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Multienzymatic preparation of 3-[(1*R*)-1-hydroxyethyl]benzoic acid and (2*S*)-hydroxy(phenyl)ethanoic acid

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ABSTRACT

The use of two oxidoreductases (an aldoketo reductase from *Escherichia coli* JM109 and an alcohol dehydrogenase from *Lactobacillus brevis*) has demonstrated that it is possible to prepare enatiomerically pure diols in a one-pot operation. The reactions were applied to the synthesis of (1*R*)-1-[3-(hydroxymethyl)phenyl]ethanol and (1*S*)-1-phenylethane-1,2-diol, using a two-step procedure. The yield is nearly quantitative and the enantiomeric purity is greater than 95%. A third step has been introduced by adding a cell biocatalyst showing dihydrodiol dehydrogenase activity from *Pseudomonas fluorescens* N3. This allows for the preparation of 3-[(1*R*)-1-hydroxyethyl]benzoic acid and (2*S*)-hydroxy(phenyl)ethanoic acid.

1. Introduction

The preparation of enantiomerically pure compounds is a typical objective for organic chemists.[1–6] We have been working in this field for many years[7] and we were able to prepare many different molecules. More recently, we began to study the possibility of using multienzymatic synthesis to attain this target.[8] In this framework we considered the preparation of enantiomerically pure diols and hydroxy acids; in particular, we would like to synthesize (2*S*)-hydroxy(phenyl)ethanoic acid **7** and 3-[(1*R*)-1-hydroxyethyl]benzoic acid **9**. A second point was represented by our intention to test the possibility to finely distinguish between the reactivity of a ketone and an aldehyde. This task can be rarely accomplished by chemical methods, in contrast to enzymatic processes. We selected three enzyme activities: an aldoketo reductase from *Escherichia coli*, an alcohol dehydrogenase from *Lactobacillus brevis*, and a dihydrodiol dehydrogenase cloned from *Pseudomonas fluorescens* N3.[9,10] The substrates chosen were 3-acetyl benzaldehyde and phenylglyoxal, both of which are commercially available. The three activities are shown in Figure 1, where it is possible to note the substrate-specific transformations. However, we already knew that all of these enzymes have a wide scope and can function with different substrates; therefore, we decided to test all activities with the corresponding selected substrates.

Each transformation is possible by chemical synthesis. The reduction of ketones and aldehydes to the corresponding alcohols is very well known, even in the enantioselective version.[11] The oxidation of alcohols to the corresponding acids is also well known. However, some problems can occur when these simple reactions are performed on substrates containing more than one carbonyl or alcohol group. In fact, it is often difficult to selectively react only one group. This is clearly demonstrated by the reported synthetic routes to our compounds.

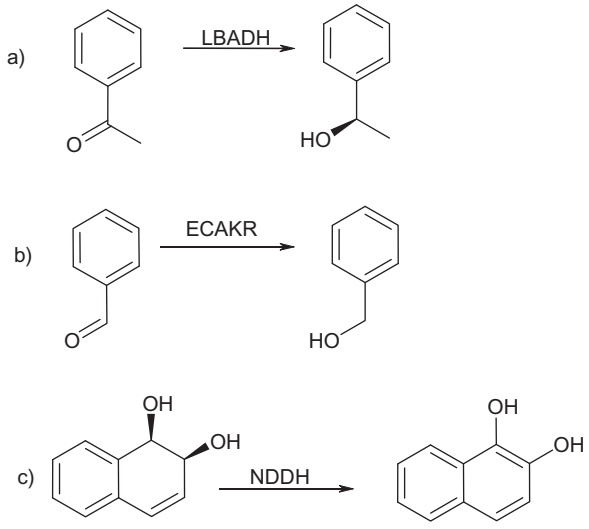

Figure 1. Reactions performed by *L. brevis* alcohol dehydrogenase (LBADH), *E. coli* JM109 aldoketoreductase (ECAKR), and *P. fluorescens* N3 dihydrodiol dehydrogenase (NDDH).

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Concerning the reactivity of 3-acetyl benzaldehyde, only the synthesis of 3-acetyl benzylic alcohol is reported in the literature;[12] this is obtained using a quite complicated catalyst. It is clear that 1-[3-(hydroxymethyl)phenyl]ethanol can be obtained using an excess of reducing agent, and that 3-[1-hydroxyethyl]benzoic acid can be synthesized by the reduction of 3-acetyl benzoic acid. However, the synthesis of the same products from the precursors that we have used is much more difficult.

