Total syntheses and re-assignment of configurations of the cyclopeptides lissoclinamide 4 and lissoclinamide 5 from *Lissoclinum patella*

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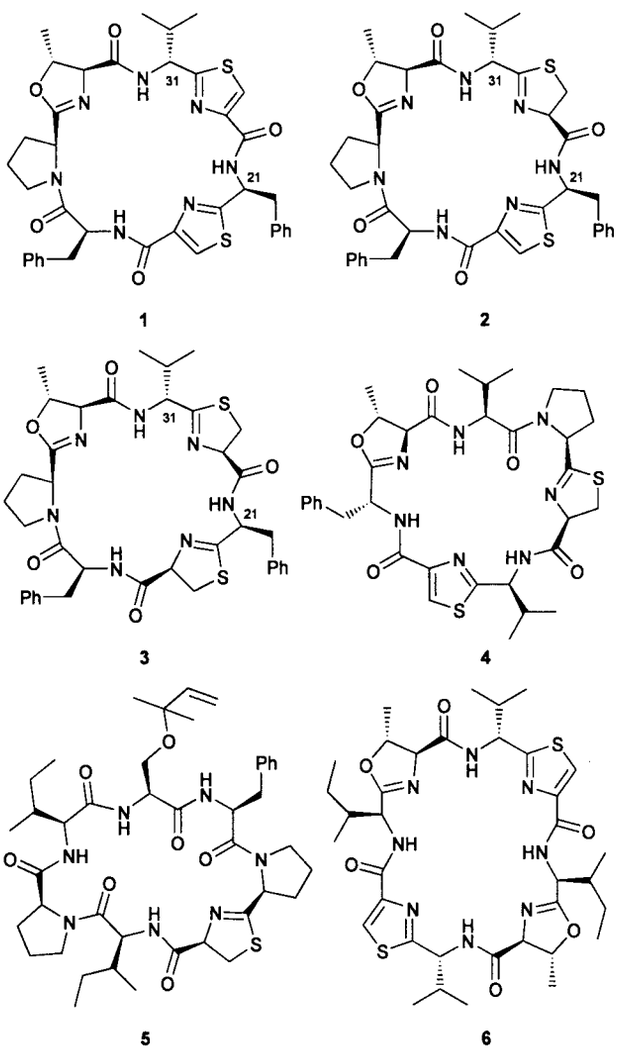
The total synthesis of lissoclinamide 4 and lissoclinamide 5, which are novel oxazoline/thiazole/thiazoline-based cyclopeptides isolated from the ascidian ("sea squirt") *Lissoclinum patella*, are described. The synthesis of the bis-thiazole based lissoclinamide 5 necessitated the development of practical solutions to the elaboration of enantiomerically pure valine and phenylalanine substituted thiazoles. To overcome the problems associated with the configurational lability of enantiopure amino acid substituted thiazolines, the thiazoline-based cyclopeptide lissoclinamide 4 was synthesised using a novel strategy whereby both the oxazoline and the thiazoline rings in the natural product were formed simultaneously in a "one-pot" double cyclodehydration sequence from an appropriate thioamide/amide cyclopeptide precursor. Based on our synthetic work the stereochemistries **2** and **1** published for natural lissoclinamide 4 and lissoclinamide 5 were re-assigned to **16** and **15** respectively.

In recent years the marine environment has revealed itself as a rich source of novel and unusual secondary metabolites, many of which have shown considerable promise as lead compounds for development of therapeutic agents.[1] A particularly fascinating family of marine metabolites is the heterocycle-containing cyclopeptides known as lissoclinamides isolated from the ascidian ("sea squirt") *Lissoclinum patella*.[2] These metabolites show structures which feature an oxazoline ring together with one or more thiazoles and/or thiazolines, derived from unusual amino acid residues, incorporated in a cyclic heptapeptide *e.g.* lissoclinamide 5, **1**, lissoclinamide 4, **2**, and lissoclinamide 7, **3**.[3–5] Structurally related compounds which have also been isolated from other ascidians include cyclodidemnamide **4**,[6] mollamide **5**,[7] and ascidiacyclamide **6**.[8] All of these cyclopeptides show a range of biological properties, particularly immunoregulatory, antibiotic and antitumoral, but including enzyme-inhibitory activity. Cyclopeptides can, of course, assume a range of conformations, each of which can have significantly different biological properties. Many marine organisms are well known to accumulate inorganic salts in considerable quantities and we,[9] and others,[10–13] have intimated that lissoclinamide–metal conjugates, involving the various heteroatom ligands associated with their macrocyclic cavities, could play a significant role in the biological properties of these metabolites. With the aim of investigating the capacity of lissoclinamides and related cyclopeptides to bind metals, and correlating these data with the biological profiles of the metabolites and their metal conjugates, we have examined synthetic routes to the 31-Val and 21-Phe cycloheptapeptides lissoclinamide 4 **2**[14] and lissoclinamide 5 **1**.[15]



In the light of the potential for cyclopeptides as therapeutic agents, it is not surprising that synthetic investigations within this family of natural products have been intensive.[16] Spectroscopic methods, together with X-ray studies in the cases of mollamide **5** and ascidiacyclamide **6** have played a crucial role in the assignment of the structures of marine cyclopeptides, but in several other instances these structures have needed significant revision following synthetic investigations.[16] The correct assignment of the chiral centres adjacent to the thiazole, and especially the thiazoline, rings in structures **1**→**6** is particularly problematic since these centres are prone to undergo epimerisation during the usual methods of cyclopeptide hydrolytic degradation and during their synthesis.[17] The design we adopted for the synthesis of the structure **1** proposed for the bisthiazole-based cyclopeptide lissoclinamide 5 is shown in

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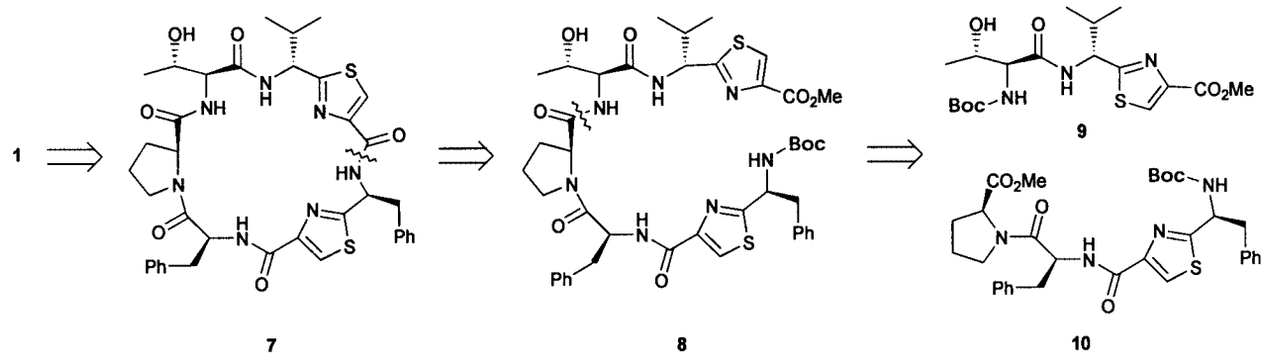
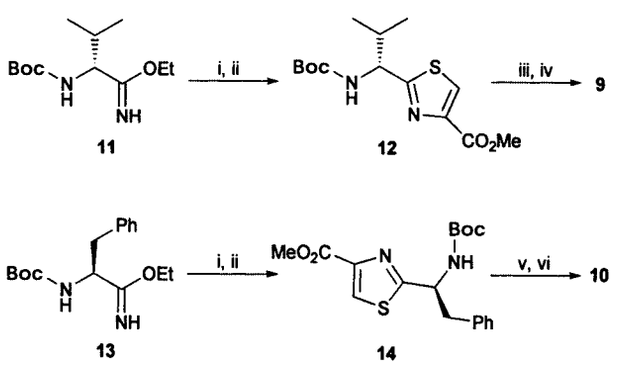


Scheme 1

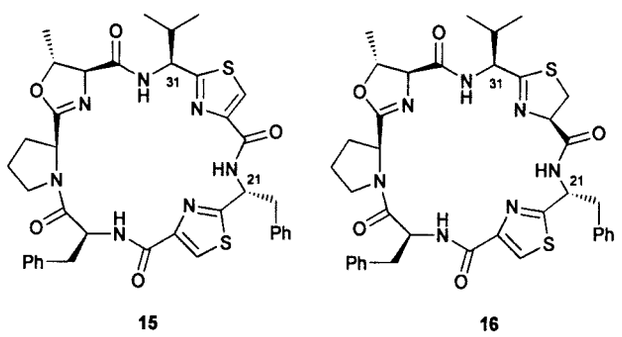
Scheme 1. Thus, we decided to prepare the thiazole-containing tri- and tetra-peptides **9** and **10** respectively, then to condense these two fragments at the Pro–Thr amide bond, leading to **8**, in readiness for macrocyclisation *via* the Thz–Phe amide bond (Thz = thiazole) producing "pre-lissoclinamide 5" **7**. Cyclodehydration of **7** using the precedent established by Shioiri and co-workers[18] and by Schmidt and Gleich[19] in their pioneering work on the syntheses of the patellamides A–D, would then be expected to lead to **1**.

