Total synthesis and assignment of configuration of the thiazoline-based cyclopeptide cyclodidemnamide isolated from the sea squirt *Didemnum molle*

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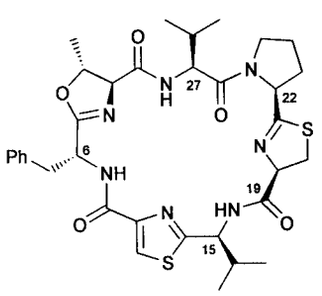
A total synthesis of the proposed structure **1** for the oxazoline/thiazoline-based cyclopeptide cyclodidemnamide, isolated from the sea squirt *Didemnum molle*, is described. The synthesis features a novel double cyclodehydration, sequential formation of chiral thiazoline and oxazoline rings in a preformed cyclopeptide intermediate, as a key stratagem. The NMR spectroscopic data for synthetic **1** and natural cyclodidemnamide did not correlate. The configuration of the natural product was re-assigned as **15**, the C-15-(*R*)-Val epimer, which was confirmed by total synthesis.

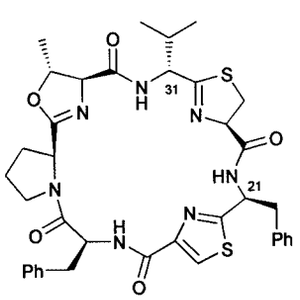
Cyclodidemnamide **1** is a thiazoline/oxazoline containing cyclopeptide which has been isolated from the sea squirt *Didemnum molle*.[1] The compound exhibits moderate toxicity toward human colon cells, and it is related structurally to other thiazoline-based marine cyclopeptides *e.g.* lissoclinamide 4 **2**[2] and mollamide **3**.[3] Chiral amino acid substituted thiazolines of the type contained within the structures **1**, **2**, and **3**, are well known to be configurationally labile under a variety of acid and base conditions.[4] In earlier work we described a total synthesis of the thiazoline-containing cyclopeptide lissoclinamide 4 **2**, whereby the thiazoline ring was produced simultaneously with the oxazoline ring in **2** *via* a novel "one-pot" double dehydration sequence from a preformed cyclothioamide intermediate as a key step.[5] We have now developed this overall strategy towards the synthesis of both cyclodidemnamide **1**[6] and mollamide **3**.[7] In this paper we report the full details of our synthesis of the structure **1** reported for cyclodidemnamide leading to the reassignment of its configuration to **15**, and the total synthesis of the latter.

In their structure elucidation of cyclodidemnamide Toske and Fenical assigned the *S*-stereochemistries at both C15 and C27 based on hydrolysis of the cyclopeptide followed by GC-MS analysis of the configuration of the resulting (*S*)-valine derivative.[1] As mentioned earlier and elsewhere,[4] however, there can be problems with this analysis owing to the ease with which thiazole and thiazoline based amino acid units of the type displayed in structures **1**–**3** undergo epimerisation during hydrolysis. Nevertheless, we accepted the assignment of the structure proposed for cyclodidemnamide and based our synthetic strategy to **1** on double dehydration from the cyclothioamide precursor **4**, as a key step, which we planned to obtain from the known (*S*)-Val substituted thiazole **7**[8] *via* the acyclic (*endo*-thio) tetra- and hepta-peptides **6** and **5**, respectively (Scheme 1). Thus, removal of the Boc group in **7** followed by acylation of the resulting amine with Boc-(*S*)-Ser-OH in the presence of DCC–HOBt first gave the tripeptide **8** in 91% yield. The Boc group in **8** was next deblocked and the free amine was then thioacylated with the proline-derived reagent **9**[9] in 56% yield from **8**. The three amino acid residues (*S*)-Val, (*S*)-*a*Thr,† and (*R*)-Phe were next introduced to the chain in sequence, by a cycle of Boc removal followed by acylations using the DCC–HOBt–iPr$_2$NEt coupling method, leading to the compounds **10**, **11** and **5** in 68%, 73% and 71% yield respectively (Scheme 2). Saponification of **5** and removal of the Boc group now gave the amino acid **12**, but all attempts to effect macrocyclisation of **12** using diphenylphosphorazide (DPPA) or pentafluorophenyl diphenylphosphinate (FDPP) failed, and instead led to products of decomposition. We suspected that the problem was due to the poor solubility of the amino acid **12** in DMF, occasioned by H-bonding involving the serine and threonine hydroxy groups. We therefore decided to convert the heptapeptide **5** into the diacetate **13** corresponding to **12** prior to macrocyclisation, and this was smoothly accomplished in three straightforward steps (Scheme 3). Treatment of the diacetate **13** with DPPA–iPr$_2$NEt in DMF (0–25 °C), and work-up after 3 days, then produced the diacetylated cyclopeptide **14a** in a highly satisfying 60–70% overall yield. Finally, saponification of the acetate groups in **14a** followed by treatment of the resulting diol **14b**

† *a*Thr = L-*allo*-threonine.



1



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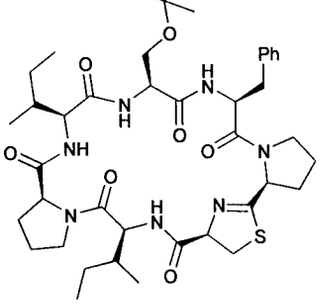