In contrast, mandelic acid can be prepared by several methods. Three routes were chosen to illustrate the different approaches; they are not exhaustive, but they cover some interesting alternatives. The first approach is based on the enantioselective reduction of phenyl pyruvic acid by enzymes.[13] Here, the substrate is transformed by yeast with complete geometric selection in high yield. The second approach shows the efficiency of the conversion of the benzaldehyde into the product by CN addition.[14] This reaction can also be performed using enzymes.[15] In both cases the yield and the geometric selection are good. The last example describes the oxidation of 1-phenylethane-1,2-diol using a gold catalyst with good yield; here, there is no geometric selection.[16]

1-Phenylethane-1,2-diol can also be prepared in several ways. We selected two examples. Both describe the catalytic hydrogenation of 2-hydroxy-1-phenylethanone using a chiral catalyst with the yield and ee being very good.[17]

Finally, the preparation of 2-hydroxy-1-phenylethanone is also reported. In this case, we selected three examples that start from three different precursors. The first uses α-chloroketones that by cesium-catalyzed hydrolysis give the product in high yield.[18] The second starts from acyl chlorides, which are transformed into the hydroxy ketone in the presence of tris(trimethylsiloxy)ethylene, triethylamine, and microwaves, with the addition of one carbon atom.[19] The third is the reduction of glyoxal by titanium tetraiodide; selectivity is good, but the yield is moderate (40%).[20] However, we were unable to find any example that reports the 2–3-step syntheses that we describe herein.

2. Results and discussion

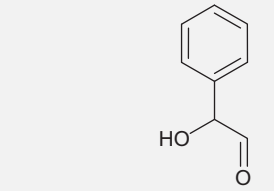
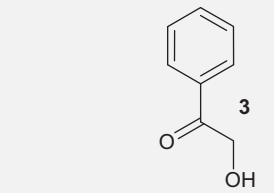
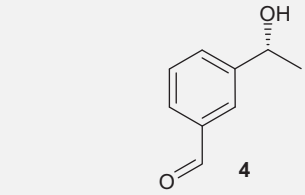
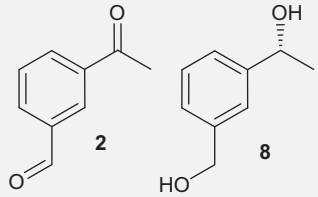
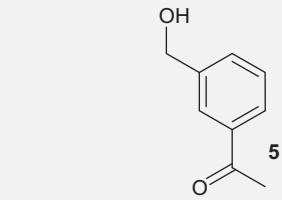
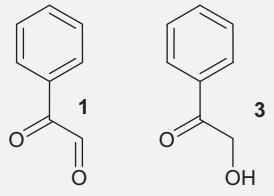
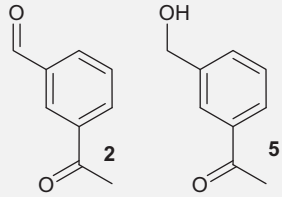
We used a general approach: first, we tested each single reaction step and then we combined two or three steps to obtain the final product in one-pot procedures. We studied each enzymatic activity separately. First, we considered *L. brevis* alcohol dehydrogenase (LBADH) activity on both substrates (Table 1). Using phenylglyoxal **1** we could expect three different products: 2-hydroxy-1-phenylethanone, hydroxy(phenyl)acetaldehyde, and 1-phenylethane-1,2-diol. Unfortunately, the reaction only gave a mixture of unidentified products.

Using 3-acetylbenzaldehyde **2** we expected three different products: 1-[3-(hydroxymethyl)phenyl]ethanone, 3-(1-hydroxyethyl) benzaldehyde **4**, and 1-[3-(hydroxymethyl)phenyl]ethanol **8**. In this case, the only product is **4** with trace amounts of diol **8**; the yield was very good (~90%) and **4** was enantiomerically pure. The direct preparation of **8** by this procedure is unfeasible because the reaction does not proceed in an acceptable amount of time.

