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

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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-steps multienzymatic stereoselective cascade reduction of α-bromo-α,β-unsaturated ketones to give the corresponding bromohydrins in good yields, with high *ee*s and *de*s. 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 tetrahydrofurn-2-ol. Finaly, 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 decades a considerable attention has been devoted to the development of efficient and stereocontrolled methodologies for the synthesis of 2,5-disubsituted tetrahydrofuranic derivates,[1] key structural fragments of many natural products. However, no so much effort 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 knowledge of the authors no enantioselective syntheses of the very useful bromoderivative[5] **1** have never 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*s ranged between 62% and 80%). Then, the two enantiomers of *trans*-**2** were obtained by Mitsunobu inversion of C(3) stereocentres of the *cis* enantiomers, conserving most of their original optical purity.[2]

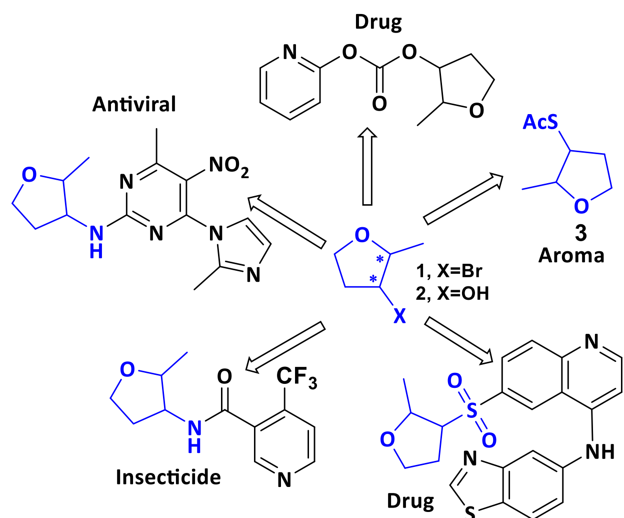


Figure 1. Selected examples of relevant industrial products bearing a 2-methyl-3-substitued tetrahydrofuran moiety.

RESULTS AND DISCUSSION

In Figure 2 is shown 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 is then ring-closed affording **1**. Often, diastereo- and/or enantiocomplementary asymmetric catalytic transformations require completely new and time consuming set-up 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**.

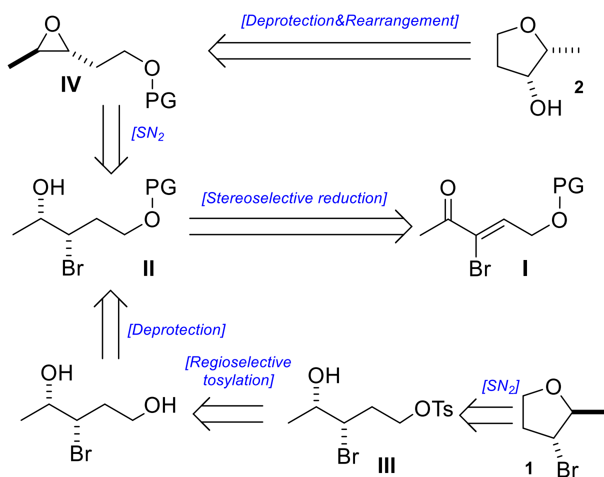
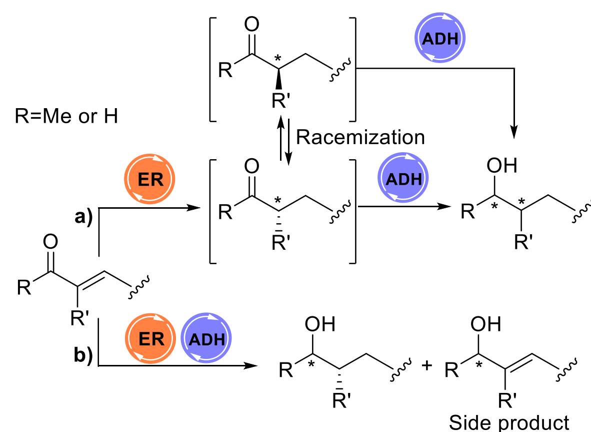


Figure 2. Retrosynthesis of the diastereocomplementary synthons **1** and **2**.

