

Design and preparation of serine–threonine protein phosphatase inhibitors based upon the nodularin and microcystin toxin structures. Part 3†

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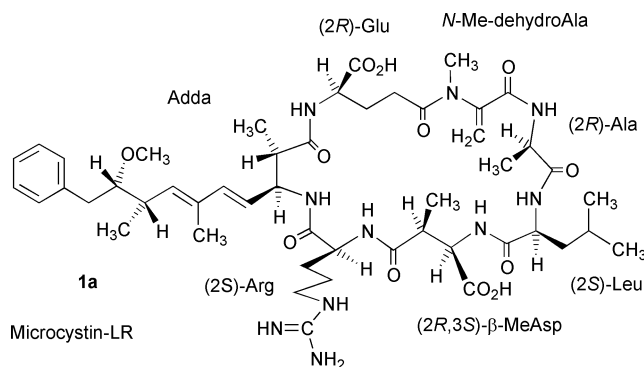
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Nodularin and microcystins are complex natural cyclic isopeptidic hepatotoxins that serve as subnanomolar inhibitors of the eukaryotic serine–threonine protein phosphatases PP1 and PP2A, enzymes that are intimately involved in controlling cellular metabolism. Previously we described a solution-phase synthesis of stripped-down nodularin analogues; cyclo[-β-Ala-(*R*)-Glu-α-OMe-γ-Sar-(*R*)-Asp-α-OMe-β-(*S*)-Phe-] **3** and cyclo[-(3*R*)-3-hydroxy-methyl-β-Ala-(*R*)-Glu-α-OMe-γ-Sar-(*R*)-Asp-α-OMe-β-(*S*)-Phe-] **5**. The synthetic strategy was designed to allow post-macrocyclisation elaboration. Here we examine alternative methods for introducing diversity and achieving macrolactamisation and compare the relative efficiency of solution- vs. solid-phase peptide syntheses of the macrocycles. Syntheses and the biological activities of the macrocycles cyclo{-(2*R*)-α-4-benzylpiperidinylamido-Asp]-β-[(*R*)-Glu]-γ-Sar-[(*R*)-Asp]-β-(*S*)-Phe-} **29** and cyclo{-(2*S*)-Phe-[(2*R*)-α-4-benzylpiperidinylamido-Asp]-(*R*)-Glu-γ-(*S*)-Pro-β-(*R*)-Asp-} **65** are compared. Both compounds contain sufficient side-chain functionality to interact with a hydrophobic groove at the enzyme active site. The proline containing analogues **30**, **31** ($R^3 = \text{CH}_3$) where sarcosine is replaced in macrocycles **3** and **4**, were also synthesised in order to correlate conformational properties with biological activity. In accord with predictions macrocycles **29** and **65** were found to be weak inhibitors of PP1 with IC_{50} 2.9 and 2.7 mM respectively.

Introduction

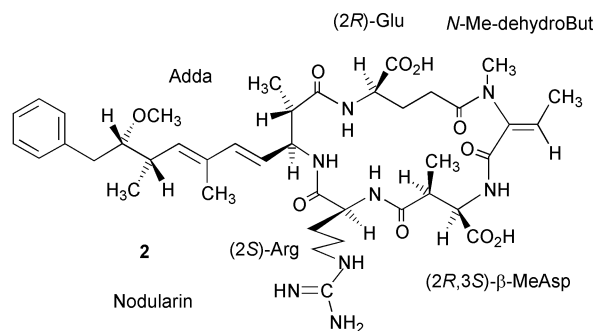
The natural cyclic isopeptide toxins microcystin **1** and nodularin **2** are known to inhibit the catalytic subunit of the mammalian serine–threonine protein phosphatases PP1 and PP2A



1b Microcystin-LA (2*S*-Ala replaces (2*S*)-Arg in **1a**)

1c Microcystin-RR (2*S*-Arg replaces (2*S*)-Leu in **1a**)

(but not PP2B or 2C) at subnanomolar levels, as determined for IC_{50} values.¹ Each of these catalytic activities, together with serine–threonine protein kinases, are involved in the maintenance of a delicate balance of pools of phosphorylated and dephosphorylated proteins which affect cellular metabolism



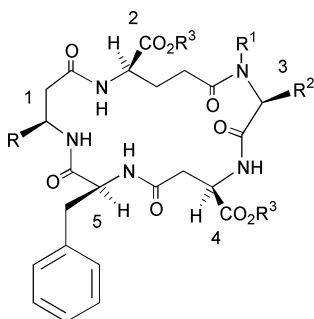
and communication.^{2–4} The toxin sensitive enzymes, PP1_{cat} and PP2A_{cat} , are highly homologous and display ~50% identity in their primary structure.^{5,6} The microcystins **1** and nodularin **2** differ considerably from other cyclic peptides. Both groups are cyclic triisopeptides and contain two free carboxylic acid groups, an *N*-methyl dehydro amino acid moiety and a large rigid lipophilic side-chain which forms part of the Adda residue [(2*S*,3*S*,4*E*,6*E*,8*S*,9*S*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid]. These five motifs are the only structural features common to the two toxin families.

Other naturally occurring competitive inhibitors of PP1_{cat} and PP2A_{cat} include the powerful tumour promoter okadaic acid, which is responsible for diarrhetic shellfish poisoning, tautomycin,⁷ cantharidin⁸ and the calyculins.⁹ However, these compounds show little specificity towards either of the two enzyme types. This lack of specificity can be rationalised by the analysis of the aligned amino acid sequences for the enzymes within the context of the published X-ray crystal structures available for PP1_{cat} .^{10,11} Essentially this analysis indicates that

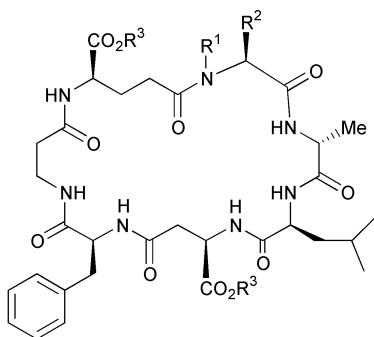
† For Part 2, see ref. 21.

the structure and composition of the active-site cleft is extremely highly conserved and that the inhibitors only interact with the conserved regions.¹²

It has been shown that the macrocyclic structure, the Adda residue and the two free carboxylic acids are essential for the inhibitory effect.^{13–16} Sodium borohydride reduction of the dehydroalanine residue in microcystin-LR gives dihydro-microcystin-LR diastereomers containing either (2*R*)- or (2*S*)-alanine.¹⁷ Each of these diastereoisomers was found to be equipotent with the parent compound when tested against PP2A.¹⁸ Using this information, and data available for natural variants, we were able to identify a simplified macrocycle which should serve as a framework for attaching specific functionalities.¹⁸ Since one of our goals was to synthesise minimal analogues to probe the active-site binding interactions and then identify specific inhibitors for each catalytic subunit type, PP1_{cat} and PP2A_{cat}, we opted initially for a convergent synthetic strategy. Here it was expected that the macrocycle and the lipophilic side-chain precursor could be separately preformed and then brought together towards the end of the synthesis. It was reasoned that such a strategy would enable the preparation of libraries of both macrocycles and side-chains which could be connected to give a diverse array of synthetic toxin analogues.



- 3** R = H; R¹ = CH₃; R² = H; R³ = CH₃
5 R = CH₂OH; R¹ = CH₃; R² = H; R³ = CH₃
6 R = CHO; R¹ = CH₃; R² = H; R³ = CH₃
7 R = CO₂H; R¹ = CH₃; R² = H; R³ = CH₃
30 R = H; R¹ = R² = -(CH₂CH₂CH₂); R³ = CH₃ or H



- 4** R¹ = CH₃; R² = H; R³ = CH₃
31 R¹ = R² = -(CH₂CH₂CH₂); R³ = CH₃ or H

The viability of this strategy rested on our ability to prepare stripped down macrocycles lacking the side chain functionalities. Various cyclisation protocols were tested on a model nodularin macrocycle target, cyclo[-β-Ala-(*R*)-Glu-α-OMe-γ-NMeGly-(*R*)-(Asp)-α-OMe-β-(*S*)-Phe-], **3**.^{18,19} These studies revealed that while macrolactamisation between an activated sarcosine (or glycine) carboxy group and the amino group of the (2*R*)-aspartic acid residue in suitably protected linear peptides did not occur, displacement of the β-pentafluorophenyl ester of the (2*R*)-aspartate α-methyl ester residue by the

free amino group of the (2*S*)-phenylalanine residue proceeded in excellent yield (89%).^{18,19} A similar approach was employed in the synthesis of the nodularin analogue motuporin.²⁰ Extension of this chemistry allowed us to prepare the 25-membered microcystin macrocycle, cyclo[-β-Ala-(*R*)-Glu-α-OMe-γ-NMeGly-(*R*)-Ala-(*S*)-Leu-(*R*)-Asp-α-OMe-β-(*S*)-Phe-], **4** (69% yield) and the functionalised 19-membered nodularin macrocycle, cyclo[-(3*R*)-hydroxymethyl-β-Ala-(*R*)-Glu-α-OMe-γ-NMeGly-(*R*)-Asp-α-OMe-β-(*S*)-Phe-], **5** (41% yield).²¹ We believed that the lengthy sequences and relatively low overall yield obtained for the preparation of macrocycle **5** warranted further investigation. In essence we wished to assess the potential advantages offered by use of such a preformed functionalised macrocycle for post-cyclisation elaboration against linear and divergent solid-phase methods where the cyclisation step is performed either off-resin, in solution, or on-resin in the gel-phase.

A pre-formed functionalised nodularin macrocycle

In the synthesis of the model nodularin macrocycle, cyclo[-β-Ala-(*R*)-Glu-α-OMe-γ-Sar-(*R*)-Asp-α-OMe-β-(*S*)-Phe-] **3** described earlier,^{18,19} the lipophilic Adda side-chain was totally omitted through the use of a β-alanine residue. Since we wished to introduce a group into the 3-position of β-alanine that could be easily modified to provide a range of lipophilic side-chains, including those containing diene functionalities, to provide rigidity, and lipophilic amides, we chose to use a 3-formyl group, the aldehyde **6**, or a 3-carboxy group, compound **7**. A similar strategy using appropriately protected aspartic α-semialdehydes and Wittig or Julia chemistry has been successfully employed in the synthesis of the Adda residue by other groups in the recent past.^{16,20,22–27}

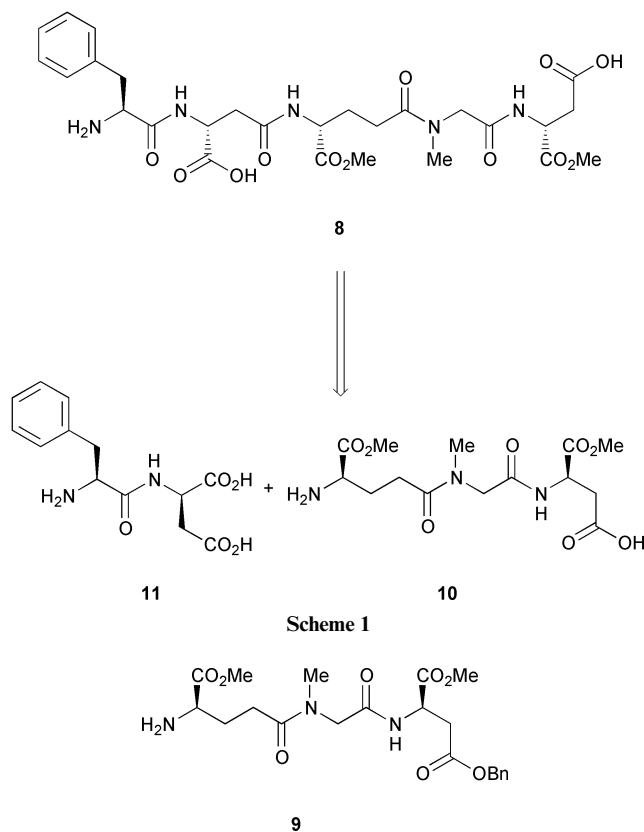
As it was important to retain the stereochemical integrity at the (2*R*)-α-centre of the aspartic α-semialdehyde residue and, therefore, to avoid racemisation through enol formation, a route to the reduced macrocyclic analogue **5** was devised in which the (3*R*)-3-amino-4-hydroxybutanoate residue was incorporated directly at the alcohol oxidation level. In order to stream-line the synthesis and provide both the aldehyde **6** and the carboxylic acid **7** a new pathway was devised to give direct access to the mono acid diester **7**. This approach required the incorporation of a (2*R*)-aspartic acid residue in place of the β-Ala residue in the peptidic macrocycle **3** and was expected only to present challenges in selecting suitably orthogonal ester group protections. It was seen as a potential additional advantage to introduce lipophilic side-chain surrogates for the Adda residue last through exo-macrocyclic amide formation with the α-carboxy group of such a (2*R*)-aspartic acid residue. Such a strategy was expected to give access to the aldehyde **6** and its ene derivatives and, at the same time, allow efficient access to a diverse range of exocyclic amide derivatives. Moreover, our modelling work had indicated that the natural protein substrates, which are of course polyamides, should employ the Adda binding pocket for substrate recognition. Any information on substrate or inhibitor binding was worthy of pursuit given that no structural information is yet available to define the manner in which the substrates recognise the Ser-Thr protein phosphatase 1 and 2A enzymes. While such information is always inaccessible directly due to the catalytic lability of the substrate, it was anticipated that the same information might be obtained, indirectly, through the preparation and evaluation of exocyclic amide derivatives of the natural inhibitors. Given these objectives we set out to prepare precursors for exocyclic ene and amide derivatives. We wished, within the current study, to assess both post- and pre-macrocyclisation elaboration strategies and also the potential for using solid-phase synthesis in the construction of the peptide and for macrocyclisation. It was also intended to gain sufficient biological information on structural variants to focus future synthetic work on the preparation of selective inhibitors for PPI and PP2A.

In order to refer to specific residues within the macrocycle of nodularin analogues, the surrogate for the Adda residue will be referred to as residue number 1. Other residues are then numbered in a conventional sense from the C-terminal of the Adda surrogate such that for nodularin analogues, there is an amide bond between residues 5 and 1.

Results and discussion

Solution-phase synthesis

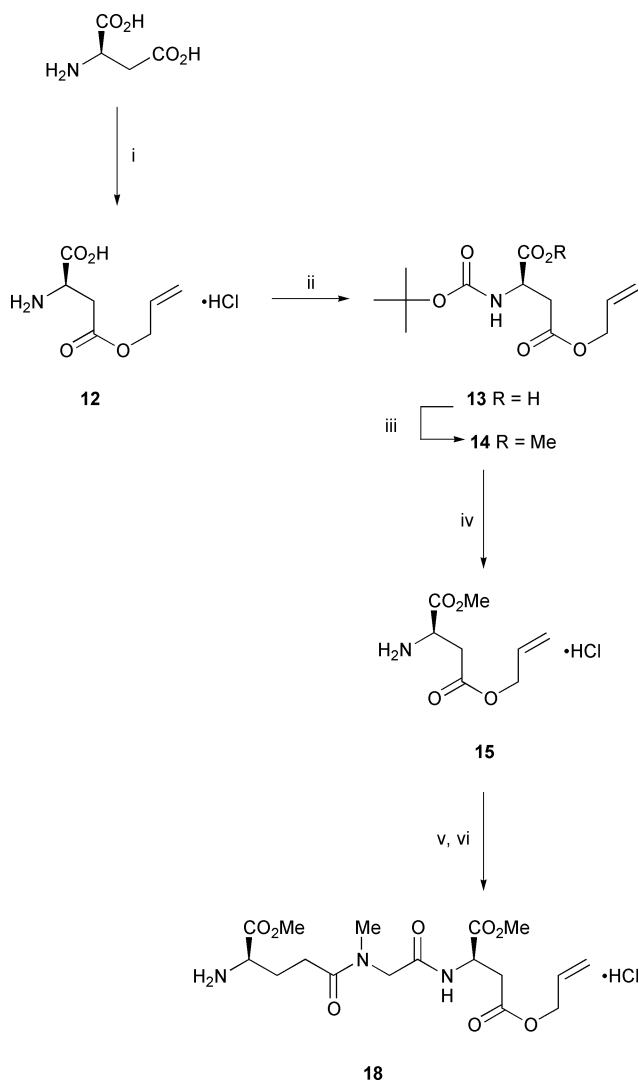
Disconnection of cyclo[-(*R*)-Asp- α -OH- β -(*R*)-Glu- α -OMe- γ -Sar-(*R*)-Asp- α -OMe- β -(*S*)-Phe-] **7** at the peptide bond between the amino group of the (*S*)-Phe residue and the β -carboxy group of the (*R*)-aspartate α -methyl ester, residues 4 and 5, using the same strategy as employed previously,^{18,19,21} gives the linear triisopentapeptide **8**. The three C-terminal residues (residues 2, 3 and 4) had been incorporated into macrolactams **3** and **5** previously as the tripeptide triester (*R*)-Glu- α -OMe- γ -NMeGly-(*R*)-Asp- α -OMe- β -OBn **9** without incident. Therefore, the isopentapeptide (*S*)-Phe-(*R*)-Asp- α -OH- β -(*R*)-Glu- α -OMe- γ -Sar-(*R*)-Asp- α -OMe- γ -OH **8** was disconnected between the (*R*)-Asp- α -OH and (*R*)-Glu residues to give the tripeptide diester **10** and the dipeptide (*2S*)-Phe-(*2R*)-Asp- α -benzyl ester **11** (Scheme 1).



The success of this strategy hinged on the availability of an ester protecting group which is orthogonal to the Boc, benzyl and methyl ester or urethane groups that might be employed in the preparation of molecules based upon the routes used for the macrolactams **3** and **5**. Allyl ester groups can be selectively removed by the use of tetrakis(triphenylphosphine)palladium(0) in the presence of a suitable allyl acceptor or by treatment with tris(triphenylphosphine)rhodium(I) chloride.^{28–33} However, whilst an allyl ester might be removed without affecting a benzyl ester in the same molecule, catalytic hydrogenolysis of a benzyl ester would give concomitant hydrogenation of the allyl group. It was decided, therefore, to protect the β -carboxylic acid groups of the two (*2R*)-aspartic acid residues (residues 1 and 4) as their allyl esters, given the ready

availability of aspartic acid β -allyl ester.^{34–36} With respect to the α -carboxy groups, it was important to be able to selectively deprotect the α -carboxy group of one of these (*2R*)-Asp residues without affecting the protection of either the other (*2R*)-Asp residue or the (*2R*)-Glu residue. Methyl ester protection had been employed in the precursor Boc-(*2R*)-Glu- α -OMe- γ -NMeGlyOH **16** for macrocycles **3** and **5**.^{18,19,21} Therefore, we chose to use the same protection for the α -carboxy group of the (*2R*)-Asp residue, number 4, since this would be coupled to the peptide fragment **16**. Orthogonal benzyl ester protection could then be used for the α -carboxy of the other (*2R*)-Asp residue, number 1. Thus, access to both (*2R*)-Asp- α -OBn- β -Oallyl and (*2R*)-Asp- α -OMe- β -Oallyl would be required.

Accordingly, (*2R*)-aspartic acid was esterified by treatment with HCl in allyl alcohol in a modification of the procedure of Lajoie³⁶ to give (*2R*)-aspartic acid β -allyl ester hydrochloride **12** (mp 176–178 °C). The amino group was protected as its *N*-*tert*-butoxycarbonyl derivative, under standard conditions, to give *N*-Boc-(*2R*)-aspartic acid β -allyl ester **13** (Scheme 2). The

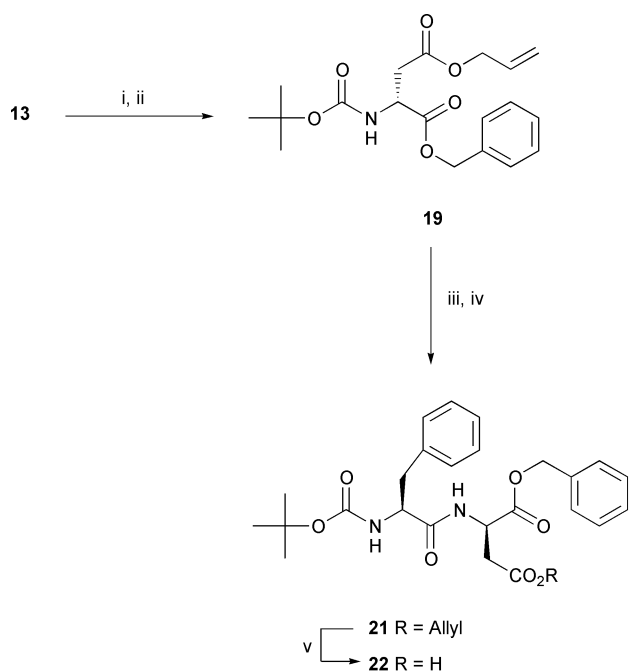


Scheme 2 Reagents and conditions: i) HCl, allyl alcohol, 0 °C→rt, 89%; ii) Boc₂O, Et₃N, H₂O, dioxane, 91%; iii) CH₂N₂, Et₂O, 0 °C, 94%; iv) HCl, EtOAc, 0 °C, 96%; v) (*2R*)-*N*-(*tert*-butoxycarbonyl)-[α -methylglutamyl]- γ -sarcosine **16**, IBCF, NMM, THF, -40 °C→rt, 69%; vi) HCl, EtOAc, 0 °C, 100%.

α -carboxylic acid was then converted to its methyl ester by treatment with ethereal diazomethane to give fully protected α -methyl β -allyl *N*-*tert*-butoxycarbonyl (*2R*)-aspartate diester **14**. The amino protecting group was removed by treatment with a solution of HCl in ethyl acetate to afford α -methyl β -allyl

(2*R*)-aspartate diester hydrochloride **15**. This was coupled to α -methyl *N*-Boc-(*R*)- γ -glutamyl-*N*-methylglycine **16**, prepared as described previously,^{18,19,21} to give the tripeptide, *N*-Boc-(*R*)-Glu- α -OMe- γ -*N*MeGly-(*R*)-Asp- α -OMe- β -Oallyl **17**, in 69% yield after purification by flash chromatography on silica. Acidolytic removal of the *N*-Boc protecting group gave a suitably protected form of the 2,3,4-tripeptide, [α -methyl (2*R*)- γ -glutamyl]-*N*-methylglycyl- [α -methyl β -allyl (2*R*)-aspartate] triester hydrochloride **18** (Scheme 2), which was ready for coupling to the 5,1-dipeptide.

To prepare the 5,1-dipeptide fragment, (*S*)-Phe-(*R*)-Asp- α -OBn- β -OH **22**, the doubly protected (2*R*)-aspartic mono acid derivative **13** was neutralised by treatment with 20% caesium carbonate solution in aqueous methanol. The resulting caesium salt was dissolved in dry DMF and was reacted with benzyl bromide according to the procedure of Gisin and co-workers.³⁷ The required protected diester *N*-Boc-(2*R*)-Asp- α -OBn- β -Oallyl **19** was obtained as a colourless oil in 91% yield and the *N*-Boc group was removed using HCl in ethyl acetate to afford α -benzyl β -allyl (2*R*)-aspartate diester hydrochloride **20**. The free amine was coupled to *N*-Boc-(2*S*)-Phe to give the 5,1-dipeptide diester, *N*-Boc-(2*S*)-phenylalanyl- [α -benzyl β -allyl (2*R*)-aspartate] **21** in 83% yield after flash chromatography on silica. This compound was isolated as a viscous oil which solidified on prolonged standing. The allyl ester was selectively cleaved through reaction with catalytic quantities of tetrakis-(triphenylphosphine)palladium(0) and pyrrolidine in dichloromethane to give the required 5,1-dipeptide free acid **22** in 56% yield after flash chromatography on silica (Scheme 3). This

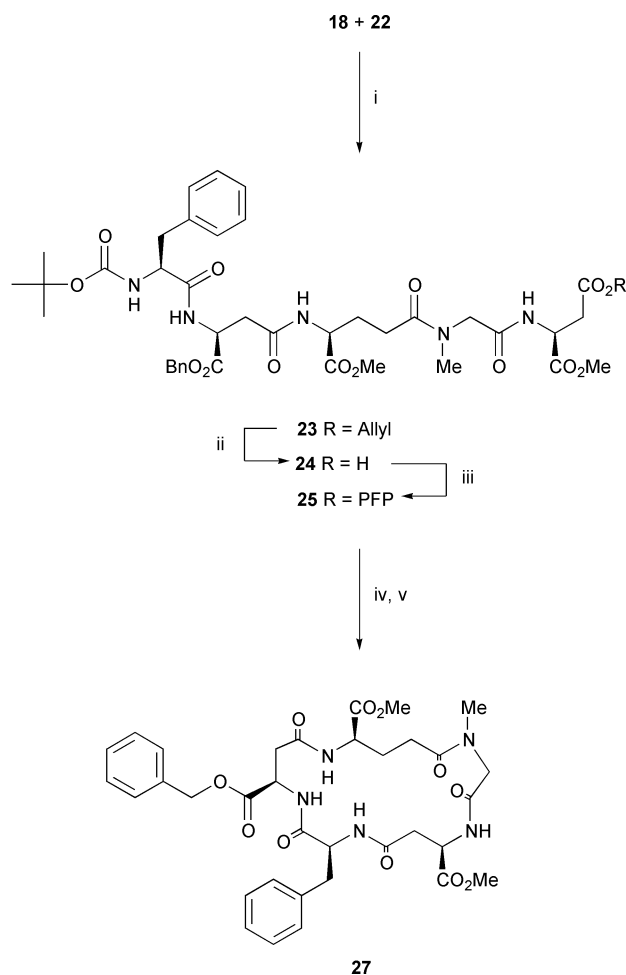


Scheme 3 Reagents and conditions: i) Cs₂CO₃, H₂O, MeOH; ii) BnBr, DMF, 0 °C \rightarrow rt, 91%; iii) HCl, EtOAc, 0 °C, 89%; iv) IBCF, NMM, Boc-(*S*)-Phe, THF -40 °C \rightarrow rt, 83%; v) (Ph₃P)₄Pd(0), pyrrolidine, CH₂Cl₂, 95%.

purification was not routinely repeated. Instead the crude material (isolated in 95% recovery) was carried forward directly to the next stage, with no significant diminution of the yield for the coupling of the two peptide fragments.

The two protected peptides **18** and **22** were coupled using mixed anhydride methodology in a mixture of dry DMF and THF to give the fully protected linear pentapeptide **23** in 81% yield after flash chromatography on silica. The allyl ester was selectively removed using a similar protocol to that described above, to give the free acid **24** which displayed the expected signals in its ¹H-NMR spectrum. Without purification acid

24 was esterified with pentafluorophenol, using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide (EDCI) activation, to give the *N*-Boc-pentapeptide pentafluorophenyl (PFP) ester **25** in 86% yield over the two steps, after chromatographic purification. The *N*-terminal *tert*-butoxycarbonyl protecting group was removed by treatment with trifluoroacetic acid in dichloromethane and the resulting ammonium trifluoroacetate salt **26** was thoroughly dried under high vacuum. Treatment with *N,N*-diisopropylethylamine (DIPEA), under conditions of high dilution in dichloromethane, allowed cyclisation to proceed. After 7 days at room temperature, when periodic TLC analysis showed that the conversion was complete, the reaction was terminated to afford the fully protected macrocyclic pentapeptide **27** in 75% yield as a white solid (Scheme 4). The

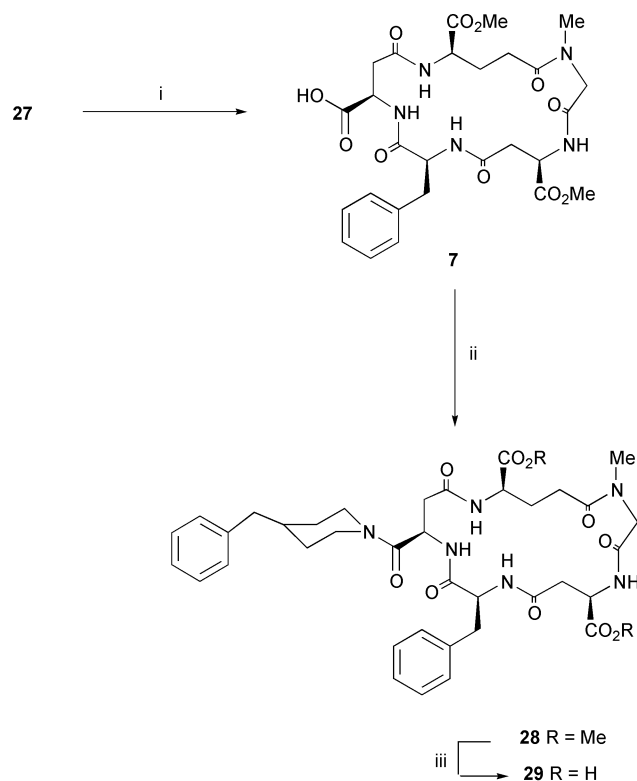


Scheme 4 Reagents and conditions: i) IBCF, NMM, THF, DMF, -40 °C \rightarrow rt, 81%; ii) (Ph₃P)₄Pd, pyrrolidine, CH₂Cl₂; iii) EDCI, pentafluorophenol, CH₂Cl₂, 0 °C \rightarrow rt, 86% over two steps; iv) TFA, CH₂Cl₂; v) DIPEA, CH₂Cl₂, 75% over two steps.

compound existed as a 3:1 mixture of conformers/rotoisomers in DMSO solution, in accord with the properties of other synthetic nodularin analogues lacking an α -methyl group in the first β -amino acid residue, see below.