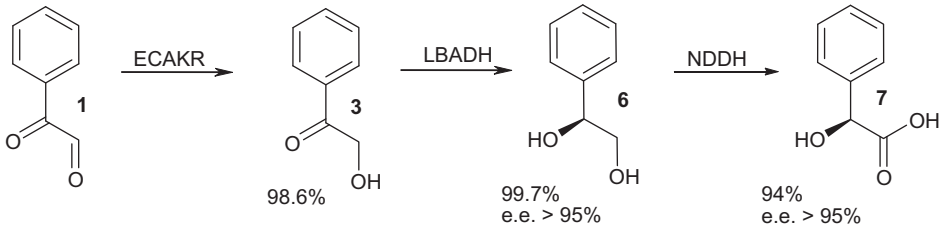
As already described in the literature,[21] *E. coli* JM109, our usual host organism, expresses a reductive activity (ECAKR) that chemoselectively transforms aldehydes into the corresponding alcohols. When used with our substrates, this catalyst only gives the products shown in Table 1, without any trace of by-products. This activity is highly efficient and it is impossible to prevent its action on any kind of the aldehyde that we have tested so far.

As a result, two sequences were considered (Schemes 1 and 2). We knew that the last transformation could be performed only after the previous two reactions (unpublished results). Before

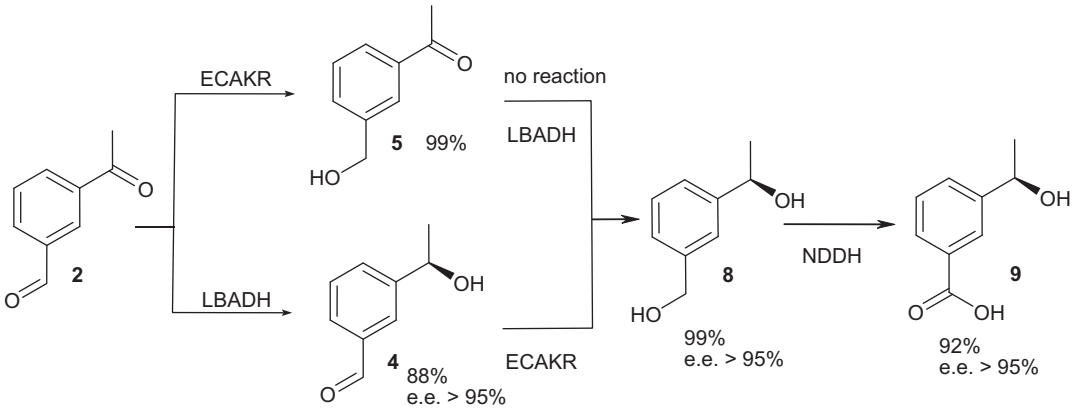
Table 1
Product obtained using LBADH[a] and ECAKR[b] biocatalysts on phenylglyoxal and 3-acetylbenzaldehyde

Substrate	Product	Experimental conditions	Yield	ee
		LBADH (25 U), 0.3731 mmol, 50 mL M9 + 1 mM $MgSO_4$, 3 mL *iso*-propanol, 3 mg NADP, 30 °C, 20 h	Unidentified mixture	
				
			88%	>95%
		LBADH (25 U), 0.3375 mmol, 50 mL M9 + 1 mM $MgSO_4$, 3 mL *iso*-propanol, 3 mg NADP, 30 °C, 20 h	<10%	n.d.
			–	
		*E. coli* JM109 (1 g/L CDW[c]) (ECAKR), 0.3731 mmol, 50 mL M9, 30 °C, 2 h	98.6%	n.a.
		*E. coli* JM109 (1 g/L CDW[c]) (ECAKR), 0.3375 mmol, 50 mL M9, 30 °C, 0.5 h	99%	n.a.

[a] *L. brevis* alcohol dehydrogenase.
[b] *E. coli* aldoketoreductase.
[c] Cell dry weight.



Scheme 1. Three-step preparation of (2*S*)-hydroxy(phenyl)ethanoic acid **7**.



Scheme 2. Three-step preparation of 3-[(1*R*)-1-hydroxyethyl]benzoic acid **9**.

performing the one-pot reactions, we tested the sequences step by step, isolating and characterizing all intermediates.

Compound **1** was readily transformed into **3** by ECAKR; the reaction was very fast (less than 2 h), complete, and no side products were present. This result confirms the selectivity of ECAKR toward aldehydes. Hydroxyketone **3** was then converted into **6** by LBADH; herein, the reaction was slower (10–16 h), yet also complete. The diol obtained was enantiopure, as confirmed by chiral GLC comparison with a commercial sample and has the expected (*S*)-configuration. Finally, the last conversion of **6** to **7** by NDDH was also complete and selective (no trace of the keto acid); it requires 20 h and the oxidation did not touch the benzyl alcohol configuration (chiral GLC and comparison to a commercial compound).

