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Exploitation of a Multienzymatic Stereoselective Cascade Process in the Synthesis of 2-Methyl-3-Substituted Tetrahydrofuran Precursors

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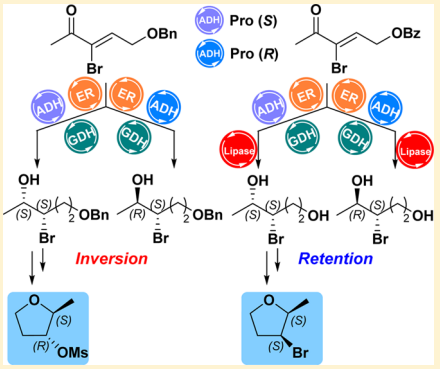
Supporting Information

ABSTRACT: Enantiopure 2-methyl-3-substituted tetrahydrofurans are key precursors of several biologically active products (drugs, flavors, and agrochemicals). Thus, a stereocontrolled and efficient methodology for the obtainment of these synthons is highly desirable. We exploited a two-step multienzymatic stereoselective cascade reduction of α-bromo-α,β-unsaturated ketones to give the corresponding bromohydrins in good yields, with high *ee* and *de* values. The cascade process is catalyzed by an ene-reductase and an alcohol dehydrogenase. Further manipulations of these bromohydrins, by two diastereodivergent routes, allowed the preparation of the tetrahydrofuran synthons. One route is based on a lipase catalyzed cleavage of the protecting group. The second route is characterized by a camphor sulfonic acid mediated isomerization of a β-hydroxyepoxide to give the tetrahydrofuran-2-ol. Finally, the synthesis of the most odorous and pleasant stereoisomer of the roasted meat aroma, i.e., (2*S*,3*R*)-2-methyl-3-thioacetate tetrahydrofuran, is reported as well.



INTRODUCTION

Chiral tetrahydrofurans are important building blocks of organic chemistry; indeed, in the last several decades considerable attention has been devoted to the development of efficient and stereocontrolled methodologies for the synthesis of 2,5-disubstituted tetrahydrofuranic derivates,[1] key structural fragments of many natural products. However, less attention has been dedicated to the 2,3-disubstituted tetrahydrofurans, which, however, are largely employed in the preparation of many products of industrial relevance such as aromas,[2] drugs,[3] and agrochemicals[4] (Figure 1).

Thus, the development of efficient strategies for the stereoselective synthesis of the key synthons 3-bromo-2-methyl tetrahydrofuran **1** and 3-hydroxy-2-methyl tetrahydrofuran **2** (Figure 1) is highly desirable. For example, these synthons may find a valuable application in the preparation of the most odorous and pleasant stereoisomer of the roasted meat aroma, i.e., (2*S*,3*R*)-**3**.

To the authors' knowledge, no enantioselective syntheses of the very useful bromoderivative[5] **1** have ever been reported, whereas each enantiomer of the *cis*-diastereoisomer of **2** was prepared by Sharpless asymmetric dihydroxylation (AD-mix-β), but not with a very high stereoselectivity (*ee* values ranged between 62% and 80%). Then, the two enantiomers of *trans*-**2** were obtained by Mitsunobu inversion of C(3) stereocenters of the *cis* enantiomers, conserving most of their original optical purity.[2]

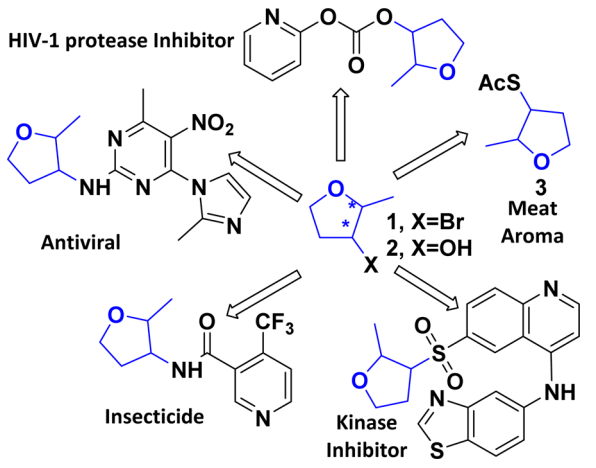


Figure 1. Selected examples of biologically active products bearing a 2-methyl-3-substituted tetrahydrofuran moiety.

RESULTS AND DISCUSSION

Figure 2 shows the retrosynthetic analysis of **1** and **2**. It relies on the stereospecific reduction of the (*Z*)-bromoketone **I** to give the corresponding bromohydrin **II**; the latter might be converted by regioselective tosylation of the primary alcohol into **III**, which

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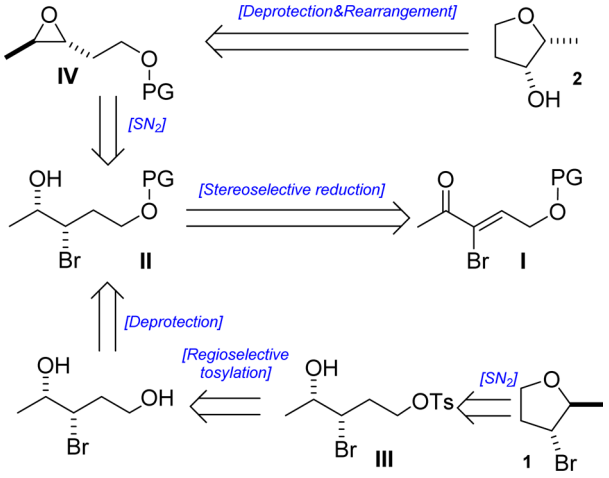


Figure 2. Retrosynthesis of the diastereocomplementary synthons **1** and **2**.

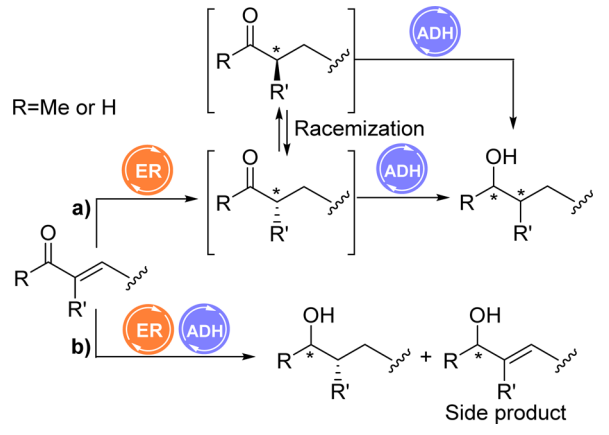
is then transformed into **1** by ring closure. Diastereo- and/or enantiocomplementary asymmetric catalytic transformations often require completely new and time-consuming setup of the experimental conditions; moreover, it is not always so trivial to prepare the enantioform of the catalyst. Thus, in our synthetic strategy, we show that, starting from the same bromohydrin **II**, it is possible to obtain **2**, diastereocomplementary synthon of **1**, by means of a ring expansion of epoxide **IV**.

According to our retrosynthetic plan, especially for the crucial stereoselective transformations, a biocatalytic approach[6] seems to be the best choice; indeed, it is well-known that the bromo substituent is extremely labile to the metal-catalyzed reduction, making this extremely valuable catalysis unfeasible with our strategy.

In the past few years, several one-pot multienzymatic systems[7,8] have been investigated. In relation to the chemoselectivity of the enzymes involved, the latter can be added to the reaction mixture either step-by-step or simultaneously.

In particular, systems based on the combination of an enzyme with ene-reductase (ER) activity with a second one with alcohol dehydrogenase (ADH) activity have proven to be extremely efficient[9] (Scheme 1). Especially, these systems are well suited for the stereoselective reduction of both functionalities of prochiral

Scheme 1. ADH and ER Multienzymatic System for the Synthesis of Optically Enriched Alcohols: (a) Sequential Enzyme Addition; (b) Cascade Procedure

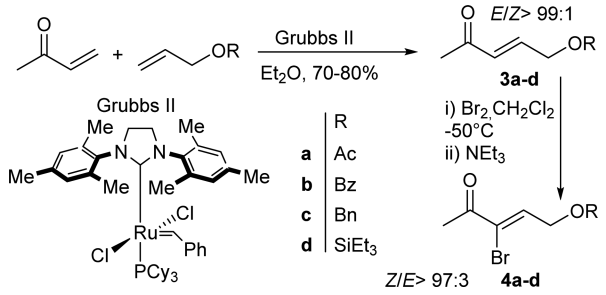


α,β-unsaturated ketones/aldehydes: the C=C double bond by ERs and the CO carbonyl group by ADHs.

