JOC*Note*

Practical and Convenient Enzymatic Synthesis of Enantiopure α-Amino Acids and Amides

Mei-Xiang Wang* and Shuang-Jun Lin

Center for Molecular Science, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, China

mxwang@infoc3.icas.ac.cn

Received February 19, 2002

Abstract: Catalyzed by the nitrile hydratase and the amidease in *Rhodococcus* sp. AJ270 cells under very mild conditions, a number of α-aryl- and α-alkyl-substituted DL-glycine nitriles **1** rapidly underwent a highly enantioselective hydrolysis to afford D-(−)-α-amino acid amides **2** and L-(+)-α-amino acids **3** in high yields with excellent enantiomeric excesses in most cases. The overall enantioselectivity of the biotransformations of nitriles originated from the combined effects of a high L-enantioselective amidase and a low enantioselective nitrile hydratase. The influence of the substrates on both reaction efficiency and enantioselectivity was also discussed in terms of steric and electronic effects. Coupled with chemical hydrolysis of D-(−)-α-phenylglycine amide, biotransformation of DL-phenylglycine nitrile was applied in practical scale to produce both D- and L-phenylglycines in high optical purity.

Biotransformations of nitriles,[1] through either a direct nitrilase-catalyzed conversion of a nitrile to a carboxylic acid[2] or a nitrile hydratase-catalyzed hydration of a nitrile followed by the hydrolysis of amide to acid by the action of the amidase,[3] have been demonstrated as being unique and environmentally benign methods for the synthesis of chiral carboxylic acids and their amide derivatives because of the excellent selectivity and very mild reaction conditions. Our earlier work has shown that *Rhodococcus* sp. AJ270, a robust nitrile hydratase/amidase-containing microorganism,[4] was able to hydrolyze a wide range of structurally diverse mono-[5] and dinitriles[6] with excellent chemo- and regioselectivieties. Very recently, we have found that *Rhodococcus* sp. AJ270 could efficiently and enantioselectively catalyze the hydrolysis of a number of racemic α-substituted phenylacetonitriles[7] and 2-arylcyclopropanecarbonitriles[8] to produce the corresponding enantiopure carboxylic acids and amides in high yields. Our continuous interest in understanding reaction scope and applications of both nitrile hydratase and amidase involved in *Rhodococcus* AJ270 and in synthesizing enantiopure amino acid and amide derivatives[9] led us to undertake the study of biotransformation of α-amino nitriles.[10]

At the outset of our project, few examples of enantioselective biotransformations of α-amino nitriles had been reported. The nitrilase of *Rhodococcus rhodochrous* PA-34[11] and a nitrilase-containing *Acinetobacter* sp. culture[12] had the ability to convert several aliphatic DL-α-amino nitriles into L-amino acids in moderate to high enantiomeric excesses, while the nitrilase-catalyzed hydrolysis of DL-phenylglycine nitrile with *Aspergillus* furmigatus[13] had been shown to form L-phenylglycine in 80% ee. Quite recently, Sheldon and co-workers[14] screened a microbial cell able to catalyze the enantioselective hydrolysis of DL-phenylglycine nitrile to give L-acid and D-amide with high ee.

Considering the optimal working pH for nitrile hydratase of *Rhodococcus* sp. AJ270 and to prevent the decomposition of α-amino nitriles in aqueous solution, the biotransformation was conducted in a phosphate buffer with pH 7.62. As described in Table 1, *Rhodococcus* sp. AJ270 catalyzed effectively the biotransformations of a number of racemic arylglycine nitriles **1a**−**h** and alkylglycine nitriles **1i**−**k** to give the corresponding optically active D-(−)-glycine amide **2** and L-(+)-glycine **3** derivatives (Scheme 1). The reaction rate and enantiomeric excesses, however, were dependent upon both the electronic and steric effects of the substituent. In the case of aromatic α-amino nitriles, the hydrolysis proceeded rapidly (within 4 h) under identical conditions except 2-methyl-substituted substrate **1e** took 12 h to transform its cyano function group into the amide **2e** and then into the acid **3e** (Entry 7). Good to excellent enantiocontrol was obtained for almost all aromatic α-amino nitriles and therefore some enantiopure D-(−)-glycine amides such as **2b**−**d**,**f** and L-(+)-glycines such as **3d**,**e**,**g** were produced in very high yield. Attempts were made to improve the enantioselectivity of the biotransformation of phenylglycine nitrile **1a** by altering the buffer pH from 7.62 to

---

[1] For recent reviews, see: (a) Sugai, T.; Yamazaki, T.; Yokoyama, M.; Ohta, H. *Biosci. Biotechnol. Biochem*. **1997**, *61*, 1419. (b) Crosby, J.; Moiliet, J.; Parratt, J. S.; Turner, N. J. *J. Chem. Soc., Perkin Trans. 1* **1994**, 1679.

[2] (a) Harper, D. B. *Biochem. Soc. Trans*. **1976**, *4*, 502. (b) Harper, D. B. *Biochem. J*. **1977**, *165*, 309. (c) Kobayashi, M.; Shimizu, S. *FEMS Microbiol. Lett*. **1994**, *120*, 217.

