

Biocatalysed Concurrent Production of Enantioenriched Compounds through Parallel Interconnected Kinetic Asymmetric Transformations

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Parallel interconnected kinetic asymmetric transformations were performed in order to obtain enantioenriched derivatives starting from a set of racemic or prochiral compounds. Thus, in a *one-*
10 *pot* reaction using two redox biocatalysts (a BVMO and an ADH) and a catalytic amount of cofactor that acts as a mediator, enantioenriched ketones, sulfoxides, and *sec*-alcohols were *concurrently* obtained in a strict *parallel* way minimising the quantity of reagents employed. By selecting the appropriate biocatalysts, this methodology is a potential tool for performing stereodivergent transformations.

15 Introduction

Enantioenriched alcohols, ketones, and esters are important synthons in the pharmaceutical and (agro)chemical industry.¹ To obtain them, several routes catalysed by (organo)metallic complexes have extensively been explored.² Enantiopure
20 sulfoxides play an important role as chiral auxiliaries as well as in medicinal and pharmaceutical chemistry.³ Different methodologies have been described for the preparation of these derivatives as, for instance, the asymmetric oxidation of the corresponding prochiral sulfides.⁴

25 In the last few years, several biocatalytic processes using redox enzymes such alcohol dehydrogenases (ADHs) and Baeyer-Villiger monooxygenases (BVMOs) have been developed due to the high selectivities obtained employing mild and environmental friendly conditions.⁵ The synthesis of
30 chiral secondary alcohols *via* reduction of ketones or oxidative kinetic resolutions of *rac*-alcohols using ADHs have attracted attention.⁶ Additionally, BVMOs represent a very promising family of redox biocatalysts which are able to catalyse the oxidation of carbonyl compounds as well as
35 certain heteroatoms with excellent regio- and/or enantioselectivities.⁷ In despite of all the advantages provided by these enzymes, their implementation at industrial scale usually remains impeded due to the high costs related to the nicotinamide cofactor needed by these redox biocatalysts.
40 Therefore, various chemical,^{8,9} electrochemical,¹⁰ and enzymatic¹¹ methods have been explored in order to regenerate the coenzymes NAD(P)H or NAD(P)⁺. However, these methodologies have several drawbacks such as harmful effects on the biocatalyst, high costs and loss of material,
45 which diminishes the *atom efficiency*¹² of the process.

In nature a huge number of chemically interconnected processes concurrently take place and the products are shared by different metabolic routes, forming a complex and effective metabolic network.¹³ For instance, redox trans-
50 formations share electron acceptors/donors such as NAD(P)⁺/NAD(P)H to connect oxidative and reductive

reactions. In an attempt to mimic such efficient natural processes, concurrent catalytic concepts are being developed for the synthesis of enantioenriched derivatives.¹⁴ A nice
55 example of this methodology was recently developed,¹⁵ by using three biocatalysts to simultaneously obtain morphinone and hydromorphone with a dehydrogenase and a reductase, respectively. The critical step was the employment of a transhydrogenase that permitted the internal cofactor-
60 recycling necessary to achieve each transformation (NADH and NADPH). Herein we describe a methodology which allows the concurrent preparation of enantioenriched derivatives in a strict parallel fashion, thereby minimising the quantity of reagents employed and maximising the *redox*
65 *economy*¹⁶ of the process by coupling two asymmetric transformations: a) *via* two kinetic resolutions or b) *via* a kinetic resolution and a desymmetrization reaction (Scheme 1). By a proper selection of both catalysts (an ADH and a BVMO) and using a catalytic amount of coenzyme (which
70 acts as connector) all possible enantiomers can be obtained in a one-pot process. xxx

<Scheme 1 here>

Scheme 1. Obtaining of enantioenriched compounds by interconnecting two asymmetric processes.

75 Results and discussion

When developing the system, several practical criteria were taken into consideration in advance. Since two different catalysts were used, parameters like pH, temperature or cofactor preference must be optimised to find compatible reaction conditions.
80 Another criterion to take into account is that each substrate should be converted by only one of the biocatalysts, since an undesired side reaction would yield a by-product, and more importantly, an incomplete process as result of ineffective cofactor recycling (see below).