The products are usually obtained in good yields, with excellent enantioselectivities, and for the ketones, with good diastereoselectivities and high optical purity. In addition, we found that the ADHs, quite often, are not able to reduce the carbonyl group of α,β-unsaturated ketones. This uncommon chemoselectivity for a reducing agent[10] makes the combination of ADHs with ERs particularly advantageous in a cascade process. In fact, possible spontaneous product racemizations, side-product formations, and intermediate product inhibition effects can be mitigated by this approach.[9b−d]

The synthesis of the starting materials **4a−d** is shown in Scheme 2. First, the α,β-unsaturated ketones **3a−d** with

Scheme 2. Synthesis of α-Bromo-α,β-unsaturated Ketones 4a−d



orthogonal protective groups of the terminal alcohol (acetyl and benzoate esters vs benzyl and triethylsilyl ethers) were easily prepared in a good yield and with an excellent diastereoselectivity (*E*/*Z*, > 99:1) by cross-metathesis:[11] we used an excess of the cheap methylvinyl ketone with respect to the *O*-protected allylic alcohol, in the presence of the second generation of Grubbs catalyst[12,13] (see the SI for the preparation of alcohols). Then, the ketones were transformed into the (*Z*)-α-bromoderivative (**4a−d**) by a one-pot two-step procedure: (i) stereospecific addition of bromine at low temperature (−50 °C), (ii) followed by *in situ* elimination with NEt_3, affording the products in fairly good yields (60−70%) and with a good *de* (*Z*/*E*, >97:3).[14] Remarkably, compound **4b** was isolated by crystallization in hexane/Et_2O (9:1, −30 °C), whereas the other bromoderivatives (**4a** and **4c−d**) required column chromatography purification.

Thus, the C=C double bond reduction of the substrates **4a−d** was tested with a set of different recombinant ERs belonging to the Old Yellow Enzymes family[15] (OYE1-3), using for the NADPH cofactor regeneration a glucose dehydrogenase[16] (GDH) and glucose as sacrificial cosubstrate. The results and the experimental conditions are summarized in Table 1.

First of all, substrates **4a−c** are very well accepted by all tested ERs, since the conversions using the standard amount of enzyme (≈100 μg/mL for a 5 mM concentration of substrate) were quantitative (entries 2, 12, 13) independently of the ER used (for instance, entries 1−3 for **4a**). An exception was ketone **4d** that did not react at all (entry 14), this result being likely due to the presence of the more sterically demanding $-OSiEt_3$ protective group.[17] Then, in view of a reaction scale-up, we optimized the amount of ER to be added, using **4c** as model compound. By lowering the amount of enzyme to 12 μg/mL, it was shown that OYE3 was the ER with the highest catalytic activity (entry 5 vs 4 and 6), but a significant decrease of conversions was observed. A good compromise between conversion

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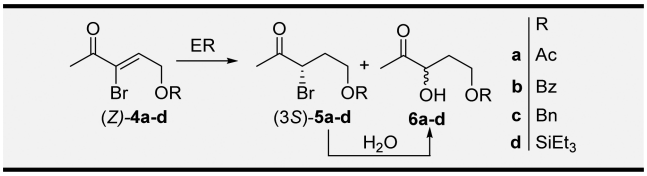
Table 1. ER Catalyzed Reduction of (*Z*)-4a−d[a]



entry	R	ER	pH	conv.[b] (%)	ratio[b] 5/6
1	Bn	OYE1	7.0	>99	88:12
2	Bn	OYE2	7.0	>99	90:10
3	Bn	OYE3	7.0	>99	87:13
4	Bn	OYE1	7.0	20[c]	88:12
5	Bn	OYE2	7.0	16[c]	90:10
6	Bn	OYE3	7.0	24[c]	87:13
7	Bn	OYE3	7.0	>99[d]	85:15
8	Bn	OYE2	6.0	>99[d]	94:6
9	Bn	OYE3	5.2	88[d]	94:6
10	Bn	OYE3	4.0	73[d]	96:4
11	Bn	OYE3	6.0	85[e,f]	93:7
12	Ac	OYE3	6.0	95	94:6
13	Bz	OYE3	6.0	>99	96:4
14	$SiEt_3$	OYE3	6.0	–[g]	–[g]
15	Ac	OYE3	6.0	53[d,f]	93:7
16	Bz	OYE3	6.0	86[d,f]	96:4

[a]Typical experimental conditions of the ER screening on 1 mL scale: substrate conc. 5 mM, 1% DMSO cosolvent, ER: 100 μg/mL, GDH 5U, 0.1 mM $NADP^+$, 20 mM glucose, pH = 7 phosphate buffer 50 mM, 30 °C, reaction time: 1 day, 180 rpm. [b]By GC−MS. [c]ER: 12 μg/mL. [d]ER: 65 μg/mL. [e]Yield after column chromatography. [f]On a preparative scale: 1 mmol of substrate. [g]No conversion.

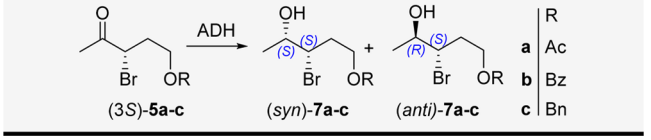
and enzyme consumption was reached by using 65 μg/mL of OYE3 (entry 7); similar results were achieved with **4a−b** (data not shown). The stereochemical configuration of the products (*vide infra*) was assigned (*S*), in agreement with a flipped binding mode of the substrate into the catalytic site of the protein.[18] Unfortunately, any attempt to determine the *ee* by chiral GC or HPLC was unsuccessful.

However, at the end of each biotransformation together to the α-bromoketone product (**5a−c**) and to the unreacting (*E*) diastereoisomer[19] ((*E*)-**4a−c**), a small amount of α-hydroxyketone, i.e., **6a−c** (detected by GC−MS, Table 1), was always present. This result can be related to the fact that, even in such very mild experimental conditions, it is not uncommon to have side reactions for halocarbonyl derivatives. For instance, Faber et al. have shown that 2,3,3-trihaloesters, products of the ER catalyzed reduction of trihaloderivatives of the methyl acrylic ester, undergo to complete and spontaneous elimination of HX at neutral pH and at 30 °C.[20] In our case, we hypothesized that, as soon as the product is formed during the biotransformation, a nucleophilic displacement of the bromo substituent[21] with water could occur to give the corresponding hydroxyl ketone. This hypothesis was confirmed by comparing the GC retention time of a sample of **6c** on purpose synthesized (see the SI). The product/side-product ratio was around 1:10, independently of the protective group and to the type of ER used. Thus, even if it is well-known that the ERs of the OYE family exhibit their highest activity at neutral pH,[22] we repeated the reductions at lower pH values with the aim of minimizing the hydrolysis, since these reactions are usually carried out in the presence of inorganic bases. By performing the reactions at pH = 5.2 and pH = 4.0 (entries 9 and 10, respectively), a gradual increase of the **5c**/**6c** ratio was observed, but this fact was accompanied by a corresponding decrease of the conversions. Eventually, the best results on a preparative scale (≈ 1 mmol) in terms of conversion, enzyme consumption, and side-product formation were achieved at pH = 6.0 (entry 11). Under these experimental conditions, both **5b** and **5c** could be isolated after column chromatography purification in a good yield (around 85%). In the case of the *O*-Ac protected ketone, the yield was lower (around 53%), because the higher hydrophilicity and volatility of **5a** made its isolation more difficult and, therefore, not quantitative.

