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Synthesis and pharmacological evaluation of glycine-modified analogues of the neuroprotective agent glycyl-L-prolyl-L-glutamic acid (GPE)

Michelle Y. H. Lai, Margaret A. Brimble,* David J. Callis, Paul W. R. Harris, Mark S. Levi and Frank Sieg

Neuren Pharmaceuticals Medicinal Chemistry Group, Department of Chemistry, University of Auckland, 23 Symonds Street, Auckland 1000, New Zealand

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Abstract—The synthesis of 10 G*PE analogues, wherein the glycine residue has been modified, is described by coupling readily accessible dibenzyl-L-prolyl-L-glutamate **2** with various analogues of glycine. Pharmacological evaluation of the novel compounds was undertaken to further understand the role of the glycine residue on the observed neuroprotective properties of the endogenous tripeptide GPE.

1. Introduction

As described in our previous papers,[1] insulin-like growth factor-1 (IGF-1) is a potent neurotrophic factor[2,3] distributed within the mammalian central nervous system[4] (CNS), which is thought to be proteolytically cleaved into des-*N*(1-3)-IGF-1, a truncated IGF-1 comprised of 67 amino acids, and the *N*-terminal tripeptide Gly-Pro-Glu-OH (GPE **1**) (Scheme 3)[5–11] in damaged regions of the brain.[12–17] IGF-1 is thought to function as a prohormone for GPE **1**, which acts on a yet unknown receptor.[18–20]

GPE **1** was suggested to possess a neuromodulatory role in the CNS, possibly through interaction with glutamate receptors,[5,6,11,18,19,21–23] which provide a potential target for the rational design of neuroprotective agents.[24–26] It has therefore been proposed that GPE **1**, or analogues thereof, may provide a novel class of pharmaceutical agents for the treatment of CNS injuries, neurodegenerative diseases including Alzheimer's, Parkinson's and Huntington's Disease, multiple sclerosis and general aging-induced cognitive dysfunction.[27–30] Analogues of GPE are of sufficiently low molecular weight and are lipophilic enough to cross the blood–brain barrier. Their metabolic stability and oral bioavailability can be improved by synthetic modification of the parent tripeptide structure.

We therefore herein report the preparation and pharmacological evaluation of several analogues of general structure Gly-Pro-Glu-OH (G*PE), in which the Gly residue (G*) has been modified. This work is part of a structure–activity relationship study to determine the significance of the Gly residue of this tripeptide on neuroprotective activity.

2. Results and discussion

In order to ascertain the importance of the glycine functionality on GPE, 10 analogues with modifications at this site were synthesized and evaluated for their neuroprotective effects. The synthetic strategy employed involved preparations of known dibenzyl-L-prolyl-L-glutamate[31] **2** (Scheme 1) and effecting peptide bond construction with several modified glycine residues. After assembly of the new peptide bond, a simple deprotection procedure furnished the desired G*PE analogues.

Keywords: Neuroprotective agents; Peptides; Glycine; GPE.
* Corresponding author. Tel.: +64 09 3737599; fax: +64 09 3737422; e-mail: m.brimble@auckland.ac.nz

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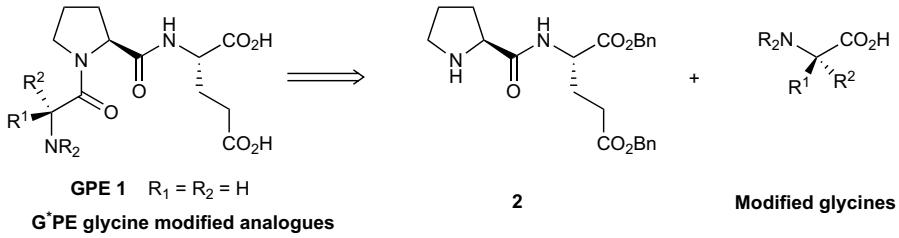


Scheme 1. General retrosynthesis for G*PE glycine-modified analogues.

The synthesis of dibenzyl-L-prolyl-L-glutamate[31] **2** was achieved in 98% yield over two steps from readily available Boc-L-proline **3** (Scheme 2). Thus, coupling of **3** with L-glutamic acid dibenzyl ester *p*-toluenesulfonate **4** using the mixed anhydride method gave protected dipeptide[31] **5**. Finally, Boc-deprotection using trifluoroacetic acid afforded amide **2**.

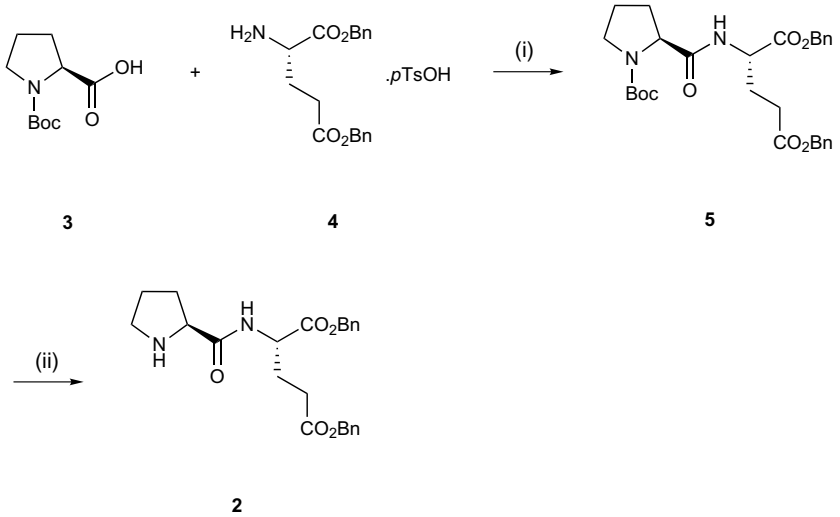
Based on the structure of GPE **1**, the first two analogues **6** and **7** involved individual replacement of one of the α-hydrogens of glycine with a methyl group having D- or L-stereochemistry. The synthesis of **6** and **7** began with alanines **8** and **9**, respectively (Scheme 3). The alanine, while increasing lipophilicity and adding a small amount of bulk, could also bypass proteases specific for Gly-Pro.

The commercially available D-**8** and L-**9** alanines were initially protected as benzyl carbamates **10** and **11**, respectively, by reaction with benzyl chloroformate under basic conditions. Coupling of amine **2** with amino ester **10** using 1-hydroxybenzotriazole hydrate (HOBt) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) gave the fully protected tripeptide, which upon hydrogenolysis afforded analogue **6** as an 82:18 *trans:cis* mixture of rotamers in 40% yield over the two steps. Similarly, amino ester **11** afforded G*PE analogue **7** as a 72:28 *trans:cis* mixture of rotamers.

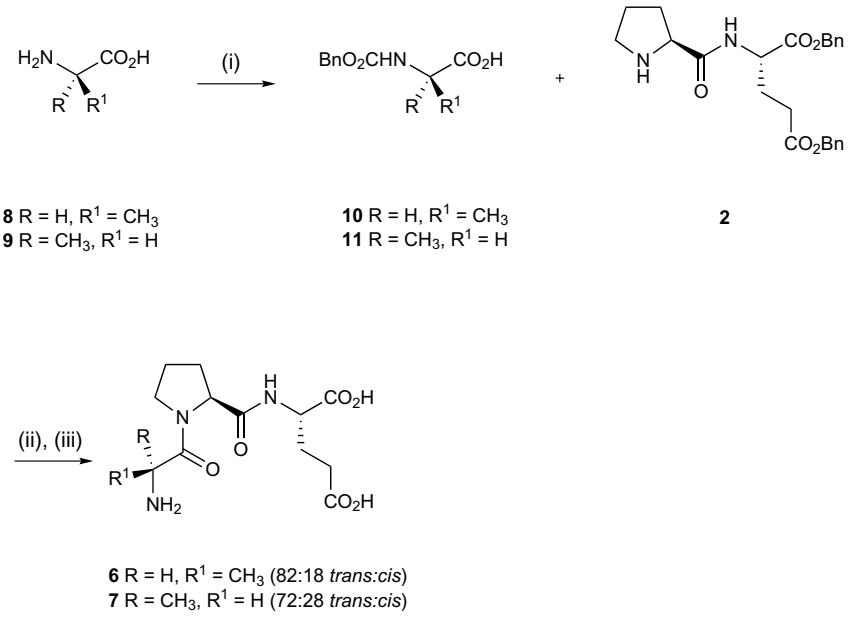
Another strategy involved replacement of both α-hydrogens of glycine with two methyl groups **12** (Scheme 4), a cyclopentyl ring **13** and a cyclohexyl ring **14** (Scheme 5). The methyl groups and the saturated rings increased lipophilicity while the dimethyl substitution produced exclusive *trans* configuration of the amide.

Analogue **12**, prepared from the previously synthesized compound L-proline methyl ester hydrochloride[1,32,33] **15**, was achieved in 18% yield over four steps. Initially, proline ester **15** was coupled with *N*-CBz-protected aminoisobutyric acid[34,35] **16** using coupling reagent, (benzotriazole-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BoP) and triethylamine as the base to afford dipeptide **17**, which, upon hydrolysis, gave acid **18**. Analogue **12** was then realized as its hydrochloride salt by subsequent coupling of acid **18** with amino ester **4**, followed by deprotection by hydrogenolysis.

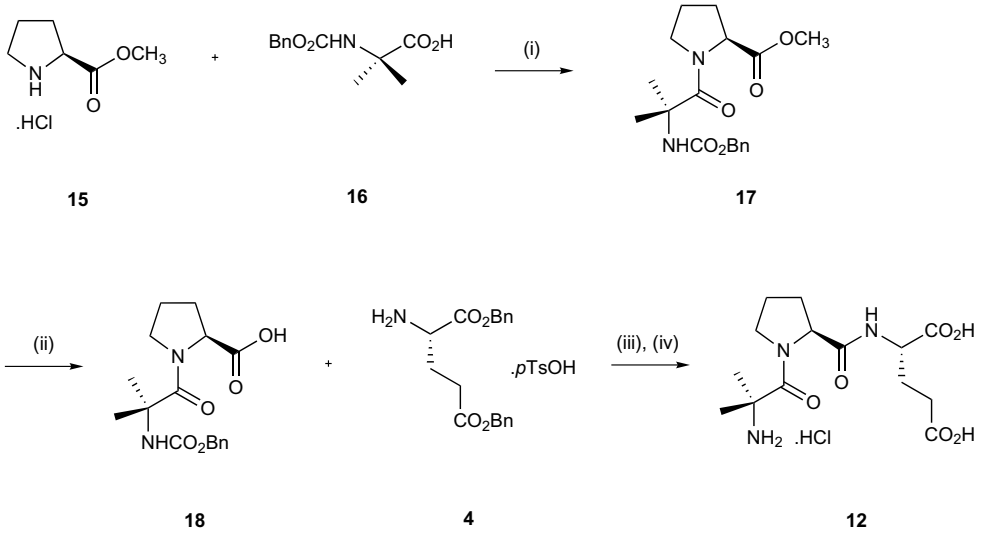
The commercially available 1-aminocyclopentanecarboxylic acid **19** and 1-aminocyclohexanecarboxylic acid **20**, were initially protected as benzyl carbamates **21** and **22**, by reaction with benzyl chloroformate under basic conditions. Coupling of the aforementioned amine **15** with acids **21** and **22** using BoP and triethylamine produced dipeptides **23** and **24**, which, upon hydrolysis, gave acids **25** and **26**, respectively. Upon coupling with amino ester **4**, acids **25** and **26** gave the respective amides



Scheme 2. Reagents and conditions: (i) Et_3N, EtOCOCl, CH_2Cl_2, 0 °C to rt, 20 h (98%); (ii) TFA, 0 °C, 1 h (100%).



Scheme 3. Reagents and conditions: For **6**: (i) Na_2CO_3, H_2O, BnOCOCl, dioxane, 0 °C to rt, 20 h (100%); (ii) HOBt, EDCI·HCl, THF, Et_3N, rt, 20 h, (46%); (iii) H_2, 10% Pd/C, MeOH, 20 h (86%). For **7**: (i) 4 M aq NaOH, BnOCOCl, 0 °C, 1 h (20%); (ii) HOBt, EDCI·HCl, THF, Et_3N, rt, 20 h (60%); (iii) H_2, 10% Pd/C, 9:1 MeOH–H_2O, 20 h (76%).