Concerning the second sequence **2** to **9**, we tested both initial steps: **2** to **5** using ECAKR and **2** to **4** using LBADH. The conversion of **2** to **5** was very fast, as usual, (less than 1 h), complete and selective in giving **5** as the sole product. In contrast, the conversion of **2** to **4** was slower (16 h), yet complete. In this case, a small amount of **8** (less than 10%) was also identified in the products. The reaction was enantioselective, as shown by chiral GLC (vide infra), and the (*R*)-configuration was presumed from the usual selectivity of LBADH. We separately tested the second step, using either LBADH (on **5**) or ECAKR (on **4**). The route that passes through **5** did not work; thus, the only possibility is the sequential use of LBADH first, and ECAKR second.

The conversion of **4** was very effective; we obtained only compound **8** in 5 h. Also in this case the enantioselectivity was confirmed by chiral GLC and the (*R*)-configuration presumed. It should be noted that the enantiopurity of **4** was presumed using two indications: the presence of only one GLC peak and the enantiopurity of **8**, because we could not prepare a racemic sample of **4**. The final conversion of **8** to **9** by NDDH was complete in 20 h; we could not find any trace of the keto acid, thus confirming the faster reaction of primary alcohols in comparison to secondary alcohols. Here again we found only one peak by chiral GLC and we presumed that the configuration of the benzylic carbon was untouched by NDDH; thus, we assign to **9** the (*R*)-configuration.

At this point, we performed the two reaction sequences in a one-pot cascade reaction: **1** to **3** to **6** to **7**; **2** to **4** to **8** to **9**. The sequences were carried out by adding the biocatalysts one after the other at the end of the preceding reaction, monitored by HPLC analysis. This implies that we never separated either the biocatalyst or the intermediates. At the end of both sequences, we were able to recover the final product essentially pure. Both sequences took approximately 48 h to complete.

3. Conclusion

The developed procedure allows for the preparation of both 3-[(1*R*)-1-hydroxyethyl]benzoic acid and (2*S*)-hydroxy(phenyl)ethanoic acid. It is also possible to prepare all the intermediates in enantiopure form, by stopping the catalyst addition when the desired product is reached. Both products are the sole compounds present at the end of the respective reaction sequences; they are easily recoverable from the cultures, and they are both enantiomerically pure. In comparison to chemical syntheses we can state that the multienzymatic procedures are very easy, user friendly, and compatible; in addition, they are highly chemo- and enantioselective. As can be seen, some transformations are simply unavailable in classical chemistry (e.g., ketone reduction in the presence of an aldehyde). The most important point is the possibility to operate cascade reactions without the need for the isolation and purification of intermediates. In contrast, the volume yield of some enzymatic reactions should be still optimized.

4. Experimental

4.1. General

All chemicals were purchased from Sigma–Aldrich. *L. brevis* ADH and $NADP^+$ were purchased from Codexis. Cells were prepared

from our stocks by usual cultivation procedures[10] and used as resting cells.

4.2. Analytical methods

Substrates and products were monitored by analyzing the water phase by HPLC, Hitachi-Merck, UV–visible detector at 220 nm, reverse phase column C18 (Hibar LICHROSORB 50334, 10 μm, 25 cm), H_2O/CH_3CN 1:1 eluent, 1 mL/min flow, Hitachi D2500 integrator. The absolute (*S*)-configuration of biocatalytically prepared (2*S*)-hydroxy(phenyl)ethanoic acid was proven via comparison with commercially available enantiopure (2*S*)-hydroxy (phenyl)ethanoic acid (Aldrich). The absolute (*R*)-configuration of biocatalytically prepared 3-[(1*R*)-1-hydroxyethyl]benzoic acid was presumed considering the known selectivity of *Lactobacillus brevis* ADH. Enantiomeric excesses were measured by GLC using a Chrompack ChiralDex-CB column. ^1H NMR and ^{13}C NMR spectra were obtained in $CDCl_3$ or DMSO-d_6 (Merck) using Bruker AC-200 instrument. All signals are expressed as ppm downfield from tetramethylsilane. Optical rotation was obtained in $CHCl_3$ or CH_3OH using JASCO P-1030 polarimeter.

