Advanced Synthesis & Catalysis

Accepted Article

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To be cited as: *Adv. Synth. Catal.* 10.1002/adsc.201801094

Link to VoR: http://dx.doi.org/10.1002/adsc.201801094

10.1002/adsc.201801094

FULL PAPER

DOI: 10.1002/adsc.201((will be filled in by the editorial staff))

Enantioselective Bio-Hydrolysis of Geranyl-Derived *rac*-Epoxides: A Chemoenzymatic Route to *trans*-Furanoid Linalool Oxide

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Received: ((will be filled in by the editorial staff))

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/adsc.201

Abstract. In contrast to many chemical dihydroxylation methods, enzymatic epoxide hydrolysis provides an environmentally benign route to vicinal diols, which are important intermediates in the synthesis of fine chemicals and pharmaceuticals. Using epoxide hydrolases, enantiopure diols are accessible under mild conditions. In order to assess the selectivity of epoxide hydrolases on geraniol-derived oxiranes, a range of derivatives were screened against a large variety of enzyme preparations.
For nearly all substrates, a matching hydrolase with excellent enantioselectivity (≥95% *ee*) could be found. In addition, a chemoenzymatic approach for the stereoselective synthesis of furanoid linalool oxide was developed. Combination of enzymatic enantioselective hydrolysis with stereoselective Tsuji-Trost reaction granted diastereoselective access to *trans*-(2*R*,5*R*)-configured linalool oxide with high diastereomeric and enantiomeric excess (97% *de* and 97% *ee*).

Keywords: Biocatalysis; Epoxide hydrolases; Furanoid linalool oxide; Geraniol epoxide; Tsuji-Trost reaction

Introduction

Biocatalytic reactions often provide an environmentally benign alternative to heavy metal-catalyzed reactions. Especially for dihydroxylation of olefins, green alternatives to hazardous Os-based functionalization are desirable.[1] Alternatively, peroxide-based olefin epoxidation followed by catalytic hydrolysis may be used to generate *vic*-diols.[2] These compounds are versatile intermediates in the synthesis of industrial products, such as chiral drugs and pesticides, while chiral vicinal diols often serve as intermediates in total syntheses.[3]

In recent years, stereoselective hydrolysis of epoxides to yield enantiopure diol products has been thoroughly investigated.[4] Besides recently developed organometallic and organocatalytic methods, enzymes have also received substantial interest[5] and a large number of microbial strains and isolated enzymes have been classified according to their substrate preference.[6] These epoxide hydrolases (EHs) have been used on a variety of substrates with significant success,[7] and excellent regioselectivity, as well as notable stereoselectivity, were typically observed.[8] The broad substrate scope of EHs was further exploited in the total synthesis of marmin,[7b] vitamin E-related chromane-systems[9] and *cis*-gigantrionenin.[10]

In addition to high regio- and stereoselectivities, easy access and straightforward implementation of these biocatalysts is an appealing aspect. Epoxide hydrolase activity of crude cell lyophilisates can often be used directly without prior protein purification, providing a cost-effective and practical catalytic platform for the stereoselective generation of *vic*-diols from corresponding epoxides.

Encouraged by promising results obtained in previous studies,[7b] the potential of epoxide hydrolases for the stereoselective hydrolysis of isoprenoid-based oxiranes bearing various functional groups, such as alcohols, ketones, esters and ethers, was assessed. Several of these compounds constitute juvenile hormones and are used in insect pest control.[11]

Results and Discussion

Our first goal was to assess the effect of substitution of racemic geraniol oxide ethers on enzymatic hydrolysis performance. Ethyl ether *rac*-**1** (Scheme 1)

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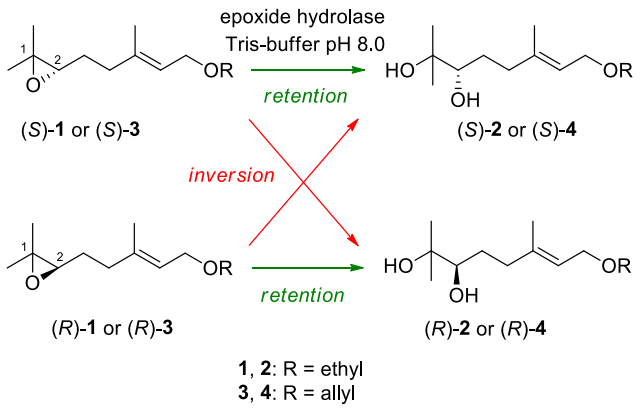


Scheme 1. Epoxide hydrolase (EH) mediated hydrolysis of racemic ethyl geraniol-6,7-oxide ether (**1**) and allyl geraniol-6,7-oxide ether (**3**). Inversion: nucleophilic attack at C2; retention: nucleophilic attack at C1.

was subjected to an array of epoxide hydrolases from different sources and in various forms (lyophilized cells and isolated enzymes, Table S1). Most enzyme preparations showed moderate to good activity after 24 h with varying selectivity (see Table 1). In most cases, the obtained enantiomeric excess of the remaining epoxide (**1**) was higher than that of the product (**2**), providing >99% *ee* for some substrates.

Depending on the type of enzyme and substitution pattern of the substrate, epoxide hydrolases can follow up to four hydrolysis pathways: Hydrolysis of both enantiomers can proceed either *via* retention (nucleophilic attack at 'C1') or inversion (nucleophilic attack at 'C2') of absolute configuration (Scheme 1). Provided the enzyme exhibits different reaction rates for the two enantiomers (i.e. enantiopreference), kinetic resolution may occur. In some cases however, an enantioconvergent process can take place, which is caused by hydrolysis of one enantiomer *via* retention and of the other *via* inversion.[12] Given the possible occurrence of all four pathways simultaneously, calculation of enantioselectivities (E-values) is not possible.[13]

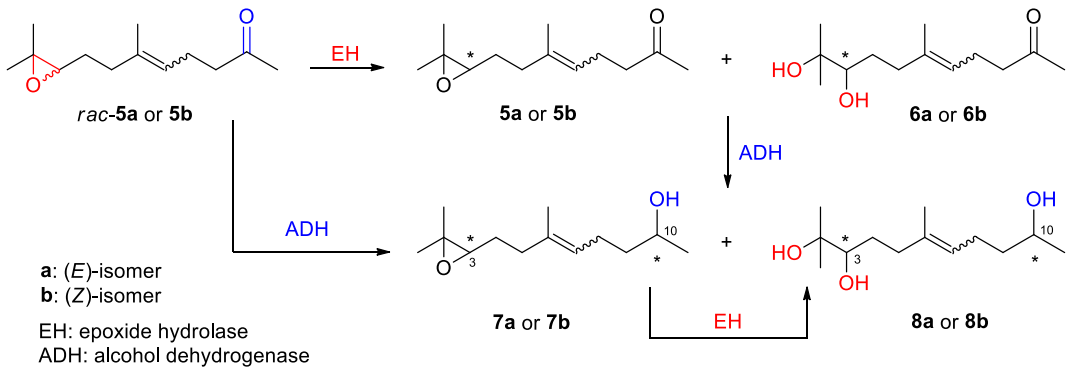
Detailed analysis of the data (conversion, ee_S and ee_P) indeed revealed mixed stereochemical pathways, which were highly strain-dependent. With **1**, apparent kinetic resolution was observed primarily among *Rhodococcus ruber* strains (Table 1, entries 4-7), through dominating inversion (i.e. remaining substrate and product share same absolute configuration), yielding (*R*)-**1** in up to 99% *ee*. Partial enantioconvergent hydrolysis [(*R*)-**1** -> (*R*)-**2** along with (*S*)-**1** -> (*R*)-**2**, Scheme 1] could be identified with E-1 and E-9 as well as isolated epoxide hydrolase E-22 from *Deinococcus radiodurans* (Table 1, entries 1, 8 & 18, almost full conversion along with moderate to good enantiopurity of product **2**, ee_p up to 83%). The absolute configuration of *vic*-diol **2** was determined by independent synthesis of the respective (*S*)-enantiomer by asymmetric Sharpless dihydroxylation using AD-mix-α (see experimental section).[14] For determination of absolute configuration of unreacted epoxide **1**, the latter was treated with aqueous acid to produce the corresponding diol **2** with retention of configuration.[15] Next, the influence of the substrate type on enzyme enantioselectivity was studied with allyl ether *rac*-**3** (Scheme 1 and Table 1). The results generally indicated no significant influence of the allyl moiety compared to the ethyl moiety of *rac*-**1** on enantioselectivity and activity, except in a few cases (Table 1, entries 11 & 17). As with **2**, nearly enantiopure diol **4** (98% *ee*) could be generated using isolated epoxide hydrolase E-23 from *Synechocystis* sp. PCC 6803 (Table 1, entry 19). Notably, a remarkable drop in activity was found using epoxide hydrolase E-21 from *Aspergillus niger*, together with a different selectivity pattern (i.e. overall preference for retention of (*S*)-**1** and inversion of (*R*)-**3**, Table 1, entry 17). E-15 on the other hand displayed opposite stereopreference on **3** but followed the same pathway (apparent inversion) as with **1** (Table 1, entry 11).

