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Synthesis of proline-modified analogues of the neuroprotective agent glycyl-L-prolyl-glutamic acid (GPE)

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Abstract—The synthesis of ten proline-modified analogues of the neuroprotective tripeptide GPE is described. Five of the analogues incorporate a proline residue with a hydrophobic group at C-2 and two further analogues have this side chain locked into a spirolactam ring system. The pyrrolidine ring was also modified by replacing the γ-CH_2 group with sulfur and/or incorporation of two methyl groups at C-5.

1. Introduction

The tripeptide Gly-Pro-Glu (GPE) is a naturally occurring peptide, which is proteolytically cleaved from insulin-like growth factor-1 (IGF-1).[1–7] IGF-1 is a potent neurotrophic factor[8,9] produced endogenously in damaged regions of the brain.[10] It has been postulated that some of the neuroprotective actions of IGF-1 are mediated by GPE[11] although the precise mechanism of action remains unclear. GPE has a different mode of action to IGF-1 as GPE does not bind to the IGF-1 receptor,[12,13] rather GPE has been shown to bind with low affinity to the *N*-methyl-D-aspartate (NMDA) receptor and also elicit a biological response via other mechanisms. GPE facilitates the release of dopamine through interaction with the NMDA receptor[16] but GPE stimulated acetyl choline release is via an unknown, non NMDA pathway.[14,16]

It has been demonstrated that GPE can act as a neuronal rescue agent following hypoxic-ischemic brain injury,[11,14] NMDA challenge[15] and in animal models of Parkinson's and Alzheimer's disease.[16,17] Analogues of GPE are thus of interest in the development of novel pharmaceutical agents for the treatment of central nervous system (CNS) injuries and neurodegenerative disorders.[18–21]

In our previous work, we have prepared GPE peptidomimetics modified at the glycine and glutamate residues in order to investigate structure–activity relationships and in an attempt to improve properties such as metabolic stability and oral bioavailability.[22–24] We herein, report the synthesis of analogues of GPE modified at the proline residue.

2. Results and discussion

In order to investigate the importance of the proline residue in GPE, ten analogues modified at either Pro or at the Pro-Glu bond were synthesized. In particular conformationally restricted analogues were prepared in order to gain insight into the receptor bound conformation. The general synthetic strategy employed involved the preparation of several modified proline residues that were then coupled to a glycine derivative and a glutamic acid di-ester (Scheme 1).

The presence of (*S*)-2-methylproline (2-MePro) is known to stabilize turns[25,26] and may also prevent peptidases recognizing the Pro-Glu amide bond resulting in resistance to proteolytic degradation.[27] Hence, the synthesis of glycyl-L-2-methylprolyl-L-glutamic acid (G-2MePE) **1** was undertaken. In order to further explore the influence of modifications at this position, four other 2-alkylproline analogues **2**–**5** were also synthesized (Scheme 2).

The 2-alkylproline derivatives were synthesized using Wang and Germanas's modification[28] of Seebach's method of self-reproducing chirality.[29,30] Condensation of L-proline **6** with choral (trichloroacetaldehyde) gave oxazolidinone **7**,[31] which was used for the synthesis of the five

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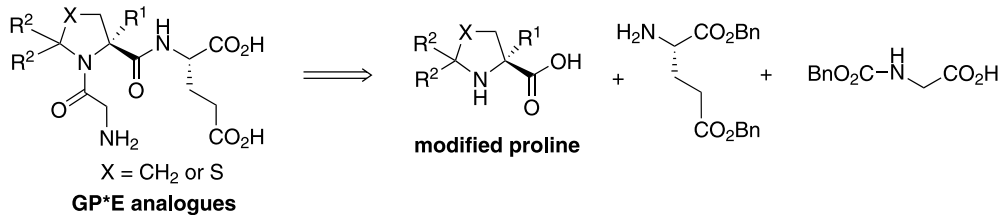
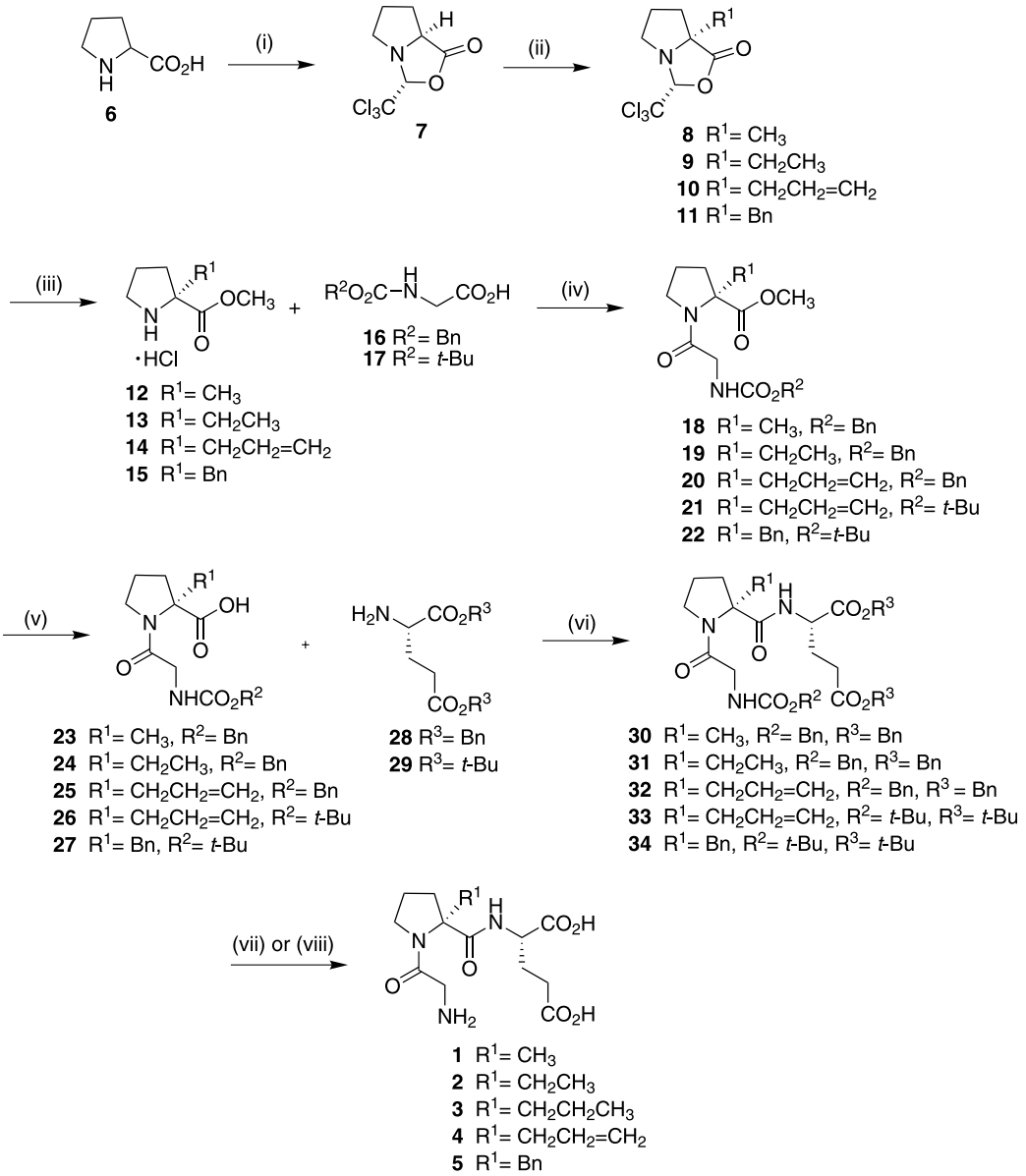
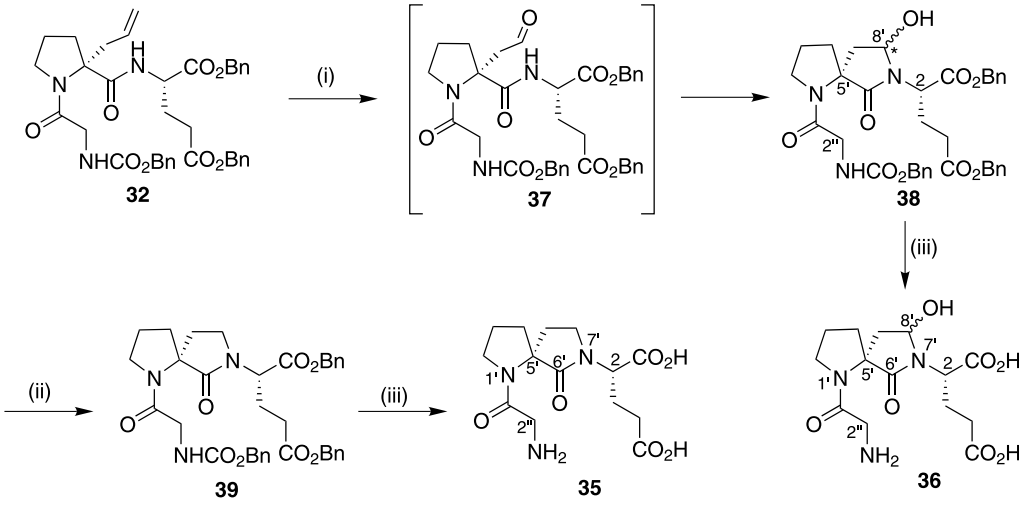


Scheme 1. General retrosynthesis for GP*E analogues.

2-alkylproline modified tripeptides **1**–**5**. Treatment of **7** with LDA to effect enolate formation followed by alkylation with iodomethane, iodoethane, allyl bromide or benzyl bromide, respectively, afforded alkylated oxazolidinones **8**–**11**. Esterification with thionyl chloride (for **8**,**9**) or acetyl chloride (for **10**,**11**) in methanol gave the methyl ester hydrochlorides **12**–**15**, which were coupled with *N*-benzyloxycarbonyl–glycine **16** (for **12**–**14**) or *N*-*tert*-butyloxycarbonyl-glycine **17** (for **14** and **15**) to give the dipeptides **18**–**22**.



Scheme 2. Reagents, conditions and yields: (i) chloral, $CHCl_3$, reflux, 6 h (77%); (ii) LDA, THF, −78 °C, MeI, EtI, $CH_3CH_2{=}CH_2Br$ or $PhCH_2Br$, −78–> −30 °C, 4 h, **8**, 63%, **9**, 46%, **10**, 60%, **11**, 2.5 h, 32%; (iii) $SOCl_2$, CH_3OH, reflux, 3 h, **12**, 100%, **13**, 71%, AcCl, CH_3OH, reflux, 24 h, **14**, 63%, **15**, 48%; (iv) for **18**, **19**, **20**: Et_3N, BoPCl, **16**, CH_2Cl_2, rt, **18**, 20.5 h, 92%, **19**, 19.5 h, 46%, **20**, 20 h, 30%; for **21**, **22**: Et_3N, BoPCl, **17**, CH_2Cl_2, rt, 19.5 h, **21**, 45%, **22**, 18.5 h, 22%; (v) dioxane, 1 M aqueous NaOH, rt, 15–20 h, **23**, 90%, **24**, 95%, **25**, 92%, **26**, 83%, **27**, 95%; (vi) for **30**, **31**, **32**: Et_3N, BoPCl, **28**, CH_2Cl_2, rt, 17 h, **30**, 89%, **31**, 17.5 h, 70%, **32**, 19.5 h, 76%; for **33**, **34**: Et_3N, BoPCl, **29**, CH_2Cl_2, rt, 17.5 h, **33**, 77%, **34**, 17 h, 68%; (vii) H_2, 10% Pd/C, CH_3OH/H_2O (90:10), rt, 23 h, **1**, 86%, **2**, 20 h, 99%, **3**, 19 h, 100%; (viii) CF_3CO_2H, CH_2Cl_2, rt, 6.5 h, **4**, 96%, **5**, 3.5 h, 100%.



Scheme 3. Reagents, conditions and yields: (i) O_3, $MeOH/CH_2Cl_2$ (1:1), 15 min then PPh_3, 24 h, then silica gel, 63%; (ii) $CF_3CO_2H/Et_3SiH/CH_2Cl_2$ (1:1:1), rt, 45 min, 96%; (iii) 10% Pd/C, CH_3OH/H_2O (88:12), 18 h, **35**, 78%, **36**, 99%.

The optimal conditions for the amide bond formation were investigated using the coupling between **12** and **16**; bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BoPCl) was found to be superior (92% yield compared to 66% with DCC) and was used for all subsequent coupling reactions. Hydrolysis of the methyl esters **18**,**19**,**20** (NaOH in dioxane) to the carboxylic acids **23**,**24**,**25** followed by coupling (BoPCl) with dibenzyl glutamate **28** afforded benzyl protected tripeptides **30**,**31**,**32**. Finally, global deprotection of the benzyl groups gave tripeptides **1**[32] and **2** whilst concomitant hydrogenolysis of the allyl group in **25** gave tripeptide **3**.

For the synthesis of the tripeptides **4** and **5** incorporating a 2-allylproline and a 2-benzylproline unit, respectively, Boc and *t*-butyl protecting groups were used. In these cases coupling of acids **26** and **27** with di-*tert*-butyl glutamate **29** gave tripeptides **33** and **34** affording tripeptides **4** and **5** as trifluoroacetate salts after deprotection with TFA.

In contrast to most peptide bonds that adopt exclusively the trans conformation, the amide bond between Xaa-Pro can exist as a mixture of cis and trans isomers.[33] The nature of the conformation about this bond can affect the biological activity of a peptide and there is evidence that some proteases only recognize the trans peptide bond.[34,35] The existence of specific peptidyl-prolyl cis-trans isomerases would seem to corroborate this evidence.[36] GPE is present as a 20:80 cis-trans mixture of isomers in D_2O solution as established by NMR analysis.[22] When an alkyl group is substituted at the 2-position of proline the trans conformation is preferred. Compounds **1**–**4** adopt the all trans conformation and the trans population also increased in compound **5** with only 10% adopting the cis conformation. The prevalence of the trans isomer in 2-methylproline compounds has been attributed to the bulky methyl group destabilizing the cis conformation.[37]

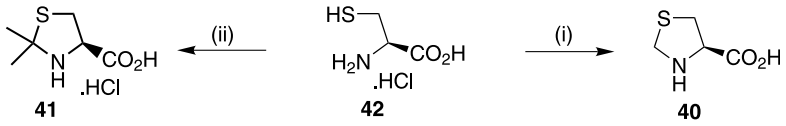
Another method of conformationally constraining a peptide is to synthesize a peptidomimetic containing a spirolactam ring system. It has been suggested that a spirolactam ring system may lock a compound into predominantly one conformation and different ring systems have been shown to mimic both β-[38] and γ-turns.[39] A spirocyclic γ-lactam bridge can be formed between the 2-position of the proline residue and the nitrogen of the glutamate residue in GPE thus, presenting an opportunity to investigate the effect of such conformational restriction in GPE analogues.

The synthesis of GP-[5.5]spirolactamE **35** and the corresponding GP-[5.5]hydroxyspirolactamE **36** is summarized in Scheme 3.

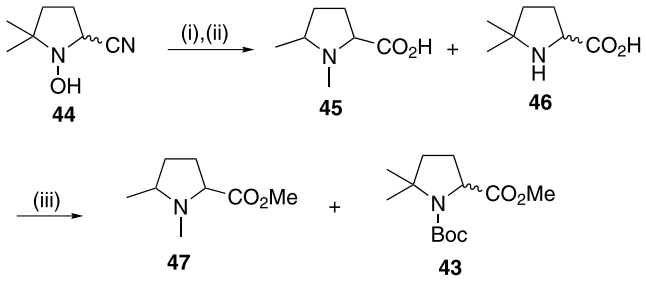
Ozonolysis of alkene **32** followed by treatment with triphenylphosphine proceeded via the intermediacy of aldehyde **37** to give alcohols **38** as a 1:1 mixture of diastereoisomers. Direct hydrogenation of **38** gave the hydroxyspirolactam **36** whereas initial reduction of the hydroxyl group (trifluoroacetic acid–triethylsilane–dichloromethane) to **39** before the hydrogenolysis step afforded spirolactam **35**. Both spirolactams adopted exclusively the trans conformation about the proline ring.

The pyrrolidine ring of proline is capable of adopting two distinct conformations. These down- and up-puckered conformations are defined as occurring when C^{γ} and the carbonyl group of proline lie on the same and opposite sides, respectively, of the plane defined by C^{δ}, N and C^{α}. The presence of a sulfur atom in the proline ring can affect the bond angles and bond lengths, in some cases altering the proline ring conformation. Kang found that replacement of the proline residue in AcProNHMe with 4-thiaproline **40** [(*R*)-thiozolidine-4-carboxylic acid (Thz)] resulted in a more puckered conformation.[40] Further conformational changes in the proline ring can be promoted by the addition of methyl groups at the 5 position of proline or Thz.[41–43] The next set of analogues incorporated such pseudo-proline moieties where the γ-CH_2 of Pro was replaced with sulfur and/or with dimethyl substitution at C^{δ}.

The pseudo-prolines: 4-thiaproline **40** [(*R*)-thiozolidine-4-carboxylic acid (Thz)] and 2,2-dimethylthiazolidine-4-carboxylic acid **41** were easily accessed by the reaction of



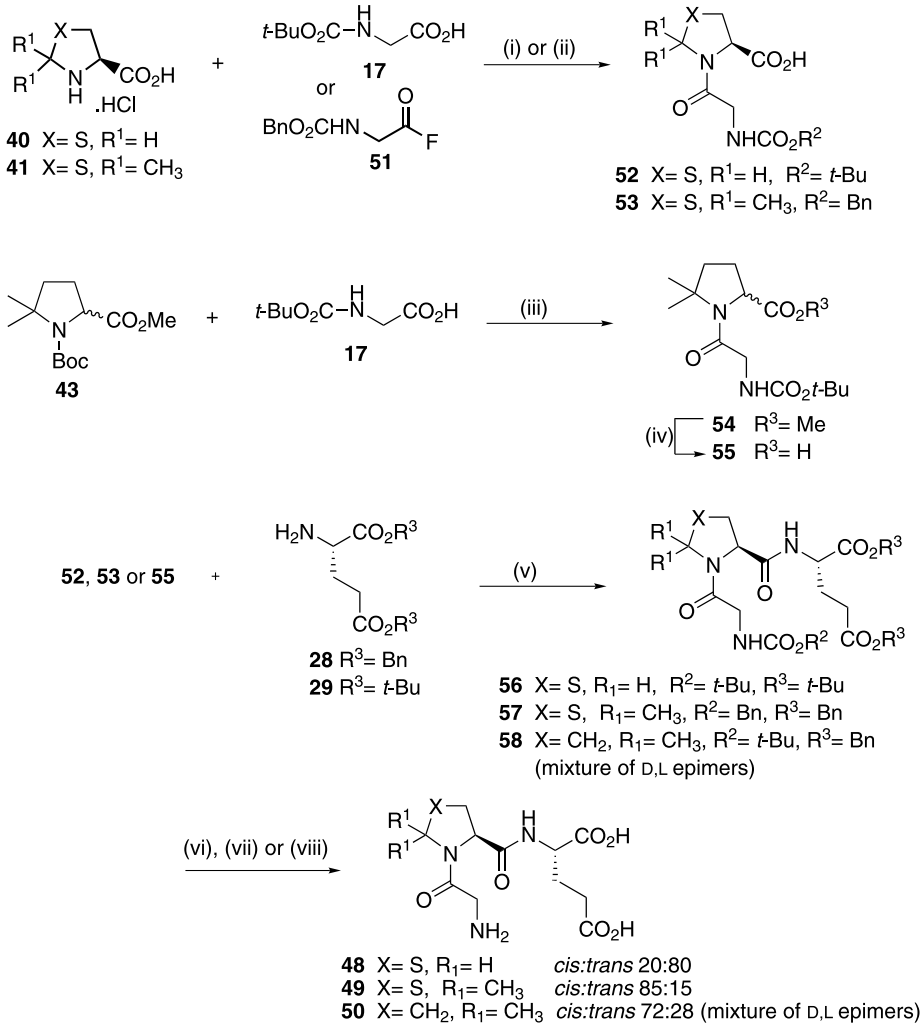
Scheme 4. Reagents, conditions and yields: (i) 37% aqueous HCHO, H_2O, rt, 22 h, then pyridine, 56%; (ii) dimethoxypropane, acetone, reflux, 2 h, 58%.



