Design and preparation of serine–threonine protein phosphatase inhibitors based upon the nodularin and microcystin toxin structures: Part 2.[1] Synthesis of a functionalised nodularin macrocycle and a stripped-down microcystin macrocycle

Antony B. Maude, Amit P. Mehrotra and David Gani*

School of Chemistry and Centre for Biomolecular Sciences, The Purdie Building, The University, St. Andrews, Fife, UK KY16 9ST

Nodularins and microcystins are complex natural isopeptidic hepatotoxins that serve as subnanomolar inhibitors of the eukaryotic serine–threonine protein phosphatases, PP1 and PP2A. In Part 1 (A. P. Mehrotra, K. L. Webster and D. Gani, *J. Chem. Soc., Perkin Trans. 1*, 1997, preceding paper) each of the key structural or potentially reactive motifs within each macrocycle type was assessed as a contributor towards phosphatase inhibitory efficacy and a stripped-down nodularin-type macrocycle was identified as a suitable precursor to potentially active synthetic inhibitors. Subsequently, synthetic routes to the 19-membered nodularin macrocyclic system were developed, using solution-phase chemistry, which demonstrated that only certain cyclisation protocols were viable. Here we describe an extension of this chemistry to provide a 19-membered nodularin macrocycle, *cyclo*-[(3*R*)-3-hydroxymethyl-β-Ala-(*R*)-Glu-α-OMe-γ-Sar-(*R*)-Asp-α-OMe-β-(*S*)-Phe-], appropriately functionalised with a hydroxymethyl group for the incorporation of lipophilic side-chains. We also demonstrate that the 25-membered microcystin macrocycle, *cyclo*-[β-Ala-(*R*)-Glu-α-OMe-γ-Sar-(*R*)-Ala-(*S*)-Leu-(*R*)-Asp-α-OMe-β-(*S*)-Phe-], can be prepared in good yield using similar protocols in which macrocyclisation is effected through the reaction of the amino group of the (2*S*)-phenylalanine residue with the β-pentafluorophenyl ester of the (2*R*)-aspartic acid residue.

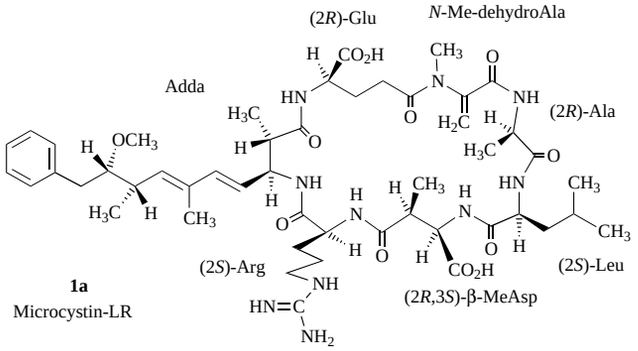
Introduction

The natural cyclic isopeptide toxins microcystin **1** and nodularin **2**, previously established as potent hepatotoxins,[2] are now known to inhibit the catalytic subunit of the mammalian serine/threonine protein phosphatases PP1 and PP2A (but not PP2B or 2C) with subnanomolar inhibition constants.[3] Each of these catalytic activities, together with serine/threonine protein kinase enzymes, are involved in controlling and maintaining the delicate balance of pools of phosphorylated and dephosphorylated proteins which effect cellular metabolism and communication.[4–6] The two toxin-sensitive enzymes, cat-PP1 and cat-PP2A, are highly homologous and display ~50% amino acid sequence identity.[7,8] Both families of toxin, microcystins and nodularins, differ considerably from other cyclic peptides. Both groups are cyclic tri-isopeptides and contain two free carboxylic acid groups, an *N*-methyldehydroamino acid moiety, and a large, rigid, lipophilic side-chain which forms part of an Adda residue (see structures **1** and **2**). These five principal motifs are the only structural features that are conserved between the two families.

Other naturally occurring competitive inhibitors of cat-PP1 and cat-PP2A include the powerful tumour promoter okadaic acid, responsible for diarrhetic shellfish poisoning, and the calyculins, tautomycin and cantharidin.[9] However, none of these compounds shows any specificity towards either of the two enzyme types. This lack of specificity can now be rationalised by an analysis of the aligned amino acid sequences for the enzymes within the context of the published X-ray crystal structures available for cat-PP1.[10] Essentially this analysis indicates that the structure and composition of the active-site cleft is extremely highly conserved and that the inhibitors interact only with conserved regions.[11]

As part of a larger programme to prepare and identify specific inhibitors for each enzyme type, we recently demonstrated that the dehydroamino acid residue present in microcystin was not essential in conferring activity as an inhibitor.[1,12] Subsequently we proceeded to evaluate the functional role of each of the remaining conserved structural motifs within the two classes of toxin, using data available for natural variants, and concluded that the cyclic structure, the two free carboxylic acid groups and elements of the large rigid lipophilic side-chain would need to be retained in active inhibitors. This work led to the identification of a stripped down and simpler macrocycle (than that present in each of the natural products **1** and **2**) which should serve as a framework for attaching specific functionalities.[11] Since our longer term interests were to synthesise minimal analogues to probe the active-site binding interactions and then to identify specific inhibitors for each catalytic subunit type, PP1 and PP2A, we decided to opt for a convergent synthetic strategy in which 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 also be suitable for preparing libraries of macrocycles and libraries of lipophilic side-chain precursors that could be connected to give a diverse array of synthetic toxin analogues.

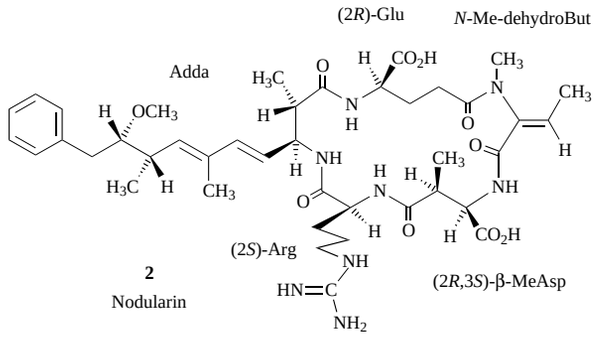
To assess the viability of this strategy and, in particular, to determine whether or not it would be possible to prepare stripped down nodularin analogues lacking the side-chain functionalities, we tested various cyclisation protocols on a model nodularin macrocycle target, *cyclo*-[β-Ala-(*R*)-Glu-α-OMe-γ-Sar-(*R*)-Asp-α-OMe-β-(*S*)-Phe-], **3**. 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



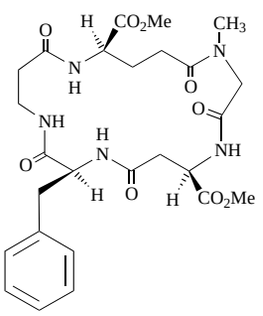
1a
Microcystin-LR

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

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



2
Nodularin



3

group of the (2*S*)-phenylalanine residue proceeded in excellent yield (89%).[1,12] In this article we describe the extension of this chemistry to provide a 19-membered nodularin macrocycle, *cyclo*-[(3*R*)-3-hydroxymethyl-β-Ala-(*R*)-Glu-α-OMe-γ-Sar-(*R*)-Asp-α-OMe-β-(*S*)-Phe-], **4**, appropriately functionalised with a hydroxymethyl group for the incorporation of lipophilic side-chains. We also show that it is possible to prepare the 25-membered microcystin macrocycle, *cyclo*-[β-Ala-(*R*)-Glu-α-OMe-γ-Sar-(*R*)-Ala-(*S*)-Leu-(*R*)-Asp-α-OMe-β-(*S*)-Phe-], **5**, in good yield, effecting macrolactamisation through the reaction of the amino group of the (2*S*)-phenylalanine residue with the β-pentafluorophenyl ester of the (2*R*)-aspartic acid residue.

Results and discussion

Synthesis of a functionalised nodularin macrocycle

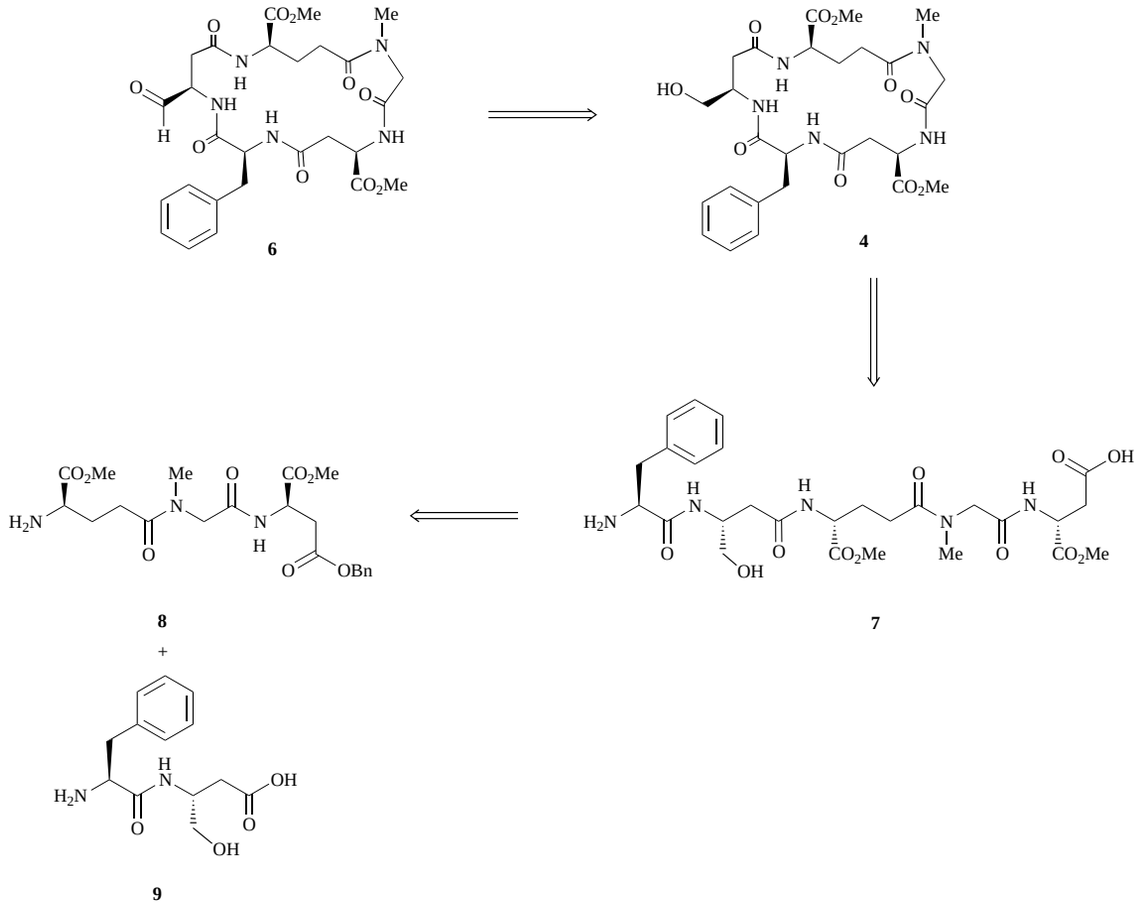
In the synthesis of the model nodularin macrocycle, *cyclo*-[β-Ala-(*R*)-Glu-α-OMe-γ-Sar-(*R*)-Asp-α-OMe-β-(*S*)-Phe-] **3** described earlier,[1,12] the lipophilic Adda side-chain was totally omitted through the use of a β-alanine residue. Since we wished to introduce a group into the 3-position of β-alanine that could be easily modified to provide a range of lipophilic side-chains, including those containing functionalities to provide rigidity, we chose to use a 3-formyl group, *i.e.* the aldehyde **6**, Scheme 1. 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.[13–18] Mann and co-workers have also very recently reported on a synthesis of the Adda residue utilising the α-semialdehyde derived from (2*R*,3*S*)-3-methylaspartic acid as an intermediate in which the lipophilic side-chain was introduced using a Stille coupling reaction.[19,20]

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 **4** was devised in which the formyl group would be masked as a primary alcohol. It was also reasoned, by comparison to the well established peptide chemistry of serine and threonine residues, that such an alcohol group could be introduced early in the synthesis of the linear peptide precursors and could be subjected to all of the conditions required for peptide coupling (masking, unmasking and acyl-group activation) without the need for protection.[21] Thus, access to a (3*R*)-3-amino(hydroxymethyl)-β-alanine [(3*R*)-3-amino-4-hydroxybutyric acid (AHB)] residue would be required.

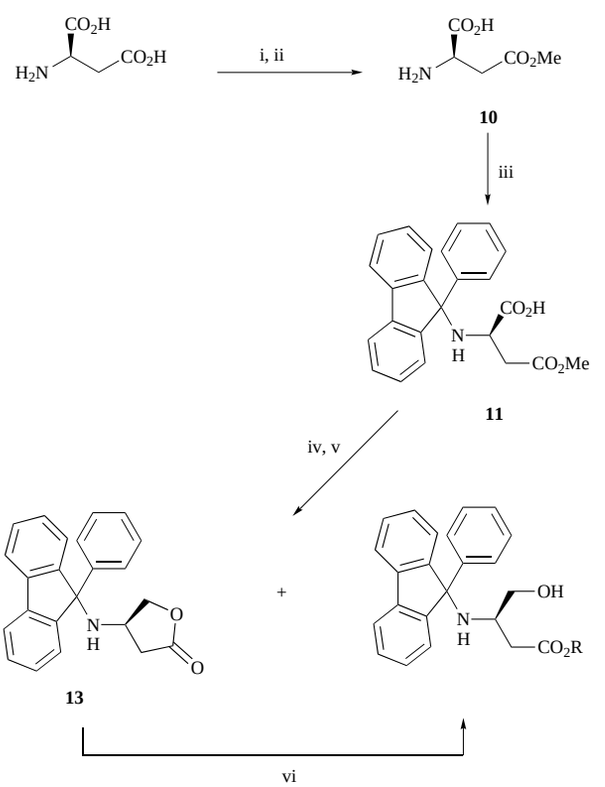
Disconnection of *cyclo*-[(3*R*)-3-hydroxymethyl-β-Ala-(*R*)-Glu-α-OMe-γ-Sar-(*R*)-Asp-α-OMe-β-(*S*)-Phe-] **4** at the peptide bond between the Phe residue and the β-carbonyl moiety of the Asp residue, using the same strategy[1,12] as employed for the non-functionalised macrocycle **3**, gives the linear isopentapeptide **7**. The three C-terminal residues had been incorporated into macrolactam **3** previously as the tripeptide triester (2*R*)-Glu-α-OMe-γ-Sar-(2*R*)-Asp-α-OMe-β-OBn **8** without incident and, therefore, the isopentapeptide (2*S*)-Phe-(3*R*)-3-hydroxymethyl-β-Ala-(2*R*)-Glu-α-OMe-γ-Sar-(2*R*)-Asp-α-OMe-β-OH **7** was disconnected between the (3*R*)-3-hydroxymethyl-β-alanine and Glu residues to give tripeptide triester **8** and the dipeptide (2*S*)-Phe-(3*R*)-3-hydroxymethyl-β-Ala-OH **9**, Scheme 1.

Two routes towards an appropriately protected hydroxymethyl dipeptide **9** were pursued. In the first route, the β-methyl ester hydrochloride of (2*R*)-aspartic acid was prepared by the method of Schwarz *et al.*[22,23] Refluxing a solution of the hydrochloride in ethanol with propylene oxide gave the enantiomerically pure free-base form of the β-methyl ester **10**, mp 194–195 °C [lit.,[24] 194–195 °C for the (2*S*) antipode], which was converted into its α-trimethylsilyl ester. Without isolation the diester was protected on nitrogen with a phenylfluorenyl group,[24] introduced using 9-bromo-9-phenylfluorene, and the α-silyl ester was hydrolysed during the work-up to give the required secondary amine **11**, mp 160 °C [lit.,[24] 160–161 °C for (2*S*) antipode], which displayed the expected properties, Scheme 2. Preparation of the mixed α-carboxy isobutyl carbonic anhydride followed by reduction with sodium borohydride in very dry THF gave the required alcohol **12** (R = Me) in 91% yield, mp 135–137 °C. This reduction protocol was unreliable and often yielded significant amounts of lactone **13**, mp 156–158 °C. While the lactone could be efficiently converted into alcohol **12** (R = H) by using sodium hydroxide in aq. methanol the overall yields were only moderate and we wished to assess an alternative strategy before committing the material to the synthesis of the macrolactam.

