DOI: 10.1002/adsc.200606164

Enzymatic Transformations 62. Preparative Scale Synthesis of Enantiopure Glycidyl Acetals using an *Aspergillus niger* Epoxide Hydrolase-Catalysed Kinetic Resolution

Bastien Doumèche,[a] Alain Archelas,[b] and Roland Furstoss[b,*]

[a] Present address: ERRMECe, EA1391, UFR Sciences et Techniques, Université de Cergy-Pontoise, 2 avenue Adolphe Chauvin, 95302 Cergy-Pontoise Cedex, France
[b] Groupe Biocatalyse et Chimie Fine, UMR CNRS 6517, Université de la Méditerranée, Faculté des Sciences de Luminy, Case 901, 163 avenue de Luminy, 13288 Marseille Cedex 9, France
Fax: (+33)-(0)491-82-91-45; e-mail: furstoss@luminy.univ-mrs.fr

Received: April 7, 2006; Accepted: July 6, 2006

Abstract: The hydrolytic kinetic resolution of five glycidaldehyde acetal derivatives was examined using the recombinant *Aspergillus niger* epoxide hydrolase as biocatalyst. This could successfully be performed, at room temperature, using solely demineralised water as solvent and following a two-phase methodology allowing us to operate at a global substrate concentration as high as 200 g/L in the reactor. The observed *E* values were shown to be modest to excellent, depending on the structure of the acetal moiety, indicating that it is possible to achieve this resolution very efficiently just by choosing the right substituents. Both the unreacted (*R*)-epoxide and the formed (*S*)-diol could thus be obtained in good to excellent *ee* (*ee* > 99% for the epoxide). For the best substrates, the reaction could be performed within a few hours by using a biocatalyst over substrate molecular ratio of about 9 to 10×10^{-4} mol%. The turnover frequency (TOF) as well as the total turnover number (TON) of the enzyme proved to be excellent as compared to chemical catalysts – reaching respectively values in the order of 6×10^{2} mol sub/mol enz/min and 6×10^{4} mol sub/mol enz. The space-time yield of the best (two-phase) reactor could thus reach a value as high as 56 g/L/hour. As a demonstration experiment, a 50-g scale resolution of glycidaldehyde 2,2-dimethyltrimethylene acetal was performed.

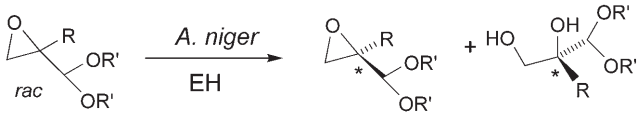
Keywords: biotransformation; chiral resolution; enzyme catalysis; epoxide hydrolase; glycidyl acetals

Introduction

Over the last decade, we (and others) have amply demonstrated that the resolution of various structurally different racemic epoxides could be very efficiently performed using dedicated microbial enzymes, i.e., epoxide hydrolases (EHs) as biocatalyst.[1,2] Following this approach, it is possible to prepare, simultaneously, both the enantiomerically enriched epoxides and the corresponding vicinal diols, which have been shown to be interesting synthetic equivalents of these epoxides.[3] We have, in particular, developed studies using the *Aspergillus niger* epoxide hydrolase (*A. niger* EH), a recombinant enzyme which can be prepared in large quantity and is now commercially available.[4] The use of this natural, biodegradable and environmentally gentle catalyst interestingly provides an important "sustainable chemistry" alternative to potentially toxic transition metal-based methodologies. It allows one to perform the hydrolytic kinetic resolution (HKR) of a given racemic epoxide under very mild, cheap, easy to use and highly efficient experimental conditions, at room temperature, using solely demineralised water as solvent (i.e., following a salt-free methodology) and by running the reaction in a two-phase reactor at a "global substrate concentration" as high as several hundred grams per litre.[5,6]

With a few exceptions,[7] most of the epoxides we have studied up to now were aromatic substrates, and we recently decided to explore the possibility to operate this resolution on some selected alkyl substrates, as for instance glycidyl derivatives. Indeed such chirons, i.e., C-3 chiral building blocks bearing one stereogenic centre and two different and chemically differentiable functions located on a short carbon skeleton, are of particular interest due to their high chemical versatily. Acetal-protected glycidaldehyde derivatives are important and widely used building blocks allowing for the synthesis of a variety of bioactive compounds like, for instance nucleoside analogues

(antiviral and cytotoxic agents),[8] leukotriene B_4 (an anti-inflammatory drug),[9] phosphonate or dihydroxyacetone phosphate analogues[10] (aldolase inhibitors), sugar analogues,[11] bicyclomycin[12] (antibiotic), brevetoxin B[13] (neurotoxin) or α-tocopherol (vitamin E).[14] Surprisingly enough, methodologies enabling the preparation of these chirons are very scarce and are not easy.[15] We here describe a simple, novel and efficient methodology allowing for the preparation of five glycidaldehyde acetal derivatives (**1–5**) and of their corresponding vicinal diols (**1d–5d**), in enantiomerically enriched form. This was performed by using the epoxide hydrolase-catalysed HKR approach illustrated in Scheme 1.



1: R = H ; R' = C_2H_5 **1d**
2: R = H ; R' = isopropyl **2d**
3: R = H ; R'/R' = CH_2-$C(CH_3)_2CH_2$ **3d**
4: R = CH_3 ; R' = C_2H_5 **4d**
5: R = CH_3 ; R'/R' = CH_2-$C(CH_3)_2CH_2$ **5d**

Scheme 1. Hydrolytic kinetic resolution of glycidaldehyde acetals *rac*-**1–5**.

Results and Discussion

Synthesis of Substrates *rac*-2–5

Except for glycidaldehyde diethyl acetal *rac*-**1** which was commercially available, *rac*-**2–5** were synthesized following a straightforward two-step procedure starting from either acrolein (for **2** and **3**) or methacrolein (for **4** and **5**). The first step implied protection of the aldehyde function by formation of the corresponding acetal, using either triethyl or triisopropyl orthoformate or 2,2-dimethylpropane-1,3-diol. The thus obtained acetal was further epoxidised following either Payne's procedure[16] or by using *meta*-chloroperbenzoic acid/NaF oxidation,[17] as described in the Experimental Section. The yields of these syntheses were reasonable to good, but were not optimized.

Preliminary Controls

Before attempting to perform the *A. niger* EH-catalysed HKR of *rac*-**1–5**, the stability of these substrates had to be checked in the experimental conditions used. These control experiments indicated that all were stable at the employed pH range (6–8) and room temperature. In particular, no noticeable hydrolysis of either the acetal or the epoxide function could be detected. Under these conditions, the aqueous solubility of these substrates was also evaluated, and was shown to be in the range of 30–60 g/L.

