Synthesis of macrocyclic analogues of the neuroprotective agent glycyl-L-prolyl-L-glutamic acid (GPE)†

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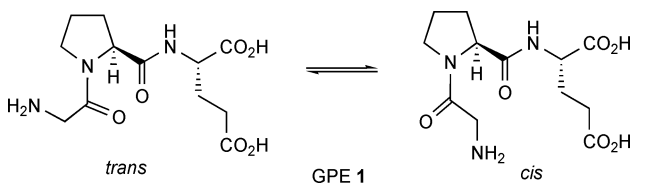
The syntheses of seven macrocyclic analogues of the neuroprotective tripeptide glycyl-L-prolyl-L-glutamic acid (GPE) **1** are described. Macrocycles **6** and **7** mimic the *cis* conformer of GPE whereas macrocycles **2–5**, **8**, and **9** mimic the *trans* conformer of GPE. The macrocyclic peptides of well-defined geometry were prepared *via* Grubbs ring closing metathesis of an appropriate diene precursor. In turn each of the diene precursors were prepared from the readily available allyl-substituted amino acid building blocks **12**, **13**, **14**, **27**, **36** and **51**.

Introduction

Protease-mediated metabolism of IGF-1 (insulin-like growth factor type-1) is reported to lead to the formation of the endogenous *N*-terminal tripeptide Gly-Pro-Glu (GPE **1**) along with the 67 amino acid des(1–3)IGF-1 fragment.[1–3] Although GPE does not bind to IGF-1 receptors and its mode of action is unclear, *in vitro* studies have demonstrated its ability to stimulate acetylcholine and dopamine release,[4,5] and to protect different types of neurons from diverse induced injuries (*e.g.* hypoxia-ischemia and glutamate).[6–8] More importantly, GPE shows neuroprotective properties in different animal models of neurodegenerative diseases such as Huntington's, Parkinson's and Alzheimer's diseases.[5,6,9] Preliminary observations suggested that GPE possibly exhibited its neuromodulatory role in the CNS through interaction with one or more glutamate receptors and it has been demonstrated[1] that it binds to the *N*-methyl-D-aspartate (NMDA) receptor but not to the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) or kainate receptors.



The structural simplicity of GPE renders it a suitable lead molecule for the development of novel strategies for the development of effective neuroprotective drugs based on non-peptide analogues. GPE peptidomimetics could also be used as pharmacological probes to investigate the mechanism of action of this tripeptide. With these aims in mind, we[10–13] and others[14,15] have embarked on a synthetic program focused on the systematic modification of the individual amino acid residues, Gly, Pro and Glu. These studies demonstrated that the neuroprotective activity of most of the GPE analogues was of lower potency than that of the endogenous tripeptide. Moreover, it was concluded that the prevention of neuronal death by these GPE analogues after NMDA injury is not directly linked to their affinity for glutamate receptors.

The use of cyclic peptides provides an elegant solution for the synthesis of peptides of restricted geometry that can be used to probe the bioactive conformation of a given peptide.[16]

The unique properties that proline[17] imparts on the overall structure of proteins indicate that proline-containing sequences act as molecular hinges, swivels, and switches and play a pivotal role in biological signaling processes.[18] The fact that the Xaa–Pro amide bond exists as a mixture of *cis* and *trans* isomers,[19] whereas most peptide bonds adopt the *trans* form, also suggests that catalysis of *cis*/*trans*-prolyl isomerization by rotamase enzymes[20] is critical for the protein folding process. Given that the conformation of the proline residue of the GPE molecule play a pivotal role in the observed biological activity we decided to synthesize a small library of GPE analogues in which the conformation of the proline ring was restricted by its incorporation into a macrocyclic structure. The synthesis of these macrocyclic analogues allows the relationship between *cis–trans* conformers and peptide bioactivity[21,22] to be probed.

Results and discussion

The ring closing metathesis reaction (RCM) is a powerful tool[23] for the synthesis of macrocyclic systems and has proven a popular method for the synthesis of cyclic peptides from an acyclic precursor.[24–32] We therefore decided to prepare a series of macrocycles **2–7** (Fig. 1) in which a short alkyl chain attached to the nitrogen atom or the alpha carbon of the glycine residue is linked to the C-5 and C-2 positions of the proline residue. Additionally, two macrocycles **8** and **9** containing a short alkyl chain linking the glycine unit (through the nitrogen atom or the alpha carbon) to the glutamate side chain were also prepared.[33]

Initially, our attention focused on the synthesis of macrocycles **2**, **3**, **4** and **5** that mimic the *trans* conformation of GPE **1**. Macrocycles **2** and **3** were prepared *via* ring closing metathesis of dienes **10** and **11** that are, in turn, derived from the union

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† Electronic supplementary information (ESI) available: general experimental details together with full experimental procedures, ^{1}H NMR, ^{13}C NMR and mass spectral data for compounds **10**, **11**, **13**, **16**, **23**, **28**, **29**, **37**, **50**, **52**, **54**, **55** and **56**. See DOI: 10.1039/b605293b

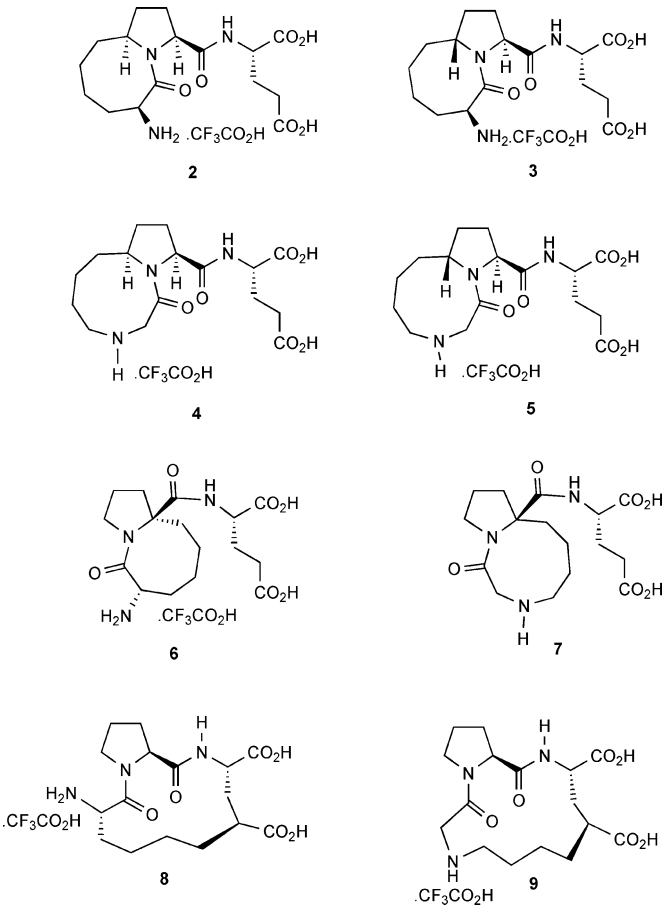


Fig. 1

of *cis*-5-allylproline **12a** or *trans*-5-allylproline **13** with Boc-(*S*)-allylglycine **14** respectively (Schemes 1 and 2). The preparation of the *trans* mimic of GPE with *cis* stereochemistry at C-2 and C-5 of the proline ring, namely macrocycle **2**, commenced with the union of *cis*-5-allylproline **12a** with Boc-(*S*)-allylglycine **14**. Pyroglutamic acid **15** was converted to a Boc protected ethyl ester derivative **16**.[34] Selective reduction of the lactam carbonyl group with lithium triethylborohydride yielded a mixture of aminols that underwent $BF_3{\cdot}Et_2O$ mediated allylation with allyltributylstannane to afford an inseparable 66 : 33 mixture of *cis*-5-allylproline **17a** : *trans*-5-allylproline **17b**.[35] Liberation of the free amines **12** upon removal of the Boc group followed by direct coupling with Boc-(*S*)-allylglycine **14**[36,37] using DCC afforded an inseparable 77 : 23 mixture of *cis*-diene **10a** : *trans*-diene **10b**. Subjection of this mixture of dienes to ring closing metathesis using Grubbs' first generation catalyst with heating in dichloromethane for 24 h followed by stirring overnight in dimethyl sulfoxide[38] resulted in exclusive formation of olefin **18** with *cis* stereochemistry between C-2 and C-5 of the proline ring due to the much slower rate of *trans*-diene **10b** to undergo the ring closing metathesis reaction. Hydrolysis of the ethyl ester afforded acid **19**, which was immediately reacted with di-*tert*-butyl (*S*)-glutamate **20** using BoP–Cl to afford olefin **21** in 70% yield over two steps. Finally hydrogenation of the olefin over PtO_2 in THF followed by immediate deprotection of the Boc group and *tert*-butyl esters afforded the desired macrocycle **2** with *cis* stereochemistry between C-2 and C-5 on the proline ring but with *trans* stereochemistry about the glycine C(O)–NPro bond.

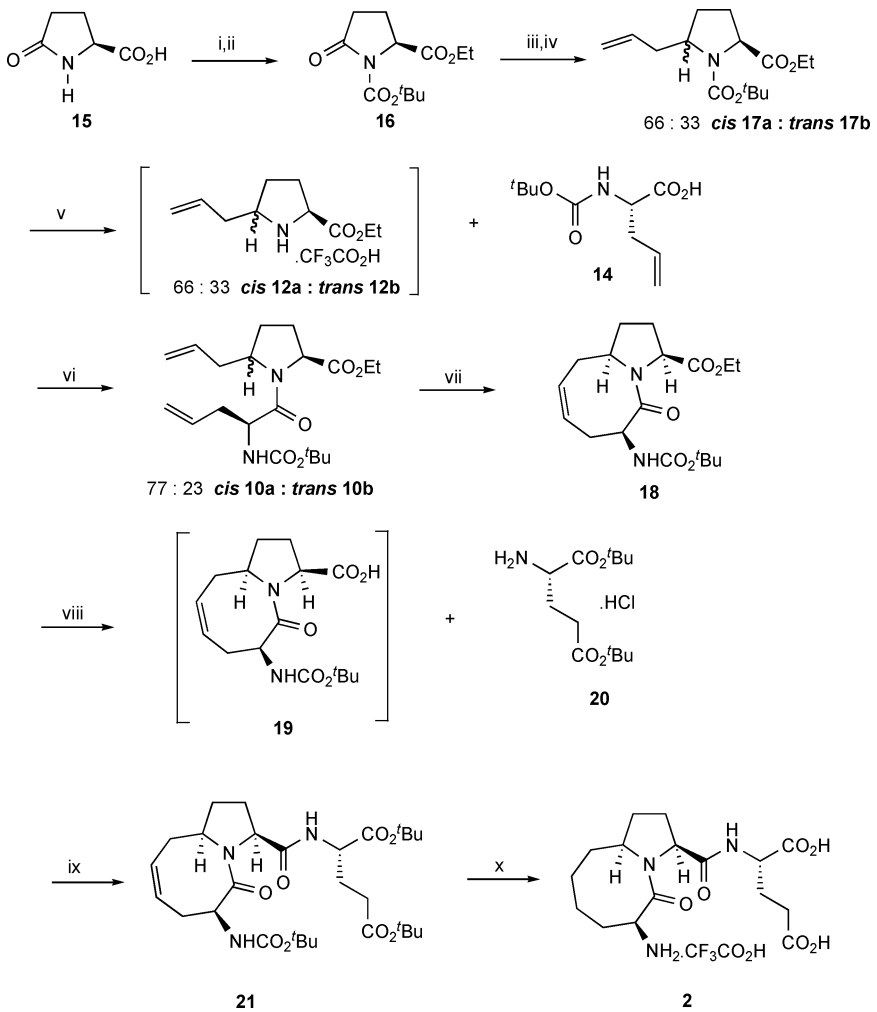
The synthesis of the analogous macrocycle **3** that also exhibits *trans* stereochemistry about the glycine C(O)–NPro bond but with *trans* stereochemistry between C-2 and C-5 on the proline ring, followed a similar strategy to macrocycle **2** (Scheme 2). In this case, allylation[39] of the mixture of aminols formed upon reduction of the lactam carbonyl group of *tert*-butyl ester **22**[40] afforded a 57 : 43 mixture of *cis*-5-allylproline **23a** : *trans*-5-allylproline **23b** that, upon deprotection of the Boc group, afforded separable amines **13**. The *trans* amine **13b** was subjected to DCC coupling with Boc-(*S*)-allylglycine **14** to afford diene **11** that underwent sluggish ring closing metathesis, despite using a high catalyst loading, affording the cyclic olefin **24** in low yield. Hydrolysis of the *tert*-butyl ester afforded acid **25** that was immediately coupled with di-*tert*-butyl (*S*)-glutamate (HCl salt) **20** using BoP–Cl to afford olefin **36** in 60% yield over two steps. Finally hydrogenation and global deprotection afforded the desired macrocycle **3** albeit in low yield.

Our attention next focused on the synthesis of macrocycles **4** and **5** in which an alkyl chain linked the glycine nitrogen to the C-5 on the proline ring. These two macrocycles also mimic the *trans* conformation of GPE **1** and were both accessible *via* ring closing metathesis of dienes **28** and **29** followed by appendage of a glutamate fragment. Dienes **28** and **29** in turn were available from the union of 5-allylproline **13** with *N*-allylglycine **27** (Schemes 3, 4 and 5).

