Microbiological Transformations. 47. A Step toward a Green Chemistry Preparation of Enantiopure (*S*)-2-, -3-, and -4-Pyridyloxirane via an Epoxide Hydrolase Catalyzed Kinetic Resolution

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The biocatalyzed hydrolytic kinetic resolution of 2-, 3-, and 4-pyridyloxirane by the *Aspergillus niger* epoxide hydrolase (EH) has been explored. This was used to perform a gram scale preparation of these epoxides of (*S*) absolute configuration using a process performed at a concentration as high as 10 g/L (82 mM). All three epoxides have been obtained in a nearly enantiopure form (ee > 98%). Interestingly, it was shown that this biotransformation could be achieved using plain water instead of buffer solution, an important improvement as far as downstream processing of an eventual industrial process is concerned. Neither of these substrates could be obtained in reasonable enantiomeric purity and yield using the nowadays most efficient metal-based catalysts.

Introduction

Enantiopure epoxides are nowadays recognized as highly valuable chiral synthons. Therefore, some elegant conventional chemistry methods, mainly based on the use of transition-metal-based catalysts, have been developed lately in order to synthesize these important building blocks in enantiopure form.[1] As a result of, in particular, increased environmental as well as regulatory pressure on chemical processes, other approaches using, for instance, biological catalysts may constitute interesting alternative approaches to these techniques.

Recently novel methods have been described that allow the preparation of enantiopure epoxides using a biocatalyzed hydrolytic kinetic resolution approach. This strategy is based on the use of "new enzymes", i.e., microbial epoxide hydrolases (EHs) (EC 3.3.2.3).[2] In the recent years, such biocatalysts have been shown to be ubiquitous in nature, to be cofactor-independent, and to catalyze the enantioselective addition of water to an epoxide, thus leading to the corresponding diol and to the recovery of the less reactive substrate enantiomer. Both products can thus be, ideally, obtained in enantiopure form. Although not yet available on preparative scale and therefore not commercially available, such enzymes are highly promising for synthetic, i.e., industrial application. Indeed, various examples of EHs have been recently shown to exhibit complementary substrate- as well as enantio- and/or regioselectivity. Moreover, some of them were described to even display an opposite regioselectivity for each enantiomer of one particular substrate. Combination of some of these aspects led, in certain cases, to the possibility to elaborate highly valuable enantioconvergent processes allowing one to overcome the 50% yield limitation intrinsic to a resolution process, as illustrated for the synthesis of some enantiomerically pure bioactive compounds.[3,5] Of course, such methods may be even more attractive if they allow the preparation of epoxides that cannot be obtained in enantiopure form by using the chemical approaches mentioned above.[6,7]

Other general advantages of the use of an epoxide hydrolase based process are the facts that it can in some cases be performed at high substrate concentration,[4a,5a,8] uses water as the only solvent and reactant, and only implies an enzyme, i.e., a natural and biodegradable

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(6) As can be seen in the above cited reference, numerous examples of enantioselective epoxidation of prochiral substrates have been described, most of them using heavy-metal-based catalysts. However, the obtained product rarely show excellent ee's (>98%). Only few applications of the new chemical HKR strategy have been described up to now. For a direct comparison of such strategies on one particular compound, see for instance: Brandes, B. D.; Jacobsen, N. E. *Tetrahedron: Asymmetry* **1997**, *23*, 3927−3993.

(7) Recently, the biocatalyzed direct epoxidation of **1** and **3** with a styrene monooxygenase overexpressing bacteria was also described, albeit in very low yield: Di Gennaro, P.; Colmegna, A.; Galli, E.; Sello, G.; Pelizzoni, F.; Bestetti, G. *Appl. Environ. Microbiol*. **1999**, *65*, 2794.

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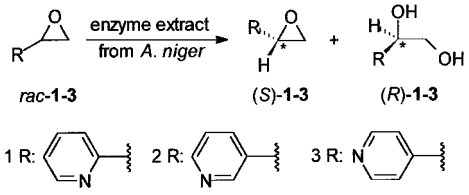
Scheme 1



protein, as catalyst. This can theoretically be produced in unlimited amount. This strategy thus constitutes an attractive "green chemistry" alternative to chemical methodologies. Moreover, significant improvement of such a native enzyme can be achieved using new (and presently fast developing) so-called directed evolution techniques.