Analytical Scale Hydrolytic Kinetic Resolution

We have previously shown that, depending on the substrate structure, the *A. niger* EH-catalysed HKR can be efficiently conducted at a global substrate concentration as high as several hundred grams per litter.[5] Preliminary experiments aimed at exploring the possible substrate concentration to be used with *rac*-**1–5** indicated that a 200 g/L (i.e., about 1.3 M) was a reasonable – although not optimized – value. Therefore, the experiments described in this work were performed using this value. Owing to our aim to allow further preparative (industrial) scale implementation of this process at low cost, one additional important parameter to be defined was the optimum substrate over enzyme ratio (S/E) to be used. Therefore, analytical scale experiments were conducted, at room temperature (controlled at 27 °C), on *rac*-**1–5** at different, that is, 10, 50, 100 and 200, S/E weight ratios. As an example, the kinetic profile observed with *rac*-**2** is illustrated in Figure 1. Similar profiles

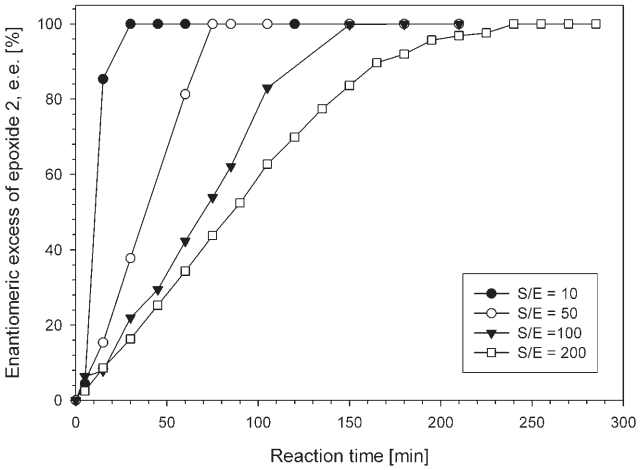


Figure 1. Kinetic profile of the *A. niger* EH-catalysed HKR resolution of *rac*-**2** at various (10, 50, 100 and 200) S/E ratios.

were obtained using the other substrates. Our experiments indicated clearly that, for *rac*-**1–3**, the HKR process could be very satisfactorily achieved, within only a few hours, using an S/E ratio of 100. In order to achieve the resolution of *rac*-**4** and **5** (bearing an additional geminal methyl group) within the same time period, a smaller S/E ratio (i.e., more enzyme) had to be used to perform the resolution.

Determination of Various Reaction Parameters. Semi-Preparative 2-g Scale Experiments

In order to fully characterise a catalytic resolution, one of the important parameters to be determined is the enantioselectivity – *E* value.[18] Due to the heterogeneous nature of our two-phase process, it was impossible to accurately evaluate this parameter by using the conversion ratio (c) value. Determination of the *ee* of the formed diol (*ee*p) was therefore necessary. Thus, the HKR of each substrate *rac*-**1**–**3** was performed at a 2-gram semi-preparative scale, using an S/E ratio of 100. In a typical experiment, an emulsion of 2 g of substrate in demineralised water (8 mL) was processed with 20 mg of (partly purified) *A. niger* EH, at 27 °C. Owing to their limited reactivity, *rac*-**4** and *rac*-**5** were processed using an S/E value of 19 and 25, respectively. The resolutions were monitored by chiral GC analysis of the remaining epoxide *ee*, the reaction being conducted until it reached a value of about 99 %. Depending on the substrate, this necessitated about 2 to 6 h. The reaction was then stopped by addition of acetonitrile. Selective extraction of the aqueous reaction medium with pentane essentially afforded the unreacted epoxide, whereas further extraction with dichloromethane allowed isolation of the formed diol. The *ee* of a given diol was determined by chiral GC analysis after either derivatisation into its acetonide (in the case of **1d**, **2d**, **3d**) or recyclisation into the starting epoxide (in the case of **4d**, **5d**).

Another important aspect to be determined for an EH-catalysed reaction is the regioselectivity of the (formal) water attack. This can formally occur at both carbon atoms of the oxirane ring, and we have previously shown that, depending on the regioselectivity coefficients, this will govern the stereochemical outcome of the reaction.[19,20] In order to check this point the *E* value was calculated, at different conversion values of *rac*-**1**, on the basis of the *ee* of the residual epoxide and of the formed diol. This led to very similar values ($E = 14$, 14, 15 and 15), indicating that the oxirane ring was attacked with a high regioselectivity on one single carbon atom. Indeed, as we have previously demonstrated, a repartition of the water attack on the two carbon atoms would have led to a variation of the calculated *E* value versus the reaction time.[19] Further determination of the absolute configuration of the recovered epoxide and formed diol **1d** (see below) indicated that, in coherence with the generally observed regioselectivity exhibited by the *A. niger* EH, this was very preferably (if not totally) directed to the terminal – less substituted – carbon atom. In the case of *rac*-**2** and **3**, the excellent *ee* of the formed diol and absolute configuration determination indicated that the regioselectivity again was essentially directed to the terminal atom for the fastest enantiomer. As far as the trisubstituted epoxides *rac*-**4** and **5** are concerned it is most improbable, owing to the mechanism of this enzyme,[21,22] that the nucleophilic attack would occur at the most substituted carbon atom. This was further confirmed by the coherency of the yields and *ee* values obtained for the remaining epoxide and the formed diol.

The measurements performed during these semi-preparative experiments allowed us to estimate various important parameters of each specific resolution: (a) its *E* value and the absolute configuration of the obtained products; (b) the efficiency of the biocatalyst (i.e., the specific activity of the enzyme against each substrate) as well as the turnover frequency (TOF) and the total turnover number (TON) of the enzyme;[23] (c) the efficiency of the process (i.e., the optimum percentage of biocatalyst to be used) (E/S mol %) as well as the space-time yield of the reactor. All these results are summarised in Table 1.

Enantioselectivity and Absolute Configuration of the Obtained Products

As can be seen on Table 1, the enantioselectivity of the *A niger* EH against *rac*-**1–5** was highly dependent

Table 1. Parameters of the HKR of *rac*-**1** to *rac*-**5** using the *A. niger* EH.

Substrate[a]	Reaction time [h]	Yield of epoxide	Yield of diol	*E*	*ee* epoxide % [Abs. conf.]	*ee* diol % [Abs. conf.]	Activity [U·mg^{-1}]	TOF[b] [mol sub/mol enz/min]	TON[b] [mol sub/mol enz]	Space-time yield[c] [g/Lh]$^{-1}$	E/S mol %[b]
1	3.5	39.4	41	16	>99 [*R*]	54 [*S*]	4.1	$3\cdot10^2$	$>6.1\cdot10^4$	20	$8.1\cdot10^{-4}$
2	4.5	46	34	>200	>99 [*R*]	97 [*S*]	2.3	$2\cdot10^2$	$>5\cdot10^4$	22	$9.6\cdot10^{-4}$
3	1.7	43.5	38	126	>99 [*R*]	92 [*S*]	14	$6\cdot10^2$	$>6\cdot10^4$	56	$8.8\cdot10^{-4}$
4	2.2	12	23	12	>99 [*R*]	45 [*S*]	1.3	70	$>10^4$	29	$4.8\cdot10^{-3}$
5	5.8	30	34	28	>99 [*R*]	70 [*S*]	0.7	34	$>12\cdot10^3$	14.5	$3.7\cdot10^{-3}$

[a] All reactions were performed at a 200 g·L^{-1} global substrate concentration, at 27 °C and using an S/E (w/w) ratio of 100, except for *rac*-**4** and *rac*-**5** for which S/E ratios of 19 and 25 were used, respectively.
[b] Calculated assuming a 25 % content of EH in the enzymatic extract and a molecular weight of 45 kDa of the protein.
[c] These values were calculated using the theoretically obtained amount of enantiopure epoxide (*ee* > 99 %). The corresponding conversion ratio was calculated from *ee*s and *ee*p [c = *ee*s/(*ee*s+*ee*p)].

on the specific acetal structure. Thus, whereas an only moderate E value was observed for diethyl acetals **1** and **4** ($E=16$ and 12, respectively), increasing the bulkiness of the acetal moiety also improved the E value. The best enantioselectivity was thus observed for the diisopropyl acetal **2** for which E value was excellent (>200). As usual, it was however possible to obtain each unreacted epoxide enantiomer in enantiopure form (ee $>99\%$) by fine-tuning the conversion ratio.

