Synthesis of (2*S*)-2-amino-3-(2′,6′-dibromo-4′-hydroxy)phenylpropionic acid (2,6-dibromo-L-tyrosine)

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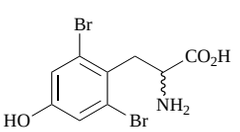
(2*S*)-2-Amino-3-(2′,6′-dibromo-4′-hydroxy)phenylpropionic acid (2,6-dibromo-L-tyrosine) 1a has been synthesized by two methods. The first is by enantioselective hydrolysis of the *N*-trifluoroacetyl derivative of the racemic 2,6-dibromotyrosine with carboxypeptidase A. The kinetic parameters of activity of carboxypeptidase A against *N*-trifluoroacetyl-2,6-dibromo-L-tyrosine have also been estimated (V_{max}, 0.32 mM min^{-1} mg^{-1}; K_m 16.15 mM). The second is an asymmetric synthesis by Schöllkopf's bis-lactim ether method. Alkylation of the lithiated bis-lactim ether of cyclo(-D-Val-Gly) 6 with 2,6-dibromo-4-methoxybenzyl bromide and subsequent hydrolysis with aqueous trifluoroacetic acid gives 2,6-dibromo-4-methoxy-L-tyrosine methyl ester 8. Demethylation of the ester 8 with boron tribromide affords the amino acid 1a. The enantiomeric purity of 2,6-dibromo-L-tyrosine 1a obtained by both methods has been determined by HPLC with a chiral stationary column and is found to be more than 95% (ee).

Introduction

Ready availability of tritium labelled peptides would be of obvious value for a variety of chemical and biological studies. The specific tritiation of peptides has predominantly been carried out by tritium–halogen exchange of precursor peptides containing halogen substituted into histidine,[1,2] phenylalanine[3] and tyrosine[4] residues. The risk of metabolic instability of tritium labels makes it necessary to explore the most metabolically stable position. We chose, therefore, the 2- and 6-ring positions of the tyrosine residue for the labelling sites because of their chemical stability and the facility with which they undergo tritiation. Earlier, we reported the syntheses of tritiated Leu-enkephalin and [D-Ala^2,D-Leu^5]enkephalin labelled in these positions.[5,6] Instead of using the required (2*S*)-2-amino-3-(2′,6′-dibromo-4′-hydroxy)phenylpropionic acid (2,6-dibromo-L-tyrosine) **1a** in our preparations, its racemate **1** was directly



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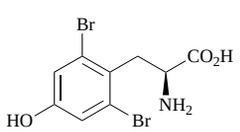
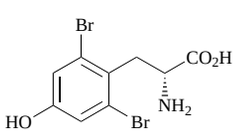


1a



1b

incorporated into the peptide by solid-phase peptide synthesis based on Fmoc-strategy (Fmoc = fluoren-9-ylmethoxycarbonyl). After cleavage of the desired epimeric dibromo peptide from the resin, the peptide was separated into epimers by HPLC. Although this method was convenient in practice, its disadvantage was that the maximum theoretical yield was 50% and this was dependent on separation of epimers: for example, HPLC analysis of [2,6-dibromo-DL-Tyr^1]Leu-enkephalin allowed partial separation,[5] whereas that of [2,6-dibromo-DL-Tyr^1,D-Ala^2,D-Leu^5]enkephalin provided baseline separation.[6] It was, therefore, desirable to obtain optically pure 2,6-dibromo-L-tyrosine **1a**. In the present study, we describe the synthesis of amino acid **1a** by enzymatic resolution of the *N*-acyl derivative of the racemate **1** and by enantioselective synthesis using Schöllkopf's bis-lactim ether method.[7,8]

Table 1 Enzymatic hydrolysis of *N*-acyl derivatives **2**–**4**

	Yield (%)[a]		
Substrate	acylase (porcine kidney)	acylase (*Aspergillus melleus*)	carboxypeptidase A (bovine pancrea)
2	1.8 ± 0.3	3.7 ± 0.5	3.0 ± 0.5
3	2.2 ± 0.8	4.8 ± 1.4	34.1 ± 1.1
4	24.3 ± 1.1	4.4 ± 0.4	97.3 ± 0.5

[a] n = 3, Mean ± S.D.

