Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 13 (2005) 519–532
Bioorganic & Medicinal Chemistry

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

Margaret A. Brimble,* Nicholas S. Trotter, Paul W. R. Harris and Frank Sieg

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

Received 11 August 2004; revised 1 October 2004; accepted 4 October 2004

Abstract—The synthesis of eight GPE* analogues, wherein the γ-carboxylic moiety of the glutamic residue has been modified, is described by coupling readily accessible *N*-benzyloxycarbonyl-glycyl-L-proline with various analogues of glutamic acid. Pharmacological evaluation of the novel compounds was undertaken to further understand the role of the glutamate residue on the observed neuroprotective properties of the endogenous tripeptide GPE.

1. Introduction

As described in our previous paper[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 1)[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] Although there is no direct evidence that GPE **1** is naturally present or produced in the brain,[21] it was observed to act as a neuronal rescue factor following transient HI brain injury for cerebral, cortical, striatal and hippocampal neurons,[22] with peripheral activity, producing wider neuroprotective effects than local administration.[20]

In addition to in vitro studies by Sara and colleagues,[5,18] GPE **1** was suggested to possess a neuromodulatory role in the CNS, possibly through interaction with glutamate receptors,[5,6,11,18,19,22–25] which provide a potential target for the rational design of neuroprotective agents.[26–28] 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.[29–32] 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.

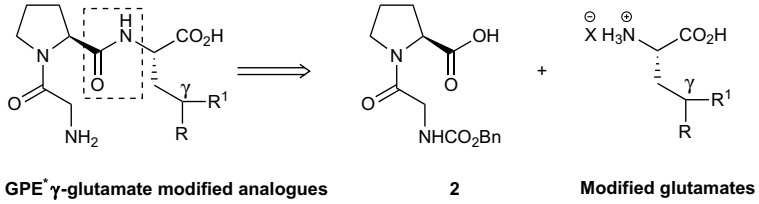
We therefore herein report the preparation and pharmacological evaluation of several analogues of general structure Gly-Pro-Glu-OH (GPE*), in which the γ-position of Glu (E*) has been modified. We also utilised our in vitro brain cell culture in the screening of these analogues.[1] This work is part of a structure–activity relationship study to determine the significance of the Glu residue of this tripeptide on neuroprotective activity, aiding our selection of suitable candidates for continued drug development.

2. Results and discussion

So as to ascertain the importance of the γ-carboxylic acid functionality on the glutamate residue of GPE, eight analogues with modifications at this site were synthesised and evaluated for their neuroprotective effects.

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

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.10.006

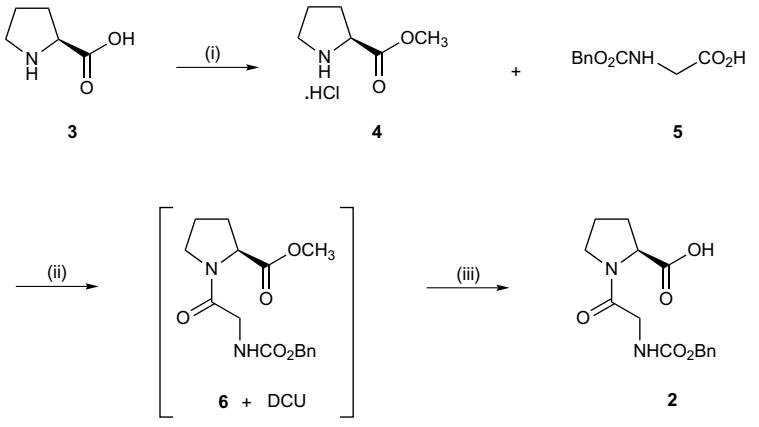


Scheme 1. General retrosynthesis for GPE* γ-glutamate modified analogues.

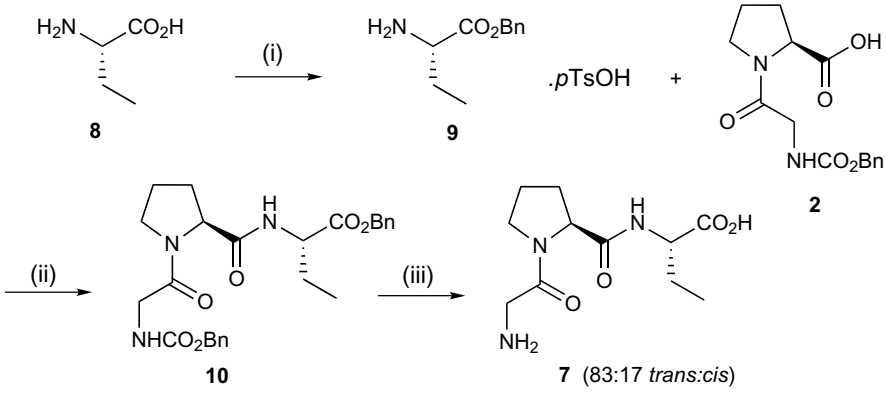
The synthetic strategy employed involved preparation of known *N*-benzyloxycarbonyl-glycyl-L-proline[33,34] **2**, and effecting peptide bond construction with several modified benzyl-protected glutamic acid residues (Scheme 1). After assembly of the new pseudo-peptide bond, a simple deprotection procedure furnished the desired GPE* analogues.

The synthesis of *N*-CBz peptide[33,34] **2** was achieved in 82% yield over two steps from readily available L-proline methyl ester **4** (Scheme 2). Thus, treatment of **3** with thionyl chloride in methanol gave quantitatively hydrochloride salt[35,36] **4**, which was subsequently coupled with commercial *N*-benzyloxycarbonylglycine **5**, using 1,3-dicyclohexylcarbodiimide (DCC) to realise protected dipeptide[37] **6**. Finally, saponification of the methyl ester afforded acid **2**.

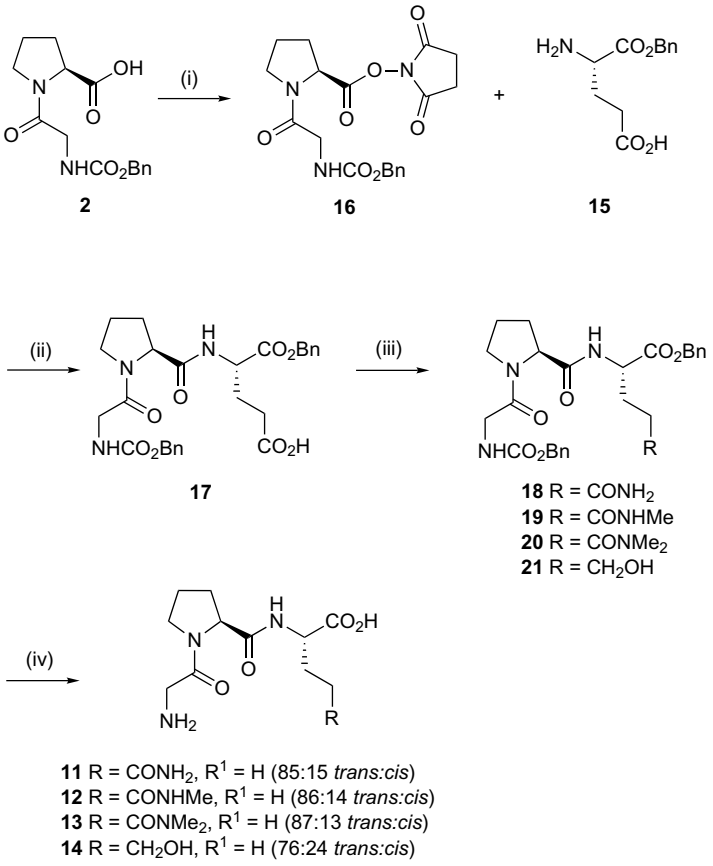
Based on the structure of GPE **1**, the first analogue **7** involved replacement of the γ-carboxylic acid group by a hydrogen, starting from the modified glutamic acid **8** (Scheme 3). Elimination of the carboxylic acid group in analogue **7** removes the amino acid character from the glutamate residue of GPE **1**, potentially suppressing



Scheme 2. Reagents and conditions: (i) $SOCl_2$, MeOH, −5 °C to rt, N_2, 19 h (98%); (ii) Et_3N, 1,3-dicyclohexylcarbodiimide (DCC), CH_2Cl_2, 0 °C to rt, N_2, 18 h; (iii) 1 M NaOH (aq), 1,4-dioxane, rt, 21 h (82% in two steps from **4**).



Scheme 3. Reagents and conditions: For **7**: (i) *p*TsOH, BnOH, benzene, reflux, 17 h (95%); (ii) DIPEA, EtOCOCl, CH_2Cl_2, 0 °C to rt, 17 h (78%); (iii) H_2, 10% Pd/C, 2:1 MeOH–H_2O, 18 h (84%). For **8**: (i) *p*TsOH, BnOH, benzene, reflux, 17 h (93%); (ii) Et_3N, EtOCOCl, CH_2Cl_2, 0 °C to rt, 17 h (94%); (iii) H_2, 10% Pd/C, 2:1 MeOH–H_2O, 18 h (73%).



Scheme 4. Reagents and conditions: (i) DME, *N*-hydroxysuccinimide, DCC, N_2, 0 °C, 23 h (85%); (ii) Et_3N, H_2O, THF, rt, 16 h (95%); For **11**: (iii) Boc_2O, NH_4HCO_3, pyridine, 1,4-dioxane, N_2, rt, 21 h (89%); (iv) H_2, 10% Pd/C, 2:1 MeOH–H_2O, rt, 18 h (82%). For **12**: (iii) Et_3N, MeNH·HCl, CH_3CN, TBTU, N_2, rt, 16.5 h (79%); (iv) H_2, 10% Pd/C, 2:1 MeOH–H_2O, rt, 18 h (67%). For **13**: (iii) Et_3N, Me_2N·HCl, CH_2Cl_2, EtOCOCl, N_2, 0 °C to rt, 17 h (89%); (iv) H_2, 10% Pd/C, 2:1 MeOH–H_2O, rt, 18 h (95%). For **14**: (iii) (a) Et_3N, THF, EtOCOCl, N_2, −5 °C, 30 min; (b) $NaBH_4$, MeOH, −5 °C to rt, 30 min (63%); (iv) H_2, 10% Pd/C, 82:16:2 THF–H_2O–Et_3N, rt, 18 h (100%).

the action of proteases, thereby imparting improved metabolic stability. Additionally, the overall polarity of the molecule is reduced, thus increasing lipophilicity and membrane permeability of the analogue.

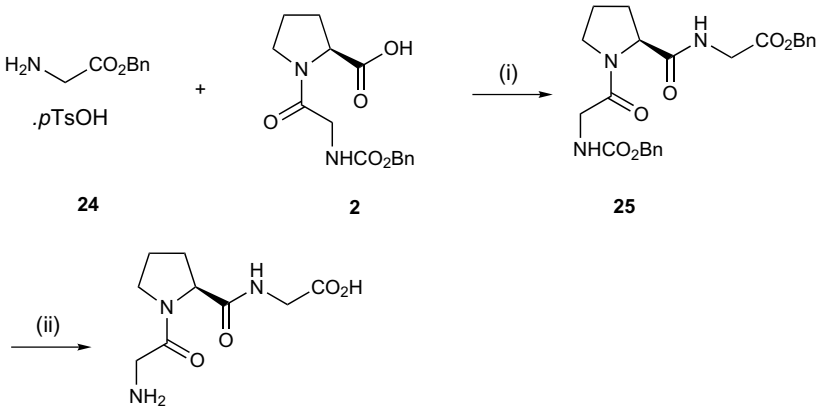
The commercial amino acid (2*S*)-aminobutanoic acid **8**, was initially protected as a benzyl ester **9** by heating with benzyl alcohol and *p*-toluenesulfonic acid in benzene. Coupling of the aforementioned acid **2** with protected amino ester **9** using peptide coupling produced protected tripeptide **10**. Upon deprotection, GPE* analogue **7** was produced as an 83:17 *trans*:*cis* mixture of rotamers.

Another strategy for reducing the overall charge in the GPE molecule whilst maintaining the steric bulk of the glutamate γ-position involves replacement of the side chain carboxylic acid with an amide (analogues **11**–**13**) or an alcohol (analogue **14**) (Scheme 4).

