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Nitrile biotransformations for the synthesis of enantiomerically enriched β^2-, and β^3-hydroxy and -alkoxy acids and amides, a dramatic O-substituent effect of the substrates on enantioselectivity

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Abstract—*Rhodococcus erythropolis* AJ270, a nitrile hydratase/amidase-containing microbial whole cell catalyst, is able to catalyze the hydrolysis of a number of β-hydroxy and β-alkoxy nitriles under very mild conditions. Both the efficiency and enantioselectivity of the biocatalysis, however, were strongly dependent upon the structures of both nitrile and amide substrates. When biotransformations of racemic 3-hydroxy-3-phenylpropionitrile and 2-hydroxymethyl-3-phenylpropionitrile gave low enantioselectivity, their O-methylated isomers underwent highly efficient and enantioselective biocatalytic reactions to afford highly enantioenriched β^2- and β^3-hydroxy amide and acid derivatives in excellent yield. The study has provided an example of simple and very convenient substrate engineering method to increase the enantioselectivity of the biocatalytic reaction.

1. Introduction

Optically active β^3- and β^2-hydroxy carboxylic acids, β-hydroxy carboxylic acids that contain, respectively, a substituent at the 3- and 2-position, and their derivatives are key intermediates in the synthesis of natural products and biologically important compounds.[1] 3-Hydroxy-3-phenylpropionic acid and its amide derivatives, for example, have been used as the precursors to chiral drugs such as tomoxetine and fluorine and nisoxetine.[2] Much effort has been devoted to the synthesis of chiral β-hydroxy carboxylic acids and their derivatives. The synthetic methods documented so far include catalytic asymmetric hydrogenation,[1a,3] reduction,[4] Mukaiyama-aldol reaction,[5] and Reformatsky reaction.[6] Biocatalytic reduction of β-ketoesters,[7] enzyme-catalyzed kinetic resolution of secondary alcohols,[8] and microbial deracemization of racemic ethyl 3-aryl-3-hydroxy propionic acid ester[9] have also been reported.

Biotransformations of nitriles, either through direct conversion from a nitrile to a carboxylic acid catalyzed by a nitrilase or through the nitrile hydratase-catalyzed hydration of a nitrile followed by the amide hydrolysis catalyzed by the amidase,[10] are an effective and environmentally benign method for the production of carboxylic acids and their amide derivatives.[11] Studies have demonstrated that the biotransformations of nitriles complement the existing asymmetric chemical and enzymatic methods for the synthesis of chiral carboxylic acids and their derivatives.[12,13] The distinct features of enzymatic transformations of nitriles are the formation of enantiopure carboxylic acids and the straightforward generation of enantiopure amides, which are valuable organonitrogen compounds in synthetic chemistry. Recently, we have shown that *Rhodococcus erythropolis* AJ270,[14] a nitrile hydratase/amidase-containing whole cell catalyst, is able to efficiently and enantioselectively transform a number of racemic nitriles including α-alkylated and functionalized nitriles,[15] α-aminonitriles,[16] cyclopropanecarbonitriles,[17] oxiranecarbonitriles,[18] and aziridinecarbonitriles[19] into chiral carboxylic acids and amide derivatives. Racemic nitriles bearing a β-stereogenic center were also biocatalytically transformed into the corresponding enantiopure acids and amides, provided the substrates were designed carefully.[20] For example, *R. erythropolis* AJ270-catalyzed hydrolysis of racemic β-benzyloxy alkanenitriles led to the formation of highly enantiopure β-benzyloxy alkanoic acids and amides whereas the biotransformations of racemic β-hydroxy alkanenitriles

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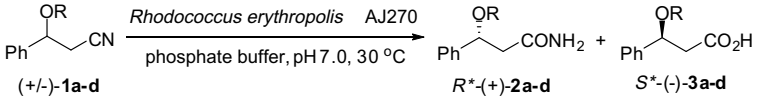
yielded very low enantioselectivity.[20b] The intention of further exploring the synthetic applications and understanding the mechanism of the powerful *R. erythropolis* AJ270 whole cell catalyst in organic chemistry led us to the current study. Having considered the ready availability of starting nitriles and the high catalytic efficiency and enantioselectivity of the biocatalyst, we envisaged that *R. erythropolis* AJ270-catalyzed biotransformations of nitriles might offer us a novel approach to optically active β^3- and β^2-hydroxy and alkoxy acids and amide derivatives. Herein, we report the microbial hydrolysis of two types of racemic β-hydroxy and β-alkoxy nitriles and the observation of a dramatic O-substituent effect on the enantioselectivity of biocatalytic reactions.

2. Results and discussion

To start our investigation, we first examined the *R. erythropolis* AJ270 whole cell catalyzed hydrolysis of racemic 3-phenyl-3-hydroxypropionitrile (±)-**1a**, a β^3-hydroxy carbonitrile substrate. Under very mild conditions, nitrile (±)-**1a** underwent a highly efficient hydrolysis to afford amide **2a** and acid **3a** in almost quantitative yields. Disappointingly, the enantiomeric excess (ee) values for amide and acid products were found to be extremely low, indicating that the nitrile hydratase and amidase enzymes involved within *R. erythropolis* AJ270 cell were almost non-enantioselective against racemic nitrile (±)-**1a** and amide (±)-**2a** substrates, respectively (Table 1, entry 1). Such a low enantioselectivity is not fully unexpected, however, since most of the biotransformations of nitriles bearing a β-stereogenic center gave very low enantioselectivity. As reported in the literature, for example, biotransformations of β-substituted β-hydroxy-α-methylenepropiononitriles,[21] 3-hydroxy-4-aryloxy-butyronitriles,[22] and 3-methyloxycarbonyl-3-benzyloxy propionitrile[23] formed amide and acid products with low ee values. It is widely believed that both chemical and enzymatic reactions generally show poor chiral induction when the substrates contain a stereogenic center remote from the reaction site. To circumvent this problem, we developed very recently a simple and powerful protection/docking strategy.[20] By the protection of a free β-hydroxy of β-hydroxy alkanenitrile with a benzyl group, for example, the enantioselectivity of an enzyme-catalyzed reaction was increased dramatically. The benzyl group probably acts as a docking moiety to enhance the chiral interaction between the substrate and the enzyme, leading therefore to a highly enantioselective biotransformation. We applied this strategy in the current study by converting substrate **1a** into its O-benzylated form **1b**. Unfortunately, biotransformation of (±)-**1b** proceeded very sluggishly, and only amide **2b** and starting nitrile were isolated in 56% and 31% yields, respectively, after a week's interaction with *R. erythropolis* AJ270. No acid product **3b** was observed (Table 1, entry 2). The sharp contrast to the very efficient biotransformations of many β-benzyloxy alkanenitriles[20] clearly suggests that the presence of a phenyl group rather than an alkyl group in the substrates poses steric hindrance to both nitrile hydratase and amidase within a microbial cell. In other words, 3-benzyloxy-3-phenylpropionitrile **1b** and 3-benzyloxy-3-phenylpropionamide **2b** were not accepted as good substrates by the nitrile hydratase and the amidase, respectively, most probably due to their steric bulkiness. Having realized the steric requirement of the enzymes toward the substrates, we then varied the O-substitu-