In model studies we compared and contrasted a range of methods for the synthesis of enantiopure amino acid based thiazoles, including the (*R*)-Val and (*S*)-Phe derived thiazoles **12** and **14**.[20,21] Both of these compounds were easily prepared, with



Scheme 2 *Reagents and conditions*: i) Cys-OMe hydrochloride, EtOH, 25 °C; ii) MnO_2, CH_2Cl_2, 25 °C; iii) CF_3CO_2H, CH_2Cl_2, 25 °C; iv) Boc-Pro-aThr-OH, DCC, HOBt, NMM, CH_2Cl_2, 0–5 °C; v) LiOH, THF–H_2O; vi) Phe-Pro-OMe TFA salt, FDPP, NMM, MeCN, 25 °C.

>98% ee, by straightforward condensation reactions between cysteine ester HCl and the *N*-Boc protected imino ethers **11** and **13** derived from (*R*)-valine and from (*S*)-phenylalanine respectively. Deprotection of the Boc residue in **12** and coupling of the free amine to Boc-Pro-aThr-OH† produced the tetrapeptide **9** in 84% yield, and saponification of **14** and coupling of the resulting carboxylic acid to (*S*)-Phe-Pro-OMe led to the corresponding tripeptide **10** (Scheme 2). Saponification of the ester **10** and condensation with the amine produced after Boc-removal from **9**, using DCC–HOBt conditions, next led to the heptapeptide **8** in a gratifying 70% yield based on **10**. Hydrolysis of the thiazole ester in **8** with lithium hydroxide, followed by removal of the Boc group from the crude carboxylic acid and treatment of the resulting amino acid TFA salt with diphenylphosphorazide (DPPA) and *N*-methylmorpholine (NMM) in DMF (2 days) then afforded the cyclopeptide **7** in 35–40% yield from **9**. Dehydration of the allothreonine residue in **7** by treatment with excess thionyl chloride at 0–4 °C for 36 h then gave the "lissoclinamide 5" **1** in 73% yield, as a foam. Comparison of the ^{1}H NMR and ^{13}C NMR spectroscopic data of the synthetic and natural lissoclinamide showed, however, that the two compounds were not identical! With knowledge of the ease with which thiazole (and thiazoline) based cyclopeptides are known to undergo epimerisation during hydrolytic degradation, alongside further analysis of the NMR data recorded for our synthetic **1** and for natural lissoclinamide 5, we re-assigned the stereochemistry of the natural product to (21*R*,31*S*) *i.e.* **15**.



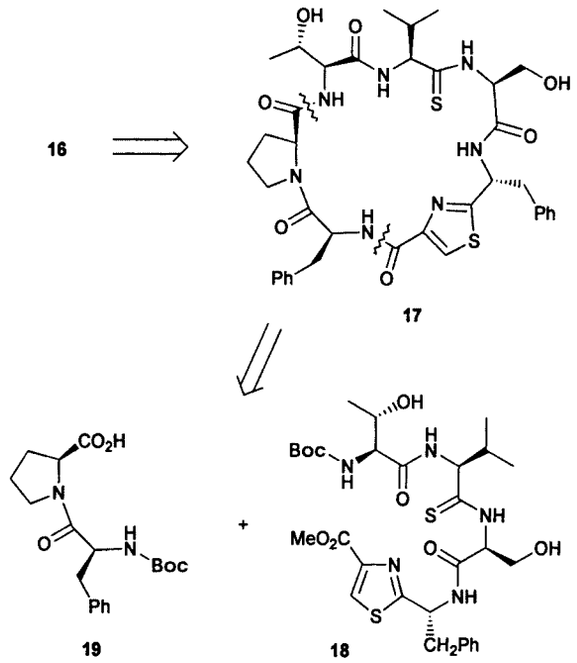
Furthermore we carried out a total synthesis of this stereostructure, starting from (*S*)-valine and from (*R*)-phenylalanine, using the strategy described previously. This synthesis gave lissoclinamide 5 **15**, which displayed ^{1}H NMR and ^{13}C NMR spectroscopic data which were identical to those found for the natural product isolated from *L. patella*.

Although the thiazoline/thiazole-based cyclopeptide lissoclinamide 4 **2** had earlier been assigned the (21*S*,31*R*) stereostructure, in light of our synthetic studies with lissoclinamide 5 (*i.e.* **15** *versus* **1**), we re-assigned its stereochemistry to that shown in structure **16**, *i.e.* (21*R*,31*S*) before embarking on its total synthesis. Hitherto there had been no reported total synthesis of any thiazoline-based cyclopeptide.[22] Our synthetic plan for the synthesis of **16** envisaged the simultaneous formation of the thiazoline and oxazoline rings in **16** from a suitable dihydroxy precursor **17**, *via* a "one-pot" double cyclodehydration sequence in the final step. The cyclothiopeptide **17**, in turn, was to be derived from macrocyclisation of the heptapeptide thioamide produced from the fragments **18** and **19** (Scheme 3).

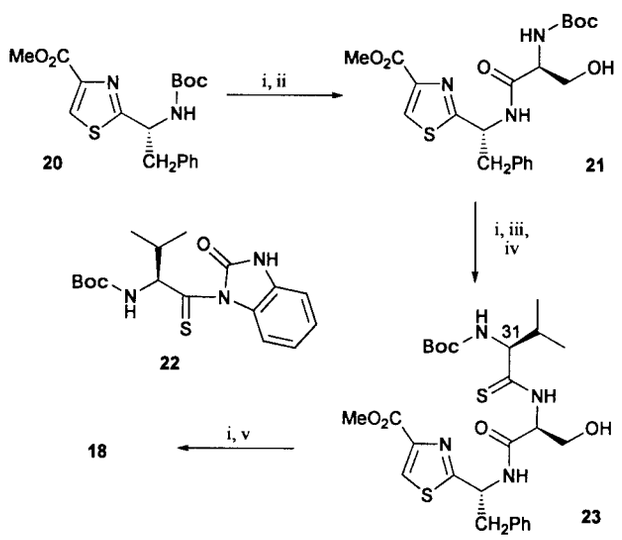
Thus, the Boc-protected (*R*)-thiazole **20** was first deblocked using TFA in CH_2Cl_2 and the resulting amine was then coupled with Boc-(*S*)-Ser-OH using DCC–HOBt leading to the tripeptide **21** in 71% yield. The tripeptide **21** was next deblocked with TFA, and the free amine generated was thioacylated with the (*S*)-valine derived thioacylating reagent **22** introduced by Zacharie *et al.*[23] producing the tetrapeptide **23** with no detectable racemisation of the centre at C-31 (Scheme 4). Boc-deprotection of **23**, by the usual method, and coupling of the resulting amine with Boc-allothreonine, finally gave the key intermediate pentapeptide **18** in 76% yield. Further deprotection of **18** and coupling to the Boc-(*S*)-Phe-Pro-OH **19** using DCC–HOBt in DMF next gave the heptapeptide **24**. Saponification of **24** to the carboxylic acid **25**, followed by Boc deprotection and cyclisation using DPPA–NMM–DMF at room temperature for 48 h, then gave the cyclopeptide **17**, whose

† aThr = L-allothreonine.

NMR spectroscopic data showed that it existed as a mixture of several rotamers (Scheme 5). Finally, treatment of **17** with Burgess' reagent[17] in refluxing THF produced the lissoclinamide **16** in 71% yield, which showed ^{1}H NMR and ^{13}C NMR spectroscopic data, together with chiroptical data, which matched corresponding data reported for natural lissoclinamide 4.



Scheme 3



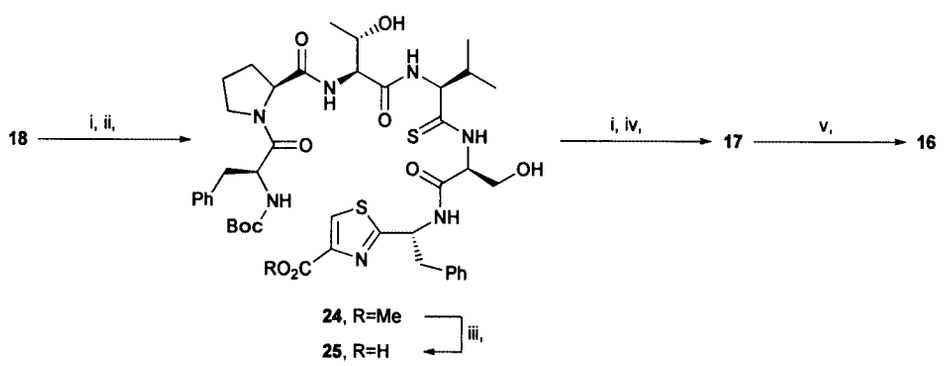
Scheme 4 *Reagents and conditions*: i, 50% TFA–CH_2Cl_2, 25 °C; ii, Boc-Ser-OH (2.5 eq), DCC + HOBt (1.25 eq), Et_3N (1 eq), CH_2Cl_2, 0 °C; iii, $NaHCO_3$, CH_2Cl_2–H_2O; iv, **22**, DMF, 0–5 °C; v, Boc-aThr-OH, DCC-HOBt, *i*Pr_2NEt, DMF, 0–25 °C.