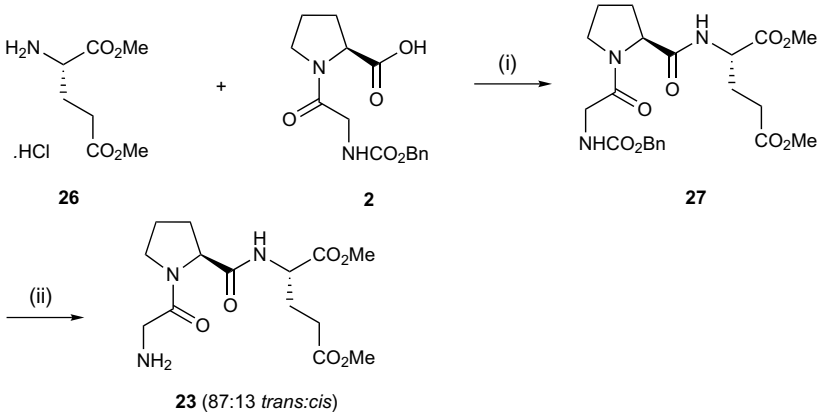
The syntheses of all four analogues **11**–**14** started from L-glutamic acid α-benzyl ester **15**. Reaction of amine **15** with succinimide **16** yielded dipeptide **17** in 95% yield. Subsequent modifications of the γ-carboxylic acid group on dipeptide **17** and deprotection provided analogues **11**–**14**.

In the case of the primary amide **11**, the precursor **17** was treated with di-*tert*-butyl dicarbonate and ammonolysis of the intermediate anhydride with ammonium hydrogen carbonate under basic conditions afforded fully protected tripeptide **18**. Subsequent deprotection yielded analogue **11** as an 85:15 *trans*:*cis* mixture of rotamers in 82% yield over the two steps. Analogues **12** and **13** were afforded by coupling of acid **17** with either methylamine hydrochloride using the coupling reagent, 2-(*N*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) or with dimethylamine hydrochloride using the mixed anhydride method, respectively, followed by hydrogenolysis of the resultant amides **19** and **20**. In the case of the alcohol **14**, reduction of the mixed anhydride formed from acid **17** with sodium borohydride followed by hydrogenolysis yielded analogue **14**. Analogues **11**–**14** all existed predominantly as the *trans* proline–glycine amide bond conformers.

Two further GPE* analogues **22** and **23** are also reported herein in which the carboxylic acid side chain of the glutamate residue is completely removed and the two carboxylic acid groups of the glutamate are converted to more lipophilic methyl esters (Schemes 5 and 6).



Scheme 5. Reagents and conditions: (i) Et_3N, EtOCOCl, CH_2Cl_2, 0 °C to rt, 17 h (94%); (ii) H_2, 10% Pd/C, MeOH, 18 h (85%).



Scheme 6. Reagents and conditions: (i) Et_3N, EtOCOCl, CH_2Cl_2, 0 °C to rt, 17 h (97%); (ii) H_2, 10% Pd/C, 2:1 MeOH–H_2O, 18 h (97%).

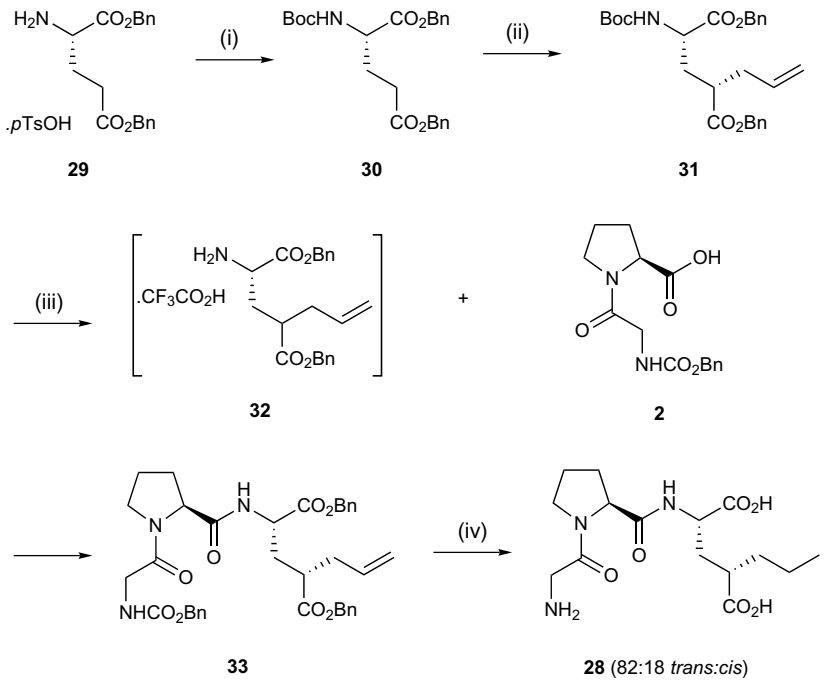
Analogue **22** was afforded by coupling of acid **2** with protected glycine **24** to give amide **25** followed by deprotection to afford GPG **22** as a 76:24 *trans*:*cis* mixture of rotamers (Scheme 5). Diester **23**, was obtained in 97% yield as an 87:13 *trans*:*cis* mixture of rotamers via condensation of dimethyl L-glutamate hydrochloride **26** with acid **2** and subsequent hydrogenolysis of the product **27** (Scheme 6).

One further analogue **28** was prepared in which the two carboxylic acid functionalities of the glutamic acid residue were left intact but however an additional propyl group was introduced to the γ-position in order to increase the lipophilic properties of the molecule (Scheme 7). Commercially available L-glutamic acid dibenzyl ester *p*-toluenesulfonate **29** was transformed into Boc-protected amino acid **30** by reaction with di-*tert*-butyl dicarbonate. Subsequent alkylation[38] with lithium hexamethyldisilazide and allyl bromide afforded the γ-alkylated product **31** as a single diastereomer in 73% yield, which then underwent Boc-deprotection to **32** and coupling with acid **2** to yield fully protected tripeptide **33** in 87% yield. The sequence was completed by hydrogenolysis of amide **33** under standard conditions affording analogue **28** in 77% yield as an 82:18 *trans*:*cis* mixture of conformers.

The eight GPE* analogues synthesised were subjected to in vitro evaluation for prevention of neuronal cell death. Of the eight analogues, GPG **22**, lacking any γ side chain, showed neuroprotective activity at 1 mM with a recovery value of 20% (Fig. 1). To compare, GPE **1** recovery values range from 25% to 40%. Lower neuroprotective values were obtained with 1 mM of the unsubstituted amide **11**, the alcohol **14** and the diesterified **23** where the recovery values ranged from 10% to 15%. None of the other analogues exhibited neuroprotective activity.

3. Conclusion

To summarise, we described herein the synthesis of eight analogues of GPE and their biological evaluation against striatal cell survival post-apoptosis-induced injury. Compound **22** exhibited the best recovery value, 20%, while **11**, **14** and **23** had lower recovery values but all four exhibited neuroprotective activity. The structure and



Scheme 7. Reagents and conditions: (i) Et_3N, Boc_2O, CH_2Cl_2, 0 °C to rt, 19 h (97%); (ii) (a) lithium hexamethyldisilazide, THF, −78 °C, N_2, 40 min; (b) allyl bromide, −78 °C, 1.5 h then −78 °C to rt, 1.5 h (73%); (iii) (a) TFA, CH_2Cl_2, 0 °C, 2 h; (b) CH_2Cl_2, Et_3N, EtOCOCl, 0 °C to rt, N_2, 17 h (87%); (iv) H_2, 10% Pd/C, 2:1 MeOH–H_2O, 18 h (77%).

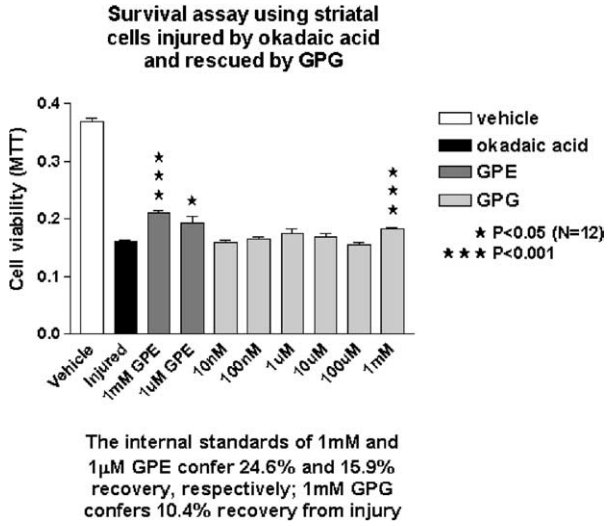


Figure 1. Cellular survival bioassay. MTT values were taken 24 h after injury when apoptotic nuclei become apparent within the cell culture. GPG (1 mM) **22** confers a significant increased cellular protection of 10.4%.

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 precoated silica gel plates (ALUGRAM® SIL G/UV_{254}). Products were visualised 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 hybridisation 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* isomerisation about the glycine–proline amide bond, the chemical shift for the minor *cis* conformer is asterisked (*). 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 CH_3CN as eluent at a flow rate of 1 mL/min. L-Proline methyl ester hydrochloride[35,36] (**4**) and *N*-benzyloxycarbonyl-glycyl-L-proline[33,34] (**2**) were prepared according to experimental procedures given in the previous paper.[1] All chemicals utilised 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 anaesthetised 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 sulphate (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.

Representative procedure A for the benzylation of carboxylic acids is as follows:

4.4. (2*S*)-Benzyl 2-aminobutanoate *p*-toluenesulfonate (10)

A mixture of L-(+)-2-aminobutanoic acid **8** (0.48 g, 4.56 mmol), *p*-toluenesulfonic acid monohydrate (98.5%) (1.29 g, 6.68 mmol) and benzyl alcohol (4.99 g, 46.1 mmol) in benzene (30 cm^3) was heated under reflux, using a Dean Stark distilling receiver, for 22 h, after which the reaction mixture was reduced in volume by removal of benzene (15 cm^3) by distillation. The solution was cooled to room temperature, anhydrous diethyl ether (20 cm^3) was added and the mixture left for 1 h at 0 °C to afford *p*-toluenesulfonate **9** (1.55 g, 93%) as a crystalline white solid: mp 125–127 °C; $[\alpha]_D$ −5.7 (*c* 0.26 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3579, 2963 br, 1748, 1602, 1523, 1497, 1450, 1400, 1367, 1302, 1259, 1212, 1188, 1125, 1038, 1013, 948, 811, 731 and 677; δ_H (300 MHz; $CDCl_3$; Me_4Si) 0.83 (3H, t, *J* 7.5, 4-H_3), 1.83 (2H, quintet, *J* 7.1, 3-H_2), 2.30 (3H, s, Ar–CH_3), 3.87–4.02 (1H, m, 2-H), 5.00 (1H, d, *J* 12.3, OCH_AH$_B$Ph), 5.12 (1H, d, *J* 12.2, OCH$_A$$H_B$Ph), 7.08 (2H, d, *J* 8.0, 2 × 3′-H), 7.22–7.34 (5H, m, Ph), 7.74 (2H, d, *J* 8.1, 2 × 2′-H) and 8.25 (3H, br s, N^+H_3); δ_C (75 MHz; $CDCl_3$) 9.0 (CH_3, 4-C), 21.2 (CH_3, Ar–*C*H_3), 23.6 (CH_2, 3-C), 54.2 (CH, 2-C), 67.6 (CH_2, O*C*H_2Ph), 126.1 (CH, Ar), 128.2 (CH, Ar), 128.3 (CH, Ar), 128.4 (CH, Ar), 128.7 (CH, Ar), 134.9 (quat., Ph), 140.2 (quat., Ar), 141.5 (quat., Ar) and 169.2 (quat., CO); *m*/*z* (FAB+) 559.2483 [M_2·*p*TsOH·H$^+$: $(C_{11}H_{15}NO_2)_2$. $C_7H_8O_3S$·H requires 559.2478].

