β-Amino Acid Isomers of a Natural Substrate of the Enzyme γ-Glutamyl-amino Acid Cyclotransferase. Synthesis of (3*S*)-3-Aminoglutaryl-(*S*)-alanine and (3*R*)-3-Aminoglutaryl-(*S*)-alanine

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(3*S*)-3-Aminoglutaryl-(*S*)-alanine (**8**) and (3*R*)-3-aminoglutaryl-(*S*)-alanine (**14**), β-amino acid isomers of the dipeptide γ-glutamyl-(*S*)-alanine, a natural substrate of the enzyme γ-glutamyl-amino acid cyclotransferase, have been synthesized by two different routes for studies of the enzyme. The better route allowed compounds (**8**) and (**14**) to be prepared in diastereoselective sequences from the common intermediate, methyl hydrogen (3*S*)-3-benzyloxycarbonylaminoglutarate (**4**); (3*S*)-3-aminoglutaryl-(*S*)-alanine (**8**) by direct coupling of the half acid (**4**) to (*S*)-alanine followed by deprotection, and (3*R*)-3-aminoglutaryl-(*S*)-alanine (**14**) by a similar reaction sequence that also included an initial chemical reversal of the acid and ester ends, thereby 'inverting' the configuration at C-3, of the glutarate component.

γ-Glutamyl-amino acid cyclotransferase (EC 2.3.2.4) (GCT) is an enzyme found widely distributed in humans and animals. GCT catalyses the cleavage of many (*S*)-γ-glutamyl-L-amino acids and (*S*)-γ-glutamyl-(*S*)-γ-glutamyl-L-amino acids by an intramolecular rearrangement of the substrate to (*S*)-pyroglutamic acid (5-oxoproline) and a free L-amino acid or (*S*)-γ-glutamyl-L-amino acid.[1] All substrates, whether di- or tripeptides, have the free α-carboxy group on the second amino acid residue, suggesting that this group is important for binding of the substrate to the enzyme.[2]

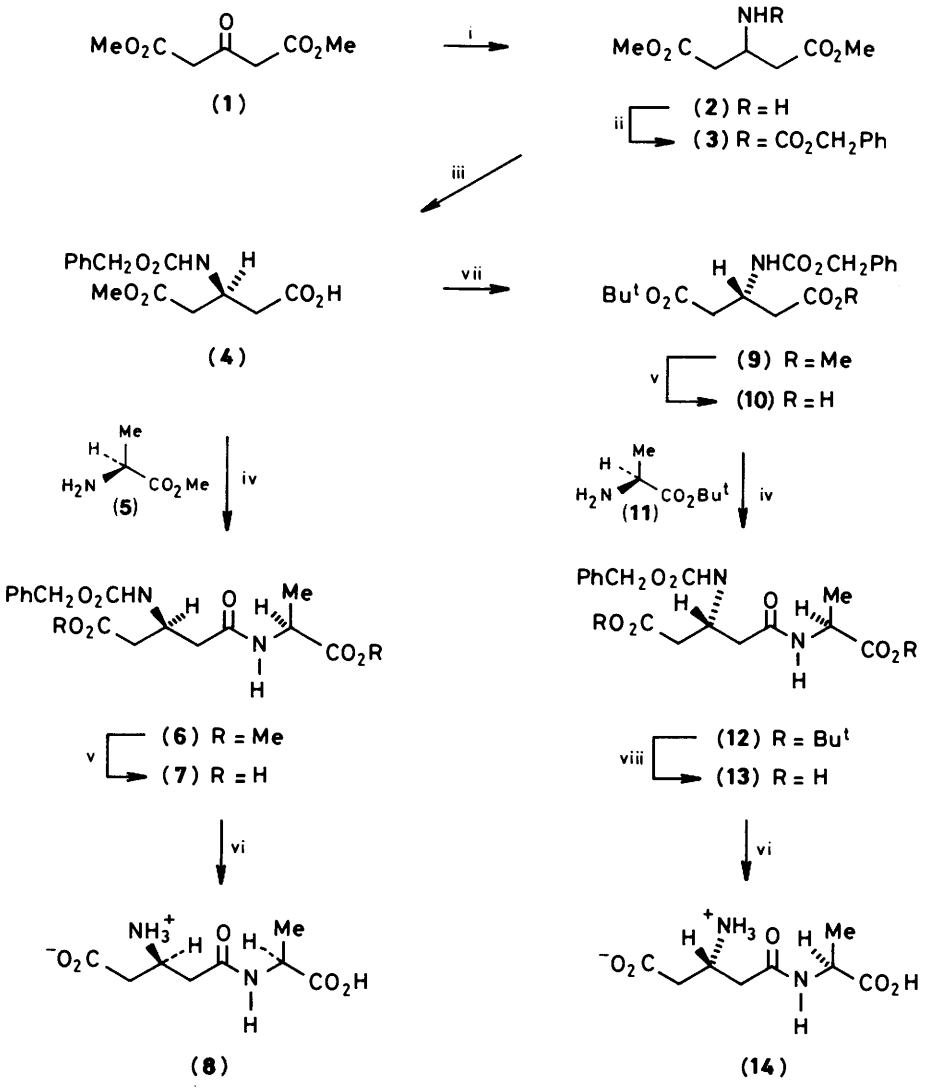
This paper reports the synthesis of (3*S*)-3-aminoglutaryl-(*S*)-alanine (**8**), (3*R*)-3-aminoglutaryl-(*S*)-alanine (**14**), and (3*R*)-3-aminoglutaryl-(*S*)-alaninol (**22**). These three dipeptides were required for structure–activity and inhibition studies on GCT. They are analogues of (*S*)-γ-glutamyl-(*S*)-alanine, a natural substrate of GCT, and should still be capable of being bound to the enzyme. However, we expected that these synthetic dipeptides would not be cleaved by the enzyme as this would require the formation of a 4-membered cyclic intermediate which is thermodynamically unlikely.[3,4] The dipeptides (**8**) and (**14**), in particular, were therefore potential inhibitors of GCT. Inhibition studies on GCT using the carboxy-modified dipeptide, (3*R*)-3-aminoglutaryl-(*S*)-alaninol, (**22**), would test the hypothesis that the free α-carboxy group on the second amino acid residue is important for binding to the enzyme.

Two routes to the dipeptides (**8**) and (**14**) have been investigated in this work. The preferred route is selective and makes use of an enzyme-catalysed cleavage of the prochiral diester (**3**) to the chiral half-ester (**4**) (Scheme 1). Porcine liver esterase (PLE) catalyses the hydrolysis of dimethyl 3-benzyloxycarbonylaminoglutarate (**3**) to give methyl hydrogen (3*S*)-3-benzyloxycarbonylaminoglutarate (**4**)[5] thereby differentiating the two carboxy functionalities in (**4**). This distinction can be exploited chemically to 'reverse' the acid and ester ends and thus bring about a reversal of stereochemistry at the 3-position. In this way both (3*S*)-3-aminoglutaryl- and (3*R*)-3-aminoglutaryl-amino acids can be formed from compound (**4**) with complete stereochemical control.