In summary, we have shown by total synthesis that the structures assigned to lissoclinamide 5 and lissoclinamide 4 have the (21*R*,31*S*) stereochemistries, **15** and **16** respectively, and not the corresponding stereochemistries, **1** and **2**, published previously. Furthermore, by establishing practical solutions to the synthesis of enantiopure amino acid substituted thiazoles and to the elaboration of chiral thiazoline cyclopeptides, it should be possible to effect the synthesis of any stereodefined thiazole/thiazoline based cyclopeptide, whether it be naturally occurring or not, in the future.

In previous publications we have expressed the view that metal cation complexation by cyclopeptides *in vivo* may facilitate the transport of such cations across hydrophobic membranes. If this hypothesis is correct, then we would expect any natural cyclopeptide to be formed from amino acids with stereochemistries which lead to epimers of the cyclopeptide which: a) adopt an "open" conformation in aqueous media, so as to allow the various O- and N-ligands in the macrocycle to attract a solvated metal cation which can then enter the cavity without undue steric hindrance, and b) are capable of forming a hydrophobic "shield" around the complexed cation (metal–ligand conjugate) so as to allow transport through cell membranes.

We decided to examine the compliance of the predicted and revised structures for lissoclinamide 4, *viz* **2** and **16**, with these criteria by carrying out conformational searches for the uncomplexed natural products in water using the MMFF94s forcefield of Halgren[24] and the GB/SA solvation model as implemented in MacroModel 5.5.[25] As can be seen from Fig. 1, a comparison of the preferred global minima for structures **2** and **16** in the aqueous phase clearly shows that both structures have an "open" lower face which would facilitate metal entry and bindings. However, calculations on hypothetical Zn^{2+} complexes, carried out using the same forcefield in the vacuum phase show a remarkable difference in the "shielding" abilities of the two structures (Fig. 2). Thus, although the upper face of the macrocyclic ring can be covered by a combination of the 31-Val isopropyl group and the 11-Phe aromatic ring in both **2** and **16**, the 21-(*R*)-Phe structure **16** is able to cap the lower face of the molecule as well with the aryl ring of the 21-(*R*)-Phe residue, whereas **2** is prevented from doing so by the "equatorial" orientation of the 21-(*S*)-Phe side chain. It is notable that the 21-(*R*)-Phe configuration is also present in the even more cytotoxic bis-thiazoline lissoclinamide 7, **26**.[26]

In contemporaneous synthetic studies we have developed efficient syntheses of the related cyclopeptides cyclodidemnamide **4**[27] and mollamide **5**,[28] carried out metal binding studies (particularly to Cu^{2+} and Zn^{2+}) of selected cyclopeptides,[29] and examined the cyclooligomerisation of thiazole and oxazole



Scheme 5 *Reagents and conditions*: i, TFA, CH_2Cl_2; ii, **19**, DCC–HOBt, *i*Pr_2NEt, DMF, 25 °C; iii, NaOH, MeOH–H_2O; iv, NMM, DPPA, DMF, 25 °C, 48 h; v, Burgess reagent, THF, 65 °C, 30 min.

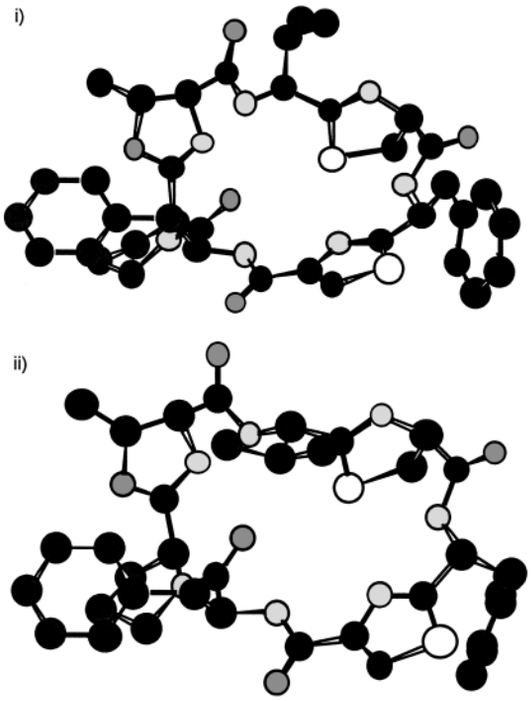
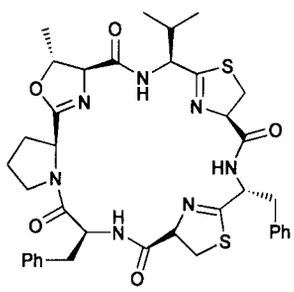


Fig. 1 Global minima for i) **2** and ii) **16** in aqueous phase, calculated with the MMFFs forcefield and GB/SA solvation model.



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based amino acids leading to novel synthetic heterocyclic-based cyclopeptides.[30] These studies are presented in other publications and, in the case of cyclodidemnamide, in the accompanying paper.

Experimental

General details

Optical rotations were measured on a JASCO DIPA-370 polarimeter; $[a]_D$ values were determined at 25 °C and are recorded in units of 10^{-1} deg cm^2 g^{-1}. Infrared spectra were obtained using a Perkin-Elmer 1600 series FT-IR instrument as dilute solutions in spectroscopic grade chloroform. Proton NMR spectra were recorded on either a Bruker DRX500 (500 MHz), a Bruker AM400 (400 MHz) or a Bruker DPX360 (360 MHz) spectrometer as dilute solutions in deuterochloroform or d_6-dimethyl sulfoxide. Chemical shifts are recorded relative to a solvent standard and the multiplicity of a signal is designated by one of the following abbreviations: s = singlet; d = doublet; t = triplet; q = quartet; br = broad; m = multiplet; app = apparent. All coupling constants, *J*, are reported in hertz. Carbon-13 NMR spectra were obtained using the instruments indicated for ^{1}H above, at frequencies of 125 MHz, 100.6 MHz and 90 MHz respectively. The spectra were recorded as dilute solutions in deuterochloroform or d_6-dimethyl sulfoxide with chemical shifts reported relative to a solvent standard on a broad band decoupled mode and the multiplicities obtained using a DEPT sequence. Where required, assignment for ^{1}H and ^{13}C NMR spectra were confirmed by two dimensional homonuclear (^{1}H) and/or heteronuclear (^{1}H/^{13}C) correlation spectroscopy. Matrix dimensions for two dimensional spectra were either 1024 points × 256 columns (homonuclear ^{1}H) or 2048 points × 128 columns (heteronuclear ^{1}H/^{13}C), and were recorded on a Bruker DRX500 instrument. Mass spectra were recorded on an AE1 MS-902, MM-70E or VG Autospec spectrometer using electron ionisation (EI) or fast atom bombardment (FAB) techniques. Flash chromatography was performed on Merck silica gel 60 as the stationary phase and the solvents employed were either of analytical grade or were distilled before use. Solvents were removed on a Büchi rotary evaporator using water aspirator pressure.

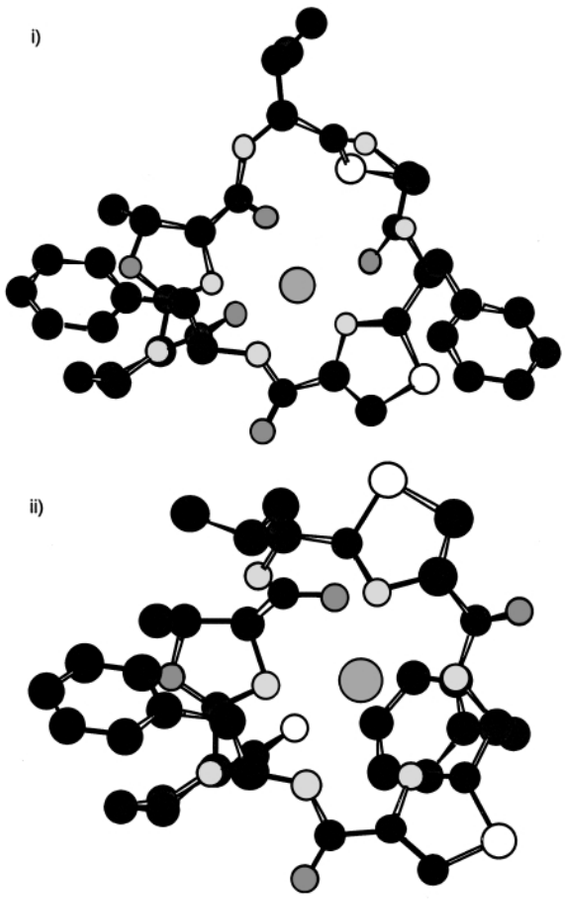

Fig. 2 Complexes of i) **2** and ii) **16** with Zn^{2+}, as calculated using the MMFFs forcefield in vacuum phase.

