Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 18 (2007) 1338–1344

# Enzymatic kinetic resolution of racemic ketones catalyzed by Baeyer–Villiger monooxygenases

Cristina Rodríguez,[a] Gonzalo de Gonzalo,[a] Marco W. Fraaije[b] and Vicente Gotor[a,*]

[a]*Departamento de Química Orgánica e Inorgánica, Instituto Universitario de Biotecnología de Asturias, Universidad de Oviedo, 33006 Oviedo, Asturias, Spain*

[b]*Laboratory of Biochemistry, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands*

Received 10 April 2007; accepted 29 May 2007

**Abstract**—A set of racemic cyclic and linear ketones, as well as 2-phenylpropionaldehyde, were tested as substrates in the enzymatic Baeyer–Villiger oxidation catalyzed by two Baeyer–Villiger monooxygenases: phenylacetone monooxygenase (PAMO) and 4-hydroxyacetophenone monooxygenase (HAPMO). Excellent enantioselectivites ($E > 200$) can be obtained in the kinetic resolution processes depending on the substrate structure and the reaction conditions. The parameters affecting the biocatalytic properties of these enzymes were also studied, in order to establish a deeper understanding of these novel biocatalysts.


## 1. Introduction

Nowadays, the Baeyer–Villiger oxidation of ketones is one of the key reactions in synthetic processes.[1] The transformation of ketones into the corresponding esters and lactones, valuable intermediates in organic chemistry and frequently used precursors in enantioselective synthesis, can be carried out chemically by employing peroxides or peracids. While these oxidants are very effective, they also represent toxic and labile reagents. To avoid the use of these reactive substances, transition metal catalysts and organocatalytic compounds have been developed,[2] which use hydrogen peroxide or oxygen as milder oxidants. However, these novel methodologies in most cases have demonstrated a lack of selectivity. In recent years, the enzymatic approach to perform Baeyer–Villiger reactions has been successfully employed.[3] By applying these novel biocatalytic methods, optically active lactones and esters can be obtained in processes carried out in water and worked with under mild and environmental friendly conditions, with high regio- and/or enantioselectivities. All of these advantages make the biocatalytic approach most appealing for the industrial application of the Baeyer–Villiger oxidation.

Baeyer–Villiger monooxygenases (BVMOs) represent a class of enzymes widely used in the oxidation of linear and cyclic ketones. BVMOs are nicotinamide cofactor dependent flavoproteins that catalyze a set of oxidative reactions, in addition to the nucleophilic oxidation of the carbonyl group and boron atoms, electrophilic oxygenations of different heteroatoms, such as sulfur, nitrogen, selenium and phosphorous are also catalyzed.[4] Over the last few decades, a large number of these biocatalysts have been discovered but only a few BVMOs were employed and studied for synthetic purposes. The favorite enzyme for BVMO-mediated catalysis was cyclohexanone monooxygenase from *Acinetobacter calcoaceticus* NCIMB 9871, a versatile enzyme, which is able to oxidize a wide range of substrates.[5]

Due to advances in biochemistry and the availability of new (recombinant) enzymes, BVMOs have received increasing interest in recent years.[6] Clear examples of this trend are the newly cloned and overexpressed BVMOs phenylacetone monooxygenase (PAMO)[7] and 4-hydroxyacetophenone monooxygenase (HAPMO).[8]

PAMO (EC 1.14.13.92) is a monomeric and thermostable NADPH-dependent BVMO isolated from *Thermobifida fusca*. Previous studies reported phenylacetone as its best substrate, but PAMO is also able to oxidize aliphatic

* Corresponding author. Tel./fax: +34 985 103448; e-mail: vgs@fq.uniovi.es

0957-4166/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2007.05.033

ketones, sulfides, racemic sulfoxides, amines and the boron atom.[9] This biocatalyst can even perform oxidations when working in non-conventional reaction media (mixtures of aqueous buffer–water miscible or immiscible organic cosolvent), which is accompanied by interesting changes in its biocatalytic properties (e.g., improvement or reversal of enantioselectivity).[10] PAMO is the first BVMO for which the crystal structure has been elucidated by X-ray analysis, which has provided details on the catalyst performance at an atomic level.[11] HAPMO (EC 1.14.13.84), obtained from *Pseudomonas fluorescens* ACB, is a homodimeric NADPH-dependent BVMO, that is, primarily active with a wide range of aromatic ketones, especially when presenting an electron-donating group at the *para*-position.[12] This enzyme can also oxidize aliphatic ketones and also catalyze enantioselective sulfoxidations.[13]

Herein we report the Baeyer–Villiger oxidation of different racemic carbonylic compounds catalyzed by the above-mentioned BVMOs, in order to obtain enantiopure ketones and corresponding chiral lactones or esters.

## 2. Results and discussion

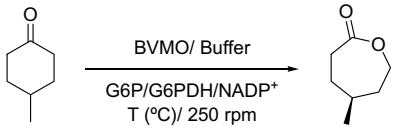
### 2.1. Enzymatic oxidation of cyclic ketones

The enzymatic Baeyer–Villiger oxidations of ketones to the corresponding esters and lactones by recombinant PAMO and HAPMO were coupled to an auxiliary enzymatic reaction in order to regenerate the nicotinamide cofactor NADPH. As the NADPH recycling system, glucose-6-phosphate and glucose-6-phosphate dehydrogenase (G6PDH) were employed.[14]

Our first efforts were devoted to studying the Baeyer Villiger oxidation of a set of cyclic ketones. Both (±)-2-methylcyclopentanone and (±)-2-methylcyclohexanone were oxidized by PAMO and HAPMO, but in processes with low selectivity (data not shown). Different reaction conditions were employed, by modifying the pH and temperature, leading in all cases to the (*S*)-lactone, with only *E* values lower than 3.[15] Oxidations conducted with PAMO were generally faster than those performed with HAPMO.

