Improved enzymatic syntheses of valuable β-arylalkyl-β-amino acid enantiomers†

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The enantioselective ($E \sim 200$) *Burkholderia cepacia*-catalysed hydrolyses of β-amino esters with H_2O (0.5 equiv.) in *t*-BuOMe or in *i*-Pr_2O at 45 °C are described. The enantiomers of biologically relevant β-arylalkyl-substituted β-amino acids, and especially (*R*)-3-amino-3-(2,4,5-trifluorophenyl)butanoic acid, the intermediate of the new antidiabetic drug sitagliptine, were prepared with high enantiomeric excesses (*ee* ≥96%) and in good yields (≥42%).

Introduction

In recent years, extensive investigations have been carried out on the chemistry of β-amino acids, in particular because of their importance in pharmaceutical research.[1] Optically pure β-arylalkyl-substituted β-amino acids have wide-ranging applications, *e.g.* β-peptides containing an (*S*)-homo-β-phenylalanine unit (**5c**), such as a matrix metalloproteinase-2 inhibitor β-tetrapeptide, utilized for the diagnosis of cancer and atherosclerosis,[2] or β-dipeptides, used for studying the conformational behaviour of foldamers.[3] Moreover, various heterocyclic compounds have been tested as modulators of protein kinase B, a potential therapeutic target for diseases associated with abnormal cell growth, cancer, inflammation or metabolic disorders.[4] (*S*)-β-Phenylethyl-β-alanine (**6c**) has been built into a heat shock protein 70 (Hsp70) modulator.[5] Hsp70 probably contributes to a number of diseases, including cancer and neurodegeneration. **6c** has also been applied in an α-helical peptide mimetic compound,[6] while a derivative of the (*R*) enantiomer (**6d**) has been tested as a hepatitis C virus inhibitor.[7] Type 2 diabetes is a major health problem in the 21st century. Unfortunately, the current modes of therapy are associated with undesirable side-effects, such as hypoglycaemia or cardiovascular abnormalities. Dipeptidyl peptidase IV is a new therapeutic target for the treatment of this form of diabetes. Inhibition of this peptidase results in increased levels of incretins (glucagon-like peptide 1 and gastric inhibitory polypeptide), which control the blood glucose concentration.[8] Januvia™ (sitagliptin phosphate) (Fig. 1), the first approved drug for the inhibition of dipeptidyl peptidase IV, contains a β-amino acid subunit, (*R*)-3-amino-4-(2,4,5-trifluorophenyl)butanoic acid (**11b**).[9] Besides Januvia™, numerous derivatives of **11b** have been synthesized and tested as potential antidiabetic drugs.[10]

Since β-arylalkyl-β-amino acids are of considerable importance, many asymmetric synthetic routes have been developed for their preparation, *e.g.* (i) enantioselective hydrogenation of an enamine,[10e,11] (ii) cross-coupling of an enantiomeric organozinc reagent with aryl iodides,[12] (iii) homologation of an optically pure α-amino acid by the Arndt–Eistert method,[10a,13] (iv) homologation of an optically pure α-amino acid by the formation of a β-amino alcohol following substitution of the OH group with a CN group,[2,14] (v) conversion of L- or D-aspartic acid to a lactone, followed by reduction and substitution,[15] (vi) conjugate addition of an amine to an α,β-unsaturated carbonyl compound,[16] (vii) S_N2 ring opening of an enantiopure β-lactone with a nitrogen-based nucleophile,[17] or (viii) ring opening of an enantiopure β-lactam.[18]

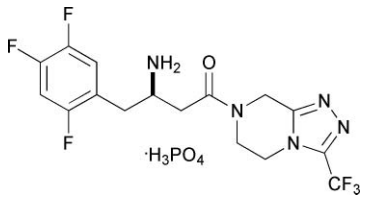

Fig. 1 Januvia™ (sitagliptin phosphate).

Enzymatic methods have also been used for the preparation of enantiopure β-amino acids. Indirect methods are based on acylation of the corresponding *N*-hydroxymethyl-β-lactam or hydrolysis of the *N*-acyloxymethyl-β-lactam.[19] However, indirect methods have some disadvantages, such as the addition and elimination of the hydroxymethyl group or the separation of the product enantiomers by column chromatography.[20] These additional steps can cause relatively low yields. We recently developed an efficient direct enzymatic method for the synthesis of carbocyclic and aryl-substituted β-amino acid enantiomers through the selective ($E > 200$) ring opening of racemic β-lactams.[21] On extension of this method to 4-arylalkyl-substituted β-lactams, surprisingly, low *E* values (≤12) were observed.[22] Preparative-scale resolutions were carried out in two steps, which led to relatively low yields (≤36%). Later, we devised a highly selective direct enzymatic method (E usually >100) through the lipase-catalysed hydrolysis of carbocyclic-,[23a] aryl-[23b] and heteroaryl[23c]-substituted β-amino esters.

