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Baeyer–Villiger monooxygenase-catalyzed kinetic resolution of racemic α-alkyl benzyl ketones: enzymatic synthesis of α-alkyl benzylketones and α-alkyl benzylesters

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ABSTRACT

The application of three BVMOs for the enantioselective oxidation of 3-phenylbutan-2-ones with different substituents in the aromatic moiety is described. By choosing the appropriate biocatalyst and substrate combination, chiral ketones and esters can be obtained with excellent enantiopurities. This methodology could also be applied to the resolution of racemic α-alkyl benzylketones with longer alkyl chains as well as with two substituted α-substituted benzylacetones. A kinetic analysis revealed that the BVMOs studied effectively convert all tested compounds showing that the enzymes are tolerant towards the substrate structure while being highly enantioselective. These properties render BVMOs as valuable biocatalysts for the preparation of compounds with high interest in organic synthesis.

1. Introduction

The use of enzymes in organic synthesis is very promising, since these biocatalysts often show remarkable chemo-, regio- and enantioselectivities that are difficult to achieve by conventional chemical methods. Moreover, enzyme-catalyzed processes occur under mild and environmentally friendly conditions.[1] One of the reactions in which enzymes have shown to be an attractive choice is the Baeyer–Villiger oxidation. This process involves the oxidative cleavage of a carbon–carbon bond adjacent to a carbonyl group in concert with the insertion of an oxygen atom between these two carbons.[2] The reaction can be performed using organic peroxyacids or alkyl hydroperoxides as oxidants. Unfortunately, the employment of these compounds presents several disadvantages. To avoid their use, different organocatalytic compounds have been tested in Baeyer–Villiger oxidations using water or hydrogen peroxide as oxidants.[3] However, these approaches have led to catalysts that display moderate or poor selectivities. As a result, the development of biocatalytic methods has become highly attractive.

More than 50 years ago, it was reported for the first time that enzymes could catalyze the Baeyer–Villiger oxidation after which time it took 20 years to isolate and characterize the first Baeyer–Villiger monooxygenase (BVMO).[4] From then on, various microbial BVMOs have been reported.[5] These enzymes not only catalyze the oxidation of carbonyl compounds, but also the oxygenation of different heteroatoms. BVMOs show high regio- and/or enantioselectivity while accepting a broad range of substrates. Oxidations catalyzed by BVMOs are carried out in aqueous buffer at mild pH using molecular oxygen as oxidant.

The identification and isolation of novel BVMOs have led to an increased interest of their synthetic potential in chemistry.[6] A recent example of this development is phenylacetone monooxygenase (PAMO) from *Thermobifida fusca*, a thermostable BVMO that is able to catalyze Baeyer–Villiger reactions and other heteroatom oxidations.[7] This enzyme is the first BVMO whose structure has been determined, allowing a deeper understanding of this biocatalyst and the development of several mutagenesis studies.[8] As a result, the M446G PAMO mutant has been designed which possesses a widened substrate active site when compared with the wild-type (wt) enzyme.[9] Another BVMO studied recently is 4-hydroxyacetophenone monooxygenase (HAPMO) from *Pseudomonas fluorescens* ACB. This protein is primarily active on aromatic compounds.[10]

Previously, it has been reported that both wt PAMO and HAPMO can perform highly enantioselective resolutions of some racemic α-alkyl benzylketones.[11] Herein we report the enzymatic kinetic resolution of a new set of aromatic ketones catalyzed by these novel BVMOs with two main objectives: firstly, extending our knowledge on these biocatalysts, and secondly, establishing a novel method to prepare enantiomerically pure α-alkyl benzyl ketones and esters by biocatalysis.

2. Results and discussion

All enzymatic Baeyer–Villiger oxidations were performed by employing a BVMO in combination with a NADPH-regeneration

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system consisting of glucose-6-phosphate dehydrogenase (G6PDH) and glucose-6-phosphate.[12] By using this system only catalytic amounts of the expensive nicotinamide coenzyme are required. For all the substrates tested, the enzymes preferentially oxidized the (*S*)-enantiomer, while the corresponding (*R*)-ketones remained unaltered. The oxidation processes were performed at 20 °C and pH 8.0, in which the kinetic resolution of model substrate (±)-**1a** was carried out with good conversions and high enantioselectivities using wt PAMO and HAPMO.[11] When we applied M446G PAMO as a biocatalyst, the same enantiopreference was observed, obtaining an excellent *E* value as shown in Table 1 (entry 1, *E* = 112)[13] and a 27% conversion.

2.1. Biocatalyzed oxidation of substituted 3-phenylbutan-2-ones

Initially, we investigated the ability of these three BVMOs to catalyze the kinetic resolution of 3-phenylbutan-2-ones bearing different substituent groups on the aromatic ring. When wt PAMO was employed as a biocatalyst, the presence of a substituent on the aromatic ring generally resulted in a decreased enantioselectivity. Only the biooxidation of 3-(3-methylphenyl)butan-2-one (±)-**4a** occurred with an excellent *E* value (*E* = 119) leading to a 30% of (*S*)-**4b** after 1 h. Good enantioselectivities were obtained in the resolution of the methoxyphenylbutan-2-ones (±)-**2a** (*E* = 32) and (±)-**5a** (*E* = 46), thereby noting that the reaction occurred faster for the 4-methoxy derivative than for the 3-methoxy case. The oxidation of 3-(3-chlorophenyl)butan-2-one (±)-**8a** yielded (*R*)-**8a** and (*S*)-**8b** with a good *E* value, as well as the wt PAMO biocatalyzed resolution of 3-(4-nitrophenyl)butan-2-one (±)-**10a** (*E* = 40). The oxidations of the remaining ketones tested were carried out with poor enantioselectivities (*E* <20). Wt PAMO presented a similar activity for all the substrates, conversions being obtained between 19% and 33% after 1 h, except for the ketones bearing strong electron-withdrawing groups. The oxidation of these compounds, (±)-**9a** and (±)-**10a,** was performed with high conversions ($c \approx 50\%$ after 30 min).

