Efficient Biocatalytic Synthesis of Highly Enantiopure α-Alkylated Arylglycines and Amides

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Abstract: A number of racemic α-alkylarylglycine amides including 1-amino-1-carbamoyl-1,2,3,4-tetrahydronaphthalene underwent efficient biocatalytic hydrolysis under very mild conditions to afford the corresponding (*S*)-α-alkylarylglycines and (*R*)-α-alkylarylglycine amides in excellent yields with enantiomeric excesses higher than 99.5%. Both the reaction rate and enantioselectivity of biocatalytic kinetic resolution were strongly dependent upon the nature of the substituent and the substitution pattern on the benzene ring of the substrate. In contrast, no effective biotransformation of the Strecker nitrile derived from acetophenone was observed under the catalysis of a nitrile hydratase/amidase-containing microbial *Rhodococcus* sp. AJ270 whole-cell catalyst. Coupled with the chemical hydrolysis of amide, this biotransformation process provided efficient syntheses of α-substituted arylglycines in both enantiomeric forms from readily available racemic amides.

Keywords: (*S*)-α-alkylarylglycines; (*R*)-α-alkylarylglycine amides; amidase; enzyme catalysis; kinetic resolution; nitrile hydratase

Introduction

The importance of optically active α-substituted α-amino acids and their derivatives in medicinal chemistry and pharmacology, and the challenge of constructing highly functionalized quaternary stereocenters have prompted chemists to establish and develop various approaches to these unusual amino acid targets. Most of the methods reported are based on stereoselective syntheses utilizing chiral auxiliaries[1] or involving a process of self-reproduction of chirality of α-hydrogenamino acid derivatives.[2] Due to the harsh conditions generally required in deprotection steps, the methods are limited to the production of α-substituted α-amino acids without labile substituents. Catalytic asymmetric synthesis of optically active amino acids with a tetrasubstituted carbon center with a nitrogen atom has not been explored until recently. For example, Trost[3] has reported enantiomeric synthesis of γ,δ-unsaturated α-substituted α-amino acids from the asymmetric allylic alkylation reaction of azlactones. γ,δ-Unsaturated α-substituted α-amino acid derivatives have also been prepared in moderate to excellent enantiomeric purities by Ito[4] who conducted the palladium(II)-catalyzed allylic alkylation of α-acetamido-β-ketoesters in the presence of (*R*)-BINAP. *C*-Alkylation of the Schiff bases derived from alanine esters under phase-transfer conditions using a pseudo-enantiomeric cinchonidine-derived quaternary ammonium salt,[5] (4*R*,5*R*)-2,2-dimethyl-α,α,α′,α′-tetraphenyl-1,3-dioxolane-4,5-dimethanol (TADDOL),[6] or enantiopure 2-hydroxy-2′-amino-1,1′-binaphthyl (NOBIN)[7] as catalysts has been demonstrated to form enantiomerically enriched α-substituted α-amino acids but with only moderate enantiomeric excesses. Very recently, Jacobsen[8] has shown a straightforward Strecker synthesis of α-methylarylglycines from enantioselective catalytic addition of HCN to ketoimines employing recyclable Schiff base catalysts, followed by a formylation and hydrolysis sequence. The enantiomeric excess of the α-aminonitrile intermediates varied dramatically from moderate to excellent, however, depending on the structure of the ketoimines. Enzyme-catalyzed kinetic resolution constitutes another useful protocol to produce optically active α-substituted α-amino acids. Hydrolytic enzymes such as acylases, esterases and lipases, for example, have been used to resolve racemic *N*-acylated α-substituted α-amino acids[9] and α-substituted α-amino acid esters,[10] respectively, to yield enantiomerically enriched α,α-dialkylamino acids. It should also be noted that the amidase from *Mycobacterium neoaurum* ATCC 25795 has been shown to catalyze kinetic resolution of α,α-dialkyl-substituted amino acid amides with excellent enantiocontrol while only moderate to good enantioselectivities were obtained for α-methyl- or α-ethylphenylglycine amide substrates.[11]

Biotransformations of nitriles, either through a direct conversion from a nitrile to a carboxylic acid catalyzed

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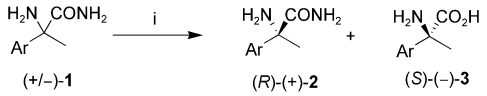
by a nitrilase[12] or through the nitrile hydratase-catalyzed hydration of a nitrile followed by the amide hydrolysis catalyzed by the amidase,[13] are effective and environmentally benign methods for the production of carboxylic acids and their amide derivatives. The microbial hydration of acrylonitrile to acrylamide, for instance, is one of the largest industrial biotransformations in the world.[14] Recent studies[15,16] have demonstrated that biotransformations of nitriles also complement the existing asymmetric chemical and enzymatic synthetic methods for carboxylic acids and their derivatives. 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. Very recently, we[17] have found that the combination of the nitrile hydratase and the amidase in *Rhodococcus* sp. AJ270 microbial cells[18] is a powerful biocatalytic system to transform a variety of racemic α-amino acid nitriles into highly enantiopure D-amino acid amides and L-amino acids. Our interest[16] in understanding of the scope and mechanism of the nitrile hydratase and the amidase, and in the synthesis of optically active α-substituted α-amino acids have led us to investigate biotransformations of α-substituted α-amino acid nitriles and amides. We report herein an efficient and convenient synthesis of highly enantiopure (*S*)-α-arylalanines and their (*R*)-amide derivatives from the kinetic resolution of amides catalyzed by the amidase within *Rhodococcus* sp. AJ270 cells.

