Enzyme-Triggered Enantioconvergent Transformation of Haloalkyl Epoxides

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Biocatalytic hydrolysis of 2,3-disubstituted *rac*-*cis*- and *rac*-*trans*-haloalkyl epoxides **1a**–**8a** using the epoxide hydrolase activity of whole bacterial cells furnished the corresponding vicinal diols **1b**–**8b** as intermediates; these (spontaneously) underwent ring closure to yield cyclic products **1c**–**6c** through an enzyme-triggered cascade reaction. In particular, *cis*-configured substrates (**1a**, **3a**, **5a**, **7a**) were transformed in an enantioconvergent fashion, which resulted in the formation of single stereoisomeric products in 100% *de*s and up to 92% *ee*s from the racemates.

Introduction

Reactions that proceed through more than a single step in a concurrent fashion − often designated as "domino" or "cascade" reactions − display the advantage that final products can be obtained in good yield despite the fact that the sequence may involve reactive (and thus often unstable) intermediates.[1] If the cascade is triggered by a chiral catalyst, such as an enzyme, the stereochemical course of the whole cascade may be directed in an asymmetric fashion, resulting in a nonracemic product.[2]

During our study of the asymmetric biohydrolysis of (±)-2,3-dialkyloxiranes by bacterial epoxide hydrolases, we discovered that (depending on the stereochemistry of the substrate) only single stereoisomers of the corresponding vicinal diols were formed, by enantioconvergent pathways and in 100% theoretical yield from the racemates.[3] In comparison with kinetic resolution, such "deracemization" processes show a considerably improved economic balance and thus have recently attracted considerable attention.[4] From previous studies, we knew that functional groups, as long as they are lipophilic, are tolerated well by bacterial epoxide hydrolases.[5,6] As an attractive alternative, halogenated compounds seemed to be ideally suited, particularly in view of the facilitated C−C coupling due to umpolung at this position. Much to our surprise, biohydrolysis did not produce the expected haloalkyl-substituted vicinal diols, but furnished epoxides and tetrahydrofuran derivatives in nonracemic forms.[7]

Results and Discussion

In order to provide insight into the factors governing the stereochemical outcome of the reaction, 2,3-disubstituted haloalkyl oxiranes (*rac*-**1a**−**8a**) were chosen as substrates in order to study the influence of the following parameters:

(i) relative *cis* and *trans* configuration of the oxirane moiety
(ii) length of the haloalkyl spacer
(iii) choice of halogen
(iv) length of the alkyl chain

Substrates were prepared as outlined in Scheme 1. Compounds *rac*-**1a**−**4a**, bearing *n*-butyl groups as the alkyl moieties, were synthesized from acetylenes **9** and **10**. Nucleophilic addition of the lithium acetylide of 1-hexyne (**9**) onto formaldehyde gave the (hydroxymethyl)acetylene **11**; the hydroxyethyl analogue **12** was prepared by alkylation of 3-butyne-1-ol (**10**). Reduction of both compounds by catalytic hydrogenation (Lindlar) or by $LiAlH_4$ gave stereoisomerically pure (*Z*)- and (*E*)-alkenes **13a**, **13b**, **15a**, and **15b**. Exchange of the primary hydroxy groups in **13a**, **13b**, **15a**, and **15b** by Cl was accomplished via the corresponding mesylates, to furnish **14a**, **14b**, **16a**, and **16b**, respectively. Haloalkenes **17b**, **17c**, **18b**, and **18c** were accessed from hydroxyalkenes **17a** and **18a** by replacement of the hydroxy group with the desired halogen as mentioned above. Finally, epoxidation of haloalkenes **14a**, **14b**, **16a**, **16b**, **17b**, **17c**, **18b**, and **18c** resulted in stereoisomerically pure substrates *rac*-*cis*-**1a**, **3a**, **5a**, and **7a** and *rac*-*trans*-**2a**, **4a**, **6a**, and **8a** in good overall yields.

