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Synthesis of novel proline–thiazole based cyclic hexa- and octapeptides

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Abstract—Novel proline–thiazole based cyclopeptides were produced by cyclooligomerisation of an *L*-proline thiazole amino acid HCl in the presence of pentafluorophenyl diphenylphosphinate (FDPP) or diphenyl phosphorazidate (DPPA).

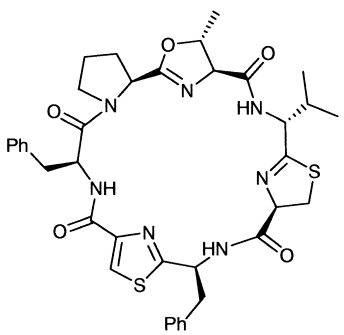
1. Introduction

Nature is a rich source of structurally novel thiazole and oxazole based cyclopeptidic structures, several of which are beginning to show scope for development as potential chemotherapeutic agents. Some representative examples include the lissoclinums, e.g. **1**, from ascidians (sea squirts),[1] telomestatin **2** isolated from *Streptomyces*,[2] wewakazole **3** from the marine cyanobacterium *Lyngbya majuscula*[3] and the ceratospongamides **4** and **5** produced by a marine alga/sponge symbiont.[4] In earlier studies we have demonstrated the scope for cyclooligomerisation and metal-templated assembly of unusual cyclopeptidic constituents from heterocyclic based amino acids.[5,6] We have also evaluated the metal ion chelating properties and cell-membrane transport phenomena of a range of natural and non-natural thiazole based cyclopeptides.[7,8]

A feature of many bioactive cyclic peptides is the presence of proline, in particular, and also N-methylated amino acids, which exercise profound effects on the conformational preferences these molecules can assume.[9] A striking example is the case of ceratospongamide which has been isolated as two stable *cis*,*cis*- and *trans*,*trans*- conformational isomers, **4** and **5** respectively.[4] Not unexpectedly the two isomers **4** and **5** have different biological activities and each can be produced from the other on heating (5:1 equilibrium mixture in favour of the *trans*,*trans*-isomer **5**).[10] As part of our ongoing interests in the biological activity of proline thiazole based cyclic peptides and poly oxazole natural products, e.g. telomestatin **2**, we have evaluated the scope for cyclooligomerisation of the proline thiazole amino acid **10** to synthesise novel cyclic constructs similar to the natural products **1**–**5**. These studies and their outcomes are described here.

2. Results and discussion

The known *N*-Boc protected *L*-proline thiazole amino acid



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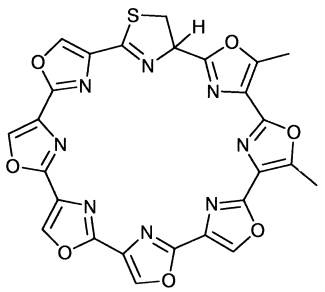

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Keywords: cyclopeptides; amino acid; thiazole.
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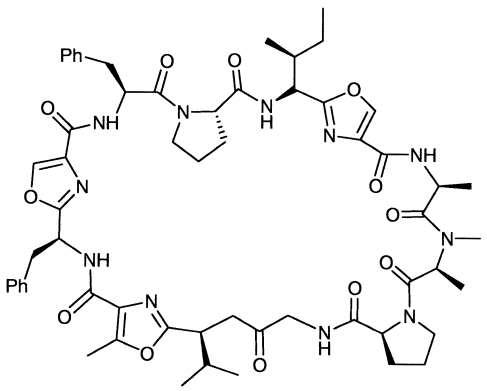

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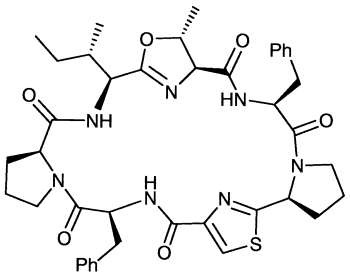

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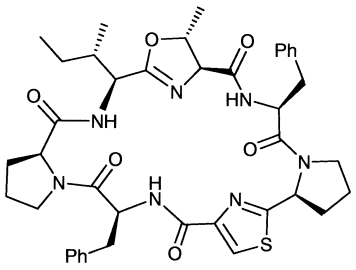

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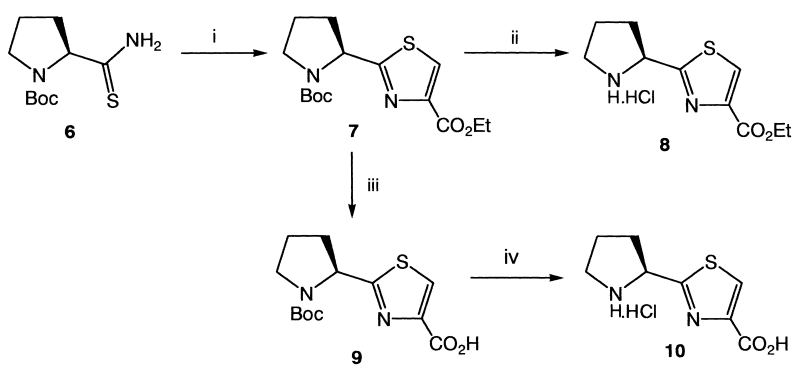
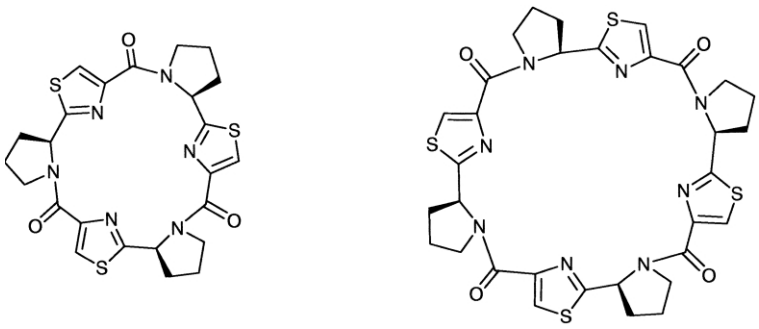
ester **7** was first synthesised starting from commercially available Boc-*L*-proline following conversion to the corresponding thioamide **6**, and a modified Hantzsch reaction with ethyl bromopyruvate.[10,11] Saponification of the ethyl ester **7**, followed by *N*-Boc deprotection of the resulting carboxylic acid **9** then produced the proline thiazole acid hydrochloride **10** as fine colourless crystals (Scheme 1).

Treatment of the amino acid HCl **10** with benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) and *N*,*N*-diisopropylethylamine (DIPEA) in DMF at room temperature for 3 days led only to the cyclic trimer **11** but in poor yield (5%). However, treatment of **10** with either of the coupling reagents pentafluorophenyl diphenylphosphinate (FDPP) or diphenyl phosphorazidate (DPPA), under similar conditions, produced both the trimer **11** and the corresponding tetramer **12**, in a 3:1 ratio and in similar overall yields of 15–20%. Both the trimer **11** and the tetramer **12** were obtained as crystalline solids, and their structures and stereochemistry were confirmed by X-ray crystallography, Figures 1 and 2 respectively. The ^{1}H NMR spectrum of **11** showed only one set of resonances for its three proline thiazole dipeptide units reflecting its C_3 symmetry in analogy with the X-ray crystal structure. The tetramer **12**, however, displayed multiple resonances for its four dipeptide units in the ^{1}H NMR spectrum, suggesting interaction of its nitrogen donor atoms with the NMR solvent.

In order to obtain larger amounts of each of the trimer **11** and the tetramer **12** it was found to be expedient to synthesize each of them in a linear fashion from **9**, and from the amino ester **8** produced from **7**, following straightforward *N*-Boc deprotection. Thus, a coupling reaction between **9** and **8** in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and 1-hydroxy benzotriazole (HOBt) first gave the proline thiazole 'dimer' **13**.



Scheme 1. *Reagents*: (i) $KHCO_3$, DME, ethyl bromopyruvate, −12°C, then TFAA, collidine, −12°C, 61%; (ii) 4 M HCl in 1,4-dioxane, 97%; (iii) NaOH, THF–H_2O (3:1), 93%; (iv) 4 M HCl in 1,4-dioxane, 91%.