The starting diester (**3**) was produced in two steps from dimethyl 3-oxoglutarate (**1**). The first step was the reductive amination of ketone (**1**) using sodium cyanoborohydride and ammonium acetate[6] which afforded the amino compound (**2**), reliably, in moderate yield. Other reported methods for the synthesis of the diester (**2**), involving the addition of ammonia to dimethyl glutaconate,[7] and the hydrogenation of methyl 3-aminoglutaconate,[8] proved to be tedious and unreliable in our hands. The benzyloxycarbonyl derivative of the diester, compound (**3**), was hydrolysed using PLE in pH 8 phosphate buffer[5] to give the half ester (**4**), in 82% yield in optically pure form. Direct coupling of (**4**) with methyl (*S*)-alaninate (**5**) by means of 2-isobutyloxy-1-isobutyloxycarbonyl-1,2-dihydroquinoline (IIDQ) gave the fully protected peptide (3*S*)-3-benzyloxycarbonylaminoglutaryl-(*S*)-alanine dimethyl ester (**6**) in 80% yield after recrystallization. Base hydrolysis of (**6**) gave a quantitative yield of the diacid (**7**). Hydrogenolysis of compound (**7**) using 10% Pd–C as the catalyst gave the free peptide, (3*S*)-3-aminoglutaryl-(*S*)-alanine (**8**).

Reversal of configuration at C-3 of the 3-aminoglutaryl residue to give the other diastereoisomer (**14**) was achieved from compound (**4**) using the procedure shown in Scheme 1. The half-ester (**4**) was esterified using t-butyl alcohol and dicyclohexylcarbodiimide with the aid of 4-dimethylaminopyridine[9] to give methyl t-butyl (3*R*)-3-benzyloxycarbonylaminoglutarate (**9**) in 83% yield. Selective hydrolysis of the methyl ester of (**9**) using sodium hydroxide in methanol (1.5 h) afforded in 81% yield the half-ester (**10**) with (*R*)-configuration at C-3. A similar process has been used by Ohno *et al.*[10] for the reversal of configuration of the half-ester. The fully protected peptide (**12**) was then obtained by coupling the half-ester (**10**) with t-butyl (*S*)-alaninate (**11**) using IIDQ. Treatment of the diester (**12**) with trifluoroacetic acid gave the free acid peptide (**13**). Hydrogenolysis of compound (**13**) using 10% Pd–C as the catalyst gave the desired free peptide, (3*R*)-3-aminoglutaryl-(*S*)-alanine (**14**).

These sequences provided each of the dipeptides unambiguously and in pure form (as revealed by reverse-phase HPLC and high field ^1H and ^{13}C NMR spectroscopy). The order of elution on reverse-phase HPLC was the only means by which the two disastereoisomers of 3-aminoglutaryl-(*S*)-alanine could be readily differentiated, the peptide (**8**) having a shorter retention time than peptide (**14**) in two different solvent systems (see Experimental section). ^1H NMR spectra of the



Scheme 1. *Reagents and conditions*: i, $NaBH_3CN$, NH_4OAc; ii, $PhCH_2O_2CCl$; iii, PLE, pH 8 phosphate buffer; iv, IIDQ; v, NaOH, MeOH; vi, H_2, Pd–C, MeOH; vii, Bu^tOH, DCC, DMAP; viii, CF_3CO_2H.

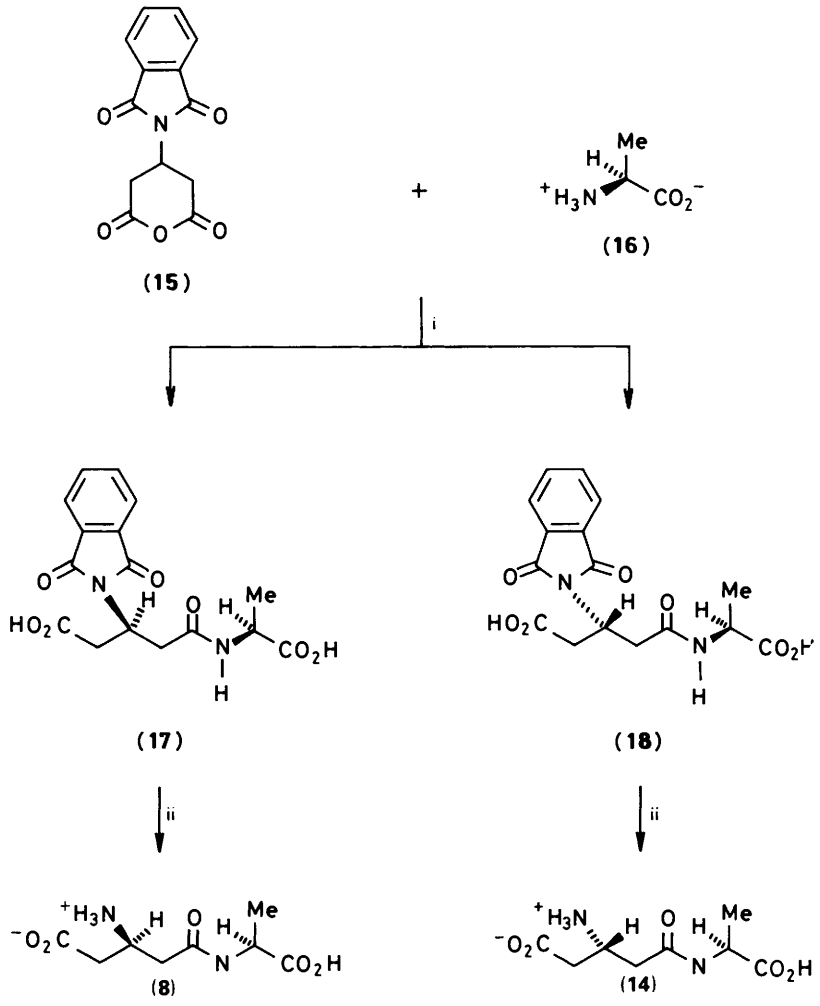
diastereoisomers are different but very similar and as such not suitable as a means of easily distinguishing between the two compounds (**8**) and (**14**) without the aid of reference spectra for comparison.

