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# Shifting the equilibrium of a biocatalytic cascade synthesis to enantiopure epoxides using anion exchangers

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ABSTRACT

Hydroxide-loaded anion exchangers have been successfully employed to shift the equilibrium of a one-pot, two-step, two-enzyme cascade reaction affording enantiopure epoxides starting from prochiral α-chloroketones. The α-chloroketones were asymmetrically reduced employing an alcohol dehydrogenase and then transformed further to the corresponding epoxides employing halohydrin dehalogenases. Each epoxide enantiomer could be obtained with up to 93% conversion in enantiomerically pure form (>99% ee). In contrast to previous studies the amount of hydride donor (2-propanol) could be reduced due to favoured halohydrin formation in the reduction of α-chloroketones.

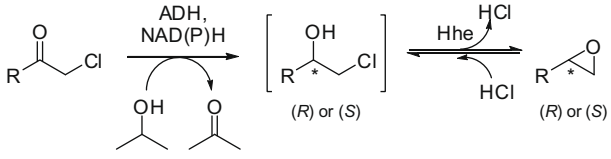


## 1. Introduction

Enantiopure epoxides and their ring-opening products (β-substituted alcohols) are important building blocks for the synthesis of high-value chiral compounds (e.g., pharmaceuticals).[1–5] While a considerable number of methods exist for the organo- or metal-catalysed epoxidation of olefins,[5–8] biocatalytic approaches to enantiomerically pure epoxides often rely on kinetic resolution of the racemate, with the major drawback that the maximum theoretical yield is limited to 50% for each enantiomer.[9–13]

A more straightforward approach is the biocatalytic asymmetric reduction of the α-chloroketone coupled with chemical ring-closure under basic conditions in a second step.[14] We have recently shown that the asymmetric bioreduction of prochiral α-chloroketones to β-chlorohydrins using an alcohol dehydrogenase (ADH) can be coupled with a biocatalytic ring closure to the epoxide employing halohydrin dehalogenase HheB from *Mycobacterium* sp. in a cascade process (Scheme 1).[15]

However, its practicability was limited by the unfavourable equilibrium of the (reversible) epoxide ring-closure reaction yielding just 57% of the epoxide in the best example. As a first attempt to overcome this problem, Ag(I) salts were used for precipitating the chloride ion formed as a byproduct, but this was not successful, since deactivation of the halohydrin dehalogenase (Hhe) occurred.[15] In this study we have employed hydroxide-loaded anion exchangers in order to bind the chloride ion liberated in the reaction, resulting in a shift of the equilibrium to the product side.




**Scheme 1.** Biocatalytic cascade sequence for the synthesis of enantiopure epoxides. ADH = alcohol dehydrogenase; Hhe = halohydrin dehalogenase.

## 2. Results and discussion

In the first step of the cascade, α-chloroketones were stereoselectively reduced to afford the enantiopure β-chlorohydrin intermediates. The stereocomplementary alcohol dehydrogenases ADH-'A' from *Rhodococcus ruber* DSM 44541[16,17] and LBADH from *Lactobacillus brevis*[18] were used to synthesise both enantiomers of the product by choosing the appropriate ADH (with either *Prelog*[19] or *anti-Prelog* selectivity). Regeneration of the nicotinamide cofactor was implemented via hydrogen transfer, the 'coupled substrate' approach[20] using 2-propanol as a hydrogen source. Two different halohydrin dehalogenases were investigated for epoxide formation: HheB from *Mycobacterium* sp. GP1,[21,22] and HheC from *Agrobacterium radiobacter* AD1.[22,23] The latter was applicable only for the conversion of a single enantiomer due to its higher stereoselectivity, which is in contrast to HheB which showed low enantioselectivity and could therefore transform both enantiomers.[22]

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While the applicability of HheB for this type of cascade process has been shown previously,[15] the tolerance of HheC towards the required cosubstrate 2-propanol and the coproduct acetone remained to be investigated. In a test experiment the enzyme HheC showed 50% residual activity in the presence of 25% v/v of acetone using *rac*-1-chloro-3-phenoxy-2-propanol **2a** as substrate. In the presence of 2-propanol a steeper decrease in activity with increasing 2-propanol concentration was observed compared to acetone, showing 21% residual activity at 25% v/v 2-propanol (Fig. 1).