[3] (a) Asano, Y.; Tani, Y.; Yamada, H. *Agric. Biol. Chem*. **1980**, *44*, 2251. (b) Asano, Y.; Fujishiro, K.; Tani, Y.; Yamada, H. *Agric. Biol. Chem*. **1982**, *46*, 1165. (c) Asano, Y.; Tachibana, M.; Tani, Y.; Yamada, H. *Agric. Biol. Chem*. **1982**, *46*, 1175.

[4] Blakey, A. J.; Colby, J.; Williams, E.; O'Reilly, C. *FEMS Microbiol. Lett*. **1995**, *129*, 57.

[5] (a) Meth-Cohn, O.; Wang, M.-X. *Tetrahedron Lett*. **1995**, *36*, 9561. (b) Meth-Cohn, O.; Wang, M.-X. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1099.

[6] (a) Meth-Cohn, O.; Wang, M.-X. *Chem. Commun*. **1997**, 1041. (b) Meth-Cohn, O.; Wang, M.-X. *J. Chem. Soc., Perkin Trans. 1* **1997**, 3197.

[7] (a) Wang, M.-X.; Lu, G.; Ji, G. J.; Huang, Z.-T.; Meth-Cohn, O.; Colby, J. *Tetrahedron: Asymmetry* **2000**, *11*, 1123. (b) Wang, M.-X.; Li, J.-J.; Ji, G.-J.; Li, J.-S. *J. Mol. Catal. B: Enzym*. **2001**, *14*, 77.

[8] Wang, M.-X.; Feng, G.-Q. *Tetrahedron Lett*. **2000**, *41*, 6501.

[9] For reviews of chemical and enzymatic syntheses, see: (a) Kochetkov K.; Belikov, V. *Russ. Chem. Rev*. **1987**, *56*, 1045. (b) Williams, R. M. *Synthesis of Optically Active* α*-Amino Acids*; Pergamon: Oxford, UK, 1989. (c) Williams, R. M.; Hexdrix, J. A. *Chem. Rev*. **1992**, *92*, 889. (d) Duthaler, R. *Tetrahedron*, **1994**, *50*, 1539. (e) Calms, M.; Daunis, J. *Amino Acids* **1999**, *16*, 215. (f) Miyazawa, T. *Amino Acids* **1999**, *16*, 191. (g) *Enzyme Catalyst in Organic Synthesis*; Drauz, K., Waldmann, H., Eds.; VCH: Weinheim, Germany, 1995. (h) Wong, C.-H.; Whitesides, G. M. *Enzymes in Synthetic Organic Chemistry*; Pergamon: New York, 1994. (i) *Preparative Biotransformation: Whole Cell and Isolated Enzymes in Organic Chemistry*; Roberts, S. M., Ed.; Wiley: New York, 1993. (j) *Stereoselective Biocatalysis*, Patel, R. N., Ed.; Marcel Dekker: New York, 2000.

[10] Part of this work has appeared as a preliminary communication: Wang, M.-X.; Lin, S.-J *Tetrahedron Lett*. **2001**, *42*, 6925.

[11] Bhalla, T. C.; Miura, A.; Wakamoto, A.; Furuhashi, K. *Appl. Microbiol. Biotechnol*. **1992**, *37*, 184.

[12] Macdam, A. M.; Knowles, C. J. *Biotechnol. Lett*. **1985**, *7*, 865.

[13] Choi, S. Y.; Goo, Y. M. *Arch. Pharm. Res*. **1986**, *9*, 45.

[14] Wegman, M. A.; Heinemann, U.; van Rantuijk, F.; Stolz, A.; Sheldon, R. A. *J. Mol. Catal. B*: *Enzym*. **2001**, *11*, 249.

10.1021/jo0256282 CCC: $22.00 © 2002 American Chemical Society

Published on Web 07/30/2002

TABLE 1. Enantioselective Biotransformations of DL-α-Amino Nitriles 1

				amide **2**		acid **3**	
entry	substrate **1**	R	pH; time (h)[a]	yield[b] (%)	ee[c] (%)	yield[b] (%)	ee[c] (%)
1	**1a**	C_6H_5	pH 7.62; 2.5	49	74	47	93
2	**1a**	C_6H_5	pH 7.13; 8	43	>99	52	>99
3	**1a**	C_6H_5	pH 7.13; 25[d]			93	<5
4	**1b**	4-ClC_6H_4	pH 7.62; 4	42	>99	52	97
5	**1c**	3-ClC_6H_4	pH 7.62; 2.7	48	>99	50	95
6	**1d**	4-MeC_6H_4	pH 7.62; 3	42	86	50	>99
7	**1e**	2-MeC_6H_4	pH 7.62; 12	45	80	42	>99
8	**1f**	4-$MeOC_6H_4$	pH 7.62; 1.5	45	>99	51	96
9	**1g**	3-$MeOC_6H_4$	pH 7.62; 1.5	47	77	46	>99
10	**1h**	3,4-$OCH_2OC_6H_3$	pH 7.62; 2.2	39	>99	48	99
11	**1i**	cyclohexyl	pH 7.62; 21[e]	45	88[f]	47	>99[f]
12	**1j**	$C_6H_5CH_2$	pH 7.62; 0.6[d]	17	96	81	13
13	**1j**	$C_6H_5CH_2$	pH 7.62; 0.7[d,e]	48	77	42	51
14	**1k**	$(CH_3)_2CH$	pH 7.62; 1.2[e]	48	22[f]	47	34[f]