Encouraged by selective hydrolysis of geranyl oxide-based ethers, the epoxide of the pheromone geranylacetone (*rac*-**5a**)[16] was subjected to the enzyme library, in parallel with its *Z*-isomer, nerylacetone (*rac*-**5b**). The results of this bioassay revealed dual activity for the lyophilisates. As anticipated with whole-cell preparations, the methyl ketone moiety was readily reduced by endogenous alcohol dehydrogenases (ADHs, Tables 2-3), while some strains and isolated enzymes displayed chemoselectivity for epoxide hydrolysis (Table 4). This resulted in a range of products (Scheme 2) with unknown stereochemistry, rendering elucidation of pathways and absolute configurations challenging. The latter were determined by independent synthesis of enantioenriched **5b** followed by stereoselective reduction of the methyl ketone using Prelog (ADH-A) and anti-Prelog (ADH200) ADHs to give **7b**.[17] Comparison with independently synthesized reference material indicated that *Rhodococcus* strains mainly showed Prelog-type alcohol dehydrogenase activity, while opposite enantiopreference was only observed for E-3 (Table 2, entry 2). In line with previous results, the overall stereoselectivity of epoxide hydrolases was moderate to good (substrate (*S*)-**5a** obtained in 87% *ee* and product (*R*)-**6a** in up to 97% *ee*, Table 4, entries 3 and 12, respectively). Four enzyme preparations showed complete hydrolysis within 24 h (Table 2, entries 1 & 8 and Table 4, entries 5 & 11), while E-3 and E-10, as with **1a** and **3a**, did not show any epoxide hydrolysis activity (Tables 2-3, entries 2 & 9). This simplified assignment of stereochemistry, as *syn*- and *anti*-diastereoisomers of **7a** (and **7b**) were produced in equal amounts as sole products. Since the ADH enantiopreference of E-3 was found opposite of that of E-10, both diastereoisomers were easily recognized.

Table 1. Conversion and selectivity of epoxide hydrolase preparations in biocatalytic hydrolysis of *rac*-**1** and *rac*-**3**.

Entry	Enzyme preparation	Conv. of **1** (%)	**1** ee_s (%)	**2** ee_p (%)	Conv. of **3** (%)	**3** ee_s (%)	**4** ee_p (%)
Lyophilized cells							
1	E-1	87	85 (*R*)	67 (*R*)	>99	n.a.	74 (*R*)
2	E-2	17	37 (*R*)	80 (*R*)	7	5 (*R*)	89 (*R*)
3	E-4	n.d.	n.a.	n.a.	43	58 (*R*)	90 (*R*)
4	E-5	53	96 (*R*)	83 (*R*)	65	89 (*R*)	86 (*R*)
5	E-6	37	84 (*R*)	86 (*R*)	55	80 (*R*)	88 (*R*)
6	E-7	65	68 (*R*)	80 (*R*)	76	38 (*R*)	82 (*R*)
7	E-8	69	>99 (*R*)	67 (*R*)	75	88 (*R*)	62 (*R*)
8	E-9	>99	n.a.	74 (*R*)	>99	n.a.	65 (*R*)
9	E-12	n.d.	n.a.	n.a.	25	64 (*S*)	67 (*S*)
10	E-14	>99	n.a.	10 (*R*)	>99	n.a.	*rac*
11	E-15	94	>99 (*R*)	27 (*R*)	85	67 (*S*)	20 (*S*)
12	E-16	79	>99 (*R*)	*rac*	80	>99 (*R*)	*rac*
13	E-17	61	>99 (*R*)	8 (*S*)	61	>99 (*R*)	30 (*S*)
14	E-18	n.d.	n.a.	n.a.	5	16 (*S*)	*rac*
Isolated enzymes							
15	E-19	17	>99 (*R*)	34 (*S*)	35	93 (*R*)	28 (*S*)
16	E-20	>99	n.a.	5 (*R*)	>99	n.a.	*rac*
17	E-21	33	20 (*R*)	59 (*S*)	8	33 (*S*)	58 (*S*)
18	E-22	>99	n.a.	55 (*R*)	>99	n.a.	57 (*R*)
19	E-23	18	77 (*R*)	95 (*R*)	22	40 (*R*)	98 (*R*)

Standard conditions: Incubation of 5 µL substrate and 50 mg epoxide hydrolase preparation in Tris-HCl buffer (1 mL, 50 mM, pH 8.0) at 30 °C for 24 h; Origin of all epoxide hydrolases can be found in the Supporting Information (Table S1); n.d. not determined; n.a. not applicable; no conversion with E-3, E-10 and E-11.



Scheme 2. Enzymatic hydrolysis (red) and concomitant ADH-catalyzed carbonyl reduction (blue) of *rac*-geranylacetone 9,10-oxide (*rac*-**5a**) and *rac*-nerylacetone 9,10-oxide (*rac*-**5b**) by epoxide hydrolase preparations (numbering of C-atoms 3 and 10 only for labelling purpose, see Tables 2-3).

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Table 2. Conversion of *rac*-**5a** with epoxide hydrolase preparations containing ADH[a] activity and product distribution.

Entry	Enzyme Preparation	**5a** Conv. (%)	*ee* (%)	**6a** *ee* (%)	*de* (%)	**7a** *ee* (%)[b] (3*R*,10*S*)	*ee* (%)[c] (3*S*,10*S*)	*de* (%)	**8a** *ee* (%)[b] (3*R*,10*S*)	*ee* (%)[c] (3*R*,10*R*)	**5a/6a/7a/8a** distribution (%)
1	E-1	>99	n.a.	66 (*R*)	n.a.	n.a	n.a.	60	63	61	0:20:0:80
2	E-3	54	*rac*	n.a.	0	66[d]	51[e]	n.a.	n.a.	n.a.	46:0:54:0
3	E-4	89	83 (*R*)	87 (*R*)	74	83	87	89	87	65	11:13:39:37
4	E-5	93	88 (*R*)	87 (*R*)	85	94	73	61	86	88	7:21:26:46
5	E-6	89	75 (*R*)	84 (*R*)	72	94	41	79	84	84	11:21:29:39
6	E-7	78	20 (*R*)	81 (*R*)	20	81	26	88	84	73	22:52:10:16
7	E-8	93	96 (*R*)	80 (*R*)	94	98	82	56	76	76	7:17:31:45
8	E-9	>99	n.a.	83 (*R*)	n.a.	n.a.	n.a.	58	77	81	0:23:0:76
9	E-10	39	*rac*	n.a.	3	69	77	n.a.	n.a.	n.a.	61:0:39:0

Standard conditions: 5 µL substrate and 50 mg epoxide hydrolase were incubated in Tris-HCl buffer (1 mL, 50 mM, pH 8.0) at 30 °C for 24 h; [a] alcohol dehydrogenase selectivity was determined by comparison with authentic material obtained with Prelog (*S*-selective) ADH-A and anti-Prelog (*R*-selective) ADH200; [b] major enantiomer; [c] minor enantiomer; [d] (3*S*,10*R*); [e] (3*R*,10*R*); n.a.: not applicable.

Table 3. Conversion of *rac*-**5b** with epoxide hydrolase preparations containing ADH[a] activity and product distribution.

Entry	Enzyme Preparation	**5b** Conv. (%)	*ee* (%)	**6b** *ee* (%)	*de* (%)	**7b** *ee* (%)[b] (3*R*,10*S*)	*ee* (%)[c] (3*S*,10*S*)	*de* (%)	**8b** *ee* (%)[b] (3*R*,10*S*)	*ee* (%)[c] (3*R*,10*R*)	**6b/7b/8b** distribution (%)
1	E-1	58	*rac*	45 (*R*)	6	41	24	40	77	72	42:11:41:10
2	E-3	71	*rac*	n.a.	0	43[d]	57[e]	n.a.	n.a.	n.a.	29:0:71:0
3	E-4	24	*rac*	49 (*R*)	9	87	87	33	73	94	76:7:11:6
4	E-5	74	4 (*R*)	33 (*R*)	5	76	85	47	63	94	26:4:55:15
5	E-6	71	*rac*	44 (*R*)	2	85	92	27	79	97	29:3:57:11
6	E-7	40	3 (*R*)	61 (*R*)	4	79	88	14	70	96	60:10:23:7
7	E-8	80	93 (*R*)	70 (*R*)	83	66	94	63	44	65	20:40:24:16
8	E-9	89	5	60 (*R*)	10	11	35	56	71	64	11:8:49:32
9	E-10	10	*rac*	n.a.	0	83	83	n.a.	n.a.	n.a.	90:0:10:0

Standard conditions: 5 µL substrate and 50 mg epoxide hydrolase were incubated in Tris-HCl buffer (1 mL, 50 mM, pH 8.0) at 30 °C for 24 h; [a] alcohol dehydrogenase selectivity was determined by comparison with authentic material obtained with Prelog (*S*-selective) ADH-A and anti-Prelog (*R*-selective) ADH200; [b] major enantiomer; [c] minor enantiomer; [d] (3*S*,10*R*); [e] (3*R*,10*R*); n.a.: not applicable.

