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Biocatalysed concurrent production of enantioenriched compounds through parallel interconnected kinetic asymmetric transformations†‡

Ana Rioz-Martínez,[a] Fabricio R. Bisogno,[a] Cristina Rodríguez,[a] Gonzalo de Gonzalo,[a] Iván Lavandera,[a] Daniel E. Torres Pazmiño,[b] Marco W. Fraaije[b] and Vicente Gotor*[a]

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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-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 represents a potential tool for performing stereodivergent transformations.

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 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, for instance the asymmetric oxidation of the corresponding prochiral sulfides.[4]

In the last few years, several biocatalytic processes using redox enzymes, such as alcohol dehydrogenases (ADHs) and Baeyer–Villiger monooxygenases (BVMOs), have been developed, due to the high selectivities obtained employing mild and environmentally friendly conditions.[5] The synthesis of 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 certain heteroatoms, with excellent regio- and/or enantioselectivities.[7] In spite 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. Therefore, various chemical,[8,9] electrochemical[10] and enzymatic[11] 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 cost and loss of material, 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 transformations 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 example of this methodology was recently developed,[15] 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-recycling necessary to achieve each transformation (NADH and NADPH). Herein, we describe a methodology which allows the concurrent preparation of enantioenriched derivatives in a strictly parallel fashion, thereby minimising the quantity of reagents employed and maximising the *redox 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 the proper selection of both catalysts (an ADH and a BVMO) and using a catalytic amount of coenzyme (which acts as the connector), all possible enantiomers can be obtained in a one-pot process.

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. 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 a result of ineffective cofactor recycling (see below).

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

[a]*Departamento de Química Orgánica e Inorgánica, Instituto Universitario de Biotecnología de Asturias, Universidad de Oviedo, C/Julián Clavería 8, 33006, Oviedo, Spain. E-mail: vgs@fq.uniovi.es; Fax: (+) 34 985 103448; Tel: (+) 34 985 103448*
[b]*Laboratory of Biochemistry, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands*

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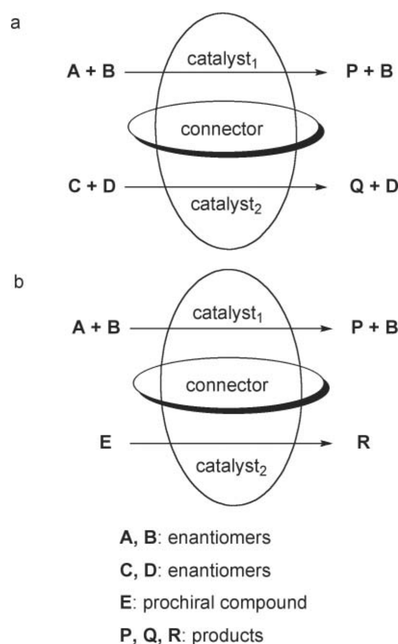
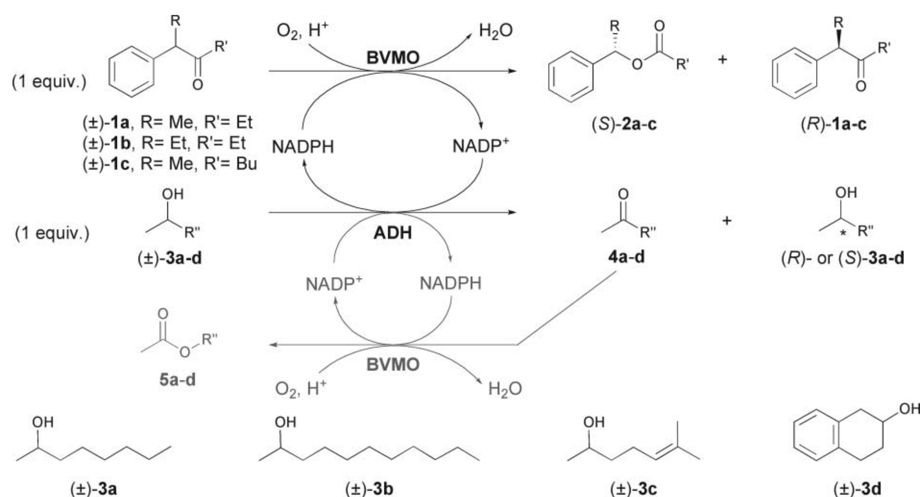


Scheme 1 Synthesis of enantioenriched compounds by interconnecting two asymmetric processes.