Our aim was to develop a direct enzymatic method for the resolution of racemic β-arylalkyl-substituted β-amino esters under non-aqueous conditions, resulting in valuable β-amino acid

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enantiomers, *e.g.* (*R*)-3-amino-4-(2,4,5-trifluorophenyl)butanoic acid (**11b**), an intermediate of sitagliptin.

Results and discussion

Syntheses of (±)-5, (±)-6 and (±)-11

β-Lactams (±)-**3** and (±)-**4** were prepared from alkenes **1** and **2** by the addition of chlorosulfonyl isocyanate (CSI), according to known literature methods.[19b,24] β-Amino esters (±)-**5** and (±)-**6** were synthesized by the ring opening of (±)-**3** and (±)-**4** with 22% HCl/EtOH, followed by treatment with aqueous K_2CO_3 (Scheme 1).

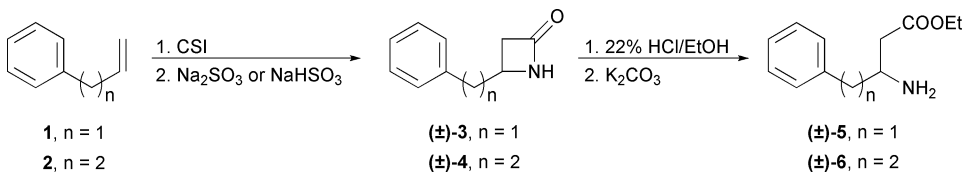
Starting from acid **7**, enamine **10** was prepared by a slightly modified literature method.[10e] β-Amino ester (±)-**11** was obtained by the reduction of enamine **10** with $NaCNBH_3$ in the presence of AcOH (Scheme 2).

Enzymatic hydrolysis of (±)-5, (±)-6 and (±)-11

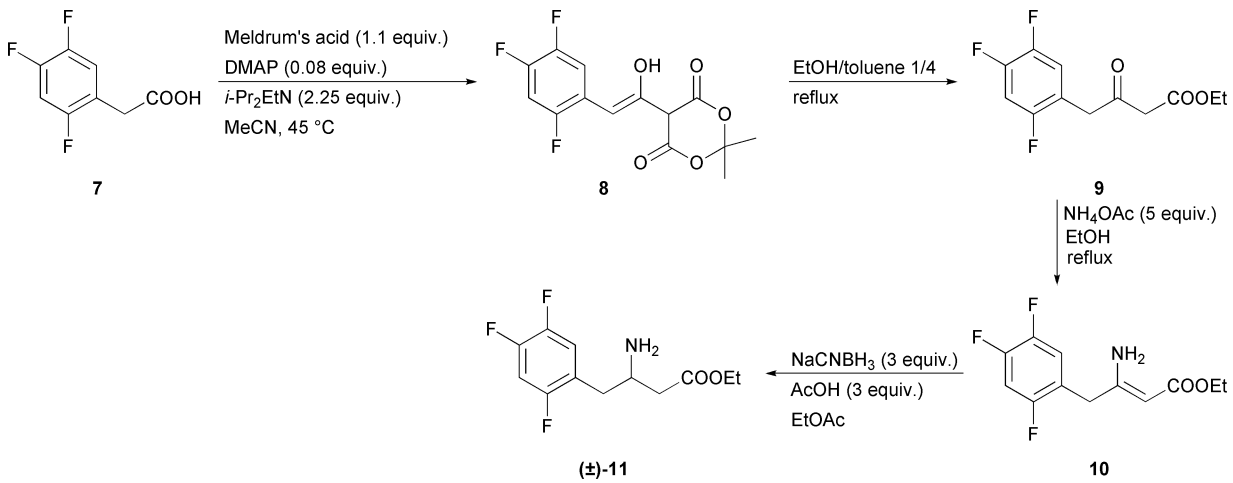
Preliminary experiments. Preliminary experiments were performed on the hydrolysis of model compound (±)-**5** (Scheme 3). First, a number of lipases were tested in *t*-BuOMe at 25 °C with 0.5 equiv. of H_2O (the quantity of the racemic compound is considered as 1 molar equivalent). The use of organic solvents in enzymatic reactions has numerous advantages, and lipases are able to hydrolyse ester and amide bonds in organic solvents with high enantioselectivity.[25] CAL-A (*Candida antarctica* lipase A) and Lipolase (*Candida antarctica* lipase B) catalysed the reaction with low *E* values (Table 1, entries 1 and 2), while PPL (porcine pancreas lipase) and lipase AK (*Pseudomonas fluorescens*) displayed moderate *E* values (entries 3 and 4). Lipase PS IM (*Burkholderia cepacia*) proved to be the best enzyme for the hydrolysis of (±)-**5** (entry 5), therefore it was chosen for further experiments and for the preparative-scale resolutions.