An alternative synthesis of the dipeptides (**8**) and (**14**) is that shown in Scheme 2. This route is analogous to that used by King and Kidd[11] for the synthesis of γ-glutamyl-amino acid dipeptides. In this work the desired peptides were obtained by the reaction of the free amino acid with 3-phthalimidoglutaric anhydride (**15**), followed by deprotection. This synthesis is non-specific as coupling of the anhydride (**15**) and (*S*)-alanine (**16**) produces both possible diastereoisomers of the dipeptide, (**17**) and (**18**), owing to the creation of the new chiral centre at C-3 of the 3-aminoglutaryl residue. This route apparently follows a method used by Griffith and Meister for the synthesis of the dipeptide mixture, *ambo*-3-aminoglutaryl-L-α-aminobutyric acid, although these workers have not provided specific details of their synthesis.[3]

The two diastereoisomers of 3-phthalimidoglutaryl-(*S*)-alanine, (**17**) and (**18**), were obtained by heating the anhydride (**15**) and (*S*)-alanine (**16**) in glacial acetic acid for 24 hours. The two diastereoisomers were produced in a ratio of 3:2 as revealed by reverse-phase analytical HPLC. The diastereoisomers were separated by reverse-phase preparative HPLC using a methanol–water–acetic acid solvent system. The major diastereoisomer, compound (**17**) [as shown by subsequent conversion to dipeptide (**8**)], was obtained pure in 46% yield, and the minor, less polar, diastereoisomer (**18**), was obtained pure in 32% yield. Once separated by HPLC, the diastereoisomers were individually deprotected.

(3*S*)-3-Phthalimidoglutaryl-(*S*)-alanine (**17**) was treated with sodium borohydride in aqueous propan-2-ol followed by treatment with acetic acid.[12] The deprotected peptide thus obtained was purified by ion-exchange chromatography and reverse-phase HPLC; this gave in good yield (3*S*)-3-aminoglutaryl-(*S*)-alanine (**8**). Deprotection of (**18**) in a similar fashion gave (3*R*)-3-aminoglutaryl-(*S*)-alanine (**14**), also in good yield. The absolute configurations of the peptides prepared by the non-specific method were assigned on the basis of comparison with the peptides of known configuration obtained by the specific synthesis.

To test the hypothesis that the free α-carboxy group on the second amino acid residue is important for binding, we wished to carry out inhibition studies using a peptide with the modified α-carboxy group. Our intention was to synthesize methyl 3-aminoglutaryl-(*S*)-alaninate to fulfill this aim. Synthesis of



Scheme 2. *Reagents and conditions*: i, Heat in AcOH; ii, $NaBH_4$, propan-2-ol, H_2O, then AcOH.

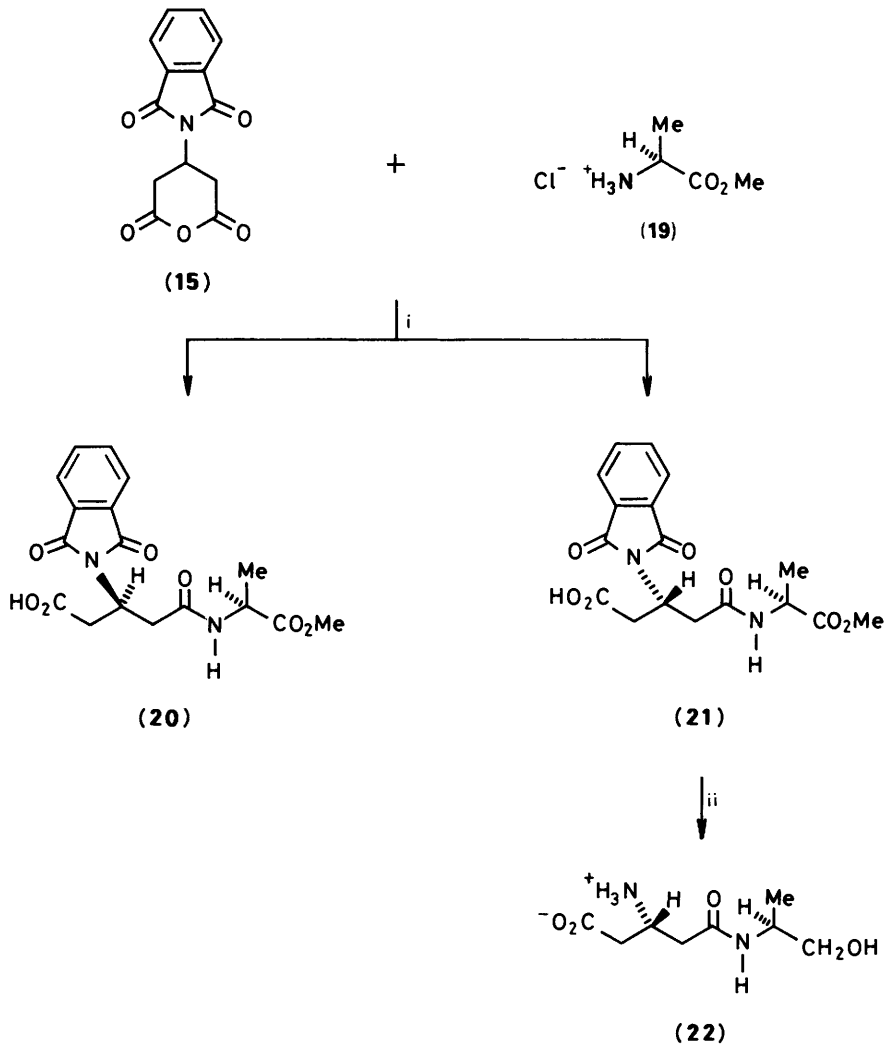
this peptide could be achieved by coupling of the anhydride (**15**) to methyl (*S*)-alaninate followed by removal of the phthaloyl protecting group. As outlined in Scheme 3, the protected peptides (**20**) and (**21**) were obtained by the reaction of the anhydride (**15**) with methyl (*S*)-alaninate hydrochloride (**19**) in the presence of triethylamine in 1,2-dimethoxyethane. The two diastereoisomers were produced in a ratio of 3:2 and separation was achieved by reverse-phase HPLC. The major diastereoisomer (**21**) obtained in 43% yield (retention time, t_R 24.2 min) was deprotected using sodium borohydride in aqueous propan-2-ol followed by treatment with acetic acid. Purification was achieved by ion-exchange chromatography and reverse-phase HPLC to yield (3*R*)-3-aminoglutaryl-(*S*)-alaninol (**22**) (77%) as a very hygroscopic solid. The ^1H NMR spectrum of the product indicated that during deprotection the methoxycarbonyl group had also been reduced, to a primary alcohol, as evidenced by the lack of appropriate proton resonances for the methyl ester. The appearance of an extra methylene group at δ 3.47–3.64 was apparent and consistent with the chemical shift of methylene protons directly attached to a hydroxy group. Each methylene proton appeared as a doublet of doublets with a small vicinal coupling to the adjacent methine protons. Usually esters are reduced only slowly by sodium borohydride. However, peptide esters have been known to undergo sodium borohydride reduction to the corresponding alcohols rapidly at room temperature.[13] Reduction of the ester has been attributed to the neighbouring complex-forming functional groups.[13]

In order to assign the stereochemistry of the alcohol (**22**), the half-ester (**21**) was converted to the free acid peptide by acid hydrolysis. The product of this reaction was found to be (3*R*)-3-phthalimidoglutaryl-(*S*)-alanine (**18**). The absolute configuration of the alcohol (**22**) is therefore (3*R*)-3-aminoglutaryl-(*S*)-alaninol.

