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Total synthesis of trunkamide A, a novel thiazoline-based prenylated cyclopeptide metabolite from *Lissoclinum* sp.

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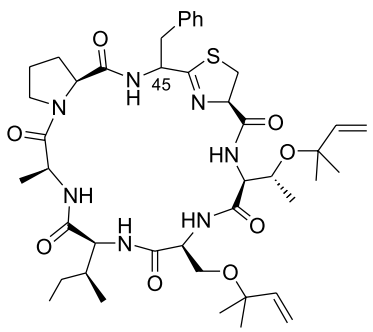
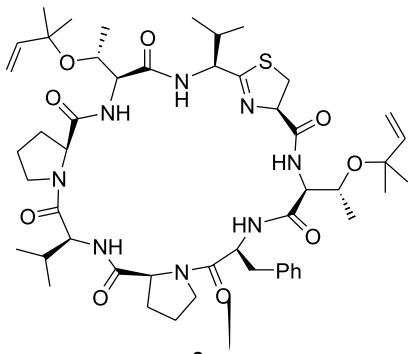
Abstract—Full details of a total synthesis of the doubly prenylated cyclic peptide trunkamide A of marine origin, and also its C45 epimer, are described.

1. Introduction

The marine environment has provided a rich source of structurally novel and biologically important secondary metabolites in recent years, many of which have attracted chemists to their total synthesis and an evaluation of their potential as chemotherapeutic agents.[1] Amongst some of the most intriguing marine natural products are heterocyclic-based cyclopeptides which have been isolated from a variety of organisms. Ascidians (sea squirts) of the genus *Lissoclinum* have proven to be a particularly rich source of these novel compounds.[2] Trunkamide A **1** was isolated from the colonial ascidian *Lissoclinum* sp. located in the Great Barrier Reef in Australia.[3] At the same time, the isolation of the related patellins, e.g. patellin 6 **2**, were also reported. Most cyclopeptides isolated from ascidians show only moderate cytotoxicity. Trunkamide A, however, is reported to have promising antitumour activity.[4] The structures of trunkamide A **1**, patellin 6 **2**, along with other marine cyclopeptides such as mollamide **3**,[5] are characterised by the presence of serine/threonine residues which have been modified as reverse prenyl ethers. In the previous paper we described the successful synthesis of mollamide **3** which demonstrated a strategy for the construction of these compounds. In contemporaneous studies Uto and Wipf described a total synthesis of the stereostructure **1b** assigned to trunkamide A by Bowden et al.[3] However, the spectroscopic data recorded for the synthetic and natural materials did not correlate. In a subsequent report these authors showed that **1a** was the correct stereostructure for natural trunkamide A.[6] More recently, a solid phase approach to



1a; C45 *R*
1b; C45 *S*



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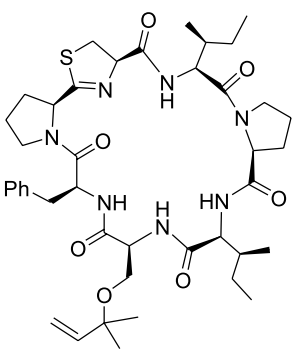

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Keywords: trunkamide A; thiazoline cyclopeptide; *Lissoclinum* sp..

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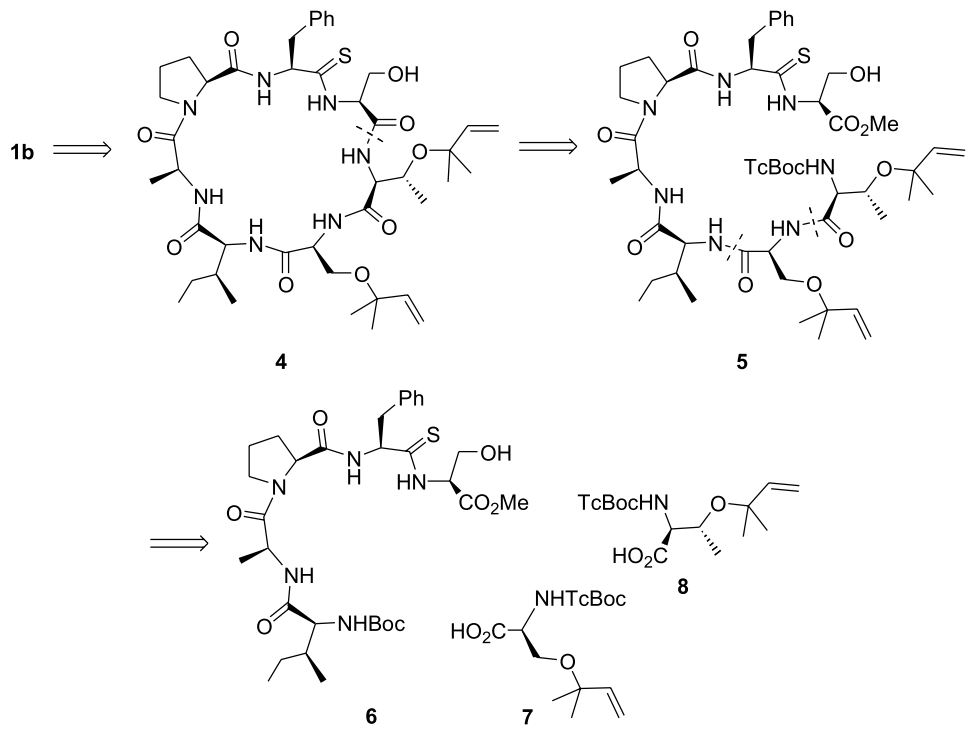


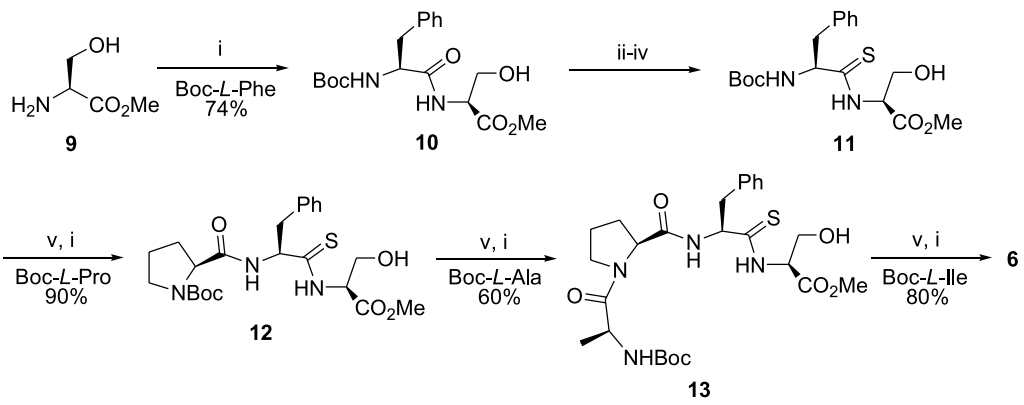
Scheme 1.

trunkamide A has been reported.[7] In this paper we report the full details of our own studies leading to a total synthesis of trunkamide A **1a** along with its C45 epimer **1b**.[8]

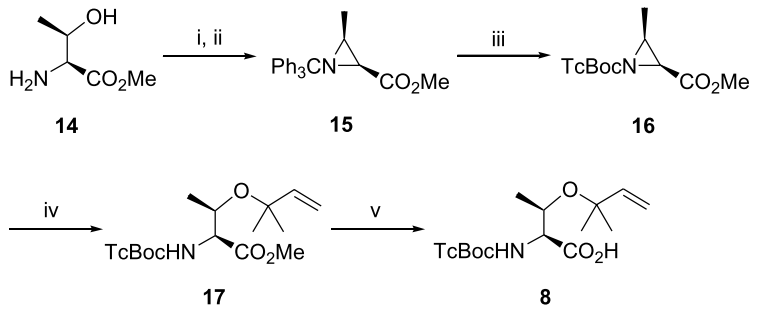
2. Results and discussion

Following our successful synthesis of mollamide **3**, we designed a synthesis of the structure **1b** reported for naturally derived trunkamide A using a similar strategy. Hence, the thiazoline ring in the natural product was to be produced in the final step of the synthesis from the thioamide-based cyclopeptide **4** (Scheme 1). We chose to elaborate **4** from the heptapeptide **5** which we planned to synthesise in a linear manner via the pentapeptide **6**. The introduction of the acid-labile reverse prenyl ether amino acids **7** and **8** at a late stage in the synthesis should permit us to construct the pentapeptide **6** by our preferred method of peptide synthesis, i.e. from the amino terminus using acid-labile Boc protecting groups.

Thus, our synthesis began with the elaboration of the pentapeptide **6** via the thiodipeptide **11** (Scheme 2). Boc-L-phenylalanine was first coupled with L-serine methyl ester **9** using DCC and HOBt as coupling reagents, leading to the dipeptide **10** which was next protected as its TBS ether and thionated using Lawesson's reagent,[9] and finally deprotected using TBAF to give **11** in good yield (57%, four steps). Removal of the Boc protecting group from **11** and reaction of the resulting free amine with Boc-L-proline in the presence of DCC and HOBt next gave the tripeptide **12**. Removal of the Boc group in **12**, followed by coupling with Boc-L-alanine then provided the tetrapeptide **13**. Finally, removal of the Boc group in **13** and coupling of the resulting amine with Boc-L-isoleucine led to the pentapeptide **6**.



Scheme 2. *Reagents*: (i) DCC, HOBt, DIPEA, DCM, 0°C→rt; (ii) TBSCl, imidazole, DMF, rt, 90%; (iii) Lawesson's reagent, benzene, 80°C→rt, 92%; (iv) TBAF, THF, rt, 93%; (v) AcCl, MeOH, rt.

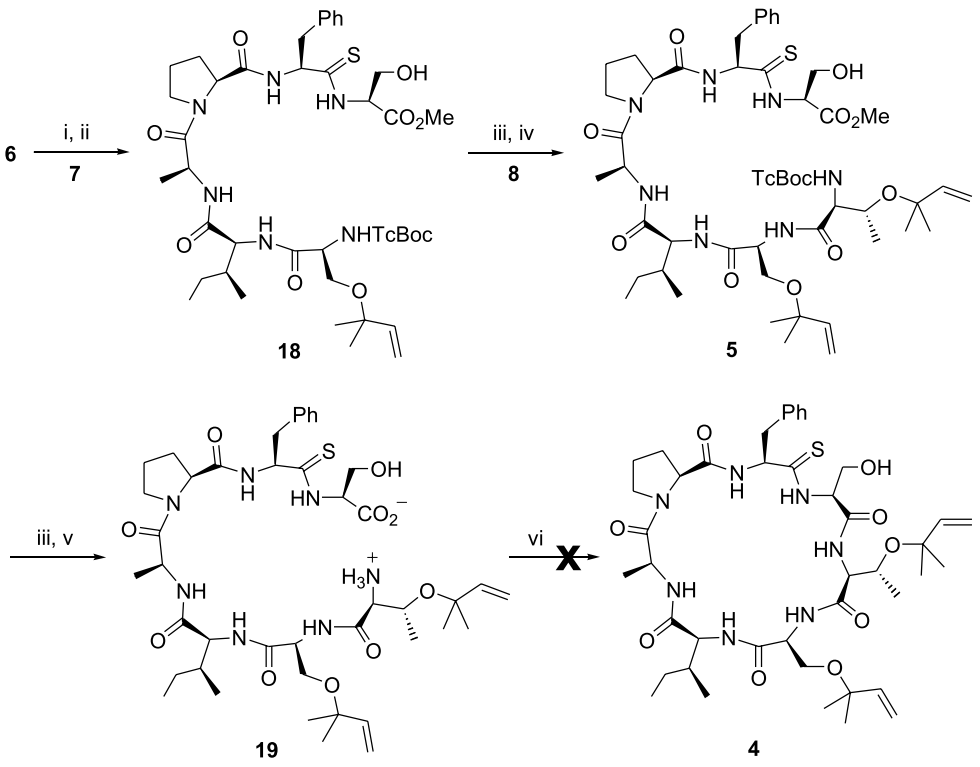


Scheme 3. *Reagents*: (i) Ph_3CCl, Et_3N, DCM, rt, 96%; (ii) MsCl, Et_3N, THF, 0°C→65°C, 97%; (iii) (a) TFA, MeOH, DCM, 0°C; (b) TcBoc-OSu, Et_3N, rt, 89%; (iv) $BF_3{\cdot}OEt_2$, 2-methyl-3-buten-2-ol, rt, 69%; (v) 1.5 M LiOH, THF/MeOH, rt, 93%.

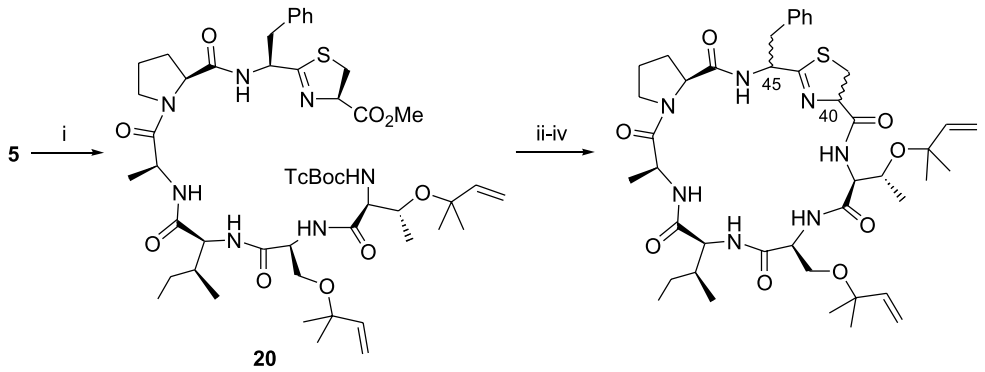
The synthesis of the amino acid **7** has been described previously,[10] and the amino acid **8** was synthesised using an identical strategy (Scheme 3). Hence, following the formation of the trityl-protected aziridine **15** from L-threonine methyl ester **14**, protecting group interconversion led to the TcBoc (2,2,2-trichloro-*tert*-butyloxycarbonyl)-protected aziridine **16**. Ring opening of **16** by reaction with 2-methyl-3-buten-2-ol in the presence of boron trifluoride diethyl etherate then gave the amino acid **17** which was saponified leading to **8**.

The synthesis of the heptapeptide **5** from **6** was achieved following removal of the Boc protecting group in **6**, coupling of the resulting amine with **7** in the presence of EDC and HOBt, removal of the TcBoc protecting group from the resulting hexapeptide **18** and a coupling reaction with **8** under similar conditions (Scheme 4). Having successfully constructed the macrocyclisation precursor **5**, we were disappointed, however, to find that, following the removal of the TcBoc[11] and methyl ester protecting groups in **5**, we were unable to induce macrolactamisation of the zwitterion **19** to the desired cyclopeptide **4**.