Catalytic hydrogenolysis of the fully protected macrocyclic pentapeptide **27** in glacial acetic acid-methanol (50:50) gave the macrocyclic dimethyl ester mono acid **7**. The ¹H-NMR spectrum of the acid showed the expected omissions in the aromatic region and the presence of multiple conformations in methanol, but in DMSO, one conformer was dominant. Without further purification the acid **7** was coupled with 4-benzylpiperidine in a mixture of dry THF and DMF, *via* the intermediacy of the mixed isobutyl carbonic anhydride. The macrocyclic amide dimethyl ester **28** was obtained in an optimum isolated recovery of 44% following an aqueous

work-up, after very many attempts. No other coupling procedures offered any improvement. The $^1\text{H-NMR}$ spectrum of the amide **28** showed the presence of the benzylpiperidine moiety and an extremely complex mixture of conformers in chloroform. However, in DMSO solution the $^1\text{H-NMR}$ showed the presence of two major conformers in a 1:1 ratio. In view of the very poor yields for the reaction and the amount of material consumed in attempting to optimise the reaction, and the need to assess biological activity, the methyl ester groups were removed using lithium hydroxide in aqueous methanol, as described by Chamberlain.²⁴ The free diacid **29** was obtained in approximately 50% isolated yield after HPLC purification (Scheme 5). The $^1\text{H-NMR}$ spectrum of the diacid showed



Scheme 5 Reagents and conditions: i) H_2 , Pd/C, MeOH-AcOH (1:1), 85%; ii) IBCF, NMM, THF, DMF -40°C , then 4-benzylpiperidine, THF, -40°C →rt, 44%; iii) 0.1 M LiOH_(aq), MeOH, 50%.

the absence of methyl ester groups and two predominant Sar *N*-methyl signals consistent with the existence of two major conformers. The diacid **29** displayed the required mass spectrum but no other data were obtained in order to use the remaining material for biological testing. [Note that a comparison of the $^1\text{H-NMR}$ spectrum of **29**, with that for an analogue in which the Sar residue was replaced by (2*S*)-Pro (compound **63**), using a different synthesis, see below, was completely consistent with the expected structure.] Diacid **29** was tested as an inhibitor for PPI_{cat}, as described below, and was found to be weakly active, in accord with expectations.

A disappointing and unsatisfactory feature of the synthesis of the macrocyclic peptide **29** was the poor optimised yield (44%) obtained for the formation of the exocyclic amide **28** in the penultimate step. Analogous reactions using acyclic aspartic acid derivatives gave much better yields (see below) and it seemed probable that the poor yield of amide **28** resulted from inaccessibility of the electrophilic α -carboxy group within the 3-D conformational structure of the macrocyclic activated acid. Thus, the post-macrocyclisation elaboration strategy seemed flawed. In light of the result, and in order to assess the synthetic efficiency of alternative strategies, we turned our attention to

solid-phase protocols for construction of the linear peptide precursors and also for macrocyclisation.

Preparation of nodularin type macrocycles via solid-phase synthesis

The principal advantages of solid-phase peptide synthesis (SPPS) over solution-phase approaches are well established and lead to shorter synthesis times and higher yields of products requiring minimal chromatographic purification. This efficiency is achieved by using excess reagents in both the peptide coupling reactions and in the deprotection steps. Thus, yields are high, and by supporting the growing peptide on a polymer resin, unreacted reagents are easily removed by washing. Given our requirements to prepare a range of nodularin analogues as potential protein phosphatase inhibitors, a solid-phase strategy designed to allow the introduction of structural diversity into the lipophilic side-chain of residue 1, as well as into the macrocycle itself seemed ideal.

We had consistently been able to perform macrolactamisation reactions between the N- and C-terminals of 5,1,2,3,4-pentapeptides of the type H-(2*S*)-Phe- X_{1aa} -[(2*R*)-Glu α -OMe]- γ - X_{3aa} -[(2*R*)-Asp α -OMe β -OH] where X_{1aa} is either β -alanine, (3*R*)-3-amino-4-hydroxybutyrate, or (2*R*)-aspartate α -benzyl ester (see above) and X_{3aa} is sarcosine.^{18,19,21} Since we could rationalise why some other points of disconnection had failed when macrocyclisations were attempted,¹⁸ by examining the lowest energy conformations for activated esters of the precursors, we opted to continue to employ the formation of the residue 4 to residue 5 amide bond in the macrocyclisation step. In principle, for strategies that allow both the linear peptide precursor to be constructed and the precursor to be cyclised on the resin, there were two obvious ways of connecting the peptide to the polymer support. Specifically, either through the α -carboxy group of the (2*R*)-Asp residue (No. 4) or through the α -carboxy group of the (2*R*)-Glu residue (No. 2). We opted to use the α -carboxy group of the (2*R*)-Asp residue, residue 4, for our initial experiments. We chose to incorporate a Pro residue in place of sarcosine at position 3 for three reasons. First, to increase the populations of conformers in the linear precursors which might possess proximal reacting termini. The intrinsic conformational restraint provided by the pyrrolidine ring in the Pro residue was expected to increase the ease of macrocycle formation. Second, the constraints provided by the Pro residue were expected to restrict the number of accessible conformations in the ground state of the macrocycle such that conformational analysis would be much easier. Third, our work on the differences of the two enzymes, PPI and PP2A, in the vicinity of the sarcosine binding pocket close to Cys-273 in PPI suggested that Pro derivatives at position 3 might allow differentially selective interactions with the two enzymes (see following article). As a prelude to solid-phase studies, a solution synthesis of each diastereomer of the target macrocycle, the proline epimers, **30** and **31** was undertaken. The targets contained a β -alanine residue at position 1 to facilitate comparison of the ease of macrolactamisation with the previously prepared sarcosine derivatives **3**, **5** and **27**.^{18,19,21}

Using solution-phase methods similar to those employed for the synthesis of the sarcosine containing macrocycle **3**,¹⁸ the (2*S*)-Pro containing linear peptide analogue **32** was prepared starting from Boc-(2*R*)-Asp- α -OMe- β -OBn **33** and Boc-(2*S*)-Phe- β -AlaOH **34**, both of which have been described previously.¹⁸ Thus Boc-(2*S*)-ProOH was activated as the mixed isobutyl carbonic anhydride and was treated with (2*R*)-Asp- α -OMe- β -OBn **35** (which was obtained by acidolysis of Boc-urethane **33**) to give the required dipeptide diester **36** in 78% yield. The amino protecting group of the Pro residue was removed using HCl in ethyl acetate and the resulting hydrochloride salt **37** was added to the mixed anhydride of Boc-(2*R*)-Glu- α -OMe- γ -OH,³⁸ in the presence of *N*-methylmorpholine to

give the required tripeptide **38** in 82% yield (see Scheme 6). Again the Boc protection was removed from tripeptide **38** under acidic conditions and the resulting hydrochloride salt **39** was reacted with activated Boc-(2*S*)-Phe-β-AlaOH **34** to afford the fully protected 5,1,2,3,4-pentapeptide **32** in 92% recovery. Recrystallisation from ethyl acetate–hexane gave the pure linear precursor in 62% yield. The compound **32** gave satisfactory analytical data and existed in both the *trans*- and *cis*-γ-Glu-Pro amide rotameric forms. Catalytic hydrogenolysis of the benzyl ester protection gave the free acid **40** in quantitative recovery and this was esterified with pentafluorophenol to give the triester **41** in the presence of EDCI in 62% yield after chromatography on silica. Treatment of the protected triester **41** with TFA, followed by thorough drying, and then treatment with DIPEA, as described above, gave the required macrocyclic diester **42** after 7 days at room temperature. As before, the reaction was monitored by TLC analysis. After purification by column chromatography on silica, the macrocyclic diester **42** was obtained in 52% yield, 13% from Boc-(2*S*)-ProOH. This yield is at least as good as those obtained for derivatives **3**, **5** and **27** containing sarcosine at position 3 in the macrocycle and indicates that the presence of a (2*S*)-Pro residue does not impede cyclisation.

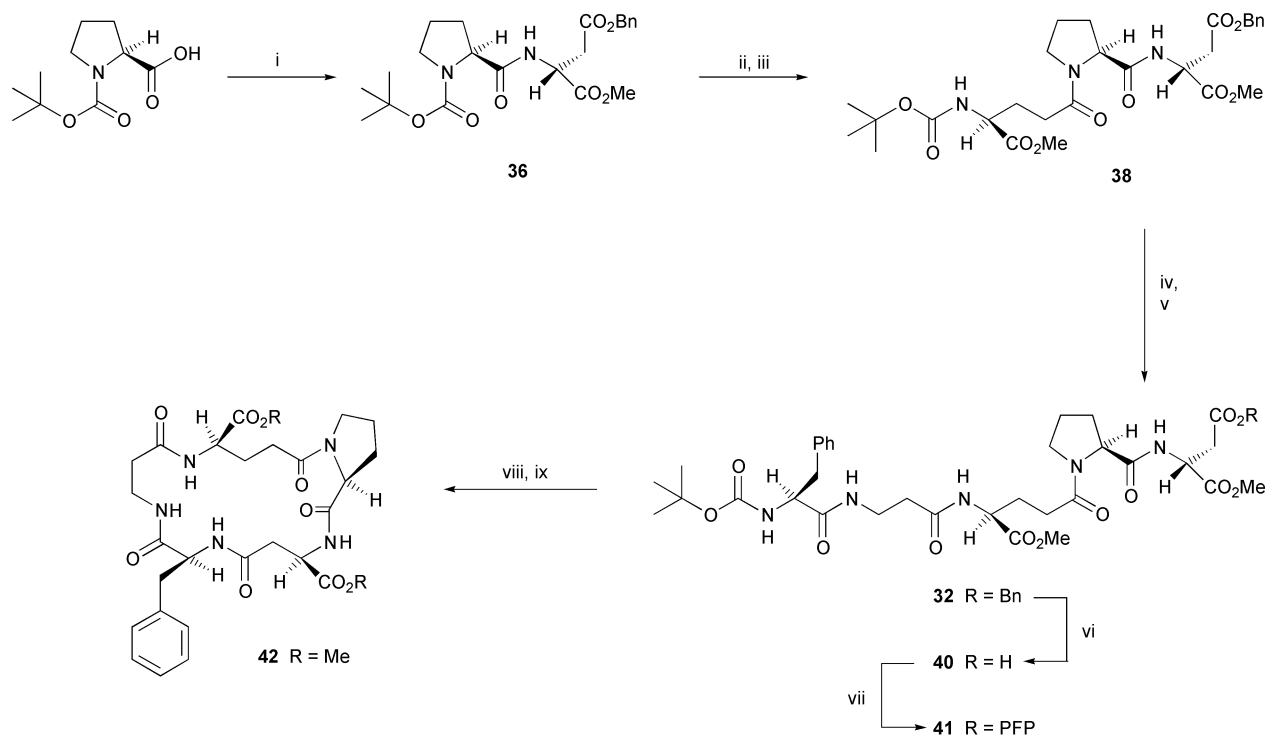
The epimer **43** (of the macrocyclic diester **42**), containing a (2*R*)-Pro residue, was prepared similarly and was obtained in 16% overall yield and the macrocyclisation step afforded 50% of the required lactam, after chromatographic purification on silica. For each macrocycle the replacement of the Sar residue by Pro allowed partial analysis of the 3-D structures of each conformer (see discussion below). After saponification of the methyl ester groups each epimer **30** and **31** ($R^3 = H$) was tested for biological activity as an inhibitor for $PP1_{cat}$, as is described below.

Having compared the influence of proline on the course of the macrocyclisation relative to sarcosine, the utilities of solid-phase synthetic approaches were assessed using the (2*S*)-Pro epimer as a model. Accordingly, the generic route (Scheme 6)

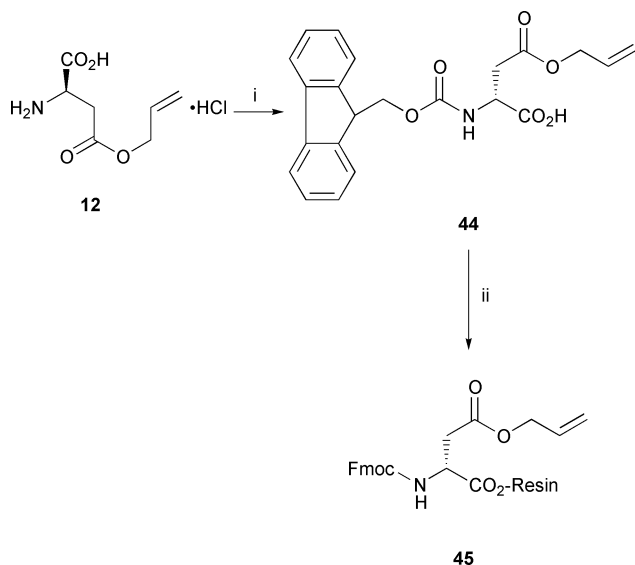
in which cyclisation was achieved through the reaction of a (2*S*)-Phe residue (No. 4) with an activated (2*R*)-Asp β-PFP ester in the linear precursor **41**,^{18,38} was adapted for use with Wang resin, Fmoc protection and benzotriazol-1-yloxytris-pyrrolidinophosphonium hexafluorophosphate (PyBOP) activation protocols.

Accordingly, *N*-Fmoc (2*R*)-Asp β-allyl ester **44** [mp 110 °C, $[\alpha]_D +3.04$ (MeOH)] was prepared in 94% yield starting from (2*R*)-Asp β-allyl ester **12**. The free α-carboxy group was activated as its 2,6-dichlorobenzoic anhydride,³⁸ and was then reacted with the 4-hydroxymethyl group of Wang resin to give the immobilised aspartate diester **45** (Scheme 7). Preparation of α-methyl *N*-fluorenylmethoxycarbonyl-(2*R*)-glutamate **49** was similar and treatment of (2*R*)-glutamic acid with chlorotrimethylsilane in allyl alcohol according to the procedure of Belshaw,³⁶ gave the γ-allyl ester hydrochloride **46**. This was sequentially *N*- and *C*-protected to give the fully protected (2*R*)-glutamate **48** which was selectively deprotected using $(Ph_3P)_4Pd(0)$ to give the required (2*R*)-glutamate derivative **49** in 53% overall yield ready for use in SPPS (Scheme 8).

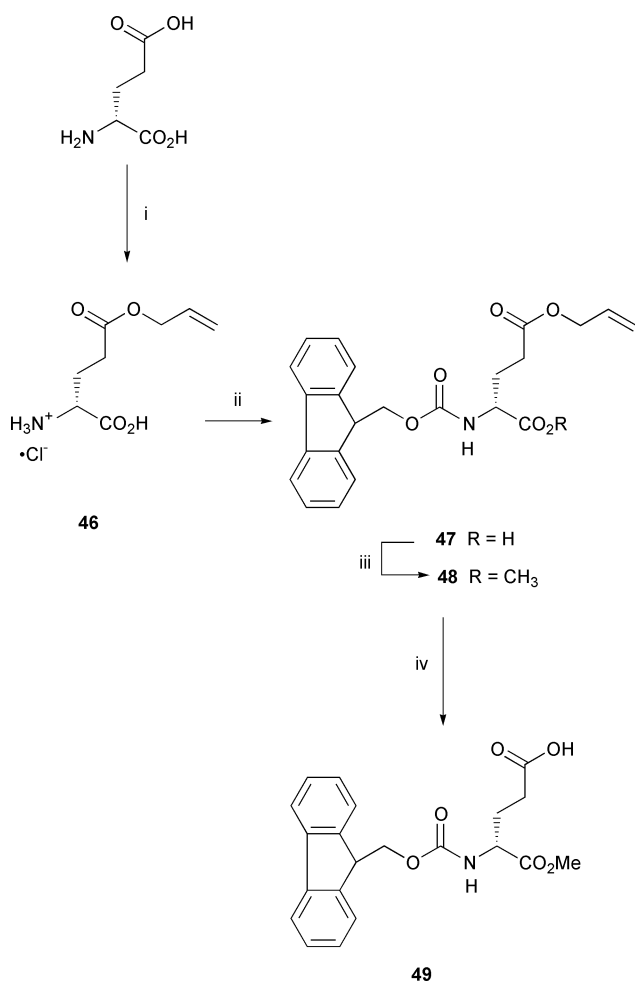
Loadings of the immobilised aspartate diester **45** ranged from 0.6 to 0.8 mmol g⁻¹ resin. Removal of the Fmoc group using 20% piperidine in DMF followed by reaction with PyBOP-activated Fmoc-(2*S*)-Pro gave the resin-bound diester **50** which was treated with piperidine to remove the Fmoc group. Fmoc-(2*R*)-Glu-α-OMe-γ-OH **49**³⁸ was activated with PyBOP then added to the free amine derived from diester **50** to give the immobilised Fmoc-tripeptide triester **51** (Scheme 9). Removal of the Fmoc group followed by reaction with PyBOP-activated Fmoc-β-alanine gave the tetrapeptide triester **52** which was deprotected and reacted with PyBOP-activated Fmoc-(2*S*)-Phe to give the Fmoc-pentapeptide triester **53**. The β-allyl ester group of Asp residue (No. 4) was unmasked using $(Ph_3P)_4Pd(0)$ to give 5,1,2,3,4-pentapeptide diester **54** ($R = Wang$) and the *N*-terminal Fmoc group of this material was removed to give the resin-bound amino acid **55** ($R = Wang$). Treatment with PyBOP and HOBt in the presence



Scheme 6 Reagents and conditions: i) IBCF, NMM, THF, DMF, -15 °C, then (2*R*)-Asp-α-OMe-β-OBn-HCl **35**, NMM, THF, DMF, -15 °C→rt, 12 h, 78%; ii) HCl_(g), EtOAc, 0 °C→rt, 4 h, 93%; iii) Boc-(2*R*)-Glu-α-OMe-γ-OH, IBCF, NMM, THF, DMF, -15 °C→rt, 12 h, 82%; iv) HCl_(g), EtOAc, 0 °C→rt, 2 h, 95%; v) Boc-(2*S*)-Phe-β-AlaOH **34**, IBCF, NMM, THF, DMF, -15 °C→rt, 12 h, 62%; vi) H₂, Pd/C, EtOH, rt, 12 h, 99%; vii) EDCI, C₆F₅OH, CH₂Cl₂, 0 °C→rt, 12 h, 62%; viii) TFA, CH₂Cl₂, rt, 45 min, 100%; ix) DIPEA, CH₂Cl₂, rt, 7 days, 52%.



Scheme 7 Reagents and conditions: i) K_2CO_3 , Fmoc-Cl, H_2O , dioxane, 94%; ii) Wang resin (0.6–0.84 mmol g^{-1}), 2,6-dichlorobenzoyl chloride, pyridine, DMF, 70% loading.



Scheme 8 Reagents and conditions: i) Me_3SiCl , allyl alcohol $0^\circ\text{C}\rightarrow\text{rt}$, 65%; ii) K_2CO_3 , Fmoc-Cl, H_2O , dioxane, 100%; iii) CH_2N_2 , EtOAc, Et_2O , 100%; iv) PhSiH_3 , $(\text{Ph}_3\text{P})_4\text{Pd}$, CH_2Cl_2 , 81%.

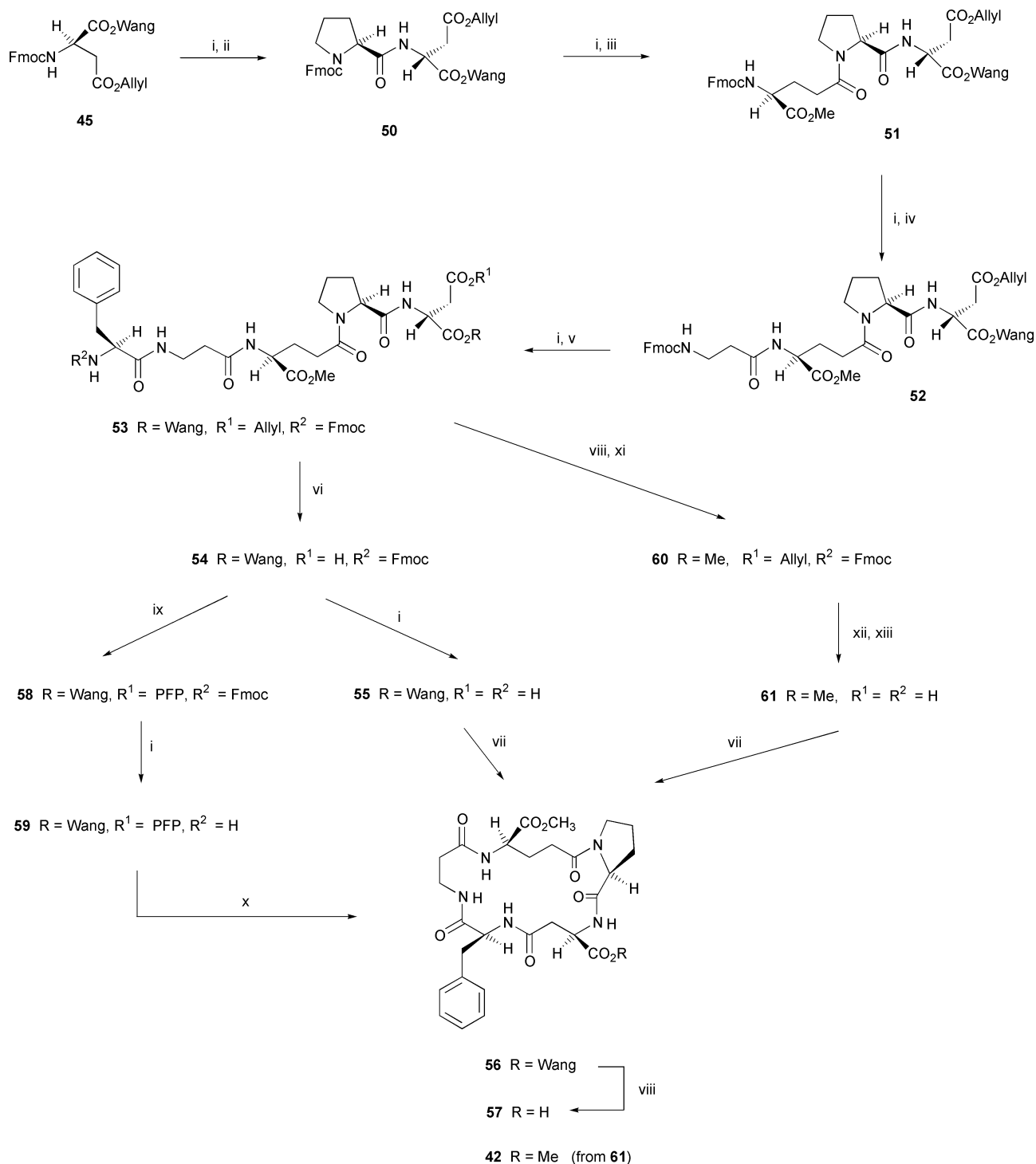
of DIPEA in DMF for 7 days gave the cyclic peptide **56** ($\text{R} = \text{Wang}$) which was removed from the resin with TFA solution to afford the crude macrocyclic monoester **57** ($\text{R} = \text{H}$) in 78% overall recovery, (Scheme 9). This displayed one major peak by reverse-phase HPLC analysis (*ca.* 50% of the total) and

several close running peaks which were subsequently removed by HPLC. The pure material (obtained in 30% yield) eluted as a single peak and gave the expected ES mass spectrum (574 Da, $[\text{M} + \text{H}]^+$). ^1H - and ^{13}C -NMR spectra recorded in DMSO and analysed using COSY, TOCSY and HSQC techniques revealed the presence of two major conformers, as expected,³⁸ corresponding to the *trans*- and *cis*-Glu- γ -Pro rotamers. Saponification of the monoester **57** ($\text{R} = \text{H}$) using sodium hydroxide in aqueous methanol gave a diacid after acidification, which displayed an identical ES mass spectrum (560 Da, $[\text{M} + \text{H}]^+$) to that of the product derived from the saponification of the diester **42** ($\text{R} = \text{Me}$). Treatment of a sample of the crude material **3** ($\text{R} = \text{H}$) with diazomethane gave the crude diester **3** ($\text{R} = \text{Me}$) which displayed several ^1H -NMR spectral signals coincident with those for the pure solution-phase synthesised material (Scheme 6) and further analysis indicated that crude **42** ($\text{R} = \text{Me}$) was *ca.* 50% pure in keeping with the HPLC analysis of the precursor **57** ($\text{R} = \text{H}$).

In an attempt to improve upon the overall yield of 30%, the resin-bound diester **54** was converted to the PFP triester **58**, through treatment with pentafluorophenol and EDCI. The triester product **58**, was treated briefly with piperidine to remove the Fmoc protection, and the free amine **59** was immediately washed (to minimise piperidine amide formation) and then treated with DIPEA to give the resin-bound macrocycle **56** ($\text{R} = \text{Wang resin}$, Scheme 9). Removal from the resin gave crude **57** ($\text{R} = \text{H}$) in 80% recovery which was of similar purity to the material **57** ($\text{R} = \text{H}$) prepared using PyBOP-activation. In order to discover the cause of the low purity of the cyclised materials, the Fmoc-pentapeptide triester **53** was removed from the resin using TFA and the free Asp α -carboxy group was methylated to give the linear pentapeptide triester **60** (Scheme 9). The crude product **60** was >90% pure and gave the expected analytical and spectroscopic data, showing that the solid-phase cyclisation itself was the cause of the problem. The allyl and Fmoc groups were then sequentially removed (Scheme 9) to give the amino peptide acid **61** ($\text{R} = \text{Me}$) which was cyclised through activation of the carboxy group with PyBOP to afford crude **42** ($\text{R} = \text{Me}$) in quantitative recovery. HPLC, MS and NMR spectroscopic analysis indicated that this material was at least 85% pure and identical to the material obtained from the solution-phase synthesis (Scheme 6). Thus, it appeared that the resin-based synthesis gives low yields for the cyclisation step, compared to the situation in solution, but offers significant advantages in the construction of the linear isopentapeptide precursor.

A solid-phase synthesis of an analogue **64** containing the isoasparagine derivative, 4-benzylpiperidyl α -aspartic acid amide (see **68f** in Scheme 11, below) at position 1 and (2*S*)-Pro at position 3 was performed using an on-resin cyclisation protocol identical to that employed in the preparation of macrolactam **57** (Scheme 10). The required material **64** was obtained in 17% overall yield, after reverse-phase HPLC purification and was saponified to give the diacid **65**, for biological evaluation, see below. Indeed, several isoasparagine analogues **66a–g** were prepared starting from β -allyl *N*-fluorenylmethoxycarbonyl-(2*R*)-aspartate **44** (Scheme 11). Some of these are described below together with phenyl substituted (3-*R/S*)-*N*-Fmoc-3-amino-3-phenylpropanoic acids **67** ($\text{R} = \text{H}$, OMe, Br) (Scheme 12).

Preparation of the isoasparagine analogues was achieved by the slow addition of a solution of the appropriate amine to a solution of the mixed isobutyl carbonic anhydride derived from β -allyl *N*-fluorenylmethoxycarbonyl-(2*R*)-aspartate **44** in dry THF at 0°C . Although the fluorenylmethoxycarbonyl protecting group is base sensitive, it was found that under carefully controlled conditions good yields of fully protected *N*-substituted and *N,N*-disubstituted isoasparagines **66a–g** could be prepared. Selective removal of the allyl ester was achieved by the procedure of Guibe and co-workers³⁹ using tetrakis

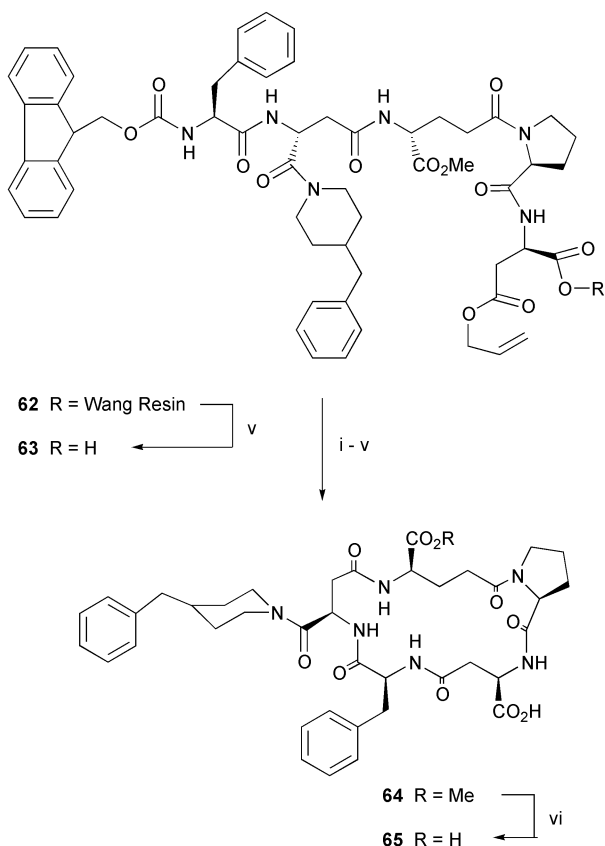


Scheme 9 Reagents and conditions: i) 20% piperidine in DMF; ii) Fmoc (2S)-Pro, PyBOP, DMF; iii) Fmoc (2R)-Glu- α -OMe- γ -OH **49**, PyBOP, DMF; iv) Fmoc- β -Ala, PyBOP, DMF; v) Fmoc (2S)-Phe, PyBOP, DMF; vi) (Ph₃P)₄Pd(0), DMSO, THF, 0.5 M HCl, NMM (2:2:1:0.1), pH 6; vii) PyBOP, HOBT, DIPEA, DMF, 7 days; viii) CH₂Cl₂-TFA-H₂O-TES (53:40:5:2); ix) C₆F₅OH, EDCl, CH₂Cl₂; x) DIPEA, CH₂Cl₂; xi) CH₂N₂, EtOAc, Et₂O, 100%; xii) PhSiH₃, (Ph₃P)₄Pd(0), CH₂Cl₂; xiii) 1.5 eq. piperidine, CH₂Cl₂.