Table 4. Conversion of *rac*-**5a** and **5b** with epoxide hydrolase preparations displaying no ADH activity.

Entry	Enzyme preparation	Conv. of **5a** (%)	**5a** *ee* (%)	**6a** *ee* (%)	Conv. of **5b** (%)	**5b** *ee* (%)	**6b** *ee* (%)
Lyophilized cells							
1	E-2	36	41 (*R*)	79 (*R*)	18	11 (*R*)	68 (*R*)
2	E-11	23	55 (*S*)	83 (*R*)	46	75 (*S*)	84 (*R*)
3	E-12	38	87 (*S*)	88 (*S*)	26	50 (*S*)	20 (*S*)
4	E-13	17	19 (*S*)	60 (*S*)	27	40 (*S*)	10 (*S*)
5	E-14	>99	n.a.	10 (*S*)	>99	n.a.	*rac*
6	E-15	98	n.a.	24 (*S*)	43	10 (*S*)	32 (*R*)
7	E-16	87	70 (*R*)	*rac*	60	73 (*R*)	20 (*S*)
8	E-17	71	70 (*R*)	*rac*	46	35 (*R*)	20 (*S*)
Isolated enzymes							
9	E-19	33	86 (*R*)	21 (*S*)	5	n.d.	n.d.
10	E-20	88	n.d.	*rac*	>99	n.a.	13 (*R*)
11	E-22	>99	n.a.	50 (*R*)	>99	n.a.	30 (*R*)
12	E-23	23	50 (*R*)	97 (*R*)	8	6 (*S*)	78 (*R*)

Standard conditions: 5 µL substrate and 50 mg epoxide hydrolase were incubated in Tris-HCl buffer (1 mL, 50 mM, pH 8.0) at 30 °C for 24 h; n.a.: not applicable; n.d.: not determined.

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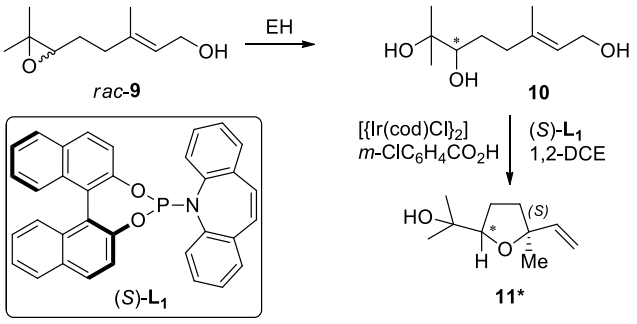
A notable finding is the stereochemistry of the minor diastereoisomer of **8a** (Tables 2-3). Two pathways are possible: **5a**->**6a**->**8a** (EH->ADH) or **5a**->**7a**->**8a** (ADH->EH). In the latter, the carbonyl is first reduced to the (*S*)-alcohol (except with E-3), further leading to formation of (3*R*,10*S*)-**8a**. Thus the presence of (3*R*,10*R*)-**8a** in the final product mixture indicates that the ADH selectivity in the first pathway is influenced by the presence of hydrolyzed epoxide.

In general, lower activities and selectivities were observed using nerylacetone (**5b**) (Tables 3-4). Diol **6b** and epoxide **5b** were obtained in 70% and 93% *ee* (max.), respectively. In some cases, the total ADH activity (formation of **7a** and **8a**) present in the lyophilisates was much lower with **5b** compared to **5a** (Table 3, entries 1, 3, 7 & 9). Combination of highest conversion and highest enantiopurity of both substrate **5b** and product was obtained through overall retaining EH activity from E-11 (Table 4, entry 2).

In analogy to EH-mediated total synthesis of marmin,[7b] a new route to furanoid natural product linalool oxide (**11**) – found in many essential oils and commercial perfumes – was proposed (Scheme 3). This route is based on a 5-*exo*-*trig* cyclization of triol **10** to form the furan ring. Based on the work of Carreira *et al.*,[18] an intramolecular enantioselective iridium-catalyzed allylic etherification of the secondary alcohol onto the allylic moiety was envisioned. The required vicinal diol **10** is available by enzymatic hydrolysis of the parent geraniol epoxide **9**, similarly to **1a**, **3a** and **5a**.



Scheme 3. Envisaged epoxide hydrolase (EH)-mediated hydrolysis of geraniol-6,7-oxide (*rac*-**9**)/iridium-catalyzed cycloetherification sequence.

In order to identify the most enantioselective enzymatic conversion, geraniol-6,7-oxide (*rac*-**9**) was subjected to the library of EHs (Table 5). Most lyophilized cell preparations catalyzed an apparent inversion process (except for E-13 and E-17, entries 11&15) with moderate enantioselectivity (max. E = 26, Table 5, entry 9), while high enantioselectivity was observed using EH of *Deinococcus radiodurans* (E-22), which catalyzed kinetic resolution of *rac*-**9** *via* inversion [(*R*)-**9** in >99% *ee* and (*R*)-**10** in 97% *ee*, E >200]. Remaining (*R*)-**9** was then subjected to acid-catalyzed hydrolysis with retention of configuration,

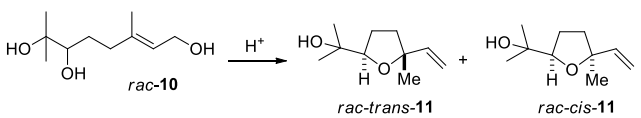
Table 5. Conversion[a] and selectivity in biocatalytic hydrolysis of *rac*-**9** to **10**.

Entry	Enzyme	Conv. (%)	ee_S (%)	ee_P (%)	E[c]
Lyophilized cells					
1	E-1	25	27 (*R*)	80 (*R*)	12
2	E-2	n.d. [b]			
3	E-4	34	45 (*R*)	87 (*R*)	22
4	E-5	34	43 (*R*)	85 (*R*)	19
5	E-6	35	47 (*R*)	87 (*R*)	23
6	E-7	24	20 (*R*)	63 (*R*)	5
7	E-8	42	59 (*R*)	82 (*R*)	18
8	E-9	34	42 (*R*)	80 (*R*)	14
9	E-11	36	50 (*S*)	88 (*R*)	26
10	E-12	24	24 (*S*)	77 (*S*)	10
11	E-13	3	1 (*R*)	34 (*S*)	2
12	E-14	>99	n.a.	45 (*R*)	n.a.
13	E-15	>99	n.a.	42 (*R*)	n.a.
14	E-16	>99	n.a.	43 (*R*)	n.a.
15	E-17	56	49 (*S*)	38 (*R*)	3
Isolated enzymes					
16	E-19	61	43 (*R*)	27 (*R*)	3
17	E-20	>99	n.a.	24 (*R*)	n.a.
18	E-22	51	>99 (*R*)	97 (*R*)	>200
19	E-23	18	21 (*R*)	93 (*R*)	34

Standard conditions: 5 µL substrate and 50 mg epoxide hydrolase were incubated in Tris-HCl buffer (1 mL, 50 mM, pH 8.0) at 30 °C for 24 h; [a] Conversion calculated by $[ee_s/(ee_s+ee_p)]$;[19] [b] Instead of product **10**, several other products were obtained, including all isomers of linalool oxide; c) apparent E-value[19] for whole-cell preparations; n.a.: not applicable; n.d.: not determined.

resulting in a fully enantioconvergent process.

Enantiopure (*R*)-**10** could finally be used in the enantioselective iridium-catalyzed ring closure reaction towards **11**. Unfortunately, the catalytic complex had little stereocontrol on the diastereoselectivity of the formation of cyclic ether **11** (data not shown). The lack of selectivity might be explained by the ease of cyclization following acid-mediated activation of the allylic alcohol in **10**. With this in mind, a short study on the effect of acid and solvent was conducted in order to achieve diastereoselective ring closure (Scheme 4). Results from acid-mediated cycloetherification test reactions (Table 6) indicate an intrinsic preference for the *trans*-product, although both solvent polarity and catalyst acidity influenced the *trans/cis* ratio.



Scheme 4. Acid-catalyzed cycloetherification of *rac*-**10** to *trans*- and *cis*-furanoid linalool oxide (**11**).

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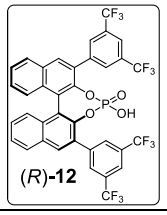
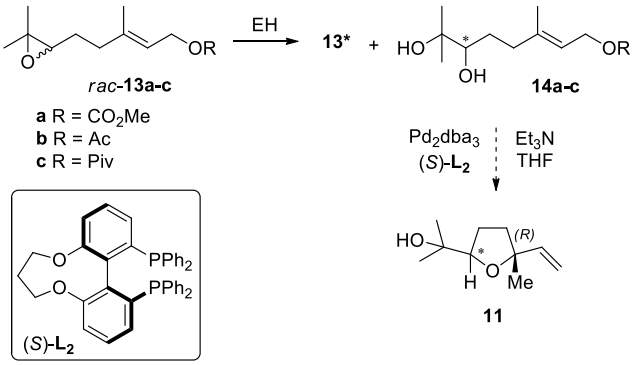
Table 6. Diastereoselectivity of acid-mediated cycloetherification of *rac*-**10** [a].

