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Chemoenzymatic asymmetric total syntheses of a constituent of Jamaican rum and of (+)-Pestalotin using an enantioconvergent enzyme-triggered cascade reaction

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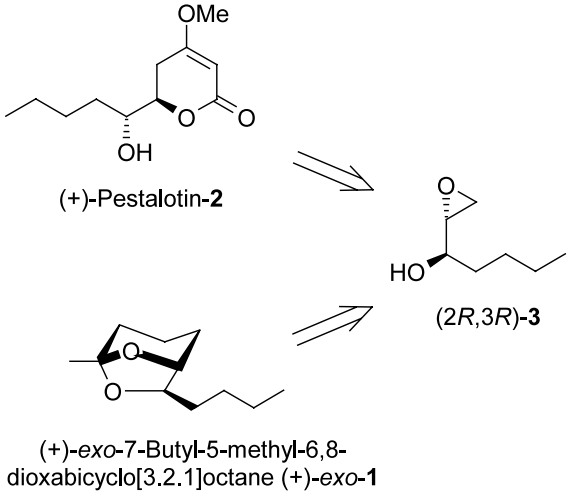
Abstract—A short chemoenzymatic route to two natural products—the first, a constituent of Jamaican rum and the second the (+)-antipode of the gibberelin synergist (−)-Pestalotin—was accomplished based on an enzyme-triggered cascade-reaction. Thus, a racemic halomethyl oxirane was hydrolyzed by bacterial epoxide hydrolases to furnish the corresponding *vic*-halomethyl-diol, which underwent spontaneous ring-closure to furnish an epoxy alcohol in up to 93% e.e. and ≥99 d.e. Due to the fact that this process was enantioconvergent, the occurrence of the undesired enantiomer was entirely avoided.

1. Introduction

exo-7-Butyl-5-methyl-6,8-dioxabicyclo[3.2.1]octane(*exo*-**1**) is a known constituent of Jamaican rum and was first isolated from a sample of fusel oil, derived from molasses.[1] The relative *exo*-configuration of **1** was elucidated via NMR- and IR-spectroscopic and mass spectrometric analyses. Its absolute configuration has not been established so far. Herein, we report a concise synthesis of (+)-*exo*-**1** for the first time using a chemoenzymatic approach based on the epoxy alcohol building block (2*R*,3*R*)-**3**. From the same key intermediate, the (+)-antipode of the naturally occurring gibberelin synergist (−)-Pestalotin was obtained.

Our synthetic strategy was based on the epoxy alcohol (2*R*,3*R*)-**3** as the main chiral building block (Scheme 1). We envisaged that (2*R*,3*R*)-**3** could easily be prepared via a biocatalytic enantioconvergent cascade-reaction recently developed by us.[2] Thus, when *cis*-configured (±)-halomethyl- or (±)-haloethyl-oxiranes were subjected to the action of a bacterial epoxide hydrolase, both enantiomers were transformed through opposite regioselective pathways in an enantioconvergent fashion to furnish a single enantiomeric *vic*-diol as the sole product.[3] Due to the presence of a halohydrin moiety, the latter intermediate underwent spontaneous ring-closure to yield epoxy alcohols (from halomethyl) or THF derivatives (from haloethyl derivatives) as the final products in excellent d.e. and e.e.[4] Overall, this



Scheme 1. Retrosynthetic analysis.

