

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*-Pr₂O 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 ($ee \geq 96\%$) and in good yields ($\geq 42\%$).

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 JanuviaTM (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 JanuviaTM, 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

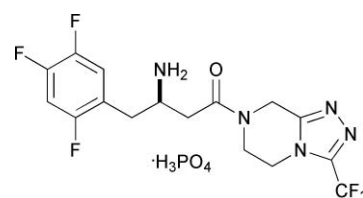


Fig. 1 JanuviaTM (sitagliptin phosphate).

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

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 ($\leq 36\%$). 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 K₂CO₃ (Scheme 1).

Starting from acid **7**, enamine **10** was prepared by a slightly modified literature method.^{10c} β-Amino ester (±)-11 was obtained by the reduction of enamine **10** with NaCNBH₃ in the presence of AcOH (Scheme 2).

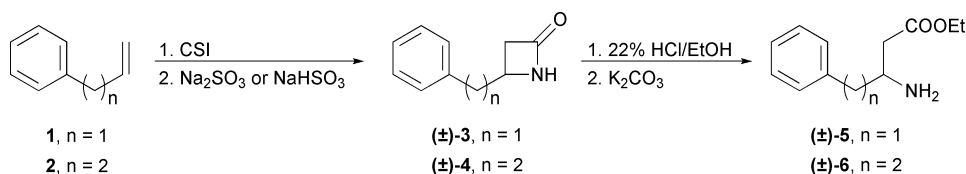
Enzymatic hydrolysis of (±)-5, (±)-6 and (±)-11

Preliminary experiments. Preliminary experiments were performed on the hydrolysis of model compound (±)-5 (Scheme 3).

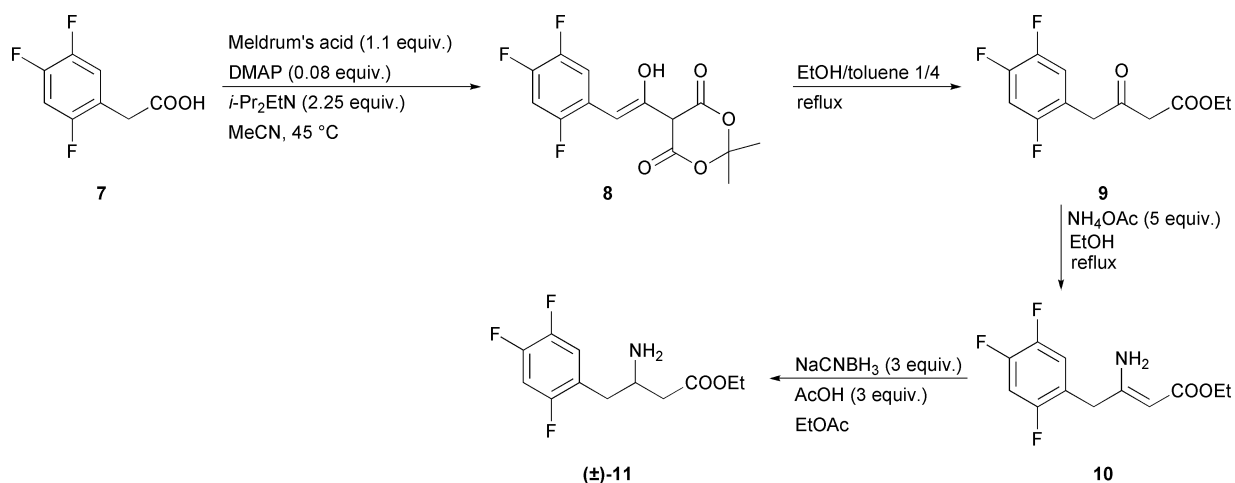
First, a number of lipases were tested in *t*-BuOMe at 25 °C with 0.5 equiv. of H₂O (the quantity of the racemic compound is considered as 1 molar equivalent). The use of organic solvents in enzymatic reactions has numerous advantages, and lipases are able to hydrolyse ester and amide bonds in organic 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.

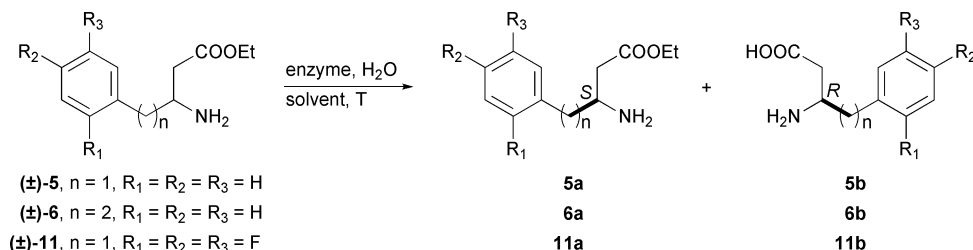
When the amount of enzyme was increased from 30 to 50 and then 75 mg mL⁻¹, the reaction rates clearly increased, while the *E*



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	<i>t</i> /h	<i>T</i> /°C	<i>ee</i> _s (%) ^b	<i>ee</i> _p (%) ^b	Conv. (%)	<i>E</i>
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 H₂O. ^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*-Pr₂O 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 H₂O was achieved by the addition of *i*-Pr₂EtN, Et₃N or 2-octanol.