Next, we focused our attention on the ADH catalyzed reduction of the carbonyl group, which, for methyl ketones, quite often proceeds with a very high stereoselectivity.[6] A panel of commercially available ADHs was screened, while, for the NAD(P)H cofactors regeneration, we used the same system employed in the biotransformations catalyzed by the ERs.

As most of the screened ADHs did not reduce the carbonyl group of the α,β-unsaturated ketones **4a−c** (see Table S1 of the SI), this very high chemoselectivity[8a,9b,c] opens the possibility to couple the two biocatalyzed reactions in a one-pot cascade process. The ADHs stereoselectivity was preliminarily tested on the model compound (3*S*)-**5c**. Conversion and diastereoselectivity are summarized in Table 2. Three ADHs showing pro (*S*)

Table 2. ADHs Catalyzed Reduction of (3*S*)-5a−c[a]



entry	R	ADH	conv.[b] (%)	*syn*[c] (%)	*anti*[c] (%)
1	Bn	CPADH	50.1	60.7	39.3
2	Bn	DRADH	66.9	62.9	37.1
3	Bn	EVO030	99.3	68.9	31.1
4	Bn	EVO270	93.3	14.4	85.6
5	Bn	EVO420	99.6	22.6	77.4
6	Bn	KRED	99.1	20.8	79.2
7	Bn	PLADH	97.2	17.8	82.1
8	Ac	EVO030	99.3	61.9	38.1
9	Bz	EVO030	98.6	65.9	34.1
10	Bz	EVO270	98.2	15.8	84.2
11	Bz	EVO420	93.7	20.4	79.6

[a]Typical experimental conditions of the ADH screening on 1 mL scale: substrate conc. 5 mM, 1% DMSO cosolvent, ADH: 200 μg/mL, GDH 5U, 0.1 mM NAD^+ + 0.1 mM $NADP^+$, 20 mM glucose, pH = 7 phosphate buffer 50 mM, 30 °C, reaction time: 12 h, 180 rpm. [b]By GC−MS. [c]By GC−MS, since the bromohydrins *syn* and *anti* are not well separated; they were transformed into the *O*-silyl derivatives by adding *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide 12 h before GC analysis.

stereoselectivity were identified (entries 1−3), but the diastereomeric excess of the *syn*-bromohydrin, i.e., (2*S*,3*S*)-**7c**, were not optimal, since, in the best case, we obtained a modest *de* = 38% with EVO030. At this stage, because we did not know the optical purity of (*S*)-**5c**, we were not able to attribute these low *de* values neither to a low stereoselectivity of the OYE3 catalyzed step nor to the ADH mediated reduction of the CO group. With the pro (*R*) ADHs (entries 4−7), the diastereoselectivity was higher, but still not satisfactory; indeed, the best result was achieved with EVO270 (entry 4, *de* = 72%). Finally, we tested the most performing pro (*S*) and pro (*R*) ADH, i.e., EVO030 and

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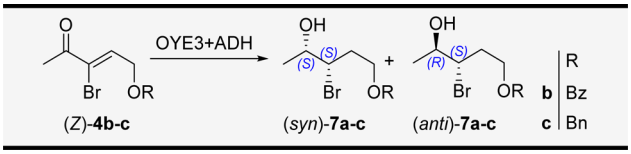
EVO270, respectively, with the other two substrates **5a−b** (entries 8−11, Table 2), the results in terms of selectivity and conversion being consistent with those obtained with **5c**.

Even if the preliminary results were not encouraging, we thought that the low *de* values might be ascribed mainly to a partial racemization of the α-bromoketone **5a−b** rather than to a low stereoselectivity of OYE3, since similar α-alkyl substituted methylenones have been reduced by the same ER but affording the products with a much higher stereoselectivity (*ee*'s > 99);[23] the stability of these ketones at the typical biotransformation conditions is likely due to the lower acidity of the proton in α to the carbonyl group relative to the corresponding proton of the α-bromo ketones. Thus, by coupling the OYE3 catalyzed reductive step with that of an ADH in a cascade process, in principle, it should be possible to minimize the racemization by converting the ketone, as soon it is formed, into the more stable alcohol. Moreover, in this case, the application of a cascade process was facilitated by the fact that the selected ADHs (EVO030 and EVO270) were completely chemoselective toward the saturated ketones, thus avoiding the detrimental formation of the allylic alcohol side-product.

In the study of coupled reactions, we focused our attention on substrates (*E*)-**4b−c**, those with the orthogonal protective groups and best conversions. The multienzymatic cascade processes were first carried out on the screening scale, as described before; conversion and diastereoselectivity are reported in Table 3.

Table 3. Multienzymatic Cascade Reduction of (*Z*)-4b−c[a]



entry	R	ADH	ADH/OYE3	conv.[b] (%)	*syn*[c] (%)	*anti*[c] (%)
1	Bn	EVO030	1:1	96.3	88	12
2	Bn	EVO030	3:1	93.3	98	2
3	Bn	EVO030	6:1	99.6	99	1
4	Bn	EVO030	3:1	89.1[d]	98	2
5	Bn	EVO270	2:1	97.2	0.3	99.7
6	Bn	EVO270	1:1	99.3	0.5	99.5
7	Bn	EVO270	0.5:1	99.3	0.5	99.5
8	Bn	EVO270	0.5:1	90.3[d]	1	99
9	Bz	EVO030	3:1	99.1	99	1
10	Bz	EVO030	3:1	87.4[d]	99	1
11	Bz	EVO270	0.5:1	90.3[d]	1	99

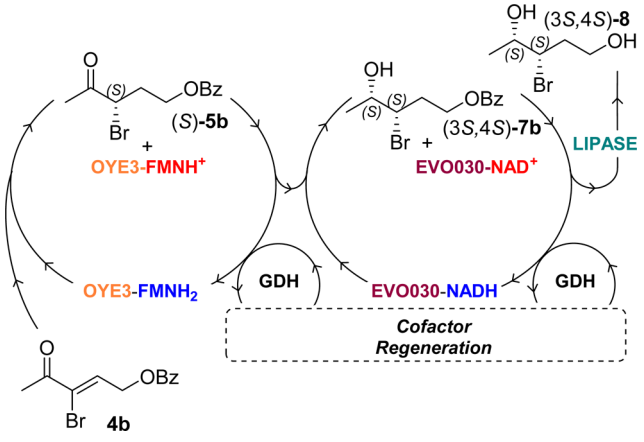
[a]Typical experimental conditions of the OYE3 + ADH screening on 1 mL scale: substrate conc. 5 mM, 1% DMSO cosolvent, OYE3: 65 μg/mL, ADH: see the table, GDH 5U, 0.1 mM NAD^+ + 0.1 mM $NADP^+$, 20 mM glucose, pH = 7 phosphate buffer 50 mM, 30 °C, reaction time: 12 h, 180 rpm. [b]By GC−MS. [c]By GC−MS: the bromohydrins *syn* and *anti* were transformed into the *O*-silyl derivatives by adding *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide 12 h before GC−MS analysis. [d]The reaction was repeated on a preparative scale (2 mmol).

The main parameter to be optimized was the ADH/OYE3 ratio. First, the cascade reduction of **4c** with OYE3 and EVO030 in a 1:1 ratio afforded the *syn*-**7c** (entry 1) with a much higher diastereoselectivity with respect to the sequential procedure (*de* = 76% vs *de* = 38%). Subsequently, by increasing the ratio to 3:1 and then to 6:1, the *de* value further increased up to 98% (entries 2−3). These results overall confirmed that OYE3 reduces these substrates with a high enantioselectivity, but the optical purity of the products is partially lost by means of a racemization occurring during the biotransformation.[24]

Then, we repeated the cascade process with the enantiocomplementary ADH, i.e., EVO270. The 1:1 enzymes ratio was already good since we obtained the *anti*-**7c** with an excellent *de* = 99% (entry 6), the same outcome being achieved even when further reducing the respective amount of EVO270 (entry 7). Thus, with the optimized experimental conditions, we scaled-up the reactions (around 2 mmol of starting material), affording the bromohydrins *syn*-**7b**, *anti*-**7b**, *syn*-**7c**, and *anti*-**7c** after column chromatography purification in quite good yields (over 80%) and with almost the same selectivity observed on the analytical scale (entries 4, 8, and 10−11). Finally, together to a lower extent of product racemization, we observed that also the formation of the side product **6b−c** diminished as well to almost negligible amounts.