When the temperature was increased from 25 to 45 °C, the conversion increased considerably (Table 1, entries 5 and 6), but a further increase of the reaction temperature did not exert any additional beneficial effect on the reaction rate (entry 10), though *E* remained high (≥56). The enantioselective ($E > 200$) hydrolysis of (±)-**5** was complete in 72 h, even at 45 °C (entry 7); accordingly, subsequent experiments were planned at 45 °C.

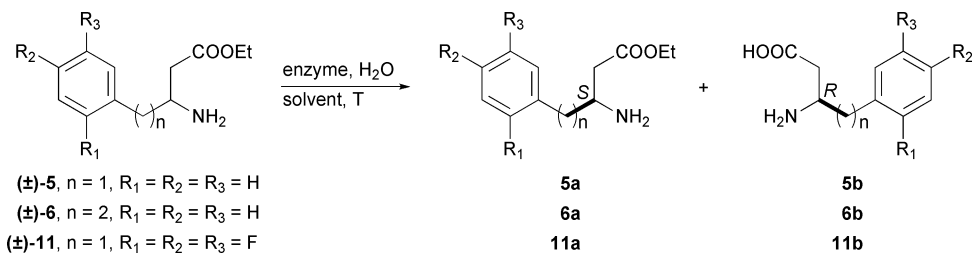
When the amount of enzyme was increased from 30 to 50 and then 75 mg mL^{-1}, the reaction rates clearly increased, while the *E*



Scheme 1 Syntheses of (±)-**5** and (±)-**6**.



Scheme 2 Synthesis of (±)-**11**.



Scheme 3 Enzyme-catalysed hydrolyses of (±)-**5**, (±)-**6** and (±)-**11**.

Table 1 Conversion, enantiomeric excesses (*ee*) and enantioselectivities (*E*) of the hydrolysis of (±)-**5**[a]

Entry	Enzyme	Enzyme/mg mL^{-1}	*t*/h	*T*/°C	ee_s (%)[b]	ee_p (%)[b]	Conv. (%)	*E*
1	CAL-A[c]	50	87	25	7	37	16	2
2	Lipolase	50	87	25	88	15	85	3
3	PPL[c]	50	87	25	2	88	2	16
4	Lipase AK[c]	50	87	25	19	88	18	19
5	Lipase PS IM	50	26	25	14	96	13	56
6	Lipase PS IM	50	26	45	37	96	28	70
7	Lipase PS IM	50	72	45	99	96	51	>200
8	Lipase PS IM	30	26	45	24	96	20	62
9	Lipase PS IM	75	26	45	48	96	33	79
10	Lipase PS IM	50	26	60	39	96	29	72

[a] 0.05 M substrate, 1 mL of *t*-BuOMe, 0.5 equiv. of H_2O. [b] According to HPLC (Experimental section). [c] Contains 20% (w/w) lipase adsorbed on Celite in the presence of sucrose.

values were apparently not affected (Table 1, entries 6, 8 and 9). For economic reasons, preparative-scale resolutions were carried out with 50 mg mL^{-1} enzyme.

We next analysed the effects of solvents on the reaction rate and *E* (Table 2). The highest *E* values and conversions were observed in *i*-Pr_2O and in *t*-BuOMe (entries 1 and 2). None of the other solvents tested were suitable for the hydrolysis of (±)-**5** (entries 3–5). In view of our earlier results on the vapour-assisted ring opening of carbocyclic *cis*-β-lactams,[26] the hydrolysis of (±)-**5** was attempted under solvent-free conditions (entry 6): the reaction rate increased considerably, while *E* decreased.

Certain additives can influence the enantioselectivity or the reaction rate of lipase-catalysed reactions (Table 3).[27] However, no enhancement relative to H_2O was achieved by the addition of *i*-Pr_2EtN, Et_3N or 2-octanol.

We then attempted to increase the reaction rate by increasing the amount of H_2O (1–10 equiv.) (Table 4). In contrast with our previous experience,[23b,c] the reaction rate decreased on increasing the amount of H_2O; moreover, the degree of hydrolysis was complete and the reaction was fastest without the addition of any H_2O (entry 1). We presume that the poorer solubility of (±)-**5** in the presence of more H_2O can cause a decrease in the reaction rate. In good correlation with our earlier observation,[21] the H_2O present in the reaction medium (<0.1%) or at the surface of the enzyme preparation (<5% w/w H_2O) was responsible for the hydrolysis of (±)-**5**.

Table 2 Effects of solvents on the hydrolysis of (±)-**5**[a]

Entry	Solvent (1 mL)	ee_s (%)[b]	ee_p (%)[b]	Conv. (%)	*E*
1	*i*-Pr_2O	60	96	38	90
2	*t*-BuOMe	50	96	34	81
3	Et_2O	8	74	10	7
4	*n*-Hexane	50	89	36	28
5	Toluene	5	82	6	11
6	Solvent-free	74	66	53	11

[a] 0.05 M substrate, 50 mg mL^{-1} lipase PS IM, 0.5 equiv. of H_2O at 45 °C after 35 h. [b] According to HPLC (Experimental section).

