Design, construction and properties of peptide *N*-terminal cap templates devised to initiate α-helices. Part 2.† Caps derived from *N*-[(2*S*)-2-chloropropionyl]-(2*S*)-Pro-(2*S*)-Pro-(2*S*,4*S*)-4-thioPro-OMe

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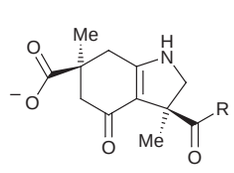
Recently, we designed 12-membered macrocyclic template caps to entrain peptides into α-helical structures, based on the covalent connection of the first and fourth residues of proline containing tetrapeptides. It was not possible to complete the synthesis of the templates from the acyclic precursors and it appeared that the generation of large molecular dipoles, caused by aligning the carbonyl groups, prevented reaction. While this work was in progress, Kemp's group published the structure of a 12-membered macrocyclic triproline template designed to initiate an α-helix that was very similar in structure to one of our own targets. However, the compound failed to cyclise in a conformation required for α-helix initiation and one or more carboxamide dipoles were not aligned. Here we provide a detailed conformational analysis of the system and test two methods for forcing the acyclic precursor into the macrocyclic conformation required for helix initiation. The first is the destabilisation of unwanted conformations in the transition state for cyclisation, and the second is the stabilisation of the favoured transition state structure through the introduction of a hydrogen-bonding interaction. Both strategies were unsuccessful and the reasons are discussed. A successful strategy which does not require the carbonyl dipoles to align in the transition state is presented in the following paper.

Introduction

The preparation of an α-helix initiating template possessing a Zimm–Bragg σ-value of 1.0, remains a considerable challenge. Effectively such a structure would align four carbonyl groups, of which the first three should be derived from carboxamides, in an α-helical conformation with the correct twist and pitch for α-helix propagation. Recently many groups have tried to devise such templates, but in each case the systems have been less effective than was hoped for. The properties of the most successful systems were discussed in the previous article where it was evident that the best of these, template **1**,[1] used several tricks to coax the templated peptide into an α-helical conformation, just as nature does. Such tricks generally include stabilising the helix dipole with charges at the termini, designing-in salt-bridges between i and $(i + 4)$ residues, using amino acids with high helix propagation values (high Zimm–Bragg s values) and using fluorophilic protic solvents such as trifluoroethanol. However, the real problem with all of the systems prepared to date is not that the polypeptide will not propagate, but that the templates do not emulate the structural and electronic properties of an α-helix closely enough.



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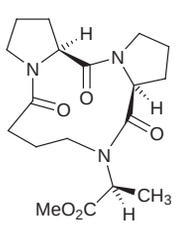

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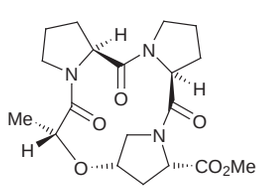

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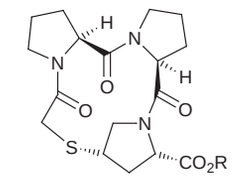
In order to circumvent some of these problems we designed template caps based on the covalent connection of the first and fourth residues of proline containing tetrapeptides which could, potentially, adopt almost perfect α-helical conformations and provide carbonyl groups properly aligned for helix propagation in templated peptide analogues. However, we were unable to complete the synthesis of the templates **2** and **3** from the acyclic precursor *N*-terminal halogenoacyl prolinyl peptide esters. Analysis of the transition states for the final cyclisation steps indicated that steric effects were unlikely to be the primary cause of the problem. Rather, it appeared that it was the generation of large molecular dipoles, caused by aligning the carbonyl groups that was further increasing the energy of the system as it approached the transition state for macrocyclisation. It was also noted that the metal ions that were present to stabilise the nucleophiles could also interact by chelation of the carbonyl O-atoms and stabilise conformations that could not further react to give the required templates.

Results and discussion

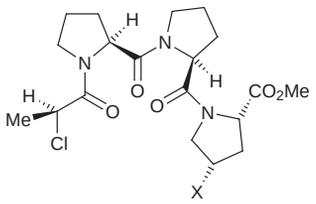
While this work was in progress, Kemp's group published the structure of a 12-membered macrocyclic triproline template **4** designed to initiate an α-helix that was very similar in structure to one of our own targets, compound **3**.[2,3] Although the template was synthesised *via* a completely different route, the 12-membered macrocycle had been prepared and differed only in

† For Part 1, see ref. 4.

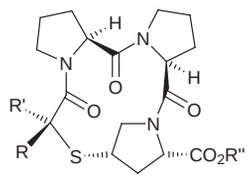


R = H or Me

4

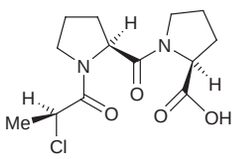


5; X = OH
7; X = SH
12; X = SAc



R'' = H or Me

6; R = H, R' = Me
8; R = Me, R' = H



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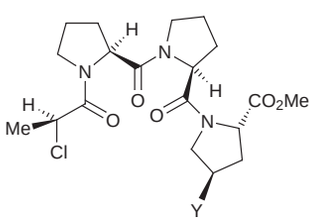
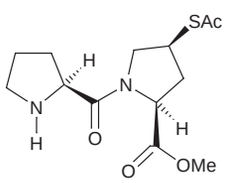


9; Y = OTs
11; Y = OH



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that a thioether linkage was used in place of the oxygen ether in system **3** and that the sulfur atom was connected to an achiral acetyl moiety rather than to the (2*R*)-centre of a propionyl residue, as in our own system **3**. Effectively the difference here amounts to the use of a glycine residue analogue by Kemp in structure **4** and a (2*R*)-alanine residue in structure **3** where, in the required all-*trans* amide forms of the templates, the heteroatom would reside in a position occupied by the amino group of the first residue in a natural α-helix. This latter difference in the structure of the first residue is of considerable importance because we had introduced the extra steric bulk specifically to destabilise the *cis* rotameric form of the first amide linkage. Moreover, Kemp actually prepared the *ctt* and *cct* forms of the template rather than the desired *ttt* form. Given that it is extremely difficult for the amide rotamers to equilibrate in the closed cycle, Kemp's system must have possessed a significant population of the first amide bond in the *cis*-form prior to cyclisation, and as such the carbonyl groups would not have been aligned in the transition state for cyclisation, Fig. 1. Thus, it seemed very likely that the reason compound **5** could not be converted to the requisite macrocycle was exactly because all of the amide groups were *trans*-oriented for significantly more time, as was determined by NMR spectroscopy,[4] and therefore that all of the carbonyl groups were aligned to give a large dipole moment and, hence, a high energy transition state for cyclisation, Fig. 2.

In view of this analysis, it seemed worthwhile to investigate the system further by first preparing the thioether version of compound **3** (*i.e.* template **6**). The replacement of the O-atom in structure **3** by sulfur was expected to facilitate the formation of an anionic nucleophilic and slightly ease crowding in the cyclised material, due to the increased length of C–S bonds. It was determined that a modification of our previous synthetic strategy would furnish the maximum amount of information

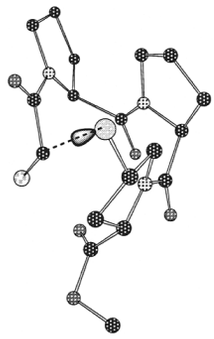


Fig. 1 Transition-state conformation required for the cyclisation to the *ctt* rotamer of Kemp's template **4**.

