Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 16 (2005) 2409–2416

Synthesis of optically active α-methylamino acids and amides through biocatalytic kinetic resolution of amides

Mei-Xiang Wang,* Jun Liu, De-Xian Wang and Qi-Yu Zheng

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

Received 25 March 2005; revised 19 June 2005; accepted 20 June 2005

Abstract—Catalyzed by *Rhodococcus* sp. AJ270, a nitrile hydratase and amidase containing microbial whole-cell catalyst, under very mild conditions, a number of racemic α-methylamino amides were resolved into the corresponding optically active (*S*)-(+)-α-methylamino acids and (*R*)-(−)-α-methylamino amides. The steric requirement of the amidase against α-amino phenylacetamides bearing methyl group(s) at α-amino nitrogen and/or α-carbon was also studied. Coupled with the chemical hydrolysis of amide, the biotransformation process provided a direct synthesis of α-methylamino acids in both enantiomeric forms from readily available racemic amides.

1. Introduction

There has been an increasing interest in the synthesis of optically active α-methylamino acids because of their significant biological properties.[1,2] A number of synthetic methods reported to date mainly involve the methylation of *N*-protected α-amino acid derivatives,[3] methylation of α-azido carboxylic acids,[4] reductive amination[5] and reduction and deprotection of oxazolidinones, which are derived from the cyclocondensation of *N*-protected α-amino acids and paraformaldehyde.[6] In addition, the resolution of racemic α-methylamino acids using chiral acids and bases[7] and the enzymatic resolution of *N*-acylated *N*-methyl α-amino acids[8] have also been reported. However, most of the methods reported suffer from at least one drawback such as low chemical yields, narrow substrate spectrum, long synthetic steps including tedious protection and deprotection, harsh reaction conditions and the problems of racemization.

Biotransformations of nitriles, either through direct conversion from a nitrile to a carboxylic acid catalyzed by a nitrilase[9] or through the nitrile hydratase-catalyzed hydration of a nitrile followed by the amide hydrolysis catalyzed by the amidase,[10] are effective and environmentally friendly 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.[11] Recent studies[12,13] have demonstrated that biotransformations of nitriles 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, it was found[14] that the combination of the nitrile hydratase and the amidase in *Rhodococcus* sp. AJ270 microbial cells[15] is a powerful biocatalytic system to transform a variety of racemic α-amino nitriles into highly enantiopure D-amino acid amides and L-amino acids. The amidase is also able to resolve α-alkylarylglycine amides to yield optically active (*S*)-α-alkylarylglycines and (*R*)-α-alkylarylglycine amides.[16] Our interests[13,14,16] in the understanding of the scope and mechanism of the nitrile hydratase and the amidase, and in the synthesis of optically active *N*-methyl α-amino acids have led us to the current study. We report herein a general and convenient synthesis of optically active (*S*)-α-methylamino acids and (*R*)-α-methylamino amides from the kinetic resolution of amides catalyzed by the amidase within *Rhodococcus*

* Corresponding author. Tel.: +86 10 62565610; fax: +86 10 62564723; e-mail: mxwang@iccas.ac.cn

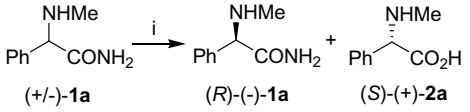
0957-4166/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2005.06.023

sp. AJ270 cells. Using structurally diversified α-amino amide substrates as probes, we are also able to define the steric requirements of the amidase action.

2. Results and discussion

We first tested the *Rhodococcus* sp. AJ270 cell-catalyzed reaction of racemic α-methylamino phenylacetonitrile. Unfortunately, in stark contrast to the efficient and highly enantioselective reaction of racemic α-amino phenylacetonitrile,[14] the α-methylamino analogue did not undergo biocatalytic hydrolysis. Efforts to improve the conversion of the nitrile, such as by varying the pH of the buffered solution, reaction temperature and concentration of the substrate were made, but all met with failure. Under the various reaction conditions including the control experiments in the absence of biocatalyst, the substrate was found to undergo a very rapid and spontaneous decomposition. The inefficient hydration of the nitrile in this case is most probably due to the instability of the substrate and the inhibition of the nitrile hydratase, a believed cobalt- or iron-containing enzyme,[10b] by the cyanide ion generated from decomposition of α-methylamino phenylacetonitrile.

Having considered the high enantioselectivity of the amidase within *Rhodococcus* sp. AJ270 and the stability of α-methylamino amides, we then concentrated on the kinetic resolution of racemic α-methylamino phenylacetamide (±)-**1a** (Scheme 1 and Table 1). In a phosphate buffer with pH ranging from 7.0 to 7.6, *Rhodococcus* sp. AJ270 readily transformed racemic (±)-**1a** into the optically active (*R*)-(−)-amide (−)-**1a** and (*S*)-(+)-acid (+)-**2a**. With the progress of reaction, the enantiomeric excess value of (−)-**1a** increased while that of (+)-**2a** decreased (entries 1–6), clearly indicating a kinetic resolution process. Under the optimal conditions, such as in a neutral buffer solution (pH 7.0) for 60 h, racemic (±)-**1a** was resolved effectively into (*R*)-(−)-**1a** and (*S*)-(+)-**2a** in excellent chemical yields with excellent enantioselectivities (entry 7).



Scheme 1. Biocatalytic kinetic resolution of racemic α-methylamino phenylacetamide (±)-**1a**. Reagents and condition: (i) *Rhodococcus* sp. AJ270, phosphate buffer, 30 °C.

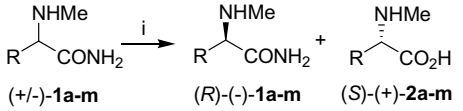
Table 1. Biocatalytic kinetic resolution of racemic α-methylamino phenylacetamide (±)-**1a**

Entry	pH	Time (h)[a]	(*R*)-(−)-**1a** (%)[b]	(*R*)-(−)-**1a** (% ee)[c]	(*S*)-(+)-**2a** (%)[b]	(*S*)-(+)-**2a** (% ee)[c]
1	7.0	48	56	73	43	96
2	7.25	48	58	64	40	93
3	7.6	48	57	64	41	92
4	7.0	72	41	>99.5	58	75
5	7.25	72	43	>99.5	56	78
6	7.6	72	43	>99.5	56	75
7	7.0	60	48	93	47	93