11 12

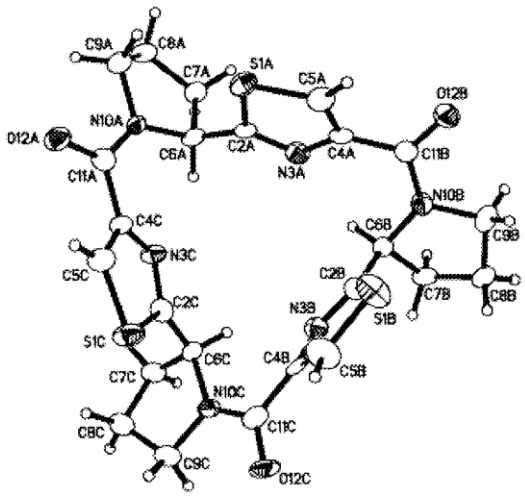


Figure 1. X-Ray crystal structure of the cyclic trimer **11**.

Saponification of **13** to **14**, followed by a second coupling reaction with **8**, next produced the linear 'trimer' **15** (Scheme 2). Saponification and *N*-Boc deprotection of **15** then gave the amino acid **17**, which underwent macrolactamisation with FDPP and DIPEA to give, finally, the target cyclic trimer **11**. In a similar sequence of ordered deprotections and coupling reactions the proline thiazole dimer **13** was elaborated to **14** and **18** and hence to the linear tetramer precursors **19** and **21** to the cyclic tetramer **12** (Scheme 3).

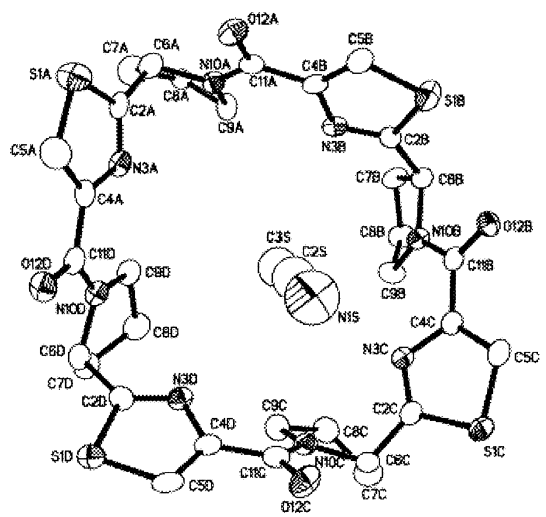
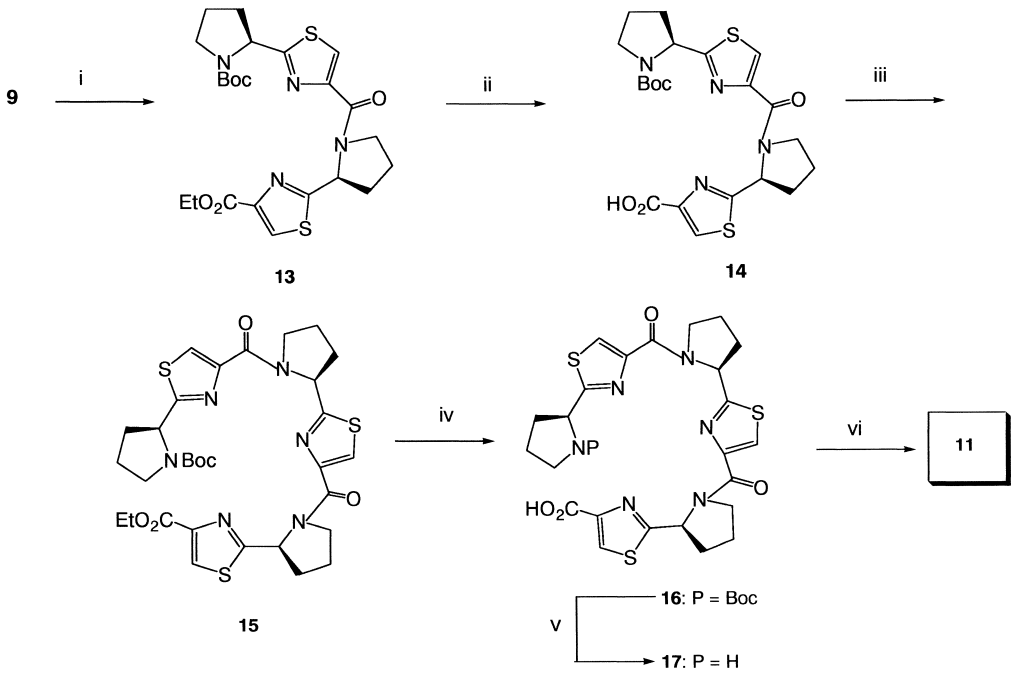


Figure 2. X-Ray crystal structure of the cyclic tetramer **12** (with encapsulation of CH_3CN).

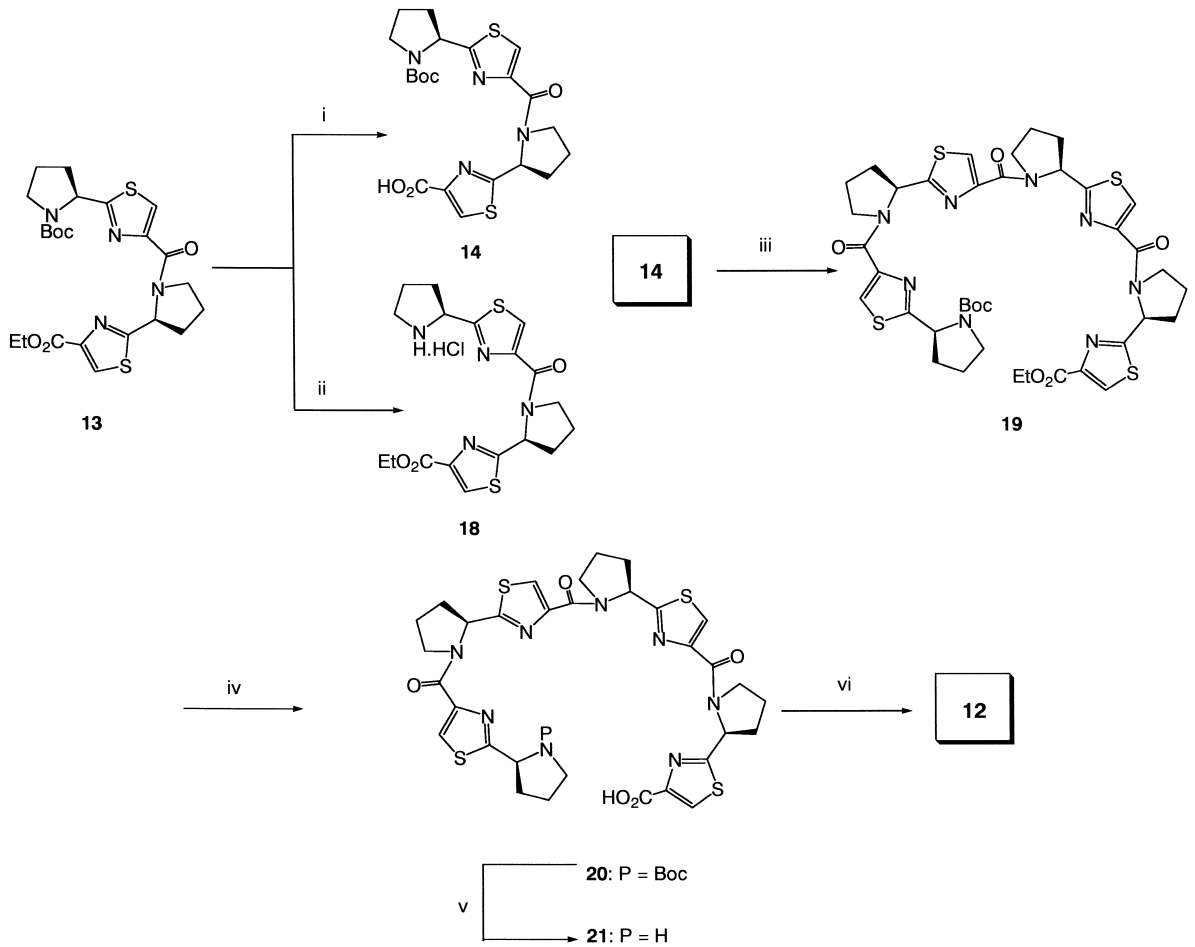
3. Conclusion

The aforementioned study has demonstrated that proline thiazole amino acids can be cyclooligomerised in reasonable yields leading to novel cyclopeptides, e.g. **11** and **12**. Similar to the naturally occurring telomerase inhibitor telemostatin **2** the cyclic octapeptide **12** has eight nitrogen donor centres inside its macrocycle cavity. This feature, together with the metal chelating capacities of these nitrogen donor ligands, suggest that the novel proline–thiazole based cyclic peptides **11** and **12** could reveal unusual ionophoric and biological properties.[†] These features are now being examined as part of our continuing studies of synthesis, metal-chelation, and ion transport phenomena with unusual heterocyclic based cyclic peptides.