85 As previously described, BVMOs catalyse irreversible oxidation processes at the expense of NADPH,¹⁷ while ADHs utilize NAD⁺ and/or NADP⁺. As these biocatalysts require stoichiometric amounts of the expensive NAD(P)H coenzyme,

we designed a coupled process that involves a BVMO-catalysed oxidation and an ADH-catalysed oxidative kinetic resolution of a *sec*-alcohol, where the coenzyme will undergo internal recycling (Scheme 1a).¹⁸ Phenylacetone monooxygenase (PAMO)¹⁹ from *Thermobifida fusca*, its M446G mutant²⁰ and 4-hydroxyacetophenone monooxygenase (HAPMO)²¹ from *Pseudomonas fluorescens* ACB were chosen as BVMOs due to their ability to catalyse enantioselective oxidations and to be readily obtainable in recombinant form.²² We selected two ADHs with the same coenzyme preference (NADP⁺) and opposite stereopreference: alcohol dehydrogenases from *Lactobacillus brevis* (LBADH)²³ and from *Thermoanaerobacter* sp. (ADH-T).²⁴ Firstly, we studied the interconnected kinetic resolution of (\pm)-2-phenylpentan-3-one **1a**^{22c} and (\pm)-2-octanol **3a** with the BVMO-ADH couple by using equimolar amounts of both compounds and a catalytic amount of the nicotinamide cofactor (Scheme 2 and Table 1). Initial experiments performed with HAPMO and ADH-T (entries 1-6) showed that the best reaction conditions were 20°C and pH 7.5 (entry 1), yielding (*R*)-**1a** and (*S*)-**2a**²⁵ in a highly selective process ($E \geq 200$).²⁶ When higher temperatures or pHs were used, lower conversions and selectivities were obtained, which is probably due to inactivation of HAPMO^{22c} and ADH-T. It is noteworthy that a catalytic amount of NADPH (0.2 mM) was sufficient to achieve a successful process. Due to the fact that ADH-T is a Prelog²⁷ enzyme, (*S*)-**3a** was selectively oxidised to **4a**, while remaining (*R*)-**3a** was obtained with high *ee*. When HAPMO was combined with LBADH at 20°C and pH 8.5²⁸ (entry 7), again an excellent double resolution was observed, although in this case alcohol (*S*)-**3a** was obtained. This shows that the selectivity of this system can simply be tuned by changing the employed biocatalysts. Reactions were also carried out using PAMO as biocatalyst

(entries 8-16). As shown in entry 8, when this enzyme was employed in combination with ADH-T, (*R*)-**1a**, (*S*)-**2a** and (*R*)-**3a** were achieved with conversions close to 50% in a process with excellent selectivity. The coupled system PAMO-LBADH was analysed in order to determine the best reaction conditions. The effect of pH when conducting the oxidations with PAMO at 20°C was studied (entries 9-12), obtaining the best conditions for the resolution at pH 8.5-9.0. Higher pHs produced a loss in both activity and selectivity (entry 12). Once established pH 8.5 as the optimum for this system, the effect of the temperature was determined. Reactions could be performed at 30-40°C with excellent selectivities and good conversions (entries 13-14), while a further increase of the temperature at 50°C led to a loss in selectivity ($E = 29$, entry 15) and finally an important decrease in the activity at 60°C (entry 16). It is important to note that the system can even work at this high temperature. Surprisingly, in some cases traces of *n*-hexyl acetate derivative **5a** were detected.²⁹ This product is formed in a cascade process by the ADH-catalysed oxidation of (\pm)-**3a** to 2-octanone **4a** followed by its BVMO-catalysed oxidation (Scheme 2).

Concurrent catalysis is relatively demanding since all the steps should work at similar rates to ensure appropriate transformations.^{6b} In this particular case, two asymmetric transformations are truly occurring in a *parallel* way. If the reaction conditions are not appropriate, even for only one of the processes, both reactions will not be completed due to the fact that they are *connected* by a recycling agent (in this case the nicotinamide coenzyme) which is the key to the whole system outcome. Considering the above mentioned features, this type of processes could be described as *parallel interconnected kinetic asymmetric transformations*.

<Scheme 2 here>

Scheme 2. Concurrent kinetic resolutions of (\pm)-**1** and (\pm)-**3** employing BVMOs and ADHs. Esters **5** are obtained as by-products through a cascade process.

