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Asymmetric Microbial Hydrolysis of Epoxides

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Abstract: Kinetic resolution of 2-mono- and 2,2-disubstituted epoxides was accomplished using epoxide hydrolases from bacterial and fungal origin by employing lyophilized whole microbial cells. In all cases investigated, the biocatalytic hydrolysis was shown to proceed with retention of configuration at the stereogenic center leading to 1,2-diols and remaining epoxides. The selectivity of the reaction was dependent on the substrate structure and the strain used with E-values ranging from low or moderate (with 2-monosubstituted epoxides) to excellent (E >100, with 2,2-disubstituted oxiranes).

Keywords: Epoxide hydrolase, biotransformation, chiral epoxide, chiral vicinal diol.

Introduction

Chiral epoxides and vicinal diols are extensively employed high-value intermediates in the synthesis of chiral compounds due to their ability to react with a broad variety of nucleophiles. In recent years a lot of research has been devoted to the development of catalytic methods for their production[1]. The Katsuki-Sharpless method reported in 1980 is a widely used catalytic procedure for the asymmetric epoxidation of allylic alcohols[2]. More recently, it has found serious competitors in the metal-catalyzed asymmetric epoxidation of unfunctionalized olefins[3,4]. Although high enantiomeric purities have been obtained for the epoxidation of *cis*-alkenes, the selectivities achieved with *trans*- and terminal olefins were less satisfactory using the latter methods.
On the other hand, biocatalytic methods[5-8] have been proven to provide a useful arsenal of methods as valuable alternatives to the above mentioned asymmetric epoxidation techniques. For instance, prochiral or racemic synthetic precursors of epoxides, such as halohydrins can be obtained *via* enantioselective ester hydrolysis or esterification by using hydrolytic enzymes[9,10] (esterases, proteases or lipases). This methodology is well developed but it is impeded by the requirement of regioisomerically pure halohydrins. Asymmetric biocatalytic reduction of α-keto-acids[11] or -alcohols[12] using D- or L-lactate dehydrogenase or glycerol dehydrogenase gives rise to chiral α-hydroxyacids and 1,2-diols, respectively, which in turn can be converted into the corresponding epoxides using conventional methodology. Although excellent selectivities are generally achieved, the need for redox-cofactors such as NAD(P)H has restricted the number of applications. Haloperoxidases catalyze the

asymmetric formation of halohydrins from alkenes and hypohalous acid, which is produced from H_2O_2 and halide. These enzymes are rare in Nature and exhibit usually low selectivities[13]. The same is true for halohydrin epoxidases, which convert a halohydrin into the corresponding epoxide[14]. Direct epoxidation of alkenes catalyzed by mono-oxygenases cannot be performed on a preparative scale with isolated enzymes due to their complex nature and their dependence on a redox cofactor. Thus whole microbial cells are used instead. Although the toxic effects of the epoxide formed and its further metabolism by the cells have successfully been minimized by employing biphasic media, this method is not trivial and requires high bioengineering skills[15]. Peroxidases, such as chloroperoxidase, on the other hand, are cofactor-independent and can be used for the enzymatic epoxidation of alkenes. Although excellent selectivities were obtained with internal *cis*-olefins, long-chain substrates and terminal alkenes were unreactive[16].

A valuable alternative to the above existing methods is the use of cofactor-independent epoxide-hydrolases [EC 3.3.2.X]. Although they have been known for a considerable time from mammalian sources such as (rat) liver tissue, they have mainly been investigated during detoxification studies[17]. Biotransformations on a preparative scale using epoxide hydrolases from rat liver are hampered by the limited supply of enzyme and they rarely surpass the millimolar range[18]. It was only recently, that a detailed search for epoxide hydrolases from microbial sources has been undertaken by the group of R. Furstoss[19,20] and in our laboratory[21,22], bearing in mind that the use of microbial enzymes allows an almost unlimited production of biocatalyst. Although several microorganisms are known to possess epoxide hydrolases, the number of applications for preparative organic transformations is remarkably small[19,23-26]. In the majority of cases, the hydrolysis of an epoxide was observed during the microbial epoxidation an alkene, where it constitutes an undesired side reaction through degradation of the product[15].

Most recently, R. Furstoss and coworkers have used the fungi *Aspergillus niger*[19,20] and *Beauveria bassiana*[20] (formerly *B. sulfurescens*) to hydrolyze racemic epoxides. During the same time, we have shown that crude bacterial enzyme preparations[21] or whole cells of *Rhodococcus* sp.[22] are equally useful. Furthermore, it was found that in addition to the 'normal' hydrolysis, also the non-natural nucleophile azide was accepted giving rise to a chiral azido-alcohol *via* an asymmetric opening of the epoxide by azide catalyzed by an enzyme[27].

Results and Discussion

Our search for microbial epoxide hydrolases was initiated with a screening employing a variety of lyophilized fungal and bacterial cells[28]. Since enzyme induction is still a largely empirical task, cells were grown on non-optimized standard media. Thus, a set of representative substrates with various structural steric requirements (**1-7**, Scheme 1) were subjected to microbial hydrolysis employing lyophilized whole cells in Tris-buffer at pH 7.0. The reaction was monitored by observing the formation of the corresponding 1,2-diol as judged by TLC by comparison with independently synthesized material. The results of the screening are summarized in Table 1. In contrast to mammalian systems, where in general epoxide hydrolases have to be induced (*e.g.* by feeding rats with phenobarbital), several strains exhibited sufficient activity even when the cells were grown on a non-optimized standard medium. 2-Mono- and 2,2-disubstituted epoxides (**1-3**) were hydrolyzed with good activity by the majority of strains. On the other hand, the more sterically hindered 3,3-dimethyl-1,2-epoxybutane (**4**) was only accepted by the fungus *Fusarium solani*. Similarly, *meso*-epoxides **5-7** were less readily accepted.