Results and Discussion

The *Rhodococcus* sp. AJ270 cell-catalyzed reaction of racemic α-phenylalanine nitrile was first tested. In contrast to the efficient and highly enantioselective reaction of racemic phenylglycine nitrile,[17] however, the α-methylated phenylglycine nitrile analogue did not undergo hydrolysis. Efforts were made to improve conversion of the nitrile by, e.g., varying the pH of the buffered solution, reaction temperature and concentration of the substrate, but all met with failure. Under the various reaction conditions tried, acetophenone was detected, indicating that decomposition of α-phenylalanine nitrile occurred. The inefficient hydration of the nitrile in this case is most probably due to the inhibition of the nitrile hydratase, a presumed cobalt- or iron-containing enzyme,[13b] by the cyanide ion generated from the decomposition of α-phenylalanine nitrile.

Having considered the high enantioselectivity of the amidase within *Rhodococcus* sp. AJ270 and the stability of α-substituted α-amino acid amides,[16] we turned our attention to kinetic resolution of racemic α-phenylalanine amide **1a**. As illustrated in Table 1, *Rhodococcus* sp. AJ270 readily transformed racemic **1a** into the optically active (*R*)-(+)-amide **2a** and (*S*)-(−)-acid **3a**[11a] with good enantioselectivity. With the progress



Scheme 1. Biocatalytic kinetic resolution of racemic α-arylalanine amides **1**.

Table 1. Amidase-catalyzed kinetic resolution of racemic α-arylalanine amides.

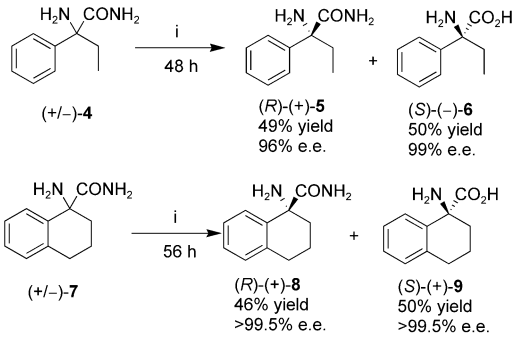
Entry	1	Ar	Time [h][a]	2		3	
				Yield [%][b]	% ee[c]	Yield [%][b]	% ee[c]
1	**1a**	C_6H_5	15	60	40	32	91
2	**1a**	C_6H_5	17.5	46	95	50	84
3	**1a**	C_6H_5	25	37	>99.5	59	80
4	**1b**	4-$MeOC_6H_4$	36	60	31	33	93
5	**1b**	4-$MeOC_6H_4$	48	37	95	61	63
6	**1c**	3-$MeOC_6H_4$	62	49	94	50	92
7	**1d**	4-MeC_6H_4	55	50	82	47	89
8	**1e**	3-MeC_6H_4	56	46	88	50	73
9	**1f**	2-MeC_6H_4	14	46	99	49	90
10	**1g**	4-FC_6H_4	18	45	>99.5	52	76
11	**1h**	4-ClC_6H_4	52	41	99.5	51	78
12	**1i**	4-BrC_6H_4	40	56	61	40	94
13	**1i**	4-BrC_6H_4	53	48	>99.5	41	93
14	**1i**	4-BrC_6H_4	66	41	>99.5	52	90

[a] The substrate **1** (1 mmol) was incubated with *Rhodococcus* sp. AJ270 whole cells (2 g wet weight) in phosphate buffer (0.1 M, pH 7.0) at 30 °C.
[b] Isolated yield.
[c] Enantiomeric excess values were determined by chiral HPLC analysis.

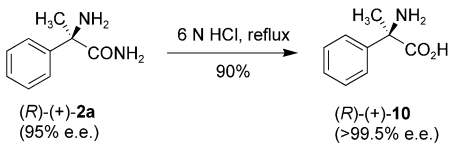
of reaction, the enantiomeric excess value of **2a** increased while that of **3a** decreased (entries 1–3), indicating clearly a kinetic resolution process. Encouraged by these results, we then extended this reaction to other racemic α-arylalanine amide substrates. In order to examine the influence of structure of the substrates on the biocatalytic kinetic resolution, a number of amino acid amides **1** bearing a different substituent at different substitution positions on the benzene ring were prepared and subjected to incubation with *Rhodococcus* sp. AJ270. The outcomes summarized in Table 1 have shown that the amidase accepted all racemic α-arylalanine amides tested as good substrates. The reaction efficiency, however, depended strongly on the nature of the substituent and the substitution pattern on the benzene ring. For example, while α-4-fluorophenylanaline amide **1 g** underwent equally efficient biohydrolysis as the parent amide **1a** (entries 2 and 10), introduction of other substituents into either the 4- or 3-position of the benzene ring generally resulted in the slow hydrolysis, and the time for *ca.* 50% conversion increased from less than 20 h for **1a** (entry 2) to 50–60 h for **1c–e** and **1h–i** (entries 6–8, 11 and 12). Another exception was the rapid hydrolysis of α-2-methylphenylalanine amide **1f**, which reached *ca.* 50% conversion within only 14 h. Although the solubility of the substrate may partly contribute to affect reaction rate, the influence of the steric effect of the substituent appeared obvious. It should also be noted that the hydrolysis of all quaternary substituted α-arylalanine amides **1** proceeded slower than their tertiary substituted α-amino arylglycine amide counterparts[17] due most probably to the steric bulkiness of the quaternary carbon-containing molecules. In generally, the larger the substituted aryl group or substrate molecule, the slower was the hydrolysis reaction. On the other hand, a scrutiny of the results in Table 1 revealed a good to excellent *S*-enantioselectivity of the amidase depending on the structure of the substrates **1**. While the hydrolysis of parent α-phenylalanine amide **1a** and its 4-methyl-, 4-fluoro- and 4-chloro-substituted phenylalanine amide analogues afforded good and comparable enantioselectivity with enantiomeric selection (E)[19] in the range of 30 to 45, the highest enantiocontrol (E = 144) was obtained from the reaction of α-4-bromophenylalanine amide **1i** (entry 13). It was interesting to note that variation of the substitution pattern led to a dramatic change of enantioselection. This was exemplified by the observation of opposite effects of decrease or increase of enantioselectivity, respectively, when a methyl substituent was shifted from the 4- to the 3- or 2-position on the benzene ring (entries 7–9). More surprisingly, in contrast to the decrease of enantioselectivity when methyl moved from the 4- to the 3-position on the benzene ring (entries 7 and 8), the same move of a methoxy group resulted in a great enhancement of enantioselectivity (entries 4–6). These demonstrated clearly that the enantioselection of the amidase-catalyzed kinetic resolution of α-arylalanine amides was determined by not only the electronic nature of the substituent but also by the substitution pattern.