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

4.5. (2*S*)-Benzyl *N*-benzyloxycarbonyl-glycyl-L-prolyl-2-aminobutanoate (10)

To a solution of acid **2** (0.65 g, 2.12 mmol) in methylene chloride (26 cm^3), cooled to 0 °C under nitrogen, was added dropwise triethylamine (0.33 cm^3, 2.37 mmol) and ethyl chloroformate (0.22 cm^3, 2.30 mmol) and the resultant mixture stirred at 0 °C for 45 min to form the mixed anhydride. Triethylamine (0.42 cm^3, 3.01 mmol) was added to a solution of benzyl 2-aminobutanoate *p*-toluenesulfonate **9** (1.02 g, 2.79 mmol) in methylene chloride (13 cm^3) under nitrogen at 0 °C and the resulting solution was added dropwise to the above mixed anhydride. The resultant mixture was warmed to room temperature and stirred for 16 h then the methylene chloride solution was extracted 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 ensuing residue by flash column chromatography (21 g SiO_2; 30–100% ethyl acetate–hexane; gradient elution) yielded *fully protected tripeptide analogue* **10** (0.96 g, 94%) as a pale yellow oil. Tripeptide **10** was shown to be a 90:10 *trans*:*cis* mixture of conformers by ^{1}H NMR analysis (the ratio was estimated from the integration of the doublets at δ 6.52 and 7.10, assigned to the Eα-NH protons of the minor and major conformers, respectively): R_f 0.50 (EtOAc); $[\alpha]_D$ −74.2 (*c* 0.15 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3312 br, 3064, 2971, 2878, 1725, 1649, 1533, 1453, 1386, 1344, 1256, 1211, 1193, 1146, 1055, 986, 917, 740 and 697; δ_H (300 MHz; $CDCl_3$; Me_4Si) 0.86 (3H, t, *J* 7.5, 4-H_3), 1.73 (1H, apparent octet, *J* 7.2, 3-H_AH_B), 1.63–2.40 (5H, m, 3-H_AH_B, Proγ-H_2 and Proβ-H_2), 3.40 (1H, dd, *J* 16.4 and 9.0, Proδ-H_AH_B), 3.47–3.70 (1H, m, Proδ-H_AH_B), 3.80* (0.10H, dd, *J* 16.8 and 4.0, Glyα-H_AH_B), 3.95 (0.90H, dd, *J* 17.1 and 4.2, Glyα-H_AH_B), 4.05 (1H, dd, *J* 17.3 and 4.6, Glyα-H_AH_B), 4.32* (0.10H, t, *J* 5.3, Proα-H), 4.44–4.63 (1.90H, m, 2-H and Proα-H), 5.05–5.25 (4H, m, 2 × OCH_2Ph), 5.59* (0.10H, br s, Gly-NH), 5.70 (0.90H, br s, Gly-NH), 6.52* (0.10H, d, *J* 7.9, 2-NH), 7.10 (0.90H, d, *J* 7.5, 2-NH) and 7.23–7.50 (10H, m, 2 × Ph); δ_C (75 MHz; $CDCl_3$) 9.4 (CH_3, 4-C), 9.8* (CH_3, 4-C), 22.1* (CH_2, Proγ-C), 24.5 (CH_2, Proγ-C), 24.8* (CH_2, 3-C), 25.0 (CH_2, 3-C), 27.7 (CH_2, Proβ-C), 31.8* (CH_2, Proβ-C), 43.0* (CH_2, Glyα-C), 43.2 (CH_2, Glyα-C), 46.1 (CH_2, Proδ-C), 46.9* (CH_2, Proδ-C), 53.4 (CH, 2-C), 59.8 (CH, Proα-C), 66.6 (CH_2, OCH_2Ph), 66.6 (CH_2, OCH_2Ph), 66.9* (CH_2, OCH_2Ph), 127.7* (CH, Ph), 127.7 (CH, Ph), 127.8 (CH, Ph), 127.9 (CH, Ph), 128.0* (CH, Ph), 128.1 (CH, Ph), 128.2 (CH, Ph), 128.3 (CH, Ph), 135.0* (quat., Ph), 135.3 (quat., Ph), 136.2 (quat., Ph), 156.1 (quat., NCO_2), 156.2* (quat., NCO_2), 167.8 (quat., Gly-CO), 167.9* (quat., Gly-CO), 170.7 (quat., Pro-CON), 171.2* (quat., Pro-CON) and 171.8 (quat., 1-CO); *m*/*z* (EI+) 481.2216 (M^+. $C_{26}H_{31}N_3O_6$ requires 481.2213).

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

4.6. (2*S*)-Glycyl-L-prolyl-2-aminobutanoic acid (7)

A mixture of protected tripeptide **10** (0.46 g, 0.96 mmol) and 10 wt% palladium on activated carbon (0.22 g, 0.21 mmol) in 2:1 methanol–water (30 cm^3) was stirred under an atmosphere of hydrogen at room temperature, protected from light, for 22 h. The reaction mixture was filtered through a Celite™ pad, and the pad washed with 50:50 methanol–water (150 cm^3). The filtrate was concentrated to dryness under reduced pressure at ambient temperature and the residue triturated with methanol to afford **7** (0.18 g, 73%) as a white amorphous solid. Compound **7** was shown to be an 83:17 *trans*:*cis* mixture of conformers by ^{13}C NMR analysis (the ratio was estimated from the relative intensities of the resonances at δ 23.9 and 26.1, assigned to the Proγ-C atoms of the minor and major conformers, respectively): mp 188–198 °C (decomp.); $[\alpha]_D$ −112.2 (*c* 0.18 in H_2O); ν_{max} (Nujol mull)/cm^{-1} 3528, 3312, 3188, 2630, 1648, 1598, 1570, 1537, 1418, 1234, 1208 and 930; δ_H (300 MHz; D_2O) 0.91 (3H, t, *J* 7.4, 4-H_3), 1.60–2.14 (4H, m, 3-H_AH_B Proγ-H_2 and Proβ-H_AH_B), 1.70 (1H, apparent octet, *J* 7.3, 3-H_AH_B), 2.14–2.48 (1H, m, Proβ-H_AH_B), 3.50–3.73 (2.17H, m, Proδ-H_2 and Glyα-H_AH_B*), 3.91–4.16 (2.83H, m, Glyα-H_AH_B* Glyα-H_2 and 2-H) and 4.49 (1H, dd, *J* 8.2 and 4.1, Proα-H); δ_C (75 MHz; D_2O) 11.4 (CH_3, 4-C), 11.6* (CH_3, 4-C), 23.9* (CH_2, Proγ-C), 26.1 (CH_2, Proγ-C), 26.5* (CH_2, 3-C), 26.9 (CH_2, 3-C), 31.3 (CH_2, Proβ-C), 33.6* (CH_2, Proβ-C), 42.1* (CH_2, Glyα-C), 42.3 (CH_2, Glyα-C), 48.8 (CH_2, Proδ-C), 49.5* (CH_2, Proδ-C), 58.6 (CH, 2-C), 58.9* (CH, 2-C), 62.0* (CH, Proα-C), 62.4 (CH, Proα-C), 167.5 (quat., Gly-CO), 168.0* (quat., Gly-CO), 174.6* (quat., Pro-CON), 175.0 (quat., Pro-CON), 180.5* (quat., 1-CO) and 180.6 (quat., 1-CO); *m*/*z* (FAB+) 258.1446 (MH^+. $C_{11}H_{20}N_3O_4$ requires 258.1454).

4.7. *N*-Benzyloxycarbonyl-glycyl-L-proline *N*-hydroxysuccinimide ester[1] (16)

To a mixture of acid **2** (4.40 g, 14.36 mmol) in anhydrous 1,2-dimethoxyethane (130 mL) under nitrogen at 0 °C, were successively added *N*-hydroxysuccinimide (1.94 g, 16.35 mmol) and a solution of 1,3-dicyclohexylcarbodiimide (3.49 g, 16.91 mmol) in 1,2-dimethoxyethane (19 mL) cooled to 0 °C. The reaction mixture was warmed to room temperature, stirred for 23 h then filtered through a Celite™ pad, to partially remove solid 1,3-dicyclohexylurea, and the filtrate concentrated to dryness under reduced pressure. The resultant residue was dissolved in methylene chloride, washed with 10% aqueous hydrochloric acid (100 mL) and saturated aqueous sodium hydrogen carbonate (100 mL), dried ($MgSO_4$), filtered and concentrated to dryness. Purification of the residue by flash column chromatography (SiO_2; 30–90% ethyl acetate–hexane; gradient elution) gave a white foam, which was dissolved in methylene chloride and triturated with diethyl ether to yield *succinimide* **16** (4.94 g, 85%) as a white solid. Compound **16** was shown to be an 85:15 *trans*:*cis* mixture of conformers: R_f 0.40 (EtOAc); mp 102–104 °C; $[\alpha]_D$ −80.7 (*c* 0.29 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3583 wk, 3406 br, 3333 br, 2952, 2883, 1815, 1784, 1738, 1660, 1522, 1439, 1368, 1245, 1206, 1077, 992, 911, 813, 734, 699 and 645; δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.98–2.25 (2H, m, Proγ-H_2), 2.28–2.39 (1.70H, m, Proβ-H_2), 2.39–2.49* (0.30H, m, Proβ-H_2), 2.82 [4H, s, C(O)CH_2CH_2C(O)], 3.38–3.56 (0.85H, m, Proδ-H_AH_B), 3.56–3.75 (1.15H, m, Proδ-H_AH_B and Proδ-H_AH_B*), 3.89* (0.15H, dd, *J* 16.8 and 3.4, Glyα-H_AH_B), 3.98 (0.85H, dd, *J* 17.2 and 3.8, Glyα-H_AH_B), 4.08 (0.85H, dd, *J* 17.3 and 5.0, Glyα-H_AH_B), 4.14* (0.15H, dd, *J* 17.0 and 6.0, Glyα-H_AH_B), 4.76* (0.15H, br t, *J* 5.1, Proα-H), 4.83 (0.85H, t, *J* 6.2, Proα-H), 5.11 (2H, s, OCH_2Ph), 5.64* (0.15H, br s, Gly-NH), 5.75 (0.85H, br s, Gly-NH) and 7.27–7.38 (5H, m, Ph); δ_C (100 MHz; $CDCl_3$) 22.0* (CH_2, Proγ-C), 24.6 (CH_2, Proγ-C), 25.4 [CH_2, C(O)CH_2CH_2C(O)], 29.1 (CH_2, Proβ-C), 31.8* (CH_2, Proβ-C), 43.1 (CH_2, Glyα-C), 45.7 (CH_2, Proδ-C), 46.7* (CH_2, Proδ-C), 56.6* (CH, Proα-C), 56.7 (CH, Proα-C), 66.6 (CH_2, OCH_2Ph), 66.7* (CH_2, OCH_2Ph), 127.8 (CH, Ph), 127.9 (CH, Ph), 128.3, (CH, Ph), 136.4 (quat., Ph), 156.2 (quat., NCO_2), 167.3 (quat., CO), 167.5 (quat., CO), 167.6* (quat., CO)

and 168.8 [quat., $C(O)CH_2CH_2C(O)$]; *m/z* (FAB+) 404.1451 (MH^+. $C_{19}H_{22}N_3O_7$ requires 404.1458).