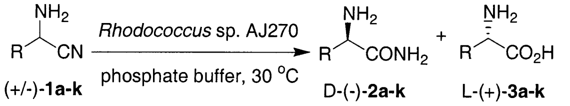
[a] Substrate (2 mmol) was incubated at 30 °C with *Rhodococcus* sp. AJ270 cells (2 g wet weight) in phosphate buffer (0.1 M, 50 mL). The reaction conditions were not optimized. [b] Isolated yield. [c] Determined by HPLC analysis. [d] 1 mmol of the substrate was used. [e] Biotransformations were performed at 20 °C. [f] Determined by HPLC analysis of the corresponding *N*-benzoylated derivatives.

TABLE 2. Kinetic Resolution of DL-α-amino Acid Amides 2

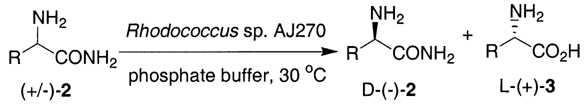
				amide **2**		acid **3**	
entry	substrate **2**	R	pH; time (h)[a]	yield[b] (%)	ee[c] (%)	yield[b] (%)	ee[c] (%)
1	**2a**	C_6H_5	pH 7.0; 2	41	84	51	85
2	**2e**	2-MeC_6H_4	pH 7.0; 9	45	61	53	60
3	**2f**	4-$MeOC_6H_4$	pH 7.0; 2	46	>99	53	90
4	**2g**	3-$MeOC_6H_4$	pH 7.0; 2	51	94	47	95
5	**2j**	$C_6H_5CH_2$	pH 7.0; 2	43	87	56	60

[a] Substrate (2 mmol) was incubated at 30 °C with *Rhodococcus* sp. AJ270 cells (2 g wet weight) in phosphate buffer (0.1 M, 50 mL). The reaction conditions were not optimized. [b] Isolated yield. [c] Determined by HPLC analysis.

SCHEME 1



SCHEME 2



7.13. Although the reaction proceeded in 8 h, much slower than that at pH 7.62, no detrimental effect such as decomposition of the substrate was observed. The enantioselectivity of the biotransformation was enhanced and both amide **2a** and acid **3a** were obtained in enantiopure forms (entry 2). It should be noted that when a pH of 7.13 was applied to the rest of the nitriles tested, the biotransformation appeared very slow and the nitrile substrates underwent a notorious decomposition to form cyanide which further inhibited the nitrile hydratase.

Under the same reaction conditions, *Rhodococcus* sp. AJ270 displayed much higher enzymatic activities against α-amino nitriles **1i**−**k** derived from alkyl aldehydes, and a very small amount of amide product was isolated from the reaction. This is in sharp contrast to the biotransformation of aromatic α-amino nitrile such as **1a** that took more than 1 day to complete its conversion to acid (entry 3). Only at a lower temperature such as 20 °C did the biotransformation afford an appreciable amount of amide. The enantiocontrol of the reaction of cyclohexylglycine nitrile **1i** was excellent, being comparable to that from the reaction of arylglycine nitriles. Nitriles **1j** and **1k**, however, led to low to moderate enantioselectivity (entries 13 and 14).

To shed further light on the biotransformation of nitriles, a selected set of racemic α-amino acid amides, prepared from the chemical hydration of the nitriles with concentrated H_2SO_4 (98%),[15] was fed to *Rhodococcus* sp. AJ270. The incubation was terminated at 50% conversion of the substrate to give optically active D-(−)-amides **2** and L-(+)-acids **3** with enantiomeric excesses ranging from 60% to >99% (Scheme 2, Table 2), indicating that a high L-enantioselective amidase[16] was involved in the cells. In general, however, the enantiocontrol of the kinetic resolution of α-amino acid amides **2** appeared less effective than that of the biotransformation of nitriles **1**. To prepare enantiopure L-(+)-α-amino acids and their D-(−)-amides, it is apparently advantageous to apply the biotransformation of nitriles rather than of amides in terms of reaction efficiency, enantioselectivity, and easy availability of the starting materials.

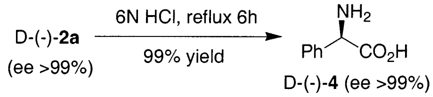
The results of the biotransformation of nitriles and of amides showed that the amidase involved in *Rhodococcus* sp. AJ270 is L- or *S*-enantioselective, while nitrile hydratase probably also exhibits L- or *S*-enantiocontrol but to a lower degree. The enantioselectivity of the overall hydrolysis of a nitrile results from the combined effects of the nitrile hydratase and the amidase, with the latter being a dominant factor. The presence of an aromatic nucleus or of a cyclohexyl moiety adjacent to the cyano or amido function gave excellent enantioselectivity of the

---

(15) Hassan, N. A.; Bayer, E.; Jochims, J. C. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3747.