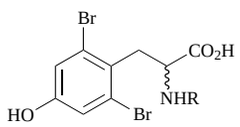
Results and discussion

Acylase (EC 3.5.1.14), isolated from porcine kidney or the fungus *Aspergillus* sp., catalyses the enantioselective hydrolysis of *N*-acyl-L-amino acids.[9–11] *N*-Acetyl- and *N*-chloroacetyl-L-tyrosine are good substrates for *Aspergillus* acylase to give L-tyrosine.[11,12] Bovine pancreatic carboxypeptidase A (EC 3.4.17.1) catalyses the enantioselective hydrolysis of *N*-acyl-L-amino acid-containing aromatic substituents on the β-carbon atom. *N*-Chloroacetyl and *N*-trifluoroacetyl-L-tyrosine were also good substrates for carboxypeptidase A to give L-tyrosine, which was uncontaminated with the opposite isomer.[13,14] We first determined the activity of acylases and carboxypeptidase A against several *N*-acyl derivatives of 2,6-dibromo-DL-tyrosine **1**. Following incubation of the *N*-acyl derivatives **2**, **3** and **4** with porcine kidney acylase, *Aspergillus* acylase and bovine pancreatic carboxypeptidase A in phosphate buffer (pH 7.4) at 37 °C for 24 h, respectively, the amounts of amino acid liberated were assessed by the colorimetric determination with ninhydrin, according to the method of Rosen[15] (Table 1). *N*-Acyl derivatives **2**, **3** and **4** were poor substrates for acylases. Hydrolysis of the *N*-trifluoroacetyl derivative **4** with carboxypeptidase A proceeded to give free amino acid in good yield (>95% based on the L-isomer of compound **4**). For determination of the optical purity of the amino acid obtained, the hydrolysate was analysed by HPLC with a chiral stationary column (Crownpak CR) eluting with 0.14% aqueous $HClO_4$–MeOH (85 : 15, v/v) to give a single peak at the retention time of 38 min. Under these conditions, HPLC analysis of 2,6-

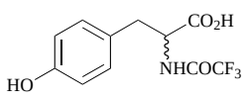
Table 2 Kinetic parameters for activity of pancreatic carboxypeptidase A against the *N*-trifluoroacetyl derivatives **4** and **5**

Substrate	V_{max} [mM min^{-1} (mg protein)$^{-1}$]	K_m (mM)	V_{max}/K_m [min^{-1} (mg protein)$^{-1}$]
4	0.32	16.15	0.02
5	13.7	3.14	4.36

dibromo-DL-tyrosine **1** provided baseline separation at the retention time of 32.6 min (**1b**) and 38 min (**1a**), respectively. These results showed that hydrolysis of compound **4** with carboxypeptidase A proceeded with high enantioselectivity. The activity of carboxypeptidase A against compound **4** was determined by measurement of the Michaelis constant (K_m) and the maximum initial velocity (V_{max}), and these values were compared with those of *N*-trifluoroacetyl-DL-tyrosine **5** (Table



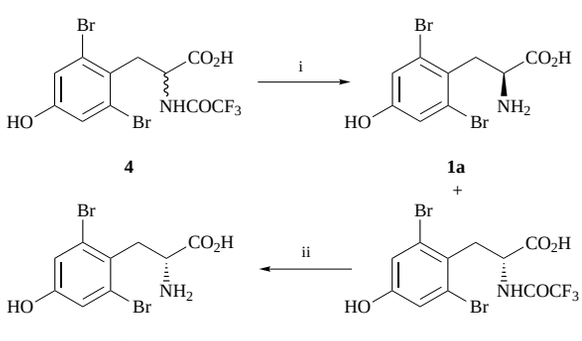
2 R = CH_3CO
3 R = $ClCH_2CO$
4 R = CF_3CO



5

2). The V_{max}/K_m value [0.02 min^{-1} (mg protein)$^{-1}$] of compound **4** was 4/1000 of that [4.36 min^{-1} (mg protein)$^{-1}$] of compound **5**. The relative low affinity of the *N*-trifluoroacetyl derivative **4** to carboxypeptidase A was probably the result of steric hindrance by the aromatic 2,6-bromine atoms of the *N*-trifluoroacetyl derivative **4** to the catalytic site of carboxypeptidase A.

Preparative resolution was performed on 1.0 g of the *N*-trifluoroacetyl derivative **4** (Scheme 1). Progress of the reaction



4 **1a** + **1b**

Scheme 1 *Reagents and conditions*: i, carboxypeptidase A, potassium phosphate buffer (pH 7.4), 37 °C; ii, LiOH, room temperature

was monitored by assaying aliquots for the amino acid liberated by colorimetric determination with ninhydrin (Fig. 1). Liberation of amino acid in the assay tubes reached a plateau after 24 h; the carboxypeptidase A in the bulk sample was then denatured by acidification with hydrochloric acid. Following extraction of the remaining *N*-acyl amino acid with diethyl ether, the acidic aqueous solution was subjected to proton ion-exchange chromatography. After washing with water, elution with 10% aqueous ammonia gave the amino acid **1a** in 75% yield based on the L-isomer of compound **4**. The *N*-acyl amino acid extracted with ether was hydrolysed in LiOH to give the amino acid **1b** in 48% yield based on the D-isomer of compound **4**. The absolute configurations of the amino acids **1a** and **1b** were confirmed upon their reduction to the corresponding known amino acids. An aliquot of each enantiomer was hydrogenated in the presence of palladium black and the product was analysed by HPLC with a chiral stationary column (Crownpak CR) eluting with 0.14% aqueous $HClO_4$ to give a single peak.