Subsequent investigations led us to suspect that the thioamide bond in **19** and its proximity to the site of macrolactamisation was the likely cause of the problem. In order to test this suggestion we decided to form the thiazoline ring in trunkamide A prior to macrolactamisation, hence removing any potential interference of the thioamide bond. This was a bold move in view of the ease with which chiral thiazolines undergo epimerisation under both acidic and basic conditions, with the likely consequence that we would lose the stereochemical integrity at C45 (and possibly C40) during the subsequent synthetic manipulations leading to the cyclopeptide target.[12] Hence, treatment of the heptapeptide thioamide **5** with Burgess' reagent[13] resulted in dehydration and cyclisation to the corresponding substituted thiazoline **20** (Scheme 5). After removal of the ester and amine protecting groups in **20**, macrolactamisation using DPPA[14] successfully produced a cyclopeptide



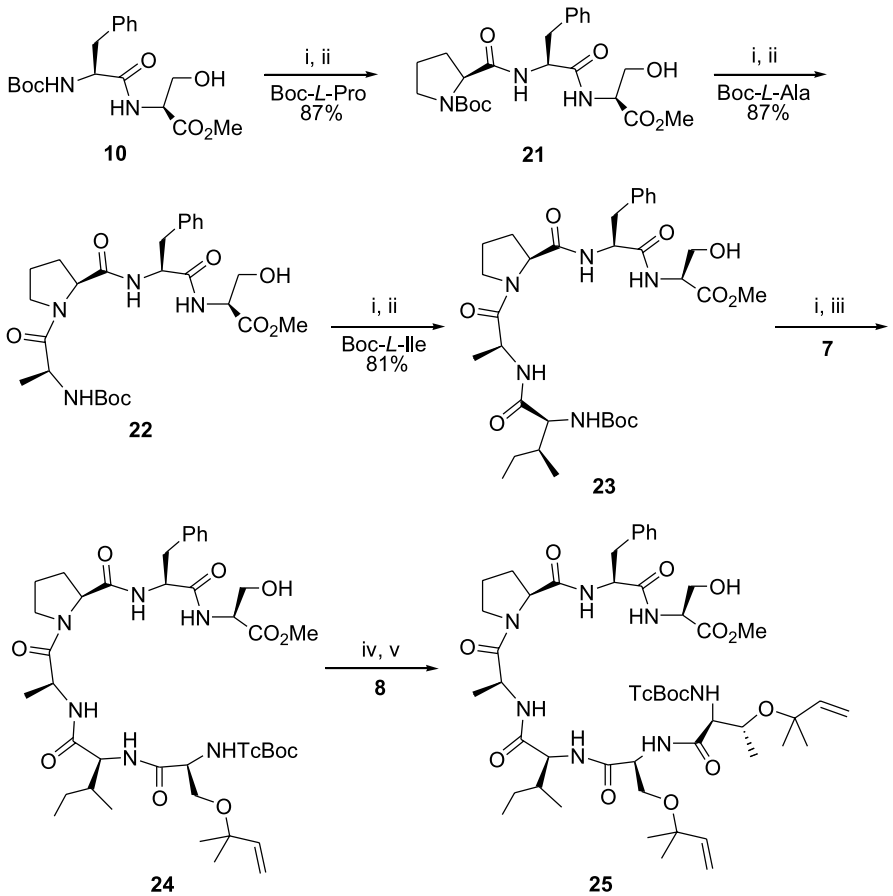
Scheme 4. *Reagents*: (i) AcCl, MeOH, rt; (ii) EDC, HOBt, DIPEA, DCM, 0°C→rt, 72%; (iii) Cd/Pb, 1 M NH_4OAc/THF (1:1), rt; (iv) EDC, HOBt, DCM, 0°C→rt, 78% (two steps); (v) 1 M NaOH, MeOH, rt; (vi) DPPA, DIPEA, DMF, −5°C→rt.



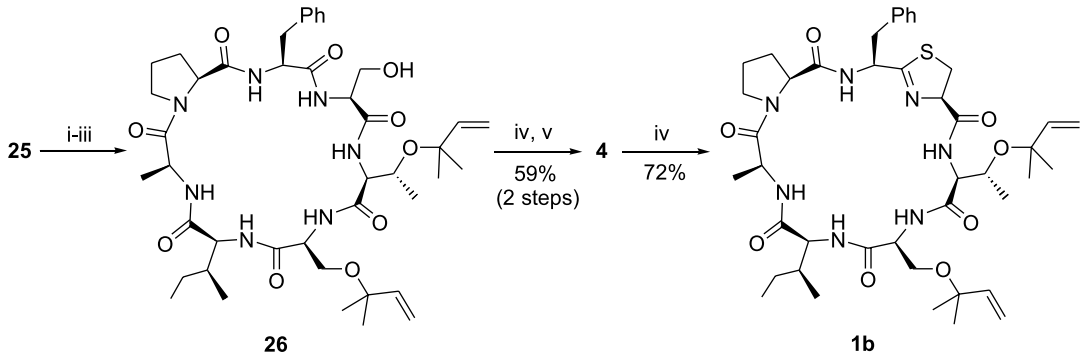
Scheme 5. *Reagents*: (i) Burgess' reagent, THF, 65°C, 92%; (ii) Cd/Pb, 1 M NH_4OAc/THF (1:1), rt, 93%; (iii) 1 M NaOH, MeOH, rt; (iv) DPPA, DIPEA, DMF, −5°C→rt.

structure. However, as anticipated, analysis of the material obtained clearly showed the presence of a mixture of diastereomeric products. Following extensive semi-preparative HPLC, we were able to show that one of the product diastereoisomers had NMR spectroscopic data which corresponded to those of the target cyclopeptide **1b** reported for naturally derived trunkamide A.[6] Furthermore, NMR data for another diastereoisomer were identical with those reported for natural trunkamide A.[3] Unfortunately, at this stage, we were unable to confirm the exact stereochemistry of this particular compound which could have been any one of the other three possible diastereoisomers.

Encouraged by the aforementioned successful macrocyclisation (Scheme 5), we speculated that if we replaced the problematic thioamide bond in **19** with an amide bond then our original macrocyclisation strategy, i.e. **5→4** (Scheme 1), may prove more successful and allow us access to diastereomerically pure material. Hence, we next decided to synthesise the heptapeptide **25** and to examine its macrocyclisation (Scheme 6). Thus, starting from the dipeptide **10**, the pentapeptide **23** was prepared by sequential addition of L-proline, L-alanine and L-isoleucine. Subsequent addition of the two reverse prenylated amino acids **7** and **8** to **23** then gave the heptapeptide **25**.



Scheme 6. *Reagents*: (i) AcCl, MeOH, rt; (ii) DCC, HOBt, DIPEA, DCM, 0°C→rt; (iii) EDC, HOBt, DIPEA, DCM, 0°C→rt, 60%; (iv) Cd/Pb, 1 M NH_4OAc/THF (1:1), rt, 94%; (v) EDC, HOBt, DCM, 0°C→rt, 72%.



Scheme 7. *Reagents*: (i) Cd/Pb, 1 M NH_4OAc/THF (1:1), rt, 98%; (ii) TBAH, THF, 0°C; (iii) DPPA, DIPEA, DMF, −5°C→rt, 35% (two steps); (iv) DAST, DCM, −15°C; (v) H_2S, Et_3N, MeOH, rt.

After our disappointment at not being able to induce macrocyclisation from the thioamide heptapeptide **5** we were delighted to find that, following removal of the TcBoc[11] and methyl ester[15] protecting groups in **25**, macrocyclisation proceeded smoothly and provided the cyclopeptide **26** in a modest 35% yield (Scheme 7). The macrocycle **26** was then elaborated to the trunkamide structure **1b** using a strategy described by Wipf et al.[6,16] i.e. cyclodehydration of **26** to the corresponding oxazoline, subsequent thiolysis with H_2S to the thioamide cyclopeptide **4**, and reaction with DAST.[17]

The cyclic peptide **1b** was shown to be identical in all respects with the trunkamide structure reported by Uto and Wipf[6] but its spectroscopic data differed significantly with those reported by Bowden et al. for natural trunkamide A.[3] Following the report of Uto and Wipf which revealed the correct structure of trunkamide A, i.e. **1a** rather than **1b**,[6a] we then decided to attempt to synthesise this epimeric structure **1a**. Instead of carrying out a new total synthesis of natural trunkamide A from D- rather than L-phenylalanine, however, we planned to effect conversion of *epi*-trunkamide A **1b** into trunkamide A **1a** by selective epimerisation of the 'incorrect' C45 stereocentre. This was a somewhat risky strategy in view of the potential for concurrent epimerisation at C40. However, we were encouraged to attempt this conversion following previous observations that, under suitably mild basic conditions, epimerisation is only seen at the stereocentre which is positioned exocyclic to the thiazoline ring.[12b] Indeed, we found that when **1b** was treated with methanolic pyridine at 50–55°C it was slowly converted into trunkamide A **1a** with no detectable formation of any other diastereoisomer (Scheme 8). After careful purification by flash chromatography followed by semi-preparative HPLC, comparison of the spectroscopic data obtained for our synthetic material with those reported for natural trunkamide A from *Lissoclinum* showed them to be identical in all respects.

3. Conclusion

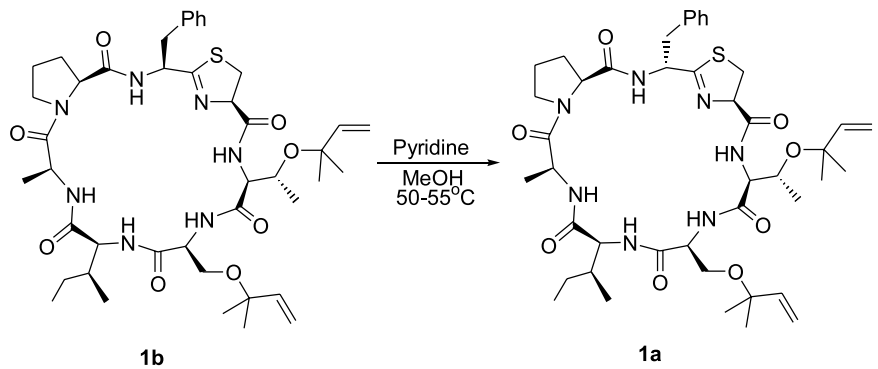
In summary, we have achieved a synthesis of trunkamide A which has not only further demonstrated our approach to the construction of this novel class of marine natural products but also its potential for accessing other members of the family. Our investigations have also shown that, in agreement with the work of Wipf and Uto,[6] the correct structure for trunkamide A is **1a**, and not **1b** as originally reported.

4. Experimental

4.1. General

For general experimental details see the previous paper.

4.1.1. Boc-Phe-Ser-OMe (10).[18] Diisopropylethylamine (19.6 ml, 112 mmol) was added dropwise over 10 min to a stirred suspension of L-serine methyl ester hydrochloride **9** (5.00 g, 32.1 mmol) in dichloromethane (200 ml) at room temperature under an atmosphere of nitrogen. On dissolution, the solution was cooled to 0°C and then



Scheme 8.

Boc-L-phenylalanine (8.53 g, 32.1 mmol) and 1-hydroxybenzotriazole (4.78 g, 35.4 mmol) were added successively, each in one portion. The suspension was stirred at 0°C for a further 15 min and then dicyclohexylcarbodiimide (7.29 g, 35.4 mmol) was added in one portion. The mixture was allowed to warm to room temperature over the course of 51 h, then filtered, and the filtrate was 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 (3×20 ml) followed by saturated aqueous sodium bicarbonate solution (5×20 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 diethyl ether as eluent to give the dipeptide (8.66 g, 74%) as a yellow solid; mp 93–95°C (ethyl acetate/hexane) (lit.[18b] mp 88–89°C); $[\alpha]_D^{22}$=+17 (*c* 1.07, $CHCl_3$) (lit.[18a] $[\alpha]_D$=+12.1 (*c* 1.02, CH_2Cl_2)); found %: C, 59.3; H, 7.3; N, 7.5; calcd for $C_{18}H_{26}N_2O_6$ %: C, 59.0, H, 7.15; N, 7.65; ν_{max} ($CHCl_3$)/cm^{-1} 3427, 2955, 1744, 1682, 1490, 1455, 1393, 1369, 1156, 1056, 908; δ_H (360 MHz, $CDCl_3$) 7.34–7.21 (5H, m, Ph-*H*), 6.80 (1H, d, *J*=7.2 Hz, N*H*), 5.02 (1H, br s, N*H*Boc), 4.60 (1H, app dt, *J*=7.1, 3.5 Hz, C*H*CO_2Me), 4.33 (1H, app dt, *J*=7.2, 6.8 Hz, C*H*CH_2Ph), 3.97–3.88 (2H, m, CH_2OH), 3.76 (3H, s, OCH_3), 3.13 (1H, dd, *J*=13.8, 6.3 Hz, CH_2Ph), 3.07 (1H, dd, *J*=13.7, 7.2 Hz, CH_2Ph), 2.81 (1H, br s, O*H*), 1.41 (9H, s, C(CH_3)$_3$); δ_C (90 MHz, $CDCl_3$) 171.8 (s), 170.7 (s), 155.8 (s), 136.6 (s), 129.4 (d), 128.7 (d), 127.0 (d), 80.5 (s), 62.8 (t), 56.0 (d), 55.0 (d), 52.7 (q), 38.2 (t), 28.3 (q); *m*/*z* (ES) 389.1682 ($[M+Na]^+$, $C_{18}H_{26}N_2O_6Na$ requires 389.1689).

4.1.2. Boc-PheΨ{(C═S)NH}-Ser-OMe (11). Imidazole (1.49 g, 21.9 mmol) was added in one portion to a stirred solution of the dipeptide **10** (4.00 g, 10.9 mmol) in dimethylformamide (50 ml) at 0°C under an atmosphere of nitrogen. The solution was stirred at 0°C for 5 min and then *tert*-butyldimethylsilyl chloride (2.47 g, 16.4 mmol) was added in one portion. The mixture was stirred at room temperature for 3 h, then diluted with ethyl acetate (50 ml) and poured into water (200 ml). The layers were separated and the aqueous fraction was then extracted with ethyl acetate (5×40 ml). The combined organic extracts were washed with water (5×50 ml), dried and evaporated in vacuo to leave the crude product which was purified by chromatography on silica using 40% diethyl ether in light petroleum (40–60°C) as eluent to give *Boc-Phe-Ser(TBS)-OMe* (4.73 g, 90%) as a white solid; mp 101–102°C (ethyl acetate/hexane); $[\alpha]_D^{23}$=+22 (*c* 1.07, $CHCl_3$); found %: C, 60.2; H, 8.45; N, 5.6; $C_{24}H_{40}N_2O_6Si$ requires %: C, 60.0; H 8.4; N 5.8; ν_{max} ($CHCl_3$)/cm^{-1} 3430, 2954, 2931, 2884, 2858, 1747, 1711, 1678, 1489, 1456, 1368, 1297, 1157, 1110, 1049, 982, 864, 840; δ_H (360 MHz, $CDCl_3$, 323 K) 7.32–7.21 (5H, m, Ph-*H*), 6.54 (1H, d, *J*=8.2 Hz, N*H*), 4.91 (1H, br s, N*H*Boc), 4.61 (1H, app dt, *J*=8.0, 3.3 Hz, C*H*CO_2Me), 4.41 (1H, app dt, *J*=7.6, 6.9 Hz, C*H*CH_2Ph), 4.03 (1H, dd, *J*=10.1, 3.0 Hz, CH_2OTBS), 3.78 (1H, dd, *J*=10.1, 3.5 Hz, CH_2OTBS), 3.73 (3H, s, OCH_3), 3.14 (1H, dd, *J*=13.9, 6.6 Hz, CH_2Ph), 3.07 (1H, dd, *J*=14.0, 6.9 Hz, CH_2Ph), 1.43 (9H, s, C(CH_3)$_3$), 0.86 (9H, s, SiC(CH_3)$_3$), 0.02 (6H, s, Si(CH_3)$_2$); δ_C (90 MHz, $CDCl_3$) 171.0 (s), 170.3 (s), 155.1 (s), 136.5 (s), 129.3 (d), 128.6 (d), 126.9 (d), 80.0 (s), 63.4 (t), 55.6 (d), 54.2 (d), 52.3 (q), 38.5 (t), 28.2 (q), 25.7 (q), 18.1 (s), −5.6 (q), −5.7 (q); *m*/*z* (ES) 503.2583 ($[M+Na]^+$, $C_{24}H_{40}N_2O_6SiNa$ requires 503.2553).

