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An efficient new enzymatic method for the preparation of β-aryl-β-amino acid enantiomers

Gábor Tasnádi [a], Enikő Forró [a,*], Ferenc Fülöp [a,b,*]

[a] *Institute of Pharmaceutical Chemistry, University of Szeged, H-6720 Szeged, Eötvös u. 6, Hungary*
[b] *Research Group for Stereochemistry, Hungarian Academy of Sciences, University of Szeged, H-6720 Szeged, Eötvös u. 6, Hungary*

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ABSTRACT

An efficient synthesis of β-aryl-β-amino acid enantiomers has been developed via the lipase-catalysed enantioselective hydrolysis of the corresponding racemic ethyl esters in an organic solvent. High enantioselectivities (E >100) were observed when the lipase PS-catalysed reactions were performed with H_2O (0.5 equiv) in diisopropyl ether at 45 °C. The products could be easily separated and were obtained in good yields of ⩾40%.

1. Introduction

β-Amino acids, including β-aryl-substituted β-amino acids, are widely used in drug research, and their pharmacological significance has been described.[1] There are several methods for the synthesis of enantiopure β-aryl-β-amino acids, such as (i) the enantioselective reduction of a β-enamino ester;[2] (ii) the addition of a chiral sulfoxide to a substituted *N*-sulfonylimine;[3] (iii) an asymmetric catalytic Mannich reaction;[4] (iv) the conjugate addition of a chiral lithium amide to a β-aryl α,β-unsaturated ester;[5] (v) the reaction of a Reformatsky reagent with an imine;[6] or (vi) the preparation of a diastereomeric salt.[7] In addition to asymmetric synthetic routes,[2–7] enzyme-catalysed processes have been developed through (i) the transesterification of an N-protected ester in isobutanol;[8] (ii) stereoselective degradation of a racemic amino acid;[9] (iii) hydrolysis of a racemic β-amino ester;[10] (iv) hydrolysis of a racemic N-acetylated β-amino acid;[11] or (v) an aminomutase-catalysed α,β-rearrangement[12] in aqueous medium.

We recently reported some direct and indirect enzymatic methods for the preparation of enantiomers of β-phenylalanine or its derivatives from racemic β-lactams.[13] Previously, we devised the first direct enzymatic highly enantioselective (E >100) hydrolysis of carbocyclic β-amino esters in *i*-Pr_2O with Lipolase at 60 °C.[14]

Herein, our goal was to extend this method to β-aryl-substituted β-amino esters, hydrolysing them to enantiopure β-aryl-β-amino acids. β-Phenylalanine was chosen as the model compound. There are complex structures which include an (*R*)- or (*S*)-β-phenylalanine **6a** or **7a** moiety. For example, **6a** has been inserted into NS5B polymerase inhibitors against the hepatitis C virus,[15] anti-inflammatory bradykinin B1 receptor antagonists,[16] anticancer matrix metalloproteinase (MMP-12) inhibitors[17] or analgesic endomorphin-1 analogue tetrapeptides.[18] (*S*)-β-Phenylalanine **7a** has been applied in the synthesis of novel antibiotics.[19] In addition to β-phenylalanine, we set out to prepare β-amino acid enantiomers with pharmaceutical potential. (*S*)-3-Amino-3-(3-fluorophenyl)propanoic acid **7b** was used to obtain chemokine receptor (CCR5) antagonists with anti-inflammatory and anti-HIV effects.[20] The (*R*)-isomer of 3-amino-3-(3,5-dichlorophenyl)propanoic acid **6c** was investigated as part of an integrin receptor $\alpha_v\beta_3$ antagonist.[21] This receptor is an interesting therapeutic target in the treatment of osteoporosis, restenosis, cancer growth and metastasis formation. Several VLA-4 integrin receptor antagonists have been synthetized, including the (*S*)-3-amino-3-(3,4-dimethoxyphenyl)propanoic acid **7d** unit, and used against inflammatory and autoimmune diseases.[22] One non-peptide bradykinin B1 receptor antagonist with anti-inflammatory and analgesic effects contains an (*R*)-3-amino-3-benzo[1,3]dioxol-5-ylpropanoic acid **6e** moiety.[23]