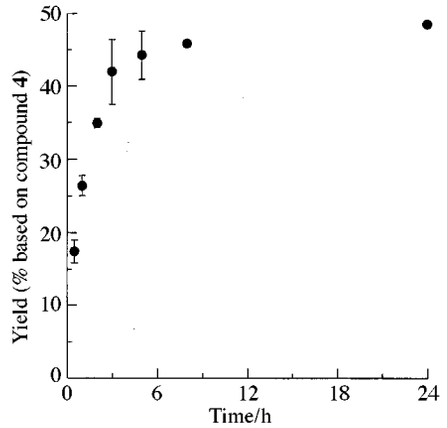
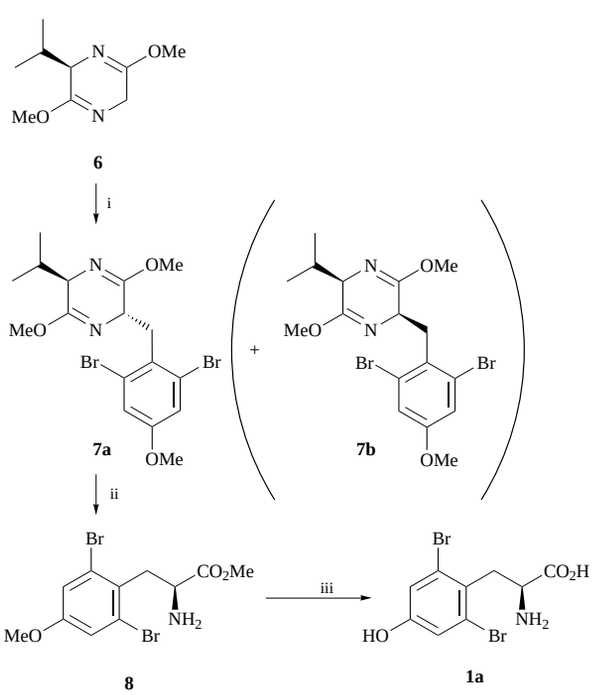


Fig. 1 Progress of hydrolysis of *N*-trifluoroacetyl-2,6-dibromo-DL-tyrosine with bovine pancreatic carboxypeptidase A (mean ± S.D., $n = 3$)

Under the same HPLC conditions, authentic L-tyrosine was eluted at 6.1 min, whereas D-tyrosine was eluted at 5.1 min. The retention time values of the reduction product of amino acids **1a** and **1b** were identical with those of L-tyrosine and D-tyrosine, respectively. These results showed that the amino acids **1a** and **1b** were identical with, respectively, 2,6-dibromo-L-tyrosine and its D-isomer. The enantiomeric purity of the amino acids **1a** and **1b**, determined by HPLC with a chiral stationary column, were found to be >95% (ee). A problem more prevalent than non-specific hydrolysis is incomplete hydrolysis of the L-enantiomer, which causes enantiomeric contamination of the D-product. The high optical purity of the amino acid **1b** showed that the hydrolysis of the *N*-trifluoroacetyl derivative **4** with carboxypeptidase A was almost complete.

Schöllkopf's bis-lactim ether methodology has proved to be of enormous utility in the preparation of a wide range of (2*R*)- and (2*S*)-amino acids[7,8] and the chiral dihydropyrazine precursors are now commercially available. The synthetic route to the amino acid **1a** is illustrated in Scheme 2. Alkylation of the lithiated anion of chiral dihydropyrazine **6** with 2,6-dibromo-4-methoxybenzyl bromide[5] proceeded smoothly to give the con-