According to our retrosynthetic plan, especially for the crucial stereoselective transformations, a biocatalytic approach 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. Even the emerging organocatalysis does not offer in this case a real alternative to biocatalysis[6] in terms of mildness of the reaction conditions and selectivity.

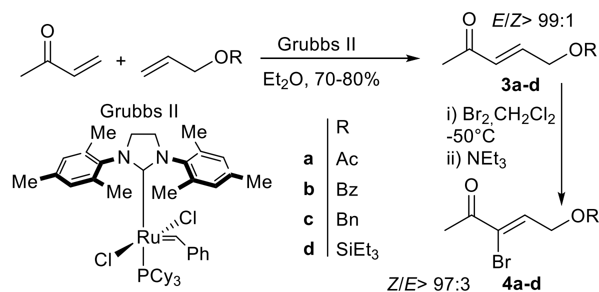
In the last few years several one-pot multienzymatic systems[7,8] have been investigated. In relation to the chemoselectivity of the enzymes involved, the latter can be added to the reaction mixture either step by step or simultaneously.

In particular, systems based on the combination of an enzyme with ene-reductase (ER) activity with a second one with alcoholdehydrogenase (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 α,β-unsaturated ketones/aldehydes: the C=C double bond by ERs and the CO carbonyl group by ADHs.



Scheme 1. ADH and ER multienzymatic system for the synthesis of optically enriched alcohols: a) sequential enzyme addition; b) cascade procedure.

The products are usually obtained in good yields, with excellent *ee*s, and for the ketones, with good *de*s 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.



Scheme 2. Synthesis of α-bromo-α,β-unsaturated ketones **4a-d**.

The synthesis of the starting materials **4a-d** is shown in Scheme 2. First, the α,β-unsaturated ketones **3a-d** with 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 very cheap methylvinyl ketone with respect the *O*-protected allylic alcohol, in presence of the 2nd generation of Grubbs catalyst[12,13] (see SI for the preparation of alcohols). Then, the ketones were transformed into the (*Z*)-α-bromoderivative (**4a-d**) by a one-pot two steps 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, with the substrates **4a-d** in our hands we tested the C=C double bond reduction 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 co-substrate. The results and the experimental conditions are summarized in Table 1.

Table 1. ER catalyzed reduction of (*Z*)-**4a-d**.[a]

(*Z*)-**4a-d** →(ER) (3*S*)-**5a-d** + **6a-d** (H_2O); R: **a** Ac, **b** Bz, **c** Bn, **d** $SiEt_3$

Entry	R	ER	Subs. (mmol)	pH	Conv.[b] (%)	Ratio[b] 5/6
1	Bn	OYE1	5 10-3	7.0	>99	88:12
2	Bn	OYE2	5 10-3	7.0	>99	90:10
3	Bn	OYE3	5 10-3	7.0	>99	87:13
4	Bn	OYE1	5 10-3	7.0	20 [c]	88:12
5	Bn	OYE2	5 10-3	7.0	16 [c]	90:10
6	Bn	OYE3	5 10-3	7.0	24 [c]	87:13
7	Bn	OYE3	5 10-3	7.0	100 [c]	85:15
8	Bn	OYE2	5 10-3	6.0	100 [d]	94:6
9	Bn	OYE3	5 10-3	5.2	88 [d]	94:6
10	Bn	OYE3	5 10-3	4.0	73 [d]	96:4
11	Bn	OYE3	1.0	6.0	85 [e]	93:7
12	Ac	OYE3	5 10-3	6.0	95	94:6
13	Bz	OYE3	5 10-3	6.0	100	96:4
14	$SiEt_3$	OYE3	5 10-3	6.0	_[f]	_
15	Ac	OYE3	1.0	6.0	53 [d]	93:7
16	Bz	OYE3	1.0	6.0	86 [d]	96:4

(a) Typical experimental conditions of the ER screening on 1 mL scale: substrate conc. 5 mM, 1 % DMSO co-solvent, 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) No conversion.