3

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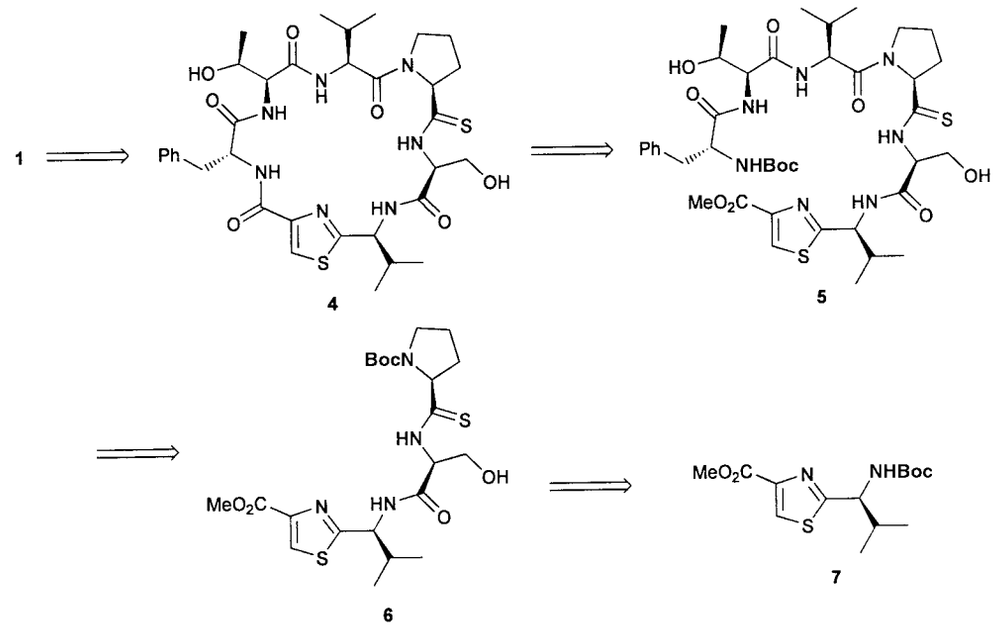
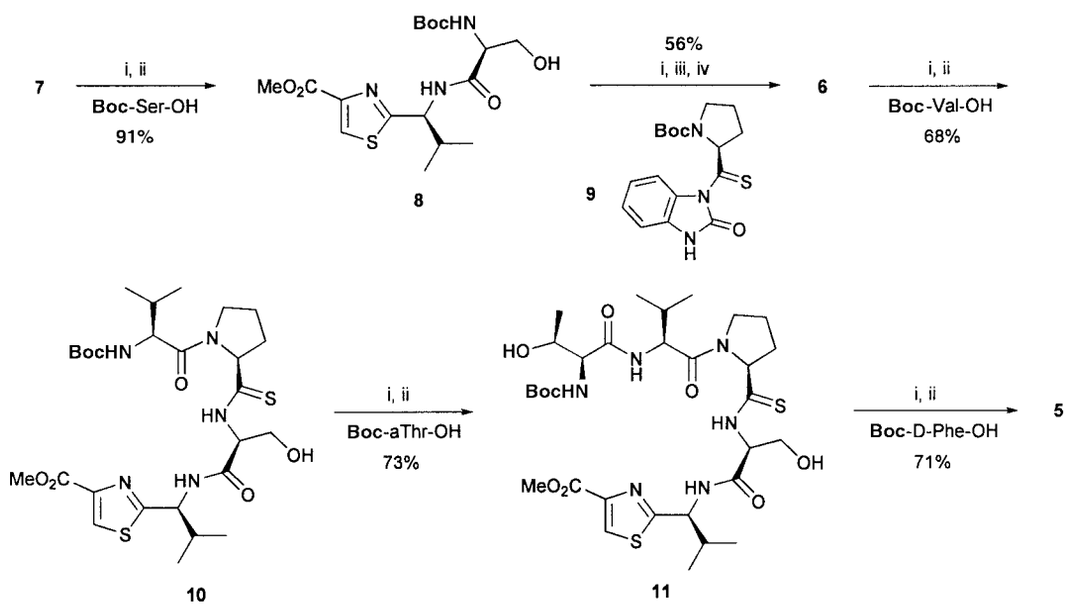


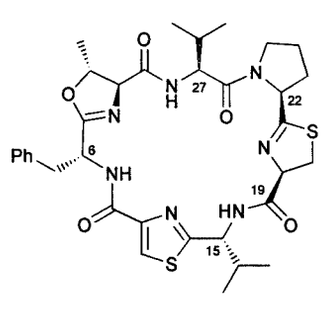
Scheme 1



Scheme 2 *Reagents*: i, 50% TFA–CH_2Cl_2, 0 °C, 1 h; ii, DCC, HOBt, iPr$_2$NEt, CH_2Cl_2, 0 °C ⟶ rt, 18 h; iii, aq. $NaHCO_3$–CH_2Cl_2; iv, **9**, DMF, 0 °C ⟶ rt, 18 h.

with Burgess' reagent[10] in *anhydrous* THF gave the cyclodidemnamide structure **1**, as a foam, in approximately 30% yield.

Much to our chagrin, although the synthetic cyclodidemnamide showed closely similar NMR spectroscopic data to those reported for the natural product, the two compounds were clearly different at one or more stereocentres. We surmised that the amino acid sequence in the natural product was assigned correctly, but that the difference between it and our synthetic compound was most likely associated with a wrongly assigned configuration at C15, the valine-derived thiazole centre, in the natural product. We therefore re-assigned this C15 centre as (*R*)-Val, and hence natural cyclodidemnamide as structure **15**, and then repeated the entire synthesis using exactly the same strategy and order of steps to those earlier described, but starting from the (*R*)-Val enantiomer of the thiazole **7**. In this manner we synthesised the stereostructure **15** which gratifyingly showed NMR spectroscopic and other data which were identical to those reported for natural cyclodidemnamide isolated from *D. molle*.