Absolute Configuration of the Obtained Epoxides 1–3

Except for (*R*)-**1**, the absolute configuration of the thus obtained products has never been described previously. Thus, they were determined either by comparison of their optical rotation sign with literature data (for **1**) or by chemical correlation with products of known absolute configuration. The positive rotation exhibited by the obtained enantiomer of **1** indicated that it was of (*R*) configuration as previously described.[24] The absolute configuration of the epoxides **2** and **3** was determined as detailed in Scheme 2, [*path (a)* or *(b)*].

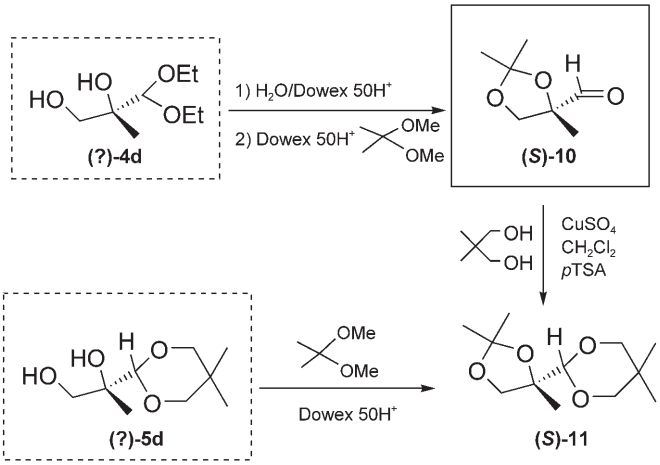
Path (a): the acetal moiety of (*R*)-**1** was hydrolysed using montmorillonite K10[25] to afford the corresponding aldehyde (*R*)-**6**. The obtained glycidaldehyde diisopropyl acetal **2** was similarly deprotected. Chiral GC comparison of the thus obtained glycidaldehyde enantiomers indicated an identical retention time, leading us to conclude that the recovered enantiomer of **2** was of (*R*) configuration.

Path (b): the absolute configuration of unreacted **3** was established as follows, using a convergent chemical correlation. Methanolysis of (*R*)-**1** afforded (*R*)-**7**, which by hydrolysis of the acetal moiety led to the aldehyde (*R*)-**8**. Further acetalisation of this aldehyde with 2,2-dimethyl-propandiol afforded (*R*)-**9**. On the other hand, the recovered epoxide **3** (of unknown absolute configuration) was submitted to methanolysis. Chiral GC analysis indicated that the thus obtained enantiomers were identical, thus allowing to attribute the *(R)* configuration to the obtained epoxide **3**.

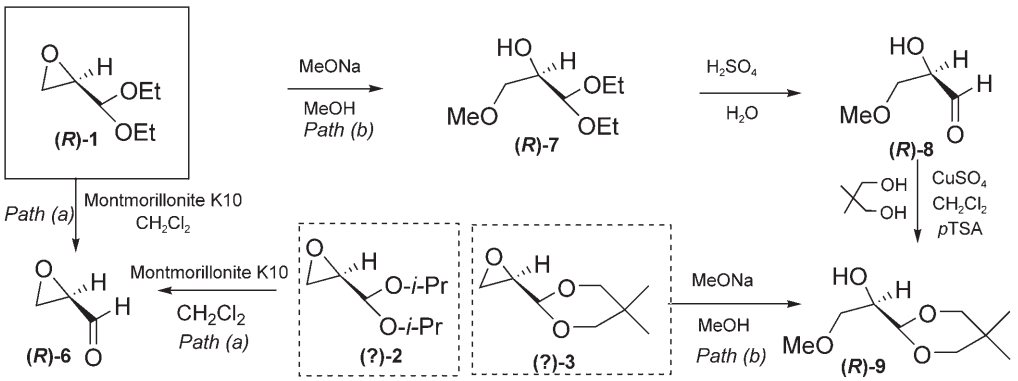
Recovered epoxides **4** and **5** were also shown (see below) to be of (*R*) absolute configuration.

Absolute Configuration of the Obtained Diols 1d–5d

As a consequence of the (*R*) configuration of the obtained epoxides and of the (formal) attack of a water molecule at the less substituted carbon atom of the oxirane ring (see above), the formed diols **1d–3d** should be of (*S*) absolute configuration. This was confirmed by chiral GC analysis of the epoxide obtained after recyclisation of diols **1d–3d** (*via* the corresponding tosylate), which proved to essentially provide the corresponding (*S*) epoxide. The absolute configuration of diols **4d** and **5d** was determined following the convergent chemical correlations detailed on Scheme 3. First, the acetal moiety of **4d** was hydrolysed into the corresponding aldehyde which was further transformed into the corresponding acetonide **10**.



Scheme 3. Determination of the absolute configuration of the obtained diols **4d** and **5d**.



Scheme 2. Determination of the absolute configuration of recovered **2** and **3**.

Chiral GC analysis and comparison with published data (chiral GC elution order of the two enantiomers on an identical column)[26] led us to conclude that **10** was of (*S*)-absolute configuration. Consequently, this same (*S*) configuration could be assigned to diol **4d**. On the other hand, the obtained enantioenriched diol **5d** (of unknown configuration) was transformed into its acetonide **11**, which was compared (chiral GC analysis) with the same product obtained by acetalisation of the corresponding (*S*)-**10** aldehyde (obtained above) by reaction with 2,2-dimethylpropandiol. This indicated that **5d** also was of (*S*) absolute configuration.

Each of the (obtained) diols (*S*)-**4d** and (*S*)-**5d** was cyclised back, without noticeable loss of stereochemical integrity, into the corresponding (*S*) epoxide. Comparative chiral GC analysis confirmed that the unreacted (recovered) epoxides **4** and **5** were, as expected, of opposite (*R*) absolute configuration.

As an overall result, it appears that, in all cases, the *A. niger* EH essentially hydrolysed the (*S*) enantiomer of epoxides *rac*-**1–5**, thus leading to the recovery of the (*R*)-epoxide and to the formation of the (*S*)-diol. At first glance, this appears to be the opposite of the general preference of this enzyme for hydrolysing (*R*) epoxides.[1a] However, this apparent switch of enantioselectivity is only due to the application of the Cahn–Ingold–Prelog nomenclature. In fact, the spatial arrangement of the recovered (*R*) **1–5** epoxides is similar to the ones of the generally obtained (*S*) epoxide enantiomers of other substrates like styrene oxide derivatives for example.

Biocatalyst Efficiency

As a general rule, one important type of parameter to be evaluated for a catalytic – and biocatalytic – process is the efficiency of the catalyst. This can be illustrated by different data, i.e., (i) the specific activity of the catalyst against each substrate, (ii) the specific turnover frequency (TOF) (which indicates the number of substrate molecules processed by one single molecule of catalyst within a given time unit), and (iii) the total turnover number (TON) (which indicates the maximum number of substrate molecules which can be processed by one single molecule of catalyst until it is exhausted).[23] All these values were determined for the resolution of *rac*-**1–5** (Table 1).

As far as the biocatalyst activity (expressed in μmol of substrate transformed per minute per mg of enzymatic powder) is concerned, it can be noticed that these were on average higher for **1–3** than for **4** and **5**, the best activity being observed for the most rigid substrate **3**. Both these observations can be explained in terms of easier (faster) positioning of the substrate into the enzymatic active site and increased steric hindrance introduced by the additional *gem*-methyl substitution.