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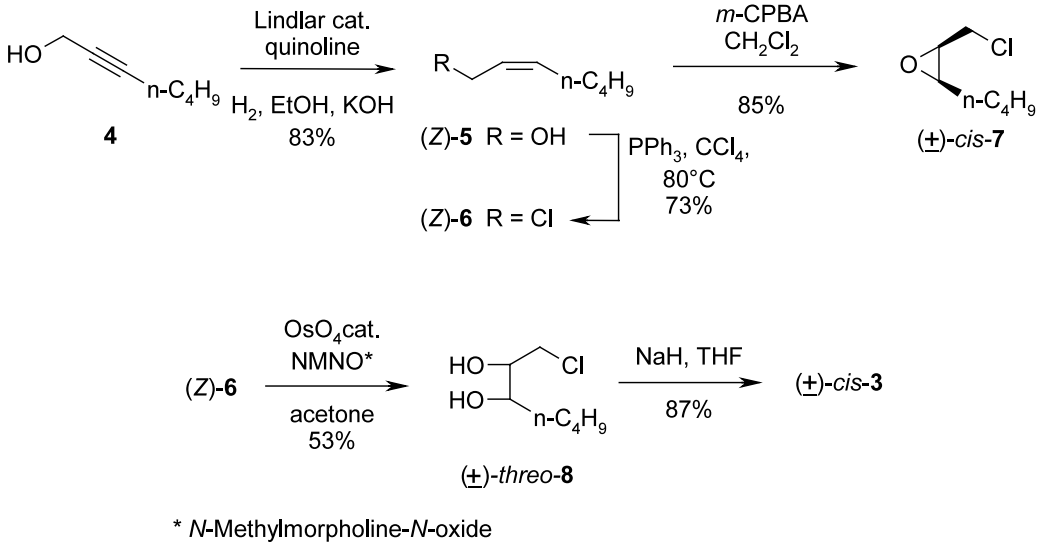
sequence represents an enzyme-catalyzed cascade-reaction.[5] Due to the intrinsic enantioconvergence of the biohydrolysis, chemical yields were markedly above the 50% limitation set for kinetic resolutions. Such 'deracemization' processes have recently gained considerable attention due to their improved economic balance.[6]

The synthetic elegance of this process prompted us to utilize the same building block (2*R*,3*R*)-**3** for the synthesis of the (+)-antipode of naturally occurring (−)-Pestalotin, *ent*-**2** (Scheme 1).[7] The latter is a gibberelin synergist isolated from culture filtrate of the phytopathogenic fungus *Pestalotia cryptomeriaecola*[8] and from an unidentified *Penicillium* species.[9]

2. Results and discussion

The key substrate for the biocatalytic transformation catalyzed by bacterial epoxide hydrolases (BEH) was epoxide (±)-*cis*-**7** (Scheme 2), which was synthesized as follows: Alcohol **4** was selectively hydrogenated with Lindlar catalyst to give the corresponding *cis*-alkene (*Z*)-**5** in 83% yield (Scheme 2). In order to achieve absolute *cis*-selectivity, poisoning of the Lindlar catalyst with quinoline alone was not sufficient (*trans*/*cis*-ratio 1:5), but required the addition of KOH. Halogenation of the hydroxyl group of (*Z*)-**5** via Appel conditions (PPh_3/CCl_4) gave haloalkene (*Z*)-**6** in 73% yield.[10] Epoxidation of the latter using *m*-CPBA afforded (±)-*cis*-**7** in 85% yield. For the identification of the expected biotransformation product, (±)-*threo*-**8** was independently synthesized as reference material. *cis*-Selective dihydroxylation of (*Z*)-**6** with a cat. amount of OsO_4 afforded (±)-*threo*-diol **8** in 53% yield. The latter compound was treated with NaH to give epoxy alcohol (±)-*cis*-**3** after ring-closure in 87% yield.

The racemic substrate (±)-*cis*-**7** was screened for biohydrolysis in Tris-buffer at pH 8.0 using resting cells of a range of bacteria, in particular *Actinomyces* sp., known to possess strong secondary metabolism and epoxide hydrolase activity. Under screening conditions it was verified that in the absence of biocatalyst no spontaneous hydrolysis of (±)-*cis*-**7** was observed within the anticipated reaction time of ~130 h. We were pleased to find that hydrolysis of (±)-*cis*-**7** furnished the corresponding diol **8**, and that the latter intermediate (detected at low concentration <5%) underwent subsequent intramolecular cyclization to yield epoxy alcohol **3** as the final product.



Scheme 2. Synthesis of substrates and reference materials.