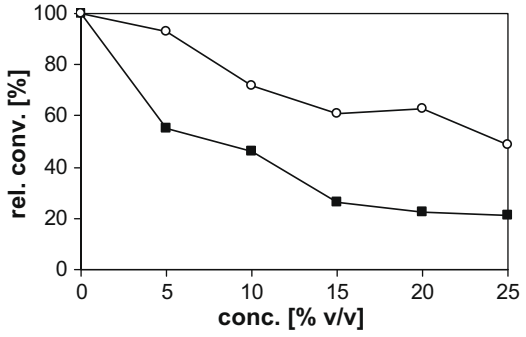
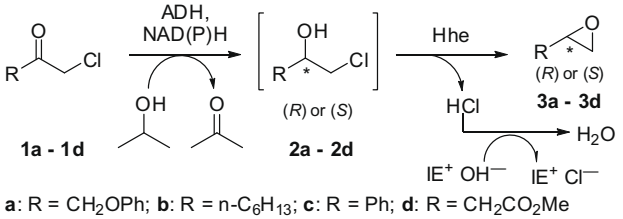



**Figure 1.** Conversion of halohydrin **2a** to epoxide **3a** employing HheC at varied concentrations of 2-propanol (–■–) or acetone (–○–) (4 h reaction time).

However, very recently we observed that the ADH-catalysed oxidation of *sec*-alkanols with α-chloroacetone as a formal oxidant was virtually irreversible,[24] thus only 1 equiv of α-chloroacetone was required in this biocatalytic hydrogen transfer oxidation reaction, which otherwise requires a 10-fold excess if acetone is employed to shift the equilibrium to the product side. We applied here for the first time the reverse concept, thus the asymmetric synthesis of halohydrins from the corresponding α-chloroketones employing theoretically only 1 equiv of 2-propanol. In order to improve the solubility of the lipophilic substrate in the aqueous phase we used 1% v/v 2-propanol, at which enzyme deactivation was negligible.

In order to test whether the application of anion exchangers would lead to higher epoxide conversions for the ring-closure reaction of the halohydrins (Scheme 2), the transformation of halohydrin **2a** to the corresponding epoxide **3a** was tested in the presence of different amounts of hydroxide-loaded anion exchanging resins employing HheC as a catalyst. Best results were obtained employing MTO-Dowex® SBR-LCNG OH-form, doubling conversion within 4 h compared to the reaction without ion exchanger (Fig. 2). Additionally it could be shown that the anion exchanger did not influence the enantiomeric purity of the outcome, thus no racemisation of the intermediate α-halohydrin occurred.




**Scheme 2.** Shifting equilibrium to enantiopure epoxide in the biocatalytic cascade sequence employing ion exchanger. The schematic reaction part involving the IE is a simplified representation. IE = ion exchanger; ADH = alcohol dehydrogenase; Hhe = halohydrin dehalogenase.

Testing the effect of the best ion exchanger on the buffer showed that the strongly basic anion exchanger MTO-Dowex® caused a slight change of the pH. For instance under the best conditions applied (60 mg of MTO-Dowex® SBR-LCNG OH-form per 1 mL of 200 mM buffer, pH 7.5) the pH shifted from 7.5 to 8.3. In order to differentiate between effects caused by the pH-shift and those caused by the exchanger itself, epoxide formation from halohydrin *rac*-**2a** with HheC was tested at pH 7.5 and pH 8.3 without ion exchanger and in the presence of ion exchanger at a final pH of 8.3. Epoxide formation without ion exchanger was rather slow (Fig. 3). In contrast, a significant increase in conversion was observed in the presence of anion exchanger. Additional experiments showed that MTO-Dowex® already catalysed epoxide formation in the absence of HheC; nevertheless epoxide formation was three times faster in the presence of HheC and anion exchanger.


**Figure 2.** Conversion of **2a** to **3a** employing HheC and different amounts of anion exchangers: Amberlite® (–■–), Amberlyst® (–○–), Ambersep® (–□–) or MTO-Dowex® (–●–) (4 h reaction time).

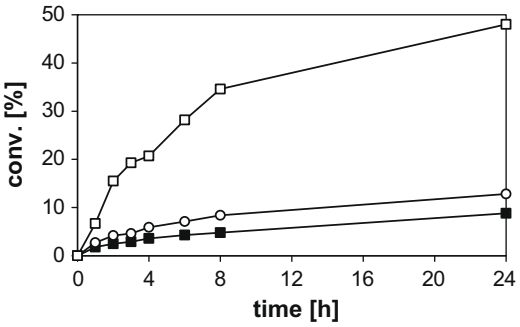



**Figure 3.** Transformation of **2a** to **3a** employing HheC in buffer at pH 7.5 (–■–), pH 8.3 (–○–), and buffer with anion exchanger MTO-Dowex® at a final pH of 8.3 (–□–).