Scheme 1. Substrates for the screening of epoxide hydrolase activity.

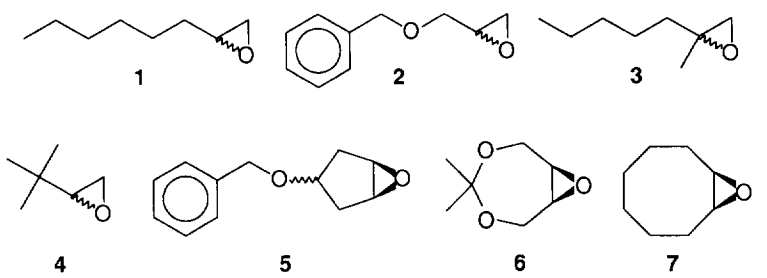
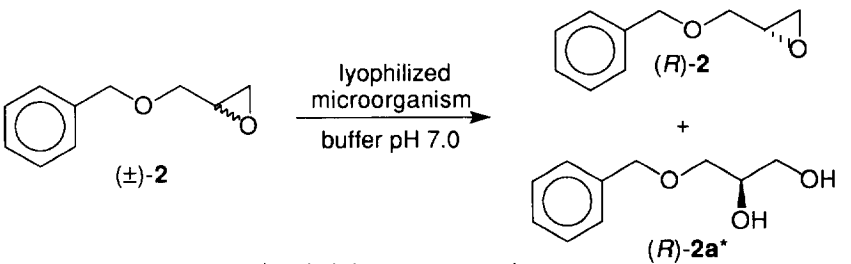


Table 1. Screening of substrates **1**-**7** for microbial epoxide hydrolases.

Microorganism	Substrate						
	(±)-**1**	(±)-**2**	(±)-**3**	(±)-**4**	(*cis*/*trans*)-**5**	**6**	**7**
Rhodococcus sp. NCIMB 11216*	+	+	+	-	-	-	-
Rhodococcus sp. NCIMB 11215*	+	+	+	-	-	-	-
Rhodococcus sp. NCIMB 11540	+	+	+	+	n.d.	-	n.d.
Corynebacterium sp. UPT 9	+	n.d.	+	n.d.	n.d.	n.d.	n.d.
Diploida gossypina ATCC 10936	+	+	+	+	-	-	-
Fusarium solani DSM 62416	+	+	+	-	-	-	-
Glomerella cingulata ATCC 10534	+	+	+	-	-	-	-

* Formerly also denoted as *Nocardia* sp.; + activity, - no activity; n.d. not determined.

Scheme 2. Resolution of benzyl glycidyl ether (±)-**2**.



* switch in sequence rule

Microorganism	Diol		Epoxide		Selectivity[29]
	e.e. [%]	Config.	e.e. [%]	Config.	(E)
Rhodococcus sp. NCIMB 11216	9	n.d.	<5	--	~1
Fusarium solani DSM 62416	<5	n.d.	<5	--	~1
Diploida gossypina ATCC 10936	22	*R**	5	*R*	1.6

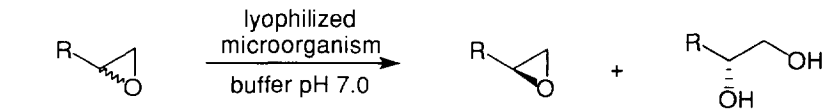
N.d. = not determined; * = switch in sequence rule.

Benzyl glycidyl ether (**2**) serves as chiral C_3-building block for the synthesis of chiral amino alcohols and several bioactive compounds such as β-blockers[30]. The selectivity of three strains was investigated on a preparative scale (Scheme 2). The initial results were disappointing. Both *Rhodococcus* sp. NCIMB 11216 and *Fusarium solani* DSM 62416 hydrolyzed (±)-**2** without enantio-discrimination, and *Diploida gossypina* ATCC 10936 gave diol (*R*)-**2a** in only 22% e.e.

1-Epoxyoctane (±)-**1** was hydrolyzed by several bacteria and fungi, all of which displayed the same enantio-preference, *i.e.* (*R*)-diol (**1a**) was formed and epoxide (*S*)-**1** was recovered. However, the selectivity remained low and enantiomeric excesses of epoxide and diol did not exceed 50%.

When the sterically more demanding 3,3-dimethyl-1,2-epoxybutane (±)-**4** was hydrolyzed with *Diploida gossypina* ATCC 10936, the results were more encouraging. When the reaction was terminated at 15% conversion, epoxide (*S*)-**4** and diol (*R*)-**4a** were obtained in 15 and 88% e.e., resp. (E = 18)[29]. In a recent study on microsomal epoxide hydrolase isolated from rabbit liver, (*R*)-**4a** was obtained from (±)-**4** in 92% e.e. (E = 38)[31].

Scheme 3. Resolution of monosubstituted oxiranes (±)-**1** and (±)-**4**.