Table 1. Selectivities from the biocatalytic transformation of chloroalkyl-oxirane (±)-*cis*-**7**

Entry	Biocatalyst	Substrate e.e.$_S$ (%)	Intermediate[a] e.e.$_I$ (%)	Product e.e.$_P$ (%)	Conversion[b] (%)
1	*M. paraffinicum* NCIMB 10420	>99 (2*R*,3*R*)	91 (2*S*,3*R*)	93 (2*R*,3*R*)	81[c]
2	*Rhodococcus* sp. R 312 (CBS 717.73)	50 (2*R*,3*R*)	67 (2*S*,3*R*)	72 (2*R*,3*R*)	66[d]
3	*Rhodococcus ruber* DSM 44540	5 (2*R*,3*R*)	62 (2*S*,3*R*)	57 (2*R*,3*R*)	48[d]
4	*Rhodococcus ruber* DSM 44539	29 (2*R*,3*R*)	59 (2*S*,3*R*)	62 (2*R*,3*R*)	52[d]
5	*Rhodococcus ruber* DSM 43338	12 (2*R*,3*R*)	69 (2*S*,3*R*)	64 (2*R*,3*R*)	39[d]
6	*Rhodococcus equi* IFO 3730	30 (2*S*,3*S*)	22 (2*R*,3*S*)	19 (2*S*,3*S*)	40[d]

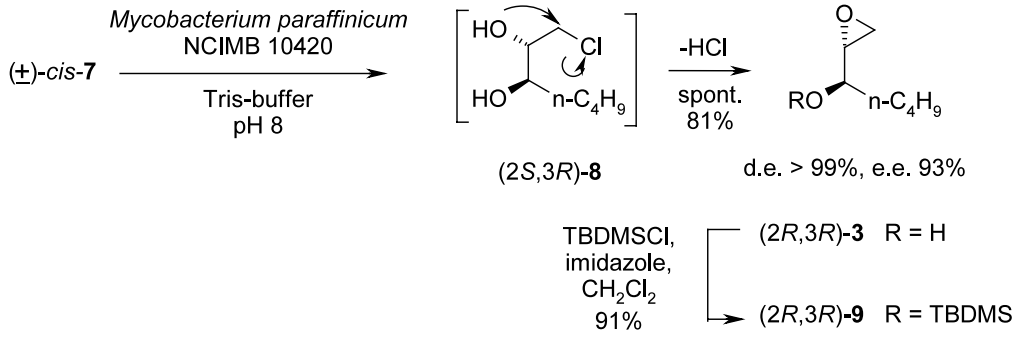
[a] Absolute configuration of intermediate was deduced from that of the product based on the S_N2-type mechanism of ring closure.[2]
[b] Conversion based on non-reacted substrate: c = [sub]–([intermediate]+[product]).
[c] After 115 h.
[d] After 130 h.

Table 1 shows the stereoselectivities [given as conversion (c) and enantiomeric purities of substrate (e.e.$_S$), intermediate (e.e.$_I$) and product (e.e.$_P$)] obtained from the biohydrolysis of (±)-*cis*-**7** using various biocatalysts. In general, (±)-*cis*-**7** was converted with moderate to good selectivities by several bacterial strains with the predominant formation of (2*R*,3*R*)-**3** in up to 93% e.e. and ≥99% d.e. except for *Rhodococcus equi* IFO 3730, which produced the mirror-image counterpart (2*S*,3*S*)-**3** albeit in low optical purity (entry 6). Several datasets of c, e.e.$_S$, e.e.$_I$ and e.e.$_P$ clearly indicated that the transformation does not follow a kinetic resolution pathway, which is most striking for entry 1.

