Deracemization of (±)-2,3-disubstituted oxiranes *via* biocatalytic hydrolysis using bacterial epoxide hydrolases: kinetics of an enantioconvergent process

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Asymmetric biocatalytic hydrolysis of (±)-2,3-disubstituted oxiranes leading to the formation of vicinal diols in up to 97% ee at 100% conversion was accomplished by using the epoxide hydrolase activity of various bacterial strains. The mechanism of this deracemization was elucidated by $^{18}OH_2$-labelling experiments using a partially purified epoxide hydrolase from *Nocardia* EH1. The reaction was shown to proceed in an enantioconvergent fashion by attack of OH^- at the (*S*)-configured oxirane carbon atom with concomitant inversion of configuration. A mathematical model developed for the description of the kinetics was verified by the determination of the four relative rate constants governing the regio- and enantio-selectivity of the process.

Introduction

Chiral epoxides and vicinal diols—the latter employed as their corresponding cyclic sulfite or sulfate esters as reactive intermediates[1]—are extensively used intermediates for the synthesis of chiral target compounds due to their ability to react with a broad variety of nucleophiles in a highly stereoselective fashion. In recent years, intense research has been devoted to the development of catalytic methods for their production.[1–4] Besides chemocatalytic methods,[3] biocatalysis[4] has been shown to provide a useful arsenal of methods as alternatives to the asymmetric epoxidation or dihydroxylation of alkenes. Among several types of enzymes employed, epoxide hydrolases [EC 3.3.2.X] are particularly attractive due to their cofactor-independence. Although enzymes from mammalian sources, such as liver tissue, have been investigated in great detail, aiming at the elucidation of detoxification mechanisms,[5] biotransformations on a preparative scale were severely impeded by the limited supply of enzyme, which is generally isolated from (rat or rabbit) liver tissue derived from phenobarbital-treated animals. As a consequence, the scale of these biotransformations did not surpass the millimolar range. It was only recently that several microbial strains from fungal[6,7] and bacterial origin[8] were identified as convenient and reliable sources of highly selective epoxide hydrolases.[9] This allows the production of biocatalysts in (almost) unlimited amounts and opens the way to the transformation of substrates on a multigram scale.[7]

During our recent studies on the substrate-structure selectivity relationship of several bacterial epoxide hydrolases, several structurally different types of substrates were investigated with varying success.

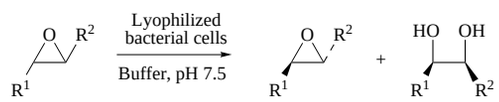
Whereas monosubstituted oxiranes were resolved with insufficient selectivity by bacterial enzymes—fungal epoxide hydrolases seem to be more suitable for these substrates[6,7]—2,2-disubstituted oxiranes were shown to be readily accepted by bacterial enzymes, and kinetic resolutions proceeded with virtually absolute enantiospecificity (*E*-values >200).[8a] For both of these types of substrates, the regioselectivity of the oxirane opening was found to be absolute, *i.e.* attack always occurred at the less substituted carbon atom and the configuration of the stereogenic centre was retained.[10] Thus, the reaction follows a kinetic resolution pattern and the enantioselectivity can accurately be described by using the 'Enantiomeric Ratio' (*E*-value).[11] In contrast, incomplete regioselectivity was observed for styrene oxide-type substrates—presumably due to the presence of a benzylic carbon atom—and for 2,3-disubstituted analogues.[10] As a consequence, *E*-values were found to be inapplicable due to the non-classical nature of the kinetics involved.

In order to broaden the applicability of bacterial epoxide hydrolases, we extended our investigation in two directions, *i.e.* by (i) using substrates bearing two stereogenic centres, and (ii) the elucidation of the kinetics underlying the reaction.[12]

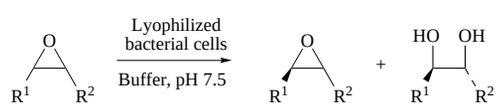
Results and discussion

Biocatalytic hydrolyses

Guided by our experience that bacterial epoxide hydrolases in general do not accept substrates bearing polar functional groups, *cis*- and *trans*-configurated 2,3-dialkyloxiranes bearing alkyl groups of varying chain length were chosen as substrates (Scheme 1). The most promising bacterial strains showing high



rac-**1** $R^1 = CH_3$, $R^2 = n\text{-}C_4H_9$ (2*R*,3*R*)-**1** (2*R*,3*S*)-**1a**
rac-**2** $R^1 = C_2H_5$, $R^2 = n\text{-}C_3H_7$ (3*R*,4*R*)-**2** (3*R*,4*S*)-**2a**
rac-**3** $R^1 = CH_3$, $R^2 = n\text{-}C_5H_{11}$ (2*R*,3*R*)-**3** (2*R*,3*S*)-**3a**
rac-**4** $R^1 = CH_3$, $R^2 = n\text{-}C_6H_{13}$ (2*R*,3*R*)-**4** (2*R*,3*S*)-**4a**



rac-**5** $R^1 = CH_3$, $R^2 = n\text{-}C_4H_9$ (2*R*,3*S*)-**5** (2*R*,3*R*)-**5a**
rac-**6** $R^1 = C_2H_5$, $R^2 = n\text{-}C_3H_7$ (3*S*,4*R*)-**6** (3*R*,4*R*)-**6a**

Scheme 1 Biocatalytic hydrolysis of epoxides (±)-**1**–**6**

epoxide hydrolase activity were selected from our previous studies: *Rodococcus* sp. NCIMB 11216,[8a] *Rhodococcus equi* IFO 3730,[13] *Rhodococcus ruber* DSM 43338,[8b] *Mycobacterium paraffinicum* NCIMB 10420,[13] *Arthrobacter* sp. DSM 312 and three *Nocardia* strains (H8, TB1 and EH1).[8b] Substrates were incubated with rehydrated lyophilized whole cells in Tris-buffer (pH = 7.5). After a certain degree of conversion had been