4.3. Preparation procedures

4.3.1. 2-Hydroxy-1-phenylethanone 3 preparation

At first, 6.58 mM of phenylglyoxal **1**, directly suspended in the medium, was allowed to react with *E. coli* JM109 (1 g/L CDW) in 50 mL of M9 medium, at 30 °C. After 2 h, the cells were separated by centrifugation (10,000 rpm, 4 °C); the supernatant was extracted using AcOEt (three 30 mL portions), and the organic phases were collected, dried over Na_2SO_4, and evaporated at reduced pressure. The crude product (43 mg, 99%) exclusively contained 2-hydroxy-1-phenylethanone **3** (42.8 mg, 99.6%). Oil; δ_H (200 MHz, $CDCl_3$) 4.92 (2H, s), 7.5–7.7 (3H, m), 7.94 (2H, d, J = 7.5 Hz). δ_C (75.5 MHz, $CDCl_3$) 65.5 (t), 127.3 (d), 128.6 (d), 133.2 (d), 134.2 (s), 198.2 (s).

4.3.2. 1,2-Dihydroxy-1-phenylethanol 6 preparation

At first, 6.47 mM of 2-hydroxy-1-phenylethanone **3** was allowed to react with *L. bacillus* ADH (25 U) in 50 mL of M9 medium, containing $MgSO_4$(1 mM), *iso*-propanol (3 mL), and NADP (3 mg), at 30 °C. After 20 h. the solution was extracted using AcOEt (three 30 mL portions), and the organic phases were collected, dried over Na_2SO_4, and evaporated at reduced pressure. The crude product (44.5 mg, 99.7%) contained exclusively 1,2-dihydroxy-1-phenylethanol **6**. Oil; δ_H (200 MHz, $CDCl_3$) 3.68 (1H, dd, J = 7.7, 11.3), 3.81 (1H, dd, J = 3.8, 7.7), 4.86 (1H, dd, J = 3.8, 11.3), 7.3–7.7 (5H, m). δ_C (75.5 MHz, $CDCl_3$) 68.3 (t), 74.9 (d), 126.3 (d), 128.2 (d), 129.4 (d), 131.3 (d), 140.7 (s). $[\alpha]_D$ = +68.23 (*c* 5.8 mg, $CHCl_3$). Ee >95%. Retention times in chiral GLC (t_0 = 80 °C for 0 min, t_f = 180 °C, 5 °C/min, P_{He} = 0.8 atm) of this enantiomer: 10.9 min.

4.3.3. (2*S*)-Hydroxy(phenyl)ethanoic acid 7 preparation

At first, 6.38 mM of 1,2-dihydroxy-1-phenylethanol **6** was allowed to react with *E. coli* JM109 (pVL2028) (1 g/L CDW) in 50 mL of M9 medium, at 30 °C. After 20 h, the cells were separated by centrifugation (10,000 rpm, 4 °C); the supernatant was acidified with HCl 3 M and extracted using AcOEt (three 30 mL portions); the organic phases were collected, washed with water, dried over Na_2SO_4, and evaporated at reduced pressure. The crude product (45.5 mg, 94%) contained exclusively (2*S*)-hydroxy(phenyl)ethanoic acid **7**. Oil; δ_H (200 MHz, DMSO-d_6) 5.0 (1H, s), 7.3–7.5 (5H, m). δ_C (75.5 MHz, DMSO-d_6) 72.3 (d), 126.6 (d), 127.4 (d), 128.4 (d), 140.3 (s), 174.2 (s). $[\alpha]_D$ = +153.4 (*c* 8.8 mg, H_2O). Ee >95%. Retention times in chiral GLC (t_0 = 80 °C for 0 min, t_f = 180 °C, 5 °C/min, P_{He} = 0.8 atm) of this enantiomer: 21.4 min.

4.3.4. (2*S*)-Hydroxy(phenyl)ethanoic acid 7 one pot preparation

At first, 6.58 mM of phenylglyoxal **1**, directly suspended in the medium, was allowed to react with *E. coli* JM109 (1 g/L CDW) in 50 mL of M9 medium, at 30 °C. After 2 h, the product was allowed to react with *L. bacillus* ADH (25 U) in the same medium, containing $MgSO_4$ (1 mM), *iso*-propanol (3 mL), and NADP (3 mg), at 30 °C. After 22 h, the product was allowed to react with *E. coli* JM109 (pVL2028) (1 g/L CDW) in the same medium, at 30 °C. After 20 h, the cells were separated by centrifugation (10,000 rpm, 4 °C); the supernatant was acidified with HCl 3 M, and extracted using AcOEt (three 30 mL portions); the organic phases were collected, washed with water, dried over Na_2SO_4, and evaporated at reduced pressure. The crude product (46 mg, 92%) contained exclusively (2*S*)-hydroxy(phenyl)ethanoic acid **7**.

