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Asymmetric reduction of substituted α- and β-ketoesters by *Bacillus pumilus* Phe-C3

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ABSTRACT

The enantioselective reduction of substituted α- and β-ketoesters using resting cells of *Bacillus pumilus* Phe-C3 was investigated. Effects of substrate concentration on the catalytic efficiency of the microorganism were studied. Preparative scale productions were carried out under the optimized conditions with 62.4–91.0% yields and 90.2–97.1% ee. The cells retained 80% of initial activity after recycling for six times.

1. Introduction

Optically active secondary alcohols are important building blocks for the production of flavors, pharmaceuticals, and agrochemicals.[1] Ethyl (*R*)-3-hydroxybutanoate and ethyl (*S*)-4-halo-3-hydroxybutanoate are key intermediates of inhibitors of angiotensin-converting enzyme,[2] and HMG-CoA reductase,[3,4] respectively. They are also valuable synthons for the production of pharmaceuticals such as L-carnitine,[5] and (*R*)-4-amino-3-hydroxybutanoic acid (GABOB).[6]

Methods for the production of α- and β-hydroxy ester include reduction of the corresponding ketoesters using either metal catalysts[7–16] or biocatalysts.[17–28] Moreover, enzymatic deracemization[29–38] and resolution[39–44] of racemic hydroxy esters have also been employed in some cases. Among these synthetic strategies problems exist with, either less than 50% yields presented for resolution methods or low overall yield for chemical methods with tedious synthetic routes. Instead of these methods, the asymmetric bioreduction of prochiral ketones with dehydrogenase is a superior way for producing secondary alcohol building blocks. Bacterial whole cells have the advantage over isolated enzymes since they contain all the necessary cofactors and the metabolic pathway for their regeneration. Moreover, all the enzymes and cofactors are well protected within their natural cellular environment.[17]

We are interested in expanding the scope of the enzymatic reduction of ketoesters in practical syntheses by developing highly active and easy handle bacterial catalysts with new substrate specificity. Ideal catalyst for this conversion could offer broad substrate acceptance, high enantioselectivity, rapid reaction rates, low cost, and maximum substrate concentration burden. The strain *Bacillus pumilus* Phe-C3 was reported to be capable of reducing β-ketoxyesters to optically active secondary alcohols. It was also identified that NADPH-dependent alcohol dehydrogenase was responsible for the reduction.[45] Herein, we have extended the substrate diversity of this strain for the biocatalytic reduction of series substituted prochiral α- and β-ketoesters to the corresponding hydroxyl esters.

2. Results and discussion

2.1. Reduction of β-ketoesters with *B. pumilus* Phe-C3

Bioreductions of β-ketoesters were carried out with the cells of *B. pumilus* Phe-C3 on a 10-mL scale in the exploratory stage.[45] Substrate tolerance of biocatalyst is a crucial factor for its potential industrial application. Higher substrate tolerance of the biocatalyst can increase its production capability in industrial process. Therefore, different substrate concentrations with the same cell density of 3 g cdw/L were explored for the determination of catalytic capacity of the strain. The bioconversions were followed by GC or HPLC analyses.

As shown in Table 1, reduction of β-ketoesters **1a**–**6a** gave the corresponding β-hydroxyesters **1b**–**6b**. High activities and high conversions were observed in the bioreduction of **2a**, **4a**, and **5a** with 19.3, 23.4, and 27.8 U/g cdw (U: μmol/min, cdw: cell dry weight), respectively. While low to moderate activities were obtained for **1a**, **3a**, and **6a** with 9.1, 6.7, and 11.8 U/g cdw, respectively. It was shown that the bioreduction of 10 mM of **2a** formed **2b** with a lower conversion of 85.3%, compared to 96.3% conversion with a substrate concentration of 15 mM. This is probably due to the further degradation of the product **2b** by other enzymes inside the strain with lower **2b** concentration.