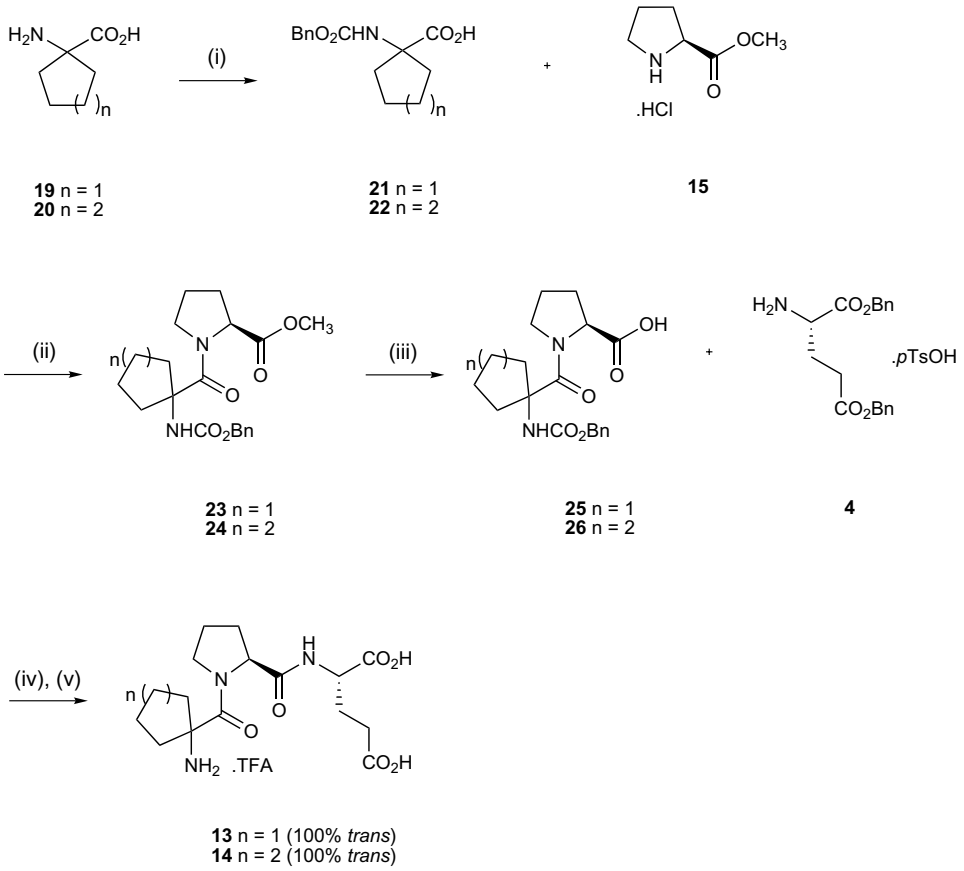
Scheme 4. Reagents and conditions: (i) Et_3N, BOP, CH_2Cl_2, rt, 4 h (68%); (ii) dioxane, 1 M NaOH, rt, 20 h (95%); (iii) Et_3N, BOPCl, CH_2Cl_2, 0 °C to rt, 20 h (53%); (iv) H_2, 10% Pd/C, 20% H_2O–THF, concd HCl, rt, 4 h (52%).

that, after hydrolysis, afforded analogues **13** (44% yield) and **14** (35% yield) as exclusively *trans* proline–glycine amide bond conformers.

A further set of four analogues **27**–**33** was next prepared that differed from the five described above in that the α-position of the glycine residue was left intact. However, the amino group of the glycine residue was modified to increase the lipophilicity and decrease polarity of the analogues, therefore enhancing transmembrane permeability (Scheme 6 and 7). In the first two analogues, the amino group was methylated to form *N*-methylglycine **27** and *N*,*N*-dimethylglycine **28** (Scheme 6).

Analogues **27** and **28** were afforded by coupling of acids **31** and **32**, respectively, with amine **2** using HOBt and EDCI followed by hydrogenolysis of the resultant tripeptides **33** and **34**. Analogue **27** was afforded in 45% yield over the two steps as an 84:16 *trans:cis* mixture of rotamers, whilst analogue **28** was afforded in 48% yield as a 79:21 *trans:cis* mixture of rotamers.

Preparation of the G*PE analogues **29** and **30** involved substitution of the amine with the secondary amines, pyrrolidine and piperidine, respectively. The pyrrolidino and piperidino amino acids **35** and **36** were prepared by reaction of bromoacetic acid with pyrrolidine or



19 n = 1
20 n = 2

21 n = 1
22 n = 2

15

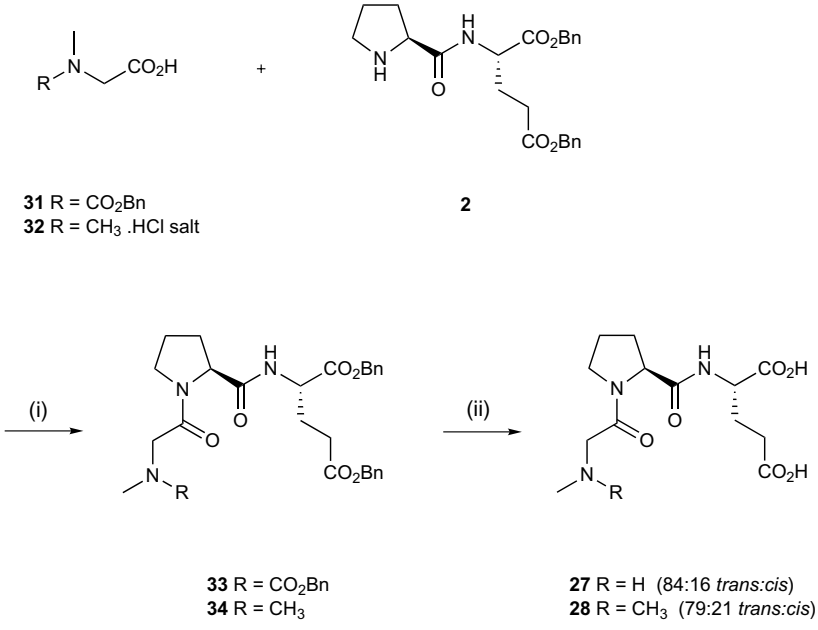
23 n = 1
24 n = 2

25 n = 1
26 n = 2

4

13 n = 1 (100% *trans*)
14 n = 2 (100% *trans*)

Scheme 5. Reagents and conditions: For **13**: (i) Na_2CO_3, H_2O, BnOCOCl, 2:1 H_2O–dioxane, 0 °C to rt, 20 h (62%); (ii) BoP, CH_2Cl_2, Et_3N, rt, 20 h (80%); (iii) 1 M aq NaOH, dioxane, rt, 21 h (ca. 95%); (iv) EtOCOCl, Et_3N, CH_2Cl_2, 0 °C, 40 min then **4**, 0 °C to rt, 17 h (83%); (v) 10% Pd/C, H_2, EtOAc, CF_3CO_2H, rt, 17 h (44%). For **14**: (i) Na_2CO_3, H_2O, BnOCOCl, 3:1 H_2O–dioxane, 0 °C to rt, 20 h (88%); (ii) BoP, CH_2Cl_2, Et_3N, rt, 20 h (74%); (iii) 1 M aq NaOH, dioxane, rt, 23.5 h (96%); (iv) Et_3N, BoPCl, CH_2Cl_2, rt, 19 h (75%); (v) 10% Pd/C, H_2, EtOAc–CF_3CO_2H (3:2), rt, 17 h (35%).



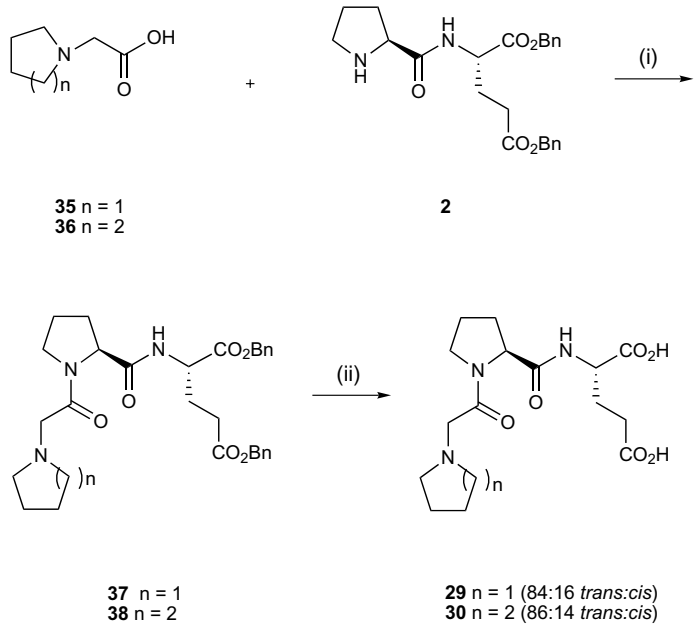
31 R = CO_2Bn
32 R = CH_3 .HCl salt

2

33 R = CO_2Bn
34 R = CH_3

27 R = H (84:16 *trans:cis*)
28 R = CH_3 (79:21 *trans:cis*)

Scheme 6. Reagents and conditions: For **27**: (i) HOBt, EDCl·HCl, THF, Et_3N, rt, 20 h (47%); (ii) H_2, 10% Pd/C, MeOH, 20 h (96%). For **28**: (i) Et_3N, HOBt, DMF, EDCl·HCl, CH_2Cl_2, 20 h (51%); (ii) H_2, 10% Pd/C, 9:1 MeOH–H_2O, 20 h (94%).



Scheme 7. Reagents and conditions: For **29**: (i) NaOH, H_2O, rt, 3 days (73%); (ii) PyBOP, Et_3N, DMF, rt, 20 h (46%); (iii) 10% Pd/C, H_2, MeOH, 20 h (86%). For **30**: (i) NaOH, H_2O, rt, 20 h (89%); (ii) HOBt, EDCl·HCl, DMF, rt, 20 h (59%); (iii) 10% Pd/C, H_2, MeOH, 20 h (78%).

piperidine, respectively.[36,37] The amino acids **35** and **36** were then coupled with amine **2** using (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBoP) and HOBt or EDCI to afford amides **37** and **38**, respectively. Subsequent deprotection gave the lipophilic analogues **29** and **30** as an 84:16 or an 86:14 *trans:cis* mixture of rotamers, respectively (Scheme 7).

The final compound in the series was analogue **39** (Scheme 8) in which an extra methylene group was introduced into the glycine residue. Following literature precedent, β-alanine was protected using benzyl chloroformate to afford carbamate **40**,[38] which was coupled with amine **2** using EDCI and additive HOBt to give tripeptide **41**. Subsequent deprotection yielded analogue **39** as an 82:18 *trans:cis* mixture of rotamers.

The 10 G*PE analogues synthesized were subjected to in vitro evaluation for prevention of neuronal cell death. Of the 10 analogues, L-APE **7**, showed neuroprotective activity at 1 mM with a recovery value of 35% (Fig. 1). To compare, GPE **1** recovery values range from 25% to 40%. Analogue **28**, having the *N*-methyl produced a recovery value 30–35%. Lower neuroprotective values (<20%) were obtained with 1 mM of the pyrrolidine **29** and D-APE **6**. None of the other analogues exhibited neuroprotective activity.

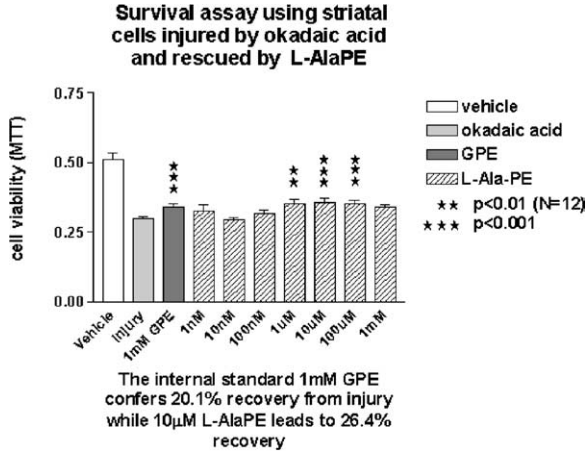
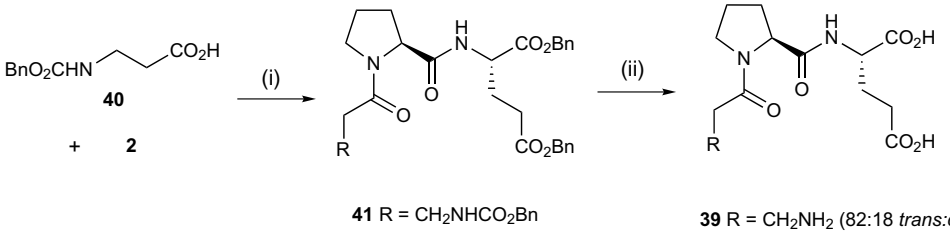


Figure 1. Cellular survival bioassay. MTT-values were taken 24 h after injury when apoptotic nuclei become apparent within the cell culture. The neuroprotective dose range for L-AlaPE **7** lies between 1 and 100 μM with the highest recovery value of 26.4%.



Scheme 8. Reagents and conditions: (i) HOBt, EDCl·HCl, THF, Et_3N, rt, 20 h (70%); (ii) H_2, 10% Pd/C, MeOH, 20 h (86%).

3. Conclusion

To summarize, we described herein the synthesis of 10 analogues of GPE and their biological evaluation against striatal cell survival post apoptosis-induced injury. Compound **7** exhibited the best recovery value, 35%, while **28** was very similar. Analogues **6** and **29** had lower recovery values but all four exhibited neuroprotective activity. The structure and spectra of the new analogues were determined by full analysis of the NMR and mass spectral data.

4. Experimental

4.1. General method

All reagents were used as supplied. Solvents were purified by standard methods.[39] Analytical thin layer chromatography (TLC) was carried out on 0.20 mm pre-coated silica gel plates (ALUGRAM® SIL G/UV$_{254}$). Products were visualized by UV fluorescence and heating of plates dipped in anisaldehyde in ethanolic sulfuric acid or alkaline potassium permanganate solution. Flash chromatography was performed using Scharlau 60 (40–60 μm mesh) silica gel. Melting points in degrees Celsius (°C) were determined on an Electrothermal® melting point apparatus and are uncorrected. Optical rotations were measured at the sodium D line (589 nm), at 20 °C, with a Perkin Elmer 341 polarimeter using a 1 dm path length cell and are given in units of 10^{-1} deg cm^2 g^{-1}. IR spectra were recorded on a Perkin Elmer Spectrum one FT-IR spectrometer and the samples were prepared as thin films between sodium chloride plates. Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker AVANCE DRX400 (^{1}H, 400 MHz; ^{13}C, 100 MHz), a Bruker AVANCE 300 (^{1}H, 300 MHz; ^{13}C, 75 MHz) or a Bruker AC200 (^{1}H, 200 MHz; ^{13}C, 50 MHz) spectrometer at 298 K. For ^{1}H 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, 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), $(CD_3)_2SO$ (δ 39.4) or externally to 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS) and are reported consecutively as position (δ_C), degree of hybridization and assignment. Assignments were aided by DEPT135, COSY, NOESY, HSQC and HMBC experiments. When two sets of peaks arise in the NMR spectra due to *cis*/*trans* isomerization about the glycine–proline amide bond, the chemical shift for the minor *cis* conformer is indicated by the symbol #. Assignment of the respective isomers is made according to Kessler[40] and NOESY experiments. Mass spectra were recorded on a VG-70SE mass spectrometer (EI, CI and FAB). High-resolution mass spectra were recorded at a nominal resolution of 5000. Analytical HPLC studies were performed on a Waters 600 system with a 2487 dual λ absorbance detector using an Altech Econosphere C_{18} Si column (150 × 4.6 mm; 5 μm), with a 5 min H_2O flush (0.05% TFA) then steady gradient over 25 min to 100% CH_3CN as eluent at a flow rate of 1 mL/min. L-Proline methyl ester hydrochloride (**15**) was prepared according to experimental procedures given in the previous paper.[32,33] All chemicals utilized in the bioassay were purchased from Invitrogen.