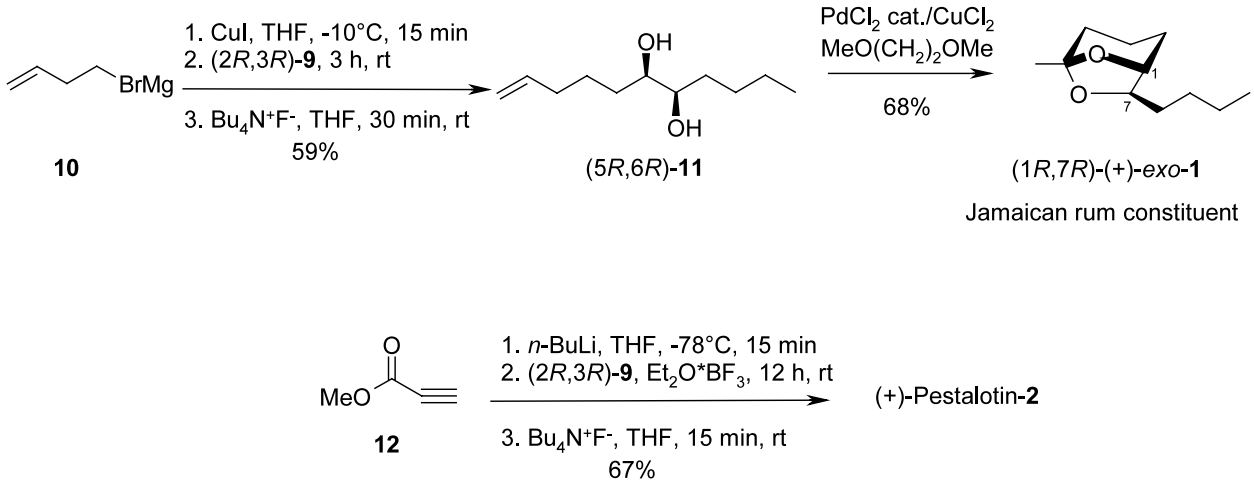
In order to provide sufficient quantities of (2*R*,3*R*)-**3** for the asymmetric syntheses of (+)-*exo*-**1** and (+)-Pestalotin-**2**, *Mycobacterium paraffinicum* NCIMB 10420 (1.7 g) was chosen for the preparative-scale biotransformation. Thus, conversion of substrate (±)-*cis*-**7** (0.9 g) in Tris-buffer (pH 8.0) gave (2*R*,3*R*)-**3** in 81% yield, 99% d.e. and 93% e.e. as the sole product (Scheme 3). Protection of the free hydroxy moiety in substrate (2*R*,3*R*)-**3** with TBDMSCl afforded (2*R*,3*R*)-**9** in 91% yield.

The remaining steps in the asymmetric syntheses of (+)-*exo*-**1** and (+)-Pestalotin-**2** are depicted in Scheme 4. In the case of the rum constituent (+)-*exo*-**1**, (2*R*,3*R*)-**9** was coupled to Grignard-reagent **10**.[11] This was accomplished by treatment of **10** with CuI in THF at −10°C and subsequent addition of (2*R*,3*R*)-**9**, followed by deprotection of the silyl ether using $Bu_4N^+F^-$ to furnish diol (5*R*,6*R*)-**11** in 59% yield. Finally, Wacker oxidation of (5*R*,6*R*)-**11** employing $PdCl_2$ as catalyst, using $CuCl_2$ as re-oxidant, gave (+)-*exo*-**1** in 94% e.e. (16% overall yield). The absolute configuration of epoxide (2*R*,3*R*)-**3** was confirmed by comparison with known specific rotation values.[4] The absolute configuration of (5*R*,6*R*)-**11** was deduced from that of the precursor (2*R*,3*R*)-**3**, since the configuration at carbon centers C(2) and C(3) of (2*R*,3*R*)-**3** are not affected during the coupling reaction. The relative configuration of (+)-*exo*-**1** was determined via NMR-spectroscopy and its absolute configuration was deduced from that of precursor (5*R*,6*R*)-**11**. Thus, (+)-*exo*-**1** was shown to posses (1*R*,7*R*)-configuration.

For the synthesis of (+)-Pestalotin-**2**, methyl propiolate **12** was treated with *n*-BuLi in THF at −78°C and $BF_3{\cdot}Et_2O$ and (2*R*,3*R*)-**9** were added subsequently. Deprotection of the silyl ether using $Bu_4N^+F^-$, which also initiated lactonization, afforded (+)-Pestalotin-**2** in 26% overall yield. The specific rotation of (+)-Pestalotin-**2** was in full accordance with literature data.[12]



Scheme 3. Enantioconvergent enzyme-triggered cascade reaction of chloroalkyl-oxirane (±)-*cis*-**7**.



Scheme 4. Synthesis of (+)-*exo*-**1** and (+)-Pestalotin-**2**.