After verifying that the ADHs were active in the presence of the ion exchangers, the complete cascade process was tested. α-Chloroketone **1b** was converted to the corresponding epoxide **3b** employing ADH-'A' and HheC as well as 30 mg of MTO-Dowex®. Following the time course of the reaction (Fig. 4), the reduction

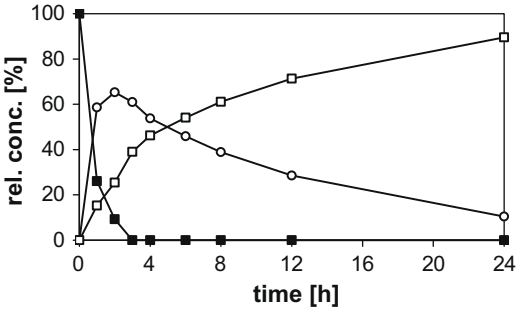



**Figure 4.** Time course for the conversion of **1b** (–■–) to **3b** (–□–) via **2b** (–○–) employing ADH-'A' and HheC in the presence of MTO-Dowex®.

of the chloroketone turned out to be much faster than the ring-closure reaction; thus the substrate was reduced within 4 h.

This showed clearly that for the reduction of α-chloroketones no huge excess of the reducing agent 2-propanol was required to shift the equilibrium to the product side; compared to the previous studies we just used one-fifth of 2-propanol, corresponding to two equivalents. Although 1 equiv would be sufficient we used an elevated amount for increasing the solubility of the lipophilic substrate in the aqueous phase. After 24 h a conversion of 91% to the enantiomerically pure epoxide **3b** was achieved.

Additionally to 1-chloro-2-octanone **1b** also 1-chloro-3-phenoxy-2-propanone **1a** was transformed to the epoxide **3a** with excellent conversion (93%) performing the reduction and ring-closure simultaneously. Subjecting 2-chloroacetophenone **1c** and methyl-4-chloro-3-hydroxybutyrate **1d** to the cascade resulted in lower yields first. This could be attributed to inhibition of HheC by the chloroketones **1c** and **1d**. Inhibition phenomena could successfully be avoided by retarded addition of the halohydrin dehalogenase and the ion exchanger to the reaction mixture after the ketone was reduced, that is, after 4 h (Table 1). This procedure ensured that epoxide ring-closure started after ketone reduction was completed, thus the two reaction steps were separated by time while still being performed in one pot without isolation of the intermediates.

**Table 1**
Retarded addition of HheC and MTO-Dowex® for the conversion of **1c** to **3c** and **1d** to **3d**

| Substrate | Reagents added after 4 h | Conv.[a] (%) | | |
|---|---|---|---|---|
| | | **1** | **2** | **3** |
| **1c** | — | 0 | 67 | 33 |
| **1c** | HheC | 0 | 58 | 42 |
| **1c** | HheC, MTO-Dowex® | 15 | 10 | 72[b] |
| **1d** | — | 0 | 79 | 21 |
| **1d** | HheC | 0 | 81 | 19 |
| **1d** | HheC, MTO-Dowex® | 2 | 61 | 37 |

[a] Determined by GC analysis after 24 h reaction time.
[b] 3% of diol formed.

Having found a solution to avoid the inhibition, optimisation of further reaction parameters such as amount of enzymes and ion exchanger was performed. Finally, employing the optimised procedure (see Section 4) both epoxide stereoisomers were obtained in high yields and excellent enantiomeric purity (Table 2).

Finally, the cascade process employing ADH-'A' and HheC was performed on a preparative scale (Table 3). The GC conversions for the preparative scale were similar to the values obtained on the analytical scale; the isolated yields were limited due to the volatility of the recovered epoxides.

**Table 3**
Preparative scale (50 mg) cascade transformation of chloroketones **1a**, **1b** and **1c** into enantiopure epoxides

| Product | Conv.[a] (%) | Yield[b] (%) | ee[c] (%) | $[\alpha]_D^{20}$ |
|---|---|---|---|---|
| **3a** | 83 | 47 | >99 (*S*) | +4.5 |
| **3b** | 87 | 40 | >99 (*R*) | +4.8 |
| **3c** | 89 | 45 | >99 (*R*) | −19.5 |

[a] Measured by GC after 24 h reaction time.
[b] Isolated yield.
[c] Determined by GC or HPLC using a chiral stationary phase.