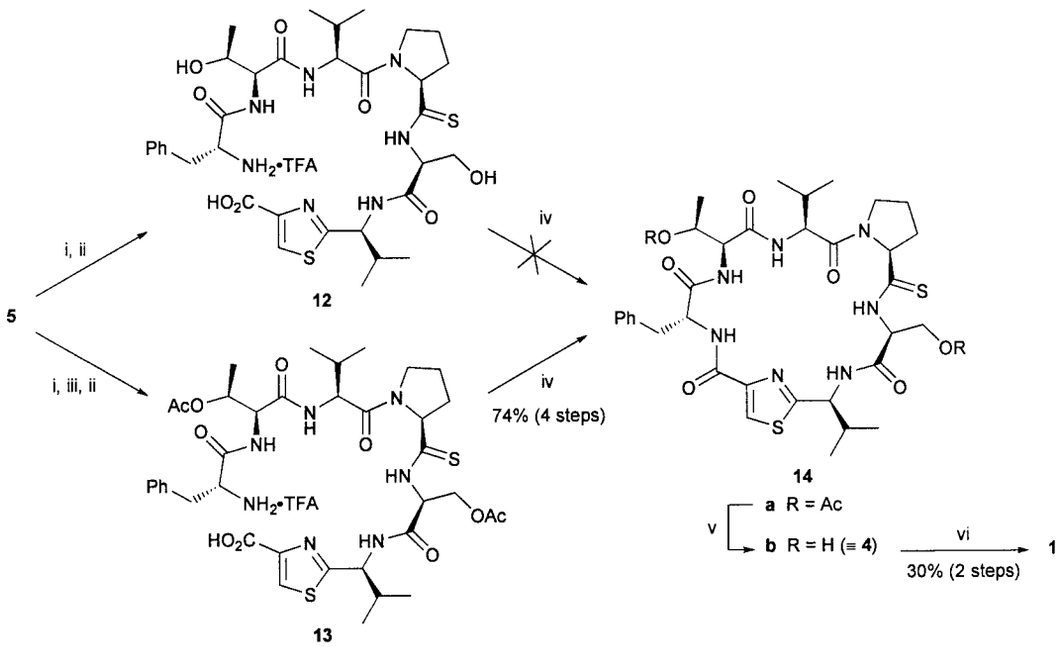


Experimental

For general experimental details see ref. 5*a*.

Boc-(*S*)-Ser-Val-Thz-OMe; (*R*)-Val epimer, 8

Trifluoroacetic acid (50 ml) was added dropwise over 0.5 h to a



Scheme 3 *Reagents*: i, aq. NaOH, THF–MeOH (3:1), 0 °C, 1 h; ii, 50% TFA–CH_2Cl_2, 0 °C, 1 h; iii, Ac_2O, Et_3N, cat. DMAP, DMF, rt, 2 h; iv, DPPA, $i Pr_2NEt$, DMF, 0 °C ⟶ rt, 3 days; v, aq. K_2CO_3, MeOH, 0 °C, 1 h; vi, Burgess' reagent, THF, reflux, 2 h.

stirred solution of the *R*-enantiomer of **7** (7.77 g, 24.71 mmol)[5] in dichloromethane (50 ml) at 0 °C and the mixture was stirred at 0 °C for 1 h. The solution was concentrated *in vacuo* and the solid residue was dissolved in dichloromethane (150 ml), cooled to 0 °C and treated dropwise over 5 min with $i Pr_2NEt$ (10.8 ml, 62.0 mmol). The mixture was stirred at 0 °C for 15 min and then Boc-(*S*)-Ser-OH (5.58 g, 27.19 mmol) was added followed by HOBt (4.01 g, 29.7 mmol). The resulting cloudy mixture was stirred at 0–4 °C for a further 15 min, then treated with DCC (6.63 g, 32.1 mmol) and stirred at 0 °C for 90 min followed by a further 18 h at room temperature. The suspension was concentrated *in vacuo* and then EtOAc (150 ml) was added to the residue. The mixture was filtered and the filtrate was washed with 10% w/v citric acid solution and saturated $NaHCO_3$, then dried ($MgSO_4$), and evaporated to dryness *in vacuo*. The residue was purified by flash chromatography on silica gel (40% EtOAc–Et_2O eluant) to give the *tripeptide* (9.04 g, 22.5 mmol, 91%) as a white foam: δ_H (500 MHz, DMSO, 90 °C): 8.36 (1H, s), 8.00 (1H, d, *J* 8.5 Hz), 6.31 (1H, br s), 5.04 (1H, dd, *J* 6.6, 8.5 Hz), 4.52 (1H, br s), 4.13 (1H, dt, *J* 8.0, 5.7 Hz), 3.87 (3H, s), 3.65 (2H, m), 2.36 (1H, app octet, *J* 6.7 Hz), 1.42 (9H, s), 0.97 (3H, d, *J* 6.8 Hz), 0.94 (3H, d, *J* 6.8 Hz); δ_C (125 MHz, DMSO, 90 °C): 172.4 (s), 170.3 (s), 160.9 (s), 154.8 (s), 145.4 (s), 127.9 (d), 78.1 (s), 61.5 (t), 56.8 (d), 56.2 (d), 51.3 (q), 31.9 (d), 27.8 (q), 18.8 (q), 17.6 (q); HRMS, *m/z* for $C_{17}H_{27}N_3O_6SNa$ (M^+ + Na), calcd: 424.1520; found 424.1518.

NMR data for **8**, (*S*)-Val epimer: δ_H (400 MHz, DMSO, 80 °C): 8.37 (1H, s), 8.00 (1H, br s), 6.31 (1H, br s), 5.04 (1H, dd, *J* 6.6, 8.5 Hz), 4.53 (1H, br s), 4.13 (1H, dt, *J* 8.0, 5.7 Hz), 3.87 (3H, s), 3.66 (2H, m), 2.36 (1H, app octet, *J* 6.7 Hz), 1.42 (9H, s), 0.97 (3H, d, *J* 6.8 Hz), 0.94 (3H, d, *J* 6.8 Hz); δ_C (100 MHz, DMSO, 80 °C): 172.5 (s), 170.3 (s), 160.9 (s), 154.85 (s), 145.4 (s), 127.95 (d), 78.2 (s), 61.5 (t), 56.8 (d), 56.2 (d), 51.4 (q), 31.9 (d), 27.9 (q), 18.8 (q), 17.6 (q).