Table 3 Effects of additives on the hydrolysis of (±)-**5**[a]

Entry	Additive (1 equiv.)	ee_s (%)[b]	ee_p (%)[b]	Conv. (%)	*E*
1	H_2O	44	96	31	76
2	*i*-Pr_2EtN	41	96	30	73
3	Et_3N	43	96	31	75
4	2-Octanol	39	96	29	72

[a] 0.05 M substrate, 1 mL of *t*-BuOMe, 50 mg mL^{-1} lipase PS IM at 45 °C after 31 h. [b] According to HPLC (Experimental section).

Table 4 Effects of added H_2O on the hydrolysis of (±)-**5**[a]

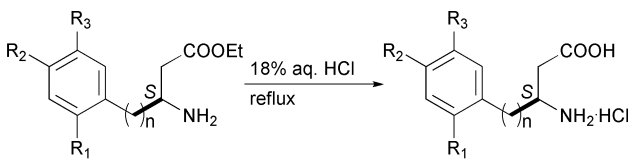
Entry	H_2O (equiv.)	ee_s (%)[b]	ee_p (%)[b]	Conv. (%)	*E*
1	0	38	96	28	71
2	0.5	35	96	27	69
3	1	31	96	24	66
4	5	22	96	19	61
5	10	8	96	8	53

[a] 0.05 M substrate, 1 mL of *t*-BuOMe, 50 mg mL^{-1} lipase PS IM at 45 °C after 25 h. [b] According to HPLC (Experimental section).

Racemic **6** and **11** were hydrolysed under the optimized conditions for (±)-**5**: with 0.5 equiv. of H_2O in the presence of 50 mg mL^{-1} lipase PS IM in *t*-BuOMe or in *i*-Pr_2O at 45 °C. When the reaction was performed in *i*-Pr_2O instead of *t*-BuOMe, higher reaction rates and better *E* values were observed (Table 5).

With regard to the results of the preliminary experiments, preparative-scale resolutions were performed with lipase PS IM in *t*-BuOMe or in *i*-Pr_2O with 0.5 equiv. of H_2O at 45 °C. The reactions were stopped at close to 50% conversion, and the products were obtained in good yields (≥42%) and with good *ee* values (≥96%).

Enantiomeric **5a**, **6a** and **11a** were hydrolysed with aqueous HCl, affording **5c**, **6c** and **11c** (*ee* ≥96%) (Scheme 4). Treatment of **5b**, **6b** and **11b** with 22% HCl/EtOH resulted in the corresponding enantiopure **5d**, **6d** and **11d** (*ee* ≥96%) (Scheme 5).



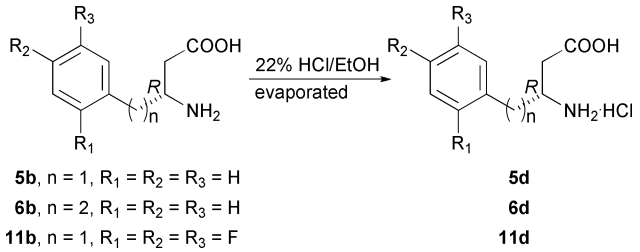
5a, n = 1, $R_1 = R_2 = R_3$ = H — **5c**
6a, n = 2, $R_1 = R_2 = R_3$ = H — **6c**
11a, n = 1, $R_1 = R_2 = R_3$ = F — **11c**

Scheme 4 Syntheses of **5c**, **6c** and **11c**.

Table 5 Effects of solvents on the hydrolyses of (±)-**6** and (±)-**11**[a]

Entry	Substrate	*t*/h	Solvent (1 mL)	ee_s (%)[b]	ee_p (%)[b]	Conv. (%)	*E*
1	(±)-**6**	43	*i*-Pr_2O	79	96	45	119
2	(±)-**6**	43	*t*-BuOMe	54	91	37	36
3	(±)-**11**	65	*i*-Pr_2O	85	97	47	179
4	(±)-**11**	65	*t*-BuOMe	61	97	39	123

[a] 0.05 M substrate, 50 mg mL^{-1} lipase PS IM, 0.5 equiv. of H_2O at 45 °C. [b] According to HPLC (Experimental section).



Scheme 5 Syntheses of **5d**, **6d** and **11d**.

The absolute configurations and *E* values were proved by comparing the [α] values with literature data (Experimental section).