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

Encouraged by these results, we then extended this reaction to other racemic α-methylamino amide substrates. In order to examine the influence of the structure of the substrates on the biocatalytic kinetic resolution, both the number of α-methylamino arylacetamides (±)-**1b–j** bearing a different substituent at different substitution positions on the benzene ring and α-methylamino alkanamides (±)-**1k–m** were prepared and subjected to incubation with *Rhodococcus* sp. AJ270 (Scheme 2 and Table 2). The results summarized in Table 2 shows that the amidase accepted all the racemic α-methylamino amides tested as substrates. The conversion of the amide, however, depended strongly on the nature of the substituent and the substitution pattern on the arene ring of the substrates. For example, while *N*-methylamino 4-fluorophenylacetamide (±)-**1b** (entry 2) and *N*-methylamino 3-chlorophenylacetamide (±)-**1d** (entry 4) underwent the equally efficient biohydrolysis as the parent amide (±)-**1a** (entry 1), the introduction of other substituents into either the 3- or 4-position of the benzene ring generally resulted in the slow hydrolysis, and the time for ca. 50% conversion increased from 1–2 days to 3–4.5 days. The presence of an *ortho* substituent such as in (±)-**1e** (entry 5) and (±)-**1i** (entry 9), led to even slower reaction. It is of interest to note that all methyl-substituted substrates (±)-**1g–i** (entries 7–9) underwent slower hydrolysis than their chloro-substituted counterparts (entries 3–5). In the case of α-methylamino-β-phenylpropionamide (±)-**1k** (entry 11) and α-methylamino-β-methylbutyramide (±)-**1l** (entry 12), biocatalytic hydrolysis proceeded very rapidly in 2–4 h to give more than 50% conversion. The conversion of cyclohexyl-substituted methylaminoacetamide (±)-**1m** (entry 13) was comparable to that of α-methylamino 4-chlorophenylacetamide (±)-**1c** (entry 3). The influence of the steric effect of the substituent appeared obvious; the smaller the steric bulkiness around the stereogenic centre that is adjacent to amide functional group, the faster the biocatalytic hydrolysis.

On the other hand, the scrutiny of the results tabulated in Table 2 revealed a varied (*S*)-enantioselectivity of the amidase depending on the structure of the substrates. For example, while the biohydrolysis of parent α-methylamino phenylacetamide (±)-**1a** (entry 1) furnished excellent enantioselectivity ($E^{17} = 94$), its 4-fluoro (±)-**1b**



Scheme 2. Amidase-catalyzed kinetic resolution of racemic α-methylamino amides (±)-**1**. Reagents and condition: (i) *Rhodococcus* sp. AJ270, phosphate buffer, 30 °C.

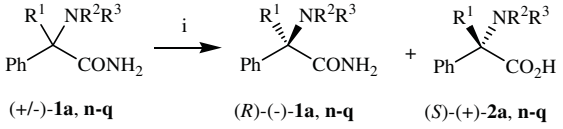
Table 2. Amidase-catalyzed kinetic resolution of racemic α-methylamino amides (±)-**1**

Entry	1	R	Time (d)[a]	(−)-**1** (%)[b]	(−)-**1** (% ee)[c]	(+)-**2** (%)[b]	(+)-**2** (% ee)[c]	*E*
1	**1a**	C_6H_5	2.5[d]	48	93	47	93	94
2	**1b**	4-FC_6H_4	1	45	93	53	83	36
3	**1c**	4-ClC_6H_4	2	57	57	37	85	21
4	**1d**	3-ClC_6H_4	1	48	5	46	15	1.4
5	**1e**	2-ClC_6H_4	7	68	<3	31	33	2
6	**1f**	4-BrC_6H_4	4.5	47	6	47	16	1.5
7	**1g**	4-MeC_6H_4	7	43	85	56	57	9.4
8	**1h**	3-MeC_6H_4	3	53	67	46	81	19
9	**1i**	2-MeC_6H_4	7[e]	72	4	23	13	1.3
10	**1j**	4-$MeOC_6H_4$	4	47	95	51	76	26
11	**1k**	$C_6H_4CH_2$	2 h[d]	38	73	60	56	7.5
12	**1l**	Me_2CH	4 h	49	21	51	13	1.6
13	**1m**	*c*-C_6H_{11}	2.5	55	39	42	96	71

[a] The substrate **1** (1 mmol) was incubated with *Rhodococcus* sp. AJ270 whole cells (2 g wet weight) in phosphate buffer (pH 7.0, 0.1 M, 50 ml) at 30 °C.
[b] Isolated yield.
[c] Enantiomeric excess values were determined by chiral HPLC analysis.
[d] 2 mmol of the substrate were used.
[e] 0.5 mmol of the substrate were used.

(entry 2), 4-chloro (±)-**1c** (entry 3), 4-methyl (±)-**1g** (entry 7) and 4-methoxy (±)-**1j** (entry 10) substituted amide analogues gave modest enantiocontrol, with *E* values ranging roughly from 10 to 36. Very low enantioselection was obtained for α-methylamino 4-bromophenylacetamide (±)-**1f** (entry 6). Variation of the substitution pattern from the *para*- to the *meta*- or *ortho*-position on the benzene ring resulted in a dramatic decrease in enantioselectivity (entries 3–5, 7 and 9) with the exception of α-methylamino 3-methylphenylacetamide (±)-**1h**, which afforded modest selection (*E* = 19) (entry 8). Low enantioselectivity was observed for alkanamides (±)-**1k** and (±)-**1l**, whereas α-cyclohexyl methylaminoacetamide (±)-**1m** showed a comparable enantioselectivity (*E* = 71) as that of aromatic analogues (±)-**1a** and (±)-**1b**. The aforementioned stereoselectivities suggested that the enantioselection of the amidase-catalyzed kinetic resolution of α-methylamino amides was determined by both electronic and steric factors of the substrate, with the latter being a pronounced one.

To shed further light on the steric requirement of the amidase involved in *Rhodococcus* sp. AJ270, more α-amino amide substrates were employed to probe the reactivity and enantioselectivity of the enzyme (Scheme 3 and Table 3). In the case of phenylglycine amides bearing a tertiary carbon stereogenic centre, increasing the steric encumbrance of the amino group from NH_2 [(±)-**1n**][14] to NHMe (±)-**1a** or to NMe_2 (±)-**1o** led to a dramatic decrease of conversion. This was shown by the inefficient hydrolysis of α-dimethylamino phenylacetamide (±)-**1o** in a prolonged incubation time with whole-cell catalyst (entry 3). Introduction of an additional methyl group to the stereogenic centre caused the biocatalytic hydrolysis of the corresponding quaternary carbon-centred substrates (±)-**1p**[16] and (±)-**1q** more sluggishly. Only under the conditions using a lower substrate concentration with a long period of interaction with *Rhodococcus* sp. AJ270, did the reaction give 50% conversion of the starting amide (entries 4 and 5). More surprisingly, the amidase displayed a clear-cut enantioselection power depending on the presence of one more methyl group either at the amino nitrogen or the stereogenic carbon centre. This was shown by the observation



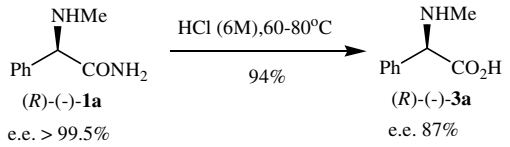
(+/-)-**1a**, **n-q** (*R*)-(-)-**1a**, **n-q** (*S*)-(+)-**2a**, **n-q**

Scheme 3. Biocatalytic hydrolysis of substituted α-amino phenylacetamides. Reagents and condition: (i) *Rhodococcus* sp. AJ270, phosphate buffer pH 7.0, 30 °C.