2. Results and discussion

2.1. Syntheses of ethyl 3-amino-3-arylpropanoates 3a–e

The racemic compounds **2a–e** were first synthesized by a modified Rodionov synthesis from the corresponding **1a–e** through condensation with malonic acid and in the presence of NH_4OAc, the mixture was heated at reflux in EtOH (Scheme 1).[11c] Compounds **3a–e**·HCl were prepared by the esterification of **2a–e** in the presence of $SOCl_2$. The free bases **3a–e** were then liberated by treatment of **3a–e**·HCl with aqueous KOH.

* Corresponding authors. Tel.: +36 62 545564; fax: +36 62 545705 (F.F.).
E-mail addresses: Forro.Eniko@pharm.u-szeged.hu (E. Forró), fulop@pharm.u-szeged.hu (F. Fülöp).

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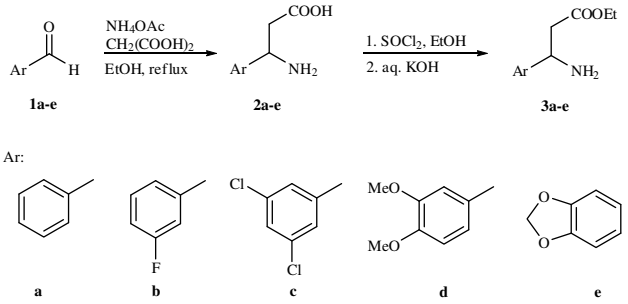


Scheme 1. Synthesis of **3a–e**.

2.2. Lipase-catalysed enantioselective ring cleavage of 3a–e

Previous results[14] on the lipase-catalysed enantioselective hydrolysis of carbocyclic β-amino esters suggested the possibility of the enantioselective hydrolysis of **3a–e** with 0.5 equiv of H_2O in the presence of Lipolase (CAL-B) in *i*-Pr_2O at 60 °C. Since Lipolase did not exhibit any selectivity towards **3a**, further enzymes were screened, including lipase PS (*Pseudomonas cepacia*), PPL (porcine pancreatic lipase), lipase AK (*Pseudomonas fluorescens*), lipase AY (*Candida rugosa*) and Chirazyme L-5 (lipase A from *Candida antarctica*) (Table 1).

Table 1
Conversion and enantioselectivity of the hydrolysis of **3a**[a]

Entry	Enzyme (50 mg mL^{-1})	H_2O (equiv)	T (°C)	t (h)	Conv. (%)	ee_s[b] (%)	ee_p[c] (%)	E
1	PPL	0.5	45	5	43	66	88	31
2	lipase AK[d]	0.5	45	5	43	67	89	35
3	Lipase PS[d]	0.5	25	6	49	92	>99	>200
4	Lipase PS[d]	0.5	45	5	49	96	>99	>200
5	Lipase PS[d]	—	45	2	46	85	>99	>200
6	Lipase PS[d]	1	45	2	48	93	>99	>200
7	Lipase PS[d]	2	45	2	50	99	>99	>200
8	Lipase PS[d]	5	45	2	50	>99	>99	>200

[a] 0.05 M substrate, 1 mL *i*-Pr_2O.
[b] According to GC after derivatization (Section 4).
[c] According to GC after double derivatization (Section 4).
[d] Contains 20% (w/w) lipase adsorbed on Celite in the presence of sucrose.

A key issue was to develop a simple analytical method allowing the progress of the reactions to be followed. The unreacted amino ester and the product amino acid were detected in the same run by GC on a chiral column, where the hydrolysed amino acid was derivatized (Section 4).

All enzymes were active in the presence of 0.5 equiv of H_2O in *i*-Pr_2O at 45 °C, but lipase AY and Chirazyme L-5 did not exhibit any selectivity towards **3a**, while PPL and lipase AK catalysed the reaction with moderate enantioselectivities (entries 1 and 2). Lipase PS directed the hydrolysis with excellent enantioselectivity (E >200) (entry 4). When the lipase PS-catalysed hydrolysis of **3a** was performed at 25 °C, a slight decrease in the reaction rate was observed (entry 3).