4.2. Striatal cell culture

Two pregnant Wistar rats (gestational day 18) underwent Caesarean section according to approved procedures from the animal ethics committee of the University of Auckland. The Wistar dams were anaesthetized in a CO_2-enriched atmosphere and sacrificed by subsequent spinal cord dislocation. The embryos were removed from their embryonic sacs and decapitated. After incisions into the skull with fine scissors, the embryonic brain was removed with a fine spatula. Striatal tissue was separated from neocortical tissue under the microscope and placed into serum-free DMEM/F-12 medium supplemented with penicillin/streptomycin (100 u/mL). Tissue underwent 15 trituration steps using a P1000 pipettor to obtain dissociated cells, which were centrifuged for 5 min at 250 G (4 °C) and the supernatant was discarded. Cells were re-suspended into 1 mL DMEM/F-12 medium and were kept on ice. Cell suspension (400,000 cells/cm^2) was applied to 0.1 mg/mL poly-L-lysine pre-coated 96-well plates (3 h at 37 °C) and the volume was increased up to 100 μL with DMEM/F-12 + 5% FBS. Cells were cultivated at 37 °C in 100% humidity and saturated 5% CO_2 atmosphere. After 24 h the medium was changed to serum-free Neurobasal/B27. Cells were fed every 3 days and maintained until 8 days in vitro (DIV).

4.3. Cellular injury, drug administration and cellular survival analysis

Striatal cells were injured after 7 DIV. The injury paradigm involved 30 nM okadaic acid treatment with simultaneous drug administration (GPE analogues) for 24 h. Afterward, 1 μL of 3 μM okadaic acid stock solution together with 1 μL of GPE analogue dissolved in PBS (vehicle received 2 μL of PBS) were incubated for 24 h with the striatal cells. Subsequently, 20 μL MTT (5 mg/mL in PBS) was added for 4 h. The reaction was terminated by addition of 100 μL 4% sodium dodecyl sulfate (SDS) solution. After 16–24 h incubation time, extinction values are read at 595 nm.

The difference between the vehicle and the okadaic acid injury condition was calculated. This value is set as the theoretical 100% recovery value. Measured values for the tested compounds are expressed in percentage of the 100% value. The unpaired Student's *t*-test is used for statistical analysis.

4.4. Dibenzyl *N*-*tert*-butoxycarbonyl-L-prolyl-L-glutamate[31] (5)

N-*tert*-Butoxycarbonyl-L-proline **3** (5.00 g, 23.20 mmol) was dissolved in dichloromethane (65 mL) under nitro-

gen. The solution was cooled to 0 °C and triethylamine (3.25 mL, 23.30 mmol) added dropwise over a period of 5 min. Ethyl chloroformate (2.2 mL, 23.20 mmol) was then added dropwise over a period of 5 min that produced a white gas and a precipitate. The mixture was stirred at 0 °C for 30 min, then a solution of L-glutamic acid dibenzyl ester *p*-toluenesulfonate **4** (11.60 g, 23.20 mmol) in dichloromethane (60 mL) and triethylamine (3.25 mL, 23.30 mmol) at 0 °C was added over a period of 5 min. The white precipitate dissolved and the solution was stirred for 2 h at 0 °C then warmed to room temperature and stirred overnight. Dichloromethane (50 mL) was added and the organic layer was washed with aqueous saturated sodium hydrogen carbonate solution (2 × 50 mL) and aqueous 2 M citric acid (50 mL). The organic layer was dried ($MgSO_4$), filtered and evaporated under reduced pressure to form crude protected *peptide* **5** (11.9 g, 98%) as a light green oil, which was used without further purification: δ_H (200 MHz; $CDCl_3$; Me_4Si) 1.42 [9H, s, $(CH_3)_3C$], 1.73–2.49 (8H, m, Proβ-H_2, Proγ-H_2, Gluβ-H_2 and Gluγ-H_2), 3.42 (2H, br s, Proδ-H_2), 4.20–4.24 (1H, br s, Proα-H), 4.58–4.68 (1H, m, Gluα-H), 5.09 (2H, s, OCH_2Ph), 5.15 (2H, s, OCH_2Ph), 6.80 (1H, br s, Glu-NH) and 7.23–7.43 (10H, m, 2 × Ph); δ_C (50 MHz; $CDCl_3$) 23.7 (CH_2, Proγ-C), 27.4 (CH_2, Gluβ-C), 28.3 [CH_3, $(CH_3)_3C$], 30.3 (CH_2, Proβ-C), 30.7 (CH_2, Gluγ-C), 47.0 (CH_2, Proδ-C), 51.5 (CH, Gluα-C), 59.8 (CH, Proα-C), 66.4 (CH_2, OCH_2Ph), 67.2 (CH_2, OCH_2Ph), 80.8 [quat., $(CH_3)_3C$], 128.2 (CH, Ph), 128.4 (CH, Ph), 128.5 (CH, Ph), 128.6 (CH, Ph), 128.8 (CH, Ph), 135.2 (quat., Ph), 135.6 (quat., Ph), 156.8 (quat., NCO_2), 171.4 (quat., Glu-CO), 172.4 (quat., Glu-CO) and 175.0 (quat., Pro-CON); *m*/*z* (EI+) 524 (M^+, 4%).

4.5. Dibenzyl L-prolyl-L-glutamate[31] (2)

Peptide **5** (5.0 g, 9.53 mmol) was dissolved in trifluoroacetic acid (10 mL) at 0 °C and the solution was stirred for 1 h, then evaporated under reduced pressure to form an orange gum. Ethyl acetate (80 mL) was added and the organic layer washed with 0.5 M aqueous potassium carbonate solution (2 × 20 mL). The organic layer was dried ($MgSO_4$) and evaporated to give crude protected *dipeptide* **2** (4.04 g, 100%) as a light orange gum, which was used without further purification: δ_H (200 MHz; $CDCl_3$; Me_4Si) 1.68–2.44 (8H, m, Proβ-H_2, Proγ-H_2, Gluβ-H_2 and Gluγ-H_2), 2.91–3.08 (2H, m, Proδ-H_2), 3.78 (1H, br s, Pro-NH), 3.91–3.95 (1H, m, Proα-H), 4.55–4.65 (1H, m, Gluα-H), 5.08 (2H, s, OCH_2Ph), 5.14 (2H, s, OCH_2Ph), 7.26–7.42 (10H, m, 2 × Ph) and 8.17–8.20 (1H, m, Glu-NH); δ_C (50 MHz; $CDCl_3$) 26.0 (CH_2, Proγ-C), 27.1 (CH_2, Gluβ-C), 30.3 (CH_2, Proβ-C), 30.7 (CH_2, Gluγ-C), 47.0 (CH_2, Proδ-C), 51.5 (CH, Gluα-C), 60.1 (CH, Proα-C), 66.5 (CH_2, OCH_2Ph), 67.2 (CH_2, OCH_2Ph), 128.0 (CH, Ph), 128.2 (CH, Ph), 128.3 (CH, Ph), 128.4 (CH, Ph), 128.5 (CH, Ph), 135.2 (quat., Ph), 135.6 (quat., Ph), 171.6 (quat., Glu-CO), 172.2 (quat., Glu-CO) and 175.3 (quat., Pro-CON); *m*/*z* (FAB+) 425 (MH^+, 100%).

Representative procedure A for the protection of amino acids is as follows:

4.6. *N*-Benzyloxycarbonyl-D-alanine[41] (10)

D-Alanine **8** (0.40 g, 4.5 mmol) and sodium carbonate (1.43 g, 13.5 mmol) were dissolved in water (15 mL) and cooled to 0 °C. Benzyl chloroformate (0.70 mL, 4.90 mmol) in dioxane (4 mL) was added dropwise over 5 min and the solution was stirred at 0 °C for 0.5 h and allowed to warm to room temperature. Stirring was continued for 20 h and the solution was washed with diethyl ether (50 mL), and the aqueous layer acidified with 10% HCl solution to pH 1–2 and extracted with ethyl acetate (2 × 50 mL). The organic layers were combined, dried ($MgSO_4$), filtered and evaporated under reduced pressure to form a clear oil, which solidified on standing to crude *carbamate* **8** (1.00 g, 100%) as a gummy white solid which was used without further purification: δ_H (200 MHz; $CDCl_3$; Me_4Si) 1.43 (3H, d, *J* 7.4, Ala-CH_3), 4.35–4.40 (1H, m, Alaα-H), 5.11 (2H, s, OCH_2Ph), 5.48 (1H, d, *J* 8.0, Ala-NH), 7.33 (5H, s, Ph) and 10.85 (1H, br s, CO_2H); δ_C (50 MHz; $CDCl_3$) 18.3 (CH_3, Ala-CH_3), 48.9 (CH, Alaα-C), 67.6 (CH_2, OCH_2Ph), 128.5 (CH, Ph), 128.7 (CH, Ph), 129.0 (CH, Ph), 136.0 (quat., Ph), 155.9 (quat., NCO_2) and 177.6 (quat., CO_2H); *m*/*z* (EI+) 223 (M^+, 4%).

Representative procedure B for the preparation of protected tripeptides and analogues is as follows:

4.7. Dibenzyl-*N*-benzyloxycarbonyl-D-alanyl-L-prolyl-L-glutamate

Amine[31] **2** (1.00 g, 2.36 mmol), acid **10** (0.53 g, 2.36 mmol) and 1-hydroxybenzotriazole hydrate (0.36 g, 2.36 mmol) were dissolved in tetrahydrofuran (40 mL) under nitrogen at room temperature. EDCI (0.45 g, 2.36 mmol) was added, followed by triethylamine (0.33 mL, 2.36 mmol) and the solution was stirred for 20 h. The solvent was evaporated under reduced pressure to dryness and the residue was dissolved in ethyl acetate (125 mL), washed successively with saturated sodium hydrogen carbonate solution (30 mL), brine (30 mL), water (30 mL) and then dried ($MgSO_4$). The solution was filtered and the solvent removed under reduced pressure to produce a white gum. Purification of the gum by flash column chromatography (20% hexane–ethyl acetate) *gave fully protected tripeptide* (0.69 g, 46%) as a clear gum: $[\alpha]_D$ −52.3 (*c* 0.04, MeOH); δ_H (200 MHz; $CDCl_3$; Me_4Si) 1.31 (3H, d, *J* 7.3, Ala-CH_3), 1.92–2.28 (6H, m, Proβ-H_2, Proγ-H_2 and Gluβ-H_2), 2.42 (2H, t, *J* 7.5, Gluβ-H_2), 3.30–3.50 (1H, m, Proδ-H), 3.70–3.80 (1H, m, Proδ-H), 4.46–4.58 (3H, m, Alaα-H, Proα-H and Gluα-H), 4.99 (2H, s, OCH_2Ph), 5.09 (2H, s, OCH_2Ph), 5.12 (2H, s, OCH_2Ph), 5.60–5.66 (1H, d, *J* 8.0, Ala-NH) and 7.26–7.38 (15H, m, 3 × Ph); δ_C (50 MHz; $CDCl_3$) 17.5 (CH_3, Ala-CH_3), 24.5 (CH_2, Proγ-C), 26.8 (CH_2, Gluβ-C), 28.1 (CH_2, Proβ-C), 30.1 (CH_2, Gluγ-C), 47.0 (CH_2, Proδ-C), 48.5 (CH, Alaα-C), 51.7 (CH, Gluα-C), 60.2 (CH, Proα-C), 66.3 (CH_2, OCH_2Ph), 66.8 (CH_2, OCH_2Ph), 67.0 (CH_2, OCH_2Ph), 127.9 (CH, Ph), 128.0 (CH, Ph), 128.2 (CH, Ph), 128.4 (CH, Ph), 135.3 (quat., Ph), 135.7 (quat., Ph), 136.3 (quat., Ph), 155.8 (quat., NCO_2), 171.0 (quat., Ala-CO), 171.2 (quat., Glu-CO),

172.0 (quat., Glu-CO) and 172.5 (quat., Pro-CON); *m/z* (EI+) 629.2734 (M^+, $C_{35}H_{39}N_3O_8$ requires 629.2737).

