Asymmetric total synthesis of (+)-*exo*-brevicomin based on enantioconvergent biocatalytic hydrolysis of an alkene-functionalized 2,3-disubstituted epoxide

Sandra F. Mayer, Harald Mang, Andreas Steinreiber, Robert Saf, and Kurt Faber

Abstract: A short total asymmetric synthesis of (+)-*exo*- and (–)-*endo*-brevicomin ((+)-*exo*-**3** and (–)-*endo*-**3**), which are components of the attracting pheromone system of several bark-beetle species belonging to the genera *Dendroctonus* and *Dryocoetes*, was accomplished via a chemoenzymatic protocol. The key step consisted of biocatalytic hydrolysis by bacterial epoxide hydrolases of *cis*-configured 2,3-disubstituted oxiranes bearing olefinic side chains. This reaction proceeded in an enantioconvergent fashion, by affording a single enantiomeric *vic*-diol from the *rac*-epoxide in up to 92% ee and 83% isolated yield.

Key words: bacterial epoxide hydrolase, 2,3-disubstituted oxirane, enantioconvergent hydrolysis, (+)-*exo*-brevicomin, (–)-*endo*-brevicomin.

Résumé : Faisant appel à un protocole chimioenzymatique, on a réalisé une courte synthèse asymétrique totale des (+)-*exo*- et (–)-*endo*-brévicomines [(+)-*exo*-**3** et (–)-*endo*-**3**], des composants du système de phéromone d'attraction de plusieurs espèces de scolytes des genres *Dendroctonus* et *Dryocoetes*. L'étape clé implique une hydrolyse biocatalytique à l'aide d'hydrolases bactériennes d'époxyde d'oxiranes 2,3-disubstitués, de configuration *cis*- et portant des chaînes latérales oléfiniques. Cette réaction s'est produite de façon énantioconvergente en ne fournissant, à partir de l'époxyde racémique, qu'un seul énantiomère de diol vicinal, avec un rendement isolé de 83% et un e.e. de 92%.

Mots clés : hydrolase bactérienne d'époxyde, oxirane 2,3-disubstitué, hydrolyse énantioconvergente, (+)-*exo*-brévicomine, (–)-*endo*-brévicomine.

[Traduit par la Rédaction]

Introduction

Bacterial epoxide hydrolases have been shown to be highly versatile biocatalysts for the preparation of nonracemic *vic*-diols. In particular, bulky 2,2-disubstituted epoxides were hydrolyzed with excellent selectivities (*E* values up to > 200) via kinetic resolution (1). In addition, we recently reported the asymmetric biohydrolysis of (±)-*cis*-2,3-dialkyl oxiranes, which led to the formation of the corresponding internal *vic*-diols in high enantiomeric purities. In the case of haloalkyl-substituted derivatives, enzyme-triggered cascade reactions were observed (2, 3). The remarkable feature of these biotransformations is the fact that — depending on the relative stereochemistry of the substrate — they did not follow a kinetic-resolution pathway but proceeded in an enantioconvergent fashion by furnishing a single enantiomeric *vic*-diol as the sole product in 100% theoretical yield. In view of their considerably improved economic balance, such "deracemization" processes have recently gained considerable attention (4).

To extend the preparative utility of this method, we investigated more synthetically useful substrates bearing various functional groups to allow for further synthetic transformations. Our previous experience indicated that bacterial epoxide hydrolases generally do not accept substrates with polar functional groups (such as OH, N_3) (5). We therefore turned our attention to 2,3-disubstituted oxiranes, *rac*-*cis*-**1** and *rac*-*trans*-**1**, bearing olefinic side chains, which would serve as functional groups for further (oxidative) transformations (Scheme 1) (6). For this reason we investigated a short synthesis of (+)-*exo*-brevicomin. In addition, (–)-*endo*-brevicomin was also synthesized in moderate optical purity.

(+)-*Exo*- and (–)-*endo*-brevicomin ((+)-*exo*-**3** and (–)-*endo*-**3**, respectively) are components of the attracting pheromone system of several bark-beetle species belonging to the genera *Dendroctonus* and *Dryocoetes* (7). In several species, *endo*-**3** is a minor component accompanying (+)-*exo*-**3**. The (–)-*endo* isomer has been reported to be an anti-aggregation pheromone for the southern pine beetle *Dendroctonus frontalis*.

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S.F. Mayer, H. Mang, A. Steinreiber, and K. Faber.[1] Department of Chemistry, Organic & Bioorganic Chemistry, University of Graz, Heinrichstrasse 28, A-8010 Graz, Austria.
R. Saf. Institute for Chemical Technology of Organic Materials, Graz University of Technology, Stremayrgasse 16, A-8010 Graz, Austria.

[1]Corresponding author: (e-mail: kurt.faber@uni-graz.at).

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Scheme 1. Retrosynthetic analysis.

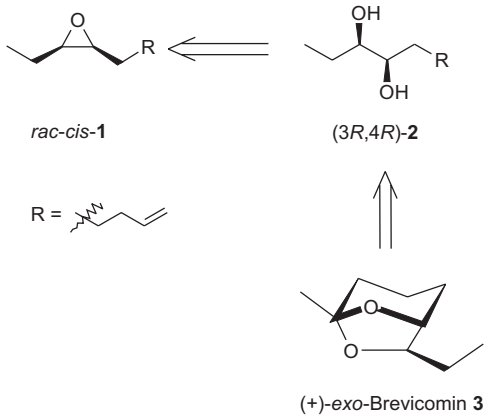
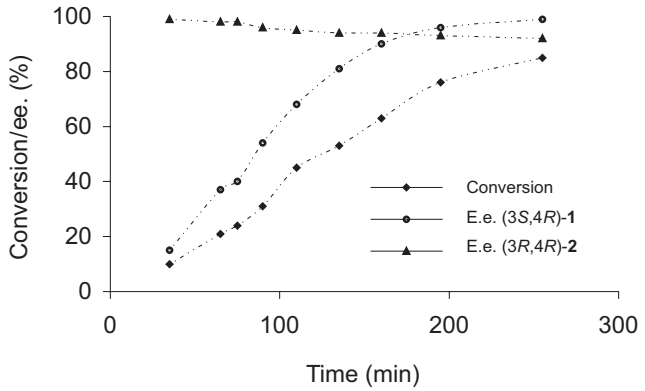


Fig. 1. Time-line of hydrolysis of *rac-cis*-**1** using *Mycobacterium paraffinicum* NCIMB 10420.