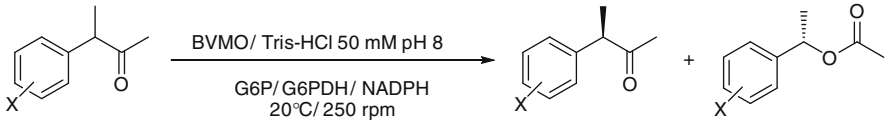
M446G PAMO seemed to prefer the presence of electron-withdrawing groups in the aromatic moiety in terms of activity, as shown in Table 1 for substrates (±)-**8a**, (±)-**9a** and (±)-**10a**. This enzyme catalyzed the resolution of the derivatives (±)-**4a** and (±)-**10a** with excellent enantioselectivties. The oxidation was very slow when resolving (±)-**4a** (9% of (*S*)-**4b** after 1 h), while for (±)-**10a** a 50% of (*S*)-**10b** was reached after only 30 min. Biocatalyzed resolution of the methoxy derivatives (±)-**2a** and (±)-**5a**, as well as the biooxidation of the *para*-halogenated ketones (±)-**6a** and (±)-**7a**, led to the (*R*)-ketones and (*S*)-esters with good *E* values. The presence of the 4-ethyl-, 3-chloro- or 3-trifluoromethyl substituents in the aromatic ring had a negative effect on the selectivity of M446G PAMO (Table 1).

Furthermore, HAPMO was explored as biocatalyst in the selective oxidation of substituted racemic benzylketones. As for the two PAMO enzymes, this BVMO was found to be able to catalyze the resolution of 3-(3-methylphenyl)butan-2-one to obtain (*R*)-**4a** and (*S*)-**4b** with high enantioselectivity (*E* = 126) and 20% conversion after 2 h. HAPMO catalyzed the formation of (*S*)-1-(3-trifluoromethylphenyl)ethyl acetate (*S*)-**9b** with high *E* value and 39% conversion after 1 h, in contrast to the low selectivity showed by wt PAMO and M446G for this ketone. The 3- and 4-chlorophenylbutan-2-ones (±)-**7a** and (±)-**8a** were also converted by HAPMO with good enantioselectivities, especially when oxidizing the 4-chloro derivative (*E* = 50 and 43% conversion after 2 h). The reaction was much slower for (±)-**8a** (12% of (*S*)-**8b** after 2 h). The HAPMO-catalyzed oxidation of (±)-**2a** occurred with good enantioselectivity (*E* = 41), resulting in 14% of (*S*)-**2b** after 2 h. As shown in entry 3,3-(4-ethylphenyl)butan-2-one (±)-**3a** seemed not to be a good substrate for the all BVMOs studied. HAPMO oxidized with moderate/poor selectivities ketone (±)-**5a** (in contrast to the results reported for both PAMO biocatalysts), as well as the 4-bromo- and 4-nitroderivatives (±)-**6a** and (±)-**10a**.

2.2. Oxidation of long alkyl chain ketones catalyzed by BVMOs

In addition to the oxidation of substituted 3-phenylbutan-2-ones, we investigated the ability of the BVMOs to catalyze the oxidation of racemic benzylketones which contain longer alkyl chains on either R_1, R_2 or both, as shown in Table 2. Regardless of which BVMO was employed, all the ketones with longer alkyl chains led to lower conversions when compared to the biooxidation of (±)-**1a.** The biocatalyzed oxidation of racemic 3-phenylheptan-2-one (±)-**11a** and (±)-3-phenylhex-5-en-2-one (±)-**12a** yielded to the corresponding (*R*)-ketones and (*S*)-esters with low or moderate conversions and poor enantioselectivities.

Table 1
Baeyer–Villiger monooxygenases-biocatalyzed oxidation of racemic ketones (±)-**1–10a** in aqueous buffer[a]

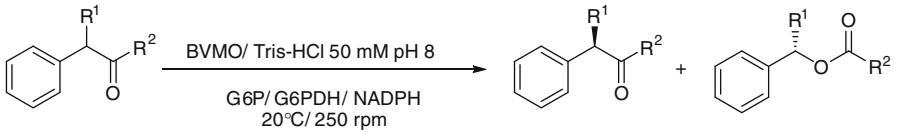


(±)-**1-10a** (*R*)-**1-10a** (*S*)-**1-10b**

Ketone	X	Wt PAMO					M446G PAMO				HAPMO				
		t (h)	ee_s[b] (%)	ee_p[b] (%)	*c*[c] (%)	*E*[d]	ee_s[b] (%)	ee_p[b] (%)	*c*[c] (%)	*E*[d]	*t* (h)	ee_s[b] (%)	ee_p[b] (%)	*c*[c] (%)	*E*[d]
1a	H	1	36	98	27	188	36	97	27	112	2	33	98	25	136
2a	4-OMe	1	44	91	33	32	9	97	8	61	2	16	95	14	41
3a	4-Et	1	36	86	30	19	29	86	25	18	2	66	68	49	10
4a	3-Me	1	42	97	30	119	10	98	9	109	2	25	98	20	126
5a	3-OMe	1	22	95	19	46	11	96	11	52	2	71	76	48	15
6a	4-Br	1	17	48	26	3	11	95	11	44	2	8	25	42	2
7a	4-Cl	1	39	82	32	15	11	96	10	54	2	70	92	43	50
8a	3-Cl	1	27	92	22	33	38	73	34	9	2	13	96	12	63
9a	3-CF_3	0.5	47	43	52	5	39	92	30	36	1	62	97	39	112
10a	4-NO_2	0.5	87	87	50	40	98	93	50	121	1	53	58	48	6

[a] For reaction conditions see Section 4.
[b] Enantiomeric excesses (ee) determined by GC, except (±)-**10b**, for which HPLC was used.
[c] Conversion determined by GC.
[d] Enantiomeric ratio, $E = \ln\{(1 - ee_s)/[1 + (ee_s/ee_p)]\}/\ln\{(1 + ee_s)/[1 + (ee_s/ee_p)]\}$.