(±)-**1** R = *n*-C_6H_{13} (*S*)-**1** (*R*)-**1a**

(±)-**4** R = *tert*-Bu (*S*)-**4** (*R*)-**4a**

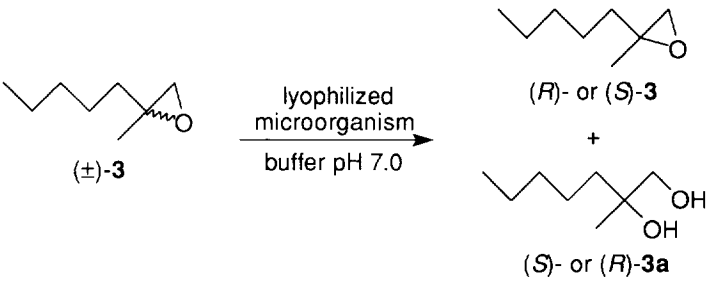
Microorganism	Substrate	Diol		Epoxide		Selectivity
		e.e. [%]	Config.	e.e. [%]	Config.	(E)[29]
Fusarium solani DSM 62416	(±)-**1**	<5	n.d.	<5	n.d.	~1
Rhodococcus sp. NCIMB 11540	(±)-**1**	26	*R*	12	*S*	1.9
Glomerella cingulata ATCC 10534	(±)-**1**	28	*R*	20	*S*	2.1
Corynebacterium sp. UPT 9	(±)-**1**	41	*R*	10	*S*	2.6
Rhodococcus sp. NCIMB 11216	(±)-**1**	39	*R*	21	*S*	2.8
Diploida gossypina ATCC 10936	(±)-**1**	46	*R*	19	*S*	3.2
Rhodococcus sp. NCIMB 11540	(±)-**4**	13	*S*	7	*R*	1.4
Diploida gossypina ATCC 10936	(±)-**4**	88	*R*	15	*S*	18

N.d. = not determined.

However, the picture became more complex and encouraging selectivities were achieved, when the 2,2-disubstituted oxirane (±)-**3** was chosen as substrate. The latter compound serves as building block for the synthesis of the ω-chain of the prostaglandin derivative Arbaprostil[32]. Fungal cells preferentially cleaved the (*R*)-epoxide by forming (*R*)-**3a**, albeit without pronounced enantiopreference. On the other hand, the bacteria exhibited the opposite enantiopreference and exhibited much better selectivities. In particular, both of the *Rhodococcus* sp. gave E-values of 29 and 104, resp. Interestingly, a structurally closely related substrate (2-

methyl-1,2-epoxyhexane) was hydrolyzed by rabbit liver epoxide hydrolase with lower selectivity (E = 4.9) by exhibiting a preference for the (*S*)-enantiomer[33].

Scheme 4. Resolution of 2-methyl-1,2-epoxyheptane (±)-**3**.



Microorganism	Diol		Epoxide		Selectivity
	e.e. [%]	Config.	e.e. [%]	Config.	(E)[29]
Fusarium solani DSM 62416	<5	n.d.	<5	n.d.	~1
Glomerella cingulata ATCC 10534	5	*R*	9	*S*	1.2
Diploida gossypina ATCC 10936	20	*R*	10	*S*	1.7
Corynebacterium sp. UPT 9	71	*S*	18	*R*	7
Rhodococcus sp. NCIMB 11540	89	*S*	51	*R*	29
Rhodococcus sp. NCIMB 11216	96	*S*	71	*R*	104

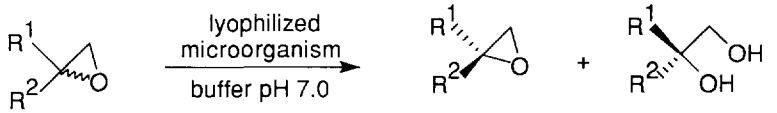
N.d. = not determined.

In order to investigate, whether the selectivity of *Rhodococcus* sp. NCIMB 11216 was of general applicability for 2,2-disubstituted oxiranes, the substitution pattern of substrates was varied by alkyl chain extension leading to substrates **8**-**10** (Scheme 5). Interestingly, when the difference in size of the 2-alkyl substituents was diminished - 2-ethyl-1,2-epoxyheptane (**8**) as compared to 2-methyl-1,2-epoxyheptane (**3**) - the selectivity dropped significantly from E = 104 to E = 7.2. The opposite phenomenon was observed upon stepwise elongation of the alkyl substituent (substrates **9**, **10**), where E-values of 126 and >200 were achieved. Thus, the stereochemical preference of the *Rhodococcus* epoxide hydrolase for 2,2-disubstituted oxiranes can be characterized by the general substrate model depicted in Scheme 5. The overall yield of formed diol and remaining epoxide were in the range of ~90%.

The absolute configuration of diols formed and remaining epoxides was determined with great care for the following reason. In contrast to the majority of kinetic resolution of esters (e.g. by ester hydrolysis and -synthesis using lipases, esterases and proteases) where the absolute configuration of the stereogenic centre(s) always remains the same throughout the reaction, the microbial hydrolysis of epoxides may take place *via* two different pathways (Scheme 6): (i) Attack of the (formal) hydroxyl ion on the less shielded oxirane carbon atom affecting *retention* of configuration or (ii) attack on the stereogenic centre, which leads to *inversion*. Although retention of configuration seems to be the more common pathway, inversion of configuration has been reported in some cases[20,34,35]. As a consequence, the absolute configuration of *both* the product and substrate has to be determined. This was accomplished by interconversion of the remaining epoxide into the corresponding diol by

basic hydrolysis and comparison of the material *via* co-injection on chiral GLC with a sample of the corresponding diol which was obtained enzymatically. In all of the cases in this study, the epoxide and the diol from an enzymatic reaction was shown to be of opposite configuration; in other words, the hydrolysis was shown to proceed *via* retention of configuration.

Scheme 5. Resolution of 2,2-disubstituted oxiranes (±)-**8**-**10** using *Rhodococcus* sp. NCIMB 11216.