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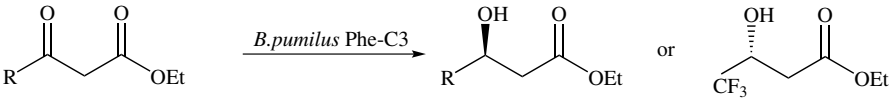
Table 1
Reduction of β-ketoesters with *B. pumilus* Phe-C3 (cells 3.0 g cdw/L)



1a-6a **1b-5b** **6b**

1: R = CH_3 **4**: R = Ph
2: R = CH_2Cl **5**: R = CH_2CN
3: R = CH_2Br **6**: R = CF_3

Substrate (mM)		Activity[a] (U/g cdw)	Conv. and ee[b,c] (%)						
			0.5 h	2 h	5 h	9 h	12 h	16 h	19 h
1a	5	9.1	16.4(92.6)	29.8	73.0	90.5	99(94.5)	—	—
1a	7	8.8	11.3(90.8)	25.3	40.1	51.9	62.4	77.3	79.5(90.8)
2a	10	18.1	16.3(93.5)	50.2	74.7	85.3(93.5)	—	—	—
2a	15	19.3	14.1(95.9)	48.1	77.7	86.5	92.1	96.3(95.9)	—
3a	3	4.8	14.4(91.4)	17.2	41.3	50.7	75.6	82.3	88.1(91.4)
3a	5	6.7	12.1(90.3)	20.8	40.3	52.5	66.3	71.5	85.4(90.3)
4a[d]	5	12.9	23.3(94.0)	50.4	76.3	91.1	98.7(94.0)	—	—
4a[d]	10	23.4	21.1(95.7)	66.6	72.4	86.7	96.9(95.7)	—	—
5a	10	20.9	18.8(94.5)	41.3	64.1	73.2	85.3	91.2	96.7(94.5)
5a	15	27.8	16.7(97.0)	39.9	62.7	70.1	85.0	90.5	95.1(97.0)
6a	5	11.8	21.3(90.2)	40.5	61.3	74.5	80.3	87.2	93.9(90.2)
6a	7	9.8	12.6(90.2)	39.6	52.8	60.4	76.1	80.9	85.3(90.2)

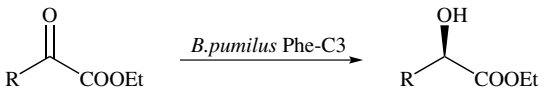
[a] The activity was determined from the product formation over the first 30 min.
[b] ee was given in brackets.
[c] Conversion and ee were determined by GC analysis; error limit: 2% of the stated values.
[d] Conversion and ee were determined by HPLC analysis; error limit: 2% of the stated values.

2.2. Reduction of α-ketoesters with *B. pumilus* Phe-C3

In order to further explore the substrate versatility of *B. pumilus* Phe-C3, substituted α-ketoesters were used as precursors. Reduction of **7a–9a** gave the corresponding α-hydroxyesters **7b–9b**. As shown in Table 2, high activities and high conversions were obtained for all of α-ketoesters tested. Extremely high activity (40.4 U/g cdw) was observed in bioreduction of **7a** at substrate concentration of 5 mM with cell density of 3 g cdw/L. With the same cell density, high activity was also obtained for **8a** and **9a** as 17.8 and 13.9 U/g cdw, respectively.

Ethyl (*R*)-2-hydroxy-4-phenylbutyrate (*R*-EHPB) **9b** is an important intermediate for the synthesis of ACE inhibitors such as cilazapril, benazepril, and enalapril.[46] Regarding its practical preparation, the reaction capacity of *B. pumilus* Phe-C3 with **9a** was further investigated. Therefore, 20–40 mM of **9a** with a cell density of 12.0 g cdw/L was used to test its influence on the catalytic efficiency of *B. pumilus* Phe-C3. Higher product concentration could be easily achieved by use of a higher cell density and substrate concentration. A concentration up to 30 mM was still well tolerated by *B. pumilus* Phe-C3 at the cell density of 12.0 g cdw/L, with 95.3% conversion at 19 h with 97.1% ee, whereas 40 mM of