The turnover frequency (TOF) value is another way to evaluate the biocatalyst efficiency, and the results obtained obviously reflected the same tendency, i.e., the lowest TOF values were for **4** and **5**, and the best one for **3**. As far as the total turnover (TON) number is concerned, it could not be calculated accurately since (although rather harsh, that is, two-phase system, no buffer to protect the enzyme, very high substrate concentration…) the experimental conditions employed did not fully exhaust the biocatalyst over the time period used. However, minimum values could be evaluated. They indicate that, for the less substituted epoxides **1–3**, one molecule of enzyme could *at least* transform about 5 to 6×10^4 molecules of substrate. A nearly ten-fold decrease of this value was observed for **4** and **5**.

Finally, evaluation of the interest of such a process also implies to examine its potential cost and overall efficiency. This can be reflected both in terms of optimum percentage of biocatalyst to be used (mol%) and by determination of the space-time yield value. Our calculations indicated that, for *rac*-**1–3**, the molecular ratio of biocatalyst necessary to perform the resolution *within a very few hours* was as low as 9 to 10×10^{-4} mol%. In the case of the less reactive substrates *rac*-**4** and *rac*-**5**, about five times more enzyme had to be used to achieve the reaction within the same time period. It should however be stressed that, by allowing the process to be run over a longer time period, an even lower ratio (i.e., amount) of biocatalyst would be sufficient, thus further lowering the overall cost of a potential (industrial) process. The calculated space time yields also were very satisfactory – if not excellent – reaching 56 g/L/h in the case of *rac*-**3**.

50-g Preparative Scale Experiment on *rac*-3

In order to demonstrate the practical feasibility of this methodology, a preparative scale resolution of *rac*-**3** was achieved on a 50-g scale. This substrate was chosen because of its easy synthesis and high activity with the enzyme. The kinetic profile of this resolution is shown in Figure 2. A total amount of 310 mg of enzyme powder (i.e., an S/E = 160 ratio) was added to the reactor in four batches, over a total reaction time of 10.3 h. The reaction was followed by chiral GC analysis of the unreacted epoxide, and was stopped when this *ee* reached a value of about 99.5%. After extraction and normal work-up, 22.8 g of unreacted (*R*)-**3** were isolated (*ee* > 99%, 45.6% preparative yield) whereas 25.4 g of the formed (*S*)-**3d** diol were also obtained (*ee* 92%, 45.8% yield). Under these conditions, the reactor space-time yield was about 9 g/L/h.

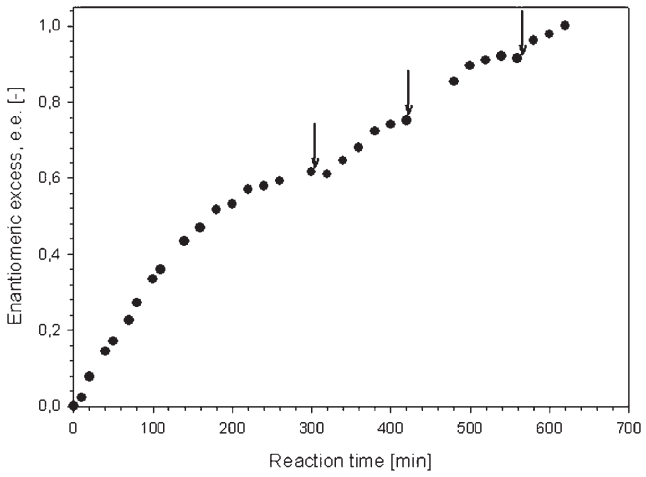


Figure 2. Kinteic profile of the 50-g scale resolution of *rac*-**3**. Arrows indicate addition of epoxide hydrolase.

Synthesis of the Corresponding (*S*)-Epoxides

In order to open the way to the preparation of either enantiomer of the epoxides and/or diols studied in enantiopure form, we have checked the possibility to cyclise the obtained enantiomerically enriched (*S*)-diols **1d–5d** back to the starting epoxide. Analytical scale experiments indicated that this could be efficiently achieved and did generally occur without noticeable (if any) loss of stereochemical integrity. This was for instance applied at a gram-scale to (*S*)-**2d** (*ee* 97%) and led to (*S*)-**2** which showed an *ee* of 90% (70% yield) using a TsCl/NaOH methodology. Similarly, the α-methyl-substituted diol (*S*)-**4d** (*ee* 72%) was converted into the corresponding acetonide on one hand or, on the other hand, cyclised back to the starting (*S*)-**4** epoxide without noticeable loss of enantiomeric integrity. Interestingly, this recyclisation strategy would allow one to resubmit the thus obtained enantiomerically enriched epoxide to a second *A. niger* EH-catalysed resolution cycle, which could afford the corresponding (*S*)-diol in nearly enantiopure form by fine-tuning the conversion ratio appropriately.[18] This diol could then be used further as a synthetic equivalent of the enantiopure (*S*) epoxide.[3]

Conclusions

In the course of this work, we have demonstrated that the resolution of five differently substituted glycidyl acetal derivatives could be achieved using the recombinant commercially available *A. niger* epoxide hydrolase. This interestingly confirmed that this enzyme – which we have shown previously to be very efficient for performing the HKR of various aromatic epoxides – can also be applied to the resolution of alkyl derivatives. This was successfully performed, at room temperature, using solely demineralised water as a solvent and by running a two-phase reactor at a global substrate concentration as high as 200 g/L. By using a biocatalyst over substrate molecular ratio as low as 9 to 10×10^{-4} mol%, the reaction could be performed within a few hours in all cases, allowing a space-time yield values of 56 g/L/h for the best reactor. All the epoxides of (*R*) absolute configuration could thus be prepared, for the first time, in high enantiomeric purity ($ee>99\%$), whereas the formed diols were of (*S*) configuration and showed moderate to excellent *ee*s (45–97%). Depending on the structure of the acetal moiety, the observed *E* values were shown to be modest to excellent, thus allowing the efficient preparation of a glycidyl acetal building block of high enantiomeric purity simply by choosing the right derivative. We have also shown that the obtained diols could be cyclised back into the corresponding epoxide without important loss of stereochemical integrity. This interestingly would allow to perform a second resolution cycle leading to the (*S*)-diols in nearly enantiopure form. The turnover frequency (TOF) as well as the total turnover number (TON) of the enzyme proved to be good to excellent against these substrates – reaching respectively values in the order of 6×10^2 mol of substrate/mol biocatalyst/minute and 6×10^4 mol of substrate/mol biocatalyst as a minimum value. As a demonstration experiment, a 50-g scale resolution of glycidaldehyde 2,2-dimethyltrimethylene acetal was performed.

Experimental Section

General Remarks

2-(Diethoxymethyl)oxirane **1** was purchased as a racemate from Interchim (Montlucon, France). Acrolein was from Acros (Noisy-Le-Grand, France). All other reagents were from Fluka (Buchs, Switzerland). Silica gel 60H (Fluka) was used for flash chromatography. GC analyses were performed with a Shimadzu GC14 A apparatus equipped with an FID detector and helium as carrier gas. Determination of the enantiomeric excesses was performed using 6 different chiral columns (0.25 mm, 25 m), i.e., Lipodex G (column I, Macherey–Nagel), Chirasil-Dex CB (column II, Varian), Cyclosil B (column III, Agilent), DA*ct*BuSilGammaCDX (column IV, MEGA), Lipodex E (column V, Macherey–Nagel), octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (column VI). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC250 instrument at 250 and 62.9 MHz, respectively. All measurements were carried out at room temperature in $CDCl_3$.