Table 1 Asymmetric hydrolysis of *trans*-epoxides (±)-**1**–**4**

Entry	Biocatalyst	Substrate	Epoxide	ee (%)	Diol	ee (%)
1	*Rhodococcus* sp. NCIMB 11216	(±)-**1**	(2*R*,3*R*)-**1**	~2	(2*R*,3*S*)-**1a**	69
2	*Rhodococcus equi* IFO 3730	(±)-**1**	(2*R*,3*R*)-**1**	~4	(2*R*,3*S*)-**1a**	65
3	*Rhodococcus ruber* DSM 43338	(±)-**1**	(2*R*,3*R*)-**1**	14	(2*R*,3*S*)-**1a**	86
4	*Mycobacterium paraffinicum* NCIMB 10420	(±)-**1**	(2*R*,3*R*)-**1**	20	(2*R*,3*S*)-**1a**	18
5	*Arthrobacter* sp. DSM 312	(±)-**1**	(2*R*,3*R*)-**1**	~5	(2*R*,3*S*)-**1a**	90
6	*Nocardia* H8	(±)-**1**	(2*R*,3*R*)-**1**	13	(2*R*,3*S*)-**1a**	83
7	*Nocardia* TB1	(±)-**1**	(2*R*,3*R*)-**1**	28	(2*R*,3*S*)-**1a**	84
8	*Nocardia* EH1	(±)-**1**	(2*R*,3*R*)-**1**	35	(2*R*,3*S*)-**1a**	90
9	*Rhodococcus* sp. NCIMB 11216	(±)-**2**	(3*R*,4*R*)-**2**	~6	(3*R*,4*S*)-**2a**	36
10	*Rhodococcus equi* IFO 3730	(±)-**2**	(3*R*,4*R*)-**2**	17	(3*R*,4*S*)-**2a**	30
11	*Rhodococcus ruber* DSM 43338	(±)-**2**	(3*R*,4*R*)-**2**	10	(3*R*,4*S*)-**2a**	40
12	*Mycobacterium paraffinicum* NCIMB 10420	(±)-**2**	(3*R*,4*R*)-**2**	~4	(3*R*,4*S*)-**2a**	~4
13	*Arthrobacter* sp. DSM 312	(±)-**2**	(3*R*,4*R*)-**2**	~4	(3*R*,4*S*)-**2a**	63
14	*Nocardia* H8	(±)-**2**	(3*R*,4*R*)-**2**	12	(3*R*,4*S*)-**2a**	47
15	*Nocardia* TB1	(±)-**2**	(3*R*,4*R*)-**2**	21	(3*R*,4*S*)-**2a**	46
16	*Nocardia* EH1	(±)-**2**	(3*R*,4*R*)-**2**	16	(3*R*,4*S*)-**2a**	38
17	*Rhodococcus* sp. NCIMB 11216	(±)-**3**	(2*R*,3*R*)-**3**	~4	(2*R*,3*S*)-**3a**	77
18	*Rhodococcus* sp. NCIMB 11216	(±)-**4**	(2*R*,3*R*)-**4**	~2	(2*R*,3*S*)-**4a**	78

Table 2 Asymmetric hydrolysis of *cis*-epoxides (±)-**5**,**6**

Entry	Biocatalyst	Substrate	Epoxide	ee (%)	Diol	ee (%)
19	*Rhodococcus* sp. NCIMB 11216	(±)-**5**	(2*R*,3*S*)-**5**	~2	(2*R*,3*R*)-**5a**	71
20	*Rhodococcus equi* IFO 3730	(±)-**5**	(2*R*,3*S*)-**5**	~4	(2*R*,3*R*)-**5a**	78
21	*Rhodococcus ruber* DSM 43338	(±)-**5**	(2*R*,3*S*)-**5**	12	(2*R*,3*R*)-**5a**	95
22	*Mycobacterium paraffinicum* NCIMB 10420	(±)-**5**	(2*R*,3*S*)-**5**	~5	(2*R*,3*R*)-**5a**	66
23	*Arthrobacter* sp. DSM 312	(±)-**5**			n.c.[a]	
24	*Nocardia* H8	(±)-**5**	(2*R*,3*S*)-**5**	~5	(2*R*,3*R*)-**5a**	78
25	*Nocardia* TB1	(±)-**5**	(2*R*,3*S*)-**5**	16	(2*R*,3*R*)-**5a**	88
26	*Nocardia* EH1	(±)-**5**	(2*R*,3*S*)-**5**	24	(2*R*,3*R*)-**5a**	97
27	*Rhodococcus* sp. NCIMB 11216	(±)-**6**	(3*S*,4*R*)-**6**	~3	(3*R*,4*R*)-**6a**	58
28	*Rhodococcus equi* IFO 3730	(±)-**6**	(3*S*,4*R*)-**6**	~1	(3*R*,4*R*)-**6a**	36
29	*Rhodococcus ruber* DSM 43338	(±)-**6**	(3*S*,4*R*)-**6**	~8	(3*R*,4*R*)-**6a**	42
30	*Mycobacterium paraffinicum* NCIMB 10420	(±)-**6**	(3*S*,4*R*)-**6**	~8	(3*R*,4*R*)-**6a**	71
31	*Arthrobacter* sp. DSM 312	(±)-**6**			n.c.[a]	
32	*Nocardia* H8	(±)-**6**	(3*S*,4*R*)-**6**	~1	(3*R*,4*R*)-**6a**	66
33	*Nocardia* TB1	(±)-**6**	(3*S*,4*R*)-**6**	~3	(3*R*,4*R*)-**6a**	77
34	*Nocardia* EH1	(±)-**6**	(3*S*,4*R*)-**6**	~1	(3*R*,4*R*)-**6a**	66

[a] n.c. = no conversion.

reached within 10–200 h, the diol formed and the remaining epoxide were isolated and analysed for enantiomeric purity and absolute configuration.

The results depicted in Tables 1 and 2 show the following trends:

(i) The *cis*- or *trans*-configuration of the epoxide has only a minor impact on the acceptance of substrates, as both series were shown to be converted by the majority of the strains. However, in general, the reaction rate was about twice as high for *trans*-epoxides as compared with their *cis*-analogues. The only notable exception was *Arthrobacter* sp. DSM 312, which was unable to hydrolyse *cis*-epoxides (±)-**5** and (±)-**6** (entries 23 and 31).

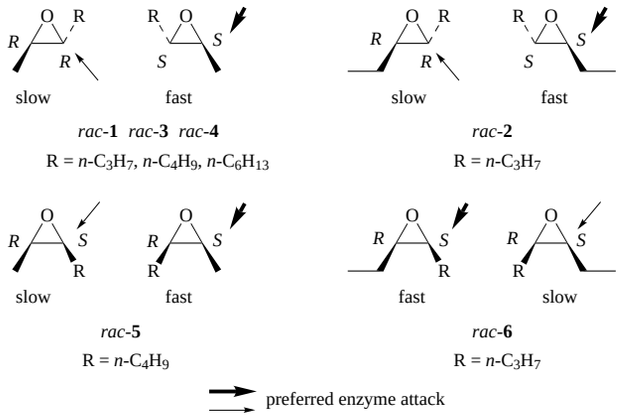
(ii) As may be deduced from the enantiomeric purity of diols formed and the remaining non-hydrolysed epoxides, the selectivity of the reaction covered a wide range, from low to excellent. In general, the selectivities obtained from *cis*-epoxides (±)-**5** and (±)-**6** were slightly higher than those for *trans*-oxiranes (±)-**1** and (±)-**2**, although this effect was not pronounced. This can also be verified by comparison of the kinetic data (see below). The only notable exception to this trend was found with substrates (±)-**1** and (±)-**5** with *Nocardia* H8 (entries 6 and 24).