Benzyloxycarbamate protected *N*-allylglycine **27** was prepared *via* slight modification of the literature preparation[41] and coupled with a mixture of diastereomeric 5-allylprolines **13** using EDCI to afford the separable dienes **28** and **29** in moderate yield (60%) (Scheme 3). The *cis*/*trans* ratio of the newly created amide (Pro) bond was 1 : 1 in both of these flexible acyclic dipeptides. Subsequent ring closing metathesis of *cis*-diene **28** afforded cyclic olefin **30** (Scheme 4). Hydrogenation of the olefin effected concomitant removal of the benzyloxycarbamate group hence the cyclic amine was reprotected as a Boc carbamate **31** after hydrolysis of the *tert*-butyl ester. Coupling acid **31** with di-*tert*-butyl (*S*)-glutamate (HCl salt) **20** using BoP–Cl afforded amide **32** that afforded the desired macrocycle **4** upon removal of the *tert*-butyl esters and the *tert*-butyl carbamate.

In a similar fashion, ring closing metathesis of *trans*-diene **29** afforded cyclic olefin **33** that was readily converted to macrocycle **5** *via* the intermediacy of acid **34** and amide **35** (Scheme 5). The ring closure was not affected by the nature of the stereochemistry of the allyl group at C-5 on the proline ring, with ring closure of dienes **28** and **29** giving similar yields of the respective cyclic olefins **30** and **33** with comparable reaction times and catalyst loadings. Very little deallylation/isomerization was observed in the ring closing metathesis reaction and both cyclic olefins **30** and **33** only adopted the *trans* conformation about the amide bond thus affording macrocycles **4** and **5** exhibiting only the *trans* conformation about the glycine C(O)–NPro bond.

Macrocycles **6** and **7**, in which a side chain linked C-2 of the proline unit to the glycine nitrogen or the α-carbon, were identified as possible mimics for the *cis* (Pro) conformation of GPE **1**. (*S*)-2-Allylproline methyl ester **36** was prepared using Seebach's procedure,[42,43] however subsequent coupling with Boc-(*S*)-allylglycine **14** using DCC with HOBt as additive only afforded diene **37** in low yield (Scheme 6). Ring closing metathesis of



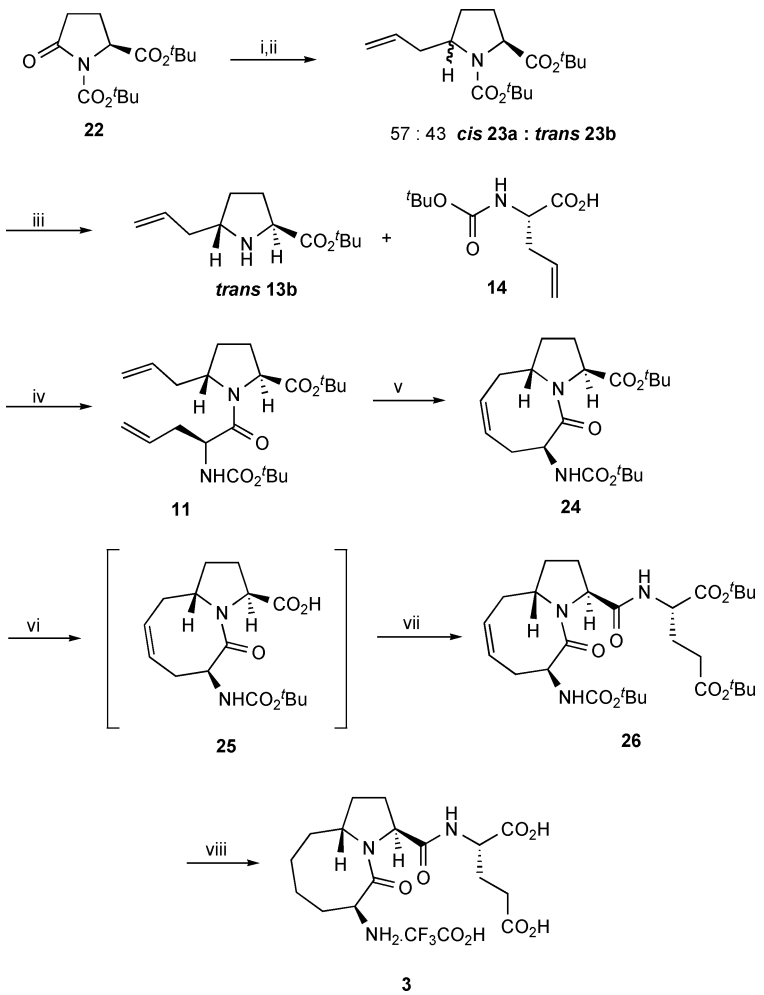
Scheme 1 *Reagents*, *conditions and yields*: (i) EtOH, $SOCl_2$, 0 °C to room temp, overnight; (ii) DMAP, Boc_2O, CH_3CN, room temp, 18 h, 85% over 2 steps; (iii) $LiEt_3BH$, THF, −78 °C, 1 h, then 30% H_2O_2, 0 °C, 0.5 h; (iv) $BF_3{\cdot}OEt_2$, allyltributylstannane, CH_2Cl_2, −78 °C, 2 h, 54% over 2 steps; (v) CF_3CO_2H, CH_2Cl_2, room temp, 4 h; (vi) DCC, Et_3N, CH_2Cl_2, 0 °C to room temp, overnight, 55% over 2 steps; (vii) $Cl_2(PCy_3)_2Ru{=}CHPh$, CH_2Cl_2, 45 °C, 24 h then DMSO, room temp, overnight, 74%; (viii) 1 M aq. NaOH, H_2O, dioxane, room temp, 23 h; (ix) BoP–Cl, Et_3N, CH_2Cl_2, 0 °C to room temp, 21 h, 70% over 2 steps; (x) PtO_2, H_2, THF, room temp, overnight, then CF_3CO_2H, CH_2Cl_2, room temp, 5 h, 60% over 2 steps.

diene **37** required the use of Grubbs' second generation catalyst[44] affording bicyclic cyclooctene **38**[45] as a single *cis* (Pro) conformer. Hydrolysis of the methyl ester and direct coupling with di-*tert*-butyl (*S*)-glutamate (HCl salt) **20** using BoP–Cl afforded olefin **39**. Finally, hydrogenation over PtO_2 followed by removal of the Boc and *tert*-butyl ester groups using trifluoroacetic acid afforded the desired macrocycle **6**.

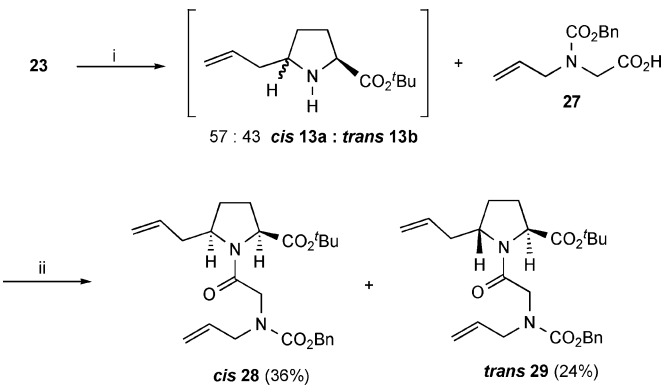
Our attention next focused on the synthesis of macrocycle **7** that contains a side chain linking C-2 of the proline unit to the glycine nitrogen. Attempts to effect ring closing metathesis of the dipeptides **43–47**, formed from the union of (*S*)-2-allylproline **36** with *N*-allylglycine derivatives **14**, **27** or **40–42**, using Grubbs' first and second generation catalysts were unsuccessful (Scheme 7). Whilst the presence of the electron-withdrawing Boc, CO_2Bn, Ac or Fmoc groups may have accelerated competing deallylation (or isomerization in the case of **42**), use of *N*,*N*′-diallylglycine **40** in an effort to overcome this problem only afforded recovered starting material possibly due to quenching of the catalyst by the more basic nitrogen atom.[46] Conversion of diallylamine **40** into its hydrochloride salt followed by ring closing metathesis was also unsuccessful.[47] The synthesis of macrocycle **7** thus required an alternative strategy.

The ring-closing metathesis of dienes contained within the GPE **1** scaffold was next examined by focusing on the preparation of macrocycles **8** and **9** in which an alkyl chain links the glutamate residue to the glycine α-carbon or nitrogen respectively. *C*-allylglycyl dipeptide **50** was prepared *via* the union of proline methyl ester **48** with Cbz-(*S*)-allylglycine **49**[48] followed by hydrolysis (Scheme 8). Subsequent union with γ-allylglutamate **51** (prepared from dibenzyl (*S*)-glutamate following a similar procedure to that reported[49] using dimethyl glutamate) afforded diene **52** that underwent ring closing metathesis to afford a mixture of isomeric cyclic olefins **53**. Hydrogenation of the olefin **53** effected global deprotection of the benzyloxycarbamate and the benzyl esters affording the desired macrocycle **8** in 58% yield as a single *trans* conformer over two steps.

In a similar fashion, *N*-allylglycyl dipeptide **55** was prepared *via* the union of proline methyl ester **48** with Cbz-*N*-allylglycine



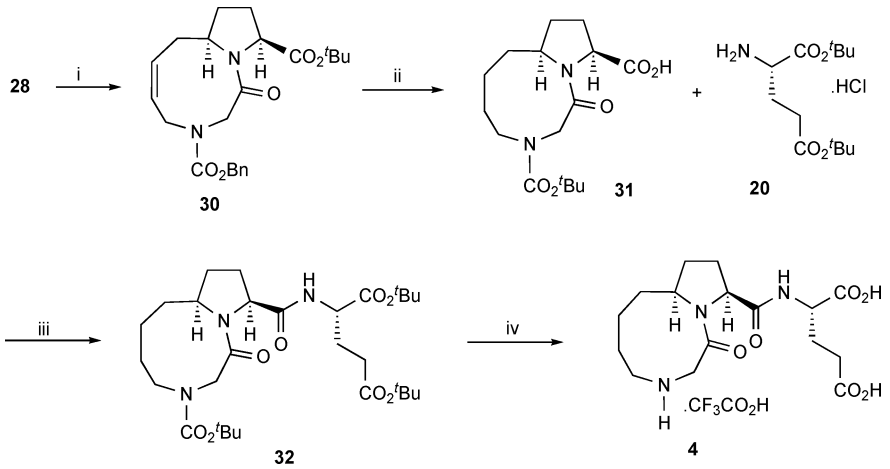
Scheme 2 *Reagents*, *conditions and yields*: (i) $LiEt_3BH$, THF, −78 °C, 1 h, then 30% H_2O_2, 0 °C, 0.5 h; (ii) allyltributylstannane, Me_3SiOTf, −78 °C, 2 h, 70% over 2 steps; (iii) 4 M HCl, dioxane, 0 °C, 1 h then room temp, 40 min, 72%; (iv) DCC, CH_2Cl_2, 0 °C to room temp, 18 h, 81%; (v) $Cl_2(PCy_3)_2Ru{=}CHPh$, CH_2Cl_2, 45 °C, 4 days, then DMSO, room temp, overnight (39%); (vi) CF_3CO_2H, CH_2Cl_2, room temp, 5 h then $NaHCO_3$, H_2O, dioxane, Boc_2O, room temp, 21 h; (vii) **20**, BoP–Cl, Et_3N, CH_2Cl_2, 0 °C to room temp, 19 h, 60% over 3 steps; (viii) PtO_2, EtOAc, H_2, CF_3CO_2H, room temp, 6 h then PtO_2, THF–MeOH, H_2, room temp, overnight, then CH_2Cl_2, CF_3CO_2H, room temp, 4 h (12%).



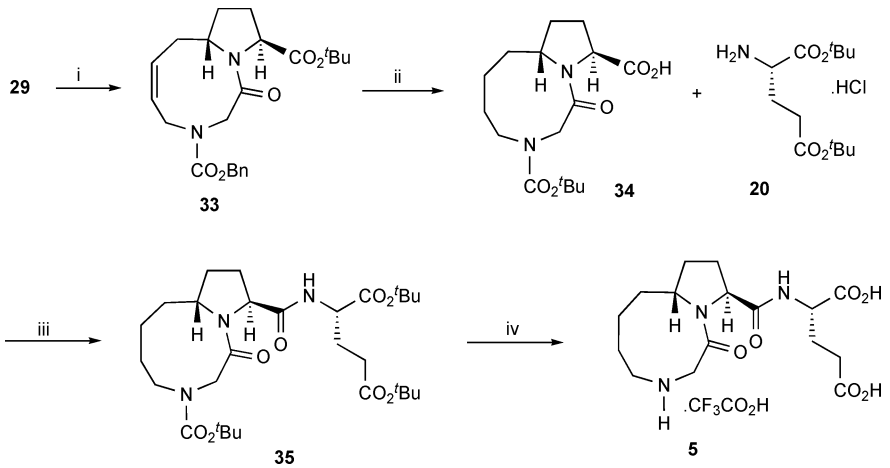
Scheme 3 *Reagents*, *conditions and yields*: (i) 4 M HCl, dioxane, 0 °C, 1 h then room temp, 40 min; (ii) **27**, EDCI, CH_2Cl_2, Et_3N, 0 °C to room temp, 16 h, 60% over 2 steps.