Table 1. Enzymatic kinetic resolution of racemic ketone **1a** and (\pm)-2-octanol **3a** by using BVMOs and ADHs in a concurrent way.^a

Entry	BVMO	ADH	pH	T (°C)	<i>ee</i> 1a (%) ^b	<i>ee</i> 2a (%) ^b	<i>c</i> (%) ^c	<i>E</i> ^d	<i>ee</i> 3a (%) ^b	3a (%) ^b	4a (%) ^b	5a (%) ^b
1	HAPMO	T	7.5	20	87	97	47	≥200	53 (<i>R</i>)	46	51	4
2	HAPMO	T	8.0	20	73	97	44	144	53 (<i>R</i>)	47	50	3
3	HAPMO	T	8.5	20	57	97	37	102	58 (<i>R</i>)	38	60	2
4	HAPMO	T	10	20	6	81	10	11	10 (<i>R</i>)	10	90	--
5	HAPMO	T	8.5	25	39	94	35	51	53 (<i>R</i>)	35	65	--
6	HAPMO	T	8.5	30	17	92	20	29	25 (<i>R</i>)	21	79	--
7	HAPMO	LB	8.5	20	92	98	48	≥200	92 (<i>S</i>)	44	52	4
8	PAMO	T	7.5	20	85	96	47	147	97 (<i>R</i>)	50	50	--
9	PAMO	LB	7.5	20	46	98	32	118	45 (<i>S</i>)	32	68	--
10	PAMO	LB	8.5	20	99	93	52	145	98 (<i>S</i>)	48	50	2
11	PAMO	LB	9.0	20	91	96	49	156	98 (<i>S</i>)	50	50	--
12	PAMO	LB	10.0	20	40	97	29	66	41 (<i>S</i>)	30	70	--
13	PAMO	LB	8.5	30	90	97	48	160	98 (<i>S</i>)	50	50	--
14	PAMO	LB	8.5	40	70	96	42	106	61 (<i>S</i>)	38	62	--
15	PAMO	LB	8.5	50	50	89	36	29	64 (<i>S</i>)	39	59	2
16	PAMO	LB	8.5	60	26	92	21	30	27 (<i>S</i>)	19	79	2

^a Reaction time: 24 h. For other reaction details, see Experimental Section. ^b Measured by GC. ^c Conversion, $c = ee_s/(ee_s+ee_p)$. ^d Enantiomeric ratio, $E = \ln\{(1-ee_s)/[1+(ee_s/ee_p)]\}/\ln\{(1+ee_s)/[1+(ee_s/ee_p)]\}$.

5 Once the best process conditions had been established at pH 8.5 and 20°C, we studied the system with other substrates (Table 2). In a first set of experiments, (\pm)-alcohol **3a** was exchanged by using (\pm)-2-undecanol **3b** or (\pm)-sulcatol **3c** in combination with (\pm)-**1a** employing BVMOs and ADHs (entries 1-8). It was observed that using (\pm)-**3b** with PAMO (entries 3 and 4) yielded better conversions and enantioselectivities than with HAPMO (entries 1 and 2), and interestingly, also a smaller amount of ester **5b** (*n*-nonyl acetate)²⁹ was produced. When alcohol (\pm)-**3c** was used, the resolution of (\pm)-**1a** was excellent, but depending on the ADH employed the obtained *ee* for **3c** varied. Thus, LBADH afforded enantiopure (*S*)-**3c** (entries 6 and 8) while ADH-T yielded the alcohol with low optical purity (entries 5 and 7). Control reactions incubating alcohols (\pm)-**3a-c** with ADH-T and an excess of acetone showed that **3a** and **3b** were enantioselectively oxidised yielding the corresponding (*R*)-alcohols. On the other hand, (\pm)-sulcatol was oxidised with a conversion higher than 95%, indicating that ADH-T was not able to differentiate between both enantiomers of alcohol **3c**. As described for (\pm)-2-undecanol, a lower quantity of ester **5c**²⁸ was obtained when PAMO was used instead of HAPMO. Another ketone such as 4-phenylhexan-3-one (\pm)-**1b** was also resolved in the presence of (\pm)-2-octanol **3a** obtaining excellent enantioselectivities ($E \geq 200$ in all cases) and high *ees* for alcohol **3a**²⁵ (entries 9 to 12). For all the tested enzymes, only traces of *n*-hexyl acetate **5a** were observed. To demonstrate the applicability of this system, this reaction was also performed at a 20-fold larger scale obtaining enantiopure (*R*)-**1b** and (*R*)-**3a** with 50% conversion and good yields. Other experiments were carried out to demonstrate that both reactions must efficiently proceed to achieve an adequate system. Starting from an appropriate model, *i.e.* (\pm)-**1a** and (\pm)-**3a**, one of these compounds was exchanged by a poor substrate for one of the enzymes. Thus, racemic **1a** was combined with 2-tetralol (\pm)-**3d** using ADH-T with the BVMOs. In both cases less than 3% of ester **2a** was obtained after 24 h. When (\pm)-**1a** was substituted by (\pm)-2-phenylheptan-3-one **1c** in the reaction catalysed by PAMO