Accordingly, commercial (2*R*)-(*tert*-*N*-butoxycarbonyl)-aspartic acid β-benzyl ester was converted into the mixed carbonic anhydride, which was reduced with sodium borohydride in THF to give the required alcohol **14** in an optimised yield of 89%, Scheme 3. This compares favourably with yields reported by Bland for the preparation of the (2*S*)-enantiomer.[25] The removal of the N-Boc protecting group using hydrogen chloride gas in ethyl acetate was accompanied by extensive cleavage of the benzyl ester moiety to give (3*R*)-3-amino-4-hydroxybutyric acid hydrochloride, mp 177–178 °C. However, this unwanted side-reaction could be largely avoided through treatment of compound **14** with trifluoroacetic acid (TFA) in dichloro-



Scheme 1



Scheme 2 *Reagents*, *conditions* (*and yields*): i, $SOCl_2$, MeOH, −30 ⟶ 10 °C (100%); ii, propylene oxide, EtOH, reflux (90%); iii, TMSCl, Et_3N, 9-bromo-9-phenylfluorene, $Pb(NO_3)_2$, CH_2Cl_2, room temp. (81%); iv, IBCF, NMM, THF, −15 °C; v, $NaBH_4$, THF, −15 °C ⟶ room temp. (91%); vi, aq. NaOH, MeOH (85%) (R = H)

methane. This modification gave the required, almost pure, deprotected amine salt **15** as judged by ^{1}H and ^{13}C NMR spectroscopy, which could be used directly for the next stage. Without further purification the amino alcohol **15** was treated with the mixed carboxylic-carbonic anhydride of *N*-Boc-(2*S*)-phenylalanine in the presence of NMM to give *N*-Boc-(2*S*)-Phe-(3*R*)-3-hydroxymethyl-β-Ala-OBn **16** in 90% overall yield from alcohol **14**. Catalytic hydrogenolysis of the benzyl ester protecting group, Scheme 3, gave the dipeptide acid **17** (mp 95–97 °C), which displayed the expected spectral and analytical properties. Importantly, the free acid **17** and its benzyl ester derivative **16** possessed only one set of signals in their ^{13}C NMR spectra, indicating that no racemisation had occurred at the α-centre of the Asp residue during the reduction of the mixed anhydride. Thus, compound **17**, a suitably protected form of compound **9** (see Scheme 1) was ready for reaction with the tripeptide triester **8**.

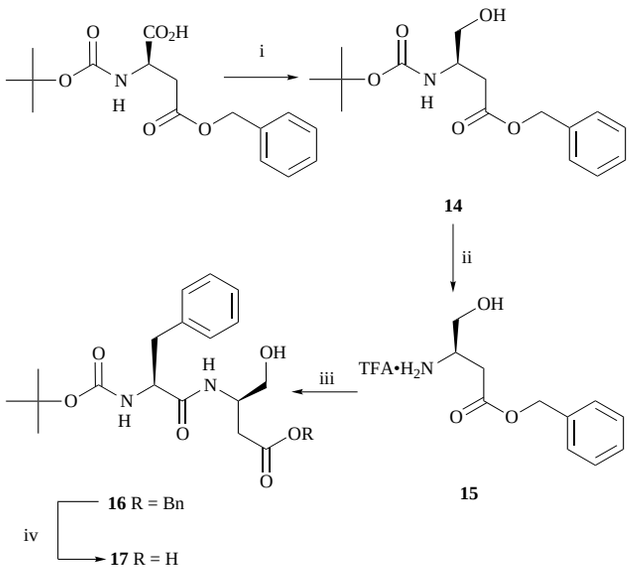
In order to construct the linear isopeptide precursor the carboxy terminal of *N*-Boc-(2*S*)-Phe-(3*R*)-3-hydroxymethyl-β-Ala-OH **17** was activated as the mixed carbonic anhydride and was treated with tripeptide triester, (2*R*)-Glu-α-OMe-γ-Sar-(2*R*)-Asp-α-OMe-β-OBn, **8** prepared as described previously[1] to give the linear pentapeptide ester **18** in 83% yield after column chromatography, {mp 89–91 °C; $[a]_D^{23}$ +9.03† (CH_2Cl_2)}. The compound gave satisfactory analytical data but, as was observed previously for sarcosine (*N*-methylglycine)-containing peptides in the synthesis of macrocycle **3**,[1,12] both rotameric forms of the compound corresponding to the *cis* and *trans* configurations at the γ-Glu–Sar amide bond were observable by ^{1}H and ^{13}C NMR spectroscopy.

Removal of the benzyl ester in compound **18** by catalytic hydrogenolysis, followed by EDCI-mediated esterification of

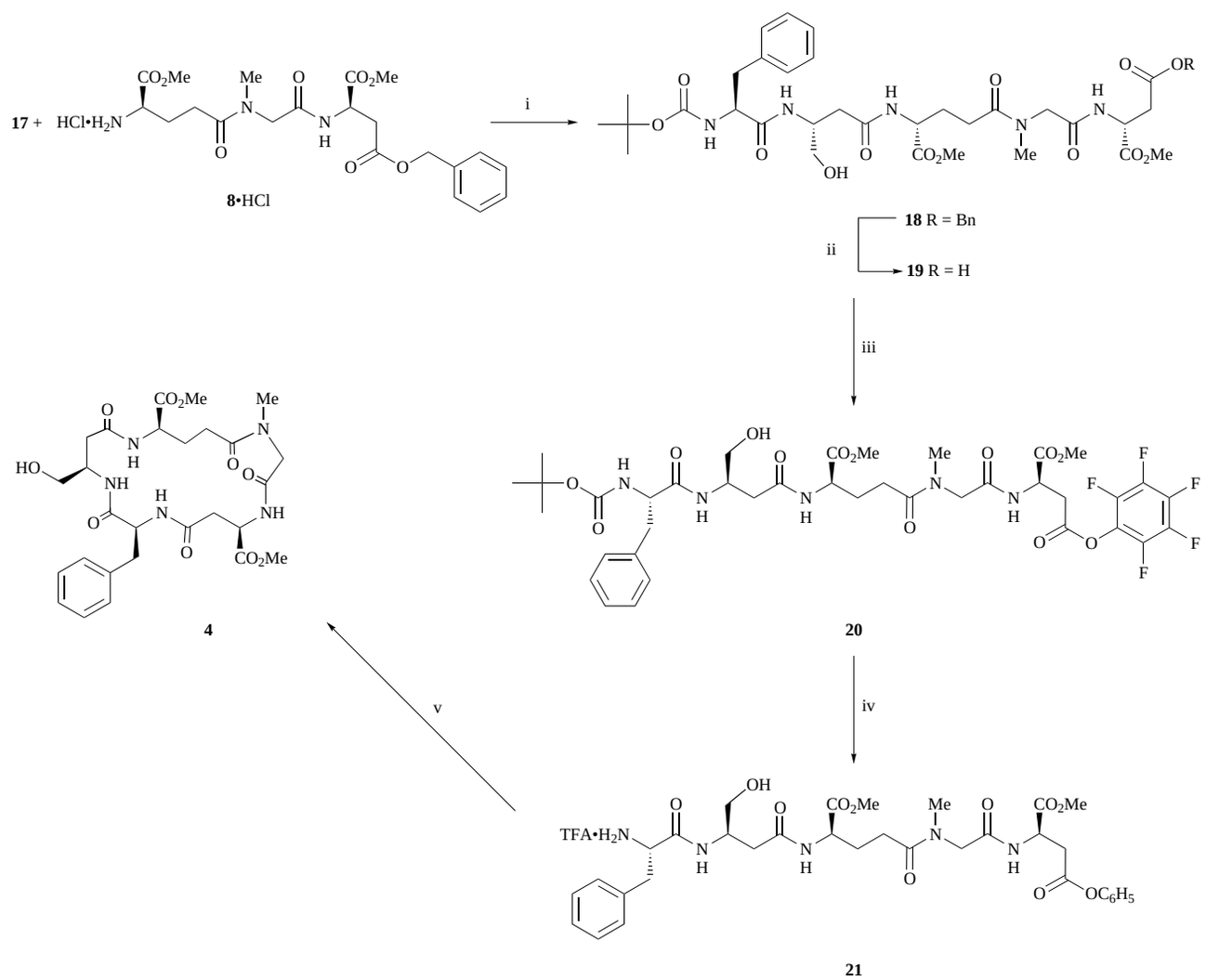
† Specific optical rotations $[a]_D$ are given in units of 10^{-1} deg cm^2 g^{-1}.

the resulting free acid **19** with pentafluorophenol, proceeded smoothly to give the *N*-Boc pentapeptide pentafluorophenyl ester **20** in 61% yield over the two steps (see Scheme 4) after purification by flash chromatography on silica gel. The N-terminal *tert*-butoxycarbonyl protecting group was removed by treatment with TFA in dichloromethane and the resulting amine trifluoroacetate salt **21** was thoroughly dried under high vacuum. Treatment with DIPEA, under conditions of high dilution in dichloromethane, allowed cyclisation to proceed and after nine days at room temperature, when periodic TLC analysis showed that the conversion of starting material was complete, the reaction was terminated. The required functionalised macrocyclic pentapeptide **4** was obtained in 41% yield after flash column chromatography on silica and elution with CH_2Cl_2–MeOH (94:6), mp 96–100 °C. No evidence for the formation of significant amounts of lactone was obtained for the crude material and the purified compound displayed all of the expected spectral and analytical properties. Macrocycle **4** showed two sets of signals in NMR spectra obtained in deuteriochloroform corresponding to two conformations in exchange on the NMR time-scale. This rate ~1–10^3 s^{-1}, would be too fast to be associated with interconverting amide rotamers[26] in small linear proline peptides,[27] but could be consistent with interconverting rotamers in a macrocycle where the attainment of amide-bond planarity in both rotamers causes other high-energy interactions.

The macrolactamisation of (2*S*)-Phe-(3*R*)-3-hydroxymethyl-β-Ala-(2*R*)-Glu-α-OMe-γ-Sar-(2*R*)-Asp-α-OMe-β-OPFP **21** to give compound **4** was noticeably more difficult than the cyclisation of (2*S*)-Phe-β-Ala-(2*R*)-Glu-α-OMe-γ-Sar-(2*R*)-Asp-α-OMe-β-OPFP to give macrocycle **3**. One possible reason is that the free hydroxy group forms a hydrogen bond with an amide or



Scheme 3 *Reagents, conditions* (*and yields*): i, IBCF, NMM, THF, −15 °C; then $NaBH_4$, THF, −15 °C ⟶ room temp. (89%); ii, TFA, CH_2Cl_2; iii, (2*S*)-*N*-Boc-PheOH, IBCF, NMM, THF, −30 °C; then **15**, NMM, THF, −30 °C ⟶ room temp. (90%); iv, H_2, Pd/C, EtOH (98%)



Scheme 4 *Reactions, conditions* (*and yields*): i, IBCF, NMM, THF, −30 °C ⟶ room temp. (83%); ii, H_2, Pd/C, EtOH (97%); iii, C_6F_5OH, EDCI, CH_2Cl_2, 0 °C ⟶ room temp. (61%); iv, TFA, CH_2Cl_2; v, DIPEA, CH_2Cl_2 (41%)

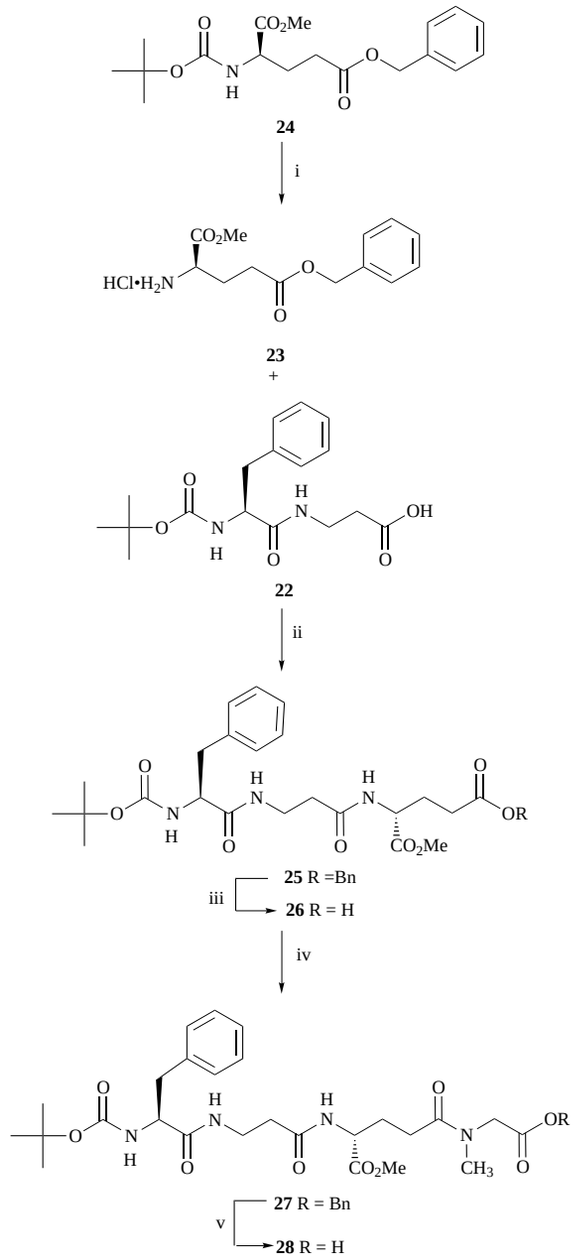
ester moiety which stabilises a conformation which is not amenable to macrolactamisation. This possibility is discussed in more detail below.

Synthesis of the stripped-down microcystin macrocycle

In view of our experiences with the nodularin macrocycle which had clearly demonstrated that certain cyclisation strategies were not viable we wished first to assess the equivalent disconnection to that which had been successfully used to prepare the non-functionalised macrocycle **3**.[1,12] Natural microcystins **1** differ from nodularins **2** in containing two extra contiguous amino acids, a (2*R*)-alanine residue and a variable (2*S*)-α-amino acid (leucine in microcystin-LR), that are inserted between the carbonyl moiety of the *N*-methyldehydroamino acid and the N-atom of the (2*R*,3*S*)-3-methylaspartate residue of nodularin. Therefore the dipeptide (2*R*)-Ala-(2*S*)-Leu was inserted into structure **3** to define the model microcystin macrocycle target as structure **5**. The equivalent disconnection to that utilised in the preparation of nodularin macrocycle **3**[1,12] applied to the synthesis of *cyclo*-[β-Ala-(2*R*)-Glu-α-OMe-γ-Sar-(2*R*)-Ala-(2*S*)-Leu-(2*R*)-Asp-α-OMe-β-(2*S*)-Phe-] **5** would require the closure to be effected between the Phe N-atom and the β-carboxy group of the (2*R*)-aspartate α-methyl ester. Note that this approach differs from that very recently described (after the completion of our own work) for the synthesis of microcystin-LA, in which the cyclisation step involved connecting the Adda N-atom to the (2*S*)-Ala carboxy group.[17] Thus, to synthesise the stripped-down microcystin-type macrocycle **5** we needed to prepare an appropriately protected and activated linear peptide of the sequence (2*S*)-Phe-β-Ala-(2*R*)-Glu-α-OMe-γ-Sar-(2*R*)-Ala-(2*S*)-Leu-(2*R*)-Asp-α-OMe-β-OH.

Accordingly, α-methyl γ-benzyl *N*-Boc-(2*R*)-glutamate diester **24** was treated with hydrogen chloride in ethyl acetate to give the deprotected hydrochloride salt **23**. This was coupled to (2*S*)-*N*-Boc-phenylalanyl-β-alanine **22**, prepared as described previously,[1,12] to give the tripeptide, *N*-Boc-(2*S*)-Phe-β-Ala-(2*R*)-Glu-α-OMe-γ-OBn, **25**, in 95% yield (see Scheme 5). Removal of the benzyl ester protection through catalytic hydrogenolysis gave the free acid **26** in 91% yield {mp 115–117 °C; $[\alpha]_D^{25}$ +14.24 (MeOH)} which displayed the expected spectral and analytical properties. Activation of the free acid **26** as the mixed isobutyl carbonic anhydride followed by reaction with sarcosine benzyl ester gave the tetrapeptide *N*-Boc-(2*S*)-Phe-β-Ala-(2*R*)-Glu-α-OMe-γ-Sar-OBn **27** in 80% yield. This material {mp 110–112 °C; $[\alpha]_D^{25}$ +9.1 (MeOH)} gave satisfactory analytical and mass data but, as is expected for sarcosine-containing peptides which exist in similarly populated *cis* and *trans* rotameric forms about the γ-Glu–Sar peptide bond, two sets of signals were observed in ^{1}H and ^{13}C NMR spectra. The benzyl ester in tetrapeptide **27** was removed by catalytic hydrogenolysis to give the free acid, *N*-Boc-(2*S*)-Phe-β-Ala-(2*R*)-Glu-α-OMe-γ-Sar-OH, **28**, in good recovery which was sufficiently pure to use in subsequent coupling reactions, as judged by an examination of ^{1}H and ^{13}C NMR spectra.

