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Non-Racemic Halohydrins *via* Biocatalytic Hydrogen-Transfer Reduction of Halo-Ketones and One-Pot Cascade Reaction to Enantiopure Epoxides

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Abstract: Biocatalytic hydrogen-transfer reduction of α-chloro-ketones furnished non-racemic chlorohydrins by employing either *Rhodococcus ruber* as lyophilized cell catalyst or an alcohol dehydrogenase preparation from *Pseudomonas fluorescens* DSM 50106 (PF-ADH). For all substrates investigated, *Rhodococcus ruber* gave strictly the "Prelog" product, whereas PF-ADH showed scattered stereopreference. One possibility for a follow-up reaction of halohydrins is the ring closure to the corresponding epoxide. A novel "one pot-one step strategy" was employed to obtain the enantiopure epoxide from the α-chloro-ketone in a cascade like fashion at pH > 12 involving biocatalytic hydrogen transfer reduction and *in situ* chemo-catalyzed ring closure.

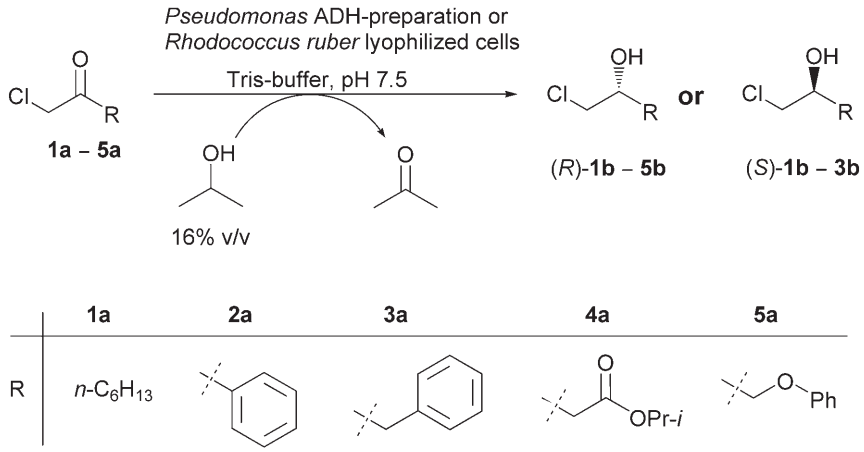
Keywords: asymmetric synthesis; cascade reaction; enzyme catalysis; epoxides; halohydrins; hydrogen-transfer reduction

Introduction

Enantiopure halohydrins are versatile intermediates for the synthesis of a vast number of bioactive compounds like natural products,[1,2] agrochemicals[2] and pharmaceuticals,[3–5] e.g., β-blockers,[6,7] HIV-protease inhibitors,[8,9] or anti-asthmatic agents.[10] Facile access to enantiopure halohydrins is achieved *via* catalytic asymmetric reduction of the corresponding α-halo-ketones *via* chiral organo catalysts[10,11] or biocatalysts. For the biocatalytic approach, the following systems were employed: (i) isolated alcohol dehydrogenases (e.g., from *Rhodococcus erythropolis*,[12] baker's yeast,[13–15] horse liver, *Thermoanaerobium brockii*, *Lactobacillus brevis*,[16] or *Pseudomonas* sp.[17]), (ii) *E. coli* transformants expressing a carbonyl reductase (e.g., from *Candida magnoliae*,[18,19]) or an alcohol dehydrogenase (e.g., from *Candida parapsilosis*[20] or *Kluyveromyces aestuarii*[21]) as well as (iii) whole cells (preferentially yeast[5,7,22–25], *Geotrichum candidum*[2,5,26–28] or various strains[9,22,26,29–32]). One reason for the high significance of chiral α-halohydrins is their broad applicability as chiral intermediates, e.g,. α-halohydrins can easily be converted to the corresponding chiral epoxides,[11,30,33] which opens a large spectrum for further transformations. We present here an asymmetric reduction by biocatalytic hydrogen transfer of α-halo-ketones to access for some substrates both enantiomers of chiral halohydrins and a novel "one-pot one-step" cascade-reaction consisting of a biocatalytic reduction of α-halo ketones and *in situ* base-induced ring closure to furnish the corresponding epoxide.