The ability of the peptides (3*S*)-3-aminoglutaryl-(*S*)-alanine (**8**), (3*R*)-3-aminoglutaryl-(*S*)-alanine (**14**), and (3*R*)-3-aminoglutaryl-(*S*)-alaninol (**22**) to inhibit the enzyme γ-glutamyl-amino acid cyclotransferase has been studied using ^1H NMR spin-echo spectroscopy. The rate of GCT-catalysed cleavage of γ-glutamyl-(*S*)-alanine in erythrocyte lysate suspensions was monitored in the absence and presence of the synthetic peptides. Both diastereoisomers of 3-aminoglutaryl-(*S*)-alanine, (**8**) and (**14**), were found to be inhibitors of GCT although (3*R*)-3-aminoglutaryl-(*S*)-alanine (**14**) was found to be twice as effective an inhibitor as the other diastereoisomer (**8**). (3*R*)-3-Aminoglutaryl-(*S*)-alaninol (**22**) was found to be a very poor inhibitor of the enzyme, supporting the contention that a free α-carboxy group on the second amino acid residue of the peptide substrates is important for binding to the enzyme. Full details and discussion of the results of the biochemical studies will be reported elsewhere.[14]

Experimental

All evaporations were carried out under reduced pressure below 40 °C; aqueous solutions were lyophilized to dryness.



Scheme 3. *Reagents and conditions*: i, Et_3N, heat in 1,2-dimethoxyethane; ii, $NaBH_4$, propan-2-ol, H_2O, then AcOH.

M.p.s were recorded on a Reichert hot-stage microscope and are uncorrected. ^1H NMR spectra were recorded on a Varian EM-390 (90 MHz), Varian XL-100 (100 MHz), or Bruker WM-400 (400 MHz) instrument for solutions in $CDCl_3$ with tetramethylsilane as internal standard unless noted otherwise; the following abbreviations are used in the assignment of resonances: Ala, alanyl residue; Agl, 3-aminoglutaryl residue; AlaOH, alaninol residue. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Fourier-transform infrared (FT IR) spectra were recorded on a Digilab Biorad 150-80 spectrometer. Mass spectra were recorded on a Finnigan TSQ-46 quadrupole mass spectrometer. All HPLC was carried out on Waters Associates equipment. Elemental analyses were performed by the Australian Microanalytical Service, Melbourne. Porcine liver esterase was obtained from the Sigma Chemical Company, St Louis, Missouri, U.S.A.

Dimethyl 3-Aminoglutarate (**2**).—A solution of dimethyl 3-oxoglutarate (26.1 g, 150 mmol) and ammonium acetate (120 g, 1.56 mol) in dry methanol (400 ml) was stirred over molecular sieves (size 3 Å; 40 g) for 2 days. The mixture was acidified to pH 3 by the addition of methanolic HCl (5M). Sodium cyanoborohydride (11.8 g, 188 mmol) was added, and the mixture reacidified to pH 3 and then stirred for 1 h at room temperature. The mixture was filtered through Celite and the methanol removed. The residual oil was basified to pH 9, with cooling, by addition of sodium hydroxide (10M); water was then added until the solution was homogeneous. The aqueous layer was extracted with dichloromethane (3 × 200 ml). The combined organic extracts were washed with saturated NaCl (2 × 100 ml) and dried (Na_2SO_4), and the solvent removed to give the crude product (**2**) as an oil (22.9 g, 87%) which was distilled under reduced pressure yielding dimethyl 3-aminoglutarate (**2**) as a colourless oil (12.6 g, 58%), b.p. 67–70 °C at 0.05 mmHg (lit.,[8] 81 °C at 0.2 mmHg); δ_H(90 MHz) 1.77 (2 H, br s, NH_2), 2.46 (4 H, d, *J* 6 Hz, CH_2), 3.37 (1 H, m, CH), and 3.60 (6 H, s, OMe).

Dimethyl 3-Benzyloxycarbonylaminoglutarate (**3**).—To dimethyl 3-aminoglutarate (**2**) (3.5 g, 20 mmol) dissolved in aqueous $NaHCO_3$ (1M; 45 ml) was added a solution of benzyl chloroformate (50% w/v in toluene) (6.8 ml, 20 mmol) at 0 °C. The mixture was stirred for 3 h at room temperature. The mixture was extracted with ether (3 × 50 ml). The combined ether extracts were washed with HCl (3M; 3 × 50 ml), saturated $NaHCO_3$ (2 × 25 ml), and brine (2 × 25 ml), and dried (Na_2SO_4), and the solvent removed to give an oil. Chromatography of the oil on silica gel and elution with 30% ethyl acetate–light petroleum gave dimethyl 3-benzyloxycarbonylaminoglutarate (**3**) as a colourless oil (5.2 g, 84%); ν_{max}(film) 3 362 (NH), 1 738 (CO), and 1 702 cm^{-1} (CO); δ_H(90 MHz) 2.67 (4 H, d, *J* 6 Hz, CH_2), 3.63 (6 H, s, OMe), 4.33 (1 H,

m, CH), 5.03 (2 H, s, CH_2Ar), 5.53 (1 H, br d, NH), and 7.23 (5 H, s, ArH).

Methyl Hydrogen (3S)-3-*Benzyloxycarbonylaminoglutarate* (**4**)—To a mixture of (**3**) (460 mg, 1.49 mmol) in phosphate buffer (0.5M; pH 8.0) (45 ml) and acetone (1.5 ml) was added pig liver esterase (PLE; 600 units). The mixture was stirred at 25 °C for 7 h. The pH of the mixture was checked periodically and NaOH (0.1M) was added by drops, if required, to maintain the pH at 8.0. The resultant solution was acidified to pH 2.0 by the addition of concentrated HCl and extracted with dichloromethane (4 × 100 ml). The combined extracts were washed with saturated NaCl (100 ml) and dried (Na_2SO_4), and the solvent removed to give the crude half-ester (**4**) (426 mg, 97%) as a white solid. Recrystallization from chloroform–light petroleum gave methyl hydrogen (3*S*)-3-benzyloxycarbonylaminoglutarate (**4**) as fine, colourless needles (360 mg, 82%), m.p. 96–98 °C (lit.,[10] 97.0–97.5 °C); $[\alpha]_D^{25}$ + 0.71° (*c* 6.12 in $CHCl_3$) {lit.,[10] $[\alpha]_D^{25}$ + 0.72° (*c* 7.5 in $CHCl_3$)}; ν_{max}($CHCl_3$) 3 431 (NH), 3 030 (OH, br), 1 728 (CO), 1 725 (CO), and 1 722 cm^{-1} (CO); δ_H(90 MHz), 2.67 (4 H, d, *J* 6 Hz, CH_2), 3.58 (3 H, s, OMe), 4.28 (1 H, m, CH), 5.02 (2 H, s, CH_2Ar), 5.73 (1 H, br d, NH), 7.27 (5 H, s, ArH), and 8.50 (1 H, br s, CO_2H).