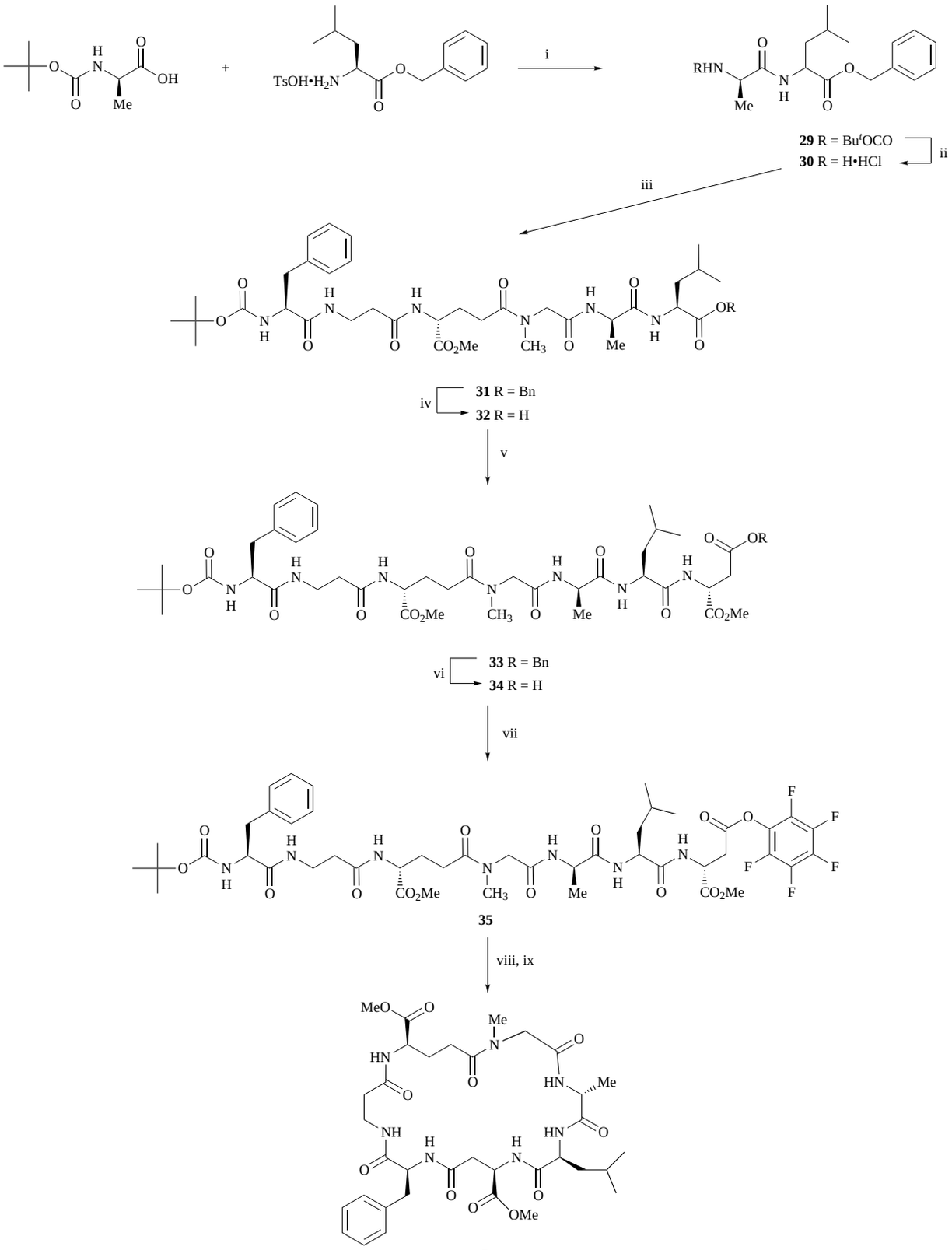
Attention was now turned to the introduction of the C-terminal tripeptide fragment (2*R*)-Ala-(2*S*)-Leu-(2*R*)-Asp-α-OMe-β-OH in the target linear heptapeptide. Accordingly, *N*-Boc-(2*R*)-alanine, activated as its mixed anhydride, was coupled to benzyl (2*S*)-leucinate to give the fully protected dipeptide **29** in 85% yield {mp 89–91 °C; $[\alpha]_D^{25}$ −8.91 (MeOH)} which displayed the expected spectral and analytical properties (see Scheme 6). Acidolysis of the N-Boc group using hydrogen chloride gas in ethyl acetate gave the amine hydrochloride **30** in 96% yield and this material was coupled to the mixed anhydride-activated tetrapeptide acid **28** to afford the hexapeptide, *N*-Boc-(2*S*)-Phe-β-Ala-(2*R*)-Glu-α-OMe-γ-Sar-(2*R*)-Ala-(2*S*)-Leu-OBn **31** in 77% yield {mp 74 °C (softening); $[\alpha]_D^{25}$ +6.83 (MeOH)} which gave satisfactory analytical data. The benzyl ester protecting group was removed by hydrogenolysis and the resulting free acid **32** was activated and then coupled



Scheme 5 *Reagents*, *conditions* (*and yields*): i, HCl, EtOAc, 0 °C (93%); ii, IBCF, NMM, THF, −30 °C ⟶ room temp. (95%); iii, H_2, Pd/C, MeOH (91%); iv, IBCF, NMM, THF, −30 °C; then MeGlyOBn·*p*TsOH, NMM, THF, −30 °C ⟶ room temp. (80%); v, H_2, Pd/C, MeOH (95%)

with β-benzyl α-methyl (2*R*)-aspartate diester hydrochloride, prepared as described previously,[1] to give the required, fully protected linear heptapeptide **33** in 76% yield after purification by column chromatography. The compound **33** {mp 137–139 °C; $[\alpha]_D^{25}$ +8.2 (MeOH)}, which now contained all of the required amino acid building blocks, gave a satisfactory micro-analysis and displayed the expected NMR and mass spectral properties. Hydrogenolysis of the benzyl ester group of protected heptapeptide **33** gave the free acid **34**, which was converted into its pentafluorophenyl ester derivative *N*-Boc-(2*S*)-Phe-β-Ala-(2*R*)-Glu-α-OMe-γ-Sar-(2*R*)-Ala-(2*S*)-Leu-(2*R*)-Asp-α-OMe-β-OPFP **35** in 91% yield by reaction with pentafluorophenol and EDCI, as described above for target **4** (see Scheme 6).

Removal of the N-Boc protecting group with TFA in dichloromethane gave the amine salt which was thoroughly dried under high vacuum. Treatment of this salt with DIPEA in dichloromethane, under high-dilution conditions, allowed the cyclisation to proceed and after 7 days the reaction was



Scheme 6 *Reagents*, *conditions* (*and yields*): i, IBCF, NMM, THF, −30 °C ⟶ room temp. (85%); ii, HCl, EtOAc, 0 °C (96%); iii, **28**, IBCF, NMM, THF, −30 °C ⟶ room temp. (77%); iv, H_2, Pd/C, MeOH (82%); v, IBCF, NMM, THF, −30 °C; then H-(2*R*)-Asp(OBn)OMe·HCl, NMM, THF, −30 °C ⟶ room temp (76%); vi, H_2, Pd/C, 2% AcOH in MeOH (95%); vii, C_6F_5OH, EDCI, CH_2Cl_2 (91%); viii, TFA, CH_2Cl_2, 0 °C; ix, DIPEA, CH_2Cl_2 (67%)

complete as judged by TLC analysis. Removal of the solvents gave a white solid, which was triturated with diethyl ether and collected by filtration to afford the 25-membered macrocyclic heptapeptide **5** in 67% yield, mp 276–278 °C (decomp.). The compound gave satisfactory analytical and mass data but displayed two sets of signals in its ^1H and ^{13}C NMR spectra, in accord with the existence of *cis*- and *trans*-γ-Glu–Sar rotamers. As for all of the macrocyclic nodularin analogue diesters that we have prepared, the microcystin-type macrocycle diester **5** did not dissolve in methanol or chloroform but was quite soluble in dimethyl sulfoxide. Thus, it was possible to prepare unfunctionalised microcystin-type macrocycles in good yield using a

similar macrolactamisation strategy to that used for the nodularin macrocycles.[1,12]

The yield obtained for the formation of macrocycle **5** compares well with the 56% yield reported by Chamberlain and coworkers for the preparation of microcystin-LA dimethyl ester.[17] The lengthy reaction times and relatively low yield of 41% obtained for the preparation of the hydroxymethyl nodularin-type macrocycle **4** compared with the model system **3** (obtained in 89% yield), several other variants containing (2*S*)- and (2*R*)-Pro in place of the sarcosine residue, and/or an α-benzyl ester of (2*R*)-Asp in place of the Adda residue (each obtained in 50–90% yield),[28,29] and for motuporin,[15] all of which were synthesised using the same approach, suggests that the potentially H-bonding hydroxy group retards the rate of macrolactamisation. Nevertheless, the potential advantages offered by preforming a functionalised macrocycle for post-cyclisation elaboration more than outweigh the yield differences.This strategy is of particular significance in the synthesis of libraries of macrocycles where it is intended to modify each macrocycle with a range of exocyclic appendages. Such synthetic studies are presently underway in the construction of inhibitor structure–activity relationships for PP1 and PP2A.

Experimental

NMR spectra were recorded on a Bruker AM-300 spectrometer (^{1}H, 300 MHz; ^{13}C, 75 MHz; ^{19}F, 282.3 MHz), a Varian Gemini spectrometer (^{1}H , 200 MHz; ^{13}C, 50.3 MHz), a Varian Gemini spectrometer (^{1}H, 300 MHz; ^{13}C, 75.4 MHz) and a Varian Unity Plus 500 spectrometer (^{1}H, 500 MHz; ^{13}C, 125.6 MHz). ^{1}H NMR were referenced internally on ^{2}HOH (δ_H 4.68), $(C^2H_3)_2SO$ (δ_H 2.52), $C^2H_3O^2H$ (δ_H 3.35) and C^2HCl_3 (δ_H 7.27). ^{13}C NMR were referenced on C^2HCl_3 (δ_C 77.5), $(C^2H_3)_2SO$ (δ_C 35.60), $C^2H_3O^2H$ (δ_C 49.15). *J*-values are given in Hz. IR spectra were recorded using a Perkin-Elmer 1710 FT-IR spectrometer. The samples were prepared as Nujol mulls or thin films between sodium chloride discs. Absorption maxima are given in wavenumbers (cm^{-1}) relative to a polystyrene standard. Mps were measured using an Electrothermal melting point apparatus and are uncorrected. Optical rotations were measured on an Optical Activity Ltd. AA-1000 polarimeter using 10 cm path length cells at room temperature. Mass spectra were recorded on a VG AutoSpec. Major fragments are given as percentages of the base peak intensity. Solvents and common reagents were purified according to the methods of Perrin, Armarego and Perrin.[30] Analytical TLC was carried out on 0.25 mm precoated silica gel plates (MN SIL G/UV_{254}), and compounds were visualised by UV fluorescence, iodine vapour, ethanolic phosphomolybdic acid or aq. potassium permanganate. Light petroleum refers to the fraction boiling at 40–60 °C.

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.

β-Methyl (2*R*)-aspartate ester 10

β-Methyl (2*R*)-aspartate ester hydrochloride (7.34 g, 40 mmol) (prepared using the procedure of Schwarz, Bumpus and Page[22]) and propylene oxide (50 cm^3) were refluxed in dry ethanol (150 cm^3) for 3 h. On cooling the resulting precipitate was filtered off, and washed with diethyl ether (25 cm^3) to yield the *title ester* as a solid (5.29 g, 90%), mp 194–195 °C (lit.,[24] 194–195 °C for the 2*S*-isomer) (Found: C, 41.0; H, 6.3; N, 9.3. $C_5H_9NO_4$ requires C, 40.8; H, 6.2; N, 9.5%) (HRMS: Found: $[M + H]^+$, 148.0610. $C_5H_{10}NO_4$ requires *m*/*z*, 148.0610); $[\alpha]_D^{23}$ −2.2 (*c* 0.5, water); ν_{max}(Nujol)/cm^{-1} 1752 (CO, acid) and 1738 (CO, ester); δ_H(200 MHz; $C^2H_3O^2H$), 2.84 (1 H, dd, *J* 8.3 and 17.7, 1 H of β-H_2) 3.05 (1 H, dd, *J* 3.9 and 17.7, 1 H of β-H_2), 3.75 (3 H, s, CH_3) and 3.88 (1 H, dd, *J* 3.9 and 38.3, α-H); δ_C(50.3 MHz; ^2H_2O) 35.43 (β-CH_2), 51.65 (CH_3), 53.61 (α-C), 173.49 (CO, ester) and 173.88 (CO, acid); *m*/*z* (EI) 149 (9%, $[M + H]^+$), 148 (100, M^+) and 102 (19, $[M - CO_2H + H]^+$).

β-Methyl (2*R*)-*N*-(9′-phenylfluoren-9′-yl)aspartate ester 11

To a stirred solution of β-methyl (2*R*)-aspartate ester **10** (2.2 g, 15 mmol) in dry CH_2Cl_2 (30 cm^3) was added chlorotrimethylsilane (TMSCl) (2.03 cm^3, 16 mmol). Triethylamine (4.46 cm^3, 32 mmol) was added after 2 h and, after another 15 min, $Pb(NO_3)_2$ (3.3 g, 10 mmol) and a solution of 9-bromo-9-phenylfluorene (6.42 g, 20 mmol) in dry CH_2Cl_2 (30 cm^3) were added. The mixture was stirred vigorously for 3 days after which time methanol (7 cm^3) was added and the reaction mixture was filtered and concentrated under reduced pressure to give a viscous residue. The residue was partioned between aq. 5% citric acid (80 cm^3) and diethyl ether (80 cm^3). The aqueous phase was separated, and then was extracted with diethyl ether (3 × 50 cm^3). The combined ethereal extracts were washed with brine (30 cm^3), dried ($MgSO_4$), and then concentrated under reduced pressure to give a pale yellow foam. Recrystallisation from CH_2Cl_2–hexane gave the *required product* as a crystalline solid (4.70 g, 81%), mp 160 °C (lit.,[24] 160–161 °C for the 2*S*-isomer) (Found: C, 74.6; H, 5.4; N, 3.5. $C_{24}H_{21}NO_4$ requires C, 74.4; H, 5.4; N, 3.6%) (HRMS: Found: $[M + H]^+$, 388.1549. $C_{24}H_{22}NO_4$ requires *m*/*z*, 388.1549); $[\alpha]_D^{23}$ +149.5 (*c* 0.75, MeOH); ν_{max}(Nujol)/cm^{-1} 3291 (NH), 1749 (CO, acid) and 1696 (CO, ester); δ_H(200 MHz; C^2HCl_3) 1.93 (1 H, dd, *J* 4.4 and 16.9, 1 H of β-H_2), 2.70 (1 H, dd, *J* 4.3 and 16.7, 1 H of β-H_2), 2.87 (1 H, t, *J* 3.7, α-H), 3.66 (3 H, s, CH_3) and 7.21–7.78 (13 H, m, ArH); δ_C(50.3 MHz; C^2HCl_3), 36.43 (β-CH_2), 52.55 (CH_3), 53.09 (α-C), 72.98 (quaternary aliphatic), 120.88, 121.09, 125.21, 125.71, 126.28, 128.23, 128.74, 129.07, 129.14, 129.62 and 129.71 (ArCH), 140.97, 143.50, 147.47 and 149.15 (ArC quaternary), 172.53 (CO, ester) and 175.58 (CO, acid); *m*/*z* (CI) 388 (3%, $[M + H]^+$), 314 (4, $[M + CH_2CO_2CH_3]^+$) and 241 (100, $PhFl^+$).