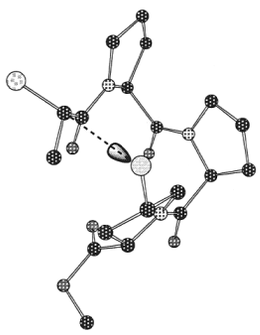


Fig. 2 Transition-state conformation required for the cyclisation of the *ttt* rotamer of compound **5**.

because the rotameric forms of each amide bond could be assessed in the precursor, by NMR spectroscopy, as before. We therefore decided that the last step should be the displacement of chloride by a Pro[4]-thiolate ion derived from a template precursor such as compound **7**.

Given that it was expected that the synthesis of the precursor **7** would not present a formidable challenge in its own right, it was of interest to examine the possible mechanism for its cyclisation to the *ttt*-form of the template, structure **6**, in more detail. We knew from our earlier work that the precursor **7** should exist primarily in its *ttt*-form. We also suspected from our analysis so far, that the alignment of carbonyl dipoles in the ground state of the required product increased its internal energy relative to the ground state of the precursor. Thus, as the ground state *ttt*-form of the precursor **7** began to approach the transition state for cyclisation, the energy of the system would increase, even before the concerted S_N2 displacement of the chloride ion became an important contributor to the energy of the system. However, the ground state of the precursor **7** is actually in equilibrium with a number of other rotameric forms including the *ctt* and the *cct* forms, both of which could cyclise to form the corresponding macrocycles. Indeed, *N*-acyl analogues of these latter compounds were the predominant products of Kemp's synthesis of the *ttt* form of template **4**. Thus, it seemed appropriate to obtain some measure of the energy penalty associated with aligning the carbonyl dipoles into a helical structure.

Application of the Hammond postulate for the endothermic cyclisation of precursors such as **7** to template structures indicates that the structures of the transition states should resemble the structures of the products. Furthermore, the relative energies of the transition states should relate to the energies of the products. It would be possible to obtain relative energies for the cyclised products by performing molecular mechanics calculations on each of the possible conformers of the template **6**. It is also reasonable to assume that the energetics for bond breaking and making in the actual displacement of chloride by thiolate in each transition state would be equal, such that for comparative purposes, these contributions to the transition

Table 1 Molecular mechanics energy calculations (in kJ mol^{-1}) for acids **4**, **6** and **8**[a]

Acid **4**			Acid **6**			Acid **8**		
Code	State[b]	Energy	Code	State[b]	Energy	Code	State[b]	Energy
G	*ctt*	299.8	*A*	*cct*	392.9	*G*	*ctt*	388.8
B	*cct*	300.6	*M*	*ttt*	405.4	*AE*	*ctt*	397.9
AE	*ctt*	307.0	*AA*	*tct*	413.1	*AG*	*ctt*	400.2
R	*ctt*	311.0	***S**** [c]	*ttt*	413.9	*R*	*ctt*	401.7
K	*ctt*	312.0	*I*	*ttt*	417.8	*AB*	*ctt*	414.4
M	*ttt*	317.5	***U**** [c]	*ttt*	419.3	*A*	*cct*	418.9
U* [c]	*ttt*	318.7	***O**** [c]	*ttt*	421.9	***U**** [c]	*ttt*	423.6
S* [c]	*ttt*	323.9	*Q*	*tct*	422.8	*D*	*ctt*	424.2
AA	*tct*	324.4	*F*	*ttt*	424.1	*I*	*ttt*	425.3
AB	*ctt*	325.1	*N*	*cct*	424.4	*M*	*ttt*	425.4
O* [c]	*ttt*	325.9	*G*	*ctt*	425.8	*Q*	*tct*	428.6
I	*ttt*	327.0	*Y*	*tcc*	426.8	***O**** [c]	*ttt*	431.4
N	*cct*	330.1	*Z*	*ttt*	431.3	*AA*	*tct*	432.0
A	*cct*	330.1	*P*	*tct*	433.8	***S**** [c]	*ttt*	433.3
Y	*tcc*	332.1	*AG*	*ctt*	436.5	*P*	*tct*	433.3

[a] Conformational energies were calculated using the AMBER all-atoms molecular mechanics force-field[28] and the Insight II program.[29] Energies are relative to an arbitrary minimum and are given in kJ mol^{-1}. [b] "State" describes the configurations of the three amide bonds in the 12-membered ring. [c] Conformers with the correct set of dihedral angles for α-helix initiation are highlighted in bold and marked with an asterisk.

state energies cancel. Thus, from transition state theory, the rates of formation of given cyclic products would be related to the concentration of each of the transition state complexes for which the relative energies should correspond to those calculated for the various conformational forms of the cyclised template. By compensating for the actual ground state concentrations of the various rotameric forms of the starting material, it should, therefore, be possible to predict the kinetic product of a given cyclisation reaction and then go on to identify, and then test, improvements in the system that would lead to the formation of a high σ value template.

Accordingly, the energies of all of the conformations of the macrocyclic thioether template systems **4**, **6** and **8** containing, respectively, acetyl-, (2*R*)-propionyl- and (2*S*)-propionyl-moieties in the first position, were calculated using the AMBER all-atoms molecular mechanics forcefield. The results are summarised in Table 1, where the fifteen computed lowest energy conformations for each macrocycle are shown. Note that for torsion about the CO–N bond, the torsional angle, ω, must be close to 0° (*cis*) or 180° (*trans*).

cis–trans Isomerisation at each of the three tertiary amide bonds generates eight possible configurational isomers where only the *ttt* form is potentially capable of α-helix initiation. Each of these eight "parent" conformations of defined ω values possesses a set of sub-states. These sub-states differ in the φ (N–C$^{\alpha}$ bond torsion) and ψ (C$^{\alpha}$–CO bond torsion) angles for each proline residue and for the α-thio acid residue, and in the torsional angle about the Pro^{4}–C4–S bond of the thioether bridge. Some of these sub-states which exist in the *ttt* form, for example, structures *M* and *I*, do not contain aligned carbonyl groups and, therefore, are not capable of α-helix initiation.

The relative conformational energies of Kemp's macrocycle **4** indicate that six isomeric forms exist which are of greater stability than the most likely α-helix-initiating *ttt* sub-state ***U****. The five lowest energy forms of these possess *cis*-stereochemistry for the first amide bond. Note that this site is adjacent to the site of structural variation in the target compounds **6** and **8**. The energy difference between the most stable forms *G* and *B* and the most stable α-helix-initiating *ttt* sub-state ***U**** is about 19 kJ mol^{-1}. While it is accepted that such calculated energy differences are not accurate, they are useful, especially when large in magnitude, and in this case the modelling study correctly predicts the structure of compound **4**, which was experimentally determined by Kemp *et al.* to assume the *ctt* and *cct* forms.[2]

Compared to compound **4**, structure **6** possesses an extra methyl group in the (*R*)-configuration at C-2 in the first residue.