Representative procedure C for the hydrogenation of protected tripeptides and analogues is as follows:

4.8. D-Alanyl-L-prolyl-L-glutamic acid (6)

A mixture of the above protected peptide (0.30 g, 0.48 mmol) and 10 wt% palladium on activated carbon (0.05 g, 0.13 mmol) in methanol (40 mL) was stirred under an atmosphere of hydrogen at room temperature for 20 h. The solution was filtered through a Celite™ pad and the pad washed with methanol (2 × 25 mL). The filtrate was evaporated to dryness, dissolved in methanol (35 mL) and refiltered through a Celite™ pad. The solution was evaporated to dryness, dried in vacuo and triturated with anhydrous ether to give *analogue* **6** (0.13 g, 86%) as a white solid. Compound **6** was shown to be an 82:18 *trans:cis* mixture of conformers by ^{13}C NMR analysis: mp 89–93 °C; $[\alpha]_D$ −49.1 (*c* 0.05, MeOH); δ_H (200 MHz; CD_3OD) 1.40–1.51 (3H, m, Ala-CH_3), 2.03–2.14 (6H, m, Proβ-H_2, Proγ-H_2 and Gluβ-H_2), 2.33–2.40 (2H, m, Gluγ-H_2), 3.54–3.58 (1H, m, Proδ-H), 3.81–3.83 (1H, m, Proδ-H), 4.16–4.20 (1H, m, Alaα-H), 4.32–4.35 (1H, m, Gluα-H) and 4.45–4.48 (1H, m, Proα-H); δ_C (50 MHz; CD_3OD) 15.9 (CH_3, Ala-CH_3), 25.5 (CH_2, Proγ-C), 28.3 (CH_2, Gluβ-C), 30.5 (CH_2, Proβ-C), 32.1 (CH_2, Gluγ-C), 48.4 (CH_2, Proδ-C), 49.4 (CH, Alaα-C), 50.1† (CH, Alaα-C), 54.8 (CH, Gluα-C), 62.0 (CH, Proα-C), 168.0 (quat., Ala-CO), 173.8 (quat., Pro-CON), 175.0 (quat., Gluα-CO) and 176.3 (quat., Gluγ-CO); *m/z* (FAB+) 316.1514 (MH^+ $C_{13}H_{22}N_3O_6$ requires 316.1509).

4.9. *N*-Benzyloxycarbonyl-L-alanine[35] (11)

Following procedure A, protection of L-alanine **9** using 4 M aqueous sodium hydroxide solution at 0 °C gave *carbamate* **11** (0.98 g, 20%) as a gummy white solid following purification by flash column chromatography (diethyl ether): mp 80–82 °C (lit.[35] 82–83 °C); δ_H (200 MHz; $CDCl_3$; Me_4Si) 1.33 (1H, d, *J* 7.2, Ala-CH_3), 4.25–4.31 (1H, m, Alaα-H), 5.03 (2H, s, OCH_2Ph), 5.46 (1H, d, *J* 8.0, Ala-NH), 7.25 (5H, s, Ph) and 7.96 (1H, br s, CO_2H); δ_C (50 MHz; $CDCl_3$) 18.3 (CH_3, Ala-CH_3), 49.4 (CH, Alaα-C), 67.0 (CH_2, OCH_2Ph), 128.0 (CH, Ph), 128.1 (CH, Ph), 128.4 (CH, Ph), 136.0 (quat., Ph), 155.9 (quat., NCO_2) and 176.8 (quat., CO_2H); *m/z* (EI+) 223 (M^+, 4%).

4.10. Dibenzyl-*N*-benzyloxycarbonyl-L-alanyl-L-prolyl-L-glutamate

Following procedure B, coupling of amine[31] **2** with carbamate **11** gave *protected tripeptide* (0.67 g, 60%) as a clear gum following purification by flash column chromatography (20% hexane–ethyl acetate): $[\alpha]_D$ −43.2 (*c* 0.05, MeOH); δ_H (200 MHz; $CDCl_3$; Me_4Si) 1.27 (3H, d, *J* 7.3, Ala-CH_3), 1.89–2.37 (8H, m, Proβ-H_2, Proγ-H_2, Gluβ-H_2 and Gluγ-H_2), 3.50–3.70 (2H, m, Proδ-H_2), 4.50–4.59 (3H, m, Proα-H, Gluα-H and Alaα-H), 5.08 (2H, s, OCH_2Ph), 5.09 (2H, s, OCH_2Ph), 5.14 (2H, s, OCH_2Ph), 5.65 (1H, d, *J* 8.0, Ala-NH) and 7.20–7.33 (15H, m, 3 × Ph); δ_C (50 MHz; $CDCl_3$) 18.3 (CH_3, Ala-CH_3), 25.0 (CH_2, Proγ-C), 27.2 (CH_2, Gluβ-C), 27.3 (CH_2, Proβ-C), 29.9 (CH_2, Gluγ-C), 47.2 (CH_2, Proδ-C_2), 48.2 (CH, Alaα-C), 51.6 (CH, Gluα-C), 59.8 (CH, Proα-C), 66.4 (CH_2, OCH_2Ph), 67.2 (CH_2, OCH_2Ph), 127.0 (CH, Ph), 128.0 (CH, Ph), 128.2 (CH, Ph), 128.5 (CH, Ph), 128.6 (CH, Ph), 135.1 (quat., Ph), 135.7 (quat., Ph), 155.6 (quat., NCO_2), 170.8 (quat., Ala-CO), 171.3 (quat., Glu-CO) 172.3 (quat., Glu-CO) and 172.5 (quat., Pro-CON); *m/z* (EI+) 630.2805 (MH^+, $C_{35}H_{40}N_3O_8$ requires 630.2815).

4.11. L-Alanyl-L-prolyl-L-glutamic acid[42–44] (7)

Following procedure C, hydrogenation of the above amide in 9:1 MeOH–H_2O afforded *analogue* **7** (0.30 g, 76%) as a white solid on trituration with anhydrous diethyl ether. Compound **7** was shown to be a 72:28 *trans:cis* mixture of conformers by ^{13}C NMR analysis: mp 51–53 °C; $[\alpha]_D$ −43.0 (*c* 0.05, MeOH); δ_H (200 MHz; CD_3OD) 1.45–1.54 (3H, m, Ala-CH_3), 1.94–2.23 (6H, m, Proβ-H_2, Proγ-H_2 and Gluβ-H_2), 2.42 (2H, t, *J* 7.5, Gluγ-H_2), 3.60–3.65 (2H, m, Proδ-H_2), 4.20–4.36 (2H, m, Alaα-H, Gluα-H) and 4.40–4.50 (1H, m, Proα-H); δ_C (50 MHz; CD_3OD) 16.4 (CH_3, Ala-CH_3), 16.8† (CH_3, Ala-CH_3), 23.4† (CH_2, Proγ-C), 26.1 (CH_2, Proγ-C), 28.6† (CH_2, Gluβ-C), 29.6 (CH_2, Gluβ-C), 30.3 (CH_2, Proβ-C), 32.8 (CH_2, Gluγ-C), 48.5 (CH_2, Proδ-C), 49.3 (CH, Gluα-C), 55.5 (CH, Alaα-C), 56.4† (CH, Alaα-C), 62.0 (CH, Proα-C), 170.4 (quat., Ala-CON), 173.2 (quat., Pro-CON), 177.7 (quat, Gluα-CO) and 178.9 (quat., Gluγ-CO); *m/z* (FAB+) 316.1500 (MH^+, $C_{13}H_{22}N_3O_6$ requires 316.1509).

4.12. *N*-Benzyloxycarbonyl aminoisobutyric acid[34,35] (16)

Aminoisobutyric acid (2.00 g, 19.40 mmol) and sodium carbonate (6.16 g, 58.12 mmol) were dissolved in water (70 mL) and the solution cooled to 0 °C. Benzyl chloroformate (3.05 mL, 21.4 mmol) in dioxane (20 mL) was added dropwise over a period of 15 min. The solution was stirred at 0 °C for 1.5 h and warmed to room temperature. Stirring continued for 20 h and the aqueous layer was extracted with diethyl ether (100 mL), acidified with hydrochloric acid (32%) to pH 1 and extracted with ethyl acetate (2 × 75 mL). The organic layers were combined, dried ($MgSO_4$), filtered and evaporated under reduced pressure to give a clear oil, which solidified on standing to yield crude *carbamate* **16** (4.59 g, 100%) as a gummy solid which was used without further purification: δ_H (200 MHz; $CDCl_3$; Me_4Si) 1.57 (6H, s, 2 × CH_3), 5.10 (2H, s, OCH_2Ph), 5.55 (1H, br s, NH), 7.26–7.33 (5H, m, Ph) and 10.53 (1H, br s, CO_2H); δ_C (50 MHz; $CDCl_3$) 25.0 (CH_3, 2 × CH_3), 56.3 [quat., $C(CH_3)_2$], 66.9 (CH_2, OCH_2Ph), 128.0 (CH, Ph), 128.4 (CH, Ph), 136.1 (quat., Ph), 155.2 (quat., NCO_2) and 179.6 (quat., CO_2H); *m/z* (EI+) 237 (M^+, 12%).

† Denotes resonances assigned to minor conformer.

4.13. Methyl-*N*-benzyloxycarbonyl-aminoisobutyryl-L-prolinate[45–48] (17)

Dry triethylamine (3.27 mL, 23.50 mmol) was added dropwise to a solution of hydrochloride **15**[32,33] (1.29 g, 7.81 mmol), *N*-benzyloxycarbonylaminoisobutyric acid **16** (2.04 g, 8.60 mmol) and BoP (3.81 g, 8.60 mmol) in dry dichloromethane (50 mL) under an atmosphere of nitrogen at room temperature, and the reaction mixture stirred for 3 h. Dichloromethane (50 mL) was added and the solution washed successively with 0.5 M aqueous hydrochloric acid (2 × 25 mL) and saturated aqueous sodium hydrogen carbonate (2 × 25 mL), dried (Na_2SO_4), filtered and evaporated in vacuo to form a light orange gum. Purification by flash column chromatography (25% hexane–ethyl acetate) yielded *ester* **17** (1.95 g, 68%) as a clear oil: $[\alpha]_D$ −98.7 (*c* 0.11, MeOH); lit.[45] −30.4 (*c* 1.20, MeOH); lit.[46] −87.5 (*c* 0.20, MeOH); δ_H (300 MHz; $CDCl_3$) 1.34 [3H, s, $(CH_3)C$], 1.41 [3H, s, $(CH_3)C$], 1.80–1.90 (2H, m, Proβ-H_2), 2.03–2.04 (2H, m, Proγ-H_2), 3.46–3.55 (1H, m, Proδ-H_AH_B), 3.70 (3H, s, CH_3O), 3.70–3.75 (1H, m, Proδ-H_AH_B), 4.55–4.56 (1H, m, Proα-H), 5.06 (2H, s, OCH_2Ph), 5.60 (1H, s br, aib-NH) and 7.27–7.36 (5H, m, Ph); δ_C (75 MHz; $CDCl_3$) 24.8 [CH_3, $(CH_3)C$], 25.1 [CH_3, $(CH_3)C$], 26.1 (CH_2, Proβ-C), 28.1 (CH_2, Proγ-C), 48.3 (CH_2, Proδ-C), 52.5 (CH_3, CH_3O), 57.2 [quat., $(CH_3)_2C$], 61.2 (CH, Proα-C), 66.9 (CH_2, OCH_2Ph), 128.5 (CH, Ph), 128.6 (CH, Ph), 128.8 (CH, Ph), 136.9 (quat., Ph), 154.7 (quat., NCO_2), 172.5 (quat., aib-CON) and 173.4 (quat., CO_2CH_3); *m/z* (EI+) 348.1681 (M^+, $C_{18}H_{24}N_2O_5$ requires 348.1685).

4.14. *N*-Benzyloxycarbonylaminoisobutyryl-L-proline[46,48] (18)

To a solution of ester **17** (1.95 g, 5.61 mmol) in dioxane (50 mL) was added dropwise 1 M aqueous NaOH (20 mL, 20 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 × 50 mL), dried (Na_2SO_4), filtered and evaporated in vacuo to give *acid* **18** (1.77 g, 95%) as a clear foam, which was used without further purification: $[\alpha]_D$ −58.0 (*c* 0.15, MeOH); δ_H (300 MHz; $CDCl_3$) 1.55 [6H, s, 2 × $(CH_3)C$], 1.80–2.09 (4H, m, Proβ-H_2 and Proγ-H_2), 3.48–3.56 (2H, m, Proδ-H_2), 4.60 (1H, m, Proα-H), 5.10 (2H, m, OCH_2Ph), 5.57 (1H, br s, aib-NH), 7.29–7.36 (5H, m, Ph) and 8.46 (1H, br s, CO_2H); δ_C (75 MHz; $CDCl_3$) 24.8 [CH_3, $(CH_3)C$], 25.1 [CH_3, $(CH_3)C$], 25.7 (CH_2, Proβ-C), 27.2 (CH_2, Proγ-C), 48.1 (CH_2, Proδ-C), 56.9 [quat., $(CH_3)_2C$], 61.4 (CH, Proα-C), 66.9 (CH_2, OCH_2Ph), 128.3 (CH, Ph), 128.5 (CH, Ph), 136.2 (quat., Ph), 154.7 (quat., NCO_2), 173.3 (quat., aib-CON) and 174.4 (quat., CO_2CH_3); *m/z* (EI+) 334.1539 (M^+, $C_{18}H_{24}N_2O_5$ requires 334.1529).