Table 1. Biotransformations of racemic β^3-hydroxy and β^3-alkoxy nitriles **1a–d**[a]



(+/-)-**1a-d** — *R**-(+)-**2a-d** — *S**-(-)-**3a-d**

Entry	(±)-**1**	Time	**2** (yield %)[b] (ee %)[c]	**3** (yield %)[b] (ee %)[c]	*E*[d]
1	**1a** R = H	0.5 h	**2a** (50) (1.6)	**3a** (49) (4.0)	1.1
2[e]	**1b** R = Bn	7 d	**2b** (56) (2.4)	**3b** (0)	—
3	**1c** R = Allyl	53 h	(*R**)-**2c** (47) (40.0)	(*S**)-**3c** (50) (29.4)	2.6
4	**1d** R = Me	5.5 h	(*R*)-(+)-**2d** (49) (77.6)	(*S*)-(−)-**3d** (51) (59.2)	8.9
5[f]	**1d** R = Me	5.5 h	(*R*)-(+)-**2d** (49) (68.8)	(*S*)-(−)-**3d** (51) (45.6)	5.3
6[g]	**1d** R = Me	5.5 h	(*R*)-(+)-**2d** (47) (70.0)	(*S*)-(−)-**3d** (51) (41.4)	4.7
7[h]	**1d** R = Me	7 h	(*R*)-(+)-**2d** (55) (69.4)	(*S*)-(−)-**3d** (44) (52.0)	6.3
8[i]	**1d** R = Me	5.5 h	(*R*)-(+)-**2d** (55) (70.2)	(*S*)-(−)-**3d** (44) (61.0)	8.4
9[j]	**1d** R = Me	11 h	(*R*)-(+)-**2d** (55) (60.8)	(*S*)-(−)-**3d** (44) (63.2)	8.1

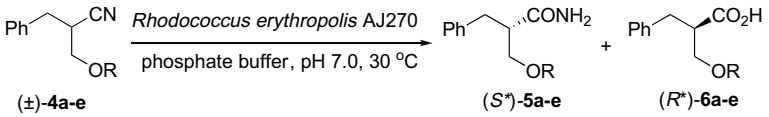
[a] Biotransformation was carried out by incubating nitrile (1 mmol) in a suspension of *Rhodococcus erythropolis* AJ270 cells (2 g wet weight) in phosphate buffer (50 mL, pH 7.0) at 30 °C.
[b] Isolated yield.
[c] Determined by HPLC analysis using a chiral column (see Section 4).
[d] Enantiomeric selection (*E*) was calculated according to a literature method.[25]
[e] Nitrile **1c** was recovered (31% yield, ee 13.4%).
[f] pH 6.5 was applied.
[g] pH 7.5 was applied.
[h] Acetone (2 mL) was used as a co-solvent.
[i] β-Cyclodextrin (100 mg) was used as an additive.
[j] Incubation temperature was 20 °C.
[*] Stereochemistry is tentatively assigned.

ent by choosing a smaller alkyl group. Introduction of an alkyl group onto the free hydroxy of **1a** resulted indeed in the effective biotransformations with a slightly increased enantioselectivity. Incubation of racemic nitrile (±)-**1c** with biocatalyst for 53 h, for example, afforded amide (*R**)-**2c** and acid (*S**)-**3c** in high yields with enantiomeric excesses being 40.0% and 29.4%, respectively (entry 3, Table 1). A further decrease in the size of the O-substituent led to faster and more enantioselective biotransformations. As indicated in entry 4, Table 1, effective biotransformations of racemic 3-methoxy-3-phenylpropionitrile (±)-**1d** finished within 5.5 h to yield quantitative yield of amide (*R*)-(+)-**2d** and acid (*S*)-(−)-**3d**[24] with enantiomeric excess values being 77.6% and 59.2%, respectively. In comparison with the biotransformations of nitrile (±)-**1a** ($E^{25} = 1.1$), the enantioselectivity of biotransformations of (±)-**1d** ($E = 8.9$) was improved more than 8-fold. Attempts were made to further increase the enantioselectivity of the reaction of **1d**. In our previous studies, a slight change of pH of the reaction media around 7.0,[16,18a] lower reaction temperature such as at 20 °C,[15e,16a,b] and the addition of acetone or β-cyclodextrin[26] were found to increase the enantioselectivity in some cases. As summarized in Table 1, however, variation of pH values of buffered reaction media (entries 5 and 6), the addition of an organic additive (entries 7 and 8), and the decrease of reaction temperature (entry 9) had only a marginal effect on enantioselectivity.

We then tuned our attention to the biotransformations of racemic 2-hydroxymethyl-3-phenylpropionitrile **4a**, a β^2-hydroxy carbonitrile species. Under identical incubation conditions for the biotransformations of nitriles (±)-**1a**–**d**, racemic nitrile (±)-**4a** underwent rapid and efficient biotransformations to produce optically active amide (*S*)-(−)-**5a** and acid (*R*)-(+)-**6a**[27] in excellent yield with moderate enantioselectivity ($E = 20$) (Table 2, entry 1). It is worth noting that the biotransformations of (±)-**4a** (Table 2, entry 1) gave higher enantioselectivity than that of (±)-**1a** (Table 1, entry 1). This is in agreement with the notion that the closer the stereogenic center is to the reaction site, the higher the chiral induction. The free hydroxy group was then protected by several different substituents, and the effect of the O-substituent on the reaction efficiency and enantioselectivity was examined. A large protection group such as a TMS **4b** or a benzyl group **4c** led to a slow reaction (Table 2, entries 2 and 3). The introduction of an allyl group onto the free hydroxy of **4a** also decreased the rate of biotransformation, especially the amidase-mediated hydrolysis of amide **5d** (Table 2, entry 4). Only under the conditions halving the concentration of (±)-**4d** did the biotransformations proceed within 36 h to afford optically active amide (*S**)-**5d** and acid (*R**)-**6d** in excellent yields (Table 2, entry 5). Surprisingly, the enantioselectivity of the reaction of O-allylated substrate (±)-**4d** ($E = 6$) decreased compared to the reaction of hydroxy-containing substrate (±)-**4a** ($E = 20$) (entries 1 and 5). Gratifyingly, when the hydroxy was protected by a methyl group, the racemic nitrile substrate (±)-**4e** underwent highly efficient and enantioselective biotransformations to furnish the corresponding amide (*S*)-(−)-**5e** and acid (*R*)-(+)-**6e**[28] both in a quantitative yield with excellent enantiomeric purity ($E = 193$) (Table 2, entry 6). The enantioselectivity of the reaction increased almost 10-fold (Table 2, entries 1 and 6).

