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Synthesis of the Isosteric L-Valine Analogues (2R,3S)- and (2R,3R)-3-Bromobutyrine

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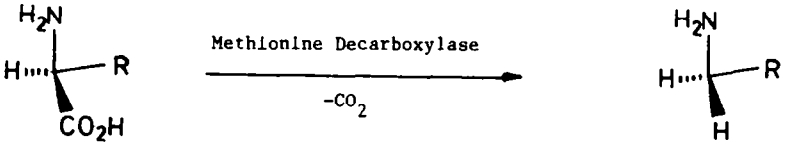
Abstract - Both diastereomers of the isosteric analogue of L-valine, in which one methyl group has been replaced by a bromine atom, have been prepared. The synthesis of the (2R,3R)-isomer provides a route to (2S,3S)-3-hydroxybutyrine (L-allo-threonine) starting from (2S,3S)-3-methylaspartic acid.

The mode of action of pyridoxal 5'-phosphate (PLP) dependent enzymes and, in particular, the mode of their mechanism-based inhibition has attracted much interest in the past five years.[1-4] Recently we have isolated the PLP-dependent enzyme L-methionine (L-alkyl amino acid) decarboxylase from two sources; from the fern Dryopteris felix-mas[5] and from the bacterium Streptomyces sp.[6] Both enzymes catalyse the decarboxylation of L-methionine (R=CH_2CH_2SMe) with retention of configuration at C^{α}.[5,6] The fern enzyme is also capable of catalysing the decarboxylation of several hydrophobic side-chain bearing amino acids including L-valine, (R=$CHMe_2$), Scheme 1.

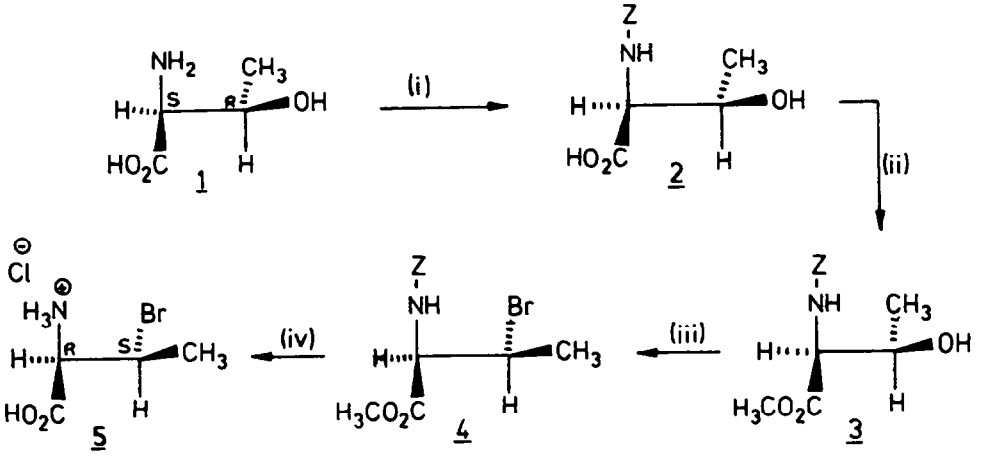


Scheme 1

In order to gain further insight into the geometry of the active-site region and the stereochemistry and mechanism of reactions catalysed by the enzymes, we decided to investigate the action of suicide substrates. As we had shown that L-valine was a substrate it seemed reasonable to expect that the products of the replacement of one of each of the methyl groups in valine by a bromine atom, each diastereomer of L-3-bromobutyrine (5 and 18) should also be substrates for the enzyme. The stereospecific introduction of a leaving group at the β-position would then facilitate suicide-type reactions and also allow direct assessment of the stereochemical course of any such inhibition process.

Here we report on the syntheses of these potential suicide substrates, the L-3-bromobutyrines (5 and 18) starting from L-threonine (1) and L-allo-threonine (12) and also on the synthesis of L-allo-threonine (12) starting from L-threo-3-methylaspartic acid (7). The syntheses are suitable for the preparation of stable isotope- and radio- labelled products.

The synthesis of L-erythro-3-bromobutyrine [the (2R,3S)-diastereomer (5)] has been described by Wieland et al.[7] Essentially we have followed their method which involves triphenylphosphine/CBr_4 mediated bromination of N-benzyloxycarbonyl protected (2S,3R)-threonine methyl ester (3) followed by acid catalysed deprotection of the product, Scheme 2. In our hands the deprotected product was retained as the hydrochloride salt which showed no tendency to cyclize to the aziridine on prolonged storage.



