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Total synthesis of the cytotoxic cyclopeptide mollamide, isolated from the sea squirt *Didemnum molle*

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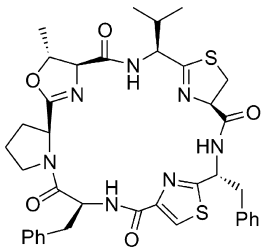
Abstract—Full details of a total synthesis of the novel reverse prenyl substituted cyclic peptide mollamide, isolated from the ascidian *Didemnum molle*, are described.

1. Introduction

The marine environment has been a hugely important source of structurally novel and biologically important natural products for more than thirty years now.[1] Among these metabolites are the fascinating family of heterocycle-containing cyclopeptides which have been isolated mainly from ascidians (sea squirts), with much current interest focusing on those isolated from the genus *Lissoclinum*.[2] The structures of these cyclopeptides are characterised by the presence of highly modified amino acid residues present in the form of thiazole, oxazole, thiazoline and oxazoline moieties, e.g. lissoclinamide 4 **1** and cyclodidemnamide **2**.[3] Mollamide **3** is a member of a rarer family of cyclopeptides where the structure has been modified by inclusion of a reverse prenyl unit associated as a serine ether residue. The cyclopeptide has been isolated from the ascidian *Didemnum molle*, and it is related to a group of about ten similar natural products, including the doubly prenylated compound trunkamide A **4**.[4,5] Like a number of these cyclopeptides, mollamide displays moderate cytotoxicity against a range of cell lines, with IC_{50} values of 1 μg/ml against P388 (murine leukaemia) and 2.5 μg/ml against A549 (human lung carcinoma), HT29 (human colon carcinoma) and CV1 (monkey kidney fibroblast) cells. Mollamide also inhibits RNA synthesis with an IC_{50} of approximately 1 μg/ml. Its unusual structure and interesting biological properties combined to make the compound a challenging synthetic target and in this paper we report the full details of our synthesis of mollamide **3**.[6]



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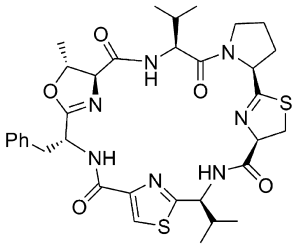

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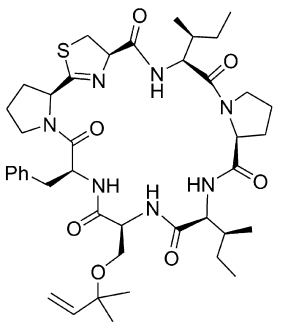

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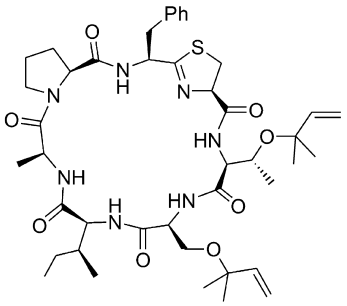

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2. Results and discussion

The structure of mollamide **3** was determined following extensive NMR and degradation studies and ultimately by X-ray crystallography. It includes a thiazoline ring, characteristic of other cyclopeptides, e.g. lissoclinamide 4 **1** and cyclodidemnamide **2**, whose synthesis we have described earlier.[7,8] With due cognisance to the ease with which thiazoline-based amino acids undergo racemisation during synthesis and handling, we decided to follow the successful synthetic strategy we developed for these related cyclopeptide structures and elaborate the thiazoline unit in

Keywords: cyclopeptide; *Didemnum molle*; ascidians.

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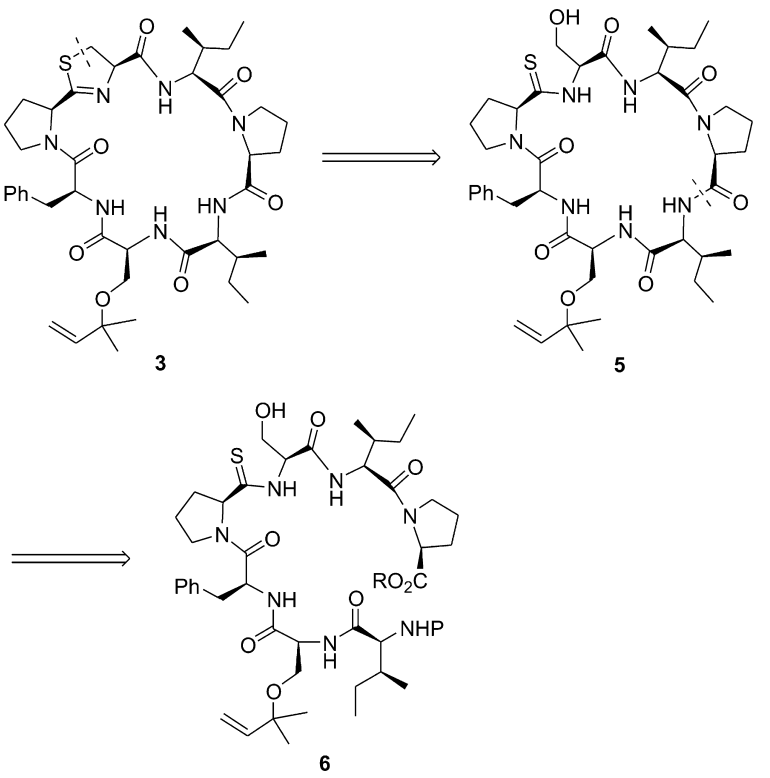
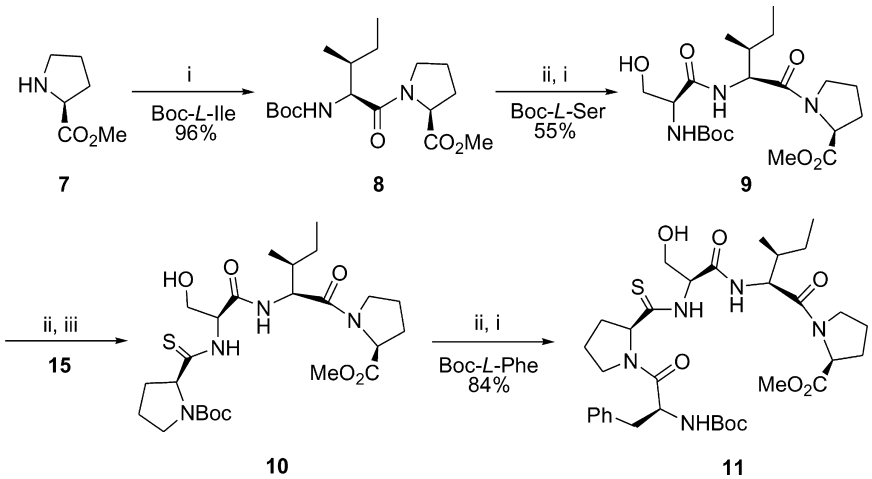


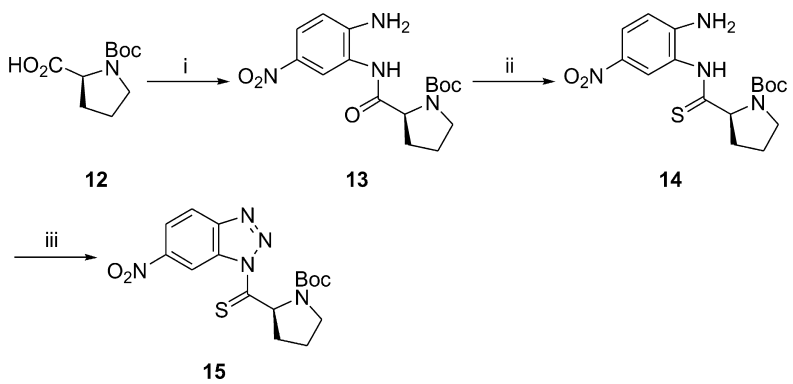
Scheme 1.

mollamide from the thioamide-based cyclopeptide **5** (Scheme 1).[9] This cyclopeptide, in turn, could be produced from the heptapeptide **6** by macrolactamisation involving the L-isoleucine/L-proline amide bond. We were encouraged to choose this particular amide bond for macrocyclisation due to the known resistance of carboxyl-activated proline residues to base-catalysed epimerisation during coupling reactions.

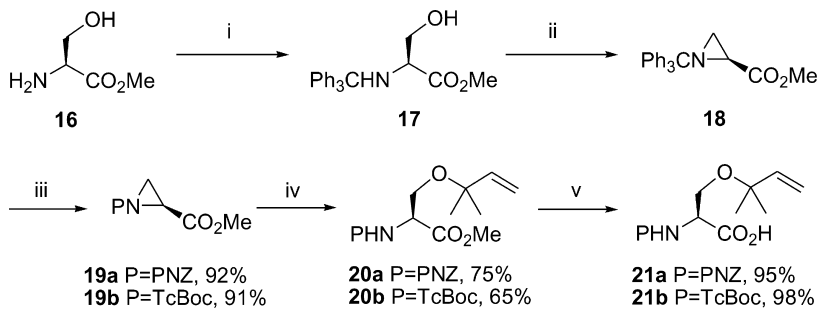
While alternative strategies were investigated, we ultimately decided to synthesise the heptapeptide **6** in a linear manner. The synthesis began with the construction of the pentapeptide **11**, which was prepared in seven steps starting from L-proline methyl ester **7** (Scheme 2). Hence, coupling of **7** with Boc-L-isoleucine first gave the dipeptide **8** in an excellent 96% yield. Removal of the Boc protecting group from **8** and a subsequent coupling with Boc-L-serine next gave the tripeptide **9**, which was then elaborated to the tetrapeptide **10** using the thioacylating agent **15**, prepared using the method described by Rapoport et al. (Scheme 3).[10] Hence, coupling of Boc-L-proline **12** with 4-nitro-1,2-phenylenediamine gave the anilide **13** in 81% yield which, on subsequent thionation led to the thioanilide **14**. Reaction of this compound with sodium nitrite under diazotisation conditions next gave **15**. After removal of the Boc group from the tripeptide **9**, reaction with **15** gave the tetrapeptide **10** and the synthesis of the pentapeptide **11** was then completed following removal of the Boc protection from **10** and a coupling with Boc-L-phenylalanine to give **11** in 84% yield.



Scheme 2. *Reagents*: (i) DCC, HOBt, DIPEA, DCM, 0°C→rt; (ii) AcCl, MeOH, rt; (iii) Et_3N, DCM, 0°C→rt, 74%.



Scheme 3. *Reagents*: (i) 4-nitro-1,2-phenylenediamine, NMM, ^{i}BuOCOCl, THF, −20°C→rt, 81%; (ii) P_4S_{10}, Na_2CO_3, THF, 0°C→rt, 95%; (iii) $NaNO_2$, AcOH, 0°C, 93%.



Scheme 4. *Reagents*: (i) Ph_3CCl, Et_3N, DCM, rt, 98%; (ii) MsCl, Et_3N, THF, 0°C→65°C, 98%; (iii) (a) TFA, MeOH, DCM, 0°C; (b) P-OSu, Et_3N, rt; (iv) 2-methyl-3-buten-2-ol, $BF_3{\cdot}OEt_2$, DCM, rt; (v) 1.5 M LiOH, THF/MeOH, rt.