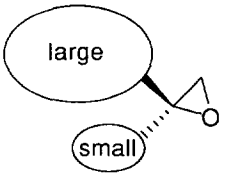


(±)-**8**-**10** (*R*)-**8**-**10** (*S*)-**8a**-**10a**

(±)-**8** $R^1 = n\text{-}C_5H_{11}$; $R^2 = C_2H_5$

(±)-**9** $R^1 = n\text{-}C_7H_{15}$; $R^2 = CH_3$

(±)-**10** $R^1 = n\text{-}C_9H_{19}$; $R^2 = CH_3$

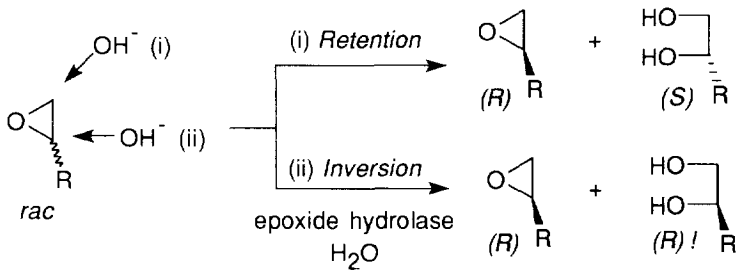


substrate model

Substrate	Diol		Epoxide		Selectivity
	e.e. [%]	Config.	e.e. [%]	Config.	(E)[29]
(±)-**8**	70	*S**	25	*R**	7.2
(±)-**9**	98	*S*	25	*R*	126
(±)-**10**	>99	*S*	55	*R*	>200

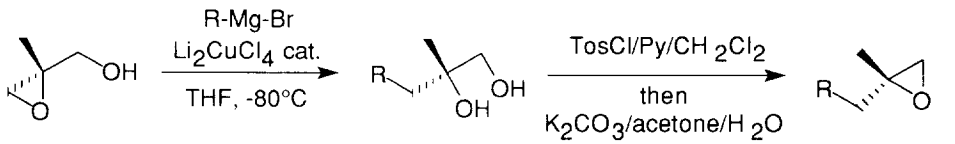
* Absolute configuration assumed by comparison of specific rotation values and elution order on chiral GCL within a homologous series of compounds (**3**, **3a**, **9**, **9a**, **10**, **10a**).

Scheme 6. Pathways of enzymatic epoxide hydrolysis *via* retention or inversion of configuration.



The absolute configuration of products was determined by comparison of optical rotation values with literature data[36] (see Experimental). Reference material for compounds **3a**, **9a** and **10a** was independently synthesized *via* Li_2CuCl_4-catalyzed addition of the corresponding Grignard-reagent onto commercially available (*S*)-2-methylglycidol following the procedure of Hosokawa *et al.*[37]. Ring closure of diols **3a**, **9a** and **10a** was accomplished by *mono*-tosylation and subsequent treatment with base. The absolute configuration of **8** was not determined due to the low selectivity of the reaction, however it was assumed to be (*R*)-**8** and (*S*)-**8a** by comparison with compounds **3**, **3a**, **9**, **9a** and **10**, **10a**, which constitute a homologous series with respect to the alkyl chain length: (i) the sense of $[\alpha]_D$-values of (*R*)-epoxides (**3**, **8**, **9**, **10**) and (*S*)-diols (**3a**, **8a**, **9a**, **10a**) is always (-), and (ii) the GLC-elution order of enzymatically obtained epoxides (**3**, **8**, **9**, **10**) and diols (**3a**, **8a**, **9a**, **10a**, analyzed as corresponding acetonides) on a γ-cyclodextrin column follows a consistent pattern, *i.e.* the enantiomers obtained enzymatically always elute first. A recently reported negative specific rotation for (*S*)-**10**[38] seems to be erroneous, which was proven by ^{1}H-NMR spectroscopy. Thus, when a sample of **10** (prepared enzymatically) was recorded in the presence of (+)-Eu(hfc)$_3$, no splitting of the 2.55 ppm signals could be achieved at 300 MHz (as described in the study), but rather only peak-broadening was observed. On the other hand, the two doublets at 9.2 and 10.2 ppm (which were used for the e.e.-measurement)[39] were absent. Final proof was achieved by independent synthesis of (*R*)-**10** from (*S*)-2-methylglycidol (see Scheme 7).

Scheme 7. Synthesis of reference material (*R*)-**3**, **9**, **10**.



(S)-2-methylglycidol *(R)*-**3a**, **9a**, **10a** *(R)*-**3**, **9**, **10**

3 R = *n*-C_4H_9 **9** R = *n*-C_6H_{13} **10** R = *n*-C_8H_{17}

Summary: This study has shown that bacterial epoxide hydrolases are highly selective biocatalysts for the preparation of optically active 2,2-disubstituted oxiranes and 2-alkyl-1,2-alkanediols. The isolation of the epoxide hydrolase from *Rhodococcus* sp. NCIMB 11216 will be reported in due course. Studies on the application of bacterial epoxide hydrolases for the synthesis of enantiopure bioactive compounds are in progress.

