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Selectivity enhancement of enantio- and stereo-complementary epoxide hydrolases and chemo-enzymatic deracemization of (±)-2-methylglycidyl benzyl ether

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Abstract—The kinetic resolution of (±)-2-methylglycidyl benzyl ether was achieved via enantioselective biohydrolysis using microbial and plant epoxide hydrolases. Depending on the type of enzyme, opposite enantiopreference and stereo-complementary mode of action (i.e., *retention* vs *inversion* of configuration) led to hetero- and homochiral product mixtures. Optimization of the reaction conditions for *Rhodococcus* sp. R312 led to significantly enhanced enantioselectivity (E >200), which enabled the deracemization of (±)-2-methylglycidyl benzyl ether via biohydrolysis (proceeding with *retention* of configuration) followed by *inverting* acid-catalyzed hydrolysis to furnish (*R*)-1-benzyloxy-2-methylpropane-2,3-diol in >97% ee and 78% yield from the racemate.

1. Introduction

Enantiopure epoxides and their corresponding vicinal diols are important building blocks for the synthesis of numerous bioactive molecules. In particular, 2,2-disubstituted oxiranes and the corresponding *vic*-diols provide easy access to sterically demanding synthons containing a fully substituted carbon atom resembling a chiral *tert*-alcohol moiety, which is difficult to obtain in its non-racemic form by conventional means.[1] In this context, enantioselective biocatalytic hydrolysis of *rac*-oxiranes using epoxide hydrolases of microbial origin has proven to be a powerful tool for obtaining these compounds.[2]

Since the presence of multiple (competing) enzymes in whole-cell biocatalysts is a common phenomenon, enantioselectivities in microbial epoxide hydrolysis are often less than perfect and selectivity enhancement is required. In this context, several procedures have been developed: substrate modification by variation of protective groups has shown to be a highly valuable tool for modulating enzyme selectivity on a range of 2,2-disubstituted oxiranes bearing an ether-functionality.[3] Alternatively, modification of an *E*- or *Z*-carbon–carbon double or triple bond was investigated,[4] as well as the adjustment of a nitro group regarding its relative position on an aromatic system.[5]

On the other hand, a general limitation in the biohydrolysis of epoxides is the poor solubility of the lipophilic oxiranes in aqueous buffer systems, causing a (apparent) low substrate concentration. One option to overcome this problem is the use of water-miscible organic co-solvents, as demonstrated by Furstoss et al. for the resolution of *p*-nitrostyrene oxide used for the synthesis of the β-blocker (*R*)-Nifénalol.[6] Subsequent studies revealed a strong influence of co-solvent concentration on enzyme activity using water-miscible organic solvents.[7,8] Likewise, the effects of two different water-soluble co-solvents on the reaction rate and selectivity of 1,2-epoxyoctane hydrolysis have been analyzed.[9] However, this procedure is not applicable to bacterial epoxide hydrolases, as the addition of even small amounts of water-soluble organic co-solvents led to rapid enzyme deactivation.[10]

However, the use of high substrate concentrations (where the substrate makes up a second 'organic' phase) has proven to increase reaction rates and enantioselectivities.[11,12] Instead of using large amounts of substrate, water-immiscible organic co-solvents have been used as

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the second liquid phase. For instance, the enantioselectivity in the resolution of glycidyl phenyl ether was improved by using a biphasic *i*-octane/aqueous system[13] and the biohydrolysis of *rac*-epichlorohydrin has been accomplished in cyclohexane supplemented with a small amount of water (2.0%, v/v) in the absence of a discrete second aqueous buffer phase.[14]

However, the benefit of two-phase solvent systems cannot be generalized, since the hydrolysis of *rac*-1-{2′,3′-dihydrobenzo[*b*]furan-4′-yl}-1,2-oxirane using 10% of methyl *tert*-butyl ether led to an improvement with *Rhodotorula glutinis*, but worse results were obtained with *Aspergillus niger*.[15] Although the exact details on the effects of the biphasic systems exert on epoxide hydrolase activity and -selectivity are not fully understood, interfacial activation (in case of *Agrobacterium radiobacter* epoxide hydrolase)[16] and mass transfer limitations[17] seem to play a major role. In an attempt to correlate the physicochemical properties of organic solvents and their biocompatibility with epoxide hydrolase activity of whole yeast cells, 40 water-miscible and -immiscible solvents have been tested with *Rhodotorula* sp. UOFS Y-0448. Among the water-miscible solvents, *mono*-alcohols (in particular 1-octanol) were considered most biocompatible, but no clear conclusions could be drawn for biphasic solvent systems.[18] Most recently, the biohydrolysis of *rac*-epoxides has also been performed in ionic liquids, with reaction rates and *E*-values being comparable to those observed in aqueous buffer systems.[19]