Entry	Solvent	Acid	pKa	d.r.[b]
1	DMSO-d_6	TFA	3.45[20]	no conv.
2	CCl_4	TFA	3.45	1.2:1
3	Acetone-d_6	TFA	3.45	2.2:1
4	$CDCl_3$	TFA	3.45	2.8:1
5	$CDCl_3$	 (*R*)-**12**	2.42-2.63[21]	4.0:1
6	Toluene-d_8			6.1:1

[a] All reactions performed in an NMR tube with excess of acid. [b] Based on *trans*-/*cis*-ratio as determined by ^{1}H NMR analysis.



Scheme 5. Epoxide hydrolase (EH)-mediated hydrolysis of geraniol-6,7-oxide derivatives **13a-c** with subsequent Tsuji-Trost allylic substitution.

Due to unsatisfactory results with the iridium-based catalyst, the strategy was altered to a Tsuji-Trost based approach,[22] in which biocatalysis could advantageously be combined with transition metal catalysis to obtain **11** stereoselectively (Scheme 5).

In order to identify the right allylic substitution for both the biocatalytic step and the cycloetherification, three leaving groups that have been already used in intramolecular Tsuji-Trost reactions[22-23] were investigated (methyl carbonate, acetate, and pivalate). First, the stability of the activation groups was tested in aqueous buffer, required for epoxide hydrolysis. Carbonate (**13a**) was largely hydrolyzed (pH 8) after 24 h at 30 °C. In contrast, acetate **13b** and pivalate **13c** derivatives were hardly affected by the buffer. Owing to the likely presence of active endogeneous lipases in whole cell preparations, **13c** was selected for further studies since pivalates are much poorer substrates for lipases compared to acetates.[24] Based on previous results with **1**, **3**, **5a-b** and **9**, a selection of epoxide hydrolases was tested (Table 7).

The most notable result was the high selectivity of *Rhodococcus* lyophilisates (E-1 and E-4-9) on **13c** (Table 7, entries 1-7). In some cases, full kinetic resolution (E >200) *via* inversion was achieved, yielding (*R*)-**14c** with *ee* up to 96% (Table 7, entries 2, 4 & 17). Remarkably, E-1 and E-9 led to almost full conversion in combination with high ee_P values, indicating a stereoconvergent process. E-4 (*Rhodococcus ruber* strain DSM44539) was selected for further optimization, with regard to enzyme loading, buffer type, and pH (Table 8). Reducing the enzyme preparation loading by 50% (final 25 mg/mL) still afforded nearly full kinetic resolution in 24 h. Buffer type and pH had only moderate influence on conversion and *ee* values.

Table 7. Conversion and selectivity in biocatalytic hydrolysis of *rac*-**13c** to **14c**.

Entry	Enzyme	Conv.[a] (%)	ee_S (%)	ee_P (%)
Lyophilized cells				
1	E-1	97	>99 (*R*)	83 (*R*)
2	E-4	51	>99 (*R*)	96 (*R*)
3	E-5	64	>99 (*R*)	89 (*R*)
4	E-6	50	>99 (*R*)	96 (*R*)
5	E-7	50	87 (*R*)	93 (*R*)
6	E-8	68	88 (*R*)	78 (*R*)
7	E-9	>99	n.a.	90 (*R*)
8	E-11	64	39 (*S*)	95 (*R*)
9	E-12	90	>99 (*S*)	63 (*S*)
10	E-13	20	7 (*S*)	85 (*S*)
11	E-14	>99	n.a.	17 (*R*)
12	E-15	92	>99 (*S*)	7 (*R*)
13	E-16	89	>99 (*S*)	18 (*S*)
14	E-17	72	53 (*S*)	35 (*S*)
Isolated enzymes				
15	E-19	89	92 (*S*)	35 (*S*)
16	E-20	65	>99 (*R*)	30 (*S*)
17	E-22	57	>99 (*R*)	95 (*R*)
18	E-23	52	62 (*R*)	96 (*R*)

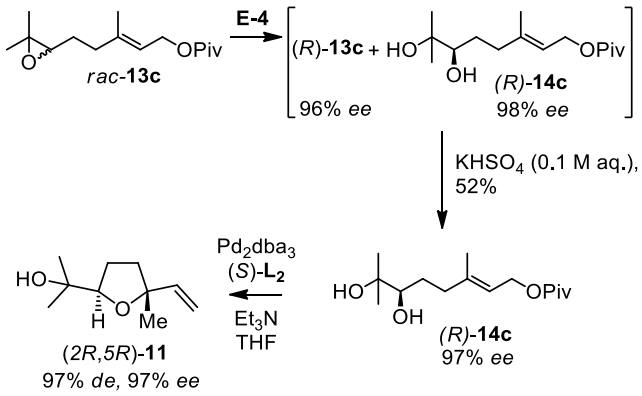
Standard conditions: 5 µL substrate and 50 mg epoxide hydrolase were incubated in Tris-HCl buffer (1 mL, 50 mM, pH 8.0) at 30 °C for 24 h; [a] based on GC integrals.

Table 8. Conversion and selectivity of E-4 mediated hydrolysis of *rac*-**13c** using various buffers and enzyme loadings.

Entry	Loading (mg/mL)	Buffer type[a]	pH	Conv. (%)	ee_S (%)	ee_P (%)
1	25	Tris·HCl	7.0	41	77	97
2	25	Tris·HCl	8.0	43	83	98
3	25	Phosphate	7.0	46	92	98
4	25	Phosphate	8.0	45	93	98
5	5	Tris·HCl	7.0	20	20	98
6	5	Tris·HCl	8.0	22	25	98
7	5	Phosphate	7.0	28	32	98
8	5	Phosphate	8.0	24	27	99
9	1	Tris·HCl	7.0	25	4	95
10	1	Tris·HCl	8.0	24	1	94
11	1	Phosphate	7.0	38	3	97
12	1	Phosphate	8.0	29	7	98

Standard conditions: 5 µL substrate and E-4 were incubated in 1 mL buffer at 30 °C for 24 h; [a] 50 mM Tris or 100 mM potassium phosphate.

Preparative scale hydrolysis of **13c** in a stereoconvergent fashion using E-4 followed by acid hydrolysis was performed (Scheme 6). Once enzymatic kinetic resolution was complete (high *ee* values for both substrate and product), the product mixture was extracted and treated with aqueous acid. This treatment resulted in full hydrolysis to the diol and yielded (*R*)-**14c** in high enantiopurity (97% *ee*). Subsequent palladium-catalyzed ring closure using *(S)*-C_3-TunePhos (**L_2**) as the ligand proceeded smoothly to give the desired *trans*-configured linalool oxide **11** with high diastereomeric and enantiomeric excess (75% yield, 97% *de* and 97% *ee*) in 39% overall yield starting from *rac*-**13c**.



Scheme 6. E-4-mediated hydrolysis of geraniol-6,7-oxide derivative **13c** followed by acid-catalyzed hydrolysis and subsequent Tsuji-Trost allylic substitution.

Conclusion

Selective hydrolysis of terpenoid geranyl-derived epoxides was established using epoxide hydrolases, allowing simple access to enantiopure vicinal diols and oxiranes. Highest enantioselectivities were obtained with epoxides derived from geranyl acetone and geraniol esters. Furthermore, a novel route to optically pure linalool oxide was developed by combining enantioselective biocatalytic epoxide hydrolysis with diastereoselective Tsuji-Trost cycloetherification, affording diastereoselective chemoenzymatic access to (2*R*,5*R*)-linalool oxide.

Experimental Section

General

Starting materials were purchased from Sigma Aldrich and were used without further treatment. Unless stated otherwise, solvents were purchased from VWR Chemicals or Biosolve and were used as such. Cyclohexane (cHex) was purified by distillation before use. Celite 512 medium was purchased from Sigma Aldrich. Column chromatography was performed on Silica-P Flash Silica Gel (40-63 µm, pore diameter 60 Å) from Silicycle. TLC was performed using TLC plates F254 (silica gel 60 on aluminium) from Merck Serono KGaA (Darmstadt) and compounds were visualized by UV detection (254 or 366 nm) and stained with basic aq. $KMnO_4$ or cerium-molybdate stain.

^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 500 (500.23 MHz for ^1H and 125.78 MHz for ^{13}C), a Bruker Avance 400 (400.13 MHz for ^1H and 100.62 MHz for ^{13}C) or a Bruker AMX 360 (360.17 MHz for ^1H and 90.56 MHz for ^{13}C) in $CDCl_3$ using the residual solvent as internal standard ($CDCl_3$: δ = 7.26 for ^1H NMR and δ = 77.16 for ^{13}C NMR). Chemical shifts (δ) are given in ppm and coupling constants (*J*) in Hz. Resonances are described as s (singlet), d (doublet), t (triplet), q (quartet), bs (broad singlet) and m (multiplet) or combinations thereof. HSQC-NMR spectra were used for the assignment of the proton and APT-NMR spectra for carbon signals. NMR data were processed using MestReNova.