Molecular mechanics calculations were carried out on a Silicon Graphics Indy R4600PC workstation using the MacroModel program, version 5.5. The global minima for the various structures were located by a 1000 conformer Monte-Carlo search using the MMFF94s (planar amide nitrogen atoms) forcefield; aqueous phase calculations utilised the GB/SA solvation model.[31]

Boc-(*R*)-Val-Thz-OMe, 12

The thiazole was prepared from cysteine ester HCl and the (*R*)-imino ether **11** according to the procedure described previously,[20] and showed: δ_H(500 MHz, DMSO, 90 °C) 8.36 (1H, s), 7.17 (1H, d, *J* 7.0 Hz), 4.59 (1H, dd, *J* 8.4, 6.9 Hz), 3.80 (3H, s), 2.25 (1H, septet, *J* 6.8 Hz), 1.40 (9H, s), 0.95 (3H, d, *J* 6.75 Hz), 0.92 (3H, d, *J* 6.75 Hz). δ_C(125 MHz, DMSO, 90 °C) 173.9 (s), 160.9 (s), 154.8 (s), 145.3 (s), 127.8 (d), 78.3 (s), 58.6 (d), 51.3 (q), 31.8 (d), 27.9 (q), 18.8 (q), 17.8 (q). *m*/*z* (ESMS) 337.1195 (M^+ + Na); $C_{14}H_{22}N_2O_4NaS$ requires 337.1198. The enantiomer of **12** was prepared in a similar manner and showed identical spectroscopic data.

Boc-(*S*)-Phe-Thz-OMe, 14

The thiazole was prepared from cysteine ester HCl and the (*S*)-imino ether **13** according to the procedure described previously,[20] and showed: δ_H(500 MHz, DMSO, 90 °C) 8.37 (1H, s), 7.29 (5H, m), 7.23 (1H, m), 5.08 (1H, ddd, *J* 9.85, 5.0, 4.2 Hz), 3.88 (3H, s), 3.37 (1H, dd, *J* 14.0, 5.0 Hz), 3.14 (1H, dd, *J* 14.0, 9.85 Hz), 1.35 (9H, s). δ_C(125 MHz, DMSO, 90 °C) 174.1 (s), 160.9 (s), 154.5 (s), 145.4 (s), 137.4 (s), 128.7 (d), 128.1 (d), 127.7 (d), 125.9 (d), 78.3 (s), 54.2 (d), 51.3 (q), 39.7 (t), 27.8 (q). *m*/*z* (ESMS) 385.1200 (M + Na); $C_{18}H_{22}N_2O_4NaS$ requires 385.1198. The enantiomer **20** was prepared in the same manner and, as expected, showed identical spectroscopic data.

Boc-aThr-(*R*)-Val-Thz-OMe, 9

Trifluoroacetic acid (2.5 ml) was added in one portion to a solution of the thiazole **12** (472 mg, 1.50 mmol) in dichloromethane (10 ml). The solution was stirred at 25 °C for 2 hours, then diluted with toluene (4 ml) and concentrated *in vacuo*. A further 4 ml of toluene was added, and the resulting mixture was then reconcentrated *in vacuo* to leave the crude amine TFA salt as a gum. Hünig's base (865 µl, 5.0 mmol) was added to a solution of the salt in dichloromethane (15 ml), and the solution was then cooled to 4 °C and stirred for 15 min. Boc-allothreonine (*ca.* 1.20 mmol) was added, followed by 1-hydroxybenzotriazole (162 mg, 1.20 mmol) and finally DCC (268 mg, 1.30 mmol). The mixture was stirred at 0–5 °C for 2 hours and then at room temperature for 5 hours. It was then filtered (to remove precipitated DCU) and washed sequentially with 10% citric acid solution (10 ml), 10% $NaHCO_3$ (10 ml), water (10 ml) and finally brine (10 ml). The solution was dried ($MgSO_4$) and concentrated *in vacuo* to leave the crude tripeptide. Purification by flash chromatography on silica gel (1 : 1 EtOAc–light petroleum eluant) gave the *tripeptide* (350 mg, 842 µmol, 70%) as a white foam: $[\alpha]_D^{25}$ +7 (*c* = 2.54, $CHCl_3$). ν_{max} ($CHCl_3$)/cm^{-1} 3429, 1716, 1369. δ_H (360 MHz, $CDCl_3$) 0.86 (3H, d, *J* 6.8 Hz, $(CH_3)_2CH$), 0.90 (3H, d, *J* 6.8 Hz, $(CH_3)_2CH$), 1.21 (3H, d, *J* 6.4 Hz, CH_3CHOH), 1.32 (9H, s, $(CH_3)_3$), 2.33–2.38 (1H, m, $(CH_3)_2CH$), 3.86 (3H, s, OCH_3), 4.00–4.07 (1H, m, CH_3-C*H*OH), 4.15 (1H, br s, C*H*NHCOO), 5.16 (1H, dd, *J* 6.1, 8.9 Hz, C*H*CH$(CH_3)_2$), 5.84 (1H, d, *J* 6.5 Hz, N*H*COO), 7.64 (1H, d, *J* 8.1 Hz, N*H*COCH), 8.04 (1H, s, SC*H*). δ_C (90.5 MHz, $CHCl_3$) 17.1 (q), 19.1 (q), 27.8 (q), 32.7 (d), 52.1 (q), 56.3 (d), 59.1 (d), 68.3 (d), 79.8 (s), 127.0 (d), 146.0 (s), 155.7 (s), 161.4 (s), 171.0 (s), 172.1 (s). *m*/*z* (FAB) 416.1862 (MH^+, $C_{18}H_{30}N_3O_6S$ requires 416.1862), 416 (29), 360 (20%).

The (*S*)-Val derived epimer of **9** showed: δ_H(500 MHz, DMSO, 100 °C) 8.35 (1H, s), 7.92 (1H, d, *J* 7.75 Hz), 6.28 (1H, d, *J* 6.75 Hz), 5.06 (1H, dd, *J* 8.4, 6.6 Hz), 4.43 (1H, br s), 4.03 (1H, dd, *J* 8.4, 6.1 Hz), 3.94 (1H, br s), 3.87 (3H, s), 2.37 (1H, septet, *J* 6.75 Hz), 1.43 (9H, s), 1.14 (3H, d, *J* 6.3 Hz), 0.99 (3H, d, *J* 6.8 Hz), 0.95 (3H, d, *J* 6.7 Hz). δ_C(125 MHz, DMSO, 100 °C) 172.2 (s), 170.2 (s), 160.8 (s), 154.8 (s), 145.4 (s), 127.7 (d), 78.1 (s), 66.5 (d), 60.2 (d), 56.2 (d), 51.2 (q), 31.8 (d), 27.8 (q), 19.2 (q), 18.7 (q), 17.5 (q). *m*/*z* (ESMS) 438.1687 (M^+ + Na); $C_{18}H_{29}N_3O_6NaS$ requires 438.167.

Boc-(*S*)-Phe-Thz-Phe-Pro-OMe, 10

Aqueous sodium hydroxide (1 M, 7.0 ml, 7.0 mmol) was added over 5 min to a stirred solution of the thiazole **14** (2.005 g, 5.54 mmol) in THF–MeOH–water (2 : 2 : 1). The solution was stirred at 25 °C for 90 min and then concentrated *in vacuo* to remove the THF and methanol; the residual aqueous solution was washed with ether (2 × 10 ml) and acidified to pH 1 using 2 M HCl. The mixture was extracted with ethyl acetate (3 × 30 ml) and the extract was then washed successively with water (5 ml) and brine (20 ml), then dried ($MgSO_4$) and concentrated *in vacuo* to leave the crude acid as an oil, which was used without further purification.