Lastly, in order to accomplish our synthetic plan, the deprotection protocol of (3*S*,4*S*)-**6b** was investigated, the classical approach by alkaline hydrolysis being unfeasible for the presence of the bromohydrin functionality. Eventually, the enzymatic hydrolysis of the benzoate[25] ester was performed at neutral pH by adding the lipase from *Candida rugosa* to the reaction mixture at the end of the cascade process[26] catalyzed by OYE3 + EVO030. Thanks to this further enzymatic step, it was possible to obtain the diol (3*S*,4*S*)-**8** in an overall yield of 77% (Scheme 3) without any column purification.

Scheme 3. Coupling of OYE3 + EVO030 Cascade with the Hydrolysis Step Catalyzed by the Lipase from *Candida rugosa* in the One-Pot Synthesis of (2*S*,3*S*)-8 from 4b



Stereoselective Synthesis of the Roasted Meat Aroma.

The 2-methyl-tetrahydrofuran-3-thioacetate **3** is one of the main aromas of the roasted meat, and it is produced in the racemic form during the cooking process by a Maillard reaction. Quite often, chiral fragrances and flavors exhibit different odor properties as a function of the relative and absolute stereochemical configuration.[27] Recently, an organoleptic study[2b] has identified (2*S*,3*R*)-**3** as the most pleasant and characterizing odor between all possible stereoisomers of **3**. In contrast, both enantiomers of *cis*-**3** diastereoisomer impart to the food an unpleasant sulfur note.

Thus, with (2*S*,3*S*)-**7c** and (3*S*,4*S*)-**8** in our hands, we prepared the stereoisomers (2*S*,3*S*)-**3** and (2*S*,3*R*)-**3** of the roasted meat flavor (Scheme 4). First, the bromohydrin (2*S*,3*S*)-**7c** was converted into the epoxide (2*R*,3*S*)-**9** with NaH in THF; with

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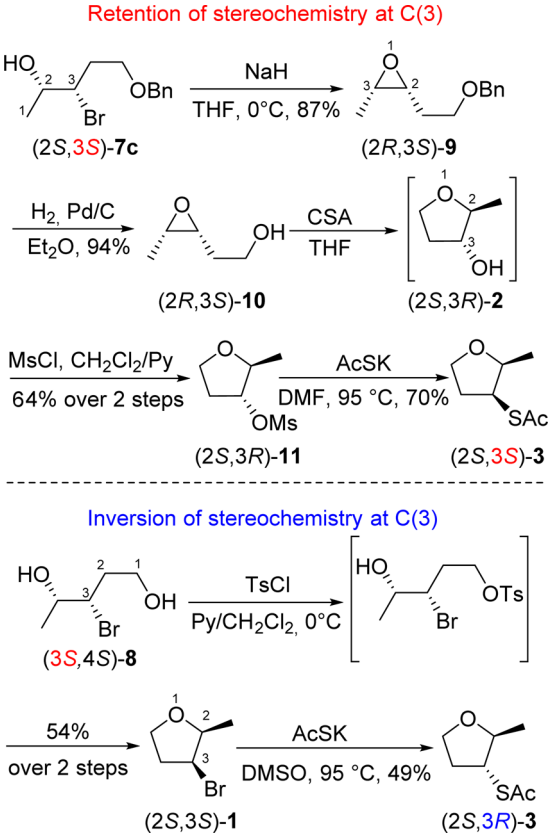
Scheme 4. Synthesis of (2*S*,3*S*)-3 and (2*S*,3*R*)-3



the same procedure but starting from *anti*-**7c**, the epoxide (2*R*,3*R*)-**9** was obtained as well. Then, the *O*Bn protective group of (2*R*,3*S*)-**9** was removed by hydrogenolysis with Pd/C, affording the epoxyalcohol (2*R*,3*S*)-**10**. The latter was rearranged in the presence of a substoichiometric amount of camphorsulfonic acid (CSA) to give the less strained tetrahydrofuranol (2*S*,3*R*)-**2**. However, this compound was not isolated due to his high volatility, but it was promptly converted into the mesyl derivative (2*S*,3*R*)-**11** in an overall yield of 64%. The rearrangement of β-hydroxy epoxides to tetrahydrofuran-2-ols proceeds with inversion of stereochemistry at the C(3) stereocenter of the epoxide ring; to our knowledge, just a few catalysts have been studied (MgI_2 and $BF_3{\cdot}OEt_2$)[28,29] compared to the extensive investigations that have been carried out for the rearrangement of γ-hydroxy epoxide homologues.[30] Thus, we studied this rearrangement on a model compound (see Table S2 of the SI); between all reagents tested, the CSA gave the best results. Finally, the mesyl derivative **11** was transformed into (2*S*,3*S*)-**3** by SN2 with AcSK in DMF,[2b] with an excellent *ee* = 99.6% (by chiral GC), which confirmed the high stereoselectivity of the cascade process.

Concerning the second stereoisomer, i.e., (2*S*,3*R*)-**3**, its synthesis was more straightforward. The diol (3*S*,4*S*)-**8** was regioselectively tosylated in pyridine at 0 °C, and the tosyl intermediate cyclized *in situ*, affording the bromoderivative (2*S*,3*S*)-**1** in an overall yield of 54% and with an excellent *de* > 97% (by GC−MS; see the SI). The almost complete conservation of the high diastereomeric excess of the starting material *syn*-**8** into the final product shows that the tosylation[28] of the primary alcohol proceeds with a remarkable regioselectivity. Then, the bromoderivative **1** was converted into (2*S*,3*R*)-**3** modifying the procedure adopted for the SN2 of **11**. Indeed, accidentally during the bulb-to-bulb distillation of (2*S*,3*S*)-**1** at high temperature (110 °C, 15 mmHg), we found that the product isomerized[31] to the *trans* diastereoisomer (*cis*/*trans* 1:1 by ^{1}H NMR; see the SI), and it decomposed as well. Thus, in order to avoid these side reactions during the nucleophilic substitution with AcSK, it is crucial to carry out the reaction at a temperature below 100 °C in DMSO and in the presence of dibenzo-18-crown-6.

CONCLUSION

The integration of at least two or more transformations in a multienzymatic cascade process is particularly appealing, since the enzymes, typically operating at very similar conditions, are intrinsically more compatible to this strategy than other types of catalysis. In this work, we demonstrated that the one-pot multienzymatic cascade process, encompassing four different enzymes (ER, ADH, GDH, and lipase) compares better in terms of stereoselectivity and side-products formation with respect to the one-pot enzyme sequential addition procedure.

Besides, one of the main drawbacks of the ERs based catalysis is the lack of wild-type enzymes with opposite stereoselectivity, and even if the C=C reduction is stereospecific, it is not always possible to prepare a regioisomer or diastereoisomer of the starting material that, once reduced, gives the opposite enantiomer (substrate engineering[9g] strategy). The generation of ER variants showing opposite stereoselectivity by protein engineering using either a (semi)-rational[32a,b] or a directed evolution[32c] approach has permitted in some cases to overcome this limitation. In this case, we bypassed this problem by devising a diastereodivergent synthesis of synthons **1** and **2**. Thus, since differently from the ERs there is large availability of ADHs with either a pro (*S*) or a pro (*R*) enantioselectivity, by means of our synthetic route, it is possible to have full access to all stereoisomers of any 2-methyl-3-substituted tetrahydrofuran. In this regard, we prepared the most pleasant roasted meat aroma (2*S*,3*R*)-2-methyl-3-thioacetate tetrahydrofuran in an overall yield of 17% and with a diastereoselectivity of 96%.