Scheme 5. Reagents, conditions and yields: (i) 32% aqueous HCl, 50 °C 5 h; (ii) H_2 (44 psi), 10% Pd/C, MeOH/H_2O (1:1), 20 h; (iii) $SOCl_2$, MeOH, 0 °C to rt, overnight then Boc_2O, *N*-methylmorpholine, CH_2Cl_2, reflux, 48 h [**43** (22%) **47** (42%)] over four steps.

cysteine **42** with formaldehyde[44] or 2,2-dimethoxypropane,[45,46] respectively (Scheme 4).

Boc-protected methyl D,L-5,5-dimethylprolinate **43** was prepared from nitrile **44** (Scheme 5). Nitrile **44** was prepared as described in the literature,[47,48] however, subsequent hydrolysis of the nitrile moiety and hydrogenation of the intermediate *N*-oxide as described[49] was concomitant with acid catalysed methyl migration yielding a mixture of **45** and **46**. (6:4 ratio, ^{1}H NMR). Extensive modification of the hydrolysis reaction could not overcome the formation of *N*-methyl compound **45**. It is interesting that this unwanted reaction has not been reported during the synthesis of 5,5-dimethylproline that has been described by several research groups.[49,50]



Scheme 6. Reagents, conditions and yields: (i) **17**, *i*-BuOCOCl, Et_3N, THF, 0 °C to rt, then **40**, Et_3N, H_2O, rt, 2 h, **52**, 81%; (ii) **51**, **41**, *i*-Pr_2EtN, DMF, rt, 18 h then MeOH, Me_3SiCl, rt, 15 h, **53**, 65%; (iii) **43**, CF_3CO_2H, CH_2Cl_2, rt, 2 h then **17**, BoPCl, *i*-Pr_2EtN, CH_2Cl_2, rt, 15 h, **54**, 52%; (iv) dioxane, 1 M aqueous NaOH, rt, 21 h, **55**, 94%; (v) for **56**: EtOCOCl, Et_3N, CH_2Cl_2, 0 °C, 35 min then **29**, Et_3N, CH_2Cl_2, 0 °C to rt, 15 h, **56**, 54%; for **57**, **58**: BoPCl, *i*-Pr_2EtN, CH_2Cl_2, **28**, rt, 7 h, **57**, 68%, **58**, 24 h, 67%; (vi) CF_3CO_2H, Et_3SiH, CH_2Cl_2, rt, 4 h, **48**, 61%; (vii) H_2(42 psi), 10% Pd/C, CH_3OH/H_2O (80:20), 24 h, **49**, 48%; (viii) CF_3CO_2H, CH_2Cl_2, rt, 75 min then H2, 10% Pd/C, CH_3OH/H_2O (80:20), 15 h, **50**, 93%.

Protection of both the acid and amine functionalities as a methyl ester and a *tert*-butyloxy carbamate (Boc), respectively, allowed facile separation and characterisation of the protected 5,5-dimethylproline **43** (22% yield over four steps) and the *N*-methyl by-product **47** (42% yield, over four steps). The desired protected 5,5-dimethylproline **43** existed as a mixture of epimers (55:45) exclusively as the cis conformer.

The tripeptides **48**–**50** were synthesized in a similar fashion to the 2-alkylproline analogues (Scheme 6). Coupling of the 4-thia-proline building block **40** to Boc-glycine **17** was carried out using a mixed anhydride activation procedure whereas the more hindered 5,5-dimethyl-4-thia-proline **41** required use of the more reactive acid fluoride **51** to afford **53**. In the case of the 5,5-dimethylproline **43** the Boc group was removed with trifluoroacetic acid before BoPCl coupling with Boc-glycine **17** to afford **54**. Hydrolysis of the methyl ester then afforded acid **55** in preparation for the second peptide coupling.

Coupling of the pseudo dipeptides **52**, **53** and **55** with either dibenzyl glutamate **28** or or di-*tert*-butyl glutamate **29** using either a mixed anhydride protocol or BoPCl gave the desired peptides **56**, **57** and **58**. The nature of the final deprotection step depended on the protecting groups employed in the synthesis thus, for **57** removal of the benzyloxycarbonyl and benzyl groups by hydrogenolysis provided tripeptide **49**. The Boc and *tert*-butyl ester groups in **56** were removed using trifluoroacetic acid to give tripeptide **48** as the trifluoroacetate salt whereas for the deprotection of **58**, treatment with trifluoroacetic acid followed by hydrogenolysis afforded tripeptide **50**.[51]

The presence of a sulfur atom at C-4 in the pyrrolidine ring of proline, by itself did not appear to significantly alter the conformation of the peptide about the Gly-Pro bond. In the GPE analogue **48** the cis:trans ratio was established to be 20:80, unchanged from the native peptide. The presence of the two methyl groups at C-5 had a more dramatic influence on the conformation with the cis:trans ratio dramatically shifted to favour the cis conformer. The population of the cis conformer in 5,5-dimethylated peptide **50** increased to 72% compared with the 20% seen with GPE. An even greater effect was observed with analogue **49**, which exhibited a 85:15 cis:trans ratio indicating that the presence of a sulphur atom at C-4 in combination with two methyl groups at C-5 in the proline ring plays a key role in determining the ratio of cis:trans isomers about the Gly-Pro bond. The high population of the cis conformer in related 5,5-dimethylprolines has been attributed to the effects of steric hindrance due to the methyl groups when the compound adopts the trans conformation.[41]

3. Conclusions

In summary, we herein report the synthesis of ten analogues of GPE. In five of these analogues the trans conformation about the Gly-Pro* bond was stabilized by either the presence of a hydrophobic alkyl group at C-2 on the proline (compounds **1**–**5**) or by a spirolactam bridge between the 2-position of the proline and the nitrogen of the glutamate (compounds **35** and **36**). In contrast, dimethylation at C-5 on the proline destabilises the trans conformation resulting in an increased population of the cis conformer (compounds **49** and **50**). These GP*E mimetics are valuable tools to provide information about the influence of the proline residue on the bioactivity of the parent peptide GPE.

4. Experimental

4.1. General details

All reactions were conducted in flame-dried or oven-dried glassware under a dry nitrogen atmosphere unless otherwise noted. All reagents were used as supplied. Tetrahydrofuran was dried over sodium/benzophenone and distilled prior to use. Flash chromatography was performed using Merck Kieselgel 60 (230–400 mesh) with the indicated solvents. Thin-layer chromatography (TLC) was carried out on precoated silica plates (Merck Kieselgel $60F_{254}$) and compounds were visualized by UV fluorescence and heating of plates dipped in anisaldehyde in ethanolic sulphuric acid or alkaline potassium permanganate solution. Melting points in degrees Celsius (°C) were measured on an Electrothermal® melting point apparatus and are uncorrected. Infrared spectra were recorded with a Perkin Elmer 1600 series Fourier-transform infrared spectrometer as thin films between sodium chloride plates. Absorption maxima are expressed in wavenumbers (cm^{-1}) with the following abbreviations: s = strong, m = medium, w = weak and br = broad nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AVANCE DRX400 (^1H, 400 MHz; ^{13}C, 100 MHz), a Bruker AVANCE 300 (^1H, 300 MHz; ^{13}C, 75 MHz) or a Bruker AC200 (^1H, 200 MHz; ^{13}C, 50 MHz) spectrometer at 298 K. For ^1H NMR data, chemical shifts are described in parts per million (ppm) relative to tetramethylsilane (δ 0.00), DOH (δ 4.75), CHD_2OD (δ 3.30) or $CHD_2S(O)CD_3$ (δ 2.50) and are reported consecutively as position (δ_H), relative integral, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, q = quintet, s = sextet, dd = doublet of doublets, m = multiplet, and where br = broad), coupling constant (*J*/Hz) and assignment. For ^{13}C NMR data, chemical shifts (ppm) are referenced internally to $CDCl_3$ (δ 77.0), CD_3OD (δ 49.1) and $(CD_3)_2S(O)$ (δ 39.4) or externally to 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS) and are reported consecutively as position (δ_C), degree of hybridisation and assignment. The asterisk* denotes resonances assigned to the minor conformer. High resolution mass spectra were recorded using a VG70-SE spectrometer operating at nominal accelerating voltage of 70 eV. Chemical ionisation (CI) mass spectra were obtained with ammonia as the reagent gas. Optical rotations were measured at 20 °C on a Perkin Elmer 341 polarimeter using 10 cm path length cells and are given in units of 10^{-1} degrees $cm^2\ g^{-1}$. Samples were prepared in the solvent indicated at the concentration specified (measured in g/100 cm^3).

4.1.1. (2*R*,5*S*)-2-Trichloromethyl-1-aza-3-oxabicyclo-[3.3.0]octan-4-one 7.[28,31] A suspension of L-proline (10.0 g, 86.8 mmol) and chloral hydrate (21.6 g, 130 mmol) were heated under reflux in chloroform

(100 cm^3) for 6 h with a reverse Dean-Stark trap. The solution was washed with water (2×30 cm^3) and the water washings were extracted with chloroform (50 cm^3). The combined organic layers were dried ($MgSO_4$), filtered and the solvent removed in vacuo to afford a light brown solid (19.8 g). The crude product was recrystallised from ethanol (80 cm^3) at 40 °C to form oxazolidinone **7** (16.1 g, 77%) as a white solid: mp 107–109 °C (lit.[31] ethanol, 107.6 °C): $[\alpha]_D$ +34.2 (*c* 2 in C_6H_6), lit.[28] $[\alpha]_D$ +33 (*c* 2.0 in C_6H_6); δ_H (200 MHz; $CDCl_3$) 1.67–2.29 (4H, m, Proβ-H_2 and Proγ-H_2), 3.08–3.20 (1H, m, Proβ-H_AH_B), 3.37–3.49 (1H, m, Proβ-H_AH_B), 4.09–4.15 (1H, m, Proα-H) and 5.17 (1H, s, NCH); δ_C (50 MHz; $CDCl_3$) 25.3 (CH_2, Proγ-C), 29.9 (CH_2, Proβ-C), 57.9 (CH_2, Proδ-C), 62.4 (CH, Proα-C), 100.6 [quat., *C*(Cl_3)], 103.6 (CH, N*C*H) and 175.5 (quat., CO); *m/z* (EI+) 244 (MH^+ 244).

4.1.2. (2*R*,5*S*)-5-Methyl-2-trichloromethyl-1-aza-3-oxabicyclo[3.3.0]octan-4-one 8. *n*-Butyllithium (1.31 M, 4.68 cm^3, 6.14 mmol) was added dropwise to a stirred solution of diisopropylamine (0.86 cm^3, 6.14 mmol) in dry tetrahydrofuran (10 cm^3) at −78 °C under an atmosphere of nitrogen. The solution was stirred for 5 min, warmed to 0 °C and stirred for 15 min. The solution was added dropwise to a solution of oxazolidinone **7** (1.00 g, 4.09 mmol) in dry tetrahydrofuran (20 cm^3) at −78 °C over 20 min (reaction mixture turned dark), stirred for a further 30 min then iodomethane (0.76 cm^3, 12.3 mmol) added dropwise over 5 min. The solution was warmed to −50 °C over 2 h. Water (15 cm^3) was added, the solution warmed to room temperature and extracted with chloroform (3×40 cm^3). The combined organic extracts were dried ($MgSO_4$), filtered and evaporated to dryness in vacuo to give a dark brown semi-solid. Purification of the residue by flash column chromatography (15% ethyl acetate–hexane) afforded oxazolidinone **8** (0.67 g, 63%) as a pale yellow solid: mp 55–57 °C (lit.[28] 57–60 °C); δ_H (300 MHz; $CDCl_3$) 1.53 (3H, s, CH_3), 1.72–2.02 (3H, m, Proβ-H and Proγ-H_2), 2.18–2.26 (1H, m, Proβ-H), 3.15–3.22 (1H, m, Proδ-H), 3.35–3.44 (1H, m, Proδ-H) and 4.99 (1H, s, NCH).

4.1.3. (2*R*,5*S*)-5-Ethyl-2-trichloromethyl-1-aza-3-oxabicyclo[3.3.0]octan-4-one 9. The reaction was carried out following a similar procedure to that described for the preparation of oxazolidinone **8** using *n*-butyllithium (1.31 M, 28.3 cm^3, 37.1 mmol), diisopropylamine (5.2 cm^3, 37.1 mmol), oxazolidinone **7** (6.0 g, 24.7 mmol) and iodoethane (5.9 cm^3, 73.8 mmol) to afford oxazolidinone **9** (3.05 g, 46%) as a light red oil that solidified on standing to a pale brown solid: mp 76–77 °C: $[\alpha]_D$ +18.5 (*c* 0.25 in $CHCl_3$); δ_H (300 MHz; $CDCl_3$) 1.04 (3H, t, *J*= 7.5 Hz, CH_3), 1.60–1.80 (1H, m, C$H_AH_B$$CH_3$), 1.72–1.99 (4H, m, $CH_AH_B$$CH_3$, Proβ-$H_AH_B$ and Proγ-H_2), 2.20–2.30 (1H, m, Proβ-H_AH_B), 3.22–3.29 (2H, m, Proδ-H_2) and 5.00 (1H, s, NCH); δ_C (75 MHz; $CDCl_3$) 8.4 (CH_3, CH_3), 25.5 (CH_2, *C*$H_2$$CH_3$), 30.9 ($CH_2$, Proγ-C), 35.6 ($CH_2$, Proβ-C), 58.6 ($CH_2$, Proδ-C), 72.5 (quat., Proα-C), 100.9 [quat., *C*(Cl_3)], 102.5 (CH, N*C*H) and 176.9 (quat., CO); *m/z* (EI+) 272.0014 (MH^+ $C_9H_{13}^{35}ClN_3O_2$ requires 272.0012).

4.1.4. (2*R*,5*R*)-5-Allyl-2-trichloromethyl-1-aza-3-oxabicyclo[3.3.0]octan-4-one 10.[28] The reaction was carried out following a similar procedure to that described for the preparation of oxazolidinone **8** using *n*-butyllithium (1.31 M, 9.93 cm^3, 13.0 mmol), diisopropylamine (1.82 cm^3, 13.0 mmol), oxazolidinone **7** (2.10 g, 8.7 mmol) and allyl bromide (2.25 cm^3, 26.0 mmol) to afford oxazolidinone **10** (1.48 g, 60%) as a light orange oil for which the NMR data were in agreement with the literature.[28]

4.1.5. (2*R*,5*R*)-5-Benzyl-2-trichloromethyl-1-aza-3-oxabicyclo[3.3.0]octan-4-one 11.[28] The reaction was carried out following a similar procedure to that described for the preparation of oxazolidinone **8** using *n*-butyllithium (1.31 M, 5.53 cm^3, 7.2 mmol), diisopropylamine (1.01 cm^3, 7.24 mmol), oxazolidinone **7** (1.18 g, 4.8 mmol) and benzyl bromide (1.72 cm^3, 14.5 mmol) to afford oxazolidinone **11** (0.52 g, 32%) as a colourless crystalline solid: mp 75–77 °C (lit.[28] 72–77); δ_H (400 MHz; $CDCl_3$) 1.32–1.55 (2H, m, Proγ-H_2), 1.93–2.13 (2H, m, Proβ-H_2), 2.58–2.65 (1H, m, Proδ-H_2), 2.92 (1H, d, *J*= 13.6 Hz, PhCH_AH_B), 2.98–3.03 (1H, m, Proδ-H_2), 3.32 (1H, d, *J*=13.6 Hz, $PhCH_AH_B$), 4.99 (1H, s, NCH) and 7.21–7.35 (5H, m, PhH); δ_C (100 MHz; $CDCl_3$) 24.8 (CH_2, Proγ-C), 34.6 (CH_2, Proβ-C), 41.6 (CH_2, Proδ-C), 58.4 (CH_2, Ph*C*H_2), 72.3 (quat., Proα-C), 100.6 (quat., CCl_3), 102.8 (CH, NCH), 127.0 (CH, Ph), 128.2 (CH, Ph), 130.9 (CH, Ph), 135.5 (quat., Ph) and 176.6 (quat., C=O); *m/z* (EI+) 333.0081 [$(M+H)^+$ $C_{14}H_{14}^{35}Cl_3NO_2$ requires 333.0090], 335.0069 [$(M+H)^+$ $C_{14}H_{14}^{35}Cl_2^{37}ClNO_2$ requires 335.0061], 337.0014 [$(M+H)^+$ $C_{14}H_{14}^{35}Cl$ $^{37}Cl_2NO_2$ requires 337.0031] and 339.0009 [$(M+H)^+$ $C_{14}H_{14}$ $^{37}Cl_3NO_2$ requires 339.0002].

4.1.6. Methyl L-2-methylprolinate hydrochloride 12. Thionyl chloride (4.30 mL, 58.9 mmol) was added dropwise cautiously to a stirred solution of **8** (7.57 g, 29.4 mmol) at 0 °C under a nitrogen atmosphere. The cooling bath was removed and mixture stirred at room temperature for 20 min then heated to reflux for 3 h. The volatiles were removed in vacuo, the residue suspended in toluene (20 mL) and concentrated at 50 °C to remove traces of thionyl chloride. Trituration with dry ether yielded a brown solid. The yellow/orange ether was decanted and the solid was shaken with dry ether, decanted and the procedure repeated until the ether was colourless. Removal of traces of ether in vacuo at 50 °C afforded **12** (ca. 5.0 g, 100%) as a free flowing, hygroscopic brown solid that was used without any further purification: mp 107–109 °C (lit.[52] 106–108 °C).

4.1.7. Methyl L-2-ethylprolinate hydrochloride 13. An ice-cooled solution of oxazolidinone **9** (2.86 g, 10.6 mmol) in dry methanol (35 cm^3) under an atmosphere of nitrogen was treated dropwise with a solution of thionyl chloride (2.3 cm^3, 31.5 mmol). The solution was heated under reflux for 3 h, cooled and the solvent removed under reduced pressure. The resultant brown oil was purified by flash column chromatography (10% methanol–dichloromethane) to afford hydrochloride **13** (1.45 g, 71%) as a light brown semi-solid: $[\alpha]_D$ −61.1 (*c* 0.3 in $CHCl_3$); δ_H (300 MHz; $CDCl_3$) 1.07 (3H, t, *J*=7.3 Hz, CH_3), 1.95–2.33 (5H, m, C$H_2$$CH_3$, Proγ-$H_2$ and Proβ-H_AH_B), 2.43–2.47 (1H, Proβ-H_AH_B), 3.63 (3H, s, OCH_3) and 6.98–7.35 (2H, br s, NH_2); δ_C (75 MHz; $CDCl_3$) 9.9 (CH_3, *C*H_3), 22.8 (CH_2, *C*$H_2$$CH_3$), 28.9 ($CH_2$, Proγ-C), 35.1 ($CH_2$, Proβ-C), 45.7 ($CH_2$, Proδ-C), 53.8 ($CH_3$, OCH_3), 73.9 (quat., Proα-C) and 171.0

(quat., CO); *m/z* (EI+) 158.1181 (MH^+ $C_8H_{16}NO_2$ requires 158.1181).