## 3. Conclusion

A two-enzyme cascade reaction system yielded enantiopure epoxides starting from prochiral α-chloroketones. The equilibrium could be shifted to the side of the epoxide by addition of hydroxide-loaded anion exchangers to remove the chloride ions liberated during epoxide formation. Ion exchangers were successfully employed to shift the equilibrium in a biocatalysed process due to ion-entrapment, and not due to enzyme stabilisation or suppression of substrate inhibition effects.[25,26] The anion exchangers did not alter the stereochemical composition of the product or intermediate; thus no racemisation was observed. Additionally it could be shown that the amount of 2-propanol as a hydride source could be kept to a minimum, since the equilibrium in the reduction step is on the side of the halohydrin. Depending on the substrate the cascade could be performed simultaneously or, in case of inhibition of the halohydrin dehalogenase by α-chloroketone, in a one-pot, two-step fashion. By applying this concept the α-chloroketones investigated were transformed to the corresponding enantiomerically pure epoxides with up to 93% conversion. By choosing the appropriate alcohol dehydrogenase both enantiomers were accessible.

## 4. Experimental

### 4.1. General remarks

NMR spectra were recorded in $CDCl_3$ using a Bruker AMX 360 at 360 ($^1$H) and 90 ($^{13}$C) MHz, respectively. Chemical shifts are reported relative to TMS ($\delta$ = 0.00) and coupling constants ($J$) are given in hertz. TLC plates were run on Silica Gel Merck 60 $F_{254}$ and compounds were visualised either by spraying with Mo reagent [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (100 g $L^{-1}$), $Ce(SO_4)_2\cdot 4H_2O$ (4 g $L^{-1}$) in $H_2SO_4$ (10%)] or by UV. Optical rotation values ($[\alpha]_D^{20}$) were measured on a Perkin–Elmer polarimeter 341 at 589 nm (Na line) in a 1 dm cuvette.

For anhydrous reactions, flasks were dried and flushed with dry argon just before use. Standard syringe techniques were applied to

**Table 2**
Results of the cascade reaction using substrates **1a–d**

| Substrate | ADH | Hhe | Ketone **1**[a] (%) | Chlorohydrin **2**[a] (%) | Epoxide **3** (%) | |
|---|---|---|---|---|---|---|
| | | | | | (%)[a] | ee[b] (%) |
| **1a** | ADH-'A' | HheC | 0 | 7 | 93 | >99 (*S*)[c] |
| **1b** | ADH-'A' | HheC | 0 | 9 | 91 | >99 (*R*) |
| **1c** | ADH-'A' | HheC | 0 | 7 | 93 | >99 (*R*) |
| **1d** | ADH-'A' | HheC | 0 | 51 | 49 | >99 (*R*) |
| **1a** | LBADH | HheB | 0 | 33 | 67 | >99 (*R*)[c] |
| **1b** | LBADH | HheB | 0 | 9 | 91 | >99 (*S*) |
| **1c** | LBADH | HheB | 0 | 11 | 84[d] | >99 (*S*) |
| **1d** | LBADH | HheB | 0 | 13 | 61[e] | >99 (*S*) |

[a] Composition determined by GC analysis after 24 h reaction time.
[b] Determined by GC or HPLC on a chiral stationary phase.
[c] Switch in CIP-priority.
[d] 5% of diol formed.
[e] 26% of diol formed.

transfer dry solvents and reagents in an inert atmosphere of dry argon. Anhydrous THF was distilled from sodium under $N_2$ atmosphere. Petroleum ether (bp 60–90 °C) and EtOAc used for chromatography were distilled prior to use.

Lyophilised cells of *Escherichia coli* Tuner™ (DE3)/pET22b-'ADH-A' containing the alcohol dehydrogenase ADH-'A' were prepared as previously described.[17] LBADH (#05.11, 500 U $mL^{-1}$, alcohol dehydrogenase from *L. brevis*), HheB (#45.30, 252 U $mL^{-1}$, halohydrin dehalogenase from *Mycobacterium* sp. GP1) and HheC (#46.30, 65 U $mL^{-1}$, halohydrin dehalogenase from *A. radiobacter* AD1) were purchased from *Codexis*. Anion exchangers Amberlyte® IRN-78 OH-form, Amberlyst® A-26 OH-form, Ambersep® 900 OH-form and MTO-Dowex® SBR LCNG OH-form were purchased from *Sigma–Aldrich*.