EXPERIMENTAL SECTION

General Remarks. Chemicals and solvents were purchased from suppliers and used without further purification, while, where required, the solvents were dried over molecular sieves (4 Å). ^{1}H and ^{13}C NMR spectra were recorded on a 400 or 500 MHz spectrometer at room temperature, using TMS as an internal standard for ^{1}H and $CDCl_3$ for ^{13}C; chemical shifts δ are expressed in ppm relative to the reference. High-resolution MS spectra were recorded with a Q-TOF mass spectrometer, equipped with an ESI source. The GC−MS analyses of all compounds were performed on a column with a low polarity stationary phase (30 m × 0.25 mm × 0.25 μm). Program temperature: 60 °C (1 min)/6 °C min^{-1}/150 °C (1 min)/12 °C min^{-1}/280 °C (5 min). For the very volatile compounds **2** and **10** was used a different method: 38 °C (9 min)/3 °C min^{-1}/90 °C (1 min)/6 °C min^{-1}/180 °C (1 min)/15 °C min^{-1}/280 °C (5 min). The enantiomeric excess values (*ee*) of compounds **3** were determined by chiral GC analysis (column DactButilSililBeta CDX) with a programmed temperature: 60 °C/1 °C min^{-1}/95 °C/90 °C min^{-1}/220 °C (2 min). TLC analyses were performed on precoated silica gel 60 F_{254} plates, and spots were visualized either by UV light (254 nm) or by spraying with phosphomolybdic acid reagent. All chromatographic separations were carried out on silica gel columns (230−400 mesh). Optical rotations were determined on a digital automatic polarimeter at 589 nm (sodium D line) and are given at rt in deg cm^3 g^{-1} dm^{-1}.

The *O*-protected allylic alcohols (OAc, OBz, OBn, and $OSiEt_3$, respectively) for the cross-metathesis were prepared following reported procedures.[33]

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J. Org. Chem. 2017, 82, 2114−2122

Enzymes and Strains. OYE1 from *Saccharomyces pastorianus*, OYE2-3 from *Saccharomyces cerevisiae*, and GDH from *Bacillus megaterium* were overexpressed in *E. coli* BL21 (DE3) strains harboring a specific plasmid, according to standard molecular biology techniques as described in ref 16. Protein concentrations were determined according to the Bradford test, using bovine serum albumine (BSA) as a standard. CPADH from *Candida parapsilosis* was purchased from Julich Chiral Solutions GmbH (now CODEXIS). PLADH from *Parvibaculum lavamentivorans*, DRADH from *Deinococcus radiodurans*, KRED (ketoreductase) from an unspecified source, and lipase from *Candida rugosa* were purchased from Sigma-Aldrich. EVO420, EVO270, and EVO030 from an unspecified source were purchased from Evocatal GmbH and used without further purifications.

General Procedure for the Preparation of Compounds 3a−d via Cross-Metathesis. To a well stirred solution of *O*-protected allylic alcohol (17 mmol), freshly distilled methyl vinyl ketone (4.8 g, 68 mmol), and CuI (96 mg, 0.51 mmol) in anhydrous and degassed Et_2O (85 mL) at 35 °C under a N_2 atmosphere was added the second gen. Grubbs' catalyst (144 mg, 0.17 mmol). After 24 h, the reaction was directly concentrated under *vacuum* and purified by column chromatography (eluent: hexane/AcOEt, 9:1), giving the product as a liquid/oil.

*(E)-4-Oxopent-2-en-1-yl Acetate (**3a**).*[10] Liquid, 1.64 g, yield 68%; tr = 9.43 min, 99% purity by GC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.76 (dt, J = 4.7 and 16.1 Hz, 1H), 6.26 (dt, J = 1.9 and 16.1 Hz, 1H), 4.77 (dd, J = 1.9 and 4.3 Hz, 2H), 2.29 (s, 3H), 2.13 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.7, 170.3, 139.8, 131.0, 62.6, 27.3, 20.7; GC−MS: m/z (%) 100 (M^+−42, 40), 83 (50), 71 (20), 43 (100).

*(E)-4-Oxopent-2-en-1-yl Benzoate (**3b**).* Oil, 2.53 g, yield 73%; tr = 22.86 min, 99% purity by GC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (dt, J = 1.4 and 7.1 Hz, 2H), 7.60 (m, 1H), 7.47 (t, J = 7.8 Hz, 2H), 6.88 (dt, J = 4.6 and 16.1 Hz, 1H), 6.36 (dt, J = 1.9 and 16.1 Hz, 1H), 5.02 (dd, J = 1.9 and 4.6 Hz, 2H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.7, 165.9, 140.0, 133.4, 131.0, 129.7, 129.5, 128.53, 63.1, 27.3; GC−MS: m/z (%) 203 (M^+−1, 1), 159 (2), 144 (2), 122 (2), 105 (100).

*(E)-5-(Benzyloxy)-pent-3-en-2-one (**3c**).*[34] Oil, 2.65 g, yield 82%; tr = 19.76 min, 99% purity by GC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (m, 5H), 6.79 (dt, J = 4.6, 9.1, and 16.2 Hz, 1H), 6.34 (dt, J = 1.6, 3.3, and 13.2 Hz, 1H), 4.58 (s, 2H), 4.20 (dd, J = 1.8 and 4.5 Hz, 2H), 2.26 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 197.9, 142.9, 137.8, 130.4, 128.5, 127.9, 127.7, 73.0, 68.9, 27.2; GC−MS: m/z (%) 190 (M^+, 1), 160 (5), 145 (10).

*(E)-5-((Triethylsilyl)oxy)pent-3-en-2-one (**3d**).* Liquid, 2.90 g, yield 80%; tr = 15.69 min, 98% purity by GC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.83 (dt, J = 3.7 and 15.8 Hz, 1H), 6.35 (dt, J = 2.1 and 15.8 Hz, 1H), 4.37 (dd, J = 2.1 and 3.7 Hz, 2H), 2.27 (s, 3H), 0.97 (t, J = 8.0, 9H), 0.62 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.4, 146.1, 128.9, 61. 9, 27.3, 6.7, 4.4; GC−MS: m/z (%) 214 (M^+, 3), 185 (75), 157 (40).

General Procedure for the Preparation of Compounds 4a−d. To a well stirred solution of **3a−d** (9.6 mmol) in CH_2Cl_2 (50 mL) at −50 °C was added dropwise a solution of Br_2 (1.54 g, 9.7 mmol) in CH_2Cl_2 (15 mL) over 1 h under a N_2 atmosphere. After complete consumption (checked by TLC) of the reactant, some drops of 1-hexene were added to remove the excess of Br_2. To the reaction mixture at −30 °C was added dropwise NEt_3 (0.99 g, 9.8 mmol), and the mixture was then left to reach 0 °C. The mixture was washed with HCl (0.2 M, 50 mL) and brine (sat., 50 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude materials of products of **4a** and **4c−d** were subjected to column chromatography purification (gradient: hexane/AcOEt from 9:1), whereas **4b** was crystallized in hexane/Et_2O (9:1; −30 °C).

*(Z)-3-Bromo-4-oxopent-2-en-1-yl Acetate (**4a**).* Yellow oil, 1.29 g, yield 61%; tr = 13.94 min, 97% purity by GC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (t, J = 4.8 Hz, 1H), 4.86 (d, J = 5.2 Hz, 2H), 2.48 (s, 3H), 2.13 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.9, 170.5, 139.3, 126.9, 64.0, 26.2, 20.7; GC−MS: m/z (%) 180 (M^+−41, 25), 178 (25), 163 (35), 161 (35),149 (30), 151 (30).

*(Z)-3-Bromo-4-oxopent-2-en-1-yl Benzoate (**4b**).* Yellow-white solid, 2.03 g, yield 75%; tr = 22.86 min, 98% purity by GC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (dt, J = 1.4 and 7.1 Hz, 2H), 7.60 (m, 1H), 7.47 (m, 2H), 7.32 (t, J = 5.2 Hz, 1H), 5.12 (d, J = 4.8 Hz, 2H), 2.50 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.00, 166.1, 139.4, 133.5, 129.8, 129.3, 128.5, 127.01, 64.5, 26.2; GC−MS: m/z (%) 283 (M^+−1, 1), 203 (10), 179 (1), 161 (15), 105 (100).