3. Conclusion

In summary, we have demonstrated that enzyme-triggered cascade reactions represent a valuable tool for the asymmetric synthesis of natural products. Thus, the first total synthesis of (+)-*exo*-7-butyl-5-methyl-6,8-dioxabicyclo[3.2.1]octane (+)-*exo*-**1**, a constituent of Jamaican rum, as well as (+)-Pestalotin-**2** was accomplished. Based on the employment of an enantioconvergent biocatalytic synthetic strategy using bacterial epoxide hydrolases, the occurrence of any undesired stereoisomer was entirely avoided, affording highly efficient syntheses.

4. Experimental

4.1. General remarks

NMR spectra were recorded in $CDCl_3$ using a Bruker AMX 360 at 360 (^{1}H) and 90 (^{13}C) MHz and a Bruker DMX Avance 500 at 500 (^{1}H) and 125 (^{13}C) MHz, respectively. 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), $Ce(SO_4)_2\cdot 4H_2O$(4 g/L) in H_2SO_4 (10%)] (detection I) or by dipping into a $KMnO_4$ reagent [2.5 g/L $KMnO_4$ 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 (25 m, 0.32 mm, 0.25 μm film). H_2 was used as a carrier gas. For programs and retention times vide infra.

High resolution mass spectra were recorded on a double focusing Kratos Profile Mass Spectrometer with electron impact ionization (EI, +70 eV). Optical rotation values were measured on a Perkin–Elmer 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. Compounds **4** and **12** were purchased from Lancaster, Lindlar catalyst and Grignard reagent **10** (0.5 M in THF) from Aldrich. For biotransformations, lyophilized bacterial cells were used. The bacteria were obtained from culture collections and strains were grown as previously described.[13]

4.2. Syntheses of substrates and reference materials

4.2.1. (*Z*)-2-Hepten-1-ol (*Z*)-5. To a solution of alkyne **4** (10 g, 89.3 mmol) in EtOH (70 mL) quinoline (3 mL), a catalytic amount of KOH and Lindlar catalyst (1.1 g) were added, and the resulting mixture was vigorously stirred under H_2 for 9 h at atmospheric pressure. The solids were removed by filtration through a plug of Celite-545 and the solvent was evaporated. Flash chromatography (pentane/MeOAc, 10:1) afforded pure (*Z*)-**5** as a colorless liquid (8.4 g, 83%). R_f (p.e./EtOAc, 1:1) = 0.58, (detection II); spectroscopic data were in full agreement with those previously reported.[4]

4.2.2. (*Z*)-1-Chloro-2-heptene (*Z*)-6. Triphenylphosphine (16 g, 61.0 mmol) and alcohol (*Z*)-**5** (7.4 g, 65 mmol) were dissolved in CCl_4 (50 mL). The reaction was complete after stirring for 12 h at 80°C. The solution was concentrated and pentane (50 mL) was added. The mixture was filtered and the filtrate was concentrated in vacuo. After flash chromatography (p.e.) chloroalkene (*Z*)-**6** (6.3 g, 73%) was isolated as a colorless liquid. R_f (p.e./EtOAc, 10:1) = 0.82 (detection II); spectroscopic data were in full agreement with those previously reported.[4]

4.2.3. *cis*-1-Chloro-2,3-epoxyheptane (±)-*cis*-7. To a vigorously stirred solution of alkene (*Z*)-**6** (4 g, 30.2 mmol) in anhydrous CH_2Cl_2 (300 mL) finely powdered NaH_2PO_4 (ca. 2.5 equiv.) was added. The mixture was stirred for 15 min at rt, it was cooled to 0°C and *m*-CPBA (1.3 equiv.) was added in portions. The mixture was allowed to warm to rt and stirring was continued for an additional 20 h. The suspension was filtered and the resulting solution was treated with 10% aqueous $Na_2S_2O_5$ (100 mL) to destroy excess peracid. The resulting two-phase system was stirred for 30 min, the layers were separated and the organic phase was washed with satd aqueous $NaHCO_3$ (100 mL), dried and evaporated. Kugelrohr distillation gave (±)-*cis*-**7** as a colorless liquid (3.6 g, 85%). R_f (p.e./EtOAc, 10:1) = 0.50, (detection I); $bp_{20\ mbar}$ (Kugelrohr): 95–105°C; NMR and HRMS data matched those previously reported.[4]