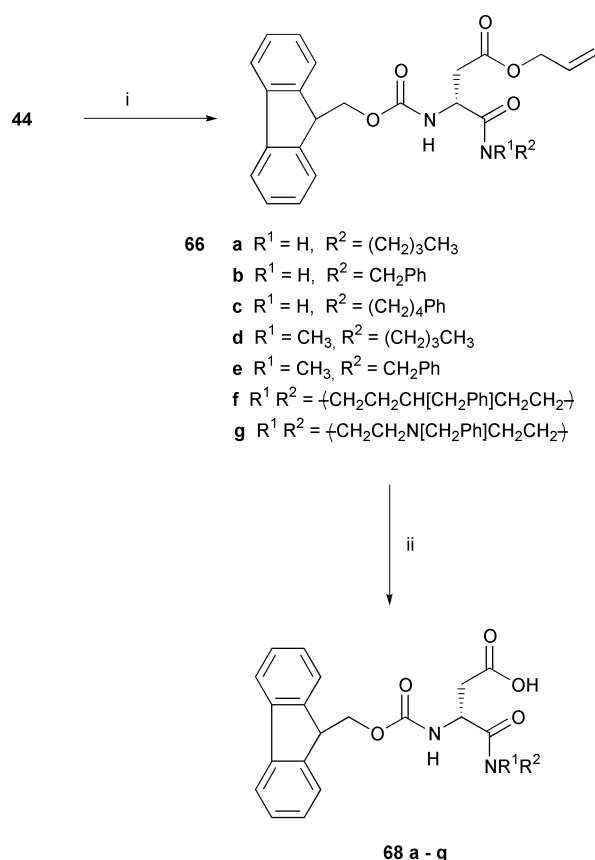
(triphenylphosphine)palladium(0) and phenylsilane in dichloromethane and gave the required (2R)-isoasparagines **68a–g**, (Scheme 11).

The 3-amino-3-phenylpropanoic acids **69** (R = H, OMe, Br) were each prepared using Rodionov chemistry to form the parent β -amino acid^{40–42} (Scheme 12). Again, it was expected that such Adda surrogates would provide information on the role of the Adda residue in binding to the enzymes. However, the poor yields obtained for the desired macrocycles **57** and **64** prepared using the on-resin cyclisation protocol prompted a final examination of the most useful preparative strategy for analogues before fully addressing the requirements for biological activity and selectivity.

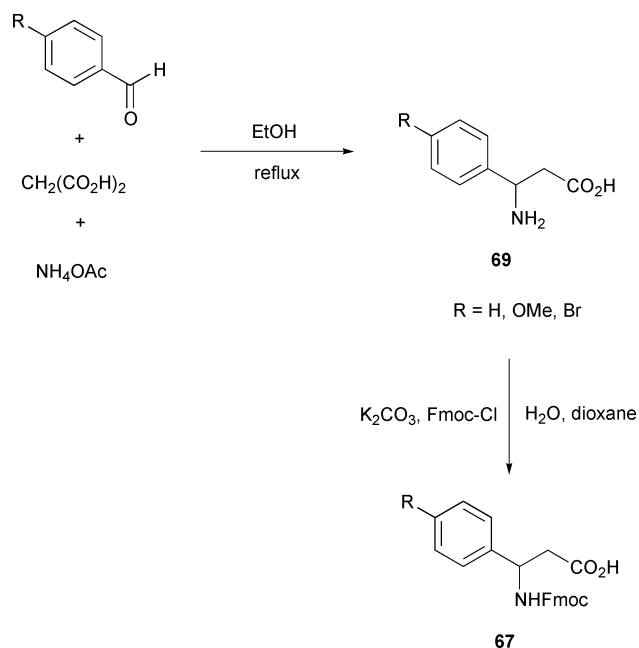
Given the problems associated in using the on-resin cyclisation strategy in which the α -carboxy group of the Asp residue was used to attach the growing peptide to the resin, attention was turned to the solid-phase preparation of appropriate linear 5,1,2,3,4-pentapeptides for solution-phase lactamisation. We wished to form the 5–4 amide bond last, in the cyclisation step, because we were confident that the reaction should work on the basis of our previous results^{18,38} (see above). Since we had already assessed use of the Asp α -carboxy group in attaching the growing polypeptide to the resin in the preparation of the linear pentapeptide **53** (see above), we reconsidered using the Asp β -carboxy group for attachment. This option became viable because it was no longer necessary to be able to activate



Scheme 10 Reagents and conditions: i) Pd(PPh₃)₄, DMSO–THF–0.5 M HCl–NMM (2:2:1:0.1), pH 6; ii) PFP, EDCl, CH₂Cl₂, 18 h; iii) 20% piperidine–DMF; iv) DIPEA, CH₂Cl₂; v) CH₂Cl₂–TFA–H₂O–TES (53:40:5:2), 17% from **45**; vi) 1 M NaOH, MeOH, 83%.



Scheme 11 Reagents and conditions: i) IBCF, NMM, THF, –40 °C, then R¹R²NH, THF, 0 °C → rt, 74–85%; ii) PhSiH₃, (Ph₃P)₄Pd, CH₂Cl₂, 54–87%.

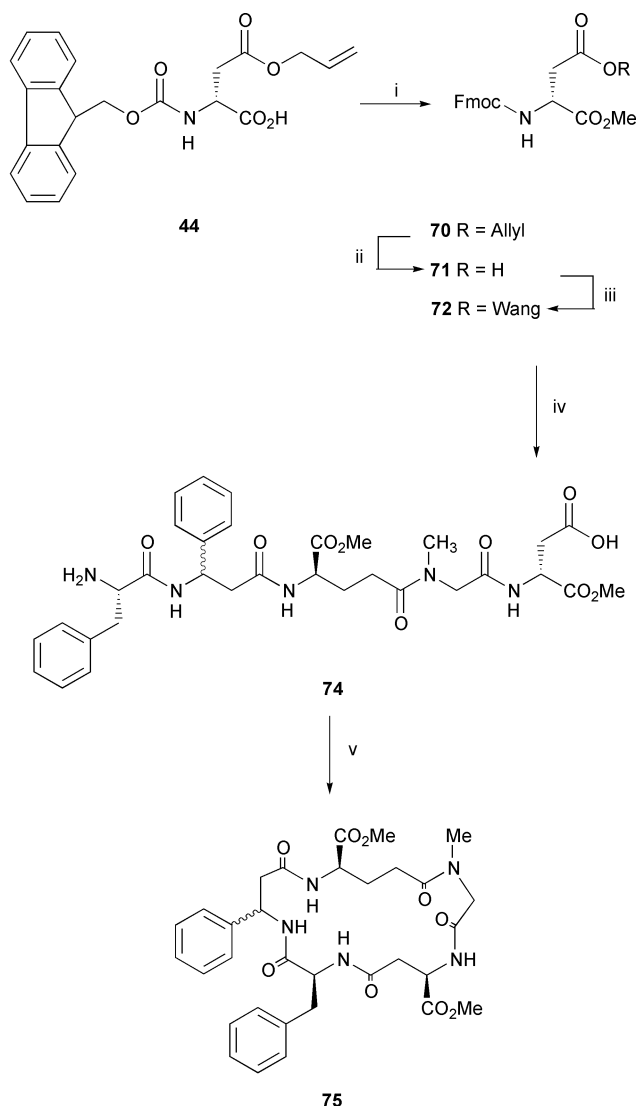


Scheme 12

the β-carboxy group to facilitate cyclisation whilst the peptide remained bound to the resin. Such a strategy also allows the resin alcohol to be used as a protecting group, thus reducing the number of preparative steps, where removal of the pentapeptide from the resin would unmask the β-carboxy group for subsequent macrolactamisation. In order to remove the protection at the α-carboxy groups of (2*R*)-Glu and (2*S*)-Asp residues after cyclisation, to allow biological evaluation, it seemed expedient to use the same ester protecting groups and it appeared that methyl esters would be most useful.

Accordingly, to anchor the β-carboxy group, β-allyl *N*-fluorenylmethoxycarbonyl-(2*R*)-aspartate **44** was treated with diazomethane to furnish the fully protected aspartate diester **70**. Selective removal of the allyl ester was achieved as before³⁹ to give α-methyl *N*-fluorenylmethoxycarbonyl-(2*R*)-aspartate ester **71** in 60% yield after purification by column chromatography. The compound was attached to the hydroxymethyl derivative of Wang resin, using the method of Sieber,⁴³ as for the α-acid β-ester **46** above, to give the required Fmoc-protected resin-bound aspartate diester **72** (loading efficiency *ca.* 80%) (Scheme 13).

In order to benchmark the Asp-β-resin ester preparative strategy, we chose to use a prototype possessing a (3*R/S*)-3-amino-3-phenylpropanoic acid residue at position 1 and a Sar residue at position 3. Hence, resin-bound aspartate **72** was treated with piperidine and the resulting free amine was treated with PyBOP activated Fmoc SarOH using standard SPPS protocols. The remaining activated Fmoc amino acids were added to the growing polypeptide chain in reverse sequence and the completed 5,1,2,3,4-polypeptide **73** was treated with piperidine to remove the N-terminal protection and then cleaved from the resin using TFA. The required pentapeptide (2*S*)-Phe-(3*R/S*)-3-amino-3-phenylpropanoyl-[(2*R*)-Glu α-OMe]-γ-Sar-[(2*R*)-Asp α-OMe]-β-OH **74** was obtained after precipitation from methanolic ether in quantitative recovery and displayed the expected signals in its ¹H- and ¹³C-NMR spectra (Scheme 13). The linear pentapeptide was cyclised in DCM–DMF (9:1) using DIPEA and BOP-Cl over a period of 7 days to give the required macrocycle **75** in 24% overall yield after an aqueous work-up and precipitation with ether. NMR spectral data indicated that each diastereomer (epimeric at C-3 of the 3-amino-3-phenylpropanoic acid residue) existed in at least two conformational/rotoisomeric forms, as was expected.



Scheme 13 Reagents and conditions: i) CH_2N_2 , EtOAc, Et₂O, 100%; ii) PhSiH_3 , $(\text{Ph}_3\text{P})_4\text{Pd}$, CH_2Cl_2 , 60%; iii) Wang resin (0.6–0.84 mmol g^{-1}), 2,6-dichlorobenzoyl chloride, pyridine, DMF, 80% loading; iv) SPPS, followed by cleavage of the pentapeptide from the resin; v) CH_2Cl_2 –DMF (9:1), DIPEA, BOP-Cl, 7 days, 24%.

Importantly, this new synthetic protocol gave comparatively pure products in good overall yield and for two other reasons was selected as the method of choice. First, the linear pentapeptide **74** and the cyclised derivative **75** did not require chromatographic purification in order to assess purity, and potentially could be screened for activity after the saponification of the two methyl esters, but before full characterisation. Second, this linear protocol did not require any post-macrocycle elaboration. We had already demonstrated that exocyclic elaborations did not proceed in good yield, for example, in the case of the elaboration of the Asp α -benzyl ester precursor **27** to give the amide **28**, as described above.

Biological activity

The four macrocyclic diacids, **29**, **30** ($\text{R}^3 = \text{H}$), **31** ($\text{R}^3 = \text{H}$) and **65** were tested for biological activity against PP1_{cat} using the substrate Lys-Arg-Thr(P)-Ile-Arg-Arg-OH at 25 °C and the malachite green assay for inorganic phosphate release.^{44,45} [Full details on the activity assay of PP1 and PP2A are given in the third paper in this series in this issue together with a description of a new assay procedure and a comparison with other procedures.] Neither of the two macrocycles (**30** and **31**; $\text{R}^3 = \text{H}$) possessing β -alanine residues displayed any activity but the two 4-benzylpiperidyl amide derivatives (**29** and **65**) gave IC_{50} values

of 2.9 and 2.7 mM for the systems containing a Sar and (2*S*)-Pro residue at position 3, respectively. These latter values are not significantly different and errors in the determinations are *ca.* $\pm 20\%$. Thus, the presence of a lipophilic side chain in the Adda surrogate confers some activity to an otherwise completely inactive macrocycle. Moreover, the similar IC_{50} values obtained for systems containing Sar and (2*S*)-Pro allows two important conclusions. First, that for PP1 there is sufficient room at the site close to the *N*-methyldehydrobutyrine residue in the bound nodularin–enzyme complex to accommodate a (2*S*)-Pro residue. Second, that the conformational restraint provided by the pyrrolidine ring does not cause the macrocycle to alter to a form that cannot bind to the enzyme. Both of these results are consistent with models for nodularin binding that mirror those that occur in the X-ray crystal structure of PP1_{cat} –microcystin complexes;^{10,11} except that there is no covalent bond between the dehydro amino acid residue, a potential Michael acceptor, and the thiol group of Cys-273. Such models indicate that the 2-methyl group in the Adda residue plays a role in controlling the conformational structure of the macrocycle and in binding to the protein in a hydrophobic pocket. It also appears that the reason that phosphothreonine containing substrates are better than those containing phosphoserine,^{46,47} derives, at least in part, from the presence of a methyl group binding pocket. Such an arrangement is consistent with recent mechanistic proposals where it is demonstrated that water attacks the phosphate ester directly, in a ternary complex mechanism⁴⁸ and with X-ray crystal data on the structure of an enzyme–tungstate complex.¹¹

None of the macrocyclic compounds described here exist in a single rotoisomeric form. Moreover, any correlation of 3-D structure with biological activity requires at least some analysis of the population distributions of conformers for each rotoisomer. The effect of the 2-methyl group at position 1 on the conformational preferences and the macrocycle and on biological activity are described in the following article,⁴⁹ using the general synthetic methods described here. In a third paper we describe a new, sensitive and reliable assay that is suitable for use with both PP1 and PP2A and also both other major Ser-Thr protein phosphatase enzyme classes.⁵⁰

Experimental

Elemental microanalyses were performed in the departmental micro-analytical laboratory. NMR spectra were recorded on a Bruker AM-300 spectrometer (¹H, 300 MHz; ¹³C, 75.4 MHz; ³¹P, 121.5 MHz; ¹⁹F, 282.3 MHz), Varian Gemini 200 spectrometer (¹H, 200 MHz; ¹³C, 50.3 MHz), Varian Gemini 300 spectrometer (¹H, 300 MHz; ¹³C, 75.4 MHz) and a Varian Unity Plus 500 spectrometer (¹H, 500 MHz; ¹³C, 125.6 MHz). Chemical shifts are described in parts per million downfield shift from SiMe₄ and are reported consecutively as position (δ_{H} or δ_{C}), relative integral, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = double of doublets, sep = septet, m = multiplet, and br = broad), coupling constant (*J*/Hz) and assignment (numbering according to the IUPAC nomenclature for the compound). ¹H-NMR were referenced internally on ²H₂O (δ 4.68), CHCl₃ (δ 7.27), C²H₃O²H (δ 3.35) or (CH₃)₂SO [δ 2.47]. ¹³C-NMR were referenced on C²HCl₃ (δ 77.5), CH₃OH (δ 49.15) or (CH₃)₂SO [δ 39.70] and ³¹P NMR spectra to external H₃PO₄ (δ 0). Pyrrolidine ring carbons and hydrogens are assigned in NMR spectra as α , β , γ , δ , going anticlockwise from the ring nitrogen, according to normal convention. Where more than one conformational isomer can be detected in the NMR spectrum due to the presence of a tertiary amide moiety, these are assigned as *c* (*cis*) or *t* (*trans*), according to the isomeric state of the amide bond. IR spectra were recorded on a Perkin-Elmer 1710 or a Nicolet Avatar 360 FT-IR spectrometer. The samples were prepared as KBr discs, Nujol mulls, solutions in chloroform or thin films between sodium chloride

discs. The frequencies (ν) as absorption maxima are given in wavenumbers (cm^{-1}) relative to a polystyrene standard. Mass spectra and accurate mass measurements were recorded on a VG 70-250 SE, a Kratos MS-50 or by the EPSRC service at Swansea using a VG AZB-E. Fast atom bombardment spectra were recorded using glycerol as a matrix. Major fragments are given as percentages of the base peak intensity (100%). Flash chromatography was performed according to the method of Still *et al.*⁵¹ using Fluka Kieselgel C60 (4–60 μm mesh) silica gel. Analytical thin layer chromatography was carried out on 0.25 mm pre-coated silica gel plates (Whatman PE SIL G/UV) and compounds were visualised using UV fluorescence, iodine vapour, ethanolic phosphomolybdic acid, aqueous potassium permanganate or ninhydrin. Melting points were taken on an Electrothermal melting point apparatus and are uncorrected. Optical rotations were measured at 23 °C on a Optical Activity AA-1000 polarimeter using 10 or 20 cm path length cells and are given in units of 10^{-1} deg $\text{cm}^2 \text{g}^{-1}$.

Preparative RP HPLC was carried out using a PerSeptive BioCAD™ SPRINT™ Perfusion® Chromatography System. Preparative RP HPLC was performed on a Luna C-18(2) 10 μm column (150 \times 21.2 mm) or on a Luna C-18(2) 10 μm column (250 \times 21.2 mm) fitted with a Luna C-18(2) 10 μm column (60 \times 21.2 mm).

Protected amino acid precursors were purchased from Calbiochem-Novabiochem (UK) Ltd (Beeston, Nottingham). All other chemicals were of analytical grade or were recrystallised or redistilled before use. The solvents used were either distilled or of Analar quality and petroleum ether refers to that portion boiling between 40–60 °C. Solvents were dried according to literature procedures. Ethanol and methanol were dried using magnesium turnings. DMF, CH_2Cl_2 , diisopropylamine and triethylamine were distilled over CaH_2 . THF and diethyl ether were dried over sodium–benzophenone and distilled under nitrogen. Thionyl chloride was distilled over sulfur and the initial fractions were always discarded. *N*-Methylmorpholine was distilled over ninhydrin.

General solid-phase synthesis and peptide removal from Wang resin

Solid-phase synthesis of the pentapeptides described below were carried out using the Rainin PPS automated peptide synthesiser. The syntheses employed Fmoc chemistry and the C-terminal amino acid residues were linked to β -allyl (2*R*)-*N*-(fluoren-9-ylmethoxycarbonyl)aspartyl-Wang resin **45** or to α -methyl (2*R*)-*N*-(fluoren-9-ylmethoxycarbonyl)aspartyl-Wang resin **72**. Amino acids and the activating agent PyBOP were purchased from Novabiochem chemicals, the solvents DMF, piperidine and *N*-methylmorpholine from Sigma-Aldrich. A four-fold excess of the amino acid was used for each coupling procedure. The *N*- α -Fmoc protecting group from the growing resin-bound peptide was removed using a 20% piperidine–DMF solution and the carboxy group was activated in the presence of 5% NMM in DMF solution. Double couplings were performed for (2*S*)-proline residues. The peptides were cleaved from the resin using a mixture of TFA–TES– H_2O – CH_2Cl_2 (TES = triethylsilane). Trituration with diethyl ether, followed by lypholisation gave the required peptides as fluffy white powders.

General cyclisation procedure using DIPEA and PFP activation

To a stirred solution of the pentapeptide activated ester (75 mg, 0.087 mmol) in CH_2Cl_2 (3 cm^3), was added trifluoroacetic acid (3 cm^3). The reaction was allowed to stir at room temperature for 1 h, after which time no starting material was detected by TLC analysis. The reaction mixture was concentrated under reduced pressure to yield a colourless oil. The residue was dissolved in CH_2Cl_2 (200 cm^3), treated with DIPEA (210 mm^3), and was stirred at room temperature for

7 days. The reaction mixture was concentrated under reduced pressure, redissolved in ethyl acetate (30 cm^3), and was washed with 1 mol dm^{-3} HCl (3 \times 20 cm^3), 5% NaHCO_3 (2 \times 20 cm^3), water (2 \times 20 cm^3) and then brine (1 \times 20 cm^3). The organic phase was dried (MgSO_4) and the solvent was removed under reduced pressure to give a colourless oil. The residue was purified by flash silica column chromatography and then by reverse-phase (RP) HPLC, as described below for each macrocyclic diester.

β -Allyl (2*R*)-aspartate ester hydrochloride **12**

Method A. Acetyl chloride (7.0 cm^3 , 97 mmol) was added dropwise to ice-cold allyl alcohol (50 cm^3). The resulting solution was stirred at 0 °C for 15 min and then at room temperature for 1 h. (2*R*)-Aspartic acid (3.33 g, 25 mmol) was added in a single portion and the suspension stirred for 18 h and then poured into ice-cold diethyl ether (250 cm^3). After stirring at 0 °C for 1 h the precipitate was collected by filtration and was washed on the pad with diethyl ether to give the hydrochloride **12** as a white powder (4.66 g, 89%), mp 176–178 °C (lit.,³⁵ 185–186 °C) (Found C, 39.85; H, 5.5; N 6.6. $\text{C}_7\text{H}_{12}\text{O}_4\text{NCl}$ requires C, 40.1; H, 5.75; N, 6.7%); ν_{max} (Nujol)/ cm^{-1} 1756 (CO acid) and 1727 (CO ester); δ_{H} (200 MHz; $^2\text{H}_2\text{O}$) 3.13 (1 H, dd, *J* 5.1 and 18.1, 1 H of β - CH_2), 3.17 (1 H, dd, *J* 5.7 and 18.1, 1 H of β - CH_2), 4.57 (1 H, t, *J* 5.4, α -H), 4.65 (2 H, d, *J* 5.7, OCH_2 -vinyl), 5.24–5.38 (2 H, m, vinyl CH_2) and 5.84–6.03 (1 H, m, vinyl CH); δ_{C} (50.3 MHz; $^2\text{H}_2\text{O}$) 36.80 (β - CH_2), 52.06 (α -C), 69.57 (OCH_3), 121.77 (vinyl CH_2), 134.19 (vinyl CH), 173.60 and 173.90 (CO); *m/z* (EI) 174 (10%, $[\text{M} - \text{Cl}]^+$), 132 (9, $[\text{M} - \text{HCl} - \text{C}_3\text{H}_6]^+$), 128 (100, $[\text{M} - \text{HCl} - \text{H}_2 - \text{CO}_2]^+$), 74 (46) and 41 (74, C_3H_5^+).

Method B. To a stirred suspension of (2*R*)-aspartic acid (5.33 g, 40 mmol) in dry allyl alcohol (30 cm^3) under N_2 , was added dropwise chlorotrimethylsilane (15.23 cm^3 , 120 mmol) and stirring was continued for 18 h. Ice-cold diethyl ether (75 cm^3) was then added and the precipitated product was collected to afford a white crystalline solid (7.3 g, 87%), mp 184–185 °C [lit.,³⁷ 185–186 °C (for the (2*S*)-isomer)]; $[\alpha]_{\text{D}} +7.7$ (*c* 8.0 in AcOH) [lit.,³⁷ –9.5 (*c* 8.0 in AcOH) (for the (2*S*)-isomer)]; ν_{max} (CH_2Cl_2)/ cm^{-1} 3364 (NH), 2965 (CH), 1751 (CO, ester) and 1536 (amide); δ_{H} (300 MHz; $^2\text{H}_2\text{O}$) 3.03–3.06 (2 H, m, β - CH_2), 4.27 (1 H, t, *J* 4.8, α -H), 4.60 (2 H, d, *J* 6, CH_2O), 4.65 (3 H, s, NH_3^+), 5.15–5.27 (2 H, m, $\text{CH}_2=\text{CH}$) and 5.77–5.90 (1 H, m, $\text{CH}_2=\text{CH}$); δ_{C} (75.4 MHz; $^2\text{H}_2\text{O}$) 34.05 (β - CH_2), 49.37 (α -C), 66.78 (CH_2O), 119.00 ($\text{CH}_2=\text{CH}$), 131.47 ($\text{CH}_2=\text{CH}$), 170.94 (CO, ester) and 171.19 (CO, acid); *m/z* (CI) 174 (100%, $[\text{M} + \text{H} - \text{HCl}]^+$).

β -Allyl (2*R*)-*N*-(*tert*-butoxycarbonyl)aspartate ester **13**

Triethylamine (6.0 cm^3 , 4.36 g, 42 mmol) was added dropwise to a stirred solution of di-*tert*-butyl dicarbonate (3.92 g, 18 mmol) and β -allyl (2*R*)-aspartate ester hydrochloride **12** (3.14 g, 15 mmol) in water (25 cm^3) and dioxane (25 cm^3). After 18 h the solution was extracted with petroleum ether (2 \times 50 cm^3) and the aqueous phase was cooled on ice and carefully acidified to pH 3 by slow addition of 10% citric acid solution. The urethane was then extracted into ethyl acetate (3 \times 50 cm^3) and the combined extracts were washed with brine (2 \times 25 cm^3), then dried (MgSO_4), filtered and concentrated under reduced pressure to give the *N*-(*tert*-butoxycarbonyl)aspartate ester **13** as a pale yellow oil (3.75 g, 91%) (HRMS: found $[\text{M} + \text{H}]^+$, 274.1275. $\text{C}_{12}\text{H}_{20}\text{NO}_6$ requires 274.1291); ν_{max} (neat)/ cm^{-1} 3364 br (NH and OH) and 1737 (CO); δ_{H} (300 MHz; C^2HCl_3) 1.41 [9 H, s, (CH_3)₃], 2.87 (1 H, dd, *J* 4.9 and 17.0, 1 H of β - CH_2), 3.01 (1 H, dd, *J* 4.4 and 17.0, 1 H of β - CH_2), 4.52–4.62 (3 H, m, OCH_2 -vinyl and α -H), 5.19–5.32 (2 H, m, vinyl CH_2), 5.59 (1 H, d, *J* 8.8, NH), 5.80–5.93 (1 H, m, vinyl CH) and 10.47 (1 H, br s, OH); δ_{C} (75.4 MHz; C^2HCl_3) 28.10 [$(\text{CH}_3)_3$], 36.43 (β - CH_2),

49.69 (α -C), 65.65 (OCH₂), 80.34 (C-O), 118.66 (vinyl CH₂), 131.65 (vinyl CH), 155.66 (CO, urethane), 170.94 and 175.60 (CO); *m/z* (FAB) 296 (7%, [M + Na]⁺), 274 (20, [M + H]⁺), 218 (90, [M + H - C₃H₄O]⁺) and 174 (100, [M + H - C₅H₈O₂]⁺).

β -Allyl α -methyl (2*R*)-*N*-(*tert*-butoxycarbonyl)aspartate diester 14

To a cooled stirred solution of β -allyl (2*R*)-*N*-(*tert*-butoxycarbonyl)aspartate **13** (2.73 g, 10 mmol) in diethyl ether (20 cm³) was added dropwise excess ethereal diazomethane. After 1 h, the solution was purged with nitrogen to remove the excess of diazomethane and was then concentrated under reduced pressure to give the aspartate diester **14** as a colourless oil (2.69 g, 94%) (HRMS: found [M + H]⁺, 288.1438. C₁₃H₂₂NO₆ requires 288.1447); ν_{\max} (neat)/cm⁻¹ 3375 (NH), 1719 (CO ester) and 1650 (CO urethane); δ_{H} (200 MHz; C²HCl₃) 1.43 [9 H, s, (CH₃)₃], 2.86 [1 H, dd, *J* 4.7 and 17.0, 1 H of β -CH₂ (Asp)], 2.99 [1 H, dd, *J* 4.7 and 16.9, 1 H of β -CH₂ (Asp)], 3.74 (3 H, s, OCH₃), 4.52–4.65 (3 H, br d, *J* 5.7, α -H and OCH₂-vinyl), 5.19–5.35 (2 H, m, vinyl CH₂), 5.49 (1 H, d, *J* 8.5, NH) and 5.78–5.98 (1 H, m, vinyl CH); δ_{C} (50.3 MHz; C²HCl₃) 28.75 [(CH₃)₃], 37.28 (β -CH₂), 50.40 (OCH₃), 53.15 (α -C), 66.09 (OCH₂-vinyl), 119.09 (vinyl CH₂), 132.15 (vinyl CH), 156.00 (CO, urethane), 171.50 and 172.10 (CO, ester); *m/z* (FAB) 288 (42%, [M + H]⁺), 232 (100, [M + H - C₃H₄O]⁺) and 188 (85, [M + H - C₅H₈O₂]⁺).