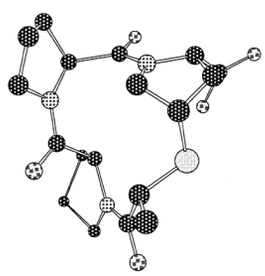


Fig. 3 The most stable form of **6**, *cct* sub-state, **A**.

The calculations show that, relative to the situation for compound **4**, the *ctt* forms of compound **6** are significantly destabilised in favour of the *ttt* forms. Indeed, six of the nine most stable sub-states for compound **6** are *ttt* forms and none of these nine most stable sub-states is a *ctt* form. The most stable form is a *cct* sub-state, *A*, in which the extra steric size of the methyl group is repealed by the two adjacent *cis*-amide bonds which place it in an equatorial position on the periphery of the macrocycle, Fig. 3. Interestingly, the orientation of the carbonyl dipoles in sub-state *A* are also randomised. Given that the *cct* rotameric form of the precursor **7** is not likely to be highly populated, since the equivalent *cct* rotameric form of **5** was not,[4] this consideration alone would not mean that *A* would be the kinetic product of any attempted macrocyclisation. However, the finding that the *M* sub-state of the *ttt* form was the most stable of the *ttt* forms, and 8.5 kJ mol^{-1} more stable than the lowest energy helix nucleating form, ***S****, was of more concern. This is because sub-state *M* possesses non-aligned carbonyl groups, in which the second carbonyl group O-atom is twisted up and in above the 5-methylene group of Pro^{4}, so that it is about 140° out of alignment with the first carbonyl group (*i.e.* nearly anti-aligned), Fig. 4. Furthermore, peptide extended analogues of the *M* sub-state of compound **6** could not be expected to untwist at accessible temperatures to give peptide extended analogues of the ***S**** form. The conformational reorganisation would require two isomerisations of the second amide bond *via* the *tct* form, *AA*, both of which are thermodynamically up-hill and both of which require large activation energies of 75 kJ mol^{-1} or more, for overcoming amide resonance and reorganising the macrocycle. These isomerisations would need to occur without the single isomerisation of the first amide bond to the *cis* form, either directly from the *M* sub-state (to give the species *G*) or, from the intermediate *AA* which would give the most stable species, *A*. Thus, it appeared that

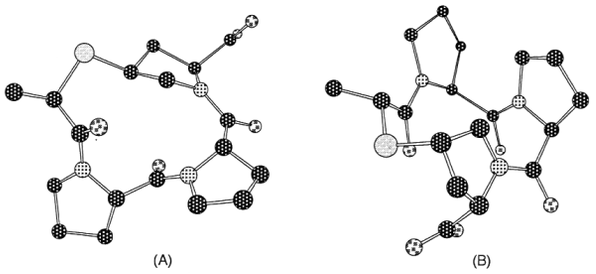


Fig. 4 The *M* sub-state (A) and the ***S**** sub-state (B) of **6**.

if cyclisation did occur from a predominantly all-*trans* form of the precursor **7**, the *M* sub-state, and possibly also the *A* sub-state, would be the predominant products.

Methyl substitution with (*S*)-stereochemistry at C-2 of the first residue gives compound **8**. In this system the six most stable sub-states possess *cis* stereochemistry in the first amide bond and five of these are *ctt* sub-states. The difference in energy of 34.8 kJ mol^{-1} between the most stable sub-state, *G*, and the lowest energy helix nucleating form, ***U****, in this case is larger than that for the diastereomer **6**, 21 kJ mol^{-1} (for *A* isomerising to ***S****). Indeed. the situation is worse for compound **8** than for the unsubstituted system **4** largely because the helix nucleating forms of **8** possess an adverse 1,3-diaxial interaction between the methyl group and the 5-methylene group of Pro[2]. It is interesting to note that the non-helix nucleating *ttt* form, sub-state *M*, is also destabilised by the same diaxial interactions. Thus, compound **8**, it appears, is not likely to cyclise to give an all *trans* form of the template.

From the comparison of the conformational energies for each compound, it is apparent that macrocycle **6** is the most likely to afford a sub-state capable of displaying α-helix-initiating properties (for example, conformer ***S****). However, it is also apparent that for the parent acid derivative conformer *A* with a *cct* arrangement of amide bonds and conformer *M* a twisted *ttt* sub-state which possesses no net dipole are the likely kinetic products. In order to validate the analysis experimentally and to test the effect of introducing modifications into structure **6**, preparative routes to the parent system **6** were devised, building upon the protocols employed in the attempted synthesis of the oxygen-containing analogue **4**.

Synthesis of macrocycle **6** (R = Me) differs from that of **4** (R = Me) in that potential precursors, for example **7**, possess a Pro[4] thiol group which it was hoped would serve as a better nucleophile in the required macrocyclisation reaction. Unlike (2*S*,4*S*)-4-hydroxyproline, which is commercially available, the thio analogue would need to be synthesised. Since it was important that the rotameric forms of the tertiary amide groups could be assessed in the precursor, it was necessary to devise syntheses that would not involve amide bond formation in the last step, see discussion above. Thus, there were two obvious ways to introduce sulfur; either into both the α-halogeno acid C2 and a 4-activated Pro[4] C4 position at the same time, for example, through reaction of **9** with sodium sulfide, or, through the separate preparation of the precursor **7** through displacement of a Pro[4] C4-leaving group with a sulfur nucleophile, followed by cyclisation. Whichever strategy was going to be used, the reaction was expected to proceed with inversion of stereochemistry at both of the electrophilic sites and thus a (2*S*)-chloropropionyl moiety and a (2*S*,4*R*)-4-substituted proline would be needed.

To assess the viability of the first strategy, the (2*S*,4*R*)-4-hydroxyproline methyl ester was prepared in almost quantitative yield from the amino acid through reaction of thionyl chloride in methanol. The amino ester was coupled to (2*S*)-2-chloropropionyl-(2*S*)-Pro-(2*S*)-Pro **10**, using thionyl chloride activation, as for the synthesis of the diastereomer **5**,[4] to give the chloropropionyl triprolyl peptide alcohol **11** in 60% yield. The compound displayed the required analytical properties and one predominant set of signals in the ^{1}H- and ^{13}C-NMR spectra corresponding to the all *trans*-isomer, as expected.

Tosylation of the secondary hydroxy group in the product to give compound **9** was accomplished using tosyl chloride in pyridine, but the low yield for the reaction (15% optimised) and the failure of the tosylated product to react with sodium sulfide to give clean products prompted abandonment in favour of a step-wise protocol for the introduction of the thioether.

The conversion of alcohols to thioethers or thioesters with the required inversion of stereochemistry is well-established.[5–7] We chose to use the Mitsunobu-type transformation of Volante,[8] which was expected to convert the alcohol **11** directly into the corresponding thioester **12** of correct stereochemistry.