Table 2
Enzymatic oxidation of ketones (±)-**11–16a** employing wt PAMO, M446G PAMO and HAPMO[a]



(±)-**11-16a** (*R*)-**11-16a** (*S*)-**11-16b**

11a: R^1: Butyl R^2: Methyl
12a: R^1: Allyl R^2: Methyl
13a: R^1: Propyl R^2: Ethyl
14a: R^1: Butyl R^2: Ethyl
15a: R^1: Allyl R^2: Ethyl
16a: R^1: Methyl R^2: Butyl

Ketone	Wt PAMO					M446G PAMO					HAPMO				
	t (h)	ee_S (%)	ee_P (%)	*c* (%)	*E*	*t* (h)	ee_S (%)	ee_P (%)	*c* (%)	*E*	*t* (h)	ee_S (%)	ee_P (%)	*c* (%)	*E*
11a	2	3	28	10	2	4	9	86	9	15	4	n.d.	n.d.	⩽3	n.d.
12a	2	26	64	29	6	4	n.d.	n.d.	⩽3	n.d.	4	n.d.	n.d.	⩽3	n.d.
13a	2	13	55	19	4	4	5	98	5	103	4	29	96	23	66
14a	2	10	86	10	15	4	10	95	9	43	4	n.d.	n.d.	⩽3	n.d.
15a	2	38	77	33	11	4	8	96	8	53	4	5	98	5	104
16a	2	n.d.	n.d.	⩽3	n.d.	4	n.d.	n.d.	⩽3	n.d.	4	n.d.	n.d.	⩽3	n.d.
17a	4	8	10	43	1	24	n.d.	n.d.	⩽3	n.d.	24	n.d.	n.d.	⩽3	n.d.
18a	4	32	95	25	52	24	n.d.	n.d.	⩽3	n.d.	24	6	97	6	69

n.d.: not determined.
[a] For reaction conditions see Experimental. For compounds (±)-**11–15a**, *E* and ee_s were calculated from ee_p and *c* values. $E = \ln[1 - c(1 + ee_p)]/\ln[1 - c(1 - ee_p)]$. Enantiomeric excesses (±)-**18a–b** were determined by GC. In case of (±)-**17**, enantiomeric ratio was calculated from ee_s and *c*. $E = \ln[(1 - c)(1 - ee_s)]/\ln[(1 - c)(1 + ee_s)]$.

4-Phenylheptan-3-one (±)-**13a** was oxidized by the three biocatalysts, with HAPMO being the enzyme most suited for the kinetic resolution (*E* = 66 and 23% conversion after 2 h). The oxidation catalyzed by M446G PAMO led to (*R*)-**13a** and (*S*)-**13b** with high selectivity but a low conversion after 4 h, while a very low *E* value was obtained when wt PAMO was employed. Mutant M446G was able to catalyze the selective oxidation of 4-phenyloctan-3-one (±)-**14a** (*E* = 43). Unfortunately, only 9% of (*S*)-**14b** was obtained after 4 h. Wt PAMO and HAPMO were found to be unsuitable biocatalysts for the kinetic resolution of this ketone. HAPMO and M446G PAMO were able to oxidize selectively racemic 4-phenylhept-6-en-3-one (±)-**15a**, but with very low conversions. On the other hand, wt PAMO converted 33% of this ketone, although with poor selectivity. Racemic 2-phenylheptan-3-one (±)-**16a** was also subjected to enzymatic oxidation by the BVMOs, but no formation of (*S*)-**16b** was achieved after long times, indicating that the presence of a long group close to the carbonyl moiety is poorly accepted by these three biocatalysts. This observation is not completely unexpected for wt PAMO, as it was previously shown that a similar substrate (1-phenylhexan-2-one) was poorly converted by this enzyme.[7b]

Finally, two α-alkyl benzylacetones were analyzed as BVMOs substrates. The enzymatic oxidation of 4-phenylpentan-2-one (±)-**17a** led to (*S*)-**17b** with a 43% conversion in a process without selectivity after 4 h when employing wt-PAMO. No reaction was observed when this compound was oxidized in the presence of M446G PAMO or HAPMO after 1 day of reaction. In contrast, when 3-methyl-4-phenylbutan-2-one (±)-**18a** was subjected to enzymatic oxidation catalyzed by wt PAMO or HAPMO, good enantioselectivities were obtained (*E* = 52 and 69, respectively). The reaction was much faster with PAMO, obtaining (*S*)-**18b** in 25% conversion after 4 h. No product formation was observed after 1 day when M446G PAMO was employed as a biocatalyst. The low activity showed by HAPMO and mutant M446G in the oxidation of this type of substrate is in agreement with recent studies in which these enzymes appeared to prefer aromatic ketones that have the carbonyl group close to the phenyl ring, for example, phenylacetone or acetophenone derivatives.[9,10a]

2.3. Kinetic parameters of BVMOs for the oxidation of racemic ketones (±)-1–10a

In order to obtain a better insight in how the different substituents of the 3-phenylbutan-2-ones affect the efficiency by which these ketones are converted, we determined their steady-state kinetic parameters (Table 3). No correlation was found between σ-parameter for the substituents (described as the Hammet relationship, which represents a contribution of factors such a resonance and both field and inductive effects) and the kinetic behaviour. Depending on the nature and the position of the substituents, different effects on both apparent affinity (K_M) and catalytic activity (k_{cat}) were achieved. The catalytic activity of wt PAMO and mutant M446G was found to be quite similar ($k_{cat} \sim 3\ s^{-1}$) for all the ketones, while HAPMO showed more variety in its k_{cat} value (from 2.2 to 6.5 s^{-1}). This suggests that in PAMO the rate limiting step is probably a kinetic event unrelated to the reactivity of the substrate, which is in line with a recently detailed kinetic study of PAMO.[8c] For HAPMO, the reaction may be limited by the chemical/sterical properties of the substrate or product.