(iii) By comparing the results obtained with *Rhodococcus* sp. NCIMB 11216 using an homologous series of *trans*-configurated substrates [(±)-**1**, (±)-**3**, (±)-**4**] the length of the alkyl chain was found to have a negligible impact on the selectivity. This is in sharp contrast to the findings obtained when 2,2-dialkyloxiranes were used as substrates.[8a]

(iv) The selectivity dropped when the epoxy moiety was moved towards the centre of the *n*-alkyl chain [compare substrates (±)-**1** and (±)-**2** or (±)-**5** and (±)-**6**]. This fact is understandable since one moves towards quasi-symmetrical substrates, which fits well with the observation that *meso*-oxiranes were found to be unsuitable substrates.[8a,9a]

(v) Despite the fact that a range of eight different bacterial strains were employed, the stereochemical course of the reaction showed a remarkable homogeneous picture: In each case, *trans*-epoxides led to the exclusive formation of *threo*-diols whereas *cis*-configurated substrates gave the corresponding *erythro*-counterparts. No trace of products from non-matching stereoselectivity could be detected. As a consequence, the reaction can be formulated as proceeding *via* S_N2-type hydrolytic opening of the epoxide, leading to inversion of configuration at the oxirane carbon atom being attacked. This fact is in line with the generally accepted proposal for the mechanism of epoxide hydrolases.[14]

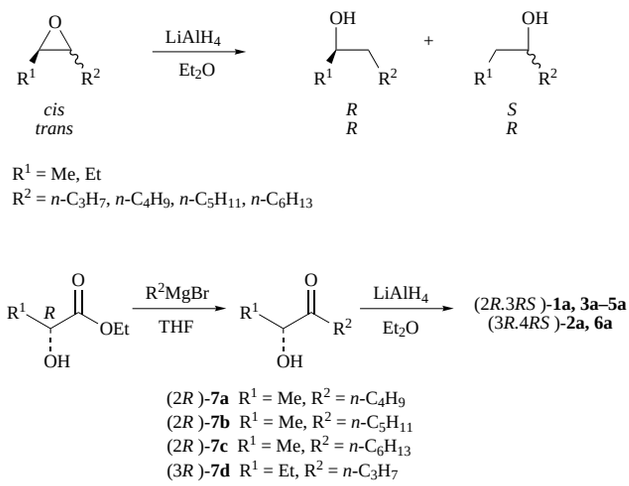
(vi) Regardless of the stereochemistry of the substrate, all of the strains showed a marked preference for attack at (*S*)-configurated oxirane carbon atoms (Scheme 2). This strict preference for the configuration of the oxirane carbon atom being attacked was coupled by a trend in the regio-selectivity in favour of the less sterically hindered position. Thus, with substrates from the *trans*-series [(±)-**1** through (±)-**4**], inversion of the more accessible (*S*)-configurated carbon atom led to (2*R*,3*S*)- and (3*R*,4*S*)-diols, respectively, while (*R*,*R*)-epoxides were hydrolysed at a slower rate. This was also true for *cis*-2,3-epoxyheptane, which led to (2*R*,3*R*)-heptanediol and remaining (2*R*,3*S*)-epoxide. The only exception to this rule was substrate (±)-**6**, where the *less accessible* (*S*)-configurated oxirane C-atom was preferred.



Scheme 2 Stereochemical course of enzymatic hydrolysis

Determination of regio- and enantio-selectivity

The absolute configuration of epoxides was determined as follows (Scheme 3): $LiAlH_4$ reduction of 2,3-epoxyalkenes **1**, **3**, **4**



Scheme 3 Determination of absolute configuration

and **5** led to a mixture of corresponding alkan-2-ols and -3-ols, respectively. The configuration of the alkan-2-ols thus obtained was determined through GLC analysis of the corresponding trifluoroacetate esters on a chiral stationary phase *via* co-injection with samples obtained from commercially available reference compounds. Once the configuration of the centre at C-2 was determined, the remaining configuration at C-3 could be elucidated from the *cis*- and *trans*-configuration of the starting epoxides, which were obtained through MCPBA-oxidation of the corresponding *Z*- and *E*-alkene. An analogous approach was chosen for 3,4-epoxyalkanes **2** and **6**. Thus, $LiAlH_4$ reduction led to a mixture of heptan-3-ol and (achiral) heptan-4-ol. The configuration of the former was elucidated *via* GLC analysis of the trifluoroacetic acid (TFA) derivative on a chiral stationary phase using reference material obtained as described above.

Enantiopure reference material for diols obtained from biocatalytic hydrolysis was synthesized as follows (Scheme 3): addition of the corresponding Grignard reagent to commercially available ethyl (*R*)-lactate or (*R*)-2-hydroxybutanoate, respectively, gave rise to (*R*)-α-hydroxyalkanones **7a**–**d**. The latter were reduced with $LiAlH_4$ to yield mixtures of the corresponding diastereomeric diols (2*R*,3*RS*)-**1a**, **-3a**, **-5a** and (3*R*,4*RS*)-**2a**, **-6a**. Comparison with independently synthesized *rac-threo*- and *rac-erythro*-diols[15] revealed their relative configuration. The configuration of all of the diols obtained from biocatalytic hydrolyses was elucidated *via* co-injection on GLC using a chiral stationary phase.

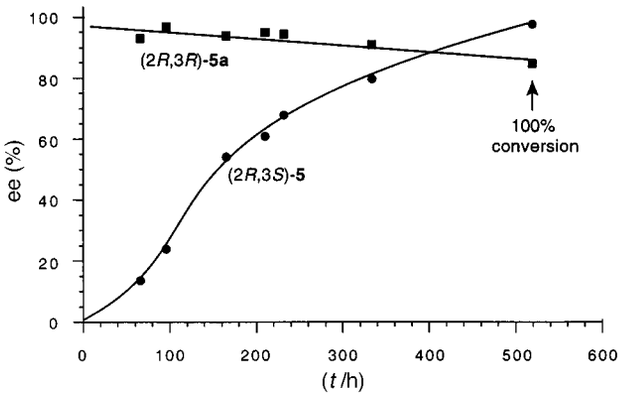
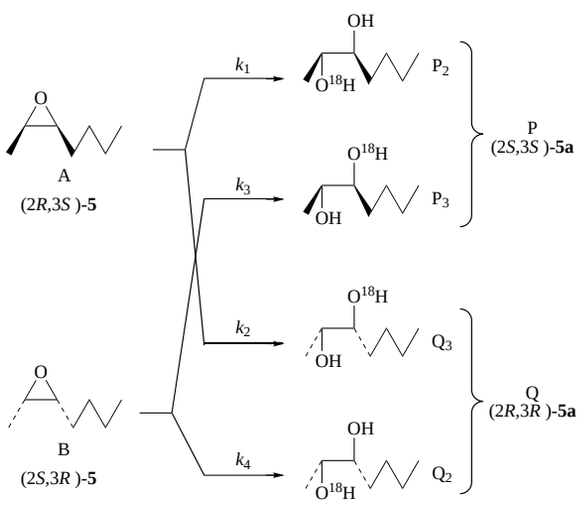