4.3.5. (2*S*)-Hydroxy(phenyl)ethanoic acid 7 one-pot preparation on a 250 mg scale

At first, 6.6 mM of phenylglyoxal **1**, directly suspended in the medium, was allowed to react with *E. coli* JM109 (1 g/L CDW) in 250 mL of M9 medium, at 30 °C. After 2 h, the product was allowed to react with *L. bacillus* ADH (125 U) in the same medium, containing $MgSO_4$ (1 mM), *iso*-propanol (15 mL), and NADP (15 mg) at 30 °C. After 24 h, the product was allowed to react with *E. coli* JM109 (pVL2028) (1 g/L CDW) in the same medium, at 30 °C. After 20 h, the cells were separated by centrifugation (10,000 rpm, 4 °C); the supernatant was acidified with HCl 3 M and extracted using AcOEt (three 100 mL portions); the organic phases were collected, washed with water, dried over Na_2SO_4, and evaporated at reduced pressure. The crude product (225 mg, 90%) contained exclusively (2*S*)-hydroxy(phenyl)ethanoic acid **7**.

4.3.6. 3-(1-Hydroxyethyl)benzaldehyde 4 preparation

At first, 6.75 mM of 3-acetylbenzaldehyde **2**, directly suspended in the medium, was allowed to react with *L. bacillus* ADH (25 U) in 50 mL of M9 medium, containing $MgSO_4$ (1 mM), *iso*-propanol (3 mL), and NADP (3 mg), at 30 °C. After 20 h, the solution was extracted using AcOEt (three 30 mL portions), and the organic phases were collected, dried over Na_2SO_4, and evaporated at reduced pressure. The crude product (44 mg, 88%) contained 3-(1-hydroxyethyl)benzaldehyde **4** with trace amounts of **8**. Oil; δ_H (200 MHz, $CDCl_3$) 5.0 (2H, s), 7.5 (2H, t, J = 7.5 Hz), 7.63 (1H, d, J = 7.5 Hz), 7.83 (2H, d, J = 7.5 Hz), 10.1 (1H, s). δ_C (75.5 MHz, $CDCl_3$) 26.1 (q), 70.5 (d), 127.1 (d), 129.6 (d), 129.9 (d), 132.2 (d), 136.8 (s), 147.1 (s), 193.0 (d). $[\alpha]_D$ = 36.84 (*c* 9.1 mg, $CHCl_3$). Ee >95%. Retention times in chiral GLC (t_0 = 80 °C for 0 min, t_f = 180 °C, 5 °C/min, P_{He} = 0.8 atm) of this enantiomer: 10.9 min.

4.3.7. 1-[3-(Hydroxymethyl)phenyl]ethanone 5 preparation

At first, 6.75 mM of 3-acetylbenzaldehyde **2**, directly suspended in the medium, was allowed to react with *E. coli* JM109 (1 g/L CDW) in 50 mL of M9 medium, at 30 °C. After 30 min, the cells were separated by centrifugation (10,000 rpm, 4 °C); the supernatant was extracted using AcOEt (three 30 mL portions), and the organic phases were collected, dried over Na_2SO_4, and evaporated at reduced pressure. The crude product (50 mg, 99%) contained exclusively 1-[3-(hydroxymethyl)phenyl]ethanone **5**. Oil; δ_H (200 MHz, $CDCl_3$) 2.1 (1H, br s), 2.6 (3H, s), 4.8 (2H, s), 7.5 (1H, t, J = 7.5), 7.6 (1H, d, J = 7.5 Hz), 7.9 (2H, d, J = 7.5 Hz), 9.0 (1H, s). δ_C (75.5 MHz, $CDCl_3$) 26.9 (q), 65 (t), 126.8 (d), 127.8 (d), 129.0 (d), 131.7 (d), 137.6 (s), 143.9 (s), 198.3 (s).

4.3.8. (1*R*)1-[3-(Hydroxymethyl)phenyl]ethanol 8 preparation

At first, 5.80 mM of 3-(1-hydroxyethyl)benzaldehyde **4**, directly suspended in the medium, was allowed to react with *E. coli* JM109 (1 g/L CDW) in 50 mL of M9 medium, at 30 °C. After 5 h, the cells were separated by centrifugation (10,000 rpm, 4 °C); the

