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Synthesis of Chiral 3-Alkyl-3,4-dihydroisocoumarins by Dynamic Kinetic Resolutions Catalyzed by a Baeyer−Villiger Monooxygenase

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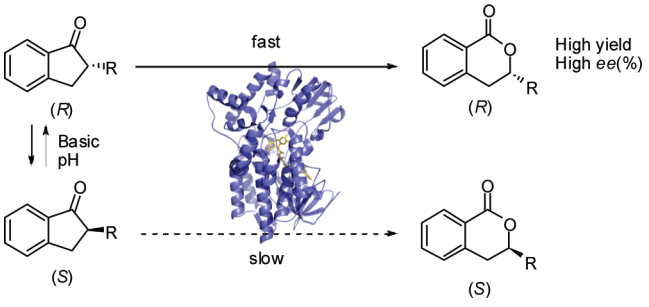

Baeyer−Villiger monooxygenases have been tested in the oxidation of racemic benzofused ketones. When employing a single mutant of phenylacetone monooxygenase (M446G PAMO) under the proper reaction conditions, it was possible to achieve 3-substituted 3,4-dihydroisocoumarins with high yields and optical purities through regioselective dynamic kinetic resolution processes.

3,4-Dihydroisocoumarins and their derivatives are compounds that widely exist in nature as key intermediates in the synthesis of biologically active molecules. As these compounds are known to have interesting activities (e.g., antifungal, antiallergenic, antiulcer, and antimalarial), they are regarded as highly attractive molecules in organic chemistry.[1] To date, several synthetic routes for these derivatives have been described. A number of methods have been reported mainly following lateral and *ortho*-lithiations along with other strategies.[2] Other recent syntheses include the alkylation of 1,2-oxiranes followed by oxidative degradation,[3] the reaction of benzocyclobutenoxides with aldehydes,[4] domino [3 + 3] cyclation/lactonization processes,[5] the CuBr-catalyzed reaction of *o*-methoxycarbonylbenzenediazonium bromides with unsaturated compounds,[6] and the Ir-catalyzed cyclation of ketoaldehydes.[7] All these methodologies suffer from some drawbacks such as harsh reaction conditions, multistep procedures, and low yields due to functional group intolerance.

Today, enzymes are recognized as efficient catalysts for the preparation of chiral compounds.[8] A clear example of the increasing interest in biocatalysis is the Baeyer−Villiger reaction, a key process for the synthesis of esters and lactones.[9] In the past few years, enzymatic methodologies employing Baeyer−Villiger monooxygenases (BVMOs) have been demonstrated to be a very useful tool to perform selective Baeyer−Villiger oxidations.[10] BVMOs are flavoproteins that are able to catalyze the oxidation of carbonylic and heteroatom-containing compounds employing atmospheric oxygen as oxidant.[11] Recent examples of these biocatalysts are 4-hydroxyacetophenone monooxygenase (HAPMO) from *Pseudomonas fluorescens* ACB[12] and a single mutant of the thermostable phenylacetone monooxygenase (M446G PAMO) from *Thermobifida fusca*.[13] This designed mutant has been shown to have a different substrate profile and selectivity when compared with the wild type (wt) enzyme.

Asymmetric BVMO-catalyzed oxidations have been developed by desymetrization of prochiral ketones or by kinetic resolution of racemic ones. Strategies to increase the theoretical yield of kinetic resolutions are of great importance. Thus, by combining an in situ racemization of substrate with an enzymatic resolution, a dynamic kinetic resolution (DKR) can

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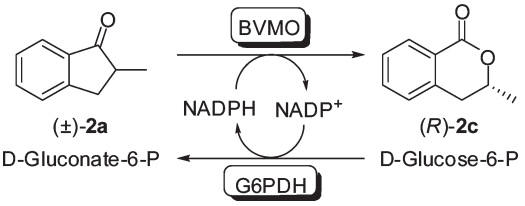
TABLE 1. Enzymatic Oxidation of 2-Methyl-1-indanone (±)-2a Catalyzed by BVMOs in Buffer Containing Hexane (5% v/v)[a]



entry	BVMO	time (h)	temp (°C)	ee of **2a**[b] (%)	ee of **2b**,**c**[b] (%)	*c*[b] (%)
1[c]	HAPMO	72	20	5	6	13
2[c]	M446G	72	30	10	92	33
3	M446G	168	40	7	82	84
4[d]	M446G	168	40	5	74	89