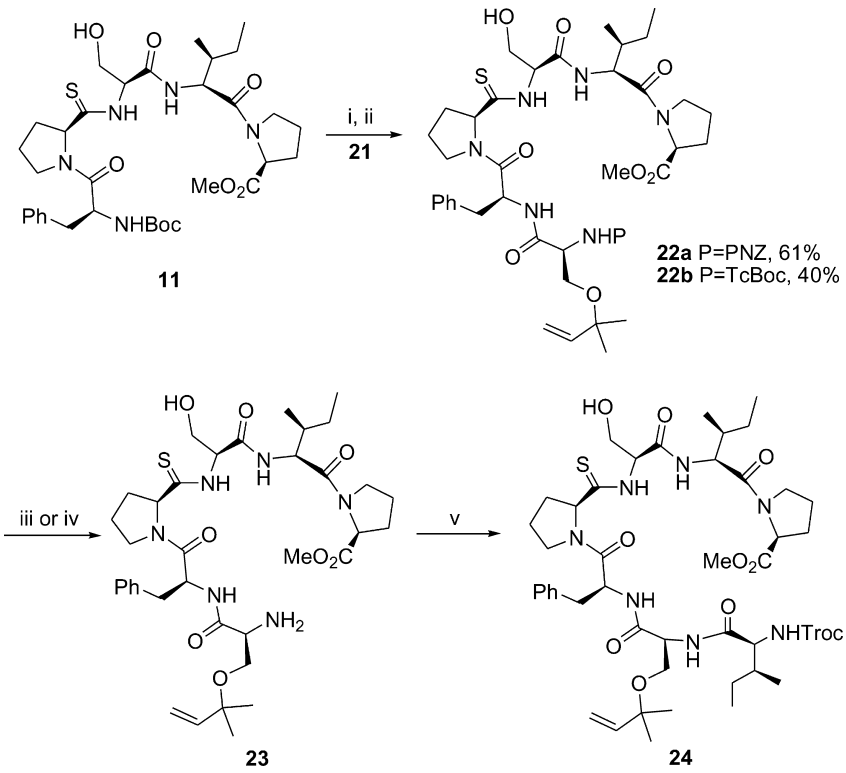
In order to synthesise the reverse prenylated amino acid **21**, we used a strategy based on extensive studies carried out earlier by Okawa et al.[11] These authors had developed a method for the synthesis of optically active β-alkoxy amino acids involving the Lewis acid-assisted ring opening of activated aziridines with alcohols. Thus, our synthesis of **21** began with formation of the chiral aziridine **18** in two steps from homochiral L-serine methyl ester **16** (Scheme 4). Selective protection of the amino group in **16** as the trityl derivative **17**, followed by treatment of **17** with methanesulfonyl chloride in THF using the procedure of Zwanenburg et al. first gave the aziridine **18** in essentially quantitative yield.[12]

In order to facilitate efficient aziridine ring opening in **18**, it was necessary to replace the trityl group with an electron withdrawing group and a protecting group had to be chosen which could be removed under conditions which would not affect the allyl ether group in any subsequent ring opened product. Having investigated a number of alternatives, we eventually identified two protecting groups, i.e. the 4-nitrobenzyloxycarbonyl (PNZ) and the 2,2,2-trichloro-*tert*-butyloxycarbonyl (TcBoc) carbamate groups, as being suitable for this purpose. The protecting group interconversion **18→19** was achieved using a one-pot strategy in order to avoid the isolation of the intermediate free aziridine, which proved problematic in our hands. Hence, following removal of the trityl protection from **18** using TFA, basification with an excess of triethylamine and in situ reprotection gave the aziridines **19a** and **19b** in 92 and 91%, respectively. The ring opening of these aziridines was then carried out by reaction with 2-methyl-3-buten-2-ol in the presence of boron trifluoride diethyl etherate, giving the amino acids **20a** and **20b** in acceptable yields. Saponification of the methyl ester in **20a** and **20b** finally led to the corresponding carboxylic acids **21a** and **21b**, respectively.

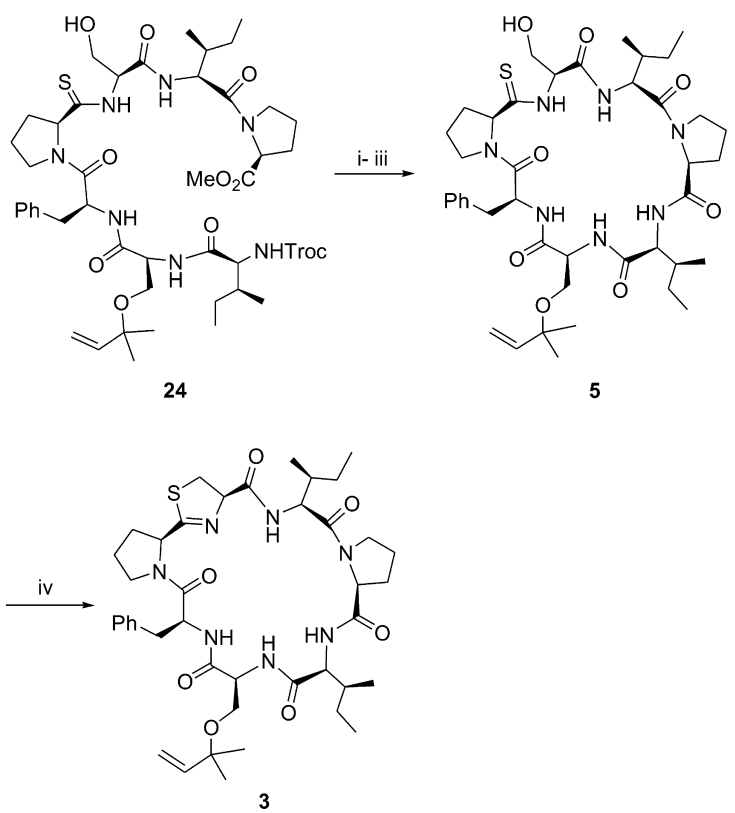
Removal of the Boc protection in the pentapeptide **11**, followed by reaction of the resulting amine with the carboxylic acids **21a** and **21b**, in the presence of 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide (EDC) and HOBt coupling reagents, smoothly gave the hexapeptides **22a** and **22b**, respectively, in modest yields (Scheme 5). Removal of the carbamate protecting group in **22** next gave the corresponding free amine **23**. At this stage in the synthesis the TcBoc group offered significant advantages. While the removal of the PNZ group in **22a** proceeded smoothly,[13] the subsequent purification proved problematic due to difficulties in separating the by-products of the reaction[†]. In contrast, removal of the TcBoc protection from **22b**, using the conditions described by Ciufolini et al. was clean and efficient and gave the amine product in an excellent 88% yield.[14]

The amine **23** was now coupled with Troc-protected isoleucine, to give the heptapeptide **24**. As with the PNZ and TcBoc protecting groups used earlier, the Troc group was chosen as protection for this residue due to the possibility of removing it under conditions which would

[†] In our initial studies we had made use of the PNZ protecting group, and the synthesis was continued with the crude amine.[6] Removal of the by-products was only achieved on completion of the synthesis of mollamide following preparative HPLC separation.



Scheme 5. *Reagents*: (i) AcCl, MeOH, rt; (ii) DIPEA, EDC, HOBt, DCM, 0°C→rt; (iii) Zn, pH 6 phosphate buffer/THF (3:1), rt; (iv) Cd/Pb, 1 M NH_4OAc/THF (1:1), rt, 88%; (v) Troc-L-Ile, EDC, HOBt, DCM, 0°C→rt, 78%.



Scheme 6. *Reagents*: (i) Cd/Pb, 1 M NH_4OAc/THF (1:1), rt, 96%; (ii) TBAH, THF, 0°C; (iii) DPPA, DIPEA, DMF, −5°C→rt 52% (2 steps); (iv) DAST, DCM, −15°C, 74%.

not simultaneously affect the allyl ether residue. Removal of the Troc group in **24**,[14] followed by saponification of the methyl ester group using tetrabutylammonium hydroxide (TBAH)[15] and macrocyclisation using diphenylphosphoryl azide (DPPA)[16] next gave the macrocycle **5** (Scheme 6). Finally, cyclodehydration of the β-hydroxy thioamide unit in **5** to the corresponding thiazoline, in the presence of diethylaminosulfur trifluoride (DAST)[17] completed the total synthesis of mollamide **3**. Comparison of the ^{1}H and ^{13}C NMR spectra as well as $[\alpha]_D$ measurement for our synthetic material, with those reported for natural mollamide isolated from *D. molle* showed the compounds to be identical.

3. Conclusion

In summary, we have achieved a synthesis of mollamide and simultaneously demonstrated a method for the construction of this important class of marine cyclopeptides. In addition, we have further illustrated a concise method for the incorporation of sensitive chiral thiazoline heterocycles into complex natural products.

4. Experimental

4.1. General details

Reactions were carried out under an atmosphere of nitrogen or argon, otherwise, where specified, in air. Reactions requiring anhydrous conditions were performed in flame-dried apparatus. Organic solvents were routinely dried and stored under a nitrogen atmosphere prior to use. Benzene, diethyl ether and toluene solvents were dried over sodium wire. Other organic solvents were dried by distillation as follows: THF (sodium benzophenone ketyl), dichloromethane (calcium hydride) and methanol (magnesium). Other organic solvents and reagents were purified according to accepted literature procedures. Organic extracts were dried over anhydrous magnesium or sodium sulphate and then filtered under gravity. Solvents were removed under reduced pressure on a Büchi rotary evaporator. All reactions were monitored by TLC using Merck silica gel 60 F_{254} pre-coated aluminium plates which were visualised with ultraviolet light and then developed with either basic potassium permanganate solution or ceric ammonium molybdate solution. Flash chromatography was performed using Merck silica gel 60 as the stationary phase. All melting points were determined on a Reichert Köfler micro hot-stage apparatus and are uncorrected. Specific rotations were measured on a JASCO DIPA-370 polarimeter. Solutions were prepared using spectroscopic grade solvents. Microanalytical data were obtained on a Perkin–Elmer 240B elemental analyser. Infrared spectra were recorded on a Perkin–Elmer 1600 series FT-IR instrument as dilute solutions using spectroscopic grade solvents.

^{1}H NMR spectra were recorded on either a Bruker DPX 360 (360 MHz), a Bruker AV 400 (400 MHz) or a Bruker DRX 500 (500 MHz) instrument as dilute solutions using deuterated solvents as specified. Chemical shifts are quoted in parts per million (ppm) on the δ scale and are recorded relative to tetramethylsilane (TMS) or residual non-deuterated solvent as internal standard. The following abbreviations are used for designation of multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; dt, double triplet; dq, double quartet; m, multiplet; app, apparent; br, broad. All coupling constants, *J*, are recorded in Hertz (Hz) and are uncorrected from peak printouts. ^{13}C NMR spectra were recorded on either a Bruker DPX 360 (90 MHz), a Bruker AV 400 (100 MHz) or a Bruker DRX 500 (125 MHz) as dilute solutions in deuterated solvents, as specified, on a broad band decoupled mode, and the multiplicities determined using a DEPT sequence. Chemical shifts are quoted in ppm on the δ scale and are recorded relative to TMS or residual non-deuterated solvent as internal standard. The following abbreviations are used to denote multiplicities: s, quaternary; d, tertiary methine; t, secondary methylene; q, primary methyl. Mass spectra were recorded either on a VG Autospec, MM-701CF, a VG Micromass 70E or a Micromass LCT spectrometer using fast atom bombardment (FAB) or electrospray (ES) techniques. Due to the soft nature of these techniques, little fragmentation of the compounds occurred and hence nominal mass and fragmentation data have not been included.

