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Dynamic kinetic resolution of α-substituted β-ketoesters catalysed by Baeyer-Villiger monooxygenases: Access to enantiopure α-hydroxy esters

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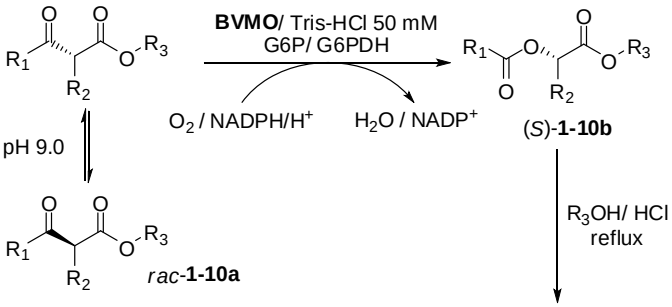
Biocatalytic procedures can circumvent some of the drawbacks that classical methodologies present in chemical synthesis.[1] One example is shown by the Baeyer-Villiger (BV) reaction, a process discovered more than 100 years ago that consists in the nucleophilic insertion of one atom of oxygen in the adjacent position of a carbonyl moiety affording esters or lactones.[2] This reaction proceeds using peroxides or peracids as oxidants with, in general, low selectivity, employing labile and shock-sensitive compounds that do not match with the principles of the Green Chemistry. Baeyer-Villiger monooxygenases (BVMOs, 1.14.13.x) represent an effective alternative to perform the BV reaction.[3] These biocatalysts are nicotinamide dependent flavoenzymes that convert linear or cyclic ketones into esters and lactones, respectively, using molecular oxygen as mild oxidant. In general, BVMOs display excellent chemo-, regio- and/or enantioselectivities while using environmentally friendly reaction conditions.

BVMOs have been widely used in the desymmetrisation or the kinetic resolution of cyclic and bicyclic ketones, as well as linear aliphatic and alkyl aryl ketones.[3] Recently, several BVMOs from different bacterial origin were employed in the enzymatic kinetic resolution of a set of aliphatic β-hydroxyketones and β-aminoketones, valuable synthons in the preparation of optically active diols and amino acids, respectively, via regioselective Baeyer–Villiger oxidation.[4] Herein we investigate whether aliphatic acyclic racemic α-alkyl-β-ketoesters are accepted as substrates by BVMOs. Interestingly, since spontaneous racemisation of the starting material occurs, this allowed us to perform an effective BVMO-catalysed dynamic kinetic resolution (DKR).[5] Such effective BVMO-based DKR provides a new catalytic pathway for the synthesis of high-valuable enantiopure α-acylated hydroxy esters (Scheme 1).[6]

These compounds are important intermediates that can easily be turned into enantioenriched α-hydroxy acids, very interesting derivatives well-known for their use in the cosmetic industry.[7] They can also be selectively hydrolysed into the corresponding α-hydroxy esters, versatile products that find application in the chemical, food and pharmaceutical industry, as e.g. anticancer drugs, antibiotics and other bioactive natural derivatives.[8] Alkyl hydroxy esters are also employed as useful building blocks of numerous highly valuable compounds.[9]

Three BVMOs were selected to perform the selective Baeyer-Villiger reaction of the starting material: phenylacetone monooxygenase (PAMO) from *Thermobifida fusca*,[10] its M446G mutant[11] and 4-hydroxyacetophenone monooxygenase (HAPMO) from *Pseudomonas fluorescens* ACB,[12] being achieved the best results with the two wild-type enzymes. It is worth noting that these biocatalysts are primarily active on aromatic compounds and have been mainly employed in the synthesis of enantioenriched aromatic sulfoxides, ketones and esters. In this study, we show that these biocatalysts also accept non-aromatic substrates.



1a: R_1: CH_3, R_2: CH_3, R_3: CH_3
2a: R_1: CH_3, R_2: CH_3, R_3: CH_2CH_3
3a: R_1: CH_3, R_2: CH_3, R_3: $CH(CH_3)_2$
4a: R_1: CH_2CH_3, R_2: CH_3, R_3: CH_3
5a: R_1: CH_3, R_2: CH_2CH_3, R_3: CH_3
6a: R_1: CH_3, R_2: CH_2CH_3, R_3: CH_2CH_3
7a: R_1: CH_3, R_2: CH_2CH_3, R_3: $CH(CH_3)_2$
8a: R_1: CH_3, R_2: $CH_2CH{=}CH_2$, R_3: CH_2CH_3
9a: R_1: CH_3, R_2: CH_2Ph, R_3: $CH(CH_3)_2$
10a: R_1: CH_3, R_2: CH_3, R_3: CH_2Ph

Scheme 1. BVMO-catalysed dynamic kinetic resolution of aliphatic α-alkyl-β-ketoesters and subsequent hydrolysis of the diesters in order to obtain the corresponding enantioenriched α-hydroxy esters.