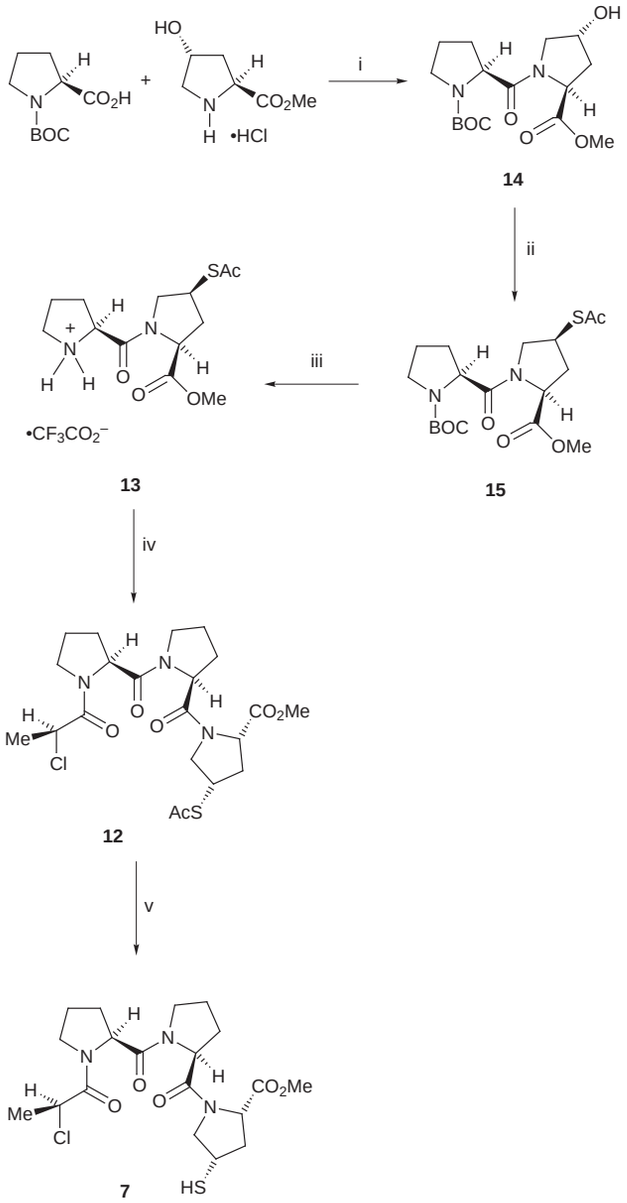
Treatment of alcohol **11** with diethyl azodicarboxylate (DEAD) or diisopropyl azodicarboxylate (DIAD) and triphenylphosphine in the presence of thioacetic *S*-acids afforded the desired thioacetate **12** in moderate yield (40%). This compound also displayed the required analytical properties and one set of signals in the ^{1}H- and ^{13}C-NMR spectra corresponding to the all-*trans* isomer.

Given that the overall yield was rather low and that modifications would be introduced into the structure which would further erode the quantities of material available for study, an alternative convergent synthesis was investigated starting from *N*-[(2*S*)-2-chloropropionyl]-(2*S*)-Pro. This molecule had been prepared earlier and so attention was focussed on the preparation of the precursor to the *C*-terminal portion of the template cap structure **6**, the prolyl acetylthioproline **13**.

(2*S*)-*N*-*tert*-Butoxycarbonylproline and (2*S*,4*R*)-4-hydroxyproline methyl ester were coupled using BOP-Cl in 62% yield to give the dipeptide **14**, Scheme 1. The secondary hydroxy group in the product was then converted to the thioester to give **15** with inversion of stereochemistry using Volante's procedure. Without purification, *tert*-butoxycarbonyl protection was removed using TFA to afford the required amine **13** as its trifluoroacetate salt in 69% yield over the two steps.

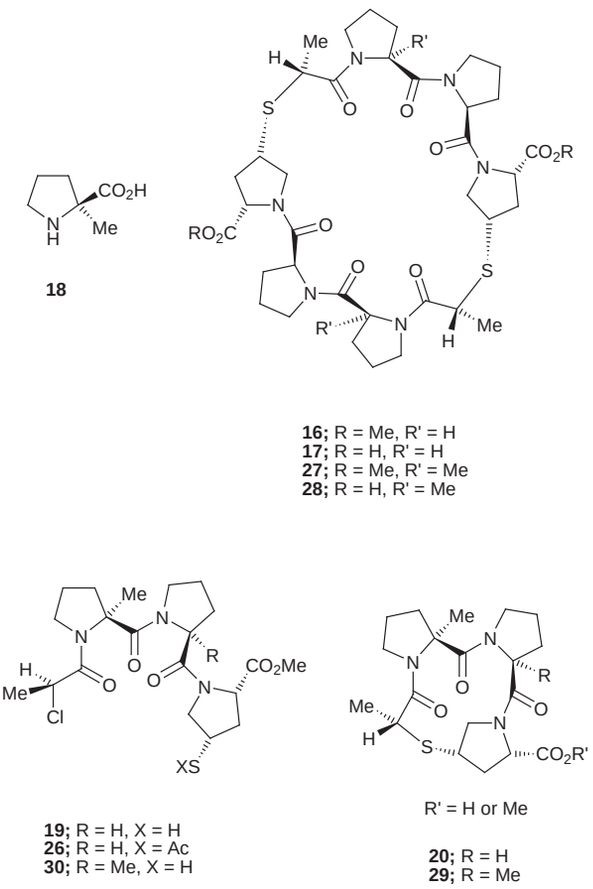
Using BOP-Cl, which was proving to be the most effective coupling reagent for connecting proline residues, *N*-[(2*S*)-2-chloropropionyl]-(2*S*)-Pro and amine **13** were peptide-coupled to give the *N*-chloropropionyl tripropyl thioester **12** in 90% yield, Scheme 1. The isolated product was identical to the material prepared earlier and the overall yield for precursor **12**, starting from *N*-[(2*S*)-2-chloropropionyl]-(2*S*)-Pro, represents a four-fold improvement over the linear strategy described above. The thiol was unmasked in 95% yield through the very mild alkaline hydrolysis of thioester **12** using a 1.6 fold excess of dilute aqueous-methanolic KOH, without saponifying the methyl ester. A thiolate anion is generated during the process, but this underwent neither intramolecular nor intermolecular reaction with the alkyl chloride. The thiol **7** displayed the required analytical properties and showed one predominant set of signals in the ^{1}H- and ^{13}C-NMR spectra corresponding to the *ttt* rotameric form.