4.1.8. Methyl L-2-allylprolinate hydrochloride 14.[28,53] An ice-cooled solution of oxazolidinone **10** (0.64 g, 2.24 mmol) in dry methanol (15 cm^3) was treated dropwise with a solution of acetyl chloride (0.36 cm^3, 5.0 mmol) in methanol (5 cm^3). The solution was heated under reflux for 24 h, cooled and the solvent removed under reduced pressure. The resultant brown oil was dissolved in toluene (40 cm^3) and concentrated to dryness to remove residual thionyl chloride and methanol, then purified by flash column chromatography (5–10% CH_3OH–CH_2Cl_2; gradient elution) to afford hydrochloride **14** (0.29 g, 63%) as a solid for which the NMR data were in agreement with that reported in the literature.[28,53] δ_H (300 MHz; $CDCl_3$) 1.72–2.25 (3H, m, Proβ-H_AH_B and Proγ-H_2), 2.32–2.52 (1H, m, Proβ-H_AH_B), 2.72–3.10 (2H, m, Proδ-H_2), 3.31–3.78 (2H, m, CH_2CH=CH_2), 3.84 (3H, s, CO_2CH_3), 5.20–5.33 (2H, m, CH=CH_2), 5.75–5.98 (1H, m, CH=CH_2) and 8.06 (1H, br s, N–H); *m/z* (CI+) 170.1183 [$(M+H)^+$ $C_9H_{16}NO_2$ requires 170.1181].

4.1.9. Methyl L-2-benzylprolinate hydrochloride 15.[54] An ice-cooled solution of oxazolidinone **11** (1.03 g, 3.07 mmol) in dry methanol (10 cm^3) was treated dropwise with a solution of acetyl chloride (0.71 cm^3, 10.0 mmol) in methanol (10 cm^3). The solution was heated under reflux for 24 h, cooled and the solvents removed under reduced pressure. The resultant brown oil was dissolved in toluene (80 cm^3), concentrated to dryness to remove residual thionyl chloride and methanol, then purified by flash column chromatography (5% CH_3OH–CH_2Cl_2) to afford hydrochloride **15**[28,53] (0.38 g, 48%) as a beige solid; δ_H (400 MHz; D_2O) 1.92–2.01 (1H, m, Proγ-H_AH_B), 2.11–2.23 (2H, m, Proβ-H_AH_B and Proγ-H_AH_B), 2.52–2.60 (1H, m, Proβ-H_AH_B), 3.19 (1H, d, J=14.3 Hz, PhCH_AH_B), 3.24–3.31 (1H, m, Proδ-H_AH_B), 3.37–3.43 (1H, m, Proδ-H_AH_B), 3.53 (1H, d, J=14.3 Hz, PhCH_AH_B), 3.83 (3H, s, CO_2CH_3) and 7.26–7.47 (5H, m, PhH); δ_C (100 MHz; D_2O) 24.4 (CH_2, Proγ-C), 36.8 (CH_2, Proβ-C), 43.8 (CH_2, PhCH_2), 47.6 (CH_2, Proδ-C), 56.0 (CH_3, OCH_3), 75.9 (quat., Proα-C), 130.4 (CH, Ph), 131.5 (CH, Ph), 131.7 (CH, Ph), 137.1 (quat., Ph) and 175.8 (quat., C=O); *m/z* (CI+) 220.1340 [$(M+H)^+$ $C_{13}H_{18}NO_2$ requires 220.1338].

4.1.10. Methyl *N*-benzyloxycarbonyl-glycyl-L-2-methylprolinate 18. Dry triethylamine (0.27 cm^3, 1.96 mmol) was added dropwise to a solution of hydrochloride **12** (0.11 g, 0.61 mmol) and *N*-benzyloxycarbonylglycine **16** (0.17 g, 0.79 mmol) in dry dichloromethane (35 cm^3) under an atmosphere of nitrogen at room temperature and the reaction mixture stirred for 10 min. Bis(2-oxo-3-oxazolidinyl)phosphinic chloride (0.196 g, 0.77 mmol) was added and the resultant colourless solution was stirred for 20.5 h. The solution was washed successively with 10% aqueous hydrochloric acid (30 cm^3) and saturated aqueous sodium hydrogen carbonate (30 cm^3), dried ($MgSO_4$), filtered and evaporated to dryness in vacuo. Purification of the resultant residue by flash column chromatography (50–80% ethyl acetate–hexane; gradient elution) yielded ester **18** (0.18 g, 92%) as a colourless oil: $[\alpha]_D$ −33.0 (*c* 1.0 in MeOH); ν_{max} (film)/cm^{-1} 3406, 2952, 1732, 1651, 1521, 1434, 1373, 1329, 1310, 1284, 1257, 1220, 1195, 1172, 1135, 1107, 1082, 1052, 1029, 986, 965, 907, 876, 829, 775, 738 and 699; δ_H (300 MHz; $CDCl_3$) 1.49 (3H, s, CH_3), 1.77–2.11 (4H, m, Proβ-H_2 and Proγ-H_2), 3.43–3.48 (2H, m, Proδ-H_2), 3.61 (3H, s, OCH_3), 3.85–3.89 (2H, m, Glyα-H_2), 5.04 (2H, s, PhCH_2), 5.76 (1H, br s, N–H) and 7.21–7.28 (5H, s, ArH); δ_C (75 MHz; $CDCl_3$) 21.1 (CH_3, Proα-CH_3), 23.5 (CH_2, Proγ-C), 38.0 (CH_2, Proβ-C), 43.3 (CH_2, Glyα-C), 46.6 (CH_2, Proδ-C), 52.1 (CH_3, OCH_3), 66.0 (quat., Proα-C), 66.3 (CH_2, PhCH_2), 127.5 (CH, Ph), 127.6 (CH, Ph), 128.1 (CH, Ph), 136.2 (quat., Ph), 155.9 (quat., NCO_2), 166.0 (quat., Gly-CON) and 173.6 (quat., CO_2CH_3); *m/z* (EI+) 334.1535 (M^+ $C_{17}H_{22}N_2O_5$ requires 334.1529).

4.1.11. Methyl *N*-benzyloxycarbonyl-glycyl-L-2-ethylprolinate 19. Dry triethylamine (2.88 cm^3, 20.7 mmol) was added dropwise to a solution of hydrochloride **13** (1.14 g, 5.9 mmol) and *N*-benzyloxycarbonylglycine **16** (2.47 g, 11.8 mmol) in dry dichloromethane (100 cm^3) under an atmosphere of nitrogen at 0 °C, and the reaction mixture stirred for 10 min. Bis(2-oxo-3-oxazolidinyl)-phosphinic chloride (3.00 g, 11.8 mmol) was added and the solution was stirred for 2 h, warmed to room temperature and further stirred for 17.5 h. Dichloromethane (50 cm^3) was added and the solution washed successively with 0.5 M aqueous hydrochloric acid (2×50 cm^3) and saturated aqueous sodium hydrogen carbonate (2×50 cm^3), dried ($MgSO_4$), filtered and evaporated in vacuo to give a light orange gum. Purification of the resultant residue by flash column chromatography (40% ethyl acetate/hexane) yielded ester **19** (0.95 g, 46%) as a clear oil: $[\alpha]_D$ −9.2 (*c* 0.13 in $CHCl_3$); δ_H (300 MHz; $CDCl_3$) 0.81 (3H, t, J=7.5 Hz, CH_3), 1.85–2.09 (5H, m, CH_2CH$_3$, Proγ-H_2 and Proβ-H_AH_B), 2.38 (1H, sextet, J=7.5 Hz, Proβ-H_AH_B), 3.43–3.47 (1H, m, Proδ-H_AH_B), 3.61–3.67 (1H, m, Proδ-H_AH_B), 3.70 (3H, s, OCH_3), 4.10–4.13 (2H, m, Glyα-H_2) 5.11 (2H, s, OCH_2Ph), 5.71 (1H, br s, Gly-NH) and 7.27–7.35 (5H, m, Ph); δ_C (75 MHz; $CDCl_3$) 8.3 (CH_3, CH_3), 24.1 (CH_2, CH_2CH_3), 26.5 (CH_2, Proγ-C), 35.3 (CH_2, Proβ-C), 44.1 (CH_2, Glyα-C), 48.2 (CH_2, Proδ-C), 52.9 (CH_3, OCH_3), 67.0 (CH_2, OCH_2Ph), 70.2 (quat., Proα-C), 128.2 (CH, Ph), 128.3 (CH, Ph), 128.7 (CH, Ph), 136.8 (quat., Ph), 156.5 (quat., NCO), 166.8 (quat., Gly-CON) and 174.5 (quat., CO_2CH_3); *m/z* (EI+) 348.1688 (MH^+ $C_{18}H_{24}N_2O_5$ requires 348.1685).

4.1.12. Methyl *N*-benzyloxycarbonyl-glycyl-L-2-allylprolinate 20. Dry triethylamine (1.07 cm^3, 7.70 mmol) was added dropwise to a solution of hydrochloride **14** (0.50 g, 2.41 mmol) and *N*-benzyloxycarbonyl-glycine **16** (0.65 g, 3.13 mmol) in dry dichloromethane (80 cm^3) under an atmosphere of nitrogen at room temperature, and the reaction mixture stirred for 10 min. Bis(2-oxo-3-oxazolidinyl)phosphinic chloride (0.772 g, 3.03 mmol) was added and the solution stirred for 20 h, then washed successively with 10% aqueous hydrochloric acid (80 cm^3) and saturated aqueous sodium hydrogen carbonate (80 cm^3), dried ($MgSO_4$), filtered and evaporated to dryness in vacuo. Purification of the resultant residue by flash column chromatography (60% ethyl acetate–hexane, seven drops of Et_3N for every 200 cm^3) yielded ester **20** (0.26 g, 30%) as a yellow oil: $[\alpha]_D$ +46.0 (*c* 0.50 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3405, 3066, 3032, 2953, 2877, 1723, 1655, 1586,

1507, 1434, 1373, 1333, 1309, 1248, 1169, 1121, 1083, 1047, 1027, 1002, 919, 866, 827, 776, 737 and 699; δ_H (300 MHz; $CDCl_3$) 1.92–2.17 (4H, m, Proβ-H_2 and Proγ-H_2), 2.60–2.67 (1H, m, C$H_A$$H_B$CH=$CH_2$), 3.09–3.16 (1H, m, $CH_A$$H_B$CH=$CH_2$), 3.35–3.42 (1H, m, Proδ-$H_A$$H_B$), 3.56–3.63 (1H, m, Proδ-$H_A$$H_B$), 3.70 (3H, s, OCH_3), 3.96 (2H, d, J=4.4 Hz, Glyα-H_2), 5.07–5.12 (4H, m, PhCH_2 and CH=CH_2), 5.58–5.70 (1H, m, CH=CH_2) and 7.27–7.35 (5H, s, PhH); δ_C (75 MHz; $CDCl_3$) 23.6 (CH_2, Proγ-C), 34.9 (CH_2, Proβ-C), 37.6 (CH_2, CH_2CH=CH_2), 43.6 (CH_2, Glyα-C), 47.5 (CH_2, Proδ-C), 52.5 (CH_3, OCH_3), 66.7 (CH_2, PhCH_2), 68.8 (quat., Proα-C), 119.4 (CH_2, CH=H_2), 127.9 (CH, Ph), 128.0 (CH, Ph), 128.4 (CH, Ph), 132.8 (CH, CH=CH_2), 136.4 (quat., Ph), 156.1 (quat., NCO_2), 166.4 (quat., Gly-CON) and 173.7 (quat., CO_2CH_3); *m/z* (EI+) 360.1682 (M^+ $C_{19}H_{24}N_2O_5$ requires 360.1685).

4.1.13. Methyl *N-tert*-butyloxycarbonyl-glycyl-L-2-allylprolinate 21. Dry triethylamine (0.28 cm^3, 2.02 mmol) was added dropwise to a solution of hydrochloride **14** (0.13 g, 0.63 mmol) and *N-tert*-butyloxycarbonylglycine **17** (0.14 g, 0.82 mmol) in dry dichloromethane (35 cm^3) under an atmosphere of nitrogen at room temperature, and the reaction mixture stirred for 10 min. Bis(2-oxo-3-oxazolidinyl)phosphinic chloride (0.20 g, 0.80 mmol) was added and the solution stirred for 19.5 h, then washed successively with 10% aqueous hydrochloric acid (35 cm^3) and saturated aqueous sodium hydrogen carbonate (35 cm^3), dried ($MgSO_4$), filtered and evaporated to dryness in vacuo. Purification of the resultant residue by flash column chromatography (40% ethyl acetate–hexane) yielded ester **21** (0.09 g, 45%) as a light yellow oil: $[\alpha]_D$ +33.8 (*c* 0.83 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3419, 3075, 2977, 2930, 2874, 1739, 1715, 1656, 1499, 1434, 1392, 1366, 1332, 1268, 1248, 1212, 1168, 1122, 1051, 1026, 1003, 943, 919, 867, 830, 779, 739, 699 and 679; δ_H (300 MHz; $CDCl_3$) 1.42 [9H, s, $C(CH_3)_3$], 1.93–2.08 (4H, m, Proβ-H_2 and Proγ-H_2), 2.59–2.67 (1H, m, C$H_A$$H_B$CH=$CH_2$), 3.09–3.16 (1H, m, $CH_A$$H_B$CH=$CH_2$), 3.35–3.44 (1H, m, Proδ-$H_A$$H_B$), 3.56–3.62 (1H, m, Proδ-$H_A$$H_B$), 3.70 (3H, s, OCH_3), 3.89 (2H, d, J=4.2 Hz, Glyα-H_2), 5.06–5.11 (2H, m, CH=CH_2), 5.42 (1H, br s, Gly-NH) and 5.58–5.72 (1H, m, CH=CH_2); δ_C (75 MHz; $CDCl_3$) 23.7 (CH_2, Proγ-C), 28.3 [CH_3, $C(CH_3)_3$], 35.0 (CH_2, Proβ-C), 37.6 (CH_2, CH_2CH=CH_2), 43.3 (CH_2, Glyα-C), 47.5 (CH_2, Proδ-C), 52.5 (CH_3, OCH_3), 68.8 (quat., Proα-C), 79.5 [quat., $C(CH_3)_3$], 119.4 (CH_2, CH=CH_2), 132.9 (CH, CH=CH_2), 155.7 (quat., NCO_2), 166.9 (quat., Gly-CON) and 173.8 (quat., CO_2CH_3); *m/z* (EI+) 326.1845 (M^+ $C_{16}H_{26}N_2O_5$ requires 326.1842).

4.1.14. Methyl *N-tert*-butyloxycarbonyl-glycyl-L-2-benzylprolinate 22. Dry triethylamine (0.59 cm^3, 4.22 mmol) was added dropwise to a solution of hydrochloride **15** (0.34 g, 1.32 mmol) and *N-tert*-butyloxycarbonylglycine **17** (0.30 g, 1.71 mmol) in dry dichloromethane (70 cm^3) under an atmosphere of nitrogen at room temperature, and the reaction mixture stirred for 10 min. Bis(2-oxo-3-oxazolidinyl)phosphinic chloride (0.42 g, 1.66 mmol) was added and the solution stirred for 18.5 h, then washed successively with 10% aqueous hydrochloric acid (70 cm^3) and saturated aqueous sodium hydrogen carbonate (70 cm^3), dried ($MgSO_4$), filtered and evaporated to dryness in vacuo. Purification of the resultant residue by flash column chromatography (50% ethyl acetate–hexane) yielded ester **22** (0.11 g, 22%) as a pale yellow oil: $[\alpha]_D$ +105.3 (*c* 0.99 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3419, 3061, 3028, 2977, 2873, 1799, 1739, 1715, 1655, 1582, 1497, 1454, 1432, 1392, 1366, 1330, 1250, 1167, 1121, 1093, 1049, 1026, 948, 915, 865, 819, 765, 736, 706 and 653; δ_H (300 MHz; $CDCl_3$) 1.08–1.12 (1H, m, Proγ-$H_A$$H_B$), 1.47 [9H, s, $C(CH_3)_3$], 1.67–1.72 (1H, m, Proγ-$H_A$$H_B$), 2.01–2.17 (2H, m, Proβ-H_2), 2.86–2.92 (1H, m, Proδ-$H_A$$H_B$), 3.08 (1H, d, J=13.8 Hz, PhC$H_A$$H_B$), 3.36–3.42 (1H, m, Proδ-$H_A$$H_B$), 3.75 (3H, s, OCH_3), 3.83 (1H, m, PhC$H_A$$H_B$), 3.90 (2H, d, J=3.6 Hz, Glyα-CH_2), 5.54 (1H, br s, N–H) and 7.06–7.30 (5H, m, PhH); δ_C (100 MHz; $CDCl_3$) 23.4 (CH_2, Proγ-C), 28.4 [CH_3, $C(CH_3)_3$], 34.7 (CH_2, Proβ-C), 37.8 (CH_2, PhCH_2), 43.6 (CH_2, Glyα-C), 47.4 (CH_2, Proδ-C), 52.6 (CH_3, OCH_3), 69.6 (quat., Proα-C), 79.6 [quat., $C(CH_3)_3$], 126.8 (CH, Ph), 128.3 (CH, Ph), 130.5 (CH, Ph), 136.7 (quat., Ph), 155.8 (quat., NCO_2), 167.2 (quat., Gly-CON) and 174.0 (quat., CO_2CH_3); *m/z* (EI+) 376.2001 (M^+ $C_{20}H_{28}N_2O_5$ requires 376.1998).

4.1.15. *N*-Benzyloxycarbonyl-glycyl-L-2-methylproline 23. To a solution of mthyl ester **18** (0.56 g, ca. 1.67 mmol) in 1,4-dioxane (33 cm^3) was added dropwise 1 M aqueous sodium hydroxide (10 cm^3, 10 mmol) and the mixture was stirred for 19 h at room temperature. Dichloromethane (100 cm^3) was then added and the organic layer extracted with saturated aqueous sodium hydrogen carbonate (2×100 cm^3). The combined aqueous layers were carefully acidified with dilute hydrochloric acid, extracted with dichloromethane (2×100 cm^3), and the combined organic layers dried ($MgSO_4$), filtered, and concentrated to dryness in vacuo. Purification of the ensuing residue (0.47 g) by flash column chromatography (50% ethyl acetate–hexane to 30% methanol–dichloromethane; gradient elution) gave acid **23** (0.68 g, 90%) as a white foam: $[\alpha]_D$ −62.3 (*c* 0.20 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3583, 3324 br, 2980, 2942, 1722, 1649, 1529, 1454, 1432, 1373, 1337, 1251, 1219, 1179, 1053, 1027, 965, 912, 735 and 698; δ_H (300 MHz; $CDCl_3$) 1.59 (3H, s, Proα-CH_3), 1.89 (1H, 6 lines, J=18.8, 6.2, 6.2 Hz, Proβ-$H_A$$H_B$), 2.01 (2H, dtt, J=18.7, 6.2, 6.2 Hz, Proγ-H_2), 2.25–2.40 (1H, m, Proβ-$H_A$$H_B$), 3.54 (2H, t, J=6.6 Hz, Proδ-H_2), 3.89 (1H, dd, J=17.1, 3.9 Hz, Glyα-$H_A$$H_B$), 4.04 (1H, dd, J=17.2, 5.3 Hz, Glyα-$H_A$$H_B$), 5.11 (2H, s, OC$H_2$Ph), 5.84 (1H, br t, J=4.2 Hz, N–H), 7.22–7.43 (5H, m, Ph) and 7.89 (1H, br s, –COOH); δ_C (75 MHz; $CDCl_3$) 21.3 (CH_3, Proα-CH_3), 23.8 (CH_2, Proγ-C), 38.2 (CH_2, Proβ-C), 43.6 (CH_2, Glyα-C), 47.2 (CH_2, Proδ-C), 66.7 (quat., Proα-C), 66.8 (CH_2, OCH_2Ph), 127.9 (CH, Ph), 127.9 (CH, Ph), 128.4, (CH, Ph), 136.4 (quat., Ph), 156.4 (quat., NCO_2), 167.5 (quat., Gly-CON) and 176.7 (quat., CO); *m/z* (EI+) 320.1368 (M^+ $C_{16}H_{20}N_2O_5$ requires 320.1372).