A rather puzzling picture emerged with regard to the effects of detergents on the reaction rate and enantioselectivity in the biohydrolysis of epoxides using *R. glutinis*. Overall, the influence of detergents seemed to be dependent on the substrate type, since the action of a certain detergent on aliphatic and aromatic substrates was found to be opposite.[20]

Enhanced enantioselectivities were also achieved by using enzyme mutants of *A. radiobacter* AD1 epoxide hydrolase.[21,22] Likewise, selectivity enhancement by enzyme modification was reported for *A. niger*[23] and *R. glutinis*[24] with varying success. Finally, rather simple selectivity enhancement was achieved by performing the biohydrolysis of an epoxide at 4 °C.[11]

During our studies toward an enantio-convergent chemo-enzymatic asymmetric total synthesis of (*all-R*)-α-tocopherol (vitamin E), we required a reliable and scalable protocol for the preparation of enantiopure 2-methylglycidyl benzyl ether. Initial attempts to apply our previously developed procedure based on the kinetic resolution of (±)-**1** to a deracemization protocol failed due to incomplete conversion (i.e., at or slightly beyond 50%), which is critical for obtaining an optimal $ee_{P(final)}$ in an enantio-convergent process.[6,25]

2. Results and discussion

From a screen for epoxide hydrolase activity including 34 bacteria, fungi, and yeasts using *rac*-methylglycidyl benzyl ether as substrate (data not shown), three bacteria and one red yeast were selected as hits for further selectivity-enhancement studies (Table 1).

In line with previous studies, *Rhodococcus* spp. showed an (*S*)-enantiopreference with promising enantioselectivities ranging from moderate to good (*E* up to 71). On the other hand, the enantioselectivity of the red yeast *Rhodotorula mucilaginosa* DSM 70404 was comparably low ($E = 8.6$), although the strain showed a rare opposite enantiopreference for (*R*)-**1** and was, therefore, included in further studies. All strains exerted a retention of configuration by directing the (formal) nucleophile [OH^-] onto the less hindered unsubstituted oxirane carbon atom during biohydrolysis, thus rendering hetero-chiral products (*R*)-**1** and (*R*)-**2** or (*S*)-**1** and (*S*)-**2**, respectively; note the switch in CIP sequence priority from epoxide **1** to diol **2**.

All attempts to improve the modest enantioselectivity of *R. mucilaginosa* DSM 70404 by variation of the reaction conditions proved disappointing (Table 2): In contrast to the trends generally observed with epoxide hydrolases from bacteria, fungi, and red yeasts,[10–12] elevated substrate concentration (entry 2) and low temperature (entry 3) had negative effects on the enantioselectivity.

More encouraging results were obtained for the selectivity enhancement using whole bacterial cells. Although *Rhodococcus ruber* DSM 44540 gave a promising

Table 1. Enantioselectivities from the biohydrolysis of (±)-**1** using selected microorganisms[a]

Microorganism	Conversion (%)	Selectivity *E*	Preferred enantiomer
Rhodococcus sp. CBS 717.73	16	16	(*S*)
Rhodococcus ruber DSM 44540	15	37	(*S*)
Rhodococcus ruber DSM 44190	8	71	(*S*)
Rhodotorula mucilaginosa DSM 70404	22	8.6	(*R*)

[a] Reaction conditions: 30 °C, substrate concentration 168 mM, catalyst/substrate ratio 2:1 (w/w).

Table 2. Enantioselectivities from the biohydrolysis of (±)-**1** using *Rhodotorula mucilaginosa* DSM 70404

Entry	Temp (°C)	Substrate concn (mM)	Catalyst/substrate (w/w)	Conversion (%)	ee_S (%)	ee_P (%)	Selectivity *E*
1	30	28	10:1	71	62	13	8.6
2	30	168	2:1	22	2	7	1.2
3	4	168	2:1	36	8	14	1.4

Table 3. Enantioselectivities from the biohydrolysis of (±)-**1** using *Rhodococcus ruber* DSM 44540

Entry	Temp (°C)	Substrate concn (mM)	Catalyst/substrate (w/w)	Conversion (%)	ee_S (%)	ee_P (%)	Selectivity *E*
1	30	28	10:1	60	95	63	15
2	30	168	2:1	15	76	94	37
3	30	786	2:1	3	3	>97	67
4	4	168	2:1	8	8	87	15
5	4	786	2:1	10	11	>97	73

enantioselectivity of $E = 37$ during the screening, scale-up experiments using an enhanced catalyst/substrate ratio of 10:1 (w/w) gave a lower value ($E = 15$, Table 3). However, when the substrate concentration was gradually enhanced, the selectivity climbed to an *E*-value of 67 (entries 2 and 3). In addition, lowering the temperature to 4 °C (entries 4 and 5) had an additional beneficial effect, which led to a final improvement of $E = 73$.