Chiral GC-FID measurements were carried out on a Shimadzu GC-2010 Plus equipped with an FID detector and a AOC-20i auto injector using a Chirasil Chiraldex Dex-CB (25 m x 0.32 mm, 0.25 µm film) column. Chiral HPLC analysis was performed using a JASCO-System equipped with a multiwavelength-detector (MD-910) and a temperature-chamber (AS-950).

Epoxide hydrolases were obtained from bacterial, yeast and fungal strains, FCC stands for in-house 'Fab-Crew-Collection' (see Supporting Information). ADH200 was from Evocatal (evo-1.1.200).

Standard Procedure for Epoxidation of Olefins[12a,25]

Finely powdered Na_2HPO_4 (2.6 eq.) was added to a vigorously stirred solution of alkene (5.8 mmol) in CH_2Cl_2 (50 mL). The mixture was stirred for 15 min at room temperature and was then cooled to 0 °C. Then *m*-CPBA (1 eq.) was added slowly. The mixture was allowed to warm to room temperature and the reaction was monitored with TLC. After the reaction was complete, the white suspension was filtered. The resulting solution was treated with $Na_2S_2O_5$ (10% aq., 15 mL) to destroy excess of peracid. The two-phase system was stirred for 30 min, the layers were separated, and the organic phase was washed twice with $NaHCO_3$ (sat. aq.). The organic phase was dried over Na_2SO_4 and evaporated. The crude product was purified by flash column chromatography.

rac-(*E*)-3-(5-Ethoxy-3-methylpent-3-en-1-yl)-2,2-dimethyloxirane (*rac*-1).

Flash column chromatography (*c*-Hex/EtOAc, 6:1) provided *rac*-**1** as a colorless liquid (0.69 g, 60%).

^1H NMR (500 MHz, $CDCl_3$) δ 5.39 (tq, *J* = 6.7, 1.3 Hz, 1H), 3.97 (d, *J* = 6.7 Hz, 2H), 3.47 (q, *J* = 7.0 Hz, 2H), 2.71 (t, *J* = 6.2 Hz, 1H), 2.25-2.08 (m, 2H), 1.68 (s, 3H), 1.67-1.58 (m, 2H), 1.30 (s, 3H), 1.25 (s, 3H), 1.21 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 139.0, 121.7, 67.2, 65.7, 64.2, 58.5, 36.3, 27.3, 25.0, 18.9, 16.6, 15.4 ppm.

rac-(*E*)-3-(5-(Allyloxy)-3-methylpent-3-en-1-yl)-2,2-dimethyloxirane (*rac*-3).

Flash column chromatography (*c*-Hex/EtOAc, 6:1) provided *rac*-**3** as a colorless liquid (0.67 g, 55%).

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.92 (ddt, *J* = 17.2, 10.3, 5.7 Hz, 1H), 5.40 (ddp, *J* = 6.7, 5.4, 1.4 Hz, 1H), 5.31-5.10 (m, 2H), 4.04-3.89 (m, 4H), 2.70 (t, *J* = 6.3 Hz, 1H), 2.29-2.06 (m, 2H), 1.68 (s, 3H), 1.67-1.62 (m, 2H), 1.29 (s, 3H), 1.25 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 139.4, 135.1, 121.5, 117.2, 71.3, 66.6, 64.1, 58.5, 36.3, 27.3, 25.0, 18.9, 16.7 ppm.

***rac*-(*E*)-8-(3,3-Dimethyloxiran-2-yl)-6-methyloct-5-en-2-one (*rac*-5a)[25]**

Flash column chromatography (*c*-Hex/EtOAc, 3:1) provided *rac*-**5a** as a colorless liquid (0.86 g, 71%).
^{1}H NMR (300 MHz, $CDCl_3$): δ 5.16 (t, *J* = 7.2 Hz, 1H), 2.70 (t, *J* = 6.2 Hz, 1H), 2.48 (t, *J* = 7.4 Hz, 2H), 2.39-2.22 (m, 2H), 2.15 (s, 3H), 2.21-2.05 (m, 2H), 1.68-1.58 (m, 2H), 1.65 (s, 3H), 1.32 (s, 3H), 1.27 (s, 3H).
NMR data in accordance with literature.[25]

***rac*-(*Z*)-8-(3,3-Dimethyloxiran-2-yl)-6-methyloct-5-en-2-one (*rac*-5b)[25]**

Flash column chromatography (*c*-Hex/EtOAc, 3:1) provided *rac*-**5b** as a colorless liquid (0.83 g, 68%).
^{1}H NMR (300 MHz, $CDCl_3$) δ 5.08 (t, *J* = 6.9 Hz, 1H), 2.67 (t, *J* = 6.3 Hz, 1H), 2.42 (t, *J* = 7.3 Hz, 2H), 2.23 (q, *J* = 7.3 Hz, 2H), 2.17-2.11 (m, 2H), 2.09 (s, 3H), 1.65 (d, *J* = 1.2 Hz, 3H), 1.62-1.52 (m, 2H), 1.27 (s, 3H), 1.23 (s, 3H).
NMR data in accordance with literature.[25]

***rac*-(*E*)-5-(3,3-Dimethyloxiran-2-yl)-3-methylpent-2-en-1-ol (*rac*-9)**

(*E*)-5-(3,3-dimethyloxiran-2-yl)-3-methylpent-2-en-1-ol (20 mmol) was dissolved in CH_2Cl_2 (100 mL). The mixture was then cooled to 0 °C and *m*-CPBA (1.1 eq.) was added. The mixture was allowed to warm to room temperature and the reaction was monitored with TLC. After the reaction was complete Na_2SO_4 was added to dry the reaction mixture, followed by addition of Na_2CO_3. After filtration the product was purified with flash column chromatography (*c*-Hex/EtOAc, 2:1), which provided *rac*-**9a** as a colorless liquid (0.89 g, 5.2 mmol, 28%).
^{1}H NMR (500 MHz, $CDCl_3$) δ 5.44 (tdt, *J* = 5.5, 2.7, 1.4 Hz, 1H), 4.15 (d, *J* = 6.8 Hz, 2H), 2.71 (t, *J* = 6.2 Hz, 1H), 2.16 (ddt, *J* = 29.8, 14.5, 7.3 Hz, 2H), 1.69 (s, 3H), 1.65 (ddd, *J* = 8.7, 4.7, 1.6 Hz, 2H), 1.30 (s, 3H), 1.25 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.78, 124.06, 64.17, 59.41, 58.54, 36.37, 27.27, 24.99, 18.88, 16.41 ppm.
NMR data in accordance with literature.[26]

***rac*-(*E*)-5-(3,3-Dimethyloxiran-2-yl)-3-methylpent-2-en-1-yl pivalate (*rac*-13c)**

(*E*)-3,7-dimethylocta-2,6-dien-1-yl pivalate (7.1 mmol) was dissolved in CH_2Cl_2 (40 mL). The mixture was then cooled to 0 °C and *m*-CPBA (1 eq.) was added. The mixture was allowed to warm to room temperature and the reaction was monitored with TLC. After the reaction was complete Na_2SO_4 was added to dry the reaction mixture, followed by addition of $NaHCO_3$. After filtration the product was purified with flash column chromatography (*c*-Hex/EtOAc, 19:1), which provided *rac*-**13c** as a colorless liquid (1.45 g, 5.7 mmol, 80%).
^{1}H NMR (500 MHz, $CDCl_3$) δ 5.37-5.33 (m, 1H), 4.56 (d, *J* = 6.8 Hz, 2H), 2.69 (td, *J* = 6.2, 1.6 Hz, 1H), 2.17 (ddt, *J* = 29.9, 14.5, 7.4 Hz, 2H), 1.71 (s, 3H), 1.65 (pd, *J* = 8.0, 7.1, 1.5 Hz, 2H), 1.29 (d, *J* = 1.7 Hz, 3H), 1.25 (d, *J* = 1.9 Hz, 3H), 1.18 (d, *J* = 2.0 Hz, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.69, 140.77, 119.46, 64.02, 61.33, 58.52, 38.85, 36.27, 27.33, 27.21, 24.99, 18.88, 16.61 ppm.
NMR data in accordance with literature.[27]

Reference material synthesis

Standard Procedure for acid catalyzed hydrolysis of epoxides[7b]

Epoxide (1 mmol) was hydrolyzed in a mixture of water (6 mL) and THF (6 mL) under acidic conditions (6 N H_2SO_4, 5 drops). The solution was extracted twice with EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by flash column chromatography. Complete conversion was obtained for substrates **2, 4, 6a** and **6b**.