β -Allyl α -methyl (2*R*)-aspartate diester hydrochloride 15

Dry ethyl acetate (100 cm³) was saturated with hydrogen chloride gas at 0 °C and after stirring for 1 h, a solution of β -allyl α -methyl (2*R*)-*N*-(*tert*-butoxycarbonyl)aspartate diester **14** (2.69 g, 9.4 mmol) in ethyl acetate (50 cm³) was added. The reaction mixture was stirred for 1.5 h at room temperature and the resulting solution was concentrated under reduced pressure to give the required hydrochloride salt **15** as a pale yellow solid (1.89 g, 96%), mp 113–114 °C; ν_{\max} (Nujol)/cm⁻¹ 1761 and 1722 (CO ester); δ_{H} (300 MHz; ²H₂O) 3.05 (1 H, dd, *J* 4.9 and 18.1, 1 H of β -CH₂), 3.11 (1 H, dd, *J* 5.8 and 18.1, 1 H of β -CH₂), 3.71 (3 H, s, OCH₃), 4.39 (1 H, dd, *J* 4.9 and 5.8, α -H), 4.55 (2 H, d, *J* 5.5, OCH₂-vinyl), 5.15–5.26 (2 H, m, vinyl-CH₂) and 5.76–5.89 (1 H, m, vinyl-CH); δ_{C} (75.4 MHz; ²H₂O) 33.73 (β -CH₂), 49.06 (OCH₃), 53.88 (α -C), 66.75 (OCH₂-vinyl), 119.08 (vinyl-CH₂), 131.73 (vinyl-CH), 169.47 (CO) and 170.95 (CO); *m/z* (FAB) 188 (100%, [M + H - HCl]⁺) and 128 (14, [M - HCl - CO₂CH₃]⁺).

β -Allyl (2*R*)-*N*-(*tert*-butoxycarbonyl)-[α -methyl glutamyl]- γ -sarcosyl-[α -methyl (2*R*)-aspartate] triester 17

To a stirred solution of *N*-(*tert*-butoxycarbonyl)-[α -methyl (2*R*)-glutamyl]- γ -sarcosine **16** (3.22 g, 10 mmol) in dry THF (50 cm³) at -40 °C was added *N*-methylemorpholine (NMM) (1.10 cm³, 10 mmol). Isobutyl chloroformate (IBCF) (1.375 cm³, 10 mmol) was then added and the suspension was stirred -15 °C for 5 min. A mixture of β -allyl α -methyl (2*R*)-aspartate diester hydrochloride **15** (2.24 g, 10 mmol) and NMM (1.10 cm³, 10 mmol) in dry THF (50 cm³) was then added. The reaction mixture was allowed to warm to room temperature and then stirred for a further 3 h. The hydrochloride salts were removed by filtration and the solution was concentrated under reduced pressure to give a yellow oil. The residue was re-dissolved in ethyl acetate (120 cm³), and the solution was washed successively with water (40 cm³), 10% citric acid (40 cm³), 5% sodium bicarbonate (40 cm³) and brine (40 cm³) and then was dried (MgSO₄), and concentrated under reduced pressure. The pale yellow oil was purified by flash chromatography on silica eluting with ethyl acetate to give the fully protected tripeptide **17** as a colourless, viscous oil (3.47 g, 69%) (HRMS: found [M + H]⁺, 502.2426. C₂₂H₃₆N₃O₁₀ requires

502.2401); ν_{\max} (neat)/cm⁻¹ 3338 br (NH), 1747 (CO ester), 1693 (CO amide) and 1645 (CO urethane); δ_{H} (300 MHz; C²HCl₃) 1.36 [9 H, s, (CH₃)₃], 1.81–1.98 [1 H, m, 1 H of β -CH₂ (Glu)], 2.22–2.36 [2 H, m, 1 H of β -CH₂ (Glu) and 1 H of γ -CH₂ (Glu)], 2.42–2.50 [1 H, m, 1 H of γ -CH₂ (Glu)], 2.84–2.93 [2 H, m, β -CH₂ (Asp)], 3.02 (3 H, s, NCH₃), 3.66 (3 H, s, OCH₃), 3.68 (3 H, s, OCH₃), 3.76 [1 H, d, *J* 15.9, 1 H of CH₂ (Sar)], 4.23–4.28 [1 H, m, α -H (Glu)], 4.31 [1 H, d, *J* 16.2, 1 H of CH₂ (Sar)], 4.52 (2 H, d, *J* 5.5, OCH₂-vinyl), 4.84–4.88 [1 H, m, α -H (Asp)], 5.16–5.27 (2 H, m, vinyl-CH₂), 5.37 [1 H, d, *J* 8.2, NH (Glu)], 5.77–5.90 (1 H, m, vinyl-CH) and 7.17 [1 H, d, *J* 8.2, NH (Asp)]; δ_{C} (75.4 MHz; C²HCl₃) 28.51 [(CH₃)₃], 28.97 [β -CH₂ (Glu)], 35.52 (NCH₃), 36.32 [γ -CH₂ (Glu)], 36.72 [β -CH₂ (Asp)], 48.74 [CH₂ (Sar)], 52.04 [α -C (Glu)], 52.72 (OCH₃), 52.80 (OCH₃), 52.99 [α -C (Asp)], 65.97 (OCH₂-vinyl), 80.25 [C(CH₃)₃], 118.94 (vinyl-CH₂), 132.18 (vinyl-CH), 156.15 (CO, urethane) and 169.20, 170.77, 171.47, 173.09 and 173.45 (CO); *m/z* (FAB) 524 (2%, [M + Na]⁺), 502 (4, [M + H]⁺), 402 (100, [M + 2H - C₅H₉O₂]⁺), 259 (85, [M + 2H - C₁₁H₁₈NO₃]⁺), 215 (41, C₁₀H₁₇NO₄⁺), 188 (53, C₈H₁₄NO₄⁺) and 144 (47, C₆H₁₀NO₃⁺).

β -Allyl (2*R*)-[α -methyl glutamyl]- γ -sarcosyl-[α -methyl (2*R*)-aspartate] triester hydrochloride 18

The deprotected hydrochloride **18** was prepared in a manner identical with that for the hydrochloride **15**, by using the Boc-protected tripeptide **17** (2.51 g, 5 mmol) to give the salt **18** as a hygroscopic white solid (2.18 g, 100%), mp 52–54 °C; δ_{H} (300 MHz; C²H₃O²H) 2.10–2.33 [2 H, m, β -CH₂ (Glu)], 2.60–2.76 [2 H, m, γ -CH₂ (Glu)], 2.84–3.02 [2 H, m, β -CH₂ (Asp)], 2.94 and 3.09 (3 H, 2 × s, NCH₃), 3.73 (3 H, s, OCH₃), 3.85 (3 H, s, OCH₃), 4.07–4.19 [3 H, m, α -H (Glu) and CH₂ (Sar)], 4.60–4.62 (2 H, m, OCH₂-vinyl), 4.82–4.87 [1 H, m, α -H (Asp)], 5.22–5.36 (2 H, m, vinyl-CH₂) and 5.88–6.01 (1 H, m, vinyl-CH); δ_{C} (75.4 MHz; C²H₃O²H) 26.60 [β -CH₂ (Glu)], 30.07 [γ -CH₂ (Glu)], 35.54 [β -CH₂ (Asp)], 37.00 (NCH₃), 50.40 [CH₂ (Sar)], 51.72 [α -C (Glu)], 53.28 [α -C (Asp)], 53.71 (OCH₃), 53.94 (OCH₃), 66.76 (OCH₂-vinyl), 118.85 (vinyl-CH₂), 133.71 (vinyl-CH) and 171.13, 171.39, 171.89, 172.70 and 174.75 (CO); *m/z* (EI) 401 (3%, [M - HCl]⁺), 342 (17, [M - HCl - CO₂CH₃]⁺), 259 (19, [M - HCl - C₆H₈NO₃]⁺), 214 (16, C₁₀H₁₆NO₄⁺), 188 (18, C₈H₁₄NO₄⁺), 170 (8, C₈H₁₀O₄⁺), 143 (10, C₆H₆NO₃⁺), 128 (18, C₆H₁₀NO₂⁺), 113 (19, C₆H₉O₂⁺), 84 (65, C₄H₄O₂⁺), 56 (27, C₆H₁₀NO₂⁺) and 44 (100).

β -Allyl α -benzyl (2*R*)-*N*-(*tert*-butoxycarbonyl)aspartate diester 19

To a cooled stirred solution of β -allyl (2*R*)-*N*-(*tert*-butoxycarbonyl)aspartate ester **13** (5.47 g, 20 mmol) in methanol (50 cm³) and water (10 cm³) was added 20% aqueous solution of caesium carbonate, dropwise, until the solution was neutral (pH 7). The mixture was concentrated under reduced pressure to give an oil which was dissolved in water (10 cm³) and lyophilised to afford a sticky white solid. The residue was then dissolved in dry DMF (60 cm³) and the cooled solution was treated with benzyl bromide (3.2 cm³, 4.60 g, 27 mmol). The reaction mixture was stirred at room temperature for 18 h, and then water (50 cm³) was added. The ester was extracted into ethyl acetate (3 × 50 cm³) and the combined extracts were washed with brine (50 cm³), dried (MgSO₄), and then concentrated under reduced pressure to give a colourless oil. This was purified by flash chromatography on silica (petroleum ether–ethyl acetate; 3:1) to give the aspartate diester **19** as a colourless oil (6.59 g, 91%) (HRMS: found [M + H]⁺, 364.1766. C₁₉H₂₆NO₆ requires 364.1760); ν_{\max} (neat)/cm⁻¹ 3384 (NH), 1722 (CO ester) and 1659 (CO urethane); δ_{H} (300 MHz; C²HCl₃) 1.42 [9 H, s, (CH₃)₃], 2.87 (1 H, dd, *J* 4.7 and 17.0, 1 H of β -CH₂), 3.02 (1 H, dd, *J* 4.7 and 17.0, 1 H of β -CH₂), 4.49–4.54 (2 H, m, OCH₂-vinyl), 4.59–4.65 (1 H, m, α -H), 5.12–5.31

(4 H, m, OCH₂Ar and vinyl CH₂), 5.52 (1 H, d, *J* 8.5, NH), 5.78–5.91 (1 H, m, vinyl CH) and 7.33 (5 H, s, Ar-H); δ_{C} (75.4 MHz; C²HCl₃) 28.04 [(CH₃)₃], 36.51 (β -CH₂), 49.88 (α -C), 65.42 (OCH₂-vinyl), 67.22 (OCH₂Ar), 79.93 [OC(CH₃)₃], 118.51 (vinyl-CH₂), 128.12, 128.30 and 128.45 (Ar-CH), 131.61 (vinyl-CH), 135.22 (Ar-C quaternary), 155.35 (CO, urethane), 170.54 and 170.88 (CO); *m/z* (FAB) 364 (7%, [M + H]⁺), 308 (25, [M + 2H - C₄H₉]⁺), 264 (17, [M + 2H - C₅H₉O₂]⁺), 228 (45, [M + H - C₈H₈O₂]⁺), 172 (59, [M + 2H - C₄H₉ - C₈H₈O₂]⁺), 128 (90, [M + 2H - C₅H₉O₂ - C₈H₈O₂]⁺), 91 (86, PhCH₂⁺) and 57 (100, C₄H₉⁺).

β -Allyl α -benzyl (2R)-aspartate diester hydrochloride 20

The deprotected hydrochloride **20** was prepared in a manner identical with that for the hydrochloride **15**, by using β -allyl α -benzyl (2R)-*N*-(*tert*-butoxycarbonyl)aspartate diester **19** (4.36 g, 12 mmol) as the starting material to give the salt **20** as a white solid (3.20 g, 89%), mp 97–98 °C; ν_{max} (Nujol)/cm⁻¹ 1731 (CO); δ_{H} (300 MHz; C²HCl₃) 3.27 (1 H, dd, *J* 5.2 and 17.9, 1 H of β -CH₂), 3.35 (1 H, dd, *J* 5.2 and 17.9, 1 H of β -CH₂), 4.49 (2 H, dd, *J* 1.1 and 5.8, OCH₂-vinyl), 4.67 (1 H, t, *J* 5.2, α -H), 5.11–5.26 (4 H, m, OCH₂Ar and vinyl CH₂), 5.71–5.82 (1 H, m, vinyl CH), 7.30 (5 H, s, Ar-H) and 8.88 (3 H, br, NH₃); δ_{C} (75.4 MHz; C²HCl₃) 33.88 (β -CH₂), 49.62 (α -C), 66.08 (OCH₂-vinyl), 68.32 (OCH₂Ar), 118.70 (vinyl CH₂), 128.44 and 128.50 (Ar-CH), 131.55 (vinyl CH), 134.54 (Ar-C quaternary), 168.26 and 169.71 (CO); *m/z* (EI) 264 (4%, [M - Cl]⁺), 178 (10, [M - Cl - C₄H₆O₂]⁺), 172 (7, [M - HCl - C₇H₇]⁺), 128 (100, C₆H₁₀NO₂⁺), 91 (64, C₇H₇⁺) and 41 (65, C₃H₅⁺).

β -Allyl α -benzyl (2S)-*N*-(*tert*-butoxycarbonyl)phenylalanyl-(2R)-aspartate diester 21

This compound was prepared in a manner identical with that described for the glutamyl-sarcosyl-aspartate tripeptide **17**, using (2S)-*N*-(*tert*-butoxycarbonyl)phenylalanine (3.18 g, 12.0 mmol) and β -allyl α -benzyl (2R)-aspartate diester hydrochloride **20** (3.60 g, 12.0 mmol) to give a colourless viscous oil. This was purified by flash chromatography on silica (petroleum ether–ethyl acetate; 3:1) to give the phenylalanyl-aspartate diester **21** as a colourless viscous oil which solidified slowly on standing (5.09 g, 83%), mp 60–61 °C (Found C, 65.95; H, 6.9; N, 5.4. C₂₈H₃₄N₂O₇ requires C, 65.85; H, 6.7; N 5.5%); ν_{max} (Nujol)/cm⁻¹ 3345 (NH), 1746 (CO ester), 1703 (CO amide) and 1669 (CO urethane); δ_{H} (300 MHz; C²HCl₃) 1.38 [9 H, s, (CH₃)₃], 2.66 [1 H, dd, *J* 4.6 and 17.5, 1 H of β -CH₂ (Asp)], 2.93–3.10 [3 H, m, 1 H of β -CH₂ (Asp) and β -CH₂ (Phe)], 4.39–4.46 [3 H, m, α -H (Phe) and OCH₂-vinyl], 4.85–4.91 [1 H, m, α -H (Asp)], 5.07–5.29 [5 H, m, vinyl CH₂, OCH₂Ar and NH (Phe)], 5.74–5.87 (1 H, m, vinyl CH), 6.94 [1 H, d, *J* 8.0, NH (Asp)] and 7.15–7.37 (10 H, m, Ar-H); δ_{C} (50.3 MHz; C²HCl₃) 28.73 [(CH₃)₃], 36.52 [β -CH₂ (Asp)], 39.14 [β -CH₂ (Phe)], 48.77 [α -C (Phe)], 56.31 [α -C (Asp)], 66.12 (OCH₂-vinyl), 67.98 (OCH₂Ar), 119.26 (vinyl CH₂), 127.37, 128.70, 128.92, 129.05, 129.11 and 129.72 (Ar-CH), 132.04 (vinyl CH), 135.64 and 137.04 (Ar-C quaternary), 170.61, 170.76 and 171.51 (CO); *m/z* (FAB) 533 (9%, [M + Na]⁺), 511 (13, [M + H]⁺), 455 (15, [M + H - C₃H₄O]⁺), 411 (100, [M + H - C₅H₈O₂]⁺), 264 (14, [M + H - C₁₄H₁₇NO₃]⁺) and 120 (82).

α -Benzyl (2S)-*N*-(*tert*-butoxycarbonyl)phenylalanyl-(2R)-aspartate ester 22

To a stirred solution of β -allyl α -benzyl *N*-(*tert*-butoxycarbonyl)-(2S)-phenylalanyl-(2R)-aspartate diester **21** (5.11 g, 10 mmol) and tetrakis(triphenylphosphine) palladium(0) (0.30 g, 0.23 mmol) in dry CH₂Cl₂ (50 cm³) under N₂, was added with freshly distilled pyrrolidine (1.5 cm³). The reaction mixture was stirred at room temperature for 1 h, and then CH₂Cl₂ (50 cm³) was added. The resulting mixture was washed

with 10% citric acid solution (40 cm³) and saturated brine (40 cm³), and then dried (MgSO₄) and the solvent removed under reduced pressure. The residue was dried under high vacuum to give the acid **22** as a flocculent yellow solid (4.47 g, 95%), mp 56–57 °C; ν_{max} (Nujol)/cm⁻¹ 3392 br (NH and OH), 1722 (CO ester) and 1659 (CO urethane); δ_{H} (300 MHz; C²HCl₃) 1.36 [9 H, s, (CH₃)₃], 2.50–2.55 [1 H, br d, 1 H of β -CH₂ (Asp)], 2.98–3.10 [3 H, m, 1 H of β -CH₂ (Asp) and CH₂ (Phe)], 4.60 [1 H, br d, α -H (Phe)], 4.81–4.86 [1 H, m, α -H (Asp)], 5.11 [3 H, br s, CH₂Ph and NH (Phe)], 5.36 [1 H, br d, NH (Asp)] and 7.14–7.36 (10 H, m, Ar-H); δ_{C} (75.4 MHz; C²HCl₃) 28.60 [(CH₃)₃], 35.96 [β -CH₂ (Asp)], 39.55 [β -CH₂ (Phe)], 48.54 [α -C (Phe)], 55.74 [α -C (Asp)], 67.90 (CH₂Ph), 81.13 [C(CH₃)₃], 127.50, 128.59, 128.87, 129.10 and 129.81 (Ar-CH), 132.75 and 135.77 [Ar-C (quaternary)], 156.29 (CO, urethane), 170.68 (CO, acid), 172.02 (CO, amide) and 174.50 (CO, ester); *m/z* (FAB) 493 (9%, [M + Na]⁺), 471 (3, [M + H]⁺), 415 (13, [M + H - C₄H₉]⁺), 371 (100, [M + H - C₅H₉O₂]⁺), 279 (33, C₁₃H₁₅N₂O₅⁺), 210 (25, C₁₀H₁₄N₂O₃⁺) and 120 (95, C₈H₈O⁺).

β -Allyl (2S)-*N*-(*tert*-butoxycarbonyl)phenylalanyl-[α -benzyl (2R)-aspartyl]- β -[α -methyl (2R)-glutamyl]- γ -sarcosyl-[α -methyl (2R)-aspartate] tetraester 23

To a stirred solution of α -benzyl (2S)-*N*-(*tert*-butoxycarbonyl)-phenylalanyl-(2R)-aspartate ester **22** (1.88 g, 4.0 mmol) in dry THF (50 cm³) and DMF (5 cm³) at -40 °C was added *N*-methylmorpholine (440 mm³, 4.0 mmol). Isobutyl chloroformate (550 mm³, 4.0 mmol) was added and the suspension was stirred at -15 °C for 5 min. A mixture of β -allyl (2R)-[α -methyl glutamyl]- γ -sarcosyl-[α -methyl (2R)-aspartate] triester hydrochloride **18** (1.75 g, 4.0 mmol) and NMM (440 mm³, 4.0 mmol) in dry THF (50 cm³) and DMF (5 cm³) was added. The reaction mixture was allowed to warm to room temperature and then stirred for a further 4 h. The hydrochloride salts were removed by filtration and the solution was concentrated under reduced pressure to ca. 15 cm³, and then diluted with ethyl acetate (150 cm³). The solution was successively washed with 10% citric acid (40 cm³), 5% sodium bicarbonate (40 cm³) and brine again (40 cm³), then dried (MgSO₄), and the solvent removed under reduced pressure. The resulting sticky yellow solid was purified by flash chromatography on silica (ethyl acetate) to give the fully protected pentapeptide **23** as a pale yellow solid (2.78 g, 81%), mp 48 °C (softening) (HRMS: found [M + H]⁺ 854.3847. C₄₂H₅₆N₅O₁₄ requires 854.3824); ν_{max} (Nujol)/cm⁻¹ 3345 (NH), 1742 (CO ester) and 1722 (CO amide); δ_{H} [500 MHz; (C²H₅)₂SO, mixture of rotamers] 1.48 [9 H, s, (CH₃)₃], 1.99–2.03 [1 H, m, 1 H of β -CH₂ (Glu)], 2.11–2.18 [1 H, m, 1 H of β -CH₂ (Glu)], 2.43–2.48 [1 H, m, 1 H of γ -CH₂ (Glu)], 2.55 [1 H, br, 1 H of γ -CH₂ (Glu)], 2.86–3.13 [6 H, m, β -CH₂ (Phe) and 2 \times β -CH₂ (Asp)], 2.95 and 3.07 (3 H, 2 \times s, NCH₃), 3.78, 3.80 and 3.82 (6 H, 3 \times s, 2 \times OCH₃), 4.13 [2 H, m, CH₂ (Sar)], 4.40–4.51 [2 H, m, α -H (Phe) and α -H (Glu)], 4.76 [2 H, s, OCH₂ (allyl)], 4.88–4.90 [2 H, m, 2 \times α -H (Asp)], 5.32 (2 H, s, CH₂Ph), 5.40 (*cis*, 1 H, d, *J* 11.0, vinyl CH₂), 5.49 (*trans*, 1 H, d, *J* 17.0, vinyl CH₂), 6.06–6.12 (1 H, m, vinyl CH), 7.09 [0.6 H, d, *J* 8.0, 0.6 \times NH (Phe)], 7.37–7.56 (10 H, m, Ar-H), 7.75 [0.4 H, d, *J* 8.0, 0.4 \times NH (Phe)], 7.82 [0.6 H, m, 0.6 \times NH (Glu)], 8.53–8.59 [1.7 H, m, 1.7 \times NH (Asp)], 8.62 [0.4 H, d, *J* 7.5, NH (Glu)] and 8.77 [0.3 H, d, *J* 8.1, NH (Asp)]; δ_{C} (75.4 MHz; C²HCl₃, mixture of rotamers) 26.84 [β -CH₂ (Glu)], 28.06 [(CH₃)₃], 28.70 [γ -CH₂ (Glu)], 35.98 [β -CH₂ (Asp)], 36.42 [β -CH₂ (Asp)], 36.99 [CH₂ (Phe)], 38.54 (NCH₃), 48.47 [CH₂ (Sar)], 48.99 (α -C), 51.72 (α -C), 52.40 (OCH₃), 52.69 (OCH₃), 55.40 [α -C (Phe)], 65.60 [CH₂ (allyl)], 67.07 (CH₂Ph), 79.73 [C(CH₃)₃], 118.60 (vinyl CH₂), 126.73, 127.89, 128.28, 128.47, 128.55 and 129.37 (Ar-CH), 131.70 (vinyl CH), 132.01 (vinyl CH), 135.45 and 136.77 (Ar-C quaternary), 155.22 (CO, urethane), 168.70, 169.98, 170.55, 170.81, 171.26, 172.39 and 173.04 (CO); *m/z* (FAB) 876 (20%, [M + Na]⁺), 854

(12, [M + H]⁺), 754 (100, [M + 2H - C₅H₉O₂]⁺) and 120 (90, C₈H₁₀N⁺).

(2S)-N-(tert-Butoxycarbonyl)phenylalanyl-[α-benzyl (2R)-aspartyl]-β-[α-methyl (2R)-glutamyl]-γ-sarcosyl-[α-methyl (2R)-aspartate] triester 24

To a stirred solution of the fully protected pentapeptide **23** (2.13 g, 2.5 mmol) and tetrakis(triphenylphosphine)palladium(0) (80 mg, 0.06 mmol) in dry CH₂Cl₂ (25 cm³) under N₂, was added distilled pyrrolidine (375 mm³). The reaction mixture was stirred at room temperature for 45 min, and then CH₂Cl₂ (75 cm³) was added. The solution was washed with 10% citric acid solution (40 cm³) and saturated brine (40 cm³), and then dried (MgSO₄) and the solvent removed under reduced pressure to give the required pentapeptide free acid **24** as a yellow solid (2.03 g, 100%), mp 66–68 °C (softening) (HRMS: found [M + H]⁺, 814.3493. C₃₉H₅₂N₅O₁₄ requires 814.3511); ν_{max}(Nujol)/cm⁻¹ 3345 br (NH and OH), 1741 (CO ester) and 1714 (CO amide); δ_H(300 MHz; C²HCl₃, mixture of rotamers) 1.32 [9 H, s, (CH₃)₃], 1.88–1.92 [1 H, m, 1 H of β-CH₂ (Glu)], 2.03–2.55 [3 H, m, 1 H of β-CH₂ (Glu) and β-CH₂ (Asp)], 2.62–2.70 [1 H, m, 1 H of CH₂ (Phe)], 2.81–3.08 [9 H, m, 1 H of CH₂ (Phe), β-CH₂ (Asp) and NCH₃], 3.67, 3.71 and 3.73 (6 H, 3 × s, 2 × OCH₃), 4.09–4.21 [2 H, m, CH₂ (Sar)], 4.47 [2 H, br, α-H (Phe) and α-H (Glu)], 4.80 [2 H, br, 2 × α-H (Asp)], 5.14 (2 H, s, CH₂Ph), 5.23 [1 H, d, J 8.2, NH (Phe)] and 7.14–7.71 (13 H, m, Ar-H and 3 × NH); δ_C(75.4 MHz; C²HCl₃, mixture of rotamers) 27.09 [(CH₃)₃], 27.86 [β-CH₂ (Glu)], 28.54 [β-CH₂ (Glu)], 36.00 [β-CH₂ (Asp)], 37.11 [β-CH₂ (Asp)], 37.51 [CH₂ (Phe)], 38.96 (NCH₃), 49.01 [CH₂ (Sar)], 49.82 (α-C), 52.48 (α-C), 52.54 (α-C), 53.00 (OCH₃), 53.13 (OCH₃), 55.43 [α-C (Phe)], 66.28 (CH₂Ph), 67.69 (CH₂Ph), 80.67 [C(CH₃)₃], 127.30, 128.48, 128.85, 129.03, 129.10, 129.86, 132.57 and 132.70 (Ar-CH), 135.57 and 135.87 (Ar-C quaternary), 156.06 (CO, urethane), 169.39, 170.87, 171.71, 172.60, 172.95, 173.64 and 173.89 (CO); m/z (FAB) 836 (10%, [M + Na]⁺), 814 (13, [M + H]⁺), 714 (86, [M + 2H - C₅H₉O₂]⁺) and 120 (100, C₈H₁₀N⁺).