Fig. 1 Time-course for hydrolysis of compound (±)-**5**

Kinetics

The experiment showing the highest apparent selectivity—*i.e.* hydrolysis of (±)-**5** with *Nocardia* EH1 (entry 26)—was selected for the investigation of the kinetics of the system. When the time-course of the hydrolysis of compound (±)-**5** was followed with respect to the enantiomeric purity of formed diol (2*R*,3*R*)-**5a** and remaining non-hydrolysed epoxide (2*R*,3*S*)-**5**, an unexpected phenomenon was observed (Fig. 1): the enantiomeric excess (ee) of the diol did not decline beyond a conversion of ~50%—which would be expected from classical kinetic resolution—but rather remained at a constant level of ⩾90% ee until the starting material was completely consumed. From these data, it was concluded that the overall process comprises four different individual reactions, characterized by the relative rate constants k_1 through k_4 (Scheme 4).[16] First, two substrate



Scheme 4 Kinetics for hydrolysis of epoxide (±)-**5**

enantiomers A and B may react at different reaction rates (corresponding to the sums of $k_1 + k_2$ and $k_3 + k_4$, respectively), leading to product enantiomers P and Q. Second, each enantiomer may be hydrolysed through two pathways of different regioselectivity (k_1 or k_2, and k_3 or k_4, respectively), involving attack at carbon 2 or 3 (Scheme 4).[10] In order to determine the individual relative rate constants, the reaction was performed in $^{18}OH_2$ using a partially purified epoxide hydrolase preparation.[17] The labelled diol products thus obtained were analysed by GLC/MS using a chiral stationary phase. The data show that (i) only one oxygen atom was introduced into the substrate, which allowed (ii) the position of the ^{18}O-label to be determined by analysis of the diol fragmentation pattern during glycol cleavage upon electron-impact ionization. Additionally, the enantiomeric composition of epoxide (A/B) and diol (corresponding to the sums of $Q_2 + Q_3/P_2 + P_3$)[16] was analysed by GLC on a chiral stationary phase (Table 3).

For the calculation of the individual relative rate constants from experimentally obtained data, a mathematical model was developed.[18] The derivations are based upon the following assumptions:

(i) The specific activity of the enzyme remains constant throughout the reaction, implying that enzyme deactivation caused by mechanical or chemical stress is negligible. (ii) Neither inhibition, (iii) nor spontaneous—and thus non-specific—hydrolysis[19] is impeding the reaction, (iv) the reactions are irreversible and (v) at the time of measurement, the starting material is still in excess.

The system described in Scheme 4 can be characterized by the following differential equations, with k_i being the first-order relative rate constants:

$$\frac{dA}{dt} = -(k_1 + k_2)A \quad (1)$$

$$\frac{dB}{dt} = -(k_3 + k_4)B \quad (2)$$

$$\frac{dP_2}{dt} = k_1 A \quad (3)$$

$$\frac{dQ_3}{dt} = k_2 A \quad (4)$$

The corresponding equations for P_3 and Q_2 are obtained in an analogous fashion. After dividing (3) by (4) and subsequent integration, the quotients of relative rate constants are obtained as (5) and (6):

$$\frac{k_1}{k_2} = \frac{P_2}{Q_3} \quad (5)$$

$$\frac{k_3}{k_4} = \frac{P_3}{Q_2} \quad (6)$$

The ratios of P_2/Q_3 and P_3/Q_2 were experimentally determined by GLC/MS *via* analysis of the peaks having an *m*/*z* of 87 (products P_2 and Q_2) and 89 (corresponding to P_3 and Q_3), which result from ^{18}O-incorporation at C_2 and C_3, respectively.[20]

To obtain the values of k_1/k_3 and k_2/k_4, $(k_1 + k_2)/(k_3 + k_4)$ has to be calculated first: thus, integration of equations (3) and (4)—with P_2 and P_3 at $t = 0$ being nil—gives equations (7)

$$P_2 = \frac{k_1 A_0}{k_1 + k_2}[1 - e^{-(k_1 + k_2)t}] \quad (7)$$

Table 3 Enantiomeric purities and label-distribution for substrate (±)-**5** using *Nocardia* EH1

	ee (%)		Intensity (counts)			
Time (*t*/h)	(2*R*,3*S*)-**5**	(2*R*,3*R*)-**5a**	P_2	P_3	Q_2	Q_3
22	3.3	95.9	62	10	2701	144
110	15.7	97.3	120	31	5309	308

$$P_3 = \frac{k_3 B_0}{k_3 + k_4}[1 - e^{-(k_3 + k_4)t}] \quad (8)$$

and (8), with A_0 and B_0 being the initial concentrations of substrates A and B at $t = 0$, respectively.[21] Equations (9) and (10) are derived in an analogous fashion:

$$Q_2 = \frac{k_4 B_0}{k_3 + k_4}[1 - e^{-(k_3 + k_4)t}] \quad (9)$$

$$Q_3 = \frac{k_2 A_0}{k_1 + k_2}[1 - e^{-(k_1 + k_2)t}] \quad (10)$$

After division of equations (1) and (2), followed by integration, equation (11) is obtained:

$$\frac{A}{B} = \frac{e^{-(k_1 + k_2)t}}{e^{-(k_3 + k_4)t}} \quad (11)$$

The quotient $(k_1 + k_2)/(k_3 + k_4)$ is calculated from the experimentally determined enantiomeric composition of the diol, which corresponds to $P_2 + P_3/Q_2 + Q_3$. If—for reasons of clarity—$e^{-(k_1 + k_2)t}$ is substituted for x, $e^{-(k_3 + k_4)t}$ can be expressed as Bx/A through equation (11). Now, the relative rate constants can be related to the enantiomeric composition of the product P and Q:

$$\frac{P_2 + P_3}{Q_2 + Q_3} = \frac{\frac{k_1}{k_1 + k_2}(1 - x) + \frac{k_3}{k_3 + k_4}\left(1 - \frac{B}{A}x\right)}{\frac{k_2}{k_1 + k_2}(1 - x) + \frac{k_4}{k_3 + k_4}\left(1 - \frac{B}{A}x\right)} \quad (12)$$

If x is expressed explicitly from equation (11), one obtains equation (13).

$$x = \frac{\frac{1}{1 + \frac{k_2}{k_1}} + \frac{1}{1 + \frac{k_4}{k_3}} - \frac{P_{2+3}}{Q_{2+3}} \cdot \left(\frac{1}{1 + \frac{k_1}{k_2}} + \frac{1}{1 + \frac{k_3}{k_4}}\right)}{\frac{1}{1 + \frac{k_2}{k_1}} + \frac{B}{A} \cdot \frac{1}{1 + \frac{k_4}{k_3}} - \frac{P_{2+3}}{Q_{2+3}} \cdot \left(\frac{1}{1 + \frac{k_1}{k_2}} + \frac{B}{A} \cdot \frac{1}{1 + \frac{k_3}{k_4}}\right)} \quad (13)$$

Now, the desired quotient can be expressed as in equation (14):

$$\frac{k_1 + k_2}{k_3 + k_4} = \frac{\ln x}{\ln\left(\frac{B}{A}x\right)} \quad (14)$$

Other quotients of relative rate constants, such as k_1/k_3, k_2/k_4, *etc.*, can be obtained from equations (14), (5) and (6). Finally—if the lowest *k*-value of a given set of four is arbitrarily set to one—the following k_is are obtained (Table 4).