To evaluate further the synthetic potential of the amidase in *Rhodococcus* sp. AJ270, substrates other than α-arylalanine amides **1** were subjected to the biotransformation. It has been found that racemic α-ethyl phenylglycine amide **4** was efficiently resolved into the high yields of (*R*)-(+)-α-ethyl phenylglycine amide **5** and (*S*)-(−)-α-ethyl phenylglycine **6** with enantiomeric excesses (ee) of 96% and 99%, respectively. More gratifyingly, the kinetic resolution of 1-aminocyclohexanecarboxylic acid amide compound (+/−)-**7**, which was derived from α-tetralone, afforded enantiopure (+)-amide **8** and (−)-acid **9** in almost quantitative yields (Scheme 2). To determine the absolute configuration of the amide products **2**, and also to prepare the antipode of the optically active (S)-(−)-α-alkyl arylglycines **3** that were directly obtained from kinetic resolution, chemical hydrolysis of biotransformed (*R*)-(+)-amides **2** was attempted. In refluxing hydrochloric acid (6 N), optically active (*R*)-(+)-α-phenylalanine amide **2a** (95% ee) was hydrolyzed to give (*R*)-(+)-α-phenylalanine **10** (> 99.5% ee) in excellent yield without racemization (Scheme 3).

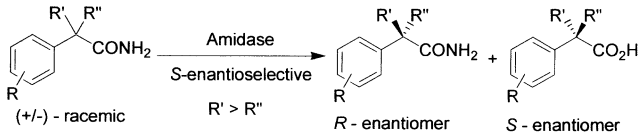
The results obtained from the current and the previous studies[16,17] have shown clearly that the amidase involved in *Rhodococcus* sp. AJ270 cells displays a predictive *S*-enantioselective against both α-monosubstituted- and α,α-disubstituted arylacetamides irrespective of the nature of the substituents attached on the



Scheme 2. Biocatalytic kinetic resolution of racemic α-ethyl phenylglycine amide (+/−)-**4** and 1-amino-1-carbamoyl-1,2,3,4-tetrahydronaphthalene (+/−)-**7**.



Scheme 3. Synthesis of (*R*)-(+)-α-phenylalanine **10**.



R' = NH_2, R" = H, Me, Et; R' = Me, Et, *n*-Pr, *i*-Pr, *n*-Bu, Allyl, MeS, R" = H

Scheme 4. Model of amidase's action against α-monosubstituted- and α,α-disubstituted arylacetamides.

benzene ring. The substitution pattern of R on the benzene ring, however, affects remarkably the reaction rate and the degree of enantioselectivity (ee of the products). Besides, the bigger the difference between two substituents R′ and R″, the higher is the enantioselectivity of the reaction (Scheme 4).

Conclusion

In conclusion, we have shown a highly efficient and convenient method for the synthesis of (*R*)-(+)-α-arylalanine amides and (*S*)-(−)-α-arylalanines with high enantiomeric purity from the kinetic resolution of racemic amides catalyzed by the amidase within *Rhodococcus* sp. AJ270 cells under very mild conditions. Both the reaction efficiency and *S*-enantioselectivity of the biocatalytic kinetic resolution are dependent on the nature of the substituent and its substitution pattern on the benzene ring. The method was also very effective for the preparation of other α-alkyl α-amino acid derivatives including (*R*)-(+)-1-amino-1-carbamoyl-1,2,3,4-tetrahydronaphthalene and (*S*)-(−)-1-amino-1-carboxy-1,2,3,4-tetrahydronaphthalene. Coupled with the chemical hydrolysis of amides, this biotransformation process provided efficient syntheses of α-substituted arylglycines in both enantiomeric forms from readily available racemic amides. The highly enantiopure α-substituted α-amino acids and their amide derivatives obtained should serve as useful chemical entities in asymmetric synthesis, and are being actively investigated in this laboratory.