***rac*-(*E*)-8-Ethoxy-2,6-dimethyloct-6-ene-2,3-diol (*rac*-2).**

Flash column chromatography (*c*-Hex/EtOAc, 1:1) provided *rac*-**2** (173 mg, 0.80 mmol, 80%) as a colorless viscous oil.
^{1}H NMR (500 MHz, $CDCl_3$): δ 5.41 (tq, *J* = 6.7, 1.3 Hz, 1H), 4.03-3.90 (m, 2H), 3.48 (q, *J* = 7.0 Hz, 2H), 3.34 (dd, *J* = 10.6, 1.9 Hz, 1H), 2.38-2.22 (m, 1H), 2.16-1.99 (m, 3H), 1.68 (s, 3H), 1.61 (dddd, *J* = 13.7, 9.8, 6.5, 2.0 Hz, 1H), 1.48-1.42 (m, 1H), 1.23-1.19 (m, 6H), 1.15 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 139.8, 121.5, 78.3, 73.2, 67.2, 65.8, 36.8, 29.6, 26.6, 23.3, 16.4, 15.4 ppm.

***rac*-(*E*)-8-(Allyloxy)-2,6-dimethyloct-6-ene-2,3-diol (*rac*-4).**

Flash column chromatography (*c*-Hex/EtOAc, 1:1) provided *rac*-**4** as a colorless viscous oil.
^{1}H NMR (500 MHz, $CDCl_3$): δ 5.96 (ddt, *J* = 17.3, 10.4, 5.8 Hz, 1H), 5.45 (tq, *J* = 6.7, 1.3 Hz, 1H), 5.36-5.16 (m, 2H), 4.05-3.98 (m, 4H), 3.39 (dd, *J* = 10.6, 1.9 Hz, 1H), 2.38-2.30 (m, 1H), 2.14 (ddd, *J* = 14.8, 9.6, 6.7 Hz, 1H), 1.72 (s, 3H), 1.65 (dddd, *J* = 13.7, 9.8, 6.5, 2.0 Hz, 1H), 1.48 (dddd, *J* = 13.7, 10.5, 9.4, 5.2 Hz, 1H), 1.23 (s, 3H), 1.19 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 140.2, 135.0, 121.3, 117.3, 78.3, 73.1, 71.3, 66.6, 36.8, 29.6, 26.6, 23.3, 16.7 ppm.

***rac*-(*E*)-9,10-Dihydroxy-6,10-dimethylundec-5-en-2-one (*rac*-6a)**

Flash column chromatography with gradient eluent (EtOAc (0% → 50%) in *c*-Hex) provided *rac*-**6a** as a colorless viscous oil.
^{1}H NMR (500 MHz, $CDCl_3$): δ 5.16 (t, *J* = 6.9 Hz, 1H), 3.33 (dd, *J* = 10.4, 1.7 Hz, 1H), 2.47 (t, *J* = 7.1 Hz, 2H) 2.29-2.16 (m, 2H), 2.14 (s, 3H), 2.10-2.03 (m, 2H), 1.63 (s, 3H), 1.61-1.55 (m, 1H), 1.44-1.36 (m, 1H), 1.20 (s, 3H), 1.16 (s, 3H); ^{13}C NMR (90 MHz, $CDCl_3$): 209.0, 136.4, 123.5, 78.3, 73.1, 43.8, 36.9, 30.1, 29.7, 26.6, 23.4, 22.6, 16.1.
NMR data in accordance with literature.[25]

***rac*-(*Z*)-9,10-Dihydroxy-6,10-dimethylundec-5-en-2-one (*rac*-6b)**

Flash column chromatography with gradient eluent (EtOAc (0% → 100%) in *c*-Hex) provided *rac*-**6b** as a colorless viscous oil.

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.07 (t, , *J* = 6.9 Hz, 1H), 3.31 (dd, *J* = 10.8, 2.1 Hz, 1H), 2.53-2.48 (m, 2H), 2.47-2.34 (m, 1H), 2.33-2.24 (m, 2H), 2.23-2.15 (m, 1H), 2.14 (s, 3H), 1.67 (s, 3H), 1.63-1.56 (m, 1H), 1.42-1.34 (m, 1H), 1.20 (s, 3H), 1.16 (s, 3H); ^{13}C NMR (90 MHz, $CDCl_3$): 209.6, 136.1, 124.5, 77.8, 73.1, 43.8, 30.1, 29.4, 28.6, 26.5, 23.4, 23.2, 22.2.
NMR data in accordance with literature.[25]

***rac*-(*E*)-3,7-Dimethyloct-2-ene-1,6,7-triol (*rac*-10)**
To a solution of epoxide (0.32 mmol) in THF (0.64 mL) was added $KHSO_4$ (1.0 M, 0.64 mL) at room temperature. After 2 h the starting material was consumed and the reaction mixture was applied on a silica gel. The mixture was purified by flash column chromatography (*c*Hex/EtOAc, 1:1) to provide *rac*-**10** as a white waxy solid (42 mg, 0.23 mmol, 70%).
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.47 (dt, 1H), 4.16 (d, *J* = 6.9 Hz, 2H), 3.35 (dd, *J* = 10.6, 2.0 Hz, 1H), 2.30 (ddd, *J* = 14.5, 9.6, 5.3 Hz, 1H), 2.15-2.06 (m, 1H), 1.69 (s, 3H), 1.61 (tdd, *J* = 9.2, 6.8, 3.3 Hz, 1H), 1.49-1.39 (m, 1H), 1.21 (s, 3H), 1.17 (s, 3H).
NMR data in accordance with literature.[26]

***rac*-(*E*)-6,7-Dihydroxy-3,7-dimethyloct-2-en-1-yl pivalate (*rac*-14c).**
To a solution of *rac*-**13c** (1.0 mmol) in THF (2.5 mL) was added $KHSO_4$ (100 mM, 2.5 mL) at rt. The reaction was vigorously stirred for 16h. When the conversion was complete, $NaHCO_3$ (5 mL, sat. aq.) was added and the mixture was diluted with EtOAc (10 mL). The aqueous phase was extracted three times with EtOAc (25 mL) and the combined organic fractions were dried over Na_2SO_4. Concentration *in vacuo* provided the crude product, which was purified by flash column chromatography (*c*Hex/EtOAc, 1:1) to provide *rac*-**14c** as a white waxy solid (233 mg, 0.86 mmol, 86%).
^{1}H NMR (500 MHz, $CDCl_3$) δ 5.37 (ddd, *J* = 8.1, 5.0, 3.4 Hz, 1H), 4.56 (dd, *J* = 6.9, 2.9 Hz, 2H), 3.33 (dd, *J* = 10.5, 2.0 Hz, 1H), 2.32-2.08 (m, 4H), 1.71 (s, 3H), 1.60 (dddd, *J* = 13.8, 9.1, 6.8, 2.0 Hz, 1H), 1.47-1.41 (m, 1H), 1.19 (s, 3H), 1.18 (s, 9H), 1.15 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 178.82, 141.67, 119.37, 78.11, 73.15, 61.40, 38.88, 36.70, 29.59, 27.33, 26.59, 23.41, 16.60. HRMS (ESI): *m/z* calcd. for $C_{15}H_{28}NaO_4$ ($[M+Na]^+$) 295.1880, found 295.1882.

Stereoselective dihydroxylation of alkenes:[1a]
(*S*,*E*)-8-Ethoxy-2,6-dimethyloct-6-ene-2,3-diol (*S*)-2.
AD-mix-α (1.41 g) was dissolved in *t*-BuOH/H_2O (1:1, 10 mL) at rt and stirred for 2 h. The reaction mixture was cooled to 0 °C and $MeSO_2NH_2$ (95 mg, 1 mmol) was added. After 5 min. (*E*)-1-ethoxy-3,7-dimethylocta-2,6-diene was added and stirred for 63 h at 0 °C. The reaction was quenched with $Na_2S_2O_3$ (sat. aq., 10 mL) and stirred until the mixture was colorless. The product was extracted three times with CH_2Cl_2 (30 mL) and the combined fractions were dried with Na_2SO_4. After filtration the extract was concentrated *in vacuo*. Flash column chromatography with gradient eluent (EtOAc (10% → 50%) in *c*-Hex) provided (*S*)-**2** (72 mg, 0.32 mmol, 32%) as a colorless viscous oil.
See *rac*-**2** for NMR data.

(*S*,*E*)-8-(Allyloxy)-2,6-dimethyloct-6-ene-2,3-diol (*S*)-4.
AD-mix-α (1.41 g) was dissolved in *t*-BuOH/H_2O (1:1, 10 mL) at rt and stirred for 1.5 h. The reaction mixture was cooled to 0 °C and $MeSO_2NH_2$ (95 mg, 1 mmol) was added. After 5 min. (*E*)-1-allyloxy-3,7-dimethylocta-2,6-diene was added and stirred for 38 h at 0 °C. The reaction was quenched with $Na_2S_2O_3$ (5.0 g) and stirred until the mixture was colorless. The product was extracted three times with CH_2Cl_2 (30 mL) and the combined fractions were dried with Na_2SO_4. After filtration the extract was concentrated *in vacuo*. Flash column chromatography with gradient eluent (EtOAc (0% → 50%) in *c*-Hex) provided (*S*)-**4** (43 mg, 0.18 mmol, 18%) as a colorless viscous oil.
See *rac*-**4** for NMR data.

