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An efficient and stereodivergent synthesis of *threo*- and *erythro*-β-methylphenylalanine. Resolution of each racemic pair by semipreparative HPLC

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Abstract—*threo* and *erythro* diastereoisomers of the constrained amino acid (βMe)Phe can be obtained separately on a multigram scale through a three-step synthesis from the corresponding *Z* and *E* isomers of 2-phenyl-4(α-phenylethylidene)-5(4*H*)-oxazolone. The 5(4*H*)-oxazolones are readily available from acetophenone and hippuric acid. The four enantiomerically pure isomers of β-methylphenylalanine, (2*R*,3*R*)-(βMe)Phe, (2*S*,3*S*)-(βMe)Phe, (2*R*,3*S*)-(βMe)Phe and (2*S*,3*R*)-(βMe)Phe, have been prepared by HPLC resolution of the racemic precursors methyl *threo* (or *erythro*)-2-benzamide-3-phenylbutanoates.

1. Introduction

The incorporation of conformationally constrained α-amino acids into peptides allows the study of structure-activity relationships and the synthesis of peptide analogues with improved pharmacological properties.[1–4]

Special mention must be made of the constrained analogues of phenylalanine, as this naturally occurring α-amino acid is directly involved in a large number of molecular recognition processes.[5–7] In all cases, the three-dimensional arrangement of the side chain moiety of the phenylalanine residue is crucial in eliciting the desired response.

The side-chain conformation can be conformationally constrained by introducing an alkyl group at the β-position of an α-amino acid residue without significantly perturbing the backbone conformation. In particular, β-methyl-α-aromatic amino acids have been incorporated into peptides[5,6,8–10] and confer on these systems a conformational side-chain rigidity that is very valuable for the study of both the specific topochemical arrays of the side chains and topochemical nature of the binding site.

The preparation of all four isomers of β-methylphenylalanine in enantiopure form has been a challenging area of synthetic organic chemistry. Several strategies have been developed and these include classical resolution,[11] the use of threonine as the starting material,[12] enzymatic resolution in conjunction with HPLC,[8,10] asymmetric synthesis,[13,14] the chiral auxiliary approach[15–19] and enantioselective hydrogenation.[20,21] When all stereoisomers are required, it may be more convenient to perform a rapid synthetic route resulting in racemates rather than a stereoselective synthesis for each isomer.

Access to the β-methylphenylalanine amino acid in its *erythro* and *threo* diastereomerically pure forms on a multigram scale is a subject of current interest. Repeated recrystallization of the hydrochloride form of the β-methylphenylalanine mixture[22] is very time consuming and especially difficult for the *threo* racemate, which requires further recrystallizations and even the use of semipreparative RP-HPLC.[10,23,24]

In the course of our work on the synthesis of conformationally restricted aspartame analogues, the availability of a simple, short and efficient method to obtain *threo*- and *erythro*-(βMe)Phe as diastereomerically pure materials was critical. We describe here a convenient route for the multigram-scale preparation of both diastereomers by catalytic hydrogenation of α,β-didehydroamino acid derivatives. *Z*- and *E*-2-Benzamide-3-phenyl-2-butenoates, obtained from *Z*- and *E*-2-phenyl-4(α-phenylethylidene)-5(4*H*)-oxazolones, are the precursors of *threo*- and *erythro*-(βMe)Phe, respectively.

Extensive work on enantiomeric separations of β-methyl amino acids using chiral derivatisations[25,26] and different chiral stationary phases,[27–31] mainly derived from macrocyclic glycopeptide antibiotics, has been reported. To the

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best of our knowledge, separation of all four stereoisomers of (βMe)Phe on HPLC has never been described on a semipreparative scale, so we would like to report here the preparation of the four isomers of β-methylphenylalanine in optically pure form by combining a racemic synthesis with an HPLC resolution procedure.

2. Results and discussion

2.1. Racemic synthesis

The *Z* and *E* isomers of 2-phenyl-4(α-phenylethylidene)-5(4*H*)-oxazolone (*Z*-**1**, *E*-**1**) proved to be convenient starting materials to afford (βMe)Phe, as demonstrated previously by our group.[32–34] The sequence of reactions leading to the amino acid involved the synthesis of pure 5(4*H*)-oxazolones, alkaline hydrolysis to 2-benzoyl-3-phenyl-2-butenoic acids followed by catalytic hydrogenation and, finally, hydrolysis to afford the amino acid. The methodology described here involves some modifications with respect to the originally reported procedure and these are described below. The synthetic route followed for the racemic synthesis of the precursors for the desired α-amino acids is outlined in Scheme 1.

The thermodynamically more stable (*Z*)-isomer, *Z*-**1**, was prepared by fractional crystallisation of an isomeric mixture (*Z*/*E*) obtained from the condensation of hippuric acid and acetophenone.[32] In the same paper, access to the less stable (*E*)-isomer *E*-**1** was effected from *Z*-**1** by treatment with HBr. In our experience, working under similar reaction conditions, isomerization was sometimes completely *E*-selective and sometimes moderately or even poorly *E*-selective. The mechanism of this reaction is apparently the same as that in the isomerization of *cis*-/*trans*-stilbene[35] and it seems to be consistent with the presence of bromine radicals.[36] We proceeded to define new reaction conditions that favour a radical reaction. After several other approaches proved unsuccessful, we were able to obtain the (*E*)-isomer in a totally controlled and reproducible manner by HBr isomerization in the presence of a catalytic amount of benzoyl peroxide.