(16) A D-enantioselective aminopeptidase from *Ochrobactrun anthropi* was reported. Asano, Y.; Nakazawa, A.; Kato, Y.; Kondo, K. *J. Biol. Chem.* **1989**, *264*, 14233.

SCHEME 3



reaction. The further increase of steric bulkiness of the aromatic group by introducing an *o*-methyl group, however, slowed the reaction rate and lowed the enantioselectivity. These results indicated clearly the dependence of effective enantioselection of the amidase and nitrile hydratase upon the steric feature of the substrates.

To test the practical use of biotransformation of nitrile in the preparation of optically active α-amino acids in both enantiomeric forms, we finally conducted a multigram scale reaction employing DL-phenylglycine nitrile **1a** as the substrate. By adding the substrate in aliquot to *Rhodococcus* sp. AJ270 culture in one flask during a 15 h time span, up to 19 mmol of **1a** was transformed into the D-(−)-phenylglycine amide **2a** (yield 46%) and L-(+)-phenylglycine **3a** (yield 53%) with enantiomeric excesses being >99% and 91%, respectively. In a well-controlled reaction, the enantiomeric excess of acid would reach >99%. In refluxing hydrochloric acid (6 N), enantiopure D-(−)-phenylglycine amide **2a** was hydrolyzed to give D-(−)-phenylglycine in almost quantitative yield without racemization (Scheme 3).

In conclusion, we have shown a very convenient and practical method for the syntheses of D-(−)-α-amino acid amides and L-(+)-α-amino acids with high optical purity from enantioselective biotransformations of DL-α-amino nitriles with *Rhodococcus* sp. AJ270 cells under very mild conditions. The overall enantioselection of the biotransformations was determined by the combined effects of a dominantly high L-enantioselective amidase and a low L-enantioselective nitrile hydratase involved in the cell. Coupled with the chemical hydrolysis of amide, this biotransformation process provided efficient syntheses of optically pure arylglycines in both enantiomeric forms from readily available Strecker nitriles. It can also be developed into a valuable synthetic alternative to other nonproteinogenic and nonnaturally occurring amino acids, which is being actively investigated in this laboratory.

Experimental Section[17]

All starting DL-amino nitriles were synthesized following a modified Strecker reaction from the corresponding aldehydes according to the literature.[18] Racemic amino amides and amino acids as standards were prepared by chemical hydrolysis of DL-amino nitriles following the literature methods.[15,19] The configurations of optically active L-amino acids **3** were determined by the comparison of their direction of optical rotation with that of authentic samples, while the configurations of D-amino amides **2** were obtained by correlating optical rotation of their chemically hydrolyzed amino acid products with that of amino acids of known configurations. All enantiomeric excess values were obtained from HPLC analysis with use of Crownpark CR(+) and Chiralcel OD and OJ columns (see Supporting Information).

General Procedure for the Biotransformations of Amino Nitriles and Amino Amides. To an Erlenmeyer flask (100 mL) with a screw cap was added *Rhodococcus* sp. AJ270 cells[6] (2 g wet weight) and potassium phosphate buffer (0.1 M, 50 mL) and the resting cells were activated at 30 or 20 °C for 30 min with orbital shaking. DL-Amino nitrile hydrochlorides **1** (2 mmol) or DL-amino acid amides **2** (2 mmol) were added in one portion to the flask and the mixture was incubated at 30 or 20 ^{o}C with use of an orbital shaker (200 rpm). The reaction, monitored by TLC, 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 concentrated. The products were isolated and purified by chromatography with consecutive use of a cationic exchange resin column (Dowex, 50 × 8), a Sephadex G-25 column, and a reverse phase silica gel column (35−70 μm). All products were characterized by their spectra data and comparison of the melting points and optical rotary power with that of the known compounds, which are listed as follows, or by full characterization.

Enzymatic Hydrolysis of DL-Phenylglycine Nitrile (1a*).* D-(−)-Phenylglycine amide (**2a**) as a white solid: 8 h (43%); $[\alpha]^{25}_{D}$ −108 (*c* 0.5, MeOH); ee >99% (HPLC); mp 126−127 °C; IR (KBr) 3378, 3339, 3269, 1679 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.49 (s, 1H, CON*H*H), 7.20−7.45 (m, 5H, ArH), 7.04 (s, 1H, CONH*H*), 4.29 (s, 1H, CH); MS (EI) *m*/*z* 106 ($[M - CONH_2]^+$, 100%). Anal. Calcd for $C_8H_{10}N_2O$: C, 63.98; H, 6.71; N, 18.65. Found: C, 63.90; H, 6.45; N, 18.30.

L-(+)-Phenylglycine **3a** as white powder: 8 h (52%); $[\alpha]^{25}_{D}$ +141 (*c* 0.95, 1 N HCl) (lit[19] $[\alpha]^{25}_{D}$ −155 (*c* 1.0, 1 N HCl, D-(−)-phenylglycine); ee >99% (HPLC); mp 252 °C (lit.[20] mp 246 °C); IR (KBr) 3130−2611, 1610 cm^{-1}; ^{1}H NMR (D_2O) δ 7.24−7.28 (m, 5H, ArH), 4.96 (s, 1H, CH).