In contrast, (+)-*endo*-**3** increases aggregation (8). *Dentroctonus ponderosae* (Mountain Pine beetle) is known to produce (+)-*exo*-brevicomin with >98% ee, whereas the optical purities of the accompanying stereoisomer *endo*-**3** were reported in the range of 65–70% ee (9). Although a number of syntheses of brevicomin stereoisomers have been reported, few are satisfactory in terms of process simplicity (9). Herein, we wish to report a short chemoenzymatic synthesis of (+)-*exo*-brevicomin ((+)-*exo*-**3**) in 92% ee starting from diol (3*R*,*4R*)-**2**, obtained via biocatalytic hydrolysis of oxirane *rac-cis*-**1**. Because the latter process proceeded in an enantioconvergent fashion, the occurrence of an undesired stereoisomer was avoided, rendering this as a highly efficient synthetic sequence. In a related approach, (–)-*endo*-brevicomin ((–)-*endo*-**3**) was obtained in 72% ee starting from *rac-trans*-**1**.

Results and discussion

To provide insight into the factors governing the stereochemical course of the biohydrolysis that would facilitate the predictability of the reaction, 2,3-disubstituted alkene-functionalized oxiranes (*rac-cis*-**1** and *rac-trans*-**1**) were chosen as substrates. Oxiranes *rac-cis*-**1** and *rac-trans*-**1** were prepared from commercially available alcohols (*Z*)-**4** and (*E*)-**4** (Scheme 2). Conversion of the primary hydroxyl group of (*Z*)-**4** and (*E*)-**4** to the corresponding bromides was accomplished via the corresponding mesylates to furnish (*Z*)-**5** and (*E*)-**5**, respectively. The latter were transformed into the corresponding Grignard reagents and then were coupled to allyl bromide to give *cis*- and *trans*-configurated dienes, (*Z*)-**6** and (*E*)-**6**. Epoxidation of the electron-rich internal C=C bond using *m*-chloroperbenzoic acid proceeded regioselectively to furnish *rac-cis*-**1** and *rac-trans*-**1** in 37 and 44% yield, respectively, in three steps.

Substrates *rac-cis*-**1** and *rac-trans*-**1** were subjected to screening for biohydrolysis in Tris-buffer at pH 8.0 using various resting cells from *Actinomyces* spp. known to possess, in particular, strong secondary metabolic activity and strong epoxide hydrolase activity (5). The absence of any undesired spontaneous hydrolysis was verified under standard conditions in the absence of a biocatalyst within the anticipated reaction time of ~21 h. The only product formed during biohydrolysis was the desired diol, *threo*-**2** or *erythro*-**2**. The stereoselectivities (denoted as conversion (c) and enantiomeric purities of substrate (ee_S) and product (ee_P)) (10) obtained from the biohydrolysis of *rac-cis*-**1** and *rac-trans*-**1** are listed in Table 1.

Oxirane *rac-cis*-**1** was hydrolyzed with moderate to good selectivities by several bacterial strains with the predominant formation of diol (3*R*,4*R*)-**2** in up to 92% ee, except for *Rhodococcus equi* IFO 3730, which produced antipode (3*S*,4*S*)-**2** in moderate optical purity (Table 1, entry 7). Several data sets of c, ee_S, and ee_P clearly indicated that the transformation did not follow a kinetic-resolution pathway as indicated by a high ee_P at high conversion. This is most striking for entries 1–4 (Table 1).

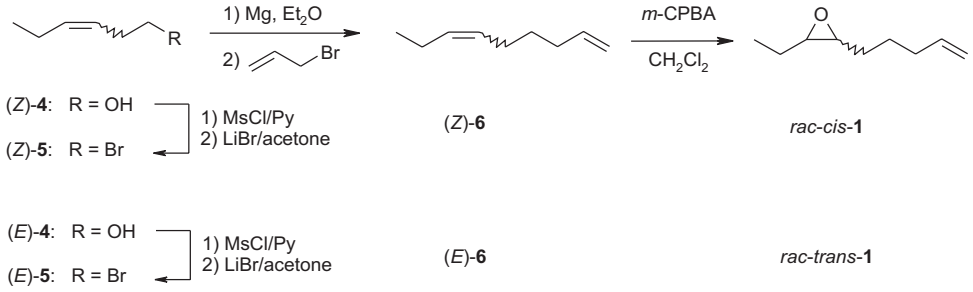
The results obtained from *rac-trans*-**1** show a different pattern. Although this substrate was rapidly converted by several species of *Rhodococci* (Table 1, entries 1, 2, 5, and 7), the selectivities were significantly reduced in comparison with those of the corresponding *cis*-analogue *rac-cis*-**1**. The best result was with diol (3*R*,4*S*)-**2**, which was obtained in a moderate 72% ee (Table 1, entry 4).

An evaluation of the data obtained reveals the following general picture. (*i*) As can be deduced from the absolute configuration of the substrate and product, the enzymatic epoxide hydrolysis results in the inversion of configuration at the attacked oxirane carbon atom, and (*ii*) the biotransformation of *trans*-oxirane is faster, but hydrolysis of the *cis*-analogue is more enantioselective.

Any enantioconvergence of the reaction — indicated as a "deviation" from the classic kinetic-resolution protocol — can easily be detected if the ee of the product is beyond the benchmark of 50% conversion. This is most striking for *rac-cis*-**1** (ee of *threo*-**2** 92% at c 83%). The molecular reason for this phenomenon is that the hydrolysis of both enantiomers proceeds with opposite regioselectivity, i.e., the enzyme shows a predominant preference to attack an (*S*)-configurated oxirane carbon atom (2). For the *cis*-oxirane, *rac-cis*-**1** that consists of (3*R*,4*S*)- and (3*S*,4*R*)-enantiomers, an enantioconvergent hydrolysis is facilitated because each enantiomer has one "matching" (*S*)-center. This is not the case for the *trans*-isomer, *rac-trans*-**1**, which consists of(3*S*,4*S*)- and (3*R*,4*R*)-enantiomers, each possessing two "matching" or two "mismatching" centers.

The time line of a typical enantioconvergent transformation by *Mycobacterium paraffinicum* NCIMB 10420 is shown in Fig. 1. At the onset of the reaction, the faster-reacting (3*R*,4*S*)-

Scheme 2. Synthesis of substrates.