β-Pentafluorophenyl (2S)-N-(tert-butoxycarbonyl)phenylalanyl-[α-benzyl (2R)-aspartyl]-β-[α-methyl (2R)-glutamyl]-γ-sarcosyl-[α-methyl (2R)-aspartate] tetraester 25

To a stirred solution of pentapeptide free acid **24** (1.63 g, 2.0 mmol) in dry CH₂Cl₂ (50 cm³) at 0 °C was added pentafluorophenol (1.10 g, 6.0 mmol) followed by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide (EDCI) (0.90 g, 3.0 mmol). The reaction mixture was allowed to warm to room temperature and then stirred for a further 15 h. The solution was concentrated under reduced pressure to give a yellow oil which was purified by flash chromatography on silica (ethyl acetate) to give the required pentafluorophenyl ester **25** as a white waxy solid (1.69 g, 86%), mp 162–164 °C (HRMS: found [M + H]⁺, 980.3333. C₄₅H₅₁N₅O₁₄F₅ requires 980.3353); ν_{max}(Nujol)/cm⁻¹ 3313 (NH), 1800 (CO ester), 1733 (CO ester), 1697 (CO amide) and 1645 (CO urethane); δ_H[500 MHz; (C²H₃)₂SO, mixture of rotamers] 1.30 [9 H, s, (CH₃)₃], 1.80–1.84 [1 H, m, 1 H of β-CH₂ (Glu)], 1.92–1.99 [1 H, m, 1 H of γ-CH₂ (Glu)], 2.27–2.40 [2 H, m, γ-CH₂ (Glu)], 2.68–2.81 [3 H, m, 1 H of β-CH₂ (Asp) and CH₂ (Phe)], 2.79 and 2.90 (3 H, 2 × s, NCH₃), 2.89–2.95 [1 H, m, 1 H of β-CH₂ (Asp)], 3.17–3.26 [1 H, m, 1 H of β-CH₂ (Asp)], 3.30–3.37 [1 H, m, 1 H of β-CH₂ (Asp)], 3.60, 3.62 and 3.68 (6 H, 3 × s, 2 × OCH₃), 3.95–4.02 [2 H, m, CH₂ (Sar)], 4.22–4.28 [1 H, m, α-H (Phe)], 4.30–4.35 [1 H, m, α-H (Glu)], 4.70–4.74 [1 H, m, α-H (Asp)], 4.82–4.88 [1 H, m, α-H (Asp)], 5.14 (2 H, s, CH₂Ph), 6.92 [0.6 H, d, J 8.5, 0.6 × NH (Phe)], 7.19–7.38 (10 H, m, Ar-H), 7.56 [0.4 H, m, 0.4 × NH (Phe)], 7.62–7.66 [0.4 H, m, 0.4 × NH (Glu)], 8.35–8.53 [2.2 H, m, 0.6 × NH (Glu) and 1.6 × NH (Asp)] and 8.76 [0.4 H, d, J 8.5, 0.4 × NH (Asp)]; δ_C(75.4 MHz; C²HCl₃, mixture of rotamers) 27.58 [β-CH₂ (Glu)], 28.49 [(CH₃)₃],

29.03 [γ-CH₂ (Glu)], 35.73 [β-CH₂ (Asp)], 36.87 [CH₂ (Phe)], 37.68 [β-CH₂ (Asp)], 38.96 (NCH₃), 48.97 [CH₂ (Sar)], 49.51 (α-C), 52.11 (OCH₃), 52.97 (OCH₃), 53.43 (α-C), 53.66 (α-C), 55.98 [α-C (Phe)], 67.66 (CH₂Ph), 80.41 [C(CH₃)₃], 127.29, 128.24, 128.41, 128.87, 129.01, 129.20, 129.83, 132.53 and 132.65 (Ar-CH), 135.81, 137.16 and 139.88 (Ar-C quaternary), 155.82 (CO urethane) and 167.32, 169.59, 170.94, 171.10, 171.26, 171.83, 172.13, 172.92 and 173.55 (CO); δ_C(282 MHz; C²HCl₃, mixture of rotamers) -153.27 (1.2 F, d, J 17.2, *o*-Ar-F), -158.74 (0.6 F, t, J 22.4, *p*-Ar-F), -162.83 (1.2 F, t, J 20.0, *m*-Ar-F), -163.51 (0.8 F, dd, J 5.1 and 17.2, *o*-Ar-F), -165.67 (0.8 F, t, J 21.4, *m*-Ar-F) and -171.10 (0.4 F, m, *p*-Ar-F); m/z (FAB) 1002 (44%, [M + Na]⁺), 980 (39, [M + H]⁺), 880 (100 [M + 2H - C₅H₉O₂]⁺) and 120 (65, C₈H₁₀N⁺).

Cyclo-[(R)-Asp α-OBn]-β-[(R)-Glu α-OMe]-γ-Sar-[(R)-Asp α-OMe]-β-(S)-Phe-} 27

To a stirred solution of pentapeptide pentafluorophenyl ester **25** (490 mg, 0.5 mmol) in dry CH₂Cl₂ (25 cm³) was added trifluoroacetic acid (TFA) (25 cm³). The mixture was stirred for 1 h and then concentrated under reduced pressure. Toluene (25 cm³) was added, and the mixture concentrated again under reduced pressure and the resulting residue was thoroughly dried *in vacuo* for several hours. The residue was dissolved in dry CH₂Cl₂ (500 cm³), then reacted with *N,N*-diisopropylethylamine (DIPEA) (1.5 cm³, 8.5 mmol), and the mixture stirred at room temperature for 7 days, when TLC analysis indicated that reaction was complete. The reaction mixture was concentrated under reduced pressure and then triturated with diethyl ether to give a solid, which was filtered off, and washed on the pad with diethyl ether to give the cyclic product **27** as a white powder (260 mg, 75%), mp 254–257 °C (decomp.) (HRMS: found [M + H]⁺, 696.2897. C₃₄H₄₂N₅O₁₁ requires 696.2881); ν_{max}(Nujol)/cm⁻¹ 3300 (NH), 1737 (CO ester), 1661 and 1639 (CO amide); δ_H[500 MHz; (C²H₃)₂SO, mixture of rotamers, only selected peaks given] 1.71–1.85 [1 H, m, 1 H of β-CH₂ (Glu)], 1.96–2.01 [1 H, m, 1 H of β-CH₂ (Glu)], 2.08–2.14 [1 H, m, 1 H of γ-CH₂ (Glu)], 2.23–2.28 [1 H, m, 1 H of γ-CH₂ (Glu)]; m/z (FAB) 696 (15%, [M + H]⁺) and 130 (100).

Cyclo-[(R)-Asp]-β-[(R)-Glu α-OMe]-γ-Sar-[(R)-Asp α-OMe]-β-(S)-Phe-} 7

To a stirred solution of the macrocyclic benzyl ester **27** (209 mg, 0.30 mmol) in glacial acetic acid–methanol (1:1, 30 cm³) was added 5% palladium on activated carbon (5 mg). The suspension was then vigorously stirred under an atmosphere of hydrogen for 18 h. The catalyst was removed by filtration on a pad of pre-washed Celite and the solvent was removed under reduced pressure to give an oily residue which was azeotropically dried using methanol and toluene. The acid **7** was obtained as a sticky white solid (154 mg, 85%) in greater than 90% purity, and was used directly, without purification, in subsequent steps to form the exocyclic amide derivatives. δ_H[500 MHz; (C²H₃)₂SO, one major rotamer] 1.62 and 1.87 [2 H, 2 × m, AB coupling of β-CH₂ (Glu)], 1.95 and 2.30 [2 H, 2 × m, AB coupling of γ-CH₂ (Glu)], 2.51–2.80 [4 H, 4 × m, 2 × AB multiplets of β-CH₂ (Asp)], 2.81 [3 H, s, N-Me (Sar)], 3.13 and 3.34 [2 H, 2 × m, AB coupling of β-CH₂ (Phe)], 3.62 (6 H, 2 × s, 2 × OCH₃), 4.10, 4.29 and 4.38 [5 H, 3 × m, α-CH of Glu, Phe and Asp, and α-CH₂ (Sar)], 4.93 [1 H, br d, α-CH (Asp)], 7.21 (5 H, m, Ar-H) and 7.60, 7.94, 8.49 and 8.63 (4 H, 4 × d, NH of Glu, Phe, Asp, Asp). The ¹H-NMR spectrum in d₄-methanol showed two major conformers in a ratio of 2:1.

Cyclo-[(2R)-α-4-benzylpiperidinyl-Asp]-β-[(R)-Glu]-γ-Sar-[(R)-Asp]-β-(S)-Phe-} 29

To a stirred solution of the macrocycle **7** (60 mg, 0.10 mmol) in dry THF (10 cm³) and DMF (2 cm³) at -40 °C was added *N*-

methylmorpholine (11 mm³, 0.1 mmol). Isobutyl chloroformate (13.75 mm³, 0.1 mmol) was added and the suspension was stirred at -15 °C for 5 min. A mixture of 4-benzylpiperidine (17.6 mm³, 0.1 mmol) and NMM (11 mm³, 0.1 mmol) in dry THF (5 cm³) was added. The reaction mixture was allowed to warm to room temperature and then stirred for a further 4 h. The hydrochloride salts were removed by filtration and the solution was concentrated under reduced pressure to ca. 2 cm³, and then diluted with ethyl acetate (10 cm³). The solution was successively washed with 10% citric acid (5 cm³), 5% sodium bicarbonate (5 cm³) and brine again (5 cm³), and then dried (MgSO₄). The solvents were removed under reduced pressure to give the required amide diester **28** as a pale brown solid (33.6 mg, 44%). δ_{H} (500 MHz; C²HCl₃) 1.16 and 1.87 [2 H, 2 × m, AB coupling of β -CH₂ (Glu)], 1.42 and 1.58 (4 H, 2 × d, *J* 8, 2 × NCH₂CH₂), 1.67 [2 H, m, γ -CH₂ (Glu)], 2.43 (2 H, m, NCH₂CH₂), 2.52 [2 H, m, β -CH₂ (Asp)], 2.57 [3 H, 2 × s, N-Me (Sar)], 2.74–3.09 [9 H, m, PhCH₂, NCH₂CH₂, PhCH₂CH, β -CH₂ (Asp) and β -CH₂ (Phe)], 3.62 (6 H, 4 × s, 2 × OCH₃), 4.32–4.65 [6 H, 3 × m, α -CH of Glu, Phe, Asp, Asp amide and α -CH₂ (Sar)], 7.03–7.38 (10 H, m, Ar-H); NH signals not assigned. The ¹H-NMR spectrum in *d*₆-DMSO solution showed at least 3 conformations. The reaction was repeated several times; 44% was the best yield.

The crude diester **28** (15 mg, 19.7 μ mol) was dissolved in methanol (2 cm³) and water (2 cm³). Aqueous 1 mol dm⁻³ NaOH solution (50 μ mol) was then added. The reaction was allowed to stir at room temperature for 2 h, after which time the methanol was removed under reduced pressure. The aqueous layer was acidified using trifluoroacetic acid and the cyclic peptide extracted using CH₂Cl₂ (2 cm³). The organic phase was dried (MgSO₄) and the solvent removed under reduced pressure. The crude material was then purified by HPLC on a C-18 reverse phase column [using isocratic reverse-phased conditions eluting with acetonitrile–water (51:49) as eluent at a flow rate of 1 cm³ min⁻¹]. The eluent was monitored by UV spectroscopy at 220 nm. The fractions corresponding to peak 1 (retention time 8.5 min) were collected and pooled together, and the solvent was removed under reduced pressure and by lyophilisation to give the desired compound in approximately 50% yield, and >95% purity. δ_{H} (500 MHz; C²HCl₃; 2 major conformers) 1.15 and 1.43 [4 H, 2 × m, β -CH₂ (Glu) and NCH₂CH₂], 1.59–1.96 [4 H, 2 × m, γ -CH₂ (Glu) and NCH₂CH₂], 2.22–2.81 [10 H, 3 × m, 2 × β -CH₂ (Asp) and 2 × NCH₂CH₂, PhCH₂CH], 2.59 [3 H, m, N-Me (Sar)], 2.95–3.30 [4 H, m, PhCH₂ and β -CH₂ (Phe)], 4.18 [4 H, m, α -CH of Glu, Phe and α -CH₂ (Sar)], 4.60 [1 H, m, α -CH (Asp)], 5.11 [1 H, m, α -CH (Asp amide)], 7.05–7.20 (10 H, m, Ar-H); NH signals not assigned; *m/z* (ES⁺) 773 (1%, [M + K]⁺) and 735 (1.5, [M + H]⁺).

β -Benzyl [(2*S*)-*N*-(*tert*-butoxycarbonyl)prolyl][α -methyl (2*R*)-aspartate] diester **36**

This compound was prepared in a manner identical to that described for pentapeptide **23**, using *N*-(*tert*-butoxycarbonyl)-(2*S*)-proline (215 mg, 1 mmol) and α -methyl β -benzyl (2*R*)-aspartate diester hydrochloride **35** (273 mg, 1 mmol), to give the required compound as a colourless oil which was refractory to crystallisation (343 mg, 78%) (Found C, 61.1; H, 7.3; N 6.2. C₂₂H₃₀N₂O₇ requires C, 60.8; H, 7.0; N, 6.4%) (HRMS: found [M + H]⁺, 435.2124. C₂₂H₃₁N₂O₇ requires 435.2131); [a]_D -5.41 (*c* 1.21 in MeOH); ν_{max} (CH₂Cl₂)/cm⁻¹ 3323 (NH), 2976 (CH), 1739 (CO, urethane) and 1699 (CO, esters); δ_{H} (300 MHz; C²HCl₃) 1.43 [9 H, s, (CH₃)₃], 1.82–2.22 [4 H, m, γ -CH₂ and β -CH₂ (Pro)], 2.85 [1 H, dd, *J* 17.1 and 4.8, 1 H of β -CH₂ (Asp)], 3.06 [1 H, dd, *J* 14.1 and 4.7, 1 H of β -CH₂ (Asp)], 3.09–3.45 [2 H, m, δ -CH₂ (Pro)], 3.66 (3 H, s, CH₃), 4.18–4.37 [1 H, m, α -H (Pro)], 4.87 [1 H, q, *J* 4.5, α -H, (Asp)], 5.09 (2 H, s, PhCH₂), 7.28–7.34 (5 H, m, Ar-H) and 7.37 (1 H, br, NH); δ_{C} (75.4 MHz;

C²HCl₃) 24.14 [*c*, γ -CH₂ (Pro)], 24.27 [*t*, γ -CH₂ (Pro)], 28.40 [*t*, (CH₃)₃], 28.75 [*c*, (CH₃)₃], 28.97 [*t*, β -CH₂ (Pro)], 29.08 [*c*, β -CH₂ (Pro)], 36.78 [β -CH₂ (Asp)], 47.25 [*t*, δ -CH₂ (Pro)], 48.49 [*c*, δ -CH₂ (Pro)], 48.68 [α -C (Asp)], 53.13 (CH₃), 60.65 [*c*, α -C (Pro)], 60.74 [*t*, α -C (Pro)], 67.29 (PhCH₂), 80.81 [C(CH₃)₃], 128.61, 128.81 and 129.04 (Ar-CH), 136.20 (Ar-C quaternary), 156.04 (CO, urethane) and 171.06, 171.11 and 171.38 (CO, esters); *m/z* (CI) 435 (100%, [M + H]⁺), 379 (55, [M + H - C₄H₉ + H]⁺) and 335 (97, [M + H - C₅H₉O₂ + H]⁺).

β -Benzyl [(2*S*)-prolyl][α -methyl (2*R*)-aspartate] diester hydrochloride **37**

This compound was prepared in a manner identical to that described for the hydrochloride **15** using the prolyl-aspartyl diester **36**, (750 mg, 1.73 mmol), to yield a white hygroscopic solid which was not purified any further (590 mg, 93%), mp 101–102 °C (Found C, 55.0; H, 6.3; N 7.4. C₁₇H₂₃ClN₂O₅ requires C, 55.1; H, 6.25; N, 7.55%) (HRMS: found [M + H - HCl]⁺, 335.1600. C₁₇H₂₃N₂O₅ requires 335.1607); [a]_D -28.3 (*c* 1.32 in MeOH); ν_{max} (CH₂Cl₂)/cm⁻¹ 3189 (NH), 2959 (CH), 1739 (CO, ester) and 1684 (CO, amide); δ_{H} (300 MHz; C²HCl₃) 1.89–1.99 [3 H, m, γ -CH₂, 1 H of β -CH₂ (Pro)], 2.38–2.58 [1 H, m, one of β -CH₂ (Pro)], 2.94 [2 H, d, *J* 5.7, β -CH₂ (Asp)], 3.43 [2 H, br, δ -CH₂ (Asp)], 3.6 (3 H, s, CH₃), 4.62–4.86 [2 H, m, α -H (Pro) and α -H, (Asp)], 5.07 [2 H, q, *J* 12.3, PhCH₂], 7.27 (5 H, s, Ph), 7.84 (1 H, br, NH) and 9.10 (1 H, d, *J* 7.5, NH⁺); δ_{C} (75.4 MHz; C²HCl₃) 24.26 [γ -CH₂ (Pro)], 30.39 [β -CH₂ (Pro)], 36.02 [β -CH₂ (Asp)], 46.74 [δ -CH₂ (Pro)], 49.45 [α -CH (Asp)], 52.84 (CH₃), 59.70 [α -C (Pro)], 66.86 (PhCH₂), 128.17, 128.3, 128.35 and 128.49 (Ar-CH), 135.45 (Ar-C quaternary) and 169.04, 170.11 and 170.86 (3 × CO, esters and amides); *m/z* (CI) 335 (39%, [M + H - HCl]⁺), 303 (82, [M - HCl - OCH₃]⁺) and 213 (100, C₁₀H₁₅NO₄⁺).

β -Benzyl [α -methyl (2*R*)-*N*-(*tert*-butoxycarbonyl)glutamyl]- γ -(2*S*)-prolyl- α -methyl (2*R*)-aspartate] triester **38**

This compound was prepared in a manner identical to that described for the pentapeptide **23**, using α -methyl (2*R*)-*N*-(*tert*-butoxycarbonyl) glutamate (277 mg, 1.19 mmol) and the hydrochloride triester **37** (440 mg, 1.19 mmol) to give the required compound as a colourless oil, which was recrystallised from ethyl acetate–hexane to give a white solid (566 mg, 82%), mp 92–93 °C (Found C, 57.9; H, 6.8; N 7.2. C₂₈H₃₉N₃O₁₀ requires C, 58.2; H, 6.8; N, 7.3%) (HRMS: found [M + H]⁺, 578.2702. C₂₈H₄₀N₃O₁₀ requires 578.2714); [a]_D -19.2 (*c* 1.25 in MeOH); ν_{max} (CH₂Cl₂)/cm⁻¹ 3354 (NH), 2956 (CH) and 1742 (CO, esters); δ_{H} (300 MHz; C²HCl₃) 1.46 [9 H, s, (CH₃)₃], 1.64–2.69 [8 H, m, γ -CH₂ and β -CH₂ (Pro and Glu)], 2.87–2.98 [2 H, m, β -CH₂ (Asp)], 3.37–3.40 [δ -CH₂ (Pro)], 3.52 and 3.58 [6 H, s, 2 × CH₃], 4.08 [1 H, br, α -H (Pro)], 4.28–4.38 [1 H, br, α -H (Glu)], 4.83 [1 H, q, *J* 4.5, α -H, (Asp)], 5.10 [1 H, d, *J* 5.7, NH (urethane)], 5.13 (2 H, s, PhCH₂), 7.29 (5 H, s, Ph) and 7.68 (1 H, d, *J* 5.7, NH); δ_{C} (75.4 MHz; C²HCl₃) 22.60 [*c*, γ -CH₂ (Pro)], 24.56 [*t*, γ -CH₂ (Pro)], 28.03 [β -CH₂ (Glu)], 28.35 [(CH₃)₃], 28.97 [γ -CH₂ (Glu)], 30.17 [*t*, β -CH₂ (Pro)], 32.13 [*c*, β -CH₂ (Pro)], 36.87 [β -CH₂ (Asp)], 46.94 [*t*, δ -CH₂ (Pro)], 47.39 [*c*, δ -CH₂ (Pro)], 48.71 [α -C (Asp)], 52.56 [α -C (Glu)], 2.76 [CH₃ (Asp)], 52.77 [CH₃ (Glu)], 60.23 [α -C (Pro)], 66.74 (PhCH₂), 80.8 [C(CH₃)₃], 128.31, 128.38, 128.59 and 128.69 (Ar-CH), 135.80 (Ar-C quaternary), 135.91 (CO, urethane) and 170.31, 170.66, 171.27, 171.51 and 171.62 (CO, esters and amides); *m/z* (CI) 578 (76%, [M + H]⁺) and 478 (100, [M + H - C₅H₉O₂ + H]⁺).

β -Benzyl [α -methyl (2*R*)-glutamyl]- γ -(2*S*)-prolyl- α -methyl (2*R*)-aspartate] triester hydrochloride **39**

The deprotected hydrochloride was prepared in a manner identical to that described for the hydrochloride **15**, using the

glutamylprolylaspartyl triester **38**, (310 mg, 0.54 mmol) as a starting material, to afford a white hygroscopic solid which was not further purified (262 mg, 95%), mp 84–85 °C (HRMS: found $[M + H - HCl]^+$, 478.2182. $C_{23}H_{32}N_3O_8$ requires 478.2189); $[a]_D -41.1$ (c 1.34 in MeOH); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3316 (NH), 2977 (CH) and 1685 (CO, amides); $\delta_H(300\text{ MHz}; C^2HCl_3)$ 1.80–2.80 [8 H, m, γ -CH₂ and β -CH₂ (Pro and Glu)], 2.86–3.04 [2 H, m, β -CH₂ (Asp)], 3.4–3.59 [δ -CH₂ (Pro)], 3.64, 3.67 and 3.78 [c and t , 6 H, s, ($2 \times CH_3$)], 4.26 [1 H, br, α -H (Pro)], 4.7 [1 H, br, α -H (Glu)], 4.86 [1 H, q, J 6, α -H (Pro)], 5.11 (2 H, s, $PhCH_2$), 7.33 (5 H, s, Ar-H), 8.15 (1 H, d, J 7.8, NH) and 8.65 (1 H, br, NH₃); $\delta_C(75.4\text{ MHz}; C^2HCl_3)$ 24.76 [γ -CH₂ (Pro)], 24.91 [β -CH₂ (Glu)], 29.29 [γ -CH₂ (Glu)], 30.53 [β -CH₂ (Pro)], 36.37 [β -CH₂ (Asp)], 47.89 [δ -CH₂ (Pro)], 48.94 [α -C (Asp)], 52.71 [α -C (Glu)], 52.92 [CH₃ (Asp)], 53.53 [CH₃ (Glu)], 60.36 [α -C (Pro)], 67.02 ($PhCH_2$), 128.43, 128.49 and 128.66 (Ar-CH), 135.67 (Ar-C quaternary) and 169.91, 170.67, 171.61, 172.01 and 172.06 (CO, esters, amides and acid); m/z (CI) 478 (42%, $[M + H - HCl]^+$), 460 (88, $[M - 2H - Me - HCl]^+$) and 144 (100, $C_6H_{10}NO_3^+$).

β -Benzyl (2S)-N-(tert-butoxycarbonyl)phenylalanyl- β -alanyl-[α -methyl (2R)-glutamyl]- γ -(2S)-prolyl-[α -methyl (2R)-aspartate] triester **32**

This compound was prepared in a manner identical to that described for the pentapeptide **23**, using the phenylalanyl- β -alanyl dipeptide **34** (246 mg, 0.73 mmol) and tripeptide **39** (376 mg, 0.73 mmol) to give the required compound as a colourless oil which was recrystallised using ethyl acetate–hexane (390 mg, 62%), mp 118–119 °C (Found C, 59.7; H, 6.7; N, 8.4. $C_{40}H_{53}N_5O_{12} \cdot 0.5 H_2O$ requires C, 59.7; H, 6.8; N, 8.7%). $[a]_D +31.67$ (c 1.2 in MeOH); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3307 (NH), 2955 (CH), 1740 (CO, urethane) and 1655 (CO, esters and amides); $\delta_H(500\text{ MHz}; C^2HCl_3)$ 1.34 [9 H, s, (CH_3)₃], 1.81–2.42 [10 H, m, γ -CH₂ and β -CH₂ (Pro and Glu) and α -CH₂ (β -Ala)], 2.92–3.13 [4 H, m, β -CH₂ (Asp) and β -CH₂ (Phe)], 3.37–3.43 [4 H, m, δ -CH₂ (Pro) and β -CH₂ (β -Ala)], 3.64, 3.69 and 3.71 (c and t , 6 H, s, $2 \times CH_3$), 4.28–4.41 [2 H, m, α -H (Phe and Pro)], 4.59–4.63 [1 H, m, α -H, (Glu)], 4.86 [1 H, q, J 5.4, α -H (Asp)], 5.14 (2 H, s, $PhCH_2$), 5.22 [1 H, d, J 5.6, NH (urethane)], 6.88 [2 H, br, $2 \times NH$ (amide)], 7.18–7.38 (10 H, m, Ar-H) and 7.76 (1 H, d, J 5.4, NH); $\delta_C(75.4\text{ MHz}; C^2HCl_3)$ 22.76 [c , γ -CH₂ (Pro)], 24.71 [t , γ -CH₂ (Pro)], 27.08 [β -CH₂ (Glu)], 28.36 [(CH_3)₃], 28.87 [γ -CH₂ (Glu)], 30.48 [t , β -CH₂ (Pro)], 31.63 [c , β -CH₂ (Pro)], 32.18 [α -CH₂ (β -Ala)], 35.48 [c , β -CH₂ (Asp)], 35.88 [c , β -CH₂ (Asp)], 36.49 [β -CH₂ (β -Ala)], 39.18 [β -CH₂ (Phe)], 46.97 [t , δ -CH₂ (Pro)], 47.51 [c , δ -CH₂ (Pro)], 48.84 [c , α -C (Asp)], 49.31 [t , α -C (Asp)], 52.02 [α -C (Glu)], 52.63 [CH₃ (Asp)], 52.82 [CH₃ (Glu)], 55.82 [α -C (Phe)], 60.11 [t , α -C (Pro)], 61.37 [c , α -C (Pro)], 66.89 ($PhCH_2$), 80.03 [C(CH_3)₃], 126.83, 128.44, 128.55, 128.67, 128.73 and 129.52 (Ar-C), 135.46, 135.63 and 135.75 (Ar-C quaternary), 155.45 (CO, urethane) and 170.53, 170.82, 171.02, 171.51, 171.65, 171.78, 171.85, 172.15 and 172.62 (c and t , CO esters and amides); m/z (FAB) 818 (72%, $[M + Na]^+$), 718 (54, $[M - C_6H_5]^+$), 696 (73, $[M + H - C_5H_9O_2 + H]^+$) and 133 (100).

(2S)-N-(tert-Butoxycarbonyl)phenylalanyl- β -alanyl-[α -methyl (2R)-glutamyl]- γ -(2S)-prolyl-[α -methyl (2R)-aspartate] diester **40**

To a stirred solution of the benzyl ester **32** (130 mg, 0.165 mmol) in ethanol (20 cm³) was added 10% palladium on carbon (20 mg) and the mixture stirred under an atmosphere of hydrogen for 3 h. The catalyst was removed by filtration through a pre-washed Celite pad and the filtrate was concentrated under reduced pressure to give the required compound as a white crystalline solid in quantitative recovery, which was not purified further, mp 86–87 °C (Found C, 54.7; H, 6.5; N, 9.3. $C_{33}H_{47}N_5O_{12} \cdot H_2O$ requires C, 54.8; H, 6.8; N, 9.7%); $[a]_D$

+31.67 (c 1.2 in MeOH); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3324 (NH), 2958 (CH), 1736 (CO, urethane) and 1653 (CO, esters and amides); $\delta_H(300\text{ MHz}; C^2HCl_3)$ 1.29 [9 H, s, (CH_3)₃], 1.78–2.48 [10 H, m, γ -CH₂ and β -CH₂ (Pro and Glu) and α -CH₂ (β -Ala)], 2.65–3.17 [4 H, m, δ -CH₂ (Asp) and β -CH₂ (Phe)], 3.32–3.58 [4 H, m, δ -CH₂ (Pro) and β -CH₂ (β -Ala)], 3.63, 3.65 and 3.68 (c and t , 6 H, s, $2 \times CH_3$), 4.21–4.4 [2 H, m, α -H (Phe and Pro)], 4.42–4.58 [1 H, m, α -H, (Glu)], 4.78 [1 H, br, α -H (Asp)], 5.22 [2 H, br, NH (urethane) and OH], 7.08–7.28 (5 H, m, Ar-H) and 7.35–7.79 (2 H, m, NH); $\delta_C(75.4\text{ MHz}; C^2HCl_3)$ 22.66 [c , γ -CH₂ (Pro)], 24.65 [t , γ -CH₂ (Pro)], 26.64 [β -CH₂ (Glu)], 28.30 [(CH_3)₃], 28.99 [γ -CH₂ (Glu)], 30.06 [t , β -CH₂ (Pro)], 31.41 [c , β -CH₂ (Pro)], 32.16 [α -CH₂ (β -Ala)], 35.65 [β -CH₂ (Asp)], 35.97 [β -CH₂ (β -Ala)], 38.85 [β -CH₂ (Phe)], 47.18 [t , δ -CH₂ (Pro)], 47.50 [c , δ -CH₂ (Pro)], 48.69 [c , α -C (Asp)], 49.10 [t , α -C (Asp)], 52.06 [α -C (Glu)], 52.62 [CH₃ (Asp)], 52.74 [CH₃ (Glu)], 55.91 [α -C (Phe)], 60.41 [t , α -C (Pro)], 61.39 [c , α -C (Pro)], 80.04 [C(CH_3)₃], 126.82, 128.54 and 129.44 (Ar-CH), 137.01 (Ar-C quaternary), 155.78 (CO, urethane) and 171.19, 171.58, 171.67, 171.95, 172.17, 172.34, 172.56, 172.72, 173.17 and 173.55 (c and t , CO); m/z (ES) 728 (5%, $[M + Na]^+$), 706 (6, $[M + H]^+$), 157 (95, $C_7H_{11}NO_3^+$) and 140 (100, $C_7H_{10}NO_2^+$).