Methyl (3*R*)-4-hydroxy-3-(9′-phenylfluoren-9′-ylamino)butyrate 12 (R = Me)

To a stirred solution of the methyl ester **11** (2.00 g, 5.17 mmol) in dry THF (25 cm^3) at −15 °C was added NMM (568 mm^3, 5.17 mmol). Isobutyl chloroformate (IBCF) (703 mm^3, 5.17 mmol) was added and the suspension was stirred at −15 °C for 5 min and then was filtered directly into a solution of sodium borohydride (118 mg, 3.10 mmol) in dry THF (15 cm^3). The reaction mixture was stirred at −15 °C for 15 min and was then allowed to warm up to room temperature. After 3 h, water (10 cm^3) was carefully added and the resulting solution was concentrated under reduced pressure until the THF had been removed. The remaining aqueous solution was extracted with ethyl acetate (3 × 25 cm^3) and the combined organic extracts were washed with brine (20 cm^3), dried ($MgSO_4$), and then concentrated under reduced pressure to give the crude alcohol. Trituration with diethyl ether gave the *required alcohol* as a solid which was collected by filtration (1.75 g, 91%), mp 135–137 °C (Found: C, 76.9; H, 6.1; N, 3.7. $C_{24}H_{23}NO_3$ requires C, 77.2; H, 6.2; N, 3.75%) (HRMS: Found: $[M + H]^+$, 374.1752. $C_{24}H_{24}NO_3$ requires *m*/*z* 374.1756); $[\alpha]_D^{23}$ +27.81 (*c* 0.52, MeOH); ν_{max}(Nujol)/cm^{-1} 3382 (NH), 3300–2800br (OH) and 1706 (CO, ester); δ_H(200 MHz; C^2HCl_3) 2.08 (1 H, dd, *J* 5.9 and 15.6, 1 H of α-H_2), 2.20 (1 H, dd, *J* 5.5 and 15.5, 1 H of α-H_2), 2.59 (1 H, quin, *J* 5.2, β-H), 2.71 (1 H, br s, OH), 2.99 (1 H, dd, *J* 4.6 and 10.8, 1 H of CH_2OH), 3.16 (1 H, dd, *J* 5.0 and 10.9, 1 H of CH_2OH), 3.63 (3 H, s, CH_3) and 7.71–7.74 (13 H, m, ArH); δ_C(50.3 MHz; C^2HCl_3) 37.98 (α-CH_2), 51.62 (β-CH), 53.00 (CH_3), 64.91 (CH_2OH), 72.91 (quaternary aliphatic), 120.62, 125.46, 125.66, 126.41, 127.75, 128.39, 128.71, 128.87, 128.98 and 129.04 (ArCH), 140.59, 141.04, 145.38, 150.14 and 150.34 (ArC quaternary) and 173.42 (CO, ester); *m*/*z* (CI) 374 (15%, $[M + H]^+$), 342 (30, $[M - OCH_3]^+$), 241 (100, $PhFl^+$), 134 (49, $[M + H - PhFl + H]^+$) and 102 (22, $[M + H - PhFl - CH_2OH]^+$).

(3*R*)-3-(9′-Phenylfluoren-9′-ylamino)butyrolactone 13

This compound could be obtained by concentration of the filtrate obtained from trituration of the hydroxy ester **12** (R = Me) (see above). The lactone **13** was obtained as a yellow foam and was then recrystallised from diethyl ether–light petroleum to give a solid. It was noted that addition of an excess of sodium borohydride in the synthesis of hydroxy ester **12** (R = Me) (see preceding experiment) resulted in reduced yields of the hydroxy ester **12** (R = Me) and the formation of larger amounts of the *lactone* **13** (5–63%), mp 156–158 °C (Found: C, 80.6; H, 5.4; N, 4.0. $C_{23}H_{19}NO_2$ requires C, 80.9; H, 5.6; N, 4.1%) (HRMS: Found: $[M + H]^+$, 342.149 021. $C_{23}H_{20}NO_2$ requires *m*/*z* 342.1494); $[\alpha]_D^{23}$ +20.0 (*c* 1.0, CH_2Cl_2); ν_{max}(Nujol)/cm^{-1} 3322 (NH) and 1784 (CO, lactone); δ_H(200 MHz; C^2HCl_3) 2.14 (2 H, d, *J* 7.8, α-H_2), 3.18 (1 H, quin, *J* 8.0, β-H), 3.77 (2 H, quin, *J* 6.8, CH_2OH) and 7.15–7.75 (13 H, m, ArH); δ_C(50.3 MHz; C^2HCl_3) 37.48 (α-CH_2), 50.50 (β-CH), 73.21 (quaternary aliphatic), 74.72 (CH_2OH), 120.84, 120.92, 125.49, 126.41, 126.52, 128.05, 128.69, 128.80, 128.96, 129.06 and 129.36 (ArCH), 140.65, 140.90, 144.50, 149.60 and 149.76 (ArC quaternary) and 176.13 (CO, lactone); *m*/*z* (CI) 342 (21%, $[M + H]^+$), 241 (17, $PhFl^+$) and 102 (29, $[M + H - PhFl + H]^+$).

(3*R*)-4-Hydroxy-3-(9′-phenylfluoren-9′-ylamino)butyric acid 12 (R = H)

To a stirred solution of the lactone **13** (1.00 g, 2.68 mmol) in methanol (30 cm^3) was added 1 mol dm^{-3} aq. NaOH (5 cm^3). The reaction mixture was stirred at room temperature for 2 h and then the solvent was evaporated off under reduced pressure. The resulting Na^+ salt was dissolved in water (25 cm^3) and the solution was washed with ethyl acetate (20 cm^3). The aqueous phase was carefully partitioned between cold 10% aq. citric acid and ethyl acetate (1 : 1; 40 cm^3) at 0 °C. The aqueous phase was extracted with ethyl acetate (3 × 25 cm^3), and the combined organic extracts were washed with brine (25 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure to yield the crude hydroxy acid. Recrystallisation from aq. methanol afforded the *hydroxy acid* **12** (R = H) as a solid (0.82 g, 85%), mp 119–122 °C (Found: C, 73.1; H, 6.1; N, 3.7. $C_{23}H_{21}NO_3 \cdot H_2O$ requires C, 73.2; H, 6.1; N, 3.7%) (HRMS: Found: $[M + H - H_2O]^+$, 342.1496. $C_{23}H_{20}NO_2$ requires *m*/*z* 342.1494); $[\alpha]_D^{23}$ +151 (*c* 0.6, MeOH); ν_{max}(Nujol)/cm^{-1} 3395 (NH), 3350–2800br (OH) and 1706 (CO, acid); δ_H(200 MHz; $C^2H_3O^2H$) 1.98 (1 H, dd, *J* 5.7 and 16.3, 1 H of α-H_2), 2.28 (1 H, dd, *J* 6.5 and 16.3, 1 H of α-H_2), 2.69 (1 H, quin, *J* 5.9, β-H), 2.99 (1 H, dd, *J* 5.4 and 11.3, 1 H of CH_2OH), 3.13 (1 H, dd, *J* 4.8 and 11.3, 1 H of CH_2OH) and 7.15–7.90 (13 H, m, ArH); δ_C(50.3 MHz; $C^2H_3O^2H$) 38.25 (α-CH_2), 54.79 (β-CH), 63.50 (CH_2OH), 74.24 (quaternary aliphatic), 121.92, 122.10, 127.21, 127.31, 127.36, 129.49, 129.68, 129.81, 129.93 130.22, 131.11 and 131.31 (ArCH), 141.94, 142.70, 143.36, 147.18 and 147.59 (ArC quaternary) and 177.93 (CO, acid); *m*/*z* (CI) 342 (100%, $[M + H - H_2O]^+$), 241 (93, $PhFl^+$) and 102 (37, $[M + H - H_2O - PhFl + H]^+$).

Benzyl (3*R*)-3-(*tert*-butoxycarbonylamino)-4-hydroxybutyrate 14

The (*tert*-butoxycarbonylamino)hydroxybutyrate ester **14** was prepared in a manner identical with that described for the hydroxy-(9′-phenylfluoren-9′-yl)butyrate **12** (R = Me), using β-benzyl (2*R*)-*N*-(*tert*-butoxycarbonyl)aspartate (3.00 g, 9.29 mmol) as the starting material. The crude alcohol was recrystallised from diethyl ether–hexane to give the *required alcohol* as a crystalline solid (2.55 g, 89%), mp 60 °C (lit.,[25] 59–62 °C for 2*S*-isomer) (Found: C, 62.1; H, 7.5; N, 4.5. $C_{16}H_{23}NO_5$ requires: C, 62.1; H, 7.5; N, 4.5%); $[\alpha]_D^{23}$ +4.60 (*c* 0.38, in MeOH) {lit.,[25] $[\alpha]_D$ −3.4 (*c* 1, MeOH (for 2*S*-isomer)}; ν_{max}(Nujol)/cm^{-1} 3555–3100br (OH), 3386 (NH), 1727 (CO, urethane) and 1697 (CO, ester); δ_H(300 MHz; C^2HCl_3) 1.42 [9 H, s, $(CH_3)_3$], 2.58–2.79 (1 H, br s, OH), 2.66 (2 H, d, *J* 6.0, α-H_2), 3.67 (2 H, d, *J* 4.8, CH_2OH), 3.94–4.04 (1 H, m, β-H), 5.12 (2 H, s, PhCH_2), 5.21 (1 H, br d, NH) and 7.34 (5 H, s, ArH); δ_C(75.4 MHz; C^2HCl_3) 28.34 [$(CH_3)_3$], 36.08 (α-CH_2), 49.48 (β-CH), 64.54 (CH_2OH), 66.69 (Ph*C*H_2), 79.95 [*C*$(CH_3)_3$], 128.31, 128.50 and 128.75 (ArCH), 135.73 (ArC quaternary), 155.99 (CO, urethane) and 171.82 (CO, ester); *m*/*z* (CI) 310 (100%, $[M + H]^+$), 291 (7, $[M + H - H_2O + H]^+$), 254 (95, $[M + H - C_4H_9 + H]^+$) and 210 (37, $[M + H - C_5H_9O_2 + H]^+$).

Benzyl (3*R*)-3-amino-4-hydroxybutyrate trifluoroacetate salt 15

To a solution of benzyl (*R*)-3-(*tert*-butoxycarbonylamino)-4-hydroxybutyrate **14** (1.10 g, 3.56 mmol) in CH_2Cl_2 (10 cm^3) was added TFA (10 cm^3). The reaction mixture was stirred at room temperature for 45 min, after which time the reaction was complete as judged by TLC. The solution was diluted with toluene (10 cm^3) and was then concentrated under reduced pressure. The residue was dissolved in water (20 cm^3) and the solution was washed with ethyl acetate (15 cm^3). The aqueous phase was concentrated under reduced pressure to give an oil, refractory to crystallisation, containing up to 10% of the lactone. This compound was used directly for the next step without further purification (0.85 g, 74%); δ_H(200 MHz; ^2H_2O) 2.67 (2 H, br, α-H_2), 3.45–3.73 (3 H, m, CH_2OH and β-H), 5.08 (2 H, s, PhCH_2) and 7.22 (5 H, s, ArH); δ_C(75.4 MHz; ^2H_2O) 33.30 (α-CH_2), 49.39 (β-CH), 60.68 (CH_2OH), 67.63 (Ph*C*H_2), 128.56, 128.90 and 128.99 (ArCH), 135.32 (ArC quaternary) and 171.78 (CO).

When the Boc deprotection was attempted using dry $HCl_{(g)}$ in ethyl acetate, hydrolysis of the benzyl ester also occurred to give the hydroxyamino acid salt, (3*R*)-3-amino-4-hydroxybutyric acid hydrochloride, mp 177–178 °C; ν_{max}(Nujol)/cm^{-1} 3250–2400br (NH and OH) and 1771 (CO); δ_H(200 MHz; ^2H_2O) 2.71–2.81 (1 H, m, 1 H of α-H_2), 3.12–3.31 (1 H, m, 1 H of α-H_2), 4.31–4.40 (1 H, m, β-H) and 4.48–4.73 (2 H, m, CH_2OH); δ_C(75.4 MHz; ^2H_2O) 33.14 (α-CH_2), 47.66 (β-CH), 71.65 (CH_2OH) and 177.84 (CO); *m*/*z* (CI) 102 (100%, $[M + H - HCl - H_2O]$).

Benzyl (3*R*)-3-[(*tert*-butoxycarbonyl)-(2*S*)-phenylalanylamino]-4-hydroxybutyrate 16

To a stirred solution of (2*S*)-*N*-Boc-phenylalanine (0.58 g, 2.19 mmol) in dry THF (25 cm^3) at −15 °C was added NMM (240 mm^3, 2.19 mmol). IBCF (300 mm^3, 2.19 mmol) was added and the suspension was stirred at −15 °C for 10 min. A mixture of benzyl (3*R*)-3-amino-4-hydroxybutyrate trifluoroacetate salt **15** (0.71 g, 2.19 mmol) and NMM (240 mm^3, 2.19 mmol) in dry THF (20 cm^3) was then added. The reaction mixture was allowed to warm up to room temperature and was then stirred for a further 2 h. The trifluoroacetate salts were removed by filtration and the solution was concentrated under reduced pressure to yield an oil. The residue was re-dissolved in ethyl acetate (30 cm^3), and the solution was washed successively with water (20 cm^3), 5% aq. $NaHCO_3$ (20 cm^3), 10% aq. citric acid (20 cm^3) and brine (20 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure to give a solid. Recrystallisation from ethyl acetate–hexane afforded the *pure dipeptide* as a crystalline solid (0.90 g, 90%), mp 72–73 °C; ν_{max}(Nujol)/cm^{-1} 3490–3220 (NH and OH), 1757 (CO, urethane), 1694 (CO, ester) and 1656 (CO, amide); δ_H[300 MHz; $(C^2H_3)_2SO$, mixture of rotamers] 1.27 and 1.29 [9 H, 2 × s, $(CH_3)_3$], 2.58–2.91 [3 H, m, 1 H of CH_2 (Phe) and α-H_2(Ahb)], 3.24–3.54 [3 H, m, 1 H of CH_2 (Phe) and CH_2OH], 4.00–4.11 [1 H, m, β-H (Ahb)], 4.38–4.48 [1 H, m, α-H (Phe)], 5.04 and 5.06 (2 H, 2 × s, OCH_2Ph), 6.80 and 6.91 (1 H, 2 × d, *J* 8.8, NH urethane), 7.17–7.36 (10 H, m, ArH) and 7.87 and 8.49 (1 H, 2 × d, *J* 5.8, NH amide); δ_C[75.4 MHz; $(C^2H_3)_2SO$, mixture of rotamers] 28.09 [$(CH_3)_3$], 33.82 [α-CH_2 (Ahb)], 35.78 and 37.73 [CH_2 (Phe)], 45.94 [β-CH (Ahb) minor), 48.07 [β-CH (Ahb) major], 55.71 [α-C (Phe)], 62.05 ($PhCH_2$ minor), 65.55 ($PhCH_2$ major), 73.16 (CH_2OH), 77.98 [*C*$(CH_3)_3$], 126.24, 126.38, 128.02, 128.07, 128.16, 128.52 and 129.35 (ArCH), 136.26 and 138.34 (ArC quaternary), 155.36 (CO, urethane) and 171.13, 171.51, 171.97 and 176.07

(CO); *m*/*z* (CI) 457 (5%, $[M + H]^+$), 381 (11, $[M + H - C_6H_5 + H]^+$), 91 (84, $PhCH_2^+$) and 57 (100, $C_4H_9^+$).

(3*R*)-3-[(*tert*-Butoxycarbonyl)-(2*S*)-phenylalanylamino]-4-hydroxybutyric acid 17

To a solution of dipeptide benzyl ester **16** (0.90 g, 1.97 mmol) in ethanol (25 cm^3) was added 10% palladium on carbon (80 mg) and the mixture was stirred under hydrogen for 4 h. The catalyst was removed by filtration through a pre-washed Celite pad and the filtrate was concentrated under reduced pressure to give the *title acid* as a solid (0.71 g, 98%), mp 95–97 °C (Found: C, 56.35; H, 6.95; N, 7.3. $C_{18}H_{26}N_2O_6 \cdot H_2O$ requires C, 56.25; H, 7.35; N, 7.3%); $[\alpha]_D^{23}$ +23.43 (*c* 0.51, MeOH); ν_{max}(Nujol)/cm^{-1} 3510–3200 (NH and OH), 1762 (CO, urethane), 1690 (CO) and 1665 (CO, amide); δ_H[200 MHz; C^2HCl_3, mixture of rotamers] 1.35 and 1.38 [9 H, 2 × s, $(CH_3)_3$], 2.12–2.18 [1 H, m, 1 H of α-H_2 (Ahb)], 2.72 [1 H, dd, *J* 8.0 and 17.9, 1 H of α-H_2 (Ahb)], 3.00 [2 H, br, CH_2 (Phe)], 4.08 (1 H, dd, *J* 2.8 and 9.9, 1 H of CH_2OH), 4.23–4.32 [1 H, m, β-H (Ahb)], 4.43 (1 H, dd, *J* 6.0 and 9.9, 1 H of CH_2OH), 4.58–4.68 [1 H, m, α-H (Phe)], 5.26 [1 H, d, *J* 8.0, NH (Phe)], 6.93 [1 H, d, *J* 6.3, NH (Ahb)] and 7.16–7.37 (5 H, m, ArH); δ_H[75.4 MHz; C^2HCl_3, mixture of rotamers] 28.12 [$(CH_3)_3$], 34.27 [α-CH_2 (Ahb) major], 35.01 [α-CH_2 (Ahb) minor], 38.63 [CH_2(Phe)], 46.12 [β-CH (Ahb) major], 48.11 [β-CH (Ahb) minor], 55.73 [α-C (Phe)], 73.62 (CH_2OH), 80.40 [*C*$(CH_3)_3$], 126.92, 127.12, 128.30, 128.58, 128.67 and 129.27 (ArCH), 136.41 (ArC quaternary), 155.66 (CO, urethane major), 155.91 (CO, urethane minor), 171.98, 172.12, 174.37 and 175.73 (CO); *m*/*z* (CI) 349 (13%, $[M + H - H_2O]^+$), 293 (100, $[M + H - H_2O - C_4H_9 + H]^+$) and 249 (25, $[M + H - H_2O - C_5H_9O_2 + H]^+$).