Table 3. Biocatalytic hydrolysis of substituted α-amino phenylacetamides

Entry	Subs.	Substituents	Conditions[a]	(−)-**1** (%)[b]	(−)-**1** (% ee)[c]	(+)-**2** (%)[b]	(+)-**2** (% ee)[c]	*E*
1	**1n**[14]	R^1 = H, R^2 = H, R^3 = H	2 mmol, 2 h	41	84	51	85	32
2	**1a**	R^1 = H, R^2 = H, R^3 = Me	2 mmol, 60 h	48	93	47	93	94
3	**1o**	R^1 = H, R^2 = Me, R^3 = Me	1 mmol, 168 h	91	14	6	6	—
4	**1p**[16]	R^1 = Me, R^2 = H, R^3 = H	1 mmol, 17.5 h	46	95	50	84	42
5	**1q**	R^1 = Me, R^2 = H, R^3 = Me	0.5 mmol, 168 h	46	17	48	32	2.3

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



Scheme 4. Preparation of optically active (*R*)-(−)-α-methylamino phenylacetic acid.

of high enantiomeric excess values for (*R*)-amides **1a**, **1n**[14] and **1p**[16] and (*S*)-acids **3a** ($E = 94$), **2n** ($E = 32$)[14] and **2p** ($E = 42$)[16] and very low ones for products **1o**, **1q**, **2o** and **2q** ($E = 2.3$). Though it is difficult at this stage to rationalize the origin of the enantioselectivity of the amidase, it is most likely that the synergetic effects of electronic and steric features of the substrates play a subtle but significant role to allow the amidase to show varied enantio-differentiating power. In other words, to achieve an efficient and highly enantioselective biotransformation of α-amino amides, it is advantageous to consider both electronic and steric effects of a substituent when engineering the substrate.

To demonstrate the practical utility of the biocatalytic synthesis of α-methyamino acids, a gram-scale biotransformation of amide was attempted. Thus, to two flasks each containing a suspension of *Rhodococcus* sp. AJ270 whole cells (4 g wet weight) in phosphate buffer (pH 7.0, 0.1 M, 100 ml) was added (±)-**1a** (24 mmol) portion-wise over 5 days. After incubation for another 4 days, highly enantiopure (*S*)-(+)-acid **2a** (1.17 g, 30% yield, ee 96%) was yielded. Chemical hydrolysis of enantiopure amide (*R*)-(−)-**1a** in hydrochloric acid (6 M) afforded acid (*R*)-(−)-**3a** with partial racemization (Scheme 4).

3. Conclusion

In conclusion, we have shown a general and convenient method for the synthesis of optically active (*R*)-(−)-α-methylamino amides and (*S*)-(+)-α-methylamino acids from the kinetic resolution of racemic amides catalyzed by the amidase within *Rhodococcus* sp. AJ270 cells under very mild conditions. The reaction results are heavily dependent upon both the nature of the substituent and its substitution pattern on the substrate. The results obtained from the current and previous studies[13,14,16] have indicated that the amidase involved in the *Rhodococcus* sp. AJ270 cells exhibit a predictive (*S*)-enantioselectivity against both α-mono-substituted- and α,α-disubstituted α-amino arylacetamides irrespective of the nature of the substituents attached on the benzene ring. Coupled with the chemical hydrolysis of amide, this biotransformation process provided a useful approach to α-methylamino acids in both enantiomeric forms from the readily available racemic amino amides. The various highly enantiopure α-amino acids and their amide derivatives obtained from biotransformation should serve as useful chemical entities in organic synthesis and bioorganic chemistry, which is being actively pursued in this laboratory.

4. Experimental

4.1. General

The configurations of optically active α-substituted α-amino acids were determined by the comparison of their direction of optical rotation with that of authentic samples, while the configurations of amides were obtained by correlating optical rotation of their chemically hydrolyzed amino acid products with that of amino acids of known configurations. For unknown optically active amino acids and amides obtained from biotransformation, their configurations were tentatively assigned by the comparison of both their direction of optical rotation and the retention times under identical HPLC analysis conditions using the same chiral stationary phase with that of (*S*)-(+)-methylamino phenylacetic acid **2a** and (*R*)-(−)-α-methylamino phenylacetamide **1a**, respectively, briefly assuming that the introduction of a substituent on the benzene ring did not change the direction of the optical rotation and the order of chromatogram. The enantiomeric excesses of all compounds were obtained with a Shimadzu LC-10AVP HPLC system. A Chirex(D)-Penicillamine column with a mixture (1:9) of 2-propanol and aqueous $CuSO_4$ solution (2 nM) as the mobile phase at a flow rate of 0.5–0.9 ml/min was employed for the analysis of all acids. For amides, a Cyclobond I™ 2000DMP column with a mixture (5:95) of methanol and acetonitrile buffered with acetic acid and triethylamine (100:0.35:0.1) as the mobile phase at a flow rate of 0.3–1.0 ml/min was used.

4.2. General procedure for the biocatalytic kinetic resolution of racemic amides

To an Erlenmeyer flask (100 ml) with a screw cap were added *Rhodococcus* sp. AJ270 cells[15] (2 g wet weight) and potassium phosphate buffer (0.1 M, pH 7.0, 50 ml) and the resting cells activated at 30 °C for 30 min with orbital shaking. Racemic amino acid amides **1** (Tables 1–3) were added in one portion to the flask and the mixture incubated at 30 °C using an orbital shaker (200 rpm). The reaction, monitored by TLC, was quenched after a specified period of time (see Tables 1–3 and Schemes 1–3) by removing the biomass through a Celite pad filtration. The resulting aqueous solution was concentrated to about 10 ml and then acidified to pH 3–4 with 2 M HCl. After the precipitate was removed by filtration, the solution was concentrated again to 5 ml. The products were isolated and purified by chromatography using consecutively a cationic exchange resin column (Dowex, 50 × 8), a reverse phase silica gel column (35–70 μm) and a Sephadex G-25 column. All products were characterized by their spectral 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.

4.2.1. Enzymatic hydrolysis of racemic α-methylamino phenylacetamide 1a. (*R*)-(−)-α-Methylamino phenylacetamide **1a**: mp 176–177 °C; $[\alpha]_D^{25} = -98.4$ (*c* 0.5, MeOH); ee 99% (HPLC, $t_R = 8.9$ min, $t_S = 11.5$ min, flow rate

0.8 ml/min); IR (KBr) ν 3286, 1690 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.43–7.32 (m, 5H), 6.93 (s, 1H), 5.44 (s, 1H), 4.06 (s, 1H), 2.47 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 174.9, 139.1, 128.9, 128.2, 127.3, 69.8, 35.4; MS (ESI) *m/z* (%) 165 $[M+1]^+$ (100). Anal. Calcd for $C_9H_{12}N_2O$: C, 65.83; H, 7.36; N, 17.06. Found: C, 65.80; H, 7.32; N, 17.06.

(*S*)-(+)-α-Methylamino phenylacetic acid **2a**: mp 207 °C (dec); $[\alpha]_D^{25} = +116.4$ (*c*, 0.5, 1 M HCl) {lit.[7] $[\alpha]_D^{25} = +130$ (*c*, 0.5, 1 M HCl)}; ee 96% (HPLC, $t_R = 12.8$ min, $t_S = 9.4$ min, flow rate 0.8 ml/min); IR (KBr) ν 2310–3061, 1628, 1593 cm^{-1}; ^1H NMR (300 MHz, $D_2O + CF_3CO_2D$) δ 7.34–7.25 (m, 5H), 4.42 (s, 1H), 2.42 (s, 3H); ^{13}C NMR ($D_2O + CF_3CO_2D$) δ 170.4, 130.0, 127.8, 127.4, 126.4, 64.7, 28.9; MS (ESI) *m/z* 166 $[M+1]^+$ (100). Anal. Calcd for $[C_9H_{12}NO_2]^+$: 166.0862. Found: 166.0863.