(*S*,*E*)-9,10-Dihydroxy-6,10-dimethylundec-5-en-2-one (*S*)-6a:
AD-mix-α (0.14 g) was dissolved in *t*-BuOH/H_2O (1:1, 1.0 mL) at rt and stirred for 1.5 h. The reaction mixture was cooled to 0 °C and $MeSO_2NH_2$ (9.5 mg, 0.1 mmol) was added. After 5 min. (*E*)-6,10-dimethylundeca-5,9-dien-2-one (20 mg, 0.10 mmol) was added and stirred for 63 h at 0 °C. The reaction was quenched with $Na_2S_2O_3$ (sat. aq., 1.0 mL) and stirred until the mixture was colorless. The product was extracted three times with CH_2Cl_2 (5 mL) and the combined fractions were dried with Na_2SO_4. After filtration the extract was concentrated *in vacuo*. Flash column chromatography with gradient eluent (EtOAc (0% → 50%) in *c*-Hex) provided (*S*)-**6a** (5 mg, 22 µmol, 20%) as a colorless viscous oil.
See *rac*-**6a** for NMR data.

(*R*,*E*)-9,10-Dihydroxy-6,10-dimethylundec-5-en-2-one (*R*)-6a:
AD-mix-*β* (0.71 g) was dissolved in *t*-BuOH/H_2O (1:1, 5.0 mL) at rt and stirred for 1.5 h. The reaction mixture was cooled to 0 °C and $MeSO_2NH_2$ (48 mg, 0.5 mmol) was added. After 5 min. (*E*)-6,10-dimethylundeca-5,9-dien-2-one (97 mg, 0.5 mmol) was added and stirred for 8 days at 0 °C. The reaction was quenched with $Na_2S_2O_3$ (sat. aq., 5 mL) and stirred until the mixture was colorless. The product was extracted three times with CH_2Cl_2 (5 mL) and the combined fractions were dried with Na_2SO_4. After filtration the extract was concentrated *in vacuo*. Flash column chromatography with gradient eluent (EtOAc (0% → 50%) in *c*-Hex) provided **(*R*)-6a** (8 mg, 35 µmol, 7%) as a colorless viscous oil.
See *rac*-**6a** for NMR-data.

(*S*,*Z*)-9,10-Dihydroxy-6,10-dimethylundec-5-en-2-one (*S*)-6b:
AD-mix-α (1.41 g) was dissolved in *t*-BuOH/H_2O (1:1, 10 mL) at rt and stirred for 2 h. The reaction mixture was cooled to 0 °C and $MeSO_2NH_2$ (95 mg, 1.0 mmol) was added. After 5 min. (*Z*)-6,10-dimethylundeca-5,9-dien-2-one (389 mg, 2.0 mmol) was added and stirred for 8 days at 0 °C. The reaction was quenched with $Na_2S_2O_3$ (sat. aq., 10 mL) and stirred until the mixture was colorless. The product was extracted three times with CH_2Cl_2 (30 mL) and the combined fractions were dried with Na_2SO_4. After

filtration the extract was concentrated *in vacuo*. Flash column chromatography with gradient eluent (EtOAc (0% → 50%) in *c*-Hex) provided (*S*)-**6b** (128 mg, 0.56 mmol, 56%) as a colorless viscous oil.
See *rac*-**6b** for NMR-data.

(*S*,*E*)-3,7-Dimethyloct-2-ene-1,6,7-triol (*S*)-10:
AD-mix-α (1.41 g) was dissolved in *t*-BuOH/H_2O (1:1, 10 mL) at rt and stirred for 3 h. The reaction mixture was cooled to 0 °C and $MeSO_2NH_2$ (95 mg, 1.0 mmol) was added. After 5 min. (*Z*)-6,10-dimethylundeca-5,9-dien-2-one (264 µL, 1.5 mmol) was added and stirred for 7 days at 0 °C. The reaction was quenched with $Na_2S_2O_3$ (sat. aq., 10 mL) and stirred until the mixture was colorless. The product was extracted three times with CH_2Cl_2 (30 mL) and the combined fractions were dried with Na_2SO_4. After filtration the extract was concentrated *in vacuo*. Flash column chromatography with gradient eluent (EtOAc (0% → 100%) in *c*-Hex) provided (*S*)-**10** (174 mg, 0.93 mmol, 93%) as a waxy solid.
See *rac*-**10** for NMR data.

Hydride reduction of ketones:
***rac*-(*E*)-8-(3,3-Dimethyloxiran-2-yl)-6-methyloct-5-en-2-ol *rac*-7a**
To a mixture of epoxide *rac*-**5a** (105 mg, 0.5 mmol) in MeOH (1.5 mL) was added $NaBH_4$ (23 mg, 0.6 mmol) in small portions at 0 °C. When all of $NaBH_4$ was added, the ice-bath was removed and stirred for 15 min. Then 10% HCl (2.5 mL) was added and the product was extracted with Et_2O. The organic phase was dried over $NaSO_4$ and concentrated *in vacuo*. The crude product was purified by flash column chromatography (*c*Hex/EtOAc, 3:1) to afford *rac*-**7a**.
^{1}H NMR (300 MHz, $CDCl_3$): δ 5.21 (t, *J* = 7.1 Hz, 1H), 3.89-3.76 (m, 1H), 2.71 (t, *J* = 6.2 Hz, 1H), 2.27-2.01 (m, 4H), 1.66 (s, 3H), 1.64-1.42 (m, 4H), 1.32 (s, 3H), 1.28 (s, 3H), 1.21 (d, *J* = 6.2 Hz, 3H).
NMR data in accordance with literature.[25]

***rac*-(*Z*)-8-(3,3-Dimethyloxiran-2-yl)-6-methyloct-5-en-2-ol *rac*-7b**
To a mixture of epoxide *rac*-**5b** (2.0 mg, 10 µmol) in MeOH (200 µL) was added a spatula tip $NaBH_4$ at 0 °C. The ice-bath was removed and stirred for 15 min. Then the reaction mixture was poured into a mixture of sat. aq. NH_4Cl (5 mL) and $CHCl_3$ (10 mL), from which the product was extracted with $CHCl_3$ (3 x 20 mL). The combined organic fractions were dried over $NaSO_4$ and concentrated *in vacuo*. The crude product was purified by flash column chromatography (*c*Hex/EtOAc, 2:1) to afford *rac*-**7b** (1.1 mg, 5 µmol, 50%).
^{1}H NMR (300 MHz, $CDCl_3$): δ 5.20 (t, *J* = 7.2 Hz, 1H), 3.89-3.74 (m, 1H), 2.74 (dd, *J* = 10.0, 4.0 Hz, 1H), 2.29-2.08 (m, 4H), 1.72 (s, 3H), 1.71-1.44 (m, 4H), 1.33 (s, 3H), 1.29 (s, 3H), 1.20 (d, *J* = 6.2 Hz, 3H).
NMR data in accordance with literature.[25]

***rac*-(*E*)-8-(3,3-Dimethyloxiran-2-yl)-6-methyloct-5-en-2-ol *rac*-8a**
rac-**7a** was hydrolyzed in a mixture of THF/water (1:1) (4 mL), which was acidified with H_2SO_4 (6 N, 2 drops). The product was extracted twice with EtOAc and dried over Na_2SO_4. The crude product was purified by flask column chromatography (EtOAc) to afford *rac*-**8a**.
^{1}H NMR (300 MHz, $CDCl_3$): δ 5.24 (t, *J* = 7.1 Hz, 1H), 3.87-3.77 (m, 1H), 3.36 (dd, *J* = 10.4, 1.7 Hz, 1H), 2.14-2.07 (m, 4H), 1.65 (s, 3H), 1.58-1.36 (m, 4H), 1.21 (s, 6H), 1.17 (s, 3H).
NMR data in accordance with literature.[28]

***rac*-(*Z*)-8-(3,3-Dimethyloxiran-2-yl)-6-methyloct-5-en-2-ol *rac*-8b**
8b was obtained similarly to **8a**, from acidic hydrolysis of **7b**:
^{1}H NMR (300 MHz, $CDCl_3$): δ 5.15 (t, *J* = 6.9 Hz, 1H), 3.82-3.71 (m, 1H), 3.34-3.18 (m, 4H), 2.46-1.99 (m, 4H), 1.65 (s, 3H), 1.63-1.23 (m, 4H), 1.18 (dd, *J* = 5.2, 2.6 Hz, 6H), 1.13 (s, 3H).
NMR data in accordance with literature.[28]

Stereoselective synthesis of epoxides:
(*R*,*Z*)-8-(3,3-Dimethyloxiran-2-yl)-6-methyloct-5-en-2-one (*R*)-5b (and (*R*)-**5a** as contaminant)
To a solution of (*S*)-**6b** (23 mg, 0.1 mmol) in CH_2Cl_2 (1 mL) was added Et_3N (56 µL, 0.4 mmol) at rt. The mixture was cooled to 0 °C and MsCl (15 µL, 0.2 mmol) was added. After allowing reaching rt and stirring for 8h, the reaction was complete as judged by TLC. The reaction was quenched with NH_4Cl (sat. aq., 2 mL) and extracted three times with CH_2Cl_2 (5 mL). The combined organic fractions were dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (*c*Hex/EtOAc, 2:1) afforded the intermediate mesylate (14 mg, 46 µmol, 46%), which was converted to (*R*)-**5b** in MeOH (0.5 mL) with K_2CO_3 (20 mg, 140 µmol) in 2 h at rt. The product was extracted three times with Et_2O (5 mL) after neutralizing with NH_4Cl (sat. aq., 2 mL). The combined organic fractions were dried over Na_2SO_4 and concentrated *in vacuo* after filtration. Flash column chromatography (*c*Hex/EtOAc, 9:1) afforded (*R*)-**5b** (2.0 mg, 9 µmol, 9% over two steps).
See *rac*-**5b** for NMR data.