27 followed by hydrolysis of the methyl ester **54** (Scheme 9). Subsequent union with γ-allylglutamate **51** afforded *N*-allylglycyl diene **56**. Exposure of diene **56** to second generation Grubbs' catalyst followed by hydrogenation of the resultant cyclic olefin **57** afforded macrocycle **9** in 58% yield after purification by HPLC together with the heterolysis product **58**. Cyclotetradecene **9** existed as a 65 : 35 mixture of *trans* : *cis* (Pro) conformers. The increased proportion of the *cis* conformer may reflect the increased flexibility of the Pro amide bond when it is embedded in a larger 14 membered ring. The observation of only the *trans* amide conformer may be a consequence of the smaller ring size and/or stabilisation of the structure by the formation of a γ-turn. A peptide γ-turn is a structural motif present in many biologically active cyclic peptides that occurs to reverse the orientation of the peptide chain. γ-Turns contain three residues held together in a seven membered cyclic conformation by an intramolecular hydrogen bond.[50]

In summary, the synthesis and olefin metathesis of several allylated tripeptides incorporating proline has been accomplished, affording macrocyclic structures related to the neuroprotective tripeptide GPE **1**. The proline-containing tripeptides cyclized to afford cyclic products that adopted a single conformation about the Gly–Pro amide bond (with the exception of **9**). *N*-Allyl dienes



Scheme 4 *Reagents*, *conditions and yields*: (i) $Cl_2(PCy_3)_2Ru{=}CHPh$, CH_2Cl_2, reflux, 48 h, then DMSO, room temp, 24 h, 46%; (ii) PtO_2, THF, H_2, room temp, 16 h then 30% HBr–HOAc, room temp, 2 h then $NaHCO_3$, H_2O, dixoane, Boc_2O, room temp, 4 days, 78% over 3 steps; (iii) BoP–Cl, Et_3N, CH_2Cl_2, 0 °C to room temp, 24 h, 64%; (iv) CF_3CO_2H, CH_2Cl_2, room temp, 4 h, 80%.



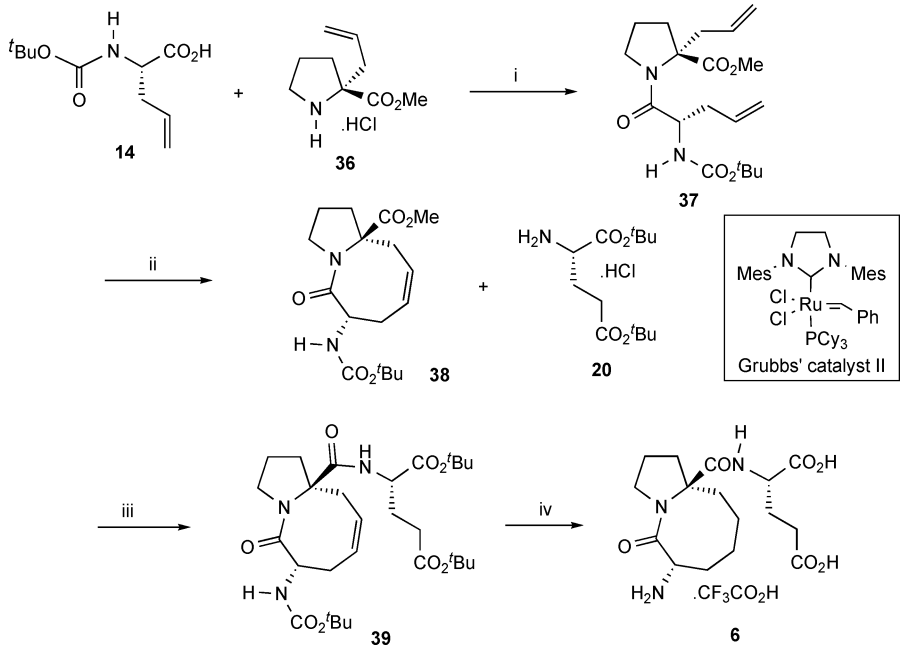
Scheme 5 *Reagents*, *conditions and yields*: (i) $Cl_2(PCy_3)_2Ru{=}CHPh$, CH_2Cl_2, reflux, 48 h, then DMSO, room temp, 24 h, 42%; (ii) PtO_2, THF, H_2, room temp, 16 h then 30% HBr–HOAc, room temp, 2 h then $NaHCO_3$, H_2O, dixoane, Boc_2O, room temp, 4 days, 58% over 3 steps; (iii) BoP–Cl, Et_3N, CH_2Cl_2, 0 °C to room temp, 17 h; (iv) CF_3CO_2H, CH_2Cl_2, room temp, 4 h, 55% over 2 steps.

28 and **29** afforded cyclononenes **30** and **33** with similar yields under identical reaction conditions. The *cis* [C-2/C-5 (Pro)] isomer of *C*-allyl diene **10a** underwent smooth cyclisation to cyclooctene **18** in good yield in contrast to the *trans* [C-2/C-5 (Pro)] isomer **11** that required high catalyst loading to achieve a moderate yield of cyclooctene **24**. In the two cases involving cyclization of dienes in which one allyl group was located at the more hindered C-2 position on proline, C-allyl diene **37** readily formed the metathesis product **38** whereas *N*-allyl dienes **43–47** failed to undergo cyclisation to the analogous diazabicyclic product.

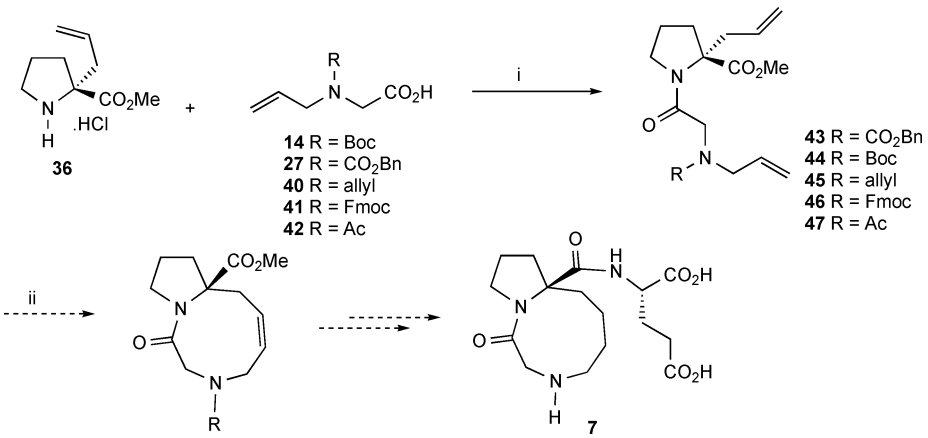
Experimental

(8*R*,3*S*,11*S*)-1-Aza-3-(*tert*-butyloxycarbonylamino)-11-ethoxycarbonyl-2-oxobicyclo[6.3.0]undec-5-ene 18

To a degassed solution of dienes **10** (0.064 g, 0.170 mmol) in dry dichloromethane (43 cm^3) was added bis(tricyclohexylphosphine)benzylidineruthenium dichloride (Grubbs' catalyst) (0.014 g, 0.0170 mmol) under a nitrogen atmosphere and the resultant purple solution heated at reflux for 24 h. The orange/brown solution was cooled to room temperature, dimethyl sulfoxide (0.160 cm^3, 2.26 mmol) was added and the solution stirred overnight. The solvent was removed *in vacuo* and the residue purified by chromatography (SiO_2, 2 : 1, 1 : 1, hexanes–ethyl acetate) to give *alkene* **18** (0.044 g, 74%) as a colourless oil. Alkene **18** existed exclusively as the *trans* C(O)–NPro conformer: $[\alpha]_D$ −93.2 (*c* 0.29 in CH_2Cl_2); δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.25 (3H, t, *J* 7.1, OCH_2CH_3), 1.42 [9H, s, $C(CH_3)_3$], 1.89–1.98 (2H, m, 10-H_AH_B and 9-H_AH_B), 2.01–2.09 (1H, m, 9-H_AH_B), 2.11–2.16 (1H, m, 10-H_AH_B), 2.24–2.33 (1H, m, 4-H_AH_B), 2.42 (1H, dq, *J* 15.2 and 3.4, 7-H_AH_B), 2.70–2.80 (2H, m, 4-H_AH_B and 7-H_AH_B), 4.15 (3H, q, *J* 7.1, OCH_2CH_3 and 8-H obscured), 4.47 (1H, dd, *J* 8.4 and 2.8, 11-H), 4.84 (3H, br q, *J* 7.8, 3-H), 5.59 (1H, d, *J* 7.3, N–H), 5.67–5.73 (1H, m, 6-H) and 5.77–5.83 (1H, m, 5-H); δ_C (100 MHz; $CDCl_3$) 14.0 (CH_3, OCH_2CH_3), 27.1 (CH_2, 10-C), 28.2 [CH_3, $C(CH_3)_3$], 32.77 (CH_2, 7-C or 9-C), 32.84 (CH_2, 9-C or 7-C), 35.2 (CH_2, 4-C), 51.7 (CH, 3-C), 58.6 (CH, 8-C), 60.2 (CH, 11-C), 60.8 (CH_2, OCH_2CH_3), 79.4 [quat., $C(CH_3)_3$], 125.7 (CH, 6-C), 129.1 (CH, 5-C), 155.1 (quat., NCO_2), 170.9 (quat., 11-CO) and



Scheme 6 *Reagents*, *conditions and yields*: (i) DCC, HOBt, Et_3N, CH_2Cl_2, 0 °C to room temp, overnight (*ca.* 23%); (ii) Grubbs' catalyst II (10 mol%), benzene, 45 °C, 48 h then DMSO overnight, 58%; (iii) 1 M aq. NaOH, dioxane, H_2O, room temp, then **39**, BoP–Cl, Et_3N, CH_2Cl_2, 0 °C to room temp, 20 h, 69% over 2 steps; (iv) PtO_2, H_2, THF, room temp, 16 h, then CF_3CO_2H, CH_2Cl_2, room temp, 5 h, 81%.

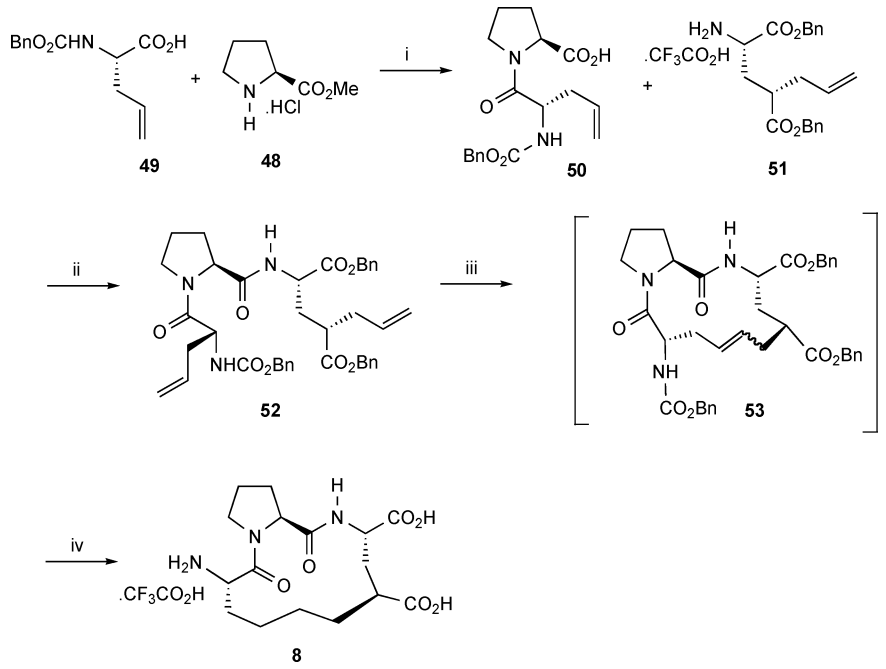


Scheme 7 *Reagents*, *conditions and yields*: (i) DCC, HOBt, Et_3N, CH_2Cl_2, 0 °C to room temp, overnight (ii) 10–30% $Cl_2(PCy_3)_2Ru{=}CHPh$, CH_2Cl_2, reflux, 24–96 h, or 30% Grubbs catalyst II, CH_2Cl_2, reflux, 48 h.