Conclusions

A simple and efficient direct enzymatic method has been developed for the synthesis of pharmacologically valuable optically active β-arylalkyl-substituted β-amino acids *via* enantioselective hydrolysis of the corresponding racemic β-amino esters in an organic medium. The *R*-selective hydrolyses of (±)-**5**, (±)-**6** and (±)-**11** were performed with H_2O (0.5 equiv.) as a nucleophile, using *Burkholderia cepacia* lipase (lipase PS IM) as an enzyme in *t*-BuOMe or in *i*-Pr_2O at 45 °C (*E* ~ 200). The enantiomers of **5a**, **6a** and **11a** (*ee* ≥96%), and **5b**, **6b** and **11b** (*ee* ≥96%) were isolated in good yields (≥42%) and could be easily separated. Ester enantiomers **5a**, **6a** and **11a** were readily hydrolysed with 18% aqueous HCl, resulting in acids **5c**, **6c** and **11c** (*ee* ≥96%).

This method offers a better choice for the preparation of β-arylalkyl-substituted β-amino acid enantiomers as compared with the ring opening of the corresponding lactam.[22] It should be mentioned that, although derivatives of **11b** have been prepared by asymmetric routes (see ref. 10*a*, 11 and 18), to the best of our knowledge they have not yet been obtained by enzymatic procedures.

Experimental section

Materials and methods

Lipase PS IM (*Burkholderia cepacia*, immobilized on diatomaceous earth) was a gift of Amano Enzyme Europe Ltd. Lipase AK (*Pseudomonas fluorescens*) was from Amano Pharmaceuticals, Lipolase (lipase B from *Candida antarctica*, produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin) and PPL (porcine pancreas lipase type II) were from Sigma, and Chyrazyme L-5 (lipase A from *Candida antarctica*) was from Novo Nordisk. Before use, lipase AK, CAL-A and PPL (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). Allylbenzene, 4-phenylbut-1-ene and Meldrum's acid were from Aldrich. (2,4,5-Trifluorophenyl)acetic acid was from Matrix Scientific. 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 (CHNS) corresponded closely (within ±0.3%) with the calculated ones in all cases.

In a typical small-scale enzyme test, (±)-**5**, (±)-**6** or (±)-**11** (0.05 M solution) in an organic solvent (1 mL) was added to the enzyme tested (30, 50 or 75 mg mL^{-1}), followed by H_2O, *i*-Pr_2EtN, Et_3N or 2-octanol (0, 0.5, 1, 5 or 10 equiv.). The mixture was shaken at 25, 45 or 60 °C.

The *ee* values for the unreacted β-amino ester and the β-amino acid enantiomers produced were determined by HPLC as follows:

5a, **6a**, **11a**, **11b**: [**11b** was pre-column derivatized with CH_2N_2[28] (**Caution!** derivatization with CH_2N_2 should be performed under a well-working hood)]: a Chiralpak IA column (4.6 mm × 250 mm); eluent: *n*-hexane (0.1% DEA)–*i*-PrOH (95 5); flow rate: 0.3 mL min^{-1}; detection at 276 nm; retention times (min) for **5a**: 35.2 (antipode: 33.9); for **6a**: 37.5 (antipode: 36.6); for **11a**: 48.5 (antipode: 39.6); for **11b**: 44.4 (antipode: 50.0).

5b: a Chirobiotic TAG column (4.6 mm × 250 mm); eluent: MeOH–AcOH–TEA (100 : 0.1 : 0.1); flow rate: 0.8 mL min^{-1}; detection at 205 nm; retention times (min): 17.2 (antipode: 19.2).

6b: an APEX Octadecyl 5 μ column (0.04 cm × 25 cm); pre-column derivatization with (*S*)-NIFE according to the literature;[29] the mobile phases were H_2O (A) and MeCN (B), both of which contained 0.1% TFA; the gradient slopes were: 95% A + 5% B at 0 min, increased to 25% A + 75% B within 60 min; flow rate: 0.8 mL min^{-1}; room temperature; detection at 205 nm; retention times (min): 42.7 (antipode: 41.9).

Syntheses of β-amino esters (±)-5 and (±)-6

Racemic β-lactams (±)-**3** and (±)-**4** were prepared by the addition of chlorosulfonyl isocyanate to allylbenzene (**1**) or 4-phenylbut-1-ene (**2**), respectively, according to known literature methods.[19b,24] (±)-**3** and (±)-**4** (6.0 mmol) were then refluxed with 22% HCl/EtOH (15 mL) for 8 h, after which the solvent was evaporated off, resulting in the corresponding β-amino ester hydrochlorides (±)-**5**·HCl and (±)-**6**·HCl, which were immediately treated with aqueous K_2CO_3 to afford (±)-**5** and (±)-**6**, as oils.