Table 2
Reduction of α-ketoesters with *B. pumilus* Phe-C3



7a-9a **7b-9b**

7: R = CH_3; **8**: R = Ph; **9**: R = CH_2CH_2Ph

Substrate (mM)		Cell density (g cdw/L)	Activity[a] (U/g cdw)	Conv. and ee[b,c] (%)						
				0.5 h	2 h	5 h	9 h	12 h	16 h	19 h
7a[d]	5	3	40.4	72.8(94.1)	83.9	92.6	>99(94.1)	—	—	—
7a[d]	7	3	23.0	29.6(94.1)	30.1	41.1	66.6	76.5	82.4(94.1)	—
8a	10	3	15.1	13.6(93.9)	41.9	70.7	81.3	90.1	95.7(93.9)	—
8a	15	3	17.8	10.7(96.6)	33.4	56.5	72.7	84.5	90.6	94.8(96.6)
9a	5	3	13.9	25.1(94.5)	62.3	76.3	85.4	88.7	93.1	99.0(94.5)
9a	20	12	14.6	26.3(96.7)	63.5	75.3	80.2	86.3	91.0	97.9(96.7)
9a	30	12	17.3	20.7(97.1)	60.1	69.8	77.2	81.2	89.9	95.3(97.1)
9a	40	12	7.4	5.6(87.3)	—	—	11.5(86.9)	—	—	29.7(86.9)

[a] The activity was determined from the product formation over the first 30 min.
[b] ee was given in brackets.
[c] Conversion and ee were determined by HPLC analysis; error limit: 2% of the stated values.
[d] Conversion and ee were determined by GC analysis; error limit: 2% of the stated values.

the substrate inhibited the catalytic activity severely; the maximum conversion obtained was only 29.7%.

2.3. The enantioselectivity and stereoselectivity of the bioreduction

The enantiomeric excess of the product was measured by chiral GC and HPLC after the first 30 min and the end of the bioreduction. β-Hydroxyesters **1b**–**6b** were obtained with a high ee of 94.5%, 95.9%, 91.4%, 95.7%, 97.0%, and 90.2%, respectively. For α-hydroxyesters **7b**–**9b**, high ee was also achieved with ees of 94.1%, 96.6%, and 97.1%, respectively. In terms of stereoselectivity, **1b** and **5b**–**9b** were obtained with the (*R*)-configuration while **2b**–**4b** formed with an (*S*)-configuration.

The stereoselectivity of the reaction indicates that the dehydrogenases inside the cell deliver hydride ion from the same side of prochiral ketoesters for all of the substrates except **6a**. This phenomenon is probably due to the CF_3 functional group in **6a**, compared with other substituted substrates, which interferes with the binding configuration strongly inside the active pocket of the dehydrogenase.

2.4. Preparative bioreduction of 1a–9a

Preparative biotransformations were performed on a 300-mL scale in a 1000-mL shaking flask (Table 3). The yields of the products were calculated after purification via column chromatography. Bioreduction of **1a**–**8a** with resting cells (3.0 g cdw/L) afforded **1b**–**8b** with 87.3%, 90.1%, 62.4%, 88.4%, 83.1%, 82.7%, 79.7%, 85.4%, and 91.0%, respectively.

Reduction of ketoesters, such as **1a**, **2a**, and **5a**, were efficiently accomplished by *B. pumilus* Phe-C3 within 24 h, and gave the corresponding hydroxyesters which are important synthetic intermediates for L-carnitine[5] and inhibitors of HMG-CoA reductase.[3,4] Interestingly, for ketoesters **2a** and **5a**, the conversion, activity, and enantioselectivity are not very much dependent on the starting concentration of substrate, which is advantageous for practical production. In the present study, the substrate concentration of **2a** and **5a** was easily increased without losing of the catalytic efficiency. These results were competitive with other reports, such as Davignon from Codexis[47] and Li et al.,[48] in terms of high product concentration and ee.

Optically active ethyl (*S*)-3-hydroxy-3-phenylpropionate **4b**, the precursor of the anti-depressant tomoxetine,[49] was obtained with 95.7% ee and a production concentration of 2.7 g/L. The result is comparable with that of reported by Salvi et al.[50] recently.

Mandelic acid and its derivatives are key intermediates for the production of various pharmaceuticals, such as semi-synthetic penicillins, cephalosporins, and antiobesity agents.[51–53] Recently, the strain *Geotrichum candidum* CIOC2.616[54] was reported to be capable of catalyzing the biosynthesis of (*R*)-methyl mandelate with high yield (92%) and ee (95%). In the present study, *B. pumilus* Phe-C3 is able to catalyze the synthesis of (*R*)-ethyl mandelate **8b** with 96.6% ee and 85.4% yield.