Results and Discussion

We have recently presented the asymmetric reduction of ketones and the enantioselective oxidation of alcohols employing *Rhodococcus ruber* DSM 44541 as lyophilized whole cell catalyst.[34–36] The broad applicability of this catalyst results from its high stability toward organic compounds and co-solvents and its ability to recycle the cofactor NADH *via* a simple hydrogen transfer



Scheme 1. Biocatalytic reduction of α-chloro-ketones *via* hydrogen transfer to access (*R*)- or (*S*)-halohydrins.

Table 1. Results of the biocatalytic reduction of **1a** – **5a** after 24 hours.

Substrate[b]	PF-ADH preparation[a]		*R. ruber* whole cells	
	Conversion [%]	ee [%][c]	Conversion [%]	ee [%][c]
1a	63	52 (*S*)-**1b**	>99	99 (*R*)-**1b**
2a	33	18 (*S*)-**2b**	21	43 (*R*)-**2b**
3a	>99	98 (*S*)-**3b**	82	73 (*R*)-**3b**
4a	97	95 (*R*)-**4b**	>99	>99 (*R*)-**4b**
5a	>99	76 (*R*)-**5b**	<1	n.a.[d]

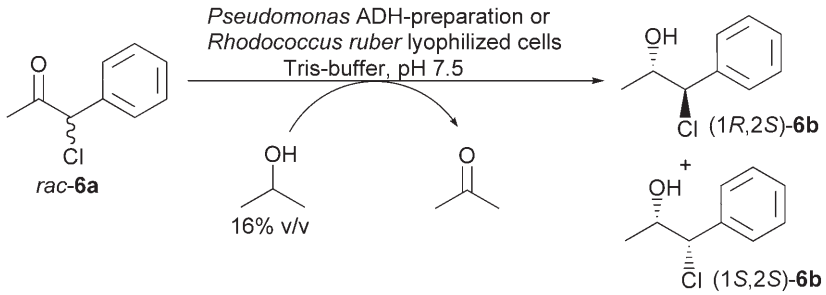
[a] Alcohol dehydrogenase preparation from *Pseudomonas fluorescens* DSM 50106.
[b] Substrate concentration was 50–58 mM, for **1a** and *R. ruber* it was 110 mM.
[c] Enantiomeric excess of the alcohol obtained, the stereoisomer denoted is in excess.
[d] n.a. = not applicable, i.e., the ee was not measured due to very low conversion.

mode using 2-propanol (for reduction) and acetone (for oxidation).[37] In order to provide access to both enantiomers of the halohydrins, stereo-complementary enzymes were required. Whereas *Rhodococcus ruber* DSM 44541 is known to follow Prelog's rule,[38] an alcohol dehydrogenase from *Pseudomonas fluorescens* DSM 50106[39,40] (PF-ADH) was expected to give the opposite enantiomer. The PF-ADH possesses also the ability to recycle the cofactor NADH using 2-propanol.

Prochiral α-halo-ketones **1a**–**5a** (Scheme 1) were chosen as model substrates for the reduction of prochiral ketones. In order to circumvent the use of highly toxic organo-cadmium compounds for the synthesis of substrate **1a**,[41] an alternative "green" reaction sequence employing zinc was adapted.[42,43] Thus, 1-bromohexane was transformed into the corresponding zinc species which was added *via* a Knochel-cuprate to chloroacetyl chloride to give **1a**. For both catalysts 2-propanol was employed as hydrogen donor and co-solvent at a concentration of 16% v/v.

Hydrogen transfer reduction of 1-chloro-2-octanone (**1a**) employing *Pseudomonas fluorescens* ADH gave access to the (*S*)-enantiomer of **1b** with moderate ee (52%), while *Rhodococcus ruber* cells yielded the opposite enantiomer (*R*)-**1b** with excellent ee (99%) and conversion (>99%), thus for substrate **1a** the two biocatalysts showed complementary enantiopreference, as expected. Derivatives of **2b** are important intermediates for a number of pharmaceuticals.[44,45] Both enantiomers of 2-chloro-1-phenylethanol (**2b**) and 3-chloro-1-phenyl-2-propanol (**3b**) were accessible in a stereocomplementary fashion using the two biocatalysts, whereby PF-ADH showed the best conversion and highest stereoselectivity for the reduction of **3a** with (*S*)-preference, while *R. ruber* gave again the (*R*)-enantiomer in excess.