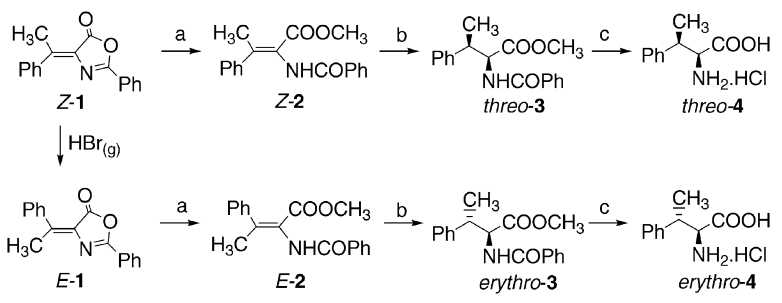
Ester derivatives are more convenient substrates for chromatography than the corresponding carboxylic acids. Thus, with the aim of resolving racemic intermediates into their enantiomers by chiral HPLC, oxazolone ring opening was performed by methanolysis of *Z*-**1** and *E*-**1** in the presence of a catalytic amount of sodium methoxide. This treatment afforded the corresponding benzamido esters *Z*-**2** and *E*-**2** in high yield and with retention of configuration, as reported previously.[37]

The next step in the synthetic route involved hydrogenation of the tetrasubstituted olefin in compounds *Z*-**2** and *E*-**2**. Initial attempts to obtain racemic methyl butanoates *threo*-**3** and *erythro*-**3** involved the use of 10% Pd/C as catalyst. The hydrogenation of *Z*-**2** was achieved by keeping the reaction at 30 °C with vigorous agitation in the presence of a small amount of Pd/C (10% w/w). The diastereomeric purity of *threo*-**3** was confirmed by ^{1}H NMR spectroscopy and HPLC. When the preparation of *erythro*-**3** was attempted under the same conditions, a mixture ranging from 95:5 to 87:13 of *erythro*-**3**:*threo*-**3** was obtained.

It is well known that the factors influencing the hydrogenation of olefins are numerous and include, among others, the nature of the solvent, hydrogen availability and catalyst.[38] These facts prompted us to modify the reaction conditions in an attempt to minimize the isomerization phenomenon.

The use of methanol/benzene mixtures as solvent had been reported to show marked inhibition of isomerization. Nevertheless, this approach did not lead to better results in our experiments. Other catalysts, such as Pt/C and PtO_2/C, did not improve on our previous results. Finally, upon increasing the amount of catalyst up to 40% catalyst, on a weight basis referred to the substrate, we managed to overcome the isomerization problem. The diastereomeric purity of *erythro*-**3** was checked by ^{1}H NMR spectroscopy and HPLC. In fact, it appears that we had indirectly increased the concentration of hydrogen on the catalyst surface. For this reason, we expected that the use of hydrogen pressures of 5–10 atm would lead to the same results, but under these conditions fragmentations occurred.

The last step of the synthetic approach involved removal of the protecting groups in *threo*-**3** and *erythro*-**3** by total hydrolysis. This step has been reported previously[34] and involves very strong conditions: heating under reflux in a mixture of hydrobromic acid and glacial acetic acid. This method led to the formation of 15% of *erythro*-**4** in the reaction mixture on starting from *threo*-**3**. Our subsequent aim was to reduce as much as possible the partial epimerization observed at Cα and, in this respect, milder deprotection conditions were assayed. Finally, the use of a mixture of 2.5 N HCl/HOAc (4:1) gave almost quantitative deprotection with no detectable epimerization by ^{1}H NMR. Nevertheless, diastereomeric purity was assessed (after



Scheme 1. Synthetic route to racemic *threo*-(βMe)Phe and *erythro*-(βMe)Phe. *Reagents and conditions*: (a) MeONa/MeOH. (b) H_2/Pd–C. (c) 2.5 N HCl/HOAc, reflux.

Table 1. Selected chromatographic data for the HPLC resolution of *threo*-**3** and *erythro*-**3** on the amylose-derived chiral stationary phase

Compound	Eluent[a] (A/B/C)	Flow (mL/min)	λ (nm)	k'_1	α	R_s
threo-**3**	95:5:0	1[b]	210	1.59	1.21	0.90
	93:5:2	1[b]	235	1.24	1.15	0.59
	93:5:2	18[c]	270	2.44	1.18	0.81
erythro-**3**	98:2:0	1[b]	210	2.79	1.33	1.67
	96:2:2	1[b]	240	2.98	1.23	1.25
	96:2:2	18[d]	265	2.87	1.19	0.80

For the definition of k', α and R_s see Section 4.2.
[a] A: *n*-hexane, B: 2-propanol, C: chloroform.
[b] Steel column, 150 mm×4.6 mm ID. Temperature: 25 °C. *c*=5 mg/mL.
[c] Steel column, 150 mm×20 mm ID. Temperature: 25 °C. *c*=100 mg/mL.
[d] Steel column, 150 mm×20 mm ID. Temperature: 25 °C. *c*=200 mg/mL.

previous transformation to the free amino acids) using a RP-HPLC protocol analogue to the method described in the literature.[24] It was found that diastereomeric purity for *threo* pair was over 99% whereas for *erythro* pair ranged 96–98% for a wide number of experiments done.