In a multigram scale biotransformation reaction, DL-phenylglycine nitrile **1a** (3.2 g, 19 mmol) was added in 10 portions (~2 mmol each) to the suspension of *Rhodococcus* sp. AJ270 (2 g wet weight) in phosphate buffer (pH 7.70, 40 mL) during a time span of 12 h. Another 30 mL of the culture suspension containing 1 g of cells were also added to the reaction flask at 4.5 and 9 h time intervals. After a total of 15 h of incubation at 30 °C, and workup, the reaction afforded D-(−)-phenylglycine amide **2a** (1.31 g, 46%) with ee >99% and L-(+)-phenylglycine **3a** (1.52 g, 53%) with ee 91%.

Enzymatic Hydrolysis of DL-4-Chlorophenylglycine Nitrile (1b). D-(−)-4-Chlorophenylglycine amide (**2b**) as white powder: 4 h (42%); $[\alpha]^{25}_{D}$ −87 (*c* 0.55, MeOH); ee >99% (HPLC); mp 94 °C; IR (KBr) 3360, 3312, 3290, 1666 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.49 (s, 1H, CON*H*H), 7.40 (d, J = 8.6 Hz, 2H, ArH), 7.35 (d, J = 8.6 Hz, 2H, ArH), 7.06 (s, 1H, CONH*H*), 4.29 (s, 1H, CH); MS (EI) *m*/*z* 142 (32), 140 ($[M - CONH_2]^+$, 100%). Aanl. Calcd for $C_8H_9ClN_2O$: C, 52.04; H, 5.91; N, 15.17. Found: C, 52.15; H, 5.01; N, 15.20.

L-(+)-4-Chlorophenylglycine (**3b**) as white powder: 4 h (52%); $[\alpha]^{25}_{D}$ +129.4 (*c* 0.34, 1 N HCl) [lit.[21] −142 (*c* 0.5, 1 N HCl), D-(−)-4-chlorophenylglycine]; ee 97% (HPLC); mp 229 °C (lit.[20] mp 238 °C); IR (KBr) 2978−2654, 1633 cm^{-1}; ^{1}H NMR (D_2O) δ 7.26 (d, J = 6.9, 2H, ArH), 7.20 (d, J = 6.9, 2H, ArH), 4.96 (s, 1H, CH).

Enzymatic Hydrolysis of DL-3-Chlorophenylglycinenitrile (1c). D-(−)-3-Chlorophenyglycine amide **2c** as white powder: 2.7 h (48%); $[\alpha]^{25}_{D}$ −80 (*c* 0.5, MeOH); ee >99% (HPLC); mp 140 °C; IR (KBr) 3392, 3352, 1685, 1669 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.52 (s, 1H, CON*H*H), 7.46 (s, 1H, ArH), 7.31−7.36 (m, 3H, ArH), 7.08 (s, 1H, CONH*H*), 4.30 (s, 1H, CH); MS (EI) *m*/*z* 142 (32), 140 ($[M - CONH_2]^+$, 100%). Anal. Calcd for $C_8H_9ClN_2O$: C, 52.04; H, 5.91; N, 15.17. Found: C, 52.20; H, 4.96; N, 14.76.

L-(+)-3-Chlorophenylglycine **3c** as white powder: 2.7 h (50%); $[\alpha]^{25}_{D}$ +137 (*c* 0.35, 1 N HCl) [lit.[22] −118 (*c* 1.0, 1 N NH_4OH), D-(−)3-chlorophenylglycine]; ee 95% (HPLC); mp 232 °C (lit.[20] mp 238 °C); IR (KBr) 2628−3058, 1615, 1602 cm^{-1}; ^{1}H NMR (D_2O) δ 6.86−7.02 (M, 4H, ArH), 4.69 (s, 1H, CH).

Enzymatic Hydrolysis of DL-4-Methyphenylglycine Nitrile 1d. D-(−)-4-Methyphenylglycine amide (**2d**) as white powder: 3 h (42%); $[\alpha]^{25}_{D}$ −100 (*c* 0.5, MeOH); ee 86% (HPLC);

---

(17) General experimental information was given in ref 6.

(18) Hanafusa, T.; Ichihara, I.; Ashida, T. *Chem. Lett.* **1987**, 687.

(19) Yokozeki, K.; Mitsugi, K.; Iwagami, H. German Patent, 285245, 1978.

(20) Zhang, Y.-M.; Li, W.-X. *Synth. Commun.* **1988**, *18*, 1685.

(21) Vermier, J. M.; Hegedus, L. S.; Miller, D. B. *J. Org. Chem.* **1992**, *57*, 6914.

(22) Garcia, M. J.; Azerad, R. *Tetrahedron: Asymmetry* **1997**, *8*, 85.

mp 104−105 °C; IR (KBr) 3370, 3309, 1661, 1654 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.40 (s, 1H, CON*H*H), 7.24 (d, *J* = 7.9 Hz, 2H, ArH), 7.08 (d, *J* = 7.9 Hz, 2H, ArH), 6.91 (s, 1H, CONH*H*), 4.21 (s, 1H, CH), 2.25 (s, 3H, CH$_3$); MS (EI) *m/z* 120 ([M − CONH$_2$]$^+$, 100%). Anal. Calcd for C$_9$H$_{12}$N$_2$O: C, 65.83; H, 7.37; N, 17.06. Found: C, 65.50; H, 7.67; N, 17.36.