Encouraged by these results, the effect of organic co-solvents was studied using a substrate concentration of 168 mM as standard (Table 4). Whereas water-miscible DMSO had a negative effect ($E = 22$, entry 2), lipophilic water-immiscible co-solvents, such as toluene (entry 3) and hydrocarbons (entries 4–7) were generally more suitable. Most striking was the difference between various hydrocarbons, where straight-chain *n*-alkanes (*n*-hexane, *n*-octane, entries 6 and 7) proved to be more suitable than branched or cyclic analogues (cyclohexane, *i*-octane, entries 4 and 5), which is contrary to the trends observed with lipases.[26] Among ethers, *i*-Pr_2O was far superior than *t*-BuOMe (entries 8 and 9).

Table 4. Enantioselectivities from the biohydrolysis of (±)-**1** using *Rhodococcus ruber* DSM 44540 in the presence of organic co-solvents

Entry	Co-solvent[a]	Conversion (%)	ee_S (%)	ee_P (%)	Selectivity *E*
1	None	15	76	94	37
2	DMSO	41	60	85	22
3	Toluene	3	3	>97	67
4	Cyclohexane	11	11	90	21
5	*i*-Octane	24	30	90	36
6	*n*-Hexane	26	33	95	53
7	*n*-Octane	27	36	93	69
8	*t*-BuOMe	1	1	85	12
9	*i*-Pr_2O	8	8	>97	71

[a] Aqueous/organic solvent 5:1 (v/v) except for dimethyl sulfoxide, which was used in a proportion of 20:1; standard conditions 168 mM substrate, catalyst/substrate 2:1 (w/w), 30 °C.

Related dependencies were observed for *R. ruber* DSM 44190 (Tables 5 and 6). Initially modest selectivities at an enhanced catalyst/substrate ratio ($E = 8.6$) could be improved by enhancing the substrate concentration, although a value of 168 mM showed a clear maximum, which dropped off again at 768 mM (entries 2 and 3). A strong positive effect was obtained by lowering the temperature to 4 °C ($E = 70$, entry 4), which largely compensated for the selectivity decrease at high substrate concentrations (768 mM). However, this went in hand with a strong decrease in the reaction rate and the conversion of 7% thus achieved within a reasonable reaction time of 24 h was by far insufficient for a deracemization protocol.

Table 6. Enantioselectivities from the biohydrolysis of (±)-**1** using *Rhodococcus ruber* DSM 44190 in the presence of organic co-solvents

Entry	Co-solvent[a]	Conversion (%)	ee_S (%)	ee_P (%)	Selectivity *E*
1	None	8	8	>97	71
2	DMSO	42	56	77	13
3	*t*-BuOMe	3	2	69	5.6
4	*i*-Pr_2O	8	7	81	10
5	Toluene	1	<1	>97	66
6	Cyclohexane	25	25	74	8.5
7	*i*-Octane	31	36	80	12
8	*n*-Octane	29	36	88	22
9	*n*-Hexane	44	75	94	73

[a] Aqueous/organic solvent 5:1 (v/v), except for dimethyl sulfoxide, which was used in a proportion of 20:1; standard conditions 168 mM substrate, catalyst/substrate 2:1 (w/w), 30 °C.

Co-solvent effects on the epoxide hydrolase activity of *R. ruber* DSM 44190 (Table 6) largely paralleled that of *R. ruber* DSM 44540. Again water-miscible DMSO lowered the *E*-value of 71 under standard conditions dramatically ($E = 13$, entry 2), similar to ethers (entries 3 and 4). No negative effects were observed in the presence of toluene ($E = 66$, entry 5). Likewise, hydrocarbons exhibited a familiar trend, that branched or cyclic solvents were less suitable than *n*-alkanes, such as *n*-octane or *n*-hexane (entries 6–9). In the latter case, the reaction rate was not markedly diminished.