In order to effect cyclisation, solutions of thiol **7** in a range of different solvents were treated with a variety of bases. The use of DBU, sodium methoxide and potassium *tert*-butoxide each failed to mediate the formation of a new product. However, when solutions of thiol **7** were heated in the presence of sodium hydride, significant changes were observed in the NMR spectra of the crude product, indicating that a reaction had occurred. Column chromatography of the crude material allowed isolation of a single product in 15–25% yield and its NMR spectra were in accord with those expected for a single conformer of macrocycle **6**. The ^{1}H NMR spectrum displayed a striking 0.3 ppm upfield shift for the doublet attributed to the propionyl methyl group at the stereogenic centre, in keeping with a change of substituent from chlorine to sulfur at the C-2 position. The ^{13}C-NMR spectrum also showed two upfield shifts; one of 3



Scheme 1 *Reagents and conditions*: i, BOP-Cl, DIEA, CH_2Cl_2, 0 °C→rt, 6 days, 62%; ii, DIAD, PPh_3, AcSH, THF, 0 °C, 2 h then rt, 15 h; iii, TFA, CH_2Cl_2, 0 °C, 2 h, 69% (2 steps); iv, *N*-[(2*S*)-2-chloropropionyl]-(2*S*)-Pro, BOP-Cl, DIEA, CH_2Cl_2, 0–5 °C, 2 h then rt, 8 days, 90%; v, 0.05 mol dm^{-3} KOH, MeOH, H_2O, 4 h, 95%.

ppm for the methyl group carbon, and; one of 10 ppm for the stereogenic carbon atom at C-2. The single set of resonances in the ^{13}C-NMR spectrum ruled out acyclic oligomers and polymers and the product lacked an S–H stretch in the infra-red spectrum further supporting the NMR evidence that a thioether was present. However, mass spectra of the compound failed to show the desired molecular ion ($M^+ = 409$ Da) and, instead, a peak of mass 819 Da of 16% intensity was observed together with a more intense peak at 841 Da, corresponding to the sodium ion complex, and a peak at 857 Da, corresponding to the potassium ion complex. Thus, the reaction product was actually the cyclic dimer **16** containing a 24-membered ring. The dimeric product **16** was indistinguishable from monomeric compound **7** by any of the other analytical techniques employed because it possesses C_2 symmetry. Conformational analysis of the product and its free acid form **17** were conducted using high-field 2D NMR experiments. From these experiments it was evident that all of the amide bonds were of *trans*-stereochemistry. The set of ROESY cross-peaks observed for the system were consistent with the structure of the dimer **16**. Interestingly, it was noted that the monomer **6** (in the *M* conformation) would show identical ROESY cross-peaks although this structure could be ruled-out on the basis of mass data.



The result is highly informative and indicates that the energies inherent in the folded conformations of the predominantly all-*trans* precursor thiol **7** which are required for intramolecular cyclisation reaction are too high. However, because the thiolate group and the α-chloroacyl moiety are inherently reactive enough, intermolecular thiol alkylation takes place, to give an acyclic dimer which has sufficient flexibility to react further to give the 24-membered macrocycle **16**. Given that the precursor thiol **7** does not populate the *cct* rotameric form significantly, this outcome is in accord with the modelling studies and provides a firm basis for the informed structural alteration of the target structure **6**.

α-Alkyl amino acids such as 2-aminoisobutyric acid (Aib) are known to favour folded conformations,[9–11] and to promote cyclisation reactions *via* the *gem*-dimethyl effect.[12] It was reasoned that if such an α-alkyl residue could be incorporated into the thiol **7**, the moiety would appreciably increase the time that such a molecule spends in folded conformations conducive to monomeric cyclisation. The minimum change which could be envisaged is the substitution of a (2*S*)-α-methylproline residue **18** for one of the proline residues in macrocycle **6**.

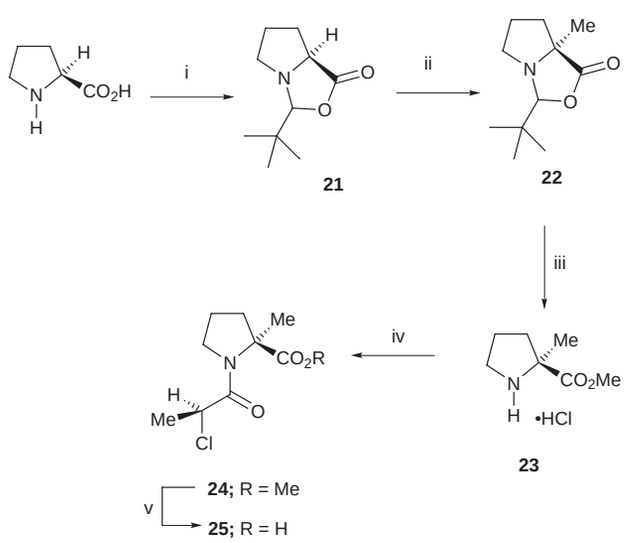
α-Methylproline (Pro^{Me}) has received little attention to date. Those studies which have appeared indicate that the amide bond preceding an α-methylproline residue is exclusively *trans*.[13,14] Likewise, the *cis*-configuration of the following amide bond is also destabilised. Substitution of Pro for Pro^{Me} at position ($i + 1$) in tetrapeptide sequences by Robinson and coworkers was found to restrict the conformational space available to the peptide so that it occupied turn-like conformations for longer periods of time;[15] exactly the type of effect that was needed to coax the precursor **7** into a helical transition state. The substitution of a (2*S*)-Pro^{Me} residue for Pro^2 in the triprolyl peptide **7** defines the new precursor target **19** which could be tested for cyclisation to the template structure **20**.

Table 2 Energy differences (in kJ mol^{-1}) between non-helical (***M***) and helical (***S***) *ttt* conformations of macrocycle acid **6** and analogues **20** and **29** containing one and two α-methylproline residues respectively

Acid	*M*	*S*	Difference
6	405.4	413.9	+8.4
20	497.2	490.7	−6.5
29	745.8	732.9	−12.9

Molecular mechanics simulations performed on the *ttt* sub-states of potential template **20** were in accord with expectations based upon the Hammond Postulate which we had invoked to link the structures and relative energies of the products to those of the transition states for their formation, see above. The results for acid **20**, Table 2, indicated that the helix-initiating ***S*** sub-state would be of *lower* energy than the non-productive ***M*** sub-state in contrast to the original thioether macrocycle **6** (Table 1).

It was envisaged that the preparative route employed for the synthesis of precursor **7** could be adopted for the preparation of the methyl homologue **19**. However, it would first be necessary to prepare a supply of (2*S*)-Pro^{Me}. α-Alkyl amino acids have been synthesised in a variety of ways,[16] but Seebach's methodology for the synthesis of α-substituted proline derivatives is the most convenient and gives products of high stereochemical integrity.[17] Thus, following Seebach, (2*S*)-proline was condensed with pivalaldehyde to give the bicyclooctanone **21** in 96% yield, which upon deprotonation α to the carbonyl group and treatment with methyl iodide gave the α-methyl derivative **22** in 57% yield, Scheme 2. The *tert*-butyl group directs the



Scheme 2 *Reagents and conditions*: i, pivalaldehyde, pentane, reflux, Dean–Stark, 7 days; ii, LDA, THF, −78 °C, 1 h; then MeI, −78 °C→−30 °C, 2 h, 55% (2 steps); iii, $SOCl_2$, MeOH, reflux, 2 h, 58%; iv, (2*S*)-2-chloropropionic acid, BOP-Cl, DIEA, CH_2Cl_2, 0–5 °C, 4 h then rt, 15 h; v, NaOH, MeOH, H_2O, 2 days, 43% (2 steps).

electrophile by controlling the conformation of the bicyclic enolate so that the alkylation must occur from the same side as deprotonation. Since the 1,3-induction results in complete retention of configuration, this procedure is termed a "self-reproduction of chirality".[17] No trace of the (2*R*)-diastereoisomer could be detected in NMR spectra of compound **22**. The oxazolidinone **22** was cleaved by the action of thionyl chloride in methanol to give (2*S*)-2-methylproline methyl ester hydrochloride **23** in 58% yield.