4.1.1. Boc-Ile-Pro-OMe (8).[18] Diisopropylethylamine (7.3 ml, 42 mmol) was added dropwise over 5 min to a stirred solution of L-proline methyl ester hydrochloride **7** (2.00 g, 12.1 mmol) in dichloromethane (20 ml) at 0°C under an atmosphere of nitrogen. The solution was stirred at 0°C for 20 min and then Boc-L-isoleucine (2.79 g, 12.1 mmol) in dichloromethane (10 ml) was added dropwise over 10 min by cannula, followed by 1-hydroxybenzotriazole (1.79 g, 13.3 mmol) in one portion. The suspension was stirred at 0°C for a further 15 min and then dicyclohexylcarbodiimide (2.74 g, 13.3 mmol) in dichloromethane (15 ml) was added dropwise over 10 min by cannula. The mixture was allowed to warm to room temperature over the course of 18 h, filtered, and then evaporated in vacuo. The residue was taken up in ethyl acetate (20 ml), filtered, and the filtrate was then washed with 10% aqueous citric acid solution (3×10 ml) followed by saturated aqueous sodium bicarbonate solution (5×10 ml), with backwashing. The combined organic extracts were dried and evaporated in vacuo to leave the crude product which was purified by chromatography on silica using 33% ethyl acetate in light petroleum (40–60°C) as eluent to give the *dipeptide* (3.97 g, 96%) as a pale yellow oil; $[\alpha]_D^{24}=-66$ (*c* 1.16, $CHCl_3$) (Lit.,[18] $[\alpha]_D^{25}=-87.2$ (*c* 0.22, MeOH)); Found %: C, 59.9; H, 8.9; N, 8.0; Calcd for $C_{17}H_{30}N_2O_5$ %: C, 59.6; H, 8.8; N, 8.2; ν_{max} ($CHCl_3$)/cm^{-1} 3437, 2966, 2934, 2878, 1744, 1704, 1644, 1495, 1455, 1392, 1368, 1318, 1156, 1090, 1045, 1022, 883; δ_H (500 MHz, $CDCl_3$) 5.14 (1H, d, *J*=9.4 Hz, N*H*Boc), 4.53 (1H, dd, *J*=8.6, 4.9 Hz, C*H*CO_2Me), 4.29 (1H, dd, *J*=9.3, 7.4 Hz, C*H*NHBoc), 3.84–3.80 (1H, m, CH_2N), 3.72 (3H, s, OCH_3), 3.68–3.64 (1H, m, CH_2N), 2.26–2.20 (1H, m, CH_2CHCO_2Me), 2.07–1.95 (3H, m, CH_2), 1.79–1.72 (1H, m, C*H*CH_3), 1.63–1.55 (1H, m, C$H_2$$CH_3$), 1.42 (9H, s, C(C$H_3$)$_3$), 1.18–1.09 (1H, m, C$H_2$$CH_3$), 1.01 (3H, d, *J*=6.8 Hz, CHCH_3), 0.91 (3H, app t, *J*=7.4 Hz, CH_2CH_3); δ_C (90 MHz, $CDCl_3$) 172.5 (s), 171.5 (s), 155.8 (s), 79.6 (s), 58.9 (d), 56.3 (d), 52.2 (q), 47.3 (t), 38.0 (d), 29.1 (t), 28.4 (q), 25.0 (t), 24.2 (t), 15.3 (q), 11.3 (q); *m/z* (FAB) 343.2233 ($[M+H]^+$, $C_{17}H_{31}N_2O_5$ requires 343.2233).

4.1.2. Boc-Ser-Ile-Pro-OMe (9). Acetyl chloride (15 ml) was added dropwise over 10 min to a stirred solution of the dipeptide **8** (6.17 g, 18.0 mmol) in methanol (150 ml) at 0°C under an atmosphere of nitrogen. The solution was warmed to room temperature, stirred for 6 h and then evaporated in vacuo to leave the corresponding hydrochloride salt (5.11 g) as a cream solid, which was used without further purification.

Diisopropylethylamine (11.2 ml, 64.2 mmol) was added dropwise over 10 min to a stirred solution of the hydrochloride salt (5.11 g, 18.3 mmol) in dichloromethane (100 ml) at 0°C under an atmosphere of nitrogen. The solution was stirred at 0°C for 15 min and then Boc-L-serine (3.76 g, 18.3 mmol) and 1-hydroxybenzotriazole (2.72 g, 20.2 mmol) were added successively in one portion. The suspension was stirred at 0°C for a further 15 min and then dicyclohexylcarbodiimide (4.16 g, 20.2 mmol) was added in one portion. The mixture was allowed to warm to room temperature over the course of 18 h, filtered, and then evaporated in vacuo. The residue was taken up in ethyl acetate (100 ml), filtered, and the filtrate was then washed with 10% aqueous citric acid solution (3×50 ml) followed by saturated aqueous sodium bicarbonate solution (3×50 ml), with backwashing. The combined organic extracts were dried and evaporated in vacuo to leave the crude product which was purified by chromatography on silica using 10% light petroleum (40–60°C) in ethyl acetate as eluent to give the *tripeptide* (4.23 g, 55%) as a colourless foam; $[\alpha]_D^{30}=-85$ (*c* 1.02, $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3432, 2968, 2935, 2879, 1743, 1704, 1644, 1489, 1455, 1393, 1368, 1319, 1158, 1097, 1056, 1001, 867; δ_H (360 MHz, $CDCl_3$) 6.94 (1H, d, *J*=8.1 Hz, N*H*), 5.48 (1H, d, *J*=6.6 Hz, N*H*Boc), 4.56 (1H, dd, *J*=8.4, 7.5 Hz, C*H*CH(CH_3)CH_2CH_3), 4.53 (1H, dd, *J*=8.6, 4.8 Hz, C*H*CO_2Me), 4.19 (1H, m, C*H*CH_2OH), 4.01–3.98 (1H, m, CH_2OH), 3.87–3.80 (1H, m, CH_2N), 3.72 (3H, s, OCH_3), 3.72–3.64 (1H, m, CH_2N), 3.59 (1H, dd, *J*=11.3, 6.1 Hz, CH_2OH), 2.29–2.20 (1H, m, CH_2CHCO_2Me), 2.10–1.94 (3H, m, CH_2), 1.91–1.84 (1H, m, C*H*CH_3), 1.63–1.53 (1H, m, CH_2CH_3), 1.45 (9H, s, C(CH_3)$_3$), 1.20–1.07 (1H, m, CH_2CH_3), 1.04 (3H, d, *J*=6.8 Hz, CHCH_3), 0.91 (3H, app t, *J*=7.4 Hz, CH_2CH_3); δ_C (90 MHz, $CDCl_3$) 172.3 (s), 171.5 (s), 171.0 (s), 155.8 (s), 80.1 (s), 63.0 (t), 59.0 (d), 55.5 (d), 55.0 (d), 52.2 (q), 47.4 (t), 37.2 (d), 29.0 (t), 28.3 (q), 25.0 (t), 24.3 (t), 15.2 (q), 11.1 (q); *m*/*z* (FAB) 430.2544 ($[M+H]^+$, $C_{20}H_{36}N_3O_7$ requires 430.2553).

4.1.3. Boc-Proψ{(C=S)NH}-Ser-Ile-Pro-OMe (10). Acetyl chloride (3 ml) was added dropwise over 5 min to a stirred solution of the tripeptide **9** (1.72 g, 4.02 mmol) in methanol (30 ml) at 0°C under an atmosphere of nitrogen. The solution was warmed to room temperature, stirred for 2.5 h and then evaporated in vacuo to leave the corresponding hydrochloride salt (1.54 g) as a cream solid, which was used without further purification.

Triethylamine (1.2 ml, 8.4 mmol) was added dropwise over 1 min to a stirred solution of the hydrochloride salt (1.54 g, 4.21 mmol) in dichloromethane (50 ml) at 0°C under an atmosphere of nitrogen. The solution was stirred at 0°C for 15 min and then a solution of the thioacylating agent **15** (1.59 g, 4.21 mmol) in dichloromethane (20 ml) was added dropwise over 15 min by cannula. The solution was allowed to warm to room temperature over the course of 23 h and then evaporated in vacuo. The crude product was purified by chromatography on silica using 20% light petroleum (40–60°C) in ethyl acetate as eluent to give the *tetrapeptide* (1.69 g, 74%) as a cream foam; $[\alpha]_D^{20}=-149$ (*c* 0.96, $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3325, 2967, 2879, 1743, 1694, 1635, 1497, 1456, 1368, 1321, 1157, 1127, 1100, 1055, 998, 972, 876; δ_H (360 MHz, d_6-DMSO, 333 K) 9.41 (1H, d, *J*=7.3 Hz, N*H*C=S), 7.76 (1H, d, *J*=8.4 Hz, N*H*), 5.01–4.98 (1H, m, C*H*CH_2OH), 4.76 (1H, app t, *J*=5.4 Hz, O*H*), 4.59 (1H, dd, *J*=8.6, 3.4 Hz, C*H*C=S), 4.46 (1H, app t, *J*=8.3 Hz, C*H*CH(CH_3)CH_2CH_3), 4.32 (1H, dd, *J*=8.5, 5.3 Hz, C*H*CO_2Me), 3.79–3.69 (3H, m, CH_2OH and CH_2N), 3.62 (3H, s, OCH_3), 3.60–3.54 (1H, m, CH_2N), 3.49–3.35 (2H, m, CH_2N), 2.25–2.13 (2H, m, CH_2CHCO_2-Me and CH_2CHC=S), 1.99–1.72 (7H, m, CH_2 and C*H*CH_3), 1.57–1.46 (1H, m, CH_2CH_3), 1.35 (9H, s, C(CH_3)$_3$), 1.17–1.04 (1H, m, CH_2CH_3), 0.90 (3H, d, *J*=6.8 Hz, CHCH_3), 0.84 (3H, app t, *J*=7.4 Hz, CH_2CH_3); δ_C (90 MHz, d_6-DMSO, 323 K) 204.9 (s), 171.8 (s), 169.5 (s), 168.1 (s), 153.2 (s), 78.6 (s), 66.6 (d), 60.4 (t), 59.6 (d), 58.3 (d), 54.3 (d), 51.4 (q), 46.8 (t), 46.6 (t), 36.2 (d), 33.2 (t), 28.4 (t), 27.8 (q), 24.3 (t), 23.7 (t), 22.7 (t), 14.7 (q), 10.6 (q); *m*/*z* (ES) 565.2697 ($[M+Na]^+$, $C_{25}H_{42}N_4O_7SNa$ requires 565.2672).

4.1.4. Boc-Phe-Proψ{(C=S)NH}-Ser-Ile-Pro-OMe (11). Acetyl chloride (2.5 ml) was added dropwise over 2 min to a stirred solution of the tetrapeptide **10** (1.58 g, 2.92 mmol) in methanol (25 ml) at 0°C under an atmosphere of nitrogen. The solution was warmed to room temperature, stirred for 5.5 h and then evaporated in vacuo to leave the corresponding hydrochloride salt (1.39 g) as a pale yellow foam, which was used without further purification.