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 -*O*$SiEt_3$ protective group.[17] Then, in view of a reaction scale-up we optimized the minimum amount of ER to be added, using **4c** as model compound. First, by lowering the amount of enzyme approximately to an eighth (12 μg/mL), it become clear that OYE3 was the ER with the highest catalytic activity (entry 5 *vs* 4 and 6), but at the same time the conversion dropped down to an unsatisfactory 24%. Thus, we found that the best compromise between conversion and minimum amount of enzyme 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 determinate the *ee* by chiral GC or HPLC was unsuccessful.

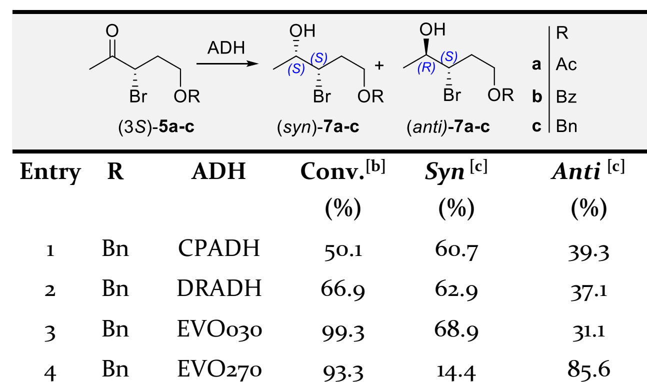
However, at the end of each biotransformation together to the α-bromoketone product (**5a-c**) and to the unreacting (*E*) diastereoisomer[19] ((*E*)-**4a-c**) a small amount of α-hydroxyketone, *i.e.* **6a-c** (detected by GC-MS, Table 1), was always present. This result can be related to the fact that, 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 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 synthetized (see SI). The product/side-product ratio was more or less 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 OYE family exhibit their highest activity at neutral pH,[22] we repeated the reductions at more acidic pH with the aim of minimizing the hydrolysis, since these reactions are usually carried out in presence of inorganic bases. Indeed, for ketone **4c** at pH=4.0 the amount of **6c** was significantly lower (94:6, entry 8) but in contrast the conversion into **5c** was worst, around 73% (entry 10) and even by adding more enzyme was not possible to reobtain a quantitative conversion.

This trend was confirmed working at less acidic conditions (pH=5.2 , entries 9); at the end we found that for a preparative scale (≈ 1 mmol) the optimum between the amount of enzyme needed to obtain the highest possible conversion and the minimization of the side-product formation was reached at pH=6.0 (entry 8). Thus, operating in such experimental conditions, each of the α-bromoketones was isolated after column chromatography purification in a good yield: around 85% for **5b**-**c**. Only 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.

Going more in details, we found that most of screened ADHs do not reduce the carbonyl group of the α,β-unsaturated ketones **4a**-**c** (see Table S1 of SI), this very high chemoselectivity[9c] 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*) stereoselectivity were identified (entries 1-3), but the diastereomeric excess of the *syn*-bromohydrin, *i.e.* (2*S*,3*S*)-**7c**, were not so exciting, 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*s 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 *de*s were higher, but still far to be 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 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**.

Table 2. ADHs catalyzed reduction of (3*S*)-**5a**-**c**.[a]