6 **7a** + **7b** **8** **1a**

Scheme 2 *Reagents and conditions*: i, BuLi, −78 °C, and then 2,6-dibromo-4-methoxybenzyl bromide, −78 °C; ii, trifluoroacetic acid, room temperature, 5 d; iii, boron tribromide, 0 °C

densation product **7a** and its diastereoisomer **7b**. The diastereoisomeric excess was determined by ^{1}H NMR spectrometric analysis of the crude product. One of the isopropylmethyl groups of compound **7a** appeared as a doublet (J 6.9 Hz) at δ 0.68 ppm, whereas that of the diastereoisomer **7b** appeared as a doublet (J 6.9 Hz) at δ 0.91 ppm. The peak area ratio between one of the isopropylmethyl groups of compound **7a** and that of its diastereoisomer **7b** indicated that the diastereoisomeric excess was 75%. Compound **7a** was separated into its diastereoisomer **7b** by flash chromatography on silica gel eluting with hexane–ethyl acetate (25 : 1, v/v) [TLC R_f in hexane–ethyl acetate (25 : 1, v/v): compound **7a**, 0.18; compound **7b**, 0.08]. ^{1}H NMR spectroscopic examination of purified compound **7a** showed the absence of the diastereoisomer **7b** as a contaminant. Hydrolysis of compound **7a** with 0.25 mmol dm^{-3} aqueous trifluoroacetic acid gave a mixture of the corresponding amino acid methyl ester **8** and D-valine methyl ester. Separation of these was achieved by flash chromatography on silica gel eluting with chloroform–methanol (50 : 1, v/v) to give the ester **8** (61%). Demethylation of the amino acid ester **8** with boron tribromide in dichloromethane gave the amino acid **1a** in 64% yield. The enantiomeric purity of the amino acid **1a** was determined by HPLC in the same manner as described above and was found to be >95% (ee).

The amino acid **1a** was synthesized both by enzymatic resolution in 21% overall yield in a 4-step process starting from 2,6-dibromo-4-methoxybenzyl bromide and by Schöllkopf's bis-lactim ether method in 26% yield, again in a 3-step process from the same starting compound. The enzymatic resolution provides both enantiomers of the amino acid having high enantiomeric purity. The present study provides the synthesis of 2,6-dibromo-L-tyrosine and the D-isomer, compounds which may be used to synthesize deuterium- or tritium-labelled tyrosine and peptides.

Experimental

All melting points were determined with a Yanaco micro-melting point apparatus MP-S3 and are uncorrected. ^{1}H NMR were determined on a Varian GEMINI-300 instrument at 300 MHz using tetramethylsilane (TMS) or 3-(trimethylsilyl)-propanesulfonate (DSS) as internal standard. J-Values are given in Hz. Optical rotations, $[a]_D$, determined on a JASCO DIP-4 polarimeter, are given in units of 10^{-1} deg cm^2 g^{-1}. Mass spectra were recorded on a ThermoQuest TSQ-700 spectrometer. Absorbance was determined on a Shimadzu UV-260 instrument. HPLC was performed on a Waters M600 multi-solvent delivery system using a Crownpak CR column (150 × 4 mm ID, Daicel Chemical, Tokyo, Japan).

Carboxypeptidase A (from bovine pancreas, type 1-DFP, 15 mg protein cm^{-3}) and acylase (from *Aspergillus melleus*) were purchased from Sigma (St. Louis, MO, USA). Acylase (from porcine kidney) was purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). (2*R*)-(−)-2,5-Dihydro-3,6-dimethoxy-2-isopropylpyrazine **6** was purchased from E. Merck (Schuchardt, Germany).

2-Acetamido-3-(2′,6′-dibromo-4′-hydroxy)phenylpropionic acid (*N*-acetyl-2,6-dibromo-DL-tyrosine) 2

Acetic anhydride (2.8 cm^3) was added to a solution of 2,6-dibromo-DL-tyrosine[5] **1** (3.3 g, 9.7 mmol) in 2 mol dm^{-3} aqueous NaOH (19.5 cm^3) at 0 °C. After the reaction mixture had been stirred at 0 °C for 3 h, further 2 mol dm^{-3} aqueous NaOH (28 cm^3) and acetic anhydride (3.0 cm^3) were added to it. The solution was then stirred at 0 °C for 3 h, after which it was adjusted to pH 2 with concentrated hydrochloric acid to produce a gummy oil, and then extracted with ethyl acetate (3 × 30 cm^3). The combined extracts were dried (Na_2SO_4) and evaporated under reduced pressure to give *N*,*O*-diacetyl-2,6-dibromo-DL-tyrosine (3.3 g, 80%) as a white solid, mp 215.5–216.5 °C (Found: C, 36.68; H, 2.92; N, 3.32. $C_{13}H_{13}NO_5Br_2$ requires C, 36.91; H, 3.10; N, 3.31%); δ_H(300 MHz; [$^2H_6$]dimethyl sulfoxide; TMS) 1.79 (3H, s, MeCON), 2.25 (3H, s, $MeCO_2$), 3.23 (1H, dd, J 8.6, 13.8, 3-*H*H), 3.41 (1H, dd, J 6.8, 13.8, 3-H*H*), 4.52 (1H, ddd, J 6.8, 8.2, 8.6, 2-H), 7.52 (2H, s, 3′,5′-H) and 8.23 (1H, d, J 8.2, NH); *m*/*z* (CI) 422, 424 and 426 (M^+ + 1, 1 : 2 : 1).