Diisopropylethylamine (1.8 ml, 10 mmol) was added dropwise over 2 min to a stirred solution of the hydrochloride salt (1.39 g, 2.90 mmol) in dichloromethane (25 ml) at 0°C under an atmosphere of nitrogen. The solution was stirred at 0°C for 15 min and then Boc-L-phenylalanine (0.77 g, 2.9 mmol) and 1-hydroxybenzotriazole (0.43 g, 3.2 mmol) were added successively in one portion. The suspension was stirred at 0°C for a further 15 min and then dicyclohexylcarbodiimide (0.66 g, 3.2 mmol) was added in one portion. The mixture was allowed to warm to room temperature over the course of 45 h and then evaporated in vacuo to leave a residue which was taken up in ethyl acetate (25 ml) and filtered. The filtrate was washed with 10% aqueous citric acid solution (3×10 ml) followed by saturated aqueous sodium bicarbonate solution (5×10 ml), with backwashing. The combined organic extracts were dried and evaporated in vacuo to leave the crude product which was purified by chromatography on silica using ethyl acetate as eluent to give the *pentapeptide* (1.68 g, 84%) as a colourless foam; $[\alpha]_D^{23}=-110$ (*c* 1.04, $CHCl_3$); Found %: C, 58.7; H, 7.4; N, 10.0; $C_{34}H_{51}N_5O_8S$ requires %: C, 59.2; H, 7.45; N, 10.15; ν_{max} ($CHCl_3$)/cm^{-1} 3429, 3349, 2970, 2879, 1744, 1704, 1644, 1493, 1454, 1393, 1368, 1321, 1159, 1058, 1000, 868; δ_H (400 MHz, d_6-DMSO, 343 K) 9.58 (1H, d, *J*=7.1 Hz, N*H*C=S), 7.64 (1H, d, *J*=7.9 Hz, N*H*), 7.29–7.20 (5H, m, Ph-*H*), 6.62 (1H, br s, N*H*Boc), 4.96 (1H, m, C*H*CH_2OH), 4.89 (1H, m, C*H*C=S), 4.73 (1H, m, O*H*), 4.47–4.43 (1H,

masked m, C*H*CH$_2$Ph), 4.45 (1H, app t, *J*=8.1 Hz, C*H*CH(CH$_3$)CH$_2$CH$_3$), 4.32 (1H, dd, *J*=8.3, 5.2 Hz, C*H*CO$_2$Me), 3.77–3.73 (5H, m, C*H*$_2$OH and C*H*$_2$N), 3.62 (3H, s, OC*H*$_3$), 3.59–3.53 (1H, m, C*H*$_2$N), 3.13–3.02 (1H, masked m, C*H*$_2$Ph), 2.76–2.67 (1H, m, C*H*$_2$Ph), 2.19–2.07 (2H, m, C*H*$_2$CHCO$_2$Me and C*H*$_2$CHC=S), 1.97–1.78 (7H, m, C*H*$_2$ and C*H*CH$_3$), 1.56–1.50 (1H, m, C*H*$_2$CH$_3$), 1.29 (9H, s, C(C*H*$_3$)$_3$), 1.16–1.07 (1H, m, C*H*$_2$CH$_3$), 0.91 (3H, d, *J*=6.8 Hz, CHC*H*$_3$), 0.84 (3H, app t, *J*=7.4 Hz, CH$_2$C*H*$_3$); δ_C (100 MHz, d_6-DMSO, 333 K) 204.5 (s), 171.8 (s), 170.2 (s), 169.4 (s), 168.1 (s), 155.0 (s), 137.9 (s), 128.9 (d), 127.7 (d), 125.8 (d), 77.8 (s), 65.7 (d), 60.4 (t), 60.0 (d), 58.3 (d), 54.3 (d), 53.5 (d), 51.3 (q), 46.9 (t), 46.6 (t), 36.1 (d), 36.1 (t), 31.4 (t), 28.3 (t), 27.8 (q), 24.3 (t), 23.9 (t), 23.7 (t), 14.6 (q), 10.5 (q); *m*/*z* (ES) 712.3372 ([M+Na]$^+$, $C_{34}H_{51}N_5O_8SNa$ requires 712.3356).

4.1.5. Boc-proline 2-amino-5-nitroanilide (13). *N*-Methylmorpholine (5.1 ml, 46 mmol) was added dropwise over 5 min to a stirred solution of Boc-L-proline **12** (5.00 g, 23.2 mmol) in tetrahydrofuran (250 ml) at −20°C under an atmosphere of nitrogen. The solution was stirred at −20°C for 5 min, then isobutyl chloroformate (3.0 ml, 23 mmol) was added dropwise over 5 min and the mixture was stirred at −20°C for a further 10 min 4-nitro-1,2-phenylenediamine (3.56 g, 23.2 mmol) was added portionwise over 5 min and the mixture was stirred at −20°C for 2 h and then at room temperature for 44 h. The solvent was removed in vacuo to leave a residue which was taken up in ethyl acetate (250 ml) and filtered. The filtrate was washed successively with 10% aqueous citric acid solution (3×100 ml), saturated aqueous sodium bicarbonate solution (3×100 ml) and brine (2×50 ml), with backwashing. The combined organic extracts were dried and evaporated in vacuo to leave the crude product which was purified by recrystallisation with ethyl acetate/hexane to give the *anilide* (6.53 g, 81%) as a yellow solid; mp 200–202°C; $[\alpha]_D^{21}=-70$ (*c* 0.75, DMSO); Found %: C, 54.95; H, 6.5; N, 15.8; $C_{16}H_{22}N_4O_5$ requires %: C, 54.85; H, 6.3; N, 16.0; ν_{max} (DMSO)/cm^{-1} 3567, 3355, 3198, 1704, 1595, 1548, 1240, 1174, 829, 776, 752; δ_H (400 MHz, d_6-DMSO, 353 K) 9.22 (1H, br s, N*H*), 8.19 (1H, br s, Ar-*H*), 7.85 (1H, dd, *J*=9.0, 2.6 Hz, Ar-*H*), 6.81 (1H, d, *J*=9.0 Hz, Ar-*H*), 6.17 (2H, br s, N*H*$_2$), 4.30 (1H, dd, *J*=8.3, 4.3 Hz, C*H*), 3.49–3.36 (2H, m, C*H*$_2$N), 2.24–2.21 (1H, m, C*H*$_2$), 2.03–1.79 (3H, m, C*H*$_2$), 1.41 (9H, s, C(C*H*$_3$)$_3$); δ_C (100 MHz, d_6-DMSO, ~1:1 mixture of rotamers) 172.2 (s), 171.7 (s), 154.1 (s), 153.1 (s), 150.1 (s), 148.4 (s), 135.7 (s), 135.4 (s), 123.5 (d), 122.5 (d), 121.4 (s), 121.1 (s), 120.7 (d), 113.9 (d), 113.4 (d), 79.1 (s), 78.7 (s), 60.0 (d), 59.9 (d), 46.8 (t), 46.6 (t), 31.1 (t), 29.9 (t), 28.1 (q), 28.0 (q), 24.1 (t), 23.3 (t); *m*/*z* (ES) 373.1511 ([M+Na]$^+$, $C_{16}H_{22}N_4O_5Na$ requires 373.1488).

4.1.6. Boc-proline 2-amino-5-nitrothioanilide (14). Phosphorus pentasulfide (318 mg, 0.714 mmol) was added in one portion to a stirred suspension of sodium carbonate (76 mg, 0.71 mmol) in tetrahydrofuran (25 ml) at room temperature under an atmosphere of nitrogen and the mixture was stirred at room temperature for 1 h. The solution was cooled to 0°C, then the anilide **13** (500 mg, 1.43 mmol) was added in one portion and the mixture was stirred at 0°C for 30 min and then at room temperature for 2.5 h. The solvent was removed in vacuo to leave a residue which was taken up in 2:1 ethyl acetate/hexane (20 ml) and washed with 5% aqueous sodium bicarbonate solution (4×5 ml) and brine (2×10 ml), with backwashing. The combined organic extracts were dried and evaporated in vacuo to leave the crude product which was purified by chromatography on silica using 40% ethyl acetate in light petroleum (40–60°C) as eluent to give the *thioanilide* (495 mg, 95%) as a yellow solid; mp 170–173°C (ethyl acetate/hexane); $[\alpha]_D^{21}=-55$ (*c* 0.65, DMSO); Found %: C, 52.3; H, 6.0; N, 15.0; $C_{16}H_{22}N_4O_4S$ requires %: C, 52.4; H, 6.05; N, 15.3; ν_{max} (DMSO)/cm^{-1} 3535, 3185, 1695, 1519, 1169, 831, 753; δ_H (360 MHz, d_6-DMSO, 343 K) 10.94 (1H, br s, N*H*C=S), 7.95 (1H, dd, *J*=9.1, 2.6 Hz, Ar-*H*), 7.92 (1H, br s, Ar-*H*), 6.84 (1H, d, *J*=9.0 Hz, Ar-*H*), 6.16 (2H, br s, N*H*$_2$), 4.72 (1H, dd, *J*=8.5, 4.6 Hz, C*H*), 3.60–3.53 (1H, m, C*H*$_2$N), 3.48–3.42 (1H, m, C*H*$_2$N), 2.40–2.30 (1H, m, C*H*$_2$), 2.15–2.03 (2H, m, C*H*$_2$), 1.91–1.80 (1H, m, C*H*$_2$), 1.43 (9H, s, C(C*H*$_3$)$_3$); δ_C (125 MHz, d_6-DMSO, 343 K) 206.9 (s), 153.7 (s), 150.2 (s), 135.5 (s), 124.3 (d), 122.3 (s), 113.7 (d), 78.9 (s), 67.0 (d), 46.8 (t), 32.9 (t), 27.8 (q), 23.2 (t); *m*/*z* (ES) 267.0893 ([(M−$C_5H_8O_2$)+H]$^+$, $C_{11}H_{15}N_4O_2S$ requires 267.0916).