Both BVMOs were also tested in the Baeyer–Villiger oxidation of a prochiral ketone, 4-methylcyclohexanone **1a**, as shown in Table 1. Compound (*S*)-**1b** was obtained with low to moderate conversions and enantiomeric excesses depending on the conditions employed. Increasing the pH and temperature led to a higher enzymatic activity, with only a slight loss in selectivity. Compound (*S*)-**1b** can be obtained with ee = 40% by performing the oxidation with PAMO at pH 9 and 20 °C (entry 2) or with ee = 44% by carrying out the Baeyer–Villiger oxidation of **1a** employing HAPMO under the same conditions (entry 7). It was observed that the addition of 1% MeOH to the reaction medium when working at pH 9 and 30 °C, can slightly improve the PAMO selectivity with a loss in conversion (entry 5), as observed previously for this enzyme.[10b] Higher contents of methanol resulted in no oxidation after long reaction times.

**Table 1.** BVMO-catalyzed oxidation of 4-methylcyclohexanone to (*S*)-4-methyl-6-hexanolactone[a]




**1a** (*S*)-**1b**

| Entry | Enzyme | pH | *T* (°C) | *c*[b] (%) | ee[b] (%) |
|---|---|---|---|---|---|
| 1 | PAMO | 8 | 30 | ⩽1 | — |
| 2 | PAMO | 9 | 20 | 6 | 40 |
| 3 | PAMO | 9 | 30 | 15 | 32 |
| 4 | PAMO | 10 | 30 | 19 | 28 |
| 5[c] | PAMO | 9 | 30 | 8 | 38 |
| 6 | HAPMO | 8 | 20 | ⩽1 | — |
| 7 | HAPMO | 9 | 20 | 9 | 44 |
| 8 | HAPMO | 9 | 30 | 14 | 42 |

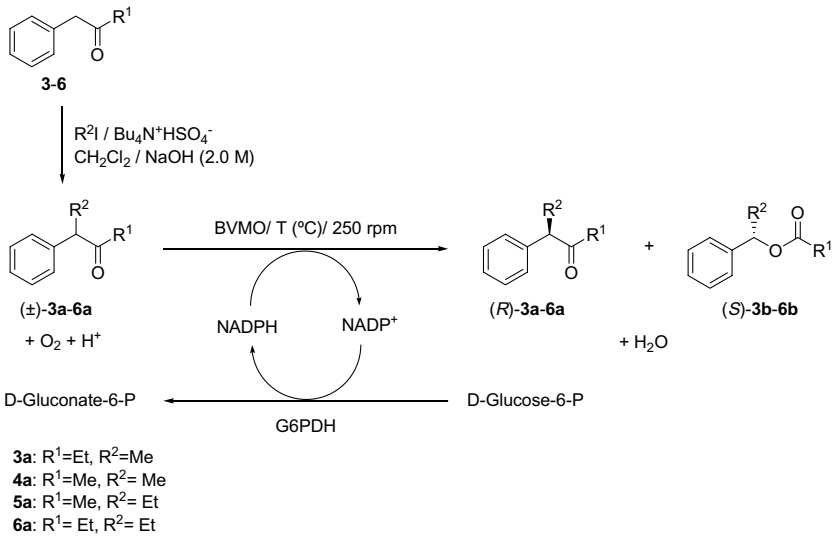
[a] Reaction time, 48 h and substrate concentration: 2 g $L^{-1}$. For other details, see Section 4.
[b] Determined by GC.
[c] Reaction performed in 1% MeOH as cosolvent.

Finally, the oxidation of phenylcyclohexanones was investigated, aided by the fact that PAMO and HAPMO are primarily active on aromatic ketones. After long incubation times, no reaction was observed with either (±)-2-phenyl- or 4-phenylcyclohexanone. This may be due to the low solubility of these substrates in aqueous buffer.

### 2.2. Oxidation of 2-phenylpropionaldehyde catalyzed by PAMO and HAPMO

After these results, we focused on the enzymatic resolution of different aromatic α-substituted carbonyl compounds (aldehyde **2a** and ketones **3a**–**6a**), catalyzed by both PAMO and HAPMO. In previous reports, it has been described that phenylacetone was the best PAMO substrate and could also be easily oxidized by HAPMO. It was observed that for this set of compounds, higher conversions were measured using PAMO. This is in agreement with the physiological substrates for both enzymes: PAMO is primarily active with phenylacetones, while HAPMO preferably oxidizes acetophenones. Racemic ketones (±)-**3a**–**6a** were prepared as shown in Scheme 1,[16] starting from the corresponding commercially available phenylacetones **3**–**6**.

Due to the structure of substrates **2a**–**6a**, presenting a hydrogen atom with a strong acidic character, we expected that spontaneous racemization could occur via a keto–enol tautomerization. Nevertheless, the enantiomeric excess of optically active compounds (*R*)-**2a**–**6a** when submitted to different reaction conditions, did not decrease after 24 h, even when carrying out the processes at high pHs (pH 9–10) and temperatures (40 °C). This excludes the possibility of performing dynamic kinetic resolutions of phenylaldehyde and phenylketones, as described in a previous paper for a similar substrate.[17] Only PAMO- or HAPMO-catalyzed kinetic resolution of the racemic carbonyl compounds was observed.