Next, we analysed the effects of the amount of added H_2O on the enantioselectivity and the reaction rate. The added H_2O amount (1–5 equiv) apparently did not affect the enantioselectivity (E >200) or the reaction rate (Table 1, entries 6, 7 and 8), but when an *i*-Pr_2O/H_2O 1/1 (v/v) mixture was used, a dramatic decrease in the enantioselectivity was observed (E = 30). In a small-scale experiment, the hydrolysis of **3a** was complete, even without the addition of H_2O (entry 5). In this case, the H_2O originated from the reaction medium (<0.1%) or from the enzyme preparation (<5%).

Next, we analysed the effect of the solvent (Table 2). All the solvents tested proved to be suitable for the enantioselective (E >200) hydrolysis of **3a**, with significant differences in the reaction rate. The highest reaction rates were observed in *i*-Pr_2O, *t*-BuOMe and *n*-hexane (entries 1, 2 and 4), while the hydrolysis was slowest in $CHCl_3$ (entry 7). We chose to continue our experiments with *i*-Pr_2O.

Table 2
Effect of solvents on the hydrolysis of **3a**[a]

Entry	Solvent (1 mL)	Conv. (%)	ee_s[b] (%)	ee_p[c] (%)	E
1	*i*-Pr2O	49	96	>99	>200
2	*t*-BuOMe	48	92	>99	>200
3	Et_2O	39	63	>99	>200
4	*n*-Hexane	50	98	>99	>200
5	Toluene	38	60	>99	>200
6	1,4-Dioxane	26	34	>99	>200
7	$CHCl_3$	4	4	>99	>200
8	THF	14	16	>99	>200
9	Me_2CO	13	15	>99	>200

[a] 0.05 M substrate, 0.5 equiv. of H_2O, 50 mg mL^{-1} lipase PS[d], after 5 h.
[b] According to GC after derivatization.
[c] According to GC after double derivatization.
[d] Contains 20% (w/w) lipase adsorbed on Celite in the presence of sucrose.

The reaction rate for the hydrolysis of **3a** clearly increased as the amount of enzyme was increased (Table 3). The highest reaction rate was observed in the presence of 75 mg mL^{-1} lipase PS (entry 6). However, for economic reasons, 30 mg mL^{-1} lipase PS was chosen for the preparative-scale resolutions of **3a–e**.

Table 3
Effect of the quantity of lipase PS on the hydrolysis of **3a**[a]

Entry	Lipase PS[d] (mg mL^{-1})	Conv. (%)	ee_s[b] (%)	ee_p[c] (%)	E
1	10	17 (45 after 24 h)	20	>99	>200
2	20	27	36	>99	>200
3	30	31	45	>99	>200
4	40	35	54	>99	>200
5	50	38	60	>99	>200
6	75	45	82	>99	>200

[a] 0.05 M substrate, 0.5 equiv of H_2O, 1 mL *i*-Pr_2O, after 1 h.
[b] According to GC after derivatization.
[c] According to GC after double derivatization.
[d] Contains 20% (w/w) lipase adsorbed on Celite in the presence of sucrose.

In view of the results of the preliminary experiments, the preparative-scale resolutions of **3a–e** were performed with 0.5 equiv of H_2O in the presence of lipase PS in *i*-Pr_2O at 45 °C. The products were characterized by a good enantiomeric excess at close to 50% conversion. The results are reported in Table 4 and in Section 4.