4.1.7. 1-(Boc-thionoprolinyl)-6-nitrobenzotriazole (15). Sodium nitrite (57 mg, 0.82 mmol) was added in one portion to a stirred solution of the thioanilide **14** (200 mg, 0.546 mmol) in glacial acetic acid (4 ml, diluted with 5% water) at 0°C. The solution was stirred at 0°C for 30 min and then ice water (50 ml) was added and the resulting suspension was extracted with ethyl acetate (10 ml then 3×5 ml). The combined organic extracts were dried and evaporated in vacuo to leave the *nitrobenzotriazole* (192 mg, 93%) as an orange gum; $[\alpha]_D^{21}=-15$ (*c* 1.00, CHCl$_3$); ν_{max} (CHCl$_3$)/cm^{-1} 2978, 2884, 1688, 1601, 1542, 1455, 1394, 1368, 1350, 1264, 1158, 1129, 1075, 1056, 983, 955, 908, 894, 875, 834; δ_H (360 MHz, CDCl$_3$, mixture of rotamers) major rotamer: 9.72 (1H, dd, *J*=2.1, 0.5 Hz, Ar-*H*), 8.44 (1H, dd, *J*=8.9, 2.1 Hz, Ar-*H*), 8.29 (1H, dd, *J*=8.9, 0.5 Hz, Ar-*H*), 6.21 (1H, dd, *J*=9.0, 3.2 Hz, C*H*), 3.78–3.60 (2H, m, C*H*$_2$N), 2.70–2.59 (1H, m, C*H*$_2$), 2.17–1.98 (3H, m, C*H*$_2$), 1.49 (9H, s, C(C*H*$_3$)$_3$); minor rotamer: 9.74 (1H, dd, *J*=2.1, 0.4 Hz, Ar-*H*), 8.48 (1H, dd, *J*=9.0, 2.1 Hz, Ar-*H*), 8.35 (1H, d, *J*=8.8 Hz, Ar-*H*), 6.24 (1H, dd, *J*=8.3, 3.1 Hz, C*H*), 3.78–3.60 (2H, m, C*H*$_2$N), 2.70–2.59 (1H, m, C*H*$_2$), 2.17–1.98 (3H, m, C*H*$_2$), 1.25 (9H, s, C(C*H*$_3$)$_3$); δ_C (100 MHz, CDCl$_3$, mixture of rotamers) 209.6 (s), 208.1 (s), 154.4 (s), 153.5 (s), 149.7 (s), 149.5 (s), 148.8 (s), 132.2 (s), 132.0 (s), 122.2 (d), 122.0 (d), 121.6 (d), 121.3 (d), 113.1 (d), 112.9 (d), 80.4 (s), 67.9 (d), 67.7 (d), 47.3 (t), 47.0 (t), 34.6 (t), 33.6 (t), 28.6 (q), 28.3 (q), 23.9 (t), 23.2 (t), which was used without further purification.

4.1.8. Tr-Ser-OMe (17).[19a] Triethylamine (8.9 ml, 64 mmol) was added dropwise over 10 min to a stirred suspension of serine methyl ester hydrochloride **16** (5.00 g, 32.1 mmol) in dichloromethane (100 ml) at room temperature under an atmosphere of nitrogen. On dissolution, the solution was cooled to 0°C and then triphenylmethyl chloride (8.96 g, 32.1 mmol) was added portionwise over 10 min. The suspension was warmed to room temperature, stirred for 21 h and then washed with 10% aqueous citric acid solution (3×20 ml) and water (2×20 ml), with backwashing. The combined organic extracts were dried and

evaporated in vacuo to leave *trityl serine methyl ester* (11.69 g) as a cream solid; δ_H (360 MHz, $CDCl_3$) 7.50–7.46 (6H, m, Ph-*H*), 7.29–7.16 (9H, m, Ph-*H*), 3.71 (1H, dd, *J*=10.2, 4.1 Hz, CH_2OH), 3.59–3.51 (2H, m, C*H*CO_2Me and CH_2OH), 3.29 (3H, s, OCH_3); δ_C (90 MHz, $CDCl_3$) 174.0 (s), 145.6 (s), 128.8 (d), 128.0 (d), 126.7 (d), 71.0 (s), 65.0 (t), 57.8 (d), 52.0 (q); *m/z* (ES) 384.1563 ($[M+Na]^+$, $C_{23}H_{23}NO_3Na$ requires 384.1576), which was used without further purification.

4.1.9. (2*S*)-Methyl 1-tritylaziridine-2-carboxylate (18).[12,19] Triethylamine (9.7 ml, 70 mmol) was added dropwise over 10 min to a stirred solution of trityl serine methyl ester **17** (11.48 g, 31.80 mmol) in tetrahydrofuran (85 ml) at 0°C under an atmosphere of nitrogen. Methanesulfonyl chloride (2.5 ml, 32 mmol) was added dropwise at 0°C over 2 min and the solution was stirred at 0°C for a further 30 min and then at reflux for 48 h. The solvent was removed in vacuo to leave a residue which was taken up in ethyl acetate (60 ml) and washed with 10% aqueous citric acid solution (3×20 ml) followed by saturated aqueous sodium bicarbonate solution (2×20 ml), with backwashing. The combined organic extracts were dried and evaporated in vacuo to leave the *aziridine* (10.60 g, 97%) as a cream solid; mp 116–118°C; δ_H (360 MHz, $CDCl_3$) 7.51–7.46 (6H, m, Ph-*H*), 7.29–7.18 (9H, m, Ph-*H*), 3.74 (3H, s, OCH_3), 2.25 (1H, dd, *J*=2.6, 1.6 Hz, CH_2), 1.89 (1H, dd, *J*=6.2, 2.7 Hz, C*H*), 1.41 (1H, dd, *J*=6.2, 1.6 Hz, CH_2); δ_C (90 MHz, $CDCl_3$) 172.0 (s), 143.7 (s), 129.4 (d), 127.7 (d), 127.0 (d), 74.5 (s), 52.2 (q), 31.8 (d), 28.8 (t); *m/z* (ES) 366.1460 ($[M+Na]^+$, $C_{23}H_{21}NO_2Na$ requires 366.1470), which was used without further purification.

4.1.10. 2(*S*)-Methyl 1-(4-nitrobenzyloxycarbonyl)aziridine-2-carboxylate (19a). Trifluoroacetic acid (0.45 ml, 5.8 mmol) was added dropwise over 1 min to a stirred solution of the aziridine **18** (1.00 g, 2.92 mmol) in dichloromethane (30 ml) and methanol (0.12 ml, 2.9 mmol) at 0°C under an atmosphere of nitrogen. The solution was stirred at 0°C for 30 min and then triethylamine (2.0 ml, 15 mmol) was added dropwise over 2 min. The mixture was stirred at 0°C for a further 5 min and then succinimidyl 4-nitrobenzyl carbonate[20] (0.86 g, 2.9 mmol) was added in one portion. The solution was warmed to room temperature, stirred for 24 h and then washed with 10% aqueous citric acid solution (3×10 ml) and water (2×10 ml), with backwashing. The combined organic extracts were dried and evaporated in vacuo to leave the crude product which was purified by chromatography on silica using 1% acetone in chloroform as eluent to give the *PNZ aziridine* (0.75 g, 92%) as a cream solid; mp 70–72°C (chloroform); $[\alpha]_D^{21}=-31$ (*c* 0.90, $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 2956, 1748, 1609, 1530, 1496, 1456, 1393, 1378, 1350, 1323, 1170, 1140, 1112, 1089, 1044, 981, 860; δ_H (500 MHz, $CDCl_3$) 8.20 (2H, d, *J*=8.8 Hz, Ar-*H*), 7.52 (2H, d, *J*=8.8 Hz, Ar-*H*), 5.25 (1H, d, *J*=13.3 Hz, Ar-CH_2), 5.20 (1H, d, *J*=13.3 Hz, Ar-CH_2), 3.74 (3H, s, OCH_3), 3.15 (1H, dd, *J*=5.2, 3.2 Hz, C*H*), 2.61 (1H, dd, *J*=3.2, 1.2 Hz, CH_2), 2.51 (1H, dd, *J*=5.3, 1.3 Hz, CH_2); δ_C (125 MHz, $CDCl_3$) 168.6 (s), 160.3 (s), 147.8 (s), 142.7 (s), 128.5 (d), 123.8 (d), 66.9 (t), 52.8 (q), 34.9 (d), 31.5 (t); *m/z* (FAB) 281.0770 ($[M+H]^+$, $C_{12}H_{13}N_2O_6$ requires 281.0774).

4.1.11. (2*S*)-Methyl 1-(2,2,2-trichloro-1,1-dimethylethoxycarbonyl)aziridine-2-carboxylate (19b). Trifluoroacetic acid (0.55 ml, 7.2 mmol) was added dropwise over 1 min to a stirred solution of the aziridine **18** (1.23 g, 3.58 mmol) in dichloromethane (20 ml) and methanol (0.15 ml, 3.6 mmol) at 0°C under an atmosphere of nitrogen. The solution was stirred at 0°C for 30 min and then triethylamine (2.5 ml, 18 mmol) was added dropwise over 2 min. The mixture was stirred at 0°C for a further 10 min and then succinimidyl 2,2,2-trichloro-1,1-dimethylethyl carbonate[20] (1.25 g, 3.94 mmol) in dichloromethane (5 ml) was added over 5 min by cannula. The solution was warmed to room temperature, stirred for 15 h and then washed with 10% aqueous citric acid solution (3×10 ml) and water (2×10 ml), with backwashing. The combined organic extracts were dried and evaporated in vacuo to leave the crude product which was purified by chromatography on silica using chloroform as eluent to give the *TcBoc aziridine* (0.90 g, 83%) as a pale yellow oil; $[\alpha]_D^{16}=-41$ (*c* 0.57, $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 2955, 1747, 1458, 1388, 1372, 1329, 1154, 1112, 1087, 1022, 971, 901, 876, 838; δ_H (400 MHz, $CDCl_3$) 3.78 (3H, s, OCH_3), 3.14 (1H, dd, *J*=5.1, 3.1 Hz, C*H*), 2.63 (1H, dd, *J*=3.2, 1.4 Hz, CH_2), 2.47 (1H, dd, *J*=5.1, 1.4 Hz, CH_2), 1.96 (3H, s, CH_3), 1.91 (3H, s, CH_3); δ_C (100 MHz, $CDCl_3$) 168.6 (s), 157.9 (s), 106.0 (s), 89.7 (s), 52.8 (q), 35.0 (d), 31.5 (t), 21.3 (q), 21.1 (q); *m/z* (FAB) 303.9934 ($[M+H]^+$, $C_9H_{13}NO_4Cl_3$ requires 303.9910).

4.1.12. PNZ-Ser(dimethylallyl)-OMe (20a). Boron trifluoride diethyl etherate (10 drops) was added to a stirred solution of the aziridine **19a** (502 mg, 1.79 mmol) in dichloromethane (5 ml) and 2-methyl-3-buten-2-ol (10 ml) at room temperature under an atmosphere of argon. The solution was stirred at room temperature for five days, then diluted with dichloromethane (20 ml) and washed with water (3×10 ml), with backwashing. The combined organic extracts were dried and evaporated in vacuo to leave the crude product which was purified by chromatography on silica using 50% diethyl ether in light petroleum (40–60°C) as eluent to give the *allyl ether* (492 mg, 75%) as a pale yellow oil; $[\alpha]_D^{23}=+13$ (*c* 1.79, $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3437, 2979, 2954, 2882, 1732, 1609, 1556, 1495, 1457, 1379, 1349, 1146, 1077, 1016, 985, 895, 862, 842; δ_H (500 MHz, $CDCl_3$) 8.23 (2H, d, *J*=8.7 Hz, Ar-*H*), 7.54 (2H, d, *J*=8.6 Hz, Ar-*H*), 5.72 (1H, dd, *J*=17.9, 10.5 Hz, C*H*–CH_2), 5.71–5.69 (1H, masked d, N*H*), 5.25 (1H, d, *J*=13.5 Hz, Ar-CH_2), 5.21 (1H, d, *J*=13.4 Hz, Ar-CH_2), 5.13–5.09 (2H, m, CH=CH_2), 4.44 (1H, app dt, *J*=8.9, 3.0 Hz, C*H*CO_2Me), 3.77 (3H, s, OCH_3), 3.77–3.75 (1H, masked dd, CH_2), 3.55 (1H, dd, *J*=9.3, 3.2 Hz, CH_2), 1.23 (3H, s, CH_3), 1.22 (3H, s, CH_3); δ_C (125 MHz, $CDCl_3$) 171.0 (s), 155.7 (s), 147.7 (s), 143.9 (s), 143.0 (d), 128.1 (d), 123.8 (d), 114.5 (t), 75.7 (s), 65.5 (t), 62.7 (t), 54.7 (d), 52.5 (q), 25.6 (q), 25.5 (q); *m/z* (FAB) 367.1512 ($[M+H]^+$, $C_{17}H_{23}N_2O_7$ requires 367.1505).