Boc-Pro-Ψ(CS-NH)-(*S*)-Ser-Val-Thz-OMe; (*R*)-Val epimer, 6

Trifluoroacetic acid (50 ml) was added dropwise over 30 min to a stirred solution of the (*R*)-Val epimer of **8** (6.21 g, 15.47 mmol) in dichloromethane (50 ml) at 0 °C and the resulting solution was stirred at 0 °C for 1 h. The solution was concentrated *in vacuo* and the solid residue was dissolved in dichloromethane (150 ml) and the solution shaken vigorously with sat. $NaHCO_3$ (50 ml) for 15 min. The separated aqueous layer was extracted a further 15 times with dichloromethane (100 ml each time) and the combined extracts were then dried ($MgSO_4$) and concentrated *in vacuo* to leave an off-white foam which was immediately dissolved in DMF (100 ml) and cooled to 0 °C. Boc-Pro-Bin[9] (Bin = benzimidazolone) was added portionwise over 10 min and the resulting bright yellow solution was stirred at 0 °C for 90 min and then for a further 18 h at room temperature. The solution was diluted with EtOAc (500 ml), then washed with water (3 × 200 ml), dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (Et_2O eluant) to give the *tetrapeptide* (4.50 g, 8.74 mmol, 56%) as a white foam: δ_H (500 MHz, DMSO, 90 °C): 9.27 (1H, d, *J* 7.0 Hz), 8.37 (1H, s), 8.18 (1H, d, *J* 8.4 Hz), 5.11 (1H, dt, *J* 7.2, 5.2 Hz), 5.06 (1H, dd, *J* 6.6, 8.4 Hz), 4.68 (1H, br t, *J* 5.2 Hz), 4.63 (1H, dd, *J* 3.8, 8.7 Hz), 3.87 (3H, s), 3.89–3.81 (2H, m), 3.52–3.42 (2H, m), 2.35 (1H, app octet, *J* 6.7 Hz), 2.31–2.23 (1H, m), 1.99–1.89 (2H, m), 1.84–1.76 (1H, m), 1.40 (9H, s), 0.97 (3H, d, *J* 6.8 Hz), 0.94 (3H, d, *J* 6.8 Hz); δ_C (125 MHz, DMSO, 90 °C): 204.9 (s), 172.3 (s), 168.5 (s), 160.9 (s), 153.4 (s), 145.3 (s), 128.1 (d), 78.7 (s), 66.9 (d), 60.5 (t), 59.8 (d), 56.5 (d), 51.3 (q), 46.8 (t), 33.1 (t), 31.9 (d), 27.8 (q), 22.7 (t), 18.8 (q), 17.6 (q); HRMS, *m/z* for $C_{22}H_{34}N_4O_6S_2$ + Na (M^+ + Na), calcd: 537.1842; found 537.1817.

NMR data for **6**, (*S*)-Val epimer: δ_H (400 MHz, DMSO, 90 °C): 9.22 (1H, d, *J* 7.0 Hz), 8.37 (1H, s), 8.10 (1H, d, *J* 8.4 Hz), 5.11 (1H, dt, *J* 7.3, 5.2 Hz), 5.06 (1H, dd, *J* 6.5, 8.4 Hz), 4.65 (1H, dd, *J* 8.7, 3.8 Hz), 4.58 (1H, br s), 3.87 (3H, s), 3.89–3.81 (2H, m), 3.52–3.42 (2H, m), 2.35 (1H, app octet, *J* 6.7 Hz), 2.31–2.23 (1H, m), 1.99–1.89 (2H, m), 1.84–1.76 (1H, m), 1.41 (9H, s), 0.99 (3H, d, *J* 6.8 Hz), 0.95 (3H, d, *J* 6.8 Hz); δ_C (100 MHz, DMSO, 90 °C): 204.9 (s), 172.2 (s), 168.5 (s), 160.8 (s), 153.4 (s), 145.4 (s), 127.8 (d), 78.7 (s), 66.9 (d), 60.5 (t), 59.7 (d), 56.5 (d), 51.2 (q), 46.8 (t), 33.0 (t), 31.85 (d), 27.7 (q), 22.7 (t), 18.7 (q), 17.6 (q).

Boc-(*S*)-Val-Pro-Ψ(CS-NH)-(*S*)-Ser-Val-Thz-OMe; C-15(*R*)-Val epimer, 10

Trifluoroacetic acid (45 ml) was added dropwise over 30 min to a stirred solution of the (*R*)-Val epimer of **6** (4.50 g, 8.74 mmol) in dichloromethane (45 ml) at 0 °C and the solution was then stirred at 0 °C for 1 h. The solution was concentrated *in vacuo*,

and the solid residue was dissolved in dichloromethane (50 ml), cooled to 0 °C and treated dropwise with *i*Pr_2NEt (3.8 ml, 21.8 mmol). The mixture was stirred at 0 °C for 15 min and then Boc-(*S*)-Val-OH (2.09 g, 9.62 mmol) was added followed by HOBt (1.42 g, 10.51 mmol). The resulting cloudy mixture was stirred for a further 15 min at 0 °C, then treated with DCC (2.34 g, 11.34 mmol) and stirred at 0 °C for 90 min followed by a further 18 h at room temperature. The suspension was concentrated *in vacuo*, EtOAc (50 ml) was added to the residue and the mixture was then filtered. The filtrate was washed with 10% w/v citric acid solution and saturated $NaHCO_3$, then dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (10% acetone–Et_2O eluant) to give the *pentapeptide* (3.65 g, 5.95 mmol, 68%) as a white foam: δ_H (500 MHz, DMSO, 90 °C): 9.45 (1H, br s), 8.38 (1H, s), 8.10 (1H, br d, *J* 7.7 Hz), 6.08 (1H, br s), 5.07 (1H, m), 5.04 (1H, dd, *J* 6.5, 8.5 Hz), 4.92 (1H, br s), 4.64 (1H, br s), 4.13 (1H, br s), 3.85 (3H, s), 3.84 (2H, m), 3.79 (1H, br s), 3.68 (1H, m), 2.35 (1H, app octet, *J* 6.7 Hz), 2.20 (1H, m), 2.14–2.00 (3H, m), 1.89 (1H, m), 1.42 (9H, s), 0.98 (3H, d, *J* 6.8 Hz), 0.97 (3H, d, *J* 6.7 Hz), 0.93 (3H, d, *J* 6.7 Hz), 0.88 (3H, d, *J* 6.7 Hz); δ_C (125 MHz, DMSO, 90 °C): 204.6 (s), 172.4 (s), 170.4 (s), 168.4 (s), 160.9 (s), 145.3 (s), 128.0 (d), 108.1 (s), 78.0 (s), 66.0 (d), 60.4 (t), 60.2 (d), 57.0 (d), 56.5 (d), 51.4 (q), 47.4 (d), 47.1 (t), 33.0 (t), 31.9 (d), 27.9 (q), 24.0 (t), 19.2 (q), 18.8 (q), 17.7 (q), 17.3 (q); HRMS, *m/z* 636.2502 (M + Na) $C_{27}H_{43}N_5O_7S_2$ requires: 636.2560.