A solution of the *N*,*O*-diacetate (3 g, 7 mmol) in 0.5 mol dm^{-3} aqueous LiOH (40 cm^3) was stirred at room temperature for 5 h, after which it was adjusted to pH 2 with concentrated hydrochloric acid to produce a gummy oil, and then extracted with ethyl acetate (3 × 30 cm^3). The combined extracts were dried (Na_2SO_4) and evaporated under reduced pressure to give the *N*-acetate **2** (2.5 g, 75%) as a white solid, mp 236–237 °C (Found: C, 34.86; H, 2.90; N, 3.67. $C_{11}H_{11}NO_4Br_2$ requires C, 34.86; H, 2.91; N, 3.68%); δ_H(300 MHz; [$^2H_6$]dimethyl sulfoxide; TMS) 1.79 (3H, s, MeCON), 3.09 (1H, dd, J 8.6, 13.9, 3-*H*H), 3.29 (1H, dd, J 6.8, 13.9, 3-H*H*), 4.48 (1H, ddd, J 6.8, 8.2, 8.6, 2-H), 7.01 (2H, s, 3′,5′-H) and 8.11 (1H, d, J 8.2, NH); *m*/*z* (EI) 380, 382 and 384 (M^+ + 1, 1 : 2 : 1).

2-Chloroacetamido-3-(2′,6′-dibromo-4′-hydroxy)phenylpropionic acid (*N*-chloroacetyl-2,6-dibromo-DL-tyrosine) 3

Chloroacetyl chloride (0.2 cm^3) was added to a solution of the racemate **1** (0.8 g, 2.4 mmol) in 2 mol dm^{-3} aqueous NaOH (2 cm^3) at 0 °C and the mixture was stirred for 2 h; it was then treated with additional 2 mol dm^{-3} aqueous NaOH (2 cm^3) and chloroacetyl chloride (0.2 cm^3) at 0 °C. The solution was stirred at 0 °C for 0.5 h and then at room temperature for 1 h. After this it was adjusted to pH 2 with concentrated hydrochloric acid to give a solid, and then extracted with ethyl acetate (3 × 10 cm^3). The combined extracts were washed with brine, dried (Na_2SO_4) and evaporated under reduced pressure to give the *N*-chloroacetate **3** (0.9 g, 90%) as a pale yellow solid, mp 203.5–205 °C (decomp.) (Found: C, 32.07; H, 2.67; N, 3.4. $C_{11}H_{10}NO_4Br_2Cl$ requires C, 31.88; H, 2.43; N, 3.38%); δ_H(300 MHz; [$^2H_6$]-dimethyl sulfoxide; TMS) 3.16 (1H, dd, J 8.8, 13.9, 3-*H*H), 3.35 (1H dd, J 6.3, 13.9, 3-H*H*), 4.06 (2H, s, CH_2Cl), 4.54 (1H, ddd, J 6.3, 8.2, 8.8, 2-H), 7.02 (2H, s, 3′,5′-H) and 8.51 (1H, d, J 8.2, NH); *m*/*z* (CI) 414, 416, 418 and 420 (M^+ + 1, 3 : 7 : 5 : 1).

2-Trifluoroacetamido-3-(2′,6′-dibromo-4′-hydroxy)phenylpropanoic acid (*N*-trifluoroacetyl-2,6-dibromo-DL-tyrosine) 4

Trifluoroacetic anhydride (4 cm^3) was added dropwise to a solution of the racemate **1** (2.0 g, 5.8 mmol) in a mixture of trifluoroacetic acid (10 cm^3) and diethyl ether (3 cm^3) at 0 °C. The solution was stirred at 0 °C for 1 h and then at room temperature for 3 h. After evaporation of the mixture under reduced pressure, the residue was dissolved in diethyl ether (20 cm^3). The ether solution was washed with brine (3 × 20 cm^3), dried (Na_2SO_4) and evaporated under reduced pressure to give the *N*-trifluoroacetate **4** (2.2 g, 86%) as a solid, mp 210–211 °C (decomp.) (Found: C, 30.38; H, 1.82; N, 3.17. $C_{11}H_8NO_4Br_2F_3$ requires C, 30.37; H, 1.85; N, 3.22%); δ_H(300 MHz; [$^2H_6$]-dimethyl sulfoxide; TMS) 3.26 (1H, dd, J 9.3, 14.3, 3-*H*H), 3.47 (1H, dd, J 5.6, 14.3, 3-H*H*), 4.50 (1H, ddd, J 5.6, 9.3, 9.8, 2-H), 7.03 (2H, s, 3′,5′-H) and 9.84 (1H, d, J 9.8, NH); *m*/*z* (EI) 433, 435 and 437 (M^+, 1 : 2 : 1).