Overall, the epoxide hydrolase system of whole cells of *Rhodococcus* sp. CBS 717.73[27] proved to be most flexible in terms of selectivity enhancement (Tables 7 and 8).

Table 5. Enantioselectivities from the biohydrolysis of (±)-**1** using *Rhodococcus ruber* DSM 44190

Entry	Temp (°C)	Substrate concn (mM)	Catalyst/substrate (w/w)	Conversion (%)	ee_S (%)	ee_P (%)	Selectivity *E*
1	30	28	10:1	71	97	40	8.6
2	30	168	2:1	8	8	>97	71
3	30	786	2:1	20	21	84	14
4	4	786	2:1	7	7	>97	70

Table 7. Enantioselectivities from the biohydrolysis of (±)-**1** using *Rhodococcus* sp. CBS 717.73

Entry	Temp (°C)	Substrate concn (mM)	Catalyst/substrate (w/w)	Conversion (%)	ee_S (%)	ee_P (%)	Selectivity *E*
1	30	28	10:1	53	>97	85	51
2	30	168	2:1	16	16	87	16
3	30	786	2:1	6	6	>97	69
4	4	168	2:1	7	6	82	10
5	4	786	2:1	7	7	>97	70

Table 8. Enantioselectivities from the biohydrolysis of (±)-**1** using *Rhodococcus* sp. CBS 717.73 in the presence of organic co-solvents

Entry	Catalyst/substrate (w/w)	Co-solvent[a]	Conversion (%)	ee_S (%)	ee_P (%)	Selectivity *E*
1	2:1	None	16	16	87	16
2	2:1	DMSO	27	34	90	26
3	2:1	*t*-BuOMe	9	7	67	5.4
4	2:1	*i*-Pr_2O	19	23	>97	82
5	2:1	Toluene	28	37	>97	94
6	2:1	*n*-Hexane	39	61	96	91
7	2:1	Cyclohexane	31	42	92	36
8	2:1	*n*-Octane	40	66	97	130
9	2:1	*i*-Octane	43	73	>97	144
10	10:1	*n*-Hexane	52	>97	90	79
11	10:1	Toluene	34	49	>97	106
12	10:1	*i*-Pr_2O	46	83	>97	171
13	10:1	*n*-Octane	51	>97	92	100
14	10:1	*i*-Octane	50	>97	>97	>200

[a] Aqueous/organic solvent 5:1 (v/v) except for dimethyl sulfoxide, which was used in a proportion of 20:1; 30 °C.

Although initial selectivities at a high catalyst loading (10:1) and low substrate concentration (28 mM) were rather good (entry 1, $E = 51$), reduced values resulted when a more realistic catalyst/substrate ratio of 2:1 was used ($E = 16$, entry 2). However, elevated substrate concentration and lower temperatures (entries 3–5) compensated these effects significantly ($E_{max} = 70$). In any case, a significant drop in reaction rate was observed again (entries 2–4).

The effects of organic co-solvents somewhat differed from those observed with the previous *Rhodococci* (Table 8). First, a weak selectivity enhancement was observed in the presence of DMSO (entry 2). The ethers behaved rather differently, as *t*-BuOMe proved to be unsuitable in contrast to *i*-Pr_2O (entries 3 and 4). Again, toluene and hydrocarbons were best. In this case, the difference between cyclic/branched and linear solvents was small, but were more profound regarding their molecular weight, as *n*- and *i*-octane gave superior results than hexanes.

In order to increase reaction rates, higher catalyst loadings were investigated (entries 10–14). Again, biphasic systems consisting of C_8-hydrocarbons proved to be best, where the critical conversion of 50% could be reached while maintaining excellent enantioselectivities (E >200, entry 14).

In order to avoid the use of whole-cell systems containing an unknown number of enzymes, the use of cloned and overexpressed epoxide hydrolases from plant and microbial origin was investigated (Table 9). Soybean epoxide hydrolase gave a modest selectivity ($E = 14$) at high catalyst loading (2:1, entry 1); when this value was decreased to 1:10, no reaction was observed. In contrast, the potato-enzyme was active at both catalyst/substrate ratios, albeit at low enantioselectivities (entries 2 and 3; E <10). With respect to the stereochemical outcome, both plant enzymes showed an opposite enantiopreference, that is, (*R*) for soybean epoxide hydrolase and (*S*) for the enzyme from potato. Both plant enzymes, however, seemed to act through the same

Table 9. Enantioselectivities from the biohydrolysis of (±)-**1** using cloned epoxide hydrolases