The configurations of biocatalytically resolved products (*R*)-3-methoxy-3-phenylpropionamide (*R*)-(+)-**2d** and (*S*)-3-methoxy-3-phenylpropionic acid (*S*)-(−)-**3d**,[24] (*S*)-2-benzyl-3-hydroxypropionamide (*S*)-(−)-**5a** and (*R*)-2-benzyl-3-hydroxypropionic acid (*R*)-(+)-**6a**,[26] and (*S*)-2-benzyl-3-methoxy-propionamide amide (*S*)-(−)-**5e** and (*R*)-2-benzyl-3-methoxy-propionic acid (*R*)-(+)-**6e**[27] were determined by comparing the optical rotatory powers of (*S*)-(−)-**3d**, (*R*)-(+)-**6a**, and (*R*)-(+)-**6e** with that of authentic samples.[24,26,27] The absolute configurations of other biotransformation products including (*R**)-**2c**, (*S**)-**3c**, (*S**)-**5c**, (*S**)-**5d**, and (*R**)-**6d** were only assigned tentatively, assuming that *R. erythropolis* AJ270 has the same sense of enantioselection or enantiodifferentiation against analogous substrates.

Table 2. Biotransformations of racemic β^2-hydroxy and β^2-alkoxy nitriles **4a-e**[a]



(±)-**4a-e** (*S**)-**5a-e** (*R**)-**6a-e**

Entry	(±)-**4** (mmol)	Time	**5** (yield %)[b] (ee %)[c]	**6** (yield %)[b] (ee %)[c]	E[d]
1	**4a** R = H (1 mmol)	1.4 h	(*S*)-(−)-**5a** (43) (71.0)	(*R*)-(+)-**6a** (47) (81.1)	20
2[e]	**4b** R = TMS (1 mmol)	3 d	**5b** (0)	**6b** (0)	—
3[f]	**4c** R = Bn (1 mmol)	7 d	(*S**)-(−)-**5c** (21) (68.4)[g]	**6c** (0)	—
4	**4d** R = Allyl (2 mmol)	7 d	(*S**)-(+)-**5d** (72) (4.3)[g]	(*R**)-**6d** (25) (62.8)[g]	—
5	**4d** R = Allyl (1 mmol)	36 h	(*S**)-(+)-**5d** (47) (57.6)[g]	(*R**)-**6d** (51) (54.6)[g]	6
6	**4e** R = Me (2 mmol)	3.5 h	(*S*)-(−)-**5e** (50) (96.2)	(*R*)-(+)-**6e** (50) (96.2)	193

[a] Biotransformation was carried out in a suspension of *Rhodococcus erythropolis* AJ270 cells (2 g wet weight) in phosphate buffer (50 mL, pH 7.0) at 30 °C.
[b] Isolated yield.
[c] Determined by HPLC analysis using a chiral column.
[d] Enantiomeric selection (E) was calculated according to a literature method.[25]
[e] Racemic nitrile (±)-**4b** (100%) was fully recovered.
[f] Nitrile **4c** (66% yield, ee 12.4%) was recovered.
[g] Absolute configuration was tentatively assigned.

The aforementioned results clearly indicated that the nature of the O-protecting group played an important role in determining the enzymatic efficiency and enantioselectivity. In both 3-hydroxy-3-phenylpropionitrile and 2-hydroxymehtyl-3-phenylpropionitrile cases, a large O-protecting group such as a benzyl or a TMS had an inhibitory effect on both nitrile hydratase and amidase, indicating the steric limitation of the substrates accepted by both enzymes. The introduction of an allyl group onto 3-hydroxy-3-phenylpropionitrile led to an increase in enantioselectivity while the same O-protection in the case of 2-hydroxymethyl-3-phenylpropionitrile had a negative effect in terms of enantioselectivity. O-Methylation in both 3-hydroxy-3-phenylpropionitrile and 2-hydroxymethyl-3-phenylpropionitrile substrates gave rise to a great increase in enantioselectivity. The varied O-substituent effect suggested the importance of choosing an O-protecting group. Based on the observation of low ee values of recovered nitriles, and also according to our early studies,[15–21] the enhancement of enantioselectivity of overall nitrile biotransformations stems predominantly the high enantioselective amidase involved in *R. erythropolis* AJ270. Although it is currently difficult to rationalize the beneficial effect of O-methylation of the substrates on the enantioselectivity because of the lack of a structure of the amidase, the favorable match or recognition between the amidase and a sterically complementary β-methoxy nitrile **1d**, or particularly **4e**, might account for the high enantioselectivity.

3. Conclusion

In conclusion, we have shown that *R. erythropolis* AJ270 whole cells can catalyze the hydrolysis of a number of β-hydroxy and β-alkoxy nitriles under very mild conditions. Both the efficiency and enantioselectivity of biocatalysis, however, were strongly dependent upon the structures of both nitrile and amide substrates. We have demonstrated an efficient approach to improve the enantioselectivity of the biotransformations of β^2- and β^3-hydroxy nitriles by simply methylating the free hydroxy group of β^2- and β^3-hydroxy nitrile substrates. When the biotransformations of 3-hydroxy-3-phenylpropionitrile and 2-hydroxymethyl-3-phenylpropionitrile gave a low enantioselectivity, their O-methylated isomers underwent highly efficient and enantioselective biocatalytic reactions to afford highly enantioenriched β^2- and β^3-hydroxy amide and acid derivatives in excellent yield. This study has provided an example of a simple and very convenient substrate engineering method to increase the performance of enzymes in terms of enantioselectivity.