4.8. α-Benzyl *N*-benzyloxycarbonyl-glycyl-L-prolyl-L-glutamate (17)

Triethylamine (1.65 mL, 11.84 mmol) and water (75 mL) were successively added to a mixture of L-glutamic acid α-benzyl ester **15** (2.21 g, 9.31 mmol) in tetrahydrofuran (150 mL) at room temperature. A mixture of succinimide **16** (3.00 g, 7.44 mmol) in tetrahydrofuran (95 mL) was slowly added to the resultant solution and the reaction mixture stirred for 16 h. The solvent was then removed in vacuo, the residue partitioned between methylene chloride (100 mL) and 10% aqueous hydrochloric acid (100 mL), and the aqueous layer extracted twice with methylene chloride. The combined organic fractions were washed with 10% aqueous hydrochloric acid (200 mL) and brine (200 mL), dried ($MgSO_4$), filtered and evaporated to dryness. Purification of the residue by flash column chromatography (SiO_2; 50–20% ethyl acetate–hexane to methanol–methylene chloride; gradient elution) afforded *acid* **17** (3.72 g, 95%) as an hygroscopic white foam. Compound **17** was shown to be a 77:23 *trans*:*cis* mixture of conformers: R_f 0.50 (10% MeOH–CH_2Cl_2); $[\alpha]_D$ −55.6 (*c* 0.48 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3583 wk, 3312 br, 2952, 1721, 1646, 1534, 1453, 1342, 1257, 1208, 1172, 1125, 1057, 983, 912, 739 and 697; δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.76–2.45 (8H, m, Proγ-H_2, Proβ-H_2, Gluβ-H_2 and Gluγ-H_2), 3.38 (0.77H, br dd, *J* 16.1 and 8.4, Proδ-H_AH_B), 3.46–3.76 (1.23H, m, Proδ-H_AH_B and Proδ-H_AH_B*), 3.76* (0.23H, dd, *J* 17.2 and 3.2, Glyα-H_AH_B), 3.96 (1H, dd, *J* 17.3 and 4.9, Glyα-H_AH_B and Glyα-H_AH_B*), 4.06 (0.77H, dd, *J* 17.1 and 4.9, Glyα-H_AH_B), 4.24–4.31* (0.23H, m, Proα-H), 4.49–4.64 (1.77H, m, Proα-H and Gluα-H), 5.03–5.21 (4H, m, 2 × OCH_2Ph), 6.01 (1H, br s, Gly-NH) and 7.27–7.39 (11H, m, 2 × Ph and Gluα-NH); δ_C (100 MHz; $CDCl_3$) 22.2* (CH_2, Proγ-C), 24.6 (CH_2, Proγ-C), 25.5* (CH_2, Gluβ-C), 26.5 (CH_2, Gluβ-C), 28.2 (CH_2, Proβ-C), 30.2 (CH_2, Gluγ-C), 30.6* (CH_2, Gluγ-C), 31.9* (CH_2, Proβ-C), 43.1* (CH_2, Glyα-C), 43.2 (CH_2, Glyα-C), 46.4 (CH_2, Proδ-C), 47.2* (CH_2, Proδ-C), 52.0 (CH, Gluα-C), 52.5* (CH, Gluα-C), 60.1 (CH, Proα-C), 66.9 (CH_2, OCH_2Ph), 67.1 (CH_2, OCH_2Ph), 67.3* (CH_2, OCH_2Ph), 127.9 (CH, Ph), 128.0 (CH, Ph), 128.1* (CH, Ph), 128.1 (CH, Ph), 128.2* (CH, Ph), 128.3 (CH, Ph), 128.4 (CH, Ph), 128.5 (CH, Ph), 135.1* (quat., Ph), 135.2 (quat., Ph), 136.1* (quat., Ph), 136.2 (quat., Ph), 156.8 (quat., NCO_2), 156.9* (quat., NCO_2), 168.7 (quat., Gly-CO), 171.2* (quat., CO), 171.3 (quat., CO), 171.3 (quat., CO), 171.5* (quat., CO), 176.0 (quat., Gluγ-CO) and 176.3* (quat., Gluγ-CO); *m/z* (FAB+) 526.2197 (MH^+. $C_{27}H_{32}N_3O_8$ requires 526.2189).

4.9. *N*-Benzyloxycarbonyl-glycyl-L-prolyl-L-glutamine α-benzyl ester (18)

To a solution of acid **17** (0.98 g, 1.86 mmol) and di-*tert*-butyl dicarbonate (0.56 g, 2.49 mmol) in 1,4-dioxane (28 mL), under nitrogen at room temperature, were added pyridine (0.21 mL, 2.60 mmol) dropwise and ammonium hydrogen carbonate (0.21 g, 2.66 mmol). The mixture was stirred for 21 h, the solvent removed in vacuo, and the resultant residue dissolved in methylene chloride, washed with 10% aqueous hydrochloric acid (50 mL) and saturated aqueous sodium hydrogen carbonate (50 mL), dried ($MgSO_4$), filtered and evaporated to dryness. Purification of the residue by flash column chromatography (SiO_2; 80–20% ethyl acetate–hexane to methanol–methylene chloride; gradient elution) gave *primary amide* **18** (0.87 g, 89%) as a white foam. Compound **18** was shown to be an 83:17 *trans*:*cis* mixture of conformers: R_f 0.50 (10% MeOH–CH_2Cl_2); $[\alpha]_D$ −53.2 (*c* 0.24 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3583 wk, 3309 br, 3065, 2952, 1720, 1655, 1536, 1453, 1342, 1255, 1212, 1172, 1129, 1052, 986, 913, 736 and 697; δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.67–2.46 (8H, m, Proγ-H_2, Proβ-H_2, Glnβ-H_2 and Glnγ-H_2), 3.27–3.75 (2H, m, Proδ-H_2), 3.86* (0.17H, dd, *J* 12.2 and 4.2, Glyα-H_AH_B), 3.95 (1H, dd, *J* 17.2 and 4.9, Glyα-H_AH_B and Glyα-$H_AH_B^*$), 4.04 (0.83H, dd, *J* 17.1 and 5.1, Glyα-H_AH_B), 4.24* (0.17H, dd, *J* 8.5 and 2.5, Proα-H), 4.47 (0.83H, br d, *J* 5.4, Proα-H), 4.57 (1H, td, *J* 8.3, 8.3 and 3.2, Glnα-H), 5.04–5.20 (4H, m, 2 × OCH_2Ph), 5.53 (0.83H, br s, $CONH_AH_B$), 5.69–5.90 (1.17H, br m, $CONH_AH_B$* and Gly-NH), 6.28 (1H, br s, CONH$_A$$H_B$) and 7.25–7.38 (11H, m, 2 × Ph and Glnα-NH); δ_C (100 MHz; $CDCl_3$) 22.2* (CH_2, Proγ-C), 24.6 (CH_2, Proγ-C), 25.9* (CH_2, Glnβ-C), 27.1 (CH_2, Glnβ-C), 28.5 (CH_2, Proβ-C), 31.0 (CH_2, Glnγ-C), 31.4* (CH_2, Glnγ-C), 31.9* (CH_2, Proβ-C), 43.1* (CH_2, Glyα-C), 43.2 (CH_2, Glyα-C), 46.3 (CH_2, Proδ-C), 47.1* (CH_2, Proδ-C), 51.8 (CH, Glnα-C), 52.4* (CH, Glnα-C), 59.7* (CH, Proα-C), 60.1 (CH, Proα-C), 66.7 (CH_2, OCH_2Ph), 67.0 (CH_2, OCH_2Ph), 67.1* (CH_2, OCH_2Ph), 127.7 (CH, Ph), 127.9 (CH, Ph), 128.0 (CH, Ph), 128.1 (CH, Ph), 128.1* (CH, Ph), 128.3 (CH, Ph), 128.3* (CH, Ph), 128.4 (CH, Ph), 128.4 (CH, Ph), 135.1* (quat., Ph), 135.2 (quat., Ph), 136.3 (quat., Ph), 156.5* (quat., NCO_2), 156.6 (quat., NCO_2), 167.6* (quat., Gly-CO), 168.2 (quat., Gly-CO), 171.4* (quat., CO), 171.5 (quat., CO), 171.6 (quat., CO), 171.7* (quat., CO) and 175.2 (quat., Glnγ-CO); *m/z* (FAB+) 525.2355 (MH^+. $C_{27}H_{33}N_4O_7$ requires 525.2349).

4.10. Glycyl-L-prolyl-L-glutamine (11)

Following procedure C, hydrogenation of amide **18** yielded *tripeptide* **11** (0.23 g, 82%) as a white solid. Compound **11** was shown to be an 85:15 *trans*:*cis* mixture of conformers: mp 167–169 °C; $[\alpha]_D$ −80.6 (*c* 0.24 in H_2O); δ_H (400 MHz; D_2O) 1.80–2.23 (5H, m, Glnβ-H_2, Proβ-H_AH_B and Proγ-H_2), 2.23–2.45 (3H, m, Proβ-H$_A$$H_B$ and Glnγ-H_2), 3.48–3.82 (2H, m, Proδ-H_2), 3.75* (0.15H, d, *J* 16.2, Glyα-H_AH_B), 3.94* (0.15H, d, *J* 16.0, Glyα-H$_A$$H_B$), 3.97 (0.85H, d, *J* 18.1, Glyα-H_AH_B), 4.01 (0.85H, d, *J* 17.4, Glyα-H$_A$$H_B$), 4.17 (1H, dd, *J* 8.4 and 4.9, Glnα-H) and 4.48 (1H, dd, *J* 7.9 and 3.7, Proα-H); δ_C (100 MHz; D_2O) 23.9* (CH_2, Proγ-C), 26.1 (CH_2, Proγ-C), 28.9* (CH_2, Glnβ-C), 29.6 (CH_2, Glnβ-C), 31.2 (CH_2, Proβ-C), 33.4 (CH_2, Glnγ-C), 33.6* (CH_2, Proβ-C), 33.6* (CH_2, Glnγ-C), 42.1* (CH_2, Glyα-C), 42.3 (CH_2, Glyα-C), 48.8 (CH_2, Proδ-C), 49.4* (CH_2, Proδ-C), 56.4 (CH, Glnα-C), 56.9* (CH, Glnα-C), 62.1*

(CH, Proα-C), 62.5 (CH, Proα-C), 167.5 (quat., Gly-CO), 168.0* (quat., Gly-CO), 174.8* (quat., Pro-CON), 175.2 (quat., Pro-CON), 179.4* (quat., Glnα-CO), 179.5 (quat., Glnα-CO), 180.2* (quat., Glnγ-CO) and 180.4 (quat., Glnγ-CO); *m/z* (FAB+) 301.1519 (MH^+. $C_{12}H_{21}N_4O_5$ requires 301.1512).

4.11. *N*-Benzyloxycarbonyl-glycyl-L-prolyl-L-*N*-methylglutamine α-benzyl ester (19)

Triethylamine (1.62 mL, 11.62 mmol) was added dropwise to a mixture of acid **17** (1.02 g, 1.94 mmol) and methylamine hydrochloride (0.30 g, 4.44 mmol) in acetonitrile (45 mL), under nitrogen at room temperature, and the reaction mixture stirred for 5 min. 2-(*N*-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) (0.64 g, 1.95 mmol) was added in one portion, the mixture stirred for 16.5 h then the solvent removed under reduced pressure. The resultant residue was partitioned between methylene chloride (50 mL) and 10% aqueous hydrochloric acid (50 mL) and the aqueous fraction extracted with methylene chloride (2 × 50 mL). The combined organic fractions were washed with 10% aqueous hydrochloric acid (100 mL) and saturated aqueous sodium hydrogen carbonate (100 mL), dried ($MgSO_4$), filtered and evaporated to dryness. Trituration of the residue with methylene chloride and diethyl ether afforded *N*-methylamide **19** (0.83 g, 79%) as a white solid. Compound **19** was shown to be an 85:15 *trans*:*cis* mixture of conformers: R_f 0.45 (10% MeOH–CH_2Cl_2); mp 137–139 °C; $[\alpha]_D$ −54.4 (*c* 0.25 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3583 wk, 3312 br, 3065, 2951, 1725, 1649, 1543, 1453, 1411, 1377, 1335, 1258, 1213, 1171, 1054, 986, 914, 734 and 698; δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.79–2.36 (8H, m, Proγ-H_2, Glnβ-H_2, Proβ-H_2 and Glnγ-H_2), 2.66–2.73* (0.45H, m, CONHCH_3), 2.69 (2.55H, d, *J* 4.7, CONHCH_3), 3.27–3.80 (2H, m, Proδ-H_2), 3.84* (0.30H, d, *J* 4.1, Glyα-H_2), 3.96 (0.85H, dd, *J* 17.3 and 4.9, Glyα-H_AH_B), 4.05 (0.85H, dd, *J* 17.3 and 4.9, Glyα-H_AH_B), 4.21* (0.15H, dd, *J* 8.5 and 2.6, Proα-H), 4.35–4.53 (0.85H, m, Proα-H), 4.54 (1H, td, *J* 8.3, 8.3 and 3.6, Glnα-H), 5.06–5.20 (4H, m, 2 × OCH_2Ph), 5.70 (0.85H, br t, *J* 4.4, Gly-NH), 5.82* (0.15H, br t, *J* 3.8, Gly-NH), 6.23–6.48 (1H, br m, CON*H*CH_3), 7.16–7.52 (10.85H, m, 2 × Ph and Gln-NH) and 7.45* (0.15H, d, *J* 7.6, Gln-NH); δ_C (75 MHz; $CDCl_3$) 22.4* (CH_2, Proγ-C), 24.8 (CH_2, Proγ-C), 26.2 (CH_3, CONH*C*H_3), 26.3* (CH_3, CONH*C*H_3), 27.8 (CH_2, Glnβ-C), 28.4 (CH_2, Proβ-C), 31.8 (CH_2, Glnγ-C), 31.9* (CH_2, Glnγ-C or Proβ-C), 32.0* (CH_2, Proβ-C or Glnγ-C), 43.1* (CH_2, Glyα-C), 43.3 (CH_2, Glyα-C), 46.3 (CH_2, Proδ-C), 47.2* (CH_2, Proδ-C), 51.8 (CH, Glnα-C), 52.4* (CH, Glnα-C), 59.8* (CH, Proα-C), 60.3 (CH, Proα-C), 66.8* (CH_2, O*C*H_2Ph), 66.9 (CH_2, O*C*H_2Ph), 67.1 (CH_2, O*C*H_2Ph), 67.2* (CH_2, O*C*H_2Ph), 127.8* (CH, Ph), 127.9 (CH, Ph), 128.1 (CH, Ph), 128.2 (CH, Ph), 128.3* (CH, Ph), 128.4 (CH, Ph), 128.4* (CH, Ph), 128.5 (CH, Ph), 128.5 (CH, Ph), 135.2 (quat., Ph), 136.2 (quat., Ph), 156.3* (quat., NCO_2), 156.4 (quat., NCO_2), 167.2* (quat., Gly-CO), 167.9 (quat., Gly-CO), 171.3 (quat., CO), 171.4* (quat., CO), 171.5 (quat., CO), 172.7 (quat., *C*ONHCH$_3$) and 172.8* (quat., *C*ONHCH$_3$); *m/z* (FAB+) 539.2508 (MH^+. $C_{28}H_{35}N_4O_7$ requires 539.2506).