We describe here our results focused on the preparation of enantiopure pyridine-type oxiranes, i.e., 2-, 3-, and 4-pyridyloxirane **1**−**3**, using the epoxide hydrolase from the filamentous fungus *Aspergillus niger*. These compounds are key-step building blocks for the synthesis of several biologically active compounds, such as β-adrenergic receptor agonists or antiobesity drugs.[9]

Results and Discussion

Independently of their potential interest as chirons for the preparation of bioactive compounds, pyridyloxiranes **1**−**3** (Scheme 1) were chosen as a target because the corresponding olefins should a priori be poor substrates for heavy-metal-based catalysts, which are essentially electrophilic reactants and therefore react more readily with electron-rich olefins. Moreover, it is not to be excluded that the nitrogen atom of the pyridine moiety would either react with the reagents used for achieving such reactions (commercial bleach or peracids, for example) and/or be complexed with the metal-based catalyst.

Chemical Asymmetric Oxidation. The above hypothesis was confirmed by the results we obtained by exploring the various methodologies presently known for the direct preparation of enantiomerically enriched epoxides or diols. Thus, using either the commercially available (salen)Mn catalyst for direct epoxidation of these olefins[10,11] or the AD-mix β-catalyzed direct dihydroxylation[12] of these olefins, only poor to moderate ee's for the resulting product were observed. Similarly, the resolution of racemic **1**−**3** using the recently discovered so-called "HKR" strategy catalyzed by (salen)Co complexes[13] proved to be ineffective. (Table 1). Moreover, the low yield obtained for the dihydroxylation procedure precluded any further use of the thus obtained diol.

Table 1. Comparison of Chemical Methods for the Asymmetric Synthesis of (*R*)-1, -2, and -3 (Analytical Scale)

	Jacobsen epoxidation[a]			Jacobsen HKR[c]			Sharpless dihydroxylation[d]		
product	ee (%)	temp (°C)	time (h)	ee (%)	temp (°C)	time (h)	ee (%)	temp (°C)	yield (%)
1	9	4	18	4	20	70	79	0	8
1[b]	23	−78	1						
2	34	4	2.5	5	20	70	72	0	nd
3	31	4	18	0	20	90	63	0	10

[a] (*S*,*S*)-salen(Mn) (0.019 mmol)/olefin (0.14 mmol)/NaOCl 14% (1.5 mL)/CH_2Cl_2 (5 mL).[10] [b] (*S*,*S*)-salen(Mn) (0.03 mmol)/olefin (0.61 mmol)/*N*-methylmorpholine *N*-oxide (2.98 mmol)/CH_2Cl_2 (5 mL).[11] [c] (*R*,*R*)-salen(CoIII)OAc was prepared from (*R*,*R*)-salen(CoII) (0.085 mmol) using acetic acid (0.17 mmol) in toluene (0.5 mL). HKR: (*R*,*R*)-salen(CoIII)OAc (0.01 mmol)/epoxides (0.42 mmol)/H_2O (0.22 mmol).[6] [d] AD-mix β (12 mg)/olefin (1.2 mL)/*t*-BuOH−H_2O (41 mL:41 mL)/23 H.[12]

Screening for Appropriate Epoxide Hydrolase Activity. Owing to the above-described difficulty, a biocatalytic equivalent of Jacobsen's HKR method was obviously of great interest. To determine the most suitable biocatalyst to achieve the resolution of **1**−**3**, we screened 14 enzyme extracts from our present collection of EH-containing enzyme preparations, i.e., nine originating from fungal strains and five obtained from various origins (overexpressed using a baculovirus/insect cells procedure). Analytical scale experiments were pursued to determine the overall reaction profile of these enzymes, including their enantio- and regioselectivity. The results obtained throughout this study showed that the EH from *A. niger* (*An*EH) was the best choice to perform the preparative scale resolution of substrates **1**−**3**.[14] We thus performed a more accurate determination of the various parameters of these BHKR by using this EH prepared from an overexpressing fungal strain we have previously elaborated.[15,16]

Determination of the Enantio- and Regioselectivity. Interestingly, the *An*EH exhibited a rather high enantioselectivity toward all three substrates, hydrolyzing preferentially the (*R*) enantiomer of **1**−**3** and thus leading to the recovery of the slow reacting (*S*) epoxide. The *E* values (calculated on the basis of the substrate ee and the percentage of conversion at about 40−50%) were shown to be respectively 96, 27, and 47 at a 5 mM substrate concentration.