β-Benzyl (3*R*)-3-[(*tert*-butoxycarbonyl)-(2*S*)-phenylalanylamino]-4-hydroxybutyryl-[α-methyl (2*R*)-glutamyl]-γ-sarcosyl-[α-methyl (2*R*)-aspartate] triester 18

To a stirred solution of (3*R*)-3-[Boc-(2*S*)-phenylalanylamino]-4-hydroxybutyric acid **17** (468 mg, 1.28 mmol) in dry THF (15 cm^3) at −15 °C was added NMM (141 mm^3, 1.28 mmol). IBCF (174 mm^3, 1.28 mmol) was added and the suspension was stirred at −15 °C for 10 min. A mixture of the tripeptide triester hydrochloride **8**·HCl (623 mg, 1.28 mmol) and NMM (141 mm^3, 1.28 mmol) in a dry mixture of DMF (2 cm^3) and THF (8 cm^3) was then added. The reaction mixture was allowed to warm up to room temperature and was stirred for a further 3 h. The hydrochloride salts were removed by filtration and the solution was concentrated under reduced pressure. The residue was re-dissolved in ethyl acetate (25 cm^3), then washed successively with water (15 cm^3), 5% aq. $NaHCO_3$ (20 cm^3), 10% aq. citric acid (20 cm^3) and brine (20 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. The crude yellow solid was chromatographed (CH_2Cl_2–MeOH, 94:6) to give the pure *pentapeptide* as a crystalline solid (849 mg, 83%), mp 89–91 °C (Found: C, 58.2; H, 6.9; N, 8.6. $C_{39}H_{53}N_5O_{13}$ requires C, 58.55; H, 6.7; N, 8.75%) (HRMS: Found: $[M + H]^+$, 800.3700. $C_{39}H_{54}N_5O_{13}$ requires *m*/*z* 800.3718); $[\alpha]_D^{23}$ +9.03 (*c* 0.31, CH_2Cl_2); ν_{max}(Nujol)/cm^{-1} 3375br (NH and OH), 1720br (CO) and 1684 (CO, amide); δ_H(300 MHz; C^2HCl_3, mixture of rotamers) 1.28 [9 H, s, $(CH_3)_3$], 1.77–1.95 [2 H, m, β-H_2 (Glu)], 2.17–2.45 [3 H, m, γ-H_2 (Glu) and 1 H of β-H_2 (Ahb)], 2.70–2.91 [5 H, m, β-H_2 (Asp), CH_2 (Phe) and 1 H of β-H_2 (Ahb)], 2.73 and 2.88 (3 H, 2 × s, NCH_3), 3.58, 3.59 and 3.60 (6 H, 3 × s, 2 × OCH_3), 3.72 [1 H, d, *J* 6.9, 1 H of CH_2 (Sar)], 3.93–4.18 [4 H, m, β-H (Ahb), α-H (Glu), α-H (Phe) and 1 H of CH_2 (Sar)], 4.41 (2 H, d, *J* 5.2, CH_2OH), 4.65–4.72 [1 H, m, α-H (Asp)], 5.09 (2 H, s, PhCH_2), 6.92 (1.4 H, d, *J* 8.2, 1.4 NH), 7.17–7.39 (10 H, m, ArH),7.52 and 7.58 (0.6 H, 2 × d, *J* 7.7 and 8.0, 0.6 NH), 8.37 (0.6 H, d, *J* 7.7, 0.6 NH), 8.50 (1 H, d, *J* 5.5, NH) and 8.61 (0.4 H, d, *J* 8.0, 0.4 NH); δ_C[75.4 MHz; $(C^2H_3)_2SO$, mixture of rotamers] 28.05 [β-CH_2 (Glu)], 28.28 [β-CH_2 (Glu)], 28.52 [$(CH_3)_3$], 28.88 [γ-CH_2 (Glu)], 29.05 [γ-CH_2 (Glu)], 34.26 [α-CH_2 (Ahb)], 34.63 (NCH_3), 36.27 [β-CH_2 (Asp)], 36.42 (NCH_3), 37.93 [CH_2 (Phe)], 46.37 [β-CH (Ahb)], 48.88 [α-C (Asp)], 48.99 [α-C (Asp)], 50.12 [CH_2 (Sar)], 52.23 (OCH_3), 52.29 (OCH_3), 52.65 (OCH_3), 53.74 [α-C (Glu)], 53.84 [α-C (Glu)], 56.18 [α-C (Phe)], 66.36 ($PhCH_2$), 73.62 (CH_2OH), 78.53 [*C*$(CH_3)_3$], 126.82, 128.53, 128.62, 129.01 and 129.78 (ArCH), 136.48 and 138.53 (ArC quaternary), 155.83 (CO, urethane), 168.95, 169.07, 170.49, 171.52, 171.65, 172.37, 172.43, 172.61 and 173.47 (CO); *m*/*z* (FAB) 822 (2%, $[M + Na]^+$), 800 (12, $[M + H]^+$), 742 (6, $[M + H - C_4H_9 + H]^+$), 700 (11, $[M + H - C_5H_9O_2 + H]^+$) and 120 (100, $[C_8H_9N + H]^+$).

(3*R*)-3-[(*tert*-Butoxycarbonyl)-(2*S*)-phenylalanylamino]-4-hydroxybutyryl-[α-methyl (2*R*)-glutamyl]-γ-sarcosyl-[α-methyl (2*R*)-aspartate] diester 19

To a solution of pentapeptide benzyl ester **18** (0.50 g, 0.626 mmol) in ethanol (20 cm^3) was added 10% palladium on carbon (75 mg) and the mixture was stirred under hydrogen for 6 h. The catalyst was removed by filtration through a pre-washed Celite pad and the filtrate was concentrated under reduced pressure to give the *title compound* **19** as a solid (0.43 g, 97%), mp 97–98 °C (HRMS: Found: $[M + H]^+$, 710.3232. $C_{32}H_{48}N_5O_{13}$ requires *m*/*z* 710.3249); ν_{max}(Nujol)/cm^{-1} 3555br (OH and NH), 1746 (CO) and 1717 (CO); δ_H(300 MHz; C^2HCl_3, mixture of rotamers) 1.41 [9 H, s, $(CH_3)_3$], 1.83–1.97 [2 H, m, β-H_2 (Glu)], 2.14–2.59 [4 H, m, γ-H_2 (Glu) OH and 1 H of α-H_2 (Ahb)], 2.74 [1 H, dd, *J* 8.0 and 17.9, 1 H of α-H_2 (Ahb)], 2.83–3.15 [4 H, m, CH_2 (Phe) and β-H_2 (Asp)], 3.20 and 3.11 (3 H, 2 × s, NCH_3), 3.73, 3.74 and 3.76 (6 H, 3 × s, 2 × OCH_3), 3.74–3.85 [2 H, br, CH_2 (Sar)], 4.07 (1 H, dd, *J* 3.3 and 9.0, 1 H of CH_2OH), 4.24–4.31 [2 H, m, α-H (Glu) and β-H (Ahb)], 4.44 (1 H, dd, *J* 6.0 and 9.9, 1 H of CH_2OH), 4.56–4.60 [1 H, br, α-H (Phe)], 4.71–4.84 [1 H, m, α-H (Asp)], 5.03 [1 H, d, *J* 8.0, NH (Phe)], 6.31 (1 H, br, NH) and 7.17–7.43 (7 H, m, ArH and 2 × NH); δ_C(75.4 MHz; C^2HCl_3, mixture of rotamers) 27.40 [β-CH_2 (Glu)], 27.80 [β-CH_2 (Glu)], 28.13 [$(CH_3)_3$], 28.47 [γ-CH_2 (Glu)], 28.54 [γ-CH_2 (Glu)], 34.33 [α-CH_2 (Ahb)], 34.99 (NCH_3), 35.30 [β-CH_2 (Asp)], 35.53 (NCH_3), 38.22 [CH_2 (Phe)], 46.29 [β-CH (Ahb)], 48.15 [α-C (Asp)], 49.94 [CH_2 (Sar)], 52.34 (OCH_3), 52.61 (OCH_3), 52.98 (OCH_3), 54.13 [α-C (Glu)], 55.88 [α-C, (Phe)], 73.00 (CH_2OH), 79.27 [*C*$(CH_3)_3$], 127.19, 128.51, 128.77, 129.08 and 129.19 (ArCH), 136.22 (ArC quaternary), 155.36 (CO, urethane), 168.87, 171.11, 171.46, 172.05, 172.63, 172.82 and 174.55 (CO); *m*/*z* (FAB) 732 ($[M + Na]^+$, 12%), 710 (12, $[M + H]^+$), 610 (14, $[M + H - C_5H_9O_2 + H]^+$), 91 (100, $PhCH_2^+$) and 120 (100, $[C_8H_9N + H]^+$).

β-Pentafluorophenyl (3*R*)-3-[(*tert*-butoxycarbonyl)-(2*S*)-phenylalanylamino]-4-hydroxybutyryl-[α-methyl (2*R*)-glutamyl]-γ-sarcosyl-[α-methyl (2*R*)-aspartate] triester 20

To a stirred solution of the pentapeptide carboxylic acid **19** (256 mg, 0.36 mmol) in CH_2Cl_2 (10 cm^3) at 0 °C was added pentafluorophenol (200 mg, 1.08 mmol) followed by EDCI (161 mg, 0.54 mmol). The reaction mixture was allowed to warm to room temperature and was then stirred for a further 12 h. The solution was concentrated under reduced pressure to give a yellow foam, which was purified by flash chromatography on silica and elution with CH_2Cl_2 to remove pentafluorophenol, then with CH_2Cl_2–MeOH (96:4) to give the required triester as an off-white solid (193 mg, 61%), mp 61–65 °C (HRMS: Found: $[M + Na]^+$, 898.2932. $C_{38}H_{46}F_5N_5NaO_{13}$ requires *m*/*z* 898.2910); ν_{max}(thin film)/cm^{-1} 3310br (NH and OH), 1752 (CO), 1720br (CO) and 1675br (CO, amide); δ_H(300 MHz; C^2HCl_3, mixture of rotamers) 1.34 [9 H, s, $(CH_3)_3$], 1.86–1.95 [1 H, m, 1 H of β-H_2 (Glu)], 2.24–2.56 [6 H, m, α-H_2 (Ahb), γ-H_2 (Glu), OH, and 1 H of β-H_2 (Glu)], 2.90–3.10 [2 H, m, CH_2 (Phe)], 2.95 and 3.03 (3 H, 2 × s, NCH_3), 3.24–3.27 [2 H, m, β-H_2 (Asp)], 3.58 (2 H, br, CH_2OH), 3.69, 3.72, 3.75 and 3.78 (6 H, 4 × s, 2 × OCH_3), 3.84 [1 H, d, *J* 16.2, 1 H of CH_2 (Sar)],

4.11–4.20 [1 H, m, α-H (Glu)], 4.25–4.33 [1 H, m, β-H (Ahb)], 4.38 [1 H, d, *J* 16.2, 1 H of CH_2 (Sar)], 4.46–4.60 [1 H, m, α-H (Phe)], 4.98–5.05 [1 H, m, α-H (Asp)], 5.29 (1 H, br, NH urethane), 7.16–7.29 (6 H, m, ArH and 1 NH), 7.32 (1 H, 2 × d, *J* 6.9, NH) and 7.55 (1 H, s × d, *J* 7.7, NH); δ_C[75.4 MHz; $(C^2H_3)_2SO$, mixture of rotamers] 28.05 [β-CH_2 (Glu)], 28.28 [β-CH_2 (Glu)], 28.54 [$(CH_3)_3$], 28.91 [γ-CH_2 (Glu)], 29.07 [γ-CH_2 (Glu)], 34.28 [α-CH_2 (Ahb)], 34.62 (NCH_3), 36.18 [β-CH_2 (Asp)], 36.30 (NCH_3), 37.92 [CH_2 (Phe)], 46.36 [β-CH (Ahb)], 48.91 [α-C (Asp)], 49.02 [α-C (Asp)], 50.08 [CH_2 (Sar)], 52.31 (OCH_3), 52.59 (OCH_3), 53.74 [α-C (Glu)], 56.18 [α-C (Phe)], 77.77 (CH_2OH), 78.53 [*C*$(CH_3)_3$], 126.82, 128.62 and 129.77 (ArCH), 138.54 (ArC quaternary), 155.82 (CO, urethane) and 168.95, 171.94, 172.11, 172.33, 172.42, 172.61 and 173.50 (CO); *m*/*z* (FAB) 898 (4%, $[M + Na]^+$), 876 (3, $[M + H]^+$), 776 (5, $[M + H - C_5H_9O_2 + H]^+$) and 147 (100, $[C_9H_9NO]^+$).

cyclo-[(*R*)-Ahb-α-OMe-(*R*)-Glu-γ-Sar-α-OMe-(*R*)-Asp-β-(*S*)-Phe] diester 4

To a solution of the *N*-*tert*-butoxycarbonyl-protected pentafluorophenyl ester **20** (165 mg, 0.189 mmol) in CH_2Cl_2 (5 cm^3) was added TFA (5 cm^3).The reaction mixture was stirred at room temperature for 45 min, after which time reaction was complete as judged by TLC. The solution containing the trifluoroacetate salt **21** was diluted with toluene (10 cm^3) and was then concentrated under reduced pressure to give an oil, which was thoroughly dried *in vacuo* for 6 h. The residue was dissolved in CH_2Cl_2 (175 cm^3), then treated with DIPEA (988 mm^3, 5.67 mmol), and the mixture was stirred at room temperature for nine days, when TLC analysis indicated that reaction was complete. The reaction mixture was concentrated under reduced pressure to give a pale brown solid. Purification by flash chromatography on silica and elution first with CH_2Cl_2, then with CH_2Cl_2–MeOH (94:6), gave an oil. This was triturated with diethyl ether to give an off-white solid, which was collected by filtration and washed on the pad with diethyl ether to give the required functionalised macrocycle **4** as an off-white powder (46 mg, 41%), mp 96–100 °C (HRMS: Found: $[M + Na - CH_3OH]^+$, 582.2150. $C_{26}H_{33}N_5NaO_9$ requires *m*/*z*, 582.2176); $[\alpha]_D^{23}$ −64.6 (*c* 0.08, CH_2Cl_2); ν_{max}(thin film)/cm^{-1} 3307br (NH and OH), 1760 (CO), 1742 (CO) and 1652 (CO, amide); δ_H[500 MHz; $(C^2H_3)_2SO$, mixture of rotamers] 1.78 and 1.90 [0.76 H, 2 × m, β-H_2 (Glu) minor], 1.79 and 1.94 [1.24 H, 2 × m, β-H_2 (Glu) major], 2.14 and 2.78 [2 H, m, α-H_2 (Ahb)], 2.27 [0.76 H, m, γ-H_2 (Glu) minor], 2.38 [1.24 H, m, γ-H_2 (Glu) major], 2.51 and 2.60 [0.76 H, 2 × m, β-H_2 (Asp) minor], 2.56 [1.24 H, m, β-H_2 (Asp) major], 2.74 (1.14 H, s, NCH_3 minor), 2.79 and 2.90 [2 H, 2 × m, CH_2 (Phe)], 2.88 (1.86 H, s, NCH_3 major), 3.52, 3.55, 3.59 and 3.62 (6 H, 4 × s, 2 × OCH_3), 3.87–3.99 and 3.90–4.01 [2 H, 2 × m, CH_2 (Sar)], 4.02 [0.38 H, m, α-H (Glu) minor], 4.05 [0.62 H, m, α-H (Glu) major], 4.39 [1 H, m, β-H (Ahb)], 4.40 [1 H, m, α-H (Phe)], 4.54 [0.38 H, m, α-H (Asp) minor], 4.56 [0.62 H, m, α-H (Asp) major], 7.14–7.30 (5 H, m, ArH), 7.47 [0.38 H, d, *J* 8.1, 0.38 NH (Glu) minor], 7.53 [0.62 H, d, *J* 6.7, 0.62 NH (Glu) major], 8.12 [0.38 H, d, *J* 8.1, 0.38 NH (Asp) minor], 8.21 [1 H, 2 × d (overlapping), *J* 5.4 and 6.7, NH (Phe)], 8.33 [0.62 H, d, *J* 8.1, 0.62 NH (Asp) major] and 8.47 [1 H, 2 × d (overlapping), *J* 5.4 and 9.4, NH (Ahb)]; δ_C(125.7 MHz; $(C^2H_3)_2SO$, mixture of rotamers] 25.7 [β-CH_2 (Glu) major], 25.9 [β-CH_2 (Glu) minor], 28.0 [γ-CH_2 (Glu) minor], 28.2 [γ-CH_2 (Glu) major], 33.4 [α-CH_2 (Ahb)], 34.9 (NCH_3 minor), 35.6 (NCH_3 major), 36.3 [β-CH_2 (Asp) major], 36.4 [β-CH_2 (Asp) minor], 37.4 [CH_2 (Phe)], 45.5 [β-CH (Ahb)], 48.3 [α-C (Asp) minor], 48.4 [α-C (Asp) major], 49.4 [CH_2 (Sar) major], 51.3 [CH_2 (Sar) minor], 51.4 [OCH_3 (Glu)], 51.5 [OCH_3 (Asp)], 52.9 [α-C (Glu) major], 53.0 [α-C (Glu) minor], 53.6 [α-C (Phe)], 76.0 (CH_2OH), 126.19, 128.01 and 129.24 (ArCH), 137.88 (ArC quaternary), 167.8 [CO (Sar) major], 167.9 [CO (Sar) minor], 168.4 [β-CO (Asp) major], 169.2 (CO), 170.7 [CO (Phe)], 171.2 [α-CO (Asp)], 171.4 (CO), 171.9 (CO), 172.2 (CO), 172.5 [α-CO (Glu)] and 172.7 (CO); *m*/*z* (FAB) 614 (3%, $[M + Na]^+$), 592 (2, $[M + H]^+$), 582 (9, $[M + Na - CH_3OH]^+$), 560 (4, $[M + H - CH_3OH]^+$) and 322 (100). The ^1H and ^{13}C NMR spectra recorded above in $(C^2H_3)_2SO$ were assigned using COSY, TOCSY, HMBC and HSQC techniques and showed the existence of two other minor conformers (~5% of the total for each). ^1H and ^{13}C NMR spectra were also obtained in C^2HCl_3 and these showed the presence of two conformations in exchange on the NMR time-scale (see Results and discussion section). Full details and conformational structural assignments will be described for this compound together with those for several other functionalised nodularin macrocycles in due course.[28]