Scheme 3. Biocatalytic hydrolysis and synthesis of (+)-*exo*- and (–)-*endo*-brevicomin.

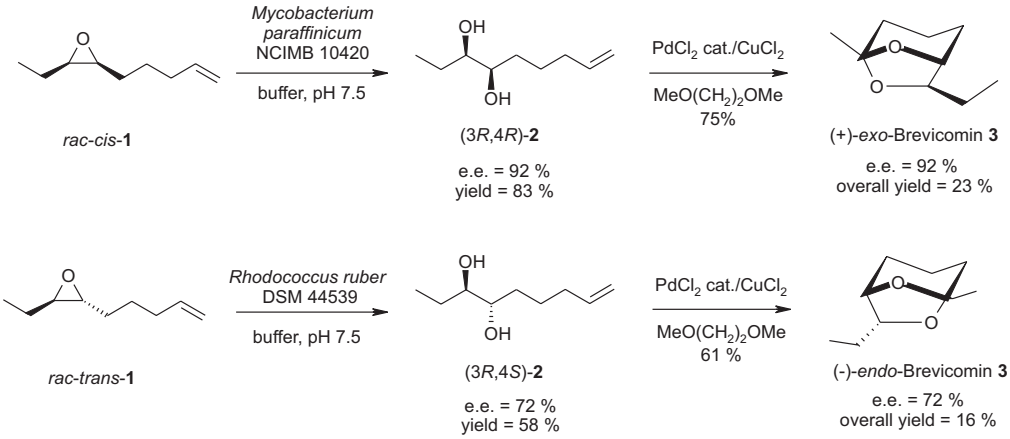


Table 1. Stereoselectivities from the biocatalytic hydrolysis of *rac-cis*-**1** and *rac-trans*-**1**.

		rac-cis-**1**			*rac-trans*-**1**		
Entry	Strain	ee_S (%)	ee_P (%)	Conversion[a] (%)	ee_S (%)[b]	ee_P (%)	Conversion[a] (%)
1	*Mycobacterium paraffinicum* NCIMB 10420	>99 (3*S*,4*R*)	92 (3*R*,4*R*)	83[c]	n.d.	36 (3*R*,4*S*)	90
2	*Rhodococcus sp.* R 312 (CBS 717.73)	59 (3*R*,4*S*)	85 (3*R*,4*R*)	79	n.d.	59 (3*R*,4*S*)	78
3	*Rhodococcus ruber* DSM 44540[d]	59 (3*R*,4*S*)	78 (3*R*,4*R*)	65	n.d.	71 (3*R*,4*S*)	54
4	*Rhodococcus ruber* DSM 44539	88 (3*R*,4*S*)	72 (3*R*,4*R*)	70	n.d.	72 (3*R*,4*S*)	58[e]
5	*Rhodococcus ruber* DSM 44541	51 (3*R*,4*S*)	76 (3*R*,4*R*)	19	n.d.	58 (3*R*,4*S*)	60
6	*Arthobacter* sp. DSM 312	22 (3*R*,4*S*)	86 (3*R*,4*R*)	10	n.d.	55 (3*R*,4*S*)	40
7	*Rhodococcus equi* IFO 3730	72 (3*S*,4*R*)	55 (3*S*,4*S*)	92	n.d.	29 (3*R*,4*S*)	96
8	*Streptomyces* sp. SM 4165	16 (3*R*,4*S*)	86 (3*R*,4*R*)	4	n.d.	67 (3*R*,4*S*)	27

[a]After 21 h.
[b]n.d. not determined (see *Experimental*).
[c]After 6 h.
[d]SM Numbers refer to the culture collection of the Institute of Biotechnology, Graz University of Technology.
[e]After 4 h.

enantiomer of *rac-cis*-**1** is preferentially hydrolyzed from the racemate, via attack at the stereochemically preferred C-3 position, to furnish diol (3*R*,4*R*)-**2** in high ee until a conversion of about 50% is reached. Beyond this point, the transformation of the slower-reacting (3*S*,4*R*)-enantiomer takes place with high but opposite regioselectivity (via attack at C-2), thus yielding the same enantiomeric diol (3*R*,4*R*)-**2**. The relative reaction rate of the enantiomers was calculated from the progress curves of c, ee_S, and ee_P vs. time, and was found to be $E = 6.7$ (11). As a consequence, the ee of *threo*-**2** remained high throughout the course of the biotransformation and (3*R*,4*R*)-**2** was formed as the sole product.

With the end goal being the asymmetric synthesis of (+)-*exo*- and (–)-*endo*-brevicomin, *Mycobacterium paraffinicum* NCIMB 10420 was chosen for the preparative-scale biohydrolysis of *rac-cis*-**1**. This reaction gave (3*R*,4*R*)-**2** as the sole product in 83% yield and 92% ee in an enantioconvergent fashion (Scheme 3). In contrast, the biotransformation of *rac-trans*-**1** underwent kinetic resolution. The hydrolysis of *rac-trans*-**1** using *Rhodococcus ruber* DSM 44539, however,

Table 2. Optical rotation values.

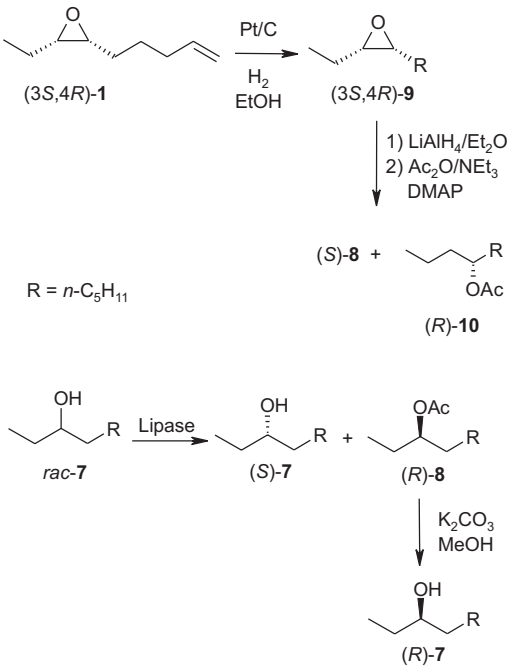
Compound	$[\alpha]_D^{20}$ [a]
(3*S*,4*R*)-**1**	–2.2 (*c* = 0.85, Et_2O, ee > 99%)
(3*R*,4*R*)-**2**	+21.8 (*c* = 0.65, CH_3Cl, ee 92%) [lit.: +25.3 (*c* = 1.0, CH_3Cl)] (16)
(3*R*,4*S*)-**2**	–11.8 (*c* = 0.42, CH_3Cl, ee 72%) [lit.: –14 (*c* = 6.0, CH_3Cl)] (20)
(+)-*exo*-**3**	+65.9 (*c* = 1.90, Et_2O, ee 92%) [lit.: 67.5 (*c* = 1.05, Et_2O, ee 98%)] (21)
(–)-*endo*-**3**	–59.3 (*c* = 0.41, Et_2O, ee 72%) [lit.: –79.5 (*c* = 1.17, Et_2O, ee 98%)] (22)
(*R*)-**7**	–8.1 (*c* = 1.87, $CHCl_3$, ee > 99%) [lit.: –8.4 (*c* = 1.5, MeOH)] (19)
(*R*)-**8**	+6.0 (*c* = 1.53, $CHCl_3$, ee > 99%)
(3*S*,4*R*)-**9**	–1.8 (*c* = 0.55, Et_2O, ee > 99%)