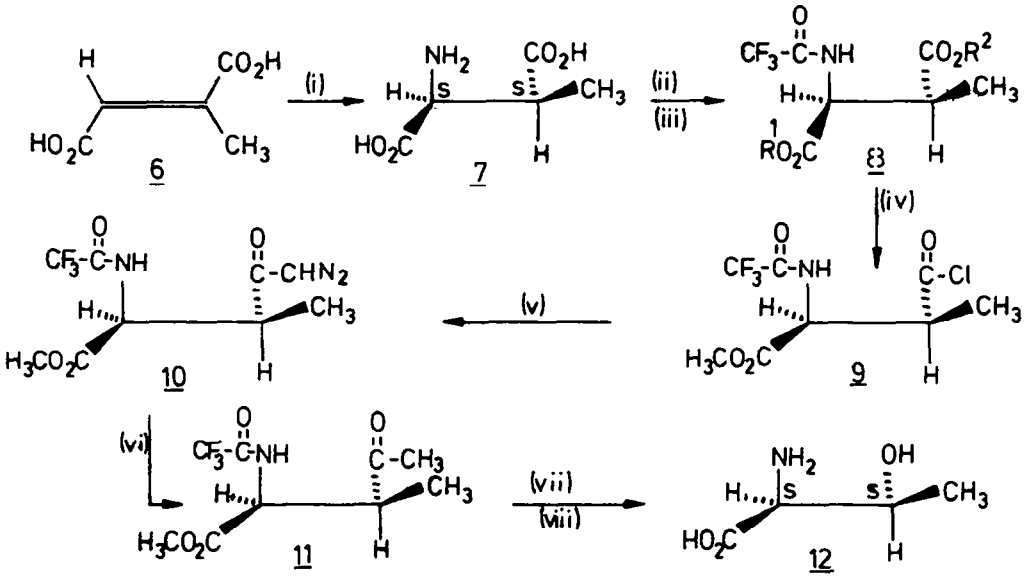
Reagents: (i) $PhCH_2OCOCl$, $NaHCO_3$, water, 25°C, 3 h;
(ii) CH_2N_2, diethyl ether, 25°C, 1 h;
(iii) CBr_4, PPh_3, benzene, 25°C, 90 min;
(iv) 12M hydrochloric acid/glacial acetic acid (1:1, v/v), 100°C, 2h.

Scheme 2

The starting material for the synthesis of (2R,3R)-3-bromobutyrine, L-allo-threonine is very expensive and is not available in a labelled form. It was, therefore, necessary to develop a synthetic route to the starting material that would facilitate the introduction of deuterium or tritium and/or ^{13}C or ^{14}C label. As we were able to prepare (2S,3S)-3-methylaspartic acid (7) in good yield by a combination of chemical and enzymic methods that allow the introduction of suitable isotopic labels, this compound was chosen as our starting material. The replacement of the β-carboxyl with a hydroxyl group with retention of stereochemistry had been achieved by Gani and Young for C-3 chirally deuteriated L-serine.[8] We therefore expected that the key reaction in their sequence, Baeyer-Villiger rearrangement at a primary migrating centre should proceed at least as well for our secondary migrating centre.

(2S,3S)-3-Methylaspartic acid was prepared through enzymic amination of mesaconic acid in 61% yield.[9] The product was treated with trifluoroacetic anhydride in THF to give (2S,3S)-N-trifluoroacetyl-3-methylaspartic anhydride. The product was treated with cold anhydrous methanol to give a 4:1 mixture of the α- and β- methyl esters as judged by ^1H-nmr spectroscopy. The pure α-methyl ester β-free acid was obtained by converting the mixture to the corresponding acid chlorides in refluxing thionyl chloride, which allowed crystallisation of the pure β-acid chloride. Hydrolysis in water yielded the pure α-ester β-acid chloride. The β-acid chloride was converted to the diazoketone through treatment with dry ethereal diazomethane. The

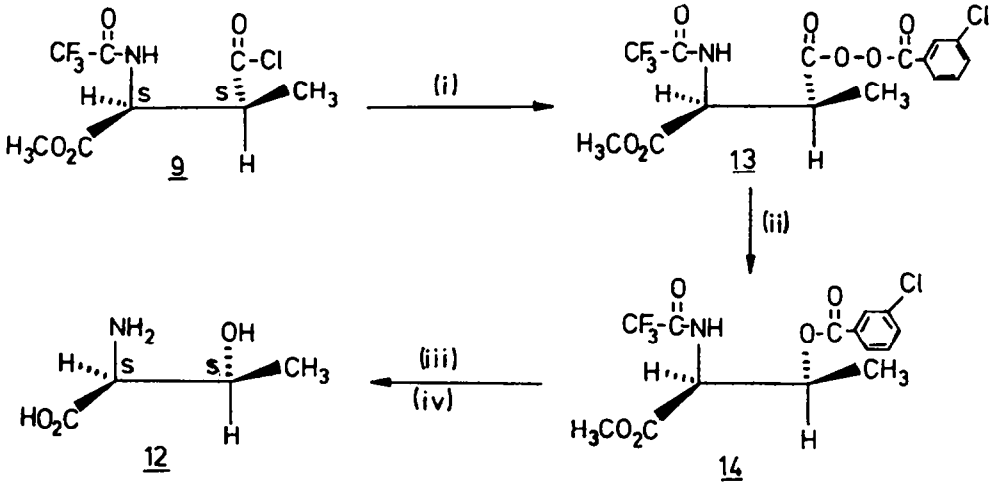
diazoketone was reduced to the ketone using hydrogen iodide in chloroform following the methods of Gani and Young.[8] Baeyer-Villiger oxidation of the ketone did not proceed smoothly under a wide variety of conditions. The highest conversion was 28% as judged by ^{1}H nmr spectroscopy of the reaction mixture after prolonged exposure to the oxidant. Indeed the reaction was less facile than that reported for the primary migrating centre rearrangement involved in the synthesis of serine, Scheme 3. A possible explanation for the poor conversion is that the migrating group in the Criegee intermediate is not able to attain the correct position with respect to the leaving group due to a conformational restriction imposed by H-bonding of the ketone oxygen atom.