The accuracy of the mathematical model and the validity of the basic assumptions discussed above were verified by hydrolysing substrate (±)-**5** with *Nocardia* EH1 epoxide hydrolase

Table 4 Relative rate constants for substrates (±)-**5** and (±)-**1**

Entry	Substrate	Biocatalyst	Time (*t*/h)	k_1	k_2	k_3	k_4
1	(±)-**5**	*Nocardia* EH1	22	6	17	1	326
2	(±)-**5**	*Nocardia* EH1	110	3.5	17	1	343
3	(±)-**5**	*Rhodococcus ruber* DSM 43338	22	14	63	1	498
4	(±)-**5**	*Rhodococcus equi* IFO 3730	22	4	6	1	39
5	(±)-**1**	*Nocardia* EH1	23	1	7	1.1	36
6	(±)-**1**	*Rhodococcus ruber* DSM 43338	23	1.2	1.4	1	34

using $^{18}OH_2$ as solvent (label content 97%). The reaction was stopped at two different intervals, after 22 and 110 h, respectively. The enantiomeric purity of the diol (2*R*,3*R*)-**5a** formed and of the remaining non-hydrolysed epoxide (2*R*,3*S*)-**5** were determined by GLC analysis and the label-distribution was analysed by GLC/MS on a chiral stationary phase (Table 3). These data were used for the calculation of the relative k_is. As may be seen from Table 4 (entries 1 and 2), the values for the relative rate constants were within a very narrow margin of experimental error, indicating the applicability of the mathematical model and the validity of the basic assumptions for the time period investigated. Analysis of the relative rate constants for substrate (±)-**5** reveals that the (2*S*,3*R*)-epoxide (**B**, Scheme 4) is the fast reacting enantiomer, which is hydrolysed with excellent regioselectivity at the more accessible C-2 atom (k_4/k_3 >300:1). On the other hand, the (2*R*,3*S*)-enantiomer (**A**) is transformed about 14 times slower ($k_3 + k_4/k_1 + k_2$ = 327:23) and low regioselectivity in favour of the *sterically more hindered C-3 atom* is observed (k_2/k_1 = 17:5). Although the selectivity for the enantiodiscrimination *per se* is relatively low (*E* ~14–17),[22] the fact that both enantiomers are hydrolysed with opposite regioselectivity yields (2*R*,3*R*)-**5a** in >95% enantiomeric excess. Since both epoxide substrate enantiomers are hydrolysed to give the same (2*R*,3*R*)-diol product, the reaction constitutes an enantioconvergent process. Thus it can be run to completion and 100% theoretical yield can be accrued.

Comparable results—*i.e.* low to moderate enantioselectivity in favour of the (2*S*,3*R*)-enantiomer of **5** (*E* ~6)[22] with matching (opposite) regioselectivity—were obtained for *Rhodococcus ruber* DSM 43338 (Table 4, entry 3). On the other hand, significantly lower regio- and enantio-selectivities were observed with *Rhodococcus equi* IFO 3730 (entry 4).

A similar situation was found for the *trans*-configurated substrate (±)-**1**. Both strains investigated showed reduced enantioselectivities, mainly due to the drop of the k_4-values by about one order of magnitude, while the regioselectivities remained within the same range. Thus, the enantiomeric purity of diol (2*R*,3*S*)-**1a** did not exceed a value of 90%.

Finally, the applicability of the enantioconvergent biocatalytic hydrolysis was demonstrated with a preparative-scale reaction. Thus, when substrate (±)-**5** was treated with lyophilized cells of *Nocardia* EH1 until the starting material had been completely consumed, diol (*R*,*R*)-**5a** was isolated in 79% chemical yield and 91% enantiomeric purity as the sole product.

Cases for a non-classical deracemization of epoxides, which leads to the highly desired formation of a single product enantiomer in 100% theoretical yield, are rare. For instance, the hydrolysis of (±)-3,4-epoxytetrahydropyran[23] and several *cis*-β-alkyl-substituted styrene oxides[24] by hepatic microsomal epoxide hydrolase proceeded in an enantioconvergent manner leading to the corresponding (*R*,*R*)-diols as the sole product. Similarly, soybean epoxide hydrolase converted (±)-*cis*-9,10-epoxyoctadec-12(*Z*)-enoic and (±)-*cis*-12,13-epoxydec-9(*Z*)-enoic acid into the corresponding (*R*,*R*)-dihydroxy acids.[25] All of the above-mentioned transformations, however, were aimed at the elucidation of enzyme mechanisms and were performed only on an analytical (<1 mmol) scale. It was only recently that preparative-scale hydrolysis of (±)-*cis*-β-methylstyrene oxide by the fungus *Beauveria bassiana*[9] was reported to afford (1*R*,2*R*)-1-phenylpropane-1,2-diol in 85% yield and 98% ee. However, in none of these studies were the kinetics of these systems investigated.

Experimental

Reactions were monitored by TLC (silica gel Merck 60 F_{254}), compounds were visualized by spraying with vanillin–conc. H_2SO_4 (5 g dm^{-3}) or Mo-reagent [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (1.1 g dm^{-3}), $Ce(SO_4)_2\cdot 4H_2O$ (4 g dm^{-3}) in H_2SO_4 (10%)]. Preparative chromatography was performed on silica gel Merck 60 (40–63 μm). The ee of epoxides and diols was determined by GLC (Shimadzu GC-14A, equipped with FID) on a CP-Chirasil-DEX CB column (25 m; 0.32 mm; 0.25 μm film; H_2). GLC/MS data were recorded on an HP 6890 GC/MS spectrometer. The EI quadrupole detector was set to 70 eV, 130 °C, auxiliary temp. 180 °C. ^{18}O-Labelled water (95–98% ^{18}O-content) was purchased from Promochem. Optical rotation values were measured on a Perkin-Elmer polarimeter 341 at 589 nm (Na-line) in a 1 dm cuvette, and are given in units of 10^{-1} deg cm^2 g^{-1}. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution on a Bruker MSL 300 (300 MHz and 75.47 MHz, respectively). Chemical shifts are reported in δ from $SiMe_4$ as internal standard; coupling constants are given in Hz. Light petroleum refers to the fraction with distillation range 60–90 °C.