(3S)-3-*Benzyloxycarbonylaminoglutaryl*-(S)-*alanine Dimethyl Ester* (**6**).—Methyl (*S*)-alaninate hydrochloride (2.0 g, 14 mmol) in water was treated with K_2CO_3 until the solution was basic. The solution was saturated with NaCl and extracted with dichloromethane (3 × 10 ml). The combined extracts were dried (Na_2SO_4) and evaporated to yield methyl (*S*)-alaninate (**5**) as an oil. To a solution of methyl hydrogen (3*S*)-3-benzyloxycarbonylaminoglutarate (147 mg, 0.5 mmol) in tetrahydrofuran (2 ml) was added IIDQ (146 mg, 0.5 mmol) and methyl (*S*)-alaninate (116 mg, 1.1 mmol). The solution was stirred for 18 h at room temperature and heated under reflux for 2 h. The solvent was removed and the residue dissolved in ethyl acetate (25 ml). The organic layer was washed with HCl (3M; 2 × 10 ml), saturated $NaHCO_3$ (2 × 10 ml) and brine (2 × 10 ml), and dried (Na_2SO_4), and the solvent removed to yield the crude product (**16**) quantitatively. Recrystallization from ethyl acetate–light petroleum gave (3S)-3-*benzyloxycarbonylaminoglutaryl*-(S)-*alanine dimethyl ester* (**6**) (139 mg, 80%), as fine needles, m.p. 145–146 °C; $[\alpha]_D^{24}$ + 3.08° (*c* 3.20 in $CHCl_3$); ν_{max}($CHCl_3$) 3 428 (NH), 1 734 (CO), 1 505 cm^{-1} (amide); δ_H (400 MHz) 1.38 (3 H, d, *J* 7.0 Hz, Ala 3-H), 2.54–2.84 (4 H, m, Agl 2- and 4-H), 3.68 (3 H, s, OMe), 3.78 (3 H, s, OMe), 4.35 (1 H, m, Agl 3-H), 4.58 (1 H, dq, *J* 7.0, 8.0 Hz, Ala 2-H), 5.12 (2 H, s, CH_2Ar), 5.93 (1 H, br d, *J* 7.0 Hz, NH), 6.35 (1 H, br d, *J* 6.0 Hz, NH), and 7.36 (5 H, br s, ArH); *m/z* 380 (M^+, 7%), 142 (7), 107 (7), 91 (100), and 44 (24) (Found: C, 57.0; H, 6.3; N, 7.7. $C_{18}H_{24}N_2O_7$ requires C, 56.8; H, 6.4; N, 7.4%).

(3S)-3-*Benzyloxycarbonylaminoglutaryl*-(S)-*alanine* (**7**).—Sodium hydroxide (1M; 1.6 ml) was added to a stirred solution of compound (**6**) (263 mg, 0.69 mmol) in methanol (5 ml), and stirring was continued for 1.5 h at 0 °C. The reaction mixture was diluted with water (10 ml) and then extracted with chloroform (10 ml). The aqueous layer was acidified to pH 2 with concentrated HCl and extracted with ethyl acetate (3 × 20 ml). The combined extracts were washed with saturated NaCl (10 ml) and dried (Na_2SO_4), and the solvent removed to yield the crude product quantitatively. The colourless solid was recrystallized from ethanol–ether to give (3S)-3-*benzyloxycarbonylaminoglutaryl*-(S)-*alanine* (**7**), as fine colourless needles (200 mg, 82%), m.p. 146–147 °C; $[\alpha]_D^{20}$ −12.9° (*c* 1.43 in MeOH); ν_{max}(KBr) 3 700–2 770 br (OH), 1 695 (CO), 1 646 (amide), and 1 536 cm^{-1} (amide); δ_H(400 MHz, $CDCl_3/CH_3OD$, $SiMe_4$) 1.39 (3 H, d, *J* 7.0 Hz, Ala 3-H), 2.49–2.73 (4 H, m, Agl 2- and 4-H), 4.25–4.34 (1 H, m, Agl 3-H), 4.44 (1 H, q, *J* 7.0 Hz, Ala 2-H), 5.06 (2 H, s, CH_2Ar), and 7.28–7.38 (5 H, m, ArH) (Found: C, 54.4; H, 5.8; N, 7.6. $C_{16}H_{20}N_2O_7$ requires C, 54.5; H, 5.7; N, 7.95%).

(3S)-3-*Aminoglutaryl*-(S)-*alanine* (**8**).—A solution of (3*S*)-3-benzyloxycarbonylaminoglutaryl-(*S*)-alanine (**7**) (31 mg, 0.09 mmol) in methanol (1.5 ml) was hydrogenated over 10% Pd–C, at 20 °C and atmospheric pressure, for 2 h. The catalyst was removed by filtration and the filtrate was evaporated to dryness to leave a white solid which was further purified by reverse-phase preparative HPLC (column: Whatman Partisil M 20 10/50 ODS-3; solvent: 0.1% formic acid in water; flow rate: 10 ml/min). This gave S-3-*aminoglutaryl*-(S)-*alanine* (**8**) as a white hygroscopic solid (9.3 mg, 47%), m.p. 217–220 °C (decomp.); $[\alpha]_D^{20}$ −28.5° (*c* 0.64 in H_2O); ν_{max}(KBr) 3 600–2 300 (NH_3^+, OH), 1 654, 1 643, 1 617 (CO), 1 550, and 1 546 cm^{-1} (NH_3^+); δ_H(400 MHz, D_2O, sodium 3-trimethylsilyltetradeuteriopropionate, TSP) 1.43 (3 H, d, *J* 7.0 Hz, Ala 3-H), 2.72–2.88 (4 H, m, Agl 2- and 4-H), 3.99 (1 H, m, Agl 3-H), and 4.34 (1 H, q, *J* 7.0 Hz, Ala 2-H) (Found: C, 43.6; H, 6.3; N, 12.5. $C_8H_{14}N_2O_5$ requires C, 44.0; H, 6.5; N, 12.8%).