[a]References given in parentheses.

Table 3. GLC-data on a chiral stationary phase.

Compound	Column	Conditions	t_R (min) (absolute configuration)
rac-*cis*-**1**	A	14 psi, 80°C (iso)	3.0 (3*S*,4*R*), 3.2 (3*R*,4*S*)
rac-*threo*-**2**	B	14 psi, 110°C (iso)	11.2 (3*R*,4*R*), 11.6 (3*S*,4*S*)
rac-*erythro*-**2**	A	14 psi, 110°C (iso)	9.2 (3*R*,4*S*), 9.5 (3*S*,4*R*)
rac-*exo*-**3**	B	14 psi, 100°C (iso)	1.5 (–)-*exo*, 1.6 (+)-*exo*
rac-*endo*-**3**	B	14 psi, 100°C (iso)	1.8 (–)-*endo*, 1.9 (+)-*endo*
rac-**8**	A	14 psi, 90°C (iso)	3.6 (*S*), 4.2 (*R*)
rac-*cis*-**9**	A	14 psi, 80°C (iso)	2.8 (3*S*,4*R*), 2.9 (3*R*,4*S*)

Scheme 4. Determination of absolute configuration of epoxide (3*S*,4*R*)-**1**.



yielded (3*R*,4*S*)-**2** in 58% yield and 72% ee (Scheme 3). Because the ee of the precursor to (–)-*endo*-brevicomin was within the suitable range of 65–70%, the material was used for further synthesis (9). Wacker oxidation of (3*R*,4*R*)-**2** or (3*R*,4*S*)-**2**, employing $PdCl_2$ as the catalyst and $CuCl_2$ as the reoxidant, gave (+)-*exo*- and (–)-*endo*-brevicomin in 92% ee (23% overall yield) and 72% ee (16% overall yield), respectively (Scheme 3).

The absolute configuration of diols (3*R*,4*R*)-**2** and (3*R*,4*S*)-**2**, and (+)-*exo*- and (–)-*endo*-brevicomin stereoisomers were confirmed by comparison of their optical rotation with known values (Table 2, vide infra). The absolute configuration of the remaining non-hydrolyzed epoxide (3*S*,4*R*)-**1** was elucidated via the following sequence (Scheme 4). Hydrogenation of the alkene-moiety of (3*S*,4*R*)-**1** (Pt/C and H_2) gave oxirane (3*S*,4*R*)-**9**. $LiAlH_4$ reduction of (3*S*,4*R*)-**9** afforded a mixture of the corresponding alcohols, alkan-3-ol and alkan-4-ol, which were converted into their acetates (*S*)-**8** and (*R*)-**10**, respectively (Ac_2O/NEt_3/DMAP cat.). The absolute configuration of (*S*)-**8** was determined via GLC analysis on a chiral stationary phase by co-injection with an independently synthesized sample of (*R*)-**8** (Scheme 4, vide infra).

Enantiopure acetate (*R*)-**8** was obtained by kinetic resolution of alcohol (±)-**7** using *Candida antarctica* lipase B. The absolute configuration of (*R*)-**8** was confirmed via deprotection of the acetate moiety (K_2CO_3/MeOH) to yield (*R*)-3-nonanol (*R*)-**7**. The absolute configuration (*R*)-**7** was confirmed by comparison of its known optical-rotation value (Table 2).

In conclusion, we have demonstrated that the biohydrolysis of *cis*-2,3-disubstituted oxiranes bearing olefinic side chains using bacterial epoxide hydrolases proceeded in a highly selective and enantioconvergent manner, which provided the corresponding *vic*-diols in high ee and in almost quantitative yield. Although the yields of the *trans*-analogues were sufficient, the selectivities were moderate. The optically enriched diols were transformed into pheromones in a straightforward manner.

Experimental

General

NMR spectra were recorded in $CDCl_3$ using a Bruker AMX 360 at 360 (^{1}H) and 90 (^{13}C) MHz and a Bruker DMX Avance 500 at 500 (^{1}H) and 125 (^{13}C) MHz. Chemical shifts are reported relative to TMS (δ 0.00) with $CHCl_3$ as internal

standard (δ 7.23 (^{1}H) and 76.90 (^{13}C)), coupling constants (*J*) are given in Hz.

TLC plates were run on silica gel Merck 60 (F_{254}) and compounds were visualized by spraying with Mo-reagent ($(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (100 g L^{-1}) and $Ce(SO_4)_2\cdot 4H_2O$ (4 g L^{-1}) in H_2SO_4 (10%)) (detection I) or by dipping into a $KMnO_4$ reagent (2.5 g L^{-1} $KMnO_4$ in H_2O) (detection II). Compounds were purified either by flash chromatography on silica gel Merck 60 (230–400 mesh) or, for volatile substances, by Kugelrohr distillation. Petroleum ether (p.e.) had a boiling range of 60–90°C. GC analyses were carried out on a Varian 3800 gas chromatograph equipped with FID and either an HP1301 or a HP1701 capillary column (both 30 m, 0.25 mm, 0.25 μm film, N_2). Enantiomeric purities were analyzed on a Varian 3800 gas chromatograph equipped with FID, using a CP-Chirasil-DEX CB column (column A, 25 m, 0.32 mm, 0.25 μm film) or an Astec Chiraldex G-PN (column B, 30 m, 0.25 mm). H_2 was used as a carrier gas. For programs and retention times vide infra.