In terms of K_M, M446G PAMO displays 5–10 times lower affinity (higher K_M) for the substrates than wt PAMO. This is in agreement with previous experiments.[9] Both wild-type and mutant PAMO showed a relative high efficiency towards the ketones (±)-**1a**, (±)-**4a,** (±)-**8a** and (±)-**10a**. On the contrary, substrates (±)-**3a**, (±)-**6a** and (±)-**7a** were converted with a much higher catalytic efficiency by HAPMO, mainly due to the high affinity of the enzyme towards these substrates. No clear correlation was found between the catalytic efficiency of the enzymes and their achieved conversion, suggesting that in some cases (enantioselective) product inhibition might occur. While the catalytic efficiencies found for PAMO and its mutant vary by less than a factor of 20, the k_{cat}/K_M values found for HAPMO showed a much higher variation (over 500-fold). Such differences have been described before for this enzyme, when substituted acetophenones were subjected to a similar study.[10b] HAPMO clearly showed a low catalytic efficiency towards 3-phenylbutan-2-ones with no or

Table 3
Steady-state kinetic parameters of wild-type PAMO, M446G PAMO and HAPMO for the oxidation of ketones (±)-**1a**–**10a**

Substrate	Wt PAMO			M446G PAMO			HAPMO		
	K_M [μM]	k_{cat} [s^{-1}]	k_{cat}/K_M [10^3 s^{-1} M^{-1}]	K_M [μM]	k_{cat} [s^{-1}]	k_{cat}/K_M [10^3 s^{-1} M^{-1}]	K_M [μM]	k_{cat} [s^{-1}]	k_{cat}/K_M [10^3 s^{-1} M^{-1}]
(±)-**1a**	16	2.9	177	168	2.7	16	457	3.2	7.1
(±)-**2a**	69	2.8	41	850	2.8	3.3	55	4.5	82
(±)-**3a**	101	2.9	29	586	2.5	4.2	7	4.7	713
(±)-**4a**	25	3.2	131	231	3.6	15	>3,500[a]	>5	1.3
(±)-**5a**	75	3.0	41	1,000	3.1	3.1	165	2.2	13
(±)-**6a**	35	2.9	84	506	2.9	6	8	6.5	831
(±)-**7a**	34	2.9	87	196	2.7	14	26	5.6	215
(±)-**8a**	8	3.2	377	60	2.8	48	437	2.7	6.2
(±)-**9a**	93	3.4	36	543	2.7	4.9	230	2.2	9.5
(±)-**10a**	40	4.5	113	180	2.2	12	40	3.5	87.5

[a] Due to limited solubility of compound (±)-**4a**, the substrate concentration could not be increased beyond this value.

meta-substituents ($k_{cat}/K_M \leqslant 13{,}000\ s^{-1}\ M^{-1}$), whereas *para*-substituted ketones were efficiently oxidized.

3. Conclusions

The enantioselective oxidation of racemic benzylketones by wt PAMO, M446G PAMO and HAPMO enables the enantioselective synthesis of various (*R*)-α-alkyl-benzylketones and (*S*)-α-alkyl-benzylesters. High enantioselectivities can be achieved by combining the right biocatalyst and substrate structure. The three enzymes showed an excellent *E* value in the resolution of 3-(3-methylphenyl)butan-2-one (±)-**4a**, while two ketones presenting electron-withdrawing groups, such as the 3-trifluoromethyl- and the 4-nitro-3-phenylbutan-2-ones, are resolved with excellent enantioselectivities by HAPMO and M446G PAMO, respectively. The other α-alkyl benzylketones tested can be resolved with good *E* values ($E \geqslant 40$) depending on the enzyme employed, with the exception of 3-(4-ethylphenyl)butan-2-one, which is a poor substrate for all the three enzymes. Most of the long alkyl chain ketones are poorly accepted by the three BVMOs. Only when M446G PAMO and HAPMO are employed in the kinetic resolution of (±)-**13** and (±)-**15**, respectively, good enantioselectivites are obtained. Wt PAMO and HAPMO were also able to oxidize 3-methyl-4-phenylbutan-2-one with good *E* values. Kinetic analysis revealed that all substrates tested are converted at a similar rate by all three biocatalysts. Differences in catalytic efficiencies were mainly caused by varying K_M values. No clear correlation was found between the properties of the different substituents and the enantioselectivity and/or kinetic parameters. The various effects of the substituents on the enzymatic properties indicate that the structural character of the 3-phenylbutan-2-ones determines whether oxidation is efficient and selective.