4.1.16. *N*-Benzyloxycarbonyl-glycyl-L-2-ethylproline 24. To a solution of methyl ester **19** (0.39 g, 1.11 mmol) in dioxane (15 cm^3) was added dropwise 1 M NaOH (7.5 cm^3, 7.50 mmol) and the mixture was stirred for 20 h at room temperature. The solution was acidified with 1 M HCl and evaporated in vacuo. The resulting aqueous layer was extracted with dichloromethane (2×30 cm^3), dried ($MgSO_4$), filtered and evaporated in vacuo to form a clear gum, which solidified on standing to acid **24** (0.35 g, 95%)

as a colourless solid, which was used without further purification: $[\alpha]_D$ −9.9 (*c* 0.11 in MeOH); δ_H (300 MHz; $CDCl_3$) 0.83 (3H, t, *J*=7.4 Hz, CH_3), 1.93–2.22 (5H, m, CH_2CH_3, Proγ-H_2 and Pro-βH_AH_B), 2.35–2.40 (1H, m, Pro-βH_AH_B), 3.43–3.46 (1H, m, Proδ-H_AH_B), 3.57–3.62 (1H, m, Proδ-H_AH_B), 4.01 (2H, dd, *J*=4.3, 17.3 Hz, Glyα-H_2), 5.11 (2H, s, OCH_2Ph), 5.82 (1H, br s, Gly-NH), 7.26–7.36 (5H, m, Ph) and 7.70 (1H, br s, CO_2H); δ_C (75 MHz; $CDCl_3$) 8.5 (CH_3, CH_3), 23.9 (CH_2, CH_2CH_3), 26.7 (CH_2, Proγ-C), 34.8 (CH_2, Proβ-C), 44.1 (CH_2, Glyα-C), 48.7 (CH_2, Proδ-C), 67.3 (CH_2, OCH_2Ph), 71.9 (quat., Proα-C), 127.3 (CH, Ph), 127.9 (CH, Ph), 128.3 (CH, Ph), 128.4 (CH, Ph), 128.9 (CH, Ph), 136.7 (quat., Ph), 156.7 (quat., NCO_2), 168.9 (quat., Gly-CON) and 175.8 (quat., CO_2H); *m*/*z* (EI^+) 334.1523 (M^+ $C_{17}H_{22}N_2O_5$ requires 334.1529).

4.1.17. *N*-Benzyloxycarbonyl-glycyl-L-2-allylproline 25. To a solution of ester **20** (0.12 g, 0.34 mmol) in dioxane (7 cm^3) was added dropwise 1 M aqueous NaOH (2.06 cm^3, 2.06 mmol) and the mixture stirred for 20 h at room temperature. Dichloromethane (25 cm^3) was then added and the organic layer extracted with saturated aqueous sodium bicarbonate (3×25 cm^3). Careful acidification of the combined aqueous layers with dilute hydrochloric acid, extraction with dichloromethane (3×25 cm^3), drying of the combined organic layers ($MgSO_4$), filtration and concentration to dryness gave the acid **25** (0.11 g, 92%) as a yellow oil: $[\alpha]_D$ −3.16 (*c* 0.95 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3408, 2957, 2923, 2850, 2622, 1715, 1650, 1531, 1453, 1375, 1333, 1259, 1214, 1173, 1121, 1083, 1056, 1028, 1002, 916, 823, 737 and 698; δ_H (300 MHz; $CDCl_3$) 1.93–2.07 (3H, m, Proβ-H_AH_B and Proγ-H_2), 2.22–2.26 (1H, m, Proβ-H_AH_B), 2.62–2.69 (1H, m, CH_AH_BCH=CH_2), 3.04–3.11 (1H, m, CH_AH_BCH=CH_2), 3.31–3.62 (2H, m, Proδ-H_2), 3.91–4.05 (2H, m, Glyα-H_2), 5.08–5.13 (3H, m, PhCH_2 and CH=CH_AH_B), 5.55–5.72 (1H, m, CH=CH_AH_B), 5.89 (1H, m, CH=CH_2), 7.29–7.45 (5H, m, PhH) and 8.12 (1H, br s, N–H); δ_C (75 MHz; $CDCl_3$) 23.5 (CH_2, Proγ-C), 34.6 (CH_2, Proβ-C), 37.5 (CH_2, CH_2CH=CH_2), 43.7 (CH_2, Glyα-C), 48.1 (CH_2, Proδ-C), 66.9 (CH_2, PhCH_2), 69.9 (quat., Proα-C), 119.8 (CH_2, CH=CH_2), 127.97 (CH, Ph), 128.04 (CH, Ph), 128.4 (CH, Ph), 132.3 (CH, CH=CH_2), 136.4 (quat., Ph), 156.4 (quat., NCO_2), 168.2 (quat., Gly-CON) and 176.1 (quat., CO_2H); *m*/*z* (EI+) 346.1526 (M^+ $C_{18}H_{22}N_2O_5$ requires 346.1529).

4.1.18. *N-tert*-Butyloxycarbonyl-glycyl-L-2-allylproline 26. To a solution of ester **21** (0.039 g, 0.12 mmol) in dioxane (2.4 cm^3) was added dropwise 1 M aqueous NaOH (0.72 cm^3, 0.72 mmol) and the mixture was stirred for 16 h at room temperature. Dichloromethane (10 cm^3) was then added and the organic layer extracted with saturated aqueous sodium bicarbonate (3×10 cm^3). Careful acidification of the combined aqueous layers with dilute hydrochloric acid, extraction with dichloromethane (3×10 cm^3), drying of the combined organic layers ($MgSO_4$), filtration and concentration to dryness gave acid **26** (0.031 g, 83%) as a yellow oil: $[\alpha]_D$ −15.8 (*c* 0.89 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3414, 3076, 2978, 2931, 2620, 1713, 1654, 1510, 1454, 1434, 1392, 1367, 1268, 1250, 1213, 1169, 1121, 1056, 1028, 920, 869, 779, 736 and 701; δ_H (300 MHz; $CDCl_3$) 1.43 [9H, s, $C(CH_3)_3$], 1.95–2.15 (3H, m, Proβ-H_AH_B and Proγ-CH_2), 2.21–2.35 (1H, m, Proβ-H_AH_B), 2.63–2.70 (1H, m, CH_AH_BCH=CH_2), 3.04–3.11 (1H, m, CH_AH_BCH=CH_2), 3.35–3.47 (1H, m, Proδ-H_AH_B), 3.53–3.62 (1H, m, Proδ-H_AH_B), 3.92 (2H, m, Glyα-H_2), 5.08–5.13 (2H, m, CH=CH_2), 5.52 (1H, br s, Gly-NH), 5.56–5.71 (1H, m, CH=CH_2) and 8.31 (1H, br s, OH); δ_C (75 MHz; $CDCl_3$) 23.5 (CH_2, Proγ-C), 28.3 [CH_3, $C(CH_3)_3$], 34.6 (CH_2, Proβ-C), 37.5 (CH_2, CH_2CH=CH_2), 43.4 (CH_2, Glyα-C), 48.1 (CH_2, Proδ-C), 69.8 (quat., Proα-C), 79.8 [quat., $C(CH_3)_3$], 119.8 (CH_2, CH=CH_2), 132.3 (CH, CH=CH_2), 155.8 (quat., NCO_2), 168.5 (quat., Gly-CON) and 175.9 (quat., CO_2H); *m*/*z* (EI+) 312.1692 (M^+ $C_{15}H_{24}N_2O_5$ requires 312.1685).

4.1.19. *N-tert*-Butyloxycarbonyl-glycyl-L-2-benzylproline 27. To a solution of ester **22** (0.098 g, 0.26 mmol) in dioxane (5.2 cm^3) was added dropwise 1 M aqueous NaOH (1.56 cm^3, 1.56 mmol) and the mixture was stirred for 15 h at room temperature. Dichloromethane (20 cm^3) was then added and the organic layer extracted with saturated aqueous sodium bicarbonate (3×20 cm^3). Careful acidification of the combined aqueous layers with dilute hydrochloric acid, extraction with dichloromethane (3×20 cm^3), drying of the combined organic layers ($MgSO_4$), filtration and concentration to dryness gave the acid **27** (0.09 g, 95%) as a colourless glass-like solid: mp 74–77 °C: $[\alpha]_D$ +69.8 (*c* 0.99 in CH_2Cl_2); ν_{max} (CH_2Cl_2)/cm^{-1} 3415, 3060, 3028, 2978, 2879, 2621, 1711, 1655, 1497, 1454, 1392, 1367, 1252, 1167, 1120, 1092, 1081, 1049, 1029, 988, 942, 915, 887, 872, 814, 764, 736, 705 and 653; δ_H (400 MHz; $CDCl_3$) 1.14–1.21 (1H, m, Proγ-H_AH_B), 1.50 [9H, s, $C(CH_3)_3$], 1.72–1.78 (1H, m, Proγ-H_AH_B), 2.11–2.29 (2H, m, Proβ-H_2), 2.94–3.00 (1H, m, Proδ-H_AH_B), 3.13 (1H, d, *J*=13.9 Hz, PhCH_AH_B), 3.42–3.48 (1H, m, Proδ-H_AH_B), 3.83 (1H, d, *J*=13.9 Hz, PhCH_AH_B), 4.13 (2H, m, Glyα-H_2), 5.69 (1H, br s, Gly-NH), 7.10–7.33 (5H, m, PhH) and 8.37 (1H, br s, OH); δ_C (100 MHz; $CDCl_3$) 23.3 (CH_2, Proγ-C), 28.3 [CH_3, $C(CH_3)_3$], 34.5 (CH_2, Proβ-C), 37.8 (CH_2, PhCH_2), 43.7 (CH_2, Glyα-C), 47.8 (CH_2, Proδ-C), 70.3 (quat., Proα-C), 79.8 [quat., $C(CH_3)_3$], 126.9 (CH, Ph), 128.4 (CH, Ph), 130.5 (CH, Ph), 136.3 (quat., Ph), 156.0 (quat., NCO_2), 168.6 (quat., Gly-CON) and 176.9 (quat., CO_2H); *m*/*z* (EI+) 362.1834 (M^+ $C_{19}H_{26}N_2O_5$ requires 362.1842).

4.1.20. Dibenzyl *N*-benzyloxycarbonyl-glycyl-L-2-methylprolyl-L-glutamate 30. Triethylamine (0.50 cm^3, 3.59 mmol) was added dropwise to a solution of dipeptide **23** (0.36 g, 1.12 mmol) and L-glutamic acid dibenzyl ester *p*-toluenesulphonate **28** (0.73 g, 1.46 mmol) in dichloromethane (60 cm^3) under nitrogen at room temperature, and the reaction mixture stirred for 10 min. Bis(2-oxo-3-oxazolidinyl)phosphinic chloride (0.37 g, 1.41 mmol) was added and the colourless solution stirred for 17 h. The dichloromethane solution was washed successively with 10% aqueous hydrochloric acid (50 cm^3) and saturated aqueous sodium hydrogen carbonate (50 cm^3), dried ($MgSO_4$), filtered, and evaporated to dryness in vacuo. Purification of the resultant residue by repeated flash column chromatography (30–70% ethyl acetate–hexane; gradient elution) yielded protected tripeptide **30** (0.63 g, 89%) as a colourless oil. Tripeptide **30** was shown to adopt exclusively the trans conformer by NMR: R_f 0.55 (EtOAc): $[\alpha]_D$ −41.9 (*c* 0.29 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3583,

3353 br, 2950, 1734, 1660, 1521, 1499, 1454, 1429, 1257, 1214, 1188, 1166, 1051, 911, 737 and 697; δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.64 (3H, s, Proα-CH_3), 1.72 (1H, dt, J= 12.8, 7.6, 7.6 Hz, Proβ-H_AH_B), 1.92 (2H, 5 lines, J=6.7 Hz, Proγ-H_2), 2.04 (1H, 6 lines, J=7.3 Hz Gluβ-H_AH_B), 2.17–2.27 (1H, m, Gluβ-H_AH_B), 2.35–2.51 (3H, m, Proβ-H_AH_B and Gluγ-H_2), 3.37–3.57 (2H, m, Proδ-H_2), 3.90 (1H, dd, J=17.0, 3.6 Hz, Glyα-H_AH_B), 4.00 (1H, dd, J=17.1, 5.1 Hz, Glyα-H_AH_B), 4.56 (1H, td, J=7.7, 4.9 Hz, Gluα-H), 5.05–5.20 (6H, m, 3×OCH_2Ph), 5.66–5.72 (1H, br m, Gly-NH), 7.26–7.37 (15H, m, 3×Ph) and 7.44 (1H, d, J= 7.2 Hz, Glu-NH); δ_C (100 MHz; $CDCl_3$) 21.9 (CH_3, Proα-CH_3), 23.4 (CH_2, Proγ-C), 26.6 (CH_2, Gluβ-C), 30.1 (CH_2, Gluγ-C), 38.3 (CH_2, Proβ-C), 43.9 (CH_2, Glyα-C), 47.6 (CH_2, Proδ-C), 52.2 (CH, Gluα-C), 66.4 (CH_2, OCH_2Ph), 66.8 (CH_2, OCH_2Ph), 67.1 (CH_2, OCH_2Ph), 68.2 (quat., Proα-C), 127.9 (CH, Ph), 128.0 (CH, Ph), 128.1, (CH, Ph), 128.2, (CH, Ph), 128.2, (CH, Ph), 128.3, (CH, Ph), 128.4, (CH, Ph), 128.5, (CH, Ph), 128.5, (CH, Ph), 135.2 (quat., Ph), 135.7 (quat., Ph), 136.4 (quat., Ph), 156.1 (quat., NCO_2), 167.3 (quat., Gly-CO), 171.4 (quat., CO), 172.9 (quat., CO) and 173.4 (quat., CO); m/z (FAB+) 630.2809 (MH^+ $C_{35}H_{40}N_3O_8$ requires 630.2815).

4.1.21. Dibenzyl *N*-benzyloxycarbonyl-glycyl-L-2-ethyl-prolyl-L-glutamate 31. Dry triethylamine (0.44 cm^3, 3.16 mmol) was added to a solution of acid **24** (0.30 g, 0.91 mmol) and L-glutamic acid dibenzyl ester *p*-toluene sulphonate **28** (0.57 g, 1.13 mmol) in dry dichloromethane (50 cm^3) under an atmosphere of nitrogen at 0 °C, and the reaction mixture stirred for 10 min. Bis(2-oxo-3-oxazolidinyl)phosphinic chloride (0.29 g, 1.14 mmol) was added and the solution stirred for 2 h, warmed to room temperature and further stirred for 17.5 h. The solution was washed successively with 0.5 M aqueous hydrochloric acid (10 cm^3) and saturated aqueous sodium hydrogen carbonate (10 cm^3), dried ($MgSO_4$), filtered and evaporated in vacuo to form a light orange gum. Purification of the resultant residue by flash column chromatography (30% ethyl acetate/hexane) yielded protected tripeptide **31** (0.41 g, 70%) as a clear oil: $[\alpha]_D$ −52.7 (c 0.16 in MeOH); δ_H (300 MHz; $CDCl_3$) 0.78 (3H, t, J=7.4 Hz, CH_3), 1.25–2.24 (7H, m, CH_2CH_3, Gluβ-H_2, Proγ-H_2 and Proβ-H_AH_B), 2.34–2.40 (2H, m, Gluγ-H_2), 2.50–2.60 (1H, m, Proβ-H_AH_B), 3.34 (1H, q, J=9.4 Hz, Proδ-H_AH_B), 3.49–3.53 (1H, m, Proδ-H_AH_B), 3.96 (2H, ddd, J=4.9, 17.3 Hz, Glyα-H_2), 4.51–4.54 (1H, td, J=5.4, 7.8 Hz, Gluα-H), 5.06–5.19 (6H, m, 3×OCH_2Ph), 5.70 (1H, br s, Gly-NH), 7.26–7.36 (15H, 3×Ph) and 8.09 (1H, d, J=7.3 Hz, Glu-NH); δ_C (75 MHz; $CDCl_3$) 8.8 (CH_3, CH_3), 23.6 (CH_2, CH_2CH_3), 27.2 (CH_2, Proγ-C), 27.7 (CH_2, Gluβ-C), 30.6 (CH_2, Gluγ-C), 34.3 (CH_2, Proβ-C), 44.5 (CH_2, Glyα-C), 49.0 (CH_2, Proδ-C), 52.6 (CH, Gluα-C), 66.9 (CH_2, OCH_2Ph), 67.3 (CH_2, OCH_2Ph), 67.5 (CH_2, OCH_2Ph), 73.9 (quat., Proα-C), 128.4 (CH, Ph), 128.5 (CH, Ph), 128.6 (CH, Ph), 128.7 (CH, Ph), 128.8 (CH, Ph), 128.9 (CH, Ph), 135.7 (quat., Ph), 136.1 (quat., Ph), 136.8 (quat., Ph), 156.6 (quat., NCO_2), 168.7 (quat., Gly-CON), 171.8 (quat., Pro-CON), 172.9 (quat., Gluα-CO) and 173.6 (quat., Gluγ-CO); m/z (FAB+) 644.2981 (MH^+ $C_{36}H_{42}N_3O_8$ requires 644.2972).