High resolution mass spectra were recorded on a double focussing Kratos Profile Mass Spectrometer with electron impact ionization (EI, +70 eV). Optical rotation values were measured on a PerkinElmer polarimeter 341 at 589 nm (Na-line) in a 1-dm cuvette and are given in units of 10^{-1} deg cm^2 g^{-1}.

Solvents were dried and freshly distilled by common practice. For anhydrous reactions, flasks were dried at 150°C and flushed with dry argon just before use. Organic extracts were dried over Na_2SO_4, and then the solvent was evaporated under reduced pressure. *m*-Chloroperbenzoic acid (*m*-CPBA, Fluka, 70%) was used. Olefinic alcohols (*Z*)-**4** and (*E*)-**4** were purchased from Lancaster. For biotransformations, lyophilized bacterial cells were used. 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 (12–15).

Syntheses of substrates and reference materials

General procedure for the synthesis of (Z)-5 and (E)-5

Compounds (*Z*)-**5** and (*E*)-**5** were obtained via mesylation of alcohols (*Z*)-**4** and (*E*)-**4**, followed by halide exchange using Method A.

Method A: Alcohol (50 mmol) was dissolved in CH_2Cl_2 (50 mL) and pyridine (100 mmol) was added. The stirred solution was cooled to 0°C and methanesulfonyl chloride (75 mmol) was added in small portions. The reaction mixture was allowed to warm to room temperature. After the reaction reached completion (16 h), water (70 mL) was added and the resulting two-phase system was stirred for 1 h to destroy excess methanesulfonyl chloride. Then the two layers were separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with 5% aq HCl (50 mL) and sat. aq $NaHCO_3$ (50 mL). The organic phase was dried and concentrated. The crude mesylate (50 mmol) was dissolved in anhydrous acetone (50 mL) and LiBr (75 mmol) was added. The reaction mixture was allowed to stir at room temperature until completion (ca. 20 h). Then, 5% aq HCl (70 mL) was added, the two layers were separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic layers were washed with sat. aq $NaHCO_3$ (70 mL) and the organic phase was dried and concentrated. Flash chromatography afforded the pure haloalkenes (*Z*)-**5** and (*E*)-**5**, details and spectroscopic data being given below.

*cis-1-Bromo-hex-3-ene ((Z)-**5**)*

Method A was employed using alcohol (*Z*)-**4** (10 g, 99.84 mmol) and LiBr for the halogenation. Flash chromatography (pentane–Et_2O, 10:1) gave *cis*-1-bromo-hex-3-ene (*Z*)-**5** as a colorless liquid (9.82 g, 61% over 2 steps). R_f (p.e.–EtOAc, 5:1) = 0.83 (detection II). ^{1}H NMR (500.13 MHz, $CDCl_3$) δ: 0.98 (t, *J* = 7.5, 3H), 2.04–2.10 (m, 2H), 2.60–2.65 (m, 2H), 3.38 (t, *J* = 7.2, 2H), 5.33–5.35 (m, 1H), 5.53–5.56 (m, 1H). ^{13}C NMR (125.77 MHz, $CDCl_3$) δ: 14.1, 20.6, 30.6, 32.5, 125.1, 134.7.

*trans-1-Bromo-hex-3-ene ((E)-**5**)*

Method A was employed using alcohol (*E*)-**4** (10 g, 99.84 mmol) and LiBr for the halogenation. Flash chromatography (pentane–Et_2O, 10:1) gave *trans*-1-bromo-hex-3-ene (*E*)-**5** as a colorless liquid (10.5 g, 65% over 2 steps). R_f (p.e.–EtOAc, 5:1) = 0.80 (detection II). ^{1}H NMR (500.13 MHz, $CDCl_3$) δ: 0.99 (t, *J* = 7.5, 3H), 2.01–2.06 (m, 2H), 2.53–2.57 (m, 2H), 3.37 (t, *J* = 7.2, 2H), 5.35–5.41 (m, 1H), 5.57–5.61 (m, 1H). ^{13}C NMR (125.77 MHz, $CDCl_3$) δ: 13.6, 25.5, 32.8, 36.0, 125.3, 135.4.

General procedure for the synthesis of (Z)-6 and (E)-6 (Method B)

Method B: To a stirred solution of allyl bromide (9.1 g, 75.5 mmol) in anhydrous Et_2O (20 mL) under an Ar atmosphere, a Grignard reagent, prepared from (*Z*)-**5** or (*E*)-**5** (50 mmol) in 15 mL of anhydrous Et_2O, was added at room temperature. The mixture was heated at reflux until the reaction was completed (16 h). Then 5% aq HCl (20 mL) was added and after 10 min the product was extracted with Et_2O. The combined organic extracts were dried and concentrated. The residue was purified by flash chromatography to give (*Z*)-**6** or (*E*)-**6**, details and spectroscopic data are given below.

*(Z)-Nona-1,6-diene ((Z)-**6**)*

Method B was employed using (*Z*)-**5** (8.2 g, 50.3 mmol). Flash chromatography (pentane) gave (*Z*)-**6** as a colorless liquid (5.4 g, 87%). R_f (p.e.) = 0.85 (detection II). ^{1}H NMR (360.13 MHz, $CDCl_3$) δ: 0.98 (t, *J* = 7.2, 3H), 1.37–1.51 (m, 2H), 1.99–2.11 (m, 6H), 4.95–5.05 (m, 2H), 5.33–5.43 (m, 2H), 5.79–5.87 (m, 1H). ^{13}C (90.6 MHz, $CDCl_3$) δ: 14.4, 20.6, 26.6, 29.1, 33.3, 114.5, 128.9, 132.0, 139.0.

*(E)-Nona-1,6-diene ((E)-**6**)*

Method B was employed using (*E*)-**5** (8.5 g, 52.1 mmol). Flash chromatography (pentane) gave (*E*)-**6** as a colorless liquid (5.9 g, 91%). R_f (p.e.) = 0.85 (detection II). ^{1}H NMR (360.13 MHz, $CDCl_3$) δ: 0.98 (t, *J* = 7.3, 3H), 1.41–1.50 (m, 2H), 1.98–2.09 (m, 6H), 4.94–5.04 (m, 2H), 5.40–5.47 (m, 2H), 5.78–5.83 (m, 1H). ^{13}C (90.56 MHz, $CDCl_3$) δ: 14.0, 25.7, 28.9, 32.0, 33.3, 114.4, 128.9, 132.4, 139.0.