Experimental Section

The configurations of the optically active α-substituted α-amino acids were determined by a comparison of their direction of optical rotation with those of authentic samples, while the configurations of the amides were obtained by correlating the optical rotations of their chemically hydrolyzed amino acid products with those of amino acids of known configurations. For unknown optically active amino acids and amides obtained from these biotransformations, the configurations were assigned by the comparison of both direction of optical rotation and the retention times on HPLC analysis on a chiral stationary phase with those of (*S*)-(−)-α-phenylalanine and (*R*)-(+)-α-phenylalanine amide, respectively, assuming that the introduction of a substituent on the benzene ring did not change the direction of the optical rotation and the order of the chromatogram. The enantiomeric excesses of all compounds were determined with a Shimadzu LC-10AVP HPLC system. A Chirobiotic Tag™ column with water as the mobile phase at a flow rate of 0.5 mL/min was employed for the analysis of all acids. For amides, a Cyclobond I™ 2000DMP column with a mixture (6:4) of methanol and aqueous buffer ($H_2O/CH_3CO_2H/Et_3N$: 100:0.15:0.1) as the mobile phase at a flow rate of 0.5 mL/min was used.

General Procedure for the Biocatalytic Kinetic Resolution of Racemic Amides

To an Erlenmeyer flask (100 mL) with a screw cap was added *Rhodococcus* sp. AJ270 cells[18] (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 30 minutes with orbital shaking. Racemic amino acid amides **1** (1 mmol) were 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, was quenched after a specified period of time (see Table 1 and Scheme 2) by removing the biomass through a Celite pad filtration. The resulting aqueous solution was concentrated. The products were isolated and purified by chromatography using consecutively 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 spectral data and comparison of melting points and optical rotary power with those of the known compounds, which are listed as follows, or by full characterization.

Enzymatic Hydrolysis of Racemic α-Phenylalanine Amide (1a)

This afforded (*R*)-(+)-α-phenylalanine amide (**2a**) after 17.5 h; yield: 46%; mp 112 – 113 °C; $[\alpha]_D^{25}$: + 44 (*c* 0.5, MeOH); ee 95% (HPLC, t_R = 5.25 min, t_S = 4.26 min); IR (KBr): ν = 3388, 3206 (NH_2), 1649 cm^{-1} (C=O); ^1H NMR (300 MHz, DMSO-d_6): δ = 7.51 (d, *J* = 7.8 Hz, 2H), 7.19 – 7.33 (m, 4H), 7.04 (s, 1H), 2.32 (br, s, 2H), 1.52 (s, 3H); ^{13}C-NMR (DMSO-d_6): δ = 178.9, 147.3, 128.6, 127.3, 126.3, 60.8, 28.9; MS (EI): *m/z* (%) = 120 [M – $CONH_2$]$^+$ (100), 119 (40), 104 (72); anal. calcd. for $C_9H_{12}N_2O$: C 65.83, H 7.37, N 17.06; found: C 65.96, H 7.42, N 17.13.

(*S*)-(−)-α-Phenylalanine (3a): Time 17.5 h; yield: 50%; mp 229 – 230 °; $[\alpha]_D^{25}$: − 44 (*c*, 0.5, 2 N HCl) [lit.[11a] $[\alpha]_D^{25}$: − 86 (*c* 1, 1 N HCl)]; ee 84% (HPLC, t_R = 11.76 min, t_S = 21.87 min); IR (KBr): ν = 2200 – 3600 (NH_3^+), 1595 cm^{-1} (COO-); ^1H NMR (300 MHz, $D_2O + CF_3CO_2D$): δ = 6.36 – 6.39 (m, 5H, ArH), 0.9 (s, 3H); ^{13}C NMR ($D_2O + CF_3CO_2D$): δ = 173.0, 134.6, 129.7, 129.1, 125.3, 61.5, 20.5; MS (FAB): *m/z* = 166 $[M + 1]^+$; anal. calcd. for $C_9H_{11}NO_2$: C 65.44, H 6.71, N 8.48; found: C 65.03, H 6.63, N, 8.33.

Enzymatic Hydrolysis of Racemic α-4-Methoxyphenylalanine Amide (1b)

This afforded (*R*)-(+)-α-4-methoxyphenylalanine amide (**2b**) after 48 h; yield: 37%; mp 130–131 °C; $[\alpha]_D^{25}$: +38 (*c* 0.5, MeOH); ee 95% (HPLC, $t_R = 6.12$ min, $t_S = 4.31$ min); IR (KBr): ν = 3545, 3400, 3323, 3268 (NH_2, $CONH_2$), 1685, 1646 1608 cm^{-1} (C=O); ^1H NMR (300 MHz, DMSO-d_6): δ = 7.41 (d, $J = 8.1$ Hz, 2H), 7.29 (br, s, 1H), 6.97 (br, s, 1H), 6.85 (d, $J = 8.1$ Hz, 2H), 3.72 (s, 3H), 1.48 (s, 3H); ^{13}C NMR (DMSO-d_6): δ = 179.1, 158.7, 139.4, 127.4, 114.0, 60.3, 55.9, 29.0; MS (EI): *m/z* (%) = 150 $[M - CONH_2]^+$ (2.7), 134 (63), 133 (53), 118 (100); anal. calcd. for $C_{10}H_{15}N_2O_2$: 195.1127; HRMS (FAB) found for MH^+: 195.1128.

***(S)*-(−)-α-4-Methoxyphenylalanine (3b):** Time 36 h; yield: 35%; mp 256–257 °C; $[\alpha]_D^{25}$: −75.6 (*c* 0.45, 2 N HCl); ee 94% (HPLC, $t_R = 15.94$ min, $t_S = 39.26$ min); IR (KBr): ν = 2400–3600 (NH_3^+), 1612 cm^{-1} (COO-); ^1H NMR (300 MHz, D_2O + CF_3CO_2D): δ = 7.00 (d, $J = 9.0$ Hz, 2H), 6.58 (d, $J = 7.2$ Hz, 2H), 3.35 (s, 3H), 1.52 (s, 3H); ^{13}C NMR: δ = 173.6, 160.0, 127.5, 114.9, 61.4, 55.6, 21.0; MS (FAB): $m/z = 196$ $[M + 1]^+$; anal. calcd. for $C_{10}H_{13}NO_3$: C 61.53, H 6.71, N 7.17; found: C 61.34, H 7.00, N, 7.08.