4.15. Dibenzyl *N*-benzyloxycarbonyl-aminoisobutyryl-L-prolyl-L-glutamate

Dry triethylamine (0.63 mL, 4.5 mmol) was added to a solution of acid **18** (0.50 g, 1.5 mmol) and L-glutamic acid dibenzyl ester *p*-toluene sulfonate **4** (0.82 g, 1.7 mmol) in dry dichloromethane (30 mL) under an atmosphere of nitrogen at 0 °C, and the reaction mixture stirred for 10 min. Bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BoP–Cl, 97%) (0.42 g, 1.7 mmol) was added and the solution stirred for 2 h, warmed to room temperature and stirred for an additional 20 h. The solution was washed successively with 1 M aqueous hydrochloric acid (2 × 30 mL) and saturated aqueous sodium hydrogen carbonate (2 × 30 mL), dried ($MgSO_4$), filtered and evaporated in vacuo to give a light orange gum. Purification of the resultant residue by flash column chromatography (ethyl acetate) yielded *fully protected tripeptide* (0.63 g, 80%) as a clear oil: $[\alpha]_D$ −36.5 (*c* 0.05, MeOH): δ_H (200 MHz; $CDCl_3$; Me_4Si) 1.41 (3H, s, CH_3), 1.51 (3H, s, CH_3), 1.68–2.29 (6H, m, Proβ-H_2, Proγ-H_2 and Gluβ-H_2), 2.44 (2H, t, *J* 7.5, Gluβ-H_2), 3.20–3.25 (1H, m, Proδ-H), 3.55–3.60 (1H, m, Proδ-H), 4.44–4.51 (1H, m, Gluα-H) 4.54–4.61 (1H, m, Proα-H), 4.97 (2H, s, OCH_2Ph), 5.06 (2H, s, OCH_2Ph), 5.15 (2H, s, OCH_2Ph), 5.48 (1H, br s, $NHCO_2$), 7.28–7.33 (15H, m, Ph) and 7.58–7.62 (1H, m, NHCO); δ_C (50 MHz; $CDCl_3$) 24.8 [CH_3, $(CH_3)_2C$], 25.6 (CH_2, Proγ-C), 26.1 [CH_3, $C(CH_3)_2$], 28.0 (CH_2, Gluβ-C and Proβ-C), 30.1 (CH_2, Gluγ-C), 48.0 (CH_2, Proδ-C), 51.8 (CH, Gluα-C), 57.0 (quat., $C(CH_3)_2$), 62.2 (CH, Proα-C), 66.1 (CH_2, OCH_2Ph), 66.9 (CH_2, OCH_2Ph), 128.0 (CH, Ph), 128.4 (CH, Ph), 128.5 (CH, Ph), 136.0 (quat., Ph), 155.1 (quat., NCO_2), 171.0 (quat., Glu-CO), 172.0 (quat., Glu-CO) and 172.7 (quat., Pro-CON); *m/z* (EI+) 644.2969 (M^+, $C_{36}H_{42}N_3O_8$ requires 644.2972).

4.16. Aminoisobutyryl-L-prolyl-L-glutamic acid hydrochloride (12)

Following procedure C, hydrogenation of the above protected amide using aqueous hydrochloric acid[49] in 20% water–tetrahydrofuran yielded *analogue* **12** (0.04 g, 52%) as a light green solid on trituration with anhydrous diethyl ether. Compound **12** was shown to be exclusively the *trans* conformer[50] by ^1H and ^{13}C NMR analysis: mp 53–55 °C; $[\alpha]_D$ −51.2 (*c* 0.04, MeOH); δ_H (400 MHz; D_2O) 1.73 [3H, s, $(CH_3)C$], 1.75 [3H, s, $(CH_3)C$], 1.94–2.04 (1H, m, Proβ-H_AH_B), 2.05–2.11 (3H, m, Proβ-H_AH_B and Gluβ-H_2), 2.23–2.34 (2H, m, Proγ-H_2), 2.55 (2H, t, *J* 7.2, Gluγ-H_2) 3.78–3.89 (2H, m, Proδ-H_2), 4.44 (1H, dd, *J* 5.4 and 8.4, Gluα-H) and 4.56 (1H, dd, *J* 5.6 and 8.4, Proα-H); δ_C (100 MHz; D_2O) 22.5 [CH_3, $(CH_3)C$], 22.7 [CH_3, $(CH_3)C$], 26.2 (CH_2, Proγ-C), 26.5 (CH_2, Gluβ-C), 29.1 (CH_2, Proβ-C), 30.7 (CH_2, Gluγ-C), 49.8 (CH_2, Proβ-C), 52.9 (CH, Gluα-C), 59.0 [quat., $(CH_3)_2C$], 63.2 (CH, Proα-C), 170.2 (quat., aib-CO), 175.2 (quat., Pro-CON), 175.7 (quat., Gluα-CO) and 177.8 (quat., Gluγ-CO); *m/z* (FAB+) 330.1664 (MH^+, $C_{14}H_{24}N_3O_6$ requires 330.1665).

4.17. *N*-Benzyloxycarbonyl-1-aminocyclopentane-1-carboxylic acid (21)

Following procedure A, protection of 1-aminocyclopentanecarboxylic acid **19** in 2:1 H_2O–dioxane afforded crude *carbamate* **21** (0.253 g, 62%) as an oil, which

solidified on standing. Carbamate **21** was shown to be a 70:30 mixture of conformers by ^{1}H NMR analysis (the ratio was estimated from the integration of the resonances at δ 5.31 and 7.29–7.40, assigned to the N–H protons of the major and minor conformers, respectively): mp 70–80 °C (lit.[51] 82–86 °C, ethyl acetate, petroleum ether); δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.83 (4H, br s, 2 × cyclopentyl-H_2), 2.04 (2H, br s, cyclopentyl-H_2), 2.20–2.40 (2H, m, cyclopentyl-H_2), 5.13 (2H, br s, OCH_2Ph), 5.31 (0.7H, br s, N–H) and 7.29–7.40 (5.3H, m, Ph and N–H$^{\#}$); δ_C (100 MHz; $CDCl_3$) 24.6 (CH_2, cyclopentyl-C), 37.5 (CH_2, cyclopentyl-C), 66.0 (quat., cyclopentyl-C), 66.8 (CH_2, OCH_2Ph), 128.0 (CH, Ph), 128.1 (CH, Ph), 128.4 (CH, Ph), 136.1 (quat., Ph), 155.8 (quat., NCO_2) and 179.5 (quat., CO_2H).

4.18. *N*-Benzyloxycarbonylcyclopentylglycyl-L-proline methyl ester (23)

To a solution of proline methyl ester hydrochloride[32,33] **15** (0.037 g, 0.22 mmol), carbamate **19** (0.064 g, 0.243 mmol) and BoP (0.107 g, 0.243 mmol) in dichloromethane (3 mL) was added triethylamine (0.057 g, 0.66 mmol) and the solution stirred at room temperature for 20 h. The reaction mixture was washed with 2 M hydrochloric acid, saturated sodium hydrogen carbonate solution, dried (Na_2SO_4), filtered and the solvent removed to give an oil (0.109 g) that was purified by chromatography (silica gel, dichloromethane–ethyl acetate, 6:1, 5:1) to afford *methyl ester* **23** (0.066 g, 80%) as a colourless oil. Methyl ester **23** was shown to be a 60:40 mixture of conformers by ^{1}H NMR analysis (the ratio was estimated from the integration of the resonances at δ 4.52 and 4.23, assigned to the Proα-H protons of the major and minor conformers, respectively): $[\alpha]_D$ −87.6 (*c* 0.46, CH_2Cl_2); δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.58–2.05 (10.4H, m, Proβ-H_2, Proγ-H_2, 3 × cyclopentyl-H_2 and cyclopentyl-H$^{\#}$), 2.1–2.25 (1.6H, m, cyclopentyl-H), 2.51–2.70 (1H, cyclopentyl-H), 3.46 (1H, m, Proδ-H_AH$_B$), 3.52–3.75 (4H, m, CO_2CH_3 and Proδ-H$_A$$H_B$), 4.23† (0.4H, br s, Proα-H), 4.52 (0.6H, br s, Proα-H), 4.92† (0.4H, d, *J* 11.7, OCH_AH$_B$Ph), 5.03 (0.6, d, *J* 11.6, OCH_AH$_B$Ph), 5.11 (0.6H, d, *J* 11.7, OCH$_A$$H_B$Ph), 5.37† (0.4H, d, *J* 11.9, OCH$_A$$H_B$Ph), 5.44 (0.6H, br s, N–H), 5.94 (0.4H, br s, N–H) and 7.29-7.34 (5H, m, Ph); δ_C (100 MHz; $CDCl_3$) 24.2 (CH_2, 2 × cyclopentyl-C), 25.3 (CH_2, Proγ-C), 27.8 (CH_2, Proβ-C), 36.5 (CH_2, cyclopentyl-C), 36.7 (CH_2, cyclopentyl-C), 37.2† (CH_2, cyclopentyl-C), 37.5† (CH_2, cyclopentyl-C), 47.5 (CH_2, Proδ-C), 51.9 (CH_3, CO_2CH_3), 60.3 (CH, Proα-C), 66.5† (CH_2, OCH_2Ph), 66.7 (CH_2, OCH_2Ph) [Glyα-C obscured], 128.1 (CH, Ph), 128.2 (CH, Ph), 128.3 (CH, Ph), 136.4 (quat., Ph), 154.4 (quat., NCO_2), 155.9† (quat., NCO_2), 171.4 (quat., Gly-CO), 171.8† (quat., Gly-CO) and 173.1 (quat., Pro-CO); *m/z* (EI+) 374.1841 (M$^+$, $C_{20}H_{26}N_2O_5$ requires 334.1842).

4.19. Dibenzyl *N*-benzyloxycarbonylcyclopentylglycyl-L-prolyl-L-glutamate

To a solution of methyl ester **23** (0.1 g) in dioxane (3 mL) was added 1 M aqueous sodium hydroxide (1.33 mL, 1.33 mmol) and the mixture was stirred for 20 h at room temperature. Water (5 mL) was added and the mixture extracted with dichloromethane. The aqueous layer was acidified with 2 M aqueous hydrochloric acid until pH 1 was attained and the mixture extracted with dichloromethane, dried (Na_2SO_4), filtered and concentrated in vacuo to afford *acid* **25** (0.092 g, 95%) that was used without further purification. Ethyl chloroformate (0.019 g, 0.183 mmol) was added dropwise to a solution of acid **25** (0.073 g, 0.2 mmol) and triethylamine (0.030 g, 0.21 mmol) in dichloromethane (4 mL) at 0 °C. The solution was stirred for 40 min, a solution of glutamic acid dibenzyl ester *p*-toluenesulfonate **4** (0.1 g, 0.2 mmol) and triethylamine (0.030 g, 0.21 mmol) in dichloromethane (2 mL) was added and stirring was continued for further 17 h at room temperature. The reaction was washed with 2 M hydrochloric acid, saturated sodium hydrogen carbonate solution, dried (Na_2SO_4), filtered and the solvent removed to give an oil (0.140 g) that was purified by chromatography (silica gel, dichloromethane–ethyl acetate, 3:1, 2:1) to afford the *title amide* (0.111 g, 83%) as a clear gum that existed exclusively as the *trans* C(O)-NPro conformer: $[\alpha]_D$ −16.5 (*c* 0.4, MeOH); δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.64–1.80 (8H, m, Proγ-H_2 and 3 × cyclopentyl-H_2), 1.89–1.94 (1H, m, Proβ-H_AH$_B$), 2.06–2.31 (4H, Gluβ-H_2, Proβ-H$_A$$H_B$ and 1 × cyclopentyl-H), 2.42–2.57 (2H, m, Gluγ-H_2), 3.20–3.35 (1H, m, Proδ-H_AH$_B$), 3.45–3.60 (1H, m, Proδ-H$_A$$H_B$), 4.46 (1H, q, *J* 4.8, Gluα-H), 4.60 (1H, t, *J* 7.0, Proα-H), 4.97–5.22 (6H, m, 3 × OCH_2Ph), 5.36 (1H, br s, N–H), 7.14–7.36 (15H, m, 3 × Ph) and 7.59 (1H, br s, N–H); δ_C (100 MHz; $CDCl_3$) 24.0 (CH_2, cyclopentyl-C), 24.2 (CH_2, cyclopentyl-C), 25.6 (CH_2, Proγ-C), 26.1 (CH_2, Gluβ-C), 28.1 (CH_2, Proβ-C), 30.0 (CH_2, Gluγ-C), 36.5 (CH_2, cyclopentyl-C), 37.7 (CH_2, cyclopentyl-C), 48.0 (CH_2, Proδ-C), 51.8 (CH, Gluα-C), 61.9 (CH, Proα-C), 66.1 (CH_2, OCH_2Ph), 66.8 (br CH_2, OCH_2Ph), 67.0 (CH_2, OCH_2Ph) [Glyα-C obscured], 128.0 (CH, Ph), 128.17 (CH, Ph), 128.22 (CH, Ph), 128.27 (CH, Ph), 128.4 (CH, Ph), 128.5 (CH, Ph), 135.6 (quat., Ph), 135.8 (quat., Ph), 136.0 (quat., Ph), 155.1 (quat., NCO_2), 171.1 (quat., Gluα-CO), 171.9 (quat., Gly-CO), 171.95 (quat., Pro-CO) and 172.7 (quat., Gluγ-CO); *m/z* (FAB+) 670.3128 (MH$^+$, $C_{38}H_{44}N_3O_8$ requires 670.3128).

4.20. Cyclopentylglycyl-L-prolyl-L-glutamic acid trifluoroacetate salt (13)

Following procedure C, hydrogenation of the above amide in 5:3 ethyl acetate–trifluoroacetic acid yielded *analogue* **13** (0.0315 g, 44%) as a hygroscopic white foam following purification by C_{18} RP HPLC [90% water (0.05% trifluoroacetic acid)–10% acetonitrile, 13 mL min^{-1}]. Compound **13** existed exclusively as the *trans* C(O)-NPro conformer: $[\alpha]_D$ −30.6 (*c* 0.17, MeOH); δ_H (400 MHz; D_2O) 1.80–2.10 (10H, m, Proγ-H_2, Proβ-H_AH$_B$, Gluβ-H_AH$_B$ and 3 × cyclopentyl-H_2), 2.22–2.33 (2H, m, Proβ-H$_A$$H_B$ and Gluβ-H$_A$$H_B$) 2.40–2.58 (4H, m, Gluγ-H_2 and cyclopentyl-H_2), 3.64–3.76 (2H, m, Proδ-H_2), 4.44 (1H, dd, *J* 9.0 and 5.3, Gluα-H) and 4.54 (1H, dd, *J* 8.6 and 6.0, Proα-H); δ_C

(100 MHz; D_2O) 25.04 (CH_2, cyclopentyl-C), 25.07 (CH_2, cyclopentyl-C), 25.3 (CH_2, Proγ-C), 25.6 (CH_2, Gluβ-C), 28.1 (CH_2, Proβ-C), 29.7 (CH_2, Gluγ-C), 34.7 (CH_2, cyclopentyl-C), 34.8 (CH_2, cyclopentyl-C), 48.5 (CH_2, Proδ-C), 51.9 (CH, Gluα-C), 62.3 (CH, Proα-C), 67.5 (quat., Glyα-C), 116.2 (quat., q, *J* 291.7, *C*F_3CO_2H), 162.7 (quat., q, *J* 36.2, CF_3CO_2H), 170.2 (quat., Gly-CO), 174.4 (quat., Pro-CO), 174.7 (quat., Gluα-CO) and 176.9 (quat., Gluγ-CO); *m/z* (FAB+) 356.1822 [M(freebase)H^+, $C_{16}H_{26}N_3O_6$ requires 356.1822]. Additionally another compound was isolated, which exhibited an identical NMR spectra but whose retention time was 2.87 min on analytical HPLC (**13** had a retention time of 15.8 min). It is assumed that this compound is a mixture of cyclopentyl-Gly-Pro-OH·TFA salt and glutamic acid·TFA salt that co-elute on analytical HPLC.