ω-Chloroacetophenone **1c**, methyl-4-chloroacetoacetate **1d**, *rac*-1,2-epoxy-3-phenoxypropane **3a**, *rac*-1,2-epoxyoctane **3b** and *rac*-styrene oxide **3c** are commercially available.

### 4.2. Synthesis of substrates and reference compounds

1-Chloro-3-phenoxy-2-propanone **1a**,[27] 1-chloro-2-octanone **1b**,[28] *rac*-1-chloro-3-phenoxy-2-propanol **2a**,[29] *rac*-1-chloro-2-octanol **2b**[29] and *rac*-methyl-3,4-epoxybutyrate **3d**[30] were synthesised as described in the literature. *rac*-2-Chloro-1-phenylethanol **2c** and *rac*-methyl-4-chloro-3-hydroxybutyrate **2d** were synthesised by $NaBH_4$-reduction of the corresponding ketones following a procedure given in the literature.[31]

### 4.3. Bioconversions

#### 4.3.1. Epoxide formation using HheC at varied 2-propanol and acetone concentrations

HheC (10 μL, 0.65 U) and *rac*-1-chloro-3-phenoxy-2-propanol **2a** (5 μL, 31 μmol) were suspended in Tris–$SO_4$ buffer (500 μL, 200 mM, pH 7.5). The appropriate amounts of 2-propanol or acetone (25–125 μL) were added. The samples were incubated for 4 h at 30 °C and 120 rpm, extracted with EtOAc (600 μL) and dried over $Na_2SO_4$. The conversion was determined by GC analysis.

#### 4.3.2. Epoxide formation using HheC and varied amounts of anion exchangers

Five samples were prepared for each anion exchanger (Amberlyte® IRN-78 OH-form, Amberlyst® A-26 OH-form, Ambersep® 900 OH-form, MTO-Dowex® SBR LCNG OH-form): HheC (10 μL, 0.65 U) and *rac*-1-chloro-3-phenoxy-2-propanol (**2a**; 5 μL, 31 μmol) were suspended in Tris–$SO_4$ buffer (500 μL, 200 mM, pH 7.5). The appropriate amounts of the anion exchange resin (5–25 mg) were added. The samples were incubated for 4 h at 30 °C and 120 rpm, extracted with EtOAc (600 μL) and dried over $Na_2SO_4$. The conversion was determined by GC analysis.

#### 4.3.3. Time study of the cascade reaction sequence using *E. coli* Tuner™ (DE3)/pET22b-'ADH-A' and HheC

Lyophilised cells of *E. coli* Tuner™ (DE3)/pET22b-'ADH-A' (5 mg) were rehydrated in Tris–$SO_4$ buffer (500 μL, 200 mM, pH 7.5) for 1 h at 30 °C and 120 rpm. 2-Propanol (5 μL, 65 μmol), HheC (30 μL, 1.95 U), MTO-Dowex® SBR LCNG OH-form (30 mg) and 1-chloro-2-octanone (**1b**; 5 μL, 31 μmol) were added. The samples were incubated for the appropriate time (1–24 h) at 30 °C and 120 rpm, extracted with EtOAc (600 μL) and dried over $Na_2SO_4$. The conversion was determined by GC analysis.

#### 4.3.4. Retarded addition of HheC and MTO-Dowex®

For the two substrates investigated (2-chloroacetophenone **1c**, methyl-4-chloroacetoacetate **1d**) three samples were prepared: Lyophilised cells of *E. coli* Tuner™ (DE3)/pET22b-'ADH-A' (5 mg) were rehydrated in Tris–$SO_4$ buffer (500 μL, 200 mM, pH 7.5) for 1 h at 30 °C and 120 rpm. 2-Propanol (5 μL, 65 μmol), the substrate (**1c**, 5.4 mg, 35 μmol or **1d**, 4 μL, 35 μmol) and either (a) HheC (30 μL, 1.95 U) and MTO-Dowex® SBR LCNG OH-form (30 mg), (b) just MTO-Dowex® SBR LCNG OH-form (30 mg) or (c) none of them were added. The samples were incubated at 30 °C and 120 rpm. After 4 h the missing reagents [HheC (30 μL, 1.95 U) and/or MTO-Dowex® SBR LCNG OH-form (30 mg)] were added to the incomplete samples. Afterwards all samples were incubated for another 20 h, extracted with EtOAc (600 μL) and dried over $Na_2SO_4$. The conversion was determined by GC analysis.