N-Terminal capping of the extremely hindered methyl ester **23** with (2*S*)-2-chloropropionic acid using isobutyl chloroformate or pyBroP gave yields of only 31 and 34% respectively of the dipeptide **24**. BOP-Cl proved to be more effective and a slight excess of the activated acid afforded a moderate 44% yield of the desired product **24**. Although a three-fold excess increased the yield to 70%, this resulted in an intolerable amount of racemisation at the C-2 halogeno-substituted chiral centre. Further optimisation established that a two-fold excess of BOP-Cl and (2*S*)-2-chloropropionic acid gave a satisfactory 62% yield with no significant racemisation.

Alkaline hydrolysis of the methyl ester **24** to the acid **25** was very slow as a consequence of the adjacent quaternary centre. With care, complete cleavage took place over 1–2 days. Increases in either the concentration of the base or reaction time led to various degrees of chloride substitution by hydroxide ion.

The next synthetic step required peptide bond formation between the acid **25** and the amine thioester **13**, an intermediate from the previous synthesis. The powerful coupling reagent HATU afforded only 21% of the desired peptide **26** but BOP-Cl was slightly more effective and gave a 31% yield. However, the reaction was very slow and required 20 h to achieve even these moderate yields. The slow acylation rate, which is a consequence of the significant steric hindrance associated with both species, may have rendered the formation of dioxopiperazine from the amine fragment **13**, a unimolecular cyclisation reaction, competitive with the acylation reaction. The dipeptide **13** is particularly prone to this cyclisation process due to the presence of proline and hence its higher propensity to contain a *cis*-amide bond. Nevertheless, useful quantities of the thioester **26** could be obtained and this was converted to the thiol **19** using mild alkaline hydrolysis. In the case of the cyclisation of thiol **19**, caesium carbonate proved to be slightly more effective than sodium hydride and afforded a cyclic product in 30% yield.

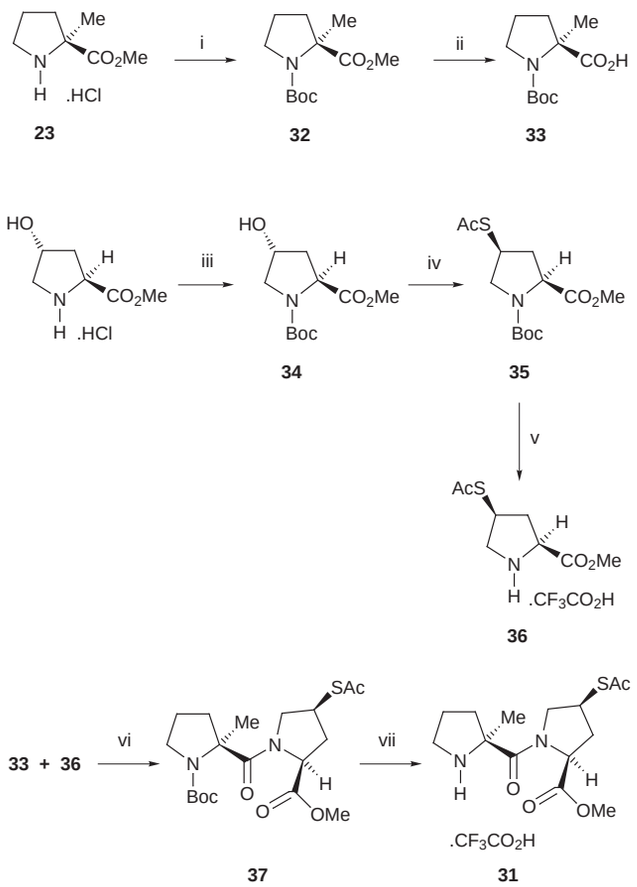
NMR spectroscopic analysis of the product showed several of the expected chemical shift changes and, once again, mass spectral analysis confirmed that the structure was a cyclic dimer **27**. It was evident that the incorporation of a Pro^{Me} residue failed to promote sufficiently the required folded forms of the acyclic precursor to allow formation of the template structure **20**. However, NMR spectra of the cyclic dimer **27** did highlight the influence that the two Pro^{Me} residues have on the conformation of the macrocycle. The dimer **16** containing proline residues which are unsubstituted at C-2, showed one predominant conformational state in both chloroform and water. In contrast, the macrocycle **27**, which contains two Pro^{Me} residues, displayed solvent-dependent conformational isomerisation. In chloroform, three sets of peaks were observed in both ^1H and ^{13}C NMR spectra, in the relative abundance 3 : 2 : 2. These signals may be due to the presence of three distinct C_2-symmetric conformations which interconvert slowly on the NMR timescale, or alternatively one C_2-symmetric conformation and one which lacks an axis of symmetry. In aqueous solution, only one set of NMR resonances was observed, suggesting either that a single conformation was preferred or that the interconversion between the various conformations was rapid on the NMR timescale.

The acid form of the dimer **27**, compound **28**, displayed isomerisation in aqueous solution and NMR spectra showed three sets of peaks in a relative abundance of 81 : 13 : 6. High-field 2D NMR experiments confirmed that the most populated conformer was the same as that found for macrocycle **16**, with six *trans*-amide bonds.

Further promotion of folded forms of the linear precursor was clearly necessary to enable cyclisation to take place. Continuing with a strategy based upon destabilising unwanted conformers in the transition state and extending the principles applied in design of the Pro^{Me} macrocycle **20** leads to the new target **29** (R = Me) which incorporates two Pro^{Me} residues. Molecular mechanics simulations for acid **29** (R = H) predicted further advantage in the form of an increased preference for the potential helix-initiating ***S*** *ttt* sub-state over the ***M*** sub-state (Table 2), as expected. Also, the introduction of a second Pro^{Me}

residue would exert an additional destabilising effect on the *cis*-configuration of the second and third amide bonds in the precursor, **30**. Hence, it appeared that if the 12-membered macrocycle **29** could be formed, its most stable conformation would be one that could initiate an α-helical conformation in attached peptides, for example, the ***S**** sub-state. Unfortunately. however, the presence of two Pro^{Me} residues was certain to pose serious synthetic problems.