Substrates *rac*-**1a**−**8a** were screened for biohydrolysis in Tris buffer at pH = 8.0, using resting cells of a variety of bacteria known to possess strong secondary metabolic activity, in particular *Actinomyces* spp. (details not shown). Close examination of the most active strains revealed a common picture (Scheme 2): Whereas (halo*methyl*)oxiranes *rac*-**1a** and **2a** gave hydroxyepoxides **1c** and **2c**, respectively, the corresponding (halo*ethyl*)analogues produced tetrahydrofuran derivatives **3c**−**6c** as the exclusive products. No other compounds were formed in notable amounts (< 5%). The formation of final products can be explained by intramolecular cyclisation of (haloalkyl)diols **1b**−**8b**, initially formed during biohydrolysis. The latter reaction shows some resemblance to a Payne-type rearrangement.[8,9] Depending on the length of the haloalkyl spacer moiety, the relative rates of hydrolysis versus cyclisation varied to a sig-

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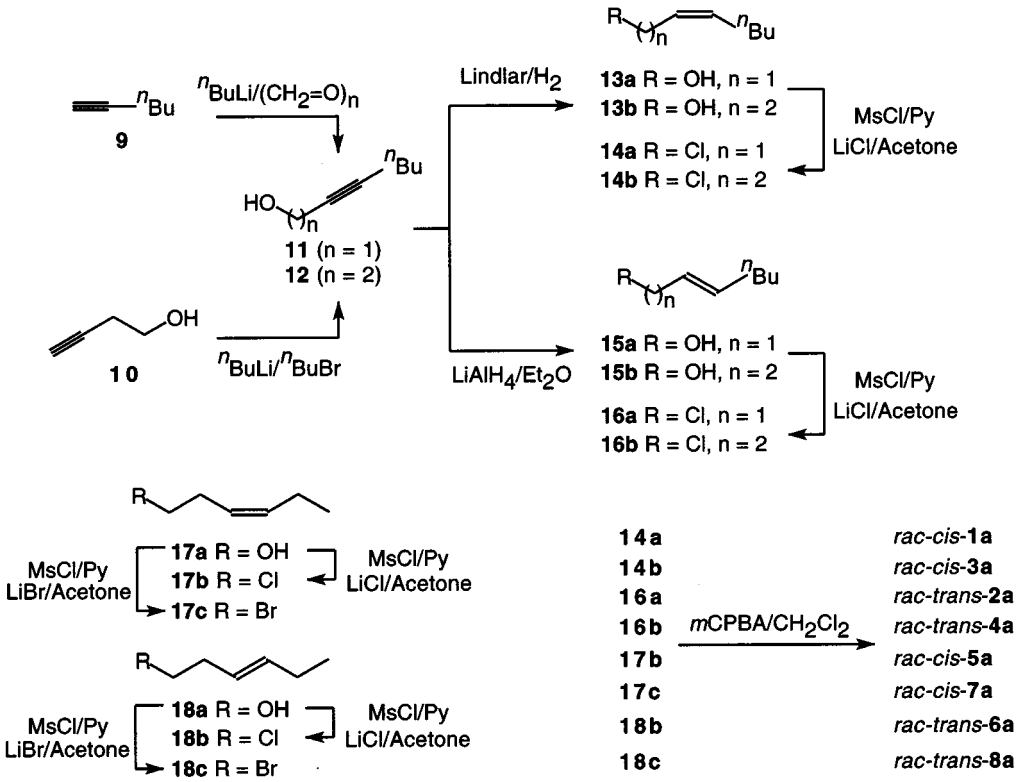
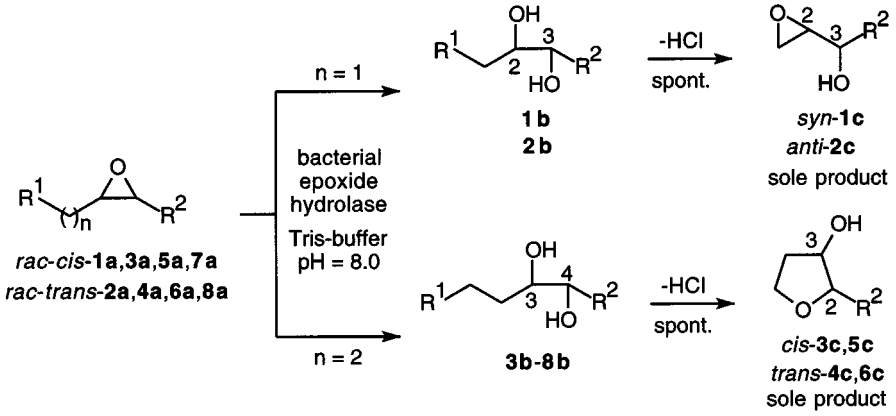