Reagents: (i) β-Methylaspartase, Mg^{2+}, K^+, water, 30°C, 40 h;
(ii) $(CF_3CO)_2O$, tetrahydrofuran, 25°C, 2 h;
(iii) Methanol, 25°C, 20 min; (iv) $SOCl_2$, 80°C, 1 h;
(v) CH_2N_2, diethyl ether, tetrahydrofuran, 25°C, 90 min;
(vi) 57% HI, chloroform, 25°C, 10 min;
(vii) CF_3CO_3H, dichloromethane, 40°C, 10 h; (viii) 6M-Hydrochloric acid, 100°C, 2 h; followed by ion-exchange [Amberlite IR45 (OH)].

Scheme 3

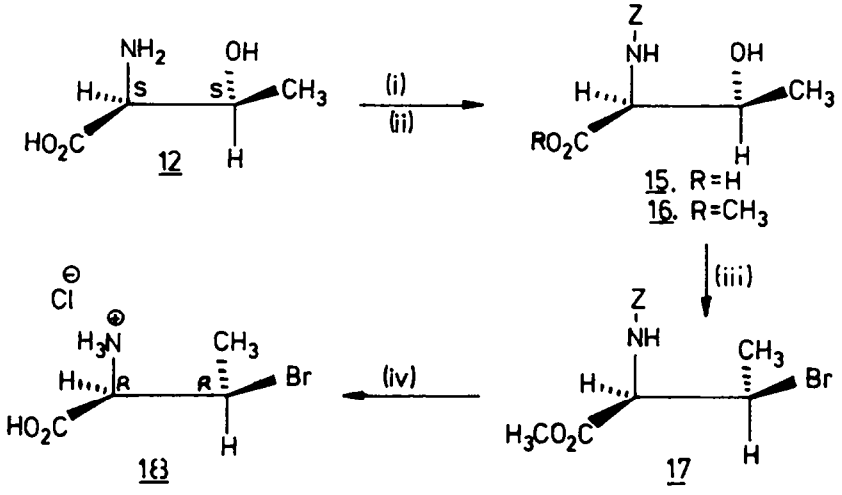
In order to prepare L-allothreonine, we now opted to use a diacylperoxide-type rearrangement which we expected to occur with retention of configuration at the secondary alkyl centre. Accordingly, the N-trifluoroacetyl-3-methylaspartyl acid chloride (9) was treated with mCPBA and pyridine in ether to give the diacyl peroxide in 60% yield.[cf.10] The product was heated to reflux in toluene to give the diester (14). Acid hydrolysis of the protecting groups followed by basic anion-exchange chromatography gave L-allo-threonine in 48% yield (from the diacylperoxide (13)), which was identical in all respects to an authentic sample, Scheme 4.



Reagents: (i) 85% mCPBA, Pyridine, diethyl ether, 0°C, 4 h;
(ii) toluene, 110°C, 3 h; (iii) 6M hydrochloric acid,
100°C, 2h; (iv) Amberlite IR45 (OH).

Scheme 4

To prepare (2R,3R)-isomer of 3-bromobutyrine, L-allo-threonine was converted to the N-benzyloxycarbonyl methyl ester derivative and the product was subjected to bromination and deprotection as outlined in Scheme 5. The product was obtained in 22% overall yield.



Reagents: (i) $PhCH_2OCOCl$, $NaHCO_3$, water, 25°C, 3h;
(ii) CH_2N_2, diethyl ether, 25°C, 1 h;
(iii) CBr_4,PPh_3, benzene, 25°C, 90 min;
(iv) 12M hydrochloric acid/glacial acetic acid (1:1; v/v)
100°C, 2 h.

Scheme 5

EXPERIMENTAL

Melting points were determined using either a Kofler hot-stage or an electrothermal melting point apparatus. Ir spectra were recorded using a Perkin Elmer 298 infra-red spectrophotometer. Nmr spectra were recorded on a Hitachi Perkin-Elmer R24(B) [60 MHz cw ^{1}H nmr], Jeol FX90Q [90 MHz ft ^{1}H-nmr], Jeol JNM-GX270 [270 MHz, ft ^{1}H nmr and 67.8 MHz, ^{13}C-nmr], and Bruker AM 360 [360 MHz ft ^{1}H-nmr and 90 MHz ^{13}C-nmr] instruments. TMS was used as external standard for ^{1}H-nmr and methanol as an internal standard for ^{13}C-nmr spectra run in $^{2}H_2O$ as a solvent. Mass spectra were obtained using an AEI-MS 30 spectrometer. Micro-analysis facilities were provided on a service basis by University College London, UK. Specific rotations were determined on an Optical Activity Ltd. AA-100 polarimeter using either 10 cm or 5 cm path-length cells. All chemicals and solvents were of analytical grade or were purified by recrystallisation or redistillation before use. Light petroleum refers to the fraction boiling at 40-60°C.