Ethyl (±)-3-amino-4-phenylbutanoate [(±)-5]. Yield: 1.07 g (86%), a pale-yellow oil; δ_H(400 MHz; $CDCl_3$; Me_4Si) 1.27-1.31

(3 H, t, *J* 7.1, CH_2CH_3), 2.32-2.38 (1 H, dd, *J* 8.7 and 15.9, CH_2COOH), 2.49-2.54 (1 H, dd, *J* 4.2 and 15.9, CH_2COOH), 2.62-2.68 (1 H, dd, *J* 8.1 and 13.4, CH_2Ar), 2.78-2.82 (1 H, dd, *J* 5.6 and 13.4, CH_2Ar), 3.49-3.52 (1 H, m, CH), 4.15-4.20 (2 H, q, *J* 7.1 and 14.3, CH_2CH_3), 7.22-7.36 (5 H, m, Ar); δ_C(100.62 MHz; $CDCl_3$; Me_4Si) 14.6, 42.4, 44.4, 50.1, 60.8, 126.9, 129.0, 129.7, 139.0, 172.8.

Ethyl (±)-3-amino-5-phenylpentanoate [(±)-6]. Yield: 1.10 g (83%), a pale-yellow oil. The ^{1}H NMR and ^{13}C NMR data are in accordance with those reported in the literature.[30]

Syntheses of β-amino ester (±)-11

To a mixture of 2,4,5-trifluorophenylacetic acid **7** (2.28 g, 12.0 mmol), 2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) (1.90 g, 13.2 mmol), *N*,*N*-dimethylaminopyridine (0.12 g, 0.96 mmol) and *N*,*N*-diisopropylethylamine (4.7 mL, 27.0 mmol) in MeCN (8 mL), trimethylacetylchloride (1.6 mL, 13.2 mmol) was added at 40 °C. The reaction mixture was stirred at 45 °C for 3 h, cooled to 0 °C, and 1 M HCl (20 mL) was then slowly added to the reaction mixture to form a solid. The resulting solid was washed with 20% CH_3CN–H_2O (50 mL) to give 5-(1-hydroxy-2-(2,4,5-trifluorophenyl)ethylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (**8**): 3.37 g (85%), white crystals; mp 99-101 °C (from MeCN) [lit.,[31] 117 °C (decomp.)]. The ^{1}H NMR and ^{13}C NMR data are in accordance with those reported in the literature.[31]

In the next step, **8** (3.30 g, 10.0 mmol) was refluxed in EtOH–toluene 1 : 4 (80 mL) for 3 h. The reaction mixture was subsequently diluted with EtOAc (35 mL), and washed with brine (2 × 50 mL). The organic layer was dried with Na_2SO_4 and evaporated to give ethyl 3-oxo-4-(2,4,5-trifluorophenyl)butanoate (**9**): 2.45 g (94%), white crystals; mp 29-30 °C (from *n*-hexane). δ_H(400 MHz; $CDCl_3$; Me_4Si) 1.30-1.34 (3 H, t, *J* 6.9, CH_2CH_3), 3.55 (2 H, s, $CH_2COOCH_2CH_3$), 3.88 (2 H, s, CH_2Ar), 4.22-4.27 (2 H, q, *J* 7.1 and 14.3, CH_2CH_3), 6.94-7.01 (1 H, m, Ar), 7.04-7.11 (1 H, m, Ar); δ_C(100.62 MHz; $CDCl_3$; Me_4Si) 14.6, 42.4, 49.1, 62.1, 106.1, 119.9, 145.8, 147.8, 155.1, 157.1, 167.1, 198.5.

A mixture of **9** (2.60 g, 10.0 mmol) and NH_4OAc (3.85 g, 50.0 mmol) in EtOH (40 mL) was refluxed for 7 h. The reaction mixture was evaporated, diluted with EtOAc (50 mL) and washed with H_2O (2 × 50 mL). The organic layer was dried with Na_2SO_4 and evaporated to give ethyl 3-amino-4-(2,4,5-trifluorophenyl)but-2-enoate (**10**): 2.31 g (89%), white crystals; mp 115-117 °C (from *n*-hexane and EtOAc). δ_H(400 MHz; $CDCl_3$; Me_4Si) 1.27-1.31 (3 H, t, *J* 7.2, CH_2CH_3), 3.44 (2 H, s, CH_2Ar), 4.12-4.17 (2 H, q, *J* 7.1 and 14.2, CH_2CH_3), 4.59 (1 H, s, CH), 6.94-7.01 (1 H, m, Ar), 7.09-7.15 (1 H, m, Ar); δ_C(100.62 MHz; $CDCl_3$; Me_4Si) 14.9, 35.1, 59.3, 85.8, 105.6, 119.2, 145.7, 147.9, 154.4, 158.8, 169.8.