4.1.22. Dibenzyl *N*-benzyloxycarbonyl-glycyl-L-2-allyl-prolyl-L-glutamate 32. Dry triethylamine (0.07 cm^3, 0.49 mmol) was added dropwise to a solution of acid **25** (0.05 g, 0.15 mmol) and L-glutamic acid dibenzyl ester *p*-toluenesulphonate **28** (0.10 g, 0.20 mmol) in dry dichloromethane (8.2 cm^3) under an atmosphere of nitrogen at room temperature, and the reaction mixture stirred for 10 min. Bis(2-oxo-3-oxazolidinyl)phosphinic chloride (0.05 g, 0.19 mmol) was added and the solution was stirred for 19.5 h. The solution was washed successively with 10% aqueous hydrochloric acid (7 cm^3) and saturated aqueous sodium hydrogen carbonate (7 cm^3), dried ($MgSO_4$), filtered and evaporated to dryness in vacuo. Purification of the resultant residue by flash column chromatography (50–80% ethyl acetate–hexane; gradient elution) yielded protected tripeptide **32** (0.08 g, 76%) as a colourless oil: $[\alpha]_D$ −27.8 (c 0.79 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3943, 3583, 3413, 3055, 3032, 2982, 2956, 2880, 2685, 2411, 2305, 1955, 1732, 1668, 1586, 1499, 1454, 1423, 1388, 1329, 1265, 1214, 1169, 1170, 1081, 1058, 1028, 994, 924, 897, 824, 737 and 701; δ_H (400 MHz; $CDCl_3$) 1.85 (3H, m, Proβ-H_AH_B and Proγ-H_2), 1.99–2.08 (1H, m, Gluβ-H_AH_B), 2.17–2.25 (1H, m, Gluβ-H_AH_B), 2.32–2.49 (3H, m, Proβ-H_AH_B and Gluγ-H_2), 2.71–2.76 (1H, m, CH_AH_BCH=CH_2), 3.05–3.10 (1H, m, CH_AH_BCH=CH_2), 3.33 (1H, m, Proδ-H_AH_B), 3.51 (1H, m, Proδ-H_AH_B), 3.96 (2H, d, J=3.9 Hz, Glyα-H_2), 4.55 (1H, dd, J=7.6, 5.1 Hz, Gluα-H), 5.07–5.19 (8H, m, 3×PhCH_2 and CH=CH_2), 5.53–5.63 (1H, m, CH=CH_2), 5.71 (1H, br s, Gly-NH), 7.32–7.36 (15H, m, 3×Ph) and 7.90 (1H, d, J=7.2 Hz, Glu-NH); δ_C (100 MHz; $CDCl_3$) 23.1 (CH_2, Proγ-C), 26.7 (CH_2, Gluβ-C), 30.2 (CH_2, Gluγ-C), 34.2 (CH_2, Proβ-C), 37.9 (CH_2, CH_2-CH=CH_2), 44.1 (CH_2, Glyα-C), 48.5 (CH_2, Proδ-C), 52.2 (CH, Gluα-C), 66.4 (CH_2, PhCH_2), 66.9 (CH_2, PhCH_2), 67.2 (CH_2, PhCH_2), 71.9 (quat., Proα-C), 119.9 (CH_2, CH=CH_2), 127.9 (CH, Ph), 128.05 (CH, Ph), 128.1 (CH, Ph), 128.2 (CH, Ph), 128.3 (CH, Ph), 128.4 (CH, Ph), 128.45 (CH, Ph), 128.5 (CH, Ph), 132.1 (CH, CH=CH_2), 135.3 (quat., Ph), 135.7 (quat., Ph), 136.4 (quat., Ph), 156.2 (quat., NCO_2), 168.1 (quat., Gly-CO), 171.3 (quat., Gluα-CO), 172.7 (quat., Gluγ-CO) and 173.0 (quat., Pro-CON); m/z (FAB+) 656.2970 (MH^+ $C_{37}H_{42}N_3O_8$ requires 656.2992).

4.1.23. Di-*tert*-butyl *N*-*tert*-butyloxycarbonyl-glycyl-L-2-allylprolyl-L-glutamate 33. Dry triethylamine (0.044 cm^3, 0.32 mmol) was added dropwise to a solution of acid **26** (0.031 g, 0.10 mmol) and L-glutamic acid di-*tert*-butyl ester hydrochloride **29** (0.038 g, 0.129 mmol) in dry dichloromethane (5.30 cm^3) under an atmosphere of nitrogen at room temperature, and the reaction mixture stirred for 10 min. Bis(2-oxo-3-oxazolidinyl)phosphinic chloride (0.032 g, 0.13 mmol) was added and the solution stirred for 17.5 h. The solution was washed successively with 10% aqueous hydrochloric acid (5 cm^3) and saturated aqueous sodium hydrogen carbonate (5 cm^3), dried ($MgSO_4$), filtered and evaporated to dryness in vacuo. Purification of the resultant residue by flash column chromatography (50% ethyl acetate–hexane) yielded protected tripeptide **33** (0.43 g, 77%) as a light yellow oil: $[\alpha]_D$ −29.9 (c 0.90 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3583, 3417, 3076, 2978, 2931, 1728, 1664, 1522, 1453, 1426, 1392, 1367, 1329, 1251, 1158, 1056, 1028, 949, 919, 846 and 735; δ_H (300 MHz; $CDCl_3$) 1.26 [9H, s, $C(CH_3)_3$], 1.42 [9H, s, $C(CH_3)_3$], 1.43 [9H, s, $C(CH_3)_3$], 1.85–1.94 (4H, m, Proβ-H_AH_B, Gluβ-H_AH_B and Proγ-H_2), 2.02–2.12 (1H, m, Gluβ-H_AH_B),

2.16–2.33 (2H, m, Gluγ-H_2), 2.48–2.53 (1H, m, Proβ-H_AH_B), 2.69–2.77 (1H, m, 0.5 CH_AH_BCH=CH_2), 3.08–3.15 (1H, m, CH_AH_BCH=CH_2), 3.29–3.38 (1H, m, Proδ-H_AH_B), 3.53–3.56 (1H, m, Proδ-H_AH_B), 3.91 (2H, d, J=4.0 Hz, Glyα-H_2), 4.33 (1H, dd, J=7.5, 5.2 Hz, Gluα-H), 5.08–5.14 (2H, m, CH=CH_2), 5.47 (1H, br s, Gly-NH), 5.52–5.66 (1H, m, CH=CH_2) and 7.77 (1H, d, J=7.4 Hz, Glu-NH); δ_C (75 MHz; $CDCl_3$) 23.1 (CH_2, Proγ-C), 27.4 (CH_2, Gluβ-C), 27.9 [CH_3, C(CH_3)$_3$], 28.0 [CH_3, C(CH_3)$_3$], 28.3 [CH_3, C(CH_3)$_3$], 31.5 (CH_2, Gluγ-C), 34.2 (CH_2, Proβ-C), 38.0 (CH_2, CH_2CH=CH_2), 43.7 (CH_2, Glyα-C), 48.4 (CH_2, Proδ-C), 52.7 (CH, Gluα-C), 71.8 (quat., Proα-C), 79.5 [quat., C(CH_3)$_3$], 80.6 [quat., C(CH_3)$_3$], 81.9 [quat., C(CH_3)$_3$], 119.8 (CH_2, CH=CH_2), 132.3 (CH, CH=CH_2), 155.7 (quat., NCO_2), 168.4 (quat., Gly-CO), 170.8 (quat., Gluα-CO), 172.3 (quat., Gluγ-CO) and 172.8 (quat., Pro-CON); m/z (EI+) 553.3359 (M^+ $C_{28}H_{47}N_3O_8$ requires 553.3363).

4.1.24. Di-*tert*-butyl *N*-*tert*-butyloxycarbonyl-glycyl-L-2-benzylprolyl-L-glutamate 34. Dry triethylamine (0.11 cm^3, 0.77 mmol) was added dropwise to a solution of acid **27** (0.09 g, 0.24 mmol) L-glutamic acid di-*tert*-butyl ester hydrochloride **29** (0.09 g, 0.31 mmol) in dry dichloromethane (13 cm^3) under an atmosphere of nitrogen at room temperature, and the reaction mixture stirred for 10 min. Bis(2-oxo-3-oxazolidinyl)phosphinic chloride (0.08 g, 0.30 mmol) was added and the solution stirred for 17 h, then washed successively with 10% aqueous hydrochloric acid (12 cm^3) and saturated aqueous sodium hydrogen carbonate (12 cm^3), dried ($MgSO_4$), filtered and evaporated to dryness in vacuo. Purification of the resultant residue by flash column chromatography (40% ethyl acetate–hexane) yielded protected tripeptide **34** (0.10 g, 68%) as a colourless oil: $[\alpha]_D$ +15.7 (c 1.15 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3418, 3357, 3060, 3028, 2978, 2933, 2875, 1727, 1663, 1582, 1519, 1497, 1454, 1426, 1392, 1367, 1330, 1251, 1158, 1093, 1051, 1029, 949, 914, 846, 736, 705 and 651; δ_H (400 MHz; $CDCl_3$) 1.28–1.39 (1H, m, Proγ-H_AH_B), 1.43 [27H, s, C(CH_3)$_3$], 1.47 [27H, s, C(CH_3)$_3$], 1.48 [27H, s, C(CH_3)$_3$], 1.69–1.74 (1H, m, Proγ-H_AH_B), 1.89–1.99 (2H, m, Proβ-H_2), 2.08–2.17 (1H, m, Gluβ-H_AH_B), 2.23–2.41 (3H, m, Gluβ-H_AH_B and Gluγ-H_2), 2.89–2.95 (1H, m, Proδ-H_AH_B), 3.13 (1H, d, J=13.4 Hz, PhCH_AH_B), 3.40–3.46 (1H, m, Proδ-H_AH_B), 3.85 (1H, d, J=13.4 Hz, PhCH_AH_B), 3.92 (2H, d, J=3.8 Hz, Glyα-H_2), 4.33 (1H, td, J=7.5, 5.2 Hz, Gluα-H), 5.58 (1H, br s, Gly-NH), 7.07–7.28 (5H, m, PhH) and 7.71 (1H, d, J=7.2 Hz, Glu-NH); δ_C (100 MHz; $CDCl_3$) 23.0 (CH_2, Proγ-C), 27.4 (CH_2, Gluβ-C), 28.0 [CH_3, C(CH_3)$_3$], 28.1 [CH_3, C(CH_3)$_3$], 28.3 [CH_3, C(CH_3)$_3$], 31.5 (CH_2, Gluγ-C), 34.2 (CH_2, Proβ-C), 38.0 (CH_2, PhCH_2), 44.1 (CH_2, Glyα-C), 48.2 (CH_2, Proδ-C), 52.9 (CH, Gluα-C), 72.4 (quat., Proα-C), 79.6 [quat., C(CH_3)$_3$], 80.7 [quat., C(CH_3)$_3$], 82.1 [quat., C(CH_3)$_3$], 126.8 (CH, Ph), 128.3 (CH, Ph), 130.4 (CH, Ph), 136.3 (quat., Ph), 155.8 (quat., NCO_2), 168.6 (quat., Gly-CO), 171.0 (quat., Gluα-CO), 172.5 (quat., Gluγ-CO) and 173.1 (quat., Pro-CON); m/z (FAB+) 604.3592 (MH^+ $C_{32}H_{50}N_3O_8$ requires 604.3598).

4.1.25. Glycyl-L-2-methylprolyl-L-glutamic acid 1. A mixture of the protected tripeptide **30** (0.63 g, 1.00 mmol) and 10% palladium on activated carbon (0.32 g, 0.30 mmol) in 90:10 methanol–water (22 cm^3) was stirred under an atmosphere of hydrogen at room temperature, protected from light, for 23 h. The reaction mixture was filtered through a Celite™ pad and the pad washed with 75:25 methanol–water (200 cm^3). The filtrate was concentrated to dryness under reduced pressure and the residue triturated with anhydrous diethyl ether to afford tripeptide **1** (0.27 g, 86%) as an hygroscopic colourless solid. Tripeptide **1** was shown to adopt the trans conformation by NMR analysis: mp 144 °: $[\alpha]_D$ −52.4 (c 0.19 in H_2O); δ_H (400 MHz; D_2O) 1.62 (3H, s, Proα-CH_3), 1.97–2.25 (6H, m, Proβ-H_2, Proγ-H_2 and Gluβ-H_2), 2.45 (2H, t, J=7.3 Hz, Gluγ-H_2), 3.62–3.70 (2H, m, Proδ-H_2), 3.96 (1H, d, J=16.5 Hz, Glyα-H_AH_B), 4.02 (1H, d, J=16.4 Hz, Glyα-H_AH_B) and 4.28 (1H, dd, J=8.4, 4.7 Hz, Gluα-H); δ_C (100 MHz; D_2O) 19.9 (CH_3, Proα-CH_3), 23.0 (CH_2, Proγ-C), 26.9 (CH_2, Gluβ-C), 30.9 (CH_2, Gluγ-C), 38.8 (CH_2, Proβ-C), 40.7 (CH_2, Glyα-C), 47.5 (CH_2, Proδ-C), 54.4 (CH, Gluα-C), 67.8 (quat., Proα-C), 164.6 (quat., Gly-CO), 175.3 (quat., Pro-CON), 177.2 (quat., Gluα-CO), and 178.5 (quat., Gluγ-CO); m/z (FAB+) 316.1508 (MH^+ $C_{13}H_{22}N_3O_6$ requires 316.1509).

4.1.26. Glycyl-L-2-ethylprolyl-L-glutamic acid 2. A mixture of protected tripeptide **31** (0.51 g, 0.80 mmol) and 10% palladium on activated carbon (0.09 g, 0.08 mmol) in 90:10 methanol–water (50 cm^3) was stirred under an atmosphere of hydrogen at room temperature for 20 h. The solution was filtered through a Celite™ pad, the pad washed with methanol (2×30 cm^3) and the filtrate evaporated to dryness to give a clear gum. The gum was placed under vacuum for 30 min then triturated with anhydrous diethyl ether to tripeptide **2** (0.26 g, 99%) as an hygroscopic colourless solid. Tripeptide **2** was shown to be exclusively the trans conformer by ^1H and ^{13}C NMR analysis: mp 82–85 °C: $[\alpha]_D$ −43.8 (c 0.1 in MeOH); δ_H (400 MHz; D_2O) 0.86 (3H, t, J=7.4 Hz, CH_3), 1.94–2.40 (8H, m, C$H_2$$CH_3$, Gluβ-$H_2$, Proβ-$H_2$ and Pro-γH_2), 2.52–2.56 (2H, m, Gluγ-H_2), 3.55–3.61 (1H, td, J=6.9, 9.7 Hz, Proδ-H_AH_B), 3.75–3.80 (1H, m, Proδ-H_AH_B), 4.08 (2H, q, J=16.6 Hz, Glyα-H_2) and 4.44 (1H, q, J=4.9 Hz, Gluα-H); δ_C (100 MHz; $CDCl_3$) 6.9 (CH_3, CH_3), 22.8 (CH_2, CH_2CH_3), 25.3 (CH_2, Proγ-C), 25.5 (CH_2, Gluβ-C), 30.11 (CH_2, Gluγ-C), 35.0 (CH_2, Proβ-C), 40.7 (CH_2, Glyα-C), 48.6 (CH_2, Proδ-C), 52.6 (CH, Gluα-C), 71.7 (quat., Proα-C), 164.9 (quat., Gly-CON), 175.2 (quat., Pro-CON) 176.0 (quat., Gluα-CO) and 177.3 (quat., Gluγ-CO); m/z (FAB+) 330.1667 (MH^+ $C_{14}H_{24}N_3O_6$ requires 330.1665).

4.1.27. Glycyl-L-2-propylprolyl-L-glutamic acid 3. A mixture of protected tripeptide **32** (64 mg, 0.097 mmol) and 10% palladium on activated carbon (20 mg, 0.19 mmol) in 90:10 methanol–water (9.8 cm^3) was stirred under an atmosphere of hydrogen at room temperature, protected from light, for 19 h. The reaction mixture was filtered through a Celite™ pad and the pad washed with 75:25 methanol–water (50 cm^3). The filtrate was concentrated to dryness under reduced pressure to afford tripeptide **3** (33 mg, 100%) as a colourless solid. Tripeptide **3** was shown to be exclusively the trans conformer by NMR analysis: mp 278–280 °C (dec.): $[\alpha]_D$ −16.7 (c 0.18 in H_2O); δ_H (300 MHz; D_2O) 0.98 (3H, t, CH_2CH_3), 1.09 (1H, m, C$H_AH_BCH_3$), 1.44 (1H, m, C$H_AH_BCH_3$), 1.82–2.31 (8H, m, Proβ-H_2, Proγ-H_2, Gluβ-H_2 and C$H_2CH_2CH_3$),

2.40 (1H, m, Gluγ-H_2), 3.55–3.63 (1H, m, Proδ-H_AH_B), 3.80 (1H, m, Proδ-H_AH_B), 4.03 (1H, d, J=16.6 Hz, Glyα-H_AH_B), 4.15 (1H, d, J=16.6 Hz, Glyα-H_AH_B) and 4.27 (1H, dd, J=8.2, 4.8 Hz, Gluα-H); δ_C (100 MHz; D_2O) 16.0 (CH_3, CH_2CH_3), 19.0 (CH_2, CH_2CH_3), 25.4 (CH_2, Proγ-C), 30.9 (CH_2, Gluβ-C), 36.3 (CH_2, Gluγ-C), 37.4 (CH_2, $CH_2CH_2CH_3$), 38.3 (CH_2, Proβ-C), 43.3 (CH_2, Glyα-C), 51.2 (CH_2, Proδ-C), 58.1 (CH, Gluα-C), 74.1 (quat., Proα-C), 167.7 (quat., NCO), 177.8 (quat., Pro-CON), 180.9 (quat., Gluα-CO) and 184.7 (quat., Gluγ-CO); m/z (FAB+) 344.1827 (MH^+ $C_{15}H_{26}N_3O_6$ requires 344.1822).

4.1.28. Glycyl-L-2-allylprolyl-L-glutamic acid trifluoroacetate 4. To a solution of the protected tripeptide **33** (41 mg, 0.073 mmol) in dichloromethane (4.5 cm^3) at room temperature was added trifluoroacetic acid (0.75 cm^3, 9.74 mmol) dropwise and the reaction mixture was stirred for 6.5 h. The solution was evaporated under reduced pressure to form tripeptide **4** (32 mg, 96%) as a pale yellow solid. Tripeptide **4** was shown to exist exclusively as the trans-conformer by NMR analysis: mp 105–108 °C: $[\alpha]_D$ −7.64 (c 0.39 in H_2O); δ_H (400 MHz; D_2O) 1.90–2.02 (1H, m, Proγ-H_AH_B), 2.03–2.14 (2H, m, Proγ-H_AH_B and Gluβ-H_AH_B), 2.19–2.32 (3H, m, Proβ-H_2 and Gluβ-H_AH_B), 2.54 (2H, ddd, J=8.1, 7.3, 2.0 Hz, Gluγ-H_2), 2.74 (1H, dd, J=13.7, 7.3 Hz, CH_AH_BCH=CH_2), 3.12 (1H, dd, J=13.7, 7.3, 0.5 Hz CH_AH_BCH=CH_2), 3.48–3.55 (1H, m, Proδ-H_AH_B), 3.72–3.77 (1H, m, Proδ-H_AH_B), 3.98 (1H, d, J=16.7 Hz, Glyα-H_AH_B), 4.10 (1H, d, J=16.7 Hz, Glyα-H_AH_B), 4.46 (1H, dd, J=4.9, 9.5 Hz, Gluα-H) 5.20–5.26 (2H, m, CH=CH_2) and 5.73–5.82 (1H, m, CH=CH_2); δ_C (100 MHz; D_2O) 25.4 (CH_2, Proγ-C), 28.1 (CH_2, Gluβ-C), 32.7 (CH_2, Gluγ-C), 38.1 (CH_2, Proβ-C), 39.1 (CH_2, CH_2CH=CH_2), 43.3 (CH_2, Glyα-C), 51.0 (CH_2, Proδ-C), 55.1 (CH, Gluα-C), 73.2 (quat., Proα-C), 120.3 (quat., CF_3), 122.6 (CH_2, CH=CH_2), 134.4 (CH, CH=CH_2), 165.4 (quat., CF_3CO_2), 167.5 (quat., Gly-CO), 177.5 (quat., Pro-CON), 178.0 (quat., Glyα-CO) and 179.8 (quat., Gluγ-CO); m/z (EI+) 342.1653 ($M^+ - CF_3CO_2$ $C_{15}H_{24}N_3O_6$ requires 342.1665).