4. Experimental

4.1. General

Recombinant histidine-tagged PAMO,[7a] its mutant M446G[9] and recombinant HAPMO[10a] were overexpressed and purified as previously described. Glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* was obtained from Fluka-Biochemika. All reagents and solvents were of the highest quality grade available and were obtained from Sigma–Aldrich–Fluka and Acros Organics. Chemical reactions were monitored by analytical TLC, performed on Merck Silica Gel 60 F_{254} plates and visualized by UV irradiation. Flash chromatography was carried out with Silica Gel 60 (230–240 mesh, Merck). IR spectra were recorded on a Perkin–Elmer 1720-X infrared Fourier transform spectrophotometer using KBr pellets. UV spectra were performed on a Perkin–Elmer Lambda Bio10 UV/vis Spectrophotometer. ^{1}H NMR, ^{13}C NMR and DEPT spectra were recorded with tetramethylsilane (TMS) as the internal standard with a Bruker AC-300 DPX (^{1}H: 300.13 MHz; ^{13}C: 75.5 MHz) spectrometer. The chemical shift values (δ) are given in ppm. APCI$^+$ and ESI$^+$ using a Hewlett–Packard 1100 chromatograph mass detector or EI$^+$ with a Hewlett–Packard 5973 mass spectrometer were used to record mass spectra (MS). GC analyses were performed on a Hewlett–Packard 6890 Series II chromatograph equipped with a Restek RtβDEXse (30 m × 0.25 mm × 0.25 μm, 1 bar N_2), a Varian CP-Chiralsil-DEX CB (25 m × 0.32 mm × 0.25 μm, 1 bar N_2) or a Mercherey-Nagel Hydrodex-β-TBOAc (30 m × 0.25 mm × 0.25 μm, 1 bar N_2) for chiral determinations or a HP-1 (crosslinked methyl siloxane, 30 m × 0.25 mm 0.25 μm, 1.0 bar N_2) from Hewlett–Packard for measuring the conversions values. For all the analyses, the injector temperature is 225 °C and the FID temperature is 250 °C. HPLC analyses were developed with a Hewlett–Packard 1100 LC liquid chromatograph equipped with a Chiralcel OD (0.46 cm × 25 cm) or Chiralpak IA (0.46 cm × 25 cm) chiral column from Daicel.

Absolute configuration of esters (*S*)-**1**–**15b** and ketones (*R*)-**17**–**18a** was determined by (i) comparison of elution order of GC with published data,[11,14] (ii) hydrolysis of the esters to the corresponding alcohols employing NaOMe in methanol, and comparison of the elution order on HPLC with published data[15] or by (iii) co-injection with commercially available material.

4.2. Synthesis of the racemic ketones (±)-1–18a and esters (±)-1–18b

The racemic ketones (±)-**1–16a** were synthesized according to the literature, using with 1-phenylpropan-2-one or 1-phenylbutan-2-one the corresponding alkyl or allyl iodide and NaOH in a biphasic medium water/CH_2Cl_2.[16] Ketones were obtained with yields from 25% to 90% depending on the substrate structure. Racemic acetates (±)-**2–5b** were synthesized by chemical Baeyer–Villiger oxidation with *m*-CPBA/dichlorometane (yields from 60% to 90%), while esters (±)-**1b** and (±)-**6–18b** were prepared by chemical acylation of the racemic alcohols employing pyridine and the corresponding anhydride in CH_2Cl_2 with high yields. Compounds **1a**,[11] **2a**,[14b] **6a**,[14b] **7a**,[17a] **9a**,[14b] **10a**,[17b] **12a**,[17c] **1b**,[11] **2b**,[14b] **4b**,[17d] **5b**,[17d] **6b**,[14b] **7b**,[14a] **9b**,[14b] **10b**,[14a] **11b**,[17e] **12b**,[17f] **16b**,[17g] **17b**[17h] and **18b**[17i] exhibit physical and spectral properties in accord with those reported.

4-Phenylpentan-2-one, (±)-**17a** and 3-methyl-4-phenylbutan-2-one, (±)-**18a** were obtained according to the literature with 30–40% yield, by Heck arylation of 3-penten-2-ol or 3-methyl-3-buten-2-ol, respectively, using iodobenzene in presence of palladium chloride, tetra-*n*-butylammonium bromide and $NaHCO_3$.[18] 3-Methyl-3-buten-2-ol was prepared by addition of methyl magnesium iodide to a methacrolein solution in diethyl ether. Compounds (±)-**17–18a** exhibit physical and spectral properties in accord with those reported.[17h,19]

4.2.1. (±)-2-Phenylheptan-3-one, (±)-16a

Colourless oil. IR (KBr): ν 3055, 2986, 1712, 1423 cm^{-1}. ^1H NMR ($CDCl_3$, 300.13 MHz): δ 0.77 (t, $^3J_{HH}$ = 7.3 Hz, 3 H), 1.08–1.20 (m, 2H), 1.34 (d, $^3J_{HH}$ = 7.0 Hz, 3 H), 1.39–1.46 (m, 2H), 2.31 (t, $^3J_{HH}$ = 7.3 Hz, 2H), 3.71 (q, $^3J_{HH}$ = 6.9 Hz, 1 H), 7.16–7.31 (m, 5H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 13.7 (CH_3), 17.4 (CH_3), 22.1 (CH_2), 25.9 (CH_2), 40.7 (CH_2), 52.9 (CH), 127.0 (2 CH), 127.8 (CH), 128.8 (2 CH), 140.7 (C), 211.0 (C=O). MS (EI^+): m/z (%) 190 (11) [M^+], 105 (81), 85 (100).

4.3. General procedure for the BVMO-catalyzed oxidation of the racemic ketones (±)-1–18a

Unless otherwise stated, the starting racemic ketones (±)-**1–18a** (20 mM) were dissolved in a 50 mM Tris/HCl buffer at pH 8.0 (1.0 mL) containing glucose-6-phosphate (40 mM), glucose-6-phosphate dehydrogenase (5.0 units), NADPH (0.2 mM) and the corresponding Baeyer–Villiger monooxygenase (5 μM). Reactions are shaken at 250 rpm and 20 °C in a rotatory shaker for the times established. Once finished, the crude reactions were extracted with AcOEt (2 × 500 μL). The organic phases were dried onto Na_2SO_4 and analyzed directly by GC or HPLC in order to determine the conversion of the oxidations and the enantiomeric excesses of the esters (*S*)-**1–18b** and the remaining ketones (*R*)-**1–18a.**