Acknowledgements

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Experimental

General:

Reactions were monitored by TLC (silica gel Merck 60 F_{254}), compounds were visualized by spraying with vanillin/H_2SO_4 conc. (5g/l) or Mo-reagent [$(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$ (1.1 g/l), $Ce(SO_4)_2 \cdot 4\ H_2O$ (4g/l) in H_2SO_4 (10%)]. Preparative chromatography was performed on silica gel Merck 60 (40-63 μm). ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ solution on a Bruker MSL 300 (300 MHz and 75.47 MHz, resp.). Chemical shifts are reported in δ from TMS as internal standard. Methyl and methylene groups were distinguished using the DEPT sequence. Optical rotation values were measured on a JASCO DIP 370 polarimeter at 589 nm (Na-line) in a 1 dm cuvette. Enzymatic reactions were monitored by GLC-analysis (DANI 8400 GC equipped with PTV-injector and FID) on a capillary column (J&W DB 1701, 30m x 0.25 mm, 0.25 μm film, N_2). Optical purities were analyzed by GLC (Shimadzu GC-14A equipped with FID) on either J&W Cyclodex B (30 m x 0.25 mm, 0.25 μm film, N_2), Astec Chiraldex G-TA (30 m x 0.25 mm, 0.25 μm film, H_2) or CP-Chirasil-DEX CB (25 m, 0.32 mm, 0.25 μm film, H_2).

Synthesis of Substrates:

1,2-Epoxyoctane (**1**) (Merck), 1,2-octanediol (**1a**) (Aldrich) and cyclooctene oxide (**7**) (Aldrich) were commercially available. Benzyl glycidyl ether (**2**) was synthesized by BF_3•Et_2O-catalyzed addition benzyl alcohol onto epichlorohydrin and subsequent treatment of the intermediate halogenhydrin with base in a one-pot procedure[40]. The crude product was chromatographed on silica prior to distillation ($Bp._2$ 85-6°C). 3,3-Dimethyl-1,2-epoxybutane (**4**) was obtained by epoxidation of 3,3-dimethylbut-1-ene following a standard procedure (*m*-CPBA/CH_2Cl_2, Bp. 88-90°C)[41]. A mixture of *cis*- and *trans*-1-benzyloxy-3,4-epoxycyclopentane (**5**)[42] was obtained as follows: $LiAlH_4$ reduction of 3,4-epoxycyclopentene[43] gave cyclopent-3-en-1-ol[44], which was O-benzylated (KH/benzyl bromide) to give 4-benzyloxycyclopent-1-ene. Epoxidation (*m*-CPBA/CH_2Cl_2) gave substrate **5** as a *cis/trans*-mixture (~1:1).

4,4-Dimethyl-3,5,8-trioxabicyclo[5.1.0]octane (**6**): To a solution of *cis*-2-butene-1,4-diol (1.5 g, 17 mmol) in 2,2-dimethoxypropane (20.8 ml, 170 mmol), a catalytic amount of *p*-toluenesulfonic acid was added with stirring. After 15 min the mixture was extracted with $NaHCO_3$ solution, the organic phase was separated, dried and excess 2,2-dimethoxypropane was evaporated. Distillation ($Bp._{13}$ 38-40°C) gave 2,2-dimethyl-4,7-dihydro-1,3-dioxepin (1.8g, 82%). ^{1}H-NMR: δ = 1.45 (s, 6H, CH_3 x 2), 4.23 (s, 4H, allylic H), 5.62 (s, 2H, vinylic H). ^{13}C-NMR: δ = 24.19 (CH_3), 61.6 (C-4 and C-7), 102.2 (C-2), 129.7 (C-5 and C-6). The latter material (1.5 g, 11.7 mmol) was epoxidized with *m*-CPBA (6g, 50-60%) in CH_2Cl_2 (80 ml) in the presence of finely powdered Na_2HPO_4 (4.9 g, 35.1 mmol). When the reaction was complete as checked by TLC, acetone (10 ml) was added to destroy excess peroxycarboxylic acid. After 2h the mixture was extracted with $NaHCO_3$ solution, the organic phase was separated and dried (Na_2SO_4/Na_2CO_3). Kugelrohr distillation ($Bp._{20}$ 175°C) gave **6** in 68% yield. ^{1}H-NMR: δ = 1.10 and 1.12 (2s, 3H, CH_3), 1.16 and 1.18 (2s, 3H, CH_3), 3.0 (s, 2H, H on C-1 and C-7), 3.47-3.90 (m, 4H, H on C-2 and C-6). ^{13}C-NMR: δ = 23.27 (CH_3), 24.58 (CH_3), 56.1 (C-2 and C-6), 59.89 (C-1 and C-7), 102.0 (C-4).

General procedure for the synthesis of 2,2-dialkyloxiranes (**3**, **8**-**10**)[45]: To a suspension of NaH (4.7 g, 156 mmol, 80% in oil) in THF (50 ml) and DMSO (30 ml) under inert gas (N_2) a solution of trimethylsulfoxonium iodide (34.3 g, 156 mmol, in 250 ml of DMSO) was added dropwise by maintaining the temperature at ~5°C. After stirring for 10 min, a solution of the corresponding ketone (136 mmol in 60 ml of DMSO) was added over a period of 10-15 min at the same temperature. After removal of the cooling bath stirring was continued for ~3h. Petroleum ether (100 ml) and NH_4Cl solution (semi-saturated, 100 ml) were added and stirring was continued for 10 min. The aqueous phase was extracted with petroleum ether and the combined organic phases were dried (Na_2SO_4/Na_2CO_3), carefully evaporated (some of the products are volatile), and the residue was purified by distillation.

2-Methyl-1,2-epoxyheptane (**3**): yield 66%, $Bp._{14}$ 48°C; ^{1}H-NMR: δ = 0.7-0.8 (t, 3H, J=7Hz, ω-CH_3), 1.10-1.13 (s, 3H, CH_3), 1.13-1.50 (m, 8H, 4 x CH_2), 2.37-2.48 (dd, 2H, J=6.6 and 5.2 Hz, CH_2-O). ^{13}C-NMR: δ = 13.8 (ω-CH_3), 20.8 (CH_2), 22.5 (CH_2), 24.8 (CH_2), 31.8 (CH_3), 36.7 (CH_2), 56.6 (C-1), 56.7 (C-2).