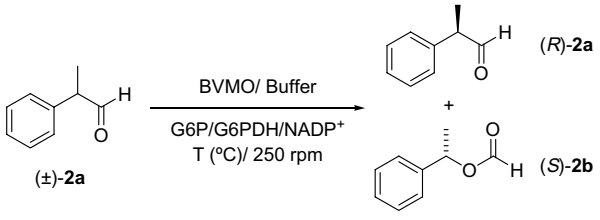



**Scheme 1.** Enzymatic resolution of different substituted phenylketones catalyzed by the Baeyer–Villiger monooxygenases PAMO and HAPMO.

First, the enzymatic Baeyer–Villiger oxidation of commercial 2-phenylpropionaldehyde (±)-**2a** catalyzed by PAMO and HAPMO was studied (Table 2). When working under the same reaction conditions, higher selectivities and activities were obtained when employing PAMO, but for both biocatalysts, moderate to low *E*-values were achieved. Oxidation of **2a** led in all cases to the formation of the (*S*)-1-phenylethyl formate **2b**, leaving the aldehyde with an (*R*)-configuration.

The effect of pH when conducting the oxidations with PAMO at 20 °C was studied (entries 1–6). It was observed that by increasing the pH of the Tris/HCl buffer, there were two effects in the enzymatic resolution of (±)-**2a**: (i) an important increase in the enzyme activity: whereas at pH 6 there was no oxidation (entry 1), (*S*)-**2b** can be obtained with 50% conversion at pH 10, and (ii) a slight decrease in the *E*-values, especially at pH values higher than 9. This correlation between the pH and enantioselective behaviour of PAMO has been thoroughly studied in a recent publication and can be attributed to (de)protonation of specific enzyme active-site moieties.[18] By increasing the temperature when performing the PAMO-catalyzed oxidations at pH 8, higher enzyme activities (at 40 °C, it was possible to obtain a $c = 28\%$ after 4 h) were accompanied by a loss in *E* (entries 7 and 8).

**Table 2.** Enzymatic oxidation of (±)-2-phenylpropionaldehyde (±)-**2a** employing novel BVMOs[a]




| Entry | BVMO | pH | *T* (°C) | ee[b] (%) (*R*)-**2a** | ee[b] (%) (*S*)-**2b** | *c*[c] (%) | *E* |
|---|---|---|---|---|---|---|---|
| 1 | PAMO | 6 | 20 | — | — | ⩽1 | — |
| 2 | PAMO | 7 | 20 | 9 | 92 | 8 | 26 |
| 3 | PAMO | 8 | 20 | 50 | 88 | 36 | 25 |
| 4 | PAMO | 9 | 20 | 62 | 85 | 42 | 23 |
| 5 | PAMO | 9.5 | 20 | 65 | 81 | 44 | 19 |
| 6 | PAMO | 10 | 20 | 76 | 76 | 50 | 17 |
| 7 | PAMO | 8 | 30 | 53 | 83 | 38 | 20 |
| 8[d] | PAMO | 8 | 40 | 30 | 75 | 28 | 9 |
| 9 | HAPMO | 8 | 20 | 9 | 88 | 9 | 17 |
| 10 | HAPMO | 9 | 20 | 12 | 77 | 13 | 9 |
| 11 | HAPMO | 9 | 30 | 14 | 83 | 15 | 10 |

[a] Reactions stopped after 8 h for PAMO and 30 h for HAPMO. [**2a**] = 2 g $L^{-1}$. For other details, see Section 4.
[b] Determined by GC.
[c] Conversion, $c = ee_s/(ee_s + ee_p)$.
[d] Reaction stopped after 4 h.

The enzymatic Baeyer–Villiger oxidation of (±)-**2a** employing HAPMO exhibited the highest enantioselectivity ($E = 17$, entry 9) at pH 8 and 20 °C. An increase in either pH (entry 10) or temperature (entry 11) led to higher conversions and less selective resolutions.

### 2.3. PAMO and HAPMO catalyzed kinetic resolution of phenylketones

The enzymatic oxidation of racemic 2-phenylpentan-3-one (±)-**3a** catalyzed by PAMO at pH 8 and 20 °C (Table 3, entry 4) led to (*S*)-**3b**, while the unreacted (*R*)-enantiomer remained, in a highly selective process ($E > 200$) with a $c = 19\%$ after 1 h.

The pH effect on this kinetic resolution was analyzed, which revealed a similar trend as observed for 2-phenylpropionaldehyde. The resolution of (±)-**3a** was carried out with high selectivities until pH 9, enabling production of enantiopure (*R*)-**3a** and (*S*)-**3b** depending on the conversion. When working under more basic Tris/HCl buffer media, there was a loss in selectivity, but even at pH 10 a good enantioselectivity can be achieved ($E = 111$, entry 7). Enzyme activity became higher by increasing the pH value. The highest conversion was observed at pH 10, ($c = 32\%$ after 1 h). Contrarily, when oxidations were performed at low pH (6 and 7, entries 1 and 2, respectively), there was an important loss in the conversions measured ($c < 10\%$ after 1 h).