The route described above allows access to diastereomerically pure *threo*-**4** and *erythro*-**4** through a three-step approach with yields of 82–86% from oxazolones and an overall yield of 31–33% from hippuric acid. These results make this methodology particularly appropriate for large-scale preparations of diastereomerically pure *erythro*-(βMe)Phe and *threo*-(βMe)Phe.

2.2. HPLC resolution

Once an efficient racemic route to the target compounds had been developed, we undertook the preparation of the products in enantiomerically pure form by HPLC resolution of a synthetic intermediate using a chiral stationary phase. The utility of polysaccharide-based phases is well documented.[39,40] Recently, a non-commercial stationary phase consisting of a mixed derivative of amylose (10-undecenoate/3,5-dimethylphenylcarbamate) covalently linked to allylsilica gel proved to be very efficient in the semipreparative separation of racemic constrained analogues of phenylalanine.[41–44] The wide applicability shown by this stationary phase together with its chemical stability make it very convenient for the resolution of racemates on a semipreparative scale.

First, analytical separation of derivatives *threo*-**3** and *erythro*-**3** was examined using mixtures of *n*-hexane/2-propanol as the mobile phase. As can be seen from the results in Table 1, chloroform leads to lower selectivity and resolution factors in the analytical assays but its presence is necessary in order to optimize the loading capacity of the column. Thus, mixtures of *n*-hexane/2-propanol/chloroform were also tested as eluents (Fig. 1).

Preparative resolutions of racemates *threo*-**3** and *erythro*-**3** were performed on a 150×20 mm ID column by successive injections using the peak shaving technique. After determination of the optimum cut points, each run was collected into three separate fractions. The first fractions and the last

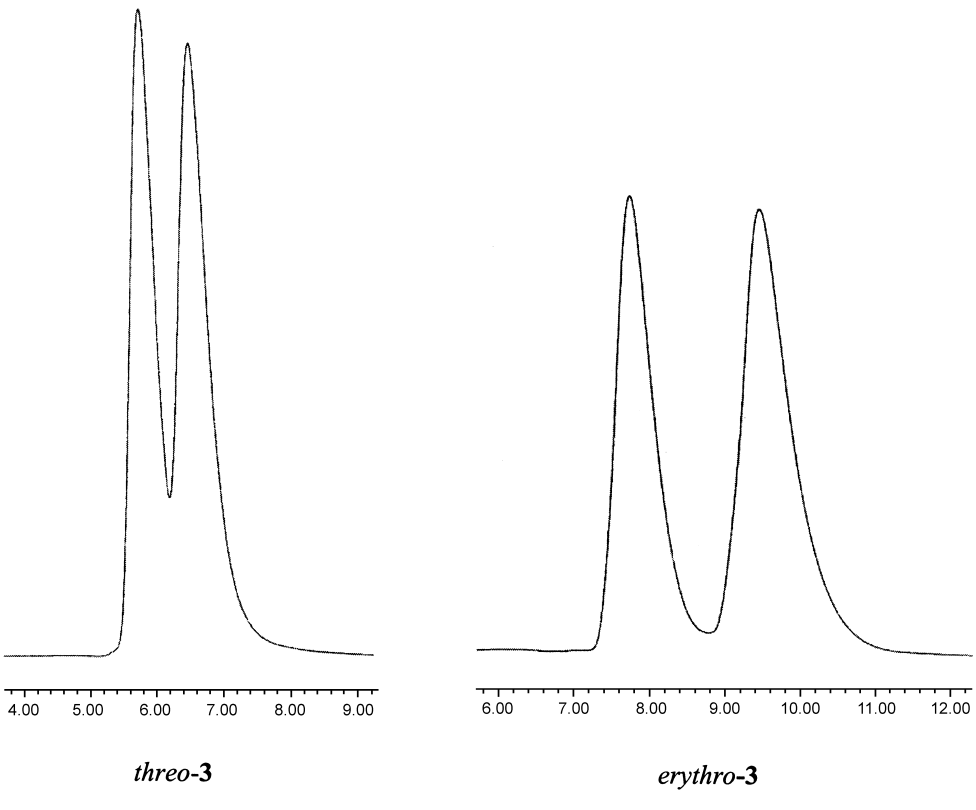


Figure 1. HPLC analytical resolution of *threo*-**3** (eluent: *n*-hexane/2-propanol 95:5) and *erythro*-**3** (eluent: *n*-hexane/2-propanol 98:2). See Table 1 for related chromatographic data.