4.12. Glycyl-L-prolyl-L-*N*-methylglutamine (12)

Following procedure C, hydrogenation of *N*-methylamide **19** yielded *analogue* **12** (0.20 g, 67%) as a white solid. Compound **12** was shown to be an 86:14 *trans*:*cis* mixture of conformers: mp 193–196 °C (dec.); $[\alpha]_D$ −77.3 (*c* 0.19 in H_2O); δ_H (300 MHz; D_2O) 1.82–2.46 (8H, m, Glnβ-H_2, Proβ-H_2, Proγ-H_2 and Glnγ-H_2), 2.72 (3H, s, CONHCH_3), 3.51–3.73 (2H, m, Proδ-H_2), 3.77* (0.14H, d, *J* 16.3, Glyα-H_AH_B), 3.96* (0.14H, d, *J* 16.4, Glyα-H_AH_B), 3.98 (0.86H, d, *J* 17.3, Glyα-H_AH_B), 4.04 (0.86H, d, *J* 17.2, Glyα-H_AH_B), 4.11–4.21* (0.14H, m, Glnα-H), 4.15 (0.86H, dd, *J* 8.5 and 4.9, Glnα-H) and 4.49 (1H, dd, *J* 8.2 and 4.0, Proα-H); δ_C (75 MHz; D_2O) 23.9* (CH_2, Proγ-C), 26.1 (CH_2, Proγ-C), 27.7 (CH_3, CONH*C*H_3), 29.2* (CH_2, Glnβ-C), 29.9 (CH_2, Glnβ-C), 31.3 (CH_2, Proβ-C), 33.6* (CH_2, Proβ-C), 34.1 (CH_2, Glnγ-C), 34.2* (CH_2, Glnγ-C), 42.1* (CH_2, Glyα-C), 42.3 (CH_2, Glyα-C), 48.8 (CH_2, Proδ-C), 49.4* (CH_2, Proδδ-C), 56.5 (CH, Glnα-C), 56.9* (CH, Glnα-C), 62.1* (CH, Proα-C), 62.5 (CH, Proα-C), 167.5 (quat., Gly-CO), 168.0* (quat., Gly-CO), 174.7* (quat., Pro-CON), 175.2 (quat., Pro-CON), 177.7* (quat., *C*ONHCH$_3$), 177.8 (quat., *C*ONHCH$_3$), 179.4* (quat., CO_2H) and 179.6 (quat., CO_2H); *m/z* (FAB+) 315.1672 (MH^+. $C_{13}H_{23}N_4O_5$ requires 315.1669).

4.13. *N*-Benzyloxycarbonyl-glycyl-L-prolyl-L-*N*,*N*-dimethylglutamine α-benzyl ester (20)

Following procedure B, coupling of acid **17** with dimethylamine hydrochloride using triethylamine as the base yielded *N*,*N*-dimethylamide **20** (0.48 g, 89%) as a colourless oil, which slowly crystallised to a white solid. Compound **20** was shown to be a 79:21 *trans*:*cis* mixture of conformers: R_f 0.50 (10% MeOH–CH_2Cl_2); mp 110–111 °C; $[\alpha]_D$ −56.9 (*c* 0.26 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3583 wk, 3297 br, 3063, 3033, 2946, 1724, 1645, 1530, 1453, 1340, 1255, 1214, 1185, 1055, 986, 915, 815, 737 and 697; δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.80–2.28 (6H, m, Proγ-H_2, Proβ-H_2 and Glnβ-H_2), 2.33 (2H, t, *J* 6.5, Glnγ-H_2), 2.82* [0.63H, s, CON(-CH_3)$_A$(CH$_3$)$_B$], 2.84 [2.37H, s, CON(CH_3)$_A$(CH$_3$)$_B$], 2.86 [2.37H, s, CON(CH$_3$)$_A$(CH_3)$_B$], 2.91* [0.63H, s, CON(CH$_3$)$_A$(CH_3)$_B$], 3.30–3.47 (0.79H, m, Proδ-H_AH_B), 3.50–3.67 (1H, m, Proδ-H_AH_B and Proδ-H_AH_B*), 3.75–3.86* (0.21H, m, Proδ-H_AH_B), 3.91* (0.42H, dd, *J* 9.5 and 4.5, Glyα-H_2), 4.03 (1.58H, d, *J* 4.2, Glyα-H_2), 4.26* (0.21H, dd, *J* 7.3 and 3.5, Proα-H), 4.39–4.56 (1.79H, m, Glnα-H and Proα-H), 5.06–5.22 (4H, m, 2 × OCH_2Ph), 5.61* (0.21H, br t, *J* 4.5, Gly-NH), 5.75 (0.79H, br t, *J* 3.8, Gly-NH), 7.27–7.39 (10H, m, 2 × Ph), 7.72 (0.79H, d, *J* 7.0, Glnα-NH) and 8.52* (0.21H, d, *J* 5.6, Glnα-NH); δ_C (75 MHz; $CDCl_3$) 22.2* (CH_2, Proγ-C), 24.5 (CH_2, Proγ-C), 24.9* (CH_2, Glnβ-C), 26.3 (CH_2, Glnβ-C), 28.5 (CH_2, Proβ-C), 29.2 (CH_2, Glnγ-C), 29.5* (CH_2, Glnγ-C), 31.8* (CH_2, Proβ-C), 35.4 [CH_3, CON(*C*H$_3$)$_A$(CH$_3$)$_B$], 35.6* [CH_3, CON(*C*H$_3$)$_A$(CH$_3$)$_B$], 36.9 [CH_3, CON(CH$_3$)$_A$(*C*H$_3$)$_B$],

43.0* (CH_2, Glyα-C), 43.3 (CH_2, Glyα-C), 46.1 (CH_2, Proδ-C), 46.9* (CH_2, Proδ-C), 52.3 (CH, Glnα-C), 52.9* (CH, Glnα-C), 60.1 (CH, Proα-C), 66.5* (CH_2, O*C*H_2Ph), 66.6 (CH_2, O*C*H_2Ph), 66.8 (CH_2, O*C*H_2Ph), 66.9* (CH_2, O*C*H_2Ph), 127.8* (CH, Ph), 127.9* (CH, Ph), 127.9 (CH, Ph), 127.9 (CH, Ph), 128.1 (CH, Ph), 128.2* (CH, Ph), 128.3 (CH, Ph), 128.3 (CH, Ph), 128.4 (CH, Ph), 135.4 (quat., Ph), 136.3 (quat., Ph), 136.4* (quat., Ph), 156.1 (quat., NCO_2), 167.4 (quat., Gly-CO), 167.7* (quat., Gly-CO), 171.1* (quat., CO), 171.2 (quat., CO), 171.4 (quat., CO), 171.5* (quat., CO), 172.1 [quat., *C*ON$(CH_3)_2$] and 172.4* [quat., *C*ON$(CH_3)_2$]; *m/z* (EI+) 552.2577 (M^+. $C_{29}H_{36}N_4O_7$ requires 552.2584).

4.14. Glycyl-L-prolyl-L-*N*,*N*-dimethylglutamine (13)

Following procedure C, hydrogenation of *N*,*N*-dimethylamide **20** yielded *analogue* **13** (0.26 g, 95%) as an extremely hygroscopic white solid, on trituration with anhydrous diethyl ether. Compound **13** was shown to be an 87:13 *trans*:*cis* mixture of conformers: mp 175 °C (dec.); $[\alpha]_D$ −65.6 (*c* 0.19 in H_2O); δ_H (300 MHz; D_2O) 1.72–2.55 (8H, m, Glnβ-H_2, Proγ-H_2, Proβ-H_2 and Glnγ-H_2), 2.86 [3H, s, CON(C*H*$_3$)$_A$(CH$_3$)$_B$], 2.99* [0.39H, s, CON(CH$_3$)$_A$(C*H*$_3$)$_B$], 3.00 [2.61H, s, CON(CH$_3$)$_A$(C*H*$_3$)$_B$], 3.43–3.67 (2H, m, Proδ-H_2), 3.71* (0.13H, d, *J* 16.5, Glyα-H_AH$_B$), 3.82–3.99 (1.87H, m, Glyα-H$_A$$H_B$* and Glyα-$H_2$), 4.10 (1H, dd, *J* 8.2 and 5.1, Glnα-H) and 4.42 (1H, dd, *J* 8.2 and 4.0, Proα-H); δ_C (75 MHz; D_2O) 23.9* (CH_2, Proγ-C), 26.1 (CH_2, Proγ-C), 28.5* (CH_2, Glnβ-C), 29.3 (CH_2, Glnβ-C), 31.3 (CH_2, Proβ-C), 31.3 (CH_2, Glnγ-C), 31.5* (CH_2, Glnγ-C), 33.6* (CH_2, Proβ-C), 37.3 [CH_3, CON(*C*H$_3$)$_A$(CH$_3$)$_B$], 39.3* [CH_3, CON(CH$_3$)$_A$(*C*H$_3$)$_B$], 39.4 [CH_3, CON(CH$_3$)$_A$(*C*H$_3$)$_B$], 42.2* (CH_2, Glyα-C), 42.3 (CH_2, Glyα-C), 48.8 (CH_2, Proδ-C), 49.4* (CH_2, Proδ-C), 56.7 (CH, Glnα-C), 57.2* (CH, Glnα-C), 62.1* (CH, Proα-C), 62.5 (CH, Proα-C), 167.6 (quat., Gly-CO), 168.2* (quat., Gly-CO), 174.8* (quat., Pro-CON), 175.2 (quat., Pro-CON), 176.7* [quat., *C*ON$(CH_3)_2$], 176.8 [quat., *C*ON$(CH_3)_2$], 179.6* (quat., CO_2H) and 179.8 (quat., CO_2H); *m/z* (FAB+) 329.1836 (MH^+. $C_{14}H_{25}N_4O_5$ requires 329.1825).