4.2.2. Enzymatic hydrolysis of racemic α-methylamino 4-fluorophenylacetamide 1b. (*R*)-(−)-α-Methylamino 4-fluorophenylacetamide **1b**: mp 135–137 °C; $[\alpha]_D^{25} = -82.0$ (*c* 0.5, MeOH); ee 93% (HPLC, $t_R = 8.2$ min, $t_S = 9.8$ min, flow rate 0.8 ml/min); IR (KBr) ν 3285, 3033, 1697 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.41–7.36 (m, 2H), 7.01 (t, $J = 4.7$ Hz, 2H), 6.94 (s, 1H), 5.54 (s, 1H), 4.04 (s, 1H), 2.46 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 173.9, 163.0, 159.8, 136.4, 129.1, 114.9, 114.6, 67.2, 34.2; MS (ESI) *m/z* (%) 183 $[M+1]^+$ (100). Anal. Calcd for $C_9H_{11}FN_2O$: C, 59.33; H, 6.09; N, 15.38. Found: C, 59.36; H, 6.08; N, 15.35.

(*S*)-(+)-α-Methylamino 4-fluorophenylacetic acid **2b**: mp 211 °C (dec); $[\alpha]_D^{25} = +78.4$ (*c* 0.5, 1 M HCl); ee 83% (HPLC, $t_R = 18.8$ min, $t_S = 12.8$ min, flow rate 0.7 ml/min); IR (KBr) ν 2319–3055, 1605, 1590 cm^{-1}; ^1H NMR (300 MHz, $D_2O + CF_3CO_2D$) δ 7.31–7.26 (m, 2H), 7.05 (t, $J = 8.8$ Hz, 2H), 4.44 (s, 1H), 2.42 (s, 3H); ^{13}C NMR ($D_2O + CF_3CO_2D$) δ 170.1, 165.9, 162.6, 131.3, 125.2, 117.5, 117.1, 63.9, 30.8; MS (ESI) *m/z* (%) 184 $[M+1]^+$ (100). Anal. Calcd for $[C_9H_{10}FNO_2]^+$: 184.0768. Found: 184.0768.

4.2.3. Enzymatic hydrolysis of racemic α-methylamino 4-chlorophenylacetamide 1c. (*R*)-(−)-α-Methylamino 4-chlorophenylacetamide **1c**: mp 150–152 °C; $[\alpha]_D^{25} = -21.0$ (*c* 0.5, MeOH); ee 57% (HPLC, $t_R = 8.0$ min, $t_S = 9.4$ min, flow rate 0.8 ml/min); IR (KBr) ν 3290, 3021, 1697 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.37–7.30 (m, 4H), 6.92 (s, 1H), 5.53 (s, 1H), 4.04 (s, 1H), 2.46 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 173.9, 136.9, 133.6, 128.5, 128.1, 68.6, 34.8; MS (ESI) *m/z* (%) 201 $[M+2+1]^+$ (36), 199 $[M+1]^+$ (100). Anal. Calcd for $C_9H_{11}ClN_2O$: C, 54.42; H, 5.58; N, 14.10. Found: C, 54.25; H, 5.63; N, 13.75.

(*S*)-(+)-α-Methyl 4-chlorophenylacetic acid **2c**: mp 219 °C (dec); $[\alpha]_D^{25} = +67.3$ (*c* 0.5, 1 M HCl); ee 85% (HPLC, $t_R = 59.5$ min, $t_S = 36.1$ min, flow rate 0.9 ml/min); IR (KBr) ν 2662–3061, 1637, 1585 cm^{-1}; ^1H NMR (300 MHz, $D_2O + CF_3CO_2D$) δ 7.34 (d, $J = 6.5$ Hz, 2H), 7.25 (d, $J = 6.7$ Hz, 2H), 4.44 (s, 1H), 2.43 (s, 3H); ^{13}C NMR ($D_2O + CF_3CO_2D$) δ 168.2, 135.7, 128.6, 125.8, 62.6, 29.1; MS (ESI) *m/z* (%) 202 (33), 200 $[M+1]^+$ (100). Anal. Calcd for $[C_9H_{12}ClNO_2]^+$: 200.0473. Found: 200.0474.

4.2.4. Enzymatic hydrolysis of racemic α-methylamino 3-chlorophenylacetamide 1d. (*R*)-(−)-α-Methylamino 3-chlorophenylacetamide **1d**: mp 120–121 °C; $[\alpha]_D^{25} = -0.8$ (*c* 1.5, MeOH); ee 5% (HPLC analysis of the corresponding acid, $t_R = 65.9$ min, $t_S = 44.9$ min, flow rate 0.7 ml/min); IR (KBr) ν 3285, 3057, 1691, 1668 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.41–7.27 (m, 4H), 6.91 (s, 1H), 5.44 (s, 1H), 4.04 (s, 1H), 2.47 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 174.1, 140.9, 134.7, 130.1, 128.5, 127.4, 125.6, 69.3, 35.3; MS (ESI) *m/z* (%) 201 $[M+2+1]^+$ (39), 199 $[M+1]^+$ (100). Anal. Calcd for $C_9H_{11}ClN_2O$: C, 54.42; H, 5.58; N, 14.10. Found: C, 54.35; H, 5.71; N, 13.79.

(*S*)-(+)-α-Methylamino 3-chlorophenylacetic acid **2d**: mp 198 °C (dec); $[\alpha]_D^{25} = +14.9$ (*c* 1.5, 1 M HCl); ee 15% (HPLC, $t_R = 65.9$ min, $t_S = 44.9$ min, flow rate 0.7 ml/min); IR (KBr) ν 2415–3061, 1590 cm^{-1}; ^1H NMR (300 MHz, $D_2O + CF_3CO_2D$) δ 7.38–7.19 (m, 4H), 4.43 (s, 1H), 2.44 (s, 3H); ^{13}C NMR ($D_2O + CF_3CO_2D$) δ 168.4, 134.2, 130.3, 130.0, 129.7, 127.7, 125.9, 62.8, 29.7; MS (ESI) *m/z* (%) 202 (68), 200 $[M+1]^+$ (100). Anal. Calcd for $[C_9H_{11}ClNO_2]^+$: 200.0473. Found: 200.0474.

4.2.5. Enzymatic hydrolysis of racemic α-methylamino 2-chlorophenylacetamide 1e. (*R*)-(−)-α-Methylamino 2-chlorophenylacetamide **1e**: mp 118–120 °C; $[\alpha]_D^{25} = -2.4$ (*c* 3.0, MeOH); ee <3% (HPLC, $t_R = 18.6$ min, $t_S = 22.9$ min, flow rate 0.3 ml/min); IR (KBr) ν 3219, 3038, 1701 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.40–7.23 (m, 4H), 7.04 (s, 1H), 6.39 (s, 1H), 4.50 (s, 1H), 2.43 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 174.4, 136.7, 133.9, 130.0, 129.4, 129.3, 127.3, 65.7, 35.3; MS (ESI) *m/z* (%) 201 $[M+2+1]^+$ (41), 199 $[M+1]^+$ (100). Anal. Calcd for $C_9H_{11}ClN_2O$: C, 54.42; H, 5.58; N, 14.10. Found: C, 54.26; H, 5.64; N, 13.73.