#### 4.3.5. Procedures for the cascade reaction sequence using *E. coli* Tuner™ (DE3)/pET22b-'ADH-A' and HheC

**4.3.5.1. Procedure A—addition of all reagents at *t* = 0 h.** Lyophilised cells of *E. coli* Tuner™ (DE3)/pET22b-'ADH-A' (5 or 7 mg) were rehydrated in Tris–$SO_4$ buffer (500 μL, 200 mM, pH 7.5) for 1 h at 30 °C and 120 rpm. 2-Propanol (5 μL, 65 μmol), HheC (30 μL, 1.95 U), MTO-Dowex® SBR LCNG OH-form (30 or 50 mg) and the substrate (**1a**, 4 μL, 27 μmol or **1b**, 5 μL, 31 μmol) were added. The samples were incubated for 24 h at 30 °C and 120 rpm, extracted with EtOAc (600 μL) and dried over $Na_2SO_4$. The conversion was determined by GC analysis.

**4.3.5.2. Procedure B—retarded addition of HheC and ion exchanger.** Lyophilised cells of *E. coli* Tuner™ (DE3)/pET22b-'ADH-A' (7 or 10 mg) were rehydrated in Tris–$SO_4$ buffer (500 μL, 200 mM, pH 7.5) for 1 h at 30 °C and 120 rpm. 2-Propanol (5 μL, 65 μmol) and the substrate (**1c**, 5.4 mg, 35 μmol or **1d**, 4 μL, 35 μmol) were added. The samples were incubated for 4 h at 30 °C and 120 rpm. After that HheC (30 μL, 1.95 U) and MTO-Dowex® SBR LCNG OH-form (35 or 50 mg) were added. Afterwards all samples were incubated for another 20 h, extracted with EtOAc (600 μL) and dried over $Na_2SO_4$. The conversion was determined by GC analysis.

The reaction conditions are summarised in Table 4.

**Table 4**
Reaction conditions for the cascade reaction sequence using *E. coli* Tuner™ (DE3)/pET22b-'ADH-A' and HheC

| Substrate | Procedure | ADH-'A' (mg) | HheC (μL) | IE[a] (mg) |
|---|---|---|---|---|
| **1a** | A | 7 | 30 | 50 |
| **1b** | A | 5 | 30 | 30 |
| **1c** | B | 10 | 30 | 35 |
| **1d** | B | 7 | 30 | 50 |

[a] IE = ion exchanger MTO-Dowex® SBR LCNG OH-form.

#### 4.3.6. Procedures for the cascade reaction sequence using LBADH and HheB

**4.3.6.1. Procedure A—addition of all reagents at *t* = 0 h.** A stock solution of LBADH (100 μL, 50 U) in Tris–$SO_4$ buffer (900 μL, 200 mM, pH 7.5, 1 mg $mL^{-1}$ NADPH) was prepared. LBADH stock solution (10 μL), 2-propanol (5 μL, 65 μmol), HheB (30 μL, 75.6 U), MTO-Dowex® SBR LCNG OH-form (30 or 50 mg) and the substrate (**1a**, 4 μL, 27 μmol or **1b**, 5 μL, 31 μmol) were added to Tris–$SO_4$ buffer (500 μL, 200 mM, pH 7.5). The samples were incubated for 24 h at 30 °C and 120 rpm, extracted with EtOAc (600 μL) and dried over $Na_2SO_4$. The conversion was determined by GC analysis.

**4.3.6.2. Procedure B—retarded addition of HheB and ion exchanger.** A stock solution of LBADH (100 μL, 50 U) in Tris–$SO_4$ buffer (900 μL, 200 mM, pH 7.5, 1 mg $mL^{-1}$ NADPH) was prepared. LBADH stock solution (20 μL), 2-propanol (5 μL, 65 μmol) and the substrate (**1c**, 5.4 mg, 35 μmol or **1d**, 4 μL, 35 μmol) were added to Tris–$SO_4$ buffer (500 μL, 200 mM, pH 7.5). The samples were

incubated for 4 h at 30 °C and 120 rpm. After that HheB (50 μL, 126 U) and MTO-Dowex® SBR LCNG OH-form (35 or 50 mg) were added. Afterwards all samples were incubated for another 20 h, extracted with EtOAc (600 μL) and dried over $Na_2SO_4$. The conversion was determined by GC analysis.