Lawesson's reagent[9d] (2.69 g, 6.65 mmol) was added in one portion to a stirred solution of the aforementioned amide (4.56 g, 9.50 mmol) in benzene (50 ml) at room temperature under an atmosphere of nitrogen. The suspension was heated under reflux for 2 h and then cooled to room temperature and stirred for 26 h. The solvent was removed in vacuo to leave a residue which was taken up in dichloromethane and filtered. The filtrate was evaporated in vacuo to leave the crude product which was purified by chromatography on silica using 25% diethyl ether in light petroleum (40–60°C) as eluent to give the corresponding thioamide (4.33 g, 92%) as a yellow oil; $[\alpha]_D^{25}$=+73 (*c* 1.33, $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3358, 2954, 2931, 2884, 2858, 1746, 1708, 1603, 1488, 1456, 1392, 1369, 1329, 1155, 1110, 1048, 1005, 981, 866, 839; δ_H (360 MHz, $CDCl_3$) 8.19 (1H, d, *J*=6.8 Hz, N*H*C═S), 7.32–7.22 (5H, m, Ph-*H*), 5.24 (1H, br s, N*H*Boc), 5.12 (1H, app dt, *J*=7.3, 2.8 Hz, C*H*CO_2Me), 4.65 (1H, app dt, *J*=7.3, 7.2 Hz, C*H*CH_2Ph), 4.06 (1H, dd, *J*=10.3, 2.6 Hz, CH_2OTBS), 3.98 (1H, dd, *J*=10.3, 3.1 Hz, CH_2OTBS), 3.72 (3H, s, OCH_3), 3.24 (1H, dd, *J*=13.9, 6.6 Hz, CH_2Ph), 3.14 (1H, dd, *J*=13.9, 7.4 Hz, CH_2Ph), 1.41 (9H, s, C(CH_3)$_3$), 0.84 (9H, s, SiC(CH_3)$_3$), 0.01 (6H, s, Si(CH_3)$_2$); δ_C (90 MHz, $CDCl_3$) 203.5 (s), 169.4 (s), 154.9 (s), 136.5 (s), 129.3 (d), 128.7 (d), 127.1 (d), 80.4 (s), 62.3 (t), 59.6 (d), 52.6 (q), 41.9 (t), 28.3 (q), 25.7 (q), 18.2 (s), −5.5 (q), −5.6 (q); *m*/*z* (ES) 519.2355 ($[M+Na]^+$, $C_{24}H_{40}N_2O_5SSiNa$ requires 519.2325).

Tetrabutylammonium fluoride (3.43 g, 13.1 mmol) was added in one portion to a stirred solution of the thioamide (4.33 g, 8.73 mmol) in tetrahydrofuran (40 ml) at 0°C under an atmosphere of nitrogen. The suspension was warmed to room temperature, stirred for 50 min and then evaporated in vacuo. The residue was taken up in dichloromethane (100 ml) and washed with water (3×30 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 β-hydroxythioamide (3.09 g, 93%) as a cream foam; $[\alpha]_D^{22}$=+64 (*c* 1.10, $CHCl_3$); found %: C, 56.5; H, 7.0; N, 7.1; $C_{18}H_{26}N_2O_5S$ requires %: C, 56.5; H, 6.85; N, 7.3; ν_{max} ($CHCl_3$)/cm^{-1} 3428, 3358, 2980, 2956, 1743, 1698, 1603, 1489, 1456, 1394, 1369, 1326, 1155, 1102, 1054, 974, 906, 863; δ_H (360 MHz, $CDCl_3$, 323 K) 8.32 (1H, d, *J*=5.8 Hz, N*H*C═S), 7.31–7.20 (5H, m, Ph-*H*), 5.21 (1H, d, *J*=6.8 Hz, N*H*Boc), 5.14 (1H, app dt, *J*=7.1, 3.5 Hz, C*H*CO_2Me), 4.60 (1H, app dt, *J*=7.3, 7.1 Hz, C*H*CH_2Ph), 4.12 (1H, ddd, *J*=11.4, 5.1, 3.6 Hz, CH_2OH), 3.94 (1H, ddd, *J*=11.5, 7.1, 3.1 Hz, CH_2OH), 3.74 (3H, s, OCH_3), 3.21 (1H, dd, *J*=13.8, 6.6 Hz, CH_2Ph), 3.16 (1H, dd, *J*=13.8, 7.2 Hz, CH_2Ph), 2.43 (1H, br s, O*H*), 1.40 (9H, s, C(CH_3)$_3$); δ_C (90 MHz, $CDCl_3$) 204.5 (s), 169.8 (s), 155.7 (s), 136.4 (s), 129.3 (d), 128.5 (d), 126.9 (d), 80.7 (s), 62.2 (d), 61.4 (t), 59.8 (d), 52.8 (q), 41.6 (t), 28.2 (q); *m*/*z* (ES) 405.1470 ($[M+Na]^+$, $C_{18}H_{26}N_2O_5SNa$ requires 405.1460).

4.1.3. Boc-Pro-PheΨ{(C═S)NH}-Ser-OMe (12). Acetyl chloride (5 ml) was added dropwise over 5 min to a stirred solution of the β-hydroxythioamide **11** (2.64 g, 6.91 mmol)

in methanol (50 ml) at 0°C under an atmosphere of nitrogen. The solution was stirred at room temperature for 3 h and then evaporated in vacuo to leave the corresponding hydrochloride salt (2.17 g, 99%) as a cream solid, which was used without further purification.

Diisopropylethylamine (4.2 ml, 24 mmol) was added dropwise over 5 min to a stirred solution of the hydrochloride salt (2.17 g, 6.81 mmol) in dichloromethane (30 ml) at 0°C under an atmosphere of nitrogen. The solution was stirred at 0°C for 10 min and then Boc-L-proline (1.47 g, 6.81 mmol) and 1-hydroxybenzotriazole (1.01 g, 7.49 mmol) were added successively in one portion. The suspension was stirred at 0°C for a further 15 min and then dicyclohexylcarbodiimide (1.55 g, 7.49 mmol) was added in one portion. The mixture was allowed to warm to room temperature over the course of 63 h and then evaporated in vacuo to leave a residue which was taken up in ethyl acetate (50 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 25% ethyl acetate in diethyl ether as eluent to give the tripeptide (2.95 g, 90%) as a cream foam; $[\alpha]_D^{24}=-94$ (*c* 0.99, $CHCl_3$); found %: C, 57.3; H, 7.0; N, 8.5; $C_{23}H_{33}N_3O_6S$ requires %: C, 57.6; H, 6.9; N 8.8; ν_{max} ($CHCl_3$)/cm^{-1} 3406, 2979, 2955, 2886, 1745, 1682, 1603, 1556, 1496, 1455, 1393, 1369, 1327, 1158, 1127, 1062, 979, 892; δ_H (360 MHz, $CDCl_3$, 323 K) 8.70 (1H, br s, N*H*C=S), 7.29–7.18 (5H, m, Ph-*H*), 6.76 (1H, d, *J*=8.2 Hz, N*H*), 5.13 (1H, app dt, *J*=7.2, 3.5 Hz, C*H*CO_2Me), 5.07 (1H, m, C*H*CH_2Ph), 4.20 (1H, dd, *J*=8.6, 3.5 Hz, C*H*NBoc), 4.05 (1H, ddd, *J*=11.7, 5.7, 3.4 Hz, CH_2OH), 3.95 (1H, ddd, *J*=11.7, 7.2, 3.4 Hz, CH_2OH), 3.75 (3H, s, OCH_3), 3.39–3.23 (4H, m, CH_2NBoc and CH_2Ph), 3.12 (1H, br s, O*H*), 2.07–1.98 (1H, m, CH_2), 1.96–1.88 (1H, m, CH_2), 1.77–1.74 (1H, m, CH_2), 1.60–1.55 (1H, m, CH_2), 1.43 (9H, s, C(CH_3)$_3$); δ_C (90 MHz, $CDCl_3$) 204.2 (s), 172.7 (s), 169.4 (s), 155.5 (s), 136.8 (s), 129.4 (d), 128.4 (d), 126.8 (d), 81.2 (s), 61.4 (t), 60.9 (d), 60.1 (d), 58.5 (d), 52.6 (q), 47.3 (t), 41.5 (t), 29.7 (t), 28.4 (q), 24.1 (t); *m*/*z* (ES) 502.1984 ([M+Na]$^+$, $C_{23}H_{33}N_3O_6SNa$ requires 502.1988).

4.1.4. Boc-Ala-Pro-PheΨ{(C=S)NH}-Ser-OMe (13). Acetyl chloride (4 ml) was added dropwise over 5 min to a stirred solution of the tripeptide **12** (2.70 g, 5.66 mmol) in methanol (40 ml) at 0°C under an atmosphere of nitrogen. The solution was stirred at room temperature for 7 h and then evaporated in vacuo to leave the corresponding hydrochloride salt (2.35 g) as a cream solid, which was used without further purification.

Diisopropylethylamine (3.4 ml, 20 mmol) was added dropwise over 5 min to a stirred solution of the hydrochloride salt (2.35 g, 5.66 mmol) in dichloromethane (25 ml) at 0°C under an atmosphere of nitrogen. The solution was stirred at 0°C for 10 min and then Boc-L-alanine (1.07 g, 5.66 mmol) and 1-hydroxybenzotriazole (0.84 g, 6.2 mmol) were added successively in one portion. The suspension was stirred at 0°C for a further 15 min and then dicyclohexylcarbodiimide (1.28 g, 6.22 mmol) was added in one portion. The mixture was allowed to warm to room temperature over 24 h and then evaporated in vacuo to leave a residue which was taken up in ethyl acetate (50 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 50% ethyl acetate in diethyl ether as eluent to give the tetrapeptide (1.85 g, 60%) as a cream foam; $[\alpha]_D^{27}=-63$ (*c* 0.77, $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3435, 3350, 2980, 1744, 1699, 1652, 1495, 1455, 1393, 1369, 1344, 1159, 1064, 865; δ_H (360 MHz, d_6-DMSO, 343 K) 9.96 (1H, br s, N*H*C=S), 7.65 (1H, br s, N*H*CH_2Ph), 7.27–7.16 (5H, m, Ph-*H*), 6.50 (1H, br s, N*H*Boc), 5.05–4.99 (2H, m, C*H*CO_2Me and C*H*CH_2Ph), 4.96 (1H, app t, *J*=5.8 Hz, O*H*), 4.35–4.33 (1H, m, C*H*$(CH_2)_3$N), 4.24 (1H, m, C*H*CH_3), 3.86 (1H, ddd, *J*=11.3, 5.7, 5.7 Hz, CH_2OH), 3.79 (1H, ddd, *J*=11.4, 5.0, 5.0 Hz, CH_2OH), 3.65 (3H, s, OCH_3), 3.57 (1H, m, CH_2N), 3.46 (1H, m, CH_2N), 3.18 (1H, dd, *J*=14.0, 4.8 Hz, CH_2Ph), 2.95–2.88 (1H, m, CH_2Ph), 1.99–1.65 (4H, m, CH_2), 1.38 (9H, s, C(CH_3)$_3$), 1.18 (3H, d, *J*=7.1 Hz, CHCH_3); δ_C (100 MHz, d_6-DMSO, 333 K) 204.6 (s), 171.4 (s), 170.3 (s), 168.8 (s), 154.6 (s), 137.1 (s), 128.9 (d), 127.6 (d), 125.9 (d), 77.8 (s), 60.4 (d), 60.2 (t), 59.3 (d), 58.7 (d), 51.6 (q), 47.5 (d), 46.2 (t), 40.4 (t), 27.9 (t), 27.9 (q), 23.9 (t), 16.9 (q); *m*/*z* (ES) 573.2357 ([M+Na]$^+$, $C_{26}H_{38}N_4O_7SNa$ requires 573.2359).

4.1.5. Boc-Ile-Ala-Pro-PheΨ{(C=S)NH}-Ser-OMe (6). Acetyl chloride (1.5 ml) was added dropwise over 2 min to a stirred solution of the tetrapeptide **13** (1.68 g, 3.06 mmol) in methanol (15 ml) at 0°C under an atmosphere of nitrogen. The solution was stirred at room temperature for 11 h and then evaporated in vacuo to leave the corresponding hydrochloride salt (1.46 g, 98%) as a cream solid, which was used without further purification.

Diisopropylethylamine (1.8 ml, 11 mmol) was added dropwise over 2 min to a stirred solution of the hydrochloride salt (1.46 g, 3.00 mmol) in dichloromethane (15 ml) at 0°C under an atmosphere of nitrogen. The solution was stirred at 0°C for 15 min and then Boc-L-isoleucine (0.69 g, 3.0 mmol) and 1-hydroxybenzotriazole (0.45 g, 3.3 mmol) were added successively in one portion. The suspension was stirred at 0°C for a further 15 min and then dicyclohexylcarbodiimide (0.68 g, 3.3 mmol) was added in one portion. The mixture was allowed to warm to room temperature over 51 h and then evaporated in vacuo to leave a residue which was taken up in ethyl acetate (50 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 20% acetone in diethyl ether as eluent to give the pentapeptide (1.59 g, 80%) as a cream foam, mp 111–113°C; $[\alpha]_D^{24}=-54$ (*c* 0.93, $CHCl_3$); found %: C, 57.6; H, 7.5; N, 10.3; $C_{32}H_{49}N_5O_8S$ requires %: C, 57.9; H, 7.4; N 10.55; ν_{max} ($CHCl_3$)/cm^{-1} 3435, 2969, 2935, 2879, 1745, 1652, 1603, 1552, 1493, 1456, 1392, 1368, 1346, 1156, 1065, 976, 864; δ_H (400 MHz, d_6-DMSO, 343 K) 10.01 (1H, br s, N*H*C=S), 7.75 (1H, d, *J*=7.4 Hz, N*H*CHCH_3), 7.66 (1H, d, *J*=7.8 Hz,

N*H*CHCH$_2$Ph), 7.29–7.17 (5H, m, Ph-*H*), 6.41 (1H, br s, N*H*Boc), 5.06–4.99 (3H, m, C*H*CO$_2$Me, C*H*CH$_2$Ph and O*H*), 4.57–4.54 (1H, m, C*H*CH$_3$), 4.32–4.30 (1H, m, C*H*(CH$_2$)$_3$N), 3.86–3.77 (3H, m, CH_2OH and C*H*NHBoc), 3.65 (3H, s, OCH_3), 3.65–3.58 (1H, m, CH_2N), 3.48–3.44 (1H, m, CH_2N), 3.17 (1H, dd, J=14.0, 4.7 Hz, CH_2Ph), 2.94–2.88 (1H, m, CH_2Ph), 1.90–1.71 (5H, m, CH_2 and C*H*(CH$_3$)CH$_2$CH$_3$), 1.44–1.34 (1H, masked m, CH_2CH$_3$), 1.39 (9H, s, C(CH_3)$_3$), 1.22 (3H, d, J=6.8 Hz, CHCH_3), 1.14–1.05 (1H, m, CH_2CH$_3$), 0.83 (3H, d, J=7.2 Hz, CH(CH_3)CH$_2$CH$_3$), 0.82 (3H, app t, J=7.3 Hz, CH$_2$CH_3); δ_C (125 MHz, d$_6$-DMSO, 333 K) 204.4 (s), 170.6 (s), 170.4 (s), 170.2 (s), 168.8 (s), 154.9 (s), 137.1 (s), 128.9 (d), 127.5 (d), 125.9 (d), 77.9 (s), 60.4 (d), 60.2 (t), 59.3 (d), 58.6 (d), 58.6 (d), 51.5 (q), 46.3 (t), 45.8 (d), 40.4 (t), 36.5 (d), 28.0 (t), 27.9 (q), 24.1 (t), 23.8 (t), 17.0 (q), 15.1 (q), 10.6 (q); *m/z* (ES) 686.3242 ([M+Na]$^+$, $C_{32}H_{49}N_5O_8SNa$ requires 686.3200).