and both ADHs in the presence of (\pm)-2-octanol, the Baeyer-Villiger oxidation of ketone **1c** did not work (only 5% of ester **2c**). However, the ADH-catalysed oxidation of **3a** to **4a** followed by cofactor regeneration provided by the BVMO-catalysed oxidation of **4a** to **5a**, allowed the resolution of (\pm)-**3a** obtaining 46% of enantiopure (*S*)-**3a** when LBADH was employed. Furthermore, we observed the formation of ketone **4a** (6%) during production of **2c**. When ADH-T was applied as biocatalyst, 8% of 2-octanone **4a** (due to formation of **2c**) and 92% of **5a** were obtained, since this ADH was able to oxidise both enantiomers of **3a**. In this approach, formation of final products (**2** and **4**) must be equal along the reaction course. If both resolutions exhibit similar enantioselectivities, remaining substrate *ees* (**1** and **3**), should be similar during the whole process. In order to verify this, we monitored the double kinetic resolution of (\pm)-**1b** and (\pm)-**3a** catalysed by PAMO and ADH-T (Figure 1). As can be seen, percentages of ester **2b** and ketone **4a** were nearly the same during the whole experiment. Furthermore, *ee* values of remaining (*R*)-**1b** and (*R*)-**3a** remained similar. After 6 hours, (*R*)-**1b** and **4a** were slowly oxidised into the corresponding esters.

<Figure 1 here>

Figure 1. Time study of the concurrent enzymatic resolution of (\pm)-**1b** and (\pm)-**3a** using PAMO and ADH-T. Percentage of: **2b** (♦); **4a** (□); **5a** (Δ); and enantiomeric excesses of: (*R*)-**1b** (■); (*R*)-**3a** (◇) are represented.

Table 2. Parallel interconnected kinetic resolutions of racemic ketones and *sec*-alcohols by combining BVMOs and ADHs.^a

Entry	BVMO	ADH	ketone	alcohol	t (h)	<i>ee</i> 1 (%) ^b	<i>ee</i> 2 (%) ^b	<i>c</i> (%) ^c	<i>E</i> ^d	<i>ee</i> 3 (%) ^b	3 (%) ^b	4 (%) ^b	5 (%) ^b
1	HAPMO	T	(±)- 1a	(±)- 3b	24	66	97	48	144	73 (<i>R</i>)	35	57	8
2	HAPMO	LB	(±)- 1a	(±)- 3b	24	52	97	46	114	66 (<i>S</i>)	33	59	8
3	PAMO	T	(±)- 1a	(±)- 3b	24	64	96	46	145	87 (<i>R</i>)	48	52	--
4	PAMO	LB	(±)- 1a	(±)- 3b	24	99	89	53	132	99 (<i>S</i>)	49	50	1
5	HAPMO	T	(±)- 1a	(±)- 3c	24	99	99	50	≥200	3 (<i>R</i>)	38	50	12
6	HAPMO	LB	(±)- 1a	(±)- 3c	24	99	95	51	≥200	99 (<i>S</i>)	46	46	8
7	PAMO	T	(±)- 1a	(±)- 3c	24	93	96	48	179	21 (<i>R</i>)	48	50	2
8	PAMO	LB	(±)- 1a	(±)- 3c	24	98	96	50	≥200	99 (<i>S</i>)	48	50	2
9	HAPMO	T	(±)- 1b	(±)- 3a	24	75	99	43	≥200	76 (<i>R</i>)	41	57	2
10	HAPMO	LB	(±)- 1b	(±)- 3a	24	87	99	47	≥200	99 (<i>S</i>)	50	50	--
11	PAMO	T	(±)- 1b	(±)- 3a	6	99	96	50	≥200	93 (<i>R</i>)	49	49	2
12	PAMO	LB	(±)- 1b	(±)- 3a	4.5	72	98	41	≥200	71 (<i>S</i>)	42	58	