The preparation of macrocycle **29** requires a modification to the Pro^3–Pro^4 fragment **13** so that the Pro^3 residue contains a C-2 methyl group, compound **31**. Accordingly, (2*S*)-2-methylproline methyl ester hydrochloride **23** was converted to the corresponding *N*-Boc derivative **32** through the action of $(Boc)_2O$ and DMAP for prolonged reaction times. Alkaline hydrolysis of the ester gave the free acid **33** in 61% yield over the two steps (Scheme 3). Usually, the *N*-protected proline residue would



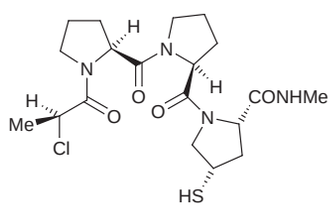
Scheme 3 *Reagents and conditions*: i, $(Boc)_2O$, DIEA, DMAP, CH_2Cl_2, 3 days; ii, 1.0 mol dm^{-3} NaOH, MeOH, H_2O, 2 days, 61% (2 steps); iii, $(Boc)_2O$, NMM, THF, 18 h, 79%; iv, DIAD, PPh_3, AcSH, THF, 0 °C, 2 h then rt, 18 h; v, TFA, CH_2Cl_2, 0 °C, 90 min, 72% (2 steps); vi, BOP-Cl, DIEA, CH_2Cl_2, 0–5 °C, 8 h then rt, 15 h, 55%; vii, TFA, CH_2Cl_2, 0 °C, 90 min, 100%.

have been coupled directly with an appropriately protected (2*S*,4*R*)-4-hydroxyproline residue, pending the subsequent introduction of the sulfur atom, as we had enacted before, Scheme 1, in the preparation of compound **13**. However, given the fact that both components of compound **31** were now synthesised, and that their coupling would be difficult, see above, we chose to incorporate the thioacetate group prior to the coupling of the two proline residues in order to avoid manipulation of the precious Pro^{Me}–Pro dipeptide homologue of compound **13**.

Accordingly, (2*S*,4*R*)-4-hydroxyproline methyl ester hydrochloride was converted to its *N*-Boc derivative **34** in 79% yield and this was in turn converted to the thioester **35** using the Mitsunobu-type inversion. Trifluoroacetic acid catalysed hydrolysis of the *N*-protecting group afforded the amine trifluoroacetate salt **36** in 57% overall yield from the free amino ester. Compound **36** and all of the intermediates displayed the expected spectral and analytical properties. The acid **33** and the amine **36** were coupled using BOP-Cl to afford the Pro^{Me}–Pro dipeptide **37** in 55% yield and this compound also displayed the expected spectral and analytical properties. Removal of the *N*-terminal protecting group gave amine trifluoroacetate salt **31**.

The subsequent coupling reaction between two Pro^{Me} residues proved to be the most difficult yet, due to both fragments, **25** and **31**, possessing extreme steric hindrance. Although BOP-Cl had proved superior to any other reagent in mediating reactions between hindered species in the past, in this case the coupling reaction failed, and instead 49% of the dioxopiperazine **38** was isolated. Clearly, the very low rate of *intermolecular* acylation allowed the *intramolecular* acylation to compete, despite the presence of a Pro^{Me} residue which disfavours the *cis* amide bond configuration of the amine **31** which is required for dioxopiperazine formation.

To assess the possibility of overcoming the problem of dioxopiperazine formation by using a smaller fragment, (2*S*)-Pro^{Me} methyl ester **23**, in place of the dipeptide amine **31**, a linear synthetic route was investigated. Thus (2*S*)-Pro^{Me} methyl ester **23** was reacted with HATU-activated *N*-[(2*S*)-2-chloropropionyl]-(2*S*)-Pro^{Me} **25**. Gratifyingly, this strategy was partially successful and the reaction afforded 21–30% of Pro^{Me}–Pro^{Me} peptide **39**. A second compound could also be isolated



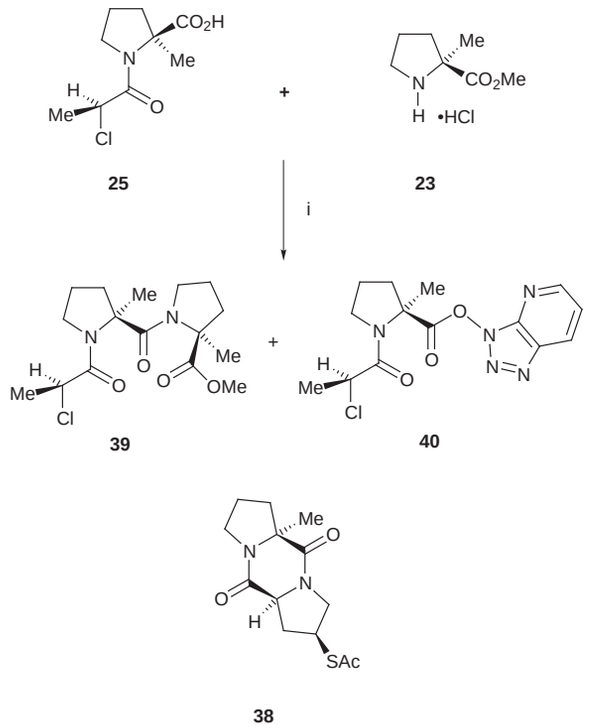
39

from the reaction mixture, and this was shown to be the azabenzotriazolyl ester **40**, Scheme 4. The acylating species survived an aqueous work-up and silica column chromatography, demonstrating the extraordinary low reactivity that the α-methyl group imparts on the adjacent carbonyl group in *N*-acyl Pro^{Me} systems.

Although it was possible to form the exceptionally crowded Pro^{Me}–Pro^{Me} sequence, the yields of reactions leading up to the synthesis of *N*-acyl-Pro^{Me}–Pro^{Me} methyl ester **39** were low, and it was clear that the production of useful amounts of more advanced intermediates would be difficult. This view was supported by an attempt to hydrolyse the methyl ester of compound **39**. This usually facile reaction did not go to completion even after a week in strongly alkaline conditions. Therefore, it was decided to abandon the idea of destabilising unwanted structures in the transition state for the formation of a helix initiating template and proceed with the first of two other strategies. Namely, these were to stabilise the required transition state structures by reducing the energy of carbonyl group dipole alignment and steric strain through a) hydrogen bond formation; and, b) removal of the need to align all of the dipoles in forming the 12-membered macrocycle.

Effect of hydrogen bonding on the conformations of linear precursors

Since our studies thus far had established that adverse steric effects alone are insufficient to promote the necessary dipole alignment in the folding of the triprolyl sequence into a template of type **8** of the correct conformation, the potential stabilising effect of hydrogen bonding in the transition state was examined. In nature, polypeptide folding is stabilised by hydrogen bonding and we wished to test whether the inclusion



Scheme 4 *Reagents and conditions*: i, HATU, DIEA, DMF, 0–5 °C, 4 h then rt, 40 h, 30% (of **39**) and 37% (of **40**).

of one H-bond would significantly alter the energies of the pathways leading to 12-membered template formation and 24-membered macrocyclisation in favour of the template structure.

Conversion of the methyl ester of the original linear precursor **7** to a methylamide gives triprolyl peptide **41**. The potential now exists for formation of intramolecular hydrogen bonds which could stabilise folded states approximating to the desired cyclic structure, thus assisting cyclisation. Possible hydrogen bonding schemes involve the terminal methyl amide $i \longrightarrow (i-5)$ (α-helical turn), $i \longrightarrow (i-4)$ (3_{10}-helical/β-turn) or $i \longrightarrow (i-3)$ (γ-turn) interactions with preceding carbonyl groups (Fig. 5). Model studies on other peptide sequences have shown that the β-turn seems to be the most stable of the three in isolation.[18,19]

These conformations may allow favourable geometry for intramolecular reaction, particularly if the α-helical turn makes a prominent contribution to the secondary structure of the linear precursor. Hydrogen bonding has found uses in favouring cyclisations in the past, for example in Wenger's synthesis of cyclosporin A.[20]

Hydrogen bonding of this sort provides an enthalpic bias towards certain folded conformations, but the actual geometry adopted depends on a fine balance between this favourable effect and other forces which act against compact structures, such as loss of entropy upon formation of hydrogen bonded rings, torsional strain, and repulsive dipolar interactions.[18,21,22] It remained to be seen whether the enthalpic gain of a hydrogen bond would be enough to overcome the repulsive steric and dipolar interactions which prohibited cyclisation of earlier linear precursors, **7** and **19**. Hydrogen bonding interactions in Pro–Pro–Ala tripeptides have been demonstrated to promote folded forms of this sequence.[23] We hoped that this might also be possible in the similar Pro–Pro–Pro sequence.