10 (1.56 g, 6.0 mmol) was dissolved in EtOAc (15 mL), the mixture was cooled to 0 °C, and $NaCNBH_3$ (1.13 g, 18.0 mmol) and glacial AcOH (1.0 mL, 18.0 mmol) were added. After 6 h, the mixture was extracted with 10% Na_2CO_3 (3 × 15 mL), and the organic phase was dried with Na_2SO_4 and evaporated, resulting in crude (±)-**11**. This was then dissolved in 22% HCl/EtOH (10 mL), and the solution was evaporated, resulting in (±)-**11**·HCl, which was immediately treated with aqueous K_2CO_3 to afford (±)-**11**: 0.85 g (54%), a pale-yellow oil; δ_H(400 MHz; $CDCl_3$; Me_4Si) 1.28-1.32 (3 H, t, *J* 7.1, CH_2CH_3), 2.32-2.39 (1 H, dd, *J* 8.5 and 15.9, CH_2COOH), 2.48-2.54 (1 H, dd, *J* 4.2 and 15.9, CH_2COOH), 2.65-2.71 (1 H, dd, *J* 7.7 and 13.7, CH_2Ar), 2.76-2.81 (1 H, dd, *J* 5.8 and 13.7, CH_2Ar), 3.46-3.52 (1 H, m, CH), 4.16.4.21 (2 H, q, *J* 7.1 and 14.2, CH_2CH_3), 6.93-6.96 (1 H, m, Ar), 7.08-7.11 (1 H, m, Ar); δ_C(100.62 MHz; $CDCl_3$; Me_4Si) 14.6, 36.8, 42.1, 49.1, 61.0, 105.6, 120.3, 145.3, 147.7, 155.0, 157.0, 172.0.

General procedure for the preparative-scale resolutions of (±)-5, (±)-6 and (±)-11

Racemic (±)-**5**, (±)-**6** and (±)-**11** (3 mmol) were dissolved in *t*-BuOMe [(±)-**5**] or in *i*-Pr_2O [(±)-**6** and (±)-**11**] (15 mL). Lipase PS IM (0.75 g, 50 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 3–5 d (Table 6). The reaction was stopped by filtering off the enzyme at close to 50% conversion. The solvent was evaporated and the residues (*S*)-**5a**, (*S*)-**6a** and (*S*)-**11a** were immediately hydrolysed by refluxing with 6 mL of 18% aqueous HCl solution for 7 h to give (*S*)-**5c**, (*S*)-**6c** and (*S*)-**11c**. The filtered-off enzyme was washed with distilled H_2O (3 × 15 mL), and the H_2O was evaporated off, yielding crystalline (*R*)-**5b**, (*R*)-**6b** and (*R*)-**11b**. When (*R*)-**5b**, (*R*)-**6b** or (*R*)-**11b** (50 mg) was treated with 22% HCl/EtOH (5 mL), then evaporated, (*R*)-**5d**, (*R*)-**6d** or (*R*)-**11d** was obtained.

(*R*)-3-Amino-4-phenylbutanoic acid (5b). Yield: 242 mg (45%), white crystals; *ee* = 96%; $[\alpha]_D^{25}$ –4.6 (*c* 0.30 in H_2O) [lit.,[22] +7 (*c* 0.20 in H_2O) for the (*S*) enantiomer]; mp 209-211 °C (from H_2O and Me_2CO) (lit.,[22] 207-210 °C) The ^{1}H NMR data are in accordance with those reported in the literature.[22] δ_C(100.62 MHz; D_2O; Me_4Si) 38.5, 38.6, 51.2, 128.1, 129.6, 130.0, 136.2, 178.3.

Hydrochloride salt of (*S*)-3-amino-4-phenylbutanoic acid (5c). Yield: 272 mg (42%), off-white crystals; *ee* = 96%; $[\alpha]_D^{25}$ +4.6 (*c* 0.36 in H_2O) [lit.,[22] +6 (*c* 0.21 in H_2O)]; mp 174-176 °C (from EtOH and Et_2O) (lit.,[22] 172-175 °C). The ^{1}H NMR and ^{13}C NMR data are in accordance with those reported in the literature.[19b]

Table 6 Lipase PS IM-catalysed hydrolyses of (±)-**5**, (±)-**6** and (±)-**11**[a]

				β-Amino acid·HCl (**5c**, **6c**, **11c**)				β-Amino acid (**5b**, **6b**, **11b**)			
	t/d	Conv. (%)	*E*	Yield (%)	Isomer	*ee* (%)[b]	$[\alpha]_D^{25}$	Yield (%)	Isomer	*ee* (%)[b]	$[\alpha]_D^{25}$
(±)-**5**	5	50	194	42	*S*	96	+4.6[c]	45	*R*	96	–4.6[d]
(±)-**6**	3	51	>200	47	*S*	98	+11.5[e]	47	*R*	96	–12.1[f]
(±)-**11**	5	50	>200	44	*S*	96	–9.8[g]	43	*R*	97	+15.5[h]

[a] 50 mg mL^{-1} lipase PS IM in *i*-Pr_2O, 0.5 equiv. of H_2O at 45 °C. [b] According to HPLC (Experimental section). [c] *c* 0.36. [d] *c* 0.30. [e] *c* 0.40. [f] *c* 0.34. [g] *c* 0.32. [h] *c* 0.35.