[†] Interestingly when the cyclooligomerisation of **10** was carried out in the presence of $LiBF_4$ or $NaBF_4$ increased amounts of the trimer **11** (ratio 2:1 of **11** to **12**) were produced, and in the presence of $AgBF_4$ the trimer **11** was formed almost exclusively in a 21% yield.



Scheme 2. *Reagents*: (i) EDCI, HOBt, **8**, DIPEA, DMF, 0°C to room temperature, 8.5 h, 91%; (ii) NaOH, THF–H_2O (3:1), room temperature, 7 h, 85%; (iii) EDCI, HOBt, **8**, DIPEA, DMF, 0°C to room temperature, 14 h, 87%; (iv) NaOH, THF–H_2O (3:1), room temperature, 9 h, 97%; (v) 4 M HCl in 1,2-dioxane, room temperature, 6 h, 94%; (vi) FDPP, DIPEA, DMF (2.1 mM), room temperature, 5 days, 67%.



Scheme 3. *Reagents*: (i) NaOH, THF–H_2O (3:1), room temperature, 7 h, 85%; (ii) 4 M HCl in 1,4-dioxane, room temperature, 6 h 96%; (iii) EDCI, HOBt, **18**, DIPEA, DMF, 0°C to room temperature, 15 h, 81%; (iv) NaOH, THF–H_2O (3:1), 30 h, room temperature, 87%; (v) 4 M HCl in 1,4-dioxane, 92%; (vi) DPPA, DIPEA, DMF (2.0 mM), room temperature, 5 days, 79%.

4. Experimental

4.1. General details

All melting points were determined on a Kofler hot stage apparatus and are uncorrected. Optical rotations were recorded in spectroscopic grade chloroform or methanol on a Jasco DIP-370 polarimeter, $[\alpha]_D$ values are recorded in units of 10^{-1} deg cm^2 g^{-1}. Infrared spectra were obtained using a Perkin–Elmer 1600 series FT-IR instrument as liquid films or as dilute solutions in spectroscopic grade chloroform. Proton NMR spectra were recorded on either a Bruker DPX 360 or a Bruker DPX 500 spectrometer as dilute solutions in either deuterochloroform or deuteromethanol unless otherwise stated. The chemical shifts are quoted in parts per million (ppm) relative to residual chloroform (δ 7.27) or residual methanol (δ 3.35) as the internal standard and the multiplicity of each signal is designated by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; quin, quintet; b, broad; m, multiplet; app., apparent. All coupling constants are quoted in Hertz. Carbon-13 NMR spectra were recorded on either a Bruker DPX 360 or a Bruker DPX 500 spectrometer as dilute solutions in either deuterochloroform or deuteromethanol unless otherwise stated. Chemical shifts are recorded relative to internal chloroform (δ 77.2) or residual methanol (δ 49.05) as standard on a broad band decoupled mode, and the multiplicities determined using a DEPT sequence. Mass spectra were recorded on a VG micromass 7070E instrument. Microanalytical data were obtained on a Perkin–Elmer 204B elemental analyzer.

Flash chromatography was performed on Merck silica gel 60 as the stationary phase and the solvents employed were either of analytical grade or were distilled before use. All reactions were monitored by TLC using Merck silica gel 60 F_{254} precoated aluminium backed plates, which were visualized with UV-light and then with either acidic ninhydrin solution or basic potassium permanganate solution.

Routinely, dry organic solvents were stored under nitrogen on 3 Å molecular sieves or purchased as solvents stored over molecular sieves. Other organic solvents were dried by distillation from the following: THF (potassium benzophenone ketyl), dichloromethane (calcium hydride), and methanol (magnesium methoxide). All organic extracts were either dried over magnesium sulphate or sodium sulphate. Solvent was removed on a Büchi rotary evaporator and reactions requiring anhydrous conditions were performed with flame-dried apparatus under nitrogen or argon atmosphere as stated.

4.1.1. Ethyl 2-(*N*-*tert*-butoxycarbonyl-2,4-pyrrolidinyl)thiazole-4-carboxylate (7). Flame-dried potassium hydrogen carbonate (21.7 g, 217.0 mmol) was added in one portion to a stirred solution of the thioprolinamide **6**[11c] (5.0 g, 21.7 mmol) in a mixture (95:5) of 1,2-dimethoxyethane and DMF (90 ml) at room temperature under a nitrogen atmosphere. The suspension was stirred at room temperature for 10 min, cooled to −12°C, and then ethyl bromopyruvate (12.7 g, 65.1 mmol) was added dropwise over 5 min. A mixture of trifluoroacetic anhydride (18.2 g, 68.8 mmol) and collidine (21.0 g, 173.7 mmol) was added to the mixture over 4 min, and the resulting orange suspension was stirred for a further 15 min at −12°C. Ice (40 ml) was added and the yellow suspension was then extracted with $CHCl_3$ (3×50 ml). The combined organic extracts were washed successively with 2 M hydrochloric acid (2×50 ml), a saturated solution of copper sulphate (2×50 ml), water (2×50 ml) and brine (100 ml), and then dried over $MgSO_4$. The filtrate was concentrated in vacuo to leave an oil which was purified by flash chromatography on silica gel with petrol–ethyl acetate (9:1)→(1:1) as eluent to give the thiazole (4.1 g, 61%) which crystallised as light yellow crystals; mp 102–104°C (diethyl ether–hexane) (the lit.[11d] quotes mp 180°C). (Found: C, 54.7; H, 6.7; N, 8.3%; calcd for $C_{15}H_{22}N_2O_4S$: C, 55.2; H, 6.8; N, 8.6%); $[\alpha]_D^{20}=-96.7$ (*c* 1.69, DMF) [the lit.[11d] quotes $[\alpha]_D^{20}=38.1$ (*c* 1, DMF)]; ν_{max} ($CHCl_3$) 3125, 1716, 1694, 1388 and 1098 cm^{-1}; δ_H (360 MHz, $CDCl_3$, *T*=298 K) two rotomers (2:1) 1.41 (9H, s, ^{t}Bu), 1.43 (3H, t, *J*=7.1 Hz, OCH_2CH_3), 1.85–1.96 (2H, m, NCH_2CH_2), 2.19–2.39 (2H, m, CH_2CH_2CH), 3.43–3.65 (2H, m, NCH_2), 4.41 (2H, q, *J*=7.1 Hz, OCH_2CH_3), 5.19 (1H, bs, NC*H*), 8.06 (1H, s, Ar *H*); δ_C (90.5 MHz; $CDCl_3$, *T*=298 K) 14.4 (q, CH_3), 23.1 (t, CH_2), 28.2 (q, $C(CH_3)_3$), 34.2 (t, CH_2), 46.7 (t, NCH_2), 59.6 (d, NCH), 61.3 (t, OCH_2), 80.4 (s, $C(CH_3)_3$), 126.7 (d, CH–S), 147.2 (s, C=*C*–C=O), 154.1 (s, CH–*C*=N), 161.4 (s, NCO–O), 177.0 (CO_2Et); *m*/*z* (FAB) 327.1382 ($M+H^+$, $C_{15}H_{23}N_2O_4S$ requires 327.1378).

4.1.2. 2-[*N*-*tert*-Butoxycarbonyl-2,4-pyrrolidinyl]thiazole-4-carboxylic acid (9). Solid sodium hydroxide (0.6 g, 15.0 mmol) was added in one portion to a stirred solution of **7** (0.7 g, 1.9 mmol) in a mixture (3:1) of THF and water (8 ml). The mixture was stirred at room temperature for 5 h, then cooled to 0°C and acidified to pH 2 with dilute hydrochloric acid (2 M). The mixture was diluted with DCM (50 ml), then washed thoroughly with brine (2×40 ml) and dried over $MgSO_4$. The filtrate was concentrated in vacuo to leave the thiazole carboxylic acid (0.55 g, 93%) which crystallised as fine colourless needles; mp 187–189°C (petrol–dichloromethane) (lit.[11d] mp 188.6°C); $[\alpha]_D^{21}=-98.0$ (*c* 1.07, DMF) [lit.[11d] $[\alpha]_D^{20}=-17.3$ (*c* 1, DMF)]; ν_{max} ($CHCl_3$) 3431, 1761, 1694, 1391 and 1368 cm^{-1}; δ_H (360 MHz, CD_3OD, *T*=298 K) 1.35 (9H, s, ^{t}Bu), 1.98–2.09 (2H, m, $CH_2CH_2CH_2$), 2.16–2.28 (1H, m, CH_2CH_2CH), 2.40–2.55 (1H, m, CH_2CH_2CH), 3.48–3.59 (1H, m, NCH_2), 3.62–3.70 (1H, m, NCH_2), 5.14–5.22 (1H, m, NC*H*), 8.18–8.32 (1H, m, Ar*H*); δ_C (90.5 MHz, CD_3OD, *T*=298 K) 24.2 (t, CH_2), 28.5 (q, $C(CH_3)_3$), 34.1 (t, CH_2), 47.9 (t, NCH_2), 60.5 (d, NCH), 81.9 (s, $C(CH_3)_3$), 128.7 (d, CH–S), 148.4 (s, $C-CO_2H$), 157.1 (s, NCO–O), 164.0 (s, CH–*C*=N), 177.8 (s, CO); *m*/*z* (FAB) 321.0833 (M^+Na^+, $C_{13}H_{18}N_2O_4SNa$ requires 321.0884).