4.15. (2*S*)-Benzyl *N*-benzyloxycarbonyl-glycyl-L-prolyl-2-amino-5-hydroxypentanoate (21)

To a solution of acid **17** (0.72 g, 1.37 mmol) in anhydrous tetrahydrofuran (50 mL), cooled to ca. −5 °C under nitrogen, were successively added dropwise triethylamine (0.32 mL, 2.30 mmol) and ethyl chloroformate (0.21 mL, 2.20 mmol) and the reaction mixture stirred at ca. −5 °C for 30 min to form the mixed anhydride. Powdered sodium borohydride (0.24 g, 6.35 mmol) was added in one portion, followed by methanol (15 mL) dropwise, keeping the temperature at ca. −5 °C. The effervescent reaction mixture was stirred for 15 min, warmed to room temperature and stirred for a further 15 min, then quenched with saturated aqueous ammonium chloride (5 mL). The organic solvents were removed in vacuo at ambient temperature, the aqueous residue extracted with ethyl acetate (50 mL) and the organic fraction washed with 10% aqueous hydrochloric acid (50 mL), saturated aqueous sodium hydrogen carbonate (50 mL) and brine (50 mL), dried ($MgSO_4$), filtered and evaporated to dryness. The resultant residue was triturated with methylene chloride and diethyl ether to yield acid-sensitive *alcohol* **21** (0.44 g, 63%) as a white solid. Compound **21** was shown to be an 85:15 *trans*:*cis* mixture of conformers: R_f 0.70 (10% MeOH–CH_2Cl_2); mp 116–117 °C; $[\alpha]_D$ −65.5 (*c* 0.20 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3583 wk, 3314 br, 3064, 3033, 2952, 2877, 1725, 1649, 1535, 1453, 1339, 1256, 1213, 1172, 1057, 987, 913, 736 and 698; δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.39–1.65 (2H, m, 4-H_2), 1.69–2.32 (6H, m, 3-H_2, Proγ-H_2 and Proβ-H_2), 2.69 (0.85H, br s, OH), 3.17* (0.15H, br s, OH), 3.39 (0.85H, br dd, *J* 16.0 and 8.4, Proδ-H_AH$_B$), 3.46–3.71 (3.15H, m, Proδ-H$_A$$H_B$, Proδ-$H_AH_B$* and 5-$H_2$), 3.90* (0.30H, d, *J* 4.7, Glyα-H_2), 3.97 (1.70H, d, *J* 4.9, Glyα-H_2), 4.28* (0.15H, t, *J* 5.6, Proα-H), 4.46–4.63 (1.85H, m, Proα-H and 2-H), 5.04–5.23 (4H, m, 2 × OC*H*$_2$Ph), 5.71* (0.15H, br t, *J* 5.0, Gly-NH), 5.75 (0.85H, br t, *J* 4.7, Gly-NH), 7.22–7.45 (10.85H, m, 2-NH and 2 × Ph) and 7.76* (0.15H, d, *J* 7.6, 2-NH); δ_C (75 MHz; $CDCl_3$) 22.3* (CH_2, Proγ-C), 24.8 (CH_2, Proγ-C), 28.0 (CH_2, Proβ-C), 28.2 (CH_2, 4-C), 28.5 (CH_2, 3-C), 32.0* (CH_2, Proβ-C), 43.3 (CH_2, Glyα-C), 46.3 (CH_2, Proδ-C), 47.1* (CH_2, Proδ-C), 52.2 (CH, 2-C), 60.1 (CH, Proα-C), 61.8 (CH_2, 5-C), 66.9 (CH_2, O*C*H_2Ph), 67.0 (CH_2, O*C*H_2Ph), 67.1* (CH_2, O*C*H_2Ph), 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), 135.4 (quat., Ph), 136.3 (quat., Ph), 156.6 (quat., NCO_2), 156.7* (quat., NCO_2), 168.0* (quat., Gly-CO), 168.2 (quat., Gly-CO), 171.0 (quat., CO), 171.4* (quat., CO) and 171.9 (quat., CO); *m/z* (FAB+) 512.2389 (MH^+. $C_{27}H_{34}N_3O_7$ requires 512.2397).

4.16. (2*S*)-Glycyl-L-prolyl-2-amino-5-hydroxypentanoic acid (14)

Following procedure C, hydrogenation of alcohol **21** in 82:16:2 tetrahydrofuran–water–triethylamine (24.5 mL; v/v) yielded *analogue* **14** (0.27 g, 100%) as an hygroscopic pale yellow solid[†] on trituration with anhydrous diethyl ether. Compound **14** was shown to be a 76:24 *trans*:*cis* mixture of conformers: $[\alpha]_D$ −80.2 (*c* 0.29 in H_2O); δ_H (300 MHz; D_2O) 1.48–2.48 (8H, m, 4-H_2, 3-H_2, Proγ-H_2 and Proβ-H_2), 3.48–3.73 (4.24H, m, Proδ-H_2, 5-H_2 and Glyα-H_AH$_B$*), 3.79–4.00 (1.76H, m, Glyα-H$_A$$H_B$* and Glyα-$H_2$), 4.11–4.26* (0.24H, m, 2-H), 4.17 (0.76H, dd, *J* 7.7 and 5.2, 2-H) and 4.40–4.57 (1H, m, Proα-H); δ_C (75 MHz; D_2O) 24.5* (CH_2, Proγ-C), 26.7 (CH_2, Proγ-C), 30.3 (CH_2, 4-C), 30.6* (CH_2), 30.7 (CH_2, 3-C), 31.9 (CH_2, Proβ-C), 34.2* (CH_2, Proβ-C), 43.3 (CH_2, Glyα-C), 49.3 (CH_2, Proδ-C), 50.0* (CH_2, Proδ-C), 57.5 (CH, 2-C), 57.7* (CH, 2-C), 62.6* (CH, Proα-C), 63.0 (CH, Proα-C), 63.6* (CH_2, 5-C), 63.7 (CH_2, 5-C), 169.5 (quat., Gly-CO), 170.3* (quat., Gly-CO), 175.4* (quat., Pro-CON), 175.7 (quat., Pro-

[†] Due to the extremely hygroscopic nature of **14**, its melting point could not be determined under standard atmospheric conditions.

CON), 180.9* (quat., 1-CO) and 181.1 (quat., 1-CO); *m/z* (FAB+) 288.1551 (MH^+. $C_{12}H_{22}N_3O_5$ requires 288.1560).

4.17. Glycine benzyl ester *p*-toluenesulfonate[41] (24)

Following procedure A, benzylation of glycine yielded *p*-toluenesulfonate[41] **24** (11.22 g, 99%) as a crystalline white solid: mp 130–132 °C (lit.,41 132–134 °C); δ_H (400 MHz; $CDCl_3$; Me_4Si) 2.23 (3H, s, Ar–CH_3), 3.69 (2H, br d, *J* 5.6, Glyα-H_2), 4.98 (2H, s, OCH_2Ph), 6.98 (2H, d, *J* 8.0, 2 × 3-H), 7.15–7.28 (5H, m, Ph), 7.67 (2H, d, *J* 8.1, 2 × 2-H) and 8.08 (3H, br s, N^+H_3); δ_C (100 MHz; $CDCl_3$) 21.2 (CH_3, Ar–*C*H_3), 40.5 (CH_2, Glyα-C), 67.6 (CH_2, O*C*H_2Ph), 125.9 (CH, Ar), 128.2 (CH, Ar), 128.3 (CH, Ar), 128.4 (CH, Ar), 128.9 (CH, Ar), 134.7 (quat., Ph), 140.3 (quat., Ar), 141.2 (quat., Ar) and 167.4 (quat., Gly-CO); *m/z* (FAB+) 166 ($BnO_2CCH_2NH_3^+$, 100%) and 91 ($C_7H_7^+$, 53).

4.18. *N*-Benzyloxycarbonyl-glycyl-L-prolyl-glycine benzyl ester (25)

Following procedure B, coupling of acid **2** with *p*-toluenesulfonate **24** using triethylamine as the base yielded *amide* **25** (1.03 g, 94%) as a slightly opaque oil. Compound **25** was shown to be an 86:14 *trans*:*cis* mixture of conformers: R_f 0.30 (EtOAc); $[\alpha]_D$ −68.7 (*c* 0.58 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3317 br, 2952, 1743, 1722, 1650, 1534, 1453, 1256, 1188, 1051, 983, 908, 739 and 698; δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.77–2.29 (3H, m, Proβ-$H_A$$H_B$ and Proγ-H_2), 2.32–2.45 (1H, m, Proβ-$H_A$$H_B$), 3.30–3.70 (2H, m, Proδ-H_2), 3.78–4.13 (4H, m, 2 × Glyα-H_2), 4.28–4.38* (0.14H, m, Proα-H), 4.61 (0.86H, dd, *J* 8.1 and 1.7, Proα-H), 5.05–5.20 (4H, m, 2 × OCH_2Ph), 5.53* (0.14H, br s, NH), 5.67 (0.86H, br s, NH), 6.78* (0.14H, br s, NH) and 7.13–7.40 (10.86H, m, 2 × Ph and NH); δ_C (75 MHz; $CDCl_3$) 21.9* (CH_2, Proγ-C), 24.4 (CH_2, Proγ-C), 27.9 (CH_2, Proβ-C), 31.8* (CH_2, Proβ-C), 41.1 (CH_2, Glyα-*C*CO_2–), 43.0* (CH_2, Glyα-C), 43.1 (CH_2, Glyα-C), 46.0 (CH_2, Proδ-C), 46.9* (CH_2, Proδ-C), 59.8 (CH, Proα-C), 66.6 (CH_2, O*C*H_2Ph), 66.7 (CH_2, O*C*H_2Ph), 66.8* (CH_2, O*C*H_2Ph), 127.6* (CH, Ph), 127.7 (CH, Ph), 127.8 (CH, Ph), 127.9* (CH, Ph), 128.0 (CH, Ph), 128.1* (CH, Ph), 128.2 (CH, Ph), 128.3 (CH, Ph), 128.6* (CH, Ph), 128.8* (CH, Ph), 135.0* (quat., Ph), 135.1 (quat., Ph), 136.2 (quat., Ph), 156.3 (quat., NCO_2), 156.5* (quat., NCO_2), 168.2 (quat., Gly-CON), 169.4 (quat., Gly-CO_2), 171.3 (quat., Pro-CON) and 171.8* (quat., Pro-CON); *m/z* (EI+) 453.1893 (M^+. $C_{24}H_{27}N_3O_6$ requires 453.1900).

4.19. Glycyl-L-prolyl-glycine[42] (22)

Following procedure C, hydrogenation of amide **25** in methanol yielded *tripeptide*[42] **22** (0.15 g, 85%) as a white solid on trituration with diethyl ether. Compound **22** was shown to be a 76:24 *trans*:*cis* mixture of conformers: mp 175–185 °C (decomp.); $[\alpha]_D$ −89.8 (*c* 0.17 in H_2O); ν_{max} (Nujol mull)/cm^{-1} 3543, 3311 br, 2659, 1649, 1595, 1570, 1533, 1317, 1230, 1201 and 930; δ_H (300 MHz; D_2O) 1.90–2.54 (4H, m, Proβ-H_2 and Proγ-H_2), 3.57–3.77 (2H, m, Proδ-H_2), 3.78–4.16 (4H, m, 2 × Glyα-H_2) and 4.53–4.63 (1H, m, Proα-H); δ_C (75 MHz; D_2O) 23.8* (CH_2, Proγ-C), 26.0 (CH_2, Proγ-C), 31.4 (CH_2, Proβ-C), 33.6* (CH_2, Proβ-C), 42.3* (CH_2, Glyα-C), 42.4 (CH_2, Glyα-C), 45.1 (CH_2, Glyα-*C*CO_2H), 45.2* (CH_2, Glyα-*C*CO_2H), 48.7 (CH_2, Proδ-C), 49.3* (CH_2, Proδ-C), 62.0* (CH, Proα-C), 62.4 (CH, Proα-C), 168.2 (quat., Gly-CON), 168.6* (quat., Gly-CON), 175.2* (quat., Pro-CON), 175.4 (quat., Pro-CON) and 178.1 (quat., Gly-CO_2H); *m/z* (FAB+) 230.1125 (MH^+. $C_9H_{16}N_3O_4$ requires 230.1141).