(*S*)-(+)-α-Methylamino 2-chlorophenylacetic acid **2e**: mp 224 °C (dec); $[\alpha]_D^{25} = +46.1$ (*c* 0.5, 1 M HCl); ee 33% (HPLC, $t_R = 33.6$ min, $t_S = 22.4$ min, flow rate 0.8 ml/min); IR (KBr) ν 2312–3056, 1632 cm^{-1}; ^1H NMR (300 MHz, $D_2O + CF_3CO_2D$) δ 7.43–7.26 (m, 4H), 4.89 (s, 1H), 2.48 (s, 3H); ^{13}C NMR ($D_2O + CF_3CO_2D$) δ 171.7, 134.2, 131.5, 130.5, 130.3, 129.9, 128.1, 63.6, 31.4; MS (ESI) *m/z* (%) 202 (33), 200 $[M+1]^+$ (100). Anal. Calcd for $[C_9H_{11}ClNO_2]^+$: 200.0473. Found: 200.0474.

4.2.6. Enzymatic hydrolysis of racemic α-methylamino 4-bromophenylacetamide 1f. (*R*)-(−)-α-Methylamino 4-bromophenylacetamide **1f**: mp 164–166 °C; $[\alpha]_D^{25} = -7.8$ (*c* 2.5, MeOH); ee 6% (HPLC, $t_R = 8.1$ min, $t_S = 9.2$ min, flow rate 0.8 ml/min); IR (KBr) ν 3420, 3288, 1669 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.48 (d, $J = 8.3$ Hz, 2H), 7.29 (d, $J = 8.4$ Hz, 2H), 6.91 (s, 1H), 5.42 (s, 1H), 4.03 (s, 1H), 2.46 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 173.6, 139.6, 130.9, 129.4, 120.2, 67.3, 34.1; MS (ESI) *m/z* (%) 245 $[M+2+1]^+$ (97), 243 $[M+1]^+$

(100). Anal. Calcd for $C_9H_{11}BrN_2O$: C, 44.47; H, 4.56; N, 11.52. Found: C, 44.19; H, 4.53; N, 11.39.

(*S*)-(+)-α-Methylamino 4-bromophenylacetic acid **2f**: mp 215 °C (dec); $[\alpha]_D^{25} = +19.4$ (*c* 1.0, 1 M HCl); ee 16% (HPLC, $t_R = 88.4$ min, $t_S = 51.4$, flow rate 0.8 ml/min); IR (KBr) *v* 2250–3078, 1586 cm^{-1}; ^1H NMR (300 MHz, $D_2O + CF_3CO_2D$) δ 7.50 (d, $J = 8.4$ Hz, 2H), 7.18 (d, $J = 8.4$ Hz, 2H), 4.43 (s, 1H), 2.42 (s, 3H); ^{13}C NMR ($D_2O + CF_3CO_2D$) δ 168.4, 131.8, 129.1, 126.6, 123.8, 62.7, 29.3; MS (ESI) *m/z* (%) 246 $[M+2+1]^+$ (96), 244 $[M+1]^+$ (100). Anal. Calcd for $C_9H_{10}BrNO_2$: C, 44.26; H, 4.10; N, 5.74. Found: C, 44.21; H, 4.27; N, 5.93.

4.2.7. Enzymatic hydrolysis of racemic α-methylamino 4-methylphenylacetamide 1g. (*R*)-(−)-α-Methylamino 4-methylacetamide **1g**: mp 166–167 °C; $[\alpha]_D^{25} = -81.6$ (*c* 0.5, MeOH); ee 85% (HPLC, $t_R = 9.7$ min, $t_S = 11.7$ min, flow rate 0.8 ml/min); IR (KBr) *v* 3291, 3025, 1697 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.34–7.14 (m, 4H), 6.91 (s, 1H), 5.42 (s, 1H), 4.02 (s, 1H), 2.46 (s, 3H), 2.33 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 174.2, 137.2, 136.2, 128.5, 127.1, 67.8, 34.2, 20.6; MS (ESI) *m/z* (%) 179 $[M+1]^+$ (100). Anal. Calcd for $C_{10}H_{14}N_2O$: C, 67.39; H, 7.92; N, 15.72. Found: C, 67.66; H, 8.01; N, 15.61.

(*S*)-(+)-α-Methylamino 4-methylphenylacetic acid **2g**: mp 196 °C (dec); $[\alpha]_D^{25} = +50.1$ (*c* 1.0, 1 M HCl); ee 57% (HPLC, $t_R = 23.2$ min, $t_S = 46.5$ min, flow rate 0.7 ml/min); IR (KBr) *v* 2319–3052, 1616, 1597 cm^{-1}; ^1H NMR (300 MHz, $D_2O + CF_3CO_2D$) δ 7.19–7.13 (m, 4H), 4.70 (s, 1H), 2.44 (s, 3H), 2.26 (s, 3H); ^{13}C NMR ($D_2O + CF_3CO_2D$) δ 169.4, 140.8, 129.5, 127.6, 124.9, 63.3, 29.6, 19.2; MS (ESI) *m/z* 180 $[M+1]^+$ (100). Anal. Calcd for $[C_{10}H_{14}NO_2]^+$: 180.1019. Found: 180.1020.

4.2.8. Enzymatic hydrolysis of racemic α-methylamino 3-methylphenylacetamide 1h. (*R*)-(+)-α-Methylamino 3-methylphenylacetamide **1h**: mp 131–132 °C; $[\alpha]_D^{25} = -68.0$ (*c* 0.5, MeOH); ee 76% (HPLC, $t_R = 4.84$ min, $t_S = 7.32$ min, flow rate 1.0 ml/min); IR (KBr) 3290, 3065, 1688, 1668 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.26–7.10 (m, 4H), 6.95 (s, 1H), 5.50 (s, 1H), 4.01 (s, 1H), 2.46 (s, 3H), 2.35 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 175.3, 139.0, 138.5, 129.0, 128.7, 128.0, 124.2, 69.8, 35.5, 21.4; MS (ESI) *m/z* (%) 179 $[M+1]^+$ (100). Anal. Calcd for $C_{10}H_{14}N_2O$: C, 67.39; H, 7.92; N, 15.72. Found: C, 67.66; H, 8.01; N, 15.83.

(*S*)-(+)-α-Methylamino 3-methylphenylacetic acid **2h**: mp 200 °C (dec); $[\alpha]_D^{25} = +83.2$ (*c* 0.5, 1 M HCl); ee 81% (HPLC, $t_R = 33.1$ min, $t_S = 21.4$ min, flow rate 0.8 ml/min); IR (KBr) *v* 2313–3061, 1606, 1584 cm^{-1}; ^1H NMR (300 MHz, $D_2O + CF_3CO_2D$) δ 7.23–7.03 (m, 4H), 4.35 (s, 1H), 2.39 (s, 3H), 2.17 (s, 3H); ^{13}C NMR ($D_2O + CF_3CO_2D$) δ 172.6, 139.8, 132.0, 130.4, 129.3, 129.0, 125.4, 66.7, 30.8, 20.3; MS (ESI) *m/z* (%) 180 $[M+1]^+$ (100). Anal. Calcd for $[C_{10}H_{14}NO_2]^+$: 180.1019. Found: 180.1019.