4.1.6. (2*S*,3*S*)-Methyl 1-trityl-3-methylaziridine-2-carboxylate (15).[19] Triethylamine (6.6 ml, 47 mmol) was added dropwise over 5 min to a stirred suspension of threonine methyl ester hydrochloride **14** (4.00 g, 23.6 mmol) in dichloromethane (70 ml) at room temperature under an atmosphere of nitrogen. On dissolution, the solution was cooled to 0°C and then triphenylmethyl chloride (6.57 g, 23.6 mmol) was added portionwise over 5 min. The suspension was stirred at room temperature for 19 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 threonine methyl ester (8.50 g, 96%)[19b] as a cream foam; δ_H (360 MHz, CDCl$_3$) 7.49–7.46 (6H, m, Ph-*H*), 7.29–7.17 (9H, m, Ph-*H*), 3.79 (1H, dq, J=7.4, 6.2 Hz, C*H*OH), 3.38 (1H, d, J=7.5 Hz, C*H*CO$_2$Me), 3.16 (3H, s, OCH_3), 1.20 (3H, d, J=6.2 Hz, CH_3); δ_C (90 MHz, CDCl$_3$) 173.7 (s), 145.5 (s), 129.0 (d), 128.0 (d), 126.7 (d), 70.8 (s), 69.8 (d), 62.6 (d), 51.8 (q), 19.0 (q); *m/z* (ES) 398.1721 ([M+Na]$^+$, $C_{24}H_{25}NO_3Na$ requires 398.1732), which was used without further purification.

Triethylamine (6.9 ml, 50 mmol) was added dropwise over 5 min to a stirred solution of trityl threonine methyl ester (8.48 g, 22.6 mmol) in tetrahydrofuran (60 ml) at 0°C under an atmosphere of nitrogen. Methanesulfonyl chloride (1.8 ml, 23 mmol) was added dropwise over 2 min and the solution was stirred at 0°C for 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 (50 ml) and washed with 10% aqueous citric acid solution (3×10 ml) followed by saturated aqueous sodium bicarbonate solution (2×10 ml), with backwashing. The combined organic extracts were dried and evaporated in vacuo to leave the *aziridine* (7.79 g, 97%) as a cream solid; δ_H (360 MHz, CDCl$_3$) 7.53–7.51 (6H, m, Ph-*H*), 7.30–7.18 (9H, m, Ph-*H*), 3.74 (3H, s, OCH_3), 1.88 (1H, d, J=6.5 Hz, C*H*CO$_2$Me), 1.63 (1H, dq, J=6.4, 5.5 Hz, C*H*CH$_3$), 1.37 (3H, d, J=5.4 Hz, CH_3); δ_C (90 MHz, CDCl$_3$) 170.8 (s), 144.0 (s), 129.5 (d), 127.7 (d), 126.9 (d), 75.1 (s), 51.9 (q), 36.0 (d), 34.9 (d), 13.4 (q); *m/z* (ES) 380.1623 ([M+Na]$^+$, $C_{24}H_{23}NO_2Na$ requires 380.1626), which was used without further purification.

4.1.7. (2*S*,3*S*)-Methyl 1-(2,2,2-trichloro-1,1-dimethylethoxycarbonyl)-3-methylaziridine-2-carboxylate (16). Trifluoroacetic acid (0.58 ml, 7.5 mmol) was added dropwise over 1 min to a stirred solution of the aziridine **15** (1.33 g, 3.73 mmol) in dichloromethane (20 ml) and methanol (0.15 ml, 3.7 mmol) at 0°C under an atmosphere of nitrogen. The solution was stirred at 0°C for 30 min and then triethylamine (2.6 ml, 19 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] (1.31 g, 4.11 mmol) in dichloromethane (5 ml) was added over 5 min by cannula. The solution was warmed to room temperature, stirred for 4.5 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 (1.06 g, 89%) as a cream solid; mp 55–58°C (chloroform); $[\alpha]_D^{16}$=−63 (*c* 0.72, CHCl$_3$); ν_{max} (CHCl$_3$)/cm^{-1} 2955, 1733, 1458, 1388, 1370, 1299, 1154, 1128, 1083, 1050, 1027, 971, 896, 877; δ_H (400 MHz, CDCl$_3$) 3.80 (3H, s, OCH_3), 3.21 (1H, d, J=6.5 Hz, C*H*CO$_2$Me), 2.85 (1H, dq, J=6.5, 5.7 Hz, C*H*CH$_3$), 1.92 (3H, s, CH_3), 1.91 (3H, s, CH_3), 1.38 (3H, d, J=5.7 Hz, CHCH_3); δ_C (100 MHz, CDCl$_3$) 167.6 (s), 158.8 (s), 105.8 (s), 89.6 (s), 52.6 (q), 40.0 (d), 39.2 (d), 21.3 (q), 21.2 (q), 12.8 (q); *m/z* (ES) 339.9918 ([M+Na]$^+$, $C_{10}H_{14}NO_4Cl_3Na$ requires 339.9886).

4.1.8. TcBoc-Thr(dimethylallyl)-OMe (17). Boron trifluoride diethyl etherate (1.6 ml, 13 mmol) was added dropwise over 2 min to a stirred solution of the aziridine **16** (4.00 g, 12.6 mmol) in 2-methyl-3-buten-2-ol (60 ml) at 0°C under an atmosphere of nitrogen. The solution was warmed to room temperature, stirred for 46 h and then diluted with dichloromethane (50 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 (3.21 g, 63%) as a pale yellow oil; $[\alpha]_D^{20}$=+13 (*c* 1.01, CHCl$_3$); ν_{max} (CHCl$_3$)/cm^{-1} 3435, 2980, 2953, 1750, 1724, 1602, 1493, 1458, 1386, 1370, 1314, 1153, 1118, 1090, 1069, 1000, 961, 899; δ_H (360 MHz, C$_6$D$_6$, 343 K) 5.67 (1H, dd, J=17.6, 10.8 Hz, C*H*=CH$_2$), 5.64 (1H, masked br s, N*H*), 4.90 (1H, dd, J=17.7, 1.0 Hz, CH=C*H*$_2$), 4.86 (1H, dd, J=10.8, 1.1 Hz, CH=C*H*$_2$), 4.33 (1H, m, C*H*CO$_2$Me), 4.02 (1H, dq, J=6.3, 2.4 Hz, C*H*CH$_3$), 3.35 (3H, s, OCH_3), 1.91 (3H, s, Cl$_3$CC(CH_3)$_2$), 1.91 (3H, s, Cl$_3$CC(CH_3)$_2$), 1.08 (3H, d, J=6.3 Hz, CHCH_3), 1.04 (3H, s, CH_3), 1.03 (3H, s, CH_3); δ_C (90 MHz, C$_6$D$_6$, 333 K) 171.0 (s), 154.8 (s), 144.2 (d), 113.5 (t), 107.1 (s), 88.7 (s), 75.9 (s), 68.6 (d), 60.3 (d), 51.5 (q), 26.5 (q), 26.2 (q), 21.9 (q), 20.6 (q); *m/z* (ES) 426.0601 ([M+Na]$^+$, $C_{15}H_{24}NO_5Cl_3Na$ requires 426.0618).

4.1.9. TcBoc-Ser(dimethylallyl)-Ile-Ala-Pro-PheΨ{(C=S)NH}-Ser-OMe (18). Acetyl chloride (0.9 ml) was added dropwise over 1 min to a stirred solution of the pentapeptide **6** (250 mg, 0.377 mmol) in methanol (9 ml) at 0°C under an atmosphere of nitrogen. The solution was warmed to room temperature, stirred for 3.5 h and then

evaporated in vacuo to leave the corresponding hydrochloride salt (226 mg) as a cream solid, which was used without further purification.

Diisopropylethylamine (229 μl, 1.32 mmol) was added dropwise over 1 min to a stirred solution of the hydrochloride salt (226 mg, 0.377 mmol) in dichloromethane (6 ml) at 0°C under an atmosphere of nitrogen. The solution was stirred at 0°C for 10 min and then the carboxylic acid **7**[10] (141 mg, 0.377 mmol) in dichloromethane (4 ml) was added dropwise over 5 min by cannula followed by 1-hydroxybenzotriazole (56 mg, 0.41 mmol) in one portion. The suspension was stirred at 0°C for a further 15 min and then 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (80 mg, 0.41 mmol) was added in one portion. The mixture was allowed to warm to room temperature over the course of 23 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 ethyl acetate as eluent to give the hexapeptide (249 mg, 72%) as a cream foam; $[\alpha]_D^{22}=-35$ (*c* 1.13, $CHCl_3$); found %: C, 51.65; H, 6.3; N, 8.8; $C_{40}H_{59}N_6O_{10}SCl_3$ requires %: C, 52.1; H, 6.45; N 9.1; ν_{max} ($CHCl_3$)/cm^{-1} 3416, 3331, 2974, 2879, 1727, 1652, 1550, 1489, 1456, 1386, 1370, 1147, 1064, 1001, 904, 872, 838; δ_H (360 MHz, d_6-DMSO, 343 K) 9.97 (1H, br s, N*H*C=S), 7.93 (1H, br s, N*H*CHCH$_3$), 7.64 (1H, br s, N*H*CHCH$_2$Ph), 7.46 (1H, br s, N*H*CHCH(CH$_3$)CH$_2$CH$_3$), 7.28–7.18 (5H, m, Ph-*H*), 6.89 (1H, br s, N*H*TcBoc), 5.83 (1H, dd, *J*=17.6, 10.8 Hz, C*H*=CH$_2$), 5.14 (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*$_2$), 5.05–4.97 (2H, m, C*H*CO$_2$Me and C*H*CH$_2$Ph), 4.95 (1H, app t, *J*=5.6 Hz, O*H*), 4.51 (1H, m, C*H*CH$_3$), 4.33–4.31 (1H, m, C*H*(CH$_2$)$_3$N), 4.26 (1H, dd, *J*=8.4, 7.4 Hz, C*H*CH(CH$_3$)CH$_2$CH$_3$), 4.11 (1H, app dt, *J*=8.3, 6.1 Hz, C*H*NHTcBoc), 3.86 (1H, ddd, *J*=11.3, 5.6, 5.6 Hz, C*H*$_2$OH), 3.79 (1H, ddd, *J*=11.3, 4.8, 4.8 Hz, C*H*$_2$OH), 3.68–3.57 (1H, m, C*H*$_2$N), 3.65 (3H, s, OC*H*$_3$), 3.52–3.44 (3H, m, C*H*$_2$OAllyl and C*H*$_2$N), 3.18 (1H, dd, *J*=14.0, 4.8 Hz, C*H*$_2$Ph), 2.95–2.89 (1H, m, C*H*$_2$Ph), 1.96–1.66 (5H, m, C*H*$_2$ and CH(CH$_3$)C*H*$_2$CH$_3$), 1.84 (6H, s, Cl$_3$CC(C*H*$_3$)$_2$), 1.48–1.38 (1H, m, C*H*$_2$CH$_3$), 1.23–1.21 (3H, masked d, CHC*H*$_3$), 1.21 (6H, s, C*H*$_3$), 1.14–1.02 (1H, m, C*H*$_2$CH$_3$), 0.84 (3H, d, *J*=6.7 Hz, CH(C*H*$_3$)CH$_2$CH$_3$), 0.82 (3H, app t, *J*=7.3 Hz, CH$_2$C*H*$_3$); δ_C (125 MHz, d_6-DMSO, 323 K) 204.7 (s), 170.6 (s), 170.3 (s), 169.9 (s), 168.9 (s), 168.7 (s), 153.3 (s), 143.4 (d), 137.2 (s), 129.0 (d), 127.6 (d), 126.0 (d), 113.5 (t), 106.3 (s), 86.9 (s), 74.9 (s), 62.2 (t), 60.5 (d), 60.3 (t), 59.3 (d), 58.6 (d), 56.3 (d), 55.1 (d), 51.7 (q), 46.4 (t), 46.0 (d), 40.5 (t), 37.0 (d), 28.1 (t), 25.3 (q), 25.1 (q), 23.9 (t), 23.9 (t), 21.2 (q), 16.8 (q), 15.0 (q), 10.7 (q); *m/z* (ES) 943.2987 ([M+Na]$^+$, $C_{40}H_{59}N_6O_{10}SCl_3Na$ requires 943.2977).

4.1.10. TcBoc-Thr(dimethylallyl)-Ser(dimethylallyl)-Ile-Ala-Pro-PheΨ{(C=S)NH}-Ser-OMe (5). An aqueous solution of lithium hydroxide (1.5 M, 0.91 ml, 1.4 mmol) was added dropwise over 1 min to a stirred solution of the allyl ether **17** (55 mg, 0.14 mmol) in tetrahydrofuran (2 ml) and methanol (0.2 ml) at 0°C. The solution was warmed to room temperature, stirred for 2 h and then evaporated in vacuo. The residue was taken up in ethyl acetate (5 ml) and water (5 ml), cooled to 0°C and then acidified with 2 M aqueous hydrochloric acid solution with vigorous stirring. The mixture was stirred at 0°C for 5 min, the layers were separated, and the aqueous layer was then extracted with ethyl acetate (7×3 ml). The combined organic extracts were dried and evaporated in vacuo to leave the corresponding carboxylic acid (46 mg, 87%) as a colourless oil, which was used without further purification.

Cadmium–lead couple[11] (709 mg, 5.70 mmol cadmium, 10% lead) was added in one portion to a rapidly stirred solution of the hexapeptide **18** (105 mg, 0.114 mmol) in tetrahydrofuran (1 ml) and 1N aqueous ammonium acetate solution (1 ml) at room temperature. The mixture was stirred vigorously at room temperature for 30 min and then filtered, washing with water (3×2 ml) and ethyl acetate (5×2 ml). The filtrate was cooled to 0°C, then basified with saturated aqueous sodium bicarbonate solution and stirred at 0°C for 5 min. The layers were then separated and the aqueous layer was extracted with ethyl acetate (6×5 ml). The combined organic extracts were dried and evaporated in vacuo to leave the amine (74 mg, 91%) as a white solid, which was used without further purification.

A solution of the carboxylic acid **8** (45 mg, 0.12 mmol) in dichloromethane (2 ml) was added dropwise over 2 min by cannula to a stirred solution of the amine (74 mg, 0.10 mmol) in dichloromethane (2 ml) at 0°C under an atmosphere of nitrogen. 1-Hydroxybenzotriazole (16 mg, 0.12 mmol) was added, and the suspension was stirred at 0°C for 15 min and then 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (22 mg, 0.12 mmol) was added. The mixture was allowed to warm to room temperature over the course of 41 h and then evaporated in vacuo. The residue was taken up in ethyl acetate (5 ml) and washed with 10% aqueous citric acid solution (3×3 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 ethyl acetate as eluent to give the heptapeptide (91 mg, 81%) as a cream foam; $[\alpha]_D^{22}=-18$ (*c* 1.06, $CHCl_3$); found %: C, 53.4; H, 6.7; N, 8.75; $C_{49}H_{74}N_7O_{12}SCl_3$ requires %: C, 53.9; H, 6.8; N 9.0; ν_{max} ($CHCl_3$)/cm^{-1} 3412, 3326, 2978, 2934, 2879, 1723, 1674, 1546, 1488, 1456, 1383, 1148, 1064, 986, 873; δ_H (360 MHz, d_6-DMSO, 343 K) 9.98 (1H, d, *J*=6.7 Hz, N*H*C=S), 7.86 (1H, br s, N*H*CHCH$_3$), 7.71–7.63 (2H, m, N*H*CHCH$_2$OAllyl and N*H*CHCH$_2$Ph), 7.47 (1H, d, *J*=8.9 Hz, N*H*CHCH(CH$_3$)-CH$_2$CH$_3$), 7.27–7.18 (5H, m, Ph-*H*), 6.40 (1H, d, *J*=8.4 Hz, N*H*TcBoc), 5.90 (1H, dd, *J*=17.6, 10.9 Hz, C*H*=CH$_2$), 5.81 (1H, dd, *J*=17.6, 10.8 Hz, C*H*=CH$_2$), 5.15 (1H, dd, *J*=17.6, 1.1 Hz, CH=C*H*$_2$), 5.13 (1H, dd, *J*=17.6, 1.2 Hz, CH=C*H*$_2$), 5.07 (1H, dd, *J*=10.8, 1.3 Hz, CH=C*H*$_2$), 5.07 (1H, dd, *J*=10.8, 1.2 Hz, CH=C*H*$_2$), 5.04–4.99 (2H, m, C*H*CO$_2$Me and C*H*CH$_2$Ph), 4.96 (1H, app t, *J*=5.9 Hz, O*H*), 4.51–4.42 (2H, m, C*H*CH$_3$ and C*H*CH$_2$OAllyl), 4.33–4.31 (1H, m, C*H*(CH$_2$)$_3$N), 4.26 (1H, dd, *J*=8.2, 7.6 Hz, C*H*CH(CH$_3$)CH$_2$CH$_3$), 4.06 (1H, dd, *J*=8.7, 4.3 Hz, C*H*NHTcBoc), 3.90–3.83 (1H, masked m, C*H*(CH$_3$)