^a For reaction details, see Experimental Section. ^b Measured by GC. ^c Conversion, $c = ee_j / (ee_s + ee_p)$. ^d Enantiomeric ratio, $E = \ln\{(1-ee_s)/[1+(ee_j/ee_p)]\} / \ln\{(1+ee_s)/[1+(ee_j/ee_p)]\}$.

5

<Scheme 3 here>

Scheme 3. Stereodivergent concurrent preparation of sulfoxide **7** and *sec*-alcohol **3** using this methodology (t= 24 h).

As BVMOs can catalyse the selective oxidation of different heteroatoms, we explored the stereodivergent and simultaneous preparation of enantioenriched methyl phenyl sulfoxide (**7**) and alcohol **3a** starting from prochiral thioanisole (**6**) and (±)-**3a** (Schemes 1b and 3). For this, we used either HAPMO, which selectively oxidised thioanisole to (*S*)-**7**,³⁰ or mutant M446G PAMO, which is able to catalyse the preparation of (*R*)-**7**,²⁰ in combination with LBADH or ADH-T. As shown in Scheme 3, both compounds were achieved with excellent conversions and selectivities, and depending on the pair of catalysts employed we could obtain all possible stereoisomer pairs. Only small amounts of *n*-hexyl acetate **5a** were formed (≤5%), and interestingly, no methyl phenyl sulfone was detected. Therefore, by simply tuning the biocatalysts employed, enantiocomplementary products could be easily obtained.

For a better understanding of the obtained ratios **2a-b/5a-c** and **5a/7**, the steady-state kinetic parameters of BVMOs toward substrates **1a-b**, **4a-c** and **6** were determined. BVMOs clearly preferred racemic ketones (±)-**1a-b** and sulfide **6** when compared with compounds **4a-c**.

Table 3. Kinetic parameters for the Baeyer-Villiger oxidation of ketones (±)-**1a-b**, (±)-**4a-c** and sulfide **6**.

Compound	BVMO	<i>K</i> _M (mM)	<i>k</i> _{cat} (s ⁻¹)	<i>k</i> _{cat} / <i>K</i> _M (M ⁻¹ s ⁻¹)
(±)- 1a	PAMO	0.03	2.90	96,700
(±)- 1a	HAPMO	4.0	14.15	3,500
(±)- 1b	PAMO	0.02	2.54	127,000
(±)- 1b	HAPMO	0.10	4.06	40,600
(±)- 4a	PAMO	0.23	0.39	1,700
(±)- 4a	HAPMO	1.0	1.48	1,480
(±)- 4a	M446G	1.34	0.25	190
(±)- 4b	PAMO	0.07	0.37	5,300
(±)- 4b	HAPMO	0.02	3.54	177,000
(±)- 4c	PAMO	2.5	1.70	680
(±)- 4c	HAPMO	1.3	0.39	300
6	HAPMO	0.36	3.02	8,400
6	M446G	1.00	0.90	900