L-(+)-4-Methyphenylglycine (**3d**) as white powder: 3 h (50%); $[\alpha]^{25}_{\mathrm{D}}$ +175 (*c* 0.9, 1 N HCl)) [lit.[15] $[\alpha]^{23}_{\mathrm{D}}$ +151 (*c* 1.0, 1N HCl); ee >99% (HPLC); mp 247 °C (lit.[20] mp 244 °C); ^{1}H NMR (D$_2$O) δ 7.14 (s, 4H, ArH), 4.95 (s, 1H, CH), 2.14 (s, 3H, CH$_3$).

Enzymatic Hydrolysis of DL-2-Methyphenylglycine Nitrile (1e). D-(−)-2-Methyphenylglycine amide **2e** as white powder: 12 h (45%); $[\alpha]^{25}_{\mathrm{D}}$ −72 (*c* 1.0, MeOH); ee 80% (HPLC); mp 117−118 °C; IR (KBr) 3351, 3274, 1661, 1618 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.39 (s, 1H, CON*H*H), 7.09−7.28 (m, 5H, ArH and CONH*H*), 4.42 (s, 1H, CH), 2.34 (s, 3H, CH$_3$); MS (EI) *m/z* 120 ([M − CONH$_2$]$^+$, 100%). Anal. Calcd for C$_9$H$_{12}$N$_2$O: C, 65.83; H, 7.37; N, 17.06. Found: C, 65.98; H, 7.78; N, 17.27.

L-(+)-2-Methyphenylglycine **3e** as white powder: 12 h (42%); $[\alpha]^{25}_{\mathrm{D}}$ +172 (*c* 0.5, 1 N HCl); ee >99% (HPLC); mp 211 °C; IR (KBr) 2620−3148, 1675, 1632, 1618 cm^{-1}; ^{1}H NMR (D$_2$O) δ 7.23−7.30 (m, 4H, ArH), 4.93 (s, 1H, CH), 2.37 (s, 3H, CH$_3$); MS (EI) *m/z* 120 ([M − CO$_2$H]$^+$, 100%). Anal. Calcd for C$_9$H$_{11}$NO$_2$: C, 65.44; H, 6.71; N, 8.48. Found: C, 65.29; H, 6.67; N, 8.32.

Enzymztic Hydrolysis of DL-4-Methoxyphenylglycine Nitrile (1f). D-(−)-4-Methoxyphenylglycine amide (**2f**) as white powder: 1.5 (45%); $[\alpha]^{25}_{\mathrm{D}}$ −80 (*c* 0.25, MeOH); ee >99% (HPLC); mp 159 °C; IR (KBr) 3404, 3368, 3359, 3205, 1634 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.39 (s, 1H, CON*H*H), 7.27 (d, *J* = 8.5 Hz, 2H, ArH), 6.94 (s, 1H, CONH*H*), 6.84 (d, *J* = 8.5 Hz, 2H, ArH), 4.19 (s, 1H, CH), 3.70 (s, 3H, CH$_3$O); MS (EI) *m/z* 136 ([M − CONH$_2$]$^+$, 100%); MS (FAB) 166 ([M + 1]$^+$). Anal. Calcd for C$_9$H$_{12}$N$_2$O$_2$: C, 59.99; H, 6.71; N, 15.55. Found: C, 59.64; H, 6.86; N, 15.34.

L-(+)-4-Methoxyphenylglycine (**3f**) as white powder: 1.5 h (51%); $[\alpha]^{25}_{\mathrm{D}}$ +153 (*c* 0.9, 1 N HCl) [lit.[21] $[\alpha]^{23}_{\mathrm{D}}$ +156 (*c* 1.0, 3N HCl)]; ee 96% (HPLC); mp 248−249 °C [lit.[15] mp 246 °C]; IR (KBr) 2508−3043, 1615 cm^{-1}; ^{1}H NMR (D$_2$O) δ 7.13 (d, *J* = 8.7 Hz, 2H, ArH), 6.78 (d, *J* = 8.7 Hz, 2H, ArH), 4.87 (s, 1H, CH), 3.56 (s, 3H, CH$_3$O).

Enzymatic Hydrolysis of DL-3-Methoxyphenylglycine Nitrile (1g). D-(−)-3-Methoxyphenylglycine amide (**2g**) as white powder: 1.5 h (47%); $[\alpha]^{25}_{\mathrm{D}}$ −64 (*c* 0.5, MeOH); ee 77% (HPLC); mp 85 °C; IR (KBr) 3382, 3348, 3326, 3284, 1674 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.47 (s, 1H, CON*H*H), 7.21 (t, *J* = 7.7 Hz, 1H, ArH), 7.02 (s, 1H, CONH*H*), 6.97 (s, 1H, ArH), 6.95 (d, *J* = 7.7 Hz, 1H, ArH), 6.79 (d, *J* = 7.7 Hz, 1H, ArH), 4.23 (s, 1H, CH), 3.73 (s, 3H, CH$_3$O); MS (EI) *m/z* 136 ([M − CONH$_2$]$^+$, 100%). Anal. Calcd for C$_9$H$_{12}$N$_2$O$_2$: C, 59.99; H, 6.71; N, 15.55. Found: C, 59.50; H, 6.78; N, 15.45.