[a]For experimental details, see the Supporting Information. [b]Determined by GC. [c][**2a**] = 4.0 mg mL^{-1}. [d]pH = 10.5.

be achieved,[14] which allows the transformation of both enantiomers of the starting material into a single enantiomeric product in 100% theoretical yield. Recently, the first BVMO-based DKRs have been described in which recombinant *Escherichia coli* cells containing cyclohexanone monooxygenase were used at relatively high pH or combined with ion-exchange resins.[15]

Previously, it has been shown than HAPMO was able to convert 1-indanones into the normal lactones, whereas M446G PAMO yielded the nonexpected ones.[16] In view of this opposite regioselectivity and attending to the structure of substituted benzofused ketones (which possess an acidic hydrogen and are therefore being able to racemize at high pH), we report herein the enantioselective oxidation of these substrates using isolated BVMOs in order to obtain the corresponding chiral lactones.

We first analyzed the kinetic resolution of racemic 2-methyl-1-tetralone (±)-**1a**. No oxidation of this compound was observed when using HAPMO. Resolutions catalyzed by M446G PAMO allowed us to recover (*S*)-**1a** and lactone (*R*)-3-methyl-4,5-dihydrobenzo[*b*]oxepin-2(3*H*)-one (**1b**), albeit with very low optical purities and conversions (data shown in the Supporting Information).

Next, we focused on the enzymatic resolution of 2-alkyl-1-indanones. Oxidation of *rac*-2-methyl-1-indanone (±)-**2a** was performed in a biphasic system, Tris-HCl buffer pH 10.0 containing 5% hexane (Table 1),[17] as these biocatalysts have been shown to work in aqueous−organic solvent media.[18] When HAPMO was employed, (*R*)-3-methyl-3,4-dihydrocoumarin

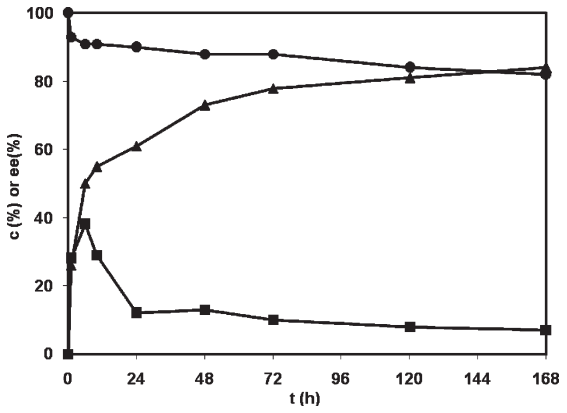


FIGURE 1. Time progress of the DKR of (±)-**2a** catalyzed by M446G PAMO in buffer pH 10 containing 5% hexane: ▲, *c* (%), ■, ee (*S*)-**2a** (%), ●, ee (*R*)-**2c** (%).

(*R*)-**2b** was obtained with low conversion and selectivity. Longer reaction times did not change the results obtained. Biooxidations catalyzed by M446G PAMO led to a different outcome. (*R*)-3-Methyl-3,4-dihydroisocoumarin (*R*)-**2c** was obtained with high optical purity (ee = 92%) and 33% conversion after 3 days (entry 2).[19] The starting ketone, presenting (*S*)-configuration, was recovered with only a 10% enantiomeric excess. This process was carried out with a smaller amount of substrate at 40 °C and stopped after 168 h, obtaining (*R*)-**2c** with 84% conversion and 82% enantiomeric excess. This indicates that a DKR process has been performed on this substrate by using high pHs and the appropriate biocatalyst. (*S*)-**2a** was achieved almost racemic (ee = 7%). More drastic conditions for the substrate racemization were achieved by using pH 10.5. After one week, conversion was improved to 89% but the optical purity of (*R*)-**2c** decreased to 74%.

The progress of the M446G PAMO-catalyzed oxidation of (±)-**2a** in buffer pH 10.0 containing 5% hexane was analyzed in more detail. As shown in Figure 1, the reaction led to 50% conversion after 6 h, presenting a classical kinetic resolution behavior with optical purities of ee = 38% for (*S*)-**2a** and ee = 92% for (*R*)-**2c**. After this first phase, racemization of starting ketone became predominant, producing an important loss in optical purity of **2a**, while the ee of (*R*)-**2c** remained almost constant. After 72 h, oxidation had reached 78% conversion and the chiral lactone was obtained with a 88% enantiomeric excess, while (*S*)-**2a** was almost racemic. Longer reaction times only resulted in a slight increase in the conversion (84%), but this was accompanied with a slight loss in optical purity of (*R*)-**2c** due to a partial racemization of this lactone.