OAllyl), 3.86 (1H, ddd, J=11.4, 5.6, 5.6 Hz, CH_2OH), 3.79 (1H, ddd, J=11.1, 5.3, 5.3 Hz, CH_2OH), 3.67–3.58 (1H, m, CH_2N), 3.65 (3H, s, OCH_3), 3.52–3.37 (2H, m, CH_2OAllyl and CH_2N), 3.50 (1H, dd, J=9.1, 4.9 Hz, CH_2OAllyl), 3.17 (1H, dd, J=14.0, 4.8 Hz, CH_2Ph), 2.95–2.88 (1H, m, CH_2Ph), 1.99–1.67 (5H, m, CH_2 and CH(CH$_3$)CH$_2$CH$_3$), 1.85 (6H, s, Cl$_3$CC(CH_3)$_2$), 1.48–1.38 (1H, m, CH_2CH$_3$), 1.25 (3H, s, CH_3), 1.23 (3H, s, CH_3), 1.23–1.20 (3H, masked d, CHCH_3), 1.20 (6H, s, CH_3), 1.13–1.02 (1H, m, CH_2CH$_3$), 1.07 (3H, d, J=6.2 Hz, CH(CH_3)OAllyl), 0.83 (3H, d, J=6.7 Hz, CH(CH_3)CH$_2$CH$_3$), 0.81 (3H, app t, J=7.3 Hz, CH$_2$CH_3); δ_C (90 MHz, d$_6$-DMSO, 323 K) 204.7 (s), 170.6 (s), 170.3 (s), 169.8 (s), 168.9 (s), 168.8 (s), 153.2 (s), 143.8 (d), 143.4 (d), 137.2 (s), 129.0 (d), 127.6 (d), 126.0 (d), 113.6 (t), 113.4 (t), 106.3 (s), 87.0 (s), 75.5 (s), 74.9 (s), 68.1 (d), 62.4 (t), 60.5 (d), 60.3 (t), 59.3 (d), 59.3 (d), 58.6 (d), 56.3 (d), 52.8 (d), 51.7 (q), 46.4 (t), 46.0 (d), 40.5 (t), 36.8 (d), 28.1 (t), 26.5 (q), 25.3 (q), 25.2 (q), 25.2 (q), 23.9 (t), 23.9 (t), 21.3 (q), 21.2 (q), 18.8 (q), 16.8 (q), 15.0 (q), 10.8 (q); m/z (ES) 1112.4109 ([M+Na]$^+$, C$_{49}$H$_{74}$N$_7$O$_{12}$SCl$_3$Na requires 1112.4079).

4.1.11. TcBoc-Thr(dimethylallyl)-Ser(dimethylallyl)-Ile-Ala-Pro-Phe-Ser(thiazoline)-OMe (20). Burgess' reagent[21] (9 mg, 0.04 mmol) was added in one portion to a stirred solution of the heptapeptide **5** (28 mg, 0.026 mmol) in tetrahydrofuran (5 ml) at room temperature under an atmosphere of nitrogen. The mixture was heated to reflux, stirred for 30 min and then evaporated in vacuo to leave the crude product which was purified by chromatography on silica using ethyl acetate as eluent to give the thiazoline (25 mg, 92%) as a cream foam; $[\alpha]_D^{20}=-19$ (c 0.93, CHCl$_3$); ν_{max} (CHCl$_3$)/cm^{-1} 3407, 2977, 2934, 2879, 1726, 1674, 1487, 1456, 1369, 1148, 1078, 1046, 986, 874; δ_H (360 MHz, d$_6$-DMSO, 343 K) 7.89 (1H, br s, NHCHCH$_2$Ph), 7.80 (1H, br s, NHCHCH$_3$), 7.64 (1H, br s, NHCHCH$_2$OAllyl), 7.44 (1H, d, J=8.4 Hz, NHCHCH(CH$_3$)CH$_2$CH$_3$), 7.27–7.18 (5H, m, Ph-H), 6.36 (1H, d, J=8.4 Hz, NHTcBoc), 5.90 (1H, dd, J=17.6, 10.8 Hz, CH=CH$_2$), 5.82 (1H, dd, J=17.6, 10.9 Hz, CH=CH$_2$), 5.15 (1H, d, J=17.6 Hz, CH=CH_2), 5.13 (1H, d, J=17.6 Hz, CH=CH_2), 5.12–5.07 (1H, masked dd, CHCH$_2$S), 5.07 (1H, d, J=10.9 Hz, CH=CH_2), 5.07 (1H, d, J=10.8 Hz, CH=CH_2), 4.85 (1H, app dt, J=7.6, 6.5 Hz, CHCH$_2$Ph), 4.50–4.44 (2H, m, CHCH$_3$ and CHCH$_2$OAllyl), 4.37–4.35 (1H, m, CH(CH$_2$)$_3$N), 4.27 (1H, dd, J=8.2, 7.2 Hz, CHCH(CH$_3$)CH$_2$CH$_3$), 4.07 (1H, dd, J=8.5, 4.2 Hz, CHNHTcBoc), 3.88 (1H, dq, J=6.2, 4.3 Hz, CH(CH$_3$)OAllyl), 3.71 (3H, s, OCH_3), 3.66–3.41 (6H, m, CH_2N, CH_2S and CH_2OAllyl), 3.17 (1H, dd, J=14.0, 5.4 Hz, CH_2Ph), 3.04–2.99 (1H, masked dd, CH_2Ph), 2.04–1.69 (5H, m, CH_2 and CH(CH$_3$)CH$_2$CH$_3$), 1.85 (6H, s, Cl$_3$CC(CH_3)$_2$), 1.45 (1H, m, CH_2CH$_3$), 1.25 (3H, s, CH_3), 1.24 (3H, s, CH_3), 1.24–1.21 (3H, masked d, CHCH_3), 1.21 (6H, s, CH_3), 1.14–1.03 (1H, m, CH_2CH$_3$), 1.08 (3H, d, J=6.2 Hz, CH(CH_3)OAllyl), 0.85–0.84 (3H, masked d, CH(CH_3)CH$_2$CH$_3$), 0.82 (3H, app t, J=7.5 Hz, CH$_2$CH_3); δ_C (125 MHz, d$_6$-DMSO, 323 K) 174.8 (s), 170.8 (s), 170.4 (s), 170.1 (s), 169.8 (s), 168.8 (s), 153.1 (s), 143.8 (d), 143.4 (d), 137.1 (s), 129.0 (d), 127.8 (d), 126.0 (d), 113.6 (t), 113.4 (t), 106.3 (s), 87.0 (s), 77.6 (d), 75.5 (s), 74.9 (s), 68.1 (d), 62.4 (t), 59.1 (d), 59.1 (d), 56.3 (d), 52.8 (d), 52.2 (d), 51.9 (q), 46.3 (t), 45.9 (d), 38.2 (t), 36.9 (d), 34.2 (t), 28.4 (t), 26.5 (q), 25.2 (q), 25.2 (q), 23.9 (t), 23.9 (t), 21.3 (q), 18.7 (q), 16.8 (q), 15.0 (q), 10.8 (q); m/z (ES) 1072.4138 ([M+H]$^+$, C$_{49}$H$_{73}$N$_7$O$_{11}$SCl$_3$ requires 1072.4154).

4.1.12. Boc-Pro-Phe-Ser-OMe (21). Acetyl chloride (5 ml) was added dropwise over 5 min to a stirred solution of the dipeptide **10** (4.24 g, 11.6 mmol) in methanol (50 ml) at 0°C under an atmosphere of nitrogen. The solution was warmed to room temperature, stirred for 3.5 h and then evaporated in vacuo to leave the corresponding hydrochloride salt (3.36 g, 96%) as a cream solid, which was used without further purification.

Diisopropylethylamine (6.8 ml, 39 mmol) was added dropwise over 5 min to a stirred solution of the hydrochloride salt (3.36 g, 11.1 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 Boc-L-proline (2.39 g, 11.1 mmol) and 1-hydroxybenzotriazole (1.65 g, 12.2 mmol) were added successively in one portion. The suspension was stirred at 0°C for a further 15 min and then dicyclohexylcarbodiimide (2.52 g, 12.2 mmol) was added in one portion. The mixture was allowed to warm to room temperature over the course of 52 h and then evaporated in vacuo to leave a residue which was taken up in ethyl acetate (50 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 50% ethyl acetate in diethyl ether as eluent to give the tripeptide (4.50 g, 87%) as a colourless foam; $[\alpha]_D^{25}=-86$ (c 1.13, CHCl$_3$); ν_{max} (CHCl$_3$)/cm^{-1} 3410, 2980, 2955, 2885, 1746, 1682, 1603, 1552, 1496, 1455, 1393, 1369, 1158, 1129, 1078, 977, 888; δ_H (360 MHz, CDCl$_3$, 323 K) 7.33–7.20 (5H, m, Ph-H), 7.04 (1H, br s, NHCHCH$_2$Ph), 6.54 (1H, d, J=8.1 Hz, NHCHCH$_2$OH), 4.76–4.71 (1H, m, CHCH$_2$Ph), 4.57 (1H, app dt, J=7.6, 3.8 Hz, CHCO$_2$Me), 4.22 (1H, dd, J=6.6, 5.6 Hz, CHNBoc), 3.92 (1H, ddd, J=11.6, 7.7, 3.9 Hz, CH_2OH), 3.86 (1H, ddd, J=11.6, 6.1, 3.9 Hz, CH_2OH), 3.76 (3H, s, OCH_3), 3.40–3.34 (1H, m, CH_2NBoc), 3.30 (1H, ddd, J=10.7, 7.8, 4.0 Hz, CH_2NBoc), 3.17–3.12 (2H, m, CH_2Ph), 2.09–2.01 (2H, m, CH_2), 1.86–1.78 (1H, m, CH_2), 1.67–1.60 (1H, m, CH_2), 1.41 (9H, s, C(CH_3)$_3$); δ_C (90 MHz, CDCl$_3$) 172.2 (s), 171.0 (s), 170.5 (s), 155.6 (s), 136.5 (s), 129.3 (d), 128.6 (d), 127.0 (d), 81.1 (s), 62.6 (t), 60.8 (d), 55.0 (d), 53.5 (d), 52.5 (q), 47.3 (t), 36.9 (t), 29.4 (t), 28.3 (q), 24.3 (t); m/z (ES) 486.2172 ([M+Na]$^+$, C$_{23}$H$_{33}$N$_3$O$_7$Na requires 486.2216).

4.1.13. Boc-Ala-Pro-Phe-Ser-OMe (22). Acetyl chloride (9 ml) was added dropwise over 10 min to a stirred solution of the tripeptide **21** (4.13 g, 8.93 mmol) in methanol (60 ml) at 0°C under an atmosphere of nitrogen. The solution was warmed to room temperature, stirred for 3 h and then evaporated in vacuo to leave the corresponding hydrochloride salt (3.53 g, 99%) as a white solid, which was used without further purification.

Diisopropylethylamine (5.4 ml, 31 mmol) was added dropwise over 5 min to a stirred solution of the hydrochloride salt (3.53 g, 8.84 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 Boc-L-alanine (1.67 g, 8.84 mmol) and 1-hydroxybenzotriazole (1.31 g, 9.72 mmol) were added successively in one portion. The suspension was stirred at 0°C for a further 15 min and then dicyclohexylcarbodiimide (2.01 g, 9.72 mmol) was added in one portion. The mixture was allowed to warm to room temperature over the course of 47 h and then evaporated in vacuo to leave a residue which was taken up in dichloromethane (150 ml) and filtered. The filtrate was washed with 10% aqueous citric acid solution (3×30 ml) followed by saturated aqueous sodium bicarbonate solution (5×30 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 tetrapeptide (4.12 g, 87%) as a colourless solid; mp 192–193°C (ethyl acetate); $[\alpha]_D^{23}=-92$ (*c* 0.51, $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3435, 2981, 2955, 1745, 1678, 1548, 1495, 1455, 1393, 1369, 1346, 1158, 1067, 863; δ_H (360 MHz, d_6-DMSO, 343 K) 7.84 (1H, d, *J*=7.6 Hz, N*H*CHCH$_2$OH), 7.56 (1H, br s, N*H*CHCH$_2$Ph), 7.28–7.16 (5H, m, Ph-*H*), 6.51 (1H, br s, N*H*Boc), 4.78 (1H, app t, *J*=5.8 Hz, O*H*), 4.55 (1H, app dt, *J*=8.3, 5.4 Hz, C*H*CH$_2$Ph), 4.37 (1H, app dt, *J*=7.7, 5.0 Hz, C*H*CH$_2$OH), 4.35–4.31 (1H, m, C*H*(CH$_2$)$_3$N), 4.25 (1H, br s, C*H*CH$_3$), 3.72 (1H, ddd, *J*=10.9, 5.5, 5.5 Hz, C*H*$_2$OH), 3.65 (1H, ddd, *J*=10.6, 5.6, 5.6 Hz, C*H*$_2$OH), 3.65 (3H, s, OC*H*$_3$), 3.59 (1H, m, C*H*$_2$N), 3.47 (1H, m, C*H*$_2$N), 3.12 (1H, dd, *J*=14.0, 5.0 Hz, C*H*$_2$Ph), 2.92–2.86 (1H, m, C*H*$_2$Ph), 2.01–1.70 (4H, m, C*H*$_2$), 1.38 (9H, s, C(C*H*$_3$)$_3$), 1.17 (3H, d, *J*=6.9 Hz, CHC*H*$_3$); δ_C (100 MHz, d_6-DMSO, 333 K) 171.4 (s), 170.8 (s), 170.5 (s), 170.4 (s), 154.7 (s), 137.4 (s), 128.9 (d), 127.6 (d), 125.9 (d), 77.8 (s), 61.0 (t), 59.5 (d), 54.5 (d), 53.2 (d), 51.4 (q), 47.5 (d), 46.3 (t), 36.8 (t), 28.2 (t), 27.9 (q), 24.1 (t), 16.7 (q); *m/z* (ES) 557.2600 ([M+Na]$^+$, $C_{26}H_{38}N_4O_8Na$ requires 557.2587).

4.1.14. Boc-Ile-Ala-Pro-Phe-Ser-OMe (23). Acetyl chloride (7.5 ml) was added dropwise over 5 min to a stirred solution of the tetrapeptide **22** (3.80 g, 7.12 mmol) in methanol (50 ml) at 0°C under an atmosphere of nitrogen. The solution was warmed to room temperature, stirred for 4 h and then evaporated in vacuo to leave the corresponding hydrochloride salt (3.34 g) as a cream solid, which was used without further purification.