4.1.29. Glycyl-L-2-benzylprolyl-L-glutamic acid trifluoroacetate 5. To a solution of the protected tripeptide **34** (98 mg, 0.16 mmol) in dichloromethane (4 cm^3) at room temperature was added trifluoroacetic acid (0.75 cm^3) dropwise and the reaction mixture was stirred for 3.5 h. The solution was evaporated under reduced pressure to give tripeptide **5** (82 mg, 100%) as an hygroscopic colourless solid. Tripeptide **5** was shown to be a 90:10 trans:cis mixture of conformers by ^{1}H NMR analysis (the ratio was estimated from the relative intensities of the double doublets and multiplet at δ 4.51 and 4.33, assigned to the Gluα-H protons of the major and minor conformers, respectively): mp 73–82 °C: $[\alpha]_D$ +41.0 (c 1.61 in MeOH); δ_H (300 MHz; D_2O) 1.27–1.39 (1H, m, Proγ-H_AH_B), 1.68–1.83 (1H, m, Proγ-H_AH_B), 2.07–2.42 (4H, m, Proβ-H_2 and Gluβ-H_2), 2.57 (2H, t, J=7.1 Hz, Gluγ-H_2), 2.82–2.92 (1H, m, Proδ-H_AH_B), 3.24 (1H, d, J=13.3 Hz, PhCH_AH_B), 3.50–3.59 (1H, m, Proδ-H_AH_B), 3.70 (1H, d, J=13.3 Hz, PhCH_AH_B), 3.91 (1H, d, J=16.7 Hz, Glyα-H_AH_B), 4.09 (1H, d, J=16.7 Hz, Glyα-H_AH_B), 4.33* (0.1H, m, Gluα-H), 4.51 (0.9H, dd, J=4.8, 9.6 Hz, Gluα-H) and 7.21–7.44 (5H, m, PhH); δ_C (100 MHz; D_2O) 25.0 (CH_2, Proγ-C), 27.9 (CH_2, Gluβ-C), 32.5 (CH_2, Gluγ-C), 37.7 (CH_2, Proβ-C), 39.2 (CH_2, PhCH_2), 43.5 (CH_2, Glyα-C), 50.6 (CH_2, Proδ-C), 54.9 (CH, Gluα-C), 56.0* (CH, Gluα-C), 73.8 (quat., Proα-C), 117.0 (quat., CF_3), 129.6 (CH, Ph), 131.1 (CH, Ph), 132.9 (CH, Ph), 138.3 (quat., Ph), 165.4 (quat., CF_3CO_2), 167.7 (quat., Gly-CO), 177.2 (quat., Pro-CON), 178.0 (quat., Gluα-CO) and 179.7 (quat., Gluγ-CO); m/z (FAB+) 392.1817 ($M^+ - CF_3CO_2$ $C_{19}H_{26}N_3O_6$ requires 392.1822).

4.1.30. Dibenzyl (2*S*,5′*R*,8′*R*)- and (2*S*,5′*R*,8′*S*)-[1′-(2″-benzyloxycarbonylamino-acetyl)-8′-hydroxy-6′-oxo-1′,7′-diazaspiro[4.4]non-7′-yl]-1,5-pentanedioate 38. Alkene **32** (96 mg, 0.15 mmol) was dissolved in dry dichloromethane–methanol (6 cm^3, 1/1) and the solution cooled to −78 °C. A slow stream of ozone was bubbled through the solution for 15 min, followed by O_2 to remove excess ozone. Triphenylphosphine (57.5 mg, 0.22 mmol) was added and the resulting mixture vigorously stirred for 24 h, then a small amount of silica was added to the reaction mixture (containing aldehyde **37**) and the solvent removed under reduced pressure. The resulting residue was purified by flash column chromatography (100% ethyl acetate) to afford hydroxyspirolactam **38** (61 mg, 63%) as a pale yellow oil. Hydroxyspirolactam **38** was shown to be a 7:3 mixture of diastereomers by ^{1}H NMR analysis. The ratio was estimated from the relative intensities of the multiplet at δ 4.76–4.79 and the doublet of doublets at δ 5.00, assigned to the 2-H protons of the minor and major isomers, respectively) with the isomers being inseparable: $[\alpha]_D$ −44.4 (c 0.90 in MeOH); ν_{max} (film)/cm^{-1} 3410, 3064, 3034, 2953, 2881, 2083, 1718, 1649, 1498, 1454, 1332, 1267, 1215, 1170, 1121, 1082, 1048, 1028, 1003, 984, 909, 776, 736 and 698; δ_H (400 MHz; $CDCl_3$) 1.74–1.77[1] (0.3H, m, 9′-H_AH_B), 1.93–2.04 (1.3H, m, 9′-H_AH_B and 3′-H_A*H_B), 2.11–2.39 [4.4H, m, 4′-H_AH_B, 3′-H_AH_B, 3-H_AH_B and 3-H_AH_B(major)], 2.42–2.66 (3.3H, m, 3-H_AH_B*, 4-H_2 and 4′-H_AH_B), 2.76 (0.7H, dd, J=12.9, 6.1 Hz, 9′-H_AH_B), 3.46–3.58 (2H, m, 2′-H_2), 3.80–3.85* (0.3H, obscured, 2″-H_AH_B), 3.87 (0.7H, dd, J=17.5, 3.3 Hz, 2″-H_AH_B), 4.02 (0.7H, dd, J=17.5, 5.1 Hz, 2″-H_AH_B), 3.99–4.05* (0.3H, obscured, 2″-H_AH_B), 4.69 (1H, s, OH), 4.76–4.79* (0.3H, m, 2-H), 5.00 (0.7H, dd, J=10.8, 4.8 Hz, 2-H), 5.09–5.21 (6H, m, 3×OCH_2Ph), 5.25* (0.3H, dd, J=12.3, 7.1 Hz, 8′-H), 5.42 (0.7H, dd, J=6.0, 2.4 Hz, 8′-H), 5.48* (0.3H, m, NH), 5.58–5.59 (0.7H, m, NH) and 7.27–7.39 (15H, m, 3× Ph); δ_C (100 MHz; $CDCl_3$) 24.1* (CH_2, 3-C), 24.2 (CH_2, 3-C), 24.5 (CH_2, 3′-C), 25.3* (CH_2, 3′-C), 30.2 (CH_2, 4′-C), 30.7* (CH_2, 4′-C), 37.9 (CH_2, 4-C), 39.4 (CH_2, 9-C), 39.7* (CH_2, 9-C), 43.0* (CH_2, 2″-C), 43.7 (CH_2, 2″-C), 47.0 (CH_2, 2-C), 47.7* (CH_2, 2-C), 54.3 (CH, 2′-C), 54.7* (CH, 2′-C), 65.6* (quat., 5-C), 66.2* (CH_2, OCH_2Ph), 66.4 (CH_2, OCH_2Ph), 66.8 (CH_2, OCH_2Ph), 67.0* (CH_2, OCH_2Ph), 67.2* (CH_2, OCH_2Ph), 67.3 (CH_2, OCH_2Ph), 68.1 (quat., 5-C), 77.7 (CH, 8-C), 80.6* (CH, 8-C), 128.05 (CH, Ph), 128.09 (CH, Ph), 128.2 (CH, Ph), 128.4 (CH, Ph), 128.5 (CH, Ph), 128.6 (CH, Ph), 128.7 (CH, Ph), 134.5 (quat., Ph), 135.3* (quat., Ph), 135.8 (quat., Ph), 136.0* (quat., Ph), 136.4 (quat., Ph), 156.1 (quat., NCO_2), 166.0 (quat., 1″-C), 167.1* (quat., 1″-C), 170.1* (quat., 1′-C), 172.5 (quat., 1′-C), 172.7* (quat., 5′-C), 173.6 (quat., 5′-C), 173.8* (quat., 6-C) and 175.1 (quat., 6-C); m/z (FAB+) 658.2749 (MH^+ $C_{36}H_{40}N_3O_9$ requires 658.2765).

4.1.31. Dibenzyl (2*S*,5′*R*)-[1′-(2″-benzyloxycarbonyl-amino-acetyl)-6′-oxo-1′,7′-diazaspiro[4.4]non-7′-yl]-1,5-pentanedioate 39. A solution of hydroxyspirolactam **38** (33 mg, 0.05 mmol) in trifluoroacetic acid–triethylsilane–dichloromethane (1.0 cm^3, 1/1/1), at room temperature, was stirred for 45 min then concentrated in vacuo to give an opaque white oil, which was purified by flash column chromatography (100% ethyl acetate) to afford spirolactam **39** (31 mg, 96%) as a colourless oil: $[\alpha]_D$ −18.0 (*c* 0.67 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3583, 3412, 3032, 2952, 1734, 1654, 1497, 1454, 1432, 1289, 1259, 1215, 1171, 1048, 982, 738 and 698; δ_H (300 MHz; $CDCl_3$) 1.76–2.19 (6H, m, 9′-H_AH_B, 4′-H_2, 3′-H_AH_B and 3-H_2), 2.31–2.63 (4H, m, 9′-H_AH_B, 3′-H_AH_B and 4-H_2), 3.31 (1H, dd, *J*=16.8, 8.1 Hz, 8′-H_AH_B), 3.40–3.47 (1H, m, 8′-H_AH_B), 3.51–3.55 (2H, m, 2′-H_2), 3.86 (1H, dd, *J*=17.1, 3.3 Hz, 2″-H_AH_B), 4.05 (1H, dd, *J*=17.1, 5.4 Hz, 2″-H_AH_B), 4.90 (1H, dd, *J*=11.3, 4.5 Hz, 2-H), 5.07–5.18 (6H, m, 3×OCH_2Ph), 5.63 (1H, br s, N–H) and 7.27–7.34 (15H, m, 3Ph); δ_C (75 MHz; $CDCl_3$) 23.4 (CH_2, 3′-C), 24.0 (CH_2, 3-C), 29.8 (CH_2, 9′-C), 30.4 (CH_2, 4-C), 36.2 (CH_2, 4′-C), 39.8 (CH_2, 8′-C), 43.7 (CH_2, 2″-C), 47.1 (CH_2, 2′-C), 53.8 (CH, 2-C), 66.3 (CH_2, OCH_2Ph), 66.9 (CH_2, OCH_2Ph), 67.2 (CH_2, OCH_2Ph), 68.2 (quat., 5-C), 128.0 (CH, Ph), 128.05 (CH, Ph), 128.1 (CH, Ph), 128.3 (CH, Ph), 128.49 (CH, Ph), 128.5 (CH, Ph), 128.7 (CH, Ph), 135.2 (quat., Ph), 136.0 (quat., Ph), 136.5 (quat., Ph), 156.2 (quat., NCO_2), 166.1 (quat., 1″-C), 170.1 (quat., 1-C), 172.7 (quat., 5-C) and 174.4 (quat., 6′-C); *m*/*z* (FAB+) 642.2802 (MH^+ $C_{36}H_{40}N_3O_8$ requires 642.2815).

4.1.32. (2*S*,5′*R*)-[1′-(2″-amino-acetyl)-6′-oxo-1′,7′-diazaspiro[4.4]non-7′-yl]-1,5-pentanedioic acid 35. A mixture of protected spirolactam **39** (30 mg, 0.047 mmol) and 10% palladium on activated carbon (9.6 mg, 0.09 mmol) in 88:12 methanol–water (6.6 cm^3) was stirred under an atmosphere of hydrogen at room temperature, protected from light, for 18 h. The reaction mixture was filtered through a Celite™ pad with 75:25 methanol–water (30 cm^3), and the filtrate concentrated to dryness under reduced pressure to give a yellow solid that was purified by reverse-phase C18 flash column chromatography (H_2O) to afford spirolactam **35** (12 mg, 78%) as a colourless solid: mp 238–239 °C (dec.): $[\alpha]_D$ −23.5 [*c* 1.15 in MeOH–H_2O (1/1)]; δ_H (400 MHz; D_2O) 1.97–2.61 (10H, m, 9′-H_2, 4′-H_2, 3′-H_2, 3-H_2 and 4-H_2), 3.44–3.78 (4H, br m, 8′-H_2 and 2′-H_2) 3.81–4.12 (2H, br m, 2″-H_2) and 4.41 (1H, m, 2-H); δ_C (100 MHz; D_2O) 25.8 (CH_2, 3′-C), 27.8 (CH_2, 3-C), 31.4 (CH_2, 9′-C), 37.3 (2×CH_2, 4′-C and 4-C), 43.1 (2×CH_2, 8′-C and 2″-C), 50.1 (CH_2, 2′-C), 60.2 (CH, 2-C), 72.5 (quat., 5′-C), 173.6 (quat., 1″-C), 178.9 (quat., 6′-C) 179.1 (quat., 1-C) and 184.9 (quat., 5-C); *m*/*z* (FAB+) 328.1521 (MH^+ $C_{14}H_{21}N_3O_6$ requires 328.1509).

4.1.33. (2*S*,5′*R*,8′*R*)- and (2*S*,5′*R*,8′*S*)-[1′-(2″-amino-acetyl)-8′-hydroxy-6′-oxo-1′,7′-diazaspiro[4.4]non-7′-yl]-1,5-pentanedioic acid 36. A mixture of protected hydroxyspirolactam **38** (27 mg, 0.041 mmol) and 10% palladium on activated carbon (8.4 mg, 0.079 mmol) in 88:12 methanol–water (5.8 cm^3) was stirred under an atmosphere of hydrogen at room temperature, protected from light, for 18 h. The reaction mixture was filtered through a Celite™ pad with 75:25 methanol–water (30 cm^3) and the filtrate concentrated to dryness under reduced pressure to afford spirolactam **36** (14 mg, 99%) as a colourless solid. Spirolactam **36** was shown to be a 7:3 mixture of two diastereomers by ^1H NMR analysis (the ratio was estimated from the relative intensities of the doublet of doublets at δ 4.47 and 4.53, assigned to 2-H of the minor and major isomers, respectively): mp 216–218 °C (dec.): $[\alpha]_D$ +0.86 [*c* 0.35 in MeOH–H_2O (1:1)]; δ_H (400 MHz; D_2O) 2.10–2.46 (9H, m, 9′-H_AH_B, 4′-H_2, 3′-H_2, 3-H_2 and 4-H_2), 2.65* (0.3H, dd, *J*=13.8, 7.0 Hz, 9′-H_AH_B), 2.75 (0.7H, dd, *J*=13.6, 6.2 Hz, 9′-H_AH_B), 3.55–3.61 (1H, m, 2′-H_AH_B), 3.69–3.73 (1H, m, 2′-H_AH_B), 3.94–4.09 (2H, d, *J*=16.5 Hz, 2″-H_2), 4.47* (0.3H, dd, *J*=9.9, 5.8 Hz, 2-H), 4.53 (0.7H, dd, *J*=10.4, 5.0 Hz, 2-H), 5.49* (0.3H, dd, *J*=6.8, 5.1 Hz, 8′-H) and 5.59 (0.7H, d, *J*=6.1 Hz, 8′-H); δ_C (100 MHz; D_2O) 26.0* (CH_2, 3′-C), 26.1 (CH_2, 3′-C), 27.4 (CH_2, 3-C), 29.6* (CH_2, 3-C), 39.3* (CH_2, 4-C), 40.1 (CH_2, 4-C), 40.7 (2×CH_2, 4′-C and 9′-C), 42.2* (CH_2, 9′-C), 43.1 (CH_2, 2″-C), 49.8 (CH_2, 2′-C), 49.9* (CH_2, 2′-C), 60.2* (CH, 2-C), 60.7 (CH, 2-C), 70.1* (quat., 5′-C), 70.9 (quat., 5′-C), 80.6 (CH, 8′-C), 83.2* (CH, 8′-C), 166.9 (quat., 1″-C), 167.1* (quat., 1″-C), 178.2* (quat., 6′-C), 179.7 (2×quat., 1-C and 6′-C), 180.4 (quat., 1-C), 182.5 (quat., 5-C) and 182.8* (quat., 5-C); *m*/*z* (FAB+) 344.1467 (MH^+ $C_{14}H_{22}N_3O_7$ requires 344.1458).

4.1.34. Methyl *N*-*tert*-butyloxycarbonyl-(D,L)-5,5-dimethylprolinate 43.[49] Nitrile **44**[49,55] (2 g, 14.3 mmol) was dissolved in 32% hydrochloric acid (6 cm^3) and heated to 50 °C for 5 h. Evaporation of the solvent afforded a residue that was dissolved in methanol–water (1/1, 30 cm^3) and hydrogenated over 10% palladium on activated carbon (0.3 g) under 44 psi of hydrogen for 20 h. The catalyst was removed by filtration through Celite™ and the solvent removed in vacuo to yield a 6:4 mixture of *N*-methyl-5-methylproline* **45**, and the desired 5,5-dimethylproline **46**; δ_H (300 MHz; D_2O) 1.34–1.40 (8.6H, m, 4×CH_3), 1.67–1.74 (1.3H, m) 1.91 (1.6H, t, *J*=12.2 Hz), 2.07–2.32 (3.5H, m), 2.40–2.54 (2H, m), 2.90* (3.6H, s, *N*-CH_3), 3.45–3.53 (1.3H, m), 4.23* (1.3H, dd, *J*=9.7, 7.5 Hz, Proα-H) and 4.47 (1H, d, *J*=8.4 Hz, Proα-H); δ_C (75 MHz; D_2O) 14.6* (CH_3), 24.5 (CH_3), 24.7 (CH_3), 25.9* (CH_2), 27.2 (CH_2), 29.9* (CH_2), 37.0 (CH_2), 39.1* (CH_3, *N*-CH_3), 58.9* (CH, Proδ-C), 67.1 (quat., Proδ-C), 67.6* (CH, Proα-C), 69.2 (CH, Proα-C), 171.5* (quat., Proα-CO) and 172.4 (quat., Proα-CO). The mixture was subsequently dissolved in dry methanol (60 cm^3), cooled to 0 °C and thionyl chloride (4.2 cm^3, 57.2 mmol) was added dropwise over 5 min. The reaction was allowed to warm to room temperature and stirred overnight. The solvent was removed, the residue was dissolved in saturated sodium hydrogen carbonate solution and the products extracted with chloroform to yield a mixture of inseparable methyl esters (1.53 g), which were dissolved in dry dichloromethane (20 cm^3). *N*-Methylmorpholine (1.56 cm^3, 14.2 mmol) and di-*tert*-butyldicarbonate (3.1 g, 14.2 mmol) were added and the reaction was heated at reflux under nitrogen for 48 h. After cooling to room temperature the reaction mixture was washed with water, 1 M aqueous hydrochloric acid (2×30 cm^3), brine and dried ($MgSO_4$). The aqueous layer was concentrated in vacuo to give methyl *N*-methyl-5-methylprolinate **47** (1.093 g, 42%, four steps) as its hydrochloride salt. This was neutralized with saturated sodium hydrogen carbonate and purified by chromatography (silica gel, 4:1,

dichloromethane/ethyl acetate); δ_H (200 MHz; $CDCl_3$; Me_4Si) 1.06 (3H, d, $J=6.0$ Hz, Proδ-CH_3), 1.35–1.55 (1H, m), 1.70–2.01 (3H, m), 2.17–2.27 (1H, m), 2.24 (3H, s, *N*-CH_3), 2.90 (1H, t, $J=7.8$ Hz, Proα-H) and 3.65 (3H, s, 2-CO_2CH_3); δ_C (75 MHz; $CDCl_3$) 18.2 (CH_3, Proδ-CH_3), 26.4 (CH_2), 31.3 (CH_2), 38.9 (CH_3, *N*-CH_3), 51.3 (CH_3, Proα-CO_2CH_3) 61.8 (CH, Proδ-C), 68.4 (CH, Proα-C) and 173.6 (quat., Proα-CO); *m/z* (CI+) 158.1176 (MH^+. $C_8H_{16}NO_2$ requires 158.1181); The organic layer was concentrated in vacuo and purified by chromatography (silica gel, dichloromethane then chloroform) to give methyl *N*-*tert*-butyloxycarbonyl-(D,L)-5,5-dimethylprolinate **43** (0.795 g, 22%, four steps) as a yellow oil. This compound existed as a mixture of epimers (55:45) almost exclusively as the cis conformer; δ_H (300 MHz; $CDCl_3$) 1.26–1.53 [15H, m, $C(CH_3)_2$, $C(CH_3)_3$], 1.60–1.90 (4H, m, Proβ-H_2 and Proγ-H_2), 3.66 (3H, CO_2CH_3), 4.24 (0.55H, dd $J=8.9$, 3.5 Hz, Proα-H) and 4.35 (0.45H, dd $J=8.5$, 2.6 Hz, Proα-H); δ_C (75 MHz; $CDCl_3$) 25.7 (CH_3, Proδ-CH_3), 25.9 (CH_2, Proγ-C), 26.0 (CH_3, Proδ-CH_3), 26.5 (CH_2, Proγ-C), 26.6 (CH_3, Proδ-CH_3), 27.2 (CH_3, Proδ-CH_3), 28.2 [CH_3, $C(CH_3)_3$], 28.3 [CH_3, $C(CH_3)_3$], 39.8 (CH_2, Proβ-C), 40.7 (CH_2, Proβ-C), 51.6 (CH_3, Proα-CO_2CH_3), 51.7 (CH_3, Proα-CO_2CH_3), 60.5 (quat., Proδ-C), 61.16 (CH, Proα-C), 61.2 (CH, Proα-C), 61.3 (quat., Proδ-C), 79.1 [quat., $C(CH_3)_3$], 79.6 [quat., $C(CH_3)_3$], 152.4 (quat., NCO_2), 154.0 (quat., NCO_2), 173.4 (quat., Proα-CO) and 173.8 (quat., Proα-CO); *m/z* (EI+) 257.1624 (M^+ $C_{13}H_{23}NO_4$ requires 257.1627).