Once it had been established that the best optical purity in combination with a reasonable conversion of lactone (*R*)-**2c** could be obtained after 48 h, several reaction parameters were varied in order to improve the reaction. First, the enzymatic resolution of (±)-**2a** was performed in different reaction media. As shown in Table 2, the oxidation catalyzed by M446G PAMO in aqueous buffer led to 76% of the (*R*)-lactone with a 79% enantiomeric excess. This is slightly

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TABLE 2. Enzymatic Oxidation of (±)-2a Catalyzed by M446G PAMO in Different Reaction Media after 48 h[a]

entry	solvent	pH	temp (°C)	ee of **2a**[b] (%)	ee of **2c**[b] (%)	c[b] (%)
1	none	10	40	≤3	79	76
2	5% MeOH	10	40	11	54	83
3	5% dioxane	10	40	10	76	73
4	5% *i*-PrOH	10	40	35	50	85
5	5% *i*-Pr_2O	10	40	22	82	73
6	5% hexane	10	40	13	88	73
7	5% MeOH	10	30	26	74	61
8	5% MeOH	10	20	5	82	13
9	5% hexane	10	30	28	81	60
10	5% hexane	10	20	6	77	20
11	5% MeOH	9	40	≤3	87	50
12	5% MeOH	8	40	16	88	38
13	5% hexane	9	40	19	83	64
14	5% hexane	8	40	34	83	60
15c	5% hexane	10	40	≤3	84	56

[a]For experimental details, see the Supporting Information. [b]Determined by GC. [c]Reaction in the presence of 5 equiv of Lewatit MP-62.

lower when compared with the optical purity obtained in 5% v/v hexane. Reaction rates were improved by employing 5% of short chain alcohols, such as methanol or 2-propanol in the reaction medium. The results are in accordance with those described for M446G PAMO in the oxidation of nonracemic indanones.[16] However, these two solvents affected negatively the enzymatic selectivity. (*R*)-**2c** was obtained only with 54% and 50% enantiomeric excess, as shown in entries 2 and 4. The use of 1,4-dioxane or *i*-Pr_2O allowed us to obtain conversions and optical purities similar to those obtained in aqueous buffer (entries 3 and 5).

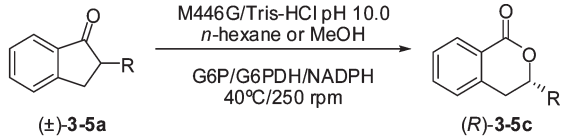
As shown in entries 7−10, the effect of the temperature was analyzed when 5% v/v hexane and methanol were used. As expected, the decrease of the reaction temperature caused a loss in the conversions, as previous studies have indicated that the wt enzyme shows a higher activity by increasing the temperature.[20] When methanol was employed at lower reaction temperatures (entries 7 and 8), an important gain in the M446G PAMO selectivity was achieved. By reducing the pH of the medium, reactions became much slower and resulted in an increase in enantioselectivity (ee = 87−88% at pH 8.0 or 9.0; entries 11 and 12) when buffer containing 5% methanol was used.

The effect of different anion-exchange resins in the dynamic kinetic resolution of (±)-**2a** using buffer with 5% hexane was also studied. For all of the resins analyzed (see the Supporting Information), racemization of the starting ketone was complete. The enzymatic selectivity was unaltered, but a partial inactivation of M446G PAMO was observed. Strong anion-exchange resins were found to be especially harmful for enzyme activity. The best result was obtained using Lewatit MP-62, a weak anion-exchange resin, which led to 56% of (*R*)-**2c** with 84% ee (entry 15). Using cell free extract containing M446G PAMO, we were also able to perform a conversion of 250 mg (±)-**2a**, resulting in 221 mg of isolated (*R*)-**2c** (80% yield). This illustrates that no enzyme purification is needed to perform these reactions on a multimilligram scale.