fractions, each containing one of the enantiomers, were collected at each passage through the column and combined. Therefore, 327 mg of *threo*-**3** dissolved in 3.3 mL of dichloromethane were fractionated to obtain 78 and 67 mg of the less and more strongly retained enantiomers, respectively. A total of 4.4 h were required (33 injections of 100 μL each with 8 min/cycle). Since the preparative column contained about 28 g of chiral stationary phase (CSP), a loading capacity of 2.7 mg racemate per gram CSP/hour was allowed in the *threo*-**3** resolution. As regards racemate *erythro*-**3**, 760 mg dissolved in 3.8 mL of dichloromethane was fractionated to afford 279 and 200 mg of the less and more strongly retained enantiomers, respectively. Six hours were required for total separation (38 injections of 100 μL each with 9.5 min/cycle) and the loading capacity was 4.5 mg racemate/g CSP per hour. Enantiomeric purity of each isomer was checked on the analytical column and only one enantiomer was detected.

2.3. Preparation of enantiomerically pure compounds

Once enantiomerically pure precursors were available, we undertook the deprotection steps under the conditions developed for the racemic material, *threo*-**3** and *erythro*-**3**, with each stereoisomer (Scheme 2). In addition, with the aim of assigning the absolute configurations, the amino acid hydrochlorides obtained after hydrolysis were treated with propylene oxide under reflux in order to obtain the optically pure free amino acids.

2.4. Assignment of absolute configurations

The absolute configurations of all four isomers of β-methylphenylalanine were determined by direct comparison of their optical rotations with the values described in the literature.[10,11,45] This allowed us to assign a (2*S*,3*R*) stereochemistry to the compounds obtained from the first eluted enantiomer of *threo*-**3** and a (2*R*,3*S*) configuration to the derivatives obtained from the last eluted enantiomer of *threo*-**3**. In a similar way, a (2*R*,3*R*) stereochemistry was assigned to the compound obtained from the first eluted enantiomer of *erythro*-**3** and a (2*S*,3*S*) configuration to the derivatives obtained from the last eluted enantiomer of *erythro*-**3**.

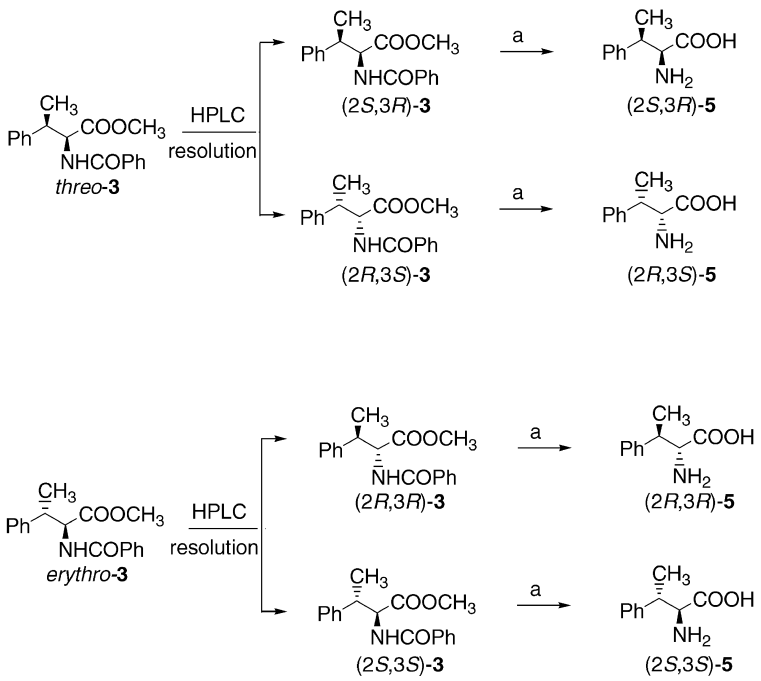
3. Conclusion

The methodology described here allows easy access to the racemic pairs (*erythro* and *threo*) of β-methylphenylalanine with high diastereomeric purity and through high yield transformations. Moreover, combining the racemic synthesis with a resolution by chiral HPLC provides all four individual isomers of β-methylphenylalanine, (2*S*,3*S*)-, (2*R*,3*R*)-, (2*S*,3*R*)- and (2*R*,3*S*)-β-methylphenylalanine, with high optical purity.

4. Experimental

4.1. General

Solvents were purified according to standard procedures. Melting points were determined on a Büchi SMP-20 apparatus and were not corrected. IR spectra were registered on a Mattson Genesis FTIR spectrophotometer; ν_{max} is given for the main absorption bands. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity-300 or a Bruker ARX-300 instrument at room temperature, unless otherwise indicated, using the residual solvent signal as the internal standard; chemical shifts are reported in ppm on the δ scale, coupling constants in Hz. Mass Spectra were obtained on a



Scheme 2. Synthetic route to enantiomerically pure (βMe)Phe stereoisomers. *Reagents and conditions*: (a) (i) 2.5 N HCl/HOAc, reflux; (ii) propylene oxide/EtOH, reflux.

high resolution VG-autospectrometer using either EI or +FAB techniques. Optical rotations were measured on a Perkin–Elmer 241 polarimeter-C in a 1 dm cell of 1 mL capacity at 25 °C. Microanalyses were carried out on a Perkin–Elmer 200 C, H, N, S analyser. Analytical TLC was performed using Merck 60 SI F_{254} precoated silica gel polyester plates and the products were examined by UV fluorescence or developed using iodine vapour. Column chromatography was performed using silica gel 60 (230–400 mesh).