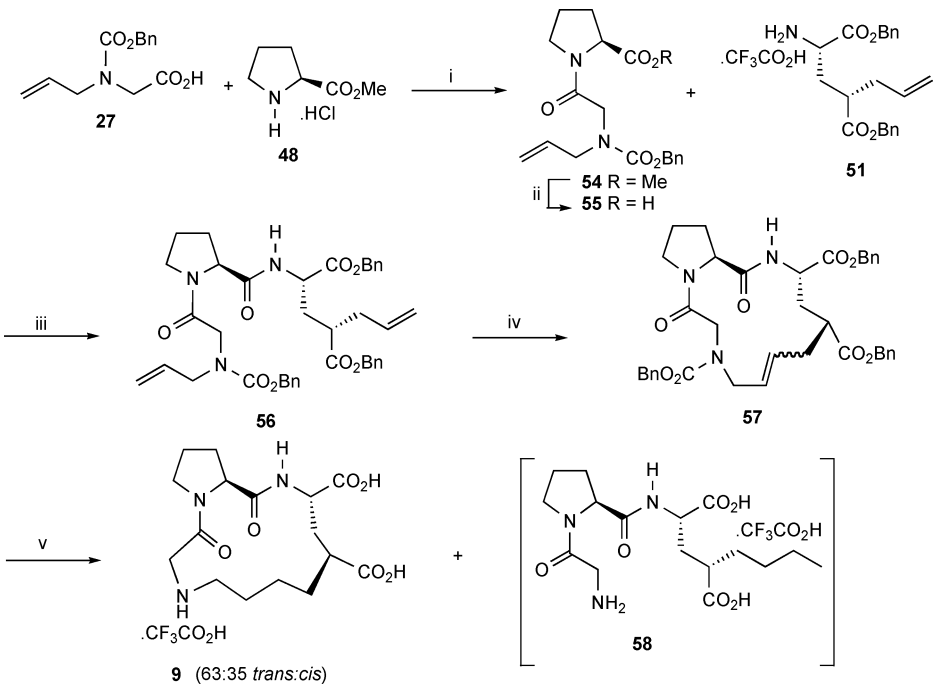
171.7 (quat., 2-C); *m/z* (EI+) 352.2001 (M^+. $C_{18}H_{28}N_2O_5$ requires 352.1998).

(2*S*,3′*S*,8′*R*,11′*S*)-Di-*tert*-butyl 2-{[1′-aza-3′-(*tert*-butyloxycarbonylamino)-2′-oxobicyclo[6.3.0]undec-5′-ene-11-carbonyl]amino}-1,5-pentanedioate 21

1 M Aqueous sodium hydroxide (0.63 cm^3, 0.63 mmol) was added to a solution of ester **18** in dioxane (1.3 cm^3) and the opaque solution stirred for 23 h at room temperature. The reaction mixture was diluted with water, extracted with dichloromethane and the aqueous layer acidified with solid citric acid and extracted with dichloromethane. The combined organic layers were dried (Na_2SO_4), filtered and the solvent removed to yield *acid* **19** (0.040 g). Hydrochloride salt **20** (0.048 g, 0.1625 mmol) was added to a solution of **19** and the solution cooled to 0 °C. Triethylamine (0.033 cm^3, 0.325 mmol) and bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BoP–Cl) (0.041 g, 0.163 mmol) were added and the mixture stirred for 21 h. The reaction mixture was washed with saturated aqueous sodium hydrogen carbonate, 2 M aqueous hydrochloric acid, dried (Na_2SO_4), filtered and the solvent removed to yield an oil (0.078 g) which was purified by chromatography (SiO_2, 1 : 1, hexane–ethyl acetate) to afford *amide* **21** (0.050 g, 70% over 2 steps) as a colourless oil. Amide **21** existed exclusively as the *trans* C(O)–NPro conformer: $[\alpha]_D$ −79.4 (*c* 0.47 in CH_2Cl_2); δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.39–1.50 [27H, m, 3 × $C(CH_3)_3$], 1.78–1.91 (3H, m, 10′-H_AH_B, 3-H_AH_B and 9′-H_AH_B), 2.0–2.33 (7H, m, 10′-H_AH_B, 3-H_AH_B, 4-H_2, 9′-H_AH_B, 7′-H_AH_B and 4′-H_AH_B), 2.80–2.95 (2H, br m, 7′-H_AH_B and 4′-H_AH_B), 4.15 (1H, br q, *J* 7.1, 8′-H), 4.42 (1H, ddd, *J* 7.8, 7.8 and 5.2, 2-H), 4.66 (1H, d, *J* 7.5, 11′-H), 4.97 (1H, br q, *J* 7.4, 3′-H), 5.57 (1H, d, *J* 7.8, N–H), 5.61–5.67 (1H, m, 6′-H), 5.81 (1H, br t, *J* 7.8, 5′-H)



Scheme 8 *Reagents*, *conditions and yields*: (i) DCC, HOBt, Et_3N, CH_2Cl_2, 0 °C to room temp, overnight, then 1 M aq. NaOH, dioxane, room temp, 24 h, 64% over 2 steps; (ii) **50**, EtOCOCl, Et_3N, CH_2Cl_2, 0 °C, 40 min, then **51**, 0 °C to room temp, overnight, 60%; (iii) $Cl_2(PCy_3)_2Ru{=}CHPh$, CH_2Cl_2, reflux, 48 h then DMSO, 24 h; (iv) 10% Pd/C, H_2, THF–H_2O (4 : 1), room temp, 21 h or 10% PtO_2, H_2, THF, room temp, 16 h, then 10% Pd/C, H_2, MeOH–H_2O (4 : 1), room temp, 5 h, 58% over 2 steps.



Scheme 9 *Reagents*, *conditions and yields*: (i) EDCI, Et_3N, CH_2Cl_2, 0 °C to room temp, 19 h, 66%; (ii) 1 M aq. NaOH, dioxane, room temp, 20 h, 55%; (ii) **55**, EtOCOCl, Et_3N, CH_2Cl_2, 0 °C then **51**, overnight, 94%; (iii) Grubbs' catalyst II, benzene, 40 °C, 65 h; (iv) 10% Pd/C, H_2, MeOH–H_2O (4 : 1), room temp, 17 h, 58% over 2 steps.

and 7.47 (1H, d, *J* 8.0, N–H); δ_C (100 MHz; $CDCl_3$) 26.1 (CH_2, 10′-C), 27.9 [CH_3, $C(CH_3)_3$], 27.95 [CH_3, $C(CH_3)_3$], 28.2 [CH_3, $C(CH_3)_3$], 28.1, (CH_2, 3-C), 31.3 (CH_2, 4-C), 32.0 (CH_2, 7′-C), 33.2 (CH_2, 9′-C), 35.7 (CH_2, 4′-C), 51.0 (CH, 3′-C), 51.9 (CH, 2-C), 58.6 (CH, 8′-C), 60.8 (CH, 11′-C), 79.6 [quat., $C(CH_3)_3$], 80.4 [quat., $C(CH_3)_3$], 81.9 [quat., $C(CH_3)_3$], 124.9 (CH, 6′-C), 130.4 (CH, 5′-C), 155.0 (quat., NCO_2), 169.9 (quat., 1-C), 170.5 (quat., 11′-CO), 171.8 (quat., 2′-C or 5-C) and 171.9 (quat., 2′-C or 5-C); *m/z* (FAB+) 566.3454 [MH^+. $C_{29}H_{48}N_3O_8$ requires 566.3441].

(2*S*,3′*S*,8′*R*,11′*S*)-2-{[(3′-Amino-1′-aza-2′-oxobicyclo-[6.3.0]undecyl)-11′-carbonyl]amino}-1,5-pentanedioic acid trifluoroacetate salt 2

PtO_2 (0.00184 g, 0.0081 mmol) was added to a stirred solution of amide **21** (0.046 g, 0.081 mmol) in tetrahydrofuran (4 cm^3) under a nitrogen atmosphere. The mixture was hydrogenated (1 atm. of hydrogen) overnight, filtered through Celite™, and the solvent removed *in vacuo.* The residue was dissolved in dichloromethane (5 cm^3), trifluoroacetic acid (3 cm^3) added and the solution stirred for 5 h at room temperature. Removal of the volatiles *in vacuo*, purification by RP HPLC [10% acetonitrile : 90% water (containing 0.05% trifluoroacetic acid)] and trituration from ether–toluene gave **2** (0.0228 g, 60%, 2 steps) as a colourless oil. Macrocycle **2** existed exclusively as the *trans* C(O)–NPro conformer: $[\alpha]_D$ −27.9 (*c* 0.33 in MeOH); δ_H (400 MHz; D_2O) 1.37–1.85 (8H, m, 5′-H_2, 6′-H_2, 4′-H_2 and 9′-H_2), 1.98–2.28 (5H, m, 3-H_2, 7′-H_2 and 10′-H_AH_B), 2.36 (1H, dt, *J* 12.4 and 7.2, 10′-H_AH_B), 2.57 (2H, t, *J* 7.6, 4-H_2), 4.23 (1H, br t, *J* 8.2, 8′-H) and 4.41 (3H, m, 2-H, 11′-H, 3′-H); δ_C (100 MHz; D_2O) 21.6 (CH_2, 5′-C or 6′-C), 24.2 (CH_2, 5′-C or 6′-C), 25.5 (CH_2, 3-C), 27.1 (CH_2, 10′-C), 29.7 (CH_2, 4-C), 31.5 (CH_2, 9′-C), 32.8 (CH_2, 7′-C), 35.0 (CH_2, 4′-C), 51.2 (CH, 3′-C), 51.9 (CH, 2-C), 59.9 (CH, 8′-C), 61.4 (CH, 11′-C), 116.2 (quat., q, *J* 291, CF_3), 162.7 (quat., q, *J* 35.2, CF_3CO_2H), 168.5 (quat., 2′-C), 173.7 (quat., 11′-CO), 174.7 (quat., 1-C) and 176.8 (quat., 5-C); *m*/*z* (FAB+) 356.1816 [MH(free base)$^+$. $C_{16}H_{26}N_3O_6$ requires 356.1822].

(8*S*,11*S*)-1-Aza-2-oxo-3-*tert*-butoxycarbonylamino-11-*tert*-butoxycarbonylbicyclo[6.3.0]undec-5-ene 24

To a degassed solution of diene **11** (0.053 g, 0.129 mmol) in dry dichloromethane (32 cm^3) was added bis(tricyclohexylphosphine)benzylidieneruthenium dichloride (Grubbs' catalyst) (0.011 g, 0.0129 mmol) under a flow of nitrogen and the resultant purple solution heated at reflux under a nitrogen atmosphere for 24 h. Further bis(tricyclohexylphosphine)benzylidieneruthenium dichloride (Grubbs' catalyst) (0.011 g, 0.0129 mmol) was added and refluxing continued for a further 48 h after which time another portion of Grubbs catalyst (0.011 g, 0.0129 mmol) was added and the solution refluxed for an additional 24 h. The orange/brown solution was cooled to room temperature, dimethyl sulfoxide (0.151 cm^3, 1.935 mmol) was added and the solution stirred overnight. The solvent was removed *in vacuo* and the residue purified by chromatography (silica gel, hexanes–ethyl acetate, 4 : 3, 3 : 1, 2 : 1) to give *alkene* **24** (0.0237 g, 39%) as a colourless solid. Alkene **24** was shown to be exclusively *trans* C(O)–NPro conformer: mp 175–195 °C; $[\alpha]_D$ −24.9 (*c* 0.237 in MeOH); δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.45 [9H, s, $C(CH_3)_3$], 1.47 [9H, s, $C(CH_3)_3$], 1.68–1.74 (1H, m, 9-H_AH_B), 1.82 (1H, td, *J* 11.0 and 5.9, 10-H_AH_B), 2.17–2.26 (2H, m, 9-H_AH_B and 10-H_AH_B), 2.27–2.40 (1H, br m, 7-H_AH_B), 2.49 (1H, br d, *J* 17.6, 7-H_AH_B), 2.55–2.63 (1H, m, 4-H_AH_B), 2.66–2.80 (1H, m, 4-H_AH_B), 4.40 (1H, dd, *J* 8.6 and 4.2, 11-H), 4.45–4.62 (2H, m, 3-H and 8-H), 5.62–5.68 (2H, m, H-6 and N–H) and 5.80–5.90 (1H, m, 5-H); δ_C (100 MHz; $CDCl_3$) 25.5 (CH_2, 10-C), 27.9 [CH_3, $C(CH_3)_3$], 28.3 [CH_3, $C(CH_3)_3$], 32.6 (CH_2, 4-C), 33.4 (CH_2, 7-C and 9-C), 55.8 (CH, 3-C), 57.2 (CH, 8-C), 61.5 (CH, 11-C), 79.5 [quat., $C(CH_3)_3$], 81.2 [quat., $C(CH_3)_3$], 128.6 (CH, 5-C and 6-C), 155.1 (quat., NCO_2), 169.6 (quat., 2-C) and 171.2 (quat., 11-CO); *m*/*z* (EI+) 380.2304 (M^+. $C_{20}H_{32}N_2O_5$ requires 380.2311).