The biocatalyzed oxidations were extended to other racemic 1-indanones (Table 3). No formation of lactones **3**−**5b** was observed when the corresponding ketones were incubated in the presence of HAPMO, while M446G PAMO was able to convert these ketones. Resolution of racemic 2-ethyl-1-indanone (±)-**3a** led to (*R*)-**3c** with a 72% conversion after 96 h and a high optical purity (ee = 91%) when employing buffer Tris−HCl pH 10.0. The use of 5% hexane led to an important decrease in enzymatic activity (entry 2), while the presence of 5% methanol improved the enzymatic activity (c = 89%) with a loss in the enantiomeric excess of (*R*)-**3c**. As shown in entry 4, oxidation of (±)-**3a** was carried out using this cosolvent at pH 8.0 in order to increase the selectivity. This resulted in 45% of (*R*)-**3c** with 85% optical purity. This oxidation was also performed at 20 °C, but the enzyme selectivity was not increased, while a lower conversion was observed after 120 h (data not shown). (±)-2-Isopropyl-1-indanone (±)-**4a** was oxidized with complete selectivity to (*R*)-**4c** (ee ≥ 97%) in the presence of M446G PAMO by employing Tris−HCl buffer as well as mixtures of buffer containing 5% hexane or methanol. Oxidation was slightly faster when 5% hexane (c = 53% after 104 h, entry 6) was added than when other reaction media (conversions lower than 50%) were used. Longer reaction times did not improve the results obtained. (*R*)-3-Butyl-3,4-dihydroisocoumarin (**5c**) can also be obtained with high enantiomeric excess (optical purities around 90%) in all of the reaction media analyzed (entries 8−10). The first set of experiments showed that the DKR of (±)-**5a** was much faster in buffer or in the presence of 5% methanol. It was possible to achieve (*R*)-**5c** with conversions close to 80%, as shown in entries 8 and 10. Results obtained indicate that alkyl chains longer than ethyl have a negative effect on enzyme activity, which is in accordance with previous results obtained for this BVMO.[20b]

Finally, we have focused on the oxidation of racemic 3-methyl-1-indanone, (±)-**6a**. For this compound, the base-induced racemization was not feasible. Therefore, a kinetic resolution was performed. Depending on the biocatalyst employed, opposite regioselectivities were again observed. After the reaction conditions (see Supporting Information)

TABLE 3. Enzymatic Resolution of Racemic 2-Alkyl-1-indanones Catalyzed by M446G PAMO[a]



(±)-**3-5a** R: Et, *i*-Pr, *n*- Bu. (*R*)-**3-5c**

entry	R	solvent	time (h)	ee of **3**−**5a**[b] (%)	ee of **3**−**5c**[b] (%)	c[b] (%)
1	Et	none	96	6	91	72
2	Et	5% hexane	96	11	91	36
3	Et	5% MeOH	96	10	60	89
4[c]	Et	5% MeOH	120	16	85	45
5	*i*-Pr	none	104	17	≥97	46
6	*i*-Pr	5% hexane	104	17	≥97	53
7	*i*-Pr	5% MeOH	104	16	≥97	42
8	*n*-Bu	none	144	17	92	80
9	*n*-Bu	5% hexane	96	11	92	26
10	*n*-Bu	5% MeOH	144	20	88	78

[a]For experimental details, see the Supporting Information. [b]Determined by GC. [c]Reaction performed at pH 8.0.

(20) See, for example: (a) Rodríguez, C.; de Gonzalo, G.; Fraaije, M. W.; Gotor, V. *Tetrahedron: Asymmetry* **2007**, *18*, 1338. (b) Rodríguez, C.; de Gonzalo, G.; Torres Pazmiño, D. E.; Fraaije, M. W.; Gotor, V. *Tetrahedron: Asymmetry* **2009**, *20*, 1168.

were optimized, the expected oxidation product (*R*)-4-methyl-3,4-dihydrocoumarin (**6b**) was achieved with 23% conversion when using HAPMO. The M446G PAMO-biocatalyzed oxidation led to (*R*)-4-methyl-3,4-dihydroisocoumarin (**6c**) in a 12% conversion. For both enzymes, high enantioselectivities ($E \geq 200$) were measured.

The enzymatic preparation of different 3-alkyl-3,4-dihydroisocoumarins has been developed with high yields and enantiomeric excesses by choosing properly the reaction parameters for enzymatic Baeyer−Villiger oxidations. These substrates were obtained from the corresponding 2-alkyl-1-indanones due to the regioselectivity displayed by M446G PAMO. The presence of an acidic hydrogen in the α-position to the carbonylic moiety can induce substrate racemization by working at high pHs (pH 10.0), allowing effective dynamic kinetic resolution of the starting ketones. Addition of 5% methanol increases the enzymatic activity with a loss of selectivity, while the use of hexane leads to the best selectivities. Higher conversions were obtained when ketones presenting short alkyl chains were oxidzed. This study shows that it is possible to obtain all targeted 3,4-dihydroisocoumarins with high optical purities. An enzymatic oxidation has been scaled up to 250 mg scale using cell-free extract.