4. Experimental

4.1. Preparation of starting nitriles and their spectroscopic data

4.1.1. Preparation of racemic 3-benzyloxy-3-phenylpropionitrile 1b. Ag_2O (24.5 g, 105 mmol) was added to a mixture of 3-hydroxy-3-phenylpropionitrile **1a** (10.37 g, 70.5mmol), which was prepared following a literature method,[29] and benzyl bromide (7.25 mL, 77.5 mmol) in dichloromethane (30 mL). The mixture was stirred at room temperature in the dark for 4 h and then filtrated. After the removal of the solvent, column chromatography using a silica gel eluted with a mixture of petroleum ether and ethyl acetate (7:1) gave 3-benzyloxy-3-phenylpropionitrile **1b** (12.4 g, 74%) as a colorless liquid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32–7.43 (10H, m), 4.64 (1H, dd, $J = 7.4$, 5.5 Hz), 4.55 (1H, d, $J = 12.0$ Hz), 4.34 (1H, d, $J = 12.0$ Hz), 2.82 (1H, dd, $J = 16.6$, 7.4 Hz), 2.70 (1H, dd, $J = 16.6$, 5.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.8, 137.3, 129.1 (2C), 129.0, 128.6 (2C), 128.0, 127.2 (2C), 126.6 (2C), 117.2, 76.3, 70.8, 27.1 ppm; IR (KBr) ν 2251 cm^{-1} (CN); MS (EI) m/z (%) 197 (14) $[M-40]^+$, 129 (14), 91 (100); Anal. Calcd for $C_{16}H_{15}NO$: C, 80.98; H, 6.37; N, 5.90. Found: C, 81.19; H, 6.37; N, 6.01.

4.1.2. Preparation of racemic 3-allyloxy-3-phenylpropionitrile 1c. A mixture of 3-hydroxy-3-phenylpropionitrile **1a** (294 mg, 2 mmol), acetone (4 mL), allyl bromide (0.51 mL, 6 mmol), and potassium carbonate (552 mg, 4 mmol) was stirred at room temperature for 3 days and then filtered. The filtrate was concentrated under vacuum and then subjected to a silica gel column chromatography eluted by petroleum ether and ethyl acetate (7:1). 3-Allyloxy-3-phenylpropionitrile **1c** (223 mg, 60%) was obtained as a colorless liquid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34–7.41 (5H, m), 5.89 (1H, m), 5.23 (2H, m), 4.63 (1H, dd, $J = 7.1$, 5.6 Hz), 3.98 (1H, m), 3.84 (1H, m), 2.76 (1H, dd, $J = 16.6$, 7.1 Hz), 2.69 (1H, dd, $J = 16.6$, 5.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.9, 133.9, 128.93 (2C), 128.88, 126.4 (2C) ($M-C_3H_4$), 117.7, 117.2, 76.3, 69.9, 27.0 ppm; IR (KBr) ν 2251 cm^{-1} (CN); MS (EI) m/z (%) 147 (100), 130 (76), 105 (58). Anal. Calcd for $C_{12}H_{13}NO$: C, 76.98; H, 7.00; N, 7.48. Found: C, 76.84; H, 6.93; N, 7.66.

4.1.3. Preparation of racemic 3-methoxy-3-phenylpropionitrile 1d. Ag_2O (4.6 g, 20 mmol) and methyl iodide (5 mL, 80 mmol) were added to 3-hydroxy-3-phenylpropionitrile **1a** (2.94 g, 20 mmol) successively and the resulting mixture was stirred in the dark overnight. The mixture was diluted with acetone (5 mL) and filtered. After the removal of solvent, column chromatography using a silica gel eluted with a mixture of petroleum ether and ethyl acetate (10:1) gave 3-methoxy-3-phenylpropionitrile **1d**[30] (2.55 g, 79%) as a colorless liquid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32–7.43 (5H, m), 4.45 (1H, dd, $J = 7.0$, 5.6 Hz), 3.29 (3H, s), 2.77 (1H, dd, $J = 16.6$, 7.1 Hz), 2.68 (1H, dd, $J = 16.6$, 5.6 Hz); IR (KBr) ν 2251 cm^{-1} (CN).

4.1.4. Preparation of racemic 2-hydroxymethyl-3-phenylpropionitrile 4a. To a suspension of methyl 2-cyano-3-phenylacrylate[31] (11.2 g, 60 mmol) in isopropanol (180 mL) cooled in a water bath was added sodium borohydride (6.84 g) and the resulting mixture was stirred overnight at ambient temperature. Acetic acid (30%) was carefully added to the reaction mixture to quench the unconsumed sodium borohydride until no bubbling was observed. 2-Propanol was then removed under vacuum and the residue was basified to pH 10 with aqueous sodium hydroxide (1 M) and extracted with diethyl ether (3 × 50 mL). After

drying over with anhydrous $MgSO_4$ and removal of solvent, 2-hydroxymethyl-3-phenylpropionitrile **4a**[32] (9.44 g, 98%) was isolated as a colorless liquid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35–7.24 (5H, m), 3.77 (2H, s), 2.97 (3H, s), 2.29 (1H, br); ^{13}C NMR (75 MHz, $CDCl_3$) δ 136.4, 129.0, 128.9, 127.4, 120.5, 61.8, 36.8, 34.5 ppm; IR (KBr) ν 3450 (OH), 2245 cm^{-1} (C≡O).

4.1.5. Preparation of racemic 3-phenyl-2-(trimethylsilylmethyl)propionitrile 4b. A mixture of HMDS (1.45 g, 9 mmol) and 2-hydroxymethyl-3-phenylpropionitrile **4a** (1.93 g, 12 mmol) was stirred at room temperature for 5 min and then passed through a silica gel column chromatography eluted with a mixture of petroleum ether and ethyl acetate (100:1). 3-Phenyl-2-(trimethylsilylmethyl)propionitrile **4b** (1.75 g, 63%) was obtained as a colorless liquid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.08–7.20 (5H, m), 3.54 (2H, d, $J = 5.1$ Hz), 2.74–2.81 (3H, m), 0.00 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.3, 129.7 (2C), 129.3 (2C), 127.8, 62.3, 37.3, 35.1, 0.0 ppm; MS (EI) m/z (%) 233 (5) $[M]^+$, 218 (100), 188 (43), 143 (37), 91 (24); Anal. Calcd for $C_{13}H_{19}NSi$ (C, H, N): C, 66.90; H, 8.21; N, 6.00. Found: C, 66.87; H, 7.80; N, 6.10.