Entry	Epoxide hydrolase	Catalyst/substrate (w/w)	Conversion (%)	ee_S (%)	ee_P (%)	Selectivity *E*	Enantiopreference
1	Soybean[a]	2:1[d]	50	74	73	14	(*R*)
2	Potato[b]	1:5	98	>97	2	2.3	(*S*)
3	Potato[b]	1:10	82	>97	22	5.1	(*S*)
4	*Rhodococcus erythropolis*[c]	1:5	86	>97	16	4.3	(*R*)
5	*Rhodococcus erythropolis*[c]	1:10	75	60	20	2.5	(*R*)

[a] *Glycine max*, expressed in *Saccharomyces cerevisae* WA6.[29]
[b] *Solanum tuberosum*, expressed in *Spodoptera frugiperda*.[30]
[c] Limonene 1,2-epoxide hydrolase from *Rhodococcus erythropolis*, expressed in *E. coli*.[31]
[d] No reaction was observed at a catalyst/substrate ratio of 1:10.

mechanism, that is with retention of configuration. A possible explanation for the low enantioselectivities of plant epoxide hydrolases might be the fact that the natural substrates of these enzymes are epoxy-fatty acids,[28] which have a rather unrelated structure to that of substrate **1**.

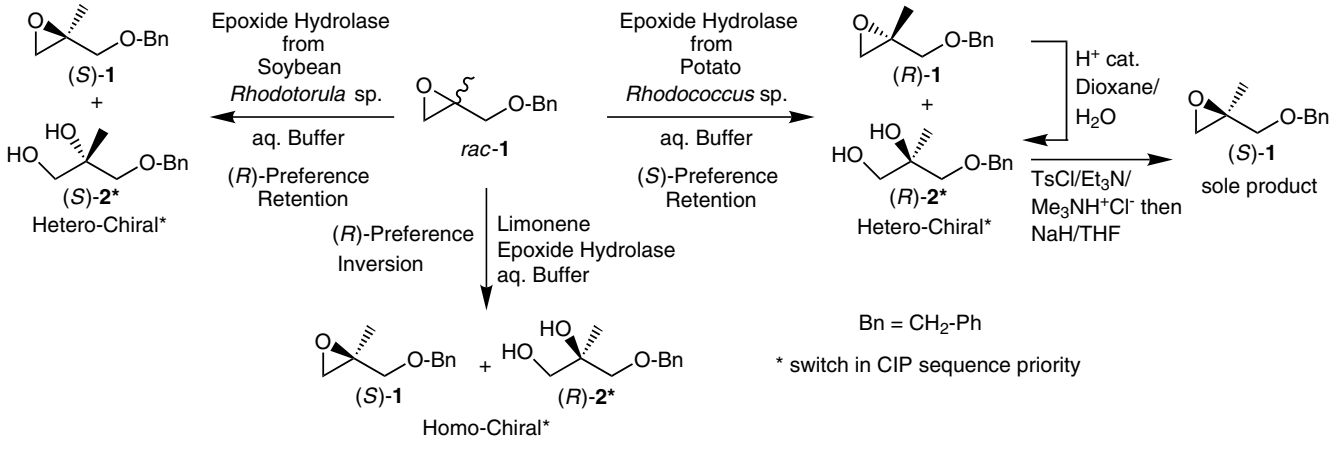
Surprising results were obtained with limonene 1,2-epoxide hydrolase from *Rhodococcus erythropolis*[31] (entries 4 and 5). Although this enzyme exhibited only low enantioselectivities with a preference for (*R*)-**1**, it formed diol (*R*)-**2**, which suggests that it acts through an inversion of configuration. These observations can be explained by taking the molecular mechanism of epoxide hydrolases into consideration (Scheme 2). The majority of enzymes investigated so far [e.g., from *A. radiobacter*[34] and *A. niger*[35]] act through clear S_N2-type displacement of the oxirane oxygen atom by (formal) hydroxyl ion. The latter is realized by nucleophilic attack of an Asp-anion within the active site of the enzyme, leading to the formation of a covalent glycol-hemiester intermediate,[32] which in turn is hydrolyzed by [OH^-] provided by an adjacent histidine-acidic-residue pair through proton-abstraction from water. Tyrosines positioned at the opposite side promote the ring opening of the oxirane by delivering a proton for the epoxy-oxygen. Overall, this mechanism represents a clear S_N2-type mechanism (Scheme 2, top).[33–35] Since nucleophilic attack at the fully substituted oxirane carbon atom of **1** bearing the chiral center is impossible for steric reasons, the [OH^-] is inserted at the unsubstituted carbon atom, which results in *retention* of configuration.