2.3. Transformations of the enantiomers

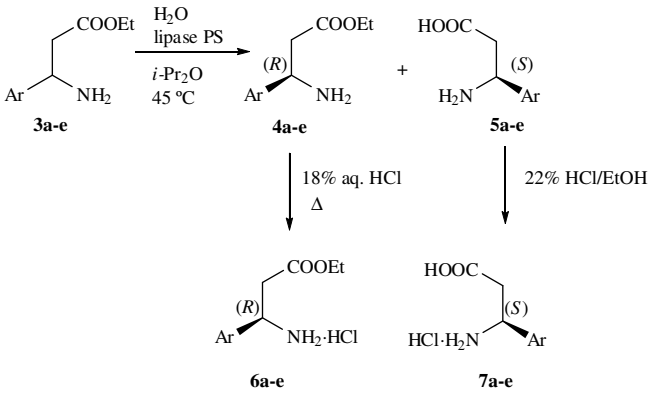
The transformations involving the hydrolysis of **4a–e** with aqueous HCl afforded **6a–e**·HCl (ee ⩾ 97%) (Scheme 2). Treatment of **5a–e** with 22% HCl/EtOH resulted in the corresponding enantiopure **7a–e**·HCl (ee >99%).

The absolute configurations in the cases of **5a**, **5d** and **5c** were proved by comparing the [α] values with literature data (Section 4), while for **5b** and **5c** the chromatograms analysed indicated the same enantiopreference for all the enzymes applied. Thus, the absolute configurations indicated the (*S*)-selective hydrolysis of **3a–e**. Although the enantiomers **5b**[10c] and the ethyl ester of **5c**[24] have been prepared, physical data for them (specific rotations and melting points) have not been described. It should be

Table 4
Lipase PS-catalysed hydrolysis of **3a–e**[a]

	Time (h)	Conv. (%)	*E*	β-Amino acid·HCl (**6a–e**)				β-Amino acid (**5a–e**)			
				Yield (%)	Isomer	ee[b] (%)	$[\alpha]_D^{25}$ (H_2O)	Yield (%)	Isomer	ee[c] (%)	$[\alpha]_D^{25}$ (H_2O)
3a	22	50	>200	44	(*R*)	>99	−4[d]	44	(*S*)	>99	−8[e]
3b	23	49	>200	40	(*R*)	>99	−6.5[f]	40	(*S*)	>99	−1.8[g]
3c	74	50	139	43	(*R*)	>99	−5.1[h]	44	(*S*)	>99	−5.5[g]
3d	18	50	>200	41	(*R*)	>99	−7.9[i]	41	(*S*)	>99	+1.3[j]
3e	16	52	110	44	(*R*)	97	−8.4[k]	46	(*S*)	>99	+4[d]

[a] 30 mg mL^{-1} enzyme in *i*-Pr_2O, 0.5 equiv H_2O, 45 °C.
[b] According to GC after derivatization.
[c] According to GC after double derivatization.
[d] *c* = 0.3.
[e] *c* = 0.27.
[f] *c* = 0.31.
[g] *c* = 0.38.
[h] *c* = 0.34.
[i] *c* = 0.32.
[j] *c* = 0.51.
[k] *c* = 0.33.



Scheme 2. Lipase PS-catalysed enantioselective hydrolysis of **3a–e** (for the meanings of **a–e**, see Scheme 1).

mentioned that lipase PS demonstrated the opposite selectivity in the hydrolysis of **3a–e** as compared with the CAL-B-catalysed enantioselective ring-opening of 4-aryl-substituted β-lactams, which was *R*-selective.[13c]

3. Conclusions

A simple and efficient direct enzymatic method has been developed for the synthesis of pharmacologically valuable, optically active β-aryl-substituted β-amino acids via the enantioselective hydrolysis of the corresponding racemic β-amino esters in an organic medium. The lipase PS-catalysed (*S*)-selective hydrolysis of **3a–e** with H_2O (0.5 equiv) as a nucleophile in *i*-Pr_2O at 45 °C led to enantiomers of **4a–e** (ee ⩾ 97%) and **5a–e** (ee >99%) with high enantioselectivities (*E* >100) in good chemical yields (40–46%). The products could be easily separated. Transformations of **4a–e** with 18% aqueous HCl resulted in the enantiomers of **6a–e**·HCl (ee ⩾ 97%).