Starting α-alkyl-β-ketoesters were synthesised following a similar methodology to that previously described, by treatment of the β-ketoesters with the corresponding alkyl halides in basic medium.[13] Initially, the BVMO-catalysed oxidation of racemic methyl 2-methyl-3-oxobutanoate *rac*-**1a** was carried out in Tris-HCl 50 mM pH 8.0. In these conditions, reactions in the presence of PAMO or HAPMO led to enantiopure (*S*)-**1b**. Although conversions were lower than 30%, racemic β-ketoester was recovered after 48 hours, showing that under these conditions substrate racemisation is feasible. As PAMO has shown higher activities in the oxidation of sulfides and ketones at high pHs,[14] this also ensured a fast substrate racemisation and therefore it was possible to obtain 62% of

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enantiopure diester (*S*)-**1b** after 48 hours by working at pH 9.0 (entry 1, Table 1). Again, the starting ketone was recovered in racemic form. So, by simply modifying the reaction pH, it was possible to perform a more suitable DKR process.

The same reaction conditions were applied to the selective oxidation of other α-methyl-β-ketoesters presenting different alkyl substituents at the ester moiety. Biooxidation of *rac*-ethyl 2-methyl-3-oxobutanoate *rac*-**2a** led to enantiopure (*S*)-**2b** with 90% conversion after 48 hours (entry 2), while complete formation of the isopropyl analogue (*S*)-**3b** (entry 4) was observed using identical conditions. For this substrate, a high conversion was even possible at pH 8.0, recovering the enantiopure diester with 72% conversion (entry 3). Thus, by increasing the size of the ester alkyl chain, faster oxidative processes were afforded by PAMO without influence on the excellent *S*-selectivity.

Table 1. DKRs of racemic β-ketoesters *rac*-**1-10a** employing BVMOs to synthesise (*S*)-**1-10b** (t=48 h).[a]

Entry	Ketone	BVMO[b]	*c* [%][c]	ee_p [%][d]
1	*rac*-**1a**	PAMO	62	≥ 99
2	*rac*-**2a**	PAMO	90	≥ 99
3[e]	*rac*-**3a**	PAMO	72	≥ 99
4	*rac*-**3a**	PAMO	≥ 99	≥ 99
5	*rac*-**4a**	PAMO	56	92
6	*rac*-**5a**	HAPMO	37	≥ 99
7	*rac*-**5a**	PAMO	89	≥ 99
8	*rac*-**6a**	HAPMO	59	≥ 99
9	*rac*-**6a**	PAMO	≥ 99	≥ 99
10	*rac*-**7a**	HAPMO	46	≥ 99
11	*rac*-**7a**	PAMO	≥ 99	≥ 99
12	*rac*-**8a**	HAPMO	91	≥ 99
13[f]	*rac*-**8a**	PAMO	≥ 99	≥ 99
14	*rac*-**9a**	PAMO	≥ 99	51
15	*rac*-**10a**	HAPMO	≥ 99	≥ 99
16	*rac*-**10a**	PAMO	≥ 99	≥ 99

[a] For reaction details, see SI. [b] Reactions were performed at 20ºC when using HAPMO and 30ºC for PAMO. [c] Determined by GC. [d] Measured by GC or HPLC. In all cases, *S* configuration was observed. [e] Reaction performed at pH 8.0. [f] Reaction time 24 h.

Baeyer-Villiger oxidation of *rac*-methyl 2-methyl-3-oxopentanoate (**4a**) led to (*S*)-**4b** with a moderate conversion and a high selectivity (entry 5). In order to improve this conversion and since PAMO has previously demonstrated its ability to catalyse oxidative processes in non-conventional media,[15] we studied the oxidation of substrate **4a** in aqueous buffer containing (non)miscible organic solvents. Thus, the use of 5% *v* v^{-1} of co-solvents presenting different physicochemical properties was analysed. As shown in Figure 1, usage of a hydrophilic solvent such as 1,4-dioxane led to a slight increase in the conversion, while the optical purity of (*S*)-**4b** remained constant. The best result was achieved in the presence of 5% *v* v^{-1} ^{t}BuOMe (TBME), yielding 72% of the final diester with 96% optical purity. The use of 5% hexane resulted in a very low conversion, but (*S*)-**4b** was recovered in enantiopure form.