In contrast, limonene 1,2-epoxide hydrolase from *R. erythropolis* was shown to possess a different mode of action.[36,37] Since its natural substrate (limonene 1,2-epoxide) is sterically very demanding, S_N2-type displacement would be energetically extremely unfavorable. As a consequence, this enzyme acts via a single push–pull step through simultaneous O-protonation and nucleophilic attack by [OH^-] without the formation of a covalent enzyme–substrate intermediate. Overall, this mechanism bears a strong resemblance to a borderline-S_N2-type mechanism, which is commonly observed in acid-catalyzed hydrolysis of epoxides, which leads to incorporation of the nucleophile at the higher substituted oxirane carbon atom (Scheme 2, bottom).[38,39] In this case, the latter bears a stereogenic center (as in the case of **1**), with inversion of configuration observed.

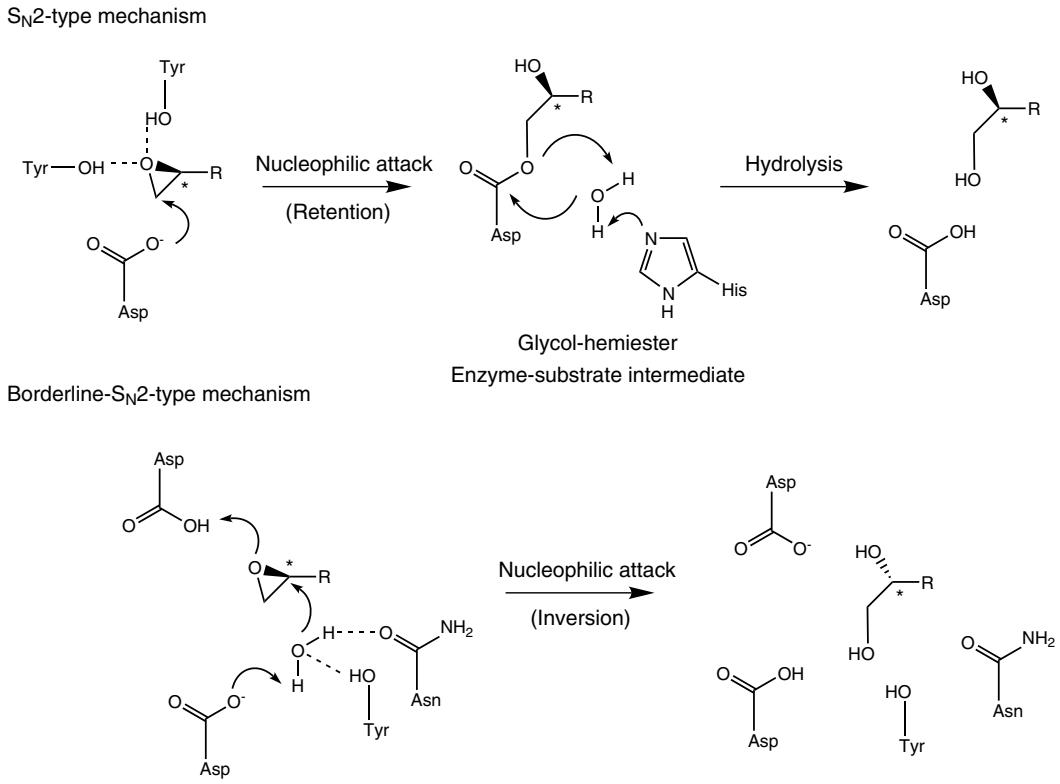
In order to obtain a single stereoisomer in 100% theoretical yield, an enantio-convergent process was envisaged as follows (Scheme 1): biohydrolysis of *rac*-**1** using lyophilized whole cells of *Rhodococcus* sp. CBS 717.73 under optimized conditions (catalyst/substrate 10:1, buffer/*i*-octane, 30 °C) proceeded with (*S*)-enantiopreference acting through *retention* of configuration to render the corresponding (*R*)-diol **2** (note the switch in CIP sequence priority) and remaining non-converted (*R*)-**1**. Acid-catalyzed hydrolysis of the latter in dioxane/water proceeded with *inversion* of configuration to furnish (*R*)-**2** as the sole product in >97% ee and 78% yield.[39] Finally, cyclization of (*R*)-**2** via tosylation of the *prim*-hydroxy moiety followed by treatment of the corresponding *mono*-tosylate with base furnished (*S*)-**1** as the sole product in 92% yield.