α-Methyl γ-benzyl (2*R*)-glutamate diester hydrochloride 23

Hydrogen chloride gas was bubbled into dry ethyl acetate (50 cm^3) at 0 °C for 1 h. To this was added γ-benzyl *N*-(*tert*-butoxycarbonyl)-α-methyl-(2*R*)-glutamate **24** (5.27 g, 15 mmol) as a solution in ethyl acetate (20 cm^3) and the solution was stirred at 0 °C for a further 1 h before being concentrated under reduced pressure to give the required product as a solid (4.02 g, 93%), mp 125–127 °C [lit.,[31] 129–135 °C for (2*S*)-isomer]; ν_{max}(Nujol)/cm^{-1} 1740 (CO ester); δ_H(200 MHz; ^2H_2O) 2.07–2.20 (2 H, m, β-H_2), 2.56 (2 H, t, *J* 6.9, γ-H_2), 3.68 (3 H, s, OCH_3), 4.07 (1 H, t, *J* 6.7, α-H), 4.73 (2 H, s, PhCH_2) and 7.34 (5 H, s, ArH); δ_C(75.3 MHz; ^2H_2O) 24.68 (β-CH_2), 29.43 (γ-CH_2), 51.93 (OCH_3), 53.53 (α-C), 67.31 (Ph*C*H_2), 128.49, 128.80 and 128.93 (ArCH), 135.52 (ArC quaternary) and 170.27 and 174.13 (CO); *m*/*z* (EI); 252 (13%, M^+), 192 (68, $[M - CH_4 - CO_2]^+$), 160 (23, $[M - PhCH_2]^+$) and 91 (100, $[PhCH_2]^+$).

γ-Benzyl (2*S*)-*N*-(*tert*-butoxycarbonyl)phenylalanyl-β-alanyl-[α-methyl (2*R*)-glutamate] diester 25

This compound was prepared in a manner identical with that described for the phenylalanyl(hydroxy)butyrate dipeptide **16**, using *N*-(*tert*-butoxycarbonyl)-(2*S*)-phenylalanyl-β-alanine **22** (2.70 g, 8.0 mol) and diester hydrochloride **23** (2.30 g, 8.0 mmol) to give the required product as a viscous gel.This was dissolved in warm ethyl acetate (200 cm^3), and the solution was washed and dried as described above to afford *compound* **25** as a solid (4.32 g, 95%), mp 127–128 °C (Found: C, 63.25; H, 7.35; N, 7.0. $C_{30}H_{39}N_3O_8$ requires C, 63.25; H, 6.9; N, 7.4%); ν_{max}(Nujol)/cm^{-1} 3310 (NH), 1770 (CO, urethane), 1740 (CO, ester), 1695 (CO, ester) and 1655br (CO, amide); δ_H(300 MHz; C^2HCl_3) 1.35 [9 H, s, $(CH_3)_3$], 1.95–2.05 [1 H, m, 1 H of β-H_2 (Glu)], 2.14–2.28 [3 H, m, 1 H of β-H_2 (Glu) and CH_2 (β-Ala)], 2.25–2.49 [2 H, m, γ-H_2 (Glu)], 2.93–3.08 [2 H, m, CH_2 (Phe)], 3.43 (2 H, m, CH_2N), 3.71 (3 H, s, OCH_3), 4.28 [1 H, br q, α-H (Phe)], 4.49–4.56 [1 H, m, α-H (Glu)], 5.10 (2 H, s, $PhCH_2$), 5.22 [1 H, d, *J* 8.2, NH (Phe)], 6.39 [1 H, d, *J* 7.7, NH (Glu)], 6.65 [1 H, t, *J* 5.8, NH (β-Ala)] and 7.16–7.41 (10 H, m, ArH); δ_C(75.7 MHz; C^2HCl_3) 26.68 [β-CH_2 (Glu)], 28.10 [$(CH_3)_3$], 30.32 [γ-CH_2 (Glu)], 35.43 [CH_2 (β-Ala) and CH_2 (Phe)], 38.60 (CH_2N), 51.65 (OCH_3), 52.49 [α-C (Glu)], 55.89 [α-C (Phe)], 66.57 ($PhCH_2$), 79.97 [*C*$(CH_3)_3$], 126.80, 128.31, 128.38, 128.53, 128.61 and 129.37 (ArCH), 135.64 and 136.89 (ArC quaternary), 155.38 (CO, urethane) and 171.64, 172.39 and 172.73 (CO); *m*/*z* (FAB) 592 (14%, $[M + Na]^+$), 570 (45, $[M + H]^+$), 470 (100, $[M + H - Boc]^+$) and 120 (62, $[C_8H_9N + H]^+$).

(2*S*)-*N*-(*tert*-Butoxycarbonyl)phenylalanyl-β-alanyl-[α-methyl (2*R*)-glutamate] ester 26

To a solution of tripeptide **25** (4.56 g, 8.0 mmol) in methanol (120 cm^3) was added 5% palladium on charcoal (10 mg) and the mixture was stirred under hydrogen for 1 h, after which time a precipitate formed. This was dissolved by the addition of glacial acetic acid (20 cm^3). The reaction mixture was stirred under hydrogen for a further 20 h, and the catalyst was then removed by filtration through a pre-washed Celite pad. The filtrate was

concentrated under reduced pressure, using toluene to remove the residual acetic acid azeotropically, to give a yellow oil. Trituration with ethyl acetate–light petroleum then gave the *required product* as an off-white solid (3.71 g, 91%), mp 115–117 °C (Found: C, 57.35; H, 6.95; N, 8.6. $C_{23}H_{33}N_3O_8$ requires C, 57.6; H, 6.95; N, 8.75%); $[\alpha]_D^{25}$ +14.24 (*c* 1.004, MeOH); ν_{max}(Nujol)/cm^{-1} 3325 (NH), 1755 (CO, urethane), 1723 (CO), 1687 (CO) and 1670 (CO); δ_H(300 MHz; C^2HCl_3) 1.34 [9 H, s, $(CH_3)_3$], 1.95–2.07 [1 H, m, 1 H of γ-H_2 (Glu)], 2.32–2.39 [3 H, m, 1 H of γ-H_2 (Glu) and CH_2 (β-Ala)], 2.87–3.01 [2 H, m, CH_2 (Phe)], 3.32–3.38 [1 H, m, 1 H of CH_2N (β-Ala)], 3.61–3.68 [1 H, m, 1 H of CH_2N (β-Ala)], 3.74 (3 H, s, OCH_3), 4.45–4.50 (1 H, m, α-H), 4.64 (1 H, br m, α-H), 5.43 [1 H, d, *J* 8.2, NH (Phe)], 6.64 [1 H, d, *J* 7.4, NH (Glu)], 6.88 [1 H, br t, NH (β-Ala)] and 7.17–7.30 (5 H, m, ArH); δ_C(75.4 MHz; C^2HCl_3) 26.47 [β-CH_2 (Glu)], 28.19 [$(CH_3)_3$], 30.22 [γ-CH_2 (Glu)], 35.31 [CH_2 (β-Ala)], 36.05 (*C*H_2Ph), 38.89 (CH_2N), 52.25 (OCH_3), 52.66 [α-C (Glu)], 55.75 [α-C (Phe)], 80.61 [*C*$(CH_3)_3$], 126.97, 128.66 and 129.39 (ArCH), 136.77 (ArC quaternary), 156.22 (CO, urethane) and 171.75, 172.15 and 175.70 (CO); *m*/*z* (FAB) 502 (30%, $[M + Na]^+$), 480 (29, $[M + H]^+$), 380 (100, $[M + H - Boc]^+$), 233 (35, $[M + 2 H - BocPhe]^+$ and 120 (57, $[C_8H_9N + H]^+$).

Benzyl (2*S*)-*N*-(*tert*-butoxycarbonyl)phenylalanyl-β-alanyl-[α-methyl (2*R*)-glutamyl]-γ-sarcosinate diester 27

This compound was prepared in a manner identical with that described for the phenylalanyl(hydroxy)butyrate dipeptide **16**, using *N*-(*tert*-butoxycarbonyl)-(2*S*)-phenylalanyl-β-alanyl-α-methyl-(2*R*)-glutamate ester **26** (3.36 g,7.0 mmol) and benzyl sarcosinate toluene-*p*-sulfonate (2.46 g, 7.0 mmol) to give an oil, which was triturated with ethyl acetate–light petroleum to afford the *desired product* as a solid (3.59 g, 80%), mp 110–112 °C (Found: C, 61.7; H, 7.15; N, 8.55. $C_{33}H_{44}N_4O_9$ requires C, 61.85; H, 6.9; N, 8.75%); $[\alpha]_D^{25}$ +9.1 (*c* 1, MeOH); ν_{max}(Nujol)/cm^{-1} 3340 (NH), 3310 (NH), 1755 (CO, urethane), 1690 (CO, ester), 1667 (CO, ester) and 1652 (CO, ester); δ_H(300 MHz; C^2HCl_3, mixture of rotamers) 1.35 [9 H, s, $(CH_3)_3$], 2.10–2.55 [6 H, m, β- and γ-H_2 (Glu) and CH_2 (β-Ala)], 2.83–2.89 (2 H, m, CH_2Ph), 2.95 and 3.03 (3 H, 2 × s, NCH_3), 3.47 [2 H, br s, CH_2N (β-Ala)], 3.71 and 3.74 (3 H, 2 × s, OCH_3), 4.14 [2 H, 2 × d, CH_2 (Sar)], 4.32 [1 H, m, α-H (Phe)], 4.49 [1 H, m, α-H (Glu)], 5.15 and 5.19 (2 H, 2 × s, $PhCH_2$), 5.30 [1 H, br s, NH (Phe)], 6.83 (1 H, br s, NH), 6.99 (1 H, br s, NH) and 7.19–7.34 (10 H, m, ArH); δ_C(75.4 MHz; C^2HCl_3, mixture of rotamers) 25.98 [β-CH_2 (Glu)], 28.13 [$(CH_3)_3$], 29.36 [γ-CH_2 (Glu)], 35.40 [CH_2 (β-Ala)], 35.53 [CH_2 (Phe)], 36.61 (NCH_3), 38.83 [CH_2N (β-Ala)], 49.73 [CH_2 (Sar)], 52.47 (OCH_3), 52.50 [α-C (Glu)], 55.79 [α-C (Phe)], 67.07 ($PhCH_2$), 79.71 [*C*$(CH_3)_3$], 126.79, 128.34, 128.54, 128.68, 128.81 and 129.44 (ArCH), 135.27 and 136.99 (ArC quaternary), 155.48 (CO, urethane) and 169.12, 171.56, 172.02 and 172.46 (CO); *m*/*z* (FAB) 663 (8%, $[M + Na]^+$), 641 (26, $[M + H]^+$), 541 (78, $[M + H - Boc]^+$), 394 (10, $[M + H - BocPhe]^+$), 323 (12, $[M + H - BocPhe-β-Ala]^+$) and 120 (100, $[C_8H_9N + H]^+$).

(2*S*)-*N*-(*tert*-Butoxycarbonyl)phenylalanyl-β-alanyl-[α-methyl (2*R*)-glutamyl]-γ-sarcosine ester 28

To a solution of tetrapeptide diester **27** (3.20 g, 5.0 mmol) in methanol (100 cm^3) was added 5% palladium on carbon (320 mg) and the mixture was stirred under hydrogen for 12 h. The catalyst was then removed by filtration through a pre-washed Celite pad and the filtrate was concentrated under reduced pressure to give the *acid* **28** as a solid (2.61 g, 95%), mp 103–105 °C; ν_{max}(Nujol)/cm^{-1} 3300br (NH and OH), 1735 (CO), 1720 (CO), 1665 (CO) and 1640 (CO); δ_H(300 MHz; $C^2H_3O^2H$, mixture of rotamers) 1.34 [9 H, s, $(CH_3)_3$], 1.94–2.00 [1 H, m, 1 H of β-H_2 (Glu)], 2.12–2.19 [1 H, m, 1 H of β-H_2 (Glu)], 2.39–2.45 [3 H, m, 1 H of CH_2 (β-Ala) and γ-CH_2 (Glu)], 2.50–2.56 [1 H, m, 1 H of CH_2 (β-Ala)], 2.76–2.83 [1 H, m, 1 H of CH_2Ph (Phe)], 3.05–3.12 [1 H, m, 1 H of CH_2Ph (Phe)], 2.93 and 3.07 (3 H, 2 × s, NCH_3), 3.39–3.45 [2 H, m, CH_2N (β-Ala)], 3.70 and 3.72 (3 H, 2 × s, OCH_3), 4.05 [1 H, d, *J* 17.6, 1 H of CH_2 (Sar)], 4.12 [1 H, d, *J* 17.6, 1 H of CH_2 (Sar)], 4.23 [1 H, m, α-H (Phe)], 4.43 [1 H, m, α-H (Glu)] and 7.23 (5 H, m, ArH); δ_C(75.4 MHz; $C^2H_3O^2H$, mixture of rotamers) 26.13 [β-CH_2 (Glu)], 27.20 [$(CH_3)_3$], 28.82 [γ-CH_2 (Glu)], 33.99 and 34.76 (NCH_3), 35.45 [CH_2 (β-Ala)], 35.58 [CH_2 (Phe)], 37.96 [CH_2N (β-Ala)], 49.02 [CH_2N (Sar)], 51.43 (OCH_3 minor), 51.92 (OCH_3 major), 52.03 [α-C (Glu)], 56.18 [α-C (Phe)], 79.27 [*C*$(CH_3)_3$], 126.41, 128.14 and 129.11 (ArCH), 137.44 (ArC quaternary), 156.33 (CO, urethane) and 171.51, 172.65, 173.19 and 173.70 (CO); *m*/*z* (FAB) 573 (57%, $[M + Na]^+$), 551 (43, $[M + H]^+$), 465 (17, $[M + H - N$-Me-Gly$]^+$), 451 (100, $[M + H - Boc]^+$), 304 (14, $[M + H - BocPhe]^+$), 233 (19, $[M + H - BocPhe-β-Ala]^+$) and 120 (51, $[C_8H_9N + H]^+$).