4.2. High performance liquid chromatography

HPLC was carried out using a Waters HPLC system equipped with a Waters 600-E pump and a Waters 991 photodiode array detector. The chiral stationary phase, which consisted of a mixed 10-undecenoate/3,5-dimethylphenylcarbamate of amylose bonded to allylsilica, was prepared according to a previously described procedure.[46,47] The analytical assays were carried out on a 150 mm×4.6 mm ID column and the semipreparative resolution was achieved on a 150 mm×20 mm ID column. All analytical assays and semipreparative chromatography were performed under the conditions given in Table 1. Diastereomeric purity of the final products was checked on a Xterra™ MS C_8 250 mm×4.6 mm ID column. The solvents used as mobile phases were of spectral grade.

The capacity (k'), selectivity (α) and resolution (R_s) values were calculated according to the equations $k'_R=(t_R-t_0)/t_0$, $\alpha=k'_2/k'_1$, $R_s=1.18\,(t_2-t_1)/(w_2+w_1)$. Subscripts 1 and 2 refer to the first and second eluted diastereoisomers, respectively; t_R ($R=1$, 2) are their retention times, and w_2 and w_1 denote their bandwidths at half height; t_0 is the dead time.

4.3. (*Z*)-2-Phenyl-4-(α-phenylethylidene)-5(4*H*)-oxazolone, *Z*-1

A mixture of acetophenone (36 g, 0.3 mol), hippuric acid (10.26 g, 0.06 mol), acetic anhydride (18.36 g, 0.18 mol), anhydrous lead(IV) acetate (13.29 g, 30 mmol) and dry THF (100 mL) was heated under reflux for 24 h. After cooling, the contents were poured onto crushed ice and extracted with CH_2Cl_2. The combined organic phases were washed with $NaHCO_3$, brine and dried over $MgSO_4$. The solvent and most of the excess acetophenone were removed in vacuo to afford a residue, which was crystallised from EtOH/H_2O to give a mixture of the geometric isomers of the oxazolone. The pure (*Z*)-isomer was obtained after a second recrystallization from EtOH/H_2O as a yellow solid (5.73 g, 38% yield). Mp 109 °C. R_f (hexanes/benzene 1:1)=0.23. IR (nujol) 1784; 1759 cm^{-1}. ^1H NMR ($CDCl_3$ 300 MHz) δ 2.78 (s, 3H); 7.42–7.54 (m, 6H); 7.86 (m, 2H); 8.05 (m, 2H). ^{13}C NMR ($CDCl_3$ 75 MHz) δ 18.37; 125.80; 127.92; 128.13; 128.77; 129.95; 131.20; 132.67; 138.88; 149.26; 160.39; 166.83. MS-EI (*m*/*z*, %) 263 [$(M)^+$, 19]; 105 (100); 77 [$(C_6H_5)^+$, 45]. Anal. calcd for $C_{17}H_{13}NO_2$: C, 77.55; H, 4.98; N 5.32. Found: C, 77.91; H, 5.04; N, 5.19.

4.4. (*E*)-2-Phenyl-4-(α-phenylethylidene)-5(4*H*)-oxazolone, *E*-1

The (*Z*)-isomer (2.5 g) was dissolved in the minimum amount of dry toluene. After adding 10 mg of benzoyl peroxide, the solution was saturated with anydrous hydrogen bromide for 15–30 min or until a precipitate had formed completely, while keeping the reaction in an ice-bath. The solid was filtered off and washed with cold toluene to afford the pure (*E*)-isomer as a pale yellow solid (2.37 g, 94% yield). Mp 115 °C. R_f (hexanes/benzene 1:1)=0.19. IR (nujol) 1788; 1651 cm^{-1}. ^1H NMR ($CDCl_3$ 300 MHz) δ 2.64 (s, 3H); 7.24–7.60 (m, 8H); 8.09 (m, 2H). ^{13}C NMR ($CDCl_3$ 75 MHz) δ 23.02; 125.91; 127.80; 128.09; 128.14; 128.41; 128.80; 129.63; 129.92; 132.68; 137.25; 152.22; 160.83; 164.06. MS-EI (*m*/*z*, %) 263 [$(M)^+$, 93]; 105 (82); 77 [$(C_6H_5)^+$, 100]. Anal. calcd for $C_{17}H_{13}NO_2$: C, 77.55; H, 4.98; N, 5.32. Found: C, 77.33; H, 5.03; N, 5.30.