Analytical HPLC (Dupont Zorbax ODS column, solvent: 0.1% formic acid in water; flow rate: 0.8 ml/min: t_R 5.9 min) (Dupont Zorbax ODS, 0.1% trifluoroacetic acid in water, 0.8 ml/min: t_R 3.6 min).

Methyl t-Butyl (3R)-3-*Benzyloxycarbonylaminoglutarate* (**9**).—To a solution of the half-ester (**4**) (629 mg, 2.13 mmol) in dry dichloromethane (2.5 ml) was added 4-dimethylaminopyridine (DMAP) (25 mg, 0.2 mmol) and t-butyl alcohol (1 ml). Dicyclohexylcarbodiimide (DCC) (456 mg, 2.2 mmol) was added to the stirred solution at 0 °C and the mixture stirred at 0 °C for 5 min and at room temperature for 3 h. The mixture was filtered and the solvent removed. The residue was taken up in ethyl acetate (50 ml), refiltered, washed with HCl (1M; 25 ml), saturated $NaHCO_3$ (25 ml), and saturated NaCl (25 ml), and dried (Na_2SO_4). The solvent was removed to give an oil. This oil was purified by chromatography on silica gel; elution with dichloromethane gave methyl t-butyl (3*R*)-3-benzyloxycarbonylaminoglutarate (**9**) as a colourless oil (622 mg, 83%); $[\alpha]_D^{19}$ −1.1° (*c* 3.5 in $CHCl_3$) {lit.,[10] $[\alpha]_D^{20}$ −1.15° (*c* 3.40 in $CHCl_3$)}; ν_{max}($CHCl_3$) 3 431 (NH), 1 730 (CO), and 1 726 cm^{-1} (CO); δ_H(90 MHz), 1.40 (9 H, s, OBu^t), 2.37 (2 H, d, *J* 6 Hz, CH_2), 2.63 (2 H, d, *J* 6 Hz, CH_2), 3.63 (3 H, s, OMe), 4.13–4.43 (1 H, br m, CH), 5.03 (2 H, s, CH_2Ar), 5.33–5.60 (1 H, br d, NH), and 7.25 (5 H, s, ArH).

t-Butyl Hydrogen (3R)-3-*Benzyloxycarbonylaminoglutarate* (**10**).—To a stirred solution of the diester (**9**) (105 mg, 0.3 mmol) in methanol (1.0 ml) was added sodium hydroxide (1M; 0.35 mmol). The solution was stirred for 2.5 h at room temperature. The reaction mixture was diluted with water (20 ml) and extracted with dichloromethane (2 × 10 ml). The aqueous layer was acidified to pH 2 by addition of concentrated HCl and then extracted with dichloromethane (4 × 20 ml). The combined extracts were washed with saturated NaCl (10 ml) and dried (Na_2SO_4), and the solvent removed to yield the half ester (**10**) as a colourless gum (82.3 mg, 81%); $[\alpha]_D^{24}$ −1.1° (*c* 2.4 in $CHCl_3$) {lit.,[10] $[\alpha]_D^{20}$ −1.4° (*c* 2.6 in $CHCl_3$)}; ν_{max}($CHCl_3$) 3 431 (NH), 3 050 (OH, br), and 1 721 cm^{-1} (CO); δ_H(90 MHz) 1.40 (9 H, s, OBu^t), 2.55 (2 H, d, *J* 6 Hz, CH_2), 2.65 (2 H, d, *J* 6 Hz, CH_2), 4.28 (1 H, m, CH), 5.03 (2 H, s, CH_2), 5.70 (1 H, br d, NH), and 7.23 (5 H, s, ArH).

(3R)-3-*Benzyloxycarbonylaminoglutaryl*-(S)-*alanine Di-t-butyl Ester* (**12**).—Coupling of (**10**) and (**11**) with the aid of IIDQ, as for the preparation of (**7**), gave the product as an oil

(80%), which was then chromatographed on silica gel with 40% ethyl acetate–light petroleum as eluant. Evaporation of the major fraction gave the crude product as a solid which was recrystallized from ethyl acetate-light petroleum to give (3R)-3-*benzyloxycarbonylaminoglutaryl*-(S)-*alanine di-t-butyl ester* (**12**) as white crystals (67%), m.p. 93–94 °C; $[\alpha]_D^{18}$ −21.1° (*c* 2.35 in MeOH); ν_{max}($CHCl_3$) 3 429 (NH), 3 424 (NH), 3 420 (NH), 1 723 (CO), and 1 504 cm^{-1} (amide); δ_H(400 MHz) 1.34 (3 H, d, *J* 7.0 Hz, Ala 3-H), 1.41 (9 H, s, OBu^t), 1.46 (9 H, s, OBu^t), 2.45–2.69 (4 H, m, Agl 2- and 4-H), 4.28 (1 H, dq, *J* 6.5, 7.0 Hz, Agl 1-H), 4.40 (1 H, m, Ala 2-H), 5.09 (2 H, s, CH_2Ar), 5.88 (1 H, br d, *J* 8.0 Hz, Agl NH), 6.40 (1 H, br d, *J* 6.0 Hz, Ala NH), and 7.25–7.35 (5 H, m, ArH); *m/z* 264 (19%), 108 (18), 107 (15), 91 (100), 79 (25), 77 (16), 57 (48), 56 (26), 55 (11), and 51 (11) (Found: C, 61.8; H, 7.65; N, 5.85. $C_{24}H_{36}N_2O_7$ requires C, 62.05; H, 7.8; N, 6.0%).

(3R)-3-*Benzyloxycarbonylaminoglutaryl*-(S)-*alanine* (**13**).—To the protected dipeptide (**12**) (69 mg, 0.15 mmol) dissolved in dichloromethane (1 ml) was added trifluoroacetic acid (0.3 ml). The reaction mixture was stirred at room temperature for 1.5 h. The solvent was removed to afford a quantitative yield of the crude product (**13**) as a white solid. Reverse-phase preparative HPLC [Whatman Partisil M20 10/50 ODS-3, methanol–water–acetic acid (27:73:1), 10 ml/min] of the solid gave (3R)-3-*benzyloxycarbonylaminoglutaryl*-(S)-*alanine* (**13**) as a white solid (40.3 mg, 76%), m.p. 171–173 °C; $[\alpha]_D^{20}$ −11.3° (*c* 1.82 in MeOH); ν_{max}(KBr) 3 600–2 700 (OH), 1 695 (CO), 1 560 (amide), and 1 532 cm^{-1} (amide); δ_H(400 MHz, $CDCl_3/CH_3OD$, $SiMe_4$) 1.39 (3 H, d, *J* 7.2 Hz, Ala 3-H), 2.48–2.73 (4 H, m, Agl 2- and 3-H), 4.28 (1 H, m, Agl 3-H), 4.68 (1 H, q, *J* 7.2 Hz, Ala 1-H), 5.09 (2 H, s, CH_2Ar), 6.31 (1 H, d, *J* 7.5 Hz, NH), 7.26–7.38 (5 H, m, ArH), and 7.45 (1 H, d, *J* 6.0 Hz, NH); *m/z* 352 (M^+, 3%), 108 (22), and 107 (16) (Found: C, 54.1; H, 5.6; N, 8.1. $C_{16}H_{20}N_2O_7$ requires C, 54.5; H, 5.7; N, 7.95%).