supernatant was extracted using AcOEt (three 30 mL portions), and the organic phases were collected, dried over Na_2SO_4, and evaporated at reduced pressure. The crude product (43 mg, 99%) contained exclusively (1*R*)-1-[3-(hydroxymethyl)phenyl]ethanol **8**. Oil; δ_H (200 MHz, $CDCl_3$) 1.47 (3H, d, J = 6.5 Hz), 3.2 (2H, br s), 4.6 (2H, s), 4.85 (1H, q, J = 6.5 Hz), 7.2–7.28 (3H, m), 7.34 (1H, s). δ_C (75.5 MHz, $CDCl_3$) 25.3 (q), 65.2 (t), 70.4 (d), 124.2 (d), 124.9 (d), 126.2 (d), 128.8 (d), 141.3 (s), 146.3 (s). $[\alpha]_D$ = 37.53 (*c* 13.0 mg, $CHCl_3$). Ee >95%. Retention times for chiral GLC (t_0 = 80 °C for 0 min, t_f = 180 °C, 5 °C/min, P_{He} = 0.8 atm) of this (*R*)-enantiomer: 12.7 min [(*S*)-enantiomer 12.4 min].

4.3.9. 3-[(1*R*)-1-Hydroxyethyl]benzoic acid 9 preparation

At first, 5.33 mM of (1*R*)-1-[3-(hydroxymethyl)phenyl]ethanol **8**, directly suspended in the medium, was allowed to react with *E. coli* JM109 (pVL2028) (1 g/L CDW) in 50 mL of M9 medium, at 30 °C. After 20 h, the cells were separated by centrifugation (10,000 rpm, 4 °C); the supernatant was acidified with HCl 3 M and extracted using AcOEt (three 30 mL portions). The organic phases were collected, washed with water, dried over Na_2SO_4, and evaporated at reduced pressure. The crude product (40 mg, 92%) contained exclusively 3-[(1*R*)-1-hydroxyethyl]benzoic acid **9**. Oil; δ_H (200 MHz, $CDCl_3$) 1.54 (3H, d, J = 6.5 Hz), 5.05 (1H, q, J = 6.5 Hz), 7.46 (1H, t, J = 7.6) 7.66 (1H, d, J = 7.6), 8.06 (1H, d, J = 7.6), 8.12 (1H, s). δ_C (75.5 MHz, $CDCl_3$) 25.4 (q), 70.2 (d), 127.4 (d), 128.9 (d), 129.5 (d), 129.7 (s), 131.0 (d), 146.4 (s), 171.8 (s). $[\alpha]_D$ = +29.9 (*c* 15.5 mg, $CHCl_3$). Ee >95%. Retention times for chiral GLC (t_0 = 80 °C for 0 min, t_f = 180 °C, 5 °C/min, P_{He} = 0.8 atm) of this (*R*)-enantiomer: 13.9 min [(*S*)-enantiomer 13.7 min].

4.3.10. 3-[(1*R*)-1-Hydroxyethyl]benzoic acid 9 one pot preparation

At first, 6.75 mM of 3-acetylbenzaldehyde **2**, directly suspended in the medium, was allowed to react with *L. bacillus* ADH (25 U) in 50 mL of M9 medium, containing $MgSO_4$ (1 mM), *iso*-propanol (3 mL), and NADP (3 mg), at 30 °C. After 20 h, the product was allowed to react with *E. coli* JM109 (1 g / L CDW) in the same medium, at 30 °C. After 5 h, the product was allowed to react with *E. coli* JM109 (pVL2028) (1 g/L CDW) in the same medium, at 30 °C. After 20 h, the cells were separated by centrifugation (10,000 rpm, 4 °C); the supernatant was acidified with HCl 3 M and extracted using AcOEt (three 30 mL portions). The organic phases were collected, washed with water, dried over Na_2SO_4, and evaporated at reduced pressure. The crude product (50.7 mg, 90%) contained exclusively 3-[(1*R*)-1-hydroxyethyl]benzoic acid **9**.

The same transformation can be performed in two steps by directly adding *E. coli* JM109 (pVL2028) after the *L. bacillus* ADH. No evident difference was visible.

4.3.11. 3-[(1*R*)-1-Hydroxyethyl]benzoic acid 9 one pot preparation on a 250 mg scale

At first, 6.75 mM of 3-acetylbenzaldehyde **2**, directly suspended in the medium, was allowed to react with *L. bacillus* ADH (125 U) in 250 mL of M9 medium, containing $MgSO_4$ (1 mM), *iso*-propanol (15 mL), and NADP (15 mg), at 30 °C. After 20 h, the product was allowed to react with *E. coli* JM109 (pVL2028) (1 g/L CDW) in the same medium, at 30 °C. After 25 h, the cells were separated by centrifugation (10,000 rpm, 4 °C); the supernatant was acidified with HCl 3 M and extracted using AcOEt (three 100 mL portions). The organic phases were collected, washed with water, dried over Na_2SO_4, and evaporated at reduced pressure. The crude product (257.7 mg, 92%) exclusively contained 3-[(1*R*)-1-hydroxyethyl]benzoic acid **9** ee >95%.