Diisopropylethylamine (4.3 ml, 25 mmol) was added dropwise over 5 min to a stirred solution of the hydrochloride salt (3.34 g, 7.10 mmol) in dichloromethane (40 ml) at 0°C under an atmosphere of nitrogen. The solution was stirred at 0°C for 15 min and then Boc-L-isoleucine (1.64 g, 7.10 mmol) and 1-hydroxybenzotriazole (1.06 g, 7.81 mmol) were added successively in one portion. The suspension was stirred at 0°C for a further 15 min and then dicyclohexylcarbodiimide (1.61 g, 7.81 mmol) was added in one portion. The mixture was allowed to warm to room temperature over the course of 28 h and then evaporated in vacuo to leave a residue which was taken up in ethyl acetate (50 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 20% acetone in ethyl acetate as eluent to give the pentapeptide (3.73 g, 81%) as a colourless solid; mp 178–180°C (ethyl acetate); $[\alpha]_D^{23}=-77$ (*c* 0.50, $CHCl_3$); ν_{max} ($CHCl_3$)/cm^{-1} 3420, 2970, 2878, 1746, 1673, 1548, 1494, 1455, 1368, 1156, 1078, 865; δ_H (360 MHz, d_6-DMSO, 343 K) 7.85 (1H, d, *J*=7.8 Hz, N*H*CHCH$_2$OH), 7.75 (1H, d, *J*=7.4 Hz, N*H*CHCH$_3$), 7.55 (1H, br s, N*H*CHCH$_2$Ph), 7.28–7.16 (5H, m, Ph-*H*), 6.37 (1H, br s, N*H*Boc), 4.77 (1H, br s, O*H*), 4.60–4.54 (1H, masked m, C*H*CH$_3$), 4.57 (1H, app dt, *J*=8.3, 5.1 Hz, C*H*CH$_2$Ph), 4.37 (1H, app dt, *J*=7.7, 5.0 Hz, C*H*CH$_2$OH), 4.30 (1H, m, C*H*(CH$_2$)$_3$N), 3.85 (1H, dd, *J*=8.2, 7.8 Hz, C*H*NHBoc), 3.72 (1H, dd, *J*=10.9, 5.3 Hz, C*H*$_2$OH), 3.68–3.65 (1H, masked dd, C*H*$_2$OH), 3.65 (3H, s, OC*H*$_3$), 3.65–3.59 (1H, m, C*H*$_2$N), 3.48 (1H, m, C*H*$_2$N), 3.12 (1H, dd, *J*=14.0, 5.2 Hz, C*H*$_2$Ph), 2.92–2.85 (1H, m, C*H*$_2$Ph), 1.99–1.68 (5H, m, C*H*$_2$ and C*H*(CH$_3$)CH$_2$CH$_3$), 1.45–1.39 (1H, m, CH$_2$CH$_3$), 1.39 (9H, s, C(C*H*$_3$)$_3$), 1.20 (3H, d, *J*=6.8 Hz, CHC*H*$_3$), 1.17–1.05 (1H, m, CH$_2$CH$_3$), 0.83 (3H, d, *J*=7.2 Hz, CH(C*H*$_3$)CH$_2$CH$_3$), 0.82 (3H, app t, *J*=7.4 Hz, CH$_2$C*H*$_3$); δ_C (100 MHz, d_6-DMSO, 333 K) 170.7 (s), 170.6 (s), 170.5 (s), 170.4 (s), 154.9 (s), 137.4 (s), 128.9 (d), 127.6 (d), 125.9 (d), 77.9 (s), 61.1 (t), 59.5 (d), 58.6 (d), 54.5 (d), 53.2 (d), 51.4 (q), 46.4 (t), 46.0 (d), 36.9 (t), 36.5 (d), 28.3 (t), 27.9 (q), 24.1 (t), 24.0 (t), 16.8 (q), 15.1 (q), 10.7 (q); *m/z* (ES) 670.3419 ([M+Na]$^+$, $C_{32}H_{49}N_5O_9Na$ requires 670.3428).

4.1.15. TcBoc-Ser(dimethylallyl)-Ile-Ala-Pro-Phe-Ser-OMe (24). Acetyl chloride (0.75 ml) was added dropwise over 1 min to a stirred solution of the pentapeptide **23** (500 mg, 0.773 mmol) in methanol (5 ml) at 0°C under an atmosphere of nitrogen. The solution was warmed to room temperature, stirred for 3.5 h and then evaporated in vacuo to leave the corresponding hydrochloride salt (450 mg) as a cream solid, which was used without further purification.

Diisopropylethylamine (0.47 ml, 2.7 mmol) was added dropwise over 1 min to a stirred solution of the hydrochloride salt (450 mg, 0.771 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 a solution of the carboxylic acid **7**[10] (277 mg, 0.736 mmol) in dichloromethane (1 ml) was added over 1 min by cannula followed by 1-hydroxybenzotriazole (115 mg, 0.848 mmol) in one portion. The suspension was stirred at 0°C for a further 15 min and then 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (163 mg, 0.848 mmol) was added in one portion. The mixture was allowed to warm to room temperature over the course of 43 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 10% acetone in ethyl acetate as eluent to give the hexapeptide (397 mg, 60%) as a colourless foam; $[\alpha]_D^{22}=-58$ (*c* 0.51, $CHCl_3$); found %: C, 52.9; H, 6.5; N, 9.1; $C_{40}H_{59}N_6O_{11}Cl_3$ requires %: C, 53.0; H, 6.6; N, 9.3; ν_{max} ($CHCl_3$)/cm^{-1} 3418, 2977, 2879, 1726, 1668, 1551, 1489, 1456, 1370, 1145, 1076, 905; δ_H (360 MHz, d_6-DMSO, 323 K) 8.06 (1H, br s, N*H*CHCH$_3$), 7.98 (1H,

d, J=7.6 Hz, N*H*CHCH$_2$OH), 7.63 (1H, d, J=8.1 Hz, N*H*CHCH$_2$Ph), 7.55 (1H, br s, N*H*CHCH(CH$_3$)CH$_2$CH$_3$), 7.27–7.16 (5H, m, Ph-*H*), 7.09 (1H, br s, N*H*TcBoc), 5.82 (1H, dd, J=17.6, 10.8 Hz, C*H*=CH$_2$), 5.13 (1H, dd, J=17.6, 1.2 Hz, CH=C*H*$_2$), 5.07 (1H, dd, J=10.8, 1.1 Hz, CH=C*H*$_2$), 4.90 (1H, app t, J=5.8 Hz, O*H*), 4.55 (1H, app dt, J=8.5, 5.0 Hz, C*H*CH$_2$Ph), 4.51–4.47 (1H, m, C*H*CH$_3$), 4.36 (1H, app dt, J=7.6, 5.0 Hz, C*H*CH$_2$OH), 4.28–4.22 (2H, m, C*H*(CH$_2$)$_3$N and C*H*CH(CH$_3$)CH$_2$CH$_3$), 4.16–4.03 (1H, m, C*H*NHTcBoc), 3.72 (1H, ddd, J=11.1, 5.6, 5.6 Hz, C*H*$_2$OH), 3.67–3.57 (2H, m, C*H*$_2$OH and C*H*$_2$N), 3.64 (3H, s, OC*H*$_3$), 3.50–3.41 (3H, m, C*H*$_2$N and C*H*$_2$OAllyl), 3.10 (1H, dd, J=14.0, 4.8 Hz, C*H*$_2$Ph), 2.86 (1H, dd, J=14.0, 8.9 Hz, C*H*$_2$Ph), 1.94–1.70 (5H, m, C*H*$_2$ and C*H*(CH$_3$)-CH$_2$CH$_3$), 1.83 (6H, s, Cl$_3$CC(C*H*$_3$)$_2$), 1.47–1.37 (1H, m, C*H*$_2$CH$_3$), 1.19 (6H, s, C*H*$_3$), 1.19 (3H, d, J=6.5 Hz, CHC*H*$_3$), 1.12–1.02 (1H, m, C*H*$_2$CH$_3$), 0.82 (3H, d, J=6.7 Hz, CH(C*H*$_3$)CH$_2$CH$_3$), 0.80 (3H, app t, J=7.3 Hz, CH$_2$C*H*$_3$); δ_C (90 MHz, d$_6$-DMSO, 323 K) 170.8 (s), 170.6 (s), 170.5 (s), 170.0 (s), 168.8 (s), 153.4 (s), 143.4 (d), 137.5 (s), 129.0 (d), 127.7 (d), 125.9 (d), 113.6 (t), 106.3 (s), 87.0 (s), 74.9 (s), 62.2 (t), 61.1 (t), 59.5 (d), 56.3 (d), 55.1 (d), 54.5 (d), 53.2 (d), 51.5 (q), 46.5 (t), 46.1 (d), 36.9 (d), 36.9 (t), 28.4 (t), 25.3 (q), 25.2 (q), 24.1 (t), 23.9 (t), 21.3 (q), 16.5 (q), 15.0 (q), 10.8 (q); *m*/*z* (ES) 927.3165 ([M+Na]$^+$, C$_{40}$H$_{59}$N$_6$O$_{11}$Cl$_3$Na requires 927.3205).

4.1.16. TcBoc-Thr(dimethylallyl)-Ser(dimethylallyl)-Ile-Ala-Pro-Phe-Ser-OMe (25). An aqueous solution of lithium hydroxide (1.5 M, 4.0 ml, 6.0 mmol) was added dropwise over 5 min to a stirred solution of the allyl ether **17** (243 mg, 0.601 mmol) in tetrahydrofuran (5 ml) and methanol (0.25 ml) at 0°C. The solution was warmed to room temperature, stirred for 4 h and then evaporated in vacuo. The residue was taken up in diethyl ether (5 ml) and water (5 ml), cooled to 0°C and then acidified with 2 M aqueous hydrochloric acid solution with vigorous stirring. The mixture was stirred at 0°C for 10 min, the layers were separated, and the aqueous layer was then extracted with diethyl ether (5×2 ml). The combined organic extracts were dried and evaporated in vacuo to leave the corresponding carboxylic acid (180 mg, 77%) as a colourless gum, which was used without further purification.

Cadmium–lead couple[11] (4.15 g, 33.6 mmol cadmium, 10% lead) was added in one portion to a rapidly stirred solution of the hexapeptide **24** (608 mg, 0.671 mmol) in tetrahydrofuran (6 ml) and 1N aqueous ammonium acetate solution (6 ml) at room temperature. The mixture was stirred vigorously at room temperature for 1.5 h and then filtered, washing with water (5×3 ml) and ethyl acetate (5×3 ml). The filtrate was cooled to 0°C, then basified with saturated aqueous sodium bicarbonate solution and stirred at 0°C for 10 min. The layers were then separated and the aqueous layer was extracted with ethyl acetate (5×3 ml). The combined organic extracts were dried and evaporated in vacuo to leave the amine (442 mg, 94%) as a colourless solid, which was used without further purification.

A solution of the carboxylic acid **8** (180 mg, 0.461 mmol) in dichloromethane (4 ml) was added dropwise over 5 min by cannula to a stirred solution of the amine (317 mg, 0.452 mmol) in dichloromethane (6 ml) at 0°C under an atmosphere of nitrogen. 1-Hydroxybenzotriazole (69 mg, 0.51 mmol) was added in one portion and the suspension was stirred at 0°C for 10 min. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (97 mg, 0.51 mmol) was added in one portion and the mixture was allowed to warm to room temperature over the course of 26 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 10% acetone in ethyl acetate as eluent to give the heptapeptide (352 mg, 72%) as a cream foam; $[\alpha]_D^{20}=-34$ (c 0.55, CHCl$_3$); found %: C, 54.55; H, 6.9; N, 8.9; C$_{49}$H$_{74}$N$_7$O$_{13}$Cl$_3$ requires %: C, 54.7; H, 6.9; N, 9.1; ν_{max} (CHCl$_3$)/cm^{-1} 3412, 2979, 2878, 1722, 1673, 1551, 1488, 1456, 1381, 1147, 1078, 986, 872; δ_H (360 MHz, d$_6$-DMSO, 343 K) 7.88–7.84 (1H, masked d, N*H*CHCH$_3$), 7.86 (1H, d, J=7.7 Hz, N*H*CHCH$_2$OH), 7.67 (1H, br s, N*H*CHCH$_2$OAllyl), 7.55 (1H, br s, N*H*CHCH$_2$Ph), 7.46 (1H, d, J=8.5 Hz, N*H*CHCH(CH$_3$)CH$_2$CH$_3$), 7.27–7.16 (5H, m, Ph-*H*), 6.39 (1H, d, J=7.1 Hz, N*H*TcBoc), 5.90 (1H, dd, J=17.6, 10.8 Hz, C*H*=CH$_2$), 5.81 (1H, dd, J=17.6, 10.8 Hz, C*H*=CH$_2$), 5.15 (1H, dd, J=17.6, 1.2 Hz, CH=C*H*$_2$), 5.13 (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*$_2$), 5.07 (1H, dd, J=10.8, 1.3 Hz, CH=C*H*$_2$) 4.76 (1H, app t, J=5.8 Hz, O*H*), 4.56 (1H, app dt, J=8.3, 5.3 Hz, C*H*CH$_2$Ph), 4.51–4.42 (2H, m, C*H*CH$_3$ and C*H*CH$_2$OAllyl), 4.37 (1H, app dt, J=7.6, 5.0 Hz, C*H*CH$_2$OH), 4.29–4.25 (2H, m, C*H*(CH$_2$)$_3$N and C*H*CH(CH$_3$)CH$_2$CH$_3$), 4.07 (1H, dd, J=8.7, 4.3 Hz, C*H*NHTcBoc), 3.88 (1H, dq, J=6.3, 4.4 Hz, C*H*(CH$_3$)OAllyl), 3.72 (1H, ddd, J=11.0, 5.6, 5.6 Hz, C*H*$_2$OH), 3.68–3.57 (2H, m, C*H*$_2$OH and C*H*$_2$N), 3.65 (3H, s, OC*H*$_3$), 3.52–3.40 (2H, m, C*H*$_2$N and C*H*$_2$OAllyl), 3.50 (1H, dd, J=9.2, 5.0 Hz, C*H*$_2$OAllyl), 3.11 (1H, dd, J=14.0, 5.1 Hz, C*H*$_2$Ph), 2.92–2.85 (1H, m, C*H*$_2$Ph), 1.99–1.67 (5H, m, C*H*$_2$ and C*H*(CH$_3$)CH$_2$CH$_3$), 1.85 (6H, s, Cl$_3$CC(C*H*$_3$)$_2$), 1.50–1.38 (1H, m, C*H*$_2$CH$_3$), 1.25 (3H, s, C*H*$_3$), 1.23 (3H, s, C*H*$_3$), 1.20 (6H, s, C*H*$_3$), 1.20–1.19 (3H, masked d, CHC*H*$_3$), 1.13–1.03 (1H, m, C*H*$_2$CH$_3$), 1.07 (3H, d, J=6.2 Hz, CH(C*H*$_3$)OAllyl), 0.84 (3H, d, J=6.7 Hz, CH(C*H*$_3$)CH$_2$CH$_3$), 0.81 (3H, app t, J=7.4 Hz, CH$_2$C*H*$_3$); δ_C (100 MHz, d$_6$-DMSO, 323 K) 170.8 (s), 170.6 (s), 170.5 (s), 169.8 (s), 168.8 (s), 153.2 (s), 143.8 (d), 143.4 (d), 137.5 (s), 129.0 (d), 127.7 (d), 125.9 (d), 113.6 (t), 113.4 (t), 106.3 (s), 87.0 (s), 75.5 (s), 74.9 (s), 68.1 (d), 62.4 (t), 61.1 (t), 59.5 (d), 59.2 (d), 56.4 (d), 54.5 (d), 53.2 (d), 52.8 (d), 51.5 (q), 46.4 (t), 46.1 (d), 36.9 (t), 36.9 (d), 28.4 (t), 26.5 (q), 25.3 (q), 25.2 (q), 25.2 (q), 24.0 (t), 23.9 (t), 21.3 (q), 21.2 (q), 18.8 (q), 16.6 (q), 15.0 (q), 10.8 (q); *m*/*z* (ES) 1096.4287 ([M+Na]$^+$, C$_{49}$H$_{74}$N$_7$O$_{13}$Cl$_3$Na requires 1096.4308).

4.1.17. Cyclo[Thr(dimethylallyl)-Ser(dimethylallyl)-Ile-Ala-Pro-Phe-Ser] (26).[6a] Cadmium–lead couple[11] (281 mg, 2.27 mmol cadmium, 10% lead) was added in one portion to a rapidly stirred solution of the heptapeptide **25** (49 mg, 0.045 mmol) in tetrahydrofuran (0.5 ml) and 1N aqueous ammonium acetate solution (0.5 ml) at room temperature. The mixture was stirred vigorously at room temperature for 1 h and then filtered, washing with water (5×2 ml) and ethyl acetate (5×2 ml). The filtrate was cooled

to 0°C, then basified with saturated aqueous sodium bicarbonate solution and stirred at 0°C for 10 min. The layers were then 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 (38 mg, 96%) as a cream solid, which was used without further purification.