4.1.3. 2-Amino-(2,4-pyrrolidinyl)thiazole-4-carboxylic acid (10). A solution of hydrochloric acid (4 M) in 1,4-dioxane (15 ml) was added in one portion to the *N*-Boc-proline thiazole amino acid **9** (1.7 g, 5.7 mmol) at room temperature under a nitrogen atmosphere. The resulting off white suspension was stirred at room temperature for 2.5 h, until TLC analysis indicated the complete consumption of starting material. The dioxane–toluene azeotrope was

removed in vacuo by repeatedly adding toluene (4×30 ml) to leave the amine hydrochloric acid salt (1.21 g, 91%) as a colourless solid; mp 255–256°C (ethyl acetate–ethanol). (Found: C, 40.8; H, 4.6; N, 11.9%; $C_8H_{11}N_2O_2SC$ requires C, 40.9; H, 4.7; N, 11.9%); $[\alpha]_D^{21}=-19.3$ (*c* 1.08, MeOH); ν_{max} (MeOH) 2331, 2130 and 1959 cm^{-1}; δ_H (360 MHz, CD_3OD, *T*=298 K) 2.24–2.35 (3H, m, $CH_2CH_2CH_2$ and CH_2CH_2CH), 2.67–2.69 (1H, m, CH_2CH_2CH), 3.50–3.66 (2H, m, NCH_2), 4.99 (1H, bs, N*H*), 5.23 (1H, t, *J*=7.3 Hz, NC*H*), 8.53 (1H, s, Ar *H*); δ_C (90.5 MHz, CD_3OD, *T*=298 K) 24.7 (t, CH_2), 32.6 (t, CH_2), 46.9 (t, CH_2), 60.7 (d, NCH)), 131.3 (d, CH–S), 148.6 (s, CH=*C*–C=O), 163.8 (s, CH–*C*=N), 166.3 (s, CO); *m/z* (FAB) 221.0343 (M^+Na^+, $C_8H_{10}O_2N_2SNa$ requires 221.0360).

4.1.4. Ethyl 2-[amino-2,4-pyrrolidinyl]thiazole-4-carboxylate (8). A solution of hydrochloric acid (4 M) in 1,4-dioxane (7.5 ml) was added to the *N*-Boc proline **7** (0.9 g, 2.8 mmol) at room temperature and the mixture was then stirred at room temperature for 6 h under a nitrogen atmosphere. The dioxane was removed in vacuo by twice forming an azeotrope with toluene to leave a waxy yellow solid which was stirred with a mixture (2:3) of ethyl acetate and petrol (20 ml) for 1 h, and then filtered. The residue was again treated with toluene to leave the amine hydrochloride salt (0.7 g, 97%) which crystallised as colourless crystals; mp 169–171°C (ethyl acetate–dichloromethane) (lit.[11d] mp 170°C). (Found: C, 45.4; H, 5.6; N, 10.4%; Calc. for $C_{10}H_{15}N_2O_2SCl$: C, 45.7; H, 5.8; N, 10.7%); $[\alpha]_D^{22}=-21.2$ (*c* 1.02, DMF) [lit.[11d] $[\alpha]_D^{20}=-35$ (*c* 1, DMF)]; ν_{max} ($CHCl_3$) 2963, 2675, 1722, 1340 and 1098 cm^{-1}; δ_H (360 MHz, CD_3OD, *T*=298 K) 1.43 (3H, t, *J*=7.2 Hz, OCH_2CH_3), 2.20–2.40 (3H, m, CH_2CH_2CH and $CH_2CH_2CH_2$), 2.62–2.73 (1H, m, CH_2CH_2CH), 3.48–3.66 (2H, m, $NHCH_2$), 4.44 (2H, q, *J*=7.2 Hz, OCH_2), 5.21 (1H, t, *J*=7.4 Hz, NC*H*), 8.56 (1H, s, Ar *H*); δ_C (90.5 MHz, CD_3OD, *T*=298 K) 14.6 (q, CH_3), 24.6 (t, CH_2), 32.6 (t, CH_2), 46.9 (t, OCH_2), 60.6 (d, NCH), 62.8 (t, NCH_2), 131.3 (d, CH–S), 148.0 (s, CH=*C*–CO_2H), 162.4 (s, CH–*C*=N), 166.5 (s, CO); *m/z* (FAB) 227.0848 ($M+H^+$, $C_{10}H_{15}N_2O_2S$ requires 227.0854).

4.1.5. Cyclic trimer (11) and cyclic tetramer (12) by cyclooligomerisation of (10). *N*,*N*-Diisopropylethylamine (0.07 ml, 0.4 mmol) was added dropwise over 5 min to a stirred solution of the proline thiazole amino acid hydrochloride **10** (0.03 g, 0.13 mmol) in *N*,*N*-dimethylformamide (30 ml, 5.0 mM) at room temperature under a nitrogen atmosphere. Pentafluorophenyl diphenylphosphinate (0.1 g, 0.26 mmol) was added in one portion, and the mixture was stirred at room temperature for 72 h. The solvent was removed in vacuo to leave a light brown oil. Chloroform (100 ml) was added and the mixture was then washed successively with 2 M HCl (2×50 ml), 2 M NaOH (2×50 ml), and brine (2×50 ml). The combined organic extracts were dried ($MgSO_4$) and concentrated in vacuo to leave a colourless oil (11 mg, 15%) consisting of a 3:1 mixture of **11** and **12**. Purification by flash chromatography (SiO_2, 5 % methanol in chloroform) gave: (i) cyclic-tris-(*S*),(*S*),(*S*)-proline thiazole **11** (eluted first) which crystallised as colourless crystals; mp >320°C (chloroform, decomp.). (Found: C, 51.4; H, 4.5; N, 14.5%; $C_{24}H_{26}N_6O_4S_3$ requires C, 51.6; H, 4.7; N, 15.0%); $[\alpha]_D^{20}=-59.8$ (*c* 1.03, $CHCl_3$); ν_{max} ($CHCl_3$) 3697, 3122, 2982, 2888, 2358, 1622, 1485, 1393 and 1325 cm^{-1}; δ_H (360 MHz, $CDCl_3$, *T*=298 K) 1.77–1.93 (3H, m, CH_2CH_2-CH_2), 1.95–2.07 (3H, m, $CH_2CH_2CH_2$), 2.08–2.17 (3H, m, $CHCH_2CH_2$) 2.50–2.64 (3H, m, $CHCH_2CH_2$), 3.72 (3H, ddd, *J*=12.1, 9.9, 7.4 Hz, NCH_2), 4.08 (3H, ddd, *J*=12.2, 9.8, 6.8 Hz, NCH_2), 6.82 (3H, d, *J*=7.9 Hz, NC*H*), 7.68 (3H, s, Ar *H*); δ_C (90.5 MHz; $CDCl_3$, *T*=298 K) 21.2 (t, CH_2), 35.1 (t, CH_2), 47.7 (t, NCH_2), 60.0 (d, NCH), 124.5 (d, CH–S), 151.5 (s, CH=*C*–C=O), 162.1 (s, CH–*C*=N), 174.5 (s, CO); *m/z* (FAB) 563.0968 ($M+Na^+$, $C_{24}H_{24}N_6S_3O_3Na$ requires 563.0969); and (ii) cyclic-tetra-(*S*),(*S*),(*S*),(*S*)-proline thiazole **12** (eluted second) which crystallised as colourless crystals; mp 238–239°C (acetonitrile–methanol); $[\alpha]_D^{20}=-108.3$ (*c* 1.01, $CHCl_3$); ν_{max} ($CHCl_3$) 3686, 3124, 2980, 2889, 2255, 1621, 1488, 1460, 1393 and 1311 cm^{-1}; δ_H (360 MHz, $CDCl_3$, *T*=298 K) 1.88–2.69 and 2.90–3.00 (16H, m, $CH_2CH_2CH_2$ and CH_2CH_2CH), 3.65–4.20 (8H, m, NCH_2), 5.34 (1H, d, *J*=7.4 Hz, NC*H*), 5.35 and 5.68–5.73 (1H, m, NC*H*), 6.42 (1H, d, *J*=7.3 Hz, NC*H*), 6.62 (1H, t, *J*=7.7 Hz, NC*H*), 7.70 (1H, s, Ar *H*), 8.06–8.25 (3H, m, Ar *H*); δ_C (90.5 MHz; $CDCl_3$, *T*=298 K) 20.9 (t, CH_2), 21.2 (t, CH_2), 21.5 (t, CH_2), 23.3 (t, CH_2), 26.1 (t, CH_2), 30.1 (t, CH_2), 32.1 (t, CH_2), 33.9 (t, CH_2), 34.6 (t, CH_2), 35.0 (t, CH_2) 47.3 (t, CH_2), 48.0 (t, CH_2), 58.7 (d, CH), 60.4 (d, CH), 61.0 (d, CH), 61.7 (d, CH), 122.5 (d, CH), 123.2 (d, CH), 127.3 (d, CH), 127.6 (d, CH), 148.8 (s, CH=*C*–C=O), 149.9 (s, CH=*C*–C=O), 150.7 (s, CH=*C*–C=O), 151.0 (s, CH=*C*–C=O), 160.4 (s, CH–*C*=N), 161.4 (s, CH–*C*=N), 162.1 (s, CH–*C*=N), 162.5 (s, CH–*C*=N), 168.7 (s, CO), 170.6 (s, CO), 172.2 (s, CO), 175.4 (s, CO); *m/z* (FAB) 721.1547 ($M+H^+$, $C_{32}H_{33}N_8O_4S_4$ requires 721.1507).