Finally, preparative scale production of (*R*)-EHPB **9b** was carried out by the reduction of 30 mM EOPB **9a** using *B. pumilus* Phe-C3 (12.0 g cdw/L) at 30 °C, 220 rpm (Table 3, entry 9). 1.7 g (*R*)-**9b** was obtained in 26 h after purification; the yield and the ee of (*R*)-**9b** were 91.0% and 97.1%, respectively. The previous reported plant cells of *Daucus carota* could also be used to catalyze the synthesis of (*R*)-**9b** with high yield (90%) and ee (>99%).[35] However, the reaction required a very long time of 10 days, and the substrate/catalyst ratio (0.1/10, w/w) was moderate. For the present study, the catalytic synthesis of (*R*)-**9b** with *B. pumilus* Phe-C3 was performed within 26 h while the substrate/catalyst ratio increased to 5.7/12 (w/w).

2.5. Reuse of *B. pumilus* Phe-C3 for the biotransformation

With 30 mM **9a** used as substrates each time, the cells of *B. pumilus* Phe-C3 could be reused for the bioreduction. In each run, the cells were collected by centrifugation at 9000 rpm, 4 °C for 15 min after the reaction. The cells were then washed by phosphate buffer (pH 7.0) and reused under the same conditions.

As shown in Figure 1, each cycle of the biotransformation resulted in a slight loss of the catalytic activity of the strain.

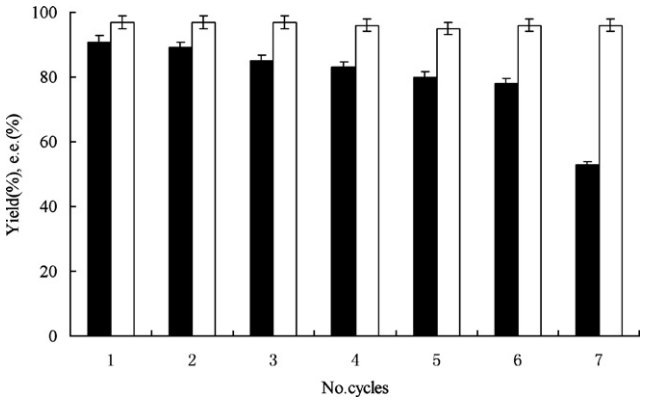


Figure 1. Reuse of *Bacillus pumilus* Phe-C3 for the biotransformation of **9a**. Reaction conditions: substrate (30 mM), phosphate buffer pH 7.0 (10 mL), glucose (2%) and cells (12 g cdw L^{-1}) were incubated with shaking at 220 rpm for 24 h of each cycle. (■)—isolation yield, (□)—ee.

Table 3
Preparation of **1b**-**9b** by bioreduction with *B. pumilus* Phe-C3

Entry	Substrate (mM)		Cell density (g cdw/L)	Time (h)	Conv.[a] (%)	ee (%)	Yield[b]		Config.[c]
							(%)	(mg)	
1	**1a**	5	3	12	99.0	94.5	87.3	172.9	(*R*)
2	**2a**	15	3	12	99.0	95.9	90.1	675.1	(*S*)
3	**3a**	3	3	24	86.0	91.4	62.4	118.5	(*S*)
4	**4a**[d]	10	3	12	99.0	95.7	88.4	514.5	(*S*)
5	**5a**	15	3	24	99.0	97.0	83.1	587.1	(*R*)
6	**6a**	5	3	24	95.2	90.2	82.7	193.5	(*R*)
7	**7a**	5	3	9	99.0	94.1	79.7	141.1	(*R*)
8	**8a**[d]	15	3	24	99.0	96.6	85.4	464.2	(*R*)
9	**9a**[d]	30	12	26	98.5	97.1	91.0	1703.5	(*R*)

[a] Conversion and ee were determined by GC analysis; error limit: 2% of the stated values.
[b] Yield of the isolated pure product.
[c] The absolute configuration was determined by comparison of the specific rotation with the literature value.
[d] Conversion and ee were determined by HPLC analysis; error limit: 2% of the stated values.

However, the cells still retained more than 80% of the initial activity after six reaction cycles. It was also observed that there was no significant change in the enantioselectivity of the bioreduction after each cycle. From a practical point of view, the reuse of biocatalysts is an effective means for cost-effective production.