4.2.4. (±)-*threo*-1-Chloro-2,3-heptanediol (±)-*threo*-8. To a solution of alkene (*Z*)-**6** (0.8 g, 6 mmol) in anhydrous acetone (25 mL) *N*-methylmorpholine-*N*-oxide (6.5 mmol) and a catalytic amount of OsO_4 were added. After 3 h solid $Na_2S_2O_5$ (10 g) was added and the resulting mixture was stirred for 30 min. Solids were removed by filtration through a plug of Celite-545 and the filtrate was washed with sat. aqueous NH_4Cl (40 mL). The combined organic phases were dried and concentrated. Flash chromatography (p.e./EtOAc, 3:1) gave (±)-*threo*-**8** (0.53 g, 53%). R_f (p.e./EtOAc, 1:1) = 0.46, (detection I); NMR and HRMS data matched those previously reported.[4]

4.2.5. *cis*-1,2-Epoxy-3-heptanol (±)-*cis*-3. Diol (±)-*threo*-**8** (0.15 g, 0.9 mmol) was dissolved in dry THF (10 mL) and NaH (1.2 mmol) was added. After 30 min water (10 mL) was added carefully. The solution was extracted three times with Et_2O (20 mL). The combined organic layers were dried and concentrated. The residue was purified by flash chromatography (p.e./EtOAc, 5:1) to afford (±)-*cis*-**3** as a colorless liquid (0.102 g, 87%). R_f (p.e./EtOAc, 1:1) = 0.58, (detection I); NMR and HRMS data matched those previously reported.[4]

4.2.6. (2*R*,3*R*)-1,2-Epoxy-3-heptanol (2*R*,3*R*)-3 via biotransformation. Lyophilized cells of *M. paraffinicum* NCIMB 10420 (1.7 g) were rehydrated in Tris-buffer (50 mL, pH 8.0, 50 mM) for 1 h and epoxide (±)-*cis*-**7** (0.9 g, 6.06 mmol) was added in one portion. The reaction was monitored by GC on a chiral stationary phase. After shaking the mixture at 30°C for 115 h, the reaction was complete and the product was continuously extracted with CH_2Cl_2 (400 mL) for 24 h. The organic phase was dried and concentrated and the residue was purified by flash chromatography (p.e./EtOAc, 5:1) to give (2*R*,3*R*)-**3** as a colorless liquid (0.64 g, 81%, e.e. = 93%, d.e. ≥99%). $[\alpha]_D^{20}$ −3.2 (*c* 0.85, $CHCl_3$). The specific rotation, NMR and HRMS data are consistent with literature data.[4]

4.2.7. (2*R*,3*R*)-3-*tert*-Butyldimethylsilyloxy-1,2-epoxyheptane (2*R*,3*R*)-9. A solution of alcohol (2*R*,3*R*)-**3** (0.60 g, 4.6 mmol), TBDMSCl (0.9 g, 5.9 mmol) and imidazole (0.4 g, 5.9 mmol) in CH_2Cl_2 (25 mL) was stirred at rt overnight. The reaction was poured into a mixture of satd $NaHCO_3$ and CH_2Cl_2 and stirred vigorously for 30 min. The layers were separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic phases were dried and concentrated. The residue was purified by flash chromatography (p.e.) to afford (2*R*,3*R*)-**9** (1.13 g, 91%). $[\alpha]_D^{20}$ +5.8 (*c* 1.50, $CHCl_3$). R_f (p.e./EtOAc, 3:1) = 0.9, (detection I); ^{1}H NMR (360.13 MHz, $CDCl_3$): δ = 0.08 (3H, s), 0.13 (3H, s), 0.89 (3H, t, J = 7.1), 0.93 (9H, s), 1.24–1.56 (6H, m), 2.57 (1H, dd, J = 2.9, 4.9), 2.79 (1H, t, J = 4.5), 2.91–2.94 (1H, m), 3.27 (1H, q, J = 6.4). ^{13}C NMR (90 MHz, $CDCl_3$): δ = −5.0, −4.3, 14.0, 18.2, 22.8, 25.9, 27.5, 34.5, 44.9, 56.1, 74.7.