*(Z)-5-(Benzyloxy)-3-bromopent-3-en-2-one (**4c**).* Yellow oil, 1.56 g, yield 60%; tr = 22.36 min, 99% purity by GC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (m, 5H), 7.23 (dd, J = 4.9, 9.8 Hz, 1H), 4.56 (s, 2H), 4.33 (d, J = 4.9, 2H), 2.41 (s, 3H); ^{13}C NMR (100 MHz, CDCl3) δ 190.6, 143.0, 137.5, 128.5, 128.0, 127.9, 126.0, 73.3, 70.4, 25.8; GC−MS: m/z (%) 269 (M^++1, 1), 252 (M^+−18, 2), 162 (4), 91 (99).

*(Z)-3-Bromo-5-((triethylsilyl)oxy)pent-3-en-2-one (**4d**).* Yellow oil, 1.13 g, yield 40%; tr = 19.24 min, 99% purity by GC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (d, J = 4.6, 1H), 4.47 (d, J = 4.6 Hz, 1H), 2.47 (s, 3H), 0.98 (t, J = 8.1, 9H), 0.65 (q, J = 8.1, 6H); ^{13}C NMR (100 MHz, CDCl3) δ 190.9, 146.5, 124.4, 63.7, 25.9, 6.5, 4.3; GC−MS: m/z (%) 292 (M^+, 1), 265 (40), 237 (20).

General Procedure for the Screening of the OYE Mediated Reduction. The substrate **4a−d** (5 μmol) dissolved in DMSO (10 μL, final 5 mM substrate, 1% DMSO cosolvent) was added to a KP_i buffer solution (0.99 mL, 50 mM, pH according to Table 1) containing glucose (4 equiv with respect to **4a−d**), $NADP^+$ (0.1 mM), GDH (5 U mL^{-1}), and an OYE1-3 (12÷100 μg mL^{-1}, according to Table 1). The mixture was stirred for 24 h in an orbital shaker (180 rpm, 30 °C). The solution was extracted with EtOAc (3 × 0.5 mL), centrifuged (15000*g*, 1.5 min). The combined organic phase was dried over Na_2SO_4 and analyzed by GC−MS; in the following, we report the GC−MS of **6a−c**. Conversions are reported in Table 1.

*3-Hydroxy-4-oxopentyl Acetate (**6a**).* tr = 11.12 min, GC−MS: m/z (%) 142 (M^+−18, 3), 118 (3), 43 (100).

*3-Hydroxy-4-oxopentyl Benzoate (**6b**).* tr = 22.00 min, GC−MS: m/z (%) 205 (M^+−18, 3), 163 (3), 122 (80).

*5-(Benzyloxy)-3-hydroxypentan-2-one (**6c**).* tr = 20.59 min, GC−MS: m/z (%) 190 (M^+−18, 3), 162 (19), 122 (80).

General Procedure for the Preparation of α-Bromo Ketone (*S*)-5a−c with OYE3. The substrate **4a−c** (1 mmol) dissolved in DMSO (1 mL, final 5 mM substrate, 0.5% DMSO cosolvent) was added to a KP_i buffer solution (200 mL, 50 mM, pH 6.00) containing glucose (4 equiv with respect to **4a−c**), $NADP^+$ (0.1 mM), GDH (5 U mL^{-1}), and OYE3 (65 μg mL^{-1}). The mixture was stirred for 24 h at room temperature. The solution was extracted with EtOAc (3 × 50 mL), the organic phase was dried over Na_2SO_4 and concentrated under reduced pressure, and the crude material was submitted to column chromatography purification (eluent: gradient hexane/AcOEt from 9:1).

*(S)-3-Bromo-4-oxopentyl Acetate ((S)-**5a**).* 118 mg, yield 56%; tr = 12.45 min, 97% purity by GC; $[\alpha]_D$ = −1.14° (*c* 1.40, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.39 (dd, J = 5.9 and 8.4 Hz, 1H), 4.20 (m, 2H), 2.39 (m, 1H), 2.39 (s, 3H), 2.20 (m, 1H), 2.05 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.9, 170.7, 61.6, 49.9, 32.4, 26.7, 20.8; GC−MS: m/z (%) 182 (M^+−41, 20), 180 (20), 164 (25), 162 (25),138 (4), 136 (4); HRMS (ESI) calcd for $C_7H_{11}BrNaO_3^+$ $[M + Na]^+$ 244.9784, found 244.9793.

*(S)-3-Bromo-4-oxopentyl Benzoate ((S)-**5b**).* 285 mg, yield 86%; tr = 22.89 min, 99% purity by GC; $[\alpha]_D$ = +1.34° (*c* 0.56, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (m, 2H), 7.58 (m, 1H), 7.45 (m, 2H), 4.47 (m, 3H), 2.56 (m, 1H), 2.41 (s, 3H), 2.35 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 200.9, 166.4, 133.2, 129.8, 129.6, 128.5, 62.1, 49. 9, 32.6, 26.8; GC−MS: m/z (%) 244 (M^+−41, 5), 242 (5), 205 (5), 163 (15); HRMS (ESI) calcd for $C_{12}H_{13}BrNaO_3^+$ $[M + Na]^+$ 306.9940, found 306.9948.

*(S)-5-(Benzyloxy)-3-bromopentan-2-one ((S)-**5c**).* 230 mg, yield 85%; tr = 21.38 min, 99% purity by GC; $[\alpha]_D$ = −114° (*c* 1.02, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (m, 5H), 4.56 (m, 1H), 4.49 (s, 2H), 3.61 (m, 2H), 2.39 (s, 1H), 2.35 (m, 4H), 2.15 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 201.4, 138.0, 129.4, 127.7, 127.7, 73.2, 67.0, 51.0, 33.8, 26.5; GC−MS: m/z (%) 244 (1), 163 (2), 131 (55), 91 (99); HRMS (ESI) calcd for $C_{12}H_{15}BrNaO_2^+$ $[M + Na]^+$ 293.0148, found 293.0152.

General Procedure for the Screening Scale of the ADH Mediated Reduction. The substrate **5a−c** (5 μmol) dissolved in DMSO (10 μL, final 5 mM substrate, 1% DMSO cosolvent) was added

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to a KP_i buffer solution (0.99 mL, 50 mM, pH 7.0) containing glucose (4 equiv with respect to **5a−c**), NAD^+ and $NADP^+$ (0.1 mM each), GDH (5 U mL^{-1}), and an ADH (200 μg mL^{-1}, according to Table 2). The mixture was stirred for 12 h in an orbital shaker (180 rpm, 30 °C). The workup was the same as that used for the OYEs screening. One day before the GC−MS analysis, the sample was derivatized by addition of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide. Conversions and diastereoselectivities are reported in Table 2.

General Procedure for the Screening of the OYE3 + ADHs Cascade Reduction. The substrate **4b−c** (5 μmol) dissolved in DMSO (10 μL, final 5 mM substrate, 1% DMSO cosolvent) was added to a KP_i buffer solution (0.99 mL, 50 mM, pH 7.0) containing glucose (4 equiv with respect to **4b−c**), NAD^+ and $NADP^+$ (0.1 mM each), GDH (5 U mL^{-1}), OYE3 (65 μg mL^{-1}), and an ADH (32÷390 μg mL^{-1}, according to the ratio indicated in Table 3). The workup and the GC−MS analysis are the same as those used for ADHs screening. Conversions and diastereoselectivities are reported in Table 3.

General Procedure for the Preparation of Bromohydrin 7b−c by OYE3 + ADHs Cascade Reduction. The substrate **4b−c** (2 mmol) dissolved in DMSO (1 mL, final 10 mM substrate, 0.5% DMSO cosolvent) was added to a KP_i buffer solution (200 mL, 50 mM, pH 7) containing glucose (8 mmol), NAD^+ or $NADP^+$ (0.1 mM according to ADH dependence), GDH (5 U mL^{-1}, 2000 U), ADH (see Table 3), and OYE3 (65 μg mL^{-1}). The mixture was stirred for 1 day at rt. The reaction mixture was extracted with EtOAc (3 × 50 mL), and the organic phase was dried over Na_2SO_4, concentrated under reduced pressure, and submitted to column chromatography purification.