4.1.6. Preparation of racemic 2-benzyloxymethyl-3-phenylpropionitrile 4c. Ag_2O (5.8 g, 25 mmol) was added to a mixture of 2-hydroxymethyl-3-phenylpropionitrile **4a** (4.03 g, 25 mmol) and benzyl bromide (4.5 mL, 37.5 mmol) in 25 mL dichloromethane. The mixture was stirred at room temperature in the dark for 28 h and then filtered. After the removal of solvent, column chromatography using a silica gel eluted with a mixture of petroleum ether and ethyl acetate (15:1) gave 2-benzyloxymethyl-3-phenylpropionitrile **4c** (2.84 g, 45%) as a colorless liquid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.23–7.38 (10H, m), 4.58 (2H, s), 3.57 (2H, d, $J = 5.4$ Hz), 2.98 (3H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.3, 136.4, 129.1 (2C), 128.8 (2C), 128.6 (2C), 128.0, 127.8 (2C), 127.3, 120.2, 73.4, 68.3, 34.8, 34.5 ppm; IR (KBr) ν 2248 cm^{-1} (CN); MS (EI) m/z (%) 251 (8) $[M]^+$, 220 (10), 181 (6), 160 (3), 143 (11), 134 (13), 130 (20), 91 (100); Anal. Calcd for $C_{17}H_{17}NO$: C, 81.24; H, 6.82; N, 5.57. Found: C, 81.15; H, 6.79; N, 5.68.

4.1.7. Preparation of racemic 3-allyloxy-3-phenylpropionitrile 4d. At 0 °C, allyl bromide (220 μL, 2.5 mmol) was added to a suspension of NaH (72 mg, 3 mmol) in dry DMF (2 mL). A solution of 2-hydroxymethyl-3-phenylpropionitrile **4a** (322 mg, 2 mmol) in dry DMF (2 mL) was added dropwise into the mixture. After stirring for 1 h, the reaction was quenched with saturated aqueous $NaHCO_3$. An additional 40 mL of water was added to the mixture and extracted with diethyl ether (3 × 15 mL). After drying over with anhydrous $MgSO_4$ and removal of solvent, 3-allyloxy-3-phenylpropionitrile **4d** (310 mg, 77%) was isolated by silica gel column chromatography eluted with petroleum ether and ethyl acetate (50:1) as a colorless liquid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.24–7.35 (5H, m), 5.90 (1H, m), 5.31 (1H, dd, $J = 17.2$, 1.6 Hz), 5.23 (1H, dd, $J = 10.4$, 1.4 Hz), 4.05 (2H, m), 3.54 (2H, d, $J = 5.3$ Hz), 2.99 (3H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 136.5, 133.9, 129.1 (2C), 128.8 (2C), 127.3, 120.2, 117.8, 72.3, 68.3, 34.8, 34.3 ppm; IR (KBr) ν 2243 cm^{-1} (CN); MS (EI) m/z (%) 201 (19) $[M]^+$, 170 (6), 143 (89), 130 (100), 91 (65); Anal. Calcd for $C_{13}H_{15}NO$: C, 77.58; H, 7.51; N, 6.96. Found: C, 77.62; H, 7.49; N, 6.93.

4.1.8. Preparation of racemic 2-methoxymethyl-3-phenylpropionitrile 4e. Ag_2O (5.8 g, 25 mmol) and methyl iodide (6.23 mL, 100 mmol) were added to a mixture of 2-hydroxymethyl-3-phenylpropionitrile **4a** (4.03 g, 25 mmol) and acetonitrile (25 mL) successively and the resulting mixture was stirred in the dark overnight after which the mixture was filtered. After the removal of the solvent, column chromatography using a silica gel eluted with a mixture of petroleum ether and ethyl acetate (20:1) gave 2-methoxymethyl-3-phenylpropionitrile **4e** (3.39 g, 77%) as a colorless liquid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.23–7.37 (5H, m), 3.48 (2H, d, $J = 5.3$ Hz), 3.40 (3H, s), 2.99 (3H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 136.5, 129.1 (2C), 128.8 (2C), 127.3, 120.3, 70.8, 59.2, 34.8, 34.2 ppm; IR (KBr) ν 2241 cm^{-1} (CN); MS (EI) m/z (%) 175 (11) $[M]^+$, 143 (100), 116 (13), 91 (65); Anal. Calcd for $C_{11}H_{13}NO$: C, 75.40; H, 7.48; N, 7.99. Found: C, 75.53; H, 7.35; N, 8.13.

4.2. General procedure for the biotransformations of nitriles

To an Erlenmeyer flask (150 mL) with a screw cap were added *R. erythropolis* AJ270 cells[14,15a] (2 g wet weight) and potassium phosphate buffer (0.1 M, pH 7.0, 50 mL), and the resting cells were activated at 30 °C for 0.5 h with orbital shaking. Racemic nitrile was added in one portion to the flask and the mixture was incubated at 30 °C using an orbital shaker (200 rpm). The reaction, monitored by TLC and HPLC, was quenched after a specified period of time (see Tables 1 and 2) by removing the biomass through a Celite pad filtration. The resulting aqueous solution was basified to pH 12 with aqueous NaOH (2 M). Extraction with ethyl acetate (3×60 mL) gave, after drying over $MgSO_4$, concentration, and a silica gel column chromatography eluted with a mixture of petroleum ether and acetone (2:1), the amide product. The aqueous solution was then acidified using aqueous HCl (2 M) to pH 2 and extracted with ethyl acetate (3×60 mL). The acid product was obtained after drying over with $MgSO_4$, removal of the solvent under vacuum, followed by silica gel column chromatography eluted with a mixture of petroleum ether and acetone (from 20:1 to 2:1). All products were characterized by their spectral data and comparison of the melting points and specific rotation values with those of the known compounds, which are listed as follows, or by full characterization. Enantiomeric excess values were obtained from HPLC analysis using a column of chiral stationary phase.