NMR data for **10**, C-15(*S*)-Val epimer: δ_H (500 MHz, DMSO, 90 °C): 9.4 (1H, br s), 8.37 (1H, s), 8.09 (1H, br d, *J* 6.8 Hz), 6.05 (1H, br s), 5.07 (1H, m), 5.05 (1H, dd, *J* 6.6, 8.4 Hz), 4.92 (1H, br s), 4.63 (1H, br s), 4.13 (1H, br s), 3.87 (3H, s), 3.84 (2H, m), 3.79 (1H, br s), 3.68 (1H, app q, *J* 6.6 Hz), 2.35 (1H, app octet, *J* 6.7 Hz), 2.20 (1H, m), 2.14–2.00 (3H, m), 1.89 (1H, m), 1.42 (9H, s), 0.98 (3H, d, *J* 6.7 Hz), 0.97 (3H, d, *J* 6.6 Hz), 0.94 (3H, d, *J* 6.8 Hz), 0.89 (3H, d, *J* 6.7 Hz); δ_C (125 MHz, DMSO, 90 °C): 204.6 (s), 172.3 (s), 170.4 (s), 168.4 (s), 160.9 (s), 145.3 (s), 128.0 (d), 108.1 (s), 78.0 (s), 66.0 (d), 60.4 (t), 60.2 (d), 57.0 (d), 56.4 (d), 51.3 (q), 47.4 (d), 47.0 (t), 33.0 (t), 31.2 (d), 27.9 (q), 24.0 (t), 19.2 (q), 18.8 (q), 17.6 (q), 17.2 (q).

Boc-(*S*)-aThr-(*S*)-Val-Pro-Ψ(CS-NH)-(*S*)-Ser-Val-Thz-OMe; C-15(*R*)-Val epimer, 11

Trifluoroacetic acid (25 ml) was added dropwise over 20 min to a stirred solution of the C-15-(*R*)-Val epimer of **10** (2.91 g, 4.74 mmol) in dichloromethane (25 ml) at 0 °C and the resulting solution was then stirred at 0 °C for 1 h. The solution was concentrated *in vacuo* and the solid residue was then dissolved in dichloromethane (25 ml), cooled to 0 °C and treated dropwise with *i*Pr_2NEt (2.1 ml, 12.06 mmol). The mixture was stirred at 0 °C for 15 min and then Boc-(*S*)-aThr-OH (1.14 g, 5.20 mmol) was added followed by HOBt (769 mg, 5.69 mmol). The resulting cloudy mixture was stirred at 0 °C for a further 15 min then treated with DCC (1.27 g, 6.16 mmol) and stirred at 0 °C for 90 min followed by a further 18 h at room temperature. The suspension was concentrated *in vacuo* and EtOAc (25 ml) was added to the residue. The mixture was filtered and the filtrate was washed with 10% w/v citric acid solution and saturated $NaHCO_3$, then dried ($MgSO_4$), concentrated *in vacuo* and the residue purified by flash chromatography on silica gel (30% acetone–Et_2O eluant) to give the *hexapeptide* (2.47 g, 3.46 mmol, 73%) as a white foam: δ_H (500 MHz, DMSO, 90 °C): 9.42 (1H, br s), 8.38 (1H, s), 8.10 (1H, br s), 7.39 (1H, br s), 6.38 (1H, br s), 5.06 (2H, m), 4.88 (1H, br s), 4.67 (1H, br s), 4.48 (2H, br s), 3.95–3.79 (5H, m), 3.86 (3H, s), 3.69 (1H, m), 2.35 (1H, app octet, *J* 6.7 Hz), 2.26–1.99 (4H, m), 1.88 (1H, br s), 1.42 (9H, s), 1.12 (3H, d, *J* 6.2 Hz), 0.98 (3H, d, *J* 6.8 Hz), 0.97 (3H, d, *J* 6.6 Hz), 0.94 (3H, d, *J* 6.8 Hz), 0.91 (3H, d, *J* 6.7 Hz); δ_C (125 MHz, DMSO, 90 °C): 204.5 (s), 172.4 (s), 170.1 (s), 170.0 (s), 168.4 (s), 160.9 (s), 154.9 (s), 145.4 (s), 128.0 (d), 78.1 (s), 66.6 (d), 64.4 (t), 60.4 (d), 60.3 (d), 56.5 (d), 55.3 (d), 52.4 (q), 47.2 (t), 32.3 (d), 31.9 (t), 29.8 (d), 27.9 (q), 23.8 (t), 19.3 (q), 18.8 (q), 17.7 (q), 17.3 (q), 14.7 (q); HRMS, *m/z* for $C_{31}H_{50}N_6O_9S_2$ + Na (M^+ + Na), calcd: 737.2964; found 737.2978.