Hydrochloride salt of (*R*)-3-amino-4-phenylbutanoic acid (5d). Quantitative yield; off-white crystals; *ee* = 96%; $[\alpha]_D^{25}$ −3.9 (*c* 0.35 in H_2O) [lit.,[22] −8 (*c* 0.11 in H_2O)]; mp 169-172 °C (lit.,[22] 182-185 °C). The ^{1}H NMR and ^{13}C NMR data for **5d** are similar to those for **5c**.

(*R*)-3-Amino-5-phenylpentanoic acid (6b). Yield: 272 mg (47%), white crystals; *ee* = 96%; $[\alpha]_D^{25}$ −12.1 (*c* 0.34 in H_2O) [lit.,[22] +24 (*c* 0.28 in H_2O) for the (*S*) enantiomer]; mp 214-216 °C (from H_2O and Me_2CO) (lit.,[22] 215-219 °C). The ^{1}H NMR data are in accordance with those reported in the literature.[22] δ_C(100.62 MHz; D_2O; Me_4Si) 31.3, 34.4, 38.7, 49.6, 127.0, 129.0, 129.4, 141.4, 178.6.

Hydrochloride salt of (*S*)-3-amino-5-phenylpentanoic acid (6c). Yield: 324 mg (47%), off-white crystals; *ee* = 98%; $[\alpha]_D^{25}$ +11.5 (*c* 0.40 in H_2O) [lit.,[22] +12 (*c* 0.21 in H_2O)]; mp 144-146 °C (from EtOH and Et_2O) (lit.,[22] 150-152 °C). The ^{1}H NMR data are in accordance with those reported in the literature.[22] δ_C(100.62 MHz; D_2O; Me_4Si) 31.1, 34.1, 36.3, 48.5, 127.1, 129.0, 129.4, 141.0, 174.8.

Hydrochloride salt of (*R*)-3-amino-5-phenylpentanoic acid (6d). Quantitative yield; off-white crystals; *ee* = 96%; $[\alpha]_D^{25}$ −10.5 (*c* 0.40 in H_2O) [lit.,[22] −15 (*c* 0.21 in H_2O)]; mp 147-149 °C (lit.,[22] 146-148 °C). The ^{1}H NMR and ^{13}C NMR data for **6d** are similar to those for **6c**.

(*R*)-3-Amino-4-phenyl(2,4,5-trifluorophenyl)butanoic acid (11b). Yield: 301 mg (43%), white crystals; *ee* = 97%; $[\alpha]_D^{25}$ +15.5 (*c* 0.35 in H_2O); mp 217-219 °C (from H_2O and Me_2CO). δ_H(400 MHz; D_2O; Me_4Si) 2.48-2.54 (1 H, dd, *J* 7.9 and 16.5, CH_2COOH), 2.59-2.65 (1 H, dd, *J* 5.0 and 16.9, CH_2COOH), 3.08-3.09 (2 H, d, *J* 6.9, CH_2Ar), 3.81-3.88 (1 H, m, C*H*), 7.22-7.27 (1 H, m, Ar), 7.31-7.37 (1 H, m, Ar); δ_C(100.62 MHz; D_2O; Me_4Si) 31.1, 38.0, 49.1, 105.8, 118.9, 145.6, 148.0, 155.1, 157.5, 177.3. To prove the absolute configuration, *N*-Boc-**11b** was prepared by a literature method[11*b*] {white crystals; $[\alpha]_D^{25}$ +24.6 (*c* 0.46 in $CHCl_3$) [lit.,[11*b*] +32.3 (*c* 1.0 in $CHCl_3$)]; mp 112-114 °C (from *n*-hexane) (lit.,[11*b*] 124-125 °C)}.

Hydrochloride salt of (*S*)-3-amino-4-(2,4,5-trifluorophenyl)-butanoic acid (11c). Yield: 356 mg (44%), off-white crystals; *ee* = 96%; $[\alpha]_D^{25}$ −9.8 (*c* 0.32 in H_2O); mp 177-179 °C (from EtOH and Et_2O). δ_H(400 MHz; D_2O; Me_4Si) 2.75-2.89 (2 H, m, CH_2COOH), 3.08-3.19 (2 H, m, CH_2Ar), 3.96-4.05 (1 H, m, C*H*), 7.21-7.28 (1 H, m, Ar), 7.32-7.38 (1 H, m, Ar); δ_C(100.62 MHz; D_2O; Me_4Si) 31.5, 36.1, 48.8, 106.5, 119.4, 146.0, 148.1, 155.4, 156.6, 174.0.

Hydrochloride salt of (*R*)-3-amino-4-(2,4,5-trifluorophenyl)-butanoic acid (11d). Quantitative yield; off-white crystals; *ee* = 97%; $[\alpha]_D^{25}$ +10.6 (*c* 0.31 in H_2O); mp 179-181 °C. The ^{1}H NMR and ^{13}C NMR data for **11d** are similar to those for **11c**.

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