(2S,3R)-N-benzyloxycarbonylthreonine (2)

To a vigorously stirred solution of threonine (0.6 g, 5 mmol) in water (50 ml) containing sodium hydrogen carbonate (1.05 g, 12.5 mmol) was added benzylchloroformate (0.80 ml, 5.5 mmol) in 5 portions over 15 mins. The reaction was stirred for 3 h, and was then extracted with ether (2 x 25 ml). The aqueous phase was acidified with concentrated HCl and, then extracted with ether (3 x 50 ml). The ethereal extracts were washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure to give a colourless oil. The oil was crystallised from ethyl acetate/light petroleum to give white crystals (800 mg, 75%) identical in all respects to an authentic sample; m.p. 100-102°C (lit.[11] 101-103°C); $[\alpha]_D^{20}$-4.3° (c 4 in glacial acetic acid) (lit.[11], $[\alpha]_D^{20}$- 4.7 ± 0.5° (c 4 in glacial acetic acid); δ_H (90 MHz, C^2HCl_3) 1.12 (3H, d, J 6.5 Hz, 3-CH_3), 4.30 (2H, m, 2 and 3-CH), 5.04 (2H, s, -OCH_2Ph), 6.07 (1H, d, J 9 Hz, -NH), 7.10 (1H, sb, -OH), and 7.29 (5H, s, -C_6H_5).

Methyl (2S,3R)-N-benzyloxycarbonylthreoninate (3)

To an ice-cold solution of (2S,3R)-N-benzyloxycarbonylthreonine (2g, 7.9 mmol) in chloroform (10 ml) was added excess diazomethane (1 g, 23.8 mmol) with stirring. The reaction was then allowed to warm to room temperature over one hour. The excess diazomethane was removed in a stream of nitrogen and the solvent removed under reduced pressure to give a waxy solid. Recrystallization from ethyl acetate/light petroleum gave the methyl ester as white crystals (1.70 g, 80%); m.p. 93-96°C (lit.[7], 88-89°C); (Found: C, 58.64; H, 6.40; N, 5.19. Calc. for $C_{13}H_{17}NO_5$: C, 58.42; H, 6.41; N, 5.24%); $[\alpha]_D^{20}$-13.7° (c 1.2 in $CHCl_3$); ν_{max} ($CHCl_3$) 3440 (NH), 3030-2970 (CH,CH_2, CH_3), 1760-1700 (C=O), 1500 (aromatic), and 1300-1100 cm^{-1} (C-O); δ_H(360 MHz, C^2HCl_3) 1.19 (3H, d, J 6.3 Hz, -CH_3), 3.03 (1H, sb, -OH), 3.70 (3H, s, -OCH_3), 4.31 (2H, d, J 8.3 Hz, 2 and 3 -CH), 5.10 (2H, s, -OCH_2Ph), 5.89 (1H, d, J 9.1 Hz, -NH), and 7.31 (5H, s, -C_6H_5); δ_C (90 MHz, {^{1}H}, C^2HCl_3) 19.83 (CH_3), 52.38 (COOCH_3), 59.53 (CH), 67.18 (OCH_2), 67.85 (CH), 128.50, 128.13 and 127.98 (aromatic), 156.35 (urethane carbonyl), and 171.70 (ester carbonyl); m/z (EI) 267 (M^+, 0.2%), 223 (4.2, [M - CO_2]$^+$), 91(100, $C_6H_5CH_2^+$).

Methyl (2R,3S)-(N-benzyloxycarbonyl)-2-amino-3-bromobutyrate (4)

Carbon tetrabromide (900 mg, 2.71 mmol) and triphenylphosphine (711 mg, 2.71 mmol) (both pre-dried in a dessicator over phosphorous pentoxide for 12 h) were dissolved in dry benzene (15 ml). To this solution was added the methyl ester (3) (500 mg, 1.87 mmol) in several portions over 30 min at room temperature with stirring. After stirring for a further 90 min, the precipitated triphenylphosphine oxide was removed by filtration and the pad washed with benzene (20 ml). The combined filtrates were concentrated under reduced pressure to give an oil. The resulting oil was purified by column chromatography on flash silica using 3:1 EtOAc:$CHCl_3$ as the eluent and then again using chloroform as the eluent. The purified material was recrystallised twice from ethyl acetate/light petroleum to give white crystals (0.38 g, 62%); m.p. 73-74°C (lit. [7], 53-54°C); (Found: C, 47.46; H, 4.88; N, 4.16. Calc. for $C_{13}H_{16}BrNO_4$: C, 47.26, H,