4.21. *N*-Benzyloxycarbonyl-1-aminocyclohexane-1-carboxylic acid (22)

Following procedure A, protection of 1-aminocyclohexanecarboxylic acid **20** in 3:1 H_2O–dioxane afforded crude *carbamate* **22** (1.23 g, 88%) as a white solid: mp 152–154 °C (lit.,[52] 148–150 °C); δ_H (400 MHz, $CDCl_3$) 1.27–1.56 (3H, m, 3 × cyclohexyl-H), 1.59–1.73 (3H, m, 3 × cyclohexyl-H), 1.85–1.91 (2H, m, 2 × cyclohexyl-H), 2.05–2.09 (2H, m, 2 × cyclopentyl-H), 5.02 (1H, br s, N–H), 5.12 (2H, s, OCH_2Ph) and 7.27–7.36 (5H, s, Ph); δ_C (100 MHz, $CDCl_3$) 21.1 (CH_2, 2 × cyclohexyl-C), 25.1 (CH_2, 2 × cyclohexyl-C), 32.3 (CH_2, cyclohexyl-C), 59.0 (quat., 1-C), 67.1 (CH_2, O*C*H_2Ph), 128.1 (CH, Ph), 128.2 (CH, Ph), 128.5 (CH, Ph), 136.1 (quat., Ph), 155.7 (quat., NCO_2) and 178.7 (quat., CO_2H).

4.22. *N*-Benzyloxycarbonylcyclohexylglycyl-L-proline methyl ester (24)

To a solution of proline methyl ester hydrochloride[32,33] **15** (0.333 g, 2.01 mmol), *N*-benzyloxycarbonylcyclohexylglycine **22** (0.613 g, 2.21 mmol) and BoP (0.978 g, 2.21 mmol) in dichloromethane (10 mL) was added triethylamine (0.615 g, 6.04 mmol) and the solution stirred at room temperature for 20 h. The reaction mixture was washed with 2 M hydrochloric acid, saturated sodium hydrogen carbonate solution, dried (Na_2SO_4), filtered and the solvent removed to give an oil (1.32 g) that was purified by chromatography (silica gel, hexane–ethyl acetate, 2:1, 1:1) to afford *methyl ester* **24** (0.578 g, 74%) as a yellow solid. Methyl ester **24** was shown to be a 70:30 *trans:cis* mixture of conformers by ^{1}H NMR analysis (the ratio was estimated from the integration of the resonances at δ 4.55 and 4.21, assigned to the Proα-H protons of the major and minor conformers, respectively): mp 148–150 °C; $[\alpha]_D$ −82.3 (*c* 0.57, CH_2Cl_2); δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.27–2.10 (14H, m, Proβ-H_2, Proγ-H_2 and 5 × cyclohexyl-H_2), 3.31–3.38 (1H, m, Proδ-$H_A$$H_B$), 3.52–3.75 (4H, m, CO_2CH_3 and Proδ-$H_A$$H_B$), 4.21† (0.3H, br s, Proα-H), 4.55 (0.7H, br s, Proα-H), 4.90† (0.3H, d, *J* 11.5, OC$H_A$$H_B$Ph), 5.02–5.20 (2.4H, m, OCH_2Ph and NH), 5.43† (0.3H, d, *J* 11.4, OC$H_A$$H_B$Ph) and 7.34–7.37 (5H, m, Ph); δ_C (100 MHz; $CDCl_3$) 21.1 (CH_2, cyclohexyl-C), 21.2 (CH_2, cyclohexyl-C), 24.9 (CH_2, cyclohexyl-C), 25.6 (CH_2, Proγ-C), 27.4 (CH_2, Proβ-C), 30.9 (CH_2, cyclohexyl-C), 32.1 (CH_2, cyclohexyl-C), 32.5† (CH_2, cyclohexyl-C), 47.5 (CH_2, Proδ-C), 51.9 (CH_3, CO_2CH_3), 59.0 (quat., Glyα-C), 60.6 (CH, Proα-C), 66.6 (CH_2, O*C*H_2Ph), 66.9† (CH_2, O*C*H_2Ph), 128.2 (CH, Ph), 128.4 (CH, Ph), 128.42 (CH, Ph), 136.3 (quat., Ph), 153.9 (quat., NCO_2), 155.6† (quat., NCO_2), 171.9 (quat., Gly-CO) and 173.2 (quat., Pro-CO); *m/z* (EI+) 388.2001 (M^+, $C_{21}H_{28}N_2O_5$ requires 334.1998).

4.23. *N*-Benzyloxycarbonylcyclohexylglycyl-L-proline (26)

To a solution of amide **24** (0.17 g, 0.44 mmol) in dioxane (5.4 mL) was added dropwise 1 M aqueous NaOH (2.63 mL, 2.63 mmol) and the mixture was stirred for 23.5 h at room temperature. Dichloromethane (30 mL) was then added and the organic layer extracted with saturated aqueous sodium bicarbonate (3 × 30 mL). Careful acidification of the combined aqueous layers with hydrochloric acid (32%), extraction with dichloromethane (3 × 30 mL), drying of the combined organic layers ($MgSO_4$), filtration and concentration to dryness gave the *acid* **26** (0.16 g, 96%) as a white solid. Protected dipeptide **26** was shown to be a 73:27 *trans:cis* mixture of conformers by ^{1}H NMR analysis: $[\alpha]_D$ −22.2 (*c* 1.58, CH_2Cl_2); ν_{max} (film)/cm^{-1} 3307, 2932, 1714, 1618, 1528, 1453, 1412, 1335, 1280, 1252, 1198, 1169, 1094, 1078, 1035, 975, 823, 736 and 699; δ_H (300 MHz, $CDCl_3$) 1.25–2.02 (14H, m, Proγ-H_2, Proγ-H_2 and 5 × cyclohexyl-H_2), 3.19–3.45 (1.73H, m, Proδ-H_2), 3.45–3.59† (0.27H, m, Proδ-H_2), 4.17–4.26† (0.27H, m, Proα-H_2), 4.58–4.62 (1.73H, m, Proα-H_2), 4.88† (0.27H, d, *J* 11.6, OC$H_2$$^{\#}$Ph), 5.09 (1.46H, br s, OCH_2Ph), 5.19 (0.73H, br s, Gly-NH), 5.39 (0.27H, d, *J* 11.6, OC$H_2$$^{\#}$Ph), 5.70† (0.27H, br s, Gly-NH) and 7.28–7.40 (5H, m, PhH); δ_C (75 MHz, $CDCl_3$) 21.16 (CH_2, cyclohexyl-C), 21.2 (CH_2, cyclohexyl-C), 24.8 (CH_2, Proγ-C), 25.6† (CH_2, cyclohexyl-C), 25.8 (CH_2, cyclohexyl-C), 26.3† (CH_2, cyclohexyl-C), 26.9 (CH_2, cyclohexyl-C), 31.2 (CH_2, cyclohexyl-C), 31.9 (CH_2, Proβ-C), 32.2† (CH_2, cyclohexyl-C), 32.5† (CH_2, Proβ-C), 48.1 (CH_2, Proδ-C), 59.3 (quat., Glyα-C), 59.4† (quat., Glyα-C), 61.4† (CH, Proα-C), 61.7 (CH, Proα-C), 67.1† (CH_2, O*C*H_2Ph), 67.3 (CH_2, O*C*H_2Ph), 128.5 (CH, Ph), 128.6 (CH, Ph), 128.8 (CH, Ph), 136.0 (quat., Ph), 139.6† (quat., Ph), 154.6 (quat., NCO_2), 156.0† (quat., NCO_2), 173.3 (quat., CO_2H), 173.9 (quat., Gly-CO), 174.2† (quat., CO_2H) and 174.5† (quat., Gly-CO); *m/z* (CI+) 375.1918 (MH^+, $C_{20}H_{27}N_2O_5$ requires 375.1920).

4.24. Dibenzyl *N*-benzyloxycarbonylcyclohexylglycyl-L-prolyl-L-glutamate

Dry triethylamine (0.18 mL, 1.26 mmol) was added dropwise to a solution of acid **26** (0.15 g, 0.40 mmol) and glutamic acid dibenzyl ester *p*-toluenesulfonate **4** (0.26 g, 0.51 mmol) in dry dichloromethane (21 mL) under an atmosphere of nitrogen at room temperature, and the reaction mixture stirred for 10 min.

Bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BoPCl, 97%) (0.13 g, 0.50 mmol) was added and the solution stirred for 19 h, then washed successively with 10% aqueous hydrochloric acid (20 mL) and saturated aqueous sodium hydrogen carbonate (20 mL). The organic extract was dried ($MgSO_4$), filtered and evaporated to dryness in vacuo. Purification of the resultant residue by flash column chromatography (60% ethyl acetate–hexane) yielded *fully protected tripeptide* (0.20 g, 75%) as a colourless oil that existed exclusively as the *trans* conformer with 6% epimerization as observed by ^{13}C NMR analysis: $[\alpha]_D$ +1.14 (*c* 4.38, CH_2Cl_2); ν_{max} (film)/cm^{-1} 3317, 3033, 2944, 2858, 1737, 1699, 1648, 1535, 1498, 1454, 1391, 1279, 1255, 1199, 1168, 1093, 1034, 973, 736 and 698; δ_H (400 MHz; $CDCl_3$) 1.21-1.35 (3H, m, 3 × cyclohexyl-H), 1.60–1.76 (6H, m, Proγ-H_2 and 4 × cyclohexyl-H), 1.82–1.89 (2H, m, Proβ-H_AH_B and 1 × cyclohexyl-H), 1.98–2.07 (3H, m, Proβ-H_AH_B and 2 × cyclohexyl-H), 2.12–2.32 (2H, m, Gluβ-H_2), 2.40–2.56 (2H, m, Gluγ-H_2), 3.15–3.21 (1H, m, Proδ-H_AH_B), 3.52–3.57 (1H, m, Proδ-H_AH_B), 4.43–4.49 (1H, m, Gluα-H), 4.58 (1H, dd, *J* 8.2 and 5.7, Proα-H), 4.90–5.20 (7H, m, NH and 3 × OCH_2Ph), 7.27–7.38 (15H, m, 3 × Ph) and 7.57 (1H, d, *J* 7.2, NH); δ_C (100 MHz; $CDCl_3$) 21.1 (CH_2, cyclohexyl-C), 21.3 (CH_2, cyclohexyl-C), 24.8 (CH_2, cyclohexyl-C), 25.8 (CH_2, Proγ-C), 26.3 (CH_2, Gluβ-C), 26.4‡ (CH_2, Gluβ-C), 28.0 (CH_2, Proβ-C), 28.6‡ (CH_2, Proβ-C), 29.7‡ (CH_2, Gluγ-C), 30.1 (CH_2, Gluγ-C), 30.6‡ (CH_2, cyclohexyl-C), 31.1‡ (CH_2, cyclohexyl-C), 31.4 (CH_2, cyclohexyl-C), 31.8 (CH_2, cyclohexyl-C), 47.9 (CH_2, Proδ-C), 48.1‡ (CH_2, Proδ-C), 51.6‡ (CH, Gluα-C), 51.8 (CH, Gluα-C), 59.3 (quat., Glyα-C), 62.4 (CH, Proα-C), 62.8‡ (CH, Proα-C), 66.0‡ (CH_2, O*C*H_2Ph), 66.2 (CH_2, O*C*H_2Ph), 66.7‡ (CH_2, O*C*H_2Ph), 66.9 (CH_2, O*C*H_2Ph), 67.1 (CH_2, O*C*H_2Ph), 67.4‡ (CH_2, O*C*H_2Ph), 127.9‡ (CH, Ph), 128.1 (CH, Ph), 128.3 (CH, Ph), 128.5 (CH, Ph), 128.6 (CH, Ph), 128.7 (CH, Ph), 128.9‡ (CH, Ph), 135.8 (quat., Ph), 135.9 (quat., Ph), 136.0 (quat., Ph), 154.8 (quat., NCO_2), 171.1 (quat., Gluα-CO), 172.0 (quat., Gly-CO), 172.2 (quat., Pro-CO) and 172.8 (quat., Gluγ-CO); *m/z* (FAB+) 684.3291 (MH^+, $C_{39}H_{46}N_3O_8$ requires 684.3285).