4.2.1. 3-Hydroxy-3-phenylpropionamide 2a.[33] Mp 122–123 °C; $[\alpha]_D^{25} = 0$ (c 2.40, $CHCl_3$); ee 1.6% (chiral HPLC analysis using a Chiralcel OJ column with a mixture of hexane and 2-propanol (95:5) at a flow rate 0.8 mL/min as the mobile phase, oven temperature 25 °C, $t_{minor} = 52.1$ min, $t_{major} = 59.6$ min); ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.15–7.35 (6H, m), 6.82 (1H, br), 5.38 (1H, br), 4.91–4.96 (1H, m), 2.41 (1H, dd, $J = 14.3$, 8.4 Hz), 2.34 (1H, dd, $J = 14.3$, 5.1 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ

172.2, 145.3, 127.9, 126.7, 125.7, 69.5, 45.2 ppm; IR (KBr) ν 3393, 3181 (OH, $CONH_2$), 1653 (C=O), 1623 cm^{-1}; MS (EI) m/z (%) 165 (3) $[M]^+$, 147 (27), 146 (54), 129 (87), 106 (100), 105 (99), 77 (81); Anal. Calcd for $C_9H_{11}NO_2$: C, 65.44; H, 6.71; N, 8.48. Found: C, 65.40; H, 6.68; N, 8.47.

4.2.2. 3-Hydroxy-3-phenylpropionic acid 3a.[34] Mp 94–95 °C; $[\alpha]_D^{25} = 0$ (c 1.40, $CHCl_3$); ee 4.0% (chiral HPLC analysis of the corresponding benzyl ester using a Chiralcel OD column with a mixture of hexane and 2-propanol (9:1) at a flow rate 0.8 mL/min as the mobile phase, oven temperature 25 °C, $t_{minor} = 17.5$ min, $t_{major} = 21.4$ min); ^1H NMR (300 MHz, $CDCl_3$) δ 7.29–7.38 (5H, m), 5.16 (1H, dd, $J = 8.9$, 4.0 Hz), 4.91 (2H, br), 2.85 (1H, dd, $J = 16.5$, 8.9 Hz), 2.76 (1H, dd, $J = 1.65$, 4.0 Hz); IR (KBr) ν 3291 (OH), 3069–2542 (COOH), 1699 cm^{-1} (C=O); MS (EI) m/z (%) 166 (2) $[M]^+$, 147 (20), 129 (17), 104 (100); Anal. Calcd for $C_9H_{10}O_3$: C, 65.05; H, 6.07. Found: C, 65.11; H, 6.12.

4.2.3. 3-Benzyloxy-3-phenylpropionamide 2b. Mp 104–105 °C; $[\alpha]_D^{25} = 0$ (c 2.50, $CHCl_3$); ee 2.4% (chiral HPLC analysis using a Chiralcel OD column with a mixture of hexane and 2-propanol (9:1) at a flow rate 0.8 mL/min as the mobile phase, oven temperature 25 °C, $t_{minor} = 32.7$ min, $t_{major} = 36.3$ min); ^1H NMR (300 MHz, $CDCl_3$) δ 7.25–7.42 (10H, m), 6.15 (1H, br), 5.62 (1H, br), 4.82 (1H, dd, $J = 9.5$, 3.5 Hz), 4.46 (1H, d, $J = 11.3$ Hz), 4.32 (1H, d, $J = 11.3$ Hz), 2.76 (1H, dd, $J = 14.9$, 9.5 Hz), 2.50 (1H, dd, $J = 14.9$, 3.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.0, 140.7, 137.7, 128.8, 128.5, 128.2, 127.94, 127.89, 126.5, 78.4, 71.0, 45.2 ppm; IR (KBr) ν 3331, 3168 ($CONH_2$), 1662 (C=O), 1641 cm^{-1}; MS (EI) m/z (%) 237 (2) $[M-18]^+$, 197 (2), 180 (2), 179 (2), 164 (6), 149 (34), 147 (34), 146 (80), 131 (29), 129 (100); Anal. Calcd for $C_{16}H_{17}NO_2$: C, 75.27; H, 6.71; N, 5.49. Found: C, 75.01; H, 6.70; N, 5.53.

4.2.4. (R^*)-3-Allyloxy-3-phenylpropionamide 2c. Mp 69–70 °C; $[\alpha]_D^{25} = 0$ (c 1.50, $CHCl_3$); ee 40.0% (chiral HPLC analysis using a Chiralcel OJH column with a mixture of hexane and 2-propanol (9:1) at a flow rate 0.5 mL/min as the mobile phase, oven temperature 25 °C, $t_{minor} = 22.4$ min, $t_{major} = 26.5$ min); ^1H NMR (300 MHz, $CDCl_3$) δ 7.26–7.39 (5H, m), 6.25 (1H, br), 5.85–5.96 (1H, m), 5.46 (1H, br), 5.16–5.26 (2H, m), 4.76 (1H, dd, $J = 9.6$, 3.4 Hz), 3.93–4.02 (1H, m), 3.80–3.86 (1H, m), 2.74 (1H, dd, $J = 14.9$, 9.6 Hz), 2.44 (1H, dd, $J = 14.9$, 3.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.9, 140.8, 134.2, 128.7, 128.1, 126.4, 117.3, 78.1, 69.7, 45.1 ppm; IR (KBr) ν 3403, 3201 ($CONH_2$), 1654 cm^{-1} (C=O); MS (EI) m/z (%) 205 (2) $[M]^+$, 164 (77), 147 (73), 146 (39), 129 (100), 105 (71); Anal. Calcd for $C_{12}H_{15}NO_2$: C, 70.22; H, 7.37; N, 6.82. Found: C, 70.35; H, 7.39; N, 6.83.

4.2.5. (S^*)-3-Allyloxy-3-phenylpropionic acid 3c. Mp 66–67 °C; $[\alpha]_D^{25} = 0$ (c 2.00, $CHCl_3$); ee 29.4% (chiral HPLC analysis of the corresponding methyl ester using a Chiralcel OD column with a mixture of hexane and 2-propanol (9:1) at a flow rate 0.8 mL/min as the mobile phase, oven temperature 25 °C, $t_{major} = 5.8$ min, $t_{minor} = 9.0$ min); ^1H NMR (300 MHz, $CDCl_3$) δ 7.31–7.40 (5H, m), 5.82–5.99 (1H, m), 5.15–5.26 (2H, m), 4.81 (1H, dd, $J = 9.3$, 4.4 Hz), 3.91–4.02 (1H, m), 3.79–3.84 (1H, m), 2.88 (1H, dd, $J = 15.7$, 9.3 Hz), 2.66 (1H, dd, $J = 15.7$, 4.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.9, 140.3, 134.3, 128.7, 128.2, 126.6, 117.3, 77.2, 69.7, 43.3 ppm; IR (KBr) ν 3068–2577 (COOH), 1706 cm^{-1} (C=O); MS (EI) m/z (%) 206 (2) $[M]^+$, 165 (6), 150 (9), 147 (12), 131 (3), 104 (100); Anal. Calcd for $C_{12}H_{14}O_3$: C, 69.88; H, 6.84. Found: C, 69.94; H, 6.88.