(3R)-3-*Aminoglutaryl*-(S)-*alanine* (**14**).—Hydrogenation of (**13**) (60 mg, 0.17 mmol) and purification of the product, as performed for the preparation of (**8**), gave (3R)-3-*aminoglutaryl*-(S)-*alanine* (**14**) as a white hygroscopic solid (11 mg, 30%), m.p. 232–234 °C (decomp.); ν_{max}(KBr) 3 623–2 362 (NH_3^+, br), 1 658, 1 643 (CO_2^-) 1 562, and 1 551 cm^{-1} (NH_3^+); δ_H(400 MHz, D_2O, TSP) 1.34 (3 H, d, *J* 7.5 Hz, Ala 3-H), 2.56 (1 H, dd, *J* 7.5, 17 Hz, Agl 2-H), 2.63 (1 H, dd, *J* 7.5, 17 Hz, Agl 2-H), 2.70 (2 H, m, Agl 4-H), 3.78 (1 H, m, Agl 3-H), and 4.17 (1 H, d, *J* 7.5 Hz, Ala 1-H) (Found: C, 44.3; H, 6.2; N, 12.9. $C_8H_{14}N_2O_5$ requires C, 44.0; H, 6.5; N, 12.8%).

Analytical HPLC (Dupont Zorbax ODS column, solvent: 0.1% formic acid in water; flow rate: 0.8 ml/min: t_R 6.6 min) (Dupont Zorbax ODS, 0.1% trifluoroacetic acid in water, 0.8 ml/min: t_R 4.2 min).

(3S)-3-*Phthalimidoglutaryl*-(S)-*alanine* (**17**) *and* (3R)-3-*Phthalimidoglutaryl*-(S)-*alanine* (**18**).—A suspension of 3-phthalimidoglutaric anhydride[15] (**15**) (200 mg, 0.772 mmol) in glacial acetic acid (4 ml) was treated with (*S*)-alanine (**16**) (138 mg, 1.55 mmol) and heated at 90 °C under nitrogen for 24 h. The reaction mixture was then evaporated to dryness to give the crude peptide. Analytical HPLC [Whatman Partisil 5 ODS-2, methanol–water–formic acid (30:70:0.1), 0.8 ml/min] revealed two products; the major component of t_R 12.8 min and the minor component of t_R 17.2 min were subsequently identified as the two diastereoisomers of 3-phthalimidoglutaryl-(*S*)-alanine. Separation of the diastereoisomers by preparative HPLC [Whatman Partisil M20 10/50 ODS-3, methanol–water–acetic acid (27:73:1), 10 ml/min] gave the major diastereoisomer (3*R*)-3-phthalimidoglutaryl-(*S*)-alanine (**18**) (124 mg, 46%) as prisms m.p. 196–198 °C; $[\alpha]_D^{24}$ +21.5° (*c* 1.0 in MeOH); δ_H(100 MHz, CH_3OD, $SiMe_4$) 1.33 (3 H, d, *J* 7.4 Hz, Ala 3-H), 2.64–3.25 (4 H, m, Agl 2- and 4-H), 4.29 (1 H, q, *J* 7.4 Hz, Ala 2-H), 4.96–5.28 (1 H, m, Agl 3-H), and 7.81 (4 H, m, ArH); *m/z* 349 (*M* + 1, 100%), 331 (10), and 260 (11); and the minor (3*S*)-diastereoisomer (**17**) (85 mg, 32%) as prisms, m.p. 185–187 °C; $[\alpha]_D^{24}$ −26.5° (*c* 1.0 in pyridine); δ_H(100 MHz, CH_3OD, $SiMe_4$) 1.21 (3 H, d, *J* 7.4 Hz, Ala 3-H), 2.64–3.26 (4 H, m, Agl 2- and 4-H), 4.26 (1 H, q, *J* 7.4 Hz, Ala 2-H), 4.96–5.26 (1 H, m, Agl 3-H), and 7.80 (4 H, m, ArH); *m/z* 349 (*M* + 1, 100%), 331 (10), and 260 (14).

Alternative Synthesis of (3S)-3-*Aminoglutaryl*-(S)-*alanine* (**8**).—To a stirred solution of diastereoisomer (**17**) (155 mg, 0.45 mmol) in propan-2-ol (4.7 ml) and water (1.0 ml) was added sodium borohydride (190 mg, 5.0 mmol). The reaction mixture was stirred for 24 h. Glacial acetic acid (1.0 ml) was added until no more foaming was observed and then the flask was stoppered and the mixture heated at 80 °C for 2 h. The crude reaction mixture was partially purified by ion-exchange chromatography [Dowex 50 (H^+ form) column (2 × 6 cm)]. The column was washed with deionized water (100 ml) and then eluted with ammonium hydroxide (1M; 100 ml). Ninhydrin-active fractions were pooled, neutralized with glacial acetic acid, and lyophilized to yield the crude free acid peptide. Reverse-phase preparative HPLC (Whatman Partisil M20 10/50 ODS-3, 0.1% formic acid in water, 10 ml/min) gave (3*S*)-3-aminoglutaryl-(*S*)-alanine (**8**) as a white, hygroscopic solid (44.9 mg, 46%). m.p. 221–222 °C (decomp.), mixed m.p., with compound (**8**) from the specific route above, 216–219 °C (decomp.); $[\alpha]_D^{19}$ −31.6° (*c* 3.06 in H_2O); ^1H NMR spectrum identical with that of compound (**8**) prepared by the specific route above.

Analytical HPLC (Dupont Zorbax ODS column, solvent: 0.1% formic acid in water; flow rate: 0.8 ml/min: t_R 5.9 min). The compound co-chromatographed with compound (**8**), prepared by the specific route, on co-injection to the column; it chromatographed differently from compound (**14**), prepared by the specific route, on co-injection.

Alternative Synthesis of (3R)-3-*Aminoglutaryl*-(S)-*alanine* (**14**).—The above procedure was repeated with (3*R*)-3-phthaloylaminoglutaryl-(*S*)-alanine (**18**) (142 mg, 0.41 mmol) to give (3*R*)-3-aminoglutaryl-(*S*)-alanine (**14**) as a white hygroscopic solid (28 mg, 31%), m.p. 232–236 °C (decomp.), mixed m.p., with compound (**14**) from the specific route above, 229–234 °C (decomp.); $[\alpha]_D^{19}$ −21.7° (*c* 1.0 in H_2O); ^1H NMR spectrum identical with that of compound (**14**) prepared by the other route.