4.4. Scale up of the BVMO-catalyzed oxidation of racemic ketones (±)-3–5a, (±)-8a, (±)-11a and (±)-13–15a

Racemic ketones (±)-**3–5a**, (±)-**8a**, (±)-**11a**, (±)-**13–15a** (20 mM) were dissolved in a 50 mM Tris/HCl buffer at pH 8.0 (12.5 mL) containing glucose-6-phosphate (40 mM), glucose-6-phosphate dehydrogenase (5.0 units), NADPH (0.2 mM) and the corresponding Baeyer–Villiger monooxygenase [wt PAMO for (±)-**3–4a**, (±)-**8a** and (±)-**15a**; HAPMO for (±)-**5a** and (±)-**13a**; M446G PAMO for (±)-**11a** and (±)-**14a** (5 μM)]. Reactions are shaken at 250 rpm and 20 °C in a rotatory shaker for the times established (1 h for (±)-**3-4a** and (±)-**8a**, 2 h for (±)-**5a** (±)-**15a** and 4 h for (±)-**13–14a**). Once finished, the crude reactions were extracted with EtOAc (4 × 5 mL). The organic phases were dried onto Na_2SO_4 filtered and evaporated under reduced pressure. The crude residues were purified by flash chromatography on silica gel using hexane/diethyl ether 8:2 (compounds **3**–**4a**), hexane/diethyl ether 7:3 (ketones **5a** and **8a**) or hexane/ethyl acetate 8:2 (compounds **13**–**15a**) to afford: (*R*)-**3a** (22.6 mg, 73% yield) and (*S*)-**3b** (10.4 mg, 71% yield); (*R*)-**4a** (19.9 mg, 68% yield) and (*S*)-**4b** (10.6 mg, 79% yield); (*R*)-**5a** (18.3 mg, 79% yield) and (*S*)-**5b** (18.0 mg, 77% yield); (*R*)-**8a** (29.8 mg, 82% yield) and (*S*)-**8b** (8.5 mg, 78% yield); (*R*)-**11a** (32.6 mg, 74% yield) and (*S*)-**11b** (3.7 mg, 79% yield); (*R*)-**13a** (25.8 mg, 70% yield) and (*S*)-**13b** (10.5 mg, 87% yield); (*R*)-**14a** (36.8 mg, 79% yield) and (*S*)-**14b** (4.4 mg, 88% yield); (*R*)-**15a** (24.6 mg, 78% yield) and (*S*)-**15b** (12.8 mg, 76% yield).

4.4.1. (*R*)-3-(4-Ethylphenyl)butan-2-one, (*R*)-3a

Colourless oil. IR (KBr): ν 3054, 2967, 1714, 1452 cm^{-1}. ^1H NMR ($CDCl_3$, 300.13 MHz): δ 1.23 (t, $^3J_{HH}$ = 7.5 Hz, 3 H), 1.37 (d, $^3J_{HH}$ = 6.9 Hz, 3H), 2.04 (s, 3H), 2.63 (q, $^3J_{HH}$ = 7.6 Hz, 2H), 3.71 (q, $^3J_{HH}$ = 6.9 Hz, 1H), 7.11–7.16 (m, 4H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 15.4 (CH_3), 17.1 (CH_3), 28.2 (CH_3), 28.3 (CH_2), 53.3 (CH), 127.7 (2 CH), 128.3 (2CH), 137.7 (C), 143.1 (C), 209.1 (C=O). MS ($APCI^+$): m/z (%) 177 (100) [M+H$^+$]. $[\alpha]_D^{25} = -12.8$ (c 0.80, $CHCl_3$), ee 36%.

4.4.2. (*S*)-1-(4-Ethylphenyl)ethyl acetate, (*S*)-3b

Colourless oil. IR (KBr): ν 3054, 2986, 1733, 1507 cm^{-1}. ^1H NMR ($CDCl_3$, 300.13 MHz): δ 1.25 (t, $^3J_{HH}$ = 7.6 Hz, 3 H), 1.54 (d, $^3J_{HH}$ = 6.5 Hz, 3H), 2.08 (s, 3H), 2.60–2.69 (m, 2H), 5.88 (q, $^3J_{HH}$ = 6.5 Hz, 1H,), 7.15–7.21 (m, 2H), 7.27–7.31 (m, 2H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 15.4 (CH_3), 21.3 (CH_3), 22.0 (CH_3), 72.2 (CH), 126.1 (2CH), 127.6 (2CH), 138.8 (C), 143.9 (C), 170.3 (C=O). MS (EI^+): m/z (%) 192 (20) [M^+], 117 (100). $[\alpha]_D^{25} = +35.9$ (c 0.74, $CHCl_3$), ee 86%.

4.4.3. (*R*)-3-(3-Methylphenyl)butan-2-one, (*R*)-4a

Yellow pale oil. IR (KBr): ν 3054, 2980, 1713, 1606, 1490 cm^{-1}. ^1H NMR ($CDCl_3$, 300.13 MHz): δ 1.37 (d, $^3J_{HH}$ = 6.9 Hz, 3H), 2.05 (s, 3H), 2.34 (s, 3H), 3.70 (q, $^3J_{HH}$ = 6.9 Hz, 1H), 7.00–7.09 (m, 3H), 7.19–7.25 (m, 1H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 17.1 (CH_3), 21.3 (CH_3), 28.3 (CH_3), 53.6 (CH), 124.8 (CH), 127.8 (CH), 128.4 (CH), 128.7 (CH), 138.6 (C), 140.4 (C), 207.1 (C=O). MS ($APCI^+$): m/z (%) 163 (100) [M+H$^+$]. $[\alpha]_D^{25} = -21.8$ (c 1.18, $CHCl_3$), ee 42%.