4.88; N, 4.24%); $[\alpha]_D^{20}$ + 31.5° (c 0.47 in $CHCl_3$); ν_{max} ($CHCl_3$) 3440 (NH), 3060-2970 (CH, CH_2, CH_3), 1760-1700 (C=0), 1515 (aromatic), and 1300-1100 cm^{-1} (C-0); δ_H (360 MHz, C^2HCl_3), 1.70 (3H, d, J 7 Hz, 3-CH_3), 3.71 (3H, s, -OCH_3), 4.27 (1H, m, 2-CH), 4.52 (1H, m, 3-CH), 5.04 (2H, s, -OCH_2Ph), 5.61 (1H, d, J 9 Hz, -NH), and 7.27 (5H, s, -C_6H_5); δ_C (90 MHz, {^1H}, C^2HCl_3), 22.52 (CH_3), 48.90 (2-CH), 52.44 (OCH_3), 59.76 (3-CH), 67.19 (OCH_2), 128.42, 128.12 and 127.98 (aromatic), 155.62 (urethane carbonyl), and 168.94 (ester carbonyl); m/z (EI) 331 and 329 (M^+, bromine isotopes, 0.6 and 0.6%), 108 (39.3, $C_7H_7OH^+$), and 91(100, $C_6H_5CH_2^+$).

(2R,3S)-2-amino-3-bromobutyric acid hydrochloride (5)

The N-protected threonine methyl ester (4) (400 mg, 1.21 mmol) was refluxed in a mixture of 12 M hydrochloric acid and glacial acetic acid (10 ml, 1:1; v/v) for 2 h. The resulting solution was concentrated under reduced pressure and the pale brown residue was dissolved in water (10 ml). The solution was decolourised with active charcoal and the solvent removed in vacuo. The resulting solid was recrystallised from methanol/diethyl ether to give white crystals (150 mg, 57%); m.p. 185°C (dec) (lit.[7], 198°C for the free amine); (Found: C, 21.66; H, 3.91; N, 6.40. $C_4H_9BrClNO_2$ requires C, 21.99; H, 4.15; N, 6.41%); $[\alpha]_D^{20}$ + 12.7° (c 0.44 in H_2O), (lit.[7], $[\alpha]_D^{21}$ + 15° (c 1 in H_2O) for the free amine); ν_{max} (nujol) 3045 ($\overset{+}{N}H_3$), 3040-2970 (CH,CH_3), 1665 (C=0), and 1300-1100 cm^{-1} (C-0); δ_H(360 MHz, ^2H_2O), 1.61 (3H, d, J 7 Hz, 3-CH_3), 4.10 (1H, d, J 2.7 Hz, 2-CH), and 4.49 (1H, q, J 2.6 Hz, 3-CH); δ_C (67.8 MHz,{^1H}, ^2H_2O), 19.28 (CH_3), 43.48 (2-CH), 57.91 (3-CH), and 167.34 (acid carbonyl); m/z (FAB) 182 and 184 (M^+- Cl, bromine isotopes, 100 and 98%), and 104 (74, $[MH - ClBr]^+$).

α-Methyl N-trifluoroacetyl-(2S,3S)-3-methylaspartate (8,R^1=CH_3, R^2 = H).

Trifluoroacetic anhydride (48.5 g, 231 mmol) was added to a stirred suspension of 3-methylaspartic acid (4 g, 27.2 mmol) in dry tetrahydrofuran (125 ml) at 0°C over 30 min under nitrogen. The reaction was allowed to warm to room temperature and was left stirring for 2 h when dissolution was complete. The solvent was removed in vacuo and the resultant anhydride was thoroughly dried. The anhydride was treated with cold dry methanol (50 ml) and the solution was allowed to warm to room temperature. The solvent was removed under reduced pressure to give a mixture of solids in quantitative recovery. The ^1H-nmr spectrum showed two methoxy singlets at 3.76 and 3.83 ppm in a ratio of 1:4 corresponding to the β- and α- methyl esters respectively. The pure α-methyl ester was obtained through hydrolysis of the pure acid chloride, prepared as described below, (250 mg, 0.97 mmol) in water (15 ml) at room temperature for 2 h. Lyophilisation gave the acid as white crystals in quantitative recovery; m.p. 117-119°C; (Found: C, 37.24; H, 3.83; N, 5.41. $C_8H_{10}NF_3O_5$ requires C, 37.36; H, 3.92; N, 5.45%); $[\alpha]_D^{20}$ + 16.2° (c 0.45 in $CHCl_3$); ν_{max} ($CHCl_3$) 3415 (NH), 3050-2950 (CH,CH_3), and 1800-1700 cm^{-1}(C=0); δ_H (360 MHz, C^2HCl_3), 1.36 (3H, d, J 7 Hz, -CH_3), 3.11 (1H, m, 3-CH), 3.83 (3H, s, 1'-OCH_3), 4.90 (1H, m, 2-CH), 7.32 (1H, d, J 10.8 Hz, -NH), and 7.46 (1H, sb, 3-CO_2H); δ_C (90 MHz, {^1H}, C^2HCl_3) 13.19 (CH_3), 41.78 (3-CH), 53.24 (OCH_3), 54.36 (2-CH), 115.80 (CF_3, q, J 288 Hz), 157.22 (amide carbonyl, q, J 38 Hz), 169.59 and 177.75 (ester and acid carbonyls); m/z (EI) 212 (M^+- CO_2H, 3.7%), 198 (100, $[M - CO_2CH_3]^+$), 180 (56.3, $[M - C_2H_5O_3]^+$), 153 (24.4, $[M - C_3H_4O_4]^+$).