3. Conclusions

The enantioselective reduction of various ketoesters by the strain *B. pumilus* Phe-C3 was investigated. The wide substrate spectrum and high chemical yield with short reaction time made *B. pumilus* Phe-C3 a promising biocatalyst in the construction of some important optically active hydroxyesters. Meanwhile, compared with previous reported plant cells of *Daucus carota*, a more efficient synthesis of (*R*)-EHPB was carried out on a preparative scale with high yield and ee. Moreover, the reuse of the cells further increased their potential for large-scale production process.

4. Experimental

β-Ketoesters **3a**[55] and **5a**[56] were prepared based on the reported procedures in our laboratory, other ketoesters were obtained from commercial suppliers and used without further purification. (±)-Ethyl hydroxyesters were synthesized by reducing the corresponding ethyl oxobutyrates with sodium borohydride.[35] All other chemicals were of analytic grade. *B. pumilus* Phe-C3 was obtained from Institute of Biotechnology, ETH Zurich. Column chromatography was carried out using silica gel. GC analyses were performed using a chiral CP-Chirasil Dex CB column, HPLC analyses were performed using a Chiralcel OB-H column. The absolute configurations were determined using a Perkin Elmer Model 343 polarimeter in $CHCl_3$, and assigned by comparison of the specific rotations with the literature values. The NMR spectra were measured on a Bruker AM 300 spectrometer or a Bruker AM 500 spectrometer.

4.1. Cultivation and preparation of the biocatalyst

B. pumilus Phe-C3 was grown on LB-agar plate for 5 days, and then inoculated into 100 mL M9 medium containing 2% (w/v) glucose in a 500-mL shaking flask. The cells were grown at 30 °C and 220 rpm for 38 h, then harvested by centrifugation at 4 °C and washed twice, stored at −80 °C in a refrigerator. M9 medium composition: Na_2HPO_4 6.78‰; KH_2PO_4 3.0‰; NaCl 0.5‰; NH_4Cl 1.0‰; $MgSO_4$ 0.24‰; $CaCl_2$ 0.01‰, pH 7.0.

4.2. General procedure for small scale bioreduction

The cells of *B. pumilus* Phe-C3 were suspended to a cell density of 3.0 g cdw/L in 10 mL of phosphate buffer (pH 7.0) containing glucose (2% w/v) in a 100-mL Erlenmeyer flask. Substrates were added as a solution in 10% MeOH to a final concentration of 3–15 mM. The mixture was shaken at 200 rpm at 30 °C. The reactions were then followed by GC or HPLC analysis. Then 300 μL aliquots were taken at predetermined time points each time for analysis. Samples for GC analysis were prepared by centrifugation to remove the cell debris, mixing of 100 μL supernatant with 400 μL phosphate buffer (pH 7.0), extracting with 500 μL chloroform and drying with anhydrous Na_2SO_4. For HPLC analysis, samples were prepared by mixing the aliquots with an equal volume of MeOH followed by centrifugation.

The specific activity of *B. pumilus* Phe-C3 was examined during the first 30 min of each reaction; one unit (U) of the activity was defined as the amount biocatalyst capable of catalyzing the reduction of 1 μmol of substrate per minute.

4.3. General procedure for preparative bioreduction

Ketoesters **1a**–**9a** (3–30 mM) were added to a suspension of *B. pumilus* Phe-C3 cells (3.0 or 12.0 g cdw/L) in 300 mL of phosphate buffer (pH 7.0) containing glucose (2%). The mixture was incubated in a 1000-mL shaking flask at 220 rpm and 30 °C. The reaction was stopped by centrifugation, and the product was extracted with $CHCl_3$. The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel to afford the corresponding hydroxyester.

4.3.1. Ethyl (*R*)-3-hydroxybutyrate 1b

$[\alpha]_D^{25} = -43.1$ (*c* 1.0, $CHCl_3$), lit.[57] for (*R*)-**1b** $[\alpha]_D^{25} = -44.5$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.25 (d, 3H), 1.27 (t, 3H), 2.48 (m, 2H), 3.06 (br, 1H), 4.16 (q, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 14.2, 22.7, 43.1, 60.6, 64.5, 173.1.