Scheme 1. Synthesis of substrates



Substrate	R^1	R^2	n	Product	R^2
rac-cis-**1a**	Cl	*n*-Bu	1	*syn*-**1c**	*n*-Bu
rac-trans-**2a**	Cl	*n*-Bu	1	*anti*-**2c**	*n*-Bu
rac-cis-**3a**	Cl	*n*-Bu	2	*cis*-**3c**	*n*-Bu
rac-trans-**4a**	Cl	*n*-Bu	2	*trans*-**4c**	*n*-Bu
rac-cis-**5a**	Cl	Et	2	*cis*-**5c**	Et
rac-trans-**6a**	Cl	Et	2	*trans*-**6c**	Et
rac-cis-**7a**	Br	Et	2	*cis*-**5c**	Et
rac-trans-**8a**	Br	Et	2	*trans*-**6c**	Et

Scheme 2. Enzyme-triggered transformation of (haloalkyl)oxiranes *rac*-**1a**−**8a**

nificant extent. On the one hand, the rate of epoxide formation in substrates **1a** and **2a** was comparable to that of biohydrolysis and, as a consequence, (halomethyl)diols **1b** and **2b** could be detected in small quantities during the course of the reaction. On the other hand, (haloethyl)diols **3b**−**8b** were found only in minute quantities, since cyclisation to

tetrahydrofuran derivatives **3c**−**6c** was considerably faster than biohydrolysis and **5b**−**8b** could neither be detected nor isolated. This behaviour is in agreement with energetic considerations and the fact that ring closure follows a (favored) *exo-tet* cyclisation.[10] The large difference in the relative rates of the cyclisations can also be attributed to differences in ring strain.

The absolute configurations of the remaining, non-hydrolyzed epoxides and final products were determined by GLC using a chiral stationary phase, by coinjection with independently synthesized reference materials (for details see Scheme 4 and the Supporting Information). The relative stereochemistry of the transformation (relative configurations were spectroscopically determined by NOE experiments) revealed a common picture: *cis*-configured epoxides (**1a**, **3a**, **5a**, **7a**) produced *anti*-(haloalkyl)diols (**1b**, **3b**, **5b**, **7b**) through an S_N2-type enzymatic epoxide hydrolysis mechanism.[11] These intermediates cyclized to furnish *cis* products once again (**1c**, **3c**, **5c**). For *trans* derivatives, analogous considerations apply. Table 1 shows the absolute configurations and the enantiomeric purities of substrates, intermediates, and products for *cis*-configured substrates **1a**, **3a**, **5a**, and **7a**.

Substrate *rac*-*cis*-**1a** was hydrolyzed by *Rhodococcus* and *Mycobacterium* strains to form (2*R*,3*R*)-**1c** in moderate to good *ee*s. Because of the presence of a polar secondary alcohol moiety, the latter product is not a substrate for bacterial epoxide hydrolases and the reaction stops at this stage. *Rhodococcus equi* IFO 3730 showed opposite enantiopreference, but low enantioselectivity. From the close proximity of the *ee*s of the intermediate and the final product, it can be seen that no enantiodiscrimination takes place during the cyclisation step and thus it can be concluded that it is spontaneous in nature.

Similar results were obtained with compound *rac*-*cis*-**3a**, which yielded (2*R*,3*R*)-**3c** as the major product in up to 92% *ee* and 93% yield. Again, *Rhodococcus equi* IFO 3730 produced the opposite enantiomer in a low *ee* and the comparable *ee*s of **3b** and **3c** indicate spontaneous ring closure. This could not be ascertained for substrates *rac*-*cis*-**5a** and *rac*-*cis*-**7a**, as no intermediate haloethyl diols (**5b** and **7b**) could be detected during the course of the reaction. With both of the latter substrates, lower reaction rates and reduced selectivities were obtained in general, which can be attributed to the reduction of the alkyl chain length from *n*-butyl to ethyl, a phenomenon that has been noted before.[3] No significant influence of the halogen atom (Cl versus Br) on the stereoselectivity could be detected.

The results obtained from *trans*-configured substrates **2a**, **4a**, **6a**, and **8a** (Table 2) show a different pattern.

Substrate *rac*-*trans*-**2a** was rapidly converted by several *Rhodococci*, and the close similarity of the enantiomeric purity of the intermediate **2b** to that of the final product **2c** again indicates spontaneous ring closure. The selectivity, however, was significantly lower than that obtained in the case of the corresponding *cis*-configured analogue **1a**, and (2*R*,3*S*)-**2c** was obtained in a moderate 55% *ee* at best.