We then attempted to increase the reaction rate by increasing the amount of H₂O (1–10 equiv.) (Table 4). In contrast with our previous experience,^{23b,c} the reaction rate decreased on increasing the amount of H₂O; moreover, the degree of hydrolysis was complete and the reaction was fastest without the addition of any H₂O (entry 1). We presume that the poorer solubility of (±)-**5** in the

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

Entry	Solvent (1 mL)	<i>ee</i> _s (%) ^b	<i>ee</i> _p (%) ^b	Conv. (%)	<i>E</i>
1	<i>i</i> -Pr ₂ O	60	96	38	90
2	<i>t</i> -BuOMe	50	96	34	81
3	Et ₂ O	8	74	10	7
4	<i>n</i> -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 H₂O 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.)	<i>ee</i> _s (%) ^b	<i>ee</i> _p (%) ^b	Conv. (%)	<i>E</i>
1	H ₂ O	44	96	31	76
2	<i>i</i> -Pr ₂ EtN	41	96	30	73
3	Et ₃ N	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 H₂O on the hydrolysis of (±)-**5**^a

Entry	H ₂ O (equiv.)	<i>ee</i> _s (%) ^b	<i>ee</i> _p (%) ^b	Conv. (%)	<i>E</i>
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).

presence of more H₂O can cause a decrease in the reaction rate. In good correlation with our earlier observation,²¹ the H₂O present in the reaction medium (<0.1%) or at the surface of the enzyme preparation (<5% w/w H₂O) was responsible for the hydrolysis of (±)-**5**.

Racemic **6** and **11** were hydrolysed under the optimized conditions for (±)-**5**: with 0.5 equiv. of H₂O in the presence of 50 mg mL⁻¹ lipase PS IM in *t*-BuOMe or in *i*-Pr₂O at 45 °C. When the reaction was performed in *i*-Pr₂O 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*-Pr₂O with 0.5 equiv. of H₂O 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).

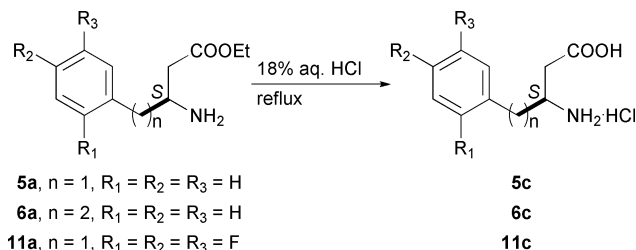
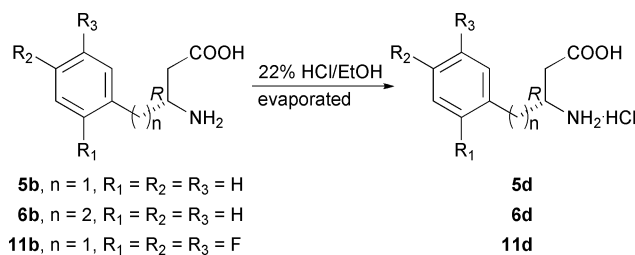
**Scheme 4** Syntheses of **5c**, **6c** and **11c**.

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

Entry	Substrate	<i>t</i> /h	Solvent (1 mL)	<i>ee</i> _s (%) ^b	<i>ee</i> _p (%) ^b	Conv. (%)	<i>E</i>
1	(±)- 6	43	<i>i</i> -Pr ₂ O	79	96	45	119
2	(±)- 6	43	<i>t</i> -BuOMe	54	91	37	36
3	(±)- 11	65	<i>i</i> -Pr ₂ O	85	97	47	179
4	(±)- 11	65	<i>t</i> -BuOMe	61	97	39	123

^a 0.05 M substrate, 50 mg mL⁻¹ lipase PS IM, 0.5 equiv. of H₂O 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 H₂O (0.5 equiv.) as a nucleophile, using *Burkholderia cepacia* lipase (lipase PS IM) as an enzyme in *t*-BuOMe or in *i*-Pr₂O 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. 10a, 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. ¹H NMR and ¹³C 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 H₂O, *i*-Pr₂EtN, Et₃N 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 CH₂N₂²⁸ (**Caution!** derivatization with CH₂N₂ 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 H₂O (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 K₂CO₃ 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; CDCl₃; Me₄Si) 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 (\pm)-3-amino-5-phenylpentanoate (\pm)-6. Yield: 1.10 g (83%), a pale-yellow oil. The ^1H NMR and ^{13}C NMR data are in accordance with those reported in the literature.³⁰