4.4.4. (*R*)-3-(3-Methoxylphenyl)butan-2-one, (*R*)-5a

Yellow pale oil. IR (KBr): ν 3055, 2983, 1712, 1609, 1487 cm^{-1}. ^1H NMR ($CDCl_3$, 300.13 MHz): δ 1.39 (d, $^3J_{HH}$ = 7.0 Hz, 3H), 2.06 (s, 3H), 3.72 (q, $^3J_{HH}$ = 7.0 Hz, 1H), 3.81 (s, 3H), 6.75–6.83 (m, 3H), 7.23–7.29 (m, 1H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 17.0 (CH_3), 28.2 (CH_3), 53.6 (CH), 55.1 (CH_3), 112.3 (CH), 113.4 (CH), 120.1 (CH), 129.8 (CH), 142.0 (C), 159.9 (C), 208.6 (C=O). MS ($APCI^+$): m/z (%) 179 (100) [M+H$^+$]. $[\alpha]_D^{25} = -31.6$ (c 1.40, $CHCl_3$), ee 71%.

4.4.5. (*R*)-3-(3-Chlorophenyl)butan-2-one, (*R*)-8a

Colourless oil. IR (KBr): ν 3054, 2985, 1714, 1422 cm^{-1}. ^1H NMR ($CDCl_3$, 300.13 MHz): δ 1.37 (d, $^3J_{HH}$ = 7.0 Hz, 3H), 2.05 (s, 3H), 3.71 (q, $^3J_{HH}$ = 7.0 Hz, 1H), 7.07–7.10 (m, 1H), 7.20–7.26 (m, 3H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 17.1 (CH_3), 28.4 (CH_3), 53.2 (CH), 125.9 (CH), 127.4 (CH), 128.0 (CH), 130.1 (CH), 134.7 (C), 142.4 (C), 208.0 (C=O). MS ($APCI^+$): m/z (%) 183 (30) [M+H$^+$]. $[\alpha]_D^{25} = -12.0$ (c 0.98, $CHCl_3$), ee 27%.

4.4.6. (*S*)-1-(3-Chlorophenyl)ethyl acetate, (*S*)-8b

Colourless oil. IR (KBr): ν 3054, 2987, 1733, 1507 cm^{-1}. ^1H NMR ($CDCl_3$, 300.13 MHz): δ 1.44 (d, $^3J_{HH}$ = 6.6 Hz, 3H), 2.01 (s, 3H), 5.75 (q, $^3J_{HH}$ = 6.6 Hz, 1H), 7.14–7.26 (m, 4H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 21.2 (CH_3), 22.1 (CH_3), 71.5 (CH), 124.2 (CH), 126.1 (CH), 127.9 (CH), 129.7 (CH), 134.3 (C), 143.7 (C), 170.1 (C=O). MS (EI^+): m/z (%) 198 (13) [M^+], 156 (70), 138 (38). $[\alpha]_D^{25} = +67.8$ (c 0.78, $CHCl_3$), ee 92%.

4.4.7. (*R*)-3-Phenylheptan-2-one, (*R*)-11a

Yellow pale oil. IR (KBr): ν 3055, 2960, 1711, 1601, 1423 cm^{-1}. ^1H NMR ($CDCl_3$, 300.13 MHz): δ 0.81 (t, $^3J_{HH}$ = 7.0 Hz, 3 H), 1.07–1.19 (m, 2H), 1.21–1.28 (m, 2H), 1.60–1.69 (m, 1H), 1.92–2.05 (m, 4H), 3.55 (t, $^3J_{HH}$ = 7.5 Hz, 1H), 7.15–7.29 (m, 5H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 13.8 (CH_3), 22.5 (CH_2), 28.9 (CH_3), 29.6 (CH_2), 31.4 (CH_2), 59.7 (CH), 127.1 (CH), 128.2 (2 CH), 128.8 (2 CH), 139.1 (C), 208.6 (C=O). MS (EI^+): m/z (%) 190 (2) [M^+] 147 (31), 91 (100).

4.4.8. (*R*)-4-Phenylheptan-3-one, (*R*)-13a

Colourless oil. IR (KBr): ν 3055, 2987, 1716, 1423 cm^{-1}. ^1H NMR ($CDCl_3$, 300.13 MHz): δ 0.90 (t, $^3J_{HH}$ = 7.2 Hz, 3 H), 0.98 (t, $^3J_{HH}$ = 7.2 Hz, 3 H), 1.15–1.29 (m, 2H), 1.64–1.77 (m, 1H), 1.97–2.10 (m, 1 H), 2.32–2.49 (m, 2H), 3.66 (t, $^3J_{HH}$ = 7.5 Hz, 1H), 7.22–7.34 (m, 5H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 7.8 (CH_3), 13.9 (CH_3), 20.6 (CH_2), 34.2 (CH_2), 35.0 (CH), 58.4 (CH), 126.9 (CH), 128.1 (2 CH), 128.7 (2 CH), 139.3 (C), 211.2 (C=O). MS (EI^+): m/z (%) 190 (9) [M^+], 105 (75), 85 (100). $[\alpha]_D^{25} = -10.8$ (c 1.12, $CHCl_3$), ee 29%.

4.4.9. (*S*)-1-Phenylbutyl propionate, (*S*)-13b

Yellow pale oil. IR (KBr): ν 3055, 2987, 1731, 1423 cm^{-1}. ^1H NMR ($CDCl_3$, 300.13 MHz): δ 0.85 (t, $^3J_{HH}$ = 7.2 Hz, 3H), 1.07 (t, $^3J_{HH}$ = 7.4 Hz, 3H), 1.15–1.34 (m, 2H), 1.61–1.73 (m, 1H), 1.77–1.90 (m, 1H), 2.24–2.33 (m, 2H), 5.69 (t, $^3J_{HH}$ = 7.0 Hz, 1H), 7.20–7.28 (m, 5H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 9.1 (CH_3), 13.7 (CH_3), 18.7 (CH_2), 27.8 (CH_2), 38.5 (CH_2), 75.6 (CH), 126.4 (2CH), 127.7

(CH), 128.3 (2CH), 140.9 (C), 173.7 (C=O). MS (ESI^+): *m*/*z* (%) 229 (100) [M+Na^+]. $[\alpha]_D^{25} = +35.8$ (*c* 0.91, $CHCl_3$), ee 96%.