4.1.35. *N*-*tert*-Butyloxyglycyl-L-4-thiaproline 52. *iso*-Butyl chloroformate (0.154 g, 1.12 mmol) was added to a stirred solution of *N*-*tert*-butyloxycarbonylglycine **17** and triethylamine (0.215 g, 1.15 mmol) in tetrahydrofuran (6 cm^3) at 0 °C. A white precipitate was observed, the cooling bath was removed and the mixture stirred at room temperature for 10 min. A solution of 4-thiaproline hydrochloride **40**[44] (0.150 g, 1.12 mmol) and triethylamine (0.215 g, 1.15 mmol) in water (2 cm^3) was added and the resultant solution was stirred for 1 h. The mixture was acidified with 2 M HCl and extracted with ethyl acetate. The combined organic extracts were dried (Na_2SO_4), filtered and concentrated in vacuo to yield an oil (0.39 g) that was purified by flash chromatography (hexane/ethyl acetate/acetic acid 2:1:0.3 then 1:1:0.2) gave dipeptide **52** (0.264 g, 81%) as a hygroscopic white foam. **52** was shown to be a 62:38 trans:cis mixture of conformers by ^1H NMR analysis (the ratio was estimated from the integration of the GlyN–H protons at δ 5.65 and 5.75 assigned to major and minor conformers, respectively): $[\alpha]_D$ −93.5 (*c* 0.25 in CH_2Cl_2); δ_H (400 MHz; $CDCl_3$) 1.43[2] [3.4H, s, $C(CH_3)_3$], 1.44 [5.6H, s, $C(CH_3)_3$], 3.31 (1.24H, d, $J=5.1$ Hz, 3-H_AH_B), 3.37* (0.38H, dd, $J=11.8$, 5.2 Hz, 3-H_AH_B), 3.49* (0.38H, dd, $J=11.7$, 1.4 Hz, 3-H_AH_B), 3.90–4.20 (2H, m, Glyα-H_2), 4.55–4.62 (1.62H, m 5-H_2, *5-H_AH_B), 4.79* (0.38H, d, $J=9.7$ Hz, 5-H_AH_B), 4.86* (0.38H, d, $J=5.7$ Hz, 2-H), 5.11 (0.62H, t, $J=4.9$ Hz, 2-H), 5.65 (0.62H, br s, GlyN–H) and 5.75* (0.38H, br s, GlyN–H); δ_C (100 MHz; $CDCl_3$) 28.2 [CH_3, $C(CH_3)_3$], 32.3 (CH_2, 3-C), 34.4* (CH_2, 3-C), 43.1 (CH_2, Glyα-C), 43.4* (CH_2, Glyα-C), 47.6 (CH_2, 5-C), 48.9* (CH_2, 5-C), 60.7* (CH, 2-C), 61.6 (CH, 2-C), 80.3* [quat., $C(CH_3)_3$], 80.7 [quat., $C(CH_3)_3$], 156.1 (quat., NCO_2), 156.4* (quat., NCO_2), 167.8 (quat., Gly-CO), 168.0* (quat., Gly-CO), 171.6 (quat., 2-CO) and 172.0* (quat., 2-CO); *m/z* (EI+) 290.0938 (M^+ $C_{11}H_{18}N_2O_5S$ requires 290.0936).

4.1.36. Di-*tert*-Butyl *N*-*tert*-butyloxycarbonylglycyl-L-4-thiaprolyl-L-glutamate 56. Ethyl chlorofomate (0.048 g, 0.445 mmol) was added dropwise to a solution of acid **52** (0.129 g, 0.445 mmol) and triethylamine (0.050 g, 0.49 mmol) in dichloromethane (3 cm^3) at 0 °C. The solution was stirred for 35 min at 0 °C then a solution of glutamic acid di-*tert*-butyl ester hydrochloride **29** (0.132 g, 0.445 mmol) and triethylamine (0.050 g, 0.49 mmol) in dichloromethane (3 cm^3) was added. The mixture was stirred overnight, washed successively with 2 M aqueous hydrochloric acid, saturated sodium hydrogen carbonate solution, dried (Na_2SO_4), filtered and the solvent removed. Purification by flash chromatography (dichloromethane/ethyl acetate, 3:1) afforded protected tripeptide **56** (0.128 g, 54%) as a colourless solid. **56** was shown to be a 66:34 trans:cis mixture of conformers by ^1H NMR analysis (the ratio was estimated from the integration of the thiaProN–H protons at δ 7.20 and 7.43 assigned to major and minor conformers, respectively): mp 99–100 °C: $[\alpha]_D$ −70 (*c* 0.6 in CH_2Cl_2); δ_H (400 MHz; $CDCl_3$) 1.25–1.45 [27H, m, $C(CH_3)_3$], 1.80–1.95 (1H, m, Gluβ-H_AH_B), 1.97–2.14 (1H, m, Gluβ-H_AH_B), 2.18–2.35 (2H, m, Gluγ-H_2), 3.14 (0.66H, dd, $J=11.2$, 7.0 Hz, 3-H_AH_B), 3.22–3.35[3] (0.34H, br m, 3-H_AH_B), 3.38 (1H, dd, $J=12.3$, 3.6 Hz, 3-H_AH_B), 3.85–4.05 (2H, m, Glyα-H_2), 4.38 (1H, br t, $J=4.8$ Hz, Gluα-H), 4.49–4.57 (1.66H, 5-H_AH_B and *5-H_AH_B), 4.69–4.77* (0.72H, *5-H_AH_B and *2-H), 4.98 (0.66H, br s, 2-H), 5.51 (1H, br s, GlyN–H), 7.20 (0.66H, d, $J=7.2$ Hz, thiaProN–H), 7.20* (0.34H, br s, thiaProN–H); δ_C (100 MHz; $CDCl_3$) 26.4* (CH_2, Gluβ-C), 27.1 (CH_2, Gluβ-C), 27.8 [CH_3, $C(CH_3)_3$], 27.9 [CH_3, $C(CH_3)_3$], 28.2 [CH_3, $C(CH_3)_3$], 31.3 (CH_2, Gluγ-C), 32.2 (CH_2, 3-C), 35.4* (CH_2, 3-C), 43.2 (CH_2, Glyα-C), 43.5* (CH_2, Glyα-C), 48.2 (CH_2, 5-C), 49.7* (CH_2, 5-C), 52.5 (CH, Gluα-C), 62.3* (CH, 2-C), 62.5 (CH, 2-C), 79.7 [quat., $C(CH_3)_3$], 80.6 [quat., $C(CH_3)_3$], 80.9* [quat., $C(CH_3)_3$], 82.1 [quat., $C(CH_3)_3$], 82.2* [quat., $C(CH_3)_3$], 155.7 (quat., NCO_2), 167.9 (quat., Gly-CO), 168.6* (quat., 2-CO), 168.9 (quat., 2-CO), 170.4 (quat., Gluα-CO) and 172.4 (quat., Gluγ-CO); *m/z* (FAB+) 532.2628 (MH^+ $C_{24}H_{42}N_3O_8S$ requires 532.2693).

4.1.37. Glycyl-L-4-thiaprolyl-L-glutamic acid trifluoroacetate 48. Trifluoroacetic acid (1 cm^3) was added to a stirred solution of protected tripeptide **56** (0.128 g, 0.241) and triethylsilane (0.084 g, 0.723 mmol) in dichloromethane (3 cm^3). The resultant solution was stirred for 4 h at room temperature and the volatiles removed in vacuo. Purification of the residue by chromatography (reverse phase C_{18}, water, 10–20% acetonitrile/water) and lyophilisation gave **48** (0.066 g, 61%) as a hygroscopic off white solid. **48** was shown to be a 80:20 trans:cis mixture of conformers by ^1H NMR analysis (the ratio was estimated from the integration of the Glyα protons at δ 3.80–3.92 and 3.58 assigned to major and minor conformers, respectively): no mp due to hygroscopic sample: $[\alpha]_D$ −88.6 (*c* 0.203 in H_2O); δ_H (400 MHz; D_2O) 1.73–1.82 (1H, m, Gluβ-H_AH_B), 1.96–2.06 (1H, m, Gluβ-H_AH_B), 2.28 (2H, t, $J=6.9$ Hz, Gluγ-H_2), 2.96 (0.8H, dd, $J=12.4$, 3.7 Hz, 3-H_AH_B), 3.13* (0.2H, d, 12.2, 3-H_AH_B), 3.20 (0.8H, dd, $J=12.4$, 7.3 Hz,

3-H_AH_B), 3.27* (0.2H, dd, J=12.4, 6.9 Hz, 3-H_AH_B), 3.58 (0.2H, d, J=16.4 Hz, Glyα-H_AH_B), 3.80–3.92 (1.8H, m, Glyα-H_2 and *Glyα-H_AH_B), 4.25 (0.8H, dd, 9.4 and 4.8, Gluα-H), 4.30* (0.2H, dd, 10.0 and 4.8, Gluα-H), 4.35–4.49 (2H, m, 5-H_2) and 4.67 (1H, dd, J=6.9 Hz, 3.8 Hz, 2-H); δ_C (100 MHz; $CDCl_3$) 24.8* (CH_2, Gluβ-C), 25.3 (CH_2, Gluβ-C), 29.5 (CH_2, Gluγ-C), 29.8* (CH_2, Gluγ-C), 32.8 (CH_2, 3-C), 34.9* (CH_2, 3-C), 40.3 (CH_2, Glyα-C), 40.5* (CH_2, Glyα-C), 48.4 (CH_2, 5-C), 49.6* (CH_2, 5-C), 51.7 (CH, Gluα-C), 51.9* (CH, Gluα-C), 61.6* (CH, 2-C), 62.6 (CH, 2-C), 115.7 (quat., q, J=290.7 Hz, CF_3CO_2H), 161.9 (quat., q, J=36.2 Hz, CF_3CO_2H), 165.0 (quat., Gly-CO), 165.6* (quat., Gly-CO), 171.0* (quat., 2-CO), 171.5 (quat., 2-CO), 174.0* (quat., Gluα-CO), 174.1* (quat., Gluα-CO) and 176.7 (quat., Gluγ-CO); m/z (FAB+) 320.0921 [M(free base)H^+ $C_{11}H_{18}N_3O_6S$ requires 320.0916].

4.1.38. *N*-Benzyloxycarbonylglycyl-L-thia-5,5-dimethylproline 53. To a stirred solution of 5,5,-dimethyl-4-thiaproline hydrochloride **41**[45,46] (0.354 g, 1.79 mmol) under nitrogen in dry dimethylformamide (35 cm^3) was added diisopropylethylamine (0.594 cm^3, 1.9 mmol) and acid fluoride **51**[56,57](0.341 g, 1.61 mmol). The solution was stirred for 18 h, the solvent was removed in vacuo and the residue was redissolved in ethyl acetate, washed with 10% citric acid solution, brine and dried ($MgSO_4$). The solvent was removed and the residue purified by flash chromatography (4:1:0.5, ethyl acetate/hexane/acetic acid, then 3:1.0.4) to give the desired compound contaminated with *N*-benzyloxycarbonylglycine **16** (14%, ^{1}H NMR). This mixture was dissolved in methanol, trimethylsilyl chloride (0.07 cm^3) added and the solution stirred overnight. Removal of the solvent in vacuo and subsequent flash chromatography (3:1, ethyl acetate/hexane [to remove *N*-benzyloxycarbonylglycine methyl ester] then 3:1.0.4 ethyl acetate/hexane/acetic acid) gave dipeptide **53** (0.320 g, 65%, based on amount of acid fluoride reacted) as a white solid. This compound existed purely as the cis conformer: mp 130–132 °C: $[\alpha]_D$ −51.7 (c 0.116 in dichloromethane); δ_H (300 MHz; $CDCl_3$) 1.85 (3H, s, [†]Pδ-CH_3) 1.91 (3H, s, Pδ-CH_3), 3.30 (1H, dd, J=12.1, 5.6 Hz, Pβ-H_AH_B), 3.40 (1H, d, J=12.1 Hz, Pβ-H_AH_B), 3.87 (1H, dd, J=16.6, 3.7 Hz, Glyα-H_AH_B), 4.09 (1H, dd, J=16.6, 3.7 Hz, Glyα-H_AH_B), 4.82 (1H, d, J=5.2 Hz, Pα-H), 5.13 (2H, s, OCH_2Ph), 6.10 (1H, br t, J=3.8 Hz, GlyN–H) and 7.29–7.39 (5H, m, Ph); δ_C (75 MHz; $CDCl_3$) 27.0 (CH_3, Pδ-CH_3) 29.4 (CH_3, Pδ-CH_3), 31.6 (CH_2, Pβ-C), 44.8 (CH_2, Glyα-C), 64.3 (CH, Pα-C), 67.3 (CH_2, OCH_2Ph), 74.2 (quat., Pδ-C), 127.9 (CH, Ph), 128.1 (CH, Ph), 128.4 (CH, Ph), 135.8 (quat., Ph), 156.8 (quat., NCO_2), 166.6 (quat., Gly-CO) and 172.0 (quat., Pα-CO); m/z (EI+) 352.1088 (M^+ $C_{16}H_{20}N_2O_5S$ requires 352.1093).

4.1.39. Dibenzyl *N*-benzyloxycarbonyl-glycyl-L-thia-5,5-dimethylprolyl-L-glutamate 57. To a stirred solution of dipeptide **53** (0.398 g, 1.11 mol), L-glutamic acid dibenzyl ester *p*-toluenesulfonate **28** (0.670 g, 1.34 mol) and diisopropylethylamine (0.51 cm^3, 2.90 mmol) in dry dichloromethane (40 cm^3) was added bis(2-oxo-3-oxazolidinyl)phosphinic chloride (0.370 g, 1.45 mmol) in one portion. The solution was stirred for 7 h under nitrogen and the solvent removed. The residue was suspended in ethyl acetate, washed with 10% citric acid solution, saturated sodium hydrogen carbonate, brine and dried ($MgSO_4$). Removal of the solvent and subsequent chromatography (2:1, hexane/ethyl acetate, then 1:1) gave protected tripeptide **57** (0.5 g, 68%) as a colourless oil. Protected tripeptide **57** was shown to be a 90:10 cis:trans mixture of conformers by ^{1}H NMR analysis (the ratio was estimated from the integration of the broad singlets at δ 5.69 and 5.80 and assigned to the GlyN–H protons of the major and minor conformers, respectively): $[\alpha]_D$ −35.6 (c 0.399 in dichloromethane); δ_H (300 MHz; $CDCl_3$) 1.85 (3H, Pδ-CH_3) 1.97 (3H, Pδ-CH_3), 2.08–2.15 (1H, m, Gluβ-H_AH_B), 2.29–2.38 (1H, m, Gluβ-H_AH_B), 2.47 (2H, t, J=5.4 Hz, Gluγ-H_2), 3.32 (2H, m, Pβ-H_2), 3.85 (1H, d, J=16.6 Hz, Glyα-H_AH_B), 3.91 (1H, dd, J=17.0, 8.0 Hz, Glyα-H_AH_B), 4.72 (2H, br s, Pα-H, Gluα-H), 5.20–5.08 (6H, m, 3×OCH_2Ph), 5.69 (0.9H, br s, Gly-NH), 5.80* (0.1H, br s, Gly-NH) and 7.33–7.39 (15H, m, Ph); δ_C (75 MHz; $CDCl_3$) 26.3 (CH_2, Gluβ-C), 26.45* (CH_2, Gluβ-C), 27.21* (CH_3, Pδ-CH_3), 27.43 (CH_3, Pδ-CH_3), 28.4 (CH_3, Pδ-CH_3), 29.91 (CH_2, Gluγ-C), 30.09* (CH_2, Gluγ-C), 32.3 (CH_2, Pβ-C), 44.7 (CH_2, Glyα-C), 45.1* (CH_2, Glyα-C), 52.1 (CH, Gluα-C), 52.3* (CH, Gluα-C), 65.8 (CH, Pα-H), 66.4 (CH_2, OCH_2Ph), 66.8 (CH_2, OCH_2Ph), 67.4 (CH_2, OCH_2Ph), 74.5 (quat., Pδ-C), 127.8 (CH, Ph), 127.9 (CH, Ph), 128.1, (CH, Ph), 128.14 (CH, Ph), 128.2 (CH, Ph), 128.3 (CH, Ph), 128.5 (CH, Ph), 134.8 (quat., Ph), 135.5 (quat., Ph), 136.2 (quat., Ph), 156.4 (quat., NCO_2), 167.1 (quat., Gly-CO), 169.6 (quat., P-CON), 170.9 (quat., Gluα-CO) and 172.7 (quat., Gluγ-CO); m/z (FAB+) 662.2536 (MH^+ $C_{35}H_{40}N_3O_8S$ requires 662.2536).

4.1.40. Glycyl-L-thia-5,5-dimethylprolyl-L-glutamic acid 49. Protected tripeptide **57** (0.44 g, 0.66 mmol) was dissolved in methanol–water (4/1, 50 cm^3), placed in a Parr bottle. The vessel was flushed with nitrogen, 10% palladium on activated carbon (70 mg, 0.066 mmol) was added and the mixture was pressurized to 40 psi with hydrogen and shaken for 3 h. Further 10% palladium on activated carbon (70 mg) was added and the reaction continued for 21 h. The reaction mixture was filtered through Celite,™ washed with methanol–water (4/1) and the solvent removed to yield an oil, which by TLC and ^{1}H NMR analysis contained the desired product and products containing varying amounts of debenzylation. The mixture was dissolved in water and passed through a C_{18} column eluting with water, then 10% methanol–water. The relevant fractions were combined, the solvent removed and the residue was triturated with dry ether to yield tripeptide **49** (0.110 g, 48%) as a white solid. Tripeptide **49** was shown to be a 85:15 cis:trans mixture of conformers by ^{1}H NMR analysis (the ratio was estimated from the integration of the doublets at δ 4.95 and 5.02 and assigned to the Pα-H protons of the major and minor conformers, respectively): mp 145–150 °C: $[\alpha]_D$ −75 (c 0.064 in water); δ_H (300 MHz; D_2O) 1.84 (2.55H, s, Pδ-CH_3). 1.90* (0.45H, s, Pδ-CH_3) 1.93 (3H, s, Pδ-CH_3), 1.96–2.05 (1H, m, Gluβ-H_AH_B), 2.18–2.27 (1H, m, Gluβ–H_AH_B), 2.42 (2H, t, J=7.5 Hz, Gluγ-H_2), 3.36 (1H, d, J=12.7 Hz, Pβ-H_AH_B) 3.59 (1H, dd, J=12.8, 6.4 Hz, Pβ-H_AH_B),, 3.70 (1H, d, J=16.2 Hz, Glyα-H_AH_B), 4.01 (1H, d, J=16.3 Hz, Glyα-CH_AH_B), 4.23* (0.15H, dd, J=9.1, 4.9 Hz, Gluα-H), 4.32 (0.85H, dd, J=9.1, 4.9 Hz,

[†] P refers to the proline analogue portion in question.