NMR data for **11**, C-15(*S*)-Val epimer: δ_H (400 MHz, DMSO, 35 °C): 9.75 (1H, d, *J* 4.0 Hz), 8.42 (1H, s), 8.38 (1H, d, 5.3 Hz), 7.60 (1H, d, *J* 8.4 Hz), 6.81 (1H, d, *J* 8.0 Hz), 5.00 (2H, m), 4.85 (1H, dd, *J* 4.4, 8.9 Hz), 4.67 (1H, d, 4.9 Hz), 4.49 (1H, app t, *J* 7.9 Hz), 3.95–3.79 (5H, m), 3.82 (3H, s), 3.65 (1H, m), 2.31 (1H, app octet, *J* 6.7 Hz), 2.26–1.99 (4H, m), 1.87 (1H, m), 1.4 (9H, s), 1.06 (3H, d, *J* 6.2 Hz), 0.95–0.82 (12H, m); δ_C (100 MHz, DMSO, 35 °C): 205.1 (s), 173.4 (s), 170.5 (s), 170.0 (s), 168.9 (s), 161.3 (s), 155.4 (s), 145.5 (s), 129.0 (d), 78.3 (s), 66.7 (d), 60.8 (d), 60.4 (d), 56.7 (d), 55.5 (d), 52.0 (q), 47.6 (t), 32.3 (d), 32.0 (t), 30.0 (d), 28.3 (q), 24.2 (t), 19.9 (q), 19.8 (q), 18.0 (q), 17.8 (q).

Boc-(*R*)-Phe-(*S*)-aThr-(*S*)-Val-Pro-Ψ(CS-NH)-(*S*)-Ser-Val-Thz-OMe; C-15(*R*)-Val epimer, 5

Trifluoroacetic acid (10 ml) was added dropwise over 15 min to a stirred solution of the C-15-(*R*)-Val epimer of **11** (920 mg, 1.29 mmol) in dichloromethane (10 ml) at 0 °C and the solution was then stirred at 0 °C for 1 h. The solution was concentrated *in vacuo* and the solid residue was dissolved in dichloromethane (10 ml), cooled to 0 °C and treated dropwise with *i*Pr_2NEt (0.56 ml, 3.21 mmol). The mixture was stirred at 0 °C for 15 min and then Boc-(*R*)-Phe-OH (376 mg, 1.42 mmol) was added followed by HOBt (209 mg, 1.55 mmol). The resulting cloudy mixture was stirred at 0 °C for a further 15 min then treated with DCC (346 mg, 1.68 mmol) and stirred at 0 °C for 90 min followed by a further 18 h at room temperature. The suspension was concentrated *in vacuo* and EtOAc (10 ml) was added to the residue. The suspension was filtered and the filtrate was then washed with 10% w/v citric acid solution and saturated $NaHCO_3$, then dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (30% acetone–Et_2O eluant) to give the *heptapeptide* (790 mg, 0.916 mmol, 71%) as a white foam: δ_H (500 MHz, DMSO, 90 °C): 9.41 (1H, br s), 8.37 (1H, s), 8.09 (1H, d, *J* 7.3 Hz), 7.68 (1H, d, *J* 8.2 Hz), 7.52 (1H, br s), 7.27–7.18 (5H, m), 6.46 (1H, br s), 5.08–5.03 (2H, m), 4.89 (1H, br s), 4.67 (1H, br s), 4.46 (2H, br s), 4.32–4.28 (2H, m), 3.88–3.76 (4H, m), 3.86 (3H, s), 3.69 (1H, m), 3.07 (1H, dd, *J* 5.1, 14.0 Hz), 2.83 (1H, dd, *J* 9.3, 14.0 Hz), 2.35 (1H, app octet, *J* 6.7 Hz), 2.21–1.99 (4H, m), 1.87 (1H, m), 1.35 (9H, s), 1.07 (3H, d, *J* 6.3 Hz), 0.98 (6H, d, *J* 6.7 Hz), 0.93 (3H, d, *J* 6.7 Hz), 0.92 (3H, d, *J* 6.3 Hz); δ_C (125 MHz, DMSO, 90 °C): 204.5 (s), 172.4 (s), 171.2 (s), 169.9 (s), 169.6 (s), 168.4 (s), 160.9 (s), 154.6 (s), 145.3 (s), 137.7 (s), 128.8 (d), 128.0 (d), 127.6 (d), 125.8 (d), 78.1 (s), 66.9 (d), 66.2 (d), 60.4 (t), 60.3 (d), 58.1 (d), 56.5 (d), 55.7 (d), 51.4 (q), 47.2 (t), 37.5 (t), 33.0 (d), 31.9 (q), 31.6 (d), 29.5 (d), 27.8 (q), 24.0 (t), 23.8 (t), 19.3 (q), 18.8 (q), 17.7 (q), 17.5 (q); HRMS, *m/z* for $C_{40}H_{59}N_7O_{10}S_2$ + Na (M^+ + Na), calcd: 884.3663; found 884.3661.

NMR data for **5**, C-15(*S*)-Val epimer: δ_H (400 MHz, DMSO, 80 °C): 9.41 (1H, br s), 8.36 (1H, s), 8.09 (1H, d, *J* 7.3 Hz), 7.68 (1H, d, *J* 8.2 Hz), 7.52 (1H, br s), 7.27–7.18 (5H, m), 6.46 (1H, br s), 5.08–5.03 (2H, m), 4.89 (1H, br s), 4.67 (1H, br s), 4.46 (2H, br s), 4.32–4.28 (2H, m), 3.88–3.76 (4H, m), 3.87 (3H, s), 3.69 (1H, m), 3.08 (1H, dd, *J* 4.0, 14.1 Hz), 2.84 (1H, dd, *J* 9.3, 14.1 Hz), 2.35 (1H, app octet, *J* 6.7 Hz), 2.21–1.99 (4H, m), 1.9 (1H, m), 1.35 (9H, s), 1.13 (3H, d, *J* 6.3 Hz), 0.98 (6H, d, *J* 6.8 Hz), 0.93 (3H, d, *J* 6.7 Hz), 0.92 (3H, d, *J* 6.3 Hz); δ_C (100 MHz, DMSO, 90 °C): 204.6 (s), 172.4 (s), 171.2 (s), 169.9 (s), 169.6 (s), 168.5 (s), 160.9 (s), 154.7 (s), 145.4 (s), 137.7 (s), 128.8 (d), 128.0 (d), 127.8 (d), 125.8 (d), 78.1 (s), 67.1 (d), 66.2 (d), 60.4 (t), 60.3 (d), 58.1 (d), 56.6 (d), 55.6 (d), 51.4 (q), 47.2 (t),

37.3 (t), 31.9 (d), 31.6 (d), 29.5 (d), 27.8 (q), 23.8 (t), 19.3 (q), 18.8 (q), 17.7 (q), 17.5 (q).