Selective oxidation of different alkyl 2-ethyl-3-oxobutanoates (*rac*-**5-7a**) was performed in order to obtain optically active alkyl 2-acetoxybutanoates (*S*)-**5-7b**. As shown in entries 6-11 of Table 1, these reactions led to the formation of enantiopure diesters (*S*)-**5-7b**, with differences in the conversions depending on the substrate structure. While the BVMO-catalysed oxidation of the methyl derivative **5a** was slightly slower than the corresponding ethyl and isopropyl analogues, PAMO afforded (*S*)-**6b** and (*S*)-**7b** quantitatively after 48 hours. HAPMO was more efficient for the preparation of enantiopure (*S*)-**6b** (59% conversion, entry 8).

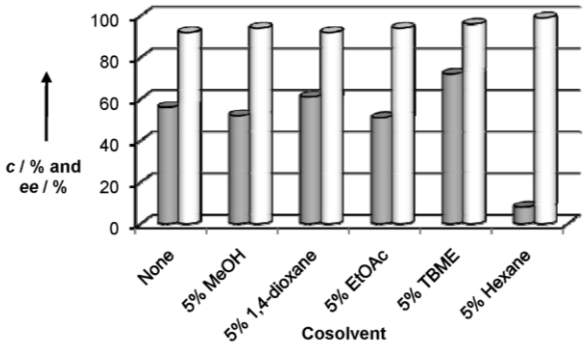

Figure 1. PAMO-biocatalysed oxidation of racemic methyl 2-methyl-3-oxopentanoate *rac*-**4a** in presence of different cosolvents. ●: Conversion. ○: Enantiomeric excess.

The influence of the *rac*-**7a** concentration on both PAMO activity and stereoselectivity was studied when employing two different reaction media: 1) Buffer Tris-HCl 50 mM pH 9.0; and 2) buffer containing 5% *v* v^{-1} TBME. Although conversions were lower at elevated substrate concentrations, the space time yield (expressed as mg of **7a** consumed per L of solution per h) increased, reaching a maximum at 20 mM (3.5 g L^{-1}) in buffer, while the presence of 5% *v* v^{-1} TBME allowed obtaining an optimal concentration of 50 mM (8.6 g L^{-1}). The stereoselectivity of the enzyme remained completely unchanged at elevated substrate concentrations.

Dynamic kinetic resolution of a bulkier substrate, *rac*-ethyl 2-acetylpent-4-enoate (**8a**), achieved enantiopure (*S*)-**8b** when employing either HAPMO or PAMO (entries 12 and 13). This afforded (*S*)-**8b** with complete enantioselectivity and high conversion with both enzymes. Since (*S*)-ethyl 2-acetoxypent-4-enoate is a trifunctionalised compound; it represents an interesting starting material to perform further transformations.

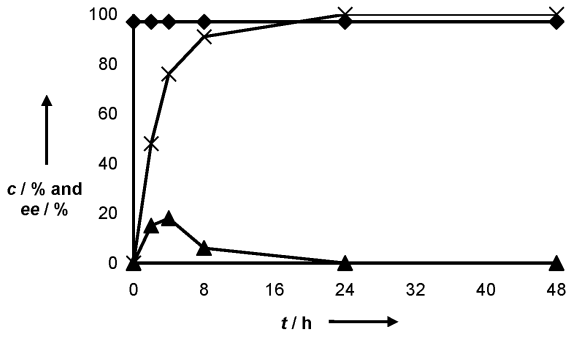

Figure 2. Time dependent conversion of *rac*-**8a** into (*S*)-**8b** using purified PAMO. ▲: Optical purity of **8a**, X: **8b** conversion, ♦: (*S*)-**8b** optical purity.

The progress of the PAMO-catalysed biooxidation of racemic **8a** was studied (Figure 2). The DKR was very fast, leading to 50% conversion after only 4 hours. Enantiomeric excess of the final diester (*S*)-**8b** was excellent during the whole oxidative process. Initially, the oxidation mainly proceeded as a kinetic resolution, with optical purities of the starting material (*R*)-**8a** close to 20% *ee* at conversions of 50%. After 4 hours, optical purity of **8a**

diminished, while the *ee* of the final diester remained constant. After 24 h, total conversion to enantiopure (*S*)-**8b** was achieved.