As PAMO has been isolated from a thermophilic microorganism, the optimal temperature in terms of activity and selectivity for the Baeyer–Villiger oxidation of (±)-**3a** was analyzed. It should be noted that the oxidations are performed in a double enzymatic system, so the temperature can also affect glucose-6-phosphate dehydrogenase. High selectivities were achieved in the range from 15 to 50 °C. Only when the oxidation was carried out at 60 °C, was there a slight decrease in the *E*-value ($E = 148$, entry 13). PAMO activity increased by working at higher temperatures, reaching a maximum value at 40 °C ($c = 20\%$ after 30 min, entry 11). Increasing the temperature from this value resulted in a negative effect on the PAMO-catalyzed resolution, obtaining lower activities. It is worth noting that oxidations with this biocatalyst can even be performed at 60 °C ($c = 11\%$ after 2 h, entry 13), demonstrating the ability of PAMO to work even under drastic conditions.

Baeyer–Villiger oxidation of (±)-**3a** with HAPMO at pH 8 and 20 °C (Table 4, entry 4) also resulted in an excellent *E*-value ($E = 165$), in a process with a conversion close to 50% ($c = 46\%$) after 14 h. The (*S*)-enantiomer was mainly

**Table 3.** Effect of pH and temperature in the enzymatic resolution of (±)-2-phenylpentan-3-one catalyzed by PAMO[a]

| Entry | pH | *T* (°C) | Time (h) | ee[b] (%) (*R*)-**3a** | ee[b] (*S*)-**3b** (%) | *c*[c] (%) | *E* |
|---|---|---|---|---|---|---|---|
| 1 | 6 | 20 | 1 | 3 | 99 | 3 | >200 |
| 2 | 7 | 20 | 1 | 6 | 99 | 6 | >200 |
| 3 | 7.5 | 20 | 1 | 13 | 99 | 12 | >200 |
| 4 | 8 | 20 | 1 | 24 | 99 | 19 | >200 |
| 5 | 8.5 | 20 | 1 | 22 | 99 | 18 | >200 |
| 6 | 9 | 20 | 1 | 26 | 99 | 21 | >200 |
| 7 | 9.5 | 20 | 1 | 35 | 98 | 27 | 155 |
| 8 | 10 | 20 | 1 | 56 | 97 | 32 | 111 |
| 9 | 8 | 15 | 1 | 13 | 99 | 12 | >200 |
| 10 | 8 | 30 | 0.75 | 27 | 99 | 21 | >200 |
| 11 | 8 | 40 | 0.5 | 24 | 99 | 20 | >200 |
| 12 | 8 | 50 | 0.75 | 24 | 98 | 20 | 193 |
| 13 | 8 | 60 | 2 | 11 | 98 | 11 | 148 |

[a] [**3a**] = 2 g $L^{-1}$. For other reaction details, see Section 4.
[b] Determined by GC.
[c] Conversion, $c = ee_s/(ee_s + ee_p)$.

**Table 4.** HAPMO catalyzed oxidation of (±)-**3a**[a]

| Entry | pH | *T* (°C) | Time (h) | ee[b] (%) (*R*)-**7a** | ee[b] (*S*)-**7b** (%) | *c*[c] (%) | *E* |
|---|---|---|---|---|---|---|---|
| 1 | 6 | 20 | 48 | — | — | ⩽1 | — |
| 2 | 7 | 20 | 48 | — | — | ⩽1 | — |
| 3 | 7.5 | 20 | 24 | 25 | 98 | 20 | 146 |
| 4 | 8 | 20 | 14 | 83 | 97 | 46 | 165 |
| 5 | 9 | 20 | 14 | 95 | 87 | 53 | 52 |
| 6 | 10 | 20 | 10 | 91 | 83 | 52 | 35 |
| 7 | 8 | 10 | 14 | 93 | 98 | 48 | >200 |
| 8 | 8 | 15 | 14 | 98 | 97 | 50 | >200 |
| 9 | 8 | 30 | 14 | 76 | 97 | 44 | 155 |
| 10 | 8 | 40 | 24 | 9 | 98 | 9 | 120 |
| 11 | 8 | 50 | 24 | 4 | 96 | 4 | 52 |

[a] [**3a**] = 2 g $L^{-1}$. For other details, see Section 4.
[b] Determined by GC.
[c] Conversion, $c = ee_s/(ee_s + ee_p)$.

oxidized in all tested conditions. The effect of pH on this enzyme for the kinetic resolution of (±)-**3a** was analyzed. No reaction was observed at pH 6 and 7, even after 48 h. When performing the reactions at pH 7.5, a high $E$ value was obtained in a slow process ($c = 20\%$ after 24 h). The highest conversions for the enzymatic resolutions were measured at pH 9 and 10 (entries 5 and 6) but in these conditions there was an important loss in HAPMO selectivity ($E = 55$–$32$ for pH 9–10, respectively). The temperature was also analyzed as an important parameter in the enzymatic resolution of (±)-**3a**. When performing the Baeyer–Villiger oxidations at 10 and 15 °C, high enantioselectivities and conversions were obtained (entries 7 and 8). Both the activity and the $E$-value became lower by increasing the temperature. This effect was especially observed at 50 °C, as shown in entry 11: the oxidation took place with only $c = 4\%$ after 24 h with good enantioselectivity ($E = 52$).