Benzyl (2*R*)-*N*-(*tert*-butoxycarbonyl)alanyl-(2*S*)-leucinate 29

This compound was prepared in a manner identical with that described for the phenylalanyl(hydroxy)butyrate dipeptide **16**, using (2*R*)-*N*-(*tert*-butoxycarbonyl)alanine (1.89 g, 10 mmol) and benzyl (2*S*)-leucinate toluene-*p*-sulfonate (3.94 g, 10 mmol) to give the required product as a solid. Recrystallisation from diethyl ether–light petroleum gave the *desired product* as fine needles (3.32 g, 85%), mp 89–91 °C (Found: C, 64.45; H, 8.5; N, 7.0. $C_{21}H_{32}N_2O_5$ requires C, 64.25; H, 8.2; N, 7.15%); $[\alpha]_D^{25}$ −8.91 (*c* 1.01, MeOH); ν_{max}(Nujol)/cm^{-1} 3345 and 3248 (NH), 1756 (CO, urethane), 1693 (CO, ester) and 1664 (CO, amide); δ_H(300 MHz; C^2HCl_3) 0.91 [3 H, d, *J* 6.0, CH_3 (Leu)], 0.92 [3 H, d, *J* 6.0, CH_3 (Leu)], 1.35 [3 H, d, *J* 7.1, CH_3 (Ala)], 1.45 [9 H, s, $(CH_3)_3$], 1.51–1.69 (3 H, m, $CHCH_2$), 4.20 [1 H, br q, α-H (Leu)], 4.65 [1 H, m, α-H (Ala)], 4.99 (1 H, br s, NH urethane), 5.13 (1 H, d, *J* 17.6, 1 H of $PhCH_2$), 5.17 (1 H, d, *J* 17.6, 1 H of $PhCH_2$), 6.67 (1 H, br s, NH amide) and 7.37 (5 H, m, ArH); δ_C(75.7 MHz; C^2HCl_3) 18.05 [CH_3 (Ala)], 21.70 [CH_3 (Leu)], 22.70 [CH_3 (Leu)], 24.70 (CH), 28.16 [$(CH_3)_3$], 41.26 (CH_2), 50.05 [α-C (Leu)], 50.69 [α-C (Ala)], 66.96 ($PhCH_2$), 128.23, 128.41 and 128.61 (ArCH), 135.44 (ArC quaternary), 155.54 (CO urethane) and 172.76 (CO); *m*/*z* (EI) 392 (17%, M^+), 336 (48, $[M - C_4H_8]^+$), 319 (32, $[M - C_4H_9O]^+$), 257 (62, $[M - C_8H_7O_2]^+$), 91 (100, $PhCH_2^+$) and 57 (80, $C_4H_9^+$).

Benzyl (2*R*)-alanyl-(2*S*)-leucinate hydrochloride 30

Hydrogen chloride gas was bubbled into dry ethyl acetate (50 cm^3) at 0 °C and the solution was stirred for 1 h. To this was added the benzyl (2*R*)-*N*-(*tert*-butoxycarbonyl)alanyl-(2*S*)-leucinate **29** (5.89 g, 15 mmol) and the solution was stirred for 1 h at room temperature. The solution was then concentrated under reduced pressure to give a sticky solid, which was dissolved in the minimum volume of diethyl ether and precipitated by addition of light petroleum. The solid was collected by filtration, and was then washed with light petroleum and dried in air to give the *desired product* as a powder (4.72 g, 96%), mp 139–140 °C; ν_{max}(Nujol)/cm^{-1} 3189 (NH), 1742, 1708 and 1678 (CO); δ_H(300 MHz; $C^2H_3O^2H$) 0.90 [3 H, m, CH_3 (Leu)], 0.95 [3 H, m, CH_3 (Leu)], 1.51 [3 H, d, *J* 6.9, CH_3 (Ala)], 1.65 [3 H, m, $CHCH_2$ (Leu)], 4.00 [1 H, q, *J* 6.9, α-H (Ala)], 4.51 [1 H, t, α-H (Leu)], 5.16 (2 H, s, $PhCH_2$) and 7.35 (5 H, s, Ph); δ_C(75.4 MHz; $C^2H_3O^2H$) 17.98 [CH_3 (Ala)], 21.77 [CH_3 (Leu)], 23.30 [CH_3 (Leu)], 26.17 (CH), 41.42 (CH_2), 50.34 [α-C (Ala)], 52.51 [α-C (Leu)], 68.20 ($PhCH_2$), 129.56, 129.65 and 129.84 (ArCH), 137.37 (ArC quaternary) and 171.46 and 173.89 (CO); *m*/*z* (FAB) 293 (100%, M^+).

Benzyl (2*S*)-*N*-(*tert*-butoxycarbonyl)phenylalanyl-β-alanyl-[α-methyl (2*R*)-glutamyl]-γ-sarcosyl-(2*R*)-alanyl-(2*S*)-leucinate diester 31

This compound was prepared in a manner identical with that described for the phenylalanyl(hydroxy)butyrate dipeptide **16**, using tetrapeptide **28** (1.65 g, 3.0 mmol) and dipeptide

hydrochloride **30** (0.99 g, 3.0 mmol) to give the *required product* as an off-white solid (1.90 g, 77%), mp 74 °C (softening) (Found: C, 61.0; H, 7.65; N, 9.9. $C_{42}H_{60}N_6O_{11}$ requires C, 61.15; H, 7.35; N, 10.2%); $[\alpha]_D^{25}$ +6.83 (*c* 1.26, MeOH); ν_{max}(Nujol)/cm^{-1} 3297 (NH), 1751 (CO, urethane), 1732 (CO, amide) and 1664 (CO, ester); δ_H[500 MHz; $(C^2H_3)_2SO$, mixture of rotamers] 0.83 [3 H, d, *J* 5.5, CH_3 (Leu)], 0.88 [3 H, d, *J* 6.5, CH_3 (Leu)], 1.23 [3 H, 2 × d, CH_3 (Ala)], 1.31 [9 H, s $(CH_3)_3$], 1.50–1.65 (3 H, m, $CHCH_2$), 1.80–1.84 [1 H, m, 1 H of β-H_2 (Glu)], 1.91–2.03 [1 H, m, 1 H of β-H_2 (Glu)], 2.28–2.32 [3 H, m, CH_2 (β-Ala) and 1 H of γ-H_2 (Glu)], 2.39–2.43 [1 H, m, 1 H of γ-H_2 (Glu)], 2.67–2.80 [2 H, m, CH_2 (Phe)], 2.78 and 2.95 (3 H, 2 × s, NCH_3), 3.23 [2 H, m, CH_2N (β-Ala)], 3.61 (3 H, 2 × s, OCH_3), 3.89–4.13 [3 H, m, CH_2 (Sar) and 1 × α-H], 4.24–4.42 (3 H, m, 3 × α-H), 5.14 (2 H, s, $PhCH_2$), 6.82 (1 H, d, *J* 9.5, NH urethane), 7.19–7.41 (10 H, m, ArH), 7.91 [1 H, br s, NH (β-Ala)] and 8.02, 8.20, 8.27, 8.30 and 8.36 (5 H, 4 × d and 1 × t, NH amide); δ_C[125.75 MHz; $(C^2H_3)_2SO$, mixture of rotamers] 18.55 [CH_3 (Ala) minor], 18.78 [CH_3 (Ala) major], 21.12 [CH_3 (Leu)], 21.23 [CH_3 (Leu)], 22.71 [CH_3 (Leu)], 24.23 [CH (Leu)], 26.43 [β-CH_2 (Glu) major], 26.58 [β-CH_2 (Glu) minor], 28.16 [$(CH_3)_3$], 28.68 [γ-CH_2 (Glu)], 34.24 [CH_2 (β-Ala)], 34.82 (NCH_3 major), 35.20 [CH_2 (Phe)], 36.11 (NCH_3 minor), 37.71 [CH_2N (β-Ala)], 42.69 [CH_2 (Leu)], 48.05 [CH_2 (Sar)], 50.29 [α-C (Ala)], 51.54 (OCH_3), 51.63 [α-C (Leu)], 51.74 (OCH_3), 51.80 [α-C (Glu)], 55.76 [α-C (Phe)], 65.92 ($PhCH_2$), 77.98 [*C*$(CH_3)_3$], 126.14, 127.80, 128.01, 128.44 and 129.20 (ArCH), 135.96 and 138.26 (ArC quaternary), 155.17 (CO, urethane) and 167.96, 170.67, 170.75, 171.54, 172.24, 172.33 and 172.50 (CO); *m*/*z* (FAB) 725 (95%, $[M + Na - C_5H_9O_2]^+$) and 120 (100, $[C_8H_9N + H]^+$).

(2*S*)-*N*-(*tert*-Butoxycarbonyl)phenylalanyl-β-alanyl-[α-methyl (2*R*)-glutamyl]-γ-sarcosyl]-(2*R*)-alanyl-(2*S*)-leucine ester 32

To a solution of hexapeptide diester **31** (1.65 g, 2.0 mmol) in methanol (50 cm^3) was added 5% palladium on charcoal (10 mg) and the mixture was stirred under hydrogen for 18 h. The catalyst was then removed by filtration through a pre-washed Celite pad and the filtrate was concentrated under reduced pressure to give the *required product* as a solid (1.21 g, 82%), mp 82 °C (softening); ν_{max}(Nujol)/cm^{-1} 3297 (NH and OH), 1747 (CO, urethane), 1722 (CO, ester) and 1664 (CO, amide); δ_H(300 MHz; $C^2H_3O^2H$, mixture of rotamers) 0.93 [6 H, m, 2 × CH_3 (Leu)], 1.34 [9 H, s, $(CH_3)_3$], 1.35 [3 H, d, CH_3 (Ala)], 1.65 [3 H, br s, $CHCH_2$ (Leu)], 1.95 [1 H, m, 1 H of β-H_2 (Glu)], 2.17 [1 H, m, 1 H of β-H_2 (Glu)], 2.42 [3 H, br s, CH_2 (β-Ala) and 1 H of γ-H_2 (Glu)], 2.53 [1 H, br t, 1 H of γ-H_2 (Glu)], 2.81 [1 H, m, 1 H of C*H*$_2$Ph (Phe)], 3.06 [1 H, m, 1 H of C*H*$_2$Ph (Phe)], 2.91 and 3.07 (3 H, 2 × s, NCH_3), 3.42 [2 H, br s, CH_2N (β-Ala)], 3.70 (3 H, s, OCH_3), 4.03 and 4.11 [2 H, 2 × s, CH_2 (Sar)], 4.24 (1 H, m, α-H), 4.40–4.48 (3 H, m, 3 × α-H) and 7.19–7.34 (5 H, m, ArH); δ_C(75.4 MHz; $C^2H_3O^2H$, mixture of rotamers) 16.65 [CH_3 (Ala) major], 17.11 [CH_3 (Ala) minor], 20.36 [CH_3 (Leu)], 22.03 [CH_3 (Leu)], 24.55 [CH (Leu)], 26.27 [β-CH_2 (Glu)], 27.22 [$(CH_3)_3$], 28.72 [γ-CH_2 (Glu)], 34.02 [CH_2 (β-Ala)], 34.75 (NCH_3 minor), 35.47 [CH_2 (Phe)], 36.11 (NCH_3 major), 37.97 [CH_2N (β-Ala)], 40.21 [CH_2 (Leu) major], 40.41 [CH_2 (Leu) minor], 48.78 [CH_2 (Sar)], 50.73 [α-C (Ala)], 51.19 [α-C (Leu)], 51.46 (OCH_3), 51.92 [α-C (Glu)], 56.16 [α-C (Phe)], 79.26 [*C*$(CH_3)_3$], 126.42, 128.14 and 129.12 (ArCH), 137.43 (ArC quaternary), 156.32 (CO, urethane) and 169.00, 169.81, 172.65, 173.19, 173.52, 173.99 and 174.92 (CO); *m*/*z* (FAB) 757 (100%, $[M + Na]^+$), 735 (6, $[M + H]^+$), 679 (8, $[M + H - C_4H_9]^+$), 657 (31, $[M + Na - C_5H_9O_2]^+$), 635 (96, $[M + H - C_5H_9O_2]^+$) and 120 (100, $[C_8H_9N + H]^+$).

β-Benzyl (2*S*)-*N*-(*tert*-butoxycarbonyl)phenylalanyl-β-alanyl-[α-methyl (2*R*)-glutamyl]-γ-sarcosyl-(2*R*)-alanyl-(2*S*)-leucyl-[α-methyl (2*R*)-aspartate] triester 33

This compound was prepared in a manner identical with that described for the phenylalanyl(hydroxy)butyrate dipeptide **16**, using hexapeptide **32** (1.18 g, 1.6 mmol) and β-benzyl α-methyl (2*R*)-aspartate hydrochloride (0.44 g, 1.6 mmol) to give a crude solid which was then dried under reduced pressure over phosphorus pentoxide. Recrystallisation from ethyl acetate–light petroleum gave the *required product* as an off-white solid (1.16 g, 76%), mp 137–139 °C (softening) (Found: C, 58.35; H, 7.15; N, 10.0. $C_{47}H_{67}N_7O_{14}\cdot H_2O$ requires C, 58.05; H, 7.15; N, 10.1%); $[\alpha]_D^{25}$ +8.2 (*c* 1.525, MeOH); ν_{max}(Nujol)/cm^{-1} 3306 (NH), 1747 (CO, urethane), 1698 (CO), 1664 (CO) and 1634 (CO); δ_H[300 MHz; $(C^2H_3)_2SO$, mixture of rotamers] 0.77 [3 H, d, *J* 6.6, CH_3 (Leu)], 0.80 [3 H, d, *J* 6.6, CH_3 (Leu)], 1.18 [3 H, 2 × d, CH_3 (Ala)], 1.26 [9 H, s, $(CH_3)_3$], 1.38–1.60 [3 H, m, $CHCH_2$ (Leu)], 1.72–1.93 [2 H, m, β-H_2 (Glu)], 2.25–2.36 [6 H, m, CH_2 (β-Ala), γ-H_2 (Glu) and β-H_2 (Asp)], 2.64–2.74 [1 H, m, 1 H of C*H*$_2$Ph (Phe)], 2.81–2.92 [1 H, m, 1 H of C*H*$_2$Ph (Phe)], 2.72 and 2.88 (3 H, 2 × s, NCH_3), 3.24 [2 H, m, CH_2N (β-Ala)], 3.57 (3 H, s, OCH_3), 3.56 and 3.60 (3 H, 2 × s, OCH_3), 3.83 [1 H, d, *J* 16.5, 1 H of CH_2 (Sar)], 3.95–4.11 [3 H, m, 2 × α-H and 1 H of CH_2 (Sar)], 4.23–4.36 (2 H, m, 2 × α-H), 4.66 [1 H, m, α-H (Asp)], 5.07 and 5.09 (2 H, 2 × s, $PhCH_2$), 6.84 and 7.17 [1 H, 2 d, *J* 8.8, NH (Phe)], 7.22 (5 H, s, ArH), 7.34 (5 H, s, ArH), 7.92 [1 H, br t, NH (β-Ala)], 8.05 (1 H, d, *J* 6.8, NH), 8.15–8.31 (2 H, m, 2 × NH) and 8.41 and 8.48 [1 H, 2 × d, *J* 8.0, NH (Asp)]; δ_C[75.4 MHz; $(C^2H_3)_2SO$, mixture of rotamers] 18.68 [CH_3 (Ala)], 21.72 [CH_3 (Leu)], 23.39 [CH_3 (Leu)], 24.59 [CH (Leu)], 27.96 [(β-CH_2 (Glu)], 28.52 [$(CH_3)_3$], 35.21 [β-CH_2 (Asp)], 35.59 [CH_2 (β-Ala)], 36.31 (NCH_3), 36.84 [CH_2 (Phe)], 38.09 [CH_2N (β-Ala)], 48.92 [CH_2 (Sar)], 51.04 (α-C), 52.25 (OCH_3), 52.62 (OCH_3), 56.21 [α-C (Phe)], 66.32 (O*C*H_2Ar), 78.46 [*C*$(CH_3)_3$], 126.70, 128.45, 128.56, 128.99 and 129.75 (ArCH), 136.46 and 138.83 (ArC quaternary), 155.79 (CO, urethane) and 168.85, 170.40, 171.39, 171.59, 172.21, 172.58 and 173.16 (CO); *m*/*z* (FAB) 976 (36%, $[M + Na]^+$), 954 (19, $[M + H]^+$), 876 (7, $[M + Na - C_5H_9O_2]^+$), 854 (100, $[M + H - C_5H_9O_2]^+$) and 120 (95, $[C_8H_9N + H]^+$).