Synthesis of Epoxides

***2-(Diethoxymethyl)oxirane* (1):** Commercial **1** was purified by flash chromatography (pentane/diethyl ether gradient) and the solvent removed under normal pressure. The resi-

due was purified by bulb-to-bulb distillation (130 °C, 0.1 mbar) to give *rac*-**1** as a translucent colourless liquid. ^{1}H NMR: $\delta = 1.22$ (6H, m), 2.78 (m, 2H), 3.10 (m, 1H), 3.60, (4H, m), 4.34 (1H, d, $J = 4.4$ Hz);^{13}C NMR: $\delta = 15.4$ ($2CH_3$); 44.0 (CH_2); 52.0 (CH); 62.5 (CH_2); 63 (CH_2); 101.6 (CH).

***2-(Diisopropoxymethyl)oxirane* (2):** *para*-Toluenesulfonic acid (250 mg) was dissolved in triisopropyl orthoformate (50 mL, 224 mmol) under a nitrogen atmosphere and the solution was refluxed at 40 °C. Distilled acrolein was added dropwise until total conversion of triisopropyl orthoformate. The reaction mixture was neutralised with solid $NaHCO_3$, filtered over celite and then diluted with pentane. The organic phase was dried over $MgSO_4$ and solvent was removed under atmospheric pressure. *3,3-Diisopropoxyprop-1-ene* was recovered as a translucent colourless liquid after distillation (80 °C, 10 mbar); yield: 38–42 %. ^{1}H NMR: $\delta = 1.18$ (12H, m), 3.89 (2H, septet $J = 6.1$ Hz), 4.93 (1H, d, $J =$ 5.4 Hz), 5.30 (2H, m), 5.86 (1H, m).

3,3-Diisopropoxyprop-1-ene (31 g, 196 mmol), $NaHCO_3$ (7 g), benzonitrile (27 g, 262 mmol) and methanol (150 mL) were mixed at 35 °C. H_2O_2 (35 %, 25 mL) was added dropwise until total conversion of benzonitrile. The reaction mixture was extracted with CH_2Cl_2 and dried over $MgSO_4$. The solvent was distilled and the formed benzamide was precipitated in cold pentane. The solvent was distilled under atmospheric pressure, then **2** was isolated by distillation (70 °C, 7 mbar) and further purified by flash chromatography (pentane/ether gradient). The solvent was evaporated at atmospheric pressure and *rac*-**2** was recovered as a translucent colourless liquid after distillation under reduced pressure; yield: 50 %. ^{1}H NMR: $\delta = 1.18$ (12H, m), 2.75 (2H, m), 3.05 (1H, m), 3.92 (2H, septet, $J = 6.1$ Hz), 4.38 (1H, d, $J =$ 4.5 Hz); ^{13}C NMR: $\delta = 22.4$ (CH_3); 22.5 (CH_3); 23.1 (CH_3); 23.2 (CH_3); 44.0 (CH_2); 52.7 (CH); 68.8 (CH); 68.9 (CH); 99.0 (CH).

***5,5-Dimethyl-2-(oxiran-2-yl)-1,3-dioxane* (3):** Acrolein (200 mL) was added dropwise to a stirred mixture of anhydrous copper sulfate (100 g, 629 mmol), 2,2-dimethylpropane-1,3-diol (200 g, 1.92 mol) and *p*TSA (500 mg) in anhydrous CH_2Cl_2 (600 mL) under a nitrogen atmosphere. After total conversion of 2,2-dimethylene-1,3-propanediol, the reaction medium was filtered over celite and washed with CH_2Cl_2. Organic phases were dried over $MgSO_4$, solvent was removed under atmospheric pressure and the olefin was purified by distillation (97 °C, 150 mbar) to furnish *5,5-dimethyl-2-vinyl-1,3-dioxane* as a translucent liquid; yield: 84 %. ^{1}H NMR: $\delta = 0.73$ (3H, s), 1.20 (3H, s), 3.43 (2H, d, $J = 11$ Hz), 3.70 (2H, d, $J = 11$ Hz), 4.87 (1H, d, $J = 4.5$ Hz), 5.50 (1H, ddd, $J = 10.5$ Hz, $J = 1.5$ Hz, $J = 1.5$ Hz), 5.50 (1H, ddd, $J = 17.2$ Hz, $J = 1.5$ Hz, $J = 1.5$ Hz), 5.88 (1H, ddd, $J =$ 17 Hz, $J = 10.5$ Hz, $J = 4.7$ Hz).

To a stirred solution of *5,5-dimethyl-2-vinyl-1,3-dioxane* (71.5 g, 503 mmol), $NaHCO_3$ (17 g) and benzonitrile (67 g, 650 mmol) in methanol (300 mL), 25 mL of H_2O_2 (35 %) were added dropwise at 40 °C until total conversion of 5,5-dimethyl-2-vinyl-1,3-dioxane and of benzonitrile. The reaction mixture was filtered over celite and Na_2SO_3 was added to destroy the remaining H_2O_2. The reaction mixture was extracted two times by CH_2Cl_2 and the organic phase dried over $MgSO_4$. Solvent was evaporated under atmospheric pressure. Benzamide was precipitated in cold pentane (−20 °C) and solvent was eliminated by evaporation at atmospheric pressure. The obtained 5,5-dimethyl-2-(oxiran-2-yl)-1,3-dioxane (**3**) was further purified by flash chromatography (pentane/ether gradient) then distilled (90 °C, 5 mbar) to provide a colourless oil; yield: 57.4 g (72 %). ^{1}H NMR: $\delta = 0.70$ (3H, s), 1.18 (3H, s), 2.77 (2H, m), 3.07 (1H, m), 3.42 (2H, m) 3.62 (2H, m), 4.31 (1H, d, $J = 4.1$ Hz); ^{13}C NMR: $\delta = 21.7$ (CH_3); 22.8 (CH_3); 30.3 (C); 43.5 (CH_2); 51.5 (CH); 76.9 ($2CH_2$); 100.3 (CH).

***2-(Diethoxymethyl)-2-methyloxirane* (4):** 2-Methyl-2-propenal (10 mL, 121 mmol) was distilled prior use and added dropwise to a stirred solution of triethyl orthoformate (18 mL, 108 mmol) and *p*TSA (70 mg) at 0 °C under a nitrogen atmosphere. After 4 h, consumption of triethyl orthoformate was total. The reaction media was washed with saturated $NaHCO_3$ solution, and extracted three times with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and the solvent removed under normal pressure before distillation. *3,3-Diethoxy-2-methylprop-1-ene* was recovered as a translucent liquid (61 °C, 100 mbar); yield: 7.47 g (43 %). ^{1}H NMR: $\delta = 1.22$ (6H, m), 1.74 (3H, s), 3.53 (4H, m), 4.69 (1H, s), 4.98 (1H, m), 5.10 (1H, m).

To a solution of *3,3-diethoxy-2-methylprop-1-ene* (7.4 g, 51 mmol) dissolved in dichloromethane were added *m*CPBA (9 g, 52 mmol) and NaF (2.2 g, 52 mmol) and this solution was stirred at room temperature. After 24 h, *m*CPBA (4.5 g, 26 mmol) and NaF (1.1 g, 26 mmol) were added to the reaction mixture to complete the reaction. After total conversion of 3,3-diethoxy-2-methylprop-1-ene, the reaction mixture was filtered over celite and *m*CPBA was destroyed by addition of an aqueous solution of Na_2SO_3 (10 %). The organic phase was isolated and the aqueous phase was saturated with $NaHCO_3$ and extracted by CH_2Cl_2. The organic phases were pooled, dried over $MgSO_4$ and the solvent was removed under atmospheric pressure. *2-(Diethoxymethyl)-2-methyloxirane* (**4**) was isolated by distillation (90 °C, 6 mbar) as a translucent colourless liquid; yield: 19 %. ^{1}H NMR: $\delta = 1.24$ (6H, m), 1.37 (3H, s), 2.60 (1H, d, $J = 5.0$ Hz), 2.82 (1H, d, $J = 5.0$ Hz), 3.67 (4H, m), 4.18 (1H, s); ^{13}C NMR: $\delta = 15.21$ (CH_3); 15.26 (CH_3); 16.01 (CH_3); 50.43 (CH_2); 57.1 (C); 63.54 ($2CH_2$); 104.0 (CH).