The intended synthetic route to the acyclic precursor **41** employed a similar strategy to the synthesis of methyl ester analogue **7**. Given that it was hoped to introduce the methylamide moiety through the direct aminolysis of a methyl ester group, and since late intermediates contain an electrophilic α-chloroacylamide moiety, it was necessary to perform the conversion before the introduction of the *N*-terminal fragment. Accordingly, **14** was converted to the corresponding methyl amide **42** in quantitative yield (Scheme 5).

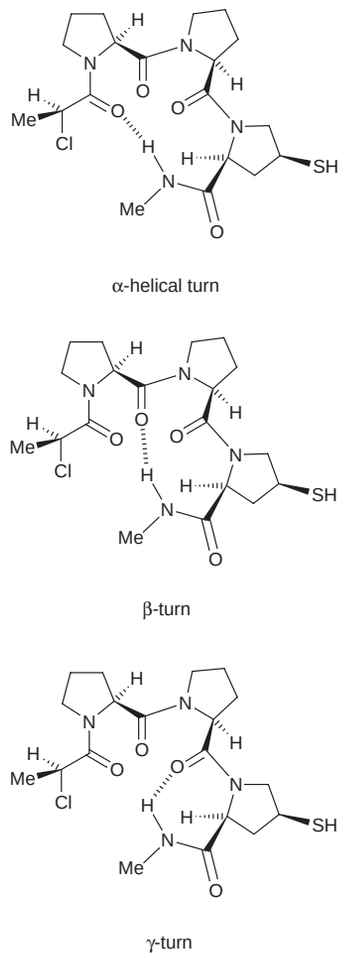


Fig. 5 Different types of turns.

The methyl ester **14** shows two conformations in the NMR spectra recorded in C^2HCl_3. Examination of the proline C^β and C^γ ^{13}C chemical shifts allows for designation of *cis*- and *trans*-isomers about the preceding urethane or amide bond. In ester **14**, the conformations present are thus identified as *trans trans* (*tt*) and *cis trans* (*ct*), in 55 and 45% abundance respectively (Fig. 6). *cis*- and *trans*-isomers of urethane bonds are of very similar energy due to the similar size of the carbonyl oxygen and the alkoxy substituent. The slight preference for the *trans*-configuration may be due to an O–H · · · O=C hydrogen bond, in a ten-membered ring, which can form between the Pro^2 γ-hydroxy substituent and the urethane carbonyl oxygen. No trace of the *cis*-configuration about the Pro–Pro amide bond could be found, indicating that it is of significantly higher energy than the *trans* form in the absence of external stabilising forces.

In contrast, the methyl amide **42** displays *three* conformational isomers. Since the only change is incorporation of a hydrogen bond donor, the additional conformation must be one stabilised by a hydrogen bond. The NMR spectra reveal that the *tc*, *tt* and *ct* configurations of the urethane and Pro–Pro amide respectively, are present, in a relative abundance of 14 : 62 : 24 (Fig. 7).

The *tc* state is not found in the methyl ester **14**, and must therefore be stabilised by an N–H · · · O=C hydrogen bond involving the methyl amide NH and the urethane carbonyl oxygen. The presence of a hydrogen bond is further supported by a 0.4 ppm upfield shift of the amide proton resonance relative to the non-hydrogen bonded form; infra-red spectra in dichloromethane solution also show two N–H stretch bands at 3448 and

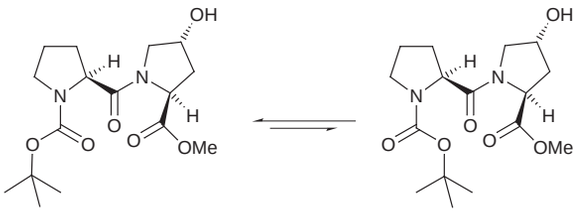
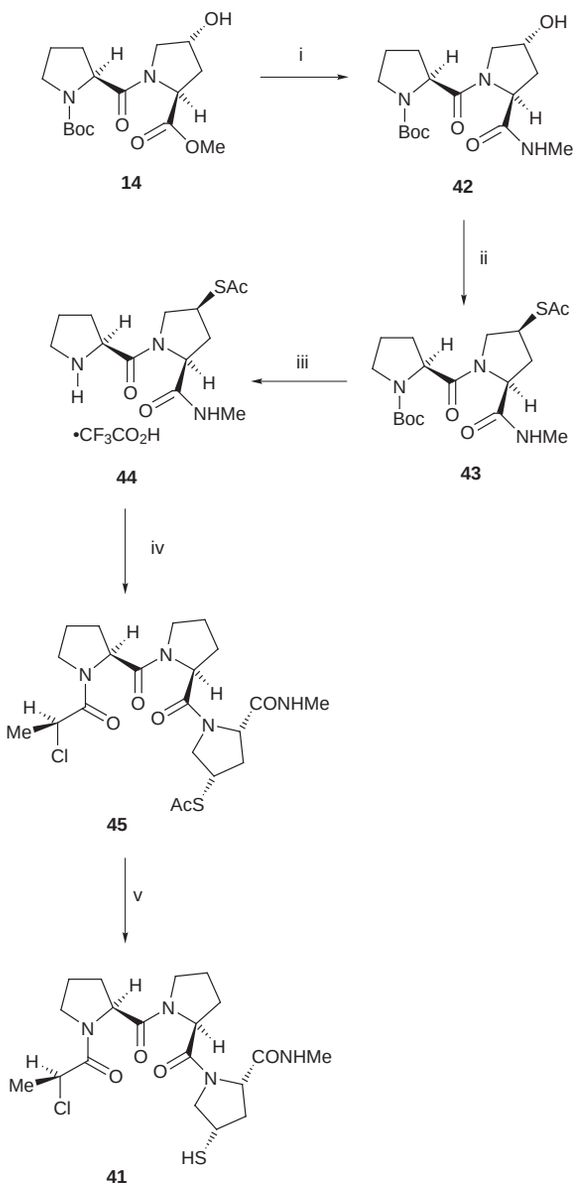


Fig. 6 Conformations adopted by compound **14**.



Scheme 5 *Reagents and conditions*: i, NH_2CH_3, MeOH, 1 day, 100%; ii, DIAD, PPh_3, AcSH, THF, 0 °C, 2.5 h then rt, 15 h, 50%; iii, TFA, CH_2Cl_2, 0 °C, 2.5 h; iv, *N*-[(2*S*)-2-chloropropionyl]-(2*S*)-proline, BOP-Cl, DIEA, CH_2Cl_2, 0–5 