii) for *Chaetomium globosum*: the mycelium and the spores present on the slant were suspended in 10 mL of physiological water (NaCl 0.9%), and 5 mL of this were used to inoculate the fermentor. At the late stationary phase *i.e* 48 h for *A. niger*, 44 h for *B. bassiana*, 72 h for *C. globosum* and 36-40 h for *M. isabellina*, the biomass was filtered off and washed with water. The determination of the dry weight was carried out in drying the wet biomass 2 days in an oven at 100°C.

General procedure for the biohydrolysis of epoxides 1-4. Determination of E and of the reaction rate. In a closed 3 mL vial, at 27°C, a stirred suspension of biomass (produced in Erlenmeyer flask as described above) in 2 mL sodium phosphate buffer 0.1 M pH 8 was prepared. 10 µL of a solution of substrate in DMF was then added (substrate concentration in the reaction medium 8 mM). The courses of the bioconversions were followed by periodically withdrawing aliquots (300 µL) at time intervals. The cells were removed by centrifugation, the remaining epoxide was extracted from the supernatant with pentane and its ee analyzed by GC. After adding MeOH to the supernatant (1/1), the formed diol was quantified by GC (OPTIMA 1701 column, 180°C) in

presence of an internal standard (diols **1a** or **2a**) after an external calibration. To control the initial concentration of epoxide, 10 µL of the substrate solution was also added in 2 mL of H_2SO_4 3 M, and, after total hydrolysis, the formed diol was quantified. The initial reaction rate was determined by the tangent for t=0 of the curve of the diol produced in function of time and expressed in µmole of diol per minute and per gram of biomass (dry weight).

Biohydrolysis of epoxides 1-4. Preparative scale applications.
The biomass produced in fermentor as described above was suspended in a 2 L fermentor filled with 1 L of sodium phosphate buffer 0.1 M pH 8. The medium was stirred from 500 to 1000 rpm and maintained at 27°C. 2 g of epoxide was added to the suspension as a solution in ethanol (10 mL). The course of the bioconversion was monitored by determining the ee of the remaining epoxide. When the ee of the epoxide reached at least 97%, the medium was filtered off. The fungal cake was washed with 500 mL of pentane, and the aqueous phase was extracted 2-3 times with pentane. The combined organic layers were dried ($MgSO_4$), the solvent eliminated, and the epoxide was purified by bulb-to-bulb distillation (13-26 mbar). The crude diol formed was obtained by continuous extraction of the aqueous phase with CH_2Cl_2. After elimination of the solvent, the diol was purified by bulb-to-bulb distillation (10^{-2} mbar). Preparative yields and ee of the residual epoxide and of the formed diol are given in Table 2.
Optical rotations of the isolated products after biohydrolysis are as follows:
(*S*)-**1** $[\alpha]_D^{23}$= -14 (c=2.1; EtOH; ee > 97%); lit.[30] (*R*)-**1** $[\alpha]_D^{23}$= +14.2 (c=2.48; EtOH; ee > 98%)
(*R*)-**1a** $[\alpha]_D^{20}$= +4 (c=2.2; EtOH; ee 35%); lit.[31] (*R*)-**1a** $[\alpha]_D^{20}$= +17.5 (c=1.16; EtOH; ee > 98%)
(*S*)-**2** $[\alpha]_D^{25}$= +8 (c=2.1; $CHCl_3$; ee > 99%); lit.[26] (*R*)-**2** $[\alpha]_D^{20}$= -6.35 (c=3.43; $CHCl_3$; ee 71%)
(*R*)-**2a** $[\alpha]_D^{25}$= +0.6 (c=3.1; $CHCl_3$; ee 32%); lit.[22] (*R*)-**2a** $[\alpha]_D^{23}$= +1.9(c=0.99; $CHCl_3$; ee 78%)
(1*S*,2*S*)-**3** $[\alpha]_J^{25}$= +5 (c=3.2; C_5H_{12}; ee > 97%); lit.[27] (1*S*,2*S*)-**3** $[\alpha]_J^{25}$= +5 (c=3; C_5H_{12}; ee > 98%)
(1*R*,2*S*)-**3a** $[\alpha]_D^{25}$= +17 (c=1.43; MeOH; ee 78%); lit.[32] (1*R*,2*S*)-**3a** $[\alpha]_D^{18}$= +22.73 (c=1.1; MeOH; ee > 98%)
(1*R*,2*S*)-**4** $[\alpha]_J^{25}$= +7 (c=2.02;C_5H_{12}; ee > 97%); lit.[27] (1*R*,2*S*)-**4** $[\alpha]_J^{25}$= +8(c=2; C_5H_{12}; ee > 98%)
(1*R*,2*R*)-**4a** $[\alpha]_D^{25}$= +9 (c=1.17; $CHCl_3$; ee 58%); lit.[33] (1*R*,2*R*)-**4a** $[\alpha]_D^{25}$= +17.5 (c=0.4; $CHCl_3$; ee > 98%)
The ^1H NMR data of the isolated epoxides were identical to those obtained for the racemates. The properties and ^1H NMR of the non commercially available diols obtained by biohydrolysis of the epoxides are as follows:
2a: colorless oil, ^1H NMR[26] δ: 0.89 (t, 3H, J = 6.9 Hz, ω-CH_3) ; 1.16 (s, 3H, CH_3); 1.20-1.50 (m, 8H, $(CH_2)_4$); 2.28 (s large, 2H, 2 OH); 3.40 and 3.47 (d, 1H, J_{gem} = 10.9 Hz).
3a: white solid, ^1H NMR[34] δ: 0.90 (t, 3H, J = 6.5 Hz, ω-CH_3); 1.15 (d, 3H, J = 6.4 Hz, CH_3); 1.22-1.51 (m, 8H, $(CH_2)_4$); 1.82 (s large, 2H, 2 OH); 3.62 (td, 1H, J_{1-2} = 3.3 Hz, J = 6.5 Hz, H_1); 3.80 (qd, 1H, J_{1-2} = 3.3 Hz, J = 6.4 Hz, H_2).
4a: colorless oil, ^1H NMR[34] δ: 0.90 (t, 3H, J = 6.4 Hz, ω-CH_3); 1.19 (d, 3H, J = 6.3 Hz, CH_3); 1.24-1.51 (m, 8H, $(CH_2)_4$); 2.05 (s large, 2H, 2 OH); 3.37 (m, 1H, H_1); 3.59 (m, 1H, H_2).