The regioselectivity of the enzymatic attack on the oxirane ring is also an important factor in such types of reactions. Indeed, it has been previously documented that

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(14) These detailed results will be described in a separate paper.

(15) This recombinant strain has been constructed by Professor J. Visser (Wageningen Agricultural University, Section Molecular Genetics of Industrial Microorganisms, Wageningen, 6703H, The Netherlands) by cloning the epoxide hydrolase gene from the wild strain *Aspergillus niger* LCP 521 into an appropriate *Aspergillus* host (unpublished results, for the corresponding patent application, see ref 16). The enzyme has been previously purified in our laboratory (see Morisseau, C.; Archelas, A.; Guitton, C.; Faucher, D.; Furstoss, R.; Baratti, J. C. *Eur. J. Biochem.* **1999**, *263*, 386) and further on sequenced and cloned in an *E. coli* host (see Arand, M.; Hemmer, H.; Durk, H.; Baratti, J.; Archelas, A.; Furstoss, R.; Oesch, F. *Biochem. J.* **1999**, *344*, 273). The X-ray crystal structure of this enzyme has also been published recently (Zou, J.-Y.; Hallberg, M.; Bergfors, T.; Oesch, F.; Arand, M.; Mowbray, S. L.; Jones, T. A. *Structure (London)* **2000**, *8*, 111). This enzyme should become available in the near future from the Fluka catalog (year 2001−2002).

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Table 2. Regioselectivity for the Conversion of 1, 2, and 3 with the AnEH

	1	2	3
$\alpha(S)/\beta(S)^a$	3/97	7/93	11/89
$\alpha(R)/\beta(R)^b$	3/97	7/93	11/89
$ee_f{}^c$	0	0	0

[a] $\alpha(S)/\beta(S)$: regioselectivity coefficient respectively for the attack at the α-carbon atom/β-carbon atom of the (*S*)-enantiomer given as percentage. [b] $\alpha(R)/\beta(R)$: regioselectivity coefficient respectively for the attack at the α-carbon atom/β-carbon atom of the (*R*)-enantiomer given as percentage. [c] ee_f: enantiomeric excess of the diol at total conversion of the racemic epoxide. These values were calculated from the equation $\alpha(R) = \alpha(S) - ee_f$ that we have established previously.[17]

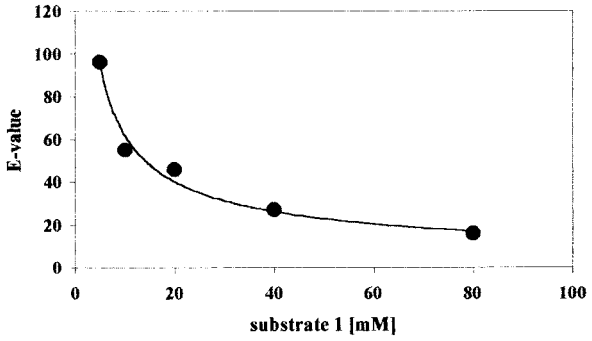

Figure 1. Calculated *E* value for the conversion of **1** with an enzyme extract of *A. niger* (strain GBCF79) at various substrate concentration.

epoxide hydrolases may exhibit the peculiar property to attack an epoxide moiety at either the less or the more substituted carbon atom. Moreover, it has been observed that such changes of regioselectivity may occur from one enantiomer to the other, even in a racemic mixture, thus leading to a (theoretically) 100% yield formation of nearly enantiopure diol.[17] Therefore, to get accurate information, it is important to establish the regioselectivity of the attack for each one of the two enantiomers of a specific substrate. We have determined the $\alpha(S)/\beta(S)$ and $\alpha(R)/\beta(R)$ ratios corresponding to each one of the three substrates **1**−**3** by studying separately the stereochemical outcome of the enzymatic hydrolysis of their enantiopure (*S*) enantiomer as well as of the racemic substrate. The overall results are described in Table 2. They indicate that attack at the β-carbon atom was highly predominant for both enantiomers, resulting in retention of configuration of the corresponding diol. This is consistent with the fact that the ee of the diol decreased to about 0% at total conversion of the racemic substrate.

High Concentration Experiments. To achieve efficient preparative scale experiments, optimum conditions had to be found, including determination of the highest possible substrate concentration. Quite unexpectedly, we observed that, for all three substrates **1**−**3**, the *E* value *decreased* when substrate concentration increased, a quite surprising result as compared to one of our previous observations where, in the case of *p*-bromostyrene oxide,[18] the *E* value was shown to *increase* upon substrate concentration increase (Figure 1).