4.2.8. (5*R*,6*R*)-10-Undecene-5,6-diol (5*R*,6*R*)-11. To a stirred solution of Grignard-reagent **10** (4.6 mL of a 0.5 M solution in THF, 2.3 mmol) under Ar, CuI (0.022 g, 0.115 mmol) was added at −10°C. After 15 min, a solution of (2*R*,3*R*)-**9** (0.28 g, 1.15 mmol) in THF (5 mL) was added dropwise at −10°C and the reaction was stirred for 3 h at rt. The reaction was quenched by addition of H_2O (20 mL) and Et_2O (30 mL). The phases were separated and the aqueous layer was extracted with Et_2O (2×25 mL). The combined organic phases were dried and evaporated. The residue was dissolved in THF (10 mL) and $Bu_4N^+F^-$ (0.57 g, 1.8 mmol) was added. After 30 min the reaction was quenched with water (10 mL) and Et_2O (20 mL). The phases were separated and the aqueous layer was extracted with Et_2O (2×20 mL). The combined organic phases were dried and evaporated. The residue was purified by flash chromatography (p.e./EtOAc, 2:1) to afford (5*R*,6*R*)-**11** as a colorless oil (0.13 g, 59%). $[\alpha]_D^{20}$ +24.3 (*c* 2.7, $CHCl_3$). R_f (p.e./EtOAc, 1:1) = 0.65, (detection I); ^{1}H NMR (360.13 MHz, $CDCl_3$): δ = 0.89 (3H, t, J = 7.1), 1.32–1.58 (10H, m), 2.06 (2H, m), 2.78 (2H, d, J = 14.2), 3.36 (2H, s), 4.91–5.02 (2H, m), 5.72–5.84 (1H, m). ^{13}C NMR (90 MHz, $CDCl_3$): δ = 14.0, 22.7, 24.9, 27.8, 32.9, 33.2, 33.6, 74.3, 74.5, 114.6, 138.5.

4.2.9. (+)-*exo*-7-Butyl-5-methyl-6,8-dioxabicyclo[3.2.1]-octane (+)-*exo*-1. Diol (5*R*,6*R*)-**11** (0.11 g, 0.59 mmol) was dissolved in anhydrous 1,2-dimethoxyethane (10 mL). The solution was stirred at rt, $PdCl_2$ (0.03 g, 0.17 mmol) and $CuCl_2$ (0.1 g, 0.74 mmol) were added and stirring was continued for 13 h. The brown solution was diluted with H_2O and Et_2O (10 mL each). After phase separation, the aqueous layer was extracted twice with Et_2O (10 mL). The combined organic phases were dried (Na_2SO_4) and concentrated. Flash chromatography (pentane/Et_2O, 5:1) gave pure (+)-*exo*-**1** (0.073 g, 68%, e.e. 93%). $[\alpha]_D^{20}$ +51.8 (*c* 0.65, $CHCl_3$). R_f (p.e./EtOAc, 5:1) = 0.50, (detection I); ^{1}H NMR (500.13 MHz, $CDCl_3$): δ = 0.90 (3H, t, J = 6.9), 1.26–1.37 (6H, m), 1.42 (3H, s), 1.46–1.63 (4H, m), 1.69–1.92 (2H, m), 3.99 (1H, t, J = 6.4), 4.13 (1H, s). ^{13}C NMR (90.56 MHz, $CDCl_3$): δ = 14.0, 17.1, 22.6, 25.1, 27.7, 27.9, 34.9, 35.4, 78.6, 79.8, 107.6. HRMS ($C_{11}H_{20}O_2$): calcd 184,1463 [M$^+$]; found 184.1476 [M$^+$].