4.2.9. Enzymatic hydrolysis of racemic α-methylamino 2-methylphenylacetamide 1i. (*R*)-(−)-α-Methylamino 2-methylphenylacetamide **1i**: mp 118–119 °C; $[\alpha]_D^{25} = -0.3$ (*c* 3.0, MeOH); ee 4% (HPLC, $t_R = 15.6$ min, $t_S = 17.4$ min, flow rate 0.3 ml/min); IR (KBr) *v* 3418, 3319, 3147, 1682 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.37–7.16 (m, 4H), 7.02 (s, 1H), 5.57 (s, 1H), 4.30 (s, 1H), 2.47 (d, $J = 6.7$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 175.0, 137.7, 136.6, 130.8, 127.9, 126.7, 126.5, 65.9, 35.8, 19.7; MS (ESI) *m/z* (%) 179 $[M+1]^+$ (100). Anal. Calcd for $C_{10}H_{14}N_2O$: C, 67.39; H, 7.92; N, 15.72. Found: C, 67.52; H, 7.93; N, 15.65.

(*S*)-(+)-α-Methylamino 2-methylphenylacetic acid **2i**: mp 235 °C (dec); $[\alpha]_D^{25} = +12.9$ (*c* 1.5, 1 M HCl); ee 13% (HPLC, $t_R = 21.6$ min, $t_S = 15.4$ min, flow rate 0.8 ml/min); IR (KBr) *v* 2342–3057, 1630, 1613, 1569 cm^{-1}; ^1H NMR (300 MHz, $D_2O + CF_3CO_2D$) δ 7.14 (s, 4H), 4.37 (s, 1H), 2.39 (s, 3H), 2.17 (s, 3H); ^{13}C NMR ($D_2O + CF_3CO_2D$) δ 169.4, 137.2, 130.8, 129.7, 126.4, 125.4, 59.5, 29.5, 17.3; MS (ESI) *m/z* 180 $[M+1]^+$ (100). Anal. Calcd for $[C_{10}H_{14}NO_2]^+$: 180.1019. Found: 180.1019.

4.2.10. Enzymatic hydrolysis of racemic α-methylamino 4-methoxyphenylacetamide 1j. (*R*)-(−)-α-Methylamino 4-methoxyphenylacetamide **1j**: mp 172–173 °C; $[\alpha]_D^{25} = -75.9$ (*c* 0.5, MeOH); ee 95% (HPLC, $t_R = 11.3$ min, $t_S = 13.4$ min, flow rate 0.8 ml/min); IR (KBr) *v* 3286, 3255, 1695, 1666 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.32 (d, $J = 8.7$ Hz, 2H), 6.89 (d, $J = 9.0$ Hz, 2H), 6.85 (s, 1H), 5.36 (s, 1H), 4.01 (s, 1H), 3.80 (s, 3H), 2.45 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 174.3, 158.4, 132.2, 128.3, 113.4, 67.4, 55.0, 34.2; MS (ESI) *m/z* (%) 195 $[M+1]^+$ (100). Anal. Calcd for $C_{10}H_{14}N_2O_2$: C, 61.84; H, 7.27; N, 14.41. Found: C, 61.47; H, 7.24; N, 14.31.

(*S*)-(+)-α-Methylamino 4-methoxyphenylacetic acid **2j**: mp 177 °C (dec); $[\alpha]_D^{25} = +131.8$ (*c* 0.5, 1 M HCl); ee 76% (HPLC, $t_R = 17.5$ min, $t_S = 11.8$ min, flow rate 0.8 ml/min); IR (KBr) *v* 2243–3059, 1615, 1584 cm^{-1}; ^1H NMR (300 MHz, $D_2O + CF_3CO_2D$) δ 7.19 (d, $J = 6.7$ Hz, 2H), 6.88 (d, $J = 6.7$ Hz, 2H), 4.33 (s, 1H), 3.67 (s, 3H), 2.37 (s, 3H); ^{13}C NMR δ 173.4, 159.9, 130.1, 125.3, 114.9, 66.5, 55.5, 31.1; MS (ESI) *m/z* 196 $[M+1]^+$ (100). Anal. Calcd for $C_{10}H_{13}NO_3$: C, 61.53; H, 6.71; N, 7.18. Found: C, 61.52; H, 6.69; N, 7.58.

4.2.11. Enzymatic hydrolysis of racemic α-methyl-β-phenylpropionamide 1k. (−)-α-3-Methylamino-β-phenylpropionamide **1k**: mp 133–134 °C; $[\alpha]_D^{25} = -66.3$ (*c* 0.5, MeOH); ee 73% (HPLC analysis of the corresponding acid, $t_{(-)} = 33.3$ min, $t_{(+)} = 28.5$ min, flow rate 0.8 ml/min); IR (KBr) 3279, 3228, 1683, 1662 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.36–7.22 (m, 5H), 7.01 (s, 1H), 5.33 (s, 1H), 3.25–3.19 (m, 2H), 2.73 (dd, $J = 14.6$, 10.4 Hz, 1H), 2.31 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 176.7, 137.5, 129.1, 128.8, 127.0, 66.1, 39.2, 35.5; MS (ESI) *m/z* (%) 179 $[M+1]^+$ (100). Anal. Calcd for $C_{10}H_{14}N_2O$: C, 67.39; H, 7.92; N, 15.72. Found: C, 67.39; H, 7.95; N, 15.81.

(+)-α-Methylamino-β-phenylpropionic acid **2k**: mp 192 °C (dec); $[\alpha]_D^{25} = +27.8$ (*c* 1.5, 1 M HCl); ee 56% (HPLC, $t_{(-)} = 33.1$ min, $t_{(+)} = 28.5$ min, flow rate 0.8 ml/min); IR (KBr) *v* 2310–3060, 1588 cm^{-1}; ^1H NMR (300 MHz, $D_2O + CF_3CO_2D$) δ 7.24–7.10 (m, 5H), 3.66 (t, $J = 6.2$ Hz, 1H), 3.03 (d, $J = 6.2$ Hz, 2H), 2.49 (s, 3H); ^{13}C NMR δ 169.2, 132.1, 128.2, 127.2, 61.1, 33.7, 30.8; MS (ESI) *m/z* (%)180 $[M+1]^+$ (100). Anal. Calcd for $[C_{10}H_{15}NO_2]^+$: 180.1019. Found: 180.1020.

4.2.12. Enzymatic hydrolysis of racemic α-methylamino-β-methylbutyramide 1l. (−)-α-Methylamino-β-methylbutyramide **1l**: mp 83–84 °C; $[\alpha]_D^{25} = -0.5$ (*c* 1.5, MeOH); ee 21% (HPLC analysis of the corresponding acid, $t_{(-)} = 20.7$ min, $t_{(+)} = 17.5$ min, flow rate 0.5 ml/min); IR (KBr) *v* 3283, 3084, 1698, 1664 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.96 (s, 1H), 5.41 (s, 1H), 2.77 (d, $J = 4.7$ Hz, 1H), 2.41 (s, 3H), 2.11–2.00 (m, 1H), 1.01 (d, $J = 7.0$ Hz, 3H), 0.94 (d, $J = 7.0$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 177.0, 70.9, 36.1, 31.3, 19.5, 18.0; MS (ESI) *m/z* (%) 131 $[M+1]^+$ (100). Anal. Calcd for $C_6H_{14}N_2O$: C, 55.35; H, 10.84; N, 21.52. Found: C, 55.15; H, 10.65; N, 21.53.