4. Experimental

4.1. Materials and methods

Lipase PS and lipase AK were from Amano Pharmaceuticals, Lipolase (lipase B from *C. antarctica* was produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin) and PPL (type II) were from Sigma, and Chirazyme L-5 (lipase B from *C. antarctica*) was from Novo Nordisk. Before use, lipase PS, lipase AK and CAL-A (5 g) were dissolved in Tris–HCl buffer (0.02 M; pH 7.8) in the presence of sucrose (3 g), followed by adsorption on Celite (17 g) (Sigma). Substituted benzaldehydes were from Aldrich, except for 3,5-dichlorobenzaldehyde, which was from Fluorochem. The solvents were of the highest analytical grade.

Optical rotations were measured with a Perkin–Elmer 341 polarimeter. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer. Melting points were determined on a Kofler apparatus. Elemental analyses (CHN) corresponded closely (within ±3%) with the calculated ones in all cases.

The ee values for the unreacted β-amino ester and the β-amino acid enantiomers produced were determined by GC on a Chirasil-L-Val column (20 m) after double derivatization with (i) CH_2N_2 [Caution! derivatization with CH_2N_2 should be performed under a well-working hood]; (ii) Ac_2O in the presence of 4-dimethylaminopyridine and pyridine [100 °C for 10 min→150 °C (temperature rise 10 °C min^{-1}), 140 kPa; retention times (min); **4a**: 23.16 (antipode: 23.65); **5a**: 21.11 (antipode: 20.80); 120 °C for 10 min→190 °C (temperature rise 10 °C min^{-1}), 140 kPa; **4b**: 16.32 (antipode: 16.45); **5b**: 15.81 (antipode: 15.69); 130 °C for 10 min→160 °C (temperature rise 10 °C min^{-1}), 140 kPa; **4c**: 40.59 (antipode: 42.29); **5c**: 37.60 (antipode: 36.06); **4d**: 56.58 (antipode: 58.20); **5d**: 48.42 (antipode: 47.39); **4e**: 43.33 (antipode: 45.00); **5e**: 38.17 (antipode: 37.06)].

4.2. General procedure for the syntheses of racemic β-amino acids 2a–e

Compounds **2a–e** were prepared by a slightly modified literature method[11c] based on the modified Rodionov synthesis from the corresponding **1a–e** (5 mmol) through condensation with malonic acid (2 equiv) in the presence of NH_4Ac (2 equiv) in EtOH at reflux for 6 h. The resulting precipitated crystals were filtered off, washed with Me_2CO and recrystallized from H_2O and Me_2CO.

4.2.1. (±)-3-Amino-3-phenylpropanoic acid 2a

Yield: 0.46 g (56%); mp 235–235 °C (lit.[25] mp 220–222 °C). The ^{1}H NMR and ^{13}C NMR data are in accordance with those reported in the literature.[2]

4.2.2. (±)-3-Amino-3-(3-fluorophenyl)propanoic acid 2b

Yield: 0.52 g (57%); mp 237–239 °C. The ^{1}H NMR and ^{13}C NMR data are in accordance with those reported in the literature.[10c]

4.2.3. (±)-3-Amino-3-(3,5-dichlorophenyl)propanoic acid 2c

Yield: 0.51 g (44%); mp 225–228 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.74–2.88 (2H, m, CH_2), 4.59–4.63 (1H, m, CH), 7.41 (2H, m, Ar), 7.53 (1H, m, Ar). ^{13}C NMR (100.62 MHz, DMSO-d_6) δ (ppm): 40.2, 51.7, 125.6, 129.0, 134.9, 139.5, 176.7.

4.2.4. (±)-3-Amino-3-(3,4-dimethoxyphenyl)propanoic acid 2d

Yield: 0.52 g (46%); mp 238–240 °C (lit.[26] mp 248–249 °C). The ^{1}H NMR and ^{13}C NMR data are in accordance with those reported in the literature.[27]

4.2.5. (±)-3-Amino-3-benzo[1,3]dioxol-5-ylpropanoic acid 2e

Yield: 0.54 g (52%); mp 230–233 °C. The ^{1}H NMR and ^{13}C NMR data are in accordance with those reported in the literature.[5b]

4.3. General procedure for the syntheses of racemic β-amino esters 3a–e

To 40 mL of EtOH was added dropwise 0.55 mL of $SOCl_2$, with the temperature being kept under −10 °C with saline ice. To this solution, **2a–e** (5 mmol) were added. The mixture was stirred at 0 °C for 30 min, and then at room temperature for 3 h, and finally heated at reflux for 1 h. The solvent was evaporated off and the resulting **3a–e**·HCl were recrystallized from EtOH and Et_2O. Treatment of **3a–e**·HCl with aqueous KOH resulted in the free **3a–e** as pale-yellow oils.