4.2.10. (6*R*,1′*R*)-(+)-5,6-Dihydro-6-(1′-hydroxypentyl)-4-methoxy-pyran-2-one (+)-Pestalotin-2. To a stirred solution of methyl propiolate **12** (0.27 g, 3.26 mmol) under Ar in dry THF (10 mL), *n*-BuLi (1.3 mL of a 2.5 M solution in hexane, 3.26 mmol) was added at −78°C. After 15 min, $Et_2O{\cdot}BF_3$ (0.46 g, 3.26 mmol) and a solution of (2*R*,3*R*)-**9** (0.4 g, 1.63 mmol) in THF (5 mL) were added dropwise at −78°C. The reaction was allowed to warm to rt and stirring was continued for further 12 h. The reaction was quenched by addition of H_2O (30 mL) and Et_2O (40 mL). The phases were separated and the aqueous layer was extracted with Et_2O (2×30 mL). The combined organic phases were dried and evaporated. The residue was dissolved in THF (10 mL) and $Bu_4N^+F^-$ (0.73 g, 2.3 mmol) was added. After 15 min the reaction was quenched by addition of water (10 mL) and Et_2O (20 mL). The phases were separated and the aqueous layer was extracted with Et_2O (2×20 mL). The combined organic phases were dried and evaporated. The residue was purified by flash chromatography (p.e./EtOAc, 1:1) to afford (+)-Pestalotin-**2** as white crystals (0.23 g, 67%). $[\alpha]_D^{20}$ +92.5 (*c* 1.3, MeOH). {In literature: $[\alpha]_D^{20}$ +91.1 (*c* 1.34, MeOH)}[12] R_f (p.e./EtOAc, 1:2) = 0.7 (detection I); ^{1}H NMR (360.13 MHz, $CDCl_3$): δ = 0.94 (3H, t, J = 6.7), 1.39–1.49 (4H, m), 1.73–1.80 (2H, m), 2.07 (1H, d, J = 4.7), 3.04 (1H, ddd, J = 18.6, 5.4, 2.5), 3.52 (1H, d, J = 18.6), 3.66 (3H, s), 4.16–4.21 (1H, m), 4.37–4.41 (1H, m), 5.35 (1H, d, J = 1.1).[12] ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.8, 22.5, 27.6, 27.9, 41.0, 50.6, 69.9, 87.4, 90.2, 169.1, 174.3. HRMS ($C_{11}H_{18}O_4$): calcd 214.1205 [M]$^+$; found 214.1214 [M]$^+$.

4.3. General procedure for the screening for biocatalytic activity of (±)-*cis*-7

Epoxide (±)-*cis*-**7** (5 μL) was 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 and GC. After 130 h the cells were removed by centrifugation and products were extracted with EtOAc (2×1 mL). The combined organic layers were dried (Na_2SO_4) and analyzed.

4.4. Chiral analysis

Enantiomeric excesses were analyzed by GC on a chiral stationary phase (Table 2).

4.5. Determination of absolute configuration

The absolute configuration of (+)-Pestalotin-**2** and epoxide (2*R*,3*R*)-**3** was confirmed by comparison with known specific rotation values.[4,12] The determination of absolute configuration of epoxide **7** and diol **8** was performed as previously reported.[4]

The relative configuration of (+)-*exo*-**1** was determined via NMR spectroscopy. The absolute configuration of the latter was deduced from that of precursor (5*R*,6*R*)-**11**, based on a related transformation.[14] The absolute configuration of (5*R*,6*R*)-**11** was deduced from precursor (2*R*,3*R*)-**3**, since the configuration at the C(2) and C(3) centers of (2*R*,3*R*)-**3** remained unchanged during the coupling reaction.

Table 2. GC-retention times on a chiral stationary phase

Compound	Conditions	Retention time [min] (configuration)
(±)-*exo*-**1**	14 psi, 100°C (iso)	5.4 (−)-*exo*, 5.6 (+)-*exo*
(±)-*cis*-**3**	10 psi, 80°C (iso)	14.7 (2*S*,3*S*), 16.9 (2*R*,3*R*)
(±)-*cis*-**7**	10 psi, 80°C (iso)	9.3 (2*S*,3*S*), 9.7 (2*R*,3*R*)
(±)-*threo*-**8**	10 psi, 125°C (iso)	9.3 (2*R*,3*S*), 10.1 (2*S*,3*R*)

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