4.5. Methyl (*Z*)-2-benzamide-3-phenyl-2-butenoate, *Z*-2

A solution of sodium methoxide (50 mg) in absolute MeOH (60 mL) was added to *Z*-**1** (2.5 g, 10 mmol) and the reaction mixture was stirred at room temperature for 30 min (check completion by TLC, hexanes/benzene 1:1). The reaction mixture was filtered and the solvent was removed in vacuo. Cold water was added dropwise to the residue until precipitation was complete. The product was collected by vacuum filtration to give *Z*-**2** as a white solid (2.67 g, 95%). Mp 153 °C. R_f (hexanes/AcOEt 8:2)=0.11. IR (nujol) 3263; 1713; 1638 cm^{-1}. ^1H NMR ($CDCl_3$ 300 MHz) δ 2.31 (s, 3H); 3.86 (s, 3H); 7.19 (brs, 1H); 7.10–7.60 (m, 11H). ^{13}C NMR ($CDCl_3$ 75 MHz) δ 20.52; 52.24; 123.79; 127.09; 127.42; 128.30; 128.61; 128.95; 131.92; 133.10; 137.01; 139.70; 165.40; 165.59. MS-EI (*m*/*z*, %) 295 [$(M)^+$, 8]; 263 [$(M-MeOH)^+$, 16]; 190 (37); 130 (8); 105 (100); 77 [$(C_6H_5)^+$, 73]. Anal. calcd for: $C_{18}H_{17}NO_3$: C, 73.20; H, 5.80; N, 4.74. Found: C, 73.01; H, 5.89; N, 4.71.

4.6. Methyl (*E*)-2-benzamide-3-phenyl-2-butenoate, *E*-2

An identical procedure to that described above was applied to the transformation of *E*-**1** (2.30 g, 8.74 mmol) to *E*-**2** (2.51 g, 97% yield). Mp 210 °C. R_f (hexanes/AcOEt 8:2)=0.11. IR (nujol) 3321; 1721; 1639 cm^{-1}. ^1H NMR ($CDCl_3$ 300 MHz) δ 2.18 (s, 3H); 3.46 (s, 3H); 7.15–7.35 (m, 5H); 7.35–7.60 (m, 4H); 7.87 (m, 2H). ^{13}C NMR ($CDCl_3$ 75 MHz) δ 22.07; 51.86; 123.72; 127.17; 127.34; 127.69; 128.07; 128.71; 132.11; 133.39; 140.87; 141.01; 165.63; 165.78. MS-EI (*m*/*z*, %) 295 [$(M)^+$, 7]; 263 [$(M-MeOH)^+$, 17]; 190 (29); 130 (6); 105 (100); 77 [$(C_6H_5)^+$, 52]. Anal. calcd for $C_{18}H_{17}NO_3$: C, 73.20; H, 5.80; N, 4.74. Found: C, 72.96; H, 5.86; N, 4.76.

4.7. Synthesis of *threo*-3 and *erythro*-3

4.7.1. Methyl *threo*-2-benzamide-3-phenylbutanoate, *threo*-3. A solution of *Z*-**2** (2.2 g, 7.46 mmol) in AcOEt (180 mL) was hydrogenated at 30 °C in the presence of 10% Pd/C (200 mg). After 6 h the catalyst was filtered off and the solvent evaporated to give diastereomerically pure *threo*-**3** as a white solid (2.12 g, 96% yield). Mp 102 °C. R_f (hexanes/AcOEt 8:2)=0.16. IR (nujol) 3327; 1739; 1631 cm^{-1}. ^1H NMR ($CDCl_3$ 300 MHz) δ 1.48 (d, 3H, J=7.1 Hz); 3.34 (q, 1H, J=6.8 Hz); 3.61 (s, 3H); 5.02 (dd, J=6.3, 8.5 Hz); 6.61 (d, 1H, J=8.3 Hz); 7.18–7.34 (m, 5H); 7.40–7.55 (m, 3H); 7.75–7.77 (m, 2H). ^{13}C NMR ($CDCl_3$

75 MHz) δ 17.10; 43.04; 52.11; 57.97; 127.01; 127.29; 127.69; 128.49; 128.63; 131.78; 134.01; 141.03; 166.86; 171.84. MS-EI (*m/z*, %) 298 [(M+1)$^+$, 23]; 238 [(M−MeOH−CO)$^+$, 11]; 193 (23); 161 (9); 105 (100); 77 [$(C_6H_5)^+$, 60]. Anal. calcd for: $C_{18}H_{19}NO_3$: C, 72.71; H, 6.44; N, 4.71. Found: C, 72.54; H, 6.52; N, 4.66.

4.7.1.1. Methyl (2*R*,3*S*)-2-benzamide-3-phenylbutanoate, (2*R*,3*S*)-3. Mp 135 °C. $[\alpha]_D^{20}$=−59.4 (*c*=0.50, $CHCl_3$). Anal. calcd for: $C_{18}H_{19}NO_3$: C, 72.71; H, 6.44; N, 4.71. Found: C, 72.54; H, 6.36; N, 4.68.

4.7.1.2. Methyl (2*S*,3*R*)-2-benzamide-3-phenylbutanoate, (2*S*,3*R*)-3. Mp 135 °C. $[\alpha]_D^{20}$=+58.2 (*c*=0.37, $CHCl_3$). Anal. calcd for: $C_{18}H_{19}NO_3$: C, 72.71; H, 6.44; N, 4.71. Found: C, 72.49; H, 6.42; N, 4.76. Spectroscopic data for both (2*R*,3*S*)-**3** and (2*S*,3*R*)-**3** were the same as those described above for *threo*-**3**.