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 mutant[20] and 4-hydroxyacetophenone monooxygenase (HAPMO)[21] from *Pseudomonas fluorescens* ACB were chosen as BVMOs, due to their ability to catalyse enantioselective oxidations and 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 (±)-2-phenylpentan-3-one **1a**[22b] and (±)-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[22b] 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 Prelog[27] enzyme, (*S*)-**3a** was selectively oxidised to **4a**, while the remaining (*R*)-**3a** was obtained with high *ee*. When HAPMO was combined with LBADH at 20 °C and pH 8.5[28] (entry 7), an excellent double resolution was observed again, although in this case alcohol (*S*)-**3a** was obtained. This shows that the selectivity of this system can simply be tuned by changing the biocatalysts employed.

Reactions were also carried out using PAMO as the 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. A higher pH produced a loss in both activity and selectivity (entry 12). Once pH 8.5 was established 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 to 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 work even 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



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

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Table 1 Enzymatic kinetic resolution of racemic ketone **1a** and (±)-2-octanol **3a** using BVMOs and ADHs in a concurrent way[a]

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

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

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oxidation of (±)-**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.[14b] 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 process could be described as *parallel interconnected kinetic asymmetric transformations*.

Once the best process conditions had been established as pH 8.5 and 20 °C, we studied the system with other substrates (Table 2). In a first set of experiments, (±)-alcohol **3a** was exchanged for (±)-2-undecanol **3b** or (±)-sulcatol **3c**, in combination with (±)-**1a**, employing BVMOs and ADHs (entries 1–8). It was observed that using (±)-**3b** with PAMO (entries 3 and 4) yielded better conversions and enantioselectivities than with HAPMO (entries 1 and 2), and interestingly, a smaller amount of ester **5b** (*n*-nonyl acetate)[29] was produced. When alcohol (±)-**3c** was used, the resolution of (±)-**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 (±)-**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, (±)-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 (±)-2-undecanol, a lower quantity of ester **5c**[29] was obtained when PAMO was used instead of HAPMO. Another ketone such as 4-phenylhexan-3-one (±)-**1b** was also resolved in the presence of (±)-2-octanol **3a**, obtaining excellent enantioselectivities ($E \geq 200$ in all cases) and high *ee*s for alcohol **3a**[25] (entries 9–12). 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

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

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

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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.* (±)-**1a** and (±)-**3a**, one of these compounds was exchanged with a poor substrate for one of the enzymes. Thus, racemic **1a** was combined with 2-tetralol (±)-**3d**, using ADH-T with the BVMOs. In both cases less than 3% of ester **2a** was obtained after 24 h. When (±)-**1a** was substituted by (±)-2-phenylheptan-3-one **1c** in the reaction catalysed by PAMO and both ADHs in the presence of (±)-2-octanol, the Baeyer–Villiger oxidation of ketone **1c** did not work (only 5% yield 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 (±)-**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 the 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 the final products (**2** and **4**) must be equal along the reaction course. If both resolutions exhibit similar enantioselectivities, the remaining substrates' *ee*s (**1** and **3**) should be similar during the whole process. In order to verify this, we monitored the double kinetic resolution of (±)-**1b** and (±)-**3a**, catalysed by PAMO and ADH-T (Fig. 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 h, (*R*)-**1b** and **4a** were slowly oxidised into the corresponding esters.

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.

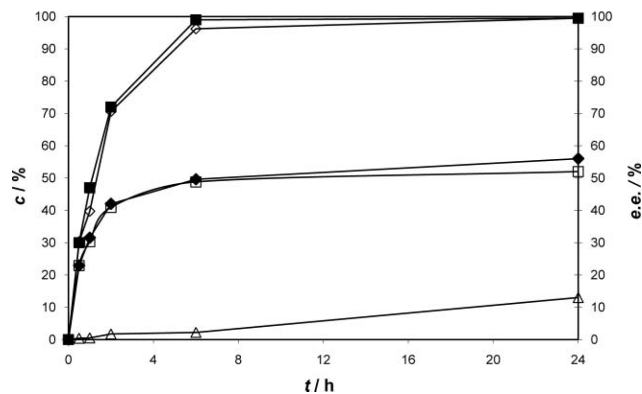
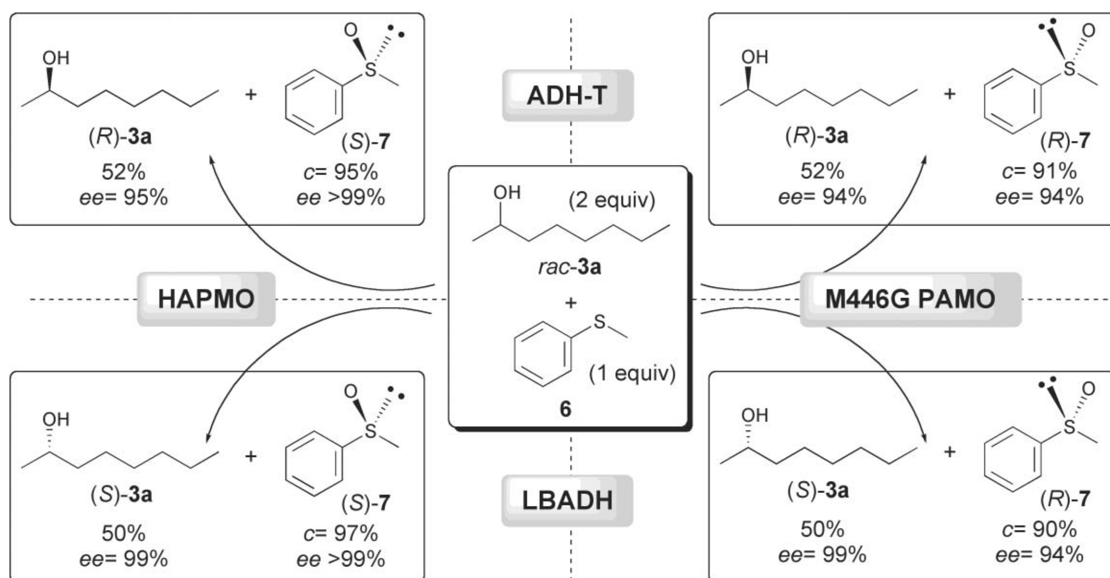