A priori, this could be due to either (a) the modification of the enzyme/substrate ratio, (b) a product inhibition, or (c) a substrate inhibition. Therefore, further experiments were performed to determine the origin of this phenomenon. This led to the following results. (a) Determination of the enantioselectivity at three different enzyme−substrate ratios versus two different substrate concentrations (20 and 40 mM) showed no modification of the *E* value, ruling out the first hypothesis. (b) A reaction was carried out at 5 mM *rac*-**1** after prior addition of *rac*-**1d** at a concentration of 3 or 41 mM. A decrease of the reaction rate could be observed at 41 mM diol concentration (factor 1.8) but again no modification of the *E* value was observed. (c) The remaining percentage of each enantiomer throughout conversion of *rac*-**1** was determined at different substrate concentrations. This is represented in Figure 2. It can be seen that, whereas we observed a normal decrease of (*R*)-**1** consumption rate upon increase of substrate concentration, the (*S*)-**1** enantiomer consumption was, surprisingly, nearly identical whatever the substrate concentration.

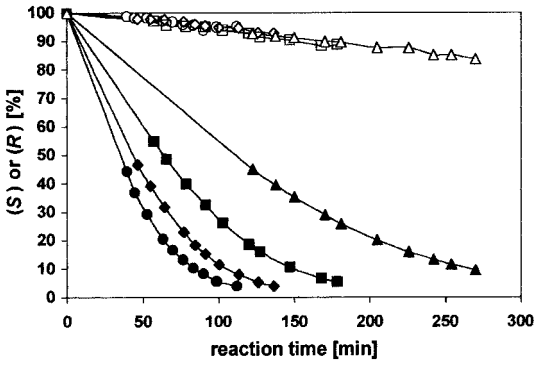

Figure 2. Remaining percentage of each enantiomer throughout the conversion of *rac*-**1** with an enzyme extract of *A. niger* (strain GBCF79) at different substrate concentrations. Filled symbols represent the (*R*)-enantiomer, unfilled symbols represent the (*S*)-enantiomer: ●, 5 mM *rac*-**1**; ◆, 10 mM *rac*-**1**; ■, 20 mM *rac*-**1**; ▲, 40 mM *rac*-**1**.

To get a better insight into this phenomenon, the initial rates[19] of the two enantiomers were determined separately at different substrate concentrations either by using the racemic compound as a substrate or by using each enantiopure enantiomer separately.[20] In theory, when using the racemic substrate, the conversion velocity of both enantiomers should be lowered (as compared with the intrinsic values of the pure enantiomer) upon substrate concentration increase as a result of competitive inhibition between enantiomers. Surprisingly, it can be seen in Figure 3 that the initial rates for (*S*)-**1**, calculated from the racemic mixture experiment on one hand and from the experiment using enantiopure (*S*)-**1** on the other hand, were nearly identical. Thus, the conversion rate of the "slow reacting" (*S*)-**1** enantiomer was only slightly influenced by the presence of the (fast reacting) (*R*)-**1** enantiomer. In contrast, the rate of (*R*)-**1** transformation was substantially lowered in the racemic substrate as compared to the pure enantiomer, indicating that hydrolysis of (*R*)-**1** was significantly inhibited by (*S*)-**1**. This discrepancy of kinetic behavior of the two enantiomers

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(19) The initial rates were determined from the slope of the derived second-order polynomal regression of the extent of conversion (0−30%). This could be done for each enantiomer, even from the racemic starting compound, as the extent of conversion was determined by using an internal standard together with chiral GC analysis.

(20) Enantiopure (*R*)-**1** was obtained by using other epoxide hydrolases; these results will be published elsewhere.

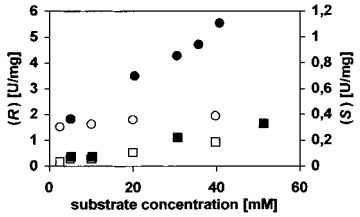

Figure 3. Initial rates for the conversion of each enantiomer of **1** with an enzyme extract of *A. niger* (strain GBCF79) at different substrate concentrations: □, (*S*)-enantiomer of *rac*-**1**; ■, enantiopure *(S)*-**1**; ○, (*R*)-enantiomer of *rac*-**1**; ●, enantiopure *(R)*-**1**.

upon substrate concentration increase clearly explains the decrease in *E* value we have observed. Further studies, i.e., determination of the corresponding kinetic constants using highly purified enzyme, would be necessary to understand the intrinsic reason of this behavior (it must be noted that the velocity increase observed in the substrate range we have used was nearly linear, indicating a much higher K_M value for both enantiomers). This will be achieved in the near future and described elsewhere.