An aqueous solution of tetrabutylammonium hydroxide (40 wt%, 57 μl, 0.087 mmol) was added dropwise over 1 min to a stirred solution of the amine (38 mg, 0.044 mmol) in tetrahydrofuran (1.5 ml) at 0°C. The solution was stirred at 0°C for 3 h and then neutralised with 2 M aqueous hydrochloric acid solution (44 μl, 0.087 mmol). The mixture was stirred at 0°C for a further 10 min, then warmed to room temperature and evaporated in vacuo, azeotroping several times with toluene, to leave the amino acid, which was used without further purification.

Diisopropylethylamine (17 μl, 0.096 mmol) was added dropwise over 1 min to a stirred solution of the amino acid in dimethylformamide (8.7 ml) at −5°C under an atmosphere of nitrogen. The solution was stirred at −5°C for 15 min and then diphenylphosphoryl azide[14] (14 μl, 0.066 mmol) was added dropwise over 1 min. The mixture was stirred at −5°C for a further 5 min, stirring was then stopped, and the solution was allowed to slowly warm to room temperature and stood at room temperature for 7 days. Ethyl acetate (10 ml) was added and the solution was then poured into ice-cold water (20 ml). The layers were separated and the aqueous layer was extracted with ethyl acetate (5×5 ml). The combined organic extracts were washed with water (3×20 ml) and brine (1×20 ml), and then dried and evaporated in vacuo. The residue was purified by chromatography on silica using 40% acetone in ethyl acetate as eluent to give the cyclopeptide (13 mg, 35%, two steps) as a colourless solid, mp 167–169°C (lit mp 167–169°C); $[\alpha]_D^{20}=-80$ (*c* 0.61, $CHCl_3$), [lit $[\alpha]_D^{23}=-94$ (*c* 0.60, $CHCl_3$]; δ_H (360 MHz, $CDCl_3$) 7.88 (1H, d, *J*=6.8 Hz, N*H*CHCH$_2$OH), 7.34–7.13 (6H, m, Ph-*H* and N*H*CHCH$_2$ OAllyl), 7.09 (1H, d, *J*=7.7 Hz, N*H*CHCH$_2$Ph), 6.97 (1H, d, *J*=8.8 Hz, N*H*CHCH(CH$_3$)CH$_2$CH$_3$), 6.67 (1H, d, *J*=6.4 Hz, N*H*CHCH(CH$_3$)OAllyl), 6.49 (1H, d, *J*=5.2 Hz, N*H*CHCH$_3$), 5.77 (1H, dd, *J*=17.6, 10.9 Hz, C*H*=CH$_2$), 5.75 (1H, dd, *J*=17.5, 10.9 Hz, C*H*=CH$_2$), 5.21–5.09 (4H, m, CH=C*H*$_2$), 4.79 (1H, app dt, *J*=6.5, 3.3 Hz, C*H*CH$_2$ OH), 4.75–4.65 (2H, m, C*H*CH$_2$OAllyl and C*H*CH$_2$Ph), 4.46 (1H, dq, *J*=7.0, 5.6 Hz, C*H*CH$_3$), 4.35–4.33 (1H, m, C*H*(CH$_2$)$_3$N), 4.23 (1H, dq, *J*=6.4, 1.2 Hz, C*H*(CH$_3$) OAllyl), 4.19–4.03 (3H, m, C*H*CH(CH$_3$)CH$_2$CH$_3$, C*H*CH(CH$_3$)OAllyl and C*H*$_2$OH), 3.97–3.90 (1H, m, C*H*$_2$OH), 3.74 (1H, dd, *J*=9.2, 1.8 Hz, C*H*$_2$OAllyl), 3.62 (1H, dd, *J*=9.3, 2.0 Hz, O*H*), 3.42–3.33 (4H, m, C*H*$_2$OAllyl, C*H*$_2$N and C*H*$_2$Ph), 3.06–2.99 (1H, m, C*H*$_2$Ph), 2.34–2.29 (1H, m, C*H*$_2$), 1.95–1.52 (4H, m, C*H*$_2$ and C*H*(CH$_3$)CH$_2$CH$_3$), 1.36–1.10 (20H, m, C*H*$_3$, CHC*H*$_3$, CH(C*H*$_3$)OAllyl and C*H*$_2$CH$_3$), 0.99 (3H, d, *J*=6.6 Hz, CH(C*H*$_3$)CH$_2$CH$_3$), 0.94 (3H, app t, *J*=7.4 Hz, CH$_2$C*H*$_3$); δ_C (90 MHz, $CDCl_3$) 172.2 (s), 171.6 (s), 170.9 (s), 170.7 (s), 170.3 (s), 170.0 (s), 169.4 (s), 143.1 (d), 142.8 (d), 136.6 (s), 129.0 (d), 128.9 (d), 127.4 (d), 115.1 (t), 114.5 (t), 76.2 (s), 75.8 (s), 66.8 (d), 64.1 (t), 62.4 (t), 61.0 (d), 60.8 (d), 57.8 (d), 55.8 (d), 54.3 (d), 53.8 (d), 48.7 (d), 46.8 (t), 38.2 (t), 36.4 (d), 31.6 (t), 29.8 (t), 29.3 (t), 26.9 (q), 25.8 (q), 25.7 (q), 24.9 (q), 24.7 (t), 21.8 (q), 21.3 (t), 16.3 (q), 15.3 (q), 11.4 (q); *m*/*z* (ES) 862.4652 ($[M+Na]^+$, $C_{43}H_{65}N_7O_{10}Na$ requires 862.4691).

4.1.18. Cyclo[Thr(dimethylallyl)-Ser(dimethylallyl)-Ile-Ala-Pro-PheΨ{(C=S)NH}-Ser] (4).[6a] Diethylaminosulfur trifluoride (14 μl, 0.10 mmol) was added dropwise over 1 min to a stirred solution of the cyclopeptide **26** (78 mg, 0.093 mmol) in dichloromethane (1.5 ml) at −15°C under an atmosphere of nitrogen. The solution was stirred at −15°C for 35 min and then additional diethylaminosulfur trifluoride (14 μl, 0.10 mmol) was added dropwise over 1 min. The mixture was stirred at −15°C for 30 min, then quenched with saturated aqueous sodium bicarbonate solution (2 ml) and warmed to room temperature. The layers were separated, the aqueous layer was extracted with dichloromethane (5×1 ml), and the combined organic extracts were then dried and evaporated in vacuo. The residue was purified by chromatography on silica using 30:1 chloroform/methanol as eluent to give the oxazoline cyclopeptide (37 mg, 50%)[6a] as a colourless solid; δ_H (360 MHz, $CDCl_3$) 8.20 (1H, d, *J*=6.9 Hz, N*H*CHCH$_2$Ph), 8.06 (1H, d, *J*=6.7 Hz, N*H*CHCH(CH$_3$)OAllyl), 7.47 (1H, d, *J*=7.0 Hz, N*H*CHCH$_2$OAllyl), 7.27 (1H, d, *J*=5.3 Hz, N*H*CHCH$_3$), 7.14–7.11 (3H, m, Ph-*H*), 7.01–6.98 (2H, m, Ph-*H*), 6.22 (1H, d, *J*=10.0 Hz, N*H*CHCH(CH$_3$)CH$_2$CH$_3$), 5.93 (1H, dd, *J*=17.6, 10.8 Hz, C*H*=CH$_2$), 5.73 (1H, dd, *J*=17.6, 10.9 Hz, C*H*=CH$_2$), 5.31 (1H, d, *J*=17.4 Hz, CH=C*H*$_2$), 5.29 (1H, d, *J*=10.8 Hz, CH=C*H*$_2$), 5.15 (1H, dd, *J*=10.9, 0.8 Hz, CH=C*H*$_2$), 5.12 (1H, dd, *J*=17.6, 0.7 Hz, CH=C*H*$_2$), 4.94 (1H, ddd, *J*=7.0, 5.2, 5.0 Hz, C*H*CH$_2$Ph), 4.89–4.87 (1H, m, C*H*(CH$_2$)$_3$N), 4.72–4.55 (5H, m, C*H*CH$_3$, C*H*CH(CH$_3$)CH$_2$CH$_3$, C*H*$_2$O and C*H*CH$_2$O), 4.49–4.45 (2H, m, C*H*CH(CH$_3$)OAllyl and C*H*CH$_2$OAllyl), 3.95 (1H, dq, *J*=6.4, 5.2 Hz, C*H*(CH$_3$) OAllyl), 3.89 (1H, dd, *J*=9.2, 2.2 Hz, C*H*$_2$OAllyl), 3.47 (1H, dd, *J*=9.2, 3.2 Hz, C*H*$_2$OAllyl), 3.44–3.37 (2H, m, C*H*$_2$N), 3.17 (1H, dd, *J*=14.0, 5.1 Hz, C*H*$_2$Ph), 2.96 (1H, dd, *J*=14.0, 5.4 Hz, C*H*$_2$Ph), 2.60–2.55 (1H, m, C*H*$_2$), 2.45–2.38 (1H, m, C*H*(CH$_3$)CH$_2$CH$_3$), 1.98–1.90 (2H, m, C*H*$_2$), 1.70–1.59 (1H, m, C*H*$_2$), 1.48 (3H, s, C*H*$_3$), 1.39 (3H, s, C*H*$_3$), 1.27 (3H, s, C*H*$_3$), 1.24 (3H, s, C*H*$_3$), 1.15 (3H, d, *J*=6.6 Hz, CHC*H*$_3$), 1.12–1.01 (2H, m, C*H*$_2$CH$_3$), 0.95 (3H, d, *J*=6.9 Hz, CH(C*H*$_3$)CH$_2$CH$_3$), 0.94 (3H, app t, *J*=7.1 Hz, CH$_2$C*H*$_3$), 0.76 (3H, d, *J*=6.5 Hz, CH(C*H*$_3$) OAllyl); δ_C (90 MHz, $CDCl_3$) 172.7 (s), 170.7 (s), 170.3 (s), 170.1 (s), 169.0 (s), 167.7 (s), 142.6 (d), 142.2 (d), 136.5 (s), 129.6 (d), 128.3 (d), 126.8 (d), 115.7 (t), 115.1 (t), 77.8 (s), 76.0 (s), 70.8 (t), 67.9 (d), 67.0 (d), 62.0 (t), 59.8 (d), 57.5 (d), 56.2 (d), 56.0 (d), 49.3 (d), 47.8 (d), 47.3 (t), 37.9 (t), 36.6 (d), 27.3 (q), 25.8 (q), 25.8 (q), 25.6 (q), 25.5 (t), 25.2 (t), 23.7 (t), 19.0 (q), 18.3 (q), 16.1 (q), 12.1 (q); *m*/*z* (ES) 844.4569 ($[M+Na]^+$, $C_{43}H_{63}N_7O_9Na$ requires 844.4585).

Hydrogen sulfide gas was bubbled through a stirred solution of the oxazoline cyclopeptide (37 mg, 0.045 mmol) in methanol/triethylamine (2:1, 3 ml) for 5 min at room temperature under an atmosphere of nitrogen. The solution was stirred at room temperature for 51 h, then evaporated in vacuo and the residue was purified by chromatography on silica using 20% acetone in ethyl acetate as eluent to give the thioamide cyclopeptide (37 mg, 97%) as a cream solid,

mp 184–186°C; $[\alpha]_D^{20}=-64$ (*c* 0.48, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 9.47 (1H, d, *J*=6.8 Hz, N*H*C=S), 7.35–7.21 (7H, m, Ph-*H*, N*H*CHCH$_2$Ph and N*H*CHCH$_2$OAllyl), 6.97 (1H, d, *J*=8.7 Hz, N*H*CHCH(CH$_3$)CH$_2$CH$_3$), 6.70 (1H, d, *J*=6.6 Hz, N*H*CHCH(CH$_3$)OAllyl), 6.56 (1H, d, *J*=5.4 Hz, N*H*CHCH$_3$), 5.77 (1H, dd, *J*=17.7, 10.5 Hz, C*H*=CH$_2$), 5.75 (1H, dd, *J*=17.6, 10.3 Hz, C*H*=CH$_2$), 5.33 (1H, app dt, *J*=6.5, 3.2 Hz, C*H*CH$_2$OH), 5.19–5.10 (3H, m, CH=C*H*$_2$), 5.18 (1H, d, *J*=17.9 Hz, CH=C*H*$_2$), 5.00–4.96 (1H, m, C*H*CH$_2$Ph), 4.76–4.74 (1H, m, C*H*CH$_2$ OAllyl), 4.61 (1H, dq, *J*=6.8, 5.8 Hz, C*H*CH$_3$), 4.36–4.31 (2H, m, C*H*(CH$_2$)$_3$N and C*H*$_2$OH), 4.26–4.23 (1H, m, C*H*(CH$_3$)OAllyl), 4.19 (1H, dd, *J*=9.8, 8.8 Hz, C*H*CH(CH$_3$)CH$_2$CH$_3$), 4.09–4.02 (2H, m, C*H*CH(CH$_3$) OAllyl and C*H*$_2$OH), 3.76 (1H, dd, *J*=9.3, 1.6 Hz, C*H*$_2$ OAllyl), 3.69 (1H, dd, *J*=14.0, 4.1 Hz, C*H*$_2$Ph), 3.38 (1H, dd, *J*=9.3, 3.5 Hz, C*H*$_2$OAllyl), 3.30–3.24 (2H, m, C*H*$_2$N and O*H*), 2.93 (1H, dd, *J*=13.8, 11.7 Hz, C*H*$_2$Ph), 2.86 (1H, app t, *J*=10.3 Hz, C*H*$_2$N), 2.36–2.30 (1H, m, C*H*$_2$), 1.89–1.81 (2H, m, C*H*$_2$ and C*H*(CH$_3$)CH$_2$CH$_3$), 1.68–1.56 (3H, m, C*H*$_2$ and C*H*$_2$CH$_3$), 1.34 (3H, d, *J*=7.1 Hz, CHC*H*$_3$), 1.29 (3H, s, C*H*$_3$), 1.26 (3H, s, C*H*$_3$), 1.25 (3H, s, C*H*$_3$), 1.22 (3H, d, *J*=6.0 Hz, CH(C*H*$_3$)OAllyl), 1.21 (3H, s, C*H*$_3$), 1.19–1.11 (1H, m, C*H*$_2$CH$_3$), 1.01 (3H, d, *J*=6.6 Hz, CH(C*H*$_3$)CH$_2$CH$_3$), 0.93 (3H, app t, *J*=7.4 Hz, CH$_2$C*H*$_3$); δ_C (125 MHz, $CDCl_3$) 201.5 (s), 172.2 (s), 171.6 (s), 170.7 (s), 170.3 (s), 169.8 (s), 168.9 (s), 143.1 (d), 142.7 (d), 137.0 (s), 129.1 (d), 128.9 (d), 127.4 (d), 115.3 (t), 114.6 (t), 76.2 (s), 75.9 (s), 66.8 (d), 64.0 (d), 62.8 (t), 62.4 (t), 61.0 (d), 60.8 (d), 59.0 (d), 57.8 (d), 53.8 (d), 48.8 (d), 46.6 (t), 41.4 (t), 36.0 (d), 31.6 (t), 27.0 (q), 25.8 (q), 25.8 (q), 24.9 (q), 24.7 (t), 21.9 (q), 21.4 (t), 16.4 (q), 15.4 (q), 11.3 (q); *m/z* (ES) 878.4479 ($[M+Na]^+$, $C_{43}H_{65}N_7O_9SNa$ requires 878.4462).