Unexpectedly, reduction of **4a** gave only the (*R*)-enantiomer (*R*)-**4b** with both catalysts at very high ee (95% for PF-ADH; >99% for *R. ruber*), thus for PF-ADH a switch in stereopreference was observed, meaning that for the β-keto ester **4a** PF-ADH followed Prelog's rule in contrast to the previous transformed substrates. Halohydrin (*R*)-**4b** is used for the synthesis of (*R*)-carnitine[46] or bioactive chiral oxazolidinones.[47] The isopropyl ester of **4a** was preferred as substrate to the methyl/ethyl ester, which underwent transesterification with the co-substrate 2-propanol when whole cells were employed as catalyst. To our surprise, substrate **5a** was not accepted by *R. ruber*, however, it was completely re-



Scheme 2. Diastereoselective reduction of *rac*-**6a**.

Table 2. Results from biocatalytic hydrogen transfer reduction of *rac*-**6a** after 24 hours.

Catalyst	Conversion [%]	ee [%][b]			de [%][b]
		6a	(1*S*,2*S*)-**6b**	(1*R*,2S)-**6b**	(1*S**,2*S**)-**6b**
PF-ADH preparation[a]	51	<1	54	75	4
R. ruber cells	78	<1	>99	97	88

[a] Alcohol dehydrogenase preparation from *Pseudomonas fluorescens* DSM 50106.
[b] The isomer denoted is in excess.

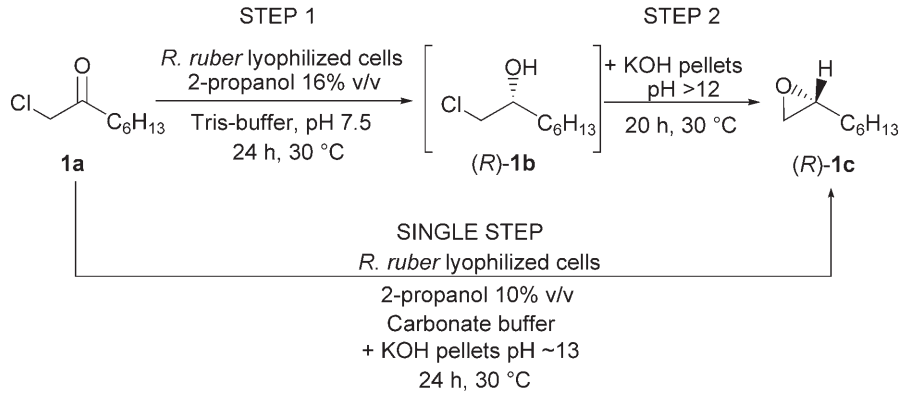
duced by PF-ADH within 24 hours to furnish the "Prelog product" (*R*)-**5b**, as already observed for **4a**.

In order to demonstrate the applicability of this process to obtain preparative amounts of halohydrins, the experiments with *Rhodococcus ruber* DSM 44541 were repeated on a larger scale (200 μL substrate) at 100 mM substrate concentration. After 24 hours, (*R*)-**1b** was obtained in 96% ee at complete conversion. This non-optimized reduction corresponds to a productivity of 3.6 mmol L^{-1} h^{-1} (=0.6 g L^{-1} h^{-1}) or 0.24 g product per gram lyophilized cells. The conversions and ee obtained in the preparative-scale reduction of **2a**–**4a** were comparable to those listed in Table 1, although the ees for (*R*)-**2b** and (*R*)-**3b** were slightly higher, namely 51% and 91%, respectively.

Substrate *rac*-**6a** (Scheme 2) bears a chiral center in the α-position of the carbonyl moiety and thus potentially gives rise to diastereomeric halohydrin products. Furthermore, the asymmetric bioreduction may proceed *via* kinetic or dynamic resolution,[48] depending on the racemization rate of the α-stereocenter.[49] Interestingly, the stereoselectivity varied considerably among both enzymes: *rac*-**6a** was reduced by PF-ADH to the "Prelog" alcohol with very low stereoselectivity (Table 2). In contrast, *R. ruber* cells gave the (1*S*,2*S*)-**6b** isomer – again the "Prelog" alcohol – as the main product in >99% ee and good de (88%). The fact that the ee of the other diastereomer (1*R*,2*S*)-**6b** produced was also very good (97%) indicated that the carbonyl moiety of both enantiomers of *rac*-**6a** was reduced to the corresponding (*S*)-alcohol with very high stereoselectivity. The good de (88%) shows that the chiral center in the α-position was recognized by *R. ruber* to a remarkable extent. At 78% conversion, the non-converted substrate **6a** was racemic, which indicated that the substrate was racemized under the conditions employed, allowing a dynamic kinetic resolution. Performing the reduction of *rac*-**6a** with *R. ruber* on a preparative scale (250 mg) a conversion of 60% was obtained within 24 hours, again the carbonyl group was exclusively reduced to the (*S*)-alcohol, thus the ee of (1*S*,2*S*)-**6b** and (1*R*,2*S*)-**6b** was >99%, however the de had somewhat decreased to 60%.