α-Methyl N-trifluoroacetyl-(2S,3S)-3-methylasparty1-β-acid-chloride (9)

The mixture of α- and β-monoesters from the above reaction (5 g, 19.4 mmol) were heated to reflux in distilled thionyl chloride (60 ml) for 1 h. The solvent was removed under reduced pressure to give a pale yellow solid which was recrystallised from diethyl ether/light petroleum to afford the pure β-chloride (3.75 g, 70%); m.p. 68-69°C; (Found: C, 34.55; H, 3.55; N, 5.32. $C_8H_9NClF_3O_4$ requires C, 34.86; H, 3.29; N, 5.08%); $[\alpha]_D^{20}$ + 25.1° (c 0.51 in $CHCl_3$); ν_{max} ($CHCl_3$), 3415 (NH), 3050-2950 (CH,CH_3), and 1800-1700 cm^{-1} (C=0); δ_H (360 MHz, C^2HCl_3), 1.39 (3H, d, J 8 Hz, -CH_3), 3.51 (1H, m, 3-CH), 3.84 (3H, s, 1'-OCH_3), 4.96 (1H, m, 2-CH), and 7.27 (1H, sb, -NH); δ_C (90 MHz, {^1H}, C^2HCl_3), 13.17 (CH_3), 41.70 (3-CH), 53.25 (OCH_3), 54.30 (2-CH), 115.98 (CF_3, q, J 288 Hz), 157.24 (amide carbonyl, q, J 38 Hz), 169.57 and 177.84 (ester

and acid chloride carbonyl's); m/z (EI) 240 ([M - Cl]$^+$, 5.7%), and 212 (46.8, [M - COCl]$^+$).

Methyl (2S,3S)-5-Diazo-4-oxo-N-trifluoroacetylisoleucinate (10)

The 3-Methylaspartyl-β-chloride (3 g, 10.9 mmol) dissolved in diethyl ether (40 ml) and tetrahydrofuran (10 ml) was added dropwise during 30 min to an excess of ethereal diazomethane (200 ml) with stirring. The solution was allowed to warm to room temperature during 90 min after which the excess diazomethane was removed under reduced pressure to yield the diazoketone (2.90 g, 92%); m/z (Found: M^+-N_2, 253.0494, $C_9H_{10}NF_3O_4$ requires 253.0562); ν_{max} ($CHCl_3$) 3415 (NH), 2900-3200 (CH, CH_3), 2120 (N=N), 1760-1710 (C=O), and 1300-1100 cm^{-1} (C-O of ester); δ_H (60 MHz, C^2HCl_3), 1.26 (3H, d, J 8Hz, -C<u>H</u>$_3$), 3.05 (1H, m, 2-C<u>H</u>), 3.72 (3H, s, -OC<u>H</u>$_3$), 4.73 (1H, m, 3-C<u>H</u>), 5.45 (1H, s, C<u>H</u>N_2), and 7.87 (1H, brds, 2-N<u>H</u>); m/z (EI) 253 ([M -N_2]$^+$, 2.7%), 212 (13.3, [M - $COCHN_2$]$^+$), and 69 (100, CF_3^+).

Methyl (2S,3S)-4-oxo-N-trifluoroacetylisoleucinate (11)

Aqueous HI (57%, excess) was added dropwise to a stirred solution of the diazoketone (2g, 7.12 mmol) in chloroform (80 ml). After 10 min the organic layer was washed with water (2 x 10 ml), 5% sodium thiosulphate (until colourless) and then dried (Na_2SO_4). The solvent was removed in vacuo to give the methyl ketone as a waxy solid (1.65 g, 86%); m/z (Found: M^+, 255.0707, $C_9H_{12}NF_3O_4$ requires 255.0718); ν_{max} ($CHCl_3$) 3415 (NH). 3050-2990 (CH, CH_3), 1760-1710 (C=O), and 1300-1100 cm^{-1} (C-O); δ_H (60 MHz, C^2HCl_3) 1.30 (3H, d, J 7 Hz, -C<u>H</u>$_3$), 2.21 (3H, s, -COC<u>H</u>$_3$), 3.12 (1H, m, 3-C<u>H</u>), 3.70 (3H, s, -OC<u>H</u>$_3$), 4.74 (1H, m, 2-C<u>H</u>), and 7.23 (1H, brds, -N<u>H</u>); m/z (EI) 255(M^+, 2%), 212 (100, [M - $COCH_3$]$^+$), and 43 (9.12, $COCH_3^+$).