Enzymatic Hydrolysis of Racemic α-3-Methoxyphenylalanine Amide (1c)

This afforded (*R*)-(+)-α-3-methoxyphenylalanine amide (**2c**) after 62 h; yield: 49%; mp 84 °C; $[\alpha]_D^{25}$: +31 (*c* 0.5, MeOH); ee 94% (HPLC, $t_R = 12.10$ min, $t_S = 9.03$ min); IR (KBr): ν = 3367, 3300, 3200 (NH_2, $CONH_2$), 1670 cm^{-1} (C=O); ^1H NMR (300 MHz, DMSO-d_6): δ = 7.34 (br, s, 1H), 7.21 (t, $J = 7.7$ Hz, 1H), 7.07 (s, 1H), 7.06 (d, $J = 8.2$ Hz, 1H), 7.00 (br, s, 1H), 6.79 (d, $J = 7.2$ Hz, 1H), 3.72 (s, 3H), 1.51 (s, 1H); ^{13}C NMR (DMSO-d_6): δ = 178.8, 159.8, 149.0, 129.7, 118.6, 112.5, 112.4, 60.9, 55.8, 28.9; MS (EI): *m/z* (%) = 150 $[M - CONH_2]^+$ (22), 149 (55), 134 (100); anal. calcd. for $C_9H_{14}N_2O_2$: C 61.84, H 7.27, N 14.42; found: C 61.91, H 7.34, N 14.45.

(S)-(−)-α-3-Methoxyphenylalanine (3c): Time 62 h; yield: 50%; mp 258–259 °C; $[\alpha]_D^{25}$: −66 (*c* 0.5, 2 N HCl); ee 92% (HPLC, $t_R = 15.74$ min, $t_S = 22.97$ min); IR (KBr): ν = 2400–3600 (NH_3^+), 1620, 1603 cm^{-1} (COO-); ^1H NMR (300 MHz, D_2O + CF_3CO_2D): δ = 6.80 (t, $J = 8.0$ Hz, 1H), 6.41–6.51 (m, 3H), 3.19 (s, 3H), 1.39 (s, 3H); ^{13}C NMR: δ = 172.8, 159.4, 136.4, 130.6, 118.0, 115.0, 111.7, 61.6, 55.3, 20.8; MS (FAB): $m/z = 196$ $[M + 1]^+$; anal. calcd. for $C_{11}H_{14}NO_3$: 196.0970; HRMS (FAB) found for MH^+: 196.0968.

Enzymatic Hydrolysis of Racemic α-4-Methylphenylalanine Amide (1d)

This afforded (*R*)-(+)-α-4-methylphenylalanine amide (**2d**) after 55 h; yield: 50%; mp 149–150.5 °C; $[\alpha]_D^{25}$: +33 (*c* 0.5, MeOH); ee 82% (HPLC, $t_R = 14.04$ min, $t_S = 8.79$ min); IR (KBr): ν = 3545, 3398, 3320, 3271 (NH_2, $CONH_2$), 1645 cm^{-1} (C=O); ^1H NMR (300 MHz, DMSO-d_6): δ = 7.38 (d, $J = 8.1$ Hz, 2H), 7.30 (s, 1H), 7.11 (d, $J = 8.1$ Hz, 2H), 6.98 (s, 1H), 2.27 (s, Me), 1.49 (s, Me); ^{13}C NMR (DMSO-d_6): δ = 179.0, 144.3, 136.2, 129.6, 129.2, 126.2, 60.6, 28.9, 21.4; MS (EI): *m/z* (%) = 134 $[M - CONH_2]^+$ (77), 113 (56), 118 (100); anal. calcd. for $C_{10}H_{14}N_2O$: C 67.39, H 7.92, N, 15.72; found: C 67.15, H 7.96, N 15.71.

(S)-(−)-α-4-Methylphenylalanine (3d): Time 55 h; yield: 47%; mp >300 °C; $[\alpha]_D^{25}$: −64 (*c* 0.45, 2 N HCl); ee 89% (HPLC, $t_R = 16.68$ min, $t_S = 39.25$ min); IR (KBr): ν = 2400–3600 (NH_3^+), 1614 cm^{-1} (COO-); ^1H NMR (300 MHz, D_2O + CF_3CO_2D): δ = 6.78 (d, $J = 8.1$ Hz, 2H), 6.86 (d, $J = 8.1$ Hz, 2H), 1.78 (s, 3H), 1.45 (s, 3H); ^{13}C NMR (D_2O + CF_3CO_2D): δ = ?173.8, 140.9, 132.4, 130.3, 125.9, 61.8, 21.2, 20.5; MS (FAB): $m/z = 180$ $[M + 1]^+$; anal. calcd. for $C_{10}H_{14}NO_2$: 180.1024; HRMS (FAB) found for MH^+: 180.1020.