Synthesis of substrates

Epoxides (±)-**1**–**6** were obtained *via* epoxidation of the corresponding alkene (17 mmol) in CH_2Cl_2 (50 cm^3) using *m*-chloroperbenzoic acid (70%; 1.2 mol equiv.) containing finely powdered K_2CO_3 (2 mol equiv.) at 0 °C (14 h). After extractive work-up (10% aq. $Na_2S_2O_5$, then saturated aq. $NaHCO_3$), the organic layer was dried (Na_2SO_4) and evaporated, and the products were distilled through a short Vigreux column; yields ranged from 58 to 96%.

***trans*-2,3-Epoxyheptane 1.** δ_H 0.88 (t, *J* 7.0, 3 H, 7-H_3), 1.12 (d, *J* 6.0, 3 H, 1-H_3), 1.21–1.44 (m, 6 H, 4-, 5- and 6-H_2), 2.41 (dt, J_t 5.3, J_d 2.2, 1 H, 3-H) and 2.55 (dq, J_q 5.2, J_d 2.2, 1 H, 2-H); δ_C 13.73 (C-7), 17.43 (C-1), 22.40 (C-6), 28.03 (C-5), 31.62 (C-4), 54.23 (C-2) and 59.46 (C-3).

***trans*-3,4-Epoxyheptane 2.** δ_H 0.82–0.93 (m, 6 H, 1- and 7-H_3), 1.43–1.55 (m, 6 H, 2-, 5- and 6-H_2) and 2.52–2.63 (m, 2 H, 3- and 4-H); δ_C 9.73, 13.75, 19.25, 25.08, 34.06, 58.18 and 59.65.

***trans*-2,3-Epoxyoctane 3.** δ_H 0.80 (t, *J* 7.0, 3 H, 8-H_3), 1.16–1.43 (m, 11 H, 1-H_3, 4–7-H_2), 2.52 (dt, J_t 5, J_d 2.0, 1 H, 3-H) and 2.64 (dq, J_q 6.0, J_d 2.0, 1 H, 2-H); δ_C 13.90, 17.61, 22.54 and 25.65 (C-5), 31.61, 31.97 (C-1 and -4), 54.45 (C-3) and 59.72 (C-2).

***trans*-2,3-Epoxynonane 4.** δ_H 0.80 (t, *J* 7.0, 3 H, 9-H_3), 1.16–1.48 (m, 13 H, 1-H_3, 4–8-H_2), 2.53 (dt, J_d 2.0, J_t 8.0, 1 H, 3-H) and 2.64 (dq, J_d 2.0, J_q 5.0, 1 H, 2-H); δ_C 13.98 (C-9), 17.61, 22.53, 25.94, 29.09, 31.74, 32.02, 54.45 (C-2) and 59.72 (C-3).

***cis*-2,3-Epoxyheptane 5.** δ_H 0.87 (t, *J* 7.0, 3 H, 7-H_3), 1.21 (d, *J* 6.0, 3 H, 1-H_3), 1.25–1.52 (m, 6 H, 4–6-H_2), 2.85 (dq, J_q 6.2, J_d 2.0, 1 H, 2-H) and 2.98 (dt, J_t 5.5, J_d 2.0, 1 H, 3-H); δ_C 13.15 (C-7), 13.98 (C-1), 22.57 (C-6), 27.21 (C-5), 28.58 (C-4), 52.60 (C-2) and 57.1 (C-3).

***cis*-3,4-Epoxyheptane 6.** δ_H 0.83–0.95 (m, 6 H, 1- and 7-H_3), 1.32–1.52 (m, 6 H, 2-, 5- and 6-H_2) and 2.71–2.86 (m, 2 H, 3- and 4-H); δ_C 10.47, 13.52, 19.86, 21.06, 29.67, 56.98 and 58.13.

Synthesis of reference material

Diols (±)-**1a**–**6a** were obtained *via* OsO_4-catalysed dihydroxylation of the corresponding *Z*- or *E*-alkene (5 mmol) in acetone (5 cm^3) using *N*-methylmorpholine *N*-oxide (6 mmol) as reoxidant (0 °C, 12 h). After extractive work-up (10% aq. $Na_2S_2O_5$, semisaturated aq. NH_4Cl) the products were purified *via* silica gel chromatography. Yields ranged from 54 to 80%.

***erythro*-Heptane-2,3-diol 1a.** δ_H 0.85 (t, *J* 7.0, 3 H, 7-H_3), 1.05 (d, *J* 7.0, 3 H, 1-H_3), 1.22–1.50 (m, 6 H, 4–6-H_2), 2.20–2.45 (s, br, 2 H, OH) and 3.55–3.65 and 3.72–3.83 (m, 2 × 1 H, 2- and 3-H); δ_C 13.99 (C-7), 16.25 (C-1), 22.73 (C-6), 28.32 (C-5), 31.51 (C-4), 70.44 (C-2) and 74.91 (C-3).

***erythro*-Heptane-3,4-diol 2a.** δ_H 0.92 (t, *J* 7.0, 3 H, 7-H_3), 0.96 (t, *J* 7.0, 3 H, 1-H_3), 1.05–1.60 (m, 6 H, 2-, 5- and 6-H_2) and 3.44–3.68 (m, 2 H, 3- and 4-H); δ_C 10.01 (C-7), 14.10 (C-1), 18.85 (C-6), 26.32 (C-2), 35.61 (C-5), 73.94 and 76.03 (C-3, -4).

***erythro*-Octane-2,3-diol 3a.** δ_H 0.80 (t, *J* 7.0, 3 H, 8-H_3), 1.18 (d, *J* 7.0, 3 H, 1-H_3), 1.05–1.50 (m, 8 H, 4–7-H_2), 2.95 (s, br, 2 H, OH), 3.64 (dt, J_t 4.0, J_d 2.0, 1 H, 3-H) and 3.80 (dq, J_q 6.5, J_d 3.0, 1 H, 2-H); δ_C 14.10 (C-8), 16.61 (C-7), 22.64 (C-1), 25.74 (C-4), 29.8 (C-5), 31.95 (C-6), 70.51 (C-3) and 75.02 (C-2).

***erythro*-Nonane-2,3-diol 4a.** δ_H 0.84 (t, *J* 7.0, 3 H, 9-H_3), 1.18 (t, *J* 7.0, 3 H, 1-H_3), 1.02–1.65 (m, 10 H, 4–8-H_2), 2.95 (s, br, 2 H, OH), 3.64 (dt, J_t 4.0, J_d 2.0, 1 H, 3-H) and 3.80 (dq, J_q 6.5, J_d 3.0, 1 H, 2-H); δ_C 14.03 (C-9), 16.64 (C-8), 22.65 (C-1), 23.40 (C-7), 25.73 (C-4), 31.82 (C-5), 31.94 (C-6), 70.53 (C-3) and 75.01 (C-2).