Experimental Section

General Methods. Recombinant histidine-tagged phenylacetone mononoxygenase mutant (M446G PAMO) and recombinant 4-hydroxyacetophenone monooxygenase (HAPMO) were overexpressed and purified as previously described.[12b,13] A 1.0 unit of BVMO will oxidize 1.0 μmol of phenylacetone to benzyl acetate per minute at pH 9.0 and room temperature in presence of NADPH. Glucose 6-phosphate dehydrogenase from *Leuconostoc mesenteroides,* racemic ketones (±)-**1**−**3a** and (±)-**5**−**6a**, and all other reagents and solvents were of the highest quality grade available and were purchased from commercial sources.

General Procedure for the Biooxidation of Racemic Ketones (±)-1−6a Catalyzed by BVMOs. The corresponding racemic ketones (±)-**1**−**6a** (14−28 mM) were dissolved in a 50 mM Tris−HCl buffer at different pH's containing 5% organic cosolvent if stated (1.0 mL), glucose 6-phosphate (28−56 mM), glucose 6-phosphate dehydrogenase (10.0 units), NADPH (0.2 mM), and the corresponding Baeyer−Villiger monooxygenase (1.0 unit). Reactions were shaken at 250 rpm, and the temperatures and times were established. Once finished, the crude reactions were extracted with EtOAc (2 × 500 μL). The organic layer were separated by centrifugation, dried onto Na_2SO_4, and analyzed directly by GC in order to determine the conversion of the oxidations and the enantiomeric excesses of the ketones (*S*)-**1**−**6a** and the lactones obtained (*R*)-**1**−**6b** or (*R*)-**2**−**6c**.

Enzymatic Preparation of Chiral 3-Alkyl-3,4-dihydroisocoumarins (±)-2−5c Catalyzed by M446G PAMO on a Multimilligram Scale. Ketones (±)-**2**−**5a** (60 mg, 0.41−0.32 mmol) were dissolved in a Tris−HCl buffer (50 mM, pH 10.0, 30 mL) containing 5% hexane for the oxidation of compound (±)-**2a** and (±)-**4a**. Then, NADPH (0.2 mM), glucose 6-phosphate (147−213 mg, 0.82−0.64 mmol), glucose 6-phosphate dehydrogenase (300 units), and M446G PAMO (30 units) were added. The mixture was shaken at 250 rpm at 40 °C during 120 h for (±)-**2a**, 96 h for (±)-**3a**, 104 h for (±)-**4a**, and 144 h for (±)-**5a**. The reaction was then stopped by extraction with ethyl acetate (4 × 15 mL), the combined organic layers were dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. Afterward, the crude residue were purified by flash chromatography using as eluent hexane/ethyl acetate 9:1 in order to obtain (*R*)-**2c** (49.9 mg, 75%), (*R*)-**3c** (42.9 mg, 65%), (*R*)-**4c** (32.8 mg, 50%), and (*R*)-**5c** (47.5 mg, 73%).

Enzymatic Preparation of Chiral 3-Methyl-3,4-dihydroisocoumarin (±)-2c Employing Cell Free Extract Containing M446G PAMO on a 250 mg Scale. Compound (±)-**2a** (250 mg, 1.71 mmol) was dissolved in a Tris−HCl buffer (50 mM, pH 10.0, 40 mL). Then, NADPH (0.2 mM), glucose 6-phosphate (890 mg, 3.4 mmol), glucose 6-phosphate dehydrogenase (1250 units), and M446G PAMO cell free extract (46 mL) were added. The mixture was shaken at 250 rpm at 40 °C and monitored by GC. After 48 h, the reaction was stopped by extraction with EtOAc (5 × 30 mL), and the organic layers were dried on Na_2SO_4 and evaporated under reduced pressure. The crude residue were purified by flash chromatography using as eluent hexane/ethyl acetate 9:1 in order to obtain (*R*)-**2c** (221 mg, 80% yield).

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Supporting Information Available: Complete results, experimental data, as well as the achiral and chiral GC data and product characterization. This material is available free of charge via the Internet at http://pubs.acs.org.