The feasibility of a "one pot-two steps" strategy to obtain the chiral epoxide from the α-chloro-ketone *via* sequential reduction to the halohydrin in a first step followed by base-mediated ring closure of the latter to furnish the epoxide in a second separate step has already been proven.[11,30,50] Applying this concept, we employed the biocatalytic hydrogen transfer concept to the reduction of **1a** in Tris-buffer (pH 7.5) as the first step to give the chiral halohydrin (*R*)-**1b** with 99% ee at >99% conversion within 24 hours. Without purification of the product, the pH was adjusted to pH >12 by addition of KOH pellets in the same reaction vessel (Scheme 3, upper pathway). After further 20 hours, complete conversion to enantiopure (*R*)-1,2-epoxyoctane (*R*)-**1c** was achieved.

An even more elegant approach than the above described "one pot-two steps" strategy from the α-chloro-ketone to the epoxide would be a "one pot-one step" strategy. In this case, the biocatalytic reduction has to be performed under strongly basic conditions. To find the optimum reaction conditions for ring closure of the halohydrin, we determined the conversion of the halohydrin to the epoxide in the absence of biocatalyst at varying pH. Since Tris-buffer is not applicable at higher pH values, phosphate and carbonate buffers were



Scheme 3. "One pot-two steps" and "one-pot-one-step" transformation of α-chloro-ketone to the corresponding epoxide by biocatalytic hydrogen-transfer reduction followed by base-mediated ring closure.

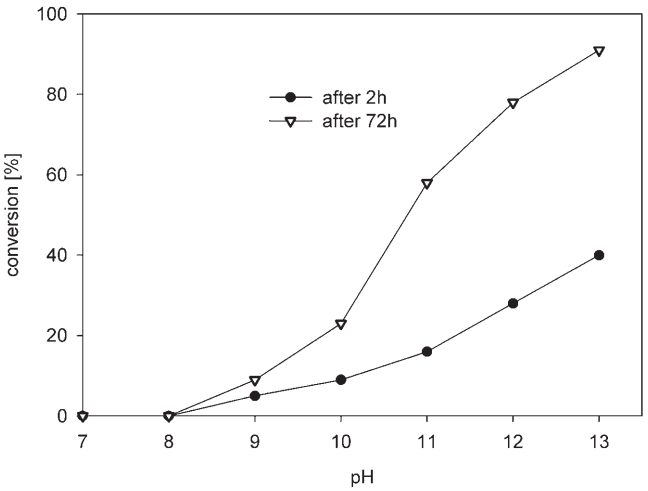


Figure 1. Base-induced formation of *rac*-1,2-epoxyoctane **1c** from *rac*-halohydrin **1b** after 2 hours (●) and 72 hours (▽) at various pH values.

used instead (up to pH 10.0 and between pH 11.0–13.0, respectively). At room temperature, ring closure of 1-chloro-2-octanol (**1b**) commenced at pH 9.0 at very low rate and increased with increasing pH (and extended reaction time, Figure 1). At pH 12.0, 78% of halohydrin **1b** were converted to epoxide **1c** after 72 hours. No hydrolysis of the epoxide to the corresponding diol was observed under the conditions employed.