***5,5-Dimethyl-2-(2-methyloxiran-2-yl)-1,3-dioxane* (5):** 2-Methyl-2-propenal (20 mL, 242 mmol) was distilled prior use and added dropwise to a stirred mixture of anhydrous copper sulfate (10 g), 2,2-dimethylpropane-1,3-diol (20 g, 192 mmol) and *p*TSA (50 mg) in anhydrous CH_2Cl_2 (100 mL) under a nitrogen atmosphere. After 24 h, conversion of the diol was complete. The reaction media was filtered over celite, washed with CH_2Cl_2 and dried over $MgSO_4$. The solvent was removed under normal pressure before isolation of *5,5-dimethyl-2-(prop-1-en-2-yl)-1,3-dioxane* by distillation (90 °C, 100 mbar) as a translucent liquid; yield: 22 g (58 %). ^{1}H NMR: $\delta = 0.74$ (3H, s), 1.21 (3H, s), 1.82 (3H, s), 3.50 (2H, d, $J = 11$ Hz), 3.66 (2H, d, $J = 11$ Hz), 4.76 (1H, s), 4.98 (1H, m), 5.15 (1H, m).

To a solution of *5,5-dimethyl-2-(prop-1-en-2-yl)-1,3-dioxane* (10 g, 64 mmol) in dichloromethane, were added *m*CPBA (22.3 g, 128 mmol) and NaF (5.5 g, 131 mmol). After stirring for 24 h at room temperature more *m*CPBA (11 g, 63 mmol) and NaF (2.7 g, 64 mmol) were added and the crude reaction mixture was stirred for 1 h. The reaction media was filtered over celite and remaining *m*CPBA was

destroyed by addition of an aqueous Na_2SO_3 solution (10%). The organic and aqueous phases were separated and the aqueous phase was saturated by $NaHCO_3$ and extracted by CH_2Cl_2. The organic phases were pooled, dried over $MgSO_4$ and the solvent was removed under atmospheric pressure. *5,5-Dimethyl-2-(2-methyloxiran-2-yl)-1,3-dioxane* (**5**) was recovered by distillation (90 °C, 60 mbar) as colourless and translucent oil; yield: 74%. ^{1}H NMR: $\delta = 0.73$ (3H, s), 1.21 (3H, s), 1.21 (3H, s), 2.64 (1H, d, $J = 5$ Hz), 2.86 (1H, d, $J = 5.3$ Hz), 3.44 (2H, m), 3.65 (2H, m), 4.17 (1H, s); ^{13}C NMR: 15.49 (CH_3); 21.76 (CH_3); 22.88 (CH_3); 30.34 (C); 51.07 (CH_2); 56.56 (C); 76.99 (CH_2); 77.05 (CH_2); 102.62 (CH).

General Procedure for Analytical Scale Biohydrolysis of *rac*-1–5

50 μL of enzymatic solution (1–10 mg mL^{-1} in phosphate buffer 10 mM, pH 7.0) were added to 450 μL of a freshly prepared aqueous solution (using demineralised water) of *rac*-**1**–**5** in order to obtain a final epoxide concentration of 5 to 200 g L^{-1}. The reaction was performed at 27 °C. Aliquots (50 μL or 1 μL for 5 g L^{-1} or 200 g L^{-1} respectively) were withdrawn at regular time intervals and immediately added to acetonitrile (50 μL). The remaining epoxide was extracted with hexane (100 μL) and its enantiomeric excess was determined by chiral GC analysis (column II, see below).

General Procedure for Semi-Preparative Biohydrolysis of 1–5

Typically, 1 mL of an *A. niger* EH solution (10 mg mL^{-1} in phosphate buffer 10 mM, pH 7.0) was added to *rac*-**1–3** (2 g) or **5** in demineralised water (7 mL) previously placed at 27 °C. The enantiomeric excess of the residual epoxide was determined by chiral GC chromatography (column II, see below) until $ee > 99\%$. The biohydrolysis of **4** (0.9 g) was performed at 27 °C in 3.7 mL of an *A. niger* EH solution (12.7 mg mL^{-1}). The biohydrolysis of **5** (2 g) was carried out at 27 °C in 8 mL of an *A. niger* EH solution (10 mg mL^{-1}). The enzymatic reaction was stopped by addition of 1 mL acetonitrile before extraction of the remaining epoxide with pentane. Pentane was removed under atmospheric pressure and remaining epoxide was purified by bulb-to-bulb distillation. The aqueous phase was extracted with ethyl acetate before being continuously extracted with CH_2Cl_2. The organic phases were removed under atmospheric pressure and crude diol was purified by bulb-to-bulb distillation.

The ^{1}H NMR spectra of the enantiopure epoxides **1–5** were identical to those of the racemates.

All diols were colourless liquids except diol **5d** which was found to be a white solid at room temperature (mp 42–43 °C).

(*R*)-**1** (ee > 99%): $[\alpha]_D^{28}$: +6.9 (*c* 1, EtOH); lit.[24] $[\alpha]_D^{28}$: +7.2 (*c* 1, EtOH); GC: Column V, 60 °C, 19.4 min (*R*) and 21.9 min (*S*).

(*S*)-**1d** ($ee = 54\%$): ^{1}H NMR: $\delta = 1.22$ (6H, m), 2.35 (1H, t, $J = 6.2$ Hz), 2.59 (1H, d, $J = 4.4$ Hz), 3.59 (3H, m), 3.78 (4H, m), 4.5 (1H, d, $J = 5.8$ Hz); ^{13}C NMR: 15.5 ($2CH_3$); 62.7(CH_2); 63.7 (CH_2); 64.4 (CH_2); 72.0 (CH); 103.5 (CH); $[\alpha]_D^{22}$: −15.3 (*c* 1.27, EtOH); lit.[24] (*R*)-**1d**: $[\alpha]_D^{28}$: +30.3 (*c* 1.04, EtOH).

(*R*)-**2** ($ee > 99\%$): $[\alpha]_D^{22}$: + 6.5 (*c* 1.08, EtOH); GC: Column I, 70 °C, 12.0 min (*S*) and 12.3 min (*R*); anal. calcd. for $C_9H_{18}O_3$ (174.24): C 62.77, H 9.36; found: C 62.51, H 10.61.

(*S*)-**2d** ($ee = 97\%$): ^{1}H NMR: $\delta = 1.21$ (12H, m), 2.38 (1H, t, $J = 6.2$ Hz), 2.55 (1H, d, $J = 5.7$ Hz), 3.52 (1H, m), 3.82 (4H, m), 4.66 (1H, d, $J = 5.0$ Hz); ^{13}C NMR: 22.1 (CH_3); 22.5 (CH_3); 23.2 (CH_3); 23.4 (CH_3); 62.3 (CH_2); 70.0(CH); 72.3 (CH); 100.2 (CH); $[\alpha]_D^{22}$: −22.2 (*c* 1.43, EtOH); GC: Column II, 115 °C, 13.2 min (*S*) and 14.5 min (*R*); anal. calcd. for $C_9H_{20}O_4$ (192.25): C 56.82, H 9.54; found: C 56.17, H 10.54.