Finally, racemic substrates presenting aromatic rings in their structure were also analysed. *rac*-Isopropyl 2-benzyl-3-oxobutanoate **9a** was not oxidised by HAPMO, while it could be converted with complete conversion using PAMO (entry 14). For this biocatalyst, moderate optical purity was obtained in the synthesis of (*S*)-**9b**. Much better results can be achieved in the biotransformation of *rac*-**10a**, presenting the aromatic ring attached to the ester group. For both HAPMO and PAMO, diester **10b** was recovered with total conversion and complete selectivity (entries 15 and 16).

Once we could obtain several α-acylated hydroxy esters with excellent conversion and selectivity, the next step was the synthesis of the corresponding optically active α-hydroxy esters. Initially, we tested a set of commercially available hydrolases in order to obtain the selective hydrolysis of the acetyl or propionyl moiety (see Supporting Information). For all the biocatalysts tested, no hydrolysis or poor regioselectivity was observed. Thus, chemical hydrolysis was performed by treatment of the starting diesters with the corresponding alcohol in the presence of a catalytic amount of hydrochloric acid. By this, enantiopure (*S*)-hydroxy esters (*S*)-**1-9c** were achieved with high yields (60-85%).

This study demonstrates that BVMOs can be used to catalyse the oxidation of a set of α-alkyl-β-ketoesters with excellent enantioselectivities and conversions in most of the cases. Indeed, due to the presence of acidic hydrogen in the substrate structure, its racemisation can be performed by working at slightly basic pH, resulting in the dynamic kinetic resolution of the starting material, affording the final products with conversions close to 100%. Furthermore, it illustrates that HAPMO and PAMO can accept not only aromatic but also aliphatic substrates. In general, higher yields are obtained by increasing the ester alkyl chain up to the isopropyl (even benzyl) group. These reactions were performed at higher scale (50 mg) in order to obtain the enantiopure final products with moderate to high yields. Employing an organic co-solvent in these biocatalysed processes, both activity and selectivity of the enzyme could be improved allowing the use of a higher substrate concentration.

Experimental Section

General procedure for the biocatalysed oxidation of the racemic α-alkyl-β-ketoesters *rac*-1-10a employing purified BVMOs. The corresponding racemic α-alkyl-β-ketoester (50 mg, 0.22-0.32 mmol) was dissolved in Tris-HCl buffer (50 mM, pH 9.0, 13 mL) containing 1% DMSO. Then, NADPH (0.2 mM), glucose-6-phosphate (40 mM), glucose-6-phosphate dehydrogenase (75 units), and PAMO (15 units) were added. The mixture was shaken at 250 rpm at 30ºC. Reactions were stopped after 24 or 36 hours by extraction with EtOAc (3 × 10 mL). The organic layer was dried over Na_2SO_4, the solvent was evaporated under reduced pressure and the conversions were measured by GC. No further purification was required, except for compounds **4-5b**, for which *flash* chromatography on silica gel was employed, using hexane/EtOAc 9:1 as eluent. Diesters **1-10b** were obtained enantiopure (except for (*S*)-**9b,** achieved with *ee*= 50%) with yields ranging from 60 to 76%.

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Dynamic kinetic resolution of α-substituted β-ketoesters catalysed by Baeyer-Villiger monooxygenases: Access to enantiopure α-hydroxy esters

Biocatalysed DKRs

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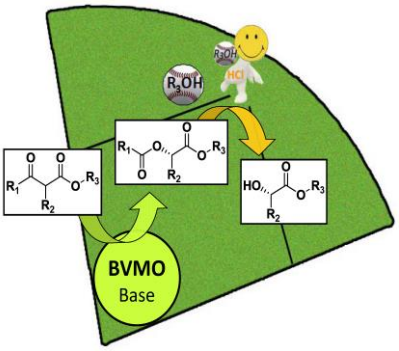

The dynamic kinetic resolution of a set of racemic α-alkyl-β-ketoesters was performed by selective Baeyer-Villiger oxidation employing different Baeyer-Villiger monooxygenases in mild basic media. Final diesters were achieved with excellent yields and enantioselectivities, resulting in interesting chiral building blocks for the synthesis of optically active α-hydroxy esters.