Enzymatic assays

The racemic *N*-acyl amino acid (400 nmol) was dissolved in 0.1 mol dm^{-3} aqueous potassium phosphate buffer (pH 7.4; 0.4 cm^3) and pre-incubated at 37 °C for 5 min. Porcine kidney acylase (240 μg) in 0.1 mol dm^{-3} aqueous potassium phosphate buffer (pH 7.4, 0.1 cm^3) was then added to the stirred solution at 37 °C. Each sample was prepared in triplicate. After incubation at 37 °C for 24 h, the solution was quenched in 1 mol dm^{-3} hydrochloric acid (0.5 cm^3). To the solution was added 0.2 mmol dm^{-3} aqueous NaCN in 2 mol dm^{-3} aqueous sodium acetate buffer (pH 5.3; 0.5 cm^3) and 3% ninhydrin in methyl

cellosolve (0.5 cm^3). The mixture was heated at 100 °C for 15 min, and then quenched with a mixture of propan-2-ol and water (1:1, v/v; 5 cm^3). The absorbances (570 nm) of the solutions were measured after allowing them to cool for 30 min.

The other assays were carried out with *Aspergillus* acylase (2 mg) and carboxypeptidase A (75 μg), respectively.

Measurement of initial velocity

The substrate (compound **4** or *N*-trifluoroacetyl-DL-tyrosine **5**) in the range of 1–10 μmol was dissolved in 0.1 mol dm^{-3} aqueous potassium phosphate buffer (pH 7.4; 1 cm^3) and pre-incubated at 37 °C for 5 min. Carboxypeptidase A (150 μg for compound **4**, 11.3 μg for compound **5**) in 0.1 mol dm^{-3} aqueous potassium phosphate buffer (pH 7.4; 0.5 cm^3) was added to the stirred solution at 37 °C. The reaction proceeded at 37 °C, six aliquots (0.2 cm^3) being periodically removed after addition of the enzyme, and quenched in 1 mol dm^{-3} hydrochloric acid (0.5 cm^3). Each sample was subjected to a ninhydrin assay as described above.

Calibration curve

To each of five standards containing known amounts of the amino acid **1** or L-tyrosine in the range of 20–500 nmol in 0.1 mol dm^{-3} aqueous potassium phosphate buffer (pH 7.4, 0.4 cm^3) was added 1 mol dm^{-3} hydrochloric acid (0.5 cm^3) and then carboxypeptidase A (11.25 μg) in 0.1 mol dm^{-3} aqueous potassium phosphate buffer (pH 7.4; 0.1 cm^3). Each sample was prepared in triplicate and subjected to ninhydrin assay as described above.

(3*R*,6*S*)-2,5-Dimethoxy-3-isopropyl-6-(2′,6′-dibromo-4′-methoxybenzyl)-3,6-dihydropyrazine 7a