Enzymatic Hydrolysis of Racemic α-3-Methylphenylalanine Amide (1e)

This afforded (R)-(+)-α-3-methylphenylalanine amide (**2e**) after 56 h; yield: 46%; mp 101–101.5 °C; $[\alpha]_D^{25}$: +92 (*c* 0.5, MeOH); ee 88% (HPLC, $t_R = 12.31$ min, $t_S = 10.59$ min); IR (KBr): ν = 2400–3500 (NH_2, $CONH_2$), 1683 cm^{-1} (C=O); ^1H NMR (300 MHz, DMSO-d_6): δ = 7.32 (br, s, 2H), 7.28 (s, 1H), 7.19 (t, $J = 7.7$ Hz, 1H), 7.03 (d, $J = 7.8$ Hz, 2H), 2.38 (br, s, 2H), 2.20 (s, 3H), 1.50 (s, 3H); ^{13}C NMR (DMSO-d_6): δ = 178.8, 147.1, 137.5, 128. 6, 127.9, 126.9, 123.4, 60.8, 28.8, 22.1; MS (EI): *m/z* (%) = 134 $[M - CONH_2]^+$ (77), 133 (56), 118 (100); anal. calcd. for $C_{10}H_{14}N_2O$: C 67.39, H 7.92, N 15.72; found: C 67.53, H 7.89, N 15.82.

***(S)*-(−)-α-3-Methylphenylalanine (3e):** Time 56 h; yield: 50%; mp >300 °C (hydrochloric acid salt); $[\alpha]_D^{25}$: −42.5 (*c* 0.4, 2 N HCl); ee 73% (HPLC, $t_R = 14.59$ min, $t_S = 21.27$ min); IR (KBr): ν = 2200–3600 (NH_3^+), 1698 cm^{-1} (COO-); ^1H NMR (300 MHz, D_2O + CF_3CO_2D): δ = 6.98–7.08 (m, 4H, Ar), 2.03 (s, 3H), 1.70 (s, 3H); ^{13}C NMR (D_2O + CF_3CO_2D): δ = 173.4, 140.0, 135.2, 130.8, 129.5, 126.3, 122.7, 61.9, 21.1, 20.6; MS (FAB): m/z 180 = $[M + 1]^+$; anal. calcd. for $C_{10}H_{13}NO_2 \cdot HCl$: C 55.36, H 6.54, N 6.49; found: C 55.18, H 6.50, N 6.28.

Enzymatic Hydrolysis of Racemic α-2-Methylphenylalanine Amide (1f)

This afforded (*R*)-(+)-α-2-methylphenylalanine amide (**2f**): Time 14 h; yield: 46%; mp 127–129°; $[\alpha]_D^{25}$: +51 (*c* 0.5, MeOH); ee 99% (HPLC, $t_R = 56.57$ min, $t_S = 53.60$ min, flow rate 0.1 mL/min); IR (KBr): ν = 3362, 3160, (NH_2, $CONH_2$), 1690 cm^{-1} (C=O); ^1H NMR (300 MHz, DMSO-d_6): δ = 7.54 (s, 1H), 7.50 (d, $J = 8.9$ Hz, 1H), 7.09–7.19 (m, 3H), 7.05 (br, s, 1H), 2.30 (s, 3H), 2.18 (s, 2H), 1.55 (s, 3H); ^{13}C NMR (DMSO-d_6): δ = 179.4, 144.5, 136.5, 131.7, 126.9, 126.1, 125.7, 60.1, 28.3, 20.5; MS (EI): *m/z* (%) = 179 $[M + 1]^+$ (58), 162 (50), 135 (31), 134 (100); anal. calcd for $C_{10}H_{14}N_2O$: C 67.39, H 7.92, N 15.72; found: C 67.05, H 7.87, N 15.71.

***(S)*-(−)-α-2-Methylphenylalanine (3f):** Time 14 h; yield: 49%; mp 236–238 °C; $[\alpha]_D^{25}$: −74.4 (*c* 0.5, 2N HCl); ee 90% (HPLC, $t_R = 54.40$ min, $t_S = 67.30$ min, flow rate 0.1 mL/min); IR (KBr): ν = 2200–3600 (NH_3^+), 1635 cm^{-1} (COO-); ^1H NMR (300 MHz, D_2O + CF_3CO_2D): δ = 7.32 (d, $J = 7.6$ Hz, 1H), 7.04–7.18 (m, 3H), 2.12 (s, 3H), 1.90 (s, 3H); ^{13}C NMR (D_2O + CF_3CO_2D): δ = 171.3, 134.7, 131.3, 130.6, 129.1, 125.5, 125.2, 60.4, 21.2, 17.6; MS (ESI): $m/z = 178$ $[M - 1]^+$; anal. calcd. for $C_{10}H_{13}NO_2$: C 67.02, H 7.31, N 7.82; found: C 67.12, H 7.34, N 7.87.

Enzymatic Hydrolysis of Racemic α-4-Fluorophenylalanine Amide (1g)

This afforded (*R*)-(+)-α-4-fluorophenylalanine amide (**2g**) after 18 h; yield: 45%; mp 110–112 °C; $[\alpha]_D^{25}$: +36 (*c* 0.5, MeOH); ee >99.5% (HPLC, t_R = 10.97 min, t_S = 8.82 min); IR (KBr): ν = 3390, 3206 (NH_2), 1652 cm^{-1} (C=O); ^1H NMR (300 MHz, DMSO-d_6): δ = 7.51 (dd, *J* = 8.7, 5.7 Hz, 2H), 7.33 (br, s, 1H), 7.11 (t, *J* = 9.0 Hz, 2H), 7.03 (br, s, 1H), 2.33 (s, 2H), 1.49 (s, 3H); ^{13}C NMR (DMSO-d_6): δ = 178.7, 162.8, 160.8, 143.4, 128.4, 128.3, 115.3, 115.1, 60.4, 29.0; MS (EI): *m/z* (%) = 138 $[M - CONH_2]^+$, (95), 137 (56), 122 (100); anal. calcd. for $C_9H_{11}FN_2O$: C 59.33, H 6.09, N 15.38; found: C 59.79, H 6.16, N 15.52.