β -Pentafluorophenyl (2S)-N-(tert-butoxycarbonyl)phenylalanyl- β -alanyl-[α -methyl (2R)-glutamyl]- γ -(2S)-prolyl-[α -methyl (2R)-aspartate] triester **41**

To a stirred solution of the pentapeptide carboxylic acid diester **40** (130 mg, 0.18 mmol) in CH_2Cl_2 (20 cm³) at 0 °C was added pentafluorophenol (102 mg, 0.55 mmol) followed by EDCI (82 mg, 0.28 mmol). The reaction mixture was allowed to warm to room temperature with stirring overnight. The solution was concentrated under reduced pressure to yield a colourless oil which was purified by flash chromatography on silica using CH_2Cl_2 –MeOH (95:5) as the eluent to give triester **41** as a white crystalline solid (100 mg, 62%), mp 79–81 °C (HRMS: found $[M + Na]^+$, 894.2978. $C_{39}H_{46}F_5N_5O_{12}Na$ requires 894.2961); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3311 (NH), 2957 (CH), 1742 (CO, urethane) and 1655 (CO, esters and amides); $\delta_H(300\text{ MHz}; C^2HCl_3)$ 1.33 [9 H, s, (CH_3)₃], 1.80–2.44 [10 H, m, γ -CH₂ and β -CH₂ (Pro and Glu) and α -CH₂ (β -Ala)], 2.84–3.57 [8 H, m, β -CH₂ (Asp), β -CH₂ (Phe), β -CH₂ (Pro) and β -CH₂ (β -Ala)], 3.69 and 3.72 (6 H, s, $2 \times CH_3$), 4.27–4.38 [2 H, m, α -H (Phe and Pro)], 4.62 [1 H, br, α -H (Glu)], 4.99 [1 H, q, J 5.7, α -CH (Asp)], 5.27 [1 H, d, J 8.1, NH (urethane)], 6.82–6.96 (2 H, m, $2 \times NH$), 7.12–7.26 (5 H, m, Ar-H) and 7.88 (1 H, d, J 7.8, NH); $\delta_C(75.4\text{ MHz}; C^2HCl_3)$ 22.73 [c , γ -CH₂ (Pro)], 24.71 [t , β -CH₂ (Pro)], 27.21 [β -CH₂ (Glu)], 28.30 [(CH_3)₃], 28.97 [γ -CH₂ (Glu)], 30.36 [t , β -CH₂ (Pro)], 31.67 [c , β -CH₂ (Pro)], 32.17 [α -CH₂ (β -Ala)], 35.50 [β -CH₂ (Asp)], 35.59 [β -CH₂ (β -Ala)], 39.04 [β -CH₂ (Phe)], 46.90 [c , δ -CH₂ (Pro)], 47.54 [t , δ -CH₂ (Pro)], 48.82 [t , α -C (Asp)], 49.48 [c , α -C (Asp)], 51.82 [α -C (Glu)], 52.63 [CH₃ (Asp)], 53.08 [CH₃ (Glu)], 55.89 [α -C (Phe)], 60.10 [t , α -C (Pro)], 61.35 [c , α -C (Pro)], 79.95 [C(CH_3)₃], 126.84, 128.54, 128.90 and 129.44 (Ar-CH), 137.04 (Ar-C quaternary), 136.40, 138.10, 139.57, 141.60 and 142.8 (C-F, PFP), 155.51 (CO, urethane), 166.69 (CO, PFP) and 170.78, 171.74, 171.79, 172.88 and 172.60 (c and t , CO, esters and amides); m/z (ES) 895 (34%, $[M + Na + H]^+$), 873 (41, $[M + 2H]^+$), 196 (27, $C_9H_{10}NO_4^+$), 158 (36, $C_6H_8NO_4^+$) and 101 (100, $C_5H_9O_2^+$).

Cyclo[β -Ala-(2R)-Glu- α -OMe- γ -(2S)-Pro-(2R)-Asp- α -OMe- β -(2S)-Phe-] **42 (R = Me)**

To a stirred solution of the *N*-(tert-butoxycarbonyl)-protected pentafluorophenyl ester **41** (78 mg, 0.09 mmol) in CH_2Cl_2 (10 cm³) was added trifluoroacetic acid (10 cm³). The reaction mixture was stirred for 1 h when the reaction was judged to be complete by TLC. The solution was concentrated under reduced pressure, triturated with diethyl ether (10 cm³), and the precipitate was collected and thoroughly dried under high

vacuum for 6 h. The residue was dissolved in CH_2Cl_2 (500 cm^3), treated with DIPEA (316 mm^3) and then left to stir under Ar for eight days. The reaction mixture was concentrated under reduced pressure and immediately purified by flash chromatography on silica using CH_2Cl_2 –MeOH (94:6) as the eluant to give a white crystalline solid which was recrystallised from acetone–diethyl ether (28 mg, 52%), mp $>220^\circ\text{C}$ (decomp.) (Found C, 55.8; H, 6.4; N, 11.3. $\text{C}_{28}\text{H}_{37}\text{N}_5\text{O}_9\cdot\text{H}_2\text{O}$ requires C, 55.5; H, 6.5; N, 11.55%) (HRMS: found $[\text{M} + \text{H}]^+$, 588.2685. $\text{C}_{28}\text{H}_{38}\text{N}_5\text{O}_9$ requires 588.2683); ν_{max} (Nujol)/ cm^{-1} 3360 (NH) and 1719 (CO, esters and amides); δ_{H} [500 MHz; $(\text{C}^2\text{H}_5)_2\text{SO}$] 1.79–1.90 [5 H, m, 1 H of β - CH_2 (*c*, Glu), β - CH_2 (*t*, Glu) and γ - CH_2 (*t*, Pro)], 1.92–2.07 [6 H, m, 1 H of β - CH_2 (*c*, Glu), γ - CH_2 (*c*, Pro), β - CH_2 (*t*, Pro) and 1 H of β - CH_2 (*c*, Pro)], 2.11–2.27 [6 H, m, 1 H of β - CH_2 (*c*, Pro), 1 H of γ - CH_2 (*c*, Glu), γ - CH_2 (*t*, Pro) and 1 H of α - CH_2 (*c* and *t*, α -Ala)], 2.34–2.61 [5 H, m, 1 H of γ - CH_2 (*c*, Glu), 1 H of β - CH_2 (*c* and *t*, β -Ala) and 1 H of β - CH_2 (*c* and *t*, Asp)], 2.68–2.82 [3 H, m, 1 H of β - CH_2 (*c* and *t*, Asp), 1 H of β - CH_2 (*t*, Phe)], 2.90–3.03 [2 H, m, 1 H of β - CH_2 (*c* and *t*, Phe)], 3.08–3.25 [3 H, m, 1 H of β - CH_2 (*c*, Phe) and 1 H of α - CH_2 (*c* and *t*, β -Ala)], 3.40–3.59 [6 H, m, δ - CH_2 (*c* and *t*, Pro) and 1 H of β - CH_2 (*c* and *t*, β -Ala)], 3.62, 3.64, 3.66, and 3.71 (12 H, s, $2 \times \text{CH}_3$, *c* and *t*), 4.13–4.25 [3 H, m, α -H (*c*, Phe), α -H (*t*, Glu) and α -H (*c*, Asp)], 4.28 [1 H, dd, *J* 8.3 and 2.7, α -H (*c*, Pro)], 4.38–4.44 [2 H, m, α -H (*c*, Glu) and α -H (*t*, Pro)], 4.47 [1 H, sep, *J* 3.1, α -H, (*t*, Asp)], 4.56 [1 H, q, *J* 5.5, α -H, (*t*, Phe)], 7.21–7.34 (10 H, m, *c* and *t*, Ph), 7.78 [1 H, t, *J* 5.5, NH (*c*, β -Ala)], 7.88 [1 H, t, *J* 5.5, NH (*t*, β -Ala)], 8.11 [1 H, d, *J* 6.7, NH (*c*, Asp)], 8.20 [1 H, d, *J* 9.2, NH (*t*, Phe)], 8.21 [1 H, d, *J* 7.9, NH (*t*, Glu)], 8.30 [1 H, d, *J* 7.3, NH (*c*, Phe)], 8.32 [1 H, d, *J* 7.9, NH (*c*, Glu)] and 8.38 [1 H, d, *J* 7.3, NH (*t*, Asp)]; δ_{C} [125.8 MHz; $(\text{C}^2\text{H}_5)_2\text{SO}$] 21.88 [*c*, γ - CH_2 (Pro)], 23.92 [*t*, γ - CH_2 (Pro)], 26.02 [*c*, β - CH_2 (Glu)], 26.66 [*t*, β - CH_2 (Glu)], 28.87 [*t*, γ - CH_2 (Glu)], 29.31 [*c*, γ - CH_2 (Glu)], 30.53 [*t*, β - CH_2 (Pro)], 31.46 [*c*, β - CH_2 (Pro)], 34.49 [α - CH_2 (β -Ala)], 34.78 [*c*, β - CH_2 (Asp)], 34.93 [*c*, β - CH_2 (Asp)], 35.57 [β - CH_2 (β -Ala)], 37.77 [*c*, β - CH_2 (Phe)], 39.18 [*t*, β - CH_2 (Phe)], 46.31 [*t*, δ - CH_2 (Pro)], 46.84 [*c*, δ - CH_2 (Pro)], 49.4 [*c*, α -C (Asp)], 50.1 [*t*, α -C (Asp)], 50.8 [α -C (Glu)], 51.79, 51.9, 52.05 and 52.14 (*c* + *t*, CH_3), 53.71 [*t*, α -C (Phe)], 55.52 [*c*, α -C (Phe)], 59.65 [*t*, α -C (Pro)], 59.71 [*c*, α -C (Pro)], 126.12, 127.91, 128.03, 128.92 and 129.03 (Ar-CH), 137.82 and 137.86 (Ar-C quaternary) and 168.88, 170.13, 170.98, 171.08, 171.14, 171.3, 171.42, 171.96, 172.18 and 172.6 (CO, ester and amides); *m/z* (CI) 588 (8%, $[\text{M} + \text{H}]^+$), 556 (25, $[\text{M} - \text{OCH}_3]^+$) and 391 (100, $[\text{M} - \text{C}_{10}\text{H}_{14}\text{NO}_3]^+$).

β -Allyl (2*R*)-*N*-(fluoren-9-ylmethoxycarbonyl)aspartate 44

To an ice-cold stirred suspension of γ -allyl (2*R*)-aspartate hydrochloride **12** (530 mg, 2.5 mmol) in water (20 cm^3) was added potassium carbonate (560 mg, 4 mmol). A pre-stirred solution of fluoren-9-ylmethoxycarbonyl chloride (717 mg, 2.75 mmol) in dioxane (20 cm^3) was added. The resulting solution was warmed to room temperature and stirred for 4 h, then poured into water (15 cm^3) and the dioxane was removed under reduced pressure. The aqueous solution was washed with diethyl ether ($2 \times 30 \text{ cm}^3$) and acidified to pH 2 at 0°C with 6 mol dm^{-3} HCl and then extracted with diethyl ether ($3 \times 30 \text{ cm}^3$). The ethereal solutions were combined, dried (MgSO_4) and the solvent was removed under reduced pressure to give the required compound as a white crystalline solid, which was not purified further (930 mg, 94%), mp 110 – 111°C (Found C, 66.3; H, 5.4; N, 3.6. $\text{C}_{22}\text{H}_{21}\text{NO}_6$ requires C, 66.8; H, 5.35; N, 3.55%) (HRMS: found $[\text{M} + \text{H}]^+$, 396.1450. $\text{C}_{22}\text{H}_{22}\text{NO}_6$ requires 396.1447); $[\alpha]_{\text{D}} + 3.04$ (*c* 0.46 in MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1} 3363 (NH), 2956 (CH) and 1741 (CO, urethane); δ_{H} (300 MHz; C^2HCl_3) 2.94 (1 H, dd, *J* 18 and 3.4, 1 H of β - CH_2), 3.13 (1 H, dd, *J* 18 and 3.45, 1 H of β - CH_2), 4.20–4.79 (6 H, m, $2 \times \text{CH}_2\text{O}$, fluorenyl CH and α -H), 5.21–5.38 (2 H, m, $\text{CH}_2=\text{CH}$), 5.82–

6.21 [2 H, m, $\text{CH}_2=\text{CH}$ and NH], 7.25–7.82 (8 H, m, Ar-H) and 9.01 (1 H, br, OH); δ_{C} (75.4 MHz; C^2HCl_3) 36.54 (β - CH_2), 47.20 (fluorenyl CH), 50.51 (α -C), 66.07 and 67.60 ($2 \times \text{CH}_2\text{O}$), 119.05 ($\text{CH}_2=\text{CH}$), 120.510, 125.28, 127.26, 127.53, 127.90 and 128.41 (Ar-CH), 131.98 ($\text{CH}_2=\text{CH}$), 141.44, 143.78 and 143.92 (Ar-C quaternary), 156.35 (CO, urethane), 170.96 (CO, ester) and 175.46 (CO, acid); *m/z* (CI) 396 (12%, $[\text{M} + \text{H}]^+$) and 179 (100, $\text{C}_{14}\text{H}_{11}^+$).

β -Allyl (2*R*)-*N*-(fluoren-9-ylmethoxycarbonyl)aspartyl-Wang resin 45

A suspension of Wang resin (500 mg, 0.84 mmol g^{-1} OH substitution) and β -allyl (2*R*)-*N*-(fluoren-9-ylmethoxycarbonyl)-aspartate **44** (660 mg, 1.67 mmol) were stirred in dry DMF (2.5 cm^3) under N_2 for 15 min. Pyridine (100 mm^3 , 2.77 mmol) and 2,6-dichlorobenzoyl chloride (105 mm^3 , 1.67 mmol) were added and the suspension was stirred for 20 h and then filtered and washed successively with DMF (25 cm^3), CH_2Cl_2 (25 cm^3) and methanol (25 cm^3). The percentage loading was checked and determined to be 70%.⁵² The remaining hydroxy groups of the resin were benzoylated with benzoyl chloride and pyridine in CH_2Cl_2 for 2 h, washed with DMF (25 cm^3), CH_2Cl_2 (25 cm^3) and methanol (25 cm^3), and dried under vacuum and checked once again for percentage loading (772 mg, 70% loading).

γ -Allyl (2*R*)-glutamate hydrochloride 46

To a stirred suspension of (2*R*)-glutamic acid (2.21 g, 15 mmol) in dry allyl alcohol (70 cm^3) under N_2 was added dropwise chlorotrimethylsilane (4.75 cm^3 , 47.5 mmol). The resulting solution was stirred at RT for 18 h, after which diethyl ether (200 cm^3) was added at 0°C to give a white precipitate which was collected by filtration, washed with diethyl ether and dried under vacuum (2.18 g, 65%), mp 131 – 132°C [lit.,⁵² 130 – 132°C (for the (2*S*)-isomer)], $[\alpha]_{\text{D}} - 22.1$ (*c* 1.1 in MeOH) {lit.,⁵² $[\alpha]_{\text{D}} + 22.5$ (*c* 1 in MeOH) [for the (2*S*) isomer]}; ν_{max} (CH_2Cl_2)/ cm^{-1} 2863 br (OH) and 1729 (CO, ester); δ_{H} (300 MHz; $^2\text{H}_2\text{O}$) 2.08–2.21 (2 H, sep, *J* 7.2, β - CH_2), 2.55 (2 H, t, *J* 6.0, γ - CH_2), 3.98 (1 H, t, *J* 6.9, α -H), 4.52 (2 H, d, *J* 5.7, CH_2O), 5.21 (2 H, dd, *J* 15.9, 1.5 $\text{CH}_2=\text{CH}$) and 5.84 (1 H, m, $\text{CH}_2=\text{CH}$); δ_{C} (75.4 MHz; $^2\text{H}_2\text{O}$) 25.00 (β - CH_2), 29.75 (γ - CH_2), 52.31 (α -C), 66.28 (CH_2O), 171.79 (CO, ester) and 174.23 (CO, acid); *m/z* (CI) 188 (84%, $[\text{M} - \text{Cl}]^+$) and 130 (100, $\text{C}_5\text{H}_8\text{NO}_3^+$).

γ -Allyl (2*R*)-*N*-(fluoren-9-ylmethoxycarbonyl)glutamate 47

To an ice-cold stirred suspension of γ -allyl (2*R*)-glutamate hydrochloride **46** (943 mg, 4.22 mmol) in water (30 cm^3) was added potassium carbonate (945 mg, 6.75 mmol). A pre-stirred solution of fluoren-9-ylmethoxycarbonyl chloride (1.21 g, 4.64 mmol) in dioxane (30 cm^3) was added. The resulting solution was warmed to room temperature and stirred for 4 hours, then poured into water (20 cm^3) and the dioxane was removed under reduced pressure. The aqueous solution was washed with diethyl ether ($2 \times 50 \text{ cm}^3$) and acidified to pH 2 at 0°C with 6 mol dm^{-3} HCl and then extracted with diethyl ether ($3 \times 50 \text{ cm}^3$). The ethereal solutions were combined, dried (MgSO_4) and the solvent was removed under reduced pressure to give the required compound as a white crystalline solid in quantitative recovery, which was not purified further, mp 113 – 114°C (HRMS: found $[\text{M} + \text{H}]^+$, 410.1595. $\text{C}_{22}\text{H}_{24}\text{NO}_6$ requires 410.1603); $[\alpha]_{\text{D}} + 30.4$ (*c* 1.3 in MeOH); ν_{max} (CH_2Cl_2)/ cm^{-1} 3364 (NH), 2965 (CH) and 1746 (CO, urethane); δ_{H} (300 MHz; C^2HCl_3) 2.01–2.58 (4 H, m, γ - CH_2 and β - CH_2), 4.18–4.62 (6 H, m, $2 \times \text{CH}_2\text{O}$, fluorenyl CH and α -H), 5.21–5.58 (2 H, m, $\text{CH}_2=\text{CH}$), 5.58 [1 H, d, *J* 7.8, NH (urethane)], 5.82–5.98 (1 H, m, $\text{CH}_2=\text{CH}$) and 7.23–7.79 (8 H, m, Ar-H); δ_{C} (75.4 MHz; C^2HCl_3) 27.42 (β - CH_2), 30.40 (γ - CH_2), 47.24 (fluorenyl CH), 53.39 (α -C), 65.67 and 67.34 ($2 \times \text{CH}_2\text{O}$), 118.68 ($\text{CH}_2=\text{CH}$), 120.13, 125.23, 127.25 and 127.89 (Ar-CH), 132.03 ($\text{CH}_2=\text{CH}$),

141.46, 143.76 and 143.97 (Ar-C quaternary), 156.42 (CO, urethane) and 172.5 and 175.66 (CO, ester and acid); m/z (CI) 424 (17%, $[M + 2H + Na]^+$), 410 (64, $[M + H]^+$), 181 (100, $C_{14}H_{13}^+$) and 130 (48, $C_5H_8NO_3^+$).

α -Methyl γ -allyl (2R)-N-(fluoren-9-ylmethoxycarbonyl)-glutamate 48

This compound was prepared in a manner identical to diester **14**, using γ -allyl ester **47** (1.04 g, 2.54 mmol) to afford the required compound as a white crystalline solid in quantitative recovery, mp 84–85 °C (Found C, 67.9; H, 5.8; N, 3.5. $C_{24}H_{25}NO_6$ requires: C, 68.1; H, 5.95; N, 3.3%) (HRMS: found $[M + H]^+$, 424.1766. $C_{24}H_{26}NO_6$ requires 424.1760); $[a]_D^{25} +22.14$ (c 0.56 in MeOH); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3364 (NH), 2965 (CH) and 1751 (CO, urethane); $\delta_H(300\text{ MHz}; C^2HCl_3)$ 1.95–2.53 (4 H, m, γ -CH₂ and β -CH₂), 3.75 (3 H, s, CH₃), 4.09–4.61 (6 H, m, 2 \times CH₂O, fluorenyl CH and α -H), 5.21–5.38 (2 H, m, CH₂=CH), 5.56 [1 H, d, J 8.1, NH (urethane)], 5.84–5.97 (1 H, m, CH₂=CH) and 7.26–7.77 (8 H, m, Ar-H); $\delta_C(75.4\text{ MHz}; C^2HCl_3)$ 27.04 (β -CH₂), 30.29 (γ -CH₂), 47.29 (fluorenyl CH), 52.70 (CH₃), 53.48 (α -C), 65.54 and 67.21 (2 \times CH₂O), 118.61 (CH₂=CH), 120.14, 125.22, 127.23 and 127.87 (Ar-CH), 132.14 (CH₂=CH), 141.46, 143.85 and 144.03 (Ar-C quaternary), 156.13 (CO, urethane) and 172.50 (CO, ester); m/z (CI) 424 (45%, $[M + H]^+$), 179 (94, $C_{14}H_{11}^+$), 144 (78, $C_6H_{10}NO_3^+$) and 57 (100, $C_4H_9^+$).

α -Methyl (2R)-N-(fluoren-9-ylmethoxycarbonyl)glutamate ester 49

To a stirred solution of diester **48** (423 mg, 1 mmol) and phenylsilane (247 mm³, 2 mmol) in dry CH₂Cl₂ (30 cm³) under Ar was added tetrakis(triphenylphosphine)Pd(0) (23 mg, 0.02 mmol). The reaction mixture was allowed to stir at room temperature for 2 h (when no starting material was detected by TLC) and was then concentrated under reduced pressure. The residue was purified by flash chromatography on silica using ethyl acetate–hexane (60:40) as the eluent to give an amorphous white solid (310 mg, 81%), mp 130–131 °C (Found C, 64.6; H, 5.45; N, 3.7. $C_{21}H_{21}NO_6 \cdot 0.5 H_2O$ requires C, 64.3; H, 5.65; N, 3.55%) (HRMS: found $[M + H]^+$, 384.1443. $C_{21}H_{22}NO_6$ requires 384.1447); $[a]_D^{25} +19.5$ (c 0.63 in MeOH); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3355 (NH), 2965 (CH) and 1732 (CO, urethane); $\delta_H(300\text{ MHz}; C^2HCl_3)$ 1.92–2.57 (4 H, m, γ -CH₂ and β -CH₂), 3.75 (3 H, s, CH₃), 4.18–4.56 (4 H, m, CH₂O, fluorenyl CH and α -H), 5.45 [1 H, d, J 7.2, NH (urethane)] and 7.25–7.77 (8 H, m, Ar-H); $\delta_C(75.4\text{ MHz}; C^2HCl_3)$ 27.57 (β -CH₂), 29.89 (γ -CH₂), 47.28 (fluorenyl CH), 52.74 (CH₃), 53.29 (α -C), 67.24 (CH₂O), 120.14, 125.19, 127.23 and 127.88 (Ar-CH), 141.47 and 143.81 (Ar-C quaternary), 156.15 (CO, urethane) and 177.54 (CO, acid); m/z (CI) 384 (7%, $[M + H]^+$), 179 (31, $C_{14}H_{11}^+$) and 144 (100, $C_6H_{10}NO_3^+$).

β -Allyl (2S)-N-(fluoren-9-ylmethoxycarbonyl)phenylalanyl- β -alanyl-[α -methyl (2R)-glutamyl]- γ -(2S)-prolyl-[(2R)-aspartate] diester 53

β -Allyl (2S)-N-(fluoren-9-ylmethoxycarbonyl)phenylalanyl- β -alanyl-[(2R)- α -methyl glutamyl]- γ -(2S)-prolyl-[(2R)-aspartate-Wang resin] diester **53** (2.1 g, 426 mg peptidyl content, 0.05 mmol) was synthesised on the peptide synthesiser and treated with cleavage mixture TFA–TES–H₂O–CH₂Cl₂ (40:2:5:53) at room temperature for 1 h. The resin was collected by filtration and the solvents were concentrated under reduced pressure (4–5 cm³). The peptide was then precipitated with excess diethyl ether to afford a white solid in quantitative recovery (426 mg), mp 115–116 °C (Found C, 59.6; H, 6.6; N, 7.9. $C_{45}H_{51}N_5O_{12} \cdot 3 H_2O$ requires C, 59.5; H, 6.35; N, 7.7%); $[a]_D^{25} -13.0$ (c 1.3 in MeOH); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3276 (NH), 2977 (CH) and 1727 br (CO, esters and amides); $\delta_H(300\text{ MHz}; C^2HCl_3)$ 1.76–2.54 [10

H, m, γ -CH₂ and β -CH₂ (Pro and Glu) and α -CH₂ (β -Ala)], 2.82–3.14 [4 H, m, β -CH₂ (Asp) and β -CH₂ (Phe)], 3.32–3.47 [4 H, m, δ -CH₂ (Pro) and β -CH₂ (β -Ala)], 3.77 (3 H, s, CH₃), 4.16–4.88 [9 H, m, 4 \times α -H, 2 \times CH₂O and fluorenyl CH], 4.97 [1 H, br, NH (urethane)], 5.14–5.36 (2 H, m, CH₂=CH), 5.76–5.94 (1 H, m, CH₂=CH), 6.18 (2 H, br, NH) and 7.00–7.23 (15 H, m, Ar-H, 2 \times NH); $\delta_C(75.4\text{ MHz}; C^2HCl_3)$ 24.53 [γ -CH₂ (Pro)], 26.86 [β -CH₂ (Glu)], 28.84 [γ -CH₂ (Glu)], 29.77 [β -CH₂ (Pro)], 34.97 [α -CH₂ (β -Ala)], 35.36 [β -CH₂ (Asp)], 36.34 [β -CH₂ (β -Ala)], 38.71 [β -CH₂ (Phe)], 46.90 [δ -CH₂ (Pro)], 47.42 [α -C (Asp)], 51.55 [α -C (Glu)], 52.51 [CH₃ (Asp)], 56.02 [α -C (Phe)], 60.00 [α -C (Pro)], 65.56 and 67.31 (2 \times CH₂O), 118.45 (CH₂=CH), 119.96, 125.14, 126.87, 127.1, 127.75, 128.49, 128.64, 129.41, 130.13, 131.83 and 133.37 (Ar-CH), 136.67 (CH₂=CH), 141.29 and 141.74 (Ar-C quaternary), 156.55 (CO, urethane) and 170.61, 171.94 and 172.7 (CO esters and amides); m/z (ES) 892 (20%, $[M + K]^+$), 876 (42, $[M + Na]^+$), 855 (28, $[M + 2H]^+$), 718 (49) and 102 (100).

(2S)-Phenylalanyl- β -alanyl-[α -methyl (2R)-glutamyl]- γ -(2S)-prolyl-[(2R)-aspartate] ester 55

To a stirred suspension of resin **53** (495 mg, 0.3 mmol) in DMSO–THF–0.5 mol dm⁻³ HCl–NMM (2:2:1:0.1), (25 cm³) under Ar was added tetrakis(triphenylphosphine)Pd(0) (104 mg, 9 \times 10⁻⁵ mol). Slow stirring was continued for 3 h, after which time the resin was collected by filtration and washed with THF (25 cm³), CH₂Cl₂ (25 cm³), and methanol (25 cm³). The resin was then treated with 20% piperidine–DMF (10 cm³) for 30 min (monitoring deprotection using the Ninhydrin Test³⁷), after which time the resin was collected by filtration and washed with DMF, CH₂Cl₂ and methanol successively. The washed resin was then treated with cleavage mixture TFA–TES–H₂O–CH₂Cl₂ (40:2:5:53) for 1 h, and the resin was removed by filtration and the solvents concentrated under reduced pressure (4–5 cm³). The required peptide was then precipitated by the addition of excess diethyl ether to give a white solid in quantitative recovery (177 mg); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3200 br (NH and OH) and 1729 br (CO, esters and amides); $\delta_H(300\text{ MHz}; ^2H_2O)$ 1.44–2.39 [10 H, m, γ -CH₂, β -CH₂ (Pro and Glu) and α -CH₂ (β -Ala)], 2.64–3.04 [4 H, m, β -CH₂ (Asp) and β -CH₂ (Phe)], 3.17–3.40 [4 H, m, δ -CH₂ (Pro) and β -CH₂ (β -Ala)], 3.57 (t , 3 H, s, CH₃), 3.59 (c , 3 H, s, CH₃), 4.00 (1 H, t , J 7.4 α -H), 4.08–4.38 (2 H, m, 2 \times α -H), 4.58–4.61 (1 H, m, α -H) and 7.02–7.38 (5 H, m, Ar-H); $\delta_C(75.4\text{ MHz}; ^2H_2O)$ 22.13 [c , γ -CH₂ (Pro)], 24.05 [t , γ -CH₂ (Pro)], 25.71 [β -CH₂ (Glu)], 29.66 [γ -CH₂ (Glu)], 29.77 [t , β -CH₂ (Pro)], 31.74 [t , β -CH₂ (Pro)], 34.23 [α -CH₂ (β -Ala)], 35.26 [β -CH₂ (Asp)], 35.45 [β -CH₂ (β -Ala)], 36.88 [β -CH₂ (Phe)], 47.93 [c , δ -CH₂ (Pro)], 47.86 [t , δ -CH₂ (Pro)], 48.98 [t , α -C (Asp)], 49.19 [c , α -C (Asp)], 52.15 [α -C (Glu)], 52.94 [CH₃ (Asp)], 54.44 [α -C (Phe)], 60.25 [t , α -C (Pro)], 60.8 [c , α -C (Pro)], 128.06, 129.23, 129.44, 130.63, 133.75 and 133.9 (Ar-CH), 135.89 (Ar-C quaternary) and 173.43, 173.64, 173.72, 173.89, 173.97, 174.27 and 174.4 (CO esters and amides); m/z (ES) 620 (5%, $[M + K]^+$), 614 (10, $[M + Na]^+$) and 592 (100, $[M + H]^+$).