Analytical HPLC (Dupont Zorbax ODS column, solvent: 0.1% formic acid in water; flow rate: 0.8 ml/min: t_R 6.6 min). The compound co-chromatographed with compound (**14**), prepared by the specific route, on co-injection to the column; it chromatographed differently from compound (**8**), prepared by the specific route, on co-injection.

Methyl (3S)-3-*Phthalimidoglutaryl*-(S)-*alaninate* (**20**) *and Methyl* (3R)-3-*Phthalimidoglutaryl*-(S)-*alaninate* (**21**).—Methyl (*S*)-alaninate hydrochloride (**19**) (1.0 g, 8.0 mmol) and triethylamine (1.1 ml, 8.0 mmol) were added to a stirred suspension of 3-phthalimidoglutaric anhydride (**15**) (1.0 g, 4.0 mmol) in 1,2-dimethoxyethane (70 ml). The reaction mixture was heated at 100 °C under nitrogen for 2 h then concentrated under reduced pressure to yield the crude peptide mixture. Analytical HPLC [Whatman Partisil 5 ODS-3, methanol–water–formic acid (30:70:0.1), 0.8 ml/min] revealed two products which were subsequently identified as the (3*R*)-ester (**21**), the major component (t_R 24.2 min), and the minor component the (3*S*)-ester (**20**) (t_R 31.2 min). Separation of the dipeptides by reverse-phase HPLC [Whatman Partisil M20

10/50 ODS-3, methanol–water–acetic acid (26 : 74 : 1), 10 ml/min] gave the major diastereoisomer (**21**) (618.4 mg, 43%), as a white solid, m.p. 182–183 °C; $[\alpha]_D^{24}$ −1.1° (*c* 1.0 in MeOH); δ_H(400 MHz, CH_3OD, $SiMe_4$) 1.83 (3 H, d, *J* 7.3 Hz, Ala 3-H) [3.28 (1 H, dd, *J* 14.3, 5.3 Hz), 3.36 (1 H, dd, *J* 15.6, 5.5 Hz), 3.51 (1 H, dd, *J* 14.3, 10.1 Hz), 3.60 (1 H, dd, *J* 15.6, 8.9 Hz) Agl 2- and 4-H], 4.02 (3 H, s, OMe), 4.81 (1 H, q, *J* 7.3 Hz, Ala 2-H), 5.53–5.62 (1 H, m, Agl 3-H), and 8.30–8.37 (4 H, m, ArH); δ_C(100 MHz, CH_3OD, $SiMe_4$) 16.5 (q), 38.5 (br t), 46.3 (d), 48.7 (d), 51.8 (q), 123.4 (d), 132.6 (s), 134.6 (d), 168.8 (s), 171.4 (s), 173.7 (s), and 174.0 (br s); and the minor diastereoisomer (**20**) was obtained as a colourless glass (286.1 mg, 20%); $[\alpha]_D^{24}$ −47.8° (*c* 1.0 in MeOH); δ_H(400 MHz, CH_3OD, $SiMe_4$) 1.78 (3 H, d, *J* 7.3 Hz, Ala 3-H) [3.35 (1 H, dd, *J* 14.0, 5.8 Hz), 3.37 (1 H, dd, *J* 14.8, 4.3 Hz), 3.49 (1 H, dd, *J* 14.3, 8.8 Hz), 3.63 (1 H, dd, *J* 15.3, 8.8 Hz), Agl 2- and 4-H], 4.18 (3 H, s, OMe), 4.81 (1 H, q, *J* 7.2 Hz, Ala 2-H), 5.55–5.62 (1 H, m, Agl 3-H), and 8.30–8.37 (4 H, m, ArH); δ_C(100 MHz, CH_3OD) 16.5 (q), 38.5 (br t), 46.2 (d), 48.5 (d), 52.09 (q), 123.4 (d), 132.55 (s), 134.7 (d), 168.7 (s), 171.5 (s), and 173.7 (s, br s).

(3R)-3-*Aminoglutaryl*-(S)-*alaninol* (**22**).—To a stirred solution of methyl (3*R*)-3-phthalimidoglutaryl-(*S*)-alaninate (**21**) (102.4 mg, 0.3 mmol) in propan-2-ol (2.6 ml) and water (0.5 ml) was added sodium borohydride (168 mg, 4.4 mmol) and stirring was continued for 24 h. After 24 h glacial acetic acid (3.5 ml) was added and the mixture heated at 80 °C for 2 h. The reaction mixture was then chromatographed by ion-exchange chromatography as for (**8**). Analytical HPLC (Dupont Zorbax ODS, 0.1% trifluoroacetic acid in water, 0.8 ml/min) revealed that the product with t_R 4.5 min required further purification. This was carried out by reverse-phase preparative HPLC [conditions as for (**8**)], which yielded (3R)-3-*aminoglutaryl*-(S)-*alaninol* (**22**) as a very hygroscopic white solid, (46.2 mg, 77%); $[\alpha]_D^{19}$ −5.3° (*c* 1.3 in H_2O); δ_H(400 MHz, D_2O, sodium 3-trimethylsilylpropanesulphonate, DSS) 1.14 (3 H, d, *J* 6.8 Hz, AlaOH 3-H), 2.69–2.85 (4 H, m, Agl 2- and 4-H), 3.50 (1 H, dd, *J* 6.6, 10.5 Hz, AlaOH 1-H), 3.62 (1 H, dd, *J* 4.0, 10.5 Hz, Ala 1-H), and 3.95–4.05 (2 H, m, AlaOH 2-H and Agl 3-H) (Found: C, 44.3; H, 7.9; N, 13.0. $C_8H_{16}N_2O_4 \cdot 0.75\ H_2O$ requires C, 44.1; H, 8.1; N, 12.9%).

Hydrolysis of Methyl (3R)-3-*Phthalimidoglutaryl*-(S)-*alaninate* (**21**).—To a solution of the major isomer (**21**) from the preceding experiment, (66 mg, 0.2 mmol) in acetone (1 ml) and water (0.5 ml) was added concentrated HCl (0.2 ml) and the solution was refluxed for 1 h. The solvent was removed to yield a white solid (30 mg, 53%), m.p. 196–198 °C. By analytical HPLC [Whatman Partisil 5 ODS-2, methanol–water–formic acid (30 : 70 : 0.1), 0.8 ml/min] the product co-chromatographed with compound (**18**), and had identical spectroscopic properties. The product is therefore (3*R*)-3-phthalimidoglutaryl-(*S*)-alanine.

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