4.3.2. Ethyl (*S*)-4-chloro-3-hydroxybutyrate 2b

$[\alpha]_D^{25} = -21.7$ (*c* 7.0, $CHCl_3$), lit.[58] for (*R*)-**2b**, $[\alpha]_D^{25} = +20.9$ (*c* 7.7, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.27 (t, 3H), 2.65 (d, 2H), 3.60 (d, 2H), 4.19 (q, 2H), 4.28 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 14.4, 38.8, 48.4, 61.1, 68.5, 172.0.

4.3.3. Ethyl (*S*)-4-bromo-3-hydroxybutyrate 3b

$[\alpha]_D^{25} = -10.9$ (*c* 1.0, $CHCl_3$), lit.[59] for (*S*)-**3b** $[\alpha]_D^{25} = -9.1$ (*c* 10.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.38 (t, 3H), 2.74 (d, 2H), 3.28 (br, 1H), 3.51 (m, 2H), 4.18 (q, 2H), 4.25 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 14.4, 37.6, 39.6, 61.3, 67.8, 172.1.

4.3.4. Ethyl (*S*)-3-hydroxy-3-phenylpropionate 4b

$[\alpha]_D^{25} = -33.1$ (*c* 1.0, $CHCl_3$), lit.[60] for (*S*)-**4b** $[\alpha]_D^{25} = -25.8$ (*c* 1.3, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.35 (t, 3H), 2.72 (d, 2H), 3.32 (br, 1H), 4.18 (q, 2H), 7.26–7.37 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): 14.1, 43.3, 60.8, 70.4, 125.7, 127.8, 128.5, 142.5, 172.4.

4.3.5. Ethyl (*R*)-4-cyano-3-hydroxybutyrate 5b

$[\alpha]_D^{25} = -32.1$ (*c* 1.0, $CHCl_3$), lit.[61] for (*R*)-**5b** $[\alpha]_D^{25} = -33.1$ (*c* 1.2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.25 (t, 3H), 2.65 (d, 2H), 3.13 (m, 1H), 3.75 (m, 2H), 4.15 (q, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 14.4, 25.2, 40.1, 61.5, 64.2, 117.3, 171.2.

4.3.6. Ethyl (*R*)-4,4,4-trifluoro-3-hydroxybutyrate 6b

$[\alpha]_D^{25} = +20.1$ (*c* 1.0, $CHCl_3$), lit.[45] for (*R*)-**6b** $[\alpha]_D^{25} = +18.7$ (*c* 1.2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.28 (t, 3H), 2.72–2.64 (m, 2H), 3.58 (m, 1H), 4.22 (q, 2H), 4.42 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 14.1, 34.7, 61.7, 66.8, 125.4, 170.8.

4.3.7. Ethyl (*R*)-2-hydroxy-propionate 7b

$[\alpha]_D^{25} = +14.3$ (*c* 1.0, $CHCl_3$), lit.[62] for (*R*)-**7b** $[\alpha]_D^{25} = +12.8$ (*c* 2.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.26 (t, 3H), 1.38 (d, 2H), 4.22 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 14.1, 20.9, 62.3, 66.9, 176.0.

4.3.8. Ethyl (*R*)-α-hydroxybenzeneacetate 8b

$[\alpha]_D^{25} = -99.3$ (*c* 1.0, $CHCl_3$), lit.[63] for (*R*)-**8b** $[\alpha]_D^{25} = -104.4$ (*c* 1.0, EtOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.20 (t, 3H), 3.63 (s, 1H), 4.15–4.25 (m, 2H), 5.20 (d, 1H), 7.33–7.42 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 13.9, 62.1, 72.8, 126.3, 128.2, 128.5, 138.3, 173.8.

4.3.9. Ethyl (*R*)-2-hydroxy-4-phenylbutyrate 9b

$[\alpha]_D^{25} = -19.5$ (*c* 1.0, $CHCl_3$), lit.[27] for (*R*)-**9b** $[\alpha]_D^{25} = -18.1$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 1.28 (t, 3H), 1.89–2.18 (m, 2H), 2.79 (m, 2H), 4.20 (q, 2H), 7.15–7.35 (m, 5H); ^{13}C NMR

(75 MHz, $CDCl_3$): δ (ppm) 14.8, 32.5, 42.9, 63.2, 72.4, 98.9, 127.9, 128.5, 129.8, 142.3, 175.2.

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