The crude trifluoroacetic acid salt from Boc-(*S*)-Phe-Pro-OMe (2.259 g, 6.00 mmol) was prepared by acidolysis with 1 : 3 TFA–dichloromethane followed by concentration *in vacuo*, and then redissolved in dichloromethane (20 ml). After cooling to 0–4 °C Hünig's base (2.55 ml, 15.0 mmol) was added, followed 10 minutes later by the crude carboxylic acid and finally pentafluorophenyl diphenylphosphinate (FDPP) (2.323 g, 6.05 mmol). The mixture was stirred at 0–4 °C for 30 minutes and then at 25 °C for 3 hours. The solvent was removed under reduced pressure and the residue was purified by flash chromatography on silica gel (EtOAc–light petroleum, 1 : 1) to give the *tetrapeptide* (2.40 g, 3.95 mmol, 71%) as a pale yellow foam: δ_H(500 MHz, DMSO, 100 °C) 8.10 (1H, s), 7.87 (1H, br s), 7.29 (8H, m), 7.23 (2H, m), 5.08 (1H, ddd, *J* 10.6, 6.8, 5.3 Hz), 5.04 (1H, br s), 4.44 (1H, br s), 3.76 (1H, br s), 3.69 (3H, br s), 3.47 (1H, ddd, *J* 10.2, 6.7, 6.5 Hz), 3.37 (1H, dd, *J* 13.9, 5.3 Hz), 3.16 (2H, 4 line m), 3.04 (1H, dd, *J* 13.7, 6.8 Hz), 2.21 (1H, br s), 1.95 (4H, br s), 1.37 (9H, s). δ_C(125 MHz, DMSO, 100 °C) 173.7 (s), 171.5 (s), 169.0 (s), 154.5 (s), 148.5 (s), 137.3 (s), 136.4 (s), 129.0 (d), 128.7 (d), 127.6 (d), 125.9 (d), 123.2 (s), 78.3 (s), 58.4 (d), 54.1 (d), 51.1 (q), 46.2 (t), 39.7 (t), 37.2 (t), 28.1 (t), 27.8 (q), 24.05 (t). *m*/*z* (ESMS) 629.2456 (M + Na); $C_{32}H_{38}N_4O_6NaS$ requires 629.2410. The 21-(*R*)-Phe epimer of **10** showed δ_H(360 MHz, DMSO, 90 °C) 8.23 (1H, d, *J* 8.1 Hz), 8.16 (1H, s), 7.83 (1H, d, *J* 8.4 Hz), 7.34–7.28 (8H, m), 7.23 (2H, m), 4.99 (2H, m), 4.38 (1H, dd, *J* 8.55, 4.9 Hz), 3.76 (1H, m), 3.66 (3H, s), 3.49 (1H, m), 3.39 (1H, m), 3.18–3.09 (2H, 5 line m), 3.04 (1H, 3 line m), 2.19 (1H, 6 line m), 1.94 (2H, m), 1.85 (2H, m), 1.33 (9H, s). δ_C(90 MHz, DMSO, 90 °C) 173.9 (s), 171.7 (s), 169.1 (s), 154.65 (s), 148.6 (s), 137.5 (s), 136.6 (s), 129.2 (d), 128.9 (d), 127.8 (d), 126.2 (d), 126.05 (d), 123.6 (s), 78.4 (s), 58.6 (d), 54.1 (d), 51.5 (q), 46.4 (t), 39.7 (t), 37.2 (t), 28.3 (t), 27.9 (q), 24.3 (t).

Boc-(*S*)-Phe-Thz-(*S*)-Phe-Pro-aThr-(*R*)-Val-Thz-OMe, 8

Aqueous sodium hydroxide (1 M, 1.05 ml, 1.05 mmol) was added dropwise over *ca.* 5 min to a stirred solution of the tetrapeptide **10** (607 mg, 1.00 mmol) in THF–water (10 ml, 4 : 1) at 0–4 °C. The solution was stirred at 0–4 °C for 30 min and then at room temperature for 1 h. Ether (15 ml) and water (5 ml) were added, and the heterogeneous mixture was then acidified to pH 1 using 2 M HCl. The aqueous layer was separated and extracted with ethyl acetate (2 × 15 ml). The combined organic layers were washed with water (15 ml) and brine (10 ml), then dried (Na_2SO_4) and concentrated *in vacuo* to leave the crude carboxylic acid.

The tripeptide **9** (312 mg, 750 µmol) was acidolysed by stirring it in a 20% TFA–dichloromethane solution at 0–4 °C for 4 hours. Work up, as previously described, gave the crude TFA salt as a gum. The salt was redissolved in DMF (5 ml), then Hünig's base (425 µl, 2.50 mmol) was added at 0–4 °C and the solution was stirred for 10 min. The crude carboxylic acid was added, followed by 1-hydroxybenzotriazole (135 mg, 1.00 mmol) and finally DCC (206 mg, 1.00 mmol). The mixture was stirred at 0 °C for 18 hours and then at room temperature for 6 hours, before being worked up as described for the tetrapeptide. The crude product was purified by flash chromatography on silica gel (methanol–ethyl acetate 1 : 39 eluant) to give the *heptapeptide* **8** (475 mg, 534 µmol, 71% based on the tripeptide) as a pale yellow foam: δ_H(500 MHz, DMSO, 100 °C) 8.32 (1H, s), 8.08 (1H, s), 8.02 (1H, d, *J* 8.1 Hz), 7.87 (1H, d, *J* 8.1 Hz), 7.67 (1H, br s), 7.28 (8H, m), 7.23 (2H, m), 5.09 (3H, m), 4.49 (2H, br s), 4.34 (3H, m), 4.00 (1H, app t, *J* 6.1 Hz), 3.74 (1H, br s), 3.60 (1H, app t, *J* 4.1 Hz), 3.50 (2H, m), 3.37 (1H, dd, *J* 14.4, 5.0 Hz), 3.17 (2H, m), 3.04 (1H, dd, *J* 12.2, 7.2 Hz), 2.36 (2H, m), 2.10–1.85 (4H, m), 1.36 (9H, s), 1.18 (3H, d, *J* 6.3 Hz), 1.00 (3H, d, *J* 6.4 Hz), 0.96 (3H, d, *J* 4.5 Hz); δ_C(125 MHz, DMSO, 90 °C) 173.8 (s), 172.2 (s), 170.8 (s), 169.7 (s), 169.3 (s), 160.3 (s), 159.4 (s), 154.5 (s), 148.6 (s), 145.7 (s), 137.3 (s), 129.9 (d), 129.7 (d), 127.6 (d), 125.9 (d), 123.2 (d), 78.3 (s), 66.7 (d),

65.7 (d), 60.1 (t), 59.5 (d), 58.5 (d), 54.7 (t), 54.1 (d), 51.5 (d), 46.5 (d), 39.7 (q), 37.3 (t), 31.85 (d), 27.75 (q), 23.9 (t), 19.4 (q), 18.7 (q), 17.6 (q), 13.6 (q). *m/z* (ESMS) 912.3433 (M + Na); $C_{44}H_{55}N_7O_9NaS_2$ requires 912.3400.

The (21*R*,31*S*) diastereomer of **8** showed: δ_H(360 MHz, DMSO, 100 °C) 8.37 (1H, s), 8.18 (1H, d, *J* 8.15 Hz), 8.09 (1H, s), 7.91 (1H, d, *J* 8.1 Hz), 7.68 (1H, br s), 7.29 (8H, m), 7.24 (2H, m), 5.07 (3H, m), 4.56 (1H, br s), 4.52 (1H, m), 4.39 (1H, m), 3.96 (1H, m), 3.86 (3H, s), 3.73 (1H, br s), 3.51 (1H, m), 3.37 (1H, dd, *J* 13.9, 5.1 Hz), 3.21 (2H, m), 3.13 (1H, dd, *J* 13.9, 9.6 Hz), 2.34 (2H, m), 2.10–1.83 (4H, m), 1.35 (9H, s), 1.18 (3H, d, *J* 6.3 Hz), 0.98 (3H, d, *J* 6.3 Hz), 0.96 (3H, d, *J* 6.3 Hz). δ_C(90 MHz, DMSO, 90 °C) 174.5 (s), 173.3 (s), 171.6 (s), 170.6 (s), 161.6 (s), 155.3 (s), 149.3 (s), 146.1 (s), 137.4 (s), 129.8 (d), 129.7 (d), 128.7 (d), 128.5 (d), 126.8 (d), 126.7 (d), 124.1 (d), 79.1 (s), 67.7 (d), 60.2 (t), 59.1 (d), 57.1 (q), 54.8 (t), 52.3 (d), 52.1 (d), 47.4 (d), 38.0 (t), 32.5 (d), 31.5 (d), 29.2 (t), 28.5 (q), 24.9 (t), 20.3 (q), 19.6 (q), 18.4 (q).