4.20. Dimethyl *N*-benzyloxycarbonyl-glycyl-L-prolyl-L-glutamate (27)

Following procedure B, coupling of acid **2** with dimethyl L-glutamate hydrochloride **26** using triethylamine as the base yielded *amide* **27** (1.91 g, 97%) as a colourless oil. Compound **27** was shown to be an 89:11 *trans*:*cis* mixture of conformers: R_f 0.30 (EtOAc); $[\alpha]_D$ −62.3 (*c* 0.23 in CH_2Cl_2); ν_{max} (film)/cm^{-1} 3305 br, 2953 br, 1732, 1650, 1532, 1440, 1256, 1213, 1173, 1054, 984 and 698; δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.84–2.48 (8H, m, Proβ-H_2, Proγ-H_2, Gluβ-H_2 and Gluγ-H_2), 3.42 (1H, br dd, *J* 16.2 and 8.6, Proδ-$H_A$$H_B$), 3.51–3.82 (1H, m, Proδ-$H_A$$H_B$), 3.64 (2.67H, s, OCH_3), 3.68* (0.33H, s, OCH_3), 3.70* (0.33H, s, OCH_3), 3.74 (2.67H, s, OCH_3), 3.98 (1H, dd, *J* 17.2 and 4.1, Glyα-$H_A$$H_B$), 4.06 (1H, dd, *J* 17.3 and 4.7, Glyα-$H_A$$H_B$), 4.29–4.37* (0.11H, m, Proα-H), 4.47–4.62 (1.89H, m, Gluα-H and Proα-H), 5.10* (0.22H, s, OCH_2Ph), 5.12 (1.78H, s, OCH_2Ph), 5.58* (0.11H, br s, Gly-NH), 5.72 (0.89H, br s, Gly-NH), 7.09–7.17* (0.11H, m, Glu-NH), 7.22 (0.89H, d, *J* 7.5, Glu-NH) and 7.28–7.41 (5H, m, Ph); δ_C (75 MHz; $CDCl_3$) 22.2* (CH_2, Proγ-C), 24.6 (CH_2, Proγ-C), 26.0* (CH_2, Gluβ-C), 26.8 (CH_2, Gluβ-C), 27.8 (CH_2, Proβ-C), 29.7 (CH_2, Gluγ-C), 30.1* (CH_2, Gluγ-C), 31.8* (CH_2, Proβ-C), 43.1* (CH_2, Glyα-C), 43.3 (CH_2, Glyα-C), 46.1 (CH_2, Proδ-C), 47.0* (CH_2, Proδ-C), 51.6 (CH_3, OCH_3), 51.8* (CH_3, OCH_3), 52.2 (CH, Gluα-C), 52.3* (CH, Gluα-C), 59.9 (CH, Proα-C), 60.1* (CH, Proα-C), 66.7 (CH_2, O*C*H_2Ph), 127.8 (CH, Ph), 127.9 (CH, Ph), 128.3 (CH, Ph), 136.3 (quat., Ph), 156.2 (quat., NCO_2), 167.9 (quat., Gly-CO), 168.2* (quat., Gly-CO), 170.9 (quat., Pro-CON), 171.5* (quat., Pro-CON), 171.7* (quat., Glu-CO), 171.9 (quat., Glu-CO), 173.1 (quat., Glu-CO) and 173.3* (quat., Glu-CO); *m/z* (EI+) 463.1958 (M^+. $C_{22}H_{29}N_3O_8$ requires 463.1955).

4.21. Dimethyl glycyl-L-prolyl-L-glutamate (23)

Following procedure C, hydrogenation of amide **27** yielded *analogue* **23** (1.31 g, 97%) as an hygroscopic white foam following purification by flash column chromatography (SiO_2; 5–40% methanol–methylene chloride; gradient elution). Compound **23** was shown to be an 87:13 *trans*:*cis* mixture of conformers: R_f 0.30 (20% MeOH–CH_2Cl_2); $[\alpha]_D$ −90.7 (*c* 0.20 in H_2O); ν_{max} (film)/cm^{-1} 3583, 3199 br, 2954 br, 1738, 1660, 1535, 1454, 1436, 1353, 1259, 1207, 1173, 1125, 987 and 917; δ_H (400 MHz; D_2O) 1.77–2.12 (4H, m, Proβ-$H_A$$H_B$ Proγ-H_2 and Gluβ-$H_A$$H_B$), 2.14–2.45 (2H, m,

Gluβ-H_AH_B and Proβ-H_AH_B), 2.45–2.61 (2H, m, Gluγ-H_2), 3.50–3.64 (2.13H, m, Proδ-H_2 and Glyα-H_AH_B*), 3.70 (3H, s, OCH_3), 3.74 (3H, s, OCH_3), 3.87–4.03 (1.87H, m, Glyα-H_2 and Glyα-H_AH_B*), 4.42–4.52 (1.87H, m, Gluα-H and Proα-H) and 4.54* (0.13H, dd, *J* 8.6 and 2.6, Proα-H); (100 MHz; D_2O) 24.7* (CH_2, Proγ-C), 27.0 (CH_2, Proγ-C), 27.9* (CH_2, Gluβ-C), 28.3 (CH_2, Gluβ-C), 32.2 (CH_2, Proβ-C), 32.6 (CH_2, Gluγ-C), 32.8* (CH_2, Gluγ-C), 34.6* (CH_2, Proβ-C), 43.1* (CH_2, Glyα-C), 43.3 (CH_2, Glyα-C), 49.6 (CH_2, Proδ-C), 50.3* (CH_2, Proδ-C), 54.8 (CH, Gluα-C), 54.9* (CH, Gluα-C), 55.1 (CH_3, OCH_3), 55.7 (CH_3, OCH_3), 55.8* (CH_3, OCH_3), 62.6* (CH, Proα-C), 63.0 (CH, Proα-C), 168.7 (quat., Gly-CO), 169.3* (quat., Gly-CO), 176.0* (quat., CO), 176.2 (quat., CO), 176.5* (quat., CO), 177.0 (quat., CO), 178.2* (quat., CO) and 178.3 (quat., CO); *m/z* (EI+) 329.1584 (M^+. $C_{14}H_{23}N_3O_6$ requires 329.1587).

4.22. Dibenzyl *N*-*tert*-butyloxycarbonyl-L-glutamate[43] (30)

Triethylamine (2.25 mL, 2.20 mmol) and di-*tert*-butyl dicarbonate (2.10 g, 9.61 mmol) were successively added to a solution of L-glutamic acid dibenzyl ester *p*-toluenesulfonate **29** (4.00 g, 8.01 mmol) in methylene chloride (80 mL) at 0 °C. The reaction mixture was warmed to room temperature, stirred for 19 h then washed with 2 M aqueous hydrochloric acid, saturated aqueous sodium hydrogen carbonate, dried ($MgSO_4$), filtered and the solvent removed in vacuo. The resultant residue was purified by crystallisation from ether–hexane to give *carbamate*[43] **30** (3.32 g, 97%) as a white solid: mp 73–76 °C; δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.46 [9H, s, $C(CH_3)_3$], 1.96–2.07 (1H, m), 2.22–2.27 (1H, m), 2.38–2.52 (2H, m), 4.40 (1H, br d, *J* 3.6, Gluα-H), 5.12–5.19 (5H, m, $2\times OCH_2Ph$ and Gluα-NH) and 7.29–7.39 (10H, m, $2\times Ph$); δ_C (100 MHz; $CDCl_3$) 27.6 (CH_2, Gluβ-C), 28.1 [CH_3, $C(CH_3)_3$], 30.1 (CH_2, Gluγ-C), 52.8 (CH, Gluα-C), 66.4 (CH_2, OCH_2Ph), 67.1 (CH_2, OCH_2Ph), 79.7 [quat., $C(CH_3)_3$], 128.1 (CH, Ph), 128.2 (CH, Ph), 128.3 (CH, Ph), 128.5 (CH, Ph), 128.5 (CH, Ph), 135.2 (quat., Ph), 135.7 (quat., Ph), 155.3 (quat., NCO_2), 172.0 (quat., CO) and 172.4 (quat., CO).

4.23. (γ*S*)-Dibenzyl *N*-*tert*-butyloxycarbonyl-L-γ-allylglutamate (31)

A solution of lithium hexamethyldisilazide (1 mol L^{-1}, 10.30 mL, 10.30 mmol) in tetrahydrofuran was added dropwise to a solution of carbamate **30** (2.00 g, 4.68 mmol) in anhydrous tetrahydrofuran (20 mL) at −78 °C under an atmosphere of nitrogen. The resultant yellow solution was stirred for 40 min, allyl bromide (1.31 mL, 14.0 mmol) was added dropwise and stirring continued for a further 1.5 h at −78 °C. Saturated aqueous ammonium chloride (20 mL) was then added and the reaction mixture warmed to room temperature over 1.5 h. The white precipitate was filtered, washed with ethyl acetate and the filtrate concentrated to dryness in vacuo. The ensuing residue was purified by flash column chromatography (SiO_2; 6:1 to 5:1 hexane–ethyl acetate) to afford *alkene* **31** (1.59 g, 73%) as a colourless oil: $[\alpha]_D$ +19.4 (*c* 0.23 in CH_2Cl_2); δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.43 [9H, s, $C(CH_3)_3$], 2.06 (2H, m, allyl-CH_2), 2.30–2.44 (2H, m, Gluβ-H_2), 2.64 (1H, quintet, *J* 6.7, Gluγ-H), 4.41 (1H, br d, *J* 7.6, Gluα-H), 5.01–5.71 (7H, m, $2\times OCH_2Ph$, $=CH_2$, Gluα-NH), 5.60–5.74 (1H, m, $CH{=}CH_2$) and 7.31–7.35 (10H, m, $2\times Ph$); δ_C (75 MHz; $CDCl_3$) 28.2 [CH_3, $C(CH_3)_3$], 33.4 (CH_2, allyl-CH_2), 36.2 (CH_2, Gluβ-C), 41.7, (CH, Gluγ-C), 52.2 (CH, Gluα-C), 66.4 (CH_2, OCH_2Ph), 67.0 (CH_2, OCH_2Ph), 80.0 [quat., $C(CH_3)_3$], 117.6 (CH_2, $=CH_2$), 128.2 (CH, Ph), 128.2 (CH, Ph), 128.3 (CH, Ph), 128.4 (CH, Ph), 128.5 (CH, Ph), 134.1 (CH, $CH{=}CH_2$), 135.2 (quat., Ph), 135.7 (quat., Ph), 155.3 (quat., NCO_2), 172.1 (quat., Gluα-CO) and 174.6 (quat., Gluγ-CO); *m/z* (FAB+) 468.2382 (MH^+. $C_{27}H_{34}NO_6$ requires 468.2386).

4.24. (γ*S*)-Dibenzyl *N*-benzyloxycarbonylglycyl-L-prolyl-L-γ-allylglutamate (33)