4.3.1. Hydrochloride salt of ethyl (±)-3-amino-3-phenylpropanoate 3a·HCl

Yield: 1.05 g (91%); mp 141–144 °C (lit.[25] mp 138–141 °C). The ^{1}H NMR and ^{13}C NMR data are in accordance with those reported in the literature.[25]

4.3.2. Hydrochloride salt of ethyl (±)-3-amino-3-(3-fluorophenyl)propanoate hydrochloride 3b·HCl

Yield: 1.14 g (92%); mp 148–151 °C. The ^{1}H NMR data are in accordance with those reported in the literature.[28] ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 13.6, 38.4, 51.5, 62.9, 114.5, 114.7, 116.9, 117.1, 131.8, 131.8, 137.2, 161.5, 164.0, 171.3.

4.3.3. Hydrochloride salt of ethyl (±)-3-amino-3-(3,5-dichlorophenyl)propanoate hydrochloride 3c·HCl

Yield: 1.22 g (82%); mp 180–185 °C. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 1.13–1.17 (3H, t, J = 7.16 Hz, CH_3), 3.06–3.21 (2H, m, CH_2CO), 4.08–4.15 (2H, m, CH_2CH_3), 4.77–4.81 (1H, t, J = 7.27 Hz, CH), 7.44–7.45 (2H, m, Ar), 7.54 (1H, m, Ar). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 13.6, 38.2, 51.0, 62.9, 126.4, 130.1, 135.8, 138.7, 171.5.

4.3.4. Hydrochloride salt of ethyl (±)-3-amino-3-(3,4-dimethoxyphenyl)propanoate hydrochloride 3d·HCl

Yield: 1.27 g (88%); mp 218–221 °C (lit.[29] mp 206–208 °C). ^{1}H NMR (400 MHz, D_2O) δ (ppm): 1.10–1.14 (3H, t, J = 7.15 Hz, CH_3), 3.05–3.21 (2H, m, CH_2CO), 3.84 (1H, s, OCH_3), 3.86 (1H, s, OCH_3), 4.07–4.12 (2H, m, CH_2CH_3), 4.73–4.76 (1H, t, J = 7.44 Hz, CH), 7.04–7.09 (3H, m, Ar). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 13.1, 37.9, 51.1, 55.8, 62.2, 110.5, 112.2, 120.5, 128.1, 148.6, 171.4.

4.3.5. Hydrochloride salt of ethyl (±)-3-amino-3-benzo[1,3]dioxol-5-ylpropanoate hydrochloride 3e·HCl

Yield: 1.26 g (92%); mp 189–191 °C. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 1.10–1.13 (3H, t, J = 7.15 Hz, CH_3), 3.00–3.15 (2H, m, CH_2CO), 4.05–4.11 (2H, m, CH_2CH_3), 4.68–4.70 (1H, t, J = 7.48 Hz, CH), 5.96 (2H, s, OCH_2O), 6.88–6.94 (3H, m, Ar). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 13.2, 37.9, 51.5, 62.5, 101.6, 107.3, 121.5, 128.6, 148.2, 171.6.

4.4. Typical small-scale enzyme tests

Racemic **3a** (0.05 M solution) in an organic solvent or H_2O (1 mL) was added to the enzyme tested (10, 20, 30, 40, 50 or 75 mg mL^{-1}), followed by H_2O (0, 0.5, 1, 2 or 15 equiv). The mixture was shaken at either 3, 25, 45 or 60 °C. The progress of the reaction was followed by taking samples from the reaction mixture at intervals and analysing them by GC.