We envisaged to perform the ring closure reaction *in situ* in combination with the biocatalytic reduction at a pH $\geq$12 due to a faster reaction rate of the epoxide formation which draws the halohydrin out of the ketone/alcohol equilibrium of the hydrogen-transfer reaction, therefore shifting the reaction to the product side. Unfortunately, neither the purified ADH from *R. ruber*[37] nor *P. fluorescens* ADH is stable at such a (biologically) extreme pH. However, when protected within the shielding environment of a whole microbial cell, the ADH from *R. ruber* in the wild-type strain is active at such a biocatalytically exceptionally high pH.[51] When the biocatalytic reduction of 1-chloro-2-octanone (**1a**) employing lyophilized cells of *R. ruber* was carried out in potassium carbonate buffer pH 12.0, **1b** was formed, but no formation of 1,2-epoxyoctane **1c** was observed, although it was expected according to Figure 1. Consequently, when an excess of powdered KOH (1 pellet mL^{-1}) was added either during the reaction or simultaneously with substrate **1a**, the formed α-chlorohydrin **1b** was immediately converted to epoxide **1c** (Scheme 3, lower pathway) in a cascade-like fashion.[52] Thus 1-chloro-2-octanone (**1a**; 20 mg) applied to whole cells of *R. ruber* DSM 44541 was transformed to enantiopure (*R*)-1,2-epoxyoctane [(*R*)-**1c**] in >99% ee at 90% conversion after already 24 hours, which is approximately one day faster than the "one pot-two steps" protocol. The use of other buffer systems (e.g., phosphate buffer) gave rise to undesired side-products. When the cascade reaction was up-scaled for 400 mg of substrate, the amount of employed cells was increased by a factor of five, so that the reaction was already finished after 4.5 hours (>99% conversion) to give (*R*)-1,2-epoxyoctane [(*R*)-**1c**; >99% ee].

Conclusion

Biocatalytic hydrogen-transfer reduction of prochiral α-chloro-ketones employing either lyophilized cells of *Rhodococcus ruber* DSM 44541 or an alcohol dehydrogenase preparation from *Pseudomonas fluorescens* DSM 50106 using 2-propanol as reducing agent gave the corresponding halohydrins in high ee. The reduction of racemic α-chloro-ketone **6a** using *R. ruber* showed moderate enantioselectivity for the two substrate enantiomers, however the carbonyl moiety of both enantiomers was reduced exclusively to the (*S*)-alcohol. For all substrates investigated, *Rhodococcus ruber* gave exclusively the "Prelog product", while *Pseudomonas fluorescens* ADH exhibited opposite stereopreference for

certain substrates. Thus, any deduction of the stereopreference of *Pseudomonas* ADH for a given substrate is not seriously possible. Furthermore, an elegant novel "one pot-one step" strategy to convert a prochiral α-chloro-ketone to the enantiopure epoxide in a cascade-like fashion is presented by employing whole cells of *R. ruber* as alkali-stable biocatalyst at pH > 12 for the hydrogen-transfer reduction at the expense of 2-propanol as hydrogen donor. The alkaline conditions pH > 12 ensured rapid ring-closure of the halohydrin to the epoxide, drawing the alcohol out of the ketone/alcohol equilibrium.

Experimental Section

General Remarks

1-Bromohexane, chloroacetyl chloride, *rac*-1,2-epoxyoctane [*rac*-**1c**], (*R*)-1,2-epoxyoctane [(*R*)-**1c**], ω-chloroacetophenone (**2a**), (2,3-epoxypropyl)-benzene, 1-phenyl-2-propanone, 1-phenyl-1-propanol, tetrapropylammonium perruthenate (TPAP), epichlorohydrin and phenol were commercially available. 2-Propyl 4-chloro-3-oxobutanoic acid ester (**4a**) as well as racemic and enantiopure 2-propyl 4-chloro-3-hydroxy-butanoic acid ester (**4b**) were gifts of CIBA SC, Basel/Switzerland.

rac-2-Chloro-1-phenylethanol (*rac*-**2b**),[53] *rac*-3-chloro-1-phenoxy-2-propanol (*rac*-**5b**)[7] and 1-chloro-1-phenyl-2-propanone (**6a**),[29] were synthesized as described in the literature.

Optical rotations were measured on a Perkin Elmer Polarimeter 341 in a 1 mL cuvette of 10 cm length. Column chromatography was performed using Merck 60 silica gel (40–63 μm). ^{1}H and ^{13}C NMR were recorded on a Bruker 360 MHz spectrometer at 360 and 90 MHz, respectively, using TMS as internal standard. THF was freshly distilled before use from sodium/potassium alloy under argon atmosphere.

Lyophilized cells of *Rhodococcus ruber* DSM 44541[54] and the alcohol dehydrogenase preparation from *Pseudomonas fluorescens* DSM 50106[40] were obtained as previously reported.