(2*S*,8′*S*,11′*S*)-Di-*tert*-butyl 2-{[(1-aza-2-oxo-3-*tert*-butoxycarbonylaminobicyclo[6.3.0]undec-5-ene)-11-carbonyl]amino}-1,5-pentanedioate 26

Alkene **24** (0.021 g, 0.055 mmol) was stirred at room temperature in dichloromethane–trifluoroacetic acid (3 : 1, v/v, 4 cm^3) for 5 h. Removal of the volatiles *in vacuo* yielded an oil that was dissolved in saturated aqueous sodium hydrogen carbonate (1.5 cm^3). Dioxane (1 cm^3) was added followed by di-*tert*-butyl dicarbonate (0.015 g, 0.066 mmol) and the milky suspension was stirred at room temperature for 21 h. The reaction was diluted with water until a solution was obtained and extracted with dichloromethane. The aqueous layer was acidified with 2 M aqueous hydrochloric acid, extracted with dichloromethane and the combined organic extracts were dried (Na_2SO_4). Removal of the solvent *in vacuo* gave *acid* **25** (0.012 g) that was dissolved in dichloromethane (3 cm^3). Glutamic acid di-*tert*-butyl ester hydrochloride **20** (0.013 g, 0.044 mmol) was added and the solution cooled to 0 °C. Triethylamine (0.013 cm^3, 0.092 mmol) and bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BoP–Cl) (0.012 g, 0.048 mmol) were added and the mixture stirred for 19 h. The reaction mixture was washed with saturated aqueous sodium hydrogen carbonate, 2 M aqueous hydrochloric acid, dried (Na_2SO_4) and the solvent removed to yield an oil (0.033 g), which was purified by chromatography (silica gel, hexane–ethyl acetate, 2 : 1, 1 : 1, 1 : 2) to afford *amide* **26** (0.0125 g, 60% over 3 steps) as a colourless oil: $[\alpha]_D$ +12.1 (*c* 0.247 in CH_2Cl_2); δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.45–1.48 [27H, s, 3 × $C(CH_3)_3$], 1.65–1.75 (1H, m, 9′-H_AH_B), 1.87–1.96 (1H, m, 3-H_AH_B), 2.02–2.41 (7H, m, 10′-H_2, 4-H_2, 3-H_AH_B, 9′-H_AH_B and 7′-H_AH_B), 2.51 (1H, br d, *J* 17.4, 7′-H_AH_B), 2.57–2.64 (1H, m, 4′-H_AH_B), 2.70–2.80 (1H, m, 4′-H_AH_B), 4.39–4.48 (2H, m, 11′-H and 2-H), 4.52–4.62 (2H, m, 8′-H and 3′-H), 5.63–5.69 (1H, m, 6′-H), 5.78–5.84 (1H, m, 5′-H), 5.91 (1H, br s, N–H) and 6.73 (1H, d, *J* 7.0, N–H); δ_C (100 MHz; $CDCl_3$) 25.4 (CH_2, 10′-C), 27.2 (CH_2, 3-C), 27.9 [CH_3, $C(CH_3)_3$], 28.0 [CH_3, $C(CH_3)_3$], 28.3 [CH_3, $C(CH_3)_3$], 31.3 (CH_2, 4-C), 32.5 (CH_2, 5′-C), 33.3 (CH_2, 9′-C), 33.7 (CH_2, 7′-C), 52.3 (CH, 2-C), 56.3 (CH, 3′-C), 57.8 (CH, 8′-C), 61.9 (CH, 11′-C), 79.7 [quat., $C(CH_3)_3$], 80.6 [quat., $C(CH_3)_3$], 82.0 [quat., $C(CH_3)_3$], 127.9 (CH, 5′-C), 128.6 (CH, 6′-C), 155.4 (quat., NCO_2), 170.4 (quat., 2′-C), 170.8 (quat., CO), 171.7 (quat., CO) and 172.6 (quat., CO); *m*/*z* (FAB+) 566.3423 [MH(free base)$^+$. $C_{29}H_{48}N_3O_8$ requires 566.3441].

(2*S*,3′*S*,8′*S*,11′*S*)-2-{[(3′-Amino-1′-aza-2′-oxobicyclo-[6.3.0]undecyl)-11′-carbonyl]amino}-1,5-pentanedioic acid trifluoroacetate salt 3

PtO_2 (0.001 g, 0.0042 mmol) was added to a stirred solution of amide **26** (0.012 g, 0.021 mmol) in ethyl acetate–trifluoroacetic acid (5 : 3, v/v, 8 cm^3) under a nitrogen atmosphere. The mixture was hydrogenated (1 atm. of hydrogen) overnight, filtered through Celite™, and the solvent removed *in vacuo.* NMR and HPLC analysis showed the reaction to be incomplete (both double bond and protecting groups remaining). Thus PtO_2 (0.0003 g, 0.0126 mmol) was added to a stirred solution of the residue in tetrahydrofuran–methanol (1 : 1, v/v, 2 cm^3) under a nitrogen atmosphere. The mixture was hydrogenated (1 atm. of hydrogen)

overnight, filtered through Celite™, and the solvent removed *in vacuo.* The residue was dissolved in dichloromethane (3 cm^3), trifluoroacetic acid (1 cm^3) added and the solution stirred for 4 h at room temperature. Removal of the volatiles *in vacuo*, purification by RP HPLC [90% water (containing 0.05% trifluoroacetic acid) : 10% acetonitrile, 13 ml min^{-1}] and drying on a freeze drier gave **3** (1.4 mg 12%) as a thin film. Due to the small amount of sample obtained an optical rotation was not recorded: δ_H (400 MHz; D_2O) 1.74–2.42 (14H, m, 5′-H_2, 6′-H_2, 4′-H_2, 9′-H_2, 3-H_2, 7′-H_2 and 10′-H_2), 2.52 (2H, t, *J* 8.8, 4-H_2), 4.3–4.4 (1H, m, 2H), 4.43–4.5 (1H, m) and 4.62–4.69 (1H, m); δ_C (100 MHz; D_2O) 22.3 (CH_2), 25.4 (CH_2), 28.4 (CH_2), 29.0 (CH_2), 30.2 (CH_2), 32.4 (CH_2), 33.7 (CH_2), 53.9 (CH), 55.1 (CH), 60.2 (CH), 62.8 (CH), 168.1 (quat., CO) and 173.7 (quat., CO); 2 quaternary carbons and trifluoroacetate signals not detected; *m/z* (FAB+) 356.1816 [MH(free base)$^+$. $C_{16}H_{26}N_3O_6$ requires 356.1822].

(6*Z*,9*R*,12*S*)-1,4-Diaza-4-benzyloxycarbonyl-12-*tert*-butoxycarbonyl-2-oxobicyclo[7.3.0]dodec-6-ene 30

To a degassed solution of diene **28** (0.11 g, 0.242 mmol) in dry dichloromethane (60 cm^3) was added bis(tricyclohexylphosphine)benzylidineruthenium dichloride (Grubbs' catalyst) (0.020 g, 0.024 mmol) under an atmosphere of nitrogen and the resultant purple solution heated at reflux for 24 h. Further bis(tricyclohexylphosphine)benzylidineruthenium dichloride (Grubbs' catalyst) (0.020 g, 0.024 mmol) was then added, and refluxing continued for a further 24 h. The solution was cooled to room temperature, dimethyl sulfoxide (0.16 cm^3, 2.26 mmol) was added and the orange/brown solution stirred for 22 h. The solvent was removed *in vacuo* and the residue purified by chromatography (SiO_2, hexanes–ethyl acetate, 3 : 1, 2 : 1, 1 : 1) to give a colourless oil which was further purified by chromatography (C_{18} RP silica, 100 : 0, 9 : 1, 9 : 3, 1 : 1, 1 : 9, water–acetonitrile,) to give *alkene* **30** (0.046 g, 46%) as a colourless oil. Alkene **30** existed exclusively as the *trans* C(O)–NPro conformer. In addition, restricted rotation about the N–CO carbamate bond was also observed resulting in a 1 : 1 mixture of conformers: $[\alpha]_D$ −243.9 (*c* 0.27 in CH_2Cl_2); δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.49 [9H, s, $C(CH_3)_3$], 1.84–2.34 (5H, m, 8-H_AH_B, 10-H_2 and 11-H_2), 2.98–3.08 (1H, m, 8-H_AH_B), 3.69–3.88 (2H, m, 5-H_AH_B and 3-H_AH_B), 4.0 (1H, p, *J* 7.6, 9-H), 4.33–4.61 (3H, m, 5-H_AH_B, 3-H_AH_B and 12-H), 5.10–5.30 (2H, m, OCH_2Ph), 5.51–5.65 (1H, m, 6-H), 5.98–6.09 (1H, m, 7-H) and 7.29–7.38 (5H, m, Ph); δ_C (100 MHz; $CDCl_3$) 27.2 (CH_2, 11-C), 28.0 [CH_3, $C(CH_3)_3$], 33.9 (CH_2, 8-C), 35.1 (CH_2, 10-C), 42.2 (CH_2, 5-C), 42.3 (CH_2, 5-C), 45.7 (CH_2, 3-C), 45.9 (CH_2, 3-C), 59.5 (CH, 9-C), 59.6 (CH, 9-C), 61.2 (CH, 12-C), 61.3 (CH, 12-C), 67.4 (CH_2, O*C*H_2Ph), 67.5 (CH_2, O*C*H_2Ph), 81.4 [quat., $C(CH_3)_3$], 81.5 [quat., $C(CH_3)_3$], 125.8 (CH, 6-C), 126.0 (CH, 6-C), 127.7 (CH, Ph), 127.8 (CH, Ph), 127.81 (CH, Ph), 127.9 (CH, Ph), 128.3 (CH, Ph), 132.0 (CH, 7-C), 132.4 (CH, 7-C), 136.5 (quat., Ph), 155.8 (quat., NCO_2), 156.2 (quat., NCO_2), 167.2 (quat., 2-C), 167.4 (quat., 2-C), 171.2 (quat., 12-CO) and 171.4 (quat., 12-CO); *m/z* (EI+) 414.2154 (M^+. $C_{23}H_{30}N_2O_5$ requires 414.2154).

(9*R*,12*S*)-1,4-Diaza-4-*tert*-butyloxycarbonyl-2-oxobicyclo[7.3.0]dodec-2-carboxylic acid 31

To a stirred solution of alkene **30** (0.12 g, 0.29 mmol) in tetrahydrofuran (4 cm^3) was added platinum oxide (0.0066 g, 0.029 mmol) under a flow of nitrogen. The mixture was hydrogenated (1 atm of H_2) for 16 h, filtered through Celite™, and the solvent removed *in vacuo.* The residue was dissolved in a solution of hydrobromic acid in acetic acid (30% w/w, 3 cm^3) and stirred at room temperature for 2 h. Removal of the volatiles *in vacuo* at 30 °C followed by addition and evaporation of methanol–water (3 : 1) several times yielded the hydrobromide salt which was dissolved in a solution of saturated sodium hydrogen carbonate (3 cm^3). Dioxane (2 cm^3) was then added and di-*tert*-butyl dicarbonate added to the opaque solution (0.076 g, 0.350 mmol). The resultant suspension was stirred for 24 h, di-*tert*-butyl dicarbonate (0.076 g, 0.3504 mmol), added and stirring continued for a further 72 h. Water (10 cm^3) was added, the solution washed with dichloromethane, and the aqueous layer acidified with 2 M aqueous hydrochloric acid and extracted with dichloromethane. The combined organic layers were dried (Na_2SO_4), filtered and the solvent removed to yield an oil (0.08 g) that was purified by chromatography (SiO_2, 2 : 1 : 0, 2 : 1 : 0.3, hexanes–ethyl acetate–acetic acid) to give *acid* **31** (0.074 g, *ca.* 78%) as a colourless oil. Acid **31** existed exclusively as the *trans* C(O)–NPro conformer. In addition, restricted rotation about the N–CO carbamate bond was also observed resulting in a 1 : 1 mixture of conformers: δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.25–1.48 [11H, m, $C(CH_3)_3$, 8-H_AH_B and 6-H_AH_B or 7-H_AH_B], 1.54–1.82 (5H, m, 8-H_AH_B, 10-H_AH_B and 6-H_AH_B or 7-H_AH_B), 1.90–2.03 (1H, m, 10-H_AH_B), 2.10–2.33 (2H, m, 11-H_2), 2.78 (1H, td, *J* 12.7 and 2.6, 5-H_AH_B), 3.78–3.95 (2H, m, 5-H_AH_B and 3-H), 4.20–4.30 (1H, m, 9-H), 4.52 (0.5H, d, *J* 17.7, 3-H_AH_B), 4.62 (1H, t, *J* 8.6, 12-H) and 4.76 (0.5H, d, *J* 17.2, 3-H_AH_B); δ_C (100 MHz; $CDCl_3$) 22.2 (CH_2, 6-C or 7-C), 27.6 (CH_2, 6-C or 7-C), 24.3 (CH_2, 11-C), 24.8 (CH_2, 11-C), 26.8 (CH_2, 6-C or 7-C), 27.9 (CH_2, 6-C or 7-C), 28.3 [CH_3, $C(CH_3)_3$], 32.6 (CH_2, 10-C), 33.1 (CH_2, 10-C), 34.8 (CH_2, 8-C), 35.3 (CH_2, 8-C), 51.1 (CH_2, 5-C), 51.7 (CH_2, 5-C), 54.7 (CH_2, 3-C), 55.9 (CH_2, 3-C), 56.6 (CH, 9-C), 56.7 (CH, 9-C), 60.8 (CH, 12-C), 61.1 (CH, 12-C), 80.7 [quat., $C(CH_3)_3$], 154.8 (quat., NCO_2), 155.6 (quat., NCO_2), 170.0 (quat., 2-C), 170.5 (quat., 2-C), 174.1 (quat., 12-CO) and 174.7 (quat., 12-CO); *m/z* (FAB+) 327.1925 (MH^+. $C_{16}H_{27}N_2O_5$ requires 327.1920).