Preparative Scale Experiments. Owing to the above-described results, the best substrate concentration/*E* value compromise was chosen to perform a gram scale preparation of each of the three epoxides **1**−**3**. Thus, as a typical example, 2 g of *rac*-**1** were submitted to biohydrolysis using 0.1 g of a partially purified enzyme extract (specific activity 23 U/mg protein).[21] The reaction was carried at a 10 g/L (82 mM) substrate concentration and at 4 °C, which we have found previously to be the best temperature choice for this enzyme.[4a] This afforded a 27% (purified) yield of (*S*)-**1**, which showed an ee of 99%, and a 55% (purified) yield of (*R*)-**1d** with an ee of 36%. The calculated turnover number (TON) could thus be estimated to be about 4500 mol substrate/mol enzyme/h and the space time yield to be about 8 g/L/day. Interestingly enough, we have recently observed that this biotransformation could also be achieved using plain (deionized) water instead of a buffer solution. This obviously constitutes an interesting improvement of the procedure, as far as "green chemistry" is concerned, thereby avoiding addition of buffering salts and thus simplifying the downstream processing for a possible industrial application. The catalytic process thus involved (a) mixing the substrate and the enzyme in plain water (b) stirring at 25 °C for 1.5 h, and (c) extraction and bulb to bulb distillation of the unreacted (*S*) epoxide. The formed diol could be obtained directly by lyophilization of the aqueous solution and was purified by simple flash chromatography. Similarly, biohydrolysis of *rac*-**2** at a 9.7 g/L substrate concentration led to (*S*)-**2** (96% ee, 6% yield) and (*R*)-**2d** (7% ee, 54% yield). For this substrate, the overall yield of epoxide was unfortunately very low as a result of the poor enantioselectivity of the epoxide hydrolase toward this substrate ($E = 3$). More interesting results were, however, obtained with *rac*-**3**. Thus 2.2 g of this substrate was resolved within about 7 h at a 10 g/L substrate concentration and afforded (*S*)-**3** (28% yield), which showed an ee of 98%, and (*R*)-**3d** (34% yield) with an ee of 56%. The calculated turn over number and space time yield were similar to the one estimated for **1**.

The absolute configuration of **1** and of the corresponding diol **1d** were assigned on the basis of their optical rotation sign by comparison with previously described data.[22,23] Those of epoxides **2** and **3** were established by chemical correlation with the corresponding pyridyl-1-ethanols **2e** and **3e**, for which the optical rotation/absolute configuration assignment was known.[24,25] Thus, reduction of (optically active) epoxides **2** and **3** with $LiAlH_4$ led to the corresponding pyridyl-1-ethanols. Since there was retention of absolute configuration at the benzylic carbon atom during this transformation, the absolute configuration of the starting epoxide could be deduced from the obtained stereochemistry of these products. Similarly, the absolute configuration of **2d** and **3d** was established by cyclization of the diol back to the corresponding epoxide.[26] We have verified that no change of stereochemical integrity occurred during this chemical step.

Conclusion

The biocatalyzed hydrolytic kinetic resolution of 2-, 3-, and 4-pyridyloxiranes **1**, **2**, and **3** was performed using an enzyme extract of the fungus *Aspergillus niger* GBCF 79. Up to now none of these products was directly accessible using presently available conventional chemical procedures, including the most effective heavy-metal-catalyzed approaches known to date. In the course of this study, we have observed that, for either of these three substrates, an increase of substrate concentration led to an unexpected decrease of the corresponding *E* value. This was shown to be due to the modification of the kinetic profile of the fast reacting enantiomer upon increase of substrate concentration. We have shown that this was due to an inhibition by the slow reacting (*S*)-**1** enantiomer, resulting in a (proportional) decrease of the hydrolysis rate of the fast reacting (*R*)-**1** enantiomer. Nevertheless, the preparative scale synthesis of each of these three targets could be performed at about 10 g/L substrate concentration, thus allowing one to obtain these compounds in nearly enantiopure form (ee > 98%). Moreover, we have shown that this biocatalyzed hydrolytic kinetic resolution could be performed using plain water instead of buffer solution, an important step in favor of the "green chemistry" aspect. Thus, this very simple procedure, which only uses water as a reactant and solvent, appears to be nowadays the best and most direct way to prepare these very valuable chiral synthons in enantiopure form.