Synthesis of Substrates and Reference Compounds

1-Chloro-2-octanone (**1a**): Naphthalene (6.86 g, 53.5 mmol) was added to lithium (0.36 g, 52 mmol, solid lumps) in THF (27 mL) under an argon atmosphere. After a few minutes, an intensive green color was observed. The solution was stirred at room temperature for 30 minutes before a solution of dry zinc(II) chloride (3.61 g, 26.5 mmol) in THF (30 mL) was added dropwise during 30 minutes. The mixture was left standing for 30 minutes, THF was removed and fresh THF was added. The washing procedure was repeated once to remove excess naphthalene, before 1-bromohexane (3.8 mL, 19.8 mmol) was added in one portion. The mixture was stirred at room temperature for three hours and left standing for one hour. After filtration, the solution was added during 60 minutes to lithium bromide (0.62 g, 7.1 mmol) and copper(I) cyanide (0.46 g, 5.1 mmol) in THF (20 mL) at −40 °C in a three-necked, round-bottom flask (500 mL). Finally chloroacetyl chloride (1.5 mL, 18.8 mmol) was added and the reaction mixture was left to warm to room temperature while stirring was continued for 16 hours. The reaction was quenched by the addition of aqueous NH_4Cl (2 M, 50 mL), the mixture extracted with diethyl ether (3 × 30 mL), the combined organic phase was dried (Na_2SO_4), the organic solvent was removed under reduced pressure and the crude product was purified by column chromatography (50 g silica gel, petroleum ether/ethyl acetate = 20:1) to give pure **1a** as a yellowish oil; yield: 533 mg (18%). The product was identified by comparison of ^{1}H and ^{13}C NMR with literature data.[42]

rac-*1-Chloro-2-octanol* (*rac*-**1b**): 1-Chloro-2-octanone (**1a**; 19.3 mg, 0.12 mmol) was dissolved in absolute ethanol (3 mL) and $NaBH_4$ (9.4 mg, 0.25 mmol) was added at −30 °C. The stirring was continued at −30 °C for 15 minutes before the mixture was stirred at room temperature for 1.5 hours. After acidification with 5% aqueous H_2SO_4 (3 mL) and extraction with dichloromethane (3 × 3 mL), the combined organic phase was dried (Na_2SO_4), the organic solvent removed under reduced pressure and the crude product was purified by column chromatography (2 g silica gel, petroleum ether/ethyl acetate = 10:1) to give pure *rac*-**1b**; yield: 7.6 mg (42%). The product was identified by comparison of ^{1}H and ^{13}C NMR with literature data.[55]

rac-*3-Chloro-1-phenyl-2-propanol* (*rac*-**3b**): $LiClO_4$ (4.54 g, 43 mmol) and NH_4Cl (2.17 g, 41 mmol) were suspended in acetonitrile (50 mL) and stirred for 10 minutes before (2,3-epoxypropyl)-benzene (3.56 g, 27 mmol) was added. The mixture was refluxed for 3 hours, then it was cooled to room temperature and water (100 mL) was added. After extraction with ethyl acetate (3 × 100 mL) the combined organic phase was dried (Na_2SO_4), the organic solvent was removed under reduced pressure to give *rac*-**3b** (98% pure by GC); yield: 3.54 g (99%). The product was identified by comparison of its ^{1}H NMR spectrum with literature data.[56]

3-Chloro-1-phenyl-2-propanone (**3a**): Under an argon atmosphere, *rac*-3-chloro-1-phenyl-2-propanol (*rac*-**3b**; 2.15 g, 12.6 mmol) dissolved in diethyl ether (10 mL) was added slowly to a mixture of *N*,*N*′-dicyclohexylcarbodiimide (DCC, 5.2 g, 25.2 mmol) in pyridine (0.2 mL), DSMO (2 mL) and ether (30 mL) at 4 °C. After 30 minutes of stirring, CF_3COOH (0.2 mL) was added and the mixture was allowed to warm to room temperature, the stirring was continued for 16 hours. The solution was again cooled to 0 °C and oxalic acid (2.3 g, 25 mmol) dissolved in absolute MeOH (5 mL) was added. The mixture was filtered through a layer of silica gel (1 cm) and the silica gel was carefully washed with ethyl acetate (3 × 20 mL). The combined organic phase was washed with water (3 × 20 mL), aqueous $NaHCO_3$ solution (5%, 1 × 20 mL) and finally with brine (1 × 20 mL). The organic phase was dried (Na_2SO_4), the organic solvent was removed under reduced pressure and the crude product was purified by column chromatography (50 g silica gel, petroleum ether/ethyl acetate = 5:1) to give pure **3a**; yield: 0.93 g (44%). The product was identified by comparison of ^{1}H and ^{13}C NMR with literature data.[57]