4.1.19. C45 *epi*-Trunkamide A (1b).[6a] Diethylaminosulfur trifluoride (9.6 μl, 0.073 mmol) was added dropwise over 1 min to a stirred solution of the thioamide cyclopeptide **4** (28 mg, 0.033 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. The layers were separated, the aqueous layer was extracted with dichloromethane (6×0.5 ml), and the combined organic extracts were then dried and evaporated in vacuo. The residue was purified by chromatography on silica using 30:1 chloroform/methanol as eluent to give *epi*-trunkamide A (20 mg, 72%) as a viscous oil; $[\alpha]_D^{24}=-10$ (*c* 0.50, $CHCl_3$) (lit.[6a] $[\alpha]_D^{22}=-10.4$ (*c* 0.5, $CHCl_3$)); δ_H (400 MHz, $CDCl_3$) 8.45 (1H, d, *J*=7.1 Hz, N*H*CHCH$_2$Ph), 8.17 (1H, d, *J*=7.1 Hz, N*H*CHCH(CH$_3$)OAllyl), 7.56 (1H, d, *J*=7.0 Hz, N*H*CHCH$_2$OAllyl), 7.30 (1H, d, *J*=5.5 Hz, N*H*CHCH$_3$), 7.18–7.14 (3H, m, Ph-*H*), 7.12–7.07 (2H, m, Ph-*H*), 6.26 (1H, d, *J*=10.0 Hz, N*H*CHCH(CH$_3$)CH$_2$CH$_3$), 5.96 (1H, dd, *J*=17.6, 10.8 Hz, C*H*=CH$_2$), 5.76 (1H, dd, *J*=17.6, 10.9 Hz, C*H*=CH$_2$), 5.32 (1H, dd, *J*=17.5, 0.4 Hz, CH=C*H*$_2$), 5.30 (1H, dd, *J*=10.8, 0.7 Hz, CH=C*H*$_2$), 5.17 (1H, dd, *J*=10.8, 0.9 Hz, CH=C*H*$_2$), 5.14 (1H, dd, *J*=17.5, 0.9 Hz, CH=C*H*$_2$), 5.07–4.97 (2H, m, C*H*CH$_2$S and C*H*CH$_2$Ph), 4.85 (1H, app d, *J*=7.5 Hz, C*H*(CH$_2$)$_3$N), 4.68–4.58 (1H, masked dq, C*H*CH$_3$), 4.67 (1H, dd, *J*=10.0, 3.2 Hz, C*H*CH(CH$_3$)CH$_2$CH$_3$), 4.61 (1H, dd, *J*=6.8, 5.5 Hz, C*H*CH(CH$_3$)OAllyl), 4.47 (1H, app dt, *J*=6.5, 2.9 Hz, C*H*CH$_2$OAllyl), 4.00 (1H, dq, *J*=6.5, 5.2 Hz, C*H*(CH$_3$) OAllyl), 3.91 (1H, dd, *J*=9.3, 2.3 Hz, C*H*$_2$OAllyl), 3.67 (1H, dd, *J*=11.4, 9.5 Hz, C*H*$_2$S), 3.62 (1H, app t, *J*=11.3 Hz, C*H*$_2$S), 3.51 (1H, dd, *J*=9.3, 3.4 Hz, C*H*$_2$OAllyl), 3.47–3.35 (2H, m, C*H*$_2$N), 3.22 (1H, dd, *J*=14.0, 5.4 Hz, C*H*$_2$Ph), 2.95 (1H, dd, *J*=14.1, 5.8 Hz, C*H*$_2$Ph), 2.59–2.55 (1H, m, C*H*$_2$), 2.46–2.40 (1H, m, CH(CH$_3$)CH$_2$CH$_3$), 2.01–1.84 (2H, m, C*H*$_2$), 1.68–1.60 (1H, m, C*H*$_2$), 1.51 (3H, s, C*H*$_3$), 1.42 (3H, s, C*H*$_3$), 1.36–1.26 (2H, m, C*H*$_2$CH$_3$), 1.29 (3H, s, C*H*$_3$), 1.26 (6H, s, CHC*H*$_3$ and C*H*$_3$), 1.19 (3H, d, *J*=6.7 Hz, CH(C*H*$_3$)OAllyl), 0.97 (3H, d, *J*=6.2 Hz, CH(C*H*$_3$)CH$_2$CH$_3$), 0.95 (3H, app t, *J*=6.4 Hz, CH$_2$C*H*$_3$); δ_C (100 MHz, $CDCl_3$) 172.6 (s), 171.8 (s), 170.7 (s), 170.4 (s), 170.3 (s), 169.8 (s), 169.2 (s), 142.7 (d), 142.2 (d), 136.4 (s), 129.6 (d), 128.2 (d), 126.8 (d), 115.8 (t), 115.1 (t), 77.9 (s), 77.8 (d), 76.0 (s), 67.3 (d), 62.0 (t), 59.7 (d), 57.6 (d), 56.4 (d), 56.1 (d), 53.6 (d), 47.8 (d), 47.3 (t), 40.5 (t), 36.5 (d), 35.8 (t), 29.8 (t), 27.4 (q), 25.9 (q), 25.7 (q), 25.5 (q), 25.2 (t), 23.9 (t), 18.9 (q), 18.4 (q), 16.2 (q), 12.2 (q); *m/z* (ES) 860.4321 ($[M+Na]^+$, $C_{43}H_{63}N_7O_8SNa$ requires 860.4357).

4.1.20. Trunkamide A (1a). Pyridine (48 μl, 0.59 mmol) was added to a solution of *epi*-trunkamide A **1b** (10 mg, 0.012 mmol) in methanol (0.1 ml) at room temperature. The solution was heated to 50°C, stood at this temperature for 9 days and then evaporated in vacuo. The residue was purified by chromatography on silica using 30:1 chloroform/methanol as eluent and then by HPLC using 25% isopropanol in hexane as eluent[†] to give trunkamide A (3 mg, 32%) as a viscous oil; $[\alpha]_D^{23}=-21$ (*c* 0.26, $CHCl_3$) (lit.[6a] $[\alpha]_D=-23.1$ (*c* 0.06, $CHCl_3$));[‡] δ_H (360 MHz, $CDCl_3$) 7.97 (1H, d, *J*=6.4 Hz, N*H*CHCH(CH$_3$)OAllyl), 7.56 (1H, d, *J*=7.8 Hz, N*H*CHCH$_2$OAllyl), 7.29–7.21 (4H, m, Ph-*H* and N*H*CHCH$_2$Ph), 7.17 (1H, d, *J*=7.7 Hz, N*H*CHCH$_3$), 7.15–7.12 (2H, m, Ph-*H*), 6.32 (1H, d, *J*=9.6 Hz, N*H*CHCH(CH$_3$)CH$_2$CH$_3$), 5.93 (1H, dd, *J*=17.6, 10.8 Hz, C*H*=CH$_2$), 5.75 (1H, dd, *J*=17.6, 10.9 Hz, C*H*=CH$_2$), 5.28 (1H, dd, *J*=17.5, 0.6 Hz, CH=C*H*$_2$), 5.25 (1H, dd, *J*=10.8, 0.7 Hz, CH=C*H*$_2$), 5.17–5.11 (1H, masked ddd, C*H*CH$_2$Ph), 5.16 (1H, dd, *J*=10.9, 0.9 Hz, CH=C*H*$_2$), 5.12 (1H, dd, *J*=17.5, 0.9 Hz, CH=C*H*$_2$), 4.93 (1H, app t, *J*=9.6 Hz, C*H*CH$_2$S), 4.63–4.56 (3H, m, C*H*CH(CH$_3$)CH$_2$ CH$_3$, C*H*CH$_2$OAllyl and C*H*CH(CH$_3$)OAllyl), 4.51 (1H, dq, *J*=6.5, 5.7 Hz, C*H*CH$_3$), 4.39 (1H, app t, *J*=6.0 Hz, C*H*(CH$_2$)$_3$N), 4.04 (1H, dq, *J*=6.5, 5.1 Hz, C*H*(CH$_3$) OAllyl), 3.91 (1H, dd, *J*=9.1, 2.1 Hz, C*H*$_2$OAllyl), 3.73 (1H, dd, *J*=11.3, 9.7 Hz, C*H*$_2$S), 3.64 (1H, dd, *J*=11.2, 9.7 Hz, C*H*$_2$S), 3.55–3.49 (2H, m, C*H*$_2$N), 3.46 (1H, dd, *J*=9.1, 3.2 Hz, C*H*$_2$OAllyl), 3.25 (1H, dd, *J*=13.9, 5.7 Hz, C*H*$_2$Ph), 2.95 (1H, dd, *J*=14.0, 5.9 Hz, C*H*$_2$Ph), 2.40–2.33 (1H, m, C*H*(CH$_3$)CH$_2$CH$_3$), 2.27–2.22 (1H, m, C*H*$_2$), 1.96–1.87 (3H, m, C*H*$_2$), 1.49 (3H, s, C*H*$_3$), 1.39 (3H, s, C*H*$_3$), 1.35–1.18 (2H, m, C*H*$_2$CH$_3$), 1.27 (3H, s, C*H*$_3$), 1.25 (3H, s, C*H*$_3$), 1.22 (3H, d, *J*=6.6 Hz, CHC*H*$_3$), 1.07 (3H, d, *J*=6.5 Hz, CH(C*H*$_3$)OAllyl), 0.96 (3H, d, *J*=6.9 Hz, CH(C*H*$_3$)CH$_2$CH$_3$), 0.94 (3H, app t, *J*=7.4 Hz, CH$_2$C*H*$_3$);

[†] HPLC separation was performed on an Agilent 1100 LC system using a Hichrom Nucleosil 100-5 ID silica column (4.6 mm×25 cm). Mobile phase: 25% IPA in hexane; detection: UV 254 nm; flow rate: 1 ml/min; retention time: 8 min (trunkamide A), 30 min (C45 *epi*-trunkamide A).

[‡] In the original report the $[\alpha]_D$ for natural trunkamide A was erroneously given as $[\alpha]_D=-231$ (*c* 0.06, $CHCl_3$).[3]

δ_C (100 MHz, $CDCl_3$) 173.3 (s), 171.1 (s), 170.9 (s), 170.6 (s), 170.2 (s), 170.1 (s), 168.7 (s), 142.7 (d), 142.1 (d), 135.7 (s), 129.7 (d), 128.4 (d), 127.2 (d), 115.8 (t), 115.0 (t), 78.2 (d), 77.9 (s), 76.0 (s), 67.3 (d), 62.3 (t), 60.0 (d), 57.9 (d), 56.5 (d), 55.4 (d), 52.8 (d), 47.8 (d), 47.1 (t), 39.9 (t), 36.5 (d), 36.4 (t), 28.6 (t), 27.4 (q), 25.8 (q), 25.8 (q), 25.7 (q), 25.6 (t), 23.8 (t), 18.6 (q), 18.1 (q), 16.1 (q), 12.0 (q); *m/z* (ES) 860.4320 ($[M+Na]^+$, $C_{43}H_{63}N_7O_8SNa$ requires 860.4357).

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References

1. For recent reviews see: (a) Faulkner, D. J. *Nat. Prod. Rep.* **2002**, *19*, 1; this is an ongoing annual series which began in 1984. (b) Jaspars, M. *Chem. Ind.* **1999**, 51.
2. (a) Wipf, P. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Pergamon: New York, 1998; pp 187–228. (b) Davidson, B. S. *Chem. Rev.* **1993**, *93*, 1771.
3. Carroll, A. R.; Coll, J. C.; Bourne, D. J.; MacLeod, J. K.; Zabriskie, T. M.; Ireland, C. M.; Bowden, B. F. *Aust. J. Chem.* **1996**, *49*, 659.
4. Bowden B. F.; Garcia G. D. WO9739025A1, October 23, 1997; *Chem. Abstr*. **1997**, *127*, 290919m.
5. Carroll, A. R.; Bowden, B. F.; Coll, J. C.; Hockless, D. C. R.; Skelton, B. W.; White, A. H. *Aust. J. Chem.* **1994**, *47*, 61.
6. (a) Wipf, P.; Uto, Y. *J. Org. Chem.* **2000**, *65*, 1037. (b) Wipf, P.; Uto, Y. *Tetrahedron Lett.* **1999**, *40*, 5165.
7. Caba, J. M.; Rodriguez, I. M.; Manzanares, I.; Giralt, E.; Albericio, F. *J. Org. Chem.* **2001**, *66*, 7568.
8. Preliminary communication: McKeever, B.; Pattenden, G. *Tetrahedron Lett.* **2001**, *42*, 2573.
9. (a) Jenson, O. E.; Senning, A. *Tetrahedron* **1986**, *42*, 6555. (b) Cava, M. P.; Levinson, M. I. *Tetrahedron* **1985**, *41*, 5061. (c) Lajoie, G.; Lépine, F.; Maziak, L.; Belleau, B. *Tetrahedron Lett.* **1983**, *24*, 3815. (d) Clausen, K.; Thorsen, M.; Lawesson, S.-O. *Tetrahedron* **1981**, *37*, 3635.
10. See preceding paper for details of the preparation of this compound; McKeever, B.; Pattenden, G. *Tetrahedron* **2003**, *59*, 2701.
11. Dong, Q.; Anderson, C. E.; Ciufolini, M. A. *Tetrahedron Lett.* **1995**, *36*, 5681.
12. (a) Boden, C. D. J.; Pattenden, G.; Ye, T. *Synlett* **1995**, 417. (b) Wipf, P.; Fritch, P. C. *Tetrahedron Lett.* **1994**, *35*, 5397. (c) Yonetani, K.; Hirotsu, Y.; Shiba, T. *Bull. Chem. Soc. Jpn* **1975**, *48*, 3302. (d) Hirotsu, Y.; Shiba, T.; Kaneko, T. *Bull. Chem. Soc. Jpn* **1970**, *43*, 1870. (e) Konigsberg, W.; Hill, R. J.; Craig, L. C. *J. Org. Chem.* **1961**, *26*, 3867.
13. Wipf, P.; Miller, C. P. *Tetrahedron Lett.* **1992**, *33*, 6267 (see also Ref. 12b).
14. Shioiri, T.; Ninomiya, K.; Yamada, S. *J. Am. Chem. Soc.* **1972**, *94*, 6203.
15. Abdel-Magid, A. F.; Cohen, J. H.; Maryanoff, C. A.; Shah, R. D.; Villani, F. J.; Zhang, F. *Tetrahedron Lett.* **1998**, *39*, 3391.
16. Wipf, P.; Miller, C. P.; Venkatraman, S.; Fritch, P. C. *Tetrahedron Lett.* **1995**, *36*, 6395.
17. (a) Phillips, A. J.; Uto, Y.; Wipf, P.; Reno, M. J.; Williams, D. R. *Org. Lett.* **2000**, *2*, 1165. (b) Lafargue, P.; Guenot, P.; Lellouche, J.-P. *Heterocycles* **1995**, *41*, 947. (c) Burrell, G.; Evans, J. M.; Jones, G. E.; Stemp, G. *Tetrahedron Lett.* **1990**, *31*, 3649.
18. (a) Dunn, M. J.; Jackson, R. F. W. *Tetrahedron* **1997**, *53*, 13905. (b) Schwyzer, R.; Karlaganis, G. *Justus Liebigs Ann. Chem.* **1973**, 1298.
19. (a) Willems, J. G. H.; Hersmis, M. C.; de Gelder, R.; Smits, J. M. M.; Hammink, J. B.; Dommerholt, F. J.; Thijs, L.; Zwanenburg, B. *J. Chem. Soc., Perkin Trans. 1* **1997**, 963. (b) Tanaka, T.; Nakajima, K.; Okawa, K. *Bull. Chem. Soc. Jpn* **1980**, *53*, 1352.
20. This reagent was prepared from the corresponding chloroformate using the method described by Lapatsanis et al; Lapatsanis, L.; Milias, G.; Froussios, K.; Kolovos, M. *Synthesis* **1983**, 671.
21. Atkins, G. M., Jr.; Burgess, E. M. *J. Am. Chem. Soc.* **1968**, *90*, 4744.