Cyclooligomerisation of **10** using PyBOP produced only **11** (5%), and DPPA produced a 3:1 mixture of **11** and **12** in an overall 20% yield.

X-Ray crystal structure determination of **11** and **12**. For each compound data were collected at 150 K on a Bruker SMART CCD area detector diffractometer equipped with an Oxford Cryosystem open-flow nitrogen cryostat, **11** on a SMART1000 and **12** on an APEX. The structures was solved by direct methods (SHELXS-97) and refined using full-matrix least squares refinement against F^2. All non-atoms were refined with anisotropic atomic displacement parameters (adps) and H atoms placed in geometrically calculated positions and refined as part of a riding model, with $U(H)_{iso}=1.5U_{eq}(C)$ unless otherwise stated.

Colourless crystals of **11** were obtained by slow crystallization ($CDCl_3$) at 0–4°C over 1 week. A crystal of dimensions 0.15×0.12×0.10 mm^3 was selected and found to crystallize in the orthorhombic space group $P2_12_12_1$ with *a*=6.446(7), *b*=18.756(7), *c*=20.357(7) Å, *V*=2461(3) $Å^3$, *Z*=4, $D_{calcd}=1.411$ g/cm^3, *T*=150 K. 15012 reflections were measured, 5604 unique ($R_{int}=0.19$), 5601 of which were used in all calculations. The final $wR(F^2)$ was 0.152 for all data, $R_1(F)$ was 0.084 for 2803 observed data where $I>2\sigma(I)$. The Flack parameter refined to −0.06(16) suggesting that the absolute configuration reported is correct

although there may be some ambiguity due to the slightly large standard uncertainty.

Colourless crystals of **12** were obtained by crystallization (CH_3CN–MeOH) at 0–4°C. A crystal of dimensions 0.36×0.28×0.06 mm^3 was selected for the study and also found to crystallize in the orthorhombic space group $P2_12_12_1$ with a=13.568(3), b=14.631(3), c=18.062(3) Å, V=3586(2) $Å^3$, Z=4, T=150 K. 21581 reflections were measured, 8075 unique (R_{int}=0.12), 8043 of which were used in all calculations. The final $wR(F^2)$ was 0.143 for all data, $R_1(F)$ was 0.058 for 6367 observed data where $I>2\sigma(I)$. The Flack parameter refined to −0.09(8) indicating the correct assignment of the absolute configuration. Disorder was observed in C7a and C8a and these were modelled over two sites with occupancies 0.60 and 0.40, suitable distance restraints were applied and the atoms refined with isotropic adps, as were those of the included MeCN molecule.

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 200095 and 200096. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ [fax: +44(0)-1223-336033 or email: deposit@ccdc.cam. ac.uk].

4.1.6. Ethyl 2-[(*N*-*tert*-butoxycarbonyl-2,4-pyrrolidinyl)thiazole]$_2$-4-carboxylate (13). 1-Hydroxybenzotriazole (0.23 g, 1.7 mmol) was added in one portion to a stirred solution of the proline thiazole amino acid **9** (0.42 g, 1.41 mmol) in dry DMF (10 ml) at 0°C under a nitrogen atmosphere. After 10 min, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.32 g, 1.7 mmol) was added, and the mixture was stirred at room temperature for a further 5 min. A solution of the amino ester **8** (0.4 g, 1.5 mmol) in dry DMF (5 ml) was added at 0°C, followed 5 min later by *N*,*N*-diisopropylethylamine (0.49 ml, 2.8 mmol) in one portion at the same temperature. The mixture was stirred at 0°C for 2.5 h, then at 10°C for 3 h and finally at room temperature for a further 3 h. The DMF was removed in vacuo and the residue was diluted with ethyl acetate (100 ml). The ethyl acetate extract was washed with a saturated aqueous solution of ammonium chloride (2×40 ml), and brine (2×40 ml), and then dried ($MgSO_4$). The solvent was removed in vacuo to leave a light brown residue which was purified by flash chromatography on silica gel using petrol–ethyl acetate (9:1)→(1:4) as eluent to give the linear proline thiazole dimer (0.65 g, 91%) as a colourless foam (1:1 mixture of two rotamers). (Found: C, 54.0; H, 5.8; N, 11.2%; $C_{23}H_{30}N_4O_5S_2$ requires C, 54.5; H, 6.0; N, 11.1%); $[\alpha]_D^{24}$=−78.9 (*c* 1.41, $CHCl_3$); ν_{max} ($CHCl_3$) 3125, 1716, 1693, 1621, 1387, 1368 and 1098 cm^{-1}; δ_H (360 MHz, $CDCl_3$, T=298 K) 1.15 (3H, t, J=7.3 Hz, OCH_2CH_3), 1.22 (9H, s, ^{t}Bu), 1.55–2.34 (8H, m, $CH_2CH_2CH_2$ and CH_2CH_2CH), 3.10–3.94 (4H, m, NCH_2), 4.19 (2H, q, J=7.3 Hz, OCH_2CH_3), 4.65 and 4.98 (1H, dd, J=31.0, 6.4 Hz, NC*H*), 5.52 (1H, d, J=4.3 Hz, NC*H*), 6.14 (1H, dd, J=40.4, 6.4 Hz, NC*H*), 7.82–7.97 (2H, m, Ar *H*); δ_C (90.5 MHz; $CDCl_3$, T=298 K) 14.0 (q, CH_3), 14.1 (q, CH_3), 20.6 (t, CH_2), 22.8 (t, CH_2), 23.5 (t, CH_2), 24.7 (t, CH_2), 28.0 (q, $C(CH_3)_3$), 31.2 (t, CH_2), 32.2 (t, CH_2), 32.8 (t, CH_2), 34.5 (t, CH_2), 46.2 (t, CH_2), 46.4 (t, CH_2), 47.4 (t, CH_2), 49.5 (t, CH_2), 57.9 (d, CH), 58.8 (d, CH), 59.5 (d, CH), 60.8 (t, 2×CH_2), 61.1 (d, CH), 79.8 (s, $C(CH_3)_3$) 110.1 (d, CH), 117.8 (d, CH), 126.5 (d, CH), 126.6 (d, CH), 147.0 (s, CH=*C*–C=O), 148.9 (s, CH=*C*–C=O), 149.3 (s, CH=*C*–C=O), 149.9 (s, CH=*C*–C=O), 153.5 (s, CH–*C*=N), 154.2 (s, CH–*C*=N), 160.9 (s, NCO–O), 161.4 (s, CH–*C*=N), 161.8 (s, CH–*C*=N), 173.2 (s, CO), 173.4 (s, CO), 173.9 (s, CO), 176.6 (s, CO); *m*/*z* (FAB) 530.1653 ($M+H^+Na^+$, $C_{23}H_{31}N_4O_5S_2Na$ requires 530.1633).