α-Methyl (2S,3S)-N-trifluoroacetyl-3-methylaspartyl-m-chlorobenzoyl peroxide (13)

The N-trifluroacetyl-(2S,3S)-3-methylaspartyl-β-chloride (9) (500 mg, 1.81 mmol) and 80-90% meta-chloroperbenzoic acid (367 mg, 1.81 mmol) were added to dry diethyl ether (15 ml), and the solution stirred under nitrogen in an ice-salt bath. Pyridine (143 mg, 1.8 mmol) in dry diethyl ether (2 ml) was added slowly to the mixture and stirring was continued for a further 4 h. The solution was filtered, washed with water and aqueous 1M-sodium carbonate, and then dried (Na_2SO_4). The solvent was removed under reduced pressure to give a white solid which crystallised from diethyl ether/light petroleum as colourless needles (450 mg, 60%); m.p. 111-112°C; (Found: C, 43.84; H, 3.24; N, 3.64. $C_{15}H_{13}ClF_3NO_7$ requires C, 43.76; H, 3.18; N, 3.40%). m/z (Found: [M -CO_2CH_3]$^+$, 352.0248, $C_{13}H_{10}{}^{35}ClF_3NO_5$ requires 352.0200); $[\alpha]_D^{20}$ + 46.6° (c 0.7 in $CHCl_3$); ν_{max} ($CHCl_3$), 3440 (NH), 3015-2995 (CH,CH_3), 1805, 1770 (peroxy-anhydride), 1740 (ester), 1728 (amide), and 1530 cm^{-1} (aromatic); δ_H (270 MHz, C^2HCl_3) 1.51 (3H, d, J 7.3 Hz, -C<u>H</u>$_3$), 3.30 (1H, m, J 4.8 and 7.3 Hz, 3-C<u>H</u>), 3.85 (3H, s, -OC<u>H</u>$_3$), 4.88 (1H, m, J 4.8 and 8.3 Hz, 2-C<u>H</u>), and 7.4-8.0 (5H, m, NH and aromatic); δ_C (67.8 MHz, {^{1}H}, C^2HCl_3), 14.03 (CH_3), 39.57 (3-CH), 53.58 (OCH_3), 54.35 (2-CH), 115.64(CF_3, q, J 288 Hz), 135.30-126.75 (aromatic), 157.19 (amide carbonyl, q, J 38 Hz), 161.98, 168.34 and 168.58 (carbonyls); m/z (EI) 354 and 352 ([M - CO_2CH_3]$^+$, Cl isotopes, 0.1 and 0.4%), 240 (12.3, [M-$C_7H_4ClO_3$]$^+$), 158 and 156 (18.7 and 59.9, Cl isotopes, [$C_7H_4ClO_2H$]$^+$),141 and 139 (31.4 , 100, Cl isotopes, [C_7H_4ClO]$^+$).

(2S,3S) Threonine (12)

The diacyl peroxide (13) (250 mg, 0.61 mmol) was heated to reflux in toluene (10 ml) for 3 h. The solvent was removed under reduced pressure to give the ester (14) as a gummy off-white solid (220 mg) [δ_H (270 MHz, C^2HCl_3) 1.31 (3H, d, J 6.6 Hz, -C<u>H</u>$_3$), 3.84 (3H, s, -OC<u>H</u>$_3$), 4.21 (1H, m, J 3.9 and 6.8 Hz, 3-C<u>H</u>), 4.65 (1H, m, J 3.57 and 7.8 Hz 2-C<u>H</u>), and 7.2-8.1 (5H, m, NH and aromatic)].

The crude (2S,3S)-methyl-N-trifluoroacetyl-O-(m-chlorobenzoyl) allo-threoninate (14) was heated to reflux in 6M hydrochloric acid under nitrogen for 2 h. The solution was cooled and washed with chloroform (3 x 15 ml). The aqueous phase was concentrated under reduced pressure

to yield L-allo-threonine hydrochloride (110 mg). The hydrochloride salt was then dissolved in water (30 ml) and was applied to a column of Amberlite 1R45(OH) weakly basic anion exchange resin eluting with water (120 ml of 1 drop/3s). Lyophilization of the eluent gave 35 mg (48%) of L-allo-threonine as a white amorphous solid; m.p. 270-274°C (dec), (lit.[12] 273-274°C (dec)); $[\alpha]_D^{20}$ + 27.5° (c 0.29 in 5M HCl), (cf.[12] +28.0° for an authentic sample of L-allo-threonine), δ_H (360 MHz, ^2H_2O, pH 1), 1.00 (3H, d, J 6.6 Hz, $-CH_3$), 3.63 (1H, d, J 4.8 Hz, 2-CH), 4.15 (1H, m, J 6.3 Hz and 5.3 Hz, 3-CH); δ_C (90 MHz, {^1H}, ^2H_2O, pH 1), 14.39 (CH_3), 59.79 (2CH), 63.44 (3CH) and 169.62 (CO_2H).

Methyl (2S,3S)-N-benzyloxycarbonylthreoninate (16)