*(3S,4S)-3-Bromo-4-hydroxypentyl Benzoate (syn-**7b**).* Oil, 501 mg, yield = 87.4%; $[\alpha]_D = -52.9°$ (*c* 1.4, CH_2Cl_2); tr = 23.63 min, 95% purity by GC; *de* = 98% by GC; ^1H NMR (400 MHz, $CDCl_3$) δ 8.03 (m, 2H), 7.57 (m, 1H), 7.44 (m, 2H), 4.66 (m, 1H), 4.48 (m, 1H), 4.19 (dt, *J* = 4.1 and 10.1 Hz, 1H), 3.83 (bs, 1H), 2.38 (m, 2H), 2.15 (bs, 1H), 1.34 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 133.1, 130.0, 129.6, 128.4, 70.5, 62.9, 61.3, 34.7, 21.4; GC−MS: *m*/*z* (%) 244 (M^+-43, 3), 242 (3), 189 (40), 163 (35); HRMS (ESI) calcd for $C_{12}H_{15}BrNaO_3^+$ $[M + Na]^+$ 309.0097, found 309.0102.

*(3S,4R)-3-Bromo-4-hydroxypentyl Benzoate (anti-**7b**).* Oil, 520 mg, yield = 90%; $[\alpha]_D = -50.8°$ (*c* 1.3, CH_2Cl_2); tr = 23.78 min, 95% purity by GC; *de* = 99% by ^1H NMR; ^1H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 7.2 Hz, 2H), 7.49 (t, *J* = 7.2 Hz, 2H), 7.36 (t, *J* = 7.2 Hz, 2H), 4.55 (m, 1H), 4.38 (m, 1H), 4.21 (dt, *J* = 4.1 and 10.1 Hz, 1H), 3.85 (m, 1H), 2.85 (bs, 1H), 2.53 (s, 3H), 2.32 (m, 1H), 2.10 (m, 1H), 1.24 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 133.1, 130.0, 129.6, 128.4, 70.2, 62.6, 60.5, 32.8, 19.4; GC−MS: *m*/*z* (%) 244 (M^+-43, 3), 242 (3), 189 (40), 163 (35); HRMS (ESI) calcd for $C_{12}H_{15}BrNaO_3^+$ $[M + Na]^+$ 309.0097, found 309.0105.

*(2S,3S)-5-(Benzyloxy)-3-bromopentan-2-ol (syn-**7c**).* Oil, 487 mg, yield = 89%; tr = 22.12 min, 99% purity by GC; $[\alpha]_D = -37.1°$ (*c* 1.6, CH_2Cl_2); *de* = 98% by GC; ^1H NMR (400 MHz, $CDCl_3$) δ 7.34 (m, 5H), 4.53 (s, 2H), 4.25 (m, 1H), 3.68 (m, 3H), 2.29 (m, 3H), 1.30 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.0, 128.5, 127.8, 73.3, 70.0, 67.8, 62.3, 35.8, 21.4; GC−MS: *m*/*z* (%) 272 (M^+-1, 1), 175 (5), 107 (45), 91 (99); HRMS (ESI) calcd for $C_{12}H_{17}BrNaO_2^+$ $[M + Na]^+$ 295.0304, found 295.0322.

*(2R,3S)-5-(Benzyloxy)-3-bromopentan-2-ol (anti-**7c**).* Oil, 493 mg, yield = 90.3%; tr = 22.42 min, 99% purity by GC; *de* = 98% by GC; $[\alpha]_D = -29.0°$ (*c* 1.0, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 7.34 (m, 5H), 4.54 (s, 2H), 4.31 (q, *J* = 5.7, 11.2 Hz, 1H), 3.92 (q, *J* = 5.5 and 11.1 Hz, 1H), 3.74 (m, 1H), 3.65 (m, 1H) 2.60 (d, *J* = 5.6 Hz, 1H), 2.15 (q, *J* = 5.6, 11.6 Hz, 2H), 1.30 (d, *J* = 6.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.9, 128.5, 127.7, 73.4, 70.5, 67.5, 61.4, 34.0, 19.6; GC−MS: *m*/*z* (%) 272 (M^+-1, 1), 175 (5), 107 (45), 91 (99); HRMS (ESI) calcd for $C_{12}H_{17}BrNaO_2^+$ $[M + Na]^+$ 295.0304, found 295.0318.

*(3S,4S)-3-Bromopentane-1,4-diol ((3S,4S)-**8**).* To the reaction mixture of the cascade process (OYE3 + EVO030) for substrate **4c** was added lipase from *Candida rugosa* (30 mg); the neutral pH was maintained with a pH controlled dosing pump (1 M NaOH). After completeness of hydrolysis, the solution was washed with Et_2O/hexane (1:1, 2 × 30 mL). Then, the aqueous phase was extracted with EtOAc (150 mL) in a continuous liquid−liquid extractor apparatus; occasionally, the pH was adjusted to the neutrality. After 12 h, the organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to give the crude material of sufficient purity for the next step. Overall yield 78%; oil, 281 mg, 94% purity by ^1H NMR; $[\alpha]_D = -23.1°$ (*c* 0.9, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 4.26 (m, 1H), 3.89 (m, 1H), 3.81 (m, 2H), 2.92 (bs, 2H), 2.15 (m, 2H), 1.31 (d, *J* = 6.3 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 70.2, 61.6, 60.2, 38.0, 21.1.

General Procedure for the Ring Closure of Bromohydrins 7c. To an ice-cooled solution of **7c** (1.0 mmol) in dry THF (5 mL) was added NaH (oil suspension 60%, 3.4 mmol of NaH). The reaction mixture was left stirring at 0 °C for 2 h; then, to it were added Et_2O (20 mL) and NH_4Cl (sat., 30 mL). The organic layer was washed several times with brine (sat., 3 × 20 mL) and NH_4Cl (sat., 20 mL); after that, it was dried over Na_2SO_4 and concentrated under reduced pressure. The crude material was submitted to column chromatography purification (eluent: gradient hexane/AcOEt, 99:1 → 7:3) to give the epoxide as a colorless oil.

*(2R,3S)-2-(2-(Benzyloxy) ethyl)-3-methyloxirane ((2R,3S)-**9**).* Yield 87%; 168 mg; tr = 18.69 min, 99% purity by GC; $[\alpha]_D = -11.0°$ (*c* 0.8, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$) δ 7.32 (m, 5H), 4.54 (s, 2H), 3.66 (dd, *J* = 5.8, 7.1 Hz, 2H), 3.06 (m, 2H), 1.88 (m, 2H), 1.28 (d, *J* = 5.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.5, 128.5, 127.7, 73.2, 67.7, 54.8, 52.6, 28.5, 13.5; GC−MS: *m*/*z* (%) 191 (M^+-1, 1), 174 (M^+-18, 2), 159 (15), 91 (99).

*(2R,3R)-2-(2-(Benzyloxy)ethyl)-3-methyloxirane ((2R,3R)-**9**).* Yield 80%; 154 mg; tr = 18.48 min, 99% purity by GC; $[\alpha]_D = 28.4°$ (*c* 0.84, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$) δ 7.34 (m, 5H), 4.53 (s, 2H), 3.61 (m, 2H), 2.79 (m, 2H), 1.91 (m, 1H), 1.77 (m, 1H), 1.31 (d, *J* = 4.9 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 138.5, 128.5, 127.7, 73.2, 67.2, 57.5, 54.8, 32.7, 17.8; GC−MS: *m*/*z* (%) 191 (M^+-1, 1), 174 (M^+-18, 2), 159 (15), 91 (99).