(*R*)-**3** ($ee > 99\%$): $[\alpha]_D^{29}$: + 13.3 (*c* 1, EtOH); GC: Column II, 100 °C, 16.3 min (*S*) and 17.0 min (*R*) or column IV: 120 °C, 11.22 min (*R*) and 11.92 min (*S*); anal. calcd. for $C_8H_{14}O_3$ (158.19): C 60.74, H 8.92; found: C 59.55, H 8.85.

(*S*)-**3d** ($ee = 92\%$): ^{1}H NMR: $\delta = 0.74$ (3H, s), 1.19 (3H, s), 2.25 (1H, dd), $J = 7.1$ Hz, $J = 5.5$ Hz), 2.57 (1H, d, $J = 5.7$ Hz), 3.46 (2H, dd, $J = 11$ Hz, $J = 3.6$ Hz), 3.65 (3H, m), 3.80 (2H, m), 4.55 (1H, d, $J = 4.3$ Hz); ^{13}C NMR: 21.7 (CH_3); 22.9 (CH_3); 30.5 (C); 62.4 (CH_2); 72.1(CH); 76.9 ($2CH_2$); 100.3 (CH); $[\alpha]_D^{22}$: −13.2 (*c* 1.25, EtOH); anal. calcd. for $C_8H_{16}O_4$ (176.21): C 54.53, H 9.15; found: C 55.09, H 9.38.

(*R*)-**4**: ($ee > 99\%$): $[\alpha]_D^{30}$: + 17.8 (*c* 1.16, EtOH); GC: Column IV, 80 °C, 10.3 min (*R*) and 11.1 min (*S*).

(*S*)-**4d** ($ee = 45\%$): ^{1}H NMR: $\delta = 1.13$ (3H, s), 1.26 (6H, m), 2.50 (1H, dd, $J = 7.7$ Hz, 5.4 Hz), 2.72 (1H, s), 3.36 to 4.0 (6H, m), 4.36 (1H, s); ^{13}C NMR: 15.01 (CH_3); 15.08 (CH_3); 19.1 (CH_3); 65.78 (CH_2); 66.09 (CH_2); 66.36 (CH_2); 72.24 (C); 107.37 (CH); $[\alpha]_D^{30}$: +3.62 (*c* 0.94, EtOH).

(*R*)-**5** ($ee > 99\%$): $[\alpha]_D^{30}$: +6.0 (*c* 1.73, EtOH); GC: Column II, 120 °C, 6.5 min (*R*) and 6.9 min (*S*).

(*S*)-**5d** ($ee = 70\%$); ^{1}H NMR: $\delta = 0.71$ (3H, s), 1.17 (3H, s), 1.56 (3H, s), 2.49 (1H, dd, $J = 4.34$ Hz), 2.74 (1H, s), 3.45 (4H, m), 3.66 (2H, d, $J = 11$ Hz), 3.74 (1H, dd, $J = 11$ Hz, $J = 5.0$ Hz), 4.40 (1H); ^{13}C NMR: 19.35 (CH_3); 21.64 (CH_3); 22.89 (CH_3); 30.42 (C); 66.80 (CH_2); 72.70 (C); 77.18 (CH_2); 77.21 (CH_2); 104.36 (CH); $[\alpha]_D^{30}$: +5.2 (c 1.05, EtOH).

Preparative Scale Biohydrolysis of 50 g of *rac*-3 at 200 g·L^{-1}

In a propelled reactor, *rac*-**3** (50 g, 316 mmol) was added in plain (demineralised) water (180 mL). An emulsion was formed by agitation at ~350 rpm before careful addition of the enzyme (20 mL of a 10 mg mL^{-1} solution in phosphate buffer 10 mM, pH 7.0). The enantiomeric excess of the remaining epoxide was followed by chiral GC analysis (column IV). Enzyme (50 mg, 50 mg and 10 mg) was further added to reach an ee > 99% in three batches at time intervals as indicated on Figure 2. The reaction was stopped by addition of acetonitrile (5 mL). (*R*)-**3** was recovered by extraction with pentane followed by removal of the solvent under normal pressure. (*R*)-**3** was purified by distillation (47 °C, 2 mbar); yield: 22.8 g (144 mmol, 45.6%). The emulsion in the aqueous reaction media was broken by centrifugation and the aqueous phase was first extracted with ethyl acetate to recover the diol and further continuously extracted by CH_2Cl_2. The combined organic phases were dried over $MgSO_4$. Solvents were removed under atmospheric pressure.

(*S*)-**3d** was recovered after distillation (77 °C, 3–4.10^{-2} mbar); yield: 25.4 g (144 mmol, 45.9 %).

Derivatisation of 1d–5d to the Corresponding Acetonide

Diol **1d–5d** (5 μL) was added to 100 μL of a *p*TSA solution (0.47 g·L^{-1}) in 2,2-dimethoxypropane at room temperature. After 30 min, the reaction was quenched by addition of 50 μL $NaHCO_3$ (saturated solution). The organic phase was diluted with hexane before chiral GC analysis.

Acetonide of **1d**: GC: column II, 80 °C, 24.7 min (*S*) and 25.9 min (*R*).

Acetonide of **2d**: GC: column II, 100 °C, 12.2 min (*R*) and 12.7 min (*S*).

Acetonide of **3d**: GC: column I, 90 °C, 33.7 min (*R*) and 34.3 min (*S*).

Acetonide of **4d** : GC: column III, 130 °C, 9.72 min (*R*) and 9.99 min (*S*).

Acetonide of **5d** (**11**): GC: column III, 110 °C, 38.8 min (*R*) and 39.5 min (*S*).

Cyclisation of 4d–5d to the Corresponding Epoxides

Diol **4d** or **5d** (8–20 mg) was dissolved in 1 mL anhydrous THF before addition of one molar equivalent of tosyl chloride. After 30–60 min, a large excess of NaH (60–65 % in oil) was added and let to react overnight. Water (1 mL) was carefully added before extraction of the epoxide **4** or **5** with hexane and chiral GC analysis.

Assignment of the Absolute Configuration of the Epoxides and Diols

Epoxides (*R*)-**1** or (?)-**2** (50 mg) were dissolved in dry CH_2Cl_2 (10 mL) and stirred with montmorillonite K10 (200 mg). After 30 min at room temperature, the organic phase was submitted to chiral GC analysis in order to compare the elution order of (*R*)-**6** obtained from (*R*)-**1** and (?)-**2**. GC: column V, 60 °C, 3.4 min (*R*) and 3.7 min (*S*).

To a stirred solution of pure (*R*)-**1** (150 mg) dissolved in methanol (5 mL) MeONa (10 mg) was added and the mixture was refluxed for 24 h. Solvent was removed under vacuum, the residue was extracted with ether (20 mL) and the formed product (*R*)-**7** was dissolved in a sulfuric acid solution (1 mL, 0.1 N) and heated at 40 °C for 12 h. After neutralisation ($NaHCO_3$ powder) and extraction (CH_2Cl_2, 10 mL) of the aqueous solution the organic phase was dried over Na_2SO_4 and evaporated to give crude (*R*)-**8**. (*R*)-**8** was further protected using 2,2-dimethyl-1,3-propanediol (100 mg, 0.96 mmol) in CH_2Cl_2 (2 mL) in presence of $CuSO_4$ (20 mg) and *p*TSA (traces). After 1 h of stirring the $CuSO_4$ was filtered off and the organic phase, containing (*R*)-**9**, was directly submitted to chiral GC (column VI). Epoxide (?)-**3** (150 mg) was treated by MeONa in methanol as described above to lead to (?)-**9** and analysed by chiral GC (column VI) in order to compare the elution order with the one of (*R*)-**9** previously obtained. GC: column VI, 100 °C, 23.8 min (*R*) and 25.2 min (*S*).