(*S*,*Z*)-8-(3,3-Dimethyloxiran-2-yl)-6-methyloct-5-en-2-one (*S*)-5b (and (*S*)-**5a** as contaminant)**.**
To a solution of (*R*)-**6b** (23 mg, 0.1 mmol) in CH_2Cl_2 (1 mL) was added Et_3N (56 µL, 0.4 mmol) at rt. The mixture was cooled to 0 °C and MsCl (12 µL, 0.15 mmol) was added. After 1h the mixture was allowed to reach rt and stirring was continued for 22 h. The reaction was quenched with NH_4Cl (sat. aq., 2 mL) and extracted three times with CH_2Cl_2 (5 mL). The combined organic fractions were dried over Na_2SO_4 and concentrated *in vacuo*. Flash column chromatography (*c*Hex/EtOAc, 2:1) afforded the intermediate mesylate (3.0 mg, 10 µmol, 10%), which was converted to (*S*)-**5b** in MeOH (250 µL) with K_2CO_3 (4 mg, 30 µmol) in 2 h at rt. The reaction mixture was directly applied on silica gel column. Flash column chromatography (*c*Hex/EtOAc, 4:1) followed by a second flash column chromatography (*c*Hex/EtOAc, 9:1) afforded (*S*)-**5b** (1.8 mg, 8 µmol, 8% over two steps).
See *rac*-**5b** for NMR data.

(*R*,*E*)-6,7-Dihydroxy-3,7-dimethyloct-2-en-1-yl pivalate (*R*)-14c

E-4 (1.5 g, whole cell lyophilisate of DSM 44539) was rehydrated for 1 h in phosphate buffer (2x 25 mL, 100mM, pH 8.0) in two plastic Greiner tubes at 30 °C. Subsequently *rac*-**13c** (254 mg, 1 mmol) was added and the conversion was carefully monitored by GC until (*S*)-**13c** was consumed. After 40 h the products were extracted three times with EtOAc, dried over Na_2SO_4 and concentrated after filtration. The crude product mixture was then redissolved in THF (5 mL) and treated with $KHSO_4$ (5 mL, 0.1 M). When the starting material was consumed after 1.5 h, $NaHCO_3$ (20 mL, sat.aq.) was added and the product was extracted three times with EtOAc (25 mL). The combined organic fractions were dried over Na_2SO_4, filtered and concentrated *in vacuo*. Flash column chromatography (*c*Hex/EtOAc, 3:1) afforded (*R*)-**14c** (143 mg, 0.52 mmol, 52%) in high optical purity (97% *ee*).
See *rac*-**14c** for NMR data.

Determination of absolute configuration of 7b

Enzymatic hydrolysis of *rac*-**5b** delivered enantioenriched (*S*)-**5b** using E-12 (Table 4), which was further reduced by ADH of known selectivity (Prelog or anti-Prelog) to produce **7b**, allowing attribution of absolute configuration to enantiomers of **7b** on chiral GC:

(*S*,*Z*)-8-(3,3-Dimethyloxiran-2-yl)-6-methyloct-5-en-2-one (*S*)-5b and (*R*,*Z*)-9,10-dihydroxy-6,10-dimethylundec-5-en-2-one (*R*)-6b:

To a rehydrated suspension of E-12 (NRRL4671, 300 mg, whole cell) in Tris-Buffer (50 mM, pH 8.0, 10 mL) was added *rac*-**5b** (14 mg, 67 µmol). After incubation of 27 h the suspension was extracted two times with EtOAc (10 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. Flash column chromatography (*c*Hex/EtOAc, 9:1) afforded (*S*)-**5b**, which was used for further reductive biotransformation, and (*R*)-**6b**.

Biocatalytic reduction of ketones:

(*S*,*E*)-8-[(*S*)-3,3-Dimethyloxiran-2-yl]-6-methyloct-5-en-2-ol (3*S*,10*S*)-7b.

One aliquot of (*S*)-**5b** obtained from enzymatic hydrolysis reaction (see above) was added to a mixture of ADH-A (10 µL) in phosphate Buffer (500 µL, 100 mM, pH 8.0) with NADH (0.1 mL, 200 mM). After incubation of 24 h, the product was extracted two times with EtOAc (1 mL), dried over Na_2SO_4 and analysed by GC for assignment of enantiomer peak to (3*S*,10*S*)-**7b**.

Linalool oxide synthesis:

2-[(*5S*)-5-Methyl-5-vinyltetrahydrofuran-2-yl]propan-2-ol (11*)

According to literature procedure[18], [{Ir(cod)Cl}$_2$] (3.4 mg, 5 µmol) was dissolved with phosphoramidite (*S*)-**L$_1$** (10.2 mg, 20 µmol) and *m*-$ClC_6H_4CO_2H$ (16 mg, 100 µmol) in anhydrous $ClCH_2CH_2Cl$ (0.4 mL) at rt. After stirring for 1 h, *rac*-**9** was added and the mixture was heated to 50 °C for 1 day. After sampling of the reaction mixture, GC analysis showed that the cycloetherification proceeded with very low selectivity. Crude NMR showed signals of both *trans*- and *cis*-isomers (data not shown) which correspond to literature data.[22]

2-[(2*R*,5*R*)-5-Methyl-5-vinyltetrahydrofuran-2-yl]propan-2-ol (2*R*,5*R*)-11[22]

To a solution of (*S*)-TunePhos (31 mg, 49 µmol) in anhydrous THF (2.0 mL) was added (*R*)-**14c** (62 mg, 216 µmol). Subsequently Pd_2dba_3 (12 mg, 13 µmol) was added followed by Et_3N (60 µL, 446 µmol). The reaction mixture was then heated to 65 °C under N_2-atmosphere for 29 days. Five aliquots (0.2 mL) were taken over the course of the reaction, which left 50% of the reaction mixture. After evaporation of the solvent the crude reaction mixture was applied on a silica gel column and purified by flash column chromatography, which provided (2*R*,5*R*)-**11** (13.8 mg, 81 µmol, 75%) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 5.88 (dd, *J* = 17.3, 10.7 Hz, 1H), 5.19 (dd, *J* = 17.3, 1.5 Hz, 1H), 4.99 (dd, *J* = 10.6, 1.5 Hz, 1H), 3.80 (t, *J* = 7.2 Hz, 1H), 1.90 (ddd, *J* = 10.9, 8.3, 6.1 Hz, 1H), 1.83 (q, *J* = 7.3 Hz, 2H), 1.73 (ddd, *J* = 10.9, 8.3, 6.1 Hz, 1H), 1.32 (s, 3H), 1.23 (s, 3H), 1.13 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 143.85, 111.47, 85.70, 83.20, 71.27, 37.63, 27.39, 26.97, 26.46, 24.31.
NMR data in accordance with literature.[22]

Relevant NMR spectra and GC methods and traces can be found in the supporting information.

Acknowledgements

The Amsterdam Institute for Molecules Medicines and Systems (AIMMS) and the University of Graz are gratefully acknowledged for financial support. We thank Elwin Janssen (VUA) for technical assistance, Dr. Andreas W. Ehlers (VUA) for NMR maintenance and Barbara Grischek (UG) for the production of the enzyme preparations. Dr. Thomas Netscher is thanked for the kind gift of starting materials. Prof. Michael Arand (University of Zürich, Switzerland) and Elisabeth Blee (University of Strasbourg, France) are thanked for the kind gift of epoxide hydrolase samples.

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10.1002/adsc.201801094

FULL PAPER

Enantioselective Bio-Hydrolysis of Geranyl-Derived *rac*-Epoxides: A Chemoenzymatic Route to *trans*-Furanoid Linalool Oxide

Adv. Synth. Catal. **Year**, *Volume*, Page – Page

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