(2*S*,9′*S*,12′*S*)-Di-*tert*-butyl 2-{[(1′,4′-diaza-4′-*tert*-butyloxycarbonyl-2′-oxobicyclo[7.3.0]dodecyl)-12′-carbonyl]amino}-1,5-pentanoate 32

Bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BoP–Cl) (0.08 g, 0.31 mmol) was added to a solution of acid **31** (0.07 g, 0.20 mmol), L-glutamic acid di-*tert*-butyl ester hydrochloride **20** (0.085 g, 0.289 mmol) and triethylamine (0.084 cm^3, 0.60 mmol) in dichloromethane (7 cm^3) at 0 °C. The mixture was stirred for 24 h, washed with saturated aqueous sodium hydrogen carbonate, 2 M aqueous hydrochloric acid, dried (Na_2SO_4), filtered and the solvent removed under reduced pressure. The resultant oil (0.141 g) was purified by chromatography (SiO_2, 1 : 1, 1 : 2, hexane–ethyl acetate) to afford *amide* **32** (0.077 g, *ca.* 64%) as a colourless oil. Amide **32** existed exclusively as the *trans* C(O)–NPro conformer. In addition, restricted rotation about the N–CO carbamate bond was also observed resulting in a 46 : 54 mixture of conformers: δ_H (300 MHz; $CDCl_3$; Me_4Si) 1.14–1.30 [2H, m, 8′-H_AH_B and H′-6(1H) or H′-7(1H)], 1.34–1.41 [27H, m, 3 × $C(CH_3)_3$], 1.54–2.28 ([11H, m, 8′-H_AH_B, 10′-H_2, 3-H_2, 4-H_2, 6′-H(3H) or 7′-H(3H)] and 11′-H_AH_B), 2.31–2.49 (1H, m, 11′-H_AH_B), 2.60–2.70 (1H, m,

5′-H_AH$_B$), 3.65–3.87 (2H, m, 5′-H$_A$$H_B$ and 3′-H_AH$_B$), 4.18 (1H, br s, 9′-H), 4.39 (1H, q, *J* 7.6, 2-H), 4.48 (0.5H, d, *J* 17.5, 3′-H$_A$$H_B$), 4.65 (1H, t, *J* 7.8, 12′-H), 4.74 (0.5H, d, *J* 17.0, 3′-H$_A$$H_B$), 7.45 (0.46H, d, *J* 7.4, N–H), and 7.64 (0.54H, d, *J* 7.7, N–H); δ_C (75 MHz; $CDCl_3$) 22.7 (CH_2, 11′-C), 23.3 (CH_2, 6′-C or 7′-C), 23.4 (CH_2, 6′-C or 7′-C), 27.0 (CH_2, 3-C, 6′-C or 7′-C), 27.7 (CH_2, 3-C, 6′-C or 7′-C), 27.8 (CH_2, 3-C, 6′-C or 7′-C), 28.2 (CH_2, 3-C, 6′-C or 7′-C), 27.9 [CH_3, $C(CH_3)_3$], 28.0 [CH_3, $C(CH_3)_3$], 28.4 [CH_3, $C(CH_3)_3$], 31.4 (CH_2, 4-C), 32.5 (CH_2, 10′-C), 32.8 (CH_2, 10′-C), 34.4 (CH_2, 8′-C, 35.1 (CH_2, 8′-C), 50.7 (CH_2, 5′-C), 51.5 (CH_2, 5′-C), 52.25 (CH, 2-C), 52.33 (CH, 2-C), 54.6 (CH_2, 3′-C), 55.0 (CH_2, 3′-C), 56.6 (CH, 9′-C), 56.8 (CH, 9′-C), 60.2 (CH, 12′-C), 60.8 (CH, 12′-C), 80.4 [quat., $C(CH_3)_3$], 80.6 [quat., $C(CH_3)_3$], 81.9 [quat., $C(CH_3)_3$], 154.7 (quat., NCO_2), 155.5 (quat., NCO_2), 169.4 (quat., 2′-C), 169.8 (quat., 2′-C), 170.6 (quat., 12′-CO, 1-C, 5-C), 170.8 (quat., 12′-CO, 1-C, 5-C) and 171.8 (quat., 12′-CO, 1-C, 5-C); *m/z* (FAB+) 568.3592 (MH^+. $C_{29}H_{50}N_3O_8$ requires 568.3598).

(2*S*,9′*R*,12′*S*)-2-{[(1′,4′-Diaza-2′-oxobicyclo[7.3.0]dodecyl)-12′-carbonyl]amino}-1,5-pentanedioic acid trifluoroacetate 4

Amide **32** (0.072 g, 0.126 mmol) was dissolved in a mixture of dichloromethane and trifluoroacetic acid (5 : 2, v/v) and stirred at room temperature for 4 h. Evaporation of the volatiles, and subsequent purification by RP-HPLC [water (0.05% trifluoroacetic acid)–acetonitrile, 90 : 10, 13 ml min^{-1}], gave **6** (0.059 g, 80%) as a colourless wax after drying on a freeze drier. Macrocycle **4** existed exclusively as the *trans* C(O)–NPro conformer: $[\alpha]_D$ −36.9 (*c* 0.195 in MeOH); δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.71–2.29 (11H, m, 6′-H_2, 7′-H_2, 8′-H_2, 10′-H_2, 3-H_2 and 11′-H_AH$_B$), 2.33–2.39 (1H, m, 11′-H$_A$$H_B$), 3.27 (1H, dt, *J* 10.2 and 4.1, 5′-H_AH$_B$), 3.39 (1H, td, *J* 12.0 and 2.2, 5′-H$_A$$H_B$), 3.76 (1H, d, *J* 13.6, 3′-H_AH$_B$), 4.0–4.25 (2H, m, 3′-H$_A$$H_B$ and 9′-H), 4.45 (1H, dd, *J* 9.3 and 5.1, 2-H) and 4.51 (1H, dd, *J* 9.2 and 8.3, 12′-H); δ_C (100 MHz; $CDCl_3$) 24.06 (CH_2, 6′-C or 7′-C), 24.1 (CH_2, 6′-C or 7′-C), 25.7 (CH_2, 3-C), 27.7 (CH_2, 11′-C), 29.6 (CH_2, 4-C), 32.5 (CH_2, 10′-C), 33.4 (CH_2, 8′-C), 43.3 (CH_2, 5′-C), 46.8 (CH_2, 3′-C), 51.8 (CH, 2-C), 61.9 (CH, 12′-C), 62.8 (CH, 9′-C), 116.2 (quat., q, *J* 290, CF_3), 162.6 (quat., q, *J* 35.2, CF_3CO_2H), 164.7 (quat., 2′-C), 173.4 (quat., 12′-CO), 174.6 (quat., 1-C), and 176.8 (quat., 5-C); *m/z* (FAB+) 356.1823 [MH(free base)$^+$. $C_{16}H_{26}N_3O_6$ requires 356.1822].

(6*Z*,9*S*,12*S*) *N*-Benzyloxycarbonyl-1,4-diaza-12-*tert*-butoxycarbonyl-2-oxobicyclo[7.3.0]dodec-6-ene 33

To a degassed solution of diene **29** (0.10 g, 0.23 mmol) in dry dichloromethane (56 cm^3) was added bis(tricyclohexylphosphine)benzylidineruthenium dichloride (Grubbs′ catalyst) (0.018 g, 0.023 mmol) under an atmosphere of nitrogen and the resultant purple solution heated at reflux for 24 h. Further bis(tricyclohexylphosphine)benzylidineruthenium dichloride (0.018 g, 0.023 mmol) was added and heating continued for 24 h, then the reaction mixture was cooled to room temperature and dimethyl sulfoxide (0.160 cm^3, 2.3 mmol) was added and the orange/brown solution stirred for 24 h. The solvent was removed *in vacuo* and the residue purified by chromatography (SiO_2, 3 : 1, 2 : 1, 1 : 1, hexanes–ethyl acetate) to give a colourless oil which was further purified by chromatography (C_{18} RP silica, 100 : 0, 9 : 1, 9 : 3, 1 : 1, 1 : 9, water–acetonitrile,) to give *alkene* **33** (0.039 g, 42%) as a colourless oil. Alkene **33** existed exclusively as the *trans* C(O)–NPro conformer. In addition, restricted rotation about the N–CO carbamate bond was also observed resulting in a 1 : 1 mixture of conformers: $[\alpha]_D$ −118.2 (*c* 0.34 in CH_2Cl_2); δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.46 [9H, s, $C(CH_3)_3$], 1.74 (1H, dd, *J* 11.8 and 5.7, 10-H_AH$_B$), 1.93 (1H, dd, *J* 12.5 and 6.5, 11-H_AH$_B$), 2.11–2.45 (4H, m, 8-H_2, 10-H$_A$$H_B$ and 11-H$_A$$H_B$), 3.53 (1H, dd, *J* 15.7 and 8.0, 5-H_AH$_B$), 4.13–4.37 (4H, m, 3-H_2, 9-H and 12-H), 4.50 (1H, d, *J* 15.4, 5-H$_A$$H_B$), 5.12–5.29 (2H, m, OC$H_2$Ph), 5.54–5.82 (2H, m, 6-H and 7-H) and 7.31–7.43 (5H, m, Ph); δ_C (100 MHz; $CDCl_3$) 26.7 (CH_2, 11-C), 27.8 [CH_3, $C(CH_3)_3$], 34.0 (CH_2), 34.2 (CH_2), 34.3 (CH_2), 34.4 (CH_2), 43.9 (CH_2, 5-C), 44.5 (CH_2, 5-C), 48.5 (CH_2, 3-C), 49.5 (CH_2, 3-C), 57.2 (CH, 9-C), 57.6 (CH, 9-C), 60.1 (CH, 12-C), 67.6 (CH_2, OCH_2Ph), 81.2 [quat., $C(CH_3)_3$], 126.8 (CH, 6-C), 126.9 (CH, 6-C), 128.0 (CH, Ph), 128.4 (CH, Ph), 128.7 (CH, Ph), 129.9 (CH, 7-C), 136.3 (quat., Ph), 136.4 (quat., Ph), 155.7 (quat., NCO_2), 167.8 (quat., 2-C), 167.9 (quat., 2-C) and 170.7 (quat., 12-CO); *m/z* (EI+) 414.2157 (M^+. $C_{23}H_{30}N_2O_5$ requires 414.2155).

(9*S*,12*S*)-1,4-Diaza-4-*tert*-butyloxycarbonyl-2-oxo-bicyclo[7.3.0]dodecyl-12-carboxylic acid 34

To a stirred solution of alkene **33** (0.11 g, 0.263 mmol) in tetrahydrofuran (4 cm^3) was added platinum oxide (0.006 g, 0.026 mmol) under a flow of nitrogen. The mixture was hydrogenated (1 atm. of hydrogen) for 16 h, filtered over Celite™, and the solvent removed *in vacuo.* The residue was dissolved in a solution of 30% hydrobromic acid in acetic acid (3 cm^3) and stirred at room temperature for 2 h. Removal of the volatiles *in vacuo* at 40 °C followed by repeated evaporation from methanol–water (3 : 1) yielded the hydrobromide salt which was dissolved in a solution of saturated sodium hydrogen carbonate (3 cm^3). Dioxane (2 cm^3) and di-*tert*-butyl dicarbonate (0.07 g, 0.32 mmol) were added and the resultant suspension was stirred for 24 h, then further di-*tert*-butyl dicarbonate (0.07 g, 0.32 mmol) was added and stirring continued for a further 48 h. Water (10 cm^3) was added, the solution washed with dichloromethane and the aqueous layer was acidified with 2 M aqueous hydrochloric acid and extracted with dichloromethane. The combined organic layers were dried (Na_2SO_4), filtered and the solvent removed to yield an oil (0.072 g) that was purified by chromatography (SiO_2, 1 : 1 : 0.3, hexanes–ethyl acetate–acetic acid) to give *acid* **34** (0.05 g, 58%) as a colourless oil. Acid **34** existed exclusively as the *trans* C(O)–NPro conformer. In addition, restricted rotation about the N–CO carbamate bond was also observed resulting in a 72 : 28 mixture of conformers: $[\alpha]_D$ +3.9 (*c* 0.23 in CH_2Cl_2); δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.30–1.48 [11H, m, $C(CH_3)_3$, 8-H_AH$_B$ and 11-H_AH$_B$], 1.63–1.79 (5H, m, 8-H$_A$$H_B$, 6-$H_2$ and 7-H_2), 2.01–2.07 (1H, m, 11-H$_A$$H_B$), 2.12–2.32 (2H, m, 10-$H_2$), 2.62–2.85 (1H, m, 5-$H_AH_B$), 3.73–3.83 (1.28 H, 3-$H_AH_B$ and 5-H$_A$$H_B$*), 4.0 (0.72H, br d, *J* 14.2, 5-H$_A$$H_B$), 4.20–4.32 (1H, m, 9-H), 4.41* (0.28H, d, *J* 8.0, 12-H), 4.50 (0.72H, d, *J* 8.8, 12-H), 4.57 (0.72H, d, *J* 17.8, 3-H$_A$$H_B$), 4.69* (0.28H, d, *J* 16.5, 3-H$_A$$H_B$) and 8.40 (1H, br s, OH); δ_C (100 MHz; $CDCl_3$) 22.1 (CH_2, 7-C), 22.6* (CH_2, 7-C), 25.1 (CH_2, 11-C), 25.7* (CH_2, 11-C), 26.6 (CH_2, 6-C), 28.1 [CH_3, $C(CH_3)_3$], 28.3* [CH_3, $C(CH_3)_3$], 32.5 (CH_2, 10-C), 32.7* (CH_2, 10-C), 35.9* (CH_2, 8-C), 36.3 (CH_2, 8-C), 50.3* (CH_2, 5-C), 51.8 (CH_2, 5-C), 54.9* (CH_2, 3-C), 55.4 (CH, 9-C), 56.0*

(CH, 9-C), 56.3 (CH_2, 3-C), 60.5* (CH, 12-C), 60.8 (CH, 12-C), 80.6* [quat., $C(CH_3)_3$], 81.0 [quat., $C(CH_3)_3$], 155.2 (quat., NCO_2), 155.5* (quat., NCO_2), 169.5* (quat., 2-C), 169.8 (quat., 2-C), 174.9 (quat., 12-CO) and 175.1* (quat., 12-CO); *m/z* (EI+) 326.1837 (M^+. $C_{16}H_{26}N_2O_5$ requires 326.1842).