General procedure for the synthesis of rac-cis-1 and rac-trans-1

Substrates *rac-cis*-**1** and *rac-trans*-**1** were prepared by epoxidation of alkenes (*Z*)-**6** and (*E*)-**6** using *m*-chloroperbenzoic acid (*m*-CPBA) following Method C.

Method C: To a vigorously stirred solution of the alkene (40 mmol) in anhydrous CH_2Cl_2 (400 mL) was added NaH_2PO_4 (ca. 2.6 equiv). After the mixture was stirred for 15 min at room temperature, it was cooled to 0°C and *m*-CPBA (1.05 equiv) was added slowly. The reaction was allowed to warm to room temperature and stirred for an additional 8 h. The white suspension was filtered and the resulting solution was treated with 10% aq $Na_2S_2O_5$ (100 mL) to destroy the excess of peracid. The resulting two-phase system was stirred for 30 min, the layers were separated and the organic phase was washed with sat. aq $NaHCO_3$ (50 mL). The organic phase was dried and evaporated. Kugelrohr distillation afforded pure oxiranes *rac-cis*-**1** and *rac-trans*-**1**. Details and spectroscopic data are given below.

*cis-3,4-Epoxynon-8-ene (rac-cis-**1**)*

Method C was employed using (*Z*)-**6** (5 g, 40.3 mmol). Kugelrohr distillation gave *rac-cis*-**1** as a colorless liquid (3.9 g, 69%). R_f (p.e.–EtOAc, 5:1) = 0.55 (detection I). bp_{20mbar} (Kugelrohr): 80–90°C. ^{1}H NMR (360.13 MHz, $CDCl_3$) δ: 1.01 (t, *J* = 7.0, 3H), 1.49–1.58 (m, 6H), 2.08–2.12 (m, 2H), 2.84–2.92 (m, 2H), 4.94–5.04 (m, 2H), 5.75–5.83 (m, 1H). ^{13}C NMR (90.56 MHz, $CDCl_3$) δ: 10.6, 21.2, 25.9, 27.2, 33.6, 57.1, 58.4, 114.9, 138.4. Anal. calcd. for $C_9H_{16}O$: C 77.05, 11.50; found: C 77.12, H 11.51.

*trans-3,4-Epoxynon-8-ene (rac-trans-**1**)*

Method C was employed using (*E*)-**6** (6 g, 48.4 mmol). Kugelrohr distillation gave *rac-trans*-**1** as a colorless liquid (5.1 g, 75%). R_f (p.e.–EtOAc, 5:1) = 0.65 (detection I). bp_{20mbar} (Kugelrohr): 85–95°C. ^{1}H NMR (360.13 MHz, $CDCl_3$) δ: 0.98 (t, *J* = 7.0, 3H), 1.48–1.58 (m, 6H), 2.09 (d, *J* = 5.9, 2H), 2.62–2.67 (m, 2H), 4.94–5.03 (m, 2H), 5.76–5.84 (m, 1H). ^{13}C NMR (90.56 MHz, $CDCl_3$) δ: 9.94, 25.2, 25.4, 31.5, 33.5, 58.4, 60.0, 114.8, 138.4. Anal. calcd. for $C_9H_{16}O$: C 77.05, 11.50; found: C 77.08, H 11.55.

General procedure for the synthesis of reference material for racemic diols rac-threo-2 and rac-erythro-2

The *rac*-diols *rac-threo*-**2** and *rac-erythro*-**2** were obtained by acid-catalysed hydrolysis of *rac-cis*-**1** and *rac-trans*-**1** following Method D.

Method D: Racemic diols *rac-threo*-**2** and *rac-erythro*-**2** were obtained by hydrolysis of the corresponding racemic oxiranes of *rac-cis*-**1** and *rac-trans*-**1** (1.1 mmol) in a mixture of water and THF (10 mL, 4:1) under acidic conditions (6 M H_2SO_4, 10 drops). After the reaction reached completion (2 h), the solution was extracted with EtOAc (2 × 10 mL). The combined organic layers were dried and concentrated. The residue was purified by flash chromatography. Details and spectroscopic data are given below.

*rac-threo-8-Nonene-3,4-diol (rac-threo-**2**)*

Method D was employed using oxirane *rac-cis*-**1** (0.3 g, 2.1 mmol). Flash chromatography (p.e.–EtOAc, 3:1) gave pure *rac-threo*-8-nonene-3,4-diol *rac-threo*-**2** (0.25 g, 74%). R_f (p.e.–EtOAc, 1:1) = 0.42 (detection I). Spectroscopic data were in full agreement with those previously reported (16). HRMS calcd. for $C_9H_{18}O_2$: 158.1307 [M^+]; found: 158.1314 [M^+].

*rac-erythro-8-Nonene-3,4-diol (rac-erythro-**2**)*

Method D was employed using oxirane *rac-trans*-**1** (0.3 g, 2.1 mmol). Flash chromatography (p.e.–EtOAc, 3:1) gave pure *erythro*-8-nonene-3,4-diol *rac-erythro*-**2** (0.21 g, 63%). R_f (p.e.–EtOAc, 1:1) = 0.45 (detection I). Spectroscopic data were in full agreement with those previously reported (16). HRMS calcd. for $C_9H_{18}O_2$: 158.1307 [M^+]; found: 158.1303 [M^+].

General procedure for preparative-scale biohydrolysis of rac-cis-1 and rac-trans-1

Racemic epoxides *rac-cis*-**1** (0.7 g, 5 mmol) and *rac-trans*-**1** (0.3 g, 2.14 mmol) were hydrolyzed using rehydrated lyophilized microbial cells (1 g *Mycobacterium paraffinicum* NCIMB 10420 for *rac-cis*-**1**, 0.4 g of *Rhodococcus ruber* DSM 44539 for *rac-trans*-**1**) in Tris-buffer (30 mL for *rac-cis*-**1**, 15 mL for *rac-trans*-**1**, 0.05 M, pH 8.0) on an orbit shaker (120 rpm) at 30°C. When the starting material was consumed (after 6 h for *rac-cis*-**1**, 4 h for *rac-trans*-**1**, as judged by TLC and GLC), the mixture was extracted continuously with CH_2Cl_2 for 24 h. The organic layers were dried and evaporated. The residue was flash chromatographed (p.e.–EtOAc, 3:1) to give (3*R*,4*R*)-**2** (0.66 g, 83%, ee 92%) or (3*R*,4*S*)-**2** (0.20 g, 58%, ee 72%). Spectroscopic data of these products matched those of material obtained by acidic hydrolysis of the corresponding *rac*-oxiranes. Elemental analysis of (3*R*,4*R*)-**2**: Anal. calcd. for $C_9H_{18}O_2$: C 68.30, H 11.45; found: C 68.35, H 11.46. Elemental analysis of (3*R*,4*S*)-**2**: Anal. calcd. for $C_9H_{18}O_2$: C 68.30, H 11.45; found: C 68.38, H 11.48.