To a stirred solution of the dihydropyrazine **6** (0.95 cm^3, 5.3 mmol) in dry tetrahydrofuran (20 cm^3) was added 1.6 mol dm^{-3} BuLi in hexane (3.5 cm^3, 5.6 mmol) at −78 °C. The solution was stirred for 30 min at −78 °C to allow formation of the anion, after which a solution of 2,6-dibromo-4-methoxybenzyl bromide[5] (2.0 g, 5.6 mmol) in dry tetrahydrofuran (10 cm^3) was added dropwise to it with stirring at −78 °C. The solution was stirred for 2 h at −78 °C and then at 0 °C for 16 h, after which it was evaporated under reduced pressure. The residual oil was dissolved in diethyl ether (50 cm^3), and the solution washed with water (3 × 10 cm^3), dried (Na_2SO_4) and concentrated under reduced pressure to give a yellow oil. Flash chromatography of the oil on silica gel eluting with hexane–ethyl acetate (20:1) gave the (3*R*,6*S*)-dihydropyrazine **7a** (1.7 g, 66%) as a colourless oil; its diastereoisomer the (3*R*,6*R*)-dihydropyrazine **7b** (0.2 g, 8%) was also obtained as a colourless oil. Each diastereoisomer had the following physical characteristics: compound **7a** (Found: C, 44.21; H, 4.85; N, 6.05. $C_{17}H_{22}N_2O_3Br_2$ requires C, 44.18; H, 4.80; N, 6.06%); $[a]_D$ +49.8 (*c* 1.0 in ethyl acetate); δ_H(300 MHz; $CDCl_3$; TMS) 0.68 (3H, d, *J* 6.9, isopropyl-Me), 1.06 (3H, d, *J* 6.9, isopropyl-Me), 2.27 (1H, m, isopropyl-CH), 3.11 (1H, dd, *J* 10.4 and 13.5, C*H*H), 3.46 (1H, dd, *J* 5.4 and 13.5, CH*H*), 3.62 (3H, s), 3.72 (3H, s), 3.77 (3H, s), 3.97 (1H, d, *J* 3.3, 3-H), 4.41 (1H, m, 6-H) and 7.10 (2H, s, 3′,5′-H); *m*/*z* (EI) 460, 462 and 464 (M^+, 1:2:1), 417, 419 and 421 [$M^+ - CH(CH_3)_2$], 381, 383 ($M^+ - Br$, 1:1); and compound **7b** (Found: C, 44.24; H, 4.89; N, 6.13. $C_{17}H_{22}N_2O_3Br_2$ requires C, 44.18; H, 4.80; N, 6.06%); $[a]_D$ −55.0 (*c* 1.0 in ethyl acetate); δ_H(300 MHz; $CDCl_3$; TMS) 0.91 (3H, d, *J* 6.9, isopropyl-Me), 1.12 (3H, d, *J* 6.9, isopropyl-Me), 2.26 (1H, m, isopropyl-CH), 3.09 (1H, dd, *J* 9.9 and 13.5, C*H*H), 3.45 (1H, dd, *J* 5.3 and 13.5, CH*H*), 3.65 (3H, s), 3.69 (3H, s), 3.78 (3H, s), 3.94 (1H, d, *J* 4.3, 3-H), 4.41 (1H, m, 6-H) and 7.11 (2H, s, 3′,5′-H); *m*/*z* (EI) 460, 462 and 464 (M^+, 1:2:1), 417, 419 and 421 (M^+ − isopropyl) and 381 and 383 ($M^+ - Br$, 1:1).

Methyl (2*S*)-2-amino-3-(2′,6′-dibromo-4-methoxy)phenylpropionate 8

The dihydropyrazine **7a** (1.8 g, 3.9 mmol) was added to 0.25 mol dm^{-3} aqueous trifluoroacetic acid (65 cm^3), and the mixture was stirred at room temperature for 5 days. The aqueous solution was then washed with diethyl ether (2 × 30 cm^3), after which it was adjusted to pH 10 with 30% aqueous ammonia, and then extracted with diethyl ether (4 × 30 cm^3). The combined extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give a yellow oil. This was purified by flash chromatography on silica gel eluting with chloroform–methanol (50:1) to yield the ester **8** (0.9 g, 61%) as a colourless oil (Found: C, 35.99; H, 3.48; N, 3.94. $C_{11}H_{13}NO_3Br_2$ requires C, 36.00; H, 3.57; N, 3.81%); δ_H(300 MHz; $CDCl_3$; TMS) 3.22 (1H, dd, *J* 9.1, 13.9, 3-*H*H), 3.35 (1H, dd, *J* 6.1, 13.9, 3-H*H*), 3.62 and 3.72 (3H × 2, s and s, OMe and CO_2Me), 3.90 (1H, dd, *J* 6.1, 9.1, 2-H) and 7.12 (2H, s, 3′,5′-H); *m*/*z* (EI) 365, 367 and 369 (M^+, 1:2:1) and 285 and 287 (M^+ − Br, 1:1).

(2*S*)-2-Amino-3-(2′,6′-dibromo-4-hydroxy)phenylpropionic acid (2,6-dibromo-L-tyrosine) 1a

Method A. To a suspension of the ester **8** (840 mg, 2.3 mmol) in dichloromethane (30 cm^3) was added 1 mol dm^{-3} boron tribromide in dichloromethane (10 cm^3) at 0 °C. The solution was stirred for 2 h at 0 °C and then for 16 h at room temperature, after which it was poured into water (50 cm^3). The aqueous layer was separated and adjusted to pH 5.5 with 10% aqueous ammonia to give a precipitate; it was then refrigerated overnight. The precipitate was collected and washed with cold water to give the amino acid **1a** (530 mg, 64%) as a colourless solid, mp 234–236 °C (decomp.) (Found: C, 30.24; H, 3.04; N, 3.98. $C_9H_9NO_3Br_2 \cdot 1H_2O$ requires C, 30.28; H, 3.11; N, 3.92%); $[a]_D$ +39.8 (*c* 1, 1 M HCl); δ_H(300 MHz; 1 M HCl; DSS) 3.48 (1H, dd, *J* 7.6, 14.4, 3-*H*H), 3.65 (1H, dd, *J* 8.8, 14.4, 3-H*H*), 4.35 (1H, dd, *J* 7.6, 8.8, 2-H) and 7.20 (2H, s, 3′,5′-H).