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References

1. Chen, G.; Fournier, R. L.; Varanasi, S. *Enzyme Microb. Technol.* **1997**, *21*, 491–495.
2. Kihumbu, D.; Stillger, T.; Hummel, W.; Liese, A. *Tetrahedron: Asymmetry* **2002**, *13*, 1069–1072.
3. (a) Glueck, S. M.; Fabian, W. M. F.; Faber, K.; Mayer, S. F. *Chem. Eur. J.* **2004**, *10*, 3467–3478; (b) Larissegger-Schnell, B.; Kroutil, W.; Faber, K. *Synlett* **2005**, 1936–1938.
4. Hecquet, L.; Bolte, J.; Demuynck, C. *Tetrahedron* **1996**, *52*, 8223–8232.
5. Guerard, C.; Alphand, V.; Archelas, A.; Demuynck, C.; Hecquet, L.; Furstoss, R.; Bolte, J. *Eur. J. Org. Chem.* **1999**, 3399–3402.
6. Zimmermann, F. T.; Schneider, A.; Schoerken, U.; Sprenger, G. A.; Fessner, W.-D. *Tetrahedron: Asymmetry* **1999**, *10*, 1643–1646.
7. Bestetti, G.; Di Gennaro, P.; Galli, E.; Leoni, B.; Pelizzoni, F.; Sello, G.; Bianchi, D. *Appl. Microbiol. Biotechnol.* **1994**, *40*, 791–793.
8. Sello, G.; Bernasconi, S.; Orsini, F.; Di Gennaro, P. *Tetrahedron: Asymmetry* **2009**, *20*, 563–565.
9. Bernasconi, S.; Orsini, F.; Sello, G.; Di Gennaro, P. *Tetrahedron: Asymmetry* **2004**, *15*, 1603–1606.
10. Sello, G.; Bernasconi, S.; Orsini, F.; Mattavelli, P.; Di Gennaro, P.; Bestetti, G. *J. Mol. Catal. B: Enzym.* **2008**, *52–53*, 67–73.
11. Ema, T.; Ura, N.; Yoshii, M.; Korenaga, T.; Sakai, T. *Tetrahedron* **2009**, *65*, 9583–9591.
12. Gnanamgari, D.; Moores, A.; Rajaseelan, E.; Crabtree, R. H. *Organometallics* **2007**, *26*, 1226–1230.
13. Guo, J.; Mu, X.; Zheng, C.; Xu, Y. *J. Chem. Technol. Biotechnol.* **2009**, *84*, 1787–1792.
14. North, M.; Omedes-Pujol, M. *Tetrahedron Lett.* **2009**, *50*, 4452–4454.
15. Mateo, C.; Chmura, A.; Rustler, S.; van Rantwijk, F.; Stolz, A.; Sheldon, R. A. *Tetrahedron: Asymmetry* **2006**, *17*, 320–323.
16. Biella, S.; Castiglioni, G. L.; Fumagalli, C.; Prati, L.; Rossi, M. *Catal. Today* **2002**, *72*, 43–49.
17. (a) Kadyrov, R.; Koenigs, R. M.; Brinkmann, C.; Voigtlaender, D.; Rueping, M. *Angew. Chem., Int. Ed.* **2009**, *48*, 7556–7559; (b) Ohkuma, T.; Utsumi, N.; Watanabe, M.; Tsutsumi, K.; Arai, N.; Murata, K. *Org. Lett.* **2007**, *9*, 2565–2567.
18. Wonga, F. F.; Chang, P.-W.; Lin, H.-C.; You, B.-J.; Huang, J.-J.; Lin, S.-K. *J. Organomet. Chem.* **2009**, *694*, 3452–3455.
19. Vaismaa, M. J. P.; Leskinen, M. V.; Lajunen, M. K. *Synth. Commun.* **2009**, *39*, 2042–2052.
20. Hayakawa, R.; Sahara, T.; Shimizu, M. *Tetrahedron Lett.* **2000**, *41*, 7939–7942.
21. Sello, G.; Orsini, F.; Bernasconi, S.; Di Gennaro, P. *Molecules* **2006**, *11*, 365–369.