Syntheses of β -amino ester (\pm)-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 ^1H 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 \times 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, $\text{CH}_2\text{COOCH}_2\text{CH}_3$), 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 \times 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 \times 15 mL), and the organic phase was dried with Na_2SO_4 and evaporated, resulting in crude (\pm)-**11**. This was then dissolved in 22% HCl/EtOH (10 mL), and the solution was evaporated, resulting in (\pm)-**11**·HCl, which was immediately treated with aqueous K_2CO_3 to afford (\pm)-**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 (\pm)-5, (\pm)-6 and (\pm)-11

Racemic (\pm)-**5**, (\pm)-**6** and (\pm)-**11** (3 mmol) were dissolved in t -BuOMe [(\pm)-**5**] or in i -Pr₂O [(\pm)-**6** and (\pm)-**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 \times 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%; $[\alpha]_{\text{D}}^{25}$ −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 ^1H 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%; $[\alpha]_{\text{D}}^{25}$ +4.6 (c 0.36 in H_2O) [lit.,²² +6 (c 0.21 in H_2O)]; mp 174–176 °C (from EtOH and Et₂O) (lit.,²² 172–175 °C). The ^1H NMR and ^{13}C NMR data are in accordance with those reported in the literature.^{19b}

Table 6 Lipase PS IM-catalysed hydrolyses of (\pm)-**5**, (\pm)-**6** and (\pm)-**11**^a

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

^a 50 mg mL^{−1} lipase PS IM in i -Pr₂O, 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%; $[\alpha]_{\text{D}}^{25}$ -3.9 (*c* 0.35 in H₂O) [lit.,²² -8 (*c* 0.11 in H₂O)]; mp 169–172 °C (lit.,²² 182–185 °C). The ¹H NMR and ¹³C 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%; $[\alpha]_{\text{D}}^{25}$ -12.1 (*c* 0.34 in H₂O) [lit.,²² +24 (*c* 0.28 in H₂O) for the (*S*) enantiomer]; mp 214–216 °C (from H₂O and Me₂CO) (lit.,²² 215–219 °C). The ¹H NMR data are in accordance with those reported in the literature.²² δ_{C} (100.62 MHz; D₂O; Me₄Si) 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%; $[\alpha]_{\text{D}}^{25}$ +11.5 (*c* 0.40 in H₂O) [lit.,²² +12 (*c* 0.21 in H₂O)]; mp 144–146 °C (from EtOH and Et₂O) (lit.,²² 150–152 °C). The ¹H NMR data are in accordance with those reported in the literature.²² δ_{C} (100.62 MHz; D₂O; Me₄Si) 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%; $[\alpha]_{\text{D}}^{25}$ -10.5 (*c* 0.40 in H₂O) [lit.,²² -15 (*c* 0.21 in H₂O)]; mp 147–149 °C (lit.,²² 146–148 °C). The ¹H NMR and ¹³C 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%; $[\alpha]_{\text{D}}^{25}$ +15.5 (*c* 0.35 in H₂O); mp 217–219 °C (from H₂O and Me₂CO). δ_{H} (400 MHz; D₂O; Me₄Si) 2.48–2.54 (1 H, dd, *J* 7.9 and 16.5, CH₂COOH), 2.59–2.65 (1 H, dd, *J* 5.0 and 16.9, CH₂COOH), 3.08–3.09 (2 H, d, *J* 6.9, CH₂Ar), 3.81–3.88 (1 H, m, CH), 7.22–7.27 (1 H, m, Ar), 7.31–7.37 (1 H, m, Ar); δ_{C} (100.62 MHz; D₂O; Me₄Si) 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^{11b} {white crystals; $[\alpha]_{\text{D}}^{25}$ +24.6 (*c* 0.46 in CHCl₃) [lit.,^{11b} +32.3 (*c* 1.0 in CHCl₃)]; mp 112–114 °C (from *n*-hexane) (lit.,^{11b} 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%; $[\alpha]_{\text{D}}^{25}$ -9.8 (*c* 0.32 in H₂O); mp 177–179 °C (from EtOH and Et₂O). δ_{H} (400 MHz; D₂O; Me₄Si) 2.75–2.89 (2 H, m, CH₂COOH), 3.08–3.19 (2 H, m, CH₂Ar), 3.96–4.05 (1 H, m, CH), 7.21–7.28 (1 H, m, Ar), 7.32–7.38 (1 H, m, Ar); δ_{C} (100.62 MHz; D₂O; Me₄Si) 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%; $[\alpha]_{\text{D}}^{25}$ +10.6 (*c* 0.31 in H₂O); mp 179–181 °C. The ¹H NMR and ¹³C NMR data for **11d** are similar to those for **11c**.

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