(+)-α-Methylamino-β-methylbutyric acid **2l**: mp 219 °C (dec); $[\alpha]_D^{25} = +8.8$ (*c* 0.5, 1 M HCl); ee 76% (HPLC, $t_{(-)} = 20.7$ min, $t_{(+)} = 17.5$ min, flow rate 0.5 ml/min); IR (KBr) *v* 2309–3050, 1580 cm^{-1}; ^1H NMR (300 MHz, $D_2O + CF_3CO_2D$) δ 3.59–3.54 (m, 1H), 2.64–2.60 (m, 3H), 2.18–2.14 (m, 1H), 0.96–0.84 (m, 6H); ^{13}C NMR ($D_2O + CF_3CO_2D$) δ 168.8, 66.0, 31.3, 27.7, 16.1, 14.9; MS (ESI) *m/z* (%) 132 $[M+1]^+$ (100). Anal. Calcd for $C_6H_{13}NO_2$: C, 54.94; H, 9.99; N, 10.68. Found: C, 54.44; H, 9.86; N, 10.61.

4.2.13. Enzymatic hydrolysis of racemic α-methylamino-α-cyclohexylacetamide 1m. (−)-α-Methylamino-α-cyclohexylacetamide **1m**: mp 160–161.5 °C; $[\alpha]_D^{25} = -2.6$ (*c* 2.5, MeOH); ee 39% (HPLC analysis of the corresponding acid, $t_{(-)} = 64.3$ min, $t_{(+)} = 53.2$ min, flow rate 0.5 ml/min); IR (KBr) *v* 3312, 3144, 1670, 1618 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.97 (s, 1H), 6.14 (s, 1H), 2.76 (d, $J = 4.3$ Hz, 1H), 2.40 (m, 3H), 1.73–1.05 (m, 11H); ^{13}C NMR ($CDCl_3$) δ 176.9, 70.8, 41.3, 36.1, 30.1, 28.5, 26.33, 26.26, 26.22; MS (ESI) *m/z* (%) 171 $[M+1]^+$ (100). Anal. Calcd for $C_9H_{18}N_2O$: C, 63.49; H, 10.66; N, 16.45. Found: C, 63.40; H, 10.58; N, 16.50.

(+)-α-Methylamino-α-cyclohexylacetic acid **2m**: mp 245 °C (dec); $[\alpha]_D^{25} = +46.2$ (*c* 1.0, 1 M HCl); ee 96% (HPLC, $t_{(-)} = 64.3$ min, $t_{(+)} = 53.2$ min, flow rate 0.5 ml/min); IR (KBr) *v* 2310–3061, 1581 cm^{-1}; ^1H NMR (300 MHz, $D_2O + CF_3CO_2D$) δ 3.51–3.50 (m, 1H), 2.63–2.50 (m, 3H), 1.80–0.90 (m, 11H); ^{13}C NMR ($D_2O + CF_3CO_2D$) δ 169.1, 65.9, 37.5, 31.6, 27.4, 26.6, 24.3, 24.1, 23.9; MS (ESI) *m/z* (%) 172 $[M+1]^+$ (100). Anal. Calcd for $[C_9H_{18}NO_2]^+$: 172.1331. Found: 172.1333.

4.2.14. Enzymatic hydrolysis of racemic α-dimethylamino phenylacetamide 1o. (*R*)-(−)-α-Dimethylamino phenylacetamide **1o**: mp 160–161.5 °C; $[\alpha]_D^{25} - 7.5$ (*c* 0.5, MeOH); ee 14% (HPLC, $t_R = 6.5$ min, $t_S = 13.4$ min, flow rate 0.75 ml/min); IR (KBr) 3349, 3177, 1655 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.37–7.28 (m 5H), 6.95 (s, 1H), 5.72 (s, 1H), 3.73 (s, 1H), 2.23 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 174.5, 136.2, 128.8, 128.5, 128.2, 43.9; MS (ESI) *m/z* (%) 179 $[M+1]^+$ (100). Anal. Calcd for $C_{10}H_{14}N_2O$: C, 67.39; H, 7.92; N, 15.72. Found: C, 67.62; H, 7.84; N, 15.57.

(*S*)-(+)-α-Dimethylamino phenylacetic acid **2o**: mp 236 °C (dec); $[\alpha]_D^{25} = +5.5$ (*c* 0.5, 1 M HCl) {lit.[7] (*R*)-(−)-α-dimethylamino-phenylacetic acid: $[\alpha]_D^{25} = -122$ (*c*, 1.0, H_2O)}; ee 6% (HPLC, $t_R = 16.5$ min, $t_S = 7.8$ min, flow rate 0.8 ml/min); IR (KBr) *v* 2320–3066, 1628 cm^{-1}; ^1H NMR (300 MHz, $D_2O + CF_3CO_2D$) δ 6.70–6.67 (m, 5H), 4.23 (s, 1H), 2.21 (s, 3H), 1.78 (s, 3H); ^{13}C NMR ($D_2O + CF_3CO_2D$) δ 167.6, 129.9, 128.3, 127.6, 125.7, 70.5, 41.6, 38.6; MS (ESI) *m/z* (%) 180 $[M+1]^+$ (100). Anal. Calcd for $[C_{10}H_{14}NO_2]^+$: 180.1019. Found: 180.1019.

4.2.15. Enzymatic hydrolysis of racemic α-methylamino-α-phenylpropionamide 1q. (−)-α-Methylamino-α-phenylpropionamide **1q**: mp 90–91 °C; $[\alpha]_D^{25} = -3.8$ (*c* 0.5, MeOH); ee 17% (HPLC analysis of the corresponding acid, $t_{(-)} = 28.5$ min, $t_{(+)} = 25.0$ min, flow rate 0.5 ml/min); IR (KBr) *v* 3419, 3212, 1643 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.61–7.25 (m, 5H), 6.92 (s, 1H), 5.53 (s, 1H), 2.30 (s, 3H), 1.73 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 173.9, 136.9, 133.6, 128.5, 128.1, 68.6, 34.8; MS (ESI) *m/z* (%) 199 $[M+1]^+$ (100). Anal. Calcd for $C_{10}H_{14}N_2O$: C, 67.39; H, 7.92; N, 15.72. Found: C, 67.62; H, 7.84; N, 15.57.

(+)-α-Methylamino-α-phenylpropionic acid **2q**: mp 208 °C (dec); $[\alpha]_D^{25} = +30.3$ (*c* 0.5, 1 M HCl); ee 32% (HPLC, $t_{(-)} = 28.5$ min, $t_{(+)} = 25.9$ min, flow rate 0.5 ml/min); IR (KBr) *v* 3476, 3186, 2494–3039, 1631, 1616 cm^{-1}; ^1H NMR (300 MHz, $D_2O + CF_3CO_2D$) δ 7.32 (s, 5H), 2.21 (s, 3H), 1.71 (s, 3H); ^{13}C NMR ($D_2O + CF_3CO_2D$) δ 171.9, 130.7, 129.4, 128.5, 125.2, 65.8, 25.9, 15.8; MS (ESI) *m/z* (%) 180 $[M+1]^+$ (100). Anal. Calcd for $[C_{10}H_{14}NO_2]^+$: 180.1019. Found: 180.1020.