This compound was prepared in an identical manner as for the diastereomeric (2S,3R)-N-benzyloxycarbonylthreonine methyl ester vide supra but starting from L-allo-threonine (500 mg, 4.2 mmol). The crude (2S,3S)-N-benzyloxycarbonylthreonine (15) [δ_H (90 MHz, C^2HCl_3) 1.18 (3H, d, J 6.8 Hz, $-CH_3$), 4.12 (2H, m, 2 and 3-CH), 5.03 (2H, s, $-OCH_2Ph$), 5.50 (1H, sb, -OH), 6.25 (1H, d, J 9 Hz, -NH) and 7.27 (5H, s, $-C_6H_5$)] was treated with excess diazomethane to give an oil. The crude oil was crystallised using ethyl acetate/light petroleum to yield 600 mg (54% over both steps) of the ester as a white crystalline solid; m.p. 58-59°C; (Found: C, 58.08; H, 6.46; N, 5.24. $C_{13}H_{17}NO_5$ requires C, 58.42; H, 6.41; N, 5.24%); $[\alpha]_D^{20}$ + 14.8° (c 0.5 in $CHCl_3$); ν_{max} ($CHCl_3$) 3440 (NH), 3030-2970 (CH, CH_2, CH_3), 1760-1700 st (C=O), 1500 m (aromatic), and 1300-1100 cm^{-1} (C-O); δ_H (360 MHz, C^2HCl_3), 1.21 (3H, d, J 6.4 Hz, $3-CH_3$), 2.78 (1H, d, J 6Hz, OH), 3.79 (3H, s, $-OCH_3$), 4.17 (1H, m, 3-CH), 4.45 (1H, d, J 4Hz, 2-CH), 5.13 (2H, s, $-OCH_2$), 5.71 (1H, d, J 6HZ, -NH), and 7.37 (5H, s, 2'-C_6H_5); δ_C (90 MHz, {^1H}, C^2HCl_3) 19.13 (CH_3), 52.58 ($COOCH_3$), 59.70 (CH), 67.51 (OCH_2), 69.11 (CH), 128.71, 128.41 and 128.27 (aromatics), 156.61 (urethane carbonyl), and 170.85 (ester carbonyl); m/z (EI) 223 ($M^+ - CO_2$, 3.6%), 162 (16.2, $C_6H_5CH_2CO_2NHC^+$), 108 (13.6, $C_6H_5CH_2OH^+$), and 91 (100, $C_6H_5CH_2$).

Methyl (2R,3R)-(N-benzyloxycarbonyl)-2-amino-3-bromobutyrate (17)

This was prepared in an identical manner to that for methyl (2R,3S)-(N-benzyloxycarbonyl)-2-amino-3-bromobutyrate. A 73% yield was obtained after chromatography. Attempts were made to crystallise the oil obtained using various solvents but no success resulted; (Found: C,46.90; H, 4.55; N, 3.90. $C_{13}H_{16}BrNO_4$ requires C, 47.26; H, 4.88; N, 4.24%). m/z (Found: M^+, 329.0242, $C_{13}H_{16}{}^{79}BrNO_4$ requires 329.0263); $[\alpha]_D^{20}$ + 3.1° (c 1.76 in $CHCl_3$); ν_{max} ($CHCl_3$), 3440 (NH), 3050-2970, (CH,CH_2,CH_3), 1760-1700 (C=O), 1515 (aromatic), and 1300-1100 cm^{-1} (C=O); δ_H (360 MHz, C^2HCl_3), 1.74 (3H, d, J 6.8 Hz, $-CH_3$), 3.78 (3H, s, $-OCH_3$), 4.67 (2H, m, 2 and 3-CH), 5.16 (2H, s, $-OCH_2$), 5.56 (1H, d, J 9 Hz, -NH), and 7.37 (5H, s, $-C_6H_5$); δ_C (90 MHz, {^1H}, C^2HCl_3), 23.24 (CH_3), 50.23 (2-CH), 52.98 (OCH_3), 59.38 (3-CH), 67.53 (OCH_2), 128.67, 128.37 and 128.18 (aromatics), 156.63 (urethane carbonyl), 169.77 (ester carbonyl), m/z (EI) 331 and 329 (M^+, bromine isotopes, 1.3 and 1.2%), 108 (86.2, $C_6H_5CH_2OH^+$), and 91 (100, $C_6H_5CH_2^+$).

(2R,3R)-2-amino-3-bromobutyric acid hydrochloride (18)

The N-protected L-allo-threonine methyl ester (17) (300 mg, 0.91 mmol) was refluxed in a mixture of concentrated hydrochloric acid/glacial acetic acid (10 ml, 1:1, v/v) for 2 h. The resulting solution was concentrated under reduced pressure, and the pale brown residue dissolved in water. The solution was decolourised with active charcoal, concentrated again and the resulting solid recrystallised from methanol/diethyl ether to give white crystals (110 mg, 56%); m.p. 185°C (dec); (Found: C, 21.52; H, 4.35; N, 6.52. $C_4H_9BrClO_2$ requires C, 21.99; H, 4.15; N, 6.41); $[\alpha]_D^{20}$-26.3° (c 0.5 in H_2O); ν_{max} (nujol) 3045 ($\overset{+}{N}H_3$), 3050-2970 (CH,CH_3), 1670 (C=O), and 1300-1100 cm^{-1} (C-O); δ_H (360 MHz, ^2H_2O), 1.61 (3H, d, J 7.1 Hz, $-CH_3$), 4.06 (1H, d, J 3Hz, -CH), 4.61 (1H, m, J 7 Hz and 3Hz, -CH); δ_C (67.8 MHz {^1H}, ^2H_2O), 20.61 (CH_3), 43.91 (2-CH), 57.40 (3-CH) and 167.93 (carbonyl carbon). m/z (FAB),184 and 182 (M^+ - Cl, bromine isotopes, 98 and 100%), and 104(34,[MH - ClBr]$^+$).

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