Experimental Section

General. NMR spectra (^1H and ^{13}C) were recorded in $CDCl_3$ at 250 and 100 MHz, respectively. Chemical shifts are reported in δ from TMS as internal standard. For gas chromatography analysis, the chiral column Chiralsil Dex CB (Chrompack) was used.

(21) The enzymatic activity was calculated using *p*-chlorostyrene oxide as a reference substrate. It is expressed in μmol/min/mg of protein. We estimate the specific activity of pure AnEH to be about 90 U/mg pure enzyme.

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(25) It should be noted that due to the rule of priority of substituents by Cahn, Ingold and Prelog rule the (*S*)-epoxides led to the corresponding (*R*)-pyridyl-1-ethanols.
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Synthesis of 2-Pyridyloxirane 1. This was synthesized following the procedure described by Hanzlik et al.[27] From 10.3 mL of 2-vinylpyridine (Fluka), 4.9 g of **1** was obtained (41% yield). Purification was by flash chromatography (hexane/ethyl acetate 4/6), followed by removal of solvent by bulb-to-bulb distillation (40 °C; 4×10^{-2} mbar). ^{1}H NMR: δ 2.78 (dd, 1H, H_{2c}, $J_{gem} = 5$ Hz, $J_{1-2c} = 2.5$ Hz); 2.99 (dd, 1H, H_{2t}, $J_{gem} = 5$ Hz, $J_{1-2t} = 3.5$ Hz); 3.84 (dd, 1H, H_1, $J_{1-2c} = 2.5$ Hz, $J_{1-2t} = 3.75$ Hz); 7.02−7.08 (m, 2H); 7.50 (t, 1H); 8.39 (d, 1H, $J = 5$ Hz).[28] ^{13}C NMR: δ 50.38; 52.81; 119.69; 123.12; 136.82; 149.39; 157.18. GC: 110 °C, 1 kg/cm^2 helium; (R) = 7.8 min; (S) = 8.6 min.

Synthesis of 3-Pyridyloxirane 2. As described by Giannini et al.[29] From 3.29 g of 3-pyridylaldehyde (Fluka), 2.1 g of **2** was obtained (56% yield). DMSO was removed by washing the organic phase four times with distilled water. Purification was by flash chromatography (hexane/ethyl acetate 4/6), followed by removal of solvent by bulb-to-bulb distillation without heating (4×10^{-2} mbar). ^{1}H NMR: δ 2.83 (dd, 1H, H_2, $J_{gem} = 5.3$ Hz, $J_{1-2} = 2.51$ Hz); 3.20 (dd, 1H, H_2, $J_{gem} = 5.26$ Hz, $J_{1-2} = 4.1$ Hz); 3.9 (dd, 1H, H_1, $J_{1-2} = 3.87$ Hz, $J_{1-2} = 2.7$ Hz); 7.25−7.31 (m, 1H); 7.52−7.57 (m, 1H); 8.55−8.59 (m, 2H).[9c,28] ^{13}C NMR: δ 50.38; 51.1; 123.46; 132.73; 147.88; 149.62. GC: 110 °C, 1 kg/cm^2 helium; R = 12.2 min; S = 12.8 min.

Synthesis of 4-Pyridyloxirane 3. As described by Corey and Chaykovsky.[30] From 23.3 g of 4-pyridylaldehyde (Fluka), 6.7 g of **3** was obtained (25% yield). DMSO was removed by washing the organic phase four times with distilled water. Purification was by flash chromatography (hexane/ethyl acetate 4/6), followed by removal of solvent by bulb-to-bulb distillation without heating (4×10^{-2} mbar). ^{1}H NMR: δ 2.77 (dd, 1H, H_2, $J_{gem} = 5.6$ Hz, $J_{1-2} = 2.5$ Hz); 3.20 (dd, 1H, H_2, $J_{gem} = 5.6$ Hz, $J_{1-2} = 4.2$ Hz); 3.85 (dd, 1H, H_1, $J_{1-2} = 4$ Hz, $J_{1-2} = 2.5$ Hz); 7.21 (dd, 2H, $J = 4.5$ Hz, $J = 1.5$ Hz); 8.58 (d, 2H, $J = 5.7$ Hz).[28,31] ^{13}C NMR: δ 50.95; 51.33; 120.36; 146.96; 149.93. GC: 110 °C, 1 kg/cm^2 helium; R = 12.9 min; S = 13.8 min.