4.1.7. 2-[(*N*-*tert*-Butoxycarbonyl-2,4-pyrrolidinyl)-thiazole]$_2$-4-carboxylic acid (14). Solid sodium hydroxide (0.38 g, 9.5 mmol) was added in one portion to a stirred solution of the proline thiazole dimer **13** (0.6 g, 1.2 mmol) in a mixture (3:1) of THF and water (12 ml), and the resulting milky suspension was stirred at room temperature for 7 h. The mixture was acidified to pH 2 with dilute hydrochloric acid (2 M) and then extracted thoroughly with ethyl acetate (3×40 ml). The combined organic extracts were washed with brine (2×50 ml), dried over $MgSO_4$, and then evaporated in vacuo to leave the *carboxylic acid* (0.52 g, 93%) which crystallised as colourless crystals; mp 196–197°C (petrol–dichloromethane); $[\alpha]_D^{24}$=−113.9 (*c* 1.05, $CHCl_3$); ν_{max} ($CHCl_3$) 3698, 2980, 1710, 1620, 1391 and 1367 cm^{-1}; δ_H (360 MHz, CD_3OD, T=298 K) 1.33 (9H, s, ^{t}Bu), 1.94–2.50 (8H, m, $CH_2CH_2CH_2$ and CH_2CH_2-CH), 3.46–4.15 (4H, m, NCH_2), 5.18 (1H, d, J=7.3 Hz, NC*H*), 5.64 (1H, m, NC*H*), 6.27–6.42 (1H, m, NC*H*), 8.13–8.30 (2H, m, Ar *H*); δ_C (90.5 MHz, CD_3OD, T=298 K) 22.2 (t, CH_2), 24.1 (t, CH_2), 28.6 (q, $C(CH_3)_3$), 33.1 (t, CH_2), 34.9 (t, CH_2), 47.8 (t, CH_2), 51.5 (t, CH_2), 60.4 (d, CH), 62.9 (d, CH), 81.8 (s, $C(CH_3)_3$), 128.0 (d, CH–S), 128.9 (d, CH–S), 148.2 (s, CH=*C*–C=O), 148.9 (s, CH=*C*–C=O), 156.0 (s, NCO–O), 164.0 (s, CH–*C*=N), 164.3 (s, CH–*C*=N), 175.7 (s, CO), 178.0 (s, CO); *m*/*z* (FAB) 501.1281 ($M+Na^+$, $C_{21}H_{26}N_4O_5S_2Na$ requires 501.1242).

4.1.8. Ethyl 2-[(*N*-*tert*-butoxycarbonyl-2,4-pyrrolidinyl)thiazole]$_3$-4-carboxylate (15). 1-Hydroxybenzotriazole (0.16 g, 1.2 mmol) was added in one portion to a stirred solution of the proline thiazole dimer acid **14** (0.47 g, 0.97 mmol) in DMF (10 ml) at 0°C under a nitrogen atmosphere. The mixture was stirred at 0°C for 5 min and then 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) (0.22 g, 1.2 mmol), the amino ester **8** (0.3 g, 1.0 mmol) and *N*,*N*-diisopropylethylamine (0.34 ml, 1.9 mmol) were added consecutively over 5 min intervals. The mixture was allowed to reach room temperature, stirred for 14 h and then the DMF was removed in vacuo. Ethyl acetate (40 ml) was added to produce a milky brown suspension which was washed with a saturated aqueous solution of ammonium chloride (2×40 ml). The separated aqueous solution was again extracted with ethyl acetate (2×40 ml) and the combined organic extracts were then washed with brine (3×40 ml), dried over $MgSO_4$, and evaporated to dryness in vacuo to leave an oily residue. Purification by chromatography on silica gel with petrol–ethyl acetate (1:1)→(0:100) and then ethyl acetate–methanol (100:0)→(92:8) gave the proline thiazole trimer (0.6 g, 87%) as colourless crystals; mp 92–94°C. (Found: C, 53.3; H, 5.5; N, 12.1%; $C_{32}H_{41}N_6O_7S_3$ requires C, 53.5; H, 5.8; N, 11.7%); $[\alpha]_D^{24}$=−135.9 (*c* 1.06, $CHCl_3$); ν_{max}

($CHCl_3$) 3124, 2980, 1720, 1692, 1620, 1389 and 1323 cm^{-1}; δ_H (360 MHz, $CDCl_3$, T=298 K) 1.24 (3H, t, J=7.1 Hz, OCH_2CH_3), 1.33 (9H, s, ^{t}Bu), 1.94–2.40 (12H, m, $CH_2CH_2CH_2$ and CH_2CH_2CH), 3.30–4.08 and 4.25–4.48 (6H, m, NCH_2), 4.10 (2H, q, J=7.1 Hz, OCH_2CH_3), 4.75–5.19 (1H, m, NC*H*), 5.39 and 5.70 (1H, bs, NC*H*), 6.00–6.43 (1H, m, NC*H*), 7.98–8.10 (3H, m, Ar *H*); δ_C (90.5 MHz; $CDCl_3$, T=298 K) 14.3 (q, CH_3), 21.2 (t, CH_2), 25.3 (t, CH_2), 28.4 (q, $C(CH_3)_3$), 30.0 (t, CH_2), 31.7 (t, CH_2), 35.0 (t, CH_2), 47.0 (t, CH_2), 48.0 (t, CH_2), 49.8 (t, CH_2), 58.9 (d, CH), 60.0 (d, CH), 60.5 (t, CH_2), 61.5 (t, CH_2), 61.8 (d, CH), 80.5 (s, $C(CH_3)_3$), 126.7 (d, CH–S), 127.1 (d, CH–S), 128.0 (d, CH–S), 146.9 (s, CH=*C*–C=O), 147.5 (s, CH=*C*–C=O), 148.9 (s, CH=*C*–C=O), 154.2 (s, NCO–O), 161.5 (s, CH–*C*=N), 162.2 (s, CH–*C*=N), 162.5 (s, CH–*C*=N), 171.3 (s, CO), 173.7 (s, CO), 177.4 (s, CO); *m*/*z* (FAB) 710.1990 ($M+H+Na^+$, $C_{31}H_{39}N_6O_6S_3Na$ requires 710.1990).

4.1.9. 2-[(*N*-*tert*-Butoxycarbonyl-2,4-pyrrolidinyl)-thiazole]$_3$-4-carboxylic acid (16). Solid sodium hydroxide (0.18 g, 0.6 mmol) was added in one portion to a stirred solution of the linear trimer **15** (0.4 g, 0.6 mmol) in a mixture (3:1) of THF and water (12 ml), and the milky suspension was stirred at room temperature for 9 h. The mixture was acidified to pH 2 by the addition of 2 M hydrochloric acid, and then extracted with chloroform (3×40 ml). The combined chloroform extracts were washed with brine (2×40 ml), dried over $MgSO_4$, and then concentrated in vacuo to leave a viscous residue. The residue was triturated with ether to leave the trimer carboxylic acid (0.37 g, 97%) as fine colourless crystals; mp 120–122°C; $[\alpha]_D^{24}=-134.4$ (c 1.03, $CHCl_3$); ν_{max} ($CHCl_3$) 3124, 2979, 1760, 1693, 1620, 1391, 1368 and 1113 cm^{-1}; δ_H (360 MHz, CD_3OD, T=298 K) 1.29–1.52 (9H, m, ^{t}Bu), 1.87–2.55 (12H, m, $CH_2CH_2CH_2$ and CH_2CH_2CH), 3.44–3.97 (6H, m, NCH_2), 4.25 and 5.15 (1H, m, NC*H*), 5.40–5.70 (1H, m, NC*H*), 6.19–6.30 (1H, m, NC*H*), 8.15–8.30 (3H, m, Ar *H*); δ_C (90.5 MHz, CD_3OD, T=298 K) 22.3 (t, CH_2), 24.83 (t, CH_2), 25.99 (t, CH_2), 28.6 (q, $C(CH_3)_3$), 32.1 (t, CH_2), 33.1 (t, CH_2), 35.7 (t, CH_2), 47.7 (t, CH_2), 48.1 (t, CH_2), 51.5 (t, CH_2), 60.4 (d, CH), 61.6 (d, CH), 63.1 (d, CH), 81.8 (s, $C(CH_3)_3$), 124.4 (d, CH), 128.6 (d, CH), 128.9 (d, CH), 148.8 (s, CH=*C*–C=O), 150.1 (s, CH=*C*–C=O), 151.1 (s, CH=*C*–C=O), 155.9 (s, NCO–O), 163.3 (s, CH–*C*=N), 164.0 (s, CH–*C*=N), 164.3 (s, CH–*C*=N), 172.4 (s, CO), 173.0 (s, CO), 178.2 (s, CO); *m*/*z* (FAB) 681.1583 (M^+Na^+, $C_{29}H_{34}N_6O_6S_3Na$ requires 681.1599).