The effect of the **3a** concentration on the biocatalytic properties of the two BVMOs was evaluated, as shown in Figure 1. When the oxidations were performed employing PAMO, no changes in enantioselectivity were observed until 12 g $L^{-1}$ of ketone. For higher values, a slight decrease in the enantioselectivity was observed ($E = 137$ for 20 g $L^{-1}$). In order to compare the activity of the enzyme, the reaction rate (expressed as grams of (±)-**3a** consumed per L of solution $h^{-1}$) was defined. When the oxidation was performed with PAMO, this parameter increased by working at higher 2-phenylpentan-3-one concentrations,

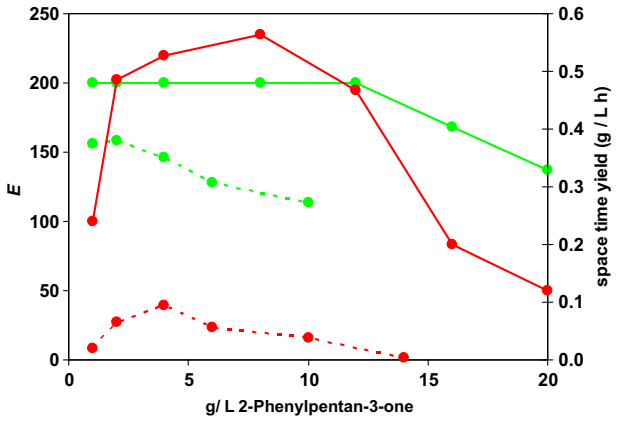



**Figure 1.** Effect of substrate concentration in the selectivity (green line) and reaction rate (red line), expressed as g of ketone consumed per L of solution and per hour of PAMO (solid line) and HAPMO (dashed line) when performing the reactions at pH 8 and 20 °C.

reaching a maximum at 8 g $L^{-1}$. After this value, the reaction rate decreased, but the biocatalyst was still active. PAMO was still able to oxidize (±)-**3a** at concentrations higher than 20 g $L^{-1}$. A very similar substrate concentration-activity profile (albeit with reduced reaction rate values) was achieved for HAPMO. This enzyme exhibited a maximum reaction rate at 4 g $L^{-1}$ while higher concentrations caused significant deactivation of the biocatalyst. At ketone concentrations higher than 10 g $L^{-1}$ no (*S*)-**3b** formation was observed even after long reaction times. A decrease in the selectivity was also observed for HAPMO when performing the oxidations at concentrations higher than 4 g $L^{-1}$, indicating that this enzyme is more sensitive to substrate concentration than PAMO.

Finally, the racemic phenylketones (±)-3-phenylbutan-2-one **4a**, (±)-3-phenylpentan-2-one **5a** and (±)-4-phenylhexan-3-one **6a**, presenting a close structure to (±)-2-phenylpentan-3-one, were also resolved with excellent selectivities by both enzymes, when the Baeyer–Villiger oxidations were performed at 20 °C and pH 8 (Table 5). (*S*)-Esters and (*R*)-ketones were obtained; the processes carried out with PAMO, were faster and more selective than those performed with HAPMO.

## 3. Conclusion

Two newly cloned and overexpressed Baeyer–Villiger monooxygenases have been employed for the enzymatic resolution of a set of carbonyl compounds. Methyl cycloketones were oxidized by PAMO and HAPMO in processes with low to moderate selectivities. No reactivity was exhibited by these two enzymes in the oxidation of phenylcyclohexanones. BVMO-catalyzed oxidation of 2-phenylpropionaldehyde led to (*S*)-1-phenylethyl formate and the (*R*)-aldehyde with moderate enantioselectivities, depending on the conditions employed. Much better results can be achieved by using linear phenylketones as substrates, especially when the kinetic resolutions were performed by PAMO. (*S*)-Esters were obtained, leaving the ketones with an (*R*)-configuration in processes with excellent selectivities. PAMO can perform oxidations at substrate concentrations close to 20 g $L^{-1}$ with high selectivity, while HAPMO seems to be more sensitive to the ketone concentration. The pH effect on the kinetic resolution of the phenylaldehyde and the phenylketones was studied, revealing that by increasing the pH of the medium, higher activities and

**Table 5.** BVMO catalyzed oxidation of substrates (±)-**4a**–**6a**[a]

| Ketone | BVMO | Time (h) | ee[b] (*R*)-ket (%) | ee[b] (*S*)-ester (%) | $c$[c] (%) | $E$ |
|---|---|---|---|---|---|---|
| **4a** | PAMO | 1 | 36 | 98 | 27 | 188 |
| **4a** | HAPMO | 14 | 43 | 97 | 30 | 137 |
| **5a** | PAMO | 1 | 45 | 99 | 32 | >200 |
| **5a** | HAPMO | 14 | 78 | 96 | 45 | 117 |
| **6a** | PAMO | 1 | 98 | 95 | 51 | 179 |
| **6a** | HAPMO | 14 | 98 | 90 | 52 | 87 |

[a] [**4a**–**6a**] = 2 g $L^{-1}$. For more reaction details, see Section 4.
[b] Determined by GC.
[c] Conversion, $c = ee_s/(ee_s + ee_p)$.

lower enantioselectivities could be obtained for both PAMO and HAPMO. It was also found that PAMO is an effective catalyst at higher temperatures (40 °C), with it able to catalyze Baeyer–Villiger reactions at 60 °C. The best results were achieved by employing HAPMO at low temperatures (10–20 °C).

## 4. Experimental

### 4.1. General

Recombinant histidine-tagged phenylacetone monooxygenase and recombinant 4-hydroxyacetophenone monooxygenase were overexpressed and purified according to previously described methods.[7,8] The oxidation reactions were performed using the purified enzymes. One unit of Baeyer–Villiger monooxygenase oxidizes 1.0 μmol of 2-phenylpentan-3-one to the ester, per minute at pH 8 and 20 °C, in the presence of NADPH. Glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* was obtained from Fluka-BioChemika. Glucose-6-phosphate and NADPH was purchased by Sigma–Aldrich.