Fig. 1 Time study of the concurrent enzymatic resolution of (±)-**1b** and (±)-**3a** using PAMO and ADH-T. Percentage of: **2b** (◆); **4a** (□); **5a** (△); and enantiomeric excesses of: (*R*)-**1b** (■); (*R*)-**3a** (◇) are represented.

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**, **1b**, **4a–c** and **6** were determined. BVMOs clearly preferred racemic ketones (±)-**1a**, **1b** and sulfide **6** when compared with compounds **4a–c**.

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



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

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

Compound	BVMO	K_M/mM	k_{cat}/s^{-1}	k_{cat}/K_M/M^{-1} s^{-1}
(±)-**1a**	PAMO	0.03	2.90	96 700
(±)-**1a**	HAPMO	4.0	14.15	3500
(±)-**1b**	PAMO	0.02	2.54	127 000
(±)-**1b**	HAPMO	0.10	4.06	40 600
(±)-**4a**	PAMO	0.23	0.39	1700
(±)-**4a**	HAPMO	1.0	1.48	1 480
(±)-**4a**	M446G	1.34	0.25	190
(±)-**4b**	PAMO	0.07	0.37	5300
(±)-**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	8400
6	M446G	1.00	0.90	900

not depend on the chemical nature of the substrate.[17,22c] On the contrary, a wide range of k_{cat} (0.39–14.15 s^{-1}) and K_M (0.02–4.0 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 to be the rate limiting step), the transformations occur in a strictly 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 the 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*. 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 (±)-**1a** and (±)-**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 2320 U mL^{-1}). Racemic alcohols (±)-**3a–3d**, ketones **4a–4d**, methyl phenyl sulfide (**6**), racemic phenyl methyl sulfoxide [(±)-**7**] as well as other reagents and solvents were of the highest quality grade available, supplied by Sigma-Aldrich-Fluka. Racemic compounds (±)-**1a–1c** were prepared according to the literature.[31] Racemic esters (±)-**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. ^{1}H NMR, ^{13}C NMR and DEPT spectra were recorded with TMS (tetramethylsilane) as the internal standard with a Bruker AC-300-DPX (^{1}H, 300.13 MHz and ^{13}C, 75.4 MHz) spectrometer. The chemical shift values (δ) are given in ppm and the coupling constants (J) in Hertz (Hz). For ESI$^+$, an 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 (±)-**1a** or **1b** (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 (±)-**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 × 0.5 mL) and the organic layer was dried over Na_2SO_4. Conversions and enantiomeric excesses of compounds **1a**, **1b**, **2a**, **2b**, **3a–c**, **4a–c**, and **5a–c** were determined by GC analysis.

Reaction of ketone (±)-1a and alcohol (±)-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

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250 rpm for 6 h. Then, the reaction was stopped by extraction with ethyl acetate (5 × 5 mL) and the organic layer was dried over Na_2SO_4. Afterwards, the organic solvents and remaining ketone **4a** were removed under reduced pressure and the crude residue was purified using *flash* chromatography (hexane–CH_2Cl_2 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). The chemical purity of reaction products was determined by both GC and NMR.

General procedure for the oxidation of thioanisole (6) and (±)-2-octanol (3a) 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 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 × 0.5 mL) and the organic layer was dried over Na_2SO_4. 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 (±)-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 DMSO). Then, NADPH (0.2 mM), $MgCl_2$ (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 × 5 mL) and the organic layer was dried over Na_2SO_4. Afterwards, the organic solvents were carefully removed under reduced pressure at low temperature and the crude residue was purified using *flash* chromatography (hexane–CH_2Cl_2 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 the reaction products was determined by both GC and NMR.

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