4.5. General procedure for the preparative-scale resolutions of 3a-e

Racemic compounds **3a–e** (3 mmol) were dissolved in *i*-Pr_2O (25 mL). Lipase PS (0.75 g, 30 mg mL^{-1}) and H_2O (27 μL, 1.5 mmol) were added and the mixture was shaken in an incubator shaker at 45 °C for 16–74 h (Table 4). The reaction was stopped by filtering off the enzyme at 50% conversion. The solvent was evaporated off and the residues (*R*)-**4a–e** were immediately hydrolysed by refluxing (5 h) with 18% aqueous HCl solution (6 mL) to give (*R*)-**6a–e**·HCl (in the case of **3e**, the unreacted **4e** was hydrolysed by shaking at room temperature for 12 h with 3.6% aqueous HCl solution). The filtered off enzyme was washed with distilled H_2O (3 × 15 mL), and the H_2O was evaporated off, yielding the crystalline (*S*)-**5a–e**.

When (*S*)-**5a–e** (50 mg) were treated with 22% HCl/EtOH (5 mL), (*S*)-**7a–e**·HCl were obtained.

4.5.1. Hydrochloride salt of (*R*)-3-amino-3-phenylpropanoic acid 6a

Yield: 266 mg (44%); recrystallized from EtOH and Et_2O; $[\alpha]_D^{25} = -4$ (*c* 0.3, H_2O) {lit.[13c] $[\alpha]_D^{25} = -3$ (*c* 0.3, H_2O)}; mp 195–198 °C (lit.[13c] mp 195–198 °C); ee >99%. The ^{1}H NMR data for **6a** are in accordance with those reported in the literature.[13c] ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 38.3, 52.0, 127.5, 129.8, 130.1, 135.6, 174.1.

4.5.2. (*S*)-3-Amino-3-phenylpropanoic acid 5a

Yield: 218 mg (44%); recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25} = -8$ (*c* 0.27; H_2O) {lit.[13c] $[\alpha]_D^{25} = +7$ (*c* 0.27, H_2O) for (*R*)-**5a**}; mp 251–253 °C (lit.[13c] mp 242–246 °C); ee >99%. The ^{1}H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **5a** are similar to those for **2a**.

4.5.3. Hydrochloride salt of (*S*)-3-amino-3-phenylpropanoic acid 7a

Quantitative yield; $[\alpha]_D^{25} = +4$ (*c* 0.3, H_2O) {lit.[13c] $[\alpha]_D^{25} = +3$ (*c* 0.28; H_2O)}; mp 197–200 °C (lit.[13c] mp 197–201 °C); ee >99%. The ^{1}H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **7a** are similar to those for **6a**.

4.5.4. Hydrochloride salt of (*R*)-3-amino-3-(3-fluorophenyl)propanoic acid 6b

Yield: 264 mg (40%); recrystallized from EtOH and Et_2O; $[\alpha]_D^{25} = -6.5$ (*c* 0.31, H_2O); mp 181–190 °C (slow melting); ee >99%. The ^{1}H NMR data for **6b** are in accordance with those reported in the literature.[30] ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 38.1, 51.5, 114.4, 114.7, 116.8, 117.1, 123.4, 131.7, 131.8, 137.9, 164.3, 173.9.

4.5.5. (*S*)-3-Amino-3-(3-fluorophenyl)propanoic acid 5b

Yield: 220 mg (40%); recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25} = -1.8$ (*c* 0.38, H_2O); mp 226–229 °C; ee >99%. The ^1H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **5b** are similar to those for **2b**.

4.5.6. Hydrochloride salt of (*S*)-3-amino-3-(3-fluorophenyl)propanoic acid 7b

Quantitative yield; $[\alpha]_D^{25} = +5.7$ (*c* 0.31, H_2O); mp 185–194 °C (slow melting); ee >99%. The ^1H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **7b** are similar to those for **6b**.

4.5.7. Hydrochloride salt of (*R*)-3-amino-3-(3,5-dichlorophenyl)propanoic acid 6c

Yield: 349 mg (43%); recrystallized from EtOH and Et_2O; $[\alpha]_D^{25} = -5.1$ (*c* 0.34, H_2O); mp 208–210 °C; ee = 97%. ^1H NMR (400 MHz, D_2O) δ (ppm): 3.07–3.13 (2H, m, CH_2), 4.74–4.78 (1H, t, *J* = 7.04 Hz, CH), 7.45 (2H, m, Ar), 7.56 (1H, m, Ar). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 37.7, 50.8, 125.8, 129.7, 135.2, 138.5, 173.4.