Cyclo-(*R*)-Phe-(*S*)-aThr(Ac)-(*S*)-Val-Pro-Ψ(CS-NH)-(*S*)-Ser(Ac)-Val-Thz; C15(*R*)-Val epimer, 14a

Aqueous NaOH (1 M, 1.8 ml, 1.8 mmol) was added to a solution of the C-15-(*R*)-Val epimer of **5** (314 mg, 0.36 mmol) in 3:1 THF–MeOH (8 ml) at 0 °C and the solution was stirred at 0 °C for 1 h. The solution was concentrated *in vacuo*, and the residue was then treated with EtOAc (10 ml) and cooled to 0 °C. HCl (2 M, 5 ml) was added dropwise over 10 min and the layers were separated. The aqueous layer was extracted with EtOAc (3 × 25 ml) and the combined organic layers were washed with brine, dried ($MgSO_4$) and concentrated to leave a white solid. The solid was dissolved in DMF (4 ml) and the solution was treated successively with Et_3N (0.30 ml, 2.15 mmol), Ac_2O (0.12 ml, 1.27 mmol) and DMAP (2.6 mg, 0.021 mmol). The resulting solution was stirred at room temperature for 2 h, cooled to 0 °C, then treated with water (5 ml) and allowed to warm to room temperature and stirred for a further 1 h. The solution was diluted with EtOAc (25 ml), then recooled to 0 °C and treated dropwise with HCl (2 M, 2 ml). The two layers were separated and the aqueous layer was extracted with EtOAc (3 × 25 ml). The combined organic layers were washed with water (3 × 10 ml) and brine, then dried ($MgSO_4$) and concentrated *in vacuo* to leave a solid. The residue was dissolved in dichloromethane (3 ml) and the solution was cooled to 0 °C. TFA (3 ml) was added dropwise over 5 min and the resulting solution was stirred at 0 °C for 1 h and then concentrated *in vacuo* to leave the diacetate **13** [C-15-(*R*)-Val epimer] as a solid. The solid was dissolved in DMF (80 ml), and the solution was cooled to 0 °C and treated with *i*Pr_2NEt (0.22 ml, 1.26 mmol). The mixture was stirred at 0 °C for 15 min and then DPPA (0.12 ml, 0.557 mmol) was added and stirring was continued at 0 °C for a further 90 min. The solution was then allowed to warm to room temperature, stirring was stopped and the mixture was allowed to stand at room temperature for 3 days. It was then diluted with EtOAc (250 ml), poured into ice-cold water (50 ml) and the two layers were separated. The aqueous layer was extracted with EtOAc (3 × 100 ml) and then the combined organic extracts were washed with water (5 × 50 ml), dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (30% acetone–Et_2O eluant) to give the *diacetylated cyclopeptide* **14a** (175 mg, 0.215 mmol, 59%) as a foam: δ_H (500 MHz, $CDCl_3$): 8.32 (1H, d, *J* 8.4 Hz), 8.21 (1H, d, *J* 7.4 Hz), 7.95 (1H, s), 7.87 (1H, d, *J* 7.4 Hz), 7.30–7.20 (5H, m), 6.78 (1H, d, *J* 9.1 Hz), 6.49 (1H, d, *J* 8.9 Hz), 5.44 (1H, app dt, *J* 2.8, 7.6 Hz), 5.28 (1H, dq, *J* 3.3, 6.6 Hz), 5.03 (2H, m), 4.88 (1H, app dt, *J* 5.9, 8.7 Hz), 4.77 (1H, dd, *J* 7.8, 12.3 Hz), 4.72 (1H, dd, *J* 3.2, 8.9 Hz), 4.50 (1H, dd, *J* 3.3, 7.4 Hz), 4.40 (1H, dd, *J* 2.8, 12.3 Hz), 3.78–3.68 (2H, m), 3.39 (1H, dd, *J* 5.9, 14.4 Hz), 3.08 (1H, dd, *J* 9.0, 14.4 Hz), 2.46 (1H, m), 2.39 (1H, m), 2.18 (1H, m), 2.07 (3H, s), 2.04 (3H, s), 2.02–1.92 (2H, m), 1.80 (1H, m), 1.36 (3H, d, *J* 6.6 Hz), 1.04 (3H, d, *J* 6.8 Hz), 0.88 (3H, d, *J* 6.7 Hz), 0.84 (3H, d, *J* 6.7 Hz), 0.12 (3H, d, *J* 6.8 Hz); δ_C (125 MHz, $CDCl_3$): 205.0 (s), 173.3 (s), 172.4 (s), 171.8 (s), 170.2 (s), 168.1 (s), 167.6 (s), 165.0 (s), 162.4 (s), 149.1 (s), 136.9 (s), 129.7 (d), 128.8 (d), 127.0 (d), 123.5 (d), 69.8 (d), 69.3 (d), 63.7 (t), 59.3 (d), 57.8 (d), 56.1 (d), 54.1 (d), 53.7 (d), 48.3 (t), 35.0 (t), 33.7 (d), 33.0 (t), 30.7 (d), 24.7 (t), 21.2 (q), 21.0 (q), 20.2 (q), 19.5 (q), 18.9 (q), 16.3 (q), 14.7 (q); a satisfactory MS could not be obtained for this compound.