L-(+)-3-Methoxyphenylglycine (**3g**) as white powder: 1.5 h (46%); $[\alpha]^{25}_{\mathrm{D}}$ +176 (*c* 0.5, 1 N HCl) (lit.[22] $[\alpha]^{23}_{\mathrm{D}}$ −115 (*c* 1.0, 1 N NH$_4$OH), D-(−)-3-methoxyphenylglycine); ee >99% (HPLC); mp 202 °C; IR (KBr) 2607−3157, 1610 cm^{-1}; ^{1}H NMR (D$_2$O) δ 7.33 (t, *J* = 7.9 Hz, 1H, ArH), 6.95−6.97 (m, 3H, ArH), 4.61 (s, 1H, CH), 3.76 (s, 3H, CH$_3$O); MS (EI) *m/z* 120 ([M − CO$_2$ − NH$_3$]$^+$, 100%). Anal. Calcd for C$_9$H$_{11}$N$_2$O$_2$: C, 59.66; H, 6.12; N, 7.73. Found: C, 59.32; H, 6.21; N, 7.79.

Enzymatic Hydrolysis of DL-3,4-Methylenedioxyphenylglycine Nitrile (1h). D-(−)-3,4-Methylenedioxyphenylglycine amide (**2h**) as a yellowy powder: 2.2 h (39%); $[\alpha]^{25}_{\mathrm{D}}$ −45 (*c* 0.4, MeOH); ee >99% (HPLC); mp 128−129 °C; IR (KBr) 3381, 3309, 3293 (sh), 3013, 1665, 1653 cm^{-1};^{1}H NMR (DMSO-d_6) δ 7.38 (s, 1H, CON*H*H), 6.96 (s, 1H, CONH*H*), 6,93 (s, 1H, ArH), 6.82 (d, *J* = 8.0 Hz, 1H, ArH), 6.79 (d, *J* = 8.0 Hz, 1H, ArH), 5.94 (s, 2H, OCH$_2$O), 4.17 (s, 1H, CH); MS (EI) *m/z* 150 ([M − CONH$_2$]$^+$, 100%). Anal. Calcd for C$_9$H$_{10}$N$_2$O$_3$: C, 55.67; H, 5.19; N, 14.43. Found: C, 55.62; H, 5.36; N, 14.28.

L-(−)-3,4-Methylenedioxyphenylglycine (**3h**) as a yellow powder: 2.2 h (48%); $[\alpha]^{25}_{\mathrm{D}}$ +120 (*c* 0.4, 1 N HCl); ee 99% (HPLC); mp 229 °C; IR (KBr) 2598−3155, 1610 cm^{-1}; ^{1}H NMR (D$_2$O) δ 6.73−6.78 (m, 3H, ArH), 5.78 (s, 2H, OCH$_2$O), 4.88 (s, 1H, CH); MS (EI) *m/z* 195 (M$^+$, 5%), 150 ([M − CO$_2$H]$^+$, 100). Anal. Calcd for C$_9$H$_9$NO$_4$: C, 55.39; H, 4.65; N, 7.18. Found: C, 55.29; H, 4.68; N, 7.11.

Enzymatic Hydrolysis of DL-Cyclohexylglycine Nitrile (1i). D-(−)-Cyclohexylglycine amide **2i** as a white solid: 21 h (45%); $[\alpha]^{25}_{\mathrm{D}}$ −20 (*c* 0.5, MeOH); ee 88% (HPLC analysis of the corresponding *N*-benzoylated compound); mp 145−146 °C; IR (KBr) 3349, 3258 (sh) 3195 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.23 (s, 1H, CON*H*H), 6.88 (s, 1H, CONH*H*), 2.83 (d, *J* = 5.1 Hz, 1H, CH), 1.43−1.67 (m, 6H), 0.91−1.18 (m, 5H); ^{13}C NMR (DMSO-d_6) δ 177.2, 59.8, 41.8, 29.8, 27.5, 26.24, 26.20, 26.0; MS (FAB) *m/z* 156 (M$^+$), 155 ([M − 1]$^+$). Anal. Calcd for C$_8$H$_{16}$N$_2$O: C, 61.51; H, 10.32; N, 17.93. Found: C, 61.18; H, 10.30; N, 17.74.

L-(+)-Cyclohexylglycine **3i** as a white solid: 21 h (47%); $[\alpha]^{25}_{\mathrm{D}}$ +28 (*c* 0.5, 1 N HCl) [lit.[23] $[\alpha]^{25}_{\mathrm{D}}$ +31.3 (*c* 1.0 1N HCl)]; ee >99% (HPLC analysis of the corresponding *N*-benzoylated compound); mp 255 °C dec (lit.[23] mp >300 °C); IR (KBr) 2611−3020, 1595 (sh), 1584 cm^{-1}; ^{1}H NMR (D$_2$O) δ 3.32 (d, *J* = 5.2, 1H, CH), 0.52−1.16 (m, 11H); MS (FAB) *m/z* 157 (M$^+$), 156 ([M − 1]$^+$). Anal. Calcd for C$_8$H$_{15}$NO$_2$: C, 61.12; H, 9.62; N, 8.91. Found: C, 60.90; H, 9.71; N, 8.91.