3-Chloro-1-phenoxy-2-propanone (**5a**): *rac*-3-Chloro-1-phenoxy-2-propanol (*rac*-**5b**; 187 mg, 1 mmol) was dissolved in acetone (5 mL) before tetrapropylammonium perruthenate (TPAP, 35 mg, 0.1 mmol) and *N*-methylmorpholine *N*-oxide (246 mg, 2.1 mmol) were added and the mixture was stirred at room temperature for 18 hours.[58] The mixture was filtered through 1 cm of silica gel and the organic solvent was removed

under reduced pressure. The crude product was purified by column chromatography (4 g silica gel, petroleum ether/ethyl acetate = 25:1) to give pure **5a**; yield: 107 mg (58%). The product was identified by comparison of ^{1}H and ^{13}C NMR with literature data.[7]

Reference materials of the isomers of **6b** were obtained by $NaBH_4$/EtOH reduction of the corresponding racemic ketone *rac*-**6a** similar to the procedure described for *rac*-**1b**. NMR data were identical to literature values.[29]

Epoxide Formation Depending on pH

The following buffer solutions (100 mL each) were prepared: 50 mM K_2HPO_4/NaH_2PO_4 at pH 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5 and 10.0 as well as 50 mM K_2CO_3 at pH 11.0, 12.0 and > 12.0. *rac*-1-Chloro-2-octanol (*rac*-**1b**; 10 mg, 60 μmol) was added (a) directly and (b) dissolved in 200 μL acetone to give 1 mL and 800 μL, respectively, of each buffer solution followed by shaking the reaction mixture at room temperature at 130 rpm for 2 and 72 hours, respectively. Afterwards, the reaction was stopped by extraction with ethyl acetate (0.5 mL), the organic phase was dried (Na_2SO_4) and analyzed by GC.

Biocatalytic Transformations

Conversions were measured on an achiral GC column, before the ee was measured on a chiral GC column (in some cases after derivatization), for details see the Supporting Information.

Representative Procedure for Small-Scale Reduction using *Rhodococcus ruber* DSM 44541

Lyophilized cells of *Rhodococcus ruber* DSM 44541 (20 mg) were suspended in Tris/HCl buffer (600 μL, 50 mM, pH 7.5) and rehydrated at 30 °C for one hour at 130 rpm. 1-Chloro-2-octanone (**1a**; 10 μL, 0.06 mmol) and 2-propanol (120 μL) were added. After 45 hours of shaking at 30 °C and 130 rpm, the reaction was stopped by extraction with ethyl acetate (3 × 200 μL), the combined organic phase was dried (Na_2SO_4) and analyzed by GC.

Representative Procedure for Small-Scale Reduction using Alcohol Dehydrogenase from *Pseudomonas fluorescens* DSM 50106

PF-ADH-preparation (lyophilized, 20 mg) was rehydrated in Tris/HCl-buffer (600 μL, 50 mM, pH 7.5) at 15 °C for one hour at 130 rpm. Substrate (5 mg) and 2-propanol (120 μL) were added. After 24/48 hours of shaking at 15 °C and 130 rpm, the reaction was stopped by extraction with ethyl acetate (3 × 200 μL), the combined organic phase was dried (Na_2SO_4) and analyzed by GC.

Preparative-Scale Biotransformation

Lyophilized cells of *Rhodococcus ruber* DSM 44541 (770 mg) were rehydrated in Tris/HCl buffer (10 mL, 50 mM, pH 7.5) at 30 °C for one hour at 130 rpm. 1-Chloro-2-octanone (**1a**; 200 μL, 1.2 mmol) and 2-propanol (2 mL) were added. After 26 h of shaking at 30 °C and 130 rpm, the reaction was stopped by extraction with ethyl acetate (3 × 2 mL), the combined organic phase was dried (Na_2SO_4), analyzed by GC (99% conversion) and purified by column chromatography (2.5 g silica gel, petroleum ether/ethyl acetate = 10:1) to afford (*R*)-**1b** (ee 96%); isolated yield: 128 mg (68%).