All the starting ketones and 2-phenylpropionaldehyde, as well as all other reagents and solvents were of the highest quality grade available, supplied by Sigma–Aldrich–Fluka–Fluka. The corresponding racemic compounds (±)-**3a**–**6a** were prepared according to the literature, using either methyl or ethyl iodide and NaOH in a biphasic medium (46–60% yields)[16] and exhibit physical and spectral properties in accordance with those reported. Racemic lactones and formate (±)-**2b** were synthesized with high yields from the corresponding ketones employing *m*-CPBA in $CH_2Cl_2$. Racemic esters (±)-**3b**–**6b** were prepared by the chemical acylation of commercial 1-phenylethanol or 1-phenylpropanol (yields higher than 80%), which cause the direct oxidation with *m*-CPBA or $H_2O_2$ to give low yields and side products. All compounds synthesized exhibit physical and spectral properties in accordance with those reported.[16,19]

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. Optical rotations were measured using a Perkin–Elmer 241 polarimeter and are quoted in units of $10^{-1}$ deg cm$^2$ g$^{-1}$. $^1$H NMR, $^{13}$C NMR and DEPT spectra were recorded with TMS (tetramethylsilane) as the internal standard with Bruker AC-300 ($^1$H, 300.13 MHz and $^{13}$C, 75.4 MHz) and Bruker AC-300-DPX ($^1$H, 300.13 MHz and $^{13}$C: 75.4 MHz) spectrometers. The chemical shift values ($\delta$) are given in ppm and the coupling constants ($J$) in Hertz (Hz). ESI$^+$ using a HP1100 chromatograph mass detector or EI with a Finigan MAT 95 spectrometer was used to record mass spectra (MS).

GC analyses were performed on a Hewlett Packard 6890 Series II chromatograph equipped with the following columns: Scientific Agilent Technologies HP-1 (30 m × 0.25 mm × 0.25 μm, 1.0 bar $N_2$) for achiral analyses and Restek RtβDEXse (30 m × 0.25 mm × 0.25 μm, 1.0 bar $N_2$) for chiral determinations. For all the analyses, the injector temperature was 225 °C and the FID temperature was 250 °C.

The absolute configuration of chiral methyl cycloketones and lactones and formate **2b** was determined by comparison of the GC chromatograms with the patterns described in previous experiments for the known configurations.[19c,20] For esters **3b**–**6b** the absolute configuration was established by comparison with an authentic sample, prepared from the chemical acylation of the corresponding commercial chiral alcohols.

### 4.2. General procedure for the BVMO-catalyzed oxidation of cyclic ketones, linear ketones (±)-3a–6a and aldehyde (±)-2a

In a typical experiment, the substrates (10–15 mM) were dissolved in a Tris/HCl buffer (50 mM, different pH, 1.0 mL), containing glucose-6-phosphate (1.5 equiv), glucose-6-phosphate dehydrogenase (10.0 units), NADPH (0.02 mM) and 1.0 unit of phenylacetone monooxygenase or 4-hydroxyacetophenone monooxygenase. The mixtures were shaken at 250 rpm in a rotatory shaker at temperatures selected for the times established. The reactions were then stopped, extracted with dichloromethane or ethyl acetate (3 × 0.5 mL), dried over $Na_2SO_4$ and analyzed by gas chromatography in order to determine the conversion and enantiomeric excesses of the (*S*)-esters and the corresponding enantiomer of the remaining substrates. Control experiments in the absence of enzyme were performed for all substrates, not observing the reaction after long times.

### 4.3. General procedure for the HAPMO-catalyzed oxidation at multimilligram scale of (±)-2-phenylpentan-3-one and (±)-4-phenylhexan-3-one

Racemic ketones **3a** or **6a** (0.5 mmol) were dissolved in a TRIS/HCl buffer (50 mM, pH 9.0 for **3a** and 8.0 for **6a**, 20 mL) containing glucose-6-phosphate (1.5 mmol), glucose-6-phosphate dehydrogenase (10.0 units), NADPH (0.02 mM) and HAPMO (1 unit). The mixtures were shaken at 20 °C and 250 rpm in a rotatory shaker for 60 h for compound **3a** and 72 h for ketone **6a**. Once finished, the reactions were extracted with ethyl acetate (4 × 15 mL) and the combined organic layers were dried over $Na_2SO_4$, filtered and evaporated under reduced pressure. The crude residues were purified by flash chromatography on silica gel, with hexane/diethyl ether 8:2 to afford: (*R*)-**3a** (colourless oil, 48.6 mg, 60% yield) and (*S*)-**3b** (colourless oil, 26.0 mg, 29% yield); (*R*)-**6a** (colourless oil, 45.8 mg, 52% yield) and (*S*)-**6b** (colourless oil, 37.2 mg, 39% yield).

**4.3.1. (*S*)-4-Methyl-6-hexanelactone, 1b.** Determination of the ee by GC analysis: RtβDEXse, 50 °C (5 min), 3 °C/min, 200 °C (10 min). $t_R$ (*S*) 37.2 min; $t_R$ (*R*) 38.0 min.

**4.3.2. (*R*)-2-Phenylpropionaldehyde, 2a.** Determination of the ee by GC analysis: RtβDEXse, 70 °C (5 min), 3 °C/min, 200 °C (5 min). $t_R$ (*R*) 24.9 min; $t_R$ (*S*) 25.4 min.