General procedure for the synthesis of (+)-exo- and (–)-endo-brevicomin [(+)-exo- and (–)-endo-3, respectively] via Wacker-oxidation of diols (3R,4R)-2 and (3R,4S)-2, respectively, following Method E

Method E: Nonracemic diol (3*R*,4*R*)-**2** or (3*R*,4*S*)-**2** (3.2 mmol) was dissolved in anhydrous 1,2-dimethoxyethane (10 mL). The solution was stirred at room temperature. $PdCl_2$ (0.10 g, 0.57 mmol) and $CuCl_2$ (0.43 g, 3.2 mmol) were added and stirring was continued for 10 h. The brown solution was diluted with H_2O and Et_2O (10 mL each). After phase separation, the aqueous layer was extracted with Et_2O (2 × 10 mL). The combined organic phases were dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography. Details and spectroscopic data are given below.

*(+)-exo-Brevicomin ((+)-exo-**3**)*

Method E was employed using (3*R*,4*R*)-**2** (0.5 g, 3.2 mmol). Flash chromatography (pentane–Et_2O, 10:1) gave pure (+)-*exo*-**3** (0.37 g, 75%, ee 92%). R_f (p.e.–EtOAc, 5:1) = 0.54 (detection I). ^{1}H NMR (360.13 MHz, $CDCl_3$) δ: 0.92 (t, *J* = 7.4, 3H), 1.43 (s, 3H), 1.57–1.84 (m, 8H), 3.94 (t, *J* = 6.4, 1H), 4.15 (s, 1H,). ^{13}C NMR (90.56 MHz, $CDCl_3$) δ: 9.8, 17.2, 25.1, 28.0, 28.6, 35.0, 78.4, 81.2, 107.7. HRMS calcd. for $C_9H_{16}O_2$: 156.1150 [M^+]; found: 156.1145 [M^+].

*(–)-endo-Brevicomin ((–)-endo-**3**)*

Method E was employed using (3*R*,4*S*)-**2** (0.15 g, 0.95 mmol). Flash chromatography (pentane–Et_2O, 10:1) gave pure (–)-*endo*-**3** (0.09 g, 61%, ee 72%). R_f (p.e.–EtOAc, 5:1) = 0.57 (detection I). ^{1}H NMR (360.13 MHz, $CDCl_3$) δ: 0.96 (t,

J = 7.4, 3H), 1.40 (s, 3H), 1.53–1.92 (m, 8H), 3.93–3.97 (m, 1H), 4.14–4.18 (m, 1H). ^{13}C NMR (90.56 MHz, $CDCl_3$) δ: 11.0, 17.6, 22.0, 23.7, 25.1, 34.5, 76.6, 81.7, 107.0. HRMS calcd. for $C_9H_{16}O_2$: 156.1150 [M^+]; found: 156.1159 [M^+].

General procedure for the screening of biocatalysts for the hydrolysis of rac-cis-1 and rac-trans-1

Racemic epoxides *rac-cis*-**1** and *rac-trans*-**1** (5 μL) were hydrolyzed using rehydrated lyophilized cells (50 mg) in Tris-buffer (1 mL, 0.05 M, pH 8.0) by shaking the mixture at 30°C with 120 rpm. The reactions were monitored by TLC. After 21 h and 42 h, a 0.4 mL aliquot was extracted with EtOAc (2 × 0.4 mL). To facilitate phase separation, the cells were removed by centrifugation. The combined organic layers were dried and analyzed by GC on a chiral stationary phase.

Determination of enantiomeric purities (Table 3)

All chiral materials could be analyzed directly by GC on a chiral stationary phase (see Table 3) except 3-nonanol **7** and *rac-trans*-**1**. In the case of alcohol **7**, derivatization to the acetate **8** was optimal, which gave satisfactory enantioseparation. For epoxide *rac-trans*-**1** no enantioseparation using four different chiral columns (columns A or B; Astec Chiraldex B-TA capillary column (column C, 30 m, 0.25 mm); or Astec Chiraldex G-PH (column D, 30 m, 0.32 mm)) was possible.

Syntheses of chiral reference material and determination of absolute configuration

All chiral compounds were diastereomerically pure. The absolute configuration of oxirane *trans*-**1** was not investigated because no enantioseparation on GC (with 4 different chiral columns) was achieved.

Determination of absolute configuration of oxirane (3S,4R)-1

The remaining non hydrolyzed epoxide from the biotransformation of *rac-cis*-**1** using *Mycobacterium paraffinicum* NCIMB 10420 (Scheme 4) was separated from the formed product (3*R*,4*R*)-**2** by flash chromatography (pentane–Et_2O, 10:1). The alkene-functionality of oxirane (3*S*,4*R*)-**1** (0.027 g, 0.2 mmol, ee > 99%) was hydrogenated with Pt/C (0.02 g, Pt on activated carbon, (5% w/w)) in anhydrous EtOH (5 mL) under H_2 at atmospheric pressure (30 min). The catalyst was removed by filtration through a plug of Celite-545 and the solvent was evaporated. After flash chromatography (pentane–Et_2O, 10:1) *cis*-(3*S*,4*R*)-epoxynonane (3*S*,4*R*)-**9** (0.021 g, 74%, >99% ee) was isolated. R_f (p.e.–EtOAc, 5:1) = 0.65 (detection I). ^{1}H NMR (360.13 MHz, $CDCl_3$) δ: 0.91 (t, J = 7.4, 3H), 1.04 (t, J = 7.2, 3H), 1.33–1.70 (m, 10H), 2.86–2.93 (m, 2H). ^{13}C NMR data match those previously described (17). Without affecting the chiral centers in position 2 and 3 of the oxirane, (3*S*,4*R*)-**10** was converted into the acetates (*S*)-**8** and (*R*)-**10**, using $LiAlH_4$ (0.015 g, 0.4 mmol) in anhydrous Et_2O (5 mL) at room temperature. After the reaction was complete (1 h), the reaction was quenched by addition of sat. aq NH_4Cl (10 mL). The combined organic phases were dried and concentrated. The residue was dissolved in Ac_2O and NEt_3 (0.040 g, 0.4 mmol) and then DMAP (5 mg, cat.) was added. The mixture was heated at reflux for 4 h and quenched by addition of water and Et_2O. The organic phase was dried and evaporated. Without further purification, the resulting mixture of (*S*)-**8** and (*R*)-**10** used directly for determination of the absolute configuration via coinjection with an independently synthesized sample of (*R*)-**8** on GC using a chiral stationary phase. Analysis was focussed on acetate **8**, since enantiopure acetate **10** was not available.