(2*S*)-*N*-(*tert*-Butoxycarbonyl)phenylalanyl-β-alanyl-[α-methyl (2*R*)-glutamyl]-γ-sarcosyl-(2*R*)-alanyl-(2*S*)-leucyl-[α-methyl (2*R*)-aspartate] diester 34

To a solution of triester **33** (0.50 g, 0.52 mmol) in a mixture of methanol (50 cm^3) and glacial acetic acid (1 cm^3) was added 5% palladium on carbon (10 mg) and the mixture was stirred under hydrogen for 18 h. The catalyst was removed by filtration through a pre-washed Celite pad and the filtrate was concentrated under reduced pressure to give a yellow oil. Residual acetic acid was removed azeotropically with toluene under reduced pressure to give the *required product* as an off-white solid (0.43 g, 95%), mp 114–116 °C (softening); ν_{max}(Nujol)/cm^{-1} 3316br (NH and OH), 1727br (CO) and 1664br (CO); δ_H(300 MHz; $C^2H_3O^2H$, mixture of rotamers) 0.88–0.94 [6 H, m, 2 × CH_3 (Leu)], 1.34 [9 H, s, $(CH_3)_3$], 1.65 [3 H, br d, $CHCH_2$ (Leu)], 1.89–1.98 [1 H, m, 1 H of β-H_2 (Glu)], 2.13–2.24 [1 H, m, 1 H of β-H_2 (Glu)], 2.31–2.56 [4 H, m, γ-H_2 (Glu) and CH_2 (β-Ala)], 2.78–2.84 [2 H, m, β-H_2 (Asp)], 2.99–3.14 [2 H, m, C*H*$_2$Ph (Phe)], 2.92 and 3.07 (3 H, 2 × s, NCH_3), 3.37–3.48 [2 H, m, CH_2N (β-Ala)], 3.64, 3.68 and 3.69 (6 H, 3 × s, 2 × OCH_3), 3.97–4.11 [2 H, m, CH_2 (Sar)], 4.25–4.48 (4 H, m, 4 × α-H), 4.74 [1 H, t, *J* 5.8, α-H (Asp)] and 7.08–7.29 (5 H, m, ArH); δ_C(75.4 MHz; $C^2H_3O^2H$, mixture of rotamers) 17.64 [CH_3 (Ala) major], 18.02 [CH_3 (Ala) minor], 21.58 [CH_3 (Leu)], 21.80 [CH_3 (Leu)], 23.60 [CH (Leu)], 26.01 [β-CH_2 (Glu)], 27.83 [$(CH_3)_3$ minor], 28.75 [$(CH_3)_3$ major], 30.27 [γ-CH_2 (Glu)], 35.65 [β-*C*H_2 (Asp)], 36.28 [*C*H_2 (β-Ala)], 36.99 [CH_2 (Phe)], 37.27 (NCH_3 minor), 37.68 (NCH_3 major), 39.51 [CH_2N (β-Ala)], 41.48 [CH_2 (Leu)], 50.61 [CH_2 (Sar)], 50.86 (α-C), 52.59 (α-C), 52.99 (OCH_3), 53.14 (OCH_3), 53.15 (α-C), 57.66 [α-C (Phe)], 80.77 [*C*$(CH_3)_3$], 126.55, 127.94, 129.46, 129.66, 130.18 and 130.64 (ArCH), 138.96 (ArC quaternary), 157.81 (CO, urethane) and 171.54, 173.10, 174.16, 174.68 and 175.51 (CO); *m*/*z* (FAB) 908 (15%, $[M + 2Na - H]^+$), 886 (90, $[M + Na]^+$),

808 (7, $[M + H - C_4H_9]^+$), 786 (52, $[M + Na - C_5H_9O_2]^+$), 764 (56, $[M + H - C_5H_9O_2]^+$) and 120 (100, $[C_8H_9N + H]^+$).

β-Pentafluorophenyl (2*S*)-*N*-(*tert*-butoxycarbonyl)phenylalanyl-β-alanyl-[α-methyl (2*R*)-glutamyl]-γ-sarcosyl-(2*R*)-alanyl-(2*S*)-leucyl-[α-methyl (2*R*)-aspartate] triester 35

To a stirred solution of the heptapeptide carboxylic acid **34** (0.43 g, 0.5 mmol) in CH_2Cl_2 (15 cm^3) at 0 °C was added pentafluorophenol (0.28 g, 1.5 mmol) followed by EDCI (0.23 g, 0.75 mmol). The reaction mixture was stirred at 0 °C for 1 h, was then allowed to warm up to room temperature, and was stirred for a further 24 h. The solution was concentrated under reduced pressure and the residual brown solid was purified by flash chromatography on silica (CH_2Cl_2 to remove excess of pentafluorophenol, then with CH_2Cl_2–MeOH, 94:6) to give the *required product* as a waxy solid (0.47 g, 91%), mp 142–144 °C; ν_{max}(Nujol)/cm^{-1} 3316 (NH), 1795 (CO), 1747 (CO), 1717 (CO), 1659 (CO) and 1644 (CO); δ_H[300 MHz; $(C^2H_3)_2SO$, mixture of rotamers] 0.80 [3 H, d, *J* 6.6, CH_3 (Leu)], 0.83 [3 H, d, *J* 6.8, CH_3 (Leu)], 1.19 [3 H, 2 × d, CH_3 (Ala)], 1.26 [9 H, s, $(CH_3)_3$], 1.44–1.54 [3 H, m, $CHCH_2$ (Leu)], 1.76–1.96 [2 H, m, β-H_2 (Glu)], 2.20–2.40 [4 H, m, CH_2 (β-Ala) and γ-H_2 (Glu)], 2.68–2.75 [1 H, m, 1 H of C*H*$_2$Ph (Phe)], 2.74 and 2.91 (3 H, 2 × s, NCH_3), 3.07–3.39 [5 H, m, 1 H of C*H*$_2$Ph (Phe), CH_2N (β-Ala) and β-H_2 (Asp)], 3.56, 3.58 and 3.63 (6 H, 3 × s, 2 × OCH_3), 3.87 [1 H, d, *J* 16.3, 1 H of CH_2 (Sar)], 3.96–4.08 [2 H, m, 1 H of CH_2 (Sar) and 1 × α-H], 4.25–4.37 (3 H, m, 3 × α-H), 4.77 [1 H, m, α-H (Asp)], 6.85 [1 H, d, *J* 8.4, NH (Phe)], 7.16 (1 H, d, *J* 5.3, NH), 7.22 (5 H, s, ArH), 7.92 [1 H, br t, NH (β-Ala)], 8.08 (1 H, br t, NH), 8.19–8.31 (2 H, m, 2 × NH) and 8.59 and 8.65 [1 H 2 × d, *J* 8.1, NH (Asp)]; δ_C[75.4 MHz; $(C^2H_3)_2SO$, mixture of rotamers] 18.27 [CH_3 (Ala) major], 18.63 [CH_3 (Ala) minor], 21.19 [CH_3 (Leu)], 22.94 [CH_3 (Leu)], 24.13 [CH (Leu)], 28.08 [$(CH_3)_3$], 28.61 [γ-CH_2 (Glu)], 34.91 [CH_2 (β-Ala) and β-CH_2 (Asp)], 35.15 [CH_2 (Phe)], 36.02 (NCH_3), 37.66 [CH_2N (β-Ala)], 48.26 [CH_2 (Sar)], 50.08 (α-C), 50.58 (α-C), 51.50 (OCH_3), 51.77 (OCH_3), 52.42 (α-C), 55.78 [α-C (Phe)], 78.00 [*C*$(CH_3)_3$], 126.25, 128.10 and 129.32 (ArCH), 138.41 (ArC quaternary), 155.36 (CO, urethane), 166.79, 168.02, 168.44, 170.63, 170.93, 171.77, 172.14, 172.34 and 172.73 (CO); δ_F[282.3 MHz; $(C^2H_3)_2SO$] −154.16 (2 F, t, *J* 19.8 Ar *o*-F), −159.13 (1 F, dt, *J* 5.9 and 23.8, Ar *p*-F), −163.22 (2 F, dd, *J* 5.9 and 19.8, Ar *o*-F), −164.08 (2 F, t, *J* 21.8, Ar *m*-F), −166.91 (2 F, t, *J* 21.8, Ar *m*-F) and −173.40 (1 F, dt, *J* 5.9 and 23.8, Ar *p*-F); *m*/*z* (FAB) 1052 (25%, $[M + Na]^+$), 1030 (18, $[M + H]^+$), 930 (100, $[M + H - Boc]^+$) and 120 (83, $[C_8H_9N + H]^+$).

***cyclo*-[β-Ala-α-OMe-(*R*)-Glu-γ-Sar-(*R*)-Ala-(*S*)-Leu-α-OMe-(*R*)-Asp-β-(*S*)-Phe] 5**

To a stirred solution of the *N*-(*tert*-butoxycarbonyl)-protected pentafluorophenyl ester **35** (0.25 g, 0.24 mmol) in dry CH_2Cl_2 (10 cm^3) was added TFA (5 cm^3). The mixture was stirred for 90 min and was then diluted with toluene (5 cm^3), and concentrated under reduced pressure to give a pale brown solid, which was thoroughly dried *in vacuo* for 6 h. The resulting residue was dissolved into dry CH_2Cl_2 (250 cm^3), and the solution was treated with DIPEA (0.71 cm^3, 4.0 mmol) stirred at room temperature while the reaction was followed by TLC (94:6 CH_2Cl_2–MeOH) which indicated complete reaction after seven days. The solution was then concentrated under reduced pressure to give a solid,which was triturated with diethyl ether and then collected by filtration and washed on the pad with diethyl ether to afford the *pure macrocycle* **5** as a powder (120 mg, 67%), mp 276–278 °C (decomp.) (Found: C, 55.7; H, 6.75; N, 12.6. $C_{35}H_{51}N_7O_{11}\cdot 1/2H_2O$ requires C, 55.7; H, 6.95; N, 13.0%) (HRMS: Found: $[M + Na]^+$, 768.3524. $C_{35}H_{51}N_7NaO_{11}$ requires *m*/*z* 768.3544); ν_{max}(Nujol)/cm^{-1} 3306 (NH), 1751 (CO), 1708 (CO), 1664 (CO) and 1634 (CO); δ_H[300 MHz; $(C^2H_3)_2SO$, mixture of rotamers] 0.76–0.86 [6 H, m, 2 × CH_3 (Leu)], 1.16 and 1.19 [3 H, 2 × d, CH_3 (Ala)], 1.39–1.55 [3 H, m, $CHCH_2$ (Leu)], 1.78–1.91 [2 H, m, β-H_2 (Glu)], 2.18–2.42 [6 H, m, α-H_2 (Glu), β-H_2 (Asp) and CH_2(β-Ala)], 2.63–2.71 [1 H, m, 1 H of C*H*$_2$Ph (Phe)], 2.89–3.05 [1 H, m, 1 H of C*H*$_2$Ph (Phe)], 2.75 and 2.91 (3 H, 2 × s, NCH_3), 3.09–3.14 [1 H, m, 1 H of CH_2N (β-Ala)], 3.43 and 3.47 (3 H, 2 × s, OCH_3), 3.51–3.56 [1 H, m, 1 H of CH_2N (β-Ala)], 3.58 and 3.59 (3 H, 2 × s, OCH_3), 3.80 [1 H, d, 1 H of CH_2N (Sar)], 4.00–4.40 [6 H, m, 1 H of CH_2N (Sar) and 5 × α-H], 7.18–7.26 (5 H, m, ArH and 7.90–8.27 (6 H, m, 6 × NH); δ_C[75.4 MHz; $(C^2H_3)_2SO$, mixture of rotamers] 16.88 [CH_3 (Ala) major], 18.11 [CH_3 (Ala) minor], 21.08 [CH_3 (Leu) major], 21.18 [CH_3 (Leu) minor], 22.97 [CH_3 (Leu) major], 23.06 [CH_3 (Leu) minor], 24.25 [CH (Leu)], 26.43 [β-CH_2 (Glu)], 28.91 [γ-CH_2 (Glu) minor], 29.30 [γ-CH_2 (Glu) major], 34.27 [β-CH_2 (Asp)], 35.16 [CH_2 (β-Ala)], 36.37 (NCH_3 major), 36.44 (NCH_3 minor), 37.39 [CH_2 (Phe)], 37.59 [CH_2N (β-Ala)], 48.65 [CH_2 (Sar) minor], 48.93 [CH_2 (Sar) major], 49.52 [α-C (Ala)], 49.98 [α-C (Asp)], 50.90 [α-C (Leu)], 51.12 [α-C (Glu)], 51.53 (OCH_3), 51.73 (OCH_3), 51.93 (OCH_3), 54.07 [α-C (Phe) minor], 54.43 [α-C (Phe) major], 126.35, 128.20, 129.23 and 129.29 (ArCH), 138.08 and 138.14 (ArC quaternary) and 168.31, 169.25, 170.95, 171.08, 171.25, 171.41, 171.54, 171.62, 171.88, 172.35, 172.67, 172.74 and 172.78 (CO); *m*/*z* (FAB) 768 (25%, $[M + Na]^+$), 746 (23, $[M + H]^+$) and 120 (100, $[C_8H_9N + H]^+$).

Acknowledgements

We thank the BBSRC and EPSRC for grants GR/K64068 and B05162, Dr T. Rutherford for obtaining and interpreting NMR data, Dr J. Wilkie for performing computational analyses of enzyme-bound toxin conformations, Dr M. Akhtar for scientific support and the Wellcome Trust for an NMR equipment grant.

Abbreviations

COSY, 2-D homonuclear chemical-shift correlation spectroscopy; HMBC, 2-D heteronuclear multiple-bond correlation spectroscopy; HSQC, proton-detected single-quantum-coherence heteronuclear correlation spectroscopy; TOCSY, phase-sensitive 2-D total correlation spectroscopy; AHB, 3-amino-4-hydroxybutyric acid; DIPEA, *N*,*N*-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; EDCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide; IBCF, isobutyl chloroformate; NMM, 4-methylmorpholine; PFP, pentafluorophenyl; PP1 serine/threonine protein phosphatase type 1; PP2A, serine/threonine protein phosphatase type 2A; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TMSCl, chlorotrimethylsilane.

References

1 A. P. Mehrotra, K. L. Webster and D. Gani, *J. Chem. Soc., Perkin Trans. 1*, 1997, preceding paper.
2 W. W. Carmichael, *J. Appl. Bacteriol*., 1992, **72**, 445.
3 R. E. Honkanen, J. Zwiller, S. L. Dailly, B. S. Khatra, M. Dukelow and A. L. Boynton, *J. Biol. Chem*., 1991, **266**, 6614.
4 S. Shenolikar and A. C. Nairn, *Adv. Sec. Mess. Phosphoprotein Res*., 1991, **23**, 1.
5 M. Bollen and W. Stalmans, *Crit. Rev. Biochem. Mol. Biol*., 1992, **27**, 227.
6 A. A. Depaoli-Roach, I.-K. Park, V. Cerovsky, C. Csortos, S. D. Durbin, M. J. Kuntz, A. Sitikov, P. M. Tang, A. Verin and S. Zolnierowicz, *Adv. Enzyme Regul*., 1994, **34**, 199.
7 P. Cohen, *Annu. Rev. Biochem*., 1989, **58**, 453.
8 P. T. W. Cohen, N. D. Brewis, V. Hughes and D. J. Mann, *FEBS Lett*., 1990, **268**, 355.
9 C. F. B. Holmes and M. P. Boland, *Curr. Opin. Struct. Biol*., 1993, **3**, 934.
10 J. Goldberg, H. Huang, Y. Kwon, P. Greengard, A. C. Nairn and J. Kuriyan, *Nature*, 1995, **376**, 745.
11 J. Wilkie and D. Gani, unpublished results.
12 A. P. Mehrotra and D. Gani, *Tetrahedron Lett*., 1996, **37**, 6915.

13 M. Namikoshi, K. L. Rinehart, A. M. Dahlem, V. R. Beasley and W. W. Carmichael, *Tetrahedron Lett*., 1989, **30**, 4349.
14 T. K. Chakraborty and S. P. Joshi, *Tetrahedron Lett*., 1990, **31**, 2043.
15 M. F. Beatty, C. Jennings-White and M. A. Avery, *J. Chem. Soc., Perkin Trans. 1*, 1992, 1637.
16 R. J. Valentokovich and S. L. Schreiber, *J. Am. Chem. Soc*., 1995, **117**, 9069.
17 J. M. Humphrey, J. B. Aggen and R. A. Chamberlain, *J. Am. Chem. Soc*., 1996, **118**, 11759.
18 H. Y. Kim and P. L. Toogood, *Tetrahedron Lett*., 1996, **37**, 2349.
19 F. D'Aniello and A. Mann, *J. Org. Chem*., 1996, **61**, 4870.
20 F. D'Aniello, A. Mann, A. Schoenfelder and M. Taddei, *Tetrahedron*, 1997, **53**, 1447.
21 P. Hormozdiari and D. Gani, *Tetrahedron Lett*., 1996, **37**, 8227.
22 H. Schwarz, F. M. Bumpus and I. H. Page, *J. Am. Chem. Soc*., 1957, **79**, 5697.
23 M. Goodman and F. Boardman, *J. Am. Chem. Soc*., 1963, **85**, 2483.
24 P. Gmeiner, P. L. Feldman, M. Y. Chu-Moyer and H. Rapoport, *J. Org. Chem*., 1990, **55**, 3068.
25 J. M. Bland, *Synth. Commun*., 1995, **25**, 467.
26 K. Wüthrich, *NMR of Proteins and Nucleic Acids*, Wiley Interscience, 1986, New York.
27 M. M. Lenman, S. L. Ingham and D. Gani, *J. Chem. Soc., Chem. Commun*., 1996, 85.
28 K. L. Webster, A. P. Mehrotra, A. B. Maude, D. Gani and T. J. Rutherford, Parts 3 and 4 in this series, in preparation. See also K. L. Webster, T. J. Rutherford and D. Gani, *Tetrahedron Lett*., 1997, **38**, 5713.
29 A. B. Maude and D. Gani, unpublished results.
30 D. D. Perrin, W. L. F. Armarego and D. R. Perrin, in *Purification of Laboratory Chemicals*, Pergamon Press, Oxford, 1980.
31 G. R. Pettit and P. S. Nelson, *J. Org. Chem*., 1986, **51**, 1282.

Paper 7/02410J
Received 8*th April* 1997
Accepted 29*th April* 1997