*2-((2R,3S)-3-Methyloxiran-2-yl)ethanol ((2R,3S)-**10**).* To a stirred solution of (2*R*,3*S*)-**9** (211 mg, 1.1 mmol) in Et_2O (5 mL) was added Pd/C (5 wt %, 10 mg) under an H_2 atmosphere, and it was left to stir at rt until complete absorption of H_2. Thus, the reaction mixture was filtered on a Celite pad and concentrated slowly under reduced pressure. The crude material was submitted to column chromatography purification (eluent: gradient pentane/Et_2O, 99:1 → 1:1) to give (2*R*,3*S*)-**9** as a colorless liquid. Yield 94%; 105 mg; tr = 16.26 min, 99% purity by GC; $[\alpha]_D = +24.5°$ (*c* 1, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 3.87 (m, 2H), 3.08 (m, 2H), 1.78 (m, 3H), 1.29 (d, *J* = 5.4 Hz, 3H); 13C NMR (100 MHz, $CDCl_3$) δ 60.6, 55.0, 52.3, 30.3, 13.3; GC−MS: *m*/*z* (%) 101 (M^+-1, 5), 71 (80), 57 (30), 45 (99).

*(2S,3R)-2-Methyltetrahydrofuran-3-yl Methanesulfonate ((2S,3R)-**11**).* To a stirred solution of CSA (47 mg, 0.2 mmol) in dry CH_2Cl_2 (2 mL) was added dropwise the epoxyalcohol **10** (102 mg, 1.0 mmol) in dry CH_2Cl_2 (2 mL) at 28 °C. The reaction was completed after 6 days. Due to the high volatility of the tetrahydrofuran (2*S*,3*R*)-**2**, the reaction mixture was used without any workup for the next step; tr = 11.76 min, purity 75% by GC; GC−MS: *m*/*z* (%) 102 (M^+, 1), 87 (M^+-15, 3), 69 (2), 57 (99); we did not isolate it, since it is a very volatile product. To the ice-cooled stirred solution of **2** were added NEt_3 (202 mg, 2.0 mmol) and methanesulfonyl chloride (149 mg, 1.3 mmol), and it was left stirring overnight at rt. After 1 day, to the reaction mixture was added water (100 mg); then it was concentrated under reduced pressure and the crude material was submitted to column chromatography purification (eluent: gradient hexane/AcOEt, 8:2 → 1:1) to give the mesylate as a yellow oil. Yield 64%; 115 mg; tr = 13.15 min, 99% purity by GC; $[\alpha]_D = -34.1°$ (*c* 0.92, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 4.81 (m, 1H), 4.11 (m, 1H), 4.01 (m, 1H), 3.89 (m, 1H), 3.02 (d, *J* = 1.4 Hz, 3H), 2.27 (m, 1H), 2.16 (m, 1H), 1.25 (dd, *J* = 1.9, 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 85.1, 79,8, 66.4, 38.6, 32.6, 18.5; GC−MS: *m*/*z* (%) 165 (M^+-15, 5), 136 (5), 101 (50), 84 (50).

*(2S,3S)-2-Methyltetrahydrofuran-3-thiol Acetate ((2S,3S)-**3**).* A stirred solution of AcSK (171 mg, 1.5 mmol) and **12** (90 mg, 0.5 mmol) in dry DMF (2 mL) under a N_2 atmosphere was heated at reflux for 6 h; then, to it was added Et_2O (5 mL). The reaction mixture was filtered on a Celite pad; the Celite was washed several times with Et_2O (3 × 5 mL). Then, the combined organic phase was washed with brine (sat., 5 mL × 3), dried over Na_2SO_4, and concentrated under

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J. Org. Chem. 2017, 82, 2114−2122

reduced pressure. The crude material was submitted to column chromatography purification (eluent: gradient hexane/AcOEt, 9:1 → 7:3) to give the product as a yellow oil. Yield 70%; 56 mg; tr = 10.97 min, 99% purity by GC; *de* > 99.9 by GC, *ee* = 99.6% by chiral GC, tr = 20.95 min; $[\alpha]_D = -21.4°$ (*c* 0.78, CH_2Cl_2) vs Lett. $[\alpha]_D = -8.09°$ (*c* 4.37, $CHCl_3$, ref 2a); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.13 (m, 1H), 4.04 (m, 1H), 3.91 (dt, *J* = 8.3, 16.5, 6.2 Hz, 1H), 3.74 (dt, *J* = 8.4, 16.9, 6.2 Hz, 1H), 2.47 (m, 1H), 2.34 (s, 3H), 1.94 (m, 1H), 1.20 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.4, 76.5, 66.0, 46.3, 33.6, 30.6, 16.9; GC–MS: *m*/*z* (%) 159 (M^+-1, 1), 145 (M^+-15, 3), 116 (10), 103 (4), 84 (80).

*(2S,3S)-3-Bromo-2-methyltetrahydrofuran ((2S,3S)-**1**).* To an ice-cold and well stirred solution of **8** (300 mg, 1.6 mmol) in pyridine (0.8 mL) was added portion wise tosyl chloride (430 mg) under a N_2 atmosphere. After complete consumption of **8** (checked by TLC, AcOEt/hexane 6:4), the reaction was left at rt for 1 h. Then, the ice-cooled reaction mixture was diluted with Et_2O/pentane (1:1, 5 mL). After trituration of the gummy solid, the supernatant liquid was separated, and washed with HCl (1 M, 3 × 5 mL). The organic phase was dried over Na_2SO_4 and then concentrated under reduced pressure to give a yellow liquid of sufficient purity for the next step. Yield 54%; 143 mg; a sample was distilled with a bulb-to-bulb apparatus (50 °C, 1 mmHg); tr = 5.81 min, 94% purity by GC; $[\alpha]_D = +8.4°$ (*c* 0.25, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.46 (m, 1H), 4.14 (q, *J* = 8.4 and 15.8 Hz, 1H), 3.86 (m, 2H), 2.62 (m, 1H), 2.42 (m, 1H), 1.35 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 77.8, 65.9, 55.7, 37.7, 18.9; GC–MS: *m*/*z* (%) 166 (M^+ +1, 15), 164 (M^+ -1, 15), 149 (M^+,100), 147 (100), 120 (23), 118 (23).

*(2S,3R)-2-Methyltetrahydrofuran-3-thiol Acetate ((2S,3R)-**3**).* A stirred solution of AcSK (171 mg, 1.5 mmol), 18-crown-6 (50 mg), and **2** (102 mg, 0.6 mmol) in dry DMSO (1 mL) under a N_2 atmosphere was heated at 95 °C refluxed for 24 h; then, to it was added Et_2O (5 mL). The reaction mixture was filtered on a Celite pad; the Celite was washed several times with Et_2O (3 × 5 mL). Then, the combined organic phase was washed with brine (sat., 5 mL × 3), dried over Na_2SO_4, and concentrated under reduced pressure. The crude material was submitted to column chromatography purification (eluent: gradient hexane/AcOEt, 9:1 → 7:3) to give the product as a yellow oil. Yield 49%; 47 mg; tr = 10.07 min, 98% purity by GC; *de* = 97% by GC–MS, *ee* > 99% by chiral GC, tr = 17.17 min; $[\alpha]_D = -25.4°$ (*c* 0.78, CH_2Cl_2) Lett. $[\alpha]_D = -20.5°$ (*c* 4.49, $CHCl_3$, ref 2a); ^{1}H NMR (400 MHz, CDCl3) δ 3.97 (m, 1H), 3.79 (m, 2H), 3.53 (m, 1H), 2.48 (m, 1H), 2.34 (s, 3H), 1.86 (m, 1H), 1.28 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.3, 78.0, 66.7, 46.7, 33.3, 30.6, 19.2; GC–MS: *m*/*z* (%) 159 (M^+ -1, 1), 145 (M^+ -15, 1), 116 (10), 103 (4), 84 (80).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b02927.

All ^{1}H and ^{13}C NMR spectra, the study of the epoxide/tetrahydrofuran isomerization on a model compound, and the chiral GC of compound **1**. In addition, the screening on the chemoselectivity of ADHs (PDF)

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Notes

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