"Pre-lissoclinamide" 5, 7

Aqueous sodium hydroxide solution (1 M, 400 µl, 400 µmol) was added in one portion to the heptapeptide **8** (140 mg, 158 µmol) dissolved in THF–methanol (5 ml, 1:1) and the mixture was stirred for 90 min. It was then concentrated to a small volume (removing the THF and methanol), and the residue was diluted with water (5 ml), acidified to pH 1 using 2 M HCl and extracted with ethyl acetate (3 × 10 ml). The combined extracts were washed with water (10 ml) and brine (8 ml), dried ($MgSO_4$) and concentrated *in vacuo* to leave the crude carboxylic acid (131 mg, 95% mass recovery) as a yellow foam. The acid was dissolved in a 50% solution of TFA in dichloromethane (4 ml) and stirred at 25 °C for 2 h. The solvents were removed *in vacuo* using the toluene addition procedure described for **8** to leave a gummy residue, which was dissolved in DMF (30 ml). *N*-Methylmorpholine (70 µl, 500 µmol) was added, followed by DPPA (44 µl, 200 µmol) and the resulting solution was stirred at 0–4 °C for 2 h, and then allowed to stand at room temperature for 2 days. Work up as for the heptapeptide, followed by flash chromatography on silica gel (methanol–ethyl acetate 1:39 eluant) gave the macrolactam (42 mg, 55.5 µmol, 35%) as a white foam: $[a]_D$ +26 ($c = 0.16$, $CHCl_3$). ν_{max} ($CHCl_3$)/cm^{-1} 3382, 2927, 1675, 1631, 1546, 1493, 1463. δ_H (360 MHz, $CDCl_3$) 0.86 (3H, d, *J* 6.7 Hz, CH_3)$_2$), 1.05 (3H, d, *J* 6.7 Hz, (CH_3)$_2$), 1.25 (3H, d, *J* 6.8 Hz, CH_3CHOH), 1.78–1.98 (1H, m, CH_2CH$_2$N), 1.99–2.13 (2H, m, CH_2CH$_2$N, CH_2CH), 2.18–2.31 (1H, m, CH_2CH), 2.45–2.51 (1H, m, (CH$_3$)$_2$C*H*), 3.06–3.14 (1H, m, CH_2N), 3.16–3.23 (3H, m, C*H*H′Ph′, CH_2Ph), 3.46–3.51 (1H, m, CH*H*′Ph′), 3.63–3.69 (1H, m, CH_2N), 4.06–4.14 (3H, m, C*H*OH, OCHC*H*, CH$_2$C*H*), 4.41 (1H, br s, O*H*), 4.92 (1H, app t, *J* 8.0 Hz, (CH$_3$)$_2$CHC*H*), 5.14 (1H, ddd, *J* 6.2, 6.2, 9.5 Hz, C*H*CH$_2$Ph), 5.62–5.68 (1H, m, C*H*CH$_2$Ph), 6.68 (1H, d, *J* 8.0 Hz, OCHCHN*H*), 7.16–7.37 (10H, m, Ar*H*), 7.91 (1H, s, SC*H*), 8.06 (1H, s, SC*H*), 8.17 (1H, d, *J* 7.6 Hz, (CH$_3$)$_2$-CHCHN*H*), 8.24 (1H, d, *J* 9.5 Hz, NCOCH(CH$_2$)N*H*), 8.59 (1H, d, *J* 8.3 Hz, SCCHN*H*). δ_C (90.5 MHz, $CDCl_3$) 19.1 (q), 19.5 (q), 19.6 (q), 25.5 (t), 28.7 (t), 33.3 (d), 39.1 (t), 42.0 (t), 47.9 (t), 52.2 (d), 52.6 (d), 58.8 (d), 60.6 (d), 62.8 (d), 67.1 (d), 123.6 (d), 124.3 (d), 127.1 (d), 127.3 (d), 128.5 (d), 128.8 (d), 129.6 (d), 129.7 (d), 135.3 (s), 136.2 (s), 148.2 (s), 148.5 (s), 160.4 (s), 160.5 (s), 168.5 (s), 169.5 (s), 169.6 (s), 171.4 (s); *m/z* (FAB) 758.2798 (MH^+); $C_{38}H_{44}N_7O_6S$ requires 758.2795.

The (21*R*,31*S*) diastereomer of **7** showed: δ_H(400 MHz, $CDCl_3$, 25 °C) 9.44 (1H, d, *J* 5.6 Hz), 8.61 (1H, d, *J* 7.6 Hz), 9.16 (1H, d, *J* 9.1 Hz), 7.82 (1H, s), 7.65 (1H, s), 7.345 (4H, app dt, *J* 16.45, 7.4 Hz), 7.24 (4H, m), 6.82 (2H, br s), 5.57 (1H, br s), 5.48 (1H, ddd, *J* 5.6, 4.7, 4.2 Hz), 5.065 (1H, dd, *J* 11.2, 9.1 Hz), 4.80 (1H, t, *J* 6.5 Hz), 4.51 (1H, ddd, *J* 9.6, 7.6, 4.8 Hz), 4.17 (1H, dd, *J* 13.8, 4.15 Hz), 4.08 (1H, t, *J* 7.8 Hz), 3.75 (1H, t, *J* 6.7 Hz), 3.05 (1H, dd, *J* 13.1, 4.8 Hz), 2.85 (1H, dd, *J* 13.1, 9.6 Hz), 2.75 (1H, m), 2.69 (1H, m), 2.53 (1H, dd, *J* 13.8, 11.3 Hz), 2.10 (1H, m), 2.04 (1H, m), 1.93 (1H, m), 1.78 (1H, m), 1.63 (1H, m), 1.34 (3H, d, *J* 6.3 Hz), 1.17 (3H, d, *J* 6.6 Hz), 0.71 (3H, d, *J* 6.3 Hz). δ_C(100 MHz, $CDCl_3$, 25 °C) 170.0 (s), 168.5 (s), 167.8 (s), 165.4 (s), 158.5 (s), 157.0 (s), 147.9 (s), 146.1 (s), 134.3 (s), 134.1 (s), 128.1 (d), 128.1 (d), 127.1 (d), 127.0 (d), 125.8 (d), 125.4 (d), 121.9 (d), 120.8 (d), 65.5 (d), 60.6 (d), 54.2 (d), 51.7 (d), 51.2 (d), 45.0 (t), 40.6 (t), 39.0 (t), 29.7 (d), 27.5 (t), 23.2 (t), 18.7 (q), 18.6 (q), 18.5 (q). *m/z* (FAB) 758.2815 (MH^+); $C_{38}H_{44}N_7O_6S_2$ requires 758.2795.

Lissoclinamide 5, 1, and revised structure 15

Thionyl chloride (500 µl) was added dropwise to a stirred solution of the macrocyclic heptapeptide **7** (34 mg, 45 µmol) in dichloromethane (5 ml) at 0–4 °C, and the mixture was stirred at 0–4 °C for 30 minutes and then left to stand at 3–5 °C for 36 h. The mixture was carefully added to a rapidly stirred potassium carbonate solution (10%), and then extracted with ethyl acetate (3 × 15 ml). The combined extracts were washed with water (10 ml) and brine (10 ml), dried (Na_2SO_4) and then concentrated *in vacuo* to leave the crude product. Purification by flash chromatography on silica gel (methanol–ethyl acetate 1:99 eluant) gave the "lissoclinamide 5" **1** (23.5 mg, 31.8 µmol, 71%) as a white foam. $[a]_D^{25}$ +64 ($c = 0.54$, $CHCl_3$). ν_{max} ($CHCl_3$)/cm^{-1} 3380, 2927, 1667. δ_H (360 MHz, $CDCl_3$) 0.94 (3H, d, *J* 6.7 Hz, (CH_3)$_2$), 1.09 (3H, d, *J* 6.6 Hz, (CH_3)$_2$), 1.48 (3H, d, *J* 6.3 Hz, CH_3CHO), 1.61–2.07 (3H, m, C*HH*′CH$_2$N, CH_2N), 2.14–2.22 (1H, m, CH_2CHN), 2.72–2.82 (1H, m, (CH$_3$)$_2$C*H*), 2.83–2.90 (1H, m, CH_2N), 3.00 (1H, dd, *J* 3.1, 13.6 Hz, CH_2Ph), 3.04 (1H, dd, *J* 4.6, 13.5 Hz, CH_2Ph), 3.27 (1H, dd, *J* 7.9, 13.5 Hz, CH_2Ph), 3.54–3.58 (1H, m, CH_2N), 3.73 (1H, dd, *J* 7.0, 13.5 Hz, CH_2Ph), 4.19 (1H, d, *J* 5.8 Hz, CHC*H*NC), 4.45 (1H, app t, *J* 7.6 Hz, CH$_2$C*H*N), 4.79–4.86 (1H, m, OC*H*), 4.90 (1H, app t, *J* 8.4 Hz, (CH$_3$)$_2$CH-C*H*), 4.99–5.05 (1H, m, OCC*H*CH$_2$Ph), 5.33–5.39 (1H, app q, *J* 7.4 Hz, SCC*H*CH$_2$Ph), 7.13–7.35 (10H, m, Ar*H*), 7.43 (1H, d, *J* 7.8 Hz, (CH$_3$)$_2$CHCHN*H*), 7.92 (1H, s, SC*H*), 8.04 (1H, s, SC*H*), 8.14 (1H, d, *J* 8.8 Hz, OCCH(CH$_2$Ph)N*H*), 8.47 (1H, d, *J* 7.5 Hz, SCCH(CH$_2$Ph)N*H*). δ_C (90.5 MHz, $CDCl_3$) 19.4 (q), 19.9 (q), 21.6 (q), 25.3 (t), 29.1 (t), 31.4 (d), 39.5 (t), 41.7 (t), 47.2 (t), 51.9 (d), 54.6 (d), 56.3 (d), 58.5 (d), 75.3 (d), 80.6 (d), 123.4 (d), 124.4 (d), 127.1 (d), 127.1 (d), 128.3 (d), 128.6 (d), 129.6 (d), 129.8 (d), 135.6 (s), 136.6 (s), 148.2 (s), 148.8 (s), 159.8 (s), 161.5 (s), 169.0 (s), 169.2 (s), 170.0 (s), 171.2 (s). *m/z* (FAB) 740.2690 (MH^+); $C_{38}H_{42}N_7O_5S_2$ requires 740.2689.