Racemic 2-Pyridyldiol 1d. As described by Hanzlik et al.[27] A total of 1.6 g of **1** was hydrolyzed under acidic conditions (H_2SO_4) for 64 h to give 1.1 g of **1d** (yield 60%). ^{1}H NMR (acetone-d_6): δ 3.64 (dd, 1H, H_2, $J_{gem} = 10.8$ Hz, $J_{1-2} = 5.6$ Hz); 3.82 (dd, 1H, H_2, $J_{gem} = 11.3$ Hz, $J_{1-2} = 6.1$ Hz); 3.97−3.94 (m, 1H, H_{OH}); 4.66 (d, 1H, H_{OH}, $J = 4.95$); 4.77−4.73 (m, 1H, H_1); 7.28−7.24 (m, 1H); 7.56 (d, 1H, $J = 7.8$ Hz); 7.82−7.75 (m, 1H); 8.51 (d, 1H, $J = 4.5$ Hz).[28,31] ^{13}C NMR: δ 67.81; 75.06; 121.59; 123.07; 137.32; 149.12;162.57.

General Procedure for the Biohydrolysis of 1−3. Analytical Scale Conversion. A substrate solution (5−80 mM) in a 0.1 M pH 8 Na phosphate buffer was used and diethylendiethylglycolether (Aldrich) was added as an internal standard. Before addition of the enzymatic extract, a sample was taken for the 100%-epoxide value. The reaction was started with addition of the substrate solution to the enzyme extract. The kinetic resolution (28 °C, 750 rpm) was followed by taking aliquots from the reaction medium (1 vol sample was added to 1 vol of methanol and 2 vol of $CHCl_3$). The organic phase was analyzed by GC to calculate the degree of conversion and the ee of the remaining epoxide (GC, 110 °C; internal standard = 4.6 min). The diol was extracted (after total extraction of the epoxide with $CHCl_3$) using ethyl acetate after saturation of the aqueous phase with NaCl. To determine its ee, the diol was cyclized back to the epoxide following the procedure previously described by Golding et al.[26] (without change of its absolute configuration) as follows. To the dried diol, 100 μL of HBr in glacial acetic acid (33%) were added. After 1.25 h at room temperature, the mixture was neutralized by addition of 500 μL of water and about 200 mg of solid Na_2CO_3 and extracted twice with 300 μL of ethyl acetate. The combined organic layers were evaporated and dried under vacuum. To this, 50 μL of KOH in MeOH (1 N) was added and after 30 min at room temperature 300 μL of water was added. The epoxide was extracted with 300 μL of $CHCl_3$ and analyzed by GC.

General Procedure for the Biohydrolysis of 1−3. Preparative Scale Conversion. An amount of 2 g (16.5 mmol) of *rac*-epoxide **1**−**3**, dissolved in 200 mL of 0.1 M Na phosphate buffer at pH 8 (cooled at 4 °C), was added directly to 100 mg of the enzyme extract of *A. niger* (4 °C, 250 rpm). The reaction was stopped by adding methanol to the reaction mixture (up to a 1/1 methanol/buffer proportion) when the ee of the remaining substrate reached a value of about 96%. After extraction with $CHCl_3$ (3 × 300 mL) the aqueous phase was saturated with NaCl and extracted twice with ethyl acetate (800 mL). The aqueous phase was then further extracted by continuous extraction with CH_2Cl_2 (500 mL) for 4 days. The epoxide and the diol were purified by flash chromatography (only in the first extraction with $CHCl_3$ was the epoxide present; the diol was present in all organic phases). Flash chromatography of the epoxide was performed with hexane/ethyl acetate 2/8 and for the diol with ethyl acetate/methanol 9/1. The fractions containing the epoxide and the diol, respectively, were pooled and dried over Na_2SO_4 and the solvent was removed by stripping. The epoxide was purified via bulb-to-bulb distillation without heating (4×10^{-2} mbar).