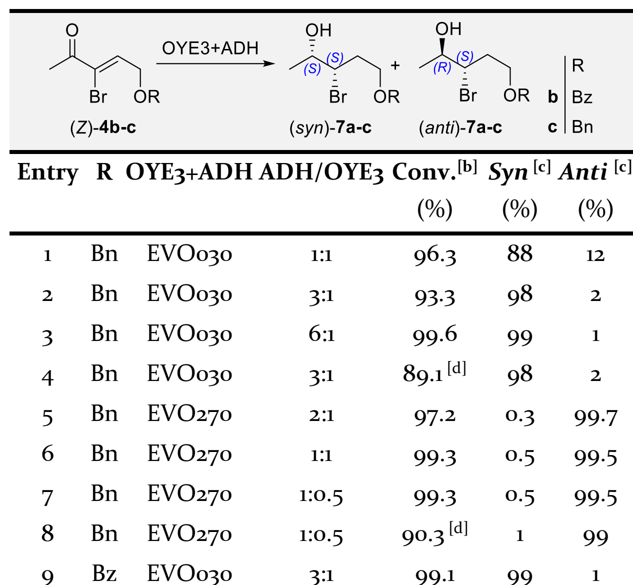


Entry	R	ADH	Conv.[b] (%)	*Syn* [c] (%)	*Anti* [c] (%)
1	Bn	CPADH	50.1	60.7	39.3
2	Bn	DRADH	66.9	62.9	37.1
3	Bn	EVO030	99.3	68.9	31.1
4	Bn	EVO270	93.3	14.4	85.6
5	Bn	EVO420	99.6	22.6	77.4
6	Bn	KRED	99.1	20.8	79.2
7	Bn	PLADH	97.2	17.8	82.1
8	Ac	EVO030	99.3	61.9	38.1
9	Bz	EVO030	98.6	65.9	34.1
10	Bz	EVO270	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 co-solvent, 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, were transformed into the *O*-silyl derivatives by adding *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide 12 hr before of GC analysis.

Even if the preliminary results were not encouraging, we thought that the low *de*s 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 with 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 set-up of a cascade procedure was not be very complicated, since the selected ADHs (EVO030 and EVO270) are completely chemoselective towards the saturated ketones, and the detrimental formation of the allylic alcohol side-product was therefore prevented.

Table 3. Multienzymatic cascade reduction of (*Z*)-**4b**-**c**.[a]



Entry	R	OYE3+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	1:0.5	99.3	0.5	99.5
8	Bn	EVO270	1:0.5	90.3 [d]	1	99
9	Bz	EVO030	3:1	99.1	99	1

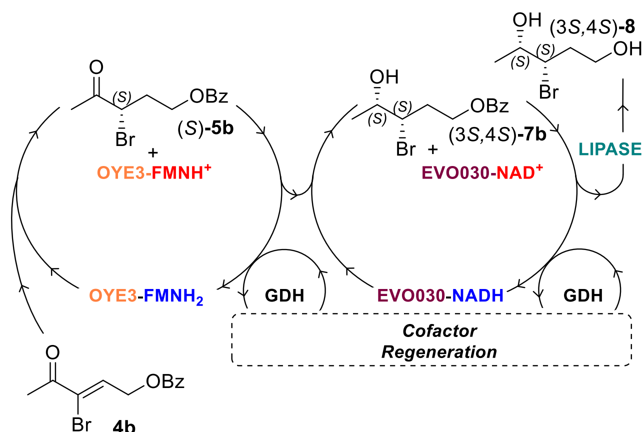
10	Bz	EVO030	3:1	87.4 [d]	99	1
11	Bz	EVO270	1:0.5	90.3 [d]	1	99

(a) Typical experimental conditions of the OYE3+ADH screening on 1 mL scale: substrate conc. 5 mM, 1 % DMSO co-solvent, 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 hr, 180 rpm; (b) By GC-MS; (c) By GC-MS, since the bromohydrins *syn* and *anti* are not well separated, were transformed into the *O*-silyl derivatives by adding *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide 12 hr before of GC-MS analysis; (d) The reaction was repeated on a preparative scale.