The reaction conditions are summarised in Table 5.

**Table 5**
Reaction conditions for the cascade reaction sequence using LBADH and HheB

| Substrate | Procedure | LBADH (μL) | HheB (μL) | IE[a] (mg) |
|---|---|---|---|---|
| **1a** | A | 10 | 30 | 50 |
| **1b** | A | 10 | 30 | 30 |
| **1c** | B | 20 | 50 | 35 |
| **1d** | B | 20 | 50 | 50 |

[a] IE = ion exchanger MTO-Dowex® SBR LCNG OH-form.

#### 4.3.7. Procedures for the preparative scale cascade reaction sequence using *E. coli* Tuner™ (DE3)/pET22b-'ADH-A' and HheC

**4.3.7.1. Procedure A—addition of all reagents at *t* = 0 h.** Lyophilised cells of *E. coli* Tuner™ (DE3)/pET22b-'ADH-A' (50 mg) were rehydrated in Tris–$SO_4$ buffer (5 mL, 200 mM, pH 7.5) for 1 h at 30 °C and 120 rpm. 2-Propanol (50 μL, 0.65 mmol), HheC (300 μL, 19.5 U), MTO-Dowex® SBR LCNG OH-form (350 or 500 mg) and the substrate (**1a**, 40 μL, 0.27 mmol or **1b**, 50 μL, 0.31 mmol) were added. The mixture was incubated for 24 h at 30 °C and 120 rpm, extracted with EtOAc (2 × 10 mL), dried over $Na_2SO_4$ and evaporated under reduced pressure to give the crude product. Flash chromatography ($Al_2O_3$, petrol ether/EtOAc = 4/1) furnished the pure epoxides.

(*S*)-**3a** (19 mg, 47%) colourless liquid. $^1$H NMR (360 MHz, $CDCl_3$) $\delta$: 2.78 (1H, dd, $J_1$ = 4.7 Hz, $J_2$ = 2.5 Hz, $CH_2$), 2.92 (1H, t, $J$ = 4.5 Hz, $CH_2$), 3.36–3.39 (1H, m, CH), 3.98 (1H, dd, $J_1$ = 11.0 Hz, $J_2$ = 5.4 Hz, $CH_2$), 4.23 (1H, dd, $J_1$ = 11.0 Hz, $J_2$ = 3.2 Hz, $CH_2$), 6.93–7.01 (3H, m, Ar), 7.29–7.33 (2H, m, Ar). $^{13}$C NMR (90 MHz, $CDCl_3$) $\delta$: 44.7, 50.1, 68.7, 114.7, 121.2, 129.5, 158.5. *m*/*z* (EI, 70 eV): 150 ($M^+$), 120, 107, 94, 77, 65, 57, 51, 39. HPLC (Chiralpak AD, *n*-Heptan): ee >99% (*S*). $[\alpha]_D^{20} = +4.7$ (*c* 1, $CHCl_3$), lit.[32] $[\alpha]_D^{20} = +4.5$ (*c* 0.4, $CHCl_3$, ee = 93.2%).

(*R*)-**3b** (16 mg, 40%) colourless liquid. $^1$H NMR (360 MHz, $CDCl_3$) $\delta$: 0.85–0.90 (3H, m, $CH_3$), 1.24–1.63 (10H, m, $CH_2$), 2.48 (1H, dd, $J_1$ = 5.0 Hz, $J_2$ = 2.7 Hz, $OCH_2$), 2.77 (1H, t, $J$ = 4.5 Hz, $OCH_2$), 2.90–2.94 (1H, m, OCH). $^{13}$C NMR (90 MHz, $CDCl_3$) $\delta$: 14.0, 22.5, 25.9, 29.1, 31.7, 32.5, 47.1, 52.4. *m*/*z* (EI, 70 eV): 128 ($M^+$), 113, 85, 57, 43. GC (Chiralsil Dex-CB): ee >99% (*R*). $[\alpha]_D^{20} = +4.8$ (*c* 1, $CHCl_3$), lit.[33] $[\alpha]_D^{20} = +7.4$ (*c* 1.15, $CHCl_3$, ee >99%).