4.3. Chemical hydrolysis of (*R*)-(−)-α-methylamino phenylacetamide 1a

A mixture of (*R*)-(−)-α-methylamino phenylacetamide (−)-**1a** (100 mg, ee >99.5%) in 6 M HCl (10 ml) was stirred at 60 °C for 5 h and then was kept at 80 °C for another 5 h. The acid product was purified using a cationic exchange resin column (Dowex, 50 × 8) and a Sephadex G-25 column to give (*R*)-(−)-α-methylamino phenylacetic acid (+)-**2a**: 94.1 mg (94%); mp 213 °C; $[\alpha]_D^{25} = -101.7$ (*c* 0.5, 1 M HCl) {lit.[7] $[\alpha]_D^{25} = -171$ (*c*, 0.5, 1 M HCl)}, ee 87% (HPLC, $t_R = 12.8$ min, $t_S = 9.4$ min, flow rate 0.8 ml/min). The spectral data are identical with those of (*S*)-(+)-**3a**.

Acknowledgements

We thank the State Major Fundamental Research Program (2003CB716005, G2000077506), the Ministry

of Science and Technology (Grant No. 2002CCA03100), the National Science Foundation of China, and the Chinese Academy of Sciences for financial support. M.-X. Wang also thanks O. Meth-Cohn, J. Colby and C. O'Reilly for discussion.

References

1. Fairlie, D. P.; Abbenante, G.; March, D. R. *Curr. Med. Chem.* **1995**, *2*, 654.
2. (a) Cody, W. L.; He, J. X.; Reily, M. D.; Haleen, S. J.; Walker, D. M.; Reyner, E. L.; Stewart, B. H.; Doherty, A. M. *J. Med. Chem.* **1997**, *40*, 2228; (b) Haviv, F.; Fitzpatrick, T. D.; Swenson, R. E.; Nicholas, C. J.; Mort, N. A.; Bush, E. U.; Diaz, G.; Barmmert, G.; Nguyen, A.; Nellans, H. N.; Hoffman, D. J.; Johnson, E. S.; Greer, J. *J. Med. Chem.* **1993**, *36*, 363; (c) Vitoux, B.; Aubry, A.; Cung, M. T.; Marraud, M. *Int. J. Pept. Protein Res.* **1986**, *26*, 617.
3. (a) Olsen, R. K. *J. Org. Chem.* **1970**, *35*, 1912; (b) Coggins, J. R.; Benoiton, N. L. *Can. J. Chem.* **1971**, *49*, 1968; (c) McDermott, J. R.; Benoiton, N. L. *Can. J. Chem.* **1973**, *51*, 2562, and references cited therein; (d) Kitagawa, I.; Lee, N. K.; Kobayashi, M.; Shibuya, H. *Tetrahedron* **1991**, *47*, 2169; (e) Prashad, M.; Har, D.; Hu, B.; Kim, H.-Y.; Repic, O.; Blacklock, T. J. *Org. Lett.* **2003**, *5*, 125.
4. Dorow, R. L.; Gingrich, D. E. *J. Org. Chem.* **1995**, *60*, 4986.
5. (a) Chu, K. S.; Negrete, G. R.; Konopelski, J. P. *J. Org. Chem.* **1991**, *56*, 5196; (b) Luke, R. W. A.; Boyce, P. G. T.; Dorling, E. K. *Tetrahedron Lett.* **1996**, *37*, 263.
6. (a) Freidinger, R. M.; Hinkle, J. S.; Perlow, D. S.; Arison, B. H. *J. Org. Chem.* **1983**, *48*, 77; (b) Aurelio, L.; Brownlee, R. T. C.; Hughes, A. B. *Aust. J. Chem.* **2000**, *53*, 425; (c) Aurelio, L.; Box, J. S.; Brownlee, R. T. C.; Hughes, A. B.; Sleebs, M. M. *J. Org. Chem.* **2003**, *68*, 2652.
7. Shiraiwa, T.; Baba, Y.; Miyazaki, H.; Sakata, S.; Kawamura, S.; Uehara, M.; Kurokawa, H. *Bull. Chem. Soc. Jpn.* **1993**, *66*, 1430, and references cited therein.
8. Groeger, U.; Drauz, K.; Klenk, H. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 195.
9. Kobayashi, M.; Shimizu, S. *FEMS Microbiol. Lett.* **1994**, *120*, 217, and references cited therein.
10. (a) Meth-Cohn, O.; Wang, M.-X. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1099–1104; (b) Meth-Cohn, O.; Wang, M.-X. *J. Chem. Soc., Perkin Trans. 1* **1997**, 3197, and references cited therein.
11. Nagasawa, T.; Schimizu, H.; Yamada, H. *Appl. Microbiol. Biotechnol.* **1993**, *40*, 189.
12. For a recent review, see: Sugai, T.; Yamazaki, T.; Yokoyama, M.; Ohta, H. *Biosci. Biotechnol. Biochem.* **1997**, *61*, 1419, and references cited therein.
13. (a) Wang, M.-X.; Lin, S.-J.; Liu, C.-S.; Zheng, Q.-Y.; Li, J.-S. *J. Org. Chem.* **2003**, *68*, 4570; (b) Wang, M.-X.; Feng, G.-Q. *J. Org. Chem.* **2003**, *68*, 621; (c) Wang, M.-X.; Wu, Y. *Org. Biomol. Chem.* **2003**, 535; (d) Wang, M.-X.; Feng, G.-Q.; Zheng, Q.-Y. *Adv. Synth. Catal.* **2003**, *345*, 695; (e) Wang, M.-X.; Zhao, S.-M. *Tetrahedron: Asymmetry* **2002**, *13*, 1695; (f) Wang, M.-X.; Li, J.-J.; Ji, G.-J.; Li, J.-S. *J. Mol. Catal. B: Enzyme* **2001**, *14*, 77; (g) Wang, M.-X.; Liu, C.-S.; Li, J.-S. *Tetrahedron: Asymmetry* **2001**, *12*, 3367; (h) Wang, M.-X.; Lu, G.; Ji, G.-J.; Huang, Z.-T.; Meth-Cohn, O.; Colby, J. *Tetrahedron: Asymmetry* **2000**, *11*, 1123; (i) Wang, M.-X.; Feng, G.-Q. *New J. Chem.* **2002**, 1575; (j) Wang, M.-X.; Feng, G.-Q.; Zheng, Q.-Y. *Tetrahedron: Asymmetry* **2004**, *15*, 347.
14. Wang, M.-X.; Lin, S.-J. *J. Org. Chem.* **2002**, *67*, 6542.
15. Blakey, A. J.; Colby, J.; Williams, E.; O'Reilly, C. *FEMS Microbiol. Lett.* **1995**, *129*, 57.
16. Wang, M.-X.; Lin, S.-J.; Liu, J.; Zheng, Q.-Y. *Adv. Synth. Catal.* **2004**, *346*, 439.
17. (a) Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294; (b) Program 'Selectivity' by Faber, K.; Hoenig, H. http://www.cis.TUGraz.at/orgc/.