Rather different results were obtained with compound *rac*-*trans*-**4a**. Despite the fact that the intermediate **4b** was formed with excellent optical purities, of up to 95% *ee*, the enantiomeric composition of the final product **4c** was rather low, which indicated some (adverse) asymmetric induction during the cyclisation step and thus its nonspontaneous character. Furthermore, in contrast to other substrates, the intermediate was detected in significant concentrations of up to 6:4 (**4b**/**4c**) during the course of the reaction.

Substrates *rac*-*trans*-**6a** and *rac*-*trans*-**8a** both failed to yield the corresponding intermediates **6b** and **8b** in detectable amounts. The final cyclized product **6c** was formed in moderate *ee*. For *Rhodococcus equi* IFO 3730, the switch from Cl (*rac*-*trans*-**6a**) to Br (*rac*-*trans*-**8a**) resulted in a slight selectivity enhancement.

Evaluation of the data obtained reveals the following general picture:

(i) The relative rate of hydrolysis versus ring closure mainly depends on the size of the ring to be formed. Tetrahydrofuran derivatives are formed quickly, whereas epoxide formation is considerably slower.

Table 1. Selectivities from *cis*-configured substrates *rac*-**1a**, **3a**, **5a**, and **7a**

Substrate	Microorganism	Conversion [%][a]	*ee* [%]	Intermediate Config.	*ee* [%]	Product Config.	*ee* [%]
rac-*cis*-**1a**	*Rhodococcus equi* IFO 3730	40[b]	30[c]	(2*R*,3*S*)-**1b**	22	(2*S*,3*S*)-**1c**	19
rac-*cis*-**1a**	*Rhodococcus sp.* R 312 CBS 717.73	66[b]	50[d]	(2*S*,3*R*)-**1b**	67	(2*R*,3*R*)-**1c**	72
rac-*cis*-**1a**	*Mycobacterium paraffinicum* NCIMB 10420	86[b]	> 99[d]	(2*S*,3*R*)-**1b**	86	(2*R*,3*R*)-**1c**	85
rac-*cis*-**3a**	*Rhodococcus equi* IFO 3730	70[e]	12	(2*S*,3*S*)-**3b**[f]	5	(2*S*,3*S*)-**3c**	4
rac-*cis*-**3a**	*Rhodococcus sp.* R 312 CBS 717.73	66[e]	15	(2*R*,3*R*)-**3b**[f]	79	(2*R*,3*R*)-**3c**	83
rac-*cis*-**3a**	*Rhodococcus ruber* DSM 44540	65[e]	42	(2*R*,3*R*)-**3b**[f]	90	(2*R*,3*R*)-**3c**	87
rac-*cis*-**3a**	*Rhodococcus ruber* DSM 44541	93[e]	54	(2*R*,3*R*)-**3b**[f]	86	(2*R*,3*R*)-**3c**	92
rac-*cis*-**5a**	*Rhodococcus equi* IFO 3730	43[b]	8	n.i.	−	(2*S*,3*S*)-**5c**	22
rac-*cis*-**5a**	*Rhodococcus ruber* DSM 44540	42[b]	29	n.i.	−	(2*R*,3*R*)-**5c**	61
rac-*cis*-**7a**	*Rhodococcus equi* IFO 3730	28[b]	< 1	n.i.	−	(2*S*,3*S*)-**5c**	25
rac-*cis*-**7a**	*Rhodococcus ruber* DSM 44540	29[b]	41	n.i.	−	(2*R*,3*R*)-**5c**	70

[a] Based on unreacted substrate: c = [sub] − ([intermediate] + [product]). − [b] After 130 h. − [c] Absolute configuration (2*S*,3*S*). − [d] Absolute configuration (2*R*,3*R*). − [e] After 64 h; n.i. = intermediate could not be isolated, due to exceedingly low concentration. − [f] Absolute configuration of the intermediate was deduced from that of the product on the assumption of an S_N2-type ring-closure mechanism.