4.4.10. (*R*)-4-Phenyloctan-3-one, (*R*)-14a

Yellow pale oil. IR (KBr): *ν* 3055, 2987, 1719, 1662, 1423 cm^{-1}. ^1H NMR ($CDCl_3$, 300.13 MHz): *δ* 0.89 (t, $^3J_{HH}$ = 7.0 Hz, 3H), 1.00 (t, $^3J_{HH}$ = 7.6 Hz, 3H), 1.16–1.21 (m, 2H), 1.22–1.37 (m, 2H), 1.69–1.83 (m, 1H), 2.02–2.14 (m, 1H), 2.38–2.49 (m, 2H), 3.66 (t, $^3J_{HH}$ = 7.4 Hz, 1H), 7.24–7.36 (m, 5H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): *δ* 13.8 (CH_3), 13.8 (CH_3), 22.5 (CH_2), 29.7 (CH_2), 31.9 (CH_2), 35.0 (CH_2), 58.7 (CH), 126.9 (CH), 128.2 (2 CH), 128.7 (2CH), 139.4 (C), 211.3 (C=O). MS (EI^+): *m*/*z* (%) 204 (1) [M^+], 148 (20), 91 (100).

4.4.11. (*S*)-1-Phenylpentyl propionate, (*S*)-14b

Yellow pale oil. IR (KBr): *ν* 3055, 2986, 1731, 1423 cm^{-1}. ^1H NMR ($CDCl_3$, 300.13 MHz): *δ* 0.81 (t, $^3J_{HH}$ = 7.0 Hz, 3H), 1.10 (t, $^3J_{HH}$ = 6.6 Hz, 3H), 1.20–1.28 (m, 4H), 1.64–1.76 (m, 1H), 1.78–1.90 (m, 1H), 2.24–2.33 (m, 2H), 5.67 (t, $^3J_{HH}$ = 6.3 Hz, 1H), 7.19–7.28 (m, 5H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): *δ* 9.0 (CH_3), 13.9 (CH_3), 22.4 (CH_2), 27.6 (CH_2), 27.8 (CH_2), 36.0 (CH_2), 75.8 (CH), 126.4 (2CH), 127.6 (CH), 128.3 (2CH), 140.9 (C), 169.8 (C=O). MS (EI^+): *m*/*z* (%) 220 (5) [M^+], 164 (28), 117 (100), 91 (55). $[\alpha]_D^{25} = +21.2$ (*c* 0.58, $CHCl_3$), ee 95%.

4.4.12. (*R*)-4-Phenylhept-6-en-3-one, (*R*)-15a

Yellow pale oil. IR (KBr): *ν* 3415, 3054, 2983, 1712, 1614, 1494 cm^{-1}. ^1H NMR ($CDCl_3$, 300.13 MHz): *δ* 1.00 (t, $^3J_{HH}$ = 7.3 Hz, 3H), 2.35–2.53 (m, 3H), 2.80–2.90 (m, 1H), 3.76 (t, $^3J_{HH}$ = 7.5 Hz, 1H), 4.97–5.09 (m, 2H), 5.64–5.78 (m, 1H), 7.23–7.30 (m, 5H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): *δ* 7.7 (CH_3), 35.1 (CH_2), 36.4 (CH_2), 58.4 (CH), 116.5 (CH_2), 127.2 (CH), 128.2 (2CH), 128.8 (2CH), 135.8 (CH), 138.6 (C), 210.3 (C=O). MS ($APCI^+$): *m*/*z* (%) 189 (100) [M+H^+], 227 (8) [M+$K]^+$. $[\alpha]_D^{25} = -7.2$ (*c* 0.85, $CHCl_3$), ee 38%.

4.4.13. (*S*)-4-Phenylbut-1-enyl propionate, (*S*)-15b

Yellow pale oil. IR (KBr): *ν* 3425, 3053, 2984, 1736, 1642, 1494 cm^{-1}. ^1H NMR ($CDCl_3$, 300.13 MHz): *δ* 1.18 (t, $^3J_{HH}$ = 7.5 Hz, 3H), 2.36–2.45 (m, 2H), 2.56–2.75 (m, 2H), 5.08–5.15 (m, 2H), 5.65–5.74 (m, 1H), 5.77–5.91 (m, 1H), 7.25–7.43 (m, 5H). ^{13}C–NMR (CDC_{l3}, 75.5 MHz): *δ* 9.3 (CH_3), 28.1 (CH_2), 41.1 (CH_2), 75.1 (CH), 118.2 (CH_2), 126.7 (2CH), 128.1 (CH), 128.6 (2CH), 133.6 (CH), 140.5 (C), 173.9 (C=O). MS ($APCI^+$): *m*/*z* (%) 227 (17) [M+Na^+]. $[\alpha]_D^{25} = +16.9$ (*c* 0.65, $CHCl_3$), ee 77%.

4.5. Determination of the kinetic parameters

For the determination of the steady-kinetic parameters of wt PAMO, M446G PAMO and HAPMO with ketones (±)-**1**–**10a**, the enzymatic activity was determined by monitoring NADPH consumption at 340 nm (ε_{340} = 6.22 mM^{-1} cm^{-1}). Stocks solutions of substrates (1.0 M) were made in dimethyl sulfoxide. A reaction mixture (1.0 mL) usually contained 50 mM Tris/HCl (pH 7.5), 100 μM NADPH, 1% (v/v) DMSO and 0.05 μM BVMO. The presence of 1% DMSO resulted in only a slight decrease in BVMO activity, while a higher solubility of certain compounds can be achieved.

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