4.2.6. (R)-3-Methoxy-3-phenylpropionamide 2d.[24] Mp 69–71 °C; $[\alpha]_D^{25} = +111.9$ (c 3.20, $CHCl_3$); ee >99% (chiral HPLC analysis using a Chiralcel OD column with a mixture of hexane and 2-propanol (9:1) at a flow rate 0.8 mL/min as the mobile phase, oven temperature 25 °C, $t_{major} = 16.7$ min, $t_{minor} = 20.7$ min); ^1H NMR (300 MHz, $CDCl_3$) δ 7.27–7.39 (5H, m), 6.25 (1H, br), 5.84 (1H, br), 4.59 (1H, dd, $J = 9.6$, 3.4 Hz), 3.25 (3H, s), 2.70 (1H, dd, $J = 14.9$, 9.6 Hz), 2.46 (1H, dd, $J = 14.9$, 3.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.2, 140.6, 128.7, 128.1, 126.4, 80.4, 56.8, 45.0 ppm; IR (KBr) ν 3369, 3195 ($CONH_2$), 1671 cm^{-1} (C=O); MS (EI) m/z (%) 179 (3) $[M]^+$, 164 (26), 147 (25), 146 (33), 129 (100), 121 (47), 103 (31), 102 (30); Anal. Calcd for $C_{10}H_{13}NO_2$: C, 67.02; H, 7.31; N, 7.82. Found: C, 66.99; H, 7.43; N, 7.75.

4.2.7. (S)-3-Methoxy-3-phenylpropionic acid 3d.[24] Mp 65–66 °C; $[\alpha]_D^{25} = -41.5$ (c 1.83, $CHCl_3$); ee 64.5% (chiral HPLC analysis using a Chiralcel OD column with a mixture of hexane and 2-propanol (9:1) and 0.1% HOAc at a flow rate 0.8 mL/min as the mobile phase, oven temperature 25 °C, $t_{major} = 8.0$ min, $t_{minor} = 10.6$ min) {lit.[24] $[\alpha] = -49.3$ (c 0.3, $CHCl_3$)}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.29–7.40 (5H, m), 4.63 (1H, dd, $J = 9.4$, 4.3 Hz), 3.25 (3H, s), 2.84 (1H, dd, $J = 15.6$, 9.4 Hz), 2.64 (1H, dd, $J = 15.6$, 4.3 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.2, 140.1, 128.7, 128.2, 126.6, 79.8, 56.8, 43.2 ppm; IR (KBr) ν 3063–2582 (COOH), 1710 (C=O), 1650 cm^{-1}; MS (EI) m/z (%) 180 (4) $[M]^+$, 165 (4), 148 (5), 147 (7), 131 (5), 121 (63), 104 (100), 103 (77); Anal. Calcd for $C_{10}H_{12}O_3$: C, 66.65; H, 6.71. Found: C, 66.73; H, 6.94.

4.2.8. (S)-2-Hydroxymethyl-3-phenylpropionamide 5a. Mp 127–130 °C; $[\alpha]_D^{25} = -31.3$ (c 6.08, EtOH); ee 65.4% (chiral HPLC analysis using a Chiralcel AD column with a mixture of hexane and 2-propanol (85:15) at a flow rate 0.6 mL/min as the mobile phase, oven temperature 25 °C, $t_{major} = 10.2$ min, $t_{minor} = 12.6$ min); ^1H NMR (300 MHz, DMSO-d_6) δ 7.14–7.43 (6H, m), 6.76 (1H, br), 4.73 (1H, t, $J = 5.1$ Hz), 3.35–3.56 (2H, m), 2.51–2.73 (3H, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ 175.2, 140.1, 128.7, 128.1, 125.8, 62.4, 50.2, 34.4 ppm; IR (KBr) ν 3385 (OH), 3221 ($CONH_2$), 1690 (C=O), 1641 cm^{-1}; MS (EI) m/z (%) 179 (12) $[M]^+$, 161 (71), 148 (100), 131 (64), 117 (54), 91 (63); Anal. Calcd for $C_{10}H_{13}NO_2$: C, 67.02; H, 7.31; N, 7.82. Found: C, 66.84; H, 7.30; N, 7.74.

4.2.9. (R)-2-Hydroxymethyl-3-phenylpropionic acid 6a.[26] Mp 65–66 °C; $[\alpha]_D^{25} = +13.5$ (c 3.10, $CHCl_3$); ee 86.4% (chiral HPLC analysis of the corresponding methyl ester using a Chiralcel ODH column with a mixture of hexane and 2-propanol (95:5) at a flow rate 0.8 mL/min as the mobile

phase, oven temperature 20 °C, $t_{major} = 21.2$ min, $t_{minor} = 23.3$ min) {lit.[26] (*R*)-2-hydroxymethyl-3-phenylpropionic acid, $[\alpha]_D^{25} = +15.9$ (*c* 1.14, $CHCl_3$)}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.25–7.35 (5H, m), 6.96 (1H, br), 3.71–3.84 (2H, m), 2.84–3.14 (3H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.8, 138.2, 129.0, 128.6, 126.7, 61.9, 48.9, 34.0 ppm; IR (KBr) *v* 3341 (OH), 3107–2519 (COOH), 1698 cm^{-1} (C=O); MS (EI) *m/z* (%) 180 (2) $[M]^+$, 162 (92), 144 (16), 131 (28), 117 (100), 91 (56); Anal. Calcd for $C_{10}H_{12}O_3$: C, 66.65; H, 6.71. Found: C, 66.67; H, 6.68.