For the conversion of **1** a different workup was used. The unreacted **1** was extracted with CH_2Cl_2 and the aqueous phase was filtered (0.45 μm) to remove the enzyme extract. The aqueous phase was then lyophilized, and **1d** was directly extracted from the resulting dry diol salt with ethyl acetate and purified by flash chromatography.

Using the above-described protocol, 2 g of *rac*-**1** afforded after about 8 h 0.55 g of (S)-**1** (27% purified yield, 99% ee) and 1.26 g (55% purified yield, 36% ee) of (*R*)-**1d.** Similarly, 1.93 g of *rac*-**2** at a 9.7 g/L substrate concentration led to 0.12 g of (*S*)-**2** (6% yield, 96% ee) and 1.2 g of (*R*)-**2d** (54% yield, 7% ee). Also, 0.61 g of (*S*)-**3** (28% yield, ee 98%) and 0.85 g of (*R*)-**3d** (34% yield, ee 56%.) were obtained from rac-**3** (2 g).

Biohydrolysis of *rac*-1 Using Plain Water Instead of a Buffer Solution. A semipreparative scale experiment was conducted using a 5 mM concentration of racemic **1** (0.61 g/L). Thus, 610 mg of **1** in 1 L of plain (deionized) water were treated with 990 mg of a crude enzymatic extract of EH. After 1.5 h at 25 °C, the medium was extracted with chloroform. Normal workup of this organic phase led to 260 mg (43% yield) of (*S*)-**2**, of which the ee was shown to be higher than 99%. Lyophilization of the aqueous phase afforded directly 300 mg of (*R*)-**3** diol (43% yield) with 62% ee, purified using flash chromatography.

Optical rotations and NMR data of the isolated products after biohydrolysis were as follows: (*S*)-**1** $[\alpha]^{19}_D$ +14 (*c* 0.56, $CHCl_3$), ee 99%; lit.[22] (*R*)-**1** $[\alpha]^{25}_D$ −15 (*c* 0.41, $CHCl_3$); (*R*)-**1d** $[\alpha]^{19}_D$ −29 (*c* 1.64, EtOH), ee 36%; lit.[32] (*S*)-**1d** $[\alpha]^{25}_D$ +80.6 (*c* 1.6, EtOH); (S)-**2** $[\alpha]^{25}_D$ +18.3 (*c* 0.91, $CHCl_3$), ee 96%; (*R*)-**2d** $[\alpha]^{25}_D$ −3.5 (*c* 0.57, MeOH), ee 7.3%; (*R*)-**2e** $[\alpha]^{25}_D$ +44 (*c* 0.94, $CHCl_3$); lit.[24c] (*R*)-**2e** $[\alpha]^{25}_D$ +52.4 (*c* 1.4, $CHCl_3$); (*S*)-**3** $[\alpha]^{25}_D$ +28.5 (*c* 1.16, $CHCl_3$), ee 98%; (*R*)-**3d** $[\alpha]^{20}_D$ −11 (*c* 0.53, EtOH), ee 56%; (*R*)-**3e** $[\alpha]^{25}_D$ +38 (*c* 0.88, MeOH); lit.[24c] (*R*)-**3e** $[\alpha]^{25}_D$ +42.5 (*c* 1.04, MeOH).

The ^{1}H NMR data for epoxides **1**−**3** and diol **1d** were identical to those obtained with the corresponding racemic substrates previously prepared. **3-Pyridyldiol 2d:** ^{1}H NMR (acetone-d_6): δ 3.69−3.58 (m, 2H, H_2); 4.10 (s, 1H, H_{OH}); 4.66 (s, 1H, H_{OH}); 4.8−4.78 (m, 1H, H_1); 7.34 (dd, 1H, $J = 7.86$ Hz, $J = 4.75$ Hz); 7.81−7.77(m, 1H); 8.60−8.44 (m, 2H). ^{13}C NMR: δ 68.45; 73.16; 123.91; 134.63; 139.00; 149.07;149.28. **4-Pyridyldiol 3d:** ^{1}H NMR (250 MHz, acetone-d_6): δ 3.67−3.58 (m, 2H, H_2); 4.03 (s, 1H, H_{OH}); 4.66 (s, 1H, H_{OH}); 4.74 (s, 1H, H_1); 7.40−7.37 (m, 2H); 8.52−8.49 (m, 2H).[33] ^{13}C NMR: δ 68.22; 73.96; 122.27; 150.13; 152.59. The ^{1}H NMR data for the corresponding pyridylethanol were identical to those described previously in the literature.[23]

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