4.1.10. 2-Amino-[(2,4-pyrrolidinyl)thiazole)]$_3$-4-carboxylic acid (17). A solution of hydrochloric acid (4 M) in 1,4-dioxane (9 ml) was added in one portion to the (Boc)-protected amino acid **16** (0.42 g, 0.63 mmol), and the resulting suspension was stirred at room temperature under a nitrogen atmosphere for 6 h. The solvent was removed in vacuo, using toluene as an azeotrope, to leave a foam which was triturated with ether to give the amino acid hydrochloride salt (0.35 g, 94%) as colourless crystals; mp 196–198°C (decomp.); $[\alpha]_D^{20}=-57.8$ (c 1.10, MeOH); ν_{max} ($CHCl_3$) 3696, 3631, 2944, 2838, 1602, 1392 and 1015 cm^{-1}; δ_H (360 MHz, CD_3OD, T=298 K) 1.63–2.70 (12H, m, $CH_2CH_2CH_2$ and CH_2CH_2CH), 3.61–4.40 (6H, m, NCH_2), 5.10–5.30 (1H, m, NC*H*), 5.60–5.90 (1H, m, NC*H*), 6.18–6.24 (1H, m, NC*H*), 8.20–8.40 (3H, m, Ar *H*); δ_C (90.5 MHz, CD_3OD, T=298 K) 22.7 (t, CH_2), 24.6 (t, CH_2), 25.8 (t, CH_2), 32.8 (t, CH_2), 33.4 (t, CH_2), 35.9 (t, CH_2), 47.1 (t, CH_2), 48.7 (t, CH_2), 51.1 (t, CH_2), 60.4 (d, CH), 60.9 (d, CH), 63.2 (d, CH), 129.0 (d, CH), 129.3 (d, CH), 129.6 (d, CH), 148.8 (s, CH=*C*–C=O), 150.0 (s, CH=*C*–C=O), 151.5 (s, CH=*C*–C=O), 162.9 (s, CH–*C*=N), 163.3 (s, CH–*C*=N), 163.9 (s, CH–*C*=N), 165.3 (s, CO), 172.4 (s, CO), 179.2 (s, CO); *m*/*z* (FAB) 559.1221 ($M+H^+$, $C_{24}H_{27}N_6O_4S_3$ requires 559.1255).

4.1.11. Cyclic-tris-(*S*),(*S*),(*S*)-proline thiazole (11). *N*,*N*-Diisopropylethylamine (0.12 ml, 0.7 mmol) and pentafluorophenyl diphenylphosphinate (0.14 g, 0.36 mmol) were added sequentially in one portion to a stirred solution of the amino acid **17** (0.10 g, 0.17 mmol) in dry DMF (80 ml), and the mixture was stirred at room temperature for 5 days under nitrogen atmosphere. The DMF was removed in vacuo to leave a semi-crystalline residue. Ethyl acetate (120 ml) was added and the mixture was washed thoroughly with a 2 M sodium hydroxide solution (5×40 ml), to remove any pentafluorophenyl diphenylphosphinic acid. The combined organic extracts were washed successively with 2 M hydrochloric acid (3×40 ml) and brine (2×40 ml), then dried ($MgSO_4$), and evaporated to dryness in vacuo. The residue was purified by flash chromatography on silica gel using ethyl acetate–methanol (100:0)→(92:8) as eluent to give the cyclic trimer (61 mg, 67%) as orthorhombic crystals (from $CHCl_3$), whose spectroscopic data were identical to those described earlier.

4.1.12. Ethyl 2-[(amino-2,4-pyrrolidinyl)thiazole]$_2$-4-carboxylate (18). A solution of hydrochloric acid (4 M) in 1,4-dioxane (8.5 ml) was added in one portion to the proline thiazole dimer **13** (1.24 g, 2.5 mmol) and the resulting light yellow suspension was stirred at room temperature for 6 h under a nitrogen atmosphere. The mixture was treated with toluene (5×20 ml), forming an azeotrope with 1,4-dioxane, to leave the amine hydrochloric acid salt (1.04 g, 96%) as colourless crystals; mp 187–189°C (ethyl acetate–dichloromethane). (Found: C, 48.3; H, 5.2; N, 12.4%; $C_{18}H_{23}N_4O_3S_2Cl$ requires C, 48.8; H, 5.2; N, 12.7%); $[\alpha]_D^{21}=-39.6$ (c 1.04, $CHCl_3$); ν_{max} ($CHCl_3$) 3698, 3605, 2958, 2526, 2304, 1723, 1626, 1458, 1387 and 1100 cm^{-1}; δ_H (360 MHz, $CDCl_3$, T=298 K) 1.38 (3H, t, J=7.2 Hz, OCH_2CH_3); 1.81–2.62 (8H, m, $CH_2CH_2CH_2$ and CH_2CH_2CH), 3.44–4.10 (4H, m, NCH_2), 4.36 (2H, q, J=7.2 Hz, OCH_2CH_3); 4.98–5.11 (1H, m, NC*H*), 5.65 and 6.67 (1H, d, J=7.5 Hz, NC*H*), 8.00–8.20 (2H, m, Ar *H*), 9.20 (1H, bs, N*H*); δ_C (90.5 MHz; $CDCl_3$, T=298 K) 14.5 (q, CH_3), 21.6 (t, CH_2), 24.2 (t, CH_2), 31.6 (t, CH_2), 35.4 (t, CH_2), 45.6 (t, NCH_2), 48.2 (t, NCH_2), 59.0 (d, NCH), 61.3 (d, NCH), 61.8 (t, OCH_2), 127.3 (d, CH–S), 128.5 (d, CH–S), 146.7 (s, CH=*C*–C=O), 150.2 (s, CH=*C*–C=O), 160.9 (s, CH–*C*=N), 161.5 (s, CH–*C*=N), 163.5 (s, CO), 177.8 (s, CO–O); *m*/*z* (FAB) 407.1133 ($M+H^+$, $C_{18}H_{23}N_4O_3S_2$ requires 407.1211).

4.1.13. Ethyl 2-[(*N*-*tert*-butoxycarbonyl-2,4-pyrrolidinyl)thiazole]$_4$-4-carboxylate (19). 1-Hydroxybenzotriazole hydrate (0.42 g, 3.2 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride

(0.60 g, 3.2 mmol) were added sequentially over a 5 min period to a stirred solution of the amino acid **14** (1.26 g, 2.6 mmol) in dry DMF (25 ml) at 0°C under a nitrogen atmosphere. The mixture was stirred at 0°C for 15 min and then a solution of the ethyl ester **18** (1.37 g, 3.1 mmol) in dry DMF (10 ml) was added in one portion. The mixture was stirred at 0°C for 5 min before adding *N*,*N*-diisopropylethylamine (0.9 ml, 5.3 mmol) over 2 min. The mixture was stirred from 0°C to room temperature for 15 h, then the DMF was removed in vacuo to leave a residue which was diluted with chloroform (80 ml). The organic extract was washed successively with a saturated aqueous solution of ammonium chloride (2×50 ml) and brine (2×50 ml), then dried over $MgSO_4$ and concentrated in vacuo to leave an oily residue. Purification by chromatography on silica gel using chloroform–methanol (100:0)→(92:8) gave the acyclic tetramer (1.83 g, 81%) as a colourless powder; mp 87–89°C. (Found: C, 52.4; H, 5.6; N, 12.8%; $C_{39}H_{48}N_6O_8S_3$ requires C, 52.9; H, 5.5; N, 12.7%); $[\alpha]_D^{21}=-167.5$ (*c* 1.21, $CHCl_3$); ν_{max} ($CHCl_3$) 2980, 2358, 1724, 1692, 1619, 1487, 1390 and 1097 cm^{-1}; δ_H (360 MHz, $CDCl_3$, *T*=298 K) 1.26 (3H, t, *J*=7.2 Hz, OCH_2CH_3), 1.39 (9H, s, ^{t}Bu), 1.74–2.43 (16H, m, $CH_2CH_2CH_2$ and CH_2CH_2CH), 3.38–4.20 (8H, m, NCH_2), 3.43 (2H, q, *J*=7.2 Hz, OCH_2CH_3), 4.90–5.52 (2H, m, NC*H*), 6.10–6.45 (2H, m, NC*H*), 7.90–8.14 (4H, m, Ar *H*); δ_C (90.5 MHz; $CDCl_3$, *T*=298 K) 14.6 (q, CH_3), 21.4 (t, CH_2), 25.4 (t, CH_2), 28.5 (q, $C(CH_3)_3$), 29.9 (t, CH_2), 31.7 (t, CH_2), 32.7 (t, CH_2), 34.4 (t, CH_2), 35.3 (t, CH_2), 47.7 (t, CH_2), 48.0 (t, CH_2), 49.9 (t, CH_2), 59.0 (d, CH), 59.3 (d, CH), 60.0 (t, CH_2), 60.2 (d, CH), 60.6 (t, CH_2), 61.0 (d, CH), 61.5 (t, CH_2), 80.5 (s, $C(CH_3)_3$), 125.9 (d, CH–S), 126.5 (d, CH–S), 126.7 (d, CH–S), 127.1 (d, CH–S), 147.0 (s, CH=*C*–C=O), 147.6 (s, CH=*C*–C=O), 148.9 (s, CH=*C*–C=O), 150.0 (s, CH=*C*–C=O), 154.8 (s, NCO–O), 161.6 (s, CH–*C*=N), 162.2 (s, CH–*C*=N), 162.6 (s, CH–*C*=N), 162.7 (s, CH–*C*=N), 171.3 (s, CO), 171.5 (s, CO), 174.5 (s, CO), 177.4 (s, CO); *m*/*z* (FAB) 889.2221 ($M+H^+$, $C_{39}H_{46}N_8S_4O_7$ requires 889.2270).