**(*S*)-1-Phenylethyl formate**, **2b**: same GC conditions: $t_R$ (*S*) 26.9 min; $t_R$ (*R*) 27.3 min.

**4.3.3. (*R*)-2-Phenylpentan-3-one, 3a.** Determination of the ee by GC analysis: RtβDEXse, 70 °C (5 min), 3 °C/min, 200 °C (5 min). $t_R$ (*R*) 28.1 min; $t_R$ (*S*) 28.4 min. $[\alpha]_D^{25} = -76.4$ (*c* 1.20, $CHCl_3$), ee 95%. **(*S*)-1-Phenylethyl propionate**, **3b**: same GC conditions: $t_R$ (*S*) 28.9 min; $t_R$ (*R*) 29.3 min.

**4.3.4. (*R*)-3-Phenylbutan-2-one, 4a.** Determination of the ee by GC analysis: RtβDEXse, 70 °C (5 min), 1 °C/min, 120 °C (5 min). $t_R$ (*R*) 44.3 min; $t_R$ (*S*) 46.4 min. **(*S*)-1-Phenylethyl acetate**, **4b**: same GC conditions: $t_R$ (*S*) 42.9 min; $t_R$ (*R*) 50.5 min.

**4.3.5. (*R*)-3-Phenylpentan-2-one, 5a.** Determination of the ee by GC analysis: RtβDEXse, 110 °C Isotherm. $t_R$ (*S*) 21.4 min; $t_R$ (*R*) 22.3 min. **(*S*)-1-Phenylpropyl acetate**, **5b**: same GC conditions: $t_R$ (*S*) 23.4 min; $t_R$ (*R*) 26.0 min.

**4.3.6. (*R*)-4-Phenylhexan-3-one, 6a.** Determination of the ee by GC analysis: RtβDEXse, 90 °C (30 min), 3 °C/min, 200 °C (5 min). $t_R$ (*R*) 47.3 min; $t_R$ (*S*) 48.4 min. $[\alpha]_D^{25} = -61.2$ (*c* 0.75, $CHCl_3$), ee 98%. **(*S*)-1-Phenylpropyl propionate**, **6b**: same GC conditions: $t_R$ (*S*) 45.5 min; $t_R$ (*R*) 47.9 min. $[\alpha]_D^{25} = -41.7$ (*c* 0.83, $CHCl_3$), ee 90%.

## Acknowledgements

Cristina Rodríguez is financed by Universidad de Oviedo. Dr. Gonzalo de Gonzalo thanks the Ministerio de Educación y Ciencia (MEC) of Spain for his financial support. We thank Daniel E. Pazmiño Torres for his technical assistance. This work was supported by the MEC (Project CTQ-04-04185).