Table 2. Selectivities from *trans*-configured substrates *rac*-**2a**, **4a**, **6a**, and **8a**

Substrate	Microorganism	Conversion [%][a]	*ee* [%]	Intermediate Config.	Product *ee* [%]	Config.	*ee* [%]
rac-trans-**2a**	*Rhodococcus sp.* R 312 CBS 717.73	59[b]	6	(2*R*,3*R*)-**2b**[c]	9	(2*S*,3*R*)-**2c**	10
rac-trans-**2a**	*Mycobacterium paraffinicum* NCIMB 10420	70[b]	68	(2*R*,3*R*)-**2b**[c]	41	(2*S*,3*R*)-**2c**	40
rac-trans-**2a**	*Rhodococcus ruber* DSM 44540	64[b]	39	(2*S*,3*S*)-**2b**[c]	54	(2*R*,3*S*)-**2c**	55
rac-trans-**4a**	*Streptomyces sp.* SM 4165	25[d] [b]	8	n.d.	62	(2*R*,3*S*)-**4c**	14
rac-trans-**4a**	*Rhodococcus ruber* DSM 44541	54[e] [b]	39	n.d.	82	(2*S*,3*R*)-**4c**	18
rac-trans-**4a**	*Rhodococcus ruber* DSM 43338	86[f] [b]	99	n.d.	95	(2*S*,3*R*)-**4c**	28
rac-trans-**6a**	*Rhodococcus equi* IFO 3730	25[g]	6	n.i.	–	(2*S*,3*R*)-**6c**	17
rac-trans-**6a**	*Rhodococcus ruber* DSM 44540	61[g]	67	n.i.	–	(2*R*,3*S*)-**6c**	67
rac-trans-**8a**	*Rhodococcus equi* IFO 3730	44[g]	48	n.i.	–	(2*S*,3*R*)-**6c**	49
rac-trans-**8a**	*Rhodococcus ruber* DSM 44541	47[g]	76	n.i.	–	(2*R*,3*S*)-**6c**	71
rac-trans-**8a**	*Rhodococcus ruber* DSM 44540	45[g]	69	n.i.	–	(2*R*,3*S*)-**6c**	78

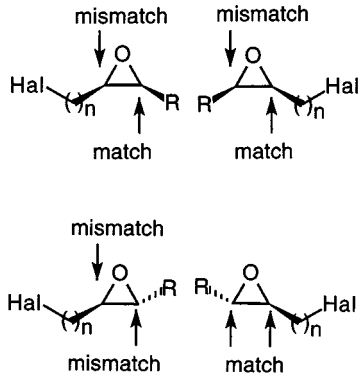
[a] Based on unreacted substrate: c = [substrate] − ([intermediate] + [product]). − [b] After 2 h. − [c] Absolute configuration of intermediate was deduced from that of the product on the assumption of an S_N2-type ring-closure mechanism. − [d] The ratio of [intermediate]/[product] was 3:7. − [e] The ratio of [intermediate]/[product] was 4:6. − [f] The ratio of [intermediate]/[product] was 6:4. − [g] After 20 h; n.d. = not determined, due to non-spontaneous nature of the ring-closure reaction, the absolute configuration cannot be deduced from that of the product; n.i. = intermediate could not be isolated, due to exceedingly low concentration.

(ii) Enzymatic epoxide hydrolysis goes in hand with inversion of configuration at the attacked oxirane carbon atom.

(iii) The selectivity of the reaction largely depends on the chain length of the substituents. *n*-Butyl derivatives **1a**−**4a** are converted with better selectivities than the corresponding ethyl analogues **5a**−**8a**.

(iv) Whereas the biotransformation of *trans*-oxiranes *rac*-**2a**, **4a**, **6a**, and **8a** is faster, *cis* analogues *rac*-**1a**, **3a**, **5a**, are **7a** are hydrolyzed more selectively.