Gluα-H), 4.95 (0.85H, d, $J=6.2$ Hz, Pα-H) and 5.02* (0.15H, d, $J=6.0$ Hz, Pα-H); δ_C (75 MHz; D_2O) 26.1* (CH_3, Pδ-CH_3), 26.3 (CH_3, Pδ-CH_3), 26.56 (CH_2, Gluβ-C), 27.6 (CH_3, Pδ-CH_3), 27.9* (CH_3, Pδ-CH_3), 31.1 (CH_2, Gluγ-C), 30.3* (CH_2, Gluγ-C), 32.1 (CH_2, Pβ-C), 32.3* (CH_2, Pβ-C), 41.1* (CH_2, Glyα-C), 41.6 (CH_2, Glyα-C), 54.4 (CH, Gluα-C), 55.0* (CH, Gluα-C), 65.3* (CH, Pα-H), 65.5 (CH, Pα-H), 74.3* (quat., Pδ-C), 74.6 (quat., Pδ-C), 164.4* (quat., Gly-CO), 164.6 (quat., Gly-CO), 170.5 (quat., P-CON), 170.8* (quat., P-CON), 176.9 (quat., Gluα-CO) and 178.3 (quat., Gluγ-CO); *m/z* (FAB+) 348.1249 (MH^+ $C_{12}H_{22}N_3O_6S$ requires 348.1229).

4.1.41. Methyl *N-tert*-buyltoxycarbonylglycyl-(D,L)-5,5-dimethylprolinate 54. Trifluoroacetic acid (1 cm^3) was added to a stirred solution of carbamate **43** (0.584 g, 2.27 mmol) in dichloromethane (6 cm^3). The solution was stirred for 2 h after which time the volatiles were removed in vacuo and traces of trifluoroacetic acid were removed by placing the sample on an oil pump for 2 h. The salt was then dissolved in dichloromethane (20 cm^3) and diisopropylethylamine (1.3 cm^3, 7.49 mmol) was added (white fumes) followed by *N-tert*-butyloxycarbonylglycine **17** (0.477 g, 2.73 mmol) and bis(2-oxo-3-oxazolidinyl)phosphinic chloride (0.691 g, 2.28 mmol). Additional dichloromethane (5 cm^3) was added and the solution was stirred overnight under nitrogen. The solvent was then removed in vacuo, the residue was dissolved in ethyl acetate and washed sequentially with 2 M aqueous hydrochloric acid, saturated sodium hydrogen carbonate and dried ($MgSO_4$). Removal of the solvent gave an oil (0.440 g) that was purified by chromatography (silica gel, hexane/ethyl acetate, 2:1, then 1:1) to give dipeptide **54** (0.368 g, 52%) as a colourless oil. Dipeptide **54** was shown to be 80:20 cis:trans mixture of conformers by ^{1}H NMR analysis (the ratio was estimated from the integration of the chemical shifts at δ 4.28 and 4.46 assigned to the Proα-H protons of the major and minor conformers, respectively); δ_H (300 MHz; $CDCl_3$) 1.26 (2.4H, s, Proδ-CH_3), 1.28 [9H, s, $C(CH_3)_3$], 1.31[5] (0.6H, s, Proδ-CH_3), 1.44* (0.6H, s, Proδ-CH_3), 1.46 (2.4H, s, Proδ-CH_3), 1.59–2.15 (4H, m, Proβ-H_2, Proγ-H_2), 3.37 (0.8H, dd, $J=16.7$, 3.3 Hz, Glyα-H_AH_B), 3.57* (0.6H, s, Proα-CO_2CH_3), 3.61 (2.4H, s, Proα-CO_2CH_3), 3.74 (0.8H, dd, $J=16.7$, 3.3 Hz, Glyα-H_AH_B), 3.84* (0.2H, dd, $J=16.9$, 3.9 Hz, Glyα-H_AH_B), 3.39–4.01* (0.2H, m, Glyα-H_AH_B), 4.28 (0.8H, d, $J=8.3$ Hz, Proα-H), 4.46* (0.2H, dd, $J=8.0$, 2.2 Hz, Proα-H) and 5.40 (1H, br s, 1H, Gly-NH); δ_H (75 MHz; $CDCl_3$) 24.5 (CH_3, Proδ-CH_3), 25.0* (CH_2, Proβ-C), 26.1 (CH_3, Proδ-CH_3), 26.9* (CH_3, Proδ-CH_3), 27.2* (CH_3, Proδ-CH_3), 27.4 (CH_2, Proβ-C), 27.9 [CH_3, $C(CH_3)_3$], 38.9 (CH_2, Proγ-C), 41.7* (CH_2, Proγ-C), 42.8* (CH_2, Glyα-C), 43.1 (CH_2, Glyα-C), 51.7* (CH_3, Proα-CO_2CH_3), 52.3 (CH_3, Proα-CO_2CH_3), 60.1 (CH, Proα-C), 60.9* (quat., Proδ-C), 61.7* (CH, Proα-C), 63.8 (CH, Proα-C), 78.0 [quat., $C(CH_3)_3$], 155.3 (quat., NCO_2), 155.4* (quat., NCO_2), 166.5 (quat., Gly-CO), 167.7* (quat., Gly-CO), 171.9 (quat., Proα-CO) and 172.4* (quat., Proα-CO); *m/z* (CI+) 315.1927 (MH^+ $C_{15}H_{27}N_2O_5$ requires 315.1920).

4.1.42. *N-tert*-Butoxycarbonylglycyl-(D,L)-5,5-dimethylproline 55. To a solution of dipeptide **54** (0.362 g, 1.16 mmol) in dioxane (12 cm^3) was added 1 M aqueous sodium hydroxide (5.91 cm^3, 5.91 mmol) and the mixture stirred for 21 h. The reaction was acidified with solid citric acid and the product was extracted with ethyl acetate. The combined organic extracts were washed with brine, dried ($MgSO_4$) and the solvent removed in vacuo to yield an oil, which was purified by chromatography (silica gel, hexane/ethyl acetate, 2:1, 1:1, 4:6), to give acid **55** (0.324 g, 94%) as a white foam, which liquified rapidly. Acid **55** was shown to be an 80:20 cis:trans mixture of conformers by ^{1}H NMR analysis (the ratio was estimated from the integration of the broad singlets at δ 5.81 and 5.66 assigned to the GlyN–H protons of the major and minor conformers, respectively); δ_H (300 MHz; $CDCl_3$) 1.40 (2.4H, s, Proδ-CH_3), 1.43 [7.2H, s, $C(CH_3)_3$], 1.44* [1.8H, s, $C(CH_3)_3$], 1.47* (0.6H, s, Proδ-CH_3), 1.58* (0.6H, s, Proδ-CH_3), 1.61 (2.4H, s, Proδ-CH_3), 1.74–2.33 (4H, m, Proβ-H_2, Proγ-H_2), 3.37 (0.8H, dd, $J=16.7$, 3.3 Hz, Glyα-H_AH_B), 3.65 (0.8H, dd, $J=16.8$, 3.6 Hz, Glyα-H_AH_B), 3.96 (1H, dd, $J=16.9$, 3.9 Hz, Glyα-H_AH_B, Glyα-H_AH_B* partially obscured), 4.21* (0.2H, dd, $J=17.0$, 5.9 Hz, Gyα-H_AH_B), 4.42 (0.8H, d, $J=7.2$ Hz, Proα-H), 4.67* (0.2H, d, $J=7.9$ Hz Proα-H), 5.66* (0.2H, br s, Gly-NH), 5.81 (0.8H, br s, Gly-NH) and 6.2 (1H, br s, OH); δ_H (75 MHz; $CDCl_3$) 24.8 (CH_3, Proδ-CH_3), 25.1* (CH_2, Proβ-C), 26.3 (CH_3, Proδ-CH_3), 27.1* (CH_3, Proδ-CH_3), 27.6* (CH_3, Proδ-CH_3), 28.0 (CH_2, Proβ-C), 28.2 [CH_3, $C(CH_3)_3$], 39.2 (CH_2, Proγ-C), 42.0* (CH_2, Proγ-C), 43.0* (CH_2, Glyα-C), 43.5 (CH_2, Glyα-C), 60.5 (CH, Proα-C), 61.8* (quat., Proδ-C), 62.2* (CH, Proα-C), 64.3 (CH, Proα-C), 79.7* [quat., $C(CH_3)_3$], 80.2 [quat., $C(CH_3)_3$], 156.0* (quat., NCO_2), 156.4 (quat., NCO_2), 166.8 (quat., Gly-CO), 169.5* (quat., Gly-CO), 173.9 (quat., Proα-CO) and 174.5* (quat., Proα-CO); *m/z* (CI+) 315.1927 (MH^+ $C_{15}H_{27}N_2O_5$ requires 315.1920).

4.1.43. Dibenzyl *N-tert*-butoxycarbonylglycyl-(D,L)-5,5-dimethylprolyl-L-glutamate 58. To a stirred solution of acid **55** (0.298 g, 1 mmol) in dry dichloromethane (40 cm^3) was added successively diisopropylethylamine (0.453 cm^3, 2.6 mmol), L-glutamic acid dibenzyl ester *p*-toluenesulphonate **28** (0.648 g, 1.3 mmol) and bis(2-oxo-3-oxazolidinyl)-phosphinic chloride (0.330 g, 1.3 mmol). The resultant solution was stirred at room temperature under nitrogen overnight and the solvent removed. The residue was dissolved in ethyl acetate, washed with 10% aqueous citric acid solution, saturated sodium hydrogen carbonate, brine and dried ($MgSO_4$). The solvent was evaporated and the product purified by chromatography (silica gel, hexane/ethyl acetate, 1:1) to give protected tripeptide **58** (0.408 g, 67%) as a yellow oil (1:1 mixture of Proα-C epimers). Tripeptide **58** was shown to be 85:15 cis:trans mixture of conformers by ^{1}H NMR analysis (the ratio was estimated from the integration of the chemical shifts at δ 5.31–5.38 and 5.48 assigned to the Gly-NH protons of the major and minor conformers, respectively); δ_H (300 MHz; $CDCl_3$) 1.41 [9H, s, $C(CH_3)_3$], 1.44 (2.55H, s, Proδ-CH_3), 1.52* (0.45H, s, Proδ-CH_3), 1.54* (0.45H, s, Proδ-CH_3), 1.64 (2.55H, s, Proδ-CH_3), 1.67 (2.55H, s, Proδ-CH_3), 1.70–2.24 (6H, m, Proβ-H_2, Proγ-H_2, Gluβ-H_2), 2.35–2.45 (2H, m, Gluγ-H_2), 3.58–3.69 (0.85H, m, Glyα-H_AH_B), 3.85 (0.85H, m, Glyα-H_AH_B), 3.88–3.98* (0.15H, m, Glyα-H_AH_B), 4.12–4.17* (0.15H, m, Glyα-H_AH_B), 4.20–4.31 (1H, m, Proα-H), 4.59–4.71 (1H, m, Gluα-H), 5.10–5.22 (m, 4H, 2×OCH_2Ph), 5.31–5.38 (0.85H, m, Gly-NH) and 5.48*

(0.15H, m, Gly-NH); δ_C (75 MHz; $CDCl_3$) 24.38 (CH_3, Proδ-CH_3), 24.46 (CH_3, Proδ-CH_3), 25.1* (CH_2, Proβ-C), 25.2* (CH_2, Proβ-C), 26.2 (CH_2, Gluβ-C), 26.3 (CH_2, Gluβ-C), 26.6 (CH_3, Proδ-CH_3), 26.8* (CH_2, Proβ-C), 27.0* (CH_3, Proδ-CH_3), 27.1* (CH_3, Proδ-CH_3), 27.9* (CH_3, Proδ-CH_3), 28.2 [CH_3, $C(CH_3)_3$], 28.5 (CH_2, Proβ-C), 28.8 (CH_2, Proβ-C), 30.05* (CH_2, Gluγ-C), 30.1* (CH_2, Gluγ-C), 30.2 (CH_2, Gluγ-C), 30.25 (CH_2, Gluγ-C), 38.9 (CH_2, Proγ-C), 39.1 (CH_2, Proγ-C), 42.4** (CH_2, Proγ-C), 43.1* (CH_2, Glyα-C), 43.3* (CH_2, Glyα-C), 43.5 (CH_2, Glyα-C), 43.6 (CH_2, Glyα-C), 51.7* (CH, Gluα-C), 52.0 (CH, Gluα-C), 52.1 (CH, Gluα-C), 61.5* (quat., Proδ-C), 61.6* (quat., Proδ-C), 61.9 (CH, Proα-C), 62.0 (CH, Proα-C), 63.0* (CH, Proα-C), 63.05* (CH, Proα-C), 64.3 (quat., Proδ-C), 64.3 (quat., Proδ-C), 66.3* (CH_2, OCH_2Ph), 66.4* (CH_2, OCH_2Ph), 66.41 (CH_2, OCH_2Ph), 66.5 (CH_2, OCH_2Ph), 67.0* (CH_2, OCH_2Ph), 67.1* (CH_2, OCH_2Ph), 67.21 (CH_2, OCH_2Ph), 67.24 (CH_2, OCH_2Ph), 79.3 [quat., $C(CH_3)_3$], 128.1 (CH, Ph), 128.2 (CH, Ph), 128.3 (CH, Ph), 128.4* (CH, Ph), 128.43 (CH, Ph), 128.5 (CH, Ph), 135.03 (quat., Ph), 135.07, (quat., Ph), 153.13* (quat., Ph), 135.18* (quat., Ph), 135.6 (quat., Ph), 135.7* (quat., Ph), 155.8* (quat., NCO_2), 155.9 (quat., NCO_2), 156.0 (quat., NCO_2), 167.6 (quat., Gly-CO), 167.7 (quat., Gly-CO), 168.8* (quat., Gly-CO), 169.3* (quat., Gly-CO), 171.1 (quat., Pro-CON), 171.3* (quat., Pro-CON), 171.4* (quat., Pro-CON), 171.6 (quat., Gluα-CO), 171.8 (quat., Gluα-CO), 172.4 (quat., Gluγ-CO), 172.5* (quat., Gluγ-CO) and 172.6 (quat., Gluγ-CO); *m/z* (EI+) 609.3035 (M^+ $C_{33}H_{43}N_3O_8$ requires 609.3050).

4.1.44. Glycyl-(D,L)-5,5-dimethylprolyl-L-glutamic acid 50. To a stirred solution of protected tripeptide **58** (0.276 g, 0.454 mmol) in dichloromethane (10 cm^3) was added trifluoroacetic acid (1 cm^3) and the mixture stirred for 75 min. The solvent was removed in vacuo, the residue was dissolved in saturated sodium hydrogen carbonate and the product extracted with ethyl acetate. The combined organic layers were washed with brine, dried ($MgSO_4$) and the solvent removed to yield an oil (0.244 g), which was dissolved in methanol–water (4/1, 50 cm^3). The flask was flushed with nitrogen, 10% palladium on activated carbon (0.048 mg, 0.454 mmol) was added and the mixture was then stirred overnight under one atmosphere of hydrogen. Filtration through Celite™ followed by removal of the solvent yielded an oil that was triturated with dry diethyl ether to yield tripeptide **50** (0.139 g, 93%) as an off white solid. Tripeptide **50** was shown to be a 72:28 cis:trans mixture of conformers by ^1H NMR analysis (the ratio was estimated from the integration of the chemical shifts at δ 3.57 and 4.15–4.16 assigned to the Glyα-H protons of the major and minor conformers, respectively). Approximately 10% of the final product was tentatively assigned as the hydrochloride salt**: mp 145–150 °C; δ_H (400 MHz; D_2O) 1.43 (2.16H, s, Proδ-CH_3), 1.49* (0.84H, s, Proδ-CH_3), 1.57* (0.84H, s, Proδ-CH_3), 1.58* (0.84H, s, Proδ-CH_3), 1.60 (2.16H, s, Proδ-CH_3), 1.61 (2.16H, s, Proδ-CH_3), 1.90–2.48 (8H, m, Proβ-H_2, Proγ-H_2, Gluβ-H_2, Gluγ-H_2), 3.57 (0.72H, dd, J=16.1, 2.8 Hz, Glyα-H_AH_B), 3.75–3.82** (0.2H, m, Glyα-H_AH_B, Gluα-H), 3.94 (0.72H, dd, J=16.1, 6.5 Hz, Glyα-H_AH_B), 4.11** (0.1H, d, J=2.5 Hz, Glyα-H_AH_B), 4.15–4.16* (0.56H, m, Glyα-H_2), 4.24–4.30 (1H, m, Gluα-H), 4.46–4.51** (0.1H, m, Proα-H), 4.60 (0.72H, t, J=10.1 Hz, Proα-H) and 4.68* (0.28H, dd, J=13.5, 4.3 Hz, Proα-H); δ_C (400 MHz; D_2O) 23.56 (CH_3, Proδ-CH_3), 23.76 (CH_3, Proδ-CH_3), 24.1** (CH_3, Proδ-CH_3), 25.0 (CH_2, Gluβ-C), 25.2 (CH_3, Proδ-CH_3), 25.6 (CH_2, Gluβ-C), 25.7** (CH_3, Proδ-CH_3), 26.0 (CH_2, Gluβ-C), 26.1* (CH_3, Proδ-CH_3), 26.2* (CH_3, Proδ-CH_3), 26.4 (CH_2, Gluβ-C), 26.8* (CH_2, Gluβ-C), 28.4, (CH_2, Proβ-C), 28.7, (CH_2, Proβ-C), 30.8* (CH_2, Gluγ-C), 31.0* (CH_2, Gluγ-C), 31.2 (CH_2, Gluγ-C), 38.7 (CH_2, Proγ-C), 38.8 (CH_2, Proγ-C), 40.6* (CH_2, Glyα-C), 40.7 (CH_2, Glyα-C), 40.8 (CH_2, Glyα-C), 41.1* (CH_2, Proγ-C), 41.2* (CH_2, Proγ-C), 46.1** (CH_2, Glyα-C), 54.1 (CH, Gluα-C), 54.5 (CH, Gluα-C), 59.7* (CH, Proα-C), 61.7 (CH, Proα-C), 61.8 (CH, Proα-C), 62.6** (quat., Proδ-C), 62.7* (quat., Proδ-C), 63.4* (CH, Proα-C), 63.9** (quat., Proδ-C), 65.0 (quat., Proδ-C), 65.1 (quat., Proδ-C), 164.8 (quat., Gly-CO), 164.9 (quat., Gly-CO), 165.6* (quat., Gly-CO), 165.8* (quat., Gly-CO), 166.0** (quat., Gly-CO), 172.3** (quat., Pro-CON), 172.8 (quat., Pro-CON), 173.1 (quat., Pro-CON), 173.3* (quat., Pro-CON), 173.6* (quat., Pro-CON), 176.9 (quat., Gluα-CO), 177.3 (quat., Gluα-CO) and 178.1 (quat., Gluγ-CO); *m/z* (FAB+) 330.1666 (MH^+ $C_{14}H_{24}N_3O_6$ requires 330.1666).

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