## References

1. (a) Krow, G. R. *Org. React.* **1993**, *43*, 251–296; (b) Renz, M.; Meunier, B. *Eur. J. Org. Chem.* **1999**, *4*, 737–750; (c) Ten Brink, G.-J.; Arends, I. W. C. E.; Sheldon, R. E. *Chem. Rev.* **2004**, *104*, 4105–4123.
2. (a) Murahashi, S.-I.; Onoi, S.; Imada, Y. *Angew. Chem., Int. Ed.* **2002**, *41*, 2366–2368; (b) Watanabe, A.; Uchida, T.; Ito, K.; Katsuki, T. *Tetrahedron Lett.* **2002**, *43*, 4481–4485; (c) Punniyamurthy, T.; Velusamy, S.; Iqbal, J. *Chem. Rev.* **2005**, *105*, 2329–2363.
3. (a) Faber, K. *Biotransformations in Organic Chemistry*; Springer: Berlin-Heidelberg, 2004, pp 242–249; (b) van Berkel, W. J. H.; Kamerbeek, N. M.; Fraaije, M. W. *J. Biotechnol.* **2006**, *124*, 670–689; (c) Mihovilovic, M. D. *Curr. Org. Chem.* **2006**, *10*, 1265–1287; (d) Urlacher, V. D.; Schmid, R. D. *Curr. Opin. Chem. Biol.* **2006**, *10*, 156–161; (e) Hollman, F.; Hofstetter, K.; Schmid, A. *Trends Biotechnol.* **2006**, *24*, 163–171.
4. (a) Fraaije, M. W.; Mattevi, A. *Trends Biochem. Sci.* **2000**, *25*, 126–132; (b) Mihovilovic, M. D.; Müller, B.; Stanetty, P. *Eur. J. Org. Chem.* **2002**, 3711–3720; (c) Kamerbeek, N. M.; Janssen, D. B.; van Berkel, W. J. H.; Fraaije, M. W. *Adv. Synth. Catal.* **2003**, *345*, 667–678; (d) Alphand, V.; Carrea, G.; Wohlgemuth, R.; Furstoss, R.; Woodley, J. M. *Trends Biotechnol.* **2003**, *21*, 318–323; (e) Kirschner, A.; Bornscheuer, U. T. *Angew. Chem., Int. Ed.* **2006**, *45*, 7004–7006.
5. (a) Colonna, S.; Gaggero, N.; Carrea, G.; Pasta, P. *Chem. Commun.* **1998**, 415–416; (b) Ottolina, G.; Bianchi, S.; Belloni, B.; Carrea, G.; Danieli, B. *Tetrahedron Lett.* **1999**, *40*, 8483–8486; (c) Sheng, D.; Ballou, D. P.; Massey, V. *Biochemistry* **2001**, *40*, 11156–11167; (d) Ottolina, G.; de Gonzalo, G.; Carrea, G.; Danieli, B. *Adv. Synth. Catal.* **2005**, *345*, 1035–1040.
6. (a) Fraaije, M. W.; Kamerbeek, N. M.; van Berkel, W. J. H.; Janssen, D. B. *FEBS Lett.* **2002**, *518*, 43–47; (b) Fraaije, M. W.; Kamerbeek, N. M.; Heidekamp, A. J.; Fortin, R. *J. Biol. Chem.* **2004**, *279*, 3354–3360; (c) Reetz, M. T.; Daligault, F.; Brunner, B.; Hinrichs, H.; Deege, A. *Angew. Chem., Int. Ed.* **2004**, *43*, 4078–4081; (d) Clouthier, C. M.; Kayser, M. M. *Tetrahedron: Asymmetry* **2006**, *17*, 2649–2653; (e) Clouthier, C. M.; Kayser, M. M.; Reetz, M. T. *J. Org. Chem.* **2006**, *71*, 8431–8437.
7. Fraaije, M. W.; Wu, J.; Heuts, D. P. H. M.; van Hellemond, E. W.; Lutje Spelberg, J. H.; Janssen, D. B. *Appl. Microbiol. Biotechnol.* **2005**, *66*, 393–400.
8. Kamerbeek, N. M.; Moonen, M. J. H.; van der Ven, J. G. M.; van Berkel, W. J. H.; Fraaije, M. W.; Janssen, D. B. *Eur. J. Biochem.* **2001**, *268*, 2547–2557.
9. (a) Bocola, M.; Schulz, F.; Leca, F.; Vogel, A.; Fraaije, M. W.; Reetz, M. T. *Adv. Synth. Catal.* **2005**, *347*, 979–986; (b) de Gonzalo, G.; Torres Pazmiño, D. E.; Ottolina, G.; Fraaije, M. W.; Carrea, G. *Tetrahedron: Asymmetry* **2005**, *16*, 3007–3083.
10. (a) Schulz, F.; Leca, F.; Hollman, F.; Reetz, M. T. *Belstein J. Org. Chem.* **2005**, *1*, 10; (b) de Gonzalo, G.; Ottolina, G.; Zambianchi, F.; Fraaije, M. W.; Carrea, G. *J. Mol. Catal. B: Enzym.* **2006**, *39*, 91–97.
11. Malito, E.; Alfieri, A.; Fraaije, M. W.; Mattevi, A. *Proc. Natl. Acad. Sci. U.S.A* **2004**, *101*, 13157–13162.
12. (a) Gutiérrez, M. C.; Alphand, V.; Furstoss, R. *J. Mol. Catal. B: Enzym.* **2003**, *21*, 231–238; (b) Kamerbeek, N. M.; Oltshoorn, A. J. J.; Fraaije, M. W.; Janssen, D. B. *Appl. Environ. Microbiol.* **2003**, 419–426; (c) Moonen, M. J. H.; Westphal, A. H.; Rietjens, I. M. C. M.; van Berkel, W. J. H. *Adv. Synth. Catal.* **2005**, *347*, 1027–1034; (d) Mihovilovic, M. D.; Kapitan, P.; Rydz, J.; Rudroff, F.; Ogink, F.; Fraaije, M. W. *J. Mol. Catal. B: Enzym.* **2005**, *32*, 135–140.
13. de Gonzalo, G.; Torres Pazmiño, D. E.; Ottolina, G.; Fraaije, M. W.; Carrea, G. *Tetrahedron: Asymmetry* **2006**, *17*, 130–135.
14. Wong, C.-H.; Whitesides, G. M. *J. Am. Chem. Soc.* **1981**, *103*, 4890–4899.
15. The enantiomeric ratio *E* is generally accepted as an adequate parameter to quantify the enantioselectivity in biochemical kinetic resolutions. See: Chen, C.-S.; Fujimoto, Y.; Girdauskas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.
16. Fry, A. J.; Bujanauskas, J. P. *J. Org. Chem.* **1978**, *43*, 3157–3163.
17. Berezina, N.; Alphand, V.; Furstoss, R. *Tetrahedron: Asymmetry* **2002**, *13*, 1953–1955.
18. Zambianchi, F.; Fraaije, M. W.; Carrea, G.; de Gonzalo, G.; Rodríguez, C.; Gotor, V.; Ottolina, G. *Adv. Synth. Catal.* **2007**, *349*, 1327–1331.
19. (a) Rahtke, M. W.; Vogiazoglou, D. *J. Org. Chem.* **1987**, *52*, 3697–3698; (b) Enders, D.; Eichenauer, H.; Baus, U.; Schubert, H.; Kremer, K. A. M. *Tetrahedron* **1984**, *40*, 1345–1359; (c) Sørbye, K.; Tautermann, C.; Carlsen, P.; Fiksdahl, A. *Tetrahedron: Asymmetry* **1998**, *9*, 681–689; (d) Birman, V. B.; Jiang, H. *Org. Lett.* **2005**, *7*, 3445–3447; (e) Berkessel, A.; Sebastian-Ibarz, M. L.; Mueller, T. N. *Angew. Chem., Int. Ed.* **2006**, *45*, 6567–6570.
20. Kyte, B. G.; Rouvière, P.; Cheng, Q.; Stewart, J. D. *J. Org. Chem.* **2004**, *69*, 12–17.