4.1.13. TcBoc-Ser(dimethylallyl)-OMe (20b). Boron trifluoride diethyl etherate (1.4 ml, 11 mmol) was added dropwise over 2 min to a stirred solution of the aziridine **19b** (1.63 g, 5.35 mmol) in 2-methyl-3-buten-2-ol (25 ml) at 0°C under an atmosphere of nitrogen. The solution was warmed to room temperature, stirred for 16 h and then

diluted with dichloromethane (25 ml) and washed with water (3×10 ml) and brine (2×10 ml), with backwashing. The combined organic extracts were dried and evaporated in vacuo to leave the crude product which was purified by chromatography on silica using 10% ethyl acetate in light petroleum (40–60°C) as eluent to give the *allyl ether* (1.36 g, 65%) as a pale yellow oil; $[\alpha]_D^{24}=+12$ (*c* 1.74, $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3436, 2979, 2954, 2882, 1728, 1602, 1492, 1458, 1386, 1351, 1302, 1146, 1097, 1065, 1001, 984, 902; δ_H (360 MHz, C_6D_6, 333 K) 5.63 (1H, masked br s, N*H*), 5.60 (1H, dd, *J*=17.6, 10.8 Hz, C*H*=CH_2), 4.92 (1H, dd, *J*=17.6, 1.1 Hz, CH=C*H*$_2$), 4.90 (1H, dd, *J*=10.8, 1.1 Hz, CH=C*H*$_2$), 4.45 (1H, m, C*H*CO_2Me), 3.58 (1H, dd, *J*=9.3, 3.4 Hz, C*H*$_2$), 3.42 (1H, dd, *J*=9.3, 3.6 Hz, C*H*$_2$), 3.33 (3H, s, OC*H*$_3$), 1.91 (3H, s, $Cl_3CC(CH_3)_2$), 1.90 (3H, s, $Cl_3CC(CH_3)_2$), 1.02 (6H, s, C*H*$_3$); δ_C (100 MHz, C_6D_6, 333 K) 170.5 (s), 154.1 (s), 143.4 (d), 114.0 (t), 107.1 (s), 88.8 (s), 75.6 (s), 63.0 (t), 55.1 (d), 51.7 (q), 25.6 (q), 25.5 (q), 21.8 (q); *m/z* (ES) 412.0443 ($[M+Na]^+$, $C_{14}H_{22}NO_5Cl_3Na$ requires 412.0461).

4.1.14. PNZ-Ser(dimethylallyl)-Phe-Proψ{(C=S)NH}-Ser-Ile-Pro-OMe (22a). An aqueous solution of lithium hydroxide (1.5 M, 0.55 ml, 0.82 mmol) was added dropwise over 1 min to a stirred solution of the allyl ether **20a** (60 mg, 0.16 mmol) in tetrahydrofuran (2 ml) and methanol (0.2 ml) at 0°C. The solution was warmed to room temperature, stirred for 1.5 h and then evaporated in vacuo. The residue was taken up in ethyl acetate (3 ml) and water (5 ml), then cooled to 0°C and acidified with 2 M aqueous hydrochloric acid solution with vigorous stirring. After stirring at 0°C for 10 min the layers were separated and the aqueous layer was then extracted with ethyl acetate (5×2 ml). The combined organic extracts were dried and evaporated in vacuo to leave the *carboxylic acid* (58 mg) as a pale yellow oil, which was used without further purification.

Acetyl chloride (0.1 ml) was added dropwise over 1 min to a stirred solution of the pentapeptide **11** (100 mg, 0.145 mmol) in methanol (1 ml) at 0°C under an atmosphere of nitrogen. The solution was warmed to room temperature, stirred for 5.5 h and then evaporated in vacuo to leave the corresponding hydrochloride salt (88 mg, 97%) as a cream solid, which was used without further purification.

Diisopropylethylamine (86 μl, 0.49 mmol) was added dropwise over 1 min to a stirred solution of the hydrochloride salt (88 mg, 0.14 mmol) in dichloromethane (2 ml) at 0°C under an atmosphere of nitrogen. The solution was stirred at 0°C for 15 min and then the carboxylic acid **21a** (58 mg, 0.16 mmol) in dichloromethane (2 ml) was added dropwise over 2 min by cannula, followed by 1-hydroxybenzotriazole (23 mg, 0.16 mmol) in one portion. The suspension was stirred at 0°C for a further 15 min and then 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (32 mg, 0.16 mmol) was added in one portion. The mixture was allowed to warm to room temperature over the course of 24 h and then evaporated in vacuo. The residue was taken up in ethyl acetate (5 ml) and washed with 10% aqueous citric acid solution (3×2 ml) followed by saturated aqueous sodium bicarbonate solution (5×2 ml), with backwashing. The combined organic extracts were dried and evaporated in vacuo to leave the crude product which was purified by chromatography on silica using 10% acetone in ethyl acetate as eluent to give the *hexapeptide* (79 mg, 61%) as a cream foam; $[\alpha]_D^{19}=-72$ (*c* 1.13, $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3411, 3338, 2956, 2933, 2879, 1738, 1643, 1558, 1491, 1454, 1349, 1320, 1144, 1077, 1000, 871; δ_H (360 MHz, d_6-DMSO, 333 K) 9.59 (1H, d, *J*=7.1 Hz, N*H*C=S), 8.20 (2H, d, *J*=8.5 Hz, Ar-*H*), 7.94 (1H, d, *J*=7.9 Hz, N*H*CHCH$_2$Ph), 7.69 (1H, d, *J*=8.5 Hz, N*H*CHCH(CH$_3$)CH$_2$CH$_3$), 7.61 (2H, d, *J*=8.3 Hz, Ar-*H*), 7.27–7.15 (5H, m, Ph-*H*), 7.06 (1H, br s, N*H*PNZ), 5.78 (1H, dd, *J*=17.5, 10.8 Hz, C*H*=CH_2), 5.17 (2H, br s, C*H*$_2$Ar), 5.14–5.09 (1H, m, CH=C*H*$_2$), 5.07 (1H, dd, *J*=10.9, 1.3 Hz, CH=C*H*$_2$), 4.99–4.97 (1H, m, C*H*CH$_2$OH), 4.86–4.77 (3H, m, C*H*CH$_2$Ph, C*H*C=S and O*H*), 4.45 (1H, app t, *J*=8.3 Hz, C*H*CH(CH$_3$)CH$_2$CH$_3$), 4.31 (1H, dd, *J*=8.4, 5.1 Hz, C*H*CO$_2$Me), 4.10 (1H, app dt, *J*=8.4, 5.9 Hz, C*H*CH$_2$OAllyl), 3.82–3.51 (6H, m, C*H*$_2$OH and C*H*$_2$N), 3.62 (3H, s, OC*H*$_3$), 3.37–3.30 (2H, m, C*H*$_2$OAllyl), 3.19–3.12 (1H, masked m, C*H*$_2$Ph), 2.81–2.74 (1H, m, C*H*$_2$Ph), 2.21–1.79 (9H, m, C*H*$_2$ and C*H*CH$_3$), 1.53–1.45 (1H, m, C*H*$_2$CH$_3$), 1.21–1.08 (1H, masked m, C*H*$_2$CH$_3$), 1.17 (6H, s, C*H*$_3$), 0.91 (3H, d, *J*=6.8 Hz, CHC*H*$_3$), 0.83 (3H, app t, *J*=7.4 Hz, CH$_2$C*H*$_3$); δ_C (100 MHz, d_6-DMSO, 323 K) 204.3 (s), 171.8 (s), 169.5 (s), 169.3 (s), 168.9 (s), 168.1 (s), 155.1 (s), 146.8 (s), 144.7 (s), 143.5 (d), 137.4 (s), 129.0 (d), 127.8 (d), 127.8 (d), 125.9 (d), 123.2 (d), 113.5 (t), 74.8 (s), 65.7 (d), 64.2 (t), 62.4 (t), 60.4 (t), 60.0 (d), 58.3 (d), 55.3 (d), 54.4 (d), 51.6 (d), 51.4 (q), 47.1 (t), 46.6 (t), 36.6 (t), 36.2 (d), 31.6 (t), 28.4 (t), 25.3 (q), 25.2 (q), 24.3 (t), 23.7 (t), 14.7 (q), 10.6 (q); *m/z* (ES) 946.3989 ($[M+Na]^+$, $C_{45}H_{61}N_7O_{12}SNa$ requires 946.3997).

4.1.15. TcBoc-Ser(dimethylallyl)-Phe-Proψ{(C=S)NH}-Ser-Ile-Pro-OMe (22b). An aqueous solution of lithium hydroxide (1.5 M, 2.5 ml, 3.8 mmol) was added dropwise over 2 min to a stirred solution of the allyl ether **20b** (148 mg, 0.379 mmol) in tetrahydrofuran (4 ml) and methanol (0.4 ml) at 0°C. The solution was warmed to room temperature, stirred for 45 min and then evaporated in vacuo. The residue was taken up in ethyl acetate (5 ml) and water (5 ml), then cooled to 0°C and acidified with 2 M aqueous hydrochloric acid solution with vigorous stirring. After stirring at 0°C for 5 min the layers were separated and the aqueous layer was then extracted with ethyl acetate (8×3 ml). The combined organic extracts were dried and evaporated in vacuo to leave the *carboxylic acid* (140 mg, 98%) as a pale yellow oil, which was used without further purification.

Acetyl chloride (0.2 ml) was added dropwise over 1 min to a stirred solution of the pentapeptide **11** (270 mg, 0.392 mmol) in methanol (2 ml) at 0°C under an atmosphere of nitrogen. The solution was warmed to room temperature, stirred for 6 h and then evaporated in vacuo to leave the corresponding hydrochloride salt (245 mg) as a cream solid, which was used without further purification.