Trifluoroacetic acid (3 mL) was added to a solution of alkene **31** (0.48 g, 1.07 mmol) in methylene chloride (15 mL) at 0 °C and the reaction mixture stirred for 2 h then the volatiles were removed in vacuo to yield tentatively *trifluoroacetate* **32** as a cream solid. To a solution of acid **2** (0.30 g, 0.94 mmol) in methylene chloride (20 mL), cooled to 0 °C under nitrogen, were successively added dropwise triethylamine (0.15 mL, 1.07 mmol) and ethyl chloroformate (0.10 mL, 1.07 mmol) and the solution stirred at 0 °C for 45 min to form the mixed anhydride. Triethylamine (0.15 mL, 1.07 mmol) was added to the crude trifluoroacetate **32** in methylene chloride (15 mL), under nitrogen at 0 °C, and the resultant solution added dropwise to the above mixed anhydride. The reaction mixture was warmed to room temperature, stirred for 17 h then washed with 10% aqueous hydrochloric acid and saturated aqueous sodium hydrogen carbonate, dried ($MgSO_4$), filtered and evaporated to dryness in vacuo. Purification of the resultant residue by flash column chromatography (SiO_2; 50–80% ethyl acetate–hexane; gradient elution) yielded *amide* **33** (0.54 g, 87%) as a colourless oil, which crystallised at 0 °C to a white solid. Compound **33** was shown to be an 88:12 *trans*:*cis* mixture of conformers: mp 42–45 °C; $[\alpha]_D$ −29.7 (*c* 0.28 in CH_2Cl_2); δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.70–1.91 (2H, m, Proβ-H_AH_B and Proγ-H_AH_B), 1.96–2.14 (4H, m, Proβ-H_AH_B, Proγ-H_AH_B and Glu-H_2), 2.30 (2H, t, *J* 6.6, allyl-CH_2), 2.64 (1H, quintet, *J* 6.7, Gluγ-H), 3.24–3.60 (2H, m, Proδ-H_2), 3.76–3.98 (2H, m, Glyα-H_2), 4.33* (0.12H, br s, Proα-H), 4.49 (0.88H, d, *J* 5.7, Proα-H), 4.60 (1H, q, *J* 7.0, Gluα-H), 4.94–5.68 (8H, m, $3\times OCH_2Ph$ and $=CH_2$), 5.55–5.68 (1H, m, $CH{=}CH_2$), 5.94 (1H, br s, Gly-NH), 7.29 (15H, s, $3\times Ph$) and 7.38 (1H, d, *J* 7.8, Glu-NH); δ_C (75 MHz; $CDCl_3$) 22.3* (CH_2, Proγ-C), 24.8 (CH_2, Proγ-C), 28.0 (CH_2, Proβ-C), 31.9* (CH_2, Proβ-C), 32.8 (CH_2, Gluβ-C), 35.9 (CH_2, allyl-CH_2), 41.4 (CH, Gluγ-C), 41.9* (CH, Gluγ-C), 43.4 (CH_2, Glyα-C), 46.2 (CH_2, Proδ-C), 47.1* (CH_2, Proδ-C), 50.8 (CH, Gluα-C), 51.2* (CH, Gluα-C), 60.0 (CH, Proα-C), 60.2* (CH, Proα-C), 66.4 (CH_2, OCH_2Ph), 66.5* (CH_2, OCH_2Ph), 66.8 (CH_2, OCH_2Ph), 67.1 (CH_2, OCH_2Ph), 67.3* (CH_2, OCH_2Ph), 67.4 (CH_2, OCH_2Ph),

67.4 (CH_2, OCH_2Ph), 117.6 (CH_2, $=CH_2$), 117.9* (CH_2, $=CH_2$), 128.0 (CH, Ph), 128.1 (CH, Ph), 128.2 (CH, Ph), 128.2 (CH, Ph), 128.4 (CH, Ph), 128.5 (CH, Ph), 128.5 (CH, Ph), 128.6 (CH, Ph), 134.2* (CH, $CH=CH_2$), 134.4 (CH, $CH=CH_2$), 135.2* (quat., Ph), 135.4 (quat., Ph), 135.7* (quat., Ph), 135.9 (quat., Ph), 136.6 (quat., Ph), 156.4 (quat., NCO_2), 156.6* (quat., NCO_2), 168.1 (quat., Gly-CO), 168.5* (quat., Gly-CO), 171.3 (quat., Pro-CON), 171.5* (quat., Pro-CON), 171.6 (quat., Gluα-CO), 171.7* (quat., Gluα-CO), 174.4* (quat., Gluγ-CO) and 174.5 (quat., Gluγ-CO); *m/z* (FAB+) 656.2964 (MH^+. $C_{37}H_{42}N_3O_8$ requires 656.2972).

4.25. (γ*S*)-Glycyl-L-prolyl-L-γ-propylglutamic acid (28)

Following procedure C, hydrogenation of amide **33** yielded *analogue* **28** (0.084 g, 77%) as an off white solid when freeze-dried from toluene–methanol. Compound **28** was shown to be an 82:18 *trans*:*cis* mixture of conformers: mp 160–165 °C; $[\alpha]_D$ −76.6 (*c* 0.06 in H_2O); δ_H (400 MHz; D_2O) 0.91 (3H, t, *J* 7.0, Gluγ-$CH_2CH_2CH_3$), 1.29–1.38 (2H, m, Gluγ-$CH_2CH_2CH_3$), 1.58 (2H, d, *J* 6.4, Gluγ-$CH_2CH_2CH_3$), 1.91–2.51 (7H, m, Gluβ-H_2, Gluγ-H, Proβ-H_2 and Proγ-H_2), 3.55–3.68 (2H, m, Proδ-H_2), 3.78* (0.18H, d, *J* 16.0, Glyα-H_AH_B), 3.97–4.08 (1.82H, m, Glyα-H_2 and Glyα-H_AH_B*), 4.31 (1H, br s, Gluα-H) and 4.53 (1H, d, *J* 5.5, Proα-H); δ_C (100 MHz; D_2O) 13.1 (CH_3, Gluγ-$CH_2CH_2CH_3$), 19.6 (CH_2, Gluγ-$CH_2CH_2CH_3$), 21.9* (CH_2, Proγ-C), 24.0 (CH_2, Proγ-C), 29.2 (CH_2, Proβ-C), 31.6* (CH_2, Proβ-C), 33.5 ($2\times CH_2$, Gluβ-C and Gluγ-$CH_2CH_2CH_3$), 33.9* (CH_2), 40.1* (CH_2, Glyα-C), 40.3 (CH_2, Glyα-C), 42.5 (CH, Gluγ-C), 43.6* (CH, Gluγ-C), 46.8 (CH_2, Proδ-C), 47.4* (CH_2, Proδ-C), 52.8 (CH, Gluα-C), 53.5* (CH, Gluα-C), 60.0* (CH, Proα-C), 60.4 (CH, Proα-C), 165.5 (quat., Gly-CO), 166.0* (quat., Gly-CO), 173.0* (quat., Pro-CON), 173.4 (quat., Pro-CON), 176.9 (quat., Gluα-CO) and 181.0 (quat., Gluγ-CO); *m/z* (FAB+) 344.1833 (MH^+. $C_{15}H_{26}N_3O_6$ requires 344.1822).

Acknowledgements

The authors thank Neuren Pharmaceuticals Ltd for financial support.

References and notes

1. Trotter, N. S.; Brimble, M. A.; Harris, P. W. R.; Callis, D. J.; Sieg, F. *Bioorg. Med. Chem.*, preceding paper, see doi:10.1016/j.bmc.2004.10.005.
2. Sara, V. R.; Carlsson-Skwirut, C. *Prog. Brain Res.* **1988**, *73*, 87.
3. De Pablo, F.; De La Rosa, E. J. *Trends Neurosci.* **1995**, *18*, 143.
4. Baskin, D. G.; Wilcox, B. J.; Figlewicz, D. P.; Dorsa, D. M. *Trends Neurosci.* **1988**, *11*, 107.
5. Sara, V. R.; Carlsson-Sdwirut, C.; Bergman, T.; Jornvall, H.; Roberts, P. J.; Crawford, M.; Hakansson, L. N.; Civalero, I.; Nordberg, A. *Biochem. Biophys. Res. Commun.* **1989**, *165*, 766.
6. Sara, V. R.; Carlsson-Skwirut, C.; Drakenberg, K.; Giacobini, M. B.; Hakansson, L.; Mirmiran, M.; Nordberg, A.; Olson, L.; Reinecke, M.; Stahlbom, P. A.; Sandberg Nordqvist, A. C. *Ann. N.Y. Acad. Sci.* **1993**, *692*, 183.
7. Yamamoto, H.; Murphy, L. J. *Endocrinology* **1994**, *135*, 2432.
8. Yamamoto, H.; Murphy, L. J. *J. Endocrinol.* **1995**, *146*, 141.
9. Sara, V. R.; Carlsson-Skwirut, C.; Andersson, C.; Hall, E.; Sjogren, B.; Holmgren, A.; Jornvall, H. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4904.
10. Carlsson-Skwirut, C.; Jornvall, H.; Holmgren, A.; Andersson, C.; Bergman, T.; Lindquist, G.; Sjogren, B.; Sara, V. R. *FEBS Lett.* **1986**, *201*, 46.
11. Bourguignon, J. P.; Gerard, A. *Brain Res.* **1999**, *847*, 247.
12. Gluckman, P. D.; Klempt, N. D.; Guan, J.; Mallard, C. E.; Sirimanne, E. S.; Dragunow, M.; Klempt, M.; Singh, K.; Williams, C. E.; Nijolics, K. *Biochem. Biophys. Res. Commun.* **1992**, *182*, 593.
13. Lee, W.-H.; Bondy, C. *Ann. N.Y. Acad. Sci. 28* **1993**, *679*, 418.
14. Galli, C.; Meucci, O.; Scorziello, A.; Werge, T. M.; Calissano, P.; Schettini, G. *J. Neurosci.* **1995**, *15*, 1172.
15. Mason, J. L.; Ye, P.; Suzuki, K.; D'Ercole, A. J.; Matsushima, G. K. *J. Neurosci.* **2000**, *20*, 5703.
16. Pons, S.; Rejas, M. T.; Torres-Aleman, I. *Dev. Brain Res.* **1991**, *62*, 169.
17. Walter, H. J.; Berry, M.; Hill, D. J.; Logan, A. *Endocrinology* **1997**, *138*, 3024.
18. Nilsson-Hakansson, L.; Civalero, I.; Zhang, X.; Carlsson-Skwirut, C.; Sara, V. R.; Nordberg, A. *Neuroreport* **1993**, *4*, 1111.
19. Ikeda, T.; Waldbillig, R. J.; Puro, D. J. *Brain Res.* **1995**, *686*, 87.
20. Sizonenko, S. V.; Sirimanne, E. S.; Williams, C. E.; Gluckman, P. D. *Brain Res.* **2001**, *922*, 42.
21. Saura, J.; Curatolo, L.; Williams, C. E.; Gatti, S.; Benatti, L.; Peters, C.; Guan, J.; Dragunow, M.; Post, C.; Faull, R. L. M.; Gluckman, P. D.; Skinner, S. J. M. *Neuroreport* **1999**, *10*, 161.
22. Guan, J.; Waldvogel, H. J.; Faull, R. L. M.; Gluckman, P. D.; Williams, C. E. *Neuroscience* **1999**, *89*, 649.
23. Guan, J.; Krishnamurthi, R.; Waldvogel, H. J.; Faull, R. L. M.; Clark, R.; Gluckman, P. *Brain Res.* **2000**, *859*, 286.
24. Bourguignon, J. P.; Alvarez Gonzalez, M. L.; Gerard, A.; Franchimont, P. *Endocrinology* **1994**, *134*, 1589.
25. Bourguignon, J. P. U.S. Patent 5,804,550, 1998.
26. Knopfel, T.; Kuhn, R.; Allgeier, H. *J. Med. Chem.* **1995**, *38*, 1417.
27. Riedel, G. *Trends Neurosci.* **1996**, *19*, 219.
28. Nicoletti, F.; Bruno, V.; Copani, A.; Casabona, G.; Knopfel, T. *Trends Neurosci.* **1996**, *19*, 267.
29. Abood, N. A.; Brimble, M. A. PCT Int. Appl. 0294856, 2002.
30. Gluckman, P.; Alexi, T. PCT Int. Appl. 0216408, 2002.
31. Gluckman, P. D.; Williams, C. E.; Guan, J.; Krishnamurthi, R. V. M. U.S. Pat. Appl. Publ. 20020035066, 2002.
32. Gluckman, P. D.; Sirimanne, E. S.; Krissansen, G. W.; Kanwar, J. R. U.S. Pat. Appl. Publ. 2003027760, 2003.
33. Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1964**, *86*, 1839.
34. Savrda, J. *J. Org. Chem.* **1977**, *42*, 3199.
35. El-Abadelah, M. M.; Hussein, A. Q.; Thaher, B. A. *Heterocycles* **1991**, *32*, 1879.
36. Williamson, D. A.; Bowler, B. E. *J. Am. Chem. Soc.* **1998**, *120*, 10902.

37. Lenman, M. M.; Lewis, A.; Gani, D. *J. Chem. Soc., Perkin Trans. 1* **1997**, 2297.
38. Hanessian, S.; Margarita, R. *Tetrahedron Lett.* **1998**, *39*, 5887–5890.
39. Perrin, D. D.; Armarego, W. L. F.; Perrin, D. R. *Purification of Laboratory Chemicals*; Pergamon: Oxford, 1980.
40. Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 512.
41. Servas, L.; Winitz, M.; Greenstein, J. P. *J. Org. Chem.* **1957**, *22*, 1515.
42. Commercially available from Indofine Chemical Company, and Bachem: Registry Number 2441-63-6.
43. Chen, S. T.; Chen, S. Y.; Wang, K. T. *J. Org. Chem.* **1992**, *57*, 6960.