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. 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%), by increasing the ratio to 3:1 and then to 6:1 the *de* further increased up to an excellent value of 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]

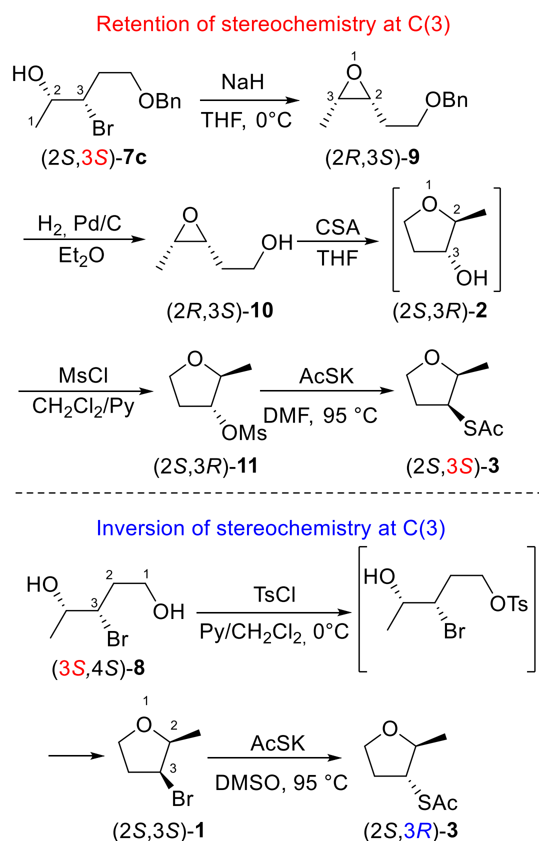
Then, we repeated the cascade process with the enantio complementary ADH, *i.e.* EVO270. The 1:1 enzymes ratio was already good since we obtained the *anti*-**7c** with an excellent *de* >99% (entry 5). In this case the activity of EVO030 toward the intermediate (3*S*)-**5c** was so high that we could halve the amount of enzyme without any significant detrimental effects (entry 7). Thus, with the optimized experimental conditions, we scale-up the reaction (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 negligible almosts.

Lastly, in order to accomplish our synthetic plan we had to cleave the protective group of (3*S*,4*S*)-**6b**; however, the classical deprotection protocol by alkaline hydrolysis is unfeasible with the presence of the bromohydryn functionality, thus, we found more convenient to carry out an enzymatic hydrolysis of the benzoate[25] ester at neutral pH. Indeed, by adding *Candida rugosa* lipase to the reaction mixture at the end of the cascade process[26] (OYE3+EVO030), it was possible to obtained the diol (3*S*,4*S*)-**8** in an overall yield of 77% (Scheme 3) without column purification.



Scheme 3. Coupling of OYE3+EVO030 and lipase from *Candida Rugosa* catalyzed hydrolysis step in the one-pot synthesis of (2*S*,3*S*)-**8**.

Stereoselective synthesis of the roasted meat aroma. The 2-methyl-tetrahydrofuran-3-thioacetate **3** is likelyone of the main aromas of the roasted meat, and it is produced in the racemic form during cooking process by a Maillard reaction. Quite often chiral fragrances and flavors exhibit different odor properties in 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 the most roasting meat characterizing odor between all possible stereoisomers of **3**. In contrast, both enantiomers of *cis*-**3** diastereoisomer imparts to the food an unpleasant sulfur note.



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

Thus, with (2*S*,3*S*)-**7c** and (3*S*,4*S*)-**8** in our hands we prepared stereoisomers (2*S*,3*S*)-**3** and (2*S*,3*R*)-**3** of the meat roasted 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, then the *O*Bn protective group was removed by hydrogenolysis with Pd/C affording the epoxyalcohol (2*R*,3*S*)-**10**. The latter, was rearranged in 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 more handy mesyl derivative (2*S*,3*R*)-**11** in an overall yield of 64%. The rearrangement of β-hydroxy epoxides to tetrahydrofuran-2-ol proceeds with inversion of stereochemistry at C(3) stereocentre of the epoxide ring; to our knowledge just 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 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 astonishing *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 bromo derivative (2*S*,3*S*)-**1** in an overall yield of 54% and with an excellent *de*>97% (by GC-MS, see 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 an astonishing regioselectivity. Then, the bromoderivative **2** 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 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 temperature below 100 °C in DMSO and in 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 type 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[9e] strategy). A remarkable work to solve this gap of ERs has been done recently by Stewart *et al* by a mutagenic approach.[32] In this case we by-passed 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 pro (*R*) enantioselectivity, by means of our synthetic route 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 anhydryficated with molecular sieves (4 Å). ^{1}H and ^{13}C NMR spectra were recorded on 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. The GC-MS analyses of all compounds were performed on a HP-5MS column (30 m x 0.25 mm x 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, Mega, Milano) with a program temperature: 60 °C / 1 °C min^{-1}/95 °C/90 °C min^{-1} / 220 °C (2 min). TLC analyses were performed on pre-coated 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 Dr. Kernchen Propol digital automatic polarimeter at 589 nm and are given at rt in °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]