Diisopropylethylamine (215 μl, 1.23 mmol) was added dropwise over 1 min to a stirred solution of the hydrochloride salt (245 mg, 0.392 mmol) in dichloromethane (5 ml) at 0°C under an atmosphere of nitrogen. The solution was stirred at 0°C for 15 min and then the carboxylic acid

21b (133 mg, 0.353 mmol) in dichloromethane (5 ml) was added dropwise over 5 min by cannula, followed by 1-hydroxybenzotriazole (52 mg, 0.39 mmol) in one portion. The suspension was stirred at 0°C for a further 15 min and then 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (74 mg, 0.39 mmol) was added in one portion. The mixture was allowed to warm to room temperature over the course of 33 h and then evaporated in vacuo. The residue was taken up in ethyl acetate (10 ml) and washed with 10% aqueous citric acid solution (3×3 ml) followed by saturated aqueous sodium bicarbonate solution (5×4 ml), with backwashing. The combined organic extracts were dried and evaporated in vacuo to leave the crude product which was purified by chromatography on silica using 20% light petroleum (40–60°C) in ethyl acetate as eluent to give the *hexapeptide* (148 mg, 40%) as a cream foam; $[\alpha]_D^{19}=-73$ (*c* 0.98, $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3414, 3336, 2974, 2879, 1729, 1643, 1489, 1455, 1386, 1364, 1322, 1147, 1077, 1001, 872, 840; δ_H (360 MHz, d_6-DMSO, 333 K) 9.61 (1H, d, *J*=7.3 Hz, N*H*C═S), 7.94 (1H, br s, N*H*CHCH$_2$Ph), 7.69 (1H, d, *J*=8.4 Hz, N*H*CHCH(CH$_3$)CH$_2$CH$_3$), 7.28–7.18 (5H, m, Ph-*H*), 6.76 (1H, br s, N*H*TcBoc), 5.79 (1H, dd, *J*=17.7, 10.8 Hz, C*H*═CH$_2$), 5.15–5.09 (1H, m, CH═C*H*$_2$), 5.08 (1H, dd, *J*=10.8, 1.3 Hz, CH═C*H*$_2$), 4.98–4.94 (1H, m, C*H*CH$_2$-OH), 4.90–4.72 (3H, m, C*H*CH$_2$Ph, C*H*C═S and O*H*), 4.45 (1H, app t, *J*=8.2 Hz, C*H*CH(CH$_3$)CH$_2$CH$_3$), 4.31 (1H, dd, *J*=8.3, 5.0 Hz, C*H*CO$_2$Me), 4.04 (1H, app dt, *J*=8.3, 6.2 Hz, C*H*CH$_2$OAllyl), 3.82–3.53 (6H, m, C*H*$_2$OH and C*H*$_2$N), 3.62 (3H, s, OC*H*$_3$), 3.33–3.32 (2H, m, C*H*$_2$-OAllyl), 3.13 (1H, masked m, C*H*$_2$Ph), 2.77 (1H, dd, *J*=14.4, 9.1 Hz, C*H*$_2$Ph), 2.20–1.83 (9H, m, C*H*$_2$ and C*H*CH$_3$), 1.83 (6H, s, Cl$_3$CC(C*H*$_3$)$_2$), 1.56–1.46 (1H, m, C*H*$_2$CH$_3$), 1.24–1.04 (1H, masked m, C*H*$_2$CH$_3$), 1.17 (6H, s, C*H*$_3$), 0.91 (3H, d, *J*=6.8 Hz, CHC*H*$_3$), 0.84 (3H, app t, *J*=7.4 Hz, CH$_2$C*H*$_3$); δ_C (90 MHz, d_6-DMSO, 323 K) 204.2 (s), 171.8 (s), 169.5 (s), 169.3 (s), 168.6 (s), 168.1 (s), 153.2 (s), 143.4 (d), 137.4 (s), 129.0 (d), 127.8 (d), 126.0 (d), 113.5 (t), 106.3 (s), 86.9 (s), 74.8 (s), 65.6 (d), 62.4 (t), 60.4 (t), 60.1 (d), 58.3 (d), 55.1 (d), 54.4 (d), 51.5 (d), 51.4 (q), 47.1 (t), 46.6 (t), 36.7 (t), 36.2 (d), 31.6 (t), 28.4 (t), 25.3 (q), 25.2 (q), 24.3 (t), 23.7 (t), 21.3 (q), 21.2 (q), 14.7 (q), 10.6 (q); *m/z* (ES) 969.3168 ([M+Na]$^+$, $C_{42}H_{61}N_6O_{10}SCl_3Na$ requires 969.3133).

4.1.16. Troc-Ile-Ser(dimethylallyl)-Phe-Proψ-{(C═S)NH}-Ser-Ile-Pro-OMe (24). Cadmium–lead couple[14] (1.17 g, 9.42 mmol cadmium, 10% lead) was added in one portion to a rapidly stirred solution of the hexapeptide **22b** (179 mg, 0.189 mmol) in tetrahydrofuran (2 ml) and 1N aqueous ammonium acetate solution (2 ml) at room temperature. The mixture was stirred vigorously at room temperature for 50 min and then filtered, washing with water (5×1 ml) and ethyl acetate (5×1 ml). The filtrate was cooled to 0°C and then basified with saturated aqueous sodium bicarbonate solution with vigorous stirring. After stirring at 0°C for 10 min the layers were separated and the aqueous layer was extracted with ethyl acetate (6×3 ml). The combined organic extracts were dried and evaporated in vacuo to leave the *amine* (124 mg, 88%) as a cream foam, which was used without further purification.

Troc-L-isoleucine (69 mg, 0.23 mmol) in dichloromethane (2 ml) was added dropwise over 2 min by cannula to a stirred solution of the amine **23** (124 mg, 0.167 mmol) in dichloromethane (1 ml) at 0°C under an atmosphere of nitrogen. 1-Hydroxybenzotriazole (25 mg, 0.18 mmol) was then added in one portion. The suspension was stirred at 0°C for 10 min and then 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (35 mg, 0.18 mmol) was added in one portion. The mixture was allowed to warm to room temperature over the course of 53 h and then evaporated in vacuo. The residue was taken up in ethyl acetate (10 ml) and washed with 10% aqueous citric acid solution (3×3 ml) followed by saturated aqueous sodium bicarbonate solution (5×3 ml), with backwashing. The combined organic extracts were dried and evaporated in vacuo to leave the crude product which was purified by chromatography on silica using 5% acetone in ethyl acetate as eluent to give the *heptapeptide* (135 mg, 78%) as a cream foam; $[\alpha]_D^{24}=-70$ (*c* 1.03, $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3415, 2966, 2933, 2879, 1741, 1644, 1556, 1492, 1454, 1364, 1322, 1141, 1079, 1000, 908, 836; δ_H (400 MHz, d_6-DMSO) 9.75 (1H, d, *J*=7.4 Hz, N*H*C═S), 8.21 (1H, d, *J*=8.5 Hz, N*H*CHCH$_2$-Ph), 7.92 (1H, d, *J*=8.4 Hz, N*H*CHCH(CH$_3$)CH$_2$CH$_3$), 7.83 (1H, d, *J*=9.0 Hz, N*H*Troc), 7.74 (1H, d, *J*=8.1 Hz, N*H*CHCH$_2$OAllyl), 7.27–7.14 (5H, m, Ph-*H*), 5.74 (1H, dd, *J*=17.6, 10.8 Hz, C*H*═CH$_2$), 5.10 (1H, dd, *J*=17.6, 1.3 Hz, CH═C*H*$_2$), 5.07 (1H, dd, *J*=10.8, 1.3 Hz, CH═C*H*$_2$), 4.96 (1H, app t, *J*=5.4 Hz, O*H*), 4.95–4.90 (1H, masked m, C*H*CH$_2$OH), 4.88–4.73 (2H, m, C*H*CH$_2$Ph and C*H*C═S), 4.83 (1H, d, *J*=12.4 Hz, C*H*$_2$CCl$_3$), 4.76 (1H, d, *J*=12.4 Hz, C*H*$_2$CCl$_3$), 4.41 (1H, app t, *J*=8.4 Hz, C*H*CH(CH$_3$)CH$_2$CH$_3$), 4.38 (1H, app dt, *J*=8.0, 5.8 Hz, C*H*CH$_2$OAllyl), 4.27 (1H, dd, *J*=8.4, 5.4 Hz, C*H*CO$_2$Me), 3.91 (1H, app t, *J*=8.5 Hz, C*H*NHTroc), 3.79–3.64 (5H, m, C*H*$_2$OH and C*H*$_2$N), 3.61 (3H, s, OC*H*$_3$), 3.61–3.52 (1H, m, C*H*$_2$N), 3.32–3.25 (2H, m, C*H*$_2$OAllyl), 3.11 (1H, dd, *J*=14.3, 4.1 Hz, C*H*$_2$Ph), 2.74 (1H, dd, *J*=14.4, 9.4 Hz, C*H*$_2$Ph), 2.20–2.01 (2H, m, C*H*$_2$CHCO$_2$Me and C*H*$_2$-CHC═S), 1.96–1.72 (8H, m, C*H*$_2$ and C*H*CH$_3$), 1.56–1.35 (2H, m, C*H*$_2$CH$_3$), 1.14 (3H, s, C*H*$_3$), 1.14 (3H, s, C*H*$_3$), 1.14–1.03 (2H, m, C*H*$_2$CH$_3$), 0.88 (3H, d, *J*=6.7 Hz, CHC*H*$_3$), 0.81 (3H, app t, *J*=7.4 Hz, CH$_2$C*H*$_3$), 0.78 (3H, app t, *J*=7.4 Hz, CH$_2$C*H*$_3$), 0.77 (3H, d, *J*=6.8 Hz, CHC*H*$_3$); δ_C (100 MHz, d_6-DMSO) 204.4 (s), 172.1 (s), 170.4 (s), 169.7 (s), 169.3 (s), 169.0 (s), 168.2 (s), 154.3 (s), 143.7 (d), 137.8 (s), 129.0 (d), 128.0 (d), 126.2 (d), 113.9 (t), 96.2 (s), 74.9 (s), 73.4 (t), 65.7 (d), 62.6 (t), 60.6 (t), 60.3 (d), 59.5 (d), 58.5 (d), 54.5 (d), 52.7 (d), 51.7 (d), 51.7 (q), 47.2 (t), 46.8 (t), 36.6 (t), 36.2 (d), 36.1 (d), 31.8 (t), 28.6 (t), 25.5 (q), 25.3 (q), 24.6 (t), 24.2 (t), 23.9 (t), 15.2 (q), 14.8 (q), 10.8 (q), 10.7 (q); *m/z* (ES) 1054.3730 ([M+Na]$^+$, $C_{46}H_{68}N_7O_{11}SCl_3Na$ requires 1054.3661).

4.1.17. Cyclo[Ile-Ser(dimethylallyl)-Phe-Proψ-{(C═S)NH}-Ser-Ile-Pro] (5). Cadmium–lead couple[14] (807 mg, 6.52 mmol cadmium, 10% lead) was added in one portion to a rapidly stirred solution of the heptapeptide **24** (135 mg, 0.131 mmol) in tetrahydrofuran (1.5 ml) and 1N aqueous ammonium acetate solution (1.5 ml) at room temperature. The mixture was stirred vigorously at room temperature for 40 min and then filtered, washing with water (5×1 ml) and ethyl acetate (5×1 ml). The filtrate was cooled to 0°C and then basified with saturated aqueous sodium bicarbonate solution with vigorous stirring. After

stirring at 0°C for 10 min, the layers were separated and the aqueous layer was extracted with ethyl acetate (6×3 ml). The combined organic extracts were dried and evaporated in vacuo to leave the *amine* (107 mg, 96%) as a cream solid, which was used without further purification.

An aqueous solution of tetrabutylammonium hydroxide (40 wt%, 162 μl, 0.250 mmol) was added dropwise over 1 min to a stirred solution of the amine (107 mg, 0.125 mmol) in tetrahydrofuran (5 ml) at 0°C. The solution was stirred at 0°C for 25 h and then additional aqueous tetrabutylammonium hydroxide solution (40 wt%, 81 μl, 0.13 mmol) was added dropwise over 1 min. After stirring at 0°C for a further 6.5 h, the solution was neutralised with 2 M aqueous hydrochloric acid solution (188 μl, 0.375 mmol). The solution was stirred at 0°C for a further 10 min, warmed to room temperature, and then evaporated in vacuo, azeotroping several times with toluene, to leave the *amino acid*, which was used without further purification.