NMR data for **14a**, C-15(*S*)-Val epimer: δ_H (500 MHz, $CDCl_3$): 8.45 (1H, d, *J* 6.2 Hz), 8.04 (1H, s), 7.87 (1H, d, *J* 8.7 Hz), 7.30–7.20 (5H, m), 7.03 (1H, d, *J* 8.5 Hz), 6.96 (1H, br s), 6.93 (1H, d, *J* 8.7 Hz), 5.42 (1H, app quintet, *J* 6.1 Hz), 5.07 (1H, dt, *J* 2.5, 5.9 Hz), 5.00 (1H, t, *J* 9.1 Hz), 4.92 (1H, app dt, *J* 5.9, 8.7 Hz), 4.71 (1H, dd, *J* 8.2, 12.1 Hz), 4.60 (1H, dd, *J* 5.2, 8.8 Hz), 4.55 (1H, dd, *J* 4.7, 8.8 Hz), 4.32 (1H, dd, *J* 2.5, 12.2 Hz), 4.03 (1H, app t, *J* 6.1 Hz), 3.88 (1H, app q, *J* 8.1 Hz), 3.73–3.63 (1H, m), 3.43 (1H, dd, *J* 4.9, 13.9 Hz), 3.13 (1H, dd, *J* 8.2, 13.9 Hz), 2.41 (1H, m), 2.32 (1H, m), 2.25 (1H, m), 2.09 (3H, s), 1.98 (3H, s), 2.06–1.92 (2H, m), 1.79 (1H, m), 1.17 (3H, d, *J* 6.4 Hz), 1.06 (3H, d, *J* 6.5 Hz), 0.95 (3H, d, *J* 6.75 Hz), 0.84 (3H, d, *J* 6.7 Hz); δ_C (125 MHz, $CDCl_3$): 204.0 (s), 173.3 (s), 173.2 (s), 171.5 (s), 170.0 (s), 168.3 (s), 167.7 (s), 166.0 (s), 161.1 (s), 148.7 (s), 136.3 (s), 129.6 (d), 129.0 (d), 127.2 (d), 124.4 (d), 69.8 (d), 69.5 (d), 63.9 (d), 61.1 (d), 61.0 (d), 56.2 (d), 55.4 (d), 55.2 (d), 48.6 (t), 37.45 (t), 32.7 (t), 32.5 (d), 30.1 (d), 24.8 (t), 21.2 (q), 21.0 (q), 20.2 (q), 19.8 (q), 19.0 (q), 17.3 (q), 14.25 (q).

Cyclodidemnamide, 15

K_2CO_3 (1 M, 1.2 ml) was added dropwise over 5 min to a solution of the C-15-(*R*)-Val epimer of **14a** (96 mg, 0.118 mmol) in MeOH (5 ml) at 0 °C and the solution was stirred at 0 °C for 1 h. Ethyl acetate (25 ml) was added and the solution was then poured into water (10 ml). The layers were separated and the aqueous layer was extracted with EtOAc (3 × 25 ml). The combined organic layer and extracts were dried ($MgSO_4$) and then concentrated *in vacuo* to leave the corresponding diol as a white powder. The powder was dissolved in THF (20 ml) and Burgess' reagent (70 mg, 0.294 mmol) was added **(Note: it was necessary to use rigorously dried THF in this reaction)**. The solution was heated under reflux for 2 h and then concentrated *in vacuo* to leave a residue which was purified by flash chromatography on silica gel (2% MeOH–dichloromethane as eluant) to give cyclodidemnamide (23 mg, 0.033 mmol, 28%) as a white foam whose NMR spectroscopic data agreed completely with literature values.[1]

Data for **1**: δ_H (500 MHz, $CDCl_3$): 8.41 (1H, d, *J* 7.9 Hz), 7.97 (1H, s), 7.48 (1H, d, *J* 7.5 Hz), 7.25 (5H, m), 7.08 (1H, d, *J* 7.5 Hz), 5.15 (1H, dd, *J* 9.8, 1.9 Hz), 5.12 (1H, m), 4.91 (1H, t, *J* 7.2 Hz), 4.83 (1H, quintet, *J* 6.3 Hz), 4.66 (1H, dd, *J* 9.9, 2.6 Hz), 4.35 (1H, t, *J* 7.5 Hz), 4.03 (1H, d, *J* 6.3 Hz), 3.70 (1H, m), 3.64 (1H, dd, *J* 11.2, 1.9 Hz), 3.52 (1H, m), 3.51 (1H, app t, *J* 10.6 Hz), 3.25 (1H, dd, *J* 13.7, 5.7 Hz), 3.18 (1H, dd, *J* 13.7, 6.5 Hz), 3.03 (1H, m), 2.32 (1H, m), 2.17 (1H, m), 2.00 (2H, m), 1.80 (1H, m), 1.39 (3H, d, *J* 6.3 Hz), 1.07 (3H, d, *J* 6.5 Hz), 0.76 (3H, d, *J* 6.7 Hz), 0.72 (3H, d, *J* 6.7 Hz), 0.14 (3H, d, *J* 6.4 Hz); δ_C (125 MHz, $CDCl_3$): 180.5 (s), 171.2 (s), 171.0 (s), 170.6 (s), 169.9 (s), 169.3 (s), 160.8 (s), 147.3 (s), 135.8 (s), 129.7 (d), 128.5 (d), 127.2 (d), 124.9 (d), 82.0 (d), 77.5 (d), 73.9 (d), 63.1 (d), 60.2 (d), 54.2 (d), 48.3 (d), 47.8 (t), 39.6 (t), 36.9 (t), 31.2 (d), 30.7 (d), 30.2 (t), 25.5 (t), 21.9 (q), 20.4 (q), 20.3 (q), 20.2 (q), 14.6 (q); MS (FAB), *m/z* (%): 716 (M^+ + Na, 55), 694 (M^+ + H, 100), 597 (14), 415 (13), 356 (6), 285 (18); HRMS, *m/z* for $C_{34}H_{44}N_7O_5S_2$ (M^+ + H), calcd: 694.2845; found: 694.2813.

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