Enzymes and Strains. OYE1 from *Saccharomices 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 Bradford test, using bovine serum albumine (BSA) as a standard. CPADH from *Candida parapsilosis* was purchased from Jülich. 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 2nd gen. Grubbs' catalyst (144 mg, 0.17 mmol). After 24 hr 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; 1H 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 drop-wise 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 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 reduce pressure. The crude material of products of **4a** and **4c-d** were subjected to column chromatography purification (gradient: hexane/AcOEt from 9:1), whereas **4b** was crystalized 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 x 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 x 50 mL), the organic phase was dried over Na_2SO_4, 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).

(*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).

(*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); ^1H 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).

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 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 work-up was the same used for the OYEs screening. One day before of 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 work-up and the GC-MS analysis are the same 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 (3x50 mL), the organic phase was dried over Na_2SO_4, concentrated under reduced pressure and submitted to column chromatography purification.

(3*S*,4*S*)-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).

(3*S*,4*R*)-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).

(2*S*,3*S*)-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).

(2*R*,3*S*)-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).

(3*S*,4*S*)-3-bromopentane-1,4-diol ((3*S*,4*S*)-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, 2x30 mL) and 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 hr the organic phase was dried over Na_2SO_4, 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 hr; then, it was added Et_2O (20 mL) and NH_4Cl (sat., 30 mL). The organic layer was washed several times with brine (sat., 3 x 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.

(2*R*,3*S*)-2-(2-(benzyloxy) ethyl)-3-methyloxirane ((2*R*,3*S*)-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).

(2*R*,3*R*)-2-(2-(Benzyloxy) ethyl)-3-methyloxirane ((2*R*,3*R*)-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-((2*R*,3*S*)-3-Methyloxiran-2-yl) ethanol ((2*R*,3*S*)-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 (elu-

ent: 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).

(2*S*,3*R*)-2-Metyltetrahydrofuran-3-yl methanesulfonate ((2*S*,3*R*)-11).
To a stirred solution of CSA (47 mg, 0.2 mmol) in dry CH_2Cl_2 (2 mL) was added dropwisely 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*S*)-**2**, the reaction mixture was used without any work-up 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** was added NEt_3 (202 mg, 2.0 mmol), 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).

(2*S*,3*S*)-2-Methyltetrahydrofuran-3-thiol acetate ((2*S*,3*S*)-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 hr; then, 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 (3x5 mL). Then, the combined organic phase was washed with brine (sat., 5 mL x 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 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); ^1H 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).

(2*S*,3*S*)-3-Bromo-2-methyltetrahydrofuran ((2*S*,3*S*)-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 chlorode (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 and washed with HCl (1 M, 3x5 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 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); ^1H 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).

(2*S*,3*R*)-2-Methyltetrahydrofuran-3-thiol acetate ((2*S*,3*R*)-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 reflux for 24 hr; then, 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 (3x5 mL). Then, the combined organic phase was washed with brine (sat., 5 mL x 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); ^1H 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. All ^1H and ^{13}C NMR spectra and the study of the epoxide/tetrahydrofuran isomerization on a model compound. In addition the screening on the chemoselectivity of ADHs. This material is available free of charge via the Internet at http://pubs.acs.org.

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SYNOPSIS TOC