(2*S*,9′*S*,12′*S*)-2-{[(1′,4′-Diaza-2′-oxobicyclo[7.3.0]dodecyl)-12′-carbonyl]amino}-1,5-pentanedioic acid trifluoroacetate salt 5

Bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BoP–Cl) (0.057 g, 0.225 mmol) was added to a solution of acid **2** (0.05 g, 0.15 mmol), L-glutamic acid di-*tert*-butyl ester hydrochloride **20** (0.062 g, 0.21 mmol) and triethylamine (0.061 cm^3, 0.44 mmol) in dichloromethane (5 cm^3) at 0 °C. The mixture was stirred for 17 h, washed with saturated aqueous sodium hydrogen carbonate, 2 M aqueous hydrochloric acid, dried (Na_2SO_4), filtered and the solvent removed to yield an oil (0.108 g) which was purified by chromatography (SiO_2, 1 : 1, 1 : 2, 1 : 3, hexane–ethyl acetate) to afford an inseparable mixture (0.074 g) of the desired amide **35** contaminated with a glutamate–bis(2-oxo-3-oxazolidinyl)phosphinic chloride adduct. The mixture was dissolved in dichloromethane (5 cm^3), trifluoroacetic acid (2 cm^3) was added and the solution stirred for 4 h. Further trifluoroacetic acid (1 cm^3) was then added and stirring continued for 2 h. The volatiles were removed *in vacuo*, the residue suspended in water and filtered through a plug of cotton wool. The filtrate was subsequently purified by RP-HPLC [water (0.05% trifluoroacetic acid) : acetonitrile, 90 : 10, 13 ml min^{-1}] to afford trifluoroacetate **5** (0.039 g, 55% from **34**) as a colourless wax after trituration from diethyl ether–toluene. Macrocycle **5** existed exclusively as the *trans* C(O)–NPro conformer: $[\alpha]_D$ −22.3 (*c* 0.35 in MeOH); δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.68–2.04 (9H, m, 7′-H_2, 6′-H_2, 10′-H_2, 3′-$H_A$$H_B$, 8′-$H_A$$H_B$ and 11′-$H_A$$H_B$), 2.19–2.26 (2H, 8′-$H_A$$H_B$ and 3′-$H_A$$H_B$), 2.42–2.48 (1H, m, 11′-$H_A$$H_B$), 2.50–2.58 (2H, m, 4-$H_2$), 3.04 (1H, td, *J* 11.2 and 2.5, 5′-$H_A$$H_B$), 3.33 (1H, dt, *J* 14.1 and 4.9, 5′-$H_A$$H_B$), 3.82 (1H, d, *J* 14.1, 3′-$H_A$$H_B$), 4.32 (1H, d, *J* 14.0, 3′-$H_A$$H_B$), 4.38–4.45 (2H, m, 9′-H and 2-H) and 4.59 (1H, dd *J* 9.6 and 1.8, 12′-H); δ_C (100 MHz; $CDCl_3$) 25.6 (CH_2, 6′-C or 7′-C), 26.0 (CH_2, 6′-C or 7′-C), 28.1 (CH_2, 3-C), 29.9 (CH_2, 11′-C), 32.3 (CH_2, 4-C), 34.0 (CH_2, 10′-C or 8′-C), 35.2 (CH_2, 10′-C or 8′-C), 46.9 (CH_2, 5′-C), 48.7 (CH_2, 3′-C), 54.3 (CH, 2-C), 62.9 (CH, 12′-C), 64.6 (CH, 9′-C), 118.6 (quat., q, *J* 291, CF_3), 165.1 (quat., q, *J* 36.2, CF_3CO_2H), 167.9 (quat., 2′-C), 175.9 (quat., 12′-CO), 177.1 (quat., 1-C), and 179.4 (quat., 5-C); *m/z* (FAB+) 356.1824 [MH(free base)$^+$. $C_{16}H_{26}N_3O_6$ requires 356.1822].

(3*S*,8*S*)-1-Aza-3-(*tert*-butyloxycarbonylamino)-8-methoxycarbonyl-2-oxobicyclo[6.3.0]undec-5-ene[45] 38

A solution of freshly sublimed potassium *tert*-butoxide (0.0018 g, 0.0157 mmol) in dry tetrahydrofuran (1 cm^3) was added to a stirred suspension of 1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazolium tetrafluoroborate (0.07 g, 0.0177 mmol) in dry tetrahydrofuran (2 cm^3) under an atmosphere of nitrogen. Dry tetrahydrofuran (1 cm^3) was used to rinse the remaining potassium *tert*-butoxide from the reaction flask. The resultant suspension was stirred for 2 min then a solution of bis(tricyclohexylphosphine)benzylidineruthenium dichloride (Grubbs′ catalyst) (0.010 g, 0.011 mmol) in dry benzene (10 cm^3) was added and the purple solution heated at 80 °C for 35 min. The dark brown solution was cooled to room temperature and a solution of diene **37** (0.041 g, 0.112 mmol) in dry benzene (40 cm^3) added and the mixture heated at 45 °C for 48 h. The brown solution was cooled to room temperature, dimethyl sulfoxide (0.043 g, 0.56 mmol) was added and the mixture stirred overnight. The solvent was removed *in vacuo* and the residue purified by chromatography (SiO_2, 2 : 1, 1 : 1, hexane–ethyl acetate) to give alkene **38** (0.022 g, 58%) as a pale yellow oil. Alkene **38** existed exclusively as the *trans* C(O)–NPro conformer: $[\alpha]_D$ −86.3 (*c* 0.183 in CH_2Cl_2) [lit.,[45] −87.4 (*c* 0.35 in $CHCl_3$]; δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.44 [9H, s, $C(CH_3)_3$], 1.66–1.86 (2H, m, 10-H_2), 1.96–2.11 (2H, m, 9-$H_A$$H_B$ and 4-$H_A$$H_B$), 2.46 (1H, dd, *J* 12.3 and 6.1, 9-$H_A$$H_B$), 2.66 (1H, dd, *J* 14.8 and 8.2, 7-$H_A$$H_B$), 2.80–2.90 (1H, m, 4-$H_A$$H_B$), 3.04 (1H, dd, *J* 15.1 and 8.6, 7-$H_A$$H_B$), 3.40 (1H, ddd, *J* 11.6, 11.1 and 7.4, 11-$H_A$$H_B$), 3.76–3.83 (1H, m, 11-$H_A$$H_B$), 3.78 (3H, s, OCH_3), 4.97–5.04 (1H, m, 3-H), 5.52–5.63 (2H, m, 6-H and N–H) and 5.74–5.79 (1H, m, 5-H); δ_C (100 MHz; $CDCl_3$) 20.6 (CH_2, 10-C), 28.2 [CH_3, $C(CH_3)_3$], 35.0 (CH_2, 7-C), 35.4 (CH_2, 4-C), 38.4 (CH_2, 9-C), 48.4 (CH_2, 11-C), 50.8 (CH, 3-C), 53.0 (CH_3, OCH_3), 69.7 (quat., 8-C), 79.4 [quat., $C(CH_3)_3$], 122.8 (CH, 5-C), 132.2 (CH, 6-C), 154.8 (quat., NCO_2), 171.5 (quat., 2-C) and 173.7 (quat., 8-CO).

(2*S*,3′*S*,8′*S*)-Di-*tert*-butyl 2-{[(1′-aza-3′-(*tert*-butyloxycarbonylamino)-2′-oxobicyclo[6.3.0]undec-5′-ene)-8′-carbonyl]amino}-1,5-pentanedioate 39

To a solution of alkene **38** (0.022 g, 0.065) in dioxane (0.7 cm^3) was added 1 M aqueous sodium hydroxide (0.32 cm^3, 0.32 mmol) and the opaque solution stirred at room temperature for 17 h. Water was added and the mixture washed with dichloromethane. The aqueous layer was acidified with citric acid and the product extracted with dichloromethane. The organic layers were pooled, dried ($MgSO_4$) and the solvent removed to afford an oil (0.020 g). To a solution of this oil in dichloromethane (4 cm^3) was added L-glutamic acid di-*tert*-butyl ester hydrochloride **20** (0.025 g, 0.085 mmol) was added and the solution cooled to 0 °C. Triethylamine (0.017 g, 0.17 mmol) and bis(2-oxo-3-oxazolidinyl)phosphinic chloride (0.021 g, 0.085 mmol) were added and the solution stirred for 20 h. The reaction mixture was washed with 2 M aqueous hydrochloric acid, saturated aqueous sodium hydrogen carbonate, dried (Na_2SO_4), filtered and the solvent removed to yield an oil (0.041 g) which was purified by chromatography (SiO_2, 1 : 1, 2 : 3, hexane–ethyl acetate) to give amide **39** (0.025 g, 69% in 2 steps) as a colourless oil. Amide **39** existed exclusively as the *cis* C(O)–NPro conformer: $[\alpha]_D$ −49.6 (*c* 0.25 in CH_2Cl_2); δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.40–1.44 [27H, m, 3 × $C(CH_3)_3$], 1.55–1.66 (1H, m), 1.78 (1H, p, *J* 6.4), 1.89 (1H, td, *J* 13.2 and 6.1), 2.05–2.39 (5H, m), 2.65–2.75 (2H, m, 4′-H_2), 2.80 (1H, dd, *J* 15.0 and 7.9, 7′-$H_A$$H_B$), 2.92 (1H, dd, *J* 14.9 and 8.9, 7′-$H_A$$H_B$), 3.55 (1H, ddd, *J* 11.8, 11.8 and 7.0, 11′-$H_A$$H_B$), 3.65–3.70 (1H, m, 11′-$H_A$$H_B$), 4.40–4.46 (1H, m, 3′-H), 4.88–4.97 (2H, m, 6′-H and 2-H), 5.67–5.79 (2H, m, 5′-H and N–H) and 8.36 (1H, d, *J* 8.4, N–H); δ_C (100 MHz; $CDCl_3$) 20.4 (CH_2, 10′-C), 25.4 (CH_2, 3-C), 27.9 [CH_3, $C(CH_3)_3$], 28.0 [CH_3, $C(CH_3)_3$], 28.2 [CH_3, $C(CH_3)_3$], 31.5 (CH_2, 4-C), 32.2 (CH_2, 4′-C), 34.6 (CH_2, 7′-C), 37.4 (CH_2, 9′-C), 49.3 (CH_2, 11′-C), 52.1 (CH, 3′-C), 52.8 (2-C), 71.8 (quat., 8′-C), 80.4 [quat., $C(CH_3)_3$], 81.1 [quat., $C(CH_3)_3$], 125.0

(CH, 5′-C), 130.2 (CH, 6′-C), 156.3 (quat., NCO_2), 170.5 (quat., 2′-C), 171.9 (quat., 8′-CO), 171.2 (quat., 1-C) and 173.5 (quat., 5-C); *m/z* (EI+) 565.3365 (M^+. $C_{29}H_{47}N_3O_8$ requires 565.3363).

(2*S*,3′*S*,8′*S*)-2-{[(1′-Aza-3′-amino-2′-oxobicyclo[6.3.0]undecyl)-8′-carbonyl]amino}-1,5-pentanedioic acid trifluoroacetate salt 6

PtO_2 (0.001 g, 0.004 mmol) was added to a stirred solution of amide **7** (0.025 g, 0.044 mmol) in tetrahydrofuran (4 cm^3) under a nitrogen atmosphere. The mixture was hydrogenated (1 atm. of hydrogen) for 17 h, filtered through Celite™, and the solvent removed *in vacuo.* The residue was dissolved in dichloromethane (3 cm^3), trifluoroacetic acid (1 cm^3) added and the solution stirred for 4 h at room temperature. Removal of the volatiles *in vacuo* and analysis by HPLC and NMR showed the reaction to be incomplete. The residue was therefore redissolved in dichloromethane–trifluoroacetic acid (3 : 1, 4 cm^3) and the solution stirred at room temperature for 2.5 h. The volatiles were removed *in vacuo*, and the residue purified by RP HPLC [20% acetonitrile–80% water (containing 0.05% trifluoroacetic acid)] to give an oil which was triturated from ether–toluene to give macrocycle **6** (0.0167 g, 81% over 2 steps) as a hygroscopic white solid. Macrocycle **6** existed exclusively as the *cis* C(O)–NPro conformer: mp 75–85 °C; $[\alpha]_D$ −4.4 (*c* 0.16 in MeOH); δ_H (400 MHz; D_2O) 1.30–1.39 (1H, m, 5′-H_AH_B), 1.57–1.65 (1H, m, 6′-H_AH_B), 1.79–1.91 (4H, m, 5′-H_AH_B, 6′-H_AH_B, 4′-H_AH_B and 10′-H_AH_B), 1.98–2.13 (4H, m, 4′-H_AH_B, 10′-H_AH_B, 7′-H_AH_B and 3-H_AH_B), 2.23–2.37 (4H, m, 9′-H_2, 3-H_AH_B and 7′-H_AH_B), 2.53 (2H, t, *J* 7.5, 4-H_2), 3.68 (1H, dt, *J* 14.2 and 7.4, 11′-H_AH_B), 3.79 (1H, dt, *J* 14.0 and 7.5, 11′-H_AH_B), 4.20 (1H, dd, *J* 11.2 and 6.3, 3-H) and 4.53 (1H, dd, *J* 10.2 and 4.9, 2-H); δ_C (100 MHz; D_2O) 20.4 (CH_2, 10′-C), 21.2 (CH_2, 6′C), 22.1 (CH_2, 5′-C), 24.8 (CH_2, 3-C), 30.3 (CH_2, 4-C), 31.1 (CH_2, 4′-C), 36.4 (CH_2, 7′-C), 40.9 (CH_2, 9′-C), 49.1 (CH_2, 11′-C), 51.2 (CH, 3′-C), 52.4 (CH, 2-C), 70.9 (quat., 8′-C), 116.1 (quat., q, *J* 291, CF_3), 162.7 (quat., q, *J* 36.0, CF_3CO_2H), 169.4 (quat., 2′-C), 174.6 (quat., 8′-CO), 175.3 (quat., 1-C) and 177.0 (quat., 5-C); *m/z* (FAB+) 356.1830 [MH(free base)$^+$. $C_{16}H_{16}N_3O_6$ requires 356.1822].