4.5.8. (*S*)-3-Amino-3-(3,5-dichlorophenyl)propanoic acid 5c

Yield: 309 mg (44%); recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25} = -5.5$ (*c* 0.38, H_2O); mp 219–221 °C; ee >99%. The ^1H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **5c** are similar to those for **2c**.

4.5.9. Hydrochloride salt of (*S*)-3-amino-3-(3,5-dichlorophenyl)propanoic acid 7c

Quantitative yield; $[\alpha]_D^{25} = +5.7$ (*c* 0.34, H_2O); mp 212–214 °C; ee >99%. The ^1H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **7c** are similar to those for **6c**.

4.5.10. Hydrochloride salt of (*R*)-3-amino-3-(3,4-dimethoxyphenyl)propanoic acid 6d

Yield: 322 mg (41%); recrystallized from EtOH and Et_2O; $[\alpha]_D^{25} = -7.9$ (*c* 0.32, H_2O); mp 178–181 °C; ee >99%. ^1H NMR (400 MHz, D_2O) δ (ppm): 2.98–3.14 (2H, m, CH_2), 3.79–3.80 (3H, d, *J* = 3.3 Hz, OCH_3), 3.81–3.82 (3H, d, *J* = 2.1 Hz, OCH_3), 4.68–4.70 (1H, m, CH), 7.02–7.04 (3H, m, Ar). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 38.3, 51.8, 56.2, 56.3, 111.0, 112.6, 120.7, 128.6, 148.9, 149.4, 174.1.

4.5.11. (*S*)-3-Amino-3-(3,4-dimethoxyphenyl)propanoic acid 5d

Yield: 277 mg (41%); recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25} = +1.3$ (*c* 0.51, H_2O) {lit.[5b] $[\alpha]_D^{25} = +0.9$ (*c* 0.7, H_2O)}; mp 224–229 °C (lit.[5b] mp 216–219 °C); ee >99%. The ^1H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **5d** are similar to those for **2d**.

4.5.12. Hydrochloride salt of (*S*)-3-amino-3-(3,4-dimethoxyphenyl)propanoic acid 7d

Quantitative yield; $[\alpha]_D^{25} = +7.2$ (*c* 0.315, H_2O); mp 177–181 °C; ee >99%. The ^1H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **7d** are similar to those for **6d**.

4.5.13. Hydrochloride salt of (*R*)-3-amino-3-benzo[1,3]dioxol-5-ylpropanoic acid 6e

Yield: 324 mg (44%); recrystallized from EtOH and Et_2O; $[\alpha]_D^{25} = -8.4$ (*c* 0.33, H_2O); mp 203–207 °C; ee = 97%. The ^1H NMR data for **6e** are in accordance with those reported in the literature.[31] ^{13}C NMR (100.62 MHz, D_2O) δ (ppm): 38.0, 51.5, 101.9, 107.5, 109.2, 121.7, 129.4, 148.4, 173.8.

4.5.14. (*S*)-3-Amino-3-benzo[1,3]dioxol-5-ylpropanoic acid 5e

Yield: 289 mg (46%); recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25} = +4$ (*c* 0.3, H_2O) {lit.[5b] $[\alpha]_D^{25} = +42.4$ (*c* 0.3, H_2O)}; mp 231–234 °C (lit.[5b] mp 219–220 °C); ee >99%. The ^1H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **5e** are similar to those for **2e**.

4.5.15. Hydrochloride salt of (*S*)-3-amino-3-benzo[1,3]dioxol-5-ylpropanoic acid 7e

Quantitative yield; $[\alpha]_D^{25} = +8.9$ (*c* 0.33, H_2O); mp 204–208 °C; ee >99%. The ^1H NMR (400 MHz, D_2O) and ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) data for **7e** are similar to those for **6e**.

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