4.1.14. 2-[(*N*-*tert*-Butoxycarbonyl-2,4-pyrrolidinyl)-thiazole]$_4$-4-carboxylic acid (20). Solid sodium hydroxide (0.7 g, 16.75 mmol) was added in one portion to a stirred solution of the tetramer **19** (1.8 g, 2.1 mmol) in a mixture (3:1) of THF and water (40 ml) at room temperature, and the mixture was then stirred at room temperature for 30 h. The mixture was diluted with water (50 ml) and then washed with ethyl acetate (60 ml) to remove the unreacted starting material. The aqueous layer was acidified to pH 2 with a 2 M hydrochloric acid solution and then extracted with chloroform (3×50 ml). The combined organic extracts were washed with brine (2×50 ml), dried over $MgSO_4$ and then concentrated in vacuo to leave the carboxylic acid (1.54 g, 87%) as a colourless solid; mp 125–127°C; $[\alpha]_D^{21}=-155.4$ (*c* 1.14, $CHCl_3$); ν_{max} ($CHCl_3$) 3696, 2979, 2886, 1731, 1693, 1620, 1488, 1391, 1346 and 1115 cm^{-1}; δ_H (360 MHz, CD_3OD, *T*=298 K) 1.47 (9H, s, ^{t}Bu), 1.82–2.35 (16H, m, $CH_2CH_2CH_2$ and CH_2CH_2CH), 3.45–4.10 (8H, m, NCH_2), 4.20 and 4.89 (1H, m, NC*H*), 5.24–5.42 (1H, m, NC*H*), 5.52–5.72 (1H, m, NC*H*), 6.02–6.45 (1H, m, NC*H*), 8.01–8.13 (4H, m, Ar *H*); δ_C (90.5 MHz; $CDCl_3$, *T*=298 K) 21.2 (t, CH_2), 21.5 (t, CH_2), 25.2 (t, CH_2), 25.3 (t, CH_2), 28.4 (q, $C(CH_3)_3$), 30.6 (t, CH_2), 31.3 (t, CH_2), 31.5 (t, CH_2), 35.0 (t, CH_2), 46.7 (t, CH_2), 47.8 (t, CH_2), 50.1 (t, CH_2), 59.1 (d, CH), 59.3 (d, CH), 59.6 (d, CH), 60.5 (t, CH_2), 61.7 (d, CH), 80.6 (s, $C(CH_3)_3$), 126.6 (d, CH–S), 126.9 (d, CH–S), 127.7 (d, CH–S), 128.2 (d, CH–S), 146.6 (s, CH=*C*–C=O), 147.3 (s, CH=*C*–C=O), 148.9 (s, CH=*C*–C=O), 149.6 (s, CH=*C*–C=O), 154.3 (s, NCO–O), 161.9 (s, CH–*C*=N), 162.2 (s, CH–*C*=N), 162.3 (s, CH–*C*=N), 163.1 (s, CH–*C*=N), 171.3 (s, CO), 173.6 (s, CO), 173.8 (s, CO), 176.9 (s, CO); *m*/*z* (FAB) 861.1970 ($M+Na^+$, $C_{37}H_{42}N_8O_7S_4$ requires 861.1957).

4.1.15. 2-Amino-[(2,4-pyrrolidinyl)thiazole]$_4$-4-carboxylic acid (21). A solution of hydrochloric acid (4 M) in 1,4-dioxane (14 ml) was added in one portion to the linear tetramer amino acid **20** (1.5 g, 1.7 mmol) and the mixture was stirred at room temperature for 1.5 h under a nitrogen atmosphere. The dioxane was removed in vacuo by forming an azeotrope with toluene to leave the amine as a sticky colourless foam. Trituration with ethyl acetate left the amino acid (1.21 g, 92%) as colourless crystals; mp 180–211°C (slow decomp.); $[\alpha]_D^{24}=-73.9$ (*c* 1.06, $CHCl_3$); ν_{max} ($CHCl_3$) 3696, 2958, 1715, 1616 and 1391 cm^{-1}; δ_H (360 MHz, CD_3OD, *T*=298 K) 1.82–2.41 (16H, m, $CH_2CH_2CH_2$ and CH_2CH_2CH), 3.43–4.20 (8H, m, NCH_2), 4.94–5.10 (1H, m, NC*H*), 5.26–5.41 (1H, m, NC*H*), 5.61–5.69 (1H, m, NC*H*), 6.08–6.50 (1H, m, NC*H*), 8.15–8.50 (4H, m, Ar *H*); δ_C (90.5 MHz, CD_3OD, *T*=298 K) 22.2 (t, CH_2), 24.6 (t, CH_2), 26.1 (t, CH_2), 26.2 (t, CH_2), 32.5 (t, CH_2), 32.7 (t, CH_2), 32.8 (t, CH_2), 35.7 (t, CH_2), 47.0 (t, CH_2), 51.2 (t, CH_2), 51.5 (t, CH_2), 54.9 (t, CH_2), 60.4 (d, CH), 60.7 (d, CH), 60.8 (d, CH), 62.4 (d, CH), 126.9 (d, CH–S), 127.2 (d, CH–S), 127.4 (d, CH–S), 128.2 (d, CH–S), 148.0 (s, CH=*C*–C=O), 148.8 (s, CH=*C*–C=O), 150.7 (s, CH=*C*–C=O), 151.4 (s, CH=*C*–C=O), 163.5 (s, CH–*C*=N), 163.9 (s, CH–*C*=N), 164.8 (s, CH–*C*=N), 165.3 (s, CH–*C*=N), 172.4 (s, CO), 175.7 (s, CO), 176.2 (s, CO), 178.4 (s, CO); *m*/*z* (FAB) 739.1644 (M^+, $C_{32}H_{35}N_8O_5S_4$ requires 739.1613).

4.1.16. Cyclic-tetra-(*S*),(*S*),(*S*),(*S*)-proline thiazole (12). *N*,*N*-Diisopropylethylamine (0.08 ml, 0.45 mmol) and diphenylphosphoryl azide (0.07 ml, 0.32 mmol) were added sequentially in one portion to a solution of the linear tetramer amino acid hydrochloride salt **21** (0.10 g, 0.13 mmol) in dry DMF (65 ml), and the resulting suspension was stirred at room temperature for 120 h under a nitrogen atmosphere. The mixture was evaporated to dryness in vacuo and the residue was then diluted with chloroform (70 ml). The chloroform solution was stirred with a saturated aqueous solution of sodium hydrogen carbonate (70 ml) for 4 h, and the separated chloroform extract was then washed with a saturated aqueous solution of ammonium chloride (3×40 ml) followed by brine (2×30 ml). The dried extract was concentrated in vacuo to leave a residue which was purified by flash chromatography on silica gel, using $CHCl_3$–MeOH (100:0)→(95:5) as eluent to give the cyclic tetramer (73.1 mg, 79%) as colourless crystals whose spectroscopic data were identical to those described earlier.

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