Data for revised stereostructure **15**: $[a]_D^{25}$ −35 ($c = 0.19$, $CHCl_3$). ν_{max} ($CHCl_3$)/cm^{-1} 3697, 2977, 2929, 2872, 1682, 1636, 1602, 1110. δ_H (500 MHz, $CDCl_3$) 0.80 (3H, d, *J* 6.6 Hz, (CH_3)$_2$), 1.09 (3H, d, *J* 6.6 Hz, (CH_3)$_2$), 1.47 (3H, d, *J* 6.3 Hz, CH_3CHO), 1.73–1.77 (2H, m, CH_2CH$_2$N), 1.86–1.92 (1H, m, C*H*H′CHN), 2.08–2.15 (2H, m, C*H*H′N, CH*H*′CHN), 2.77 (1H, dd, *J* 10.0, 13.2 Hz, CH_2Ph), 2.77–2.83 (1H, m, (CH$_3$)$_2$-C*H*), 2.91 (1H, dd, *J* 10.2, 12.5 Hz, CH_2Ph), 3.27 (1H, dd, *J* 4.5, 12.4 Hz, CH_2Ph), 3.27 (1H, obs. m, CH*H*′N), 3.90 (1H, dd, *J* 4.1, 13.2 Hz, CH_2Ph), 4.31 (1H, d, *J* 3.9 Hz, OCHC*H*), 4.58 (1H, app t, *J* 7.8 Hz, CH$_2$C*H*N), 4.86–4.91 (2H, m, CH$_3$C*H*O, OCC*H*CH$_2$Ph), 5.20 (1H, app t, *J* 10.3 Hz, (CH$_3$)CHC*H*), 5.43–5.47 (1H, m, SCC*H*CH$_2$Ph), 7.23–7.35 (10H, m, Ar*H*), 7.91 (1H, s, SC*H*), 7.93 (1H, d, *J* 9.9 Hz, (CH$_3$)CHCHN*H*), 8.08 (1H, s, SC*H*), 8.72 (1H, d, *J* 7.2 Hz, OCCH(CH$_2$Ph)N*H*), 9.24 (1H, d, *J* 5.8 Hz, SCCH(CH$_2$Ph)N*H*). δ_C (90.5 MHz, $CDCl_3$) 19.9 (q), 20.3 (q), 25.1 (t), 28.8 (t), 32.9 (d), 40.8 (t), 42.8 (t), 47.1 (t), 53.9 (d), 54.5 (d), 55.1 (d), 56.6 (d), 75.2 (d), 82.6 (d), 123.0 (d), 123.1 (d), 127.1 (d), 127.3 (d), 128.6 (d), 128.6 (d), 129.7 (d), 129.9 (d), 136.0 (d), 136.2 (d), 167.6 (s), 168.6 (s), 171.0 (s), 171.4 (s). *m/z* (FAB) 740.2690 (MH^+); $C_{38}H_{42}N_7O_5S_2$ requires 740.2689.

Boc-(*S*)-Ser-(*R*)-Phe-Thz-OMe, 21

Trifluoroacetic acid (5 ml) was added in one portion to a solution of the thiazole **20** (1.8 g, 5.0 mmol) in dichloromethane (20 ml). The resulting solution was stirred at 25 °C for 2 h, then diluted with toluene (5 ml) and concentrated to low volume. A further 5 ml of toluene was added, and the solution was then re-concentrated to leave the crude amine hydrotrifluoroacetate as a gum. The gum was redissolved in dichloromethane (30 ml) and the solution was then cooled to 4 °C and Hünig's base (2.55 ml, 15.0 mmol) was added in one portion. After 15 min Boc-(*S*)-serine (1.2 g, 6.00 mmol) was added, followed by 1-hydroxybenzotriazole (811 mg, 6.00 mmol) and finally DCC (1.341 g, 6.50 mmol). The mixture was stirred at 0–5 °C for 2 h, then at room temperature for 1 h and filtered (to remove precipitated DCU). The filtrate was washed with 10% citric acid solution (10 ml), 10% $NaHCO_3$ (10 ml), water (10 ml) and finally brine (10 ml), then dried ($MgSO_4$) and concentrated to leave the crude tripeptide. Purification by flash chromatography on silica gel (1:1 ethyl acetate–light petroleum eluant) gave the *tripeptide* (1.6 g, 3.55 mmol, 71%) as a white foam: δ_H(500 MHz, DMSO, 90 °C) 8.37 (1H, s), 8.33 (1H, d, *J* 8.1 Hz), 7.25 (4H, m), 7.21 (1H, m), 6.16 (1H, br s), 5.41 (1H, ddd, *J* 8.95, 8.1, 5.5 Hz), 4.43 (1H, br s), 4.04 (1H, m), 3.88 (3H, s), 3.52 (2H, m), 3.39 (1H, dd, *J* 14.05, 5.5 Hz), 3.24 (1H, dd, *J* 14.05, 8.95 Hz), 1.42 (9H, s). δ_C(125 MHz, DMSO, 90 °C) 172.5 (s), 170.1 (s), 160.9 (s), 154.7 (s), 145.3 (s), 137.0 (s), 128.8 (d), 128.3 (d), 127.8 (d), 126.0 (d), 78.1 (s), 61.5 (t), 56.8 (d), 52.1 (d), 51.4 (q), 39.7 (t), 27.8 (q). *m/z* (ESMS) 472.1490 (M + Na); $C_{21}H_{27}N_3O_6NaS$ requires 472.1518.

Boc-(*S*)-Val-Ψ(CS-NH)-(*S*)-Ser-(*R*)-Phe-Thz-OMe, 23

Trifluoroacetic acid (5 ml) was added in one portion to a solution of the tripeptide **21** (1.35 g, 3.00 mmol) in dichloromethane (20 ml). The resulting solution was stirred at 4 °C for 3 h then diluted with toluene (5 ml) and concentrated, leaving the crude TFA salt as a gum. The gum was redissolved in ethyl acetate (30 ml) and 10% $NaHCO_3$ (20 ml) and the two layers were then separated. The aqueous phase was extracted with more ethyl acetate (3 × 20 ml) and the combined organic phases were then washed with half-saturated brine. The combined aqueous phases were extracted continuously with 200 ml dichloromethane for 12 h, and the extract was combined with the ethyl acetate layers, then dried (Na_2SO_4) and concentrated *in vacuo* to leave the crude amino alcohol. The residue was redissolved in anhydrous DMF (10 ml) and the solution was cooled to 0–4 °C. A solution of the (*S*)-thioamidating reagent **22** (1.05 g, 3.00 mmol)[23] in DMF (10 ml) was introduced by careful dropwise addition to the stirred solution over 5–10 minutes. The resulting mixture was stirred at 4 °C for 1 h and then at 25 °C for 4 h, and finally diluted with ethyl acetate (100 ml). The organic extract was washed with water (4 × 20 ml) and brine (20 ml), then dried ($MgSO_4$) and concentrated *in vacuo* to leave the crude product. Purification by flash chromatography on silica gel (ethyl acetate–methanol, 98:2) gave the *tetrapeptide* (1.24 g, 2.2 mmol, 73%) as a white foam: δ_H(500 MHz, DMSO, 90 °C) 9.46 (1H, d, *J* 7.2 Hz), 8.53 (1H, d, *J* 8.1 Hz), 8.36 (1H, s), 7.26 (4H, m), 7.12 (1H, m), 6.27 (1H, d, *J* 8.4 Hz), 5.42 (1H, ddd, *J* 9.05, 8.1, 5.55 Hz), 5.075 (1H, dd, *J* 12.9, 5.7 Hz), 4.285 (1H, dd, *J* 8.8, 6.5 Hz), 3.88 (3H, s), 3.68 (1H, dd, *J* 10.9, 6.0 Hz), 3.63 (1H, dd, *J* 10.9, 5.2 Hz), 3.43 (1H, dd, *J* 14.1, 5.55 Hz), 3.22 (1H, dd, *J* 14.1, 9.05 Hz), 2.15 (1H, dqq, *J* 5.7, 6.8, 6.8 Hz), 1.405 (9H, s), 0.91 (3H, d, *J* 6.8 Hz), 0.87 (3H, d, *J* 6.8 Hz). δ_C(125 MHz, DMSO, 90 °C) 203.9 (s), 172.2 (s), 168.05 (s), 160.8 (s), 154.7 (s), 145.3 (s), 137.0 (s), 128.8 (d), 128.3 (d), 127.7 (d), 126.0 (d), 78.2 (s), 65.8 (d), 60.6 (t), 60.4, 52.3 (d), 51.3 (q), 39.7 (t), 32.4 (d), 27.8 (q), 19.0 (q), 17.1 (q). *m/z* (ESMS) 587.1960 (M^+ + Na); $C_{26}H_{36}N_4O_6NaS_2$ requires 587.1974.

