Biocatalysis

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Dynamic Kinetic Resolution of α-Substituted β-Ketoesters Catalyzed by Baeyer–Villiger Monooxygenases: Access to Enantiopure α-Hydroxy Esters**

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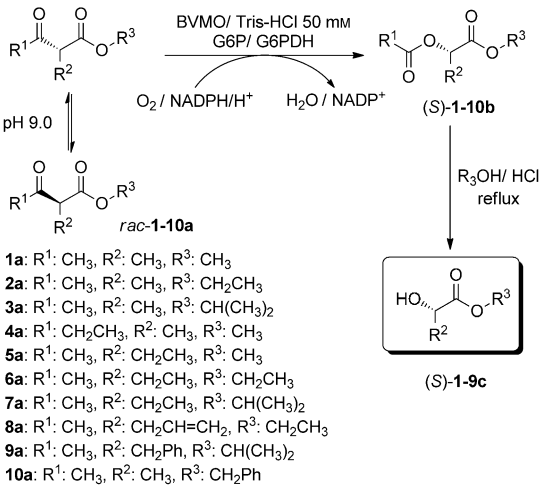
Biocatalytic procedures can circumvent some of the drawbacks that classical methodologies present in chemical synthesis.[1] One example is the Baeyer–Villiger (BV) reaction, a process that was discovered more than 100 years ago and involves the nucleophilic insertion of one atom of oxygen into the position adjacent to a carbonyl moiety, thus affording esters or lactones.[2] This reaction proceeds using peroxides or peracids as oxidants with, in general, low selectivity by employing labile and shock-sensitive compounds that do not fit with the principles of the green chemistry. Baeyer–Villiger monooxygenases (BVMOs, 1.14.13.x) represent an effective alternative for performing 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 a 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 desymmetrization 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 aliphatic β-hydroxyketones and β-aminoketones through a regioselective Baeyer–Villiger oxidation;[4] the products are valuable synthons in the preparation of optically active diols and amino acids. Herein we investigate whether aliphatic acyclic racemic α-alkyl-β-ketoesters are accepted as substrates by BVMOs. Interestingly, the spontaneous racemization of the starting material allowed us to perform an effective BVMO-catalyzed dynamic kinetic resolution (DKR).[5] Such effective BVMO-based DKR provides a new catalytic pathway for the synthesis of highly valuable, enantiopure α-acylated hydroxy esters (Scheme 1).[6]



Scheme 1. BVMO-catalyzed dynamic kinetic resolution of aliphatic α-alkyl-β-ketoesters and subsequent hydrolysis of the diesters to obtain the corresponding enantioenriched α-hydroxy esters (**10b** was not hydrolyzed).

These compounds are important intermediates that can easily be turned into enantioenriched α-hydroxy acids, which are very interesting derivatives that are well known for their use in the cosmetic industry.[7] They can also be selectively hydrolyzed into the corresponding α-hydroxy esters, which are versatile products that find application in the chemical, food, and pharmaceutical industries, as for example, 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 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] The best results were achieved with the two wild-type enzymes. Notably, these biocatalysts are primarily active on aromatic compounds and have been mainly employed in the synthesis of enantioenriched aromatic sulfoxides, ketones, and esters.

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In this study, we show that these biocatalysts also accept nonaromatic substrates.

The starting α-alkyl-β-ketoesters were synthesized using a methodology similar to one previously described, wherein the β-ketoesters were treated with the corresponding alkyl halides in basic medium.[13] Initially, the BVMO-catalyzed oxidation of racemic methyl 2-methyl-3-oxobutanoate (*rac*-**1a**) was carried out in Tris-HCl 50 mM at pH 8.0. Under these conditions, the reactions in the presence of PAMO or HAPMO led to enantiopure (*S*)-**1b**. Although the conversions were lower than 30%, the racemic β-ketoester was recovered after 48 hours, thus showing that under these reaction conditions substrate racemization is feasible. As PAMO has shown higher activities in the oxidation of sulfides and ketones at high pH values,[14] this ensured a fast substrate racemization and therefore made it possible to obtain 62% of enantiopure (*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.

Table 1: DKRs of racemic β-ketoesters (*rac*-**1**–**10a**) using BVMOs to yield (*S*)-**1**–**10b** ($t = 48$ h).[a]

Entry	β-Ketoester	BVMO[b]	Conv. [%][c]	*ee* [%][d] (Prod.)
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 the Supporting Information. [b] Reactions were performed at 20°C for HAPMO and at 30°C for PAMO. [c] Determined by GC analysis. [d] Measured by GC or HPLC analyses. In all cases, the *S*-configured product was obtained. [e] Reaction performed at pH 8.0. [f] Reaction time 24 h.