(R)-3-Nonanol ((R)-7)

Since enantioseparation for alcohol (±)-**7** was impossible, the corresponding acetate (±)-**8** was used for analysis. A reference sample of (*R*)-**7** was synthesized as follows: Lipase from *Candida antarctica* B (150 mg) was dispersed in heptane (25 mL) and after addition of (±)-**7** (1.0 g, 6.94 mmol) and vinyl acetate (2.0 g, 23.2 mmol) the mixture was agitated (120 rpm) on an orbit shaker at room temperature, while the progress of the reaction was monitored by GC. After 48 h, the conversion had reached ~50% and after filtration of the biocatalyst, the solution was concentrated in vacuo. After flash chromatography (p.e.–EtOAc, 5:1) (*R*)-3-nonyl acetate (*R*)-**8** (0.41 g, 64%, >99% ee) was isolated. R_f (p.e.–EtOAc, 3:1) = 0.80 (detection I). ^{1}H NMR (360.13 MHz, $CDCl_3$) δ: 0.86–0.90 (m, 6H), 1.23–1.27 (m, 8H), 1.42–1.54 (m, 4H), 2.04 (s, 3H), 4.77–4.82 (m, 1H). ^{13}C NMR (90.56 MHz, $CDCl_3$) δ: 9.55, 14.1, 21.2, 22.6, 25.3, 27.0, 29.3, 31.8, 33.6, 75.6, 171.0. Next, (*R*)-**8** (0.20 g, 1.07 mmol) was dissolved in MeOH (10 mL) and K_2CO_3 (0.30 g, 2.15 mmol) was added. After 30 min the reaction reached completion. The reaction was quenched by addition of water (10 mL) and Et_2O (20 mL). The combined organic phases were dried and concentrated. After flash chromatography (p.e. –EtOAc, 5:1) (*R*)-3-nonanol ((*R*)-**7**) (0.11 g, 71%, >99% ee) was isolated. R_f (p.e.–EtOAc, 3:1) = 0.50 (detection I). Spectroscopic data were in full agreement with those previously reported (18). The absolute configuration of (*R*)-**7** was confirmed by comparison of its known optical rotation value (Table 2) (19). Thus, the absolute configuration of C-3 of **8** was proven to be *R*.

The absolute configuration of diols (3*R*,4*R*)-**2** and (3*R*,4*S*)-**2**, and (+)-*exo*- and (–)-*endo*-brevicomin was determined by comparison of optical rotation values (Table 2) (16, 20–22).

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References

1. R.V.A. Orru and K. Faber. Curr. Opinion Chem. Biol. **3**, 16 (1999).
2. W. Kroutil, M. Mischitz, and K. Faber. J. Chem. Soc. Perkin Trans. 1 3629 (1997).
3. S.F. Mayer, A. Steinreiber, R.V.A. Orru, and K. Faber. Tetrahedron: Asymmetry, **12**, 41 (2001).
4. K. Faber. Chem. Eur. J. **7**, 5004 (2001).

5. A. Steinreiber, I. Osprian, S.F. Mayer, R.V.A. Orru, and K. Faber. Eur. J. Org. Chem. 3703 (2000).
6. A. Steinreiber, S.F. Mayer, and K. Faber. Synthesis, 2035 (2001).
7. S. Hu, S. Jayaraman and A.C. Oehlschlager. J. Org. Chem. **64**, 2524 (1999).
8. A.C. Oehlschlager and B.D. Johnston. J. Org. Chem. **52**, 940 (1987).
9. W. Francke and W. Schröder. Curr. Org. Chem. **3**, 407 (1999), and refs. cited therein.
10. Since the biocatalytic hydrolysis of 2,3-disubstituted epoxides is likely to proceed in an enantioconvergent fashion, the general use of *E*-values for the description of enantioselectivities is inapplicable.
11. W. Kroutil and K. Faber. In Press. A computer program will soon be released via Internet at http://borgc185.kfunigraz.ac.at; for a related mathematical treatment of the kinetics see: P. Moussou, A. Archelas, J. Baratti, and R. Furstoss. Tetrahedron: Asymmetry, **9**, 1539 (1998).
12. M. Mischitz, W. Kroutil, U. Wandel, and K. Faber. Tetrahedron: Asymmetry, **6**, 1261 (1995).
13. W. Kroutil, I. Osprian, M. Mischitz, and K. Faber. Synthesis, 156 (1997).
14. I. Osprian, W. Kroutil, M. Mischitz, and K. Faber. Tetrahedron: Asymmetry, **8**, 65 (1997).
15. W. Krenn, I. Osprian, W. Kroutil, G. Braunegg, and K. Faber. Biotechnol. Lett. **21**, 687 (1999).
16. H. Takahata, M. Kusunoki, Y. Takeda, and T. Momose. Tetrahedron: Asymmetry, **7**, 2093 (1996).
17. C. Chiappe, A. Cordoni, G.L. Moro, and C.D. Palese. Tetrahedron: Asymmetry, **9**, 341 (1998).
18. J. Spieler, O. Huttenloch, and H. Waldmann. Eur. J. Org. Chem. 391 (2000).
19. B. Weber and D. Seebach. Tetrahedron, **50**, 7473 (1994).
20. R. Bel-Rhlid, M.F. Renard, and H. Veschambre. Bull. Soc. Chim. Fr. **133**, 1011 (1996).
21. P.C. Bulman Page, C.M. Rayner, and I.O. Sutherland. J. Chem. Soc. Perkin Trans. 1, 1375 (1990).
22. H. Redlich, W. Bruns, W. Francke, V. Schurig, T.L. Payne, and J.P. Vité. Tetrahedron, **43**, 2029 (1987).