2-Ethyl-1,2-epoxyheptane (**8**): yield 74%, $Bp._{12}$ 62°C; ^{1}H-NMR: δ = 0.79.0.89 (m, 6H, 2 x CH_3), 1.16-1.34 (m, 6H, 3 x CH_2), 1.35-1.65 (m, 4H, 2 x CH_2-C-O), 2.48-2.56 (dd, 2H, J=6.6 and 5.2 Hz, CH_2-O). ^{13}C-NMR: δ = 8.9 (CH_3), 14.0 (CH_3), 22.7 (CH_2), 24.6 (CH_2), 27.1 (CH_2), 32.1 (CH_2-CO), 34.1 (CH_2), 52.1 (CH_2-O), 60.1 (C-O).

2-Methyl-1,2-epoxynonane (**9**): yield 78%, $Bp._{12}$ 79°C; ^{1}H-NMR: δ = 0.75-0.95 (m, 6H, 2 x CH_3), 1.2-1.45 (m, 10H, 5 x CH_2), 1.45-1.65 (m, 2H, CH_2-CO), 2.54-2.60 (dd, 2H, J=11.6 and 5.0 Hz, CH_2-O). ^{13}C-NMR: δ = 14.3 (CH_3), 21.1 (CH_2), 22.8 (CH_2), 25.4 (CH_3), 29.4 (CH_2), 29.8 (CH_2), 31.9 (CH_2), 36.9 (CH_2), 53.9 (CH_2-O), 57.1 (C-O).

3-Methyl-1,2-epoxyundecane (**10**): yield 80%, $Bp._{12}$ 109°C; ^{1}H-NMR: δ = 0.77-0.85 (t, J=7 Hz, 3H, CH_3), 1.10-1.40 (m, 17H, CH_3 and 7 x CH_2), 1.40-1.59 (m, 2H, CH_2-CO), 2.46-2.55 (dd, 2H, J=12 and 4.9 Hz, CH_2-O). ^{13}C-NMR: δ = 14.1 (CH_3), 20.9 (CH_2), 22.8 (CH_2), 25.4 (CH_3), 29.4 (2 x CH_2), 29.8 (2 x CH_2), 32.0 (CH_2), 36.9 (CH_2), 53.8 (CH_2-O), 56.9 (C-O).

General procedure for the base-catalyzed hydrolysis of epoxides: Epoxide (250 mg) was heated overnight (~100°C) in a mixture of aqueous DMSO (15% H_2O, 1 ml) and KOH (0.3N, 0.5 ml). When the reaction was complete (checked by TLC), the solution was neutralized by addition of sat. $NaHSO_3$ solution (10 ml). The diol was extracted with ethyl acetate (3 x 5 ml) and purified by chromatography on silica.

3-Benzyloxypropan-1,2-diol (**2a**): yield 46%, $Bp._{15}$ 140°C; ^{1}H-NMR: δ = 3.0-3.25 (br s, 2H, 2 x OH), 3.40-3.54 (m, 2H, CH_2-O), 3.54-3.71 (m, 2H, CH_2-O), 3.80-3.94 (m, 1H, CH-O), 4.43-4.57 (m, 2H, Ar-CH_2-O), 7.12-7.40 (m, 5H, Ar-H). ^{13}C-NMR: δ = 64.2 (CH_2-OH), 71.0 (CH_2-OBn), 71.9 (Ar-CH_2), 73.8 (CH-OH), 128.0 (2 x *o*-Ar-CH), 128.4 (3 x *m*- and *p*-Ar-CH), 137.9 (*i*-Ar-C).

3,3-Dimethylbutan-1,2-diol (**4a**)[46]: yield 68%, $Bp._{15}$ 97°C; ^{1}H-NMR: δ = 0.79-1.30 (m, 9H, 3 x CH_3), 3.05-3.10 (m, 1H, CH-O), 3.10-3.25 (br s, 2H, 2 x OH), 3.30-3.65 (m, 2H, CH_2-O). ^{13}C-NMR: δ = 26.1 (3 x CH_3), 33.8 [$C(CH_3)_3$], 63.2 (CH_2-O), 79.9 (C-O).

2-Methylheptan-1,2-diol (**3a**): yield 52%, $Bp._2$ 65°C; ^{1}H-NMR: δ = 0.80-0.92 (t, 3H, J=7 Hz, ω-CH_3), 1.08-1.13 (s, 3H, CH_3), 1.20-1.35 (m, 6H, 3 x CH_2), 1.35-1.50 (m, 2H, CH_2-CO), 2.02 (br s, 2H, 2 x OH), 3.30-3.43 (dd, 2H, J=20 and 11 Hz, CH_2-O). ^{13}C-NMR: δ = 14.2 (ω-CH_3), 22.7 (CH_2), 23.2 (CH_2), 23.6 (CH_2), 32.7 (CH_3), 38.9 (CH_2), 69.8 (CH_2-O), 73.4 (C-O).