(S)-(−)-α-Fluorophenylalanine (3g): Time 18 h; yield: 52%; mp 268–269 °C (hydrochloric acid salt); $[\alpha]_D^{25}$: −68 (*c* 0.5, 2 N HCl); ee 76% (HPLC, t_R = 11.46 min, t_S = 17.61 min); IR (KBr): ν = 2400–3600 (NH_3^+), 1589 cm^{-1} (COO-); ^1H NMR (300 MHz, $D_2O + CF_3CO_2D$): δ = 7.42 (dd, *J* = 8.4, 4.8 Hz, 2H), 7.10 (t, *J* = 8.1 Hz, 2H), 1.87 (s, 3H); ^{13}C NMR ($D_2O + CF_3CO_2D$): δ = 174.0, 164.4, 162.4, 131.9, 128.6, 128.5, 116.7, 116.5, 61.8, 21.6; MS (FAB): *m/z* = 184 $[M + 1]^+$; anal. calcd. for $C_9H_{10}FNO_2 \cdot HCl$: C 49.22, H 5.05, N 6.38; found: C 48.81, H 5.06, N, 6.27.

Enzymatic Hydrolysis of Racemic α-4-Chlorophenylalanine Amide (1h)

This afforded (*R*)-(+)-α-4-chlorophenylalanine amide (**2h**): Time 52 h; yield: 41%; mp 144 °C; $[\alpha]_D^{25}$: +34 (*c* 0.5, MeOH); ee 99.5% (HPLC, t_R = 21.10 min, t_S = 10.06 min); IR (KBr): ν = 3397, 3278 (NH_2), 1683, 1652 cm^{-1} (C=O); ^1H NMR (300 MHz, DMSO-d_6): d = 7.49 (d, *J* = 8.4 Hz, 2H), 7.34 (d, *J* = 8.6 Hz, 2H), 7.06 (br, s, 2H), 1.47 (s, 3H); ^{13}C NMR (DMSO-d_6): δ = 178.4, 146.3, 132.0, 128.5, 128.3, 60.5, 28.8; MS (EI): *m/z* (%) = 156 $[M + 2 - CONH_2]^+$ (21), 154 (65), 153 (46), 140 (32), 138 (100); anal. calcd. for $C_9H_{11}ClN_2O$: C 54.42, H 5.58, N 14.10; found: C 54.84, H 5.65, N 14.19.

(S)-(−)-α-4-Chlorophenylalanine (3h): Time 25 h; yield: 31%; mp 204 °C; $[\alpha]_D^{25}$: −84 (*c* 1.95, 2 N HCl); ee 89% (HPLC, t_R = 19.16 min, t_S = 36.50 min); IR (KBr) ν = 2200–3600 (NH_3^+), 1596 cm^{-1} (COO-); ^1H NMR (300 MHz, $D_2O + CF_3CO_2D$): δ = 6.59 (s, 4H), 1.12 (s, 3H); ^{13}C NMR ($D_2O + CF_3CO_2D$): δ = 172.7, 135.4, 133.5, 129.3, 126.9, 61.2, 20.9; MS (FAB): *m/z* = 200 $[M + 1]^+$; anal. calcd. for $C_9H_{11}ClNO_2$: 200.0478, 202.0449; HRMS (FAB) found for MH^+: 200.0471, 202.0443.

Enzymatic Hydrolysis of Racemic α-4-Bromophenylalanine Amide (1i)

This afforded (*R*)-(+)-α-4-bromophenylalanine amide (**2i**): Time 53 h; yield: 48%; mp 82 °C; $[\alpha]_D^{25}$: +20.8 (*c* 0.5, MeOH); ee >99.5% (HPLC, t_R = 14.86 min, t_S = 4.73 min); IR (KBr): ν = 3386, 3207 (NH_2), 1650 cm^{-1} (C=O); ^1H NMR (300 MHz, DMSO-d_6): δ = 7.49 (d, *J* = 8.7 Hz, 2H), 7.33 (d, *J* = 8.8 Hz, 2H), 7.33 (br, s, 1H), 7.05 (br, s, 1H), 2.43 (s, 2H), 1.48 (s, 3H); ^{13}C NMR (DMSO-d_6): δ = 177.8, 146.2, 130.9, 120.0, 60.1, 28.2; MS (EI): *m/z* (%) = 200 $[M + 2 - CONH_2]^+$ (92), 198 $[M - CONH_2]^+$ (96), 42 (100); anal. calcd. for $C_9H_{11}BrN_2O$: C 44.47, H 4.56, N 11.52; found: C 44.59, H 4.64, N 11.18.

(S)-(−)-α-4-Bromophenylalanine (3i): Time 53 h; yield: 41%; mp 268 °C; $[\alpha]_D^{25}$: −68 (*c* 0.5, 2 N HCl); ee 93% (HPLC, t_R = 25.70 min, t_S = 53.30 min); IR (KBr) ν = 2200–3600 (NH_3^+), 1622 cm^{-1} (COO-); ^1H NMR (300 MHz, $D_2O + CF_3CO_2D$): δ = 7.48 (d, *J* = 8.6 Hz, 2H), 7.28 (d, *J* = 8.5 Hz, 2H), 1.91 (s, 3H); ^{13}C NMR ($D_2O + CF_3CO_2D$): δ = 171.4, 132.4, 131.2, 125.9, 122.8, 60.6, 19.4; MS (FAB): *m/z* = 224 $[M + 1]^+$; anal. calcd. for $C_9H_{10}BrNO_2$: C 44.29, H 4.13, N 5.74; found: C 44.22, H 4.22, N, 5.63.