4.7.2. Methyl *erythro*-2-benzamide-3-phenylbutanoate, *erythro*-3. In a similar way to that described above, hydrogenation of *E*-**2** (2.4 g, 8.14 mmol) using 10% Pd/C (960 mg) for 10 h gave diastereomerically pure *erythro*-**3** as a white solid (82.35 g, 97% yield). Mp 118 °C. R_f (hexanes/AcOEt 8:2)=0.16. IR (nujol) 3345; 1742; 1639 cm^{-1}. ^{1}H NMR ($CDCl_3$ 300 MHz) δ 1.41 (d, 3H, *J*=7.2 Hz); 3.49 (m, 1H); 3.72 (s, 3H); 5.04 (dd, 1H, *J*=5.4, 8.7 Hz); 6.30 (d, 1H, *J*=8.4 Hz); 7.1–7.5 (m, 8H); 7.66 (m, 2H). ^{13}C NMR ($CDCl_3$ 75 MHz) δ 17.63; 42.23; 52.21; 57.47; 126.93; 127.16; 127.63; 128.05; 128.44; 128.57; 128.69; 131.74; 133.80; 140.70; 167.16; 171.96. MS-EI (*m/z*, %) 298 [(M+1)$^+$, 61]; 238 (19); 266 (6); 193 (24); 176 (40); 161 (9); 105 (100); 77 [$(C_6H_5)^+$, 54]. Anal. calcd for: $C_{18}H_{19}NO_3$: C, 72.71; H, 6.44; N, 4.71. Found: C, 72.55; H, 6.49; N, 4.76.

4.7.2.1. Methyl (2*R*,3*R*)-2-benzamide-3-phenylbutanoate, (2*R*,3*R*)-3. Mp 138 °C. $[\alpha]_D^{20}$=−74.7 (*c*=0.51, $CHCl_3$). Anal. calcd for: $C_{18}H_{19}NO_3$: C, 72.71; H, 6.44; N, 4.71. Found: C, 73.03; H, 6.36; N, 4.74.

4.7.2.2. Methyl (2*S*,3*S*)-2-benzamide-3-phenylbutanoate, (2*S*,3*S*)-3. Mp 137 °C. $[\alpha]_D^{20}$=+73.2 (*c*=0.39, $CHCl_3$). Anal. calcd for: $C_{18}H_{19}NO_3$: C, 72.71; H, 6.44; N, 4.71. Found: C, 72.43; H, 6.39; N, 4.85. Spectroscopic data for both (2*R*,3*R*)-**3** and (2*S*,3*S*)-**3** were the same as those described above for *erythro*-*3*.

4.8. Synthesis of *threo*-4 and *erythro*-4

4.8.1. *threo*-β-Methylphenylalanine hydrochloride, *threo*-4. A solution of *threo*-**3** (2 g, 6.73 mmol) in HOAc/2.5 N HCl (90 mL:360 mL) was heated for 42 h at 125 °C. The solvent was removed in vacuo and the residue partitioned between H_2O/CH_2Cl_2. The phases were separated and the aqueous layer was washed with CH_2Cl_2. Removal of water by lyophilization afforded *threo*-**4** as a white solid (1.36 g, 94% yield). Mp 214 °C. Diastereomeric purity: 99%. R_f (CH_2Cl_2/MeOH 8:2)=0. IR (nujol) 3600–2400; 1734 cm^{-1}. ^{1}H NMR (D_2O 300 MHz) δ 1.32 (d, 3H, *J*=6.5 Hz); 3.42 (q, 1H, *J*=6.5 Hz): 4.09 (d, 1H, *J*=6.4 Hz); 7.20–7.34 (m, 5H). ^{13}C NMR (D_2O 75 MHz) δ 14.83; 39.72; 58.68; 127.84; 128.16; 129.21; 139.10; 171.10. MS-FAB (*m/z*, %) 359 [(2M+1)$^+$, 10]; 202 [(M+Na)$^+$, 18]; 180 [(M+1)$^+$, 100]. Anal. calcd for $C_{10}H_{14}ClNO_2$: C, 55.69; H, 6.54; N, 6.49. Found: C, 55.89; H, 6.47; N, 6.55.

4.8.2. *erythro*-β-Methylphenylalanine hydrochloride, *erythro*-4. A similar procedure to that described above for *threo*-**4**, starting from *erythro*-**3** (2 g, 6.73 mmol), gave *erythro*-**4** as a white solid (1.35 g, 93% yield). Mp 200 °C. Diastereomeric purity: 98%. R_f (CH_2Cl_2/MeOH 8:2)=0. IR (nujol) 3600–2500; 1728 cm^{-1}. ^{1}H NMR (D_2O 300 MHz) δ 1.33 (d, 3H, *J*=7.2 Hz); 3.30 (q, 1H, *J*=7.05 Hz); 4.01 (d, 1H, *J*=7.5 Hz); 7.2–7.4 (m, 5H). ^{13}C NMR (D_2O 75 MHz) δ 16.98; 40.32; 59.02; 127.82; 128.22; 129.32; 139.27; 171.63. MS-FAB (*m/z*, %) 359 [(2M+1)$^+$, 13]; 202 [(M+Na)$^+$, 8]; 180 [(M+1)$^+$, 100]. Anal. calcd for $C_{10}H_{14}ClNO_2$: C, 55.69; H, 6.54; N, 6.49. Found: C, 57.21; H, 6.95; N, 6.13.