2-Ethylheptan-1,2-diol (**8a**): yield 62%, $Bp._{1.5}$ 79°C; ^{1}H-NMR: δ = 0.75-0.90 (m, 6H, 2 x CH_3), 1.15-1.30 (m, 6H, 3 x CH_2), 1.35-1.55 (m, 4H, 2 x CH_2-CO), 2.40-2.70 (br s, 1H, OH), 2.70-3.0 (br s, 1H, OH), 3.33-3.43 (dd, J=17 and 3.2 Hz, 2H, CH_2-O). ^{13}C-NMR: δ = 7.93 (CH_3), 14.1 (CH_3), 22.8 (CH_2), 23.3 (CH_2), 28.4 (CH_2), 32.7 (CH_2), 35.5 (CH_2), 66.7 (CH_2-O), 67.9 (C-O).

2-Methylnonan-1,2-diol (**9a**): yield 63%, $Bp._{1.4}$ 81°C; ^{1}H-NMR: δ = 0.85-0.95 (t, J=7 Hz, 3H, CH_3), 1.12-1.18 (s, 3H, CH_3), 1.20-1.42 (m, 10H, 5 x CH_2), 1.42-1.52 (m, 2H, CH_2-CO), 3.37-3.50 (dd, J=21 and 11 Hz, 2H, CH_2-O). ^{13}C-NMR: δ = 14.2 (CH_3), 18.8 (CH_2), 22.8 (CH_2), 23.9 (CH_2), 29.4 (CH_2), 30.4 (CH_2), 32.0 (CH_3), 38.9 (CH_2), 69.9 (CH_2-O), 73.3 (C-O).

2-Methylundecan-1,2-diol (**10a**): yield 68%, $Bp._{1.5}$ 93°C; ^{1}H-NMR: δ = 0.82-1.0 (t, J=7 Hz, 3H, CH_3), 1.12-1.22 (s, 3H, CH_3), 1.22-1.60 (m, 16H, 8 x CH_2), 1.60-2.20 (br s, 2H, 2 x OH), 3.38-3.52 (dd, J=11 and 6.9 Hz, 2H, CH_2-O). ^{13}C-NMR: δ = 14.3 (CH_3), 22.9 (CH_2), 23.4 (CH_2), 23.9 (CH_2), 29.0 (CH_2), 29.5 (CH_2), 29.8 (CH_2), 30.5 (CH_2), 32.1 (CH_3), 39.0 (CH_2), 69.9 (CH_2-O), 73.3 (C-O).

General procedure for the preparation of acetonides from 1,2-diols for GLC-analyses: A mixture of 1,2-diol (20 mg) and 2,2-dimethoxypropane (0.5 ml) was stirred with ion exchange resin (Amberlite IR-120, H^+-form, 200 mg) for 1h at r.t. When the reaction was complete (checked by TLC), solid $NaHCO_3$ was added, diluted with CH_2Cl_2 (5 ml) and the solution was filtered through a short pad of silica. The eluents were directly analyzed by GLC.

General procedure for the synthesis of (*R*)-**3**, **9** and **10**: A Grignard-reagent (2.2 equiv.) was prepared from the corresponding alkyl bromide (*n*-butyl-, *n*-hexyl- *n*-octylbromide, see Scheme 7) and Mg turnings in THF (N_2, r.t., ~1h). The solution was cooled to -70°C and Li_2CuCl_4 (0.08 equiv., 0.1M solution in THF) and (*S*)-2-methylglycidol (1 equiv.), were added successively. The reaction was brought to r.t. over a period of 3h and left for another 5h. Then the mixture was quenched by addition of NH_4Cl solution (semi-saturated) and ethyl ether. The organic phase was separated, washed with NH_4Cl solution, and dried (Na_2SO_4). Evaporation and distillation gave (*R*)-diols **3a**, **9a** and **10a** in 75-88% yield. A sample of diol (1.0 equiv.) was dissolved in CH_2Cl_2 and pyridine (2 equiv.). Toluenesulfonyl chloride (1.5 equiv.) was added at r.t. When the reaction was complete (~48h, checked by TLC) the solution was extracted with HCl (1N), $NaHCO_3$ (5%) and dried (Na_2SO_4). The crude product was purified by silica gel chromatography. The *mono*-tosylate was dissolved in acetone and finely powdered K_2CO_3 (5 equiv.) was added. After 24 h at r.t. the solids were filtered. Evaporation of the volatiles and distillation gave (*R*)-**3**, **9** and **10** in 70% yield.

Biocatalytic Transformations:

All strains were obtained from culture collections. Lyophilized cells of *Rhodococcus erythropolis* NCIMB 11540 were a kind gift of DSM (The Netherlands) and a lyophilized cells of *Corynebacterium* UPT 9 was purchased from Molnar Co., Berlin. The latter preparation has been developed for the biological detoxification of actonitrile-containing HPLC waste-solvents.

Fungi (*Fusarium solani* DSM 62416, *Glomerella cingulata* ATCC 10534, *Diploida gossypina* ATCC 10936) were maintained on agar slants (medium A). Shake flask cultures (250 ml each, medium A) were inoculated and maintained in an incubator (30°C, 80 rpm). At the late exponential growth phase (~72 h) the cells were filtered through a glass-fibre filter. The biomass was resuspended in Tris-buffer (0.1N, pH 7.0), centrifuged and lyophilized. Typical yields of dry cells ranged from 12-17 g/l.The cells could be stored over several months at +5°C without significant loss of activity.

Rhodococcus sp. NCIMB 11215 and NCIMB 11216 were maintained on agar slants (medium B) and grown in a 10 L fermenter at 30°C with sufficient aeration. At the late exponential growth phase (~25-35h) the pink cells were harvested by centrifugation (3000 • g), resuspended in Tris-buffer (0.1N, pH 7.0), centrifuged again and lyophilized. Typical yields of dry cells ranged from 4-5 g/l. The cells could be stored over several months at +5°C without significant loss of activity.