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

Baeyer–Villiger oxidation of methyl *rac*-2-methyl-3-oxopentanoate (**4a**) led to (*S*)-**4b** with a moderate conversion and a high selectivity (entry 5). To improve this conversion we studied the oxidation of substrate **4a** in aqueous buffer containing (non)miscible organic solvents because PAMO has previously demonstrated its ability to catalyze oxidative processes in nonconventional media.[15] Thus, the use of 5% *v*/*v* of cosolvents having different physicochemical properties was analyzed. As shown in Figure 1, usage of a hydrophilic

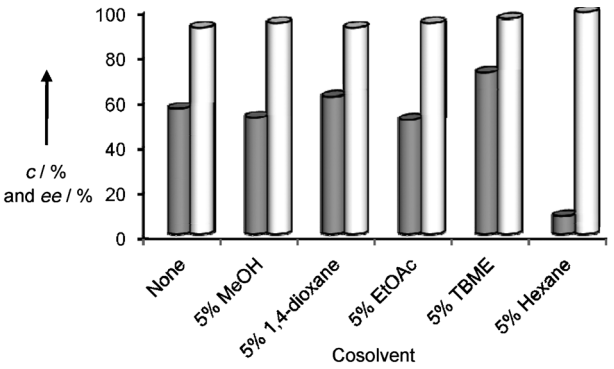

Figure 1. PAMO-biocatalyzed oxidation of racemic methyl 2-methyl-3-oxopentanoate (*rac*-**4a**) in the presence of different cosolvents. Conversion: gray bar, enantiomeric excess: white bar.

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* *t*BuOMe (TBME), which yielded 72% of the final diester with 96% optical purity. The use of 5% *n*-hexane *v*/*v* 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 to obtain the 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 that depended on the substrate structure. The HAPMO-catalyzed oxidation of the methyl derivative *rac*-**5a** was slightly slower than the corresponding ethyl (*rac*-**6a**) and isopropyl (*rac*-**7a**) analogues, and 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) than for the analogues (*S*)-**5b** and (*S*)-**7b**.

The influence of the concentration of *rac*-**7a** on both PAMO activity and stereoselectivity was studied when employing two different reaction media: 1) buffer Tris-HCl 50 mM at pH 9.0; and 2) buffer containing 5% *v*/*v* TBME. Although conversions were lower at elevated substrate concentrations, the space-time yield (expressed as mg of **7a** consumed per L of solution per hour) increased, reaching a maximum at 20 mM (3.5 $g L^{-1}$) in buffer, while the presence of 5% *v*/*v* TBME permitted 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, ethyl *rac*-2-acetylpent-4-enoate (*rac*-**8a**), led to enantiopure (*S*)-**8b**

when employing either HAPMO or PAMO (entries 12 and 13). In both cases (*S*)-**8b** was obtained with complete enantioselectivity and high conversion. Since (*S*)-**8b** contains three functional groups, it represents an interesting starting material for additional transformations.

The progress of the PAMO-catalyzed biooxidation of *rac*-**8a** was studied (Figure 2). The DKR was very fast, leading to 50% conversion after only 4 hours. Enantiomeric excess of

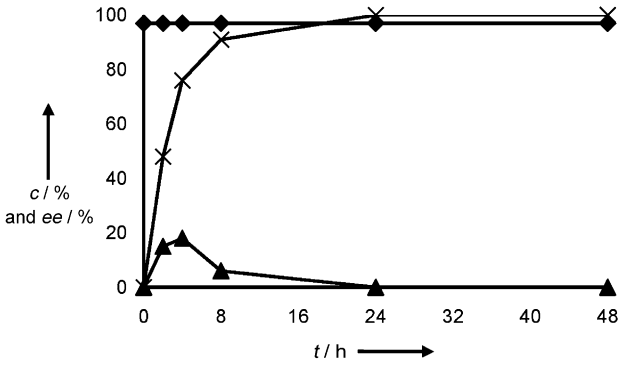

Figure 2. Time-dependent conversion of *rac*-**8a** into (*S*)-**8b** using purified PAMO. Optical purity of **8a**: ▲, conversion of **8b**: ×, optical purity of (*S*)-**8b**: ◆.

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, the optical purity of **8a** diminished and the *ee* value of the final diester remained constant. After 24 hours, total conversion to enantiopure (*S*)-**8b** was achieved.

Finally, racemic substrates having aromatic rings in their structure were also analyzed. Isopropyl *rac*-2-benzyl-3-oxobutanoate (*rac*-**9a**) was not oxidized by HAPMO, but 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**, which has the aromatic ring attached to the ester group. For both HAPMO and PAMO, diester (*S*)-**10b** was recovered with total conversion and complete selectivity (entries 15 and 16).

Once we could obtain several α-acylated hydroxy esters with excellent conversions and selectivities, the next step was the synthesis of the corresponding optically active α-hydroxy esters. Initially, we tested a set of commercially available hydrolases to obtain the selective hydrolysis of the acetyl or propionyl moiety (see the Supporting Information). For all the biocatalysts tested, either 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 using this approach, the enantiopure hydroxy esters (*S*)-**1**–**9c** were obtained with high yields (60–85%).

This study demonstrates that BVMOs can be used to catalyze the oxidation of a set of α-alkyl-β-ketoesters with excellent enantioselectivities and conversions in most of the cases. Indeed, as a result of the presence of acidic hydrogen in the substrate structure, its racemization 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 larger scale (50 mg) to obtain the enantiopure products with moderate to high yields. By employing an organic cosolvent in these biocatalyzed processes, both the activity and selectivity of the enzyme could be improved, also allowing the use of a higher substrate concentration.

Experimental Section

General procedure for the biocatalyzed 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 30°C. Reactions were stopped after 24 or 36 h 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 analysis. No additional purification was required, except for compounds **4**, **5b**, for which flash chromatography on silica gel was employed, using hexanes/EtOAc (9:1) as the eluent. Diesters **1**–**10b** were obtained in enantiopure form (except for (*S*)-**9b**, achieved with *ee* = 50%) with yields ranging from 60 to 76%.

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