Enzymatic Hydrolysis of Racemic α-Ethylphenylglycine Amide (4)

This afforded (*R*)-(+)-α-ethylphenylglycine amide (**5**) after 48 h; yield: 46%; mp 153 °C (decomp.); $[\alpha]_D^{25}$: +16 (*c* 0.5, MeOH); ee 96% (HPLC, t_R = 12.13 min, t_S = 9.11 min); IR (KBr): ν = 3394, 3351, 3275 (NH_2, $CONH_2$), 1690, 1651 cm^{-1} (C=O); ^1H NMR (300 MHz, DMSO-d_6): δ = 8.61 (s, 1H), 7.73 (s, 1H), 7.66 (s, 1H), 2.30–2.47 (m, 2H), 0.96 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (DMSO-d_6): δ = 171.8, 138.1, 129.48, 129.47, 127.4, 66.3, 28.2, 8.9; MS (EI): *m/z* (%) = 134 $[M - CONH_2]^+$ (43), 132 (47.3), 104 (100); anal. calcd. for $C_{10}H_{14}N_2O$: C 67.39, H 7.92, N 15.72; found: C 67.15, H 8.00, N 15.67.

(S)-α-Ethyl-phenylglycine (6): Time 48 h; yield: 50%; mp 250–252 °C; $[\alpha]_D^{25}$: −57 (*c* 0.5, 2 N HCl); ee >99% (HPLC, t_R = 12.36 min, t_S = 16.20 min); IR (KBr): ν = 2400–3600 (NH_3^+), 1635 cm^{-1} (COO-); ^1H NMR (300 MHz, $D_2O + CF_3CO_2D$): δ = 7.30 (s, 5H), 2.50 (q, *J* = 7.2 Hz, 2H), 1.01 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR ($D_2O + CF_3CO_2D$): δ = 170.6, 131.6, 128.5, 127.7, 123.6, 65.5, 25.9, 4.8; MS (FAB): *m/z* = 180 $[M + 1]^+$; anal. calcd. for $C_{10}H_{14}NO_2$: 180.1024; HRMS (FAB) found for MH^+: 180.1019.

Enzymatic Hydrolysis of Racemic 1-Amino-1-carbamoyl-1,2,3,4-tetrahydronaphthalene (7)

This afforded (*R*)-(+)-1-amino-1-carbamoyl-1,2,3,4-tetrahydronaphthalene (**8**): Time 56 h; yield: 46%; mp 124–125°; $[\alpha]_D^{25}$: +26 (*c* 0.5, MeOH); ee >99.5% (HPLC, t_R = 10.32 min, t_S = 8.98 min); IR (KBr): ν = 3385, 3249, 3190 (NH_2, $CONH_2$), 1671 cm^{-1} (C=O); ^1H NMR (300 MHz, DMSO-d_6): δ = 7.49 (br, s, 2H), 7.03–7.22 (m, 4H). 2.72 (t, *J* = 6.2 Hz, 2H), 2.29 (br, s, 2H), 2.13–2.19 (m, 1H), 1.82–1.87 (m, 2H), 1.67–1.80 (m, 1H); ^{13}C NMR (DMSO-d_6): δ = 174.1, 139.0, 133.1, 130.4, 129.7, 129.9, 127.2, 61.2, 32.4, 29.1, 18.5; MS (EI): *m/z* (%) = 146 $[M - CONH_2]^+$ (100), 145 (69), 117 (89); anal. calcd. for $C_{11}H_{15}N_2O$: 191.1177; HRMS (FAB) found for MH^+: 191.1179.

(*S*)-(−)-1-Amino-1-carboxy-1,2,3,4-tetrahydronaphthalene (9): Time 56 h; yield: 50%; mp 254–256 °C; $[\alpha]_D^{25}$: −94 (*c* 0.5, 2 N HCl); ee >99.5% (HPLC, t_R = 15.83 min, t_S = 24.94 min); IR (KBr): ν = 2200–3600 (NH_3^+), 1626 cm^{-1} (COO-); ^1H NMR (300 MHz, $D_2O + CF_3CO_2D$): δ = 6.83–6.99 (m, 4H), 2.46 (q, *J* = 4.7 Hz, 2H), 1.99–2.09 (m, 1H), 1.79–1.85 (m, 1H), 1.62–1.69 (m, 1H), 1.38–1.50 (m, 1H); ^{13}C NMR (D_2O): δ = 175.2, 138.6, 130.6, 130.5, 130.1, 127.5, 127.3, 61.7, 32.1, 28.4, 18.2; MS (FAB): *m/z* = 192 $[M + 1]^+$; anal. calcd. for $C_{11}H_{14}NO_2$: 192.1015; HRMS (FAB) found for MH^+: 192.1019.

Chemical Hydrolysis of (*R*)-(+)-α-Phenylalanine Amide (2a)

(*R*)-(+)-α-Phenylalanine amide (**2a**; 40 mg, ee 95%) was refluxed in 6 N HCl (5 mL) for 1 day. After removal of the solvent, the residue was dissolved in deionized water (5 mL) and then was purified using a cationic exchange resin column (Dowex, 50 × 8) to give (*R*)-(+)-α-phenylalanine (**10**); yield: 36 mg (90%); mp 245–246 °C; $[\alpha]_D^{25}$: +47 (*c* 1, 2 N HCl), ee >99.5% (HPLC, $t_R = 11.73$ min, $t_S = 21.87$ min). The spectral data are identical with those of **3a**.

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