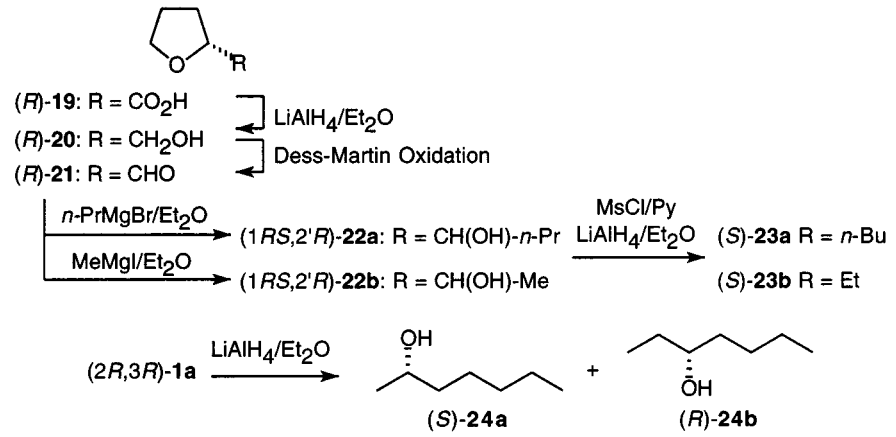
Any enantioconvergence of the reaction can be easily detected when the *ee* of the final product remains high at a high conversion rate (c), which is most striking for *rac-cis*-**1a** (*ee* of **1c**: 85%, c: 86%) and *rac-cis*-**3a** (*ee* of **3c**: 92%, c: 93%). The molecular reason for this phenomenon is the hydrolysis of both enantiomers with opposite regioselectivities: The enzyme shows a strong preference for attack at an (*S*)-configured oxirane carbon atom (Scheme 3).[3] For *cis*-configured oxiranes consisting of a (2*R*,3*S*) and a (2*S*,3*R*) enantiomer, enantioconvergent hydrolysis is facilitated because each enantiomer has one "matching" (*S*) center. On the contrary, this is not the case for *trans* isomers, which consist of a (2*S*,3*S*) and a (2*R*,3*R*) enantiomer, which possess two "matching" and two "mismatching" centers, respectively. Since the faster reacting (2*S*,3*S*) enantiomer possesses two "matching" centers, biohydrolysis proceeds with low regioselectivity, which results in a low enantiomeric excess in the product composition. {It should be kept in mind that, in the presence of a halo*methyl* substituent, the halogen atom causes a switch in the CIP priority of the chiral center to which it is attached. However, the relative steric size of the substituents (the main feature governing an enzyme's selectivity) largely remains the same. As a consequence, the configuration of the preferentially attacked oxirane carbon atom [usually (*S*)] formally switches to (*R*) for substrates bearing a halo*methyl* substituent.[12]}



Scheme 3. Stereochemical course of enzymatic hydrolysis

The time-course of a typical enantioconvergent transformation is shown in Figure S1 (see electronic Supporting Information). At the onset of the reaction, the more rapidly reacting (2*S*,3*S*) enantiomer of **1a** in the racemic mixture is preferentially hydrolyzed by *Mycobacterium paraffinicum* NCIMB 10420, by attack at the 3-position to furnish intermediate (2*S*,3*R*)-**1b** in high *ee* until a degree of conversion of about 50% is reached (ca. 5 h). Until this point, the reaction predominantly follows a kinetic resolution pattern, as indicated by the sharp drop in the curve of conversion versus time. Beyond this point, the more slowly reacting (2*R*,3*R*) enantiomer is hydrolyzed with opposite regioselectivity (by attack at C-2), thus furnishing the same (2*S*,3*R*)-configured intermediate. The latter undergoes (spontaneous) cyclisation to form (2*R*,3*R*)-**1c** as the final product.[12] As a consequence, the *ee*s of **1b** and **1c** remained high (and almost parallel) throughout the course of the biotransformation and (2*R*,3*R*)-**1c** was formed as the sole product.

In order to prove the applicability of this biotransformation for asymmetric synthesis, the biotransformations of *rac-cis*-**1a** (0.6 g), *rac-trans*-**2a** (0.3 g), and *rac-cis*-**3a** (0.6 g) were repeated on a preparative scale. Thus, (2*R*,3*R*)-**1c** (*Mycobacterium paraffinicum* NCIMB 10420), (2*R*,3*S*)-**2c** (*Rhodococcus ruber* DSM 44540), and (2*R*,3*R*)-**3c** (*Rhodococcus ruber* DSM 44541) were obtained in 85% *ee* (76% yield), 55% *ee* (61% yield), and 92% *ee* (79% yield), respectively.



Scheme 4. Synthesis of reference materials for determination of absolute configuration

All chiral products were diastereomerically pure, except for **22a** and **22b**. Absolute configurations were determined for all isolated products from the biotransformations (see below). With one exception (*rac-cis*-**1a**), the absolute configurations of oxiranes were not investigated, due to the low yields during the enantioconvergent biotransformations.