3. Conclusion

Kinetic resolution of (±)-2-methylglycidyl benzyl ether was accomplished via biohydrolysis using whole microbial cells or cloned epoxide hydrolases. While the absolute configuration was retained during biohydrolysis for the majority of biocatalysts, an opposite enantiopreference was observed depending on the type of enzyme: (*S*) for *Rhodococcus* spp. and potato epoxide hydrolase versus (*R*) for *Rhodotorula* sp. and soybean epoxide hydrolase. In contrast, limonene epoxide hydrolase acted with an inversion of configuration, thus producing a homochiral product mixture. Optimization of the reaction conditions with respect to temperature, substrate concentration, catalyst loading, and the use of aqueous/organic media led to significantly enhanced enantioselectivity for *Rhodococcus* sp. R312 (E >200), which enabled the deracemization of (±)-2-methylglycidyl benzyl ether via biohydrolysis (proceeding with *retention* of



Scheme 1. Stereo- and enantio-complementary kinetic resolution and chemo-enzymatic deracemization of (±)-2-methylglycidyl benzyl ether (**1**).



Scheme 2. Two mechanistic principles of enzymatic epoxide hydrolysis. *Center of chirality.

configuration) followed by inverting acid-catalyzed hydrolysis to furnish (*R*)-1-benzyloxy-2-methylpropane-2,3-diol in >97% ee and 78% yield from the racemate.

4. Experimental

4.1. General

Reactions were monitored by TLC (Merck silica gel 60 F_{254}) and compounds visualized by spraying with vanillin–concd H_2SO_4 (5 g L^{-1}). Column chromatography was performed using silica gel Merck 60 (230–400 mesh). Petroleum ether with a boiling range of 60–90 °C was used. Solvents were dried and freshly distilled by standard techniques. For anhydrous reactions, flasks were dried at 150 °C and flushed with dried argon just before use. For biotransformations, lyophilized bacterial cells were used. Bacteria were obtained from culture collections and grown as previously described.[40–43] (±)-2-Methylglycidyl benzyl ether **1** was synthesized as previously described.[10] Spectroscopic data were identical to those previously reported.[44] HPLC analyses were carried out on a JASCO system containing a PU-980 pump equipped with a Daicel Chiralpak AD column connected to a MD-910 multi-wavelength detector. The enantiomers of epoxide **1** were separated with *n*-heptane as eluent and a flow of 0.4 mL/min and those of diol **2** using *n*-heptane/2-propanol 90:10 at a flow of 0.5 mL/min at 18 °C. The absolute configurations of **1** and **2** were assigned according to the relative elution order of enantiomers on HPLC as previously determined.[45] Retention times were as follows: (*R*)-**1**, 16.52 min, (*S*)-**1**, 17.89 min, (*R*)-**2**, 19.05 min, (*S*)-**2**, 20.35 min; retention times for **1** could vary to some extent depending on column pretreatment.

4.2. Biocatalytic hydrolysis of (±)-2-methylglycidyl benzyl ether 1

4.2.1. Screening. Lyophilized cells (30 mg) were rehydrated in Tris/HCl buffer (500 μL, 0.05 M, pH 8) for 1 h at 30 °C and 130 rpm. Then, 15 μL (168 mM) of *rac*-**1** was added and the mixture agitated on a thermostated shaker. After 24–48 h, the reactions were stopped by extraction with ethyl acetate (twice 500 μL). To facilitate phase separation, cells were removed by centrifugation. The combined organic layers were dried over Na_2SO_4, evaporated, and chromatographed (petroleum ether/ethyl acetate 2:1) to obtain the remaining non-converted epoxide and the formed diol was eluted using ethyl acetate.

4.2.2. Optimization. The general procedure described above was modified by altering the following parameters.

4.2.2.1. Substrate concentration. Lyophilized cells (70 mg), Tris/HCl buffer (250 μL, 0.05 M, pH 8), substrate *rac*-**1** (35 μL).

4.2.2.2. Catalyst loading. Catalyst/substrate ratio 10:1: Lyophilized cells (50 mg), Tris/HCl buffer (1 mL, 0.05 M, pH 8), substrate *rac*-**1** (5 μL). Catalyst/substrate ratio 1:5: Lyophilized cells (1 mg), Tris/HCl buffer

(1 mL, 0.05 M, pH 8), substrate *rac*-**1** (5 μL). Catalyst/substrate ratio 1:10: Lyophilized cells (1 mg), Tris/HCl buffer (1 mL, 0.05 M, pH 8), substrate *rac*-**1** (10 μL).