Cyclo-[β -Ala-(2S)-Glu- α -OMe- γ -(2R)-Pro-(2R)-Asp- β -(2S)-Phe-] 57

To a stirred suspension of resin **55** (100 mg, 0.06 mmol) in DMF (5 cm³) and DIPEA (102 mm³, 0.6 mmol) under Ar was added PyBOP (157 mg, 0.3 mmol) and HOBt (41 mg, 0.3 mmol). The resin was stirred slowly for 7 days (continuous monitoring of progress using the Ninhydrin Test), and then removed by filtration and washed successively with THF (25 cm³), CH₂Cl₂ (25 cm³) and methanol (25 cm³). The resulting resin **56** was treated with cleavage mixture TFA–TES–H₂O–CH₂Cl₂ (40:2:5:53) for 1 h and then removed by filtration, and the filtrate concentrated under reduced pressure (4–5 cm³). The

peptide was precipitated by the addition of excess diethyl ether to give a white solid which was purified by reverse phase HPLC (10 mg, 30%), mp >180 °C (decomp.) (HRMS: found $[M + H - H_2O]^+$, 556.2420. $C_{27}H_{34}N_5O_8$ requires 556.2407); $\nu_{\max}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$ 3302 (NH) and 1735 and 1670 (CO, esters and amides); $\delta_{\text{H}}(500 \text{ MHz}; (\text{C}^2\text{H}_5)_2\text{SO})$ 1.72–2.02 [11 H, m, 1 H of $\beta\text{-CH}_2$ (*c* and *t*, Pro), 1 H of $\beta\text{-CH}_2$ (*c*, Pro), $\gamma\text{-CH}_2$ (*c* and *t*, Pro) and $\beta\text{-CH}_2$ (*c* and *t*, Glu)], 2.12–2.55 [15 H, m, $\gamma\text{-CH}_2$ (*c* and *t*, Glu), 1 H of $\gamma\text{-CH}_2$ (*c* and *t*, Glu), 1 H of $\beta\text{-CH}_2$ (*t*, Pro), 1 H of $\beta\text{-CH}_2$ (*c* and *t*, Asp) and $\alpha\text{-CH}_2$ (*c* and *t*, $\beta\text{-Ala}$)], 2.66–3.20 [8 H, m, 1 H of $\beta\text{-CH}_2$ (*c* and *t*, $\beta\text{-Ala}$), $\beta\text{-CH}_2$ (*c* and *t*, Phe) and 1 H of $\beta\text{-CH}_2$ (*c* and *t*, Asp)], 3.40–3.49 [6 H, m, 1 H of $\beta\text{-CH}_2$ (*c* and *t*, $\beta\text{-Ala}$) and $\delta\text{-CH}_2$ (*c* and *t*, Pro)], 3.55, 3.67, 3.78, and 3.79 [12 H, s, $2 \times \text{CH}_3$, *c* and *t*], 4.08–4.48 [8 H, m, $\alpha\text{-H}$ (*c* and *t*, Phe), $\alpha\text{-H}$ (*c* and *t*, Glu), $\alpha\text{-H}$ (*c* and *t*, Asp) and $\alpha\text{-H}$ (*c* and *t*, Pro)], 7.21–7.39 (10 H, m, Ar-H, *c* and *t*), 7.75–7.97 [3 H, m, NH (*c* and *t*, $\beta\text{-Ala}$), NH (*c*, Asp)], 8.14–8.29 [5 H, m, NH (*t*, Asp), NH (*c* and *t*, Phe) and NH (*c* and *t*, Glu)]; $\delta_{\text{C}}(75.4 \text{ MHz}; \text{C}^2\text{H}_3\text{O}^2\text{H})$ 23.89 [$\gamma\text{-CH}_2$ (Pro)], 26.18 [$\beta\text{-CH}_2$ (Glu)], 29.25 [$\gamma\text{-CH}_2$ (Glu)], 31.61 [$\beta\text{-CH}_2$ (Pro)], 34.39 [$\alpha\text{-CH}_2$ ($\beta\text{-Ala}$)], 35.11 [$\beta\text{-CH}_2$ (Asp)], 37.03 [$\beta\text{-CH}_2$ ($\beta\text{-Ala}$)], 38.76 [$\beta\text{-CH}_2$ (Phe)], 46.68 [$\delta\text{-CH}_2$ (Pro)], 46.91 [$\alpha\text{-C}$ (Asp)], 51.53 [$\alpha\text{-C}$ (Glu)], 51.73 (CH_3), 56.53 [$\alpha\text{-C}$ (Phe)], 59.5 [$\alpha\text{-C}$ (Pro)], 126.11, 127.93, 128.45, 128.96 and 129.34 (Ar-CH), 135.25 (Ar-C quaternary) and 169.12, 170.28, 170.71, 171.18 and 172.28 (CO ester, amides and acid); m/z (ES) 596 (4%, $[M + \text{Na}]^+$), 574 (16, $[M + \text{H}]^+$) and 212 (100).

Cyclo[$\beta\text{-Ala}$ -(2*R*)-Glu- γ -(2*S*)-Pro-(2*R*)-Asp- β -(2*S*)-Phe-]

To a stirred solution of the cyclic pentapeptide **57** (0.03 mmol) in methanol (2 cm³) and water (2 cm³) was added NaOH (0.065 mmol). The reaction was allowed to stir at room temperature for 2 h, after which time the methanol was removed under reduced pressure. The aqueous layer was acidified using trifluoroacetic acid and the cyclic peptide extracted using CH_2Cl_2 (2 cm³). The organic phase was dried (MgSO_4) and the solvent removed under reduced pressure. m/z (ES) 582 (3%, $[M + \text{Na}]^+$), 561 (7, $[M + 2\text{H}]^+$); >95% HPLC purity on a Poros 10 R2/H reverse phase column [using isocratic reverse-phased conditions eluting with acetonitrile–water (24:76) as eluent at a flow rate of 5 cm³ min⁻¹. The eluent was monitored by UV spectroscopy at 220 nm. The fractions corresponding to peak 1 (retention time 1.7 min) were collected and pooled together, and the solvent removed under reduced pressure and by lyophilisation].

β -Allyl (2*S*)-*N*-(fluoren-9-ylmethoxycarbonyl)phenylalanyl- β -alanyl-[α -methyl (2*R*)-glutamyl]- γ -(2*S*)-prolyl-[α -methyl (2*R*)-aspartate] triester **60**

The triester was prepared in a manner identical to diester **14**, using diester **53** (853 mg, 1 mmol) to give the required compound as a white crystalline solid in quantitative recovery. Mp 120–121 °C (Found C, 61.9; H, 6.3; N, 7.7. $C_{46}H_{53}N_5O_{12} \cdot 1.5 \text{ H}_2\text{O}$ requires C, 61.7; H, 6.3; N, 7.8%); $[a]_{\text{D}} -17.8$ (*c* 0.25 in MeOH); $\nu_{\max}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$ 3301 (NH), 2953 (CH), and 1739 and 1652 (CO, esters and amides); $\delta_{\text{H}}(300 \text{ MHz}; \text{C}^2\text{HCl}_3)$ 1.82–2.52 [10 H, m, $\gamma\text{-CH}_2$, $\beta\text{-CH}_2$ (Pro and Glu) and $\alpha\text{-CH}_2$ ($\beta\text{-Ala}$)], 2.79–3.19 [4 H, m, $\beta\text{-CH}_2$ (Asp) and $\beta\text{-CH}_2$ (Phe)], 3.25–3.80 [4 H, m, $\delta\text{-CH}_2$ (Pro) and $\beta\text{-CH}_2$ ($\beta\text{-Ala}$)], 3.51, 3.60, 3.68 and 3.72 (*c* and *t*, 6 H, $4 \times \text{s}$, CH_3), 4.08–4.97 [9 H, m, $4 \times \alpha\text{-H}$, $2 \times \text{CH}_2\text{O}$ and fluorenyl CH], 5.09–5.37 (2 H, m, $\text{CH}_2=\text{CH}$), 5.68 [1 H, d, *J* 7.9, NH, (urethane)], 5.79–5.86 (1 H, m, $\text{CH}_2=\text{CH}$), 6.90 (1 H, d, *J* 7.7, NH), 7.03 (1 H, br, NH) and 7.18–7.76 (15 H, m, Ar-H, $2 \times \text{NH}$); $\delta_{\text{C}}(75.4 \text{ MHz}; \text{C}^2\text{HCl}_3)$ 24.63 [$\gamma\text{-CH}_2$ (Pro)], 27.07 [$\beta\text{-CH}_2$ (Glu)], 29.14 [$\gamma\text{-CH}_2$ (Glu)], 30.36 [$\beta\text{-CH}_2$ (Pro)], 35.28 [$\alpha\text{-CH}_2$ ($\alpha\text{-Ala}$)], 35.73 [$\beta\text{-CH}_2$ (Asp)], 36.33 [$\beta\text{-CH}_2$ ($\beta\text{-Ala}$)], 39.07 [$\beta\text{-CH}_2$ (Phe)], 47.14 [$\delta\text{-CH}_2$ (Pro)], 47.5 [$\alpha\text{-C}$ (Asp)], 51.90 [$\alpha\text{-C}$ (Glu)], 52.59 [CH_3 (Asp)], 52.86 [CH_3 (Glu)], 56.10 [$\alpha\text{-C}$ (Phe)], 60.09 [$\alpha\text{-C}$ (Pro)],

65.73 and 67.00 ($2 \times \text{CH}_2\text{O}$), 118.60 ($\text{CH}_2=\text{CH}$), 120.05, 125.19, 126.90, 127.15, 127.79, 128.55, 129.48 and 131.86 (Ar-CH), 136.97 ($\text{CH}_2=\text{CH}$), 141.33 and 143.93 (Ar-C quaternary), 156.12 (CO, urethane) and 170.41, 171.71, 171.87 and 172.66 (CO, esters and amides); m/z (ES) 891 (12%, $[M + \text{H} + \text{Na}]^+$), 869 (50, $[M + 2\text{H}]^+$), 646 (28, $[M + 2\text{H} - \text{C}_{15}\text{H}_{11}\text{O}_2]^+$) and 111 (100).

(2*S*)-*N*-(Fluoren-9-ylmethoxycarbonyl)phenylalanyl- β -alanyl-[α -methyl (2*R*)-glutamyl]- γ -(2*S*)-prolyl-[α -methyl (2*R*)-aspartic acid]

This compound was prepared through allyl group deprotection as described for ester **22**, using the triester **60** (220 mg, 0.25 mmol). The required compound was obtained as a white crystalline solid (150 mg, 72%), mp >124 °C (decomp.); $[a]_{\text{D}} -13.5$ (*c* 1 in MeOH); $\nu_{\max}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$ 3300 (NH), 3229 br (OH), and 1801 and 1721 (CO, esters and amides); $\delta_{\text{H}}(300 \text{ MHz}; \text{C}^2\text{H}_3\text{O}^2\text{H})$ 1.87–2.45 [8 H, m, $\gamma\text{-CH}_2$, $\beta\text{-CH}_2$ (Pro and Glu) and $\alpha\text{-CH}_2$ ($\beta\text{-Ala}$)], 2.74–3.14 [4 H, m, $\beta\text{-CH}_2$ (Asp) and $\beta\text{-CH}_2$ (Phe)], 3.28–3.52 [4 H, m, $\delta\text{-CH}_2$ (Pro) and $\beta\text{-CH}_2$ ($\beta\text{-Ala}$)], 3.58, 3.59, 3.65 and 3.66 (*c* and *t*, 6 H, $4 \times \text{s}$, CH_3), 4.09–4.85 [7 H, m, $4 \times \alpha\text{-H}$, CH_2O , fluorenyl CH], and 7.18–7.77 (13 H, m, Ar-H); $\delta_{\text{C}}(75.4 \text{ MHz}; \text{C}^2\text{H}_3\text{O}^2\text{H})$ 24.12 [$\gamma\text{-CH}_2$ (Pro)], 26.53 [$\beta\text{-CH}_2$ (Glu)], 29.5 [$\gamma\text{-CH}_2$ (Glu)], 31.85 [$\beta\text{-CH}_2$ (Pro)], 34.84 [$\alpha\text{-CH}_2$ ($\beta\text{-Ala}$)], 35.53 [$\beta\text{-CH}_2$ (Asp)], 37.63 [$\beta\text{-CH}_2$ ($\beta\text{-Ala}$)], 37.92 [$\beta\text{-CH}_2$ (Phe)], 47.23 [$\delta\text{-CH}_2$ (Pro)], 47.67 [$\alpha\text{-C}$ (Asp)], 49.60 [$\alpha\text{-C}$ (Glu)], 51.97 [CH_3 (Asp)], 52.05 [CH_3 (Glu)], 56.74 [$\alpha\text{-C}$ (Phe)], 60.41 [$\alpha\text{-C}$ (Pro)], 66.72 (CH_2O), 119.66, 124.92, 125.07, 126.5, 126.92, 127.54, 128.21 and 129.13 (Ar-CH), 137.39, 141.25 and 143.90 (Ar-C quaternary), 156.87 (CO, urethane) and 171.98, 172.32, 172.66, 172.94, 173.12 and 176.14 (CO, esters, amides and acid).

(2*S*)-Phenylalanyl- β -alanyl-[α -methyl (2*R*)-glutamyl]- γ -(2*S*)-prolyl-[α -methyl (2*R*)-aspartate] diester **61**

To a stirred solution of the (2*S*)-*N*-(fluoren-9-ylmethoxycarbonyl)phenylalanyl- β -alanyl-[α -methyl (2*R*)-glutamyl]- γ -(2*S*)-prolyl-[α -methyl (2*R*)-aspartic acid] (100 mg, 0.12 mmol) in dry DMF (3 cm³) was added piperidine (17 mm³, 0.18 mmol). The reaction mixture was allowed to stir at room temperature for 45 min, and then concentrated under reduced pressure and redissolved in water (10 cm³) and acidified with TFA. The resulting solution was washed with CH_2Cl_2 and the acidic layer concentrated under reduced pressure to yield the required compound as a white hygroscopic solid (60 mg, 83%), mp 67–69 °C; $[a]_{\text{D}} +14.5$ (*c* 1.1 in MeOH); $\nu_{\max}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$ 3286 (NH), 2955 br (OH), and 1746 and 1670 (CO, esters and amides); $\delta_{\text{H}}(300 \text{ MHz}; \text{H}_2\text{O})$ 1.45–2.18 [10 H, m, $\gamma\text{-CH}_2$, $\beta\text{-CH}_2$ (Pro and Glu) and $\alpha\text{-CH}_2$ ($\beta\text{-Ala}$)], 2.64–3.04 [4 H, m, $\beta\text{-CH}_2$ (Asp) and $\beta\text{-CH}_2$ (Phe)], 3.15–3.36 [4 H, m, $\delta\text{-CH}_2$ (Pro) and $\beta\text{-CH}_2$ ($\beta\text{-Ala}$)], 3.57 and 3.60 (6 H, $2 \times \text{s}$, $2 \times \text{CH}_3$), 3.97–4.59 [4 H, m, $4 \times \alpha\text{-H}$] and 7.07–7.38 (5 H, m, Ar-H); $\delta_{\text{C}}(75.4 \text{ MHz}; \text{C}^2\text{H}_3\text{O}^2\text{H})$ 24.07 [$\gamma\text{-CH}_2$ (Pro)], 26.20 [$\beta\text{-CH}_2$ (Glu)], 29.48 [$\gamma\text{-CH}_2$ (Glu)], 31.41 [$\beta\text{-CH}_2$ (Pro)], 34.88 [$\alpha\text{-CH}_2$ ($\beta\text{-Ala}$)], 35.34 [$\beta\text{-CH}_2$ (Asp)], 36.42 [$\beta\text{-CH}_2$ ($\beta\text{-Ala}$)], 40.15 [$\beta\text{-CH}_2$ (Phe)], 46.84 [$\delta\text{-CH}_2$ (Pro)], 46.90 [$\alpha\text{-C}$ (Asp)], 49.86 [$\alpha\text{-C}$ (Glu)], 51.58 and 52.40 ($2 \times \text{CH}_3$), 56.06 [$\alpha\text{-C}$ (Phe)], 60.53 [$\alpha\text{-C}$ (Pro)], 125.11, 126.73, 126.92, 128.42, 128.59 and 129.19 (Ar-CH), 136.02 (Ar-C quaternary) and 170.55 and 172.39 (CO ester, amides and acid); m/z (ES⁺) 606 (100%, $[M + \text{H}]^+$), 590 (18, $[M - \text{CH}_3]^+$) and 324 (9, $\text{C}_{15}\text{H}_{20}\text{N}_2\text{O}_6^+$).

Cyclo[$\beta\text{-Ala}$ -(2*R*)-Glu- α -OMe- γ -(2*S*)-Pro-(2*R*)-Asp- α -OMe- β -(2*S*)-Phe-] **42** (R = Me)

To a stirred solution of the pentapeptide **61** (60 mg, 9.92×10^{-5} mol) in DMF (80 cm³) was added PyBOP (28.4 mg, 0.11 mmol) and DIPEA (102 mm³, 0.99 mmol). The resulting solution was stirred at room temperature for 7 days and then concentrated

under reduced pressure and immediately purified by flash chromatography on silica using CH_2Cl_2 -MeOH (94:6) as the eluent to give a white solid which contained PyBOP as a contaminant. Further purification by preparative HPLC on a Poros 10 R2 reverse-phase column [using isocratic reverse-phased conditions, eluting with acetonitrile-water (18:82) as eluent at a flow rate of $2 \text{ cm}^3 \text{ min}^{-1}$, with the detector set at 220 nm] gave the required compound which possessed identical analytical data to the compound prepared previously from the pentafluorophenyl ester (see compound **42** above).

N*-(Fluoren-9-ylmethoxycarbonyl)-(2*S*)-phenylalanyl-[α -4-benzylpiperidinyl-(2*R*)-aspartyl]-[α -methyl (2*R*)-glutamyl]- γ -(2*S*)-prolyl- β -allyl-(2*R*)-aspartyl-Wang resin **62**; and *N*-(fluoren-9-ylmethoxycarbonyl)-(2*S*)-phenylalanyl-[α -4-benzylpiperidinyl-(2*R*)-aspartyl]-[α -methyl (2*S*)-glutamyl]- γ -(2*S*)-prolyl- β -allyl-(2*R*)-aspartic acid **63*

N-(Fluoren-9-ylmethoxycarbonyl)-(2*S*)-phenylalanyl-[α -4-benzylpiperidinyl-(2*R*)-aspartyl]-[α -methyl (2*R*)-glutamyl]- γ -(2*S*)-prolyl- β -allyl-(2*R*)-aspartyl-Wang resin **62** was synthesised on the peptide synthesiser [using Fmoc chemistry as previously described (see general procedure)] starting from β -allyl-(2*R*)-*N*-(Fmoc)-aspartyl-Wang resin **45** (512 mg, 0.3 mmol) and incorporating the isoasparagine derivative **68f** in quantitative recovery (828 mg). For characterisation, a sample of the fully protected resin-bound pentapeptide **62** (50 mg, 18 μmol) was treated with cleavage mixture TFA-TES- H_2O - CH_2Cl_2 (40:2:5:53) at room temperature for 1 h. The polymer support was collected by filtration and the solvents were concentrated under reduced pressure (4–5 cm^3). The peptide was then precipitated with excess diethyl ether to afford the required pentapeptide **63** as a white solid in quantitative recovery, which did not require further purification, mp 134–136 °C (HRMS: found $[\text{M} + \text{H} + \text{Na}]^+$, 1077.4556. $\text{C}_{58}\text{H}_{66}\text{N}_6\text{O}_{13}\text{Na}$ requires 1077.4586); $[\alpha]_{\text{D}} + 30$ (*c* 1 in MeOH); $\nu_{\text{max}}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$ 3302 (NH), 2952 (CH), and 1739 (CO, esters and amides); δ_{H} (300 MHz; C^2HCl_3) 1.18–1.43 (4 H, m, $2 \times \text{CH}_2\text{-CH}_2\text{NH}$), 1.70 (4 H, br, $\text{CH}_2\text{-CH}_2\text{NH}$), 2.46–3.04 [5 H, m, CHCH_2Ph , CHCH_2Ph and $\beta\text{-CH}_2$ (Asp)], 1.81–2.58 [10 H, m, $\gamma\text{-CH}_2$, $\beta\text{-CH}_2$ (Pro and Glu) and $\alpha\text{-CH}_2$ (β -Ala)], 2.84–3.37 [6 H, m, $2 \times \beta\text{-CH}_2$ (Asp) and $\beta\text{-CH}_2$ (Phe)], 3.42–3.71 [4 H, m, $\delta\text{-CH}_2$ (Pro) and $\beta\text{-CH}_2$ (β -Ala)], 3.72 (*c*, 3 H, s, CH_3), 3.8 (*t*, 3 H, s, CH_3), 4.09–5.00 [10 H, m, $5 \times \alpha\text{-H}$, $2 \times \text{CH}_2\text{O}$ and fluorenyl CH], 5.01–5.37 (4 H, m, $\text{CH}_2=\text{CH}$ and CH_2Ph), 5.65 [*t*, 1 H, d, *J* 8.8, NH (urethane)], 5.75 [*c*, 1 H, d, *J* 8.4, NH (urethane)], 5.83–5.94 (1 H, m, $\text{CH}_2=\text{CH}$), 6.31 (*c*, 1 H, d, *J* 7.9, NH), 6.39 (*t*, 1 H, d, *J* 7.8, NH) and 7.11–7.77 (21 H, m, $2 \times \text{NH}$ and Ar-H); δ_{C} (75.4 MHz; C^2HCl_3) 24.51 [$\gamma\text{-CH}_2$ (Pro)], 25.53 [$\beta\text{-CH}_2$ (Glu)], 28.71 [$\gamma\text{-CH}_2$ (Glu)], 29.66 [$\beta\text{-CH}_2$ (Pro)], 35.34 [$\alpha\text{-CH}_2$ (β -Ala)], 35.43 and 35.67 [$\beta\text{-CH}_2$ (Asp)], 36.67 [$\beta\text{-CH}_2$ (β -Ala)], 39.28 [$\beta\text{-CH}_2$ (Phe)], 43.48 [$\delta\text{-CH}_2$ (Pro)], 46.90 and 47.17 [$\alpha\text{-C}$ (Asp)], 51.04 [$\alpha\text{-C}$ (Glu)], 52.81 [CH_3 (Asp)], 55.69 [$\alpha\text{-C}$ (Phe)], 60.68 [$\alpha\text{-C}$ (Pro)], 65.54, 66.95 and 67.75 ($3 \times \text{CH}_2\text{O}$), 118.46 ($\text{CH}_2=\text{CH}$), 120.13, 123.71, 125.22, 127.05, 127.26, 127.57, 127.93, 128.36, 128.57, 128.88, 129.62 and 132.12 (Ar-CH), 136.44 ($\text{CH}_2=\text{CH}$), 135.22, 136.43, 138.24, 141.41, 141.74 and 142.24 (Ar-C quaternary), 156.65 (CO, urethane) and 170.45, 170.95, 171.38, 171.84 and 173.33 (CO esters, amides and acid); *m/z* (ES) 1077 (1%, $[\text{M} + \text{Na}]^+$), 211 (100), 195 (57, $[\text{C}_{14}\text{H}_{11}\text{O}]^+$) and 157 (78, $[\text{C}_7\text{H}_9\text{O}_4]^+$).

Cyclo{-(2*S*)-Phe-[(2*R*)- α -4-benzylpiperidinyl-Asp]- (2*R*)- α -OMe-Glu- γ -(2*S*)-Pro- β -(2*R*)-Asp-} **64**

This compound was prepared in a manner identical to that for compound **57** using the resin-bound precursor **62** (750 mg, 0.27 mmol) which was selectively deprotected and cyclised to give the required compound after purification using preparative HPLC on a C-18 column [using isocratic reverse-phased

conditions, eluting with acetonitrile-water (35:65) at a flow rate of $4.5 \text{ cm}^3 \text{ min}^{-1}$. The eluent was monitored by UV spectroscopy at 220 nm. The fractions corresponding to peak 1 (retention time 2.95 min) were collected and pooled together, and the solvent removed under reduced pressure and by lyophilisation] (36 mg, 17%), mp > 167 °C (decomp.), $\nu_{\text{max}}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$ 3301 (NH), 2939 (CH), and 1732 and 1672 (CO, esters and amides) (HRMS: found $[\text{M} + \text{Na}]^+$, 797.3479. $\text{C}_{40}\text{H}_{50}\text{N}_6\text{O}_{10}\text{Na}$ requires 797.3486); δ_{H} (300 MHz; $\text{C}^2\text{H}_3\text{SO}$, mixture of rotamers) 1.40–1.73 [8 H, m, $\beta\text{-CH}_2$ (Glu), $\gamma\text{-CH}_2$ (Pro) and $2 \times \text{CH}_2\text{-CH}_2\text{NH}$], 1.84–2.05 [3 H, br, 1 H of $\gamma\text{-CH}_2$ (Glu) and $\beta\text{-CH}_2$ (Pro)], 2.10–2.57 [6 H, m, 1 H of $\gamma\text{-CH}_2$ (Glu), $2 \times \text{CH}_2\text{-CH}_2\text{NH}$ and 1 H of $\beta\text{-CH}_2$ (Asp)], 2.68–3.21 [10 H, m, 1 H of $\beta\text{-CH}_2$ (Asp), CHCH_2Ph , CHCH_2Ph , $\delta\text{-CH}_2$ (Pro), $\beta\text{-CH}_2$ (Asp) and $\beta\text{-CH}_2$ (Phe)], 3.63 and 3.72 (3 H, $2 \times$ s, OCH_3), 4.07–4.61 (5 H, m, $\alpha\text{-CH}$ of Glu, Pro, Phe and $2 \times$ Asp), 7.21–7.40 (10 H, m, Ar-H), 7.78–8.36 [3 H, m, NH (Phe) and $2 \times$ NH (Asp)] and 8.58–8.62 [1 H, m, NH (Glu)]; δ_{C} (75.4 MHz; $\text{C}^2\text{H}_3\text{SO}$) 23.47 [$\gamma\text{-CH}_2$ (Pro)], 26.09 [$\beta\text{-CH}_2$ (Glu)], 29.28 [$\beta\text{-CH}_2$ (Pro)], 30.53 [$\gamma\text{-CH}_2$ (Glu)], 31.42 ($2 \times \text{CH}_2\text{-CH}_2\text{NH}$), 35.16 [$\beta\text{-CH}_2$ (Asp)], 36.24 [$\beta\text{-CH}_2$ (Asp)], 37.97 (CHCH_2Ph), 38.15 [$\beta\text{-CH}_2$ (Phe)], 42.86 ($2 \times \text{CH}_2\text{-CH}_2\text{NH}$), 46.31 (CH_2Ph), 46.79 [$\delta\text{-CH}_2$ (Pro)], 46.91 [$\alpha\text{-CH}$ (Asp)], 47.23 [$\alpha\text{-CH}$ (Asp)], 51.03 [$\alpha\text{-CH}$ (Glu)], 52.05 (OCH_3), 56.71 [$\alpha\text{-CH}$ (Phe)], 59.62 [$\alpha\text{-CH}$ (Pro)], 126.11, 126.68, 127.19, 127.53, 127.93, 128.31, 128.85, 128.96, 129.34 and 129.58 (Ar-CH), 135.38 and 136.96 (Ar-C quaternary) and 169.93, 170.27, 170.88, 171.34, 171.76, 172.41 and 173.22 (CO esters, amides and acid); *m/z* (ES) 797 (100%, $[\text{M} + \text{Na}]^+$) and 775 (32, $[\text{M} + \text{H}]^+$).

Cyclo{-(2*S*)-Phe-[(2*R*)- α -4-benzylpiperidinyl-Asp]- (2*R*)-Glu- γ -(2*S*)-Pro- β -(2*R*)-Asp-} **65**

This compound was prepared in a manner identical to that described for cyclo[β -Ala-(2*R*)-Glu- γ -(2*S*)-Pro-(2*R*)-Asp- β -(2*S*)-Phe-] using cyclo{-(2*S*)-Phe-[(2*R*)- α -4-benzylpiperidinyl-Asp]- (2*R*)- α -OMe-Glu- γ -(2*S*)-Pro- β -(2*R*)-Asp-} **64**. *m/z* (MALDITOF) 784 (8%, $[\text{M} + \text{H} + \text{Na}]^+$), 762 (3, $[\text{M} + 2\text{H}]^+$); >95% HPLC purity on a C-18 reverse phase column [using isocratic reverse-phased conditions eluting with acetonitrile-water (51:49) at a flow rate of $1 \text{ cm}^3 \text{ min}^{-1}$. The eluent was monitored by UV spectroscopy at 220 nm. The fractions corresponding to peak 1 (retention time 8.45 min) were collected and pooled together, and the solvent removed under reduced pressure and by lyophilisation].