Enzymatic Hydrolysis of DL-Phenylalanine Nitrile (1j). D-(−)-Phenylalanine amide (**2j**) as a white powder: 0.7 h (48%); $[\alpha]^{25}_{\mathrm{D}}$ −9 (*c* 1.0, MeOH); ee 77% (HPLC); mp 135−137 °C; IR (KBr) 3338, 3182, 1667 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.16−7.31 (m, 6H, ArH and CON*H*H), 6.96 (s, 1H, CONH*H*), 3.29−3.34 (m, 1H, CH), 2.90 (dd, *J* = 13.3, 5.1 Hz, 1H), 2.58 (dd, *J* = 13.3, 8.4 Hz, 1H); MS (FAB) *m/z* 164 (M$^+$), 163 ([M − 1]$^+$). Anal. Calcd for C$_9$H$_{12}$N$_2$O: C, 65.83; H, 7.37; N, 17.06. Found: C, 65.43; H, 7.53; N, 16.98.

L-Phenylalanine (**3i**) as a white solid: 0.7 h (42%); $[\alpha]^{25}_{\mathrm{D}}$ −2 (*c* 1.0, 1 N HCl) (lit.[2424] $[\alpha]_{\mathrm{D}}^{20}$ +7.07 (*c* 1.0, 1 N HCl), D-(+)-phenylalanine); ee 51% (HPLC); mp 258 °C dec (lit.[24] mp 271 °C dec, dL-phenylalanine); IR (KBr) 2554−3339, 1589 cm^{-1}; ^{1}H NMR (D$_2$O) δ 6.98−7.13 (m, 5H, ArH), 4.04 (dd, *J* = 7.5, 5.9 Hz, 1H, CH), 3.04 (dd, *J* = 14.9, 5.6 Hz, 1H), 2.90 (dd, *J* = 14.9, 7.7 Hz, 1H).

Enzymatic Hydrolysis of DL-Valine Nitrile (1k). D-Valine amide (**2k**) as a white solid: 1.2 h (48%); ee 22% (HPLC analysis of the corresponding *N*-benzoylated compound); mp 80−83 °C; IR (KBr) 3356, 1664, 1607 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.22 (s, 1H, CON*H*H), 6.87 (s, 1H, CONH*H*), 2.82 (d, *J* = 5.0 Hz, 1H, CH), 1.72−1.88 (m, 1H, CH), 0.82 (d, *J* = 6.8 Hz, 3H, CH$_3$), 0.73 (d, *J* = 6.8 Hz, 3H, CH$_3$); MS (FAB) *m/z* 116 (M$^+$), 115 ([M − 1]$^+$). Anal. Calcd for C$_5$H$_{12}$N$_2$O: C, 51.70; H, 10.41; N, 24.12. Found: C, 51.73; H, 10.52; N, 24.02.

L-Valine **3k** as a white solid: 1.2 h (47%); ee 34% (HPLC analysis of the corresponding *N*-benzoylated compound); mp 210 °C dec [lit.[24] mp 298 °C dec, DL-valine]; IR (KBr) 2585−3164, 1595 cm^{-1}; ^{1}H NMR (D$_2$O) δ 3.48 (d, *J* = 4.4 Hz, 1H, CH), 2.13−2.16 (m, 1H), 0.91 (d, *J* = 7.0 Hz, 3H, CH$_3$), 0.85 (d, *J* = 7.0 Hz, 3H, CH$_3$).

Chemical Hydrolysis of D-(−)-Phenylglycine Amide (2a). D-(−)-Phenylglycine amide **2a** (1 mmol, ee>99%) was refluxed in hydrochloric acid solution (6 N) for 2.5 h to give, after the workup as for L-(+)-**3a**, pure D-(−)-phenylglycine **4** in 99% yield. $[\alpha]^{25}_{\mathrm{D}}$ −121 (*c* 0.8, 1 N HCl) (lit.[19] $[\alpha]^{25}_{\mathrm{D}}$ −155 (*c* 1.0, 1 N HCl), D-(−)-phenylglycine); ee >99% (HPLC); mp 239 °C (lit.[20] mp 246 °C).

Acknowledgment. This work was supported by the Major Basic Research Development Program (No. 2000077506), the National Nature Science Foundation of China, and the Chinese Academy of Sciences. M.X.W. thanks O. Meth-Cohn and J. Colby for discussions.

Supporting Information Available: Preparation of racemic α-amino nitrile hydrochlorides **1**, *N*-benzoylation of valine, cyclohexylglycine, and their amides, HPLC analysis of amino acids **3** and amides **2**, and results of biotransformation of racemic α-amino acid amides **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0256282

---

[23] Beller, M.; Eckert, M.; Geissler, H.; Napierski, B.; Rebenstock, H.-P.; Holla, E. W. *Chem.-Eur. J.* **1998**, *4*, 935.

[24] Oversly L. B.; Ingersole, A. M. *J. Am. Chem. Soc.* **1951**, *73*, 3363.