Method B. The *N*-trifluoroacetate **4** (1.0 g, 2.3 mmol) suspended in water (40 cm^3) dissolved upon addition of 2 mol dm^{-3} LiOH. The solution was then adjusted to pH 7.4 with further 2 mol dm^{-3} LiOH; the total volume of 2 mol dm^{-3} LiOH added was *ca.* 1.8 cm^3. To the solution was added carboxypeptidase A (30 mg) and then water to adjust the volume to 50 cm^3. The solution was incubated at 37 °C with progress of the reaction being monitored as follows. Aliquots of the incubation mixture (0.1 cm^3) were diluted to 2 cm^3 with water; each sample was prepared in triplicate. The solution was incubated at 37 °C along with the main bulk of the digest. Aliquots of the solution (0.1 cm^3) were removed at 0.5, 1, 2, 3, 5, 8 and 24 h after addition of the enzyme, and quenched in 1 mol dm^{-3} hydrochloric acid (0.5 cm^3). Each sample was subjected to ninhydrin assay as described above. After incubation for 24 h, the mixture was adjusted to pH 2 with 1 mol dm^{-3} hydrochloric acid, treated with charcoal and filtered through Celite. The filtrate was washed with diethyl ether (3 × 30 cm^3) to remove the remaining *N*-trifluoroacetyl derivative. The aqueous layer was concentrated under reduced pressure to a volume of 10 cm^3, after which it was adjusted to pH 5.5 with 10% aqueous ammonia to give a precipitate; the mixture was then refrigerated overnight. After this, the precipitate was collected and washed with cold water to give the amino acid **1a** as a colourless solid (310 mg, 75% based on L-isomer of *N*-trifluoroacetyl derivative **4**).

(2*R*)-2-Amino-3-(2′,6′-dibromo-4-hydroxy)phenylpropionic acid (2,6-dibromo-D-tyrosine) 1b

The ether washings in the preparation of **1a** (method B) were evaporated under reduced pressure to give the *N*-trifluoroacetyl derivative (360 mg) as a colourless solid. The solid was dissolved in 2 mol dm^{-3} aqueous LiOH (10 cm^3) and the solution stirred for 2 h at room temperature; it was then adjusted to pH 5.5 with concentrated hydrochloric acid to give a precipitate. The mixture was then refrigerated overnight. The precipitate was collected and washed with cold water to give the amino

acid **1b** (200 mg, 48% based on D-isomer of *N*-trifluoroacetyl derivative **4**) as a colourless solid, mp 227–228 °C (decomp.) (Found: C, 31.14; H, 3.20; N, 4.12. $C_9H_9NO_3Br_2 \cdot \frac{1}{2}H_2O$ requires C, 31.06; H, 2.90; N, 4.03%); $[\alpha]_D$ −37.1 (*c* 1, 1 M HCl); δ_H(300 MHz; 1 M ^{2}HCl; DSS) 3.48 (1H, dd, *J* 7.6, 14.4, 3-*H*H), 3.65 (1H, dd, *J* 8.8, 14.4, 3-H*H*), 4.38 (1H, dd, *J* 7.6, 8.8, 2-H) and 7.20 (2H, s, 3′,5′-H).

Determination of absolute configurations of the amino acids 1a and 1b

The amino acid **1a** (1 mg) was suspended in water (1 cm^3) and reduced with hydrogen in the presence of palladium black (1 mg). After the mixture had been stirred for 3 h, it was reduced for 3 h after addition of palladium black (1 mg). The catalyst was filtered off and the filtrate was subjected to HPLC [elution, 0.14% aqueous $HClO_4$ (v/v); flow rate, 0.8 cm^3 min^{-1}; temperature, 20 °C; detection, 220 nm]. The retention time value (6.1 min) of the reduction product was identical with that of authentic L-tyrosine.

The amino acid **1b** (1 mg) was also reduced and subjected to HPLC in a similar manner to that described above. The retention time value (5.1 min) of the reduction product was identical with that of authentic D-tyrosine.

Determination of the enantiomeric purity of the amino acids 1a and 1b

The enantiomeric purity of the amino acids **1a** and **1b** was analysed with HPLC [elution, 0.35% aqueous $HClO_4$–methanol (85:15, v/v); flow rate, 0.8 cm^3 min^{-1}; temperature, 20 °C; detection, 220 nm].

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