(3*S*,8*R*,10*S*,13*S*)-3-Amino-1,11-diaza-8,10-carboxy-2,12-oxobicyclo[13.3.0]hexadecane trifluoroacetate 8

To a solution of protected tripeptide **52** (0.041 g, 0.0589 mmol) in dry dichloromethane (7.5 cm^3) was added bis(tricyclohexylphosphine)benzylidineruthenium dichloride (0.015 g, 0.0126 mmol) under an atmosphere of nitrogen. The purple solution was heated at reflux for 48 h, cooled to room temperature and dimethyl sulfoxide (0.045 cm^3, 0.63 mmol) added. The orange/brown solution was stirred for 24 h, filtered through a short plug of silica gel (eluting with hexanes–ethyl acetate, 1 : 3), and purified by chromatography (C_{18} RP silica, water–acetonitrile, 20–70%) to give the metathesis product **53** (48 mg) and other unidentified product(s) as a yellow oil, [*m/z* (FAB+) 668.2977 (MH^+. $C_{38}H_{42}N_3O_8$ requires 668.2972]. The metathesis product **53** was divided into 2 samples (~25 mg each); the first sample was dissolved in tetrahydrofuran–water (4 : 1, 5 cm^3) and 10 wt% palladium on activated carbon (0.008 g, 0.00749 mmol) was added under an atmosphere of nitrogen. To the reaction flask was fitted a balloon of hydrogen and stirred for 21 h at room temperature. The reaction mixture was filtered through a pad of Celite™, washed with methanol–water (4 : 1) and concentrated *in vacuo* to yield a film. The second sample was dissolved in tetrahydrofuran (3 cm^3) and platinum(IV) oxide‡ (0.00078 g, 0.0034 mmol) added under an atmosphere of nitrogen. To the reaction flask was fitted a balloon of hydrogen and stirred for 16 h at room temperature. The reaction mixture was filtered through a pad of Celite™, washed with methanol and concentrated *in vacuo* to yield a film that was dissolved in methanol–water (4 : 1, 5 cm^3) and 10 wt% palladium on activated carbon (0.008 g 0.00749 mmol) was added under an atmosphere of nitrogen. To the reaction flask was fitted a balloon of hydrogen and stirred for 5 h at room temperature. The reaction mixture was filtered through a pad of Celite™, washed with methanol–water (4 : 1) and concentrated *in vacuo* to yield a film. Both samples were combined, purified by RP HPLC [10% acetonitrile–90% water (containing 0.05% trifluoroacetic acid) then 80 : 20, 70 : 30] and dried on a freeze drier to give macrocycle **8** (0.016 g, 58% from **53**) as a colourless wax. Macrocycle **8** existed exclusively as the *trans* conformer: $[\alpha]_D$ −39.4 (*c* 0.0864 in water); δ_H (400 MHz; D_2O), 1.20–1.62 (6H, m, 5-H_2, 14-H_2, 7-H_2), 1.81–1.90 (1H, m, 4-H_AH_B), 2.04–2.36 (7H, m, 4-H_AH_B, 9-H_2, 6-H_2, 15-H_2), 2.43 (1H, br t, *J* 6.2, 8-H), 3.68–3.79 (2H, m, 16-H_2), 4.48–4.49 (1H, m, H-3) and 4.51–4.55 (1H, m, 10-H) [13-H obscured by HOD]; δ_C (100 MHz; D_2O) 18.2 (CH_2, 5-C), 23.9 (CH_2, 14-C), 24.2 (CH_2, 15-C), 26.0 (CH_2, 6-C), 26.8 (CH_2, 4-C), 28.0 (CH_2, 7-C), 30.3 (CH_2, 9-C), 38.2 (CH, 8-C), 47.1 (CH_2, 16-C), 50.6 (CH, 10-C), 50.9 (CH, 3-C), 59.5 (CH, 13-C), 116.25 (q, *J* 291.7, CF_3), 162.8 (q, *J* 35.2, CF_3CO_2H), 169.2 (quat., 2-C), 174.0 (quat., 10-CO), and 179.8 (quat., 8-CO); *m/z* (FAB+) 356.1811 [MH(free base)$^+$. $C_{16}H_{26}N_3O_6$ requires 356.1821].

(9*R*,11*S*,14*S*)-1,4,12-Triaza-9,11-carboxy-2,13-dioxobicyclo[14.3.0]heptadecane trifluoroacetate 9

Freshly sublimed potassium *tert*-butoxide (0.009 g, 0.0792 mmol) was added to a stirred suspension of 1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazolium tetrafluoroborate (0.031 g, 0.0792 mmol) in tetrahydrofuran (6 cm^3) under an atmosphere of nitrogen. The resultant suspension was stirred for 2 min then a solution of bis(tricyclohexylphosphine)benzylideneruthenium dichloride (0.054 g, 0.066 mol) in dry benzene (6 cm^3) was added and the purple solution was heated at 80 °C for 35 min. The dark brown solution was cooled to room temperature and a solution of protected tripeptide **56** (0.155 g, 0.22 mmol) in dry benzene (85 cm^3) added and the mixture heated at 45 °C for 65 h. The solvent was removed *in vacuo* and the residue purified by chromatography (silica gel, hexane–ethyl acetate, 1 : 1, 1 : 2, 1 : 3, 1 : 4) to give the metathesis product **57** and unidentified product(s) (0.110 g) as a brown oil: [*m/z* (FAB+) 668.2982 (MH^+. $C_{38}H_{42}N_3O_8$ requires 668.2972]. The mixture was subsequently dissolved in methanol–water (4 : 1, 50 cm^3) and 10 wt% palladium on activated carbon (0.035 g, 0.033 mmol) was added under an atmosphere of nitrogen. To the reaction flask was fitted a balloon of hydrogen and the mixture stirred for 17 h at room temperature.

‡ PtO_2 cleanly reduces the double bond; the subsequent debenzylation with 10% Pd/C is much cleaner than carrying out a one pot hydrogenation/debenzylation using 10% Pd/C.

The reaction mixture was filtered through a pad of Celite™, washed with methanol–water (4 : 1, 100 cm^3) and concentrated *in vacuo* to yield a film (0.064 g) that was purified by RP HPLC [20% acetonitrile–80% water (containing 0.05% trifluoroacetic acid)]. Repeated evaporation from a toluene–ether mixture and drying on a freeze drier gave macrocycle **9** (0.045 g, 58% from **56**) as a colourless solid. Macrocycle **9** was shown to be a 65 : 35 *trans–cis* mixture of conformers by ^{1}H NMR analysis (the ratio was estimated by integration of signals at δ 2.99 and 3.17–3.33 assigned to the 5-H atoms of the minor and major conformers respectively): mp 120–130 °C; $[a]_D$ −12.5 (*c* 0.104 in H_2O); δ_H (300 MHz; D_2O), 1.24–2.68 (13.65H, m, 5-H_AH_B, 6-H_2, 7-H_2, 8-H_2, 9-H, 10-H_2, 15-H_2, 16-H_2), 2.99* (0.35H, dt, *J* 12.9 and 7.3, 5-H_AH_B), 3.17–3.33 (1H, m, 5-H), 3.40* (0.35H, d, *J* 15.6, 3-H_AH_B), 3.50–3.73 (2H, m, 17-H_2), 3.98* (0.35H, d, *J* 15.6, 3-H_AH_B), 4.01 (0.65H, d, *J* 15.6, 3-H_AH_B), 4.08 (0.65H, d, *J* 15.8, 3-H_AH_B), 4.44 (0.65H, dd, *J* 11.6 and 4.3, 11-H), 4.51 (0.65H, dd, *J* 7.5 and 5.0, 14-H) and 4.59–4.64* (0.7H, m, 11-H, 14-H); δ_C (75 MHz; D_2O) 21.0, 21.4,* 21.7,* 22.2,* 23.6, 24.8, 25.2, 28.2, 31.2,* 31.3,* 31.6 (CH_2, 6-C, 7-C, 8-C, 10-C, 15-C, 16-C), 38.4* (CH, 9-C), 40.2 (CH, 9-C), 45.5* (CH_2, 3-C, 5-C), 46.9, 47.0 (CH_2, 17-C, 5-C), 47.5*, (CH_2, 17-C), 48.2, (CH_2, 3-C), 49.6 (CH, 11-C), 50.5* (CH, 11-C), 59.9* (CH, 14-C), 61.3 (CH, 14-C), 116.2 (q, *J* 291, CF_3), 162.6 (q, *J* 35.5, CF_3CO_2H), 164.4* (quat., 2-C), 164.9 (quat., 2-C), 173.4,* 173.4* (quat., 13-C, 11-CO), 173.4, 173.4 (quat., 13-C, 11-CO), 179.3* (quat., 9-CO) and 179.8 (quat., 9-CO); *m/z* (FAB+) 356.1829 [MH(free base)$^+$. $C_{16}H_{26}N_3O_6$ requires 356.1822]; [the minor component was tentatively assigned as glycyl-L-prolyl-L-γ-butylglutamic acid trifluoroacetate **58** and was shown to be a 78 : 22 *trans–cis* mixture of conformers by ^{13}C NMR analysis (the ratio was estimated by integration of the signals at δ 51.2 and 51.8 assigned to the Gluα-C atoms of the minor and major conformers respectively): δ_H (400 MHz; D_2O), 0.90 (3H, t, *J* 6.5, Gluγ-$CH_2CH_2CH_2CH_3$), 1.33 (4H, br s, Gluγ-$CH_2CH_2CH_2CH_3$), 1.64 (2H, br q, *J* 7.0, Gluγ-$CH_2CH_2CH_2CH_3$), 2.03–2.17 (5H, m, Gluβ-H_2, Proγ-H_2, Proβ-H_AH_B), 2.31–2.35 (0.78H, m, Proβ-H_AH_B), 2.57 (1H, p, *J* 6.9, Gluγ-H), 3.59–3.75 (2.18H, m, Proδ-H_2, Glyα-H_AH_B*), 3.98–4.10 (1.82H, Glyα-H_2, Glyα-H_AH_B*), 4.47 (1H, t, *J* 7.5, Gluα-H) and 4.51–4.56 (1H, m, Proα-H); δ_C (100 MHz; D_2O) 13.0 (CH_3, Gluγ-$CH_2CH_2CH_2CH_3$), 21.7 (CH_2, Gluγ-$CH_2CH_2CH_2CH_3$), 21.8* (CH_2, Gluγ-$CH_2CH_2CH_2CH_3$), 24.0 (CH_2, Proγ-C), 28.1 (CH_2, Gluγ-$CH_2CH_2CH_2CH_3$), 28.2* (CH_2, Gluγ-$CH_2CH_2CH_2CH_3$), 29.3 (CH_2, Proβ-C), 30.9 (CH_2, Gluγ-$CH_2CH_2CH_2CH_3$), 31.3*, 31.6*, 32.1* (CH_2), 32.3 (CH_2, Gluβ-C), 40.1* (CH_2, Glyα-C), 40.3 (CH_2, Glyα-C), 42.0 (CH, Gluγ-C), 43.0* (CH, Gluγ-C), 46.8 (CH_2, Proδ-C), 47.4* (CH_2, Proδ-C), 51.2 (CH, Gluα-C), 51.8* (CH, Gluα-C), 59.9* (CH, Proα-C), 60.2 (CH, Proα-C), 115.5 (q, *J* 291, CF_3), 162.9 (q, *J* 35.2, CF_3CO_2H), 165.5 (quat., Gly-CO), 166.0* (quat., Gly-CO), 173.5* (quat., CO), 174.0 (quat., CO), 174.5* (quat., CO), 174.8 (quat., CO), 180.0 (quat., Gluγ-CO) and 180.2* (quat., Gluγ-CO); *m/z* (FAB+) 358.1978 [MH(free base)$^+$. $C_{16}H_{28}N_3O_6$ requires 358.1978].

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