Determination of the absolute configurations of the epoxides and diols. The absolute configurations of the epoxides **1-4** and diols **1a-4a** obtained by bioconversion have been determined on the basis of the optical rotation by comparison with previously described data (see above). When the reaction products were not isolated, their absolute configurations were assigned *via* GC analysis on a chiral stationary phase by comparison with these reference materials.

Chiral GC data of the epoxides **1-4** and diols **1a-4a** are as follows:
1. col. I, 40°C, t_R(*R*) 14.1 min and t_R(*S*) 14.4 min
2. col. II, 40°C, t_R(*R*) 12.7 min and t_R(*S*) 13.4 min
3. col. III, 40°C, t_R(1*S*,2*S*) 17.6 min and t_R(1*R*,2*R*) 18.4 min
4. col. II*bis*, 60°C, t_R(1*S*,2*R*) 8.3 min and t_R(1*R*,2*S*) 9.3 min
1a. acetonide derivative, col. I, 60°C, t_R(*R*) 23.6 min and t_R(*S*) 24.5 min
2a. acetonide derivative, col. I, 60°C, t_R(*S*) 12.0 min and t_R(*R*) 12.8 min
3a. acetonide derivative, col. I, 60°C, t_R(1*R*,2*S*) 13.5 min and t_R(1*S*,2*R*) 14.3 min
4a. cyclisation in epoxide **3**.

Chemoenzymatic synthesis of (*S*)-1-pentyl-1-methyl-oxirane 2. The asymmetric dihydroxylation of 3 g of 2-methyl-1-heptene (26.7 mmoles) was performed using the commercially available catalyst AD-mix-α (37.4 g) according to Sharpless et al.[22] This led, after purification by bulb-to-bulb distillation (10^{-2} mbar), to 3.73 g of the (*S*)-1-pentyl-1-methyl-ethanediol **2a** (95% yield) with an ee of 71%, $[\alpha]_D^{25}$= -2.1 (c=3.37; $CHCl_3$). Under argon, 3 g of diol (20 mmoles) and 4.6 g of tosyl chloride (1.2 equ.) were dissolved in 100 mL of anhydrous ether. After stirring for 1 h, 8 g of NaH (60% mineral oil dispersion, 10 equ.) was added. After stirring overnight, the mixture was washed with water (3 times) and dried over $MgSO_4$. After elimination of the solvent, and purification by bulb-to-bulb distillation (15 mm Hg), 2.05 g of (*S*)-**2** (80% yield) was obtained with an ee of 71%, $[\alpha]_D^{25}$= +6 (c=3.03; $CHCl_3$). 200 mg of enzymatic powder, obtained as previously described,[18] were dissolved in 45 mL of sodium phosphate buffer 0.1 M pH 7.0. 1 g of (*S*)-**2** with an ee of 71% was then added as a solution in EtOH (4 mL), and the mixture was stirred at 27°C. When the ee of **2** exhibited a value of 97% (3 h), the mixture was extracted with pentane (analytical yield determined by CPG 59%), and after elimination of the solvent, and purification by bulb-to-bulb distillation, 430 mg of (*S*)-**2** (43% yield) was isolated with an ee of 97%, $[\alpha]_D^{25}$= +9 (c=3.18; $CHCl_3$).

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