β -Allyl (2*R*)-*N*-(fluoren-9-ylmethoxycarbonyl)aspartic α -benzylamide **66b**

This compound was prepared in a manner identical to that described for pentapeptide **23**, using the ester **44** (790 mg, 2 mmol) and benzylamine (218 mmol, 2 mmol) to give the required compound as a white crystalline solid (715 mg, 74%), mp 141–142 °C (Found C, 71.3; H, 5.9; N, 6.0. $\text{C}_{29}\text{H}_{28}\text{N}_2\text{O}_5 \cdot 0.25\text{H}_2\text{O}$ requires C, 71.2; H, 5.8; N, 5.75%) (HRMS: found $[\text{M} + 2\text{H}]^+$, 486.2169. $\text{C}_{29}\text{H}_{30}\text{N}_2\text{O}_5$ requires 486.2155); $[\alpha]_{\text{D}} + 13.84$ (*c* 0.43 in MeOH); $\nu_{\text{max}}(\text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$ 3296 (NH) and 1756 and 1742 (CO, esters and amide); δ_{H} (300 MHz; C^2HCl_3) 1.91 (2 H, s, PhCH_2), 2.59 (1 H, dd, *J* 10.4 and 6.4, 1 H of $\beta\text{-CH}_2$), 2.72 (1 H, dd, *J* 10.4 and 6.2, 1 H of $\beta\text{-CH}_2$), 4.16–4.78 (4 H, m, CH_2O , fluorenyl CH and $\alpha\text{-H}$), 5.21–5.38 (2 H, m, $\text{CH}_2=\text{CH}$), 5.82–5.91 [2 H, m, $\text{CH}_2=\text{CH}$, NH (urethane)], 6.81 (1 H, br, NH) and 7.22–7.81 (13 H, m, Ar-H); δ_{C} (75.4 MHz; C^2HCl_3) 36.21 ($\beta\text{-CH}_2$), 43.71 (PhCH_2), 47.23 (fluorenyl CH), 51.19 ($\alpha\text{-C}$), 65.91 and 67.28 ($2 \times \text{CH}_2\text{O}$), 118.97 ($\text{CH}_2=\text{CH}$), 120.18, 125.08, 127.22, 127.65, 127.93 and 128.83 (Ar-CH), 131.66 ($\text{CH}_2=\text{CH}$), 141.46 and 143.74 (Ar-C quaternary), 152.89 (CO, urethane), 168.02 (CO, amide) and 170.25 (CO, ester); *m/z* (CI) 486 (92%, $[\text{M} + 2\text{H}]^+$), 263 (100, $[\text{M} + 2\text{H} - \text{C}_{14}\text{H}_{11}\text{CO}_2]^+$) and 179 (60, $\text{C}_{14}\text{H}_{11}^+$).

(2R)-N-(Fluoren-9-ylmethoxycarbonyl)aspartic α -benzylamide 68b

This compound was prepared through allyl group deprotection in a manner identical to ester **22**, using the diester **66b** (575 mg, 1.19 mmol) to give the required compound as a white crystalline solid (462 mg, 87%), mp 118–119 °C (HRMS: found $[M + H]^+$, 445.1769. $C_{26}H_{25}N_2O_5$ requires 445.1763); $[a]_D +6.88$ (*c* 0.32 in MeOH); ν_{max} (Nujol)/ cm^{-1} 3335 (NH) and 1742 and 1712 (CO, esters and amide); δ_H (300 MHz; $C^2H_3O^2H$) 1.98 (2 H, s, PhCH₂), 2.77 (1 H, dd, *J* 11.1 and 6.5, 1 H of β -CH₂), 2.91 (1 H, dd, *J* 10.7 and 6.3, 1 H of β -CH₂), 4.07–4.68 (4 H, m, CH₂O, fluorenyl CH and α -H), 4.99 (1 H, br, OH) and 7.18–7.86 (13 H, m, Ar-H); δ_C (75.4 MHz; $C^2H_3O^2H$) 35.87 (β -CH₂), 42.84 (PhCH₂), 47.03 (fluorenyl CH), 51.85 (α -C), 65.62 and 66.89 (2 \times CH₂O), 119.69, 125.0, 126.93, 127.09, 127.55 and 128.23 (Ar-CH), 138.46, 141.28 and 143.93 (Ar-C quaternary), 157.07 (CO, urethane) and 170.15 and 172.81 (CO, amide and acid); *m/z* (CI) 445 (6%, $[M + H]^+$) and 179 (100, C₁₄H₁₁⁺).

(2R)-N-(Fluoren-9-ylmethoxycarbonyl)aspartic α -4-benzylpiperidinylamide 68f

To a stirred solution of ester **44** (1 g, 2.53 mmol) in dry THF (25 cm³) at –15 °C was added *N*-methylmorpholine (276 mm³, 2.53 mmol). Isobutyl chloroformate (343 mm³, 2.53 mmol) was added and the suspension was stirred at –15 °C for a further 5 min. A solution of 4-benzylpiperidine (356 mg, 2.53 mmol) and *N*-methylmorpholine (276 mm³, 2.53 mmol) in THF (25 cm³) was added and the mixture left to stir overnight. The hydrochloride salts were removed by filtration and the filtrate concentrated under reduced pressure to give a pale yellow oil which was redissolved in ethyl acetate (15 cm³), washed successively with water (10 cm³), 5% aqueous NaHCO₃ solution (15 cm³), 10% citric acid solution (15 cm³) and then brine (15 cm³). The organic phase was dried (MgSO₄) and concentrated under reduced pressure to give the required diester **66f** which was immediately used in the next reaction.

To a stirred solution of the above diester and phenylsilane (624 mm³, 5.06 mmol) in dry CH₂Cl₂ (30 cm³) under Ar was added tetrakis(triphenylphosphine)Pd(0) (58 mg, 0.05 mmol). The reaction was allowed to stir at room temperature for 18 h, and was then concentrated under reduced pressure. The residue was immediately purified by flash chromatography on silica using ethyl acetate–hexane (60:40) as the eluent to give a white solid (700 mg, 54%), mp 79–81 °C (Found C, 71.3; H, 6.2; N, 5.1. C₃₁H₃₂N₂O₅·0.5H₂O requires C, 71.4; H, 6.4; N, 5.4%); $[a]_D +27.3$ (*c* 0.3 in MeOH); ν_{max} (CH₂Cl₂)/ cm^{-1} 3279 (NH), 3100 br (OH) and 1718 (CO, ester); δ_H (300 MHz; C^2HCl_3) 1.13–1.28 (5 H, m, CHCH₂Ph and 2 \times CH₂-CH₂NH), 1.70 (4 H, br, 2 \times CH₂-CH₂NH), 2.46–3.04 [4 H, m, PhCH₂ and β -CH₂ (Asp)], 3.98–4.59 (4 H, m, CH₂O, fluorenyl CH and α -H), 5.09 (1 H, br, OH), 6.18 [1 H, dd *J* 21 and 9, NH (urethane)] and 7.04–7.77 (13 H, m, Ar-H); δ_C (75.4 MHz; C^2HCl_3) 31.42 (2 \times CH₂-CH₂NH), 37.47 (β -CH₂), 37.95 (2 \times CHCH₂Ph), 42.94 (CH₂-CH₂NH), 46.19 (PhCH₂), 46.94 (α -C), 47.49 (fluorenyl CH), 67.09 (CH₂O), 119.84, 125.03, 125.93, 126.96, 127.58, 128.16 and 128.60 (Ar-CH), 139.59, 141.13, 143.53 and 143.59 (Ar-C quaternary), 155.68 (CO, urethane), 169.88 (CO, ester) and 173.66 (CO, acid); *m/z* (CI) 513 (1%, $[M + H]^+$) and 179 (100, C₁₄H₁₁⁺).

β -Allyl (2R)-N-(fluoren-9-ylmethoxycarbonyl)aspartic α -benzylpiperazinylamide 66g

The α -amide was prepared in a manner identical to that described for pentapeptide **23**, using aspartate monoester **44** (790 mg, 2 mmol) and benzylpiperazine (348 mm³, 2 mmol) to give the required compound as a white waxy solid (763 mg, 81%) (HRMS: found $[M + H]^+$, 554.2667. C₃₃H₃₆N₃O₅ requires 554.2655); $[a]_D +4.19$ (*c* 0.31 in MeOH); ν_{max} (CH₂Cl₂)/ cm^{-1}

3290 (NH), 2963 (CH) and 1732 and 1647 (CO, esters and amide); δ_H (300 MHz; C^2HCl_3) 2.43 (2 H, br, PhCH₂), 2.67 (1 H, dd, *J* 16.2 and 5.7, 1 H of β -CH₂), 2.87 (1 H, dd, *J* 15.9 and 6.6, 1 H of β -CH₂), 3.43–3.76 [8 H, m, 4 \times CH₂ (Piz)], 4.17–4.64 (3 H, m, CH₂O and fluorenyl CH), 5.09 (1 H, q, *J* 6.6, α -H), 5.21–5.35 (2 H, m, CH₂=CH), 5.84–5.97 (1 H, m, CH₂=CH), 6.06 [1 H, d, *J* 9.6, NH (urethane)] and 7.26–7.77 (13 H, m, Ar-H); δ_C (75.4 MHz; C^2HCl_3) 37.70 (β -CH₂), 42.45 (PhCH₂), 47.23 (fluorenyl CH), 47.54 (piperazyl CH₂), 52.62 (α -C), 65.7 and 67.23 (2 \times CH₂O), 118.67 (CH₂=CH), 120.15, 125.26, 127.23, 127.53, 127.90, 128.50 and 129.31 (Ar-CH), 132.03 (CH₂=CH), 137.43, 141.44 and 143.86 (Ar-C quaternary), 155.74 (CO, urethane) and 168.86 and 170.46 (CO, amide and ester); *m/z* (CI) 554 (100%, $[M + H]^+$), 358 (14, $[M - C_{14}H_{11}O]^+$), 179 (24, C₁₄H₁₁⁺) and 57 (57, C₃H₅O⁺).

(2R)-N-(Fluoren-9-ylmethoxycarbonyl)aspartic α -benzylpiperazinylamide 68g

This compound was prepared through allyl group deprotection in a manner identical to diester **21**, using γ -allyl-(2R)-N-(fluoren-9-ylmethoxycarbonyl)aspartic α -benzylpiperazinylamide **66g**, (575 mg, 1.04 mmol) to give the required compound as a white hygroscopic solid which still showed signs of impurity (763 mg, 69%), mp 74–75 °C; ν_{max} (CH₂Cl₂)/ cm^{-1} 3314 (NH), 3064 br (OH) and 1718 and 1635 (CO, esters and amide); δ_H (300 MHz; $C^2H_3O^2H$) 2.01 (2 H, s, PhCH₂), 2.57–3.98 (10 H, m, β -CH₂, CH₂ \times 4 Piz), 4.04–4.73 (3 H, m, CH₂O, fluorenyl CH), 4.98–5.04 (1 H, m, α -H) and 6.83–7.99 (13 H, m, Ar-H); δ_C (75.4 MHz; C^2HCl_3) 37.47 (β -CH₂), 37.95 (PhCH₂), 42.01 (Piz CH₂), 46.94 (fluorenyl CH), 47.49 (α -C), 67.24 (2 \times CH₂O), 119.84, 124.59, 125.03, 125.93, 127.58, 128.16 and 128.60 (Ar-CH), 139.59, 141.13, 143.53 and 143.59 (Ar-C quaternary), 155.68 (CO, urethane) and 168.88 and 173.66 (CO, amide and ester); *m/z* (CI) 511 (1%, $[M - 2H]^+$) and 179 (100, C₁₄H₁₁⁺).

3-Amino-3-(4'-bromophenyl)propanoic acid 69 (R = Br)

To a stirred solution of malonic acid (2.60 g, 25 mmol) and ammonium acetate (3.85 g, 50 mmol) in methanol (35 cm³) was added 4-bromobenzaldehyde (4.63 g, 25 mmol). The suspension was then refluxed for 6 h. Upon cooling the reaction mixture was filtered and then washed on the pad with cold ethanol to give the required product as a white solid (3.05 g, 50%), mp 245–247 °C (softens at 225 °C) (lit.,⁴¹ 264–265 °C) (Found C, 44.75; H, 4.0; N, 5.7. C₉H₁₀NO₂Br requires C, 44.45; H, 4.15; N, 5.75%); ν_{max} (KBr)/ cm^{-1} 3005 br (NH₃⁺), 2875 (CH's), 2546(s), 2156(s), 1630 (amino acid I), 1593 (amino acid II, CO₂⁻) and 1558 (NH₃⁺); δ_H (300 MHz; ²H₂O) 3.63 (2 H, ABX, β -CH₂), 4.18 (1 H, apparent dd, *J* 7.3, 7.4, α -CH), 7.25 (2 H, m, Ar-H) and 7.50 (2 H, m, Ar-H); δ_C (75.5 MHz; ²H₂O) 47.5 (β -CH₂), 53.1 (α -CH), 121.0 (Ar-CH *para*), 129.0 (Ar-CH *meta*), 132.2 (Ar-CH *ortho*), 144.3 (Ar-C quaternary) and 180.6 (CO, acid); *m/z* (FAB) 290 and 288 (47 and 49%, Br isotopes, $[M - H + 2Na]^+$), 137 (100) and 116 (100).

3-[N-(Fluoren-9-ylmethoxycarbonyl)amino]-3-(4'-bromophenyl)propanoic acid 67 (R = Br)

To a stirred solution of 3-amino-3-(4'-bromophenyl)propanoic acid **69** (R = Br) (1.22 g, 5 mmol) in 1 mol dm⁻³ aqueous NaOH (20 cm³) cooled in an ice-bath was added a solution of fluoren-9-ylmethoxycarbonyl chloride (1.42 g, 5.5 mmol) in dioxane (20 cm³). The reaction mixture was warmed to room temperature and stirred for 4 h and then poured into water (20 cm³). The dioxane was removed under reduced pressure and the resulting aqueous solution was washed with diethyl ether (2 \times 25 cm³), then cooled to 0 °C and carefully acidified to pH 2 with a solution of 6 mol dm⁻³ HCl. The mixture was then extracted with ethyl acetate (3 \times 20 cm³), dried (MgSO₄) and the solvent was removed under reduced pressure to give a white solid (1.11 g,

48%), mp 177–179 °C; ν_{\max} (KBr)/cm⁻¹ 3334 (NH), 3400–2800 br (OH, acid), 3042 (CH's) and 1695 (CO, urethane); δ_{H} [300 MHz; (C²H₃)₂SO] 2.68 (2 H, ABX, β -CH₂), 4.19–4.29 (3 H, m, OCH₂CH), 4.84–4.90 (1 H, m, α -CH), 7.18–7.89 (13 H, m, Ar-H), 7.98 (1 H, d, *J* 8.4, NH) and 12.23 (1 H, br s, CO₂H); δ_{C} [75.4 MHz; (C²H₃)₂SO] 40.9 (β -CH₂), 46.9 (OCH₂CH), 51.4 (α -CH), 65.6 (OCH₂), 120.3, 125.3, 127.3, 128.9, 130.4 and 131.4 (Ar-CH), 140.9, 142.5, 143.9 and 144.1 (Ar-C quaternary), 155.5 (CO, urethane) and 171.8 (CO, acid); *m/z* (ES) 488 and 490 (100 and 96%, Br isotopes, [M + Na]⁺).

3-Amino-3-(4'-methoxyphenyl)propanoic acid **69** (R = OMe)

This compound was prepared in a manner identical to that described for the 3-(4'-bromophenyl) analogue **69** (R = Br) using 4-methoxybenzaldehyde (3.40 g, 25 mmol) to give the required product as a white solid (2.78 g, 57%), mp 222–224 °C (lit.,⁴² 228–229 °C) (Found C, 65.2; H, 6.6; N, 8.55. C₉H₁₁NO₂ requires C, 65.4; H, 6.7; N, 8.5%); ν_{\max} (KBr)/cm⁻¹ 2957 br (NH₃⁺), 2932 (CH's), 2631(s), 2150(s), 1673 (amino acid I), 1610 (amino acid II, CO₂⁻) and 1548 (–NH₃⁺); δ_{H} (300 MHz; ²H₂O) 2.53 (2 H, ABX, β -CH₂), 3.81 (3 H, s, OCH₃), 4.19 (1 H, apparent dd, *J* 7.4, 7.4, α -CH), 6.97 (2 H, m, Ar-H) and 7.32 (2 H, m, Ar-H); δ_{C} (75.5 MHz; ²H₂O) 47.6 (β -CH₂), 53.0 (α -CH), 56.0 (OCH₃), 114.7 (Ar-CH *meta*), 128.3 (Ar-CH *ortho*), 137.9 (Ar-C quaternary), 158.5 (Ar-CH *para*) and 180.8 (CO, acid); *m/z* (FAB) 240 (36%, [M – H + 2Na]⁺), 137 (57) and 115 (100).

3-Amino-3-phenylpropanoic acid **69** (R = H)

This compound was prepared in a manner identical to that described for the 3-(4'-bromophenyl) analogue **69** (R = Br) using benzaldehyde (3.85 g, 25 mmol) to give the required product as a white solid (2.15 g, 52%), mp 216–218 °C (lit.,⁴² 218–219 °C) (Found C, 61.4; H, 6.95; N, 7.45. C₁₀H₁₃NO₃ requires C, 61.5; H, 6.7; N, 7.2%); ν_{\max} (KBr)/cm⁻¹ 3020 br (NH₃⁺), 2940 (CH's), 2610(s), 2206(s), 1625 (amino acid I), 1581 (amino acid II, CO₂⁻) and 1516 (–NH₃⁺); δ_{H} (300 MHz; ²H₂O) 2.57 (2 H, ABX, β -CH₂), 4.23 (1 H, apparent dd, *J* 7.3, 7.3, α -CH) and 7.29–7.43 (5 H, m, Ar-H); δ_{C} (75.5 MHz; ²H₂O) 47.6 (β -CH₂), 53.6 (α -CH), 127.0 (Ar-CH *meta*), 127.9 (Ar-CH *para*), 129.3 (Ar-CH *ortho*), 145.1 (Ar-C quaternary), and 180.7 (CO, acid); *m/z* (FAB) 210 (36%, [M – H + 2Na]⁺), 137 (54) and 116 (100).

3-[N-(Fluoren-9-ylmethoxycarbonyl)amino]-3-phenylpropanoic acid **67** (R = H)

This compound was prepared in a manner identical to that described for the 3-(4'-bromophenyl) analogue **67** (R = Br) using 3-amino-3-phenylpropanoic acid **69** (R = H) to give a white solid (1.49 g, 77%), mp 186–188 °C (Found C, 74.35; H, 5.45; N, 3.54. C₂₄H₂₁NO₄ requires C, 74.4; H, 5.45; N, 3.6%); ν_{\max} (KBr)/cm⁻¹ 3365 (NH), 3400–2800 br (OH, acid), 3043 (CH's) and 1740 (CO, urethane); δ_{H} [300 MHz; (C²H₃)₂SO] 2.68 (2 H, ABX, β -CH₂), 4.17–4.29 (3 H, m, OCH₂CH), 4.95 (1 H, apparent q, *J* 8.4, α -CH), 7.22–7.90 (13 H, m, Ar-H), 7.97 (1 H, d, *J* 8.6, NH) and 12.31 (1 H, s, CO₂H); δ_{C} [75.4 MHz; (C²H₃)₂SO] 41.2 (β -CH₂), 46.9 (OCH₂CH), 51.8 (α -CH), 65.5 (OCH₂), 120.3, 125.3, 126.5, 127.2, 127.8 and 128.5 (Ar-CH), 140.9, 143.0, 143.9 and 144.1 (Ar-C quaternary), 155.5 (CO, urethane) and 171.9 (CO, acid); *m/z* (ES) 410 (100%, [M + Na]⁺).

α -Methyl γ -allyl (2*R*)-*N*-(fluoren-9-ylmethoxycarbonyl)aspartate **70**

This compound was prepared in a manner identical to diester **14**, using γ -allyl (2*R*)-*N*-(fluoren-9-ylmethoxycarbonyl)aspartate monoester **44** (1.00 g, 2.54 mmol) to give the required compound as a white crystalline solid in quantitative recovery (1.04

g), mp 96–97 °C (Found C, 67.4; H, 5.7; N, 3.5. C₂₃H₂₃NO₆ requires C, 67.5; H, 5.65; N, 3.4%) (HRMS: found [M + H]⁺, 410.1609 C₂₃H₂₄NO₆ requires 410.1603); [α]_D –15 (*c* 0.3 in MeOH); ν_{\max} (CH₂Cl₂)/cm⁻¹ 3374 (NH), 2965 (CH) and 1751 (CO, urethane); δ_{H} (300 MHz; C²HCl₃) 2.91 (1 H, dd, *J* 17.4 and 4.1, 1 H of β -CH₂), 3.09 (1 H, dd, *J* 16.8 and 4.5, 1 H of β -CH₂), 3.77 (3 H, s, CH₃), 4.20–4.74 (6 H, m, 2 × CH₂O, fluorenyl CH and α -H), 5.21–5.38 (2 H, m, CH₂=CH), 5.82–6.21 [2 H, m, CH₂=CH and NH (urethane)] and 7.23–7.79 (8 H, m, Ar-H); δ_{C} (75.4 MHz; C²HCl₃) 36.75 (β -CH₂), 47.23 (fluorenyl CH), 50.53 (α -C), 53.01 (CH₃), 65.89 and 67.4 (2 × CH₂O), 118.92 (CH₂=CH), 120.15, 125.28, 127.23 and 127.89, 127.9 (Ar-CH), 131.76 (CH₂=CH), 141.44, 143.86 and 143.98 (Ar-C quaternary), 156.12 (CO, urethane) and 170.76 and 171.32 (CO, esters); *m/z* (CI) 410 (57%, [M + H]⁺) and 179 (100, C₁₄H₁₁⁺).

α -Methyl (2*R*)-*N*-(fluoren-9-ylmethoxycarbonyl)aspartate **71**

The monoester was prepared in a manner identical to α -methyl (2*R*)-*N*-(fluoren-9-ylmethoxycarbonyl)glutamate **46**, using methyl allyl diester **70** (242 mg, 0.50 mmol) to give the required compound as a white crystalline solid (134 mg, 60%), mp 124–125 °C (Found C, 64.8; H, 5.2; N, 3.6. C₂₀H₁₉NO₆ requires C, 65.05; H, 5.2; N, 3.8%) (HRMS: found [M + H]⁺, 370.1298. C₂₀H₂₀NO₆ requires 370.1291); [α]_D +19.05 (*c* 0.21 in MeOH); ν_{\max} (CH₂Cl₂)/cm⁻¹ 3335 (NH), 3100 br (OH) and 1727 (CO, ester); δ_{H} (300 MHz; C²H₃O²H) 2.82 (1 H, m, β -CH₂), 3.71 (3 H, s, CH₃), 4.08–4.62 (4 H, m, CH₂O, fluorenyl CH and α -H) and 7.24–7.82 (13 H, m, Ar-H); δ_{C} (75.4 MHz; C²HCl₃) 35.76 (β -CH₂), 47.03 (fluorenyl CH), 50.69 (α -C), 51.71 (CH₃), 66.85 (CH₂O), 119.64, 124.96, 126.88 and 127.50 (Ar-CH), 134.33, 141.28, 143.88 and 143.93 (Ar-C quaternary), 157.06 (CO, urethane), 171.91 (CO, ester) and 172.57 (CO, acid); *m/z* (CI) 370 (99%, [M + H]⁺), 192 (64, [M + 2H – C₁₄H₁₁]⁺), 179 (100, C₁₄H₁₁⁺) and 148 (85, C₅H₁₀NO₄⁺).

α -Methyl (2*R*)-*N*-(fluoren-9-ylmethoxycarbonyl)aspartyl-Wang resin **72**

The resin ester was prepared in a manner identical to that described for β -allyl (2*R*)-*N*-(fluoren-9-ylmethoxycarbonyl)aspartyl-Wang resin **45**, using α -methyl ester **71** (1 g, 2.71 mmol). The loading was determined to be 80%.⁵³

(2*S*)-Phenylalanyl-[3-phenylpropanoyl]-[α -methyl (2*R*)-glutamyl]-sarcosyl-[α -methyl (2*R*)-aspartate] diester **74**

(2*S*)-Phenylalanyl-[3-phenylpropanoyl]-[α -methyl (2*R*)-glutamyl]-sarcosyl-[α -methyl (2*R*)-aspartate-Wang resin] diester was synthesised on the peptide synthesiser and treated with cleavage mixture TFA–TES–H₂O–CH₂Cl₂ (40:2:5:53) at room temperature for 1 h. The resin was filtered off and the solvent concentrated under reduced pressure (4–5 cm³). Trituration with excess diethyl ether resulted in the pentapeptide **74** being obtained as a white solid (150 mg), mp >200 °C (decomp.). ν_{\max} (KBr disc)/cm⁻¹ 2500–3000 br (OH), 1730 (CO, ester) and 1670 (CO, amides); δ_{H} [300 MHz; (C²H₃)₂SO] 1.60–1.90 [2 H, m, β -CH₂ (Glu)], 2.00–2.30 [2 H, m, γ -CH₂ (Glu)], 2.60–2.80 [4 H, m, α -CH₂ (Prop) and β -CH₂ (Asp)], 2.93 and 2.96 (3 H, 2 × s, NCH₃), 2.82–3.05 [2 H, m, PhCH₂], 3.56–3.65 (6 H, m, 2 × CH₃), 3.80–4.02 [3 H, m, α -H (Phe) and CH₂ (Sar)], 4.10–4.23 [1 H, m, α -H (Glu)], 4.57–4.63 [1 H, m, α -H (Asp)], 5.15–5.25 [1 H, m, α -H (Prop)], 7.02–7.40 (10 H, m, Ar-H), 8.10 [2 H, br, NH₂ (Phe)], 8.32 [1.5 H, br, NH (Glu) and 0.5 NH (Asp)], 8.53 [0.5 H, m, 0.5 NH (Asp)], 8.78 [1 H, m, NH (Prop)].

Cyclo-[3-phenylpropanoyl-(2*R*)-Glu- α -OMe- γ -Sar-(2*R*)-Asp- α -OMe- β -(2*S*)-Phe-] **75**

To a stirred solution of the pentapeptide **74** (350 mg, 0.6 mmol), in CH₂Cl₂–DMF (9:1, 50 cm³) was added DIPEA

(150 mm³, 1.5 mmol). The mixture was cooled to 0 °C using an ice bath, and then BOP-Cl (156 mg, 0.66 mmol) was added and the mixture stirred at 0 °C for at 6 h. The solution was allowed to warm up to room temperature and stirred for a further 7 days. The resulting solution was then washed with 10% citric acid, brine, 5% sodium bicarbonate, distilled water and finally brine again. The organic layer was separated, dried (MgSO₄) and solvent removed under reduced pressure to a volume of 1 cm³. Titration with diethyl ether (10 cm³) caused the compound to precipitate out as a white solid (80 mg, 24%), mp >220 °C (decomp.) (HRMS: found [M + H]⁺, 638.2847. C₃₂H₄₀N₃O₉ requires 638.2826); ν_{max}(KBr disc)/cm⁻¹ 2500–3000 br (OH), 1730 (CO, ester) and 1670 (CO, amides); δ_H[500 MHz; (C²H₅)₂SO, mixture of rotamers] 1.65–1.95 [2 H, m, β-CH₂ (Glu)], 2.00–2.20 [2 H, m, γ-CH₂ (Glu)], 2.35–2.71 [4 H, m, α-CH₂ (Prop) and β-CH₂ (Asp)], 2.70–2.75 [1 H, m, 1 H of PhCH₂], 2.82 and 2.84 (3 H, 2 × s, NCH₃), 3.00–3.17 [1 H, m, 1 H of PhCH₂ (Phe)], 3.63, 3.65 and 3.67 (6 H, 3 × s, 2 × CH₃), 4.02 [2 H, AB, J 15.5, CH₂ (Sar)], 4.21–4.28 [1 H, m, α-H (Glu)], 4.48 [1 H, m, α-H (Asp)], 4.45 and 4.61 [1 H, 2 × m, α-H (Phe)], 5.26–5.44 [1 H, m, α-H (Prop)], 7.05–7.40 (10 H, m, Ar-H), 7.88 [0.75 H, m, NH (Phe)], 8.17 [0.75 H, m, NH (Asp)], 8.37 [0.25 H, m, NH (Phe)], 8.51 [0.75 H, m, 0.5 NH (Glu)], 8.64 [0.25 H, m, NH (Prop)] and 8.89 [1 H, m, NH (Prop)]; δ_C[75.4 MHz; (C²H₅)₂SO] 26.76 [β-CH₂ (Glu)], 29.20 [γ-CH₂ (Glu)], 34.52, 35.78 (NCH₃), 36.84 [β-CH₂ (Asp)], 36.84 and 37.98 [PhCH₂], 43.43 [β-CH₂ (Prop)], 49.61 [α-C (Asp)], 50.96 [α-C (Prop)], 51.75 [α-C (Glu)], 51.95 and 52.25 [CH₂ (Sar) and 2 × CH₃], 53.19 and 54.30 [α-C (Phe)], 126.18, 126.34, 126.87, 127.05, 128.04, 128.24, 128.34, 128.42, 128.50, 129.05, 129.20 and 129.45 (Ar-CH and Ar-C quaternary) and 168.16, 168.52, 170.24, 170.26, 171.58, 172.40 and 172.88 (CO, amides, esters); m/z (FAB) 660 (65%, [M + Na]⁺) and 638 (100, [M + H]⁺).

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