As shown in Table 3, and in accordance with the interconnected system results, PAMO and HAPMO showed different kinetic profiles. When comparing the data obtained with PAMO, it is interesting to note that *K*_M values display a relatively large variance, while *k*_{cat} values for all substrates are in the same range. This observation was already noted in previous studies with this BVMO and suggested that the maximal turnover rate did not depend on the chemical nature of the substrate.^{17,22c} On the contrary, a wide range of *k*_{cat} (from 0.39 to 14.15 s⁻¹) and *K*_M (from 0.02 to 4.0 mM) values were observed with HAPMO. These enzymes (in particular HAPMO) showed an acceptable catalytic efficiency towards aliphatic ketones **4a-c**, which explains the amount of acetates **5** obtained in these reactions. Despite the high catalytic efficiency, 2-undecanone **4b** showed inhibition of HAPMO activity at high substrate concentrations (>5 mM). Interestingly, although the catalytic efficiencies of both processes are rather different (the Baeyer-Villiger oxidation appeared as the rate limiting step), the transformations occur in a strict parallel fashion due to the NADP⁺/NADPH loop. Thus, the Baeyer-Villiger oxidation is the rate limiting step in our system considering the high catalytic efficiency of ADHs with the selected alcohols. In case of LBADH, 2-octanol was oxidised in 50 mM Tris-HCl buffer pH 8.5 at 41 s⁻¹ and showed a *K*_M value of 0.29 mM.

Conclusions

In this report we describe an integrated coenzyme regeneration cycle which allows *parallel interconnected kinetic asymmetric transformations*. By using this methodology, we could simultaneously obtain enantiopure compounds (*via* kinetic resolution or desymmetrization) and minimise the quantity of reagents employed. The reported one-pot reactions are performed using two biocatalysts and a catalytic amount of nicotinamide cofactor which is internally recycled and acts as a connector between both processes. They

strictly occur in parallel and at least one of the transformations must be (quasi)irreversible to ensure good results. In addition, the selectivity of this system can simply be tuned by changing the employed biocatalysts. As a consequence, the selection of catalysts, connector, and substrates must be carefully taken into account.

We have shown that the kinetic properties of the biocatalysts support the obtained experimental results. Both BVMOs prefer aromatic compounds (\pm)-**1a** and (\pm)-**1b** over aliphatic ketones **4a-c**. Interestingly, the oxidation of these latter compounds by BVMOs is also possible, opening new possibilities in order to oxidise such derivatives. We have demonstrated that this procedure is not only feasible with enantioselective Baeyer-Villiger oxidations but also with asymmetric sulfoxidations. This system shows the possibility of controlling biocatalytic processes in parallel with a defined purpose as occurs in nature.

Experimental

PAMO, its M446G mutant and HAPMO were overexpressed and purified according to previously described methods.¹⁹⁻²¹

The oxidation reactions were performed using purified enzymes or cell free extract preparations of the corresponding overexpressed enzyme. One unit (U) of Baeyer-Villiger monooxygenase (BVMO) oxidises 1.0 μmol of 2-phenylpentan-3-one **1a** to 1-phenylethyl propionate **2a** per minute at pH 8.0 and 20°C in the presence of NADPH. Alcohol dehydrogenases from *Lactobacillus brevis* ADH (LBADH) and *Thermoanaerobacter* sp. ADH (ADH-T) were purchased from commercial sources. The amount of ADH used in the different assays was calculated according to the activity data provided by the supplier (ADH-T 780 U mL⁻¹, LBADH 2,320 U mL⁻¹). Racemic alcohols (\pm)-**3a-3d**, ketones **4a-4d**, methyl phenyl sulfide (**6**), racemic phenyl methyl sulfoxide [(\pm)-**7**] as well as other reagents and solvents were of the highest quality grade available, supplied by Sigma-Aldrich-Fluka. Racemic compounds (\pm)-**1a-1c** were prepared according to the literature.³¹ Racemic esters (\pm)-**2a-2c** were synthesised by acylation of commercial 1-phenylethanol or 1-phenylpropanol using propionic or valeric anhydride (yields higher than 80%). Compounds **5a-5c** were prepared by conventional acetylation of the corresponding primary alcohols using acetic anhydride and DMAP.

Flash chromatography was performed using Merck silica gel 60 (230-400 mesh). IR spectra were recorded on a Perkin-Elmer 1720-X infrared Fourier transform spectrophotometer using KBr pellets. ¹H NMR, ¹³C NMR and DEPT spectra were recorded with TMS (tetramethylsilane) as the internal standard with a Bruker AC-300-DPX (¹H, 300.13 MHz and ¹³C, 75.4 MHz) spectrometer. The chemical shift values (δ) are given in ppm and the coupling constants (J) in Hertz (Hz). For ESI⁺, a HP 1100 chromatograph mass detector and for EI⁺ a Finigan MAT 95 spectrometer were used to record mass spectra (MS). GC analyses were performed on a Hewlett Packard 6890 Series II chromatograph. HPLC analyses were developed with a Hewlett Packard 1100 LC liquid chromatograph.