Determination of the Absolute Configuration of Oxirane (2*R*,3*R*)-1a

The remaining non-hydrolyzed epoxide **1a** from the biotransformation using *Mycobacterium paraffinicum* NCIMB 10420 was reduced with $LiAlH_4$ in dry THF. The absolute configuration of secondary alcohol **24a** (Scheme 4) thus obtained was elucidated via GC coinjection, using a chiral stationary phase and commercially available (*R*)-heptan-2-ol as reference material. The absolute configuration of **1b** from the biotransformation was determined by the same strategy using independently synthesized reference material.[13] The absolute configurations of the diols **2b** and **3b** were deduced from those of the products **2c** and **3c**, on the assumption of an S_N2-type mechanism of ring closure. The relative configurations of the substrates **1c** and **2c** were confirmed by their known optical rotation values (see Table S2, electronic Supporting Information). The relative *cis*/*syn* or *trans*/*anti* configurations of **3c**, **5c**, **4c**, and **6c** were elucidated by NOE experiments. Tetrahydrofuran derivatives **3c** and **4c** were converted into **23a**, and products **5c** and **6c**, in an analogous fashion, gave **23b** on removal of the hydroxy group in position 3 (MsCl/Py, then $LiAlH_4/Et_2O$), without affecting the chiral centers in positions 2. For elucidation of their absolute configurations, the compounds thus obtained were coinjected with independently synthesized materials (*S*)-**23a** and (*S*)-**23b**.

The remarkable synthetic potential of this biotransformation is evident, as single (diastereomerically pure) chiral building blocks possessing two contiguous chiral centers are produced from easily available starting materials in high enantiomeric excesses and in 100% yields.

Experimental Section

General: For general remarks, syntheses of substrates and reference materials, determination of absolute configurations, kinetics of the enantioconvergent transformation of *rac-cis*-**1a** by *Mycobacterium paraffinicum* NCIMB 10420 and analytical data (determination of optical purities, GC retention times of compounds on chiral stationary phases, optical rotation values), see the electronic Supporting Information.

General Procedure for the Biocatalytic Hydrolysis of Oxiranes: Lyophilized bacterial cells were used for biotransformations. The bacteria were obtained from culture collections, SM strain numbers refer to the culture collection of the Institute of Biotechnology, Graz University of Technology. All strains were grown as previously described.[14–17]

Lyophilized cells (50 mg) were rehydrated for 1 h in Tris buffer (1 mL, 0.05 M, pH = 8.0) by shaking at 30 °C at 120 rpm. Racemic epoxides *rac-cis*-**1a**, **3a**, **5a**, and **7a** and *rac-trans*-**2a**, **4a**, **6a**, and **8a** (5 μL) were then added, and the reaction was monitored by TLC. After intervals of 12 and 48 h, aliquots of 0.4 mL were withdrawn and extracted twice with EtOAc (0.4 mL each). To facilitate phase separation, the cells were removed by centrifugation. The combined organic layers were dried and analyzed by GC.

General Procedure for the Preparative-Scale Biotransformation: Lyophilized cells (1 g) were rehydrated for 1 h in Tris buffer (20 mL, 0.05 M, pH = 8.0) by shaking at 30 °C at 120 rpm. Racemic epoxides *rac-cis*-**1a**, *rac-trans*-**2a**, and *rac-cis*-**3a** (4 mmol) were then added, and shaking was continued. When the starting material was consumed, as judged by TLC and GLC (for *rac-cis*-**1a**, *rac-trans*-**2a**, after 98 h; for *rac-cis*-**3a** after 120 h), the products were extracted with EtOAc and the organic layers were dried and the solvents evaporated. The residue was flash-chromatographed (p.e./EtOAc, 10:1) to give (2*R*,3*R*)-**1c**, (2*R*,3*S*)-**2c**, and (2*R*,3*R*)-**3c**. The (isolated) chemical and optical yields were as follows: *rac-cis*-**1a** (0.6 g, 4.05 mmol) gave (2*R*,3*R*)-**1c** (*Mycobacterium paraffinicum* NCIMB 10420, 85% *ee*, 76% yield), *rac-trans*-**2a** (0.3 g, 2.02 mmol) furnished (2*R*,3*S*)-**2c** (*Rhodococcus ruber* DSM 44540, 55% *ee*, 61% yield), *rac-cis*-**3a** (0.6 g, 3.69 mmol) yielded (2*R*,3*R*)-**3c** (*Rhodococcus ruber* DSM 44541, 92% *ee*, 79% yield).

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