Diisopropylethylamine (48 μl, 0.28 mmol) was added dropwise over 1 min to a stirred solution of the amino acid in dimethylformamide (25 ml) at −5°C under an atmosphere of nitrogen. The solution was stirred at −5°C for 10 min and then diphenylphosphoryl azide[16] (40 μl, 0.19 mmol) was added dropwise over 1 min. After 5 min at −5°C, stirring was stopped and the solution was allowed to slowly warm to room temperature and then stood at room temperature for seven days. Ethyl acetate (20 ml) was added and the solution was poured onto ice-cold water (50 ml). The two layers were separated and the aqueous layer was then extracted with ethyl acetate (6×10 ml). The combined organic extracts were washed with water (6×30 ml) and brine (1×30 ml), dried and evaporated in vacuo to leave the crude product which was purified by chromatography on silica using ethyl acetate as eluent to give the *cyclopeptide* (53 mg, 52%, 2 steps) as a cream foam; $[\alpha]_D^{17}=-280$ (*c* 1.29, $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3334, 2967, 2932, 2879, 1651, 1551, 1494, 1456, 1381, 1345, 1306, 1145, 1128, 1076, 1048, 993, 908, 876; δ_H (360 MHz, $CDCl_3$) 8.66 (1H, d, *J*=7.7 Hz, N*H*CHCH$_2$OH), 8.49 (1H, d, *J*=4.0 Hz, N*H*CHCH(CH$_3$)CH$_2$CH$_3$), 8.08 (1H, d, *J*=9.1 Hz, N*H*CHCH$_2$Ph), 7.96 (1H, d, *J*=8.5 Hz, N*H*CHCH(CH$_3$)-CH$_2$CH$_3$), 7.37–7.28 (3H, m, Ph-*H*), 7.19 (1H, d, *J*=5.3 Hz, N*H*CHCH$_2$OAllyl), 7.14–7.11 (2H, m, Ph-*H*), 5.94 (1H, dd, *J*=17.6, 10.8 Hz, C*H*=CH$_2$), 5.20 (1H, dd, *J*=17.6, 1.1 Hz, CH=C*H*$_2$), 5.17 (1H, dd, *J*=10.8, 1.0 Hz, CH=C*H*$_2$), 5.05 (1H, app d, *J*=7.7 Hz, C*H*CH$_2$OH), 4.60 (1H, ddd, *J*=10.2, 9.4, 5.5 Hz, C*H*CH$_2$Ph), 4.51 (1H, app d, *J*=7.3 Hz, C*H*(CH$_2$)$_3$N), 4.34–4.32 (1H, m, C*H*$_2$OH), 4.26 (1H, dd, *J*=5.8, 4.3 Hz, C*H*CH(CH$_3$)CH$_2$CH$_3$), 4.10 (1H, app t, *J*=8.7 Hz, C*H*CH(CH$_3$)CH$_2$CH$_3$), 3.99 (1H, ddd, *J*=9.6, 5.5, 4.2 Hz, C*H*CH$_2$OAllyl), 3.81 (1H, app d, *J*=6.8 Hz, C*H*(CH$_2$)$_3$N), 3.72–3.64 (1H, m, C*H*$_2$N), 3.69 (1H, dd, *J*=8.7, 4.0 Hz, C*H*$_2$OAllyl), 3.58–3.31 (5H, m, C*H*$_2$N, C*H*$_2$OH and O*H*), 3.42 (1H, dd, *J*=9.9, 8.8 Hz, C*H*$_2$OAllyl), 2.97 (1H, dd, *J*=12.5, 10.4 Hz, C*H*$_2$Ph), 2.87 (1H, dd, *J*=12.6, 5.5 Hz, C*H*$_2$Ph), 2.60–2.56 (1H, m, C*H*$_2$), 2.48–2.39 (1H, m, C*H*$_2$), 2.08–1.63 (9H, m, C*H*$_2$, C*H*$_2$CH$_3$, C*H*CH$_3$), 1.54–1.40 (2H, m, C*H*$_2$ and C*H*$_2$CH$_3$), 1.41 (3H, s, C*H*$_3$), 1.40 (3H, s, C*H*$_3$), 1.29–1.14 (1H, m, C*H*$_2$CH$_3$), 1.04 (3H, d, *J*=6.9 Hz, CHC*H*$_3$), 0.97 (3H, app t, *J*=7.3 Hz, CH$_2$C*H*$_3$), 0.92 (3H, d, *J*=6.7 Hz, CHC*H*$_3$), 0.86 (3H, app t, *J*=7.4 Hz, CH$_2$C*H*$_3$); δ_C (90 MHz, $CDCl_3$) 201.4 (s), 172.8 (s), 171.3 (s), 171.3 (s), 170.5 (s), 170.4 (s), 169.2 (s), 142.8 (d), 135.8 (s), 129.2 (d), 129.1 (d), 127.7 (d), 114.8 (t), 76.9 (s), 68.0 (d), 61.2 (t), 61.1 (d), 60.9 (t), 59.4 (d), 58.1 (d), 57.4 (d), 53.2 (d), 52.4 (d), 47.0 (t), 46.6 (t), 40.1 (t), 36.5 (d), 34.7 (d), 34.6 (t), 31.7 (t), 26.1 (q), 25.3 (t), 25.1 (q), 24.9 (t), 21.9 (t), 16.0 (q), 15.9 (q), 11.7 (q), 10.5 (q); *m/z* (ES) 848.4302 ([M+Na]$^+$, $C_{42}H_{63}N_7O_8SNa$ requires 848.4357).

4.1.18. Mollamide (3). Diethylaminosulfur trifluoride (10 μl, 0.076 mmol) was added dropwise over 1 min to a stirred solution of the cyclopeptide **5** (21 mg, 0.025 mmol) in dichloromethane (0.5 ml) at −15°C under an atmosphere of nitrogen. The solution was stirred at −15°C for 1 h, then quenched with saturated aqueous sodium bicarbonate solution (1 ml) and warmed to room temperature. Dichloromethane (5 ml) and water (4 ml) were added, the layers separated, and the aqueous layer was then extracted with dichloromethane (5×1 ml). The combined organic extracts were dried and evaporated in vacuo to leave the crude product which was purified by chromatography on silica using ethyl acetate as eluent to give *mollamide* (15 mg, 74%) as a colourless solid; mp 151–153°C (Lit.,[4] mp 154–156°C); $[\alpha]_D^{17}=-40$ (*c* 0.74, $CHCl_3$) (Lit.,[4] $[\alpha]_D^{25}=-2.75$ (*c* 0.08, $CHCl_3$))[‡]; δ_H (500 MHz, $CDCl_3$) 8.18 (1H, d, *J*=8.5 Hz, N*H*CHCH(CH$_3$)CH$_2$CH$_3$), 8.09 (1H, d, *J*=8.6 Hz, N*H*CHCH$_2$Ph), 7.32–7.24 (3H, m, Ph-*H*), 7.16 (2H, d, *J*=6.9 Hz, Ph-*H*), 7.12 (1H, d, *J*=4.6 Hz, N*H*CHCH$_2$OAllyl), 7.02 (1H, br s, N*H*CHCH(CH$_3$)CH$_2$-CH$_3$), 5.88 (1H, dd, *J*=17.6, 10.9 Hz, C*H*=CH$_2$), 5.31 (1H, dd, *J*=11.4, 5.6 Hz, C*H*CH$_2$S), 5.15 (1H, d, *J*=17.8 Hz, CH=C*H*$_2$), 5.14 (1H, d, *J*=10.8 Hz, CH=C*H*$_2$), 4.85 (1H, ddd, *J*=11.2, 8.5, 4.4 Hz, C*H*CH$_2$Ph), 4.61 (1H, app d, *J*=7.3 Hz, C*H*(CH$_2$)$_3$N), 4.26 (1H, app d, *J*=8.2 Hz, C*H*CH(CH$_3$)CH$_2$CH$_3$), 4.23 (1H, app dt, *J*=10.6, 4.6 Hz, C*H*CH$_2$OAllyl), 4.16 (1H, app t, *J*=8.4 Hz, C*H*CH(CH$_3$)-CH$_2$CH$_3$), 3.94 (1H, dd, *J*=6.9, 4.6 Hz, C*H*(CH$_2$)$_3$N), 3.77 (1H, dd, *J*=7.9, 4.2 Hz, C*H*$_2$OAllyl), 3.77–3.71 (1H, masked m, C*H*$_2$N), 3.65 (1H, dd, *J*=11.4, 5.6 Hz, C*H*CH$_2$S), 3.59–3.55 (1H, m, C*H*$_2$N), 3.51 (1H, app dd, *J*=12.1, 7.1 Hz, C*H*$_2$N), 3.34 (1H, app dd, *J*=11.9, 7.2 Hz, C*H*$_2$N), 3.25 (1H, app t, *J*=11.4 Hz, C*H*$_2$S), 3.04 (1H, dd, *J*=11.9, 11.7 Hz, C*H*$_2$Ph), 2.94 (1H, dd, *J*=10.5, 7.9 Hz, C*H*$_2$OAllyl), 2.81 (1H, dd, *J*=12.3, 4.4 Hz, C*H*$_2$Ph), 2.66–2.63 (1H, m, C*H*$_2$), 2.00–1.81 (9H, m, C*H*$_2$, C*H*CH$_3$ and C*H*$_2$CH$_3$), 1.77–1.71 (1H, m, C*H*$_2$), 1.56–1.42 (2H, m, C*H*$_2$ and C*H*$_2$CH$_3$), 1.38 (3H, s, C*H*$_3$), 1.36 (3H, s, C*H*$_3$), 1.35–1.26 (1H, m, C*H*$_2$CH$_3$), 1.11 (3H, app t, *J*=7.3 Hz, CH$_2$C*H*$_3$), 1.11 (3H, d, *J*=6.8 Hz, CHC*H*$_3$), 0.94 (3H, d, *J*=6.8 Hz, CHC*H*$_3$), 0.87 (3H, app t, *J*=7.4 Hz, CH$_2$C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 176.8 (s), 172.0 (s), 171.3 (s), 170.9 (s), 170.6 (s), 169.8 (s), 143.1 (d), 136.7 (s), 129.0 (d), 128.6 (d), 127.1 (d), 114.7 (t), 79.9 (d), 76.8 (s), 61.4 (d), 61.3 (t), 60.7 (d), 59.6 (d), 57.7 (d), 52.2 (d), 52.0 (d), 46.5 (t), 46.4 (t), 40.8 (t), 36.7 (d), 36.0 (d), 33.1 (t), 31.7 (t), 31.5 (t), 25.8 (q), 25.7 (t), 25.4 (q), 25.4 (t), 23.1 (t), 22.0 (t), 15.8 (q), 15.6

[‡] While the $[\alpha]_D$ measured for our synthetic mollamide was significantly different from that reported for naturally derived material[4] it was identical to that obtained by ourselves from a sample of natural mollamide ($[\alpha]_D^{20}=-38$ (*c* 0.65, CHCl3)).

(q), 11.4 (q), 10.6 (q); *m/z* (ES) 830.4238 ($[M+Na]^+$, $C_{42}H_{61}N_7O_7SNa$ requires 830.4251).

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