4.25. Cyclohexylglycyl-L-prolyl-L-glutamic acid trifluoroacetate salt (14)

Following procedure C, hydrogenation of the above amide in 3:2 ethyl acetate–trifluoroacetic acid yielded *analogue* **14** (23 mg, 35%) as a colourless hygroscopic gummy oil following purification by C_{18} RP HPLC [90% water (0.05% trifluoroacetic acid)–10% acetonitrile, 13 mL min^{-1}]. Compound **14** existed exclusively as the *trans* conformer with 6% epimerization as observed by HPLC analysis:§ $[\alpha]_D$ −34.1 [*c* 0.18, MeOH–H_2O (1:1)]; δ_H (400 MHz; D_2O) 1.35–1.58 (3H, m, 3 × cyclohexyl-H), 1.80–1.94 (6H, m, Proβ-H_AH_B and 5 × cyclohexyl-H) 2.00–2.11 (3H, m, Proγ-H_2 and Gluβ-H_AH_B), 2.21–2.37 (4H, m, Proβ-H_AH_B, Gluβ-H_AH_B and 2 × cyclohexyl-H), 2.57 (2H, t, *J* 7.2, Gluγ-H_2), 3.83–4.05 (2H, m, Proδ-H_2), 4.44 (1H, dd, *J* 9.0 and 5.3, Gluα-H) and 4.52–4.56 (1H, m Proα-H); δ_C (100 MHz; D_2O) 22.3 (2 × CH_2, cyclohexyl-C), 25.7 (CH_2, cyclohexyl-C), 28.0 (CH_2, Proγ-C), 28.2 (CH_2, Gluβ-C), 30.5 (CH_2, Proβ-C), 31.0 (CH_2, cyclohexyl-C), 31.2 (CH_2, cyclohexyl-C), 32.3 (CH_2, Gluγ-C), 52.2 (CH_2, Proδ-C), 54.4 (CH, Gluα-C), 64.7 (quat., Glyα-C), 65.3 (CH, Proα-C), 118.8 (quat., q, *J* 290.0, *C*F_3CO_2H), 165.3 (quat., q, *J* 35.0, CF_3CO_2H), 172.6 (quat., Gly-CO), 176.9 (quat., Pro-CO), 177.3 (quat., Gluα-CO) and 179.5 (quat., Gluγ-CO); *m/z* (FAB+) 370.1983 [M(freebase)H^+, $C_{17}H_{28}N_3O_6$ requires 370.1978].

4.26. *N*-Benzyloxycarbonyl-*N*-methylglycine[53] (31)

Following procedure A, protection of sarcosine in 7:2 H_2O–dioxane afforded crude *carbamate* **31** (5.00 g, 100%) as a light green oil: δ_H (200 MHz; CD_3OD): 3.01 (3H, s, C*H*$_3$N), 4.09 (2H, s, Glyα-H_2), 5.16 (2H, s, OC*H*$_2$Ph), 5.75 (1H, br s, CO_2H) and 7.36 (5H, s, Ph); δ_C (50 MHz; CD_3OD) 35.8† (CH_3, *C*H_3N), 36.4 (CH_3, *C*H_3N), 51.1† (CH_2, *C*H_2CO_2H), 51.3 (CH_2, Glyα-C), 68.5 (CH_2, O*C*H_2Ph), 68.6 (CH_2, O*C*H_2Ph), 128.6 (CH, Ph), 128.8 (CH, Ph) 128.9 (CH, Ph), 129.5 (CH, Ph), 137.9 (quat., Ph) 158.5 (quat., NCO_2) and 172.9 (quat., CO_2H); *m/z* (EI+) 223 (M^+, 5%).

4.27. Dibenzyl-*N*-benzyloxycarbonyl-*N*-methylglycyl-L-prolyl-L-glutamate (33)

Following procedure B, coupling of amine[31] **2** with carbamate **31** using triethylamine as the base yielded *fully protected tripeptide* **33** (0.174 g, 47%) as a clear gum following purification by flash column chromatography (ethyl acetate): $[\alpha]_D$ −47.2 (*c* 0.05, MeOH); δ_H (200 MHz; $CDCl_3$; Me_4Si) 1.90–2.44 (8H, m, Proβ-H_2, Proγ-H_2, Gluβ-H_2 and Gluγ-H_2,), 2.95 (3H, s, CH_3N), 3.40–3.47 (2H, m, Proδ-H_2), 3.76–3.86 (1H, m, Gly-NCH_AH_B), 4.15–4.23 (1H, m, Gly-NCH_AH_B), 4.54–4.57 (1H, m, Proα-H), 4.57–4.59 (1H, m, Gluα-H), 5.06 (2H, s, OC*H*$_2$Ph), 5.09 (2H, s, OC*H*$_2$Ph), 5.27 (2H, s, OC*H*$_2$Ph) and 7.25–7.33 (15H, m, 3 × Ph); δ_C (50 MHz; $CDCl_3$) 25.0 (CH_2, Proγ-C), 27.0 (CH_2, Gluβ-C), 27.4 (CH_2, Proβ-C), 30.0 (CH_2, Gluγ-C), 35.6 (CH_3, *C*H_3N), 36.1† (CH_3, *C*H_3N), 46.4 (CH_2, Proδ-C), 51.3 (CH_2, Glyα-C), 51.7 (CH, Gluα-C), 60.0 (CH, Proα-C), 66.3 (CH_2, O*C*H_2Ph), 67.0 (CH_2, O*C*H_2Ph), 67.3 (CH_2, O*C*H_2Ph), 127.7 (CH, Ph), 127.9 (CH, Ph), 128.1 (CH, Ph), 128.4 (CH, Ph), 128.5 (CH, Ph), 135.0 (quat., Ph), 156.9 (quat., NCO_2), 168.3 (quat., Gly-CO), 171.0 (quat., Glu-CO), 171.3 (quat., Glu-CO) and 172.4 (quat., Pro-CON); *m/z* (EI+) 629.2726 (M^+, $C_{35}H_{39}N_3O_8$ requires 629.2737).

‡Denotes resonances assigned to epimer.

§Analytical reverse-phase HPLC studies on the mixture [Altech Econosphere C_{18} Si column, 150 × 4.6 mm, 5 mm; 5 min flush with H_2O (0.05% TFA) then steady gradient over 25 min to MeCN as eluent at flow rate of 1 cm^3/min; detection using diode array] indicated it was a 94:6 mixture of two eluting peaks with retention times of 13.53 and 14.13 min at 210 nm wavelength, respectively.

4.28. *N*-Methyl glycyl-L-prolyl-L-glutamic acid (27)

Following procedure C, hydrogenation of amide **33** in 9:1 MeOH–H_2O yielded *analogue* **27** (0.27 g, 96%) as a light orange solid on trituration with anhydrous diethyl ether. Compound **27** was shown to be an 84:16 *trans:cis* mixture of conformers by ^{13}C NMR analysis: mp 77–83 °C. $[\alpha]_D$ −42.7 (*c* 0.06, MeOH); δ_H (200 MHz; CD_3OD) 1.75–1.93 (6H, m, Gluβ-H_2, Proβ-H_2 and Proγ-H_2), 2.10–2.20 (2H, m, Gluγ-H_2), 2.48 (3H, s, CH_3N), 3.30–3.50 (2H, m, Proδ-H_2), 3.70–3.90 (2H, m, Glyα-H_2) and 4.11–4.23 (2H, m, Proα-H and Gluα-H); δ_C (50 MHz; CD_3OD) 23.5† (CH_2, Proγ-C), 25.7 (CH_2, Proγ-C), 27.5† (CH_2, GluβC), 28.1 (CH_2, Gluβ-C), 30.7 (CH_2, Proβ-C), 31.4 (CH_2, Gluγ-C), 32.0† (CH_2, Proβ-C), 33.2† (CH_2, Gluγ-C), 33.9 (CH_3, CH_3N), 47.8 (CH_2, Proδ-C), 48.5† (CH_2, Proδ-C), 50.7 (CH, Glyα-C), 53.6 (CH, Gluα-C), 61.3† (CH, Proα-C), 61.6 (CH, Proα-C), 165.5 (quat., Gly-CO), 174.1 (quat., Pro-CON), 175.0 (quat., Gluγ-CO_2) and 176.9 (quat., Gluα-CO_2); *m/z* (FAB+) 316.1500 (MH^+, $C_{13}H_{22}N_3O_6$ requires 316.1509).

4.29. Dibenzyl *N*,*N*-dimethyl glycyl-L-prolyl-L-glutamate (34)

Following procedure B, coupling of amine[31] **2** with *N*,*N*-dimethylglycine hydrochloride in dimethylformamide using triethylamine as the base yielded *fully protected tripeptide* **34** (0.31 g, 51%) as a yellow oil following purification by flash column chromatography (15% MeOH–CH_2Cl_2): $[\alpha]_D$ −55.5 (*c* 0.05, MeOH); δ_H (200 MHz; $CDCl_3$; Me_4Si) 1.85–2.45 [14H, m, Proβ-H_2, Proγ-H_2, Gluβ-H_2, Gluγ-H_2 and (CH_3)$_2$N], 3.09–3.12 (2H, m, Glyα-H_2), 3.50–3.59 (2H, m, Proδ-H_2), 4.54–4.61 (2H, m, Proα-H and Gluα-H), 5.08 (2H, s, OCH_2Ph), 5.15 (2H, s, OCH_2Ph), 7.26–7.45 (10H, m, 2 × Ph) and 7.70–7.80 (1H, br s, Glu-NH); δ_C (50 MHz; $CDCl_3$) 25.0 (CH_2, Proγ-C), 27.1 (CH_2, Gluβ-C), 27.3 (CH_2, Proβ-C), 30.0 (CH_2, Gluγ-C), 45.2 (CH_3, (*C*H_3)N), 45.3 (CH_3, (*C*H_3)N), 46.9 (CH_2, Proδ-C), 51.7 (CH, Gluα-C), 59.7 (CH, Proα-C), 61.9 (CH_2, Glyα-C), 66.3 (CH_2, O*C*H_2Ph), 67.1 (CH_2, O*C*H_2Ph), 128.2 (CH, Ph), 128.3 (CH, Ph), 128.4 (CH, Ph), 128.5 (CH, Ph), 135.2 (quat., Ph), 169.9 (quat., Glu-CO), 170.0 (quat., Glu-CO), 171.2 (quat., Gly-CO) and 172.3 (quat., Pro-CON); *m/z* (EI+) 509.2530 (M^+, $C_{28}H_{35}N_3O_6$ requires 509.2526).

4.30. *N*,*N*-Dimethyl glycine-L-proline-L-glutamic acid (28)

Following procedure C, hydrogenation of *tripeptide* **34** yielded *analogue* **28** (0.29 g, 94%) as a white solid on trituration with anhydrous diethyl ether. Compound **28** was shown to be a 79:21 *trans:cis* mixture of conformers by ^{13}C NMR analysis: mp 166–169 °C; $[\alpha]_D$ −51.0 (*c* 0.06, MeOH); δ_H (200 MHz; CD_3OD) 1.96–2.11 (6H, m, Proβ-H_2, Proγ-H_2 and Gluβ-H_2), 2.24–2.28 (2H, m, Gluγ-H_2), 2.82 (6H, s, 2 × CH_3N), 3.42–3.57 (2H, m, Proδ-H_2), 4.04–4.09 (2H, m, Glyα-H_2), 4.11–4.16 (1H, m, Gluα-H), 4.38–4.41 (1H, m, Proα-H); δ_C (50 MHz; CD_3OD) 23.4† (CH_2, Proγ-C), 25.6 (CH_2, Proγ-C), 28.4† (CH_2, Gluβ-C), 29.2 (CH_2, Gluβ-C), 30.4 (CH_2, Proβ-C), 33.0 (CH_2, Gluγ-C), 33.4† (CH_2, Gluγ-C), 44.8 (CH_3, *C*H_3N), 47.8 (CH_2, Proδ-C), 48.5† (CH_2, Proδ-C), 55.9 (CH, Gluα-C), 56.3† (CH, Gluα-C), 59.4 (CH_2, Glyα-C), 61.6† (CH, Proα-C), 62.1 (CH, Proα-C), 165.7 (quat., Gly-CO), 173.0 (quat., Pro-CON), 177.8 (quat., Gluα-CO) and 179.2 (quat., Gluα-CO_2); *m/z* (EI+) 329.1586 (M^+, $C_{14}H_{23}N_3O_6$ requires 329.1587).

4.31. Pyrrolidinoacetic acid[36,37] (35)

Bromoacetic acid (2.00 g, 14.4 mmol) was dissolved in water (8 mL) and 3.3 M aqueous sodium hydroxide solution (5 mL) was added dropwise until pH 14. The solution was cooled to 0 °C and pyrrolidine (1 mL, 11.9 mmol) was added dropwise over a period of 5 min. The solution was stirred for 1 h, warmed to room temperature and stirred for 3 days. The solution was heated on a steam bath for 2 h, cooled to room temperature and the solvent evaporated under reduced pressure. Ethanol (20 mL) was added and the solution heated to 50 °C for 15 min, filtered and evaporated under reduced pressure to form a brown glass, which solidified under vacuum to give *acid* **35** (1.13 g, 73%) as a light brown solid: mp 178–180 °C (lit.[36] mp 184–185 °C); δ_H (200 MHz; D_2O) 2.11–2.17 (4H, 2 × Pyrβ-H_2), 3.41–3.48 (4H, m, 2 × Pyrα-H_2) and 3.85 (2H, s, CH_2CO_2H); δ_C (50 MHz; D_2O) 25.4 (CH_2, Pyrβ-C), 57.2 (CH_2, Pyrα-C), 60.2 (CH_2, CH_2CO_2H) and 174.1 (quat, CO_2H); *m/z* (EI+) 129 (M^+, 10%).