Medium A: Peptone (oxoid, 10 g/l), glucose (10 g/l), malt extract (20 g/l), yeast extract (2 g/l).

Medium B: K_2HPO_4 (6 g/l), KH_2PO_4 (3 g/l), NaCl (0.5 g/l), $MgSO_4$, (0.5 g/l), $(NH_4)_2SO_4$ (1 g/l), yeast extract (2.5 g/l), peptone (2.5 g/l), glucose (10 g/l), $CaCl_2$ (50 mg/l).

General procedure for the screening of epoxide hydrolase activity: Lyophilized microbial cells (50 mg) were rehydrated in Tris-buffer (2 ml, 0.1N, pH 7.0) for 1 h in an Eppendorf-vial on a rotary shaker (180 rpm, r.t.), Then substrate (50 mg) is added and shaking was continued. Over a period of 48 h samples (0.1 ml) were withdrawn, diluted with acetone (0.1 ml) and analyzed by TLC or GLC. A blank sample (without biocatalyst) was run in parallel to check whether any spontaneous hydrolysis was present.

General procedure for the asymmetric hydrolysis of epoxides: Lyophilized microbial cells (2 g) were rehydrated in Tris-buffer (400mL, 0.1M, pH 7.0) for 30 min on a rotary shaker (180 rpm, r.t.). Substrate (1g) was then added and the mixture was agitated at room temperature while the reaction was monitored by TLC or GLC. After an appropriate degree of conversion was reached, the cells were centrifuged, and the formed diols and remaining epoxides were extracted with ethyl acetate from the buffer medium and the pellet in ~90% overall yield. With more water soluble short-chain 1,2-diols, product recovery was considerably improved using the Extrelut-system (Merck). Thus, the reaction mixture was centrifuged and the pellet was extracted with acetone. The clear supernatant was saturated with NaCl and poured onto a short column filled with Extrelute (~15 g for ~20 ml of solution). After the liquid was evenly distributed (20 min), the organic material was eluted from the column with ethyl acetate (monitored by TLC). The combined organic extracts were dried (Na_2SO_4) and evaporated. Extrelute was recycled by suspending it in excess H_2O for several days. After filtration the material was washed several times with acetone and dried (12 Torr, r.t.).

Table 2. Optical rotation values.

Compound	Optical Rotation	e.e. [%]	Reference
(*R*)-**1**	$[\alpha]_D^{21}$ +9.3 (c 3.1, EtOH)	70	47
(*S*)-**1**	$[\alpha]_D^{21}$ -2.82 (c 4.35, EtOH)	21	this study
(*S*)-**1a**	$[\alpha]_D^{20}$ -15.2 (EtOH)	81	47
(*R*)-**1a**	$[\alpha]_D^{21}$ +6.78 (c 5.0, EtOH)	46	this study
(*R*)-**2**	$[\alpha]_D^{32}$ +9.8 (c 5.13, MeOH)	95	40
(*R*)-**2**	$[\alpha]_D^{20}$ +0.515 (c 5.46, MeOH)	5	this study
(*S*)-**2a**	$[\alpha]_D^{20}$ -3.73 (c 17.6, $CHCl_3$)	not given	48
(*R*)-**2a**	$[\alpha]_D^{20}$ +0.528 (c 1.8, $CHCl_3$)	22	this study
(*R*)-**3**	$[\alpha]_D^{25}$ -7.39 (neat)	88	49
(*R*)-**3**	$[\alpha]_D^{20}$ -6.35 (c 3.43, $CHCl_3$)	71	this study
(*R*)-**3a**	$[\alpha]_D^{23}$ +1.9 (c 0.99, $CHCl_3$)	78	50
(*S*)-**3a**	$[\alpha]_D^{23}$ -1.8 (c 0.98, $CHCl_3$)	76	50
(*S*)-**3a**	$[\alpha]_D^{20}$ -3.25 (c 3.6, $CHCl_3$)	96	this study
(*S*)-**4a**	$[\alpha]_D^{20}$ +22.7 (c 1.38, $CHCl_3$)	93	51
(*R*)-**4a**	$[\alpha]_D^{20}$ -28.1 (c 0.61, $CHCl_3$)	not given	52
(*R*)-**4a**	$[\alpha]_D^{23}$ -31.0 (c 0.6, $CHCl_3$)	92	53
(*R*)-**4a**	$[\alpha]_D^{20}$ -20.5 (c 2.0, $CHCl_3$)	88	this study
(*R*)-**8***	$[\alpha]_D^{20}$ -0.33 (c 3.8, $CHCl_3$)	25	this study
(*S*)-**8a***	$[\alpha]_D^{20}$ -1.1 (c 1.5, $CHCl_3$)	70	this study
(*R*)-**9**	$[\alpha]_D^{20}$ -2.29 (c 3.5, $CHCl_3$)	25	this study
(*S*)-**9a**	$[\alpha]_D^{20}$ -5.0 (c 4.0, $CHCl_3$)	98	this study
(S)-**10**§	$[\alpha]_D$ -12.2 (c 0.3, EtOH)	90	38
(*R*)-**10**	$[\alpha]_D^{20}$ -3.65 (c 3.6, $CHCl_3$)	55	this study
(*R*)-**10**	$[\alpha]_D^{20}$ -2.82 (c 2.3, EtOH)	55	this study
(*S*)-**10a**	$[\alpha]_D^{20}$ -4.6 (c 3.6, $CHCl_3$)	>99	this study

* Absolute configuration assumed by comparison of optical rotation values and elution order on chiral GLC (see text). § Erroneous, see text.

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