4.2.2.3. Temperature. Lyophilized cells (30 mg) were rehydrated in a Tris/HCl buffer (500 μL, 0.05 M, pH 8) for 1 h at 30 °C and 130 rpm and after which for 1 h at 4 °C. Then, 15 μL of *rac*-**1** was added and the mixture agitated at 4 °C and 130 rpm for 24–48 h.

4.2.2.4. Aqueous/organic solvent systems. To a solution of rehydrated lyophilized cells (30 mg) in 500 μL of Tris/HCl buffer (0.05 M, pH 8), 100 μL of the organic co-solvent (25 μL in case of dimethyl sulfoxide) was added and the mixture was agitated for 1 h at 30 °C and 130 rpm. Then, 15 μL of *rac*-**1** was added and the reaction was agitated for 24 h under the same conditions. A catalyst/substrate ratio 10:1 in the presence of organic co-solvents: Lyophilized cells (50 mg), Tris/HCl buffer (1 mL, 0.05 M, pH 8), organic solvent (200 μL), *rac*-**1** (5 μL).

4.3. Chemo-enzymatic deracemization of (±)-1

Lyophilized cells (1 g) of *Rhodococcus* sp. CBS 717.73 were rehydrated in a mixture of Tris/HCl buffer (20 mL, 0.05 M, pH 8) and *i*-octane (4 mL) by shaking at 30 °C for 1 h. Then, 100 μL of substrate *rac*-**1** were added. The mixture was agitated on a thermostated shaker at 30 °C and 130 rpm for 24 h. The products were then extracted with ethyl acetate (four times, phase separation was facilitated by centrifugation), and the combined organic layers dried over Na_2SO_4 and evaporated. The resulting bright yellow oil consisting of (*R*)-**1** and (*R*)-**2** was subjected to acid-catalyzed epoxide opening under inversion of configuration without further purification. Thus, the mixture was dissolved in dioxane (20 mL) and cooled to 0 °C. Then, 220 μL of 93% aq H_2SO_4 was added to the solution, which was allowed to reach rt and stirred for 20 min. The reaction was neutralized with aq satd $NaHCO_3$, EtOAc was added, and the resulting biphasic mixture was stirred vigorously for 30 min. Finally, the organic layer was separated, the aqueous phase was extracted twice with ethyl acetate, and the combined organic layers were dried, filtered, and evaporated. Column chromatography rendered (*R*)-**2** (86 mg, 78% from *rac*-**1**, >97% ee) as the sole product. $[\alpha]_D^{20} = -6.6$ (*c* 1.33, CH_2Cl_2); lit.:[46] $[\alpha]_D = -6.3$ (*c* 0.87, CH_2Cl_2). Spectroscopic data were in agreement with those previously reported.[46]

4.4. Cyclization of diol (*R*)-2 to epoxide (*S*)-1

Diol (*R*)-**2** (357 mg, 1.8 mmol) was dissolved in 25 mL of CH_3CN, after which Et_3N (0.76 mL, 5.5 mmol) and $Me_3NH^+Cl^-$ (35 mg, 0.4 mmol) were added. The solution was cooled to 0 °C and *p*-toluenesulfonyl chloride (1 g, 5.5 mmol) was added. The mixture was stirred at 0 °C for 2 h and the reaction was quenched by addition of satd aq NH_4Cl solution (30 mL). The phases were separated, the aqueous layer was extracted with AcOEt (3×30 mL), and the combined organic layers were washed with saturated NH_4Cl solution (15 mL), saturated $NaHCO_3$ solution, and distilled water. After drying over Na_2SO_4, solids were filtered and the solution was evaporated to yield crude product as an oil, which was dissolved in 20 mL anhyd THF and cooled to 0 °C. NaH (132 mg, 5.5 mmol) was added and the mixture was allowed to reach room temperature while stirring for 1.5 h. It was then poured into a mixture of ice and NH_4Cl. Distilled water (10 mL) was added, the phases were separated, and the aqueous layer was extracted with CH_2Cl_2 (2×20 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated under reduced pressure to render 300 mg (92%) of (*S*)-**1**. $[\alpha]_D^{20} = +9.2$ (*c* 1.0, $CHCl_3$); lit.:[47] $[\alpha]_D^{25} = +10.9$ (*c* 1.20, MeOH).

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