General procedure for the enzymatic resolution of racemic ketones and alcohols catalysed by BVMOs and ADHs

Racemic ketones (\pm)-**1a-b** (10-15 mM) were dissolved in a Tris-HCl buffer (50 mM, different pHs, 0.5 mL). Then, NADPH (0.2 mM), the corresponding ADH (2 U), HAPMO or PAMO (2 U), and racemic alcohols (\pm)-**3a-c** (1 equiv.) were added. When LBADH was used, magnesium chloride (final concentration, 1 mM) was added to the reaction medium. The mixture was shaken at 250 rpm at selected temperatures. The reaction was stopped by extraction with ethyl acetate (2 x 0.5 mL) and the organic layer was dried over Na₂SO₄. Conversions and enantiomeric excesses of compounds **1a-b**, **2a-b**, **3a-c**, **4a-c**, and **5a-c** were determined by GC analysis.

Reaction of ketone (\pm)-**1a** and alcohol (\pm)-**3a** using PAMO and ADH-T as biocatalysts at multimilligram scale

Ketone **1a** (25 mg, 11.3 mM) was dissolved in a Tris-HCl buffer (50 mM, pH 7.5, 12.5 mL). Then, NADPH (0.2 mM), ADH-T (20 U), PAMO (20 U), and racemic alcohol **3a** (22.5 μL , 11.3 mM) were added. The mixture was shaken at 20°C and 250 rpm for 6 h. Then, the reaction was stopped by extraction with ethyl acetate (5 x 5 mL) and the organic layer was dried over Na₂SO₄. Afterwards, the organic solvents and remanent ketone **4a** were removed under reduced pressure and the crude residue was purified using flash chromatography (hexane:CH₂Cl₂ 1:1) obtaining enantiopure (*R*)-**1a** (10.1 mg, 81% yield), (*S*)-**2a** (11.7 mg, 86% yield), and (*R*)-**3a** (7.0 mg, 75% yield). Chemical purity of reaction products was determined by both GC and NMR.

General procedure for the oxidation of thioanisole and (\pm)-**2**-octanol catalysed by BVMOs and ADHs

Sulfide **6** (14.5 mM) was added to Tris-HCl buffer (50 mM, pH 7.5, 0.5 mL) containing 1% v v⁻¹ DMSO. Then, NADPH (0.2 mM), the corresponding ADH (2 U), HAPMO (2 U), and racemic alcohol **3a** (30 mM) were added. When LBADH was used, magnesium chloride (1 mM, final concentration) was added to the reaction medium. The mixture was shaken at 30°C and 250 rpm for 24 h. Then, the reactions were stopped by extraction with ethyl acetate (2 x 0.5 mL) and the organic layer was dried over Na₂SO₄. Conversions and enantiomeric excesses of final compounds were determined by GC and HPLC analysis.

HAPMO-catalysed sulfoxidation of thioanisole (**6**) coupled with the kinetic resolution of (\pm)-**2**-octanol (**3a**) catalysed by LBADH

Sulfide **6** (20 mg, 23 mM) was added into a preparation of cell free extract (in Tris-HCl buffer 50 mM pH 7.5) from recombinant *E. coli* TOP10 overexpressing HAPMO (7 mL, with a total protein concentration of approx. 10 mg mL⁻¹ containing 1% v v⁻¹ DMSO). Then, NADPH (0.2 mM), MgCl₂ (1 mM), LBADH (20 U), and racemic 2-octanol **3a** (54 μL , 47 mM) were added. The mixture was shaken at 20°C and 250 rpm for 24 h. Then, the reaction was stopped by extraction with diethyl ether (5 x 5 mL) and the organic layer was dried over Na₂SO₄. Afterwards, the organic solvents were carefully removed under reduced pressure at low temperature and the

crude residue was purified using *flash* chromatography (hexane:CH₂Cl₂ mixtures of increasing polarities) obtaining enantiopure (*S*)-**7** (18.5 mg, 84% isolated yield) and (*S*)-**3a** (20 μL, 74% isolated yield). Chemical purity of reaction products was determined by both GC and NMR.

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