Boc-aThr-(*S*)-Val-Ψ(CS-NH)-(*S*)-Ser-(*R*)-Phe-Thz-OMe, 18

Trifluoroacetic acid (2 ml) was added in one portion to a solution of the tetrapeptide **23** (565 mg, 1.00 mmol) in dichloromethane (8 ml). The resulting solution was stirred at 4 °C for 3 h then diluted with toluene (5 ml) and concentrated *in vacuo* to leave the crude TFA salt as a gum. The gum was dissolved in DMF (6 ml) and the solution was then cooled to 4 °C. Hünig's base (510 μmol) was added, followed by Boc-allothreonine (*ca.* 1.0 mmol of crude material from Boc-protection step), HOBt (142 mg, 1.05 mmol) and finally DCC (227 mg, 1.10 mmol). The mixture was stirred at 0–4 °C for 90 min and then at 25 °C for 24 h, after which the solution was diluted with ethyl acetate (40 ml) and water (20 ml). The separated aqueous layer was extracted with more ethyl acetate (20 ml), and the combined organic layers were washed with water (4 × 30 ml) and brine (10 ml), then dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (methanol–dichloromethane 1:19 eluant) to give the *pentapeptide* (507 mg, 761 μmol, 76%) as an off-white foam. δ_H(500 MHz, DMSO, 90 °C) 9.55 (1H, d, *J* 6.9 Hz), 8.37 (1H, d, *J* 8.1 Hz), 8.35 (1H, s), 7.58 (1H, d, *J* 8.4 Hz), 7.26 (4H, m), 7.21 (1H, m), 6.31 (1H, br s), 5.41 (1H, ddd, *J* 9.0, 8.1, 5.6 Hz), 5.05 (1H, dd, *J* 6.3, 6.3 Hz), 4.63 (1H, dd, *J* 8.4, 6.3 Hz), 3.97 (1H, dd, *J* 8.4, 6.3 Hz), 3.91 (1H, dq, *J* 6.3, 6.3 Hz), 3.88 (3H, s), 3.67 (1H, dd, *J* 11.0, 6.4 Hz), 3.64 (1H, dd, *J* 11.0, 5.2 Hz), 3.41 (1H, dd, *J* 14.1, 5.6 Hz), 3.23 (1H, dd, *J* 14.1, 9.0 Hz), 2.20 (1H, dqq, *J* 6.3, 6.8, 6.8 Hz), 1.43 (9H, s), 1.13 (3H, d, *J* 6.3 Hz), 0.93 (3H, d, *J* 6.8 Hz), 0.90 (3H, d, *J* 6.8 Hz). δ_C(125 MHz, DMSO, 90 °C) 203.4 (s), 172.2 (s), 170.0 (s), 167.9 (s), 160.8 (s), 154.9 (s), 145.3 (s), 137.0 (s), 128.8 (d), 128.3 (d), 127.7 (d), 126.0 (d), 78.2 (s), 66.6 (d), 63.3 (d), 60.7 (d), 60.5 (t), 60.2 (d), 52.3 (d), 51.3 (q), 39.7 (t), 32.6 (d), 27.9 (q), 19.2 (q), 19.0 (q), 17.1 (q). *m/z* (ESMS) 688.2474 (M^+ + Na); $C_{30}H_{43}N_5O_8NaS_2$ requires 688.2451.

Boc-Phe-Pro-aThr-(*S*)-Val-Ψ(CS-NH)-(*S*)-Ser-(*R*)-Phe-Thz-OMe, 24

The pentapeptide **18** (708 mg, 1.06 mmol) was converted into the corresponding TFA salt by treatment with 20% TFA in dichloromethane at 4 °C for 3 h, followed by dilution with toluene and concentration as described for previous steps. The crude TFA salt was dissolved in DMF (5 ml) at 4 °C and Hünig's base (510 μl, 3.00 mmol) was added, followed by Boc-(*S*)-Phe-Pro-OH (435 mg, 1.20 mmol), HOBt (169 mg, 1.25 mmol) and finally DCC (268 mg, 1.30 mmol). The mixture was stirred at 0–4 °C for 90 min and then 25 °C for 24 h. It was then diluted with ethyl acetate (40 ml) and water (20 ml), and the separated aqueous layer was extracted with more ethyl acetate (20 ml). The combined organic layers were washed with water (4 × 30 ml) and brine (10 ml), then dried ($MgSO_4$) and concentrated *in vacuo*. Purification by flash chromatography on silica gel (methanol–dichloromethane 1:14 eluant) gave the *heptapeptide* (643 mg, 719 μmol, 68%) as a pale yellow foam: δ_H(500 MHz, DMSO, 90 °C) 9.52 (1H, d, *J* 7.0 Hz), 8.37 (1H, d, *J* 7.8 Hz), 8.35 (1H, s), 7.68 (1H, br s), 7.56 (1H, d, *J* 8.2 Hz), 7.28 (4H, m), 7.22 (1H, m), 5.40 (1H, ddd, *J* 9.0, 8.1, 5.5 Hz), 5.05 (1H, app q, *J* 6.65 Hz), 4.63 (1H, dd, *J* 8.3, 6.3 Hz), 4.49 (2H, br s), 4.29 (1H, app t, *J* 7.1 Hz), 3.97 (1H, br s), 3.88 (3H, s), 3.66 (3H, m), 3.48 (1H, m), 3.43 (1 H, dd, *J* 14.1, 5.5 Hz), 3.23 (1H, dd, *J* 14.1, 9.0 Hz), 3.00 (1H, br s), 2.84 (1H, dd, *J* 13.9, 8.4 Hz), 2.24 (1H, m), 2.05–1.83 (4H, br m), 1.35 (9H, s), 1.16 (3H, d, *J* 6.3 Hz), 0.94 (3H, d, *J* 6.8 Hz), 0.91 (3H, d, *J* 6.8 Hz). δ_C(125 MHz, DMSO, 90 °C) 203.3 (s), 172.3 (s), 171.2 (s), 169.6 (s), 167.9 (s), 160.8 (s), 154.4 (s), 145.3 (s), 137.2 (s), 137.0 (s), 128.9 (d), 128.8 (d), 128.3 (d), 127.7 (d), 127.6 (d), 126.0 (d), 125.8 (d), 78.0 (s), 66.8 (d), 63.6 (d), 60.8 (d), 60.6 (t), 59.3 (d), 58.6 (d), 53.4 (d), 52.3 (d), 51.3 (q), 46.4 (t), 39.7 (t), 37.0 (t), 32.4 (d), 28.2 (t), 27.8 (q), 24.1, 19.4 (q), 19.9 (q), 17.1 (q). *m/z* (ESMS) 932.3682 (M^+ + Na); $C_{44}H_{59}N_7O_{10}NaS_2$ requires 932.3663.

Pre-lissoclinamide 4, 17

Aqueous sodium hydroxide solution (1 M, 400 μl, 400 μmol) was added in one portion to a solution of the heptapeptide **24** (132 mg, 147 μmol) in THF–methanol (5 ml, 1 : 1) and the mixture was stirred at 25 °C for 90 min and then concentrated to a small volume (removing the THF and methanol). The residue was diluted with water (5 ml), then acidified to pH 1 using 2 M HCl and extracted with ethyl acetate (3 × 10 ml). The combined extracts were washed with water (10 ml) and brine (10 ml), dried ($MgSO_4$) and concentrated *in vacuo* to leave the crude carboxylic acid **25** (129 mg, ~100% mass recovery) as a yellow foam. The acid was dissolved in a solution of TFA in dichloromethane (4 ml 20%) and then stirred at 0–4 °C for 4 h. The solvents were removed *in vacuo* to leave a gummy residue. The residue was dissolved in DMF (35 ml), and *N*-methylmorpholine (70 μl, 500 μmol) was then added followed by DPPA (44 μl, 200 μmol). The resulting mixture was stirred at 4 °C for 2 h, and then allowed to stand at room temperature for 2 days. Work up as described for the intermediate heptapeptide, followed by purification by flash chromatography on silica gel (methanol–dichloromethane 1 : 9 eluant) gave the *cyclopeptide diol* (34 mg, 44 μmol, 30%) as a white foam. NMR spectroscopy revealed a complex mixture of at least three rotamers, which could not be resolved to any useful extent. *m*/*z* (ESMS) 800.2854 (M^+ + Na); $C_{38}H_{47}N_7O_7NaS_2$ requires 800.2876.

Lissoclinamide 4, 16

The cyclopeptide diol **17** (21.0 mg, 27 μmol) was dissolved in THF (4 ml) and Burgess' reagent (15.0 mg, 63 μmol) was added in one portion. The resulting solution was heated under reflux in a nitrogen atmosphere for 20 minutes, then cooled to room temperature and diluted with ethyl acetate (10 ml) and water (6 ml). The separated aqueous layer was extracted with more ethyl acetate (2 × 8 ml) and the combined organic extracts were then washed with water (7 ml) and brine (10 ml), dried ($MgSO_4$) and concentrated to leave the crude product. Purification by flash chromatography on silica gel (methanol–ethyl acetate 1 : 39) gave lissoclinamide 4 (14.2 mg, 19.2 μmol, 71%) as a white foam: δ_H(400 MHz, $CDCl_3$, 25 °C); in agreement with published values. δ_C(100 MHz, $CDCl_3$, 25 °C) 173.6 (s), 171.5 (s), 170.65 (s), 170.5 (s), 169.6 (s), 168.0 (s), 159.8 (s), 148.4 (s), 136.1 (s), 136.0 (s), 130.0 (d), 128.7 (d), 127.4 (d), 127.3 (d), 123.3 (d), 82.5 (d), 80.1 (d), 77.5 (d), 75.2 (d), 56.8 (d), 55.6 (d), 54.4 (d), 54.3 (d), 47.1 (t), 42.5 (t), 40.5 (t), 34.0 (t), 33.4 (d), 28.6 (t), 25.4 (t), 21.95 (q), 20.2 (q), 19.5 (q). *m*/*z* (ESMS) 764.2641 (M^+ + Na); $C_{38}H_{43}N_7O_5NaS_2$ requires 764.2665.

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