**4.3.7.2. Procedure B—retarded addition of HheC and ion exchanger.** Lyophilised cells of *E. coli* Tuner™ (DE3)/pET22b-'ADH-A' (50 mg) were rehydrated in Tris–$SO_4$ buffer (5 mL, 200 mM, pH 7.5) for 1 h at 30 °C and 120 rpm. 2-Propanol (50 μL, 0.65 mmol) and ω-chloroacetophenone (**1c**, 54 mg, 0.35 mmol) were added. The solution was incubated for 4 h at 30 °C and 120 rpm. After that HheC (300 μL, 19.5 U) and MTO-Dowex® SBR LCNG OH-form (350 mg) were added. Afterwards the mixture was incubated for another 20 h, extracted with EtOAc (2 × 10 mL), dried over $Na_2SO_4$ and evaporated under reduced pressure to give the crude product as a yellowish liquid. Flash chromatography ($Al_2O_3$, petrol ether/EtOAc = 4/1) furnished (*R*)-**3c** (19 mg, 45%) as a colourless liquid. $^1$H NMR (360 MHz, $CDCl_3$) $\delta$: 2.82 (1H, dd, $J_1$ = 5.5 Hz, $J_2$ = 2.6 Hz, $OCH_2$), 3.17 (1H, dd, $J_1$ = 5.4 Hz, $J_2$ = 4.1 Hz, $OCH_2$), 3.88 (2H, dd, $J_1$ = 3.8, $J_2$ = 2.7, OCH), 7.29–7.39 (5H, m, Ar). $^{13}$C NMR (90 MHz, $CDCl_3$) $\delta$: 51.2, 52.3, 125.5, 128.2, 128.5, 137.6. *m*/*z* (EI, 70 eV): 120 ($M^+$), 119, 104, 91, 77, 65, 51, 39. GC (Chiralsil Dex-CB): ee >99% (*R*). $[\alpha]_D^{20} = -19.5$ (*c* 1, $CHCl_3$), lit.[34] $[\alpha]_D^{23} = +23.6$ (*c* 0.83, $CHCl_3$, ee = 94.4%).

#### 4.3.8. Analytics

**4.3.8.1. GC, achiral stationary phase.** A *Varian* CP 1301 (30 m × 250 μm × 0.25 μm) 6% cyanopropyl-phenylpolysiloxane phase column was employed and $H_2$ (column flow: 1.2 mL min$^{-1}$) was used as carrier gas.

**1a–3a**: temperature programme: 100–150 °C/25 °C min$^{-1}$–270 °C/10 °C min$^{-1}$/hold 2 min. $t_r$ [min] = 4.4 **1a**, 4.6 **2a**, 3.1 **3a**. **1b–3b**: temperature programme: 90–145 °C/5 °C min$^{-1}$–205 °C/30 °C min$^{-1}$/hold 3 min. $t_r$ [min] = 4.5 **1b**, 4.9 **2b**, 2.3 **3b**. **1c–3c**: temperature programme: 90–120 °C/10 °C min$^{-1}$–270 °C/15 °C min$^{-1}$/hold 2 min. $t_r$ [min] = 5.2 **1c**, 5.0 **2c**, 2.8 **3c**. **1d–3d**: temperature programme: 90–130 °C/5 °C min$^{-1}$–250 °C/30 °C min$^{-1}$/hold 2 min. $t_r$ [min] = 3.6 **1d**, 4.1 **2d**, 2.2 **3d**.

**4.3.8.2. GC, chiral stationary phase.** A *Varian* CP-Chiralsil-DEX CB (25 m × 320 μm × 0.25 μm) cyclodextrin on dimethylpolysiloxane phase column was employed and $H_2$ was used as carrier gas.

**3b**: column flow: 0.4 mL min$^{-1}$, temperature programme: 65 °C isothermal. $t_r$ [min] = 13.1 (*R*), 13.5 (*S*). **3c**: column flow: 0.4 mL min$^{-1}$, temperature programme: 80 °C isothermal. $t_r$ [min] = 15.1 (*R*), 16.8 (*S*). **3d**: column flow: 0.3 mL min$^{-1}$, temperature programme: 60 °C isothermal. $t_r$ [min] = 23.1 (*R*), 23.5 (*S*).

**4.3.8.3. HPLC, chiral stationary phase.** A *Daicel* Chiralpak AD column (0.46 cm × 25 cm, column oven temperature: 12 °C) was employed and *n*-heptane (flow: 0.4 mL min$^{-1}$) was used as the eluent. **3a**: $t_r$ [min] = 34.5 (*R*), 41.8 (*S*).

#### 4.3.9. Determination of absolute configurations

Absolute configurations were either assigned by comparison of specific rotation with literature data (see above), and/or comparison of elution order with the literature on a chiral stationary phase.

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