Chemoenzymatic "One-Pot" synthesis of (*R*)-1,2-Epoxyoctane (1c)

Method 1: Lyophilized cells of *Rhodococcus ruber* DSM 44541(80 mg) were rehydrated in K_2HPO_4/NaH_2PO_4 buffer (0.9 mL, 50 mM buffer pH 9.0) for 45 min at room temperature and 130 rpm on a rotary shaker in an Eppendorf tube. 1-Chloro-2-octanone (**1a**; 20 mg, 0.12 mmol) dissolved in 2-propanol (100 μL) was added and the reaction mixture was shaken at room temperature and 130 rpm for 16 hours. Afterwards, aqueous NaOH (80 μL, 2M) was added and the reaction was continued by shaking for four hours.

Method 2: Lyophilized cells of *Rhodococcus ruber* DSM 44541 (80 mg) were rehydrated in K_2CO_3 buffer (0.9 mL, 50 mM, pH 11.0 and 12.0) for 45 min at room temperature and 130 rpm on a rotary shaker in an Eppendorf tube. 1-Chloro-2-octanone (**1a**; 20 mg, 0.12 mmol) dissolved in 2-propanol (100 μL) was added and the reaction mixture was shaken at room temperature and 130 rpm for 24 hours.

Method 3: Method 2 was modified by addition of 2 pellets of KOH (each 130 mg) directly after substrate addition. The reaction was quenched by dilution of the mixture with Tris-HCl buffer (10 mM, pH 7.5) followed by neutralization using 2 M HCl and extraction with ethyl acetate, before the organic layer was dried (Na_2SO_4) and analyzed by GC analysis.

Upscaled Chemoenzymatic "one-Pot" Synthesis of (*R*)-1,2-Epoxyoctane (1c)

Lyophilized cells (4.1 g) of *Rhodococcus ruber* DSM 44541 were rehydrated in K_2CO_3 buffer (40 mL, 50 mM, pH 12.0) for 30 minutes at 24 °C and 130 rpm on a rotary shaker in a 50-mL PTFE-centrifuge tube. The suspension was cooled to 5 °C and KOH pellets (5 g) were added to the suspension under vigorous stirring. The substrate 1-chloro-2-octanone (**1a**; 400 mg, 4.8 mmol) in 2-propanol (4 mL) was added and the reaction mixture was shaken at 30 °C and 130 rpm for 4.5 h. Samples (500 μL) were taken at 0.5 h, 2.5 h and 4.5 h and extracted with Et_2O. The samples were analyzed for conversion and ee by GC.

Determination of Absolute Configuration

Absolute configurations of **1b**, **2b**, **3b** and **6b** were determined by comparison of the optical rotation values with literature data: (*R*)-**1b**, ee = 92%, $[\alpha]_D^{20}$: −2.4 ($CHCl_3$, *c* 0.89), ref.[59] (*S*)-**1b**, $[\alpha]_D^{20}$: +1.4 ($CHCl_3$, *c* 3.1); (*R*)-**2b**, ee = 50%, $[\alpha]_D^{20}$: −22.7 (*c*-C_6H_{12}, *c* 0.33), ref.[60] (*R*)-**2b**, $[\alpha]_D$: −58.9 (*c*-C_6H_{12}, *c* 1.3); (*R*)-**3b**, ee = 91%, $[\alpha]_D^{20}$: −4.8 ($CHCl_3$, *c* 0.99), ref.[61] (*R*)-**2b**, $[\alpha]_D^{20}$: −3.79 ($CHCl_3$, *c* 1.02); (1*S*,2*S*)-**6b**, ee > 99%, $[\alpha]_D^{20}$: +81 ($CHCl_3$, *c* 1.9), $[\alpha]_J^{20}$: +93 ($CHCl_3$, *c* 0.73), ref.[29]

(1*S*,2*S*)-**6b**, ee > 98%, $[\alpha]_J^{25}$: +122 ($CHCl_3$, *c* 0.03). (1*R*,2*S*)-**6b**, ee > 99%, $[\alpha]_D^{20}$: −40 ($CHCl_3$, *c* 0.5), $[\alpha]_J^{20}$: −75 ($CHCl_3$, *c* 0.5), ref.[29] (1*R*,2*S*)-**6b**, ee > 98%, $[\alpha]_J^{25}$: −80 ($CHCl_3$, *c* 0.03).

The absolute configuration of **4b** was determined by co-injection on chiral GC with independently obtained enantiopure reference material. The absolute configuration of **5b** was determined by comparison of the elution order on chiral GC with literature values.[62]

For GC data of all compounds see Supporting Information.

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