4.2.10. (*S)-2-Benzyloxymethyl-3-phenylpropionamide 5c.** Mp 80–81 °C; $[\alpha]_D^{25} = -10.0$ (*c* 2.39, $CHCl_3$); ee 68.4% (chiral HPLC analysis using a Chiralcel ADH column with a mixture of hexane and 2-propanol (9:1) at a flow rate 0.5 mL/min as the mobile phase, oven temperature 25 °C, $t_{minor} = 22.4$ min, $t_{major} = 24.6$ min); ^1H NMR (300 MHz, $CDCl_3$) δ 7.10–7.27 (10H, m), 6.11 (1H, br), 5.62 (1H, br), 4.41 (2H, s), 3.49 (2H, d, $J = 5.9$ Hz), 2.94 (1H, dd, $J = 13.2$, 6.7 Hz), 2.60–2.76 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.4, 139.0, 137.6, 129.0, 128.5 (4C), 127.9, 127.8, 126.5, 73.5, 69.8, 48.7, 34.9 ppm; IR (KBr) *v* 3376, 3197 ($CONH_2$), 1655 (C=O), 1641, 1620 cm^{-1}; MS (EI) *m/z* (%) 269 (5) $[M]^+$, 251 (5), 148 (70), 131 (24), 117 (51), 91 (100); Anal. Calcd for $C_{17}H_{19}NO_2$: C, 75.81; H, 7.11; N, 5.20. Found: C, 75.75; H, 7.13; N, 5.33.

4.2.11. (*S)-2-Allyloxymethyl-3-phenylpropionamide 5d.** Mp 46–48 °C; $[\alpha]_D^{25} = +2.8$ (*c* 4.27, $CHCl_3$); ee 57.6% (chiral HPLC analysis using a Chiralcel ADH column with a mixture of hexane and 2-propanol (9:1) at a flow rate 0.5 mL/min as the mobile phase, oven temperature 25 °C, $t_{major} = 17.9$ min, $t_{minor} = 21.9$ min); ^1H NMR (300 MHz, $CDCl_3$) δ 7.18–7.30 (5H, m), 6.28 (1H, br), 6.04 (1H, br), 5.80–5.93 (1H, m), 5.15–5.28 (2H, m), 3.94–4.04 (2H, m), 3.47–3.52 (2H, m), 2.99–3.05 (1H, m), 2.68–2.82 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.6, 139.1, 134.2, 129.0, 128.5, 126.4, 117.4, 72.2, 69.8, 48.6, 34.9 ppm; IR (KBr) *v* 3341, 3199 ($CONH_2$), 1667 cm^{-1} (C=O); MS (EI) *m/z* (%) 219 (7) $[M]^+$, 161 (32), 148 (100), 131 (39), 117 (52), 91 (24); Anal. Calcd for $C_{13}H_{17}NO_2$: C, 71.21; H, 7.81; N, 6.39. Found: C, 71.47; H, 7.89; N, 6.59.

4.2.12. (*R)-2-Allyloxymethyl-3-phenylpropionic acid 6d.** Oil; $[\alpha]_D^{25} = 0$ (*c* 3.10, $CHCl_3$); ee 54.6% (chiral HPLC analysis using a Chiralcel ADH column with a mixture of hexane and 2-propanol (9:1) at a flow rate 0.5 mL/min as the mobile phase, oven temperature 25 °C, $t_{minor} = 12.9$ min, $t_{major} = 16.0$ min); ^1H NMR (300 MHz, $CDCl_3$) δ 10.85 (1H, br), 7.22–7.35 (5H, m), 5.75–6.00 (1H, m), 5.20–5.32 (2H, m), 4.01 (2H, d, $J = 5.5$ Hz), 3.56–3.66 (2H, m), 2.88–3.11 (3H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.5, 138.5, 134.3, 129.0, 128.5, 126.6, 117.3, 72.2, 69.3, 47.5, 34.3 ppm; IR (KBr) *v* 3091–2600 (COOH), 1710 cm^{-1} (C=O); MS (EI) *m/z* (%) 220 (4) $[M]^+$, 162 (100), 144 (13), 131 (30), 117 (55), 91 (23); Anal. Calcd for $C_{13}H_{16}O_3$: C, 70.89; H, 7.32. Found: C, 70.81; H, 7.33.

4.2.13. (*S*)-2-Methoxymethyl-3-phenylpropionamide 5e. Mp 70–72 °C; $[\alpha]_D^{25} = -9.7$ (*c* 3.91, $CHCl_3$); ee 96.2% (chiral HPLC analysis using a Chiralcel AD column with a mixture of hexane and 2-propanol (9:1) at a flow rate 0.8 mL/min as the mobile phase, oven temperature 25 °C, $t_{major} = 10.2$ min, $t_{minor} = 13.9$ min); ^1H NMR (300 MHz, $CDCl_3$) δ 7.19–7.32 (5H, m), 6.12 (1H, br), 5.66 (1H, br), 3.45 (2H, d, $J = 5.6$ Hz), 3.33 (3H, s), 3.03 (1H, dd, $J = 13.4$, 6.9 Hz), 2.62–2.81 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 176.2, 139.1, 129.0, 128.5, 126.4, 72.2, 59.0, 48.7, 34.8 ppm; IR (KBr) *v* 3329, 3194 ($CONH_2$), 1666 cm^{-1} (C=O); MS (EI) *m/z* (%) 193 (14) $[M]^+$, 161 (21), 148 (100), 131 (75), 117 (36), 91 (18); Anal. Calcd for $C_{11}H_{15}NO_2$: C, 68.37; H, 7.82; N, 7.25. Found: C, 68.39; H, 7.97; N, 7.33.

4.2.14. (*R*)-2-Methoxymethyl-3-phenylpropionic acid 6e[27]. Oil; $[\alpha]_D^{25} = +4.0$ (*c* 6.74, cyclohexane); $[\alpha]_D^{25} = +7.3$ (*c* 5.74, $CHCl_3$); ee 96.2% (chiral HPLC analysis of the corresponding methyl ester using a Chiralcel OJH column with a mixture of hexane and 2-propanol (9:1) at a flow rate 0.5 mL/min as the mobile phase, oven temperature 25 °C, $t_{major} = 17.4$ min, $t_{minor} = 21.0$ min) {lit.[27] (*S*)-2-methoxymethyl-3-phenylpropionic acid, $[\alpha]_D^{25} = -4$ (*c* 1.05, cyclohexane)}; ^1H NMR (300 MHz, $CDCl_3$) δ 11.23 (1H, br), 7.18–7.31 (5H, m), 3.47–3.56 (2H, m), 3.33 (3H, s), 2.81–3.05 (3H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.6, 138.4, 129.0, 128.5, 126.6, 71.9, 59.0, 47.4, 34.2 ppm; IR (KBr) *v* 3084–2572 (COOH), 1740, 1710 (C=O) cm^{-1}; MS (EI) *m/z* (%) 194 (2) $[M]^+$, 162 (100), 148 (14), 144 (19), 131 (23), 117 (78), 91 (33); Anal. Calcd for $C_{11}H_{14}O_3$: C, 68.02; H, 7.27. Found: C, 67.64; H, 7.30.

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