Diol (?)-**4d** (30 mg, *ee* = 72 %), were stirred in water under acidic conditions for 2 h (50 mg Dowex $50H^+$). After filtration and evaporation of water in vacuum, 2,2-dimethoxypropane (300 μL) and 50 mg of Dowex $50H^+$ were added. The solution was stirred (45 min) to furnish (after filtration and evaporation) the corresponding aldehyde-acetonide (*S*)-**10** which was submitted to chiral GC analysis in order to compare the elution order with the one described in literature.[26] GC: column II, 70 °C, 10.7 min (*S*) and 11.22 min (*R*). The aldehyde moiety was then protected by 2,2-dimethyl-1,3-propanediol (35 mg) in CH_2Cl_2 in presence of anhydrous $CuSO_4$ (40 mg) and *p*TSA (traces) to lead to diacetal (*S*)-**11** (*ee* = 72 %). Diol (?)-**5d** (30 mg) was transformed to the corresponding acetonide **11** as previously described and the elution order of both acetonides was compared by chiral GC analysis (column III, see above).

Acknowledgements

This work was funded by the European Community Contract "Engineering integrated biocatalysts for the production of chiral epoxides and other pharmaceutical intermediates" (N° QLK3-CT2000–00426). Financial support, together with a fellowship of one of us (B. D.) is gratefully acknowledged.

References

[1] a) W. J. Choi, X. Y. Choi, *Biotechnol. Bioprocess Eng.* **2005**, 10, 167–179; b) E. J. de Vries, D. B. Janssen, *Curr. Opin. Biotech.* **2003**, *14*, 414–420; c) A. Archelas, R. Furstoss, *Curr. Opin. Chem. Biol.* **2001**, *5*, 112–119; d) A. Steinreiber, K. Faber, *Curr. Opin. Biotechnol.* **2001**, *12*, 552–558.

[2] A. Archelas, M. Arand, J. Baratti, R. Furstoss, *French Patent Application* No. 9905711, **1999**, and *International Patent Application* No. PCT/FR00/01217, **2000**.

[3] L. H. Byun, R. Bittman, *Tetrahedron* **2000**, *56*, 7051–7091.

[4] Fluka, ref. 71832.

[5] a) N. Monfort, A. Archelas, R. Furstoss, *Tetrahedron* **2004**, *60*, 601–604; b) K. M. Manoj, A. Archelas, J. Baratti, R. Furstoss, *Tetrahedron.* **2001**, *57*, 695–701; c) M. Cleij, A. Archelas, R. Furstoss, *J. Org. Chem.***1999**, *64*, 5029–5035.

[6] It is to be stressed that, due to the generally low aqueous solubility of most organic substrates, these experiments were all conducted in a two liquid liquid phase system. The term "global substrate concentration" therefore relates to the total amount of substrate present in the two phase reaction medium and includes both the dissolved and non-dissolved substrate.

[7] a) X.-J. Chen, A. Archelas, R. Furstoss, *J. Org. Chem.* **1993**, *58*, 5528–5532; b) A. Archelas, J.-P. Delbecque, R. Furstoss, *Tetrahedron: Asymmetry* **1993**, *4*, 2445–2446; c) P. Moussou, A. Archelas, R. Furstoss, *Tetrahedron* **1998**, *54*, 1563–1572.

[8] a) E. De Clercq, A. Holy, *J. Med. Chem.* **1979**, *22*, 510–513; b) B. Rada, M. Dragun, I. Votruba, A. Holy, *Acta Virologica (English Edition)* **1980**, *24*, 433–438.

[9] C.-Q. Han, D. DiTullio, Y.-F. Wang, C. J. Sih, *J. Org. Chem.* **1986**, *51*, 1253–1258.

[10] a) P. Page, C. Blonski, J. Périé, *Tetrahedron Lett.* **1995**, *36*, 8027–8030; b) P. Page, C. Blonski, J. Périé, *J. Bioorg. Medicinal Chem.* **1999**, *7*, 1403–14012.

[11] a) L. Hecquet, M. Lemaire, J. Bolte, C. Demuynck, *Tetrahedron Lett.* **1994**, *35*, 8791–8794; b) C.-H. Wong, E. Garcia-Junceda, L. Chen, O. Blanco, H. J. M. Gijsen, D. H. Steensma, *J. Am. Chem. Soc.* **1995**, *117*, 3333–3339.
[12] M. Yamaura, T. Nakayama, H. Hashimoto, C. G. Shin, J. Yoshimura, H. Kodama, *J. Org. Chem.* **1988**, *53*, 6035–6042.
[13] K. C. Nicolaou, E. A. Theodorakis, F. P. J. T. Rutjes, M. Sato, J. Tiebes, X.-Y. Xiao, C.-K. Hwang, M. E. Duggan, Z. Yang, E. A. Couladouros, F. Sato, J. Shin, H.-M. He, T. Bleckman, *J. Am. Chem. Soc.* **1995**, *117*, 10239–10251.
[14] C. Wattenbach, M. Maurer, H. Frauenrath, *Synlett* **1999**, 303–306.
[15] a) R. L. Peterson, K. K.-C. Liu, J. F. Rutan, L. Chen, C.-H. Wong, *J. Org. Chem.***1990**, *55*, 4897–4901; b) N. Bischofberger, H. Waldmann, T. Saito, E. S. Simon, W. Lees, M. D. Bednarsky, G. M. Whitesides, *J. Org. Chem.* **1988**, *53*, 3457–3465.
[16] G. B. Payne, P. H. Williams , *J. Org. Chem.* **1961**, *26*, 659–663.
[17] F. Camps, J. Coll, A. Messeguer, F. Pujol, *Chem. Lett.* **1983**, *7*, 971–974.
[18] C.-S. Chen, Y. Fujimoto, G. Girdaukas, C. J. Sih, *J. Am. Chem. Soc* **1982**, *104*, 7294–7299.
[19] P. Moussou, A. Archelas, J. Baratti, R. Furstoss, *J. Mol. Catal. B: Enzym.* **1998**, *5,* 213–217 [See Eq. (9) therein].
[20] P. Moussou, A. Archelas, J. Baratti, R. Furstoss, *Tetrahedron: Asymmetry* **1998**, *9*, 1539–1547.
[21] J. Zou, B. M. Hallberg, T. Bergfors, F. Oesch, M. Arand, S. L. Mowbray, T. A. Jones, *Structure* **2000**, *8*, 111–122.
[22] S. Pedragosa-Moreau, C. Morisseau, J. Zylber, A. Archelas, J. Baratti, R. Furstoss, *J. Org. Chem.* **1996**, *61*, 7402–7407.
[23] A. Liese, K. Seelbach, C. Wandrey , *Industrial Biotransformations*, Wiley-VCH, Weinheim, **2000**, pp. 57–64.
[24] N. Bischofberger, H. Waldmann, T. Saito, E. S. Simon, W. Lees, M. D. Bednarski, G. M. Whitesides, *J. Org. Chem.* **1988**, *53*, 3457–3465.
[25] E. C. L. Gautier, A. E. Graham, A. McKillop, S. P. Standen, R. J. K. Taylor, *Tetrahedron Lett.* **1997**, *38*, 1881–1884.
[26] A. Steinreiber, H. Hellström, S. F. Mayer, R. V. A. Orru, K. Faber, *Synlett* **2001**, *1*, 111–113.