***threo*-Heptane-2,3-diol 5a.** δ_H 0.85 (t, *J* 7.2, 3 H, 7-H_3), 1.22 (d, *J* 6.0, 3 H, 1-H_3), 1.22–1.50 (m, 6 H, 4–6-H_2), 2.85 (s, br, 1 H, OH), 3.25 (s, br, 1 H, OH) and 3.20–3.35 and 3.47–3.61 (m, 2 × 1 H, 2- and 3-H); δ_C 14.04 (C-7), 19.45 (C-1), 22.75 (C-6), 27.79 (C-5), 32.99 (C-4), 70.93 (C-2) and 76.77 (C-3).

***threo*-Heptane-3,4-diol 6a.** δ_H 0.91 (t, *J* 7.1, 3 H, 7-H_3), 0.97 (t, *J* 7.0, 3 H, 1-H_3), 1.20–1.63 (m, 6 H, 2-, 5- and 6-H_2) and 3.24–3.51 (m, 2 H, 3- and 4-H); δ_C 10.50, 14.12, 19.21, 24.15, 33.24, 74.35 and 76.42.

Growth of microorganisms

Bacteria were obtained from culture collections except for the *Nocardia* spp., which were a kind gift of J. de Bont (Wageningen, NL) and C. Syldatk (Stuttgart). The strains were grown in shake-flask cultures at 30 °C on the following medium: yeast extract (10 g dm^{-3}), peptone (10 g dm^{-3}), glucose (10 g dm^{-3}), NaCl (2 g dm^{-3}), $MgSO_4 \cdot 7H_2O$ (0.147 g dm^{-3}), NaH_2PO_4 (1.3 g dm^{-3}) and K_2HPO_4 (4.4 g dm^{-3}). At the late exponential growth phase (~25–40 h) the cells were harvested by centrifugation (5000 ***g***; 20 min; 25–30 g dm^{-3} wet cells), resuspended in Tris-HCl buffer (0.05 M, pH 8.0), centrifuged again and lyophilized. Typical yields of dry cells ranged from ~3 to 5 g dm^{-3}. The cells could be stored over several months at +5 °C without significant loss of activity.

Partially purified *Nocardia* EH1 epoxide hydrolase preparation

All steps of the protein purification were performed at +4 °C. Freshly grown wet cells were resuspended in Tris-HCl buffer (0.05 M, pH 8.0) and disintegrated using a glass bead mill (Vibrogen Zellmühle, shaking frequency 75 Hz; glass bead diameter 0.35 mm; 8 min). After removal of the cell debris by centrifugation (12 000 ***g***; 40 min), the supernatant was subjected to hydrophobic interaction chromatography (Phenyl Sepharose CL-4B, Sigma) using a 9.5 × 2.5 cm column. The enzyme appeared very lipophilic since it was readily adsorbed onto the stationary phase from 50 mM Tris-buffer without addition of $(NH_4)_2SO_4$. By using a linear gradient of adsorption buffer against deionized water, most of the adsorbed proteins could be removed, while the epoxide hydrolase remained still bound. Finally, the epoxide hydrolase was desorbed by using further volumes of deionized water (flow rate 10 cm^3 min^{-1}). The active fractions were pooled and adjusted with Tris-HCl buffer (0.5 M, pH 8.0) to yield a final buffer concentration of 0.05 M. This solution was then lyophilized to give a stable, partially purified epoxide hydrolase preparation which was used for $^{18}OH_2$-labelling experiments.

General procedure for the asymmetric hydrolysis of epoxides

Lyophilized bacterial cells (90 mg) were rehydrated in Tris-HCl buffer (0.05 M, pH 8.0; 5 cm^3) for 30 min on a rotary shaker (180 rpm) at ambient temperature. Substrate was then added (100 mm^3) in one portion and the mixture was agitated at room temp., while the reaction was monitored by TLC or GLC. The specific activity of the reactions was in the range ~1000–2000 μmol mg^{-1} h^{-1} (with respect to pure protein). After an appropriate degree of conversion was reached, acetone was added (1 cm^3) and the cells were removed by centrifugation (12 000 ***g***; 10 min). The organic material was extracted from the supernatant with ethyl acetate, and the organic phase was dried (Na_2SO_4) and carefully evaporated under partially reduced pressure. The ee of epoxides and diols was determined by GLC on a chiral stationary phase without further purification. For optical rotation values of products having an ee >50% see Table 6. Retention times for GLC on a chiral stationary phase are given in Table 5.

Preparative-scale reaction

Lyophilized whole cells of *Nocardia* EH1 (4 g) were rehydrated in Tris-buffer (50 cm^3; 50 mM, pH 8.0) for 1 h before addition of epoxide (±)-**5** (0.82 g, 7.1 mmol). The mixture was agitated on a rotary shaker at room temp. until the starting material was completely consumed (~64 h). After addition of acetone (30 cm^3), the mixture was centrifuged and the cells and the supernatant were separately extracted with ethyl acetate (3×). The combined organic layer was dried (Na_2SO_4), the volatiles were evaporated off, and the residue was purified by silica gel chromatography to remove minor impurities and dyes which arose from the cells, to furnish diol (*R*,*R*)-**5a** (0.75 g, 79%, ee 91%), $[\alpha]_D^{20}$ +20.45 (*c* 1, EtOH).

^{18}O-Labelling studies

A sample of partially purified lyophilized epoxide hydrolase (10 mg) was rehydrated in $^{18}OH_2$ (label content 95–97%; 120 mm^3) at 30 °C for 1 h. Then substrate (4 mm^3) was added and shaking was continued. Samples were extracted with ethyl acetate (120 mm^3), the organic layer was dried (Na_2SO_4) and analysed by GLC/MS.

Table 5 GLC data

Compound	Gas pressure (bar)	Temperature (*T*/°C)	Retention time (t_R/min)
1	0.75	40	6.5 (2*S*,3*S*)/6.7 (2*R*,3*R*)
1a	1.0	100	7.8 (2*R*,3*S*)/8.1 (2*S*,3*R*)
2	0.5	45	6.8 (3*S*,4*S*)/7.2 (3*R*,4*R*)
2a	1.0	100	7.8 (3*R*,4*S*)/8.0 (3*S*,4*R*)
3a	1.0	115	5.9 (2*R*,3*S*)/6.3 (2*S*,3*R*)
4a	1.0	120	7.5 (2*R*,3*S*)/8.2 (2*S*,3*R*)
5	0.5	60	5.5 (2*S*,3*R*)/5.6 (2*R*,3*S*)
5a	1.0	100	6.7 (2*S*,3*S*)/7.3 (2*R*,3*R*)
6	0.5	60	5.1 (3*S*,4*R*)/5.6 (3*R*,4*S*)
6a	1.0	100	6.1 (3*S*,4*S*)/6.8 (3*R*,4*R*)
Heptan-2-yl trifluoroacetate	0.75	50	4.3 (*S*)/4.5 (*R*)
Heptan-3-yl trifluoroacetate	0.75	50	3.4 (*S*)/3.6 (*R*)
Octan-2-yl trifluoroacetate	1.0	50	7.1 (*S*)/7.4 (*R*)
Nonan-2-yl trifluoroacetate	1.0	60	9.1 (*S*)/9.6 (*R*)
1[a]	1.9[b]	135	2.67 (2*R*,3*S*)/2.74 (2*S*,3*R*)
5[a]	2.4[b]	120	3.95 (2*S*,3*S*)/4.13 (2*R*,3*R*)

[a] GLC/MS analysis. [b] Flow rate (cm^3 min^{-1}).