4.9. Synthesis of enantiomerically pure 5

4.9.1. (2*R*,3*S*)-β-Methylphenylalanine, (2*R*,3*S*)-5. A solution of (2*R*,3*S*)-**3** (50 mg, 0.17 mmol) in HOAc/2.5 N HCl (2.3 mL:9 mL) was heated at 125 °C for 24 h. The solvent was removed in vacuo and the residue partitioned between H_2O/CH_2Cl_2. The phases were separated and the aqueous layer was washed with CH_2Cl_2 and evaporated to dryness to afford the amino acid hydrochloride. This compound was converted into the free amino acid by treatment with EtOH (2 mL) and propylene oxide (0.68 mL) under reflux. Removal of the solvent afforded a residue that was eluted through a C_{18} reverse-phase sep-pak cartridge. Lyophilization of the aqueous phases gave (2*R*,3*S*)-**5** as a white solid (28 mg, 93% yield). Mp 220–222 °C. $[\alpha]_D^{20}$=+6.6 (*c*=0.18, H_2O) lit.[45] (+5.1; *c*=1.1, H_2O). Diastereomeric purity: 99%. R_f (CH_2Cl_2/MeOH 8:2)=0.88. IR (nujol) 1629; 1574 cm^{-1}. ^{1}H NMR (D_2O 300 MHz) δ 1.27 (d, 3H, *J*=7.5 Hz); 3.42 (m, 1H): 3.82 (d, 1H, *J*=4.8 Hz); 7.24–7.25 (m, 5H). ^{13}C NMR (D_2O 75 MHz) δ 13.70; 39.51; 60.73; 127.73; 127.78; 129.09; 140.36; 173.54. MS-FAB (*m/z*, %) 359 [(2M+1)$^+$, 10]; 202 [(M+Na)$^+$, 18]; 180 [(M+1)$^+$, 100]. Anal. calcd for $C_{10}H_{13}NO_2$: C, 67.02; H, 7.31; N, 7.82. Found: C, 66.69; H, 7.39; N, 7.71.

4.9.2. (2*S*,3*R*)-β-Methylphenylalanine, (2*S*,3*R*)-5. An identical procedure to that described above was applied to the transformation of (2*S*,3*R*)-**3** (65 mg, 0.22 mmol) to (2*S*,3*R*)-**5** (36 mg, 92% yield). Mp 200–202 °C. $[\alpha]_D^{20}$=−7.49 (*c*=0.15, H_2O) lit.[45] (−5.3; *c*=0.75, H_2O). Diastereomeric purity: 99.5%. Anal. calcd for $C_{10}H_{13}NO_2$: C, 67.02; H, 7.31; N, 7.82. Found: C, 66.65; H, 7.42; N, 7.70.

4.9.3. (2*R*,3*R*)-β-Methylphenylalanine, (2*R*,3*R*)-5. The procedure described above was applied to the transformation of (2*R*,3*R*)-**3** (95 mg, 0.32 mmol) to (2*R*,3*R*)-**5** (52 mg, 91% yield). Mp 224 °C. $[\alpha]_D^{20}$=+27.2 (*c*=0.10, H_2O) lit.[45] (+21; *c*=1.0, H_2O). Diastereomeric purity: 97.7%. R_f (CH_2Cl_2/MeOH 8:2)=0.88. IR (nujol) 1609 cm^{-1}. ^{1}H NMR (D_2O 300 MHz) δ 1.28 (d, 3H, *J*=7.17 Hz); 3.16 (q, 1H, *J*=7.35 Hz); 3.66 (d, 1H, *J*=7.71 Hz); 7.21–7.34 (m, 5H). ^{13}C NMR (D_2O 75 MHz) δ 17.65; 40.80; 60.98; 127.89; 127.89; 127.96; 129.25; 140.20; 173.79. MS-FAB (*m/z*, %) 180 [(M+1)$^+$, 83]. Anal. calcd for $C_{10}H_{13}NO_2$: C, 67.02; H, 7.31; N, 7.82. Found: C, 66.75; H, 7.39; N, 7.73.

4.9.4. (2*S*,3*S*)-β-Methylphenylalanine, (2*S*,3*S*)-5. The method described above was used for the conversion of

(2*S*,3*S*)-**3** (110 mg, 0.37 mmol) to (2*S*,3*S*)-**5** (60 mg, 91% yield). Mp 224 °C. $[\alpha]_D^{20}=-29$ (c=0.30, H_2O) lit.[45] (−26.7; c=1.0, H_2O). Diastereomeric purity: 99.8%. Anal. calcd for $C_{10}H_{13}NO_2$: C, 67.02; H, 7.31; N, 7.82. Found: C, 66.53; H, 7.24; N, 7.77. Spectroscopic data were the same as those described above for (2*R*,3*R*)-**5**.

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