4.32. Dibenzyl pyrrolidinoglycyl-L-prolyl-L-glutamate (37)

Following procedure B, coupling of amine[31] **2** with acid **35** in dimethylformamide using PyBoP as the coupling agent and triethylamine as the base yielded *fully protected tripeptide* **37** (0.14 g, 46%) as a clear gum following purification by flash column chromatography (5% MeOH–$CHCl_3$): $[\alpha]_D$ −53.5 (*c* 0.06, MeOH); δ_H (200 MHz; $CDCl_3$; Me_4Si) 1.78–2.54 (12H, m, Proβ-H_2, Proγ-H_2, Gluβ-H_2, Gluγ-H_2 and 2 × Pyrβ-H_2), 2.68–2.77 (4H, m, 2 × Pyrα-H_2), 3.36–3.43 (2H, m, NCH_2CO), 3.48–3.59 (2H, m, Proδ-H_2), 4.53–4.60 (2H, m, Proα-H and Gluα-H), 5.08 (2H, s, OCH_2Ph), 5.14 (2H, s, OCH_2Ph), 7.27–7.32 (10H, m, 2 × Ph) and 7.49–7.52 (1H, d, *J* 8.0, Glu-NH); δ_C (50 MHz, $CDCl_3$) 23.6 (CH_2, Pyrβ-C), 25.0 (CH_2, Proγ-C), 27.0 (CH_2, Gluβ-C), 27.4 (CH_2, Proβ-C), 30.0 (CH_2, Gluγ-C), 46.9 (CH_2, Proδ-C), 51.7 (CH, Gluα-C), 54.1 (CH_2, Pyrα-C), 57.9 (CH_2, N*C*H_2CO), 59.8 (CH, Proα-C), 66.3 (CH_2, O*C*H_2Ph), 67.06 (CH_2, O*C*H_2Ph), 128.1 (CH, Ph), 128.3 (CH, Ph), 128.5 (CH, Ph), 135.2 (quat., Ph), 169.6 (quat., Pyr-CON), 171.3 (quat., Glu-CO), 171.4 (quat., Glu-CO) and 172.4 (quat., Pro-CON); *m/z* 535.2678 (M^+, $C_{30}H_{37}N_3O_6$ requires 535.2682).

4.33. Pyrrolidinoglycyl-L-prolyl-L-glutamic acid (29)

Following procedure C, hydrogenation of amide **37** yielded *analogue* **29** (0.21 g, 86%) as a white solid on trituration with anhydrous diethyl ether. Compound **29**

was shown to be an 84:16 *trans:cis* mixture of conformers by ^{13}C NMR analysis: mp 129–132 °C; $[\alpha]_D$ −49.2 (*c* 0.06, MeOH); δ_H (200 MHz; D_2O) 2.00–2.33 (10H, m, Proβ-H_2, Proγ-H_2, Gluβ-H_2 and 2 × Pyrβ-H_2), 2.52–2.59 (2H, t, *J* 7.5, Gluγ-H_2), 3.10–3.30 (2H, m, Pyrα-H_2), 3.59–3.61 (2H, m, Proδ-H_2), 3.60–3.80 (2H, m, Pyrα-H_2), 4.33 (2H, s, NCH_2CO), 4.40–4.44 (1H, m, Gluα-H) and 4.51–4.54 (1H, m, Proα-H); δ_C (50 MHz; D_2O) 23.6† (CH_2, Pyrβ-C), 24.4 (CH_2, Pyrβ-C), 25.9 (CH_2, Proγ-C), 27.2† (CH_2, Gluβ-C), 27.8 (CH_2, Gluβ-C), 30.1 (CH_2, Proβ-C), 31.7 (CH_2, Gluγ-C), 32.1† (CH_2, Proβ-C), 33.4† (CH_2, Gluγ-C), 48.6 (CH_2, Proδ-C), 54.3 (CH, Gluα-C), 56.9 (CH_2, Pyrα-C), 57.5 (CH_2, Pyrα-C), 61.6† (CH, Proα-C), 62.1 (CH, Proα-C), 166.2 (quat., Pyr-CONH), 175.4 (quat., Pro-CONH), 177.2 (quat., Gluα-CO_2H) and 179.0 (quat., Gluγ-CO_2H); *m/z* (FAB+) 356.1830 (MH^+, $C_{16}H_{26}N_3O_6$ requires 356.1822).

4.34. Piperidinoacetic acid[36,37,54] (36)

Bromoacetic acid (1.68 g, 14.4 mmol) was dissolved in water (8 mL) and 3.3 M aqueous NaOH solution (5 mL) was added dropwise until pH 14. The solution was cooled to 0 °C and piperidine (1 mL, 11.9 mmol) was added dropwise over 5 min. The solution was stirred for 1 h, warmed to room temperature and stirred for 3 days. The solution was heated on a steam bath for 2 h, cooled to room temperature and the solvent removed under reduced pressure. Ethanol (20 mL) was added and the solution heated to 50 °C for 15 min, filtered and the filtrate evaporated under reduced pressure to give a light yellow foam, which solidified under vacuum to give *acid* **36** (1.29 g, 89%) as a light yellow solid: mp 207–209 °C; (lit.[54] mp 211–212 °C); δ_H (200 MHz, D_2O) 1.58–1.66 (2H, m, Pipγ-H_2), 1.74–1.85 (4H, 2 × Pipβ-H_2), 2.97 (4H, t, *J* 8.5, 2 × Pipα-H_2) and 3.42 (2H, s, CH_2CO_2H); δ_C (50 MHz, D_2O) 22.3 (CH_2, Pipγ-C), 24.0 (CH_2, Pipβ-C), 54.1 (CH_2, Pipα-C), 60.7 (CH_2, CH_2CO_2H) and 175.0 (quat., CO_2H); *m/z* (EI+) 144 (MH^+, 45%).

4.35. Dibenzyl piperidinylglycyl-L-prolyl-L-glutamate (38)

Following procedure B, coupling of amine[31] **2** with acid **36** in dimethylformamide yielded *fully protected tripeptide* **38** (0.52 g, 59%) as a clear gum following purification by flash column chromatography (15% MeOH–$CHCl_3$): $[\alpha]_D$ −53.0 (*c* 0.05, MeOH); δ_H (200 MHz; $CDCl_3$; Me_4Si) 1.29–1.38 (2H, m, Pipγ-H_2), 1.53–1.55 (4H, m, 2 × Pipβ-H_2), 1.94–2.47 (12H, m, Proβ-H_2, Proγ-H_2, Gluβ-H_2, Gluγ-H_2 and 2 × Pipα-H_2), 3.11 (2H, s, NCH_2CO), 3.50–3.60 (2H, m, Proδ-H_2), 4.54–4.59 (2H, m, Proα-H and Gluα-H), 5.08 (2H, s, OCH_2Ph), 5.14 (2H, s, OCH_2Ph), 7.27–7.32 (10H, m, 2 × Ph) and 7.42–7.50 (1H, d, *J* 8.2, Glu-NH); δ_C (50 MHz; $CDCl_3$) 23.8 (CH_2, Pipγ-C), 25.0 (CH_2, Proγ-C), 25.7 (CH_2, Pipβ-C), 27.1 (CH_2, Gluβ-C), 27.2 (CH_2, Proβ-C), 30.0 (CH_2, Gluγ-C), 47.0 (CH_2, Proδ-C), 51.7 (CH, Gluα-C), 54.5 (CH_2, Pipα-C), 59.7 (CH, Proα-C), 61.9 (CH_2, NCH_2CO), 128.1 (CH, Ph), 128.3 (CH, Ph), 128.5 (CH, Ph), 135.2 (quat., Ph), 135.7 (quat., Ph), 170.1 (quat., Pip-CON), 171.3 (quat., Glu-CO), 172.0 (quat., Glu-CO) and 172.3 (quat., Pro-CON); *m/z* (EI+) 549.2837 (M^+, $C_{31}H_{39}N_3O_6$ requires 549.2839).

4.36. Piperidinylglycyl-L-prolyl-L-glutamic acid (30)

Following procedure C, hydrogenation of amide **38** yielded *tripeptide* **30** (0.26 g, 78%) as a white solid on trituration with anhydrous diethyl ether. Compound **30** was shown to be an 86:14 *trans:cis* mixture of conformers by ^{13}C NMR analysis: mp 132–135 °C: $[\alpha]_D$ −47.5 (*c* 0.05, MeOH); δ_H (200 MHz; D_2O) 1.80–2.40 (12H, m, Proβ-H_2, Proγ-H_2, Gluβ-H_2, Pipγ-H_2 and 2 × Pipβ-H_2), 2.43–2.50 (2H, t, *J* 7.9, Gluγ-H_2), 3.03–3.08 (2H, m, Pipα-H_2), 3.57–3.70 (4H, m, Pip-H_2, Proδ-H_2), 4.18 (2H, s, NCH_2CO), 4.25–4.29 (1H, m, Gluα-H) and 4.40–4.50 (1H, m, Proα-H); δ_C (50 MHz; D_2O) 22.3 (CH_2, Pipγ-C), 23.4† (CH_2, Pipβ-C), 23.9 (CH_2, Pipβ-C), 25.7 (CH_2, Proγ-C), 27.8† (CH_2, Gluβ-C), 28.5 (CH_2, Gluβ-C), 30.7 (CH_2, Proβ-C), 32.3 (CH_2, Gluγ-C), 32.9† (CH_2, Gluγ-C), 48.4 (CH_2, Proδ-C), 49.0† (CH_2, Proδ-C), 55.5 (CH, Gluα-C), 56.0 (CH_2, Pipα-C), 58.2 (CH_2, NCH_2CO), 61.6† (CH, Proα-C), 62.1 (CH, Proα-C), 165.2 (quat., Pip-CONH), 174.7 (quat., Pro-CON), 178.6 (quat., Gluα-CO) and 179.8 (quat., Gluγ-CO); *m/z* (FAB+) 370.1975 (MH^+, $C_{17}H_{28}N_3O_6$ requires 370.1978).

4.37. *N*-Benzyloxycarbonyl-β-alanine[38] (40)

Following procedure A, protection of β-alanine afforded *crude carbamate* **40** (4.81 g, 96%) as a gummy white solid, which was used without further purification: δ_H (200 MHz, $(CD_3)_2SO$) 2.49 (2H, t, *J* 7.7, Alaα-H_2), 3.32 (2H, t, *J* 7.6, Alaβ-H_2), 5.11 (2H, s, OCH_2Ph), 7.42 (5H, s, Ph) and 12.30 (1H, br s, CO_2H); δ_C (50 MHz, $(CD_3)_2SO$) 34.1 (CH_2, Alaα-C), 36.5 (CH_2, Alaβ-C), 65.2 (CH_2, OCH_2Ph), 127.7–128.3 (CH, Ph), 137.1 (quat., Ph), 156.0 (quat., NHCO) and 172.7 (quat., CO_2H); *m/z* (EI+) 223 (M^+, 4%).

4.38. Dibenzyl *N*-benzyloxycarbonyl β-alanyl-L-prolyl-L-glutamate[31] (41)

Following procedure B, coupling of amine[31] **2** with carbamate **40** using triethylamine as the base yielded *fully protected tripeptide* **41** (0.52 g, 70%) as a clear gum following purification by flash column chromatography (ethyl acetate): $[\alpha]_D$ −46.8 (*c* 0.06, MeOH); δ_H (200MHz; $CDCl_3$; Me_4Si) 1.90–2.50 (10H, m, Proβ-H_2, Proγ-H_2, Gluβ-H_2, Gluγ-H_2 and Alaβ-H_2), 3.35–3.49 (4H, m, Proγ-H_2 and Alaα-H_2), 4.46–4.59 (2H, m, Proα-H and Gluα-H), 5.05 (2H, s, OCH_2Ph), 5.09 (2H, s, OCH_2Ph), 5.11 (2H, s, OCH_2Ph), 5.70–5.75 (1H, m, Glu-NH) and 7.25–7.30 (15H, m, Ph); δ_C (50 MHz; $CDCl_3$) 24.7 (CH_2, Alaα-C), 26.9 (CH_2, Proγ-C), 28.0 (CH_2, Gluβ-C), 30.0 (CH_2, Proβ-C), 34.5 (CH_2, Gluγ-C), 36.5 (CH_2, Alaβ-C), 47.3 (CH_2, Proδ-C), 51.7 (CH, Gluα-C), 59.9 (CH, Proα-C), 66.4 (CH_2, OCH_2Ph), 67.2 (CH_2, OCH_2Ph), 127.9 (CH, Ph), 128.2 (CH, Ph), 128.4 (CH, Ph), 128.5 (CH, Ph), 135.1 (quat, Ph), 135.6 (quat, Ph), 136.6 (quat, Ph), 156.5 (quat, NCO_2), 171.4 (quat, Glu-CO), 171.5 (quat.,

Glu-CO) and 172.6 (quat., Pro-CON); *m/z* (EI+) 630.2807 (MH^+, $C_{35}H_{40}N_3O_8$ requires 630.2815).

4.39. β-Alanyl-L-prolyl-L-glutamic acid (39)

Following procedure C, hydrogenation of amide **41** yielded *analogue* **39** (0.25 g, 86%) as a white solid on trituration with anhydrous diethyl ether. Compound **39** was shown to be an 82:18 *trans:cis* mixture of conformers by ^{13}C NMR analysis: mp 53–56 °C; $[\alpha]_D$ −43.2 (*c* 0.05, MeOH); δ_H (200 MHz; D_2O) 2.01–2.12 (4H, m, Proβ-H_2 and Proγ-H_2), 2.23–2.34 (2H, m, Gluβ-H_2), 2.55 (2H, t, *J* 7.8, Gluγ-H_2), 2.91 (2H, t, *J* 7.5, Alaα-H_2), 3.63–3.80 (2H, m, Proγ-H_2), 4.39–4.41 (1H, m, Gluα-H) and 4.43–4.51 (1H, m, Proα-H); δ_C (50 MHz; D_2O) 26.1 (CH_2, Alaα-C), 28.1 (CH_2, Proγ-C), 31.4 (CH_2, Gluβ-C), 32.1 (CH_2, Proβ-C), 32.6 (CH_2, Gluγ-C), 33.5† (CH_2, Gluγ-C), 37.1 (CH_2, Alaβ-C), 49.1† (CH_2, Proδ-C), 49.7 (CH_2, Proδ-C), 54.8 (CH, Gluα-C), 62.1 (CH, Proα-C), 172.7 (quat., Ala-CON), 176.2 (quat., Pro-CON), 177.8 (quat., Gluα-CO_2) and 179.4 (quat., Gluγ-CO_2); *m/z* (FAB+) 316.1512 (MH^+, $C_{13}H_{22}N_3O_6$ requires 316.1509).

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