Table 6 Optical rotation values[a]

Compound	Specific rotation $[\alpha]_D^{20}$	ee (%)	(*c*, Solvent)
(2*R*,3*S*)-**1a**	−23.9	82	0.67, EtOH
(3*R*,4*S*)-**2a**	−8.8	52	0.17, EtOH
(2*R*,3*S*)-**5**	+0.7	83	0.3, Et_2O
(2*R*,3*R*)-**5a**	+21.8	97	0.66, EtOH
(3*R*,4*R*)-**6a**	+8.5	72	0.8, EtOH

[a] Compounds having an ee of <50% were not measured.

Determination of absolute configuration of epoxides

The remaining non-hydrolysed epoxide from the biotransformation was separated from the formed diol by column chromatography (light petroleum–ethyl acetate 1 : 1). The epoxide was reduced with $LiAlH_4$ (0.3 g, 8 mmol) in diethyl ether (4 cm^3) at ambient temp. After the suspension had been stirred at room temp. for 16 h, the reaction was quenched by addition of saturated aq. NH_4Cl. The secondary alcohols thus obtained were extracted with ethyl acetate and dried (Na_2SO_4). For GLC analysis, the samples were transformed into the corresponding trifluoroacetates (CH_2Cl_2, pyridine 1.25 mol equiv., trifluoroacetic anhydride, 1.25 mol equiv., room temp., 16 h). The organic layer was washed with 2 M HCl, dried (Na_2SO_4), and the absolute configuration of trifluoroacetates was determined *via* co-injection on GLC using independently synthesized reference material obtained from commercially available (*R*)-heptan-2-ol. Enantiomerically enriched reference material for heptan-3-ol was synthesized *via* reduction epoxide (2*R*,3*S*)-**5**, obtained from the biocatalytic hydrolysis of epoxide (±)-**5** using *Nocardia* EH1 (Table 2, entry 26).

Determination of absolute configuration of diols

To a Grignard reagent, prepared from the corresponding *n*-alkyl bromide (1.16 g, 8.5 mmol) and magnesium (0.205 g, 8.5 mmol) in tetrahydrofuran (20 cm^3), was added ethyl (*R*)-lactate or ethyl (*R*)-2-hydroxybutanoate[26] (4.2 mmol), dropwise (room temp.; N_2). After the solution had been stirred for 5 h at room temp., the reaction was quenched by addition of saturated aq. NH_4Cl. The phases were separated, the aqueous solution was extracted with diethyl ether (2×), the combined organic phases were dried (Na_2SO_4), and the volatiles were evaporated off to give ~90% of crude α-hydroxy ketone **7a**–**d**. The latter were reduced without further purification, using $LiAlH_4$ (70 mg, 1.8 mmol) in diethyl ether (10 cm^3) at ambient temp. The suspension was stirred at room temp. for 18 h and the reaction was quenched by addition of saturated aq. NH_4Cl. The diastereomeric mixtures of diols (2*R*,3*RS*)-**1a**, -**3a**, -**4a**, -**5a** and (3*R*,4*RS*)-**2a**, -**6a** were extracted with ethyl acetate and dried (Na_2SO_4). The relative configuration of diols was determined *via* co-injection with racemic *threo*- and *erythro*-diols (obtained *via* OsO_4-catalysed dihydroxylation of the corresponding *Z*- and *E*-alkene). The absolute configuration was elucidated from the (*R*)-lactate or (*R*)-2-hydroxybutanoate, respectively, used for the synthesis.

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15 Obtained *via* OsO_4-dihydroxylation of the corresponding *Z*- and *E*-alkene.
16 For reasons of clarity, the following nomenclature is used throughout this paper: epoxide and diol enantiomers are denoted as A/B and P/Q, respectively; the position of the oxygen incorporation in diols P and Q (as determined by ^{18}O-labelling experiments) is indicated by subscript arabic numerals.
17 Full details of the isolation and characterization of the epoxide hydrolase from *Nocardia* EH1 will be reported elsewhere. Preliminary data indicate that the enzyme is related to the epoxide hydrolase from *Rhodococcus* sp. NCIMB 11216, *i.e.* a constitutive, soluble protein having a relative molecular mass of ~35 kDa. See ref. 9*b*.
18 A related approach was elaborated by Kagan and co-workers, which allows the determination of the quotients k_1/k_2 and k_3/k_4, whereas other quotients of k_is can only be determined for *t* approaching zero. As a consequence, preparatively useful applications cannot be sufficiently treated. By using our approach, all quotients of k_is can be determined at any t_i during the whole course of the reaction. See: S. El-Baba, J.-C. Poulin and H. B. Kagan, *Tetrahedron*, 1984, **40**, 4275.
19 The absence of spontaneous hydrolysis under the reaction conditions employed was verified using blank-experiments in the absence of biocatalyst. No trace of hydrolysis was observed within ~600 h.
20 The GLC/MS data from the ^{18}O-experiments had to be subjected to background correction for the following reason: the fragmentation pattern of (unlabelled) heptane-2,3-diol shows not only a major peak with *m*/*z* 87, but also a minor signal with *m*/*z* 85 which corresponds to [(*m*/*z*) − 2 H]. Since the major *m*/*z*-peak from the ^{16}O-fragment (87) coincides with the minor [(*m*/*z*) − 2 H] signal from the ^{18}O-fragment (87), this signal was subjected to background correction by comparison with the spectra obtained from unlabelled material. The corrected values thus obtained accurately describe the regioselectivity of the ^{18}O-incorporation. Since the MS data obtained correspond to the *average of scans*, but not *absolute values*, the peak integration values were corrected with an additional factor (*f*) *via* calibration with the (absolute) values from the enantiomeric composition obtained *via* GLC. P_{GC}, Q_{GC} correspond to amounts

measured by areas, whereas P_{MS}, Q_{MS} stand for amounts *via* average counts. $f = (Q_{GC} \cdot P_{MS}/P_{GC} \cdot Q_{MS})$.
21 Since the starting material is racemic, A_0 equals B_0.
22 E corresponds to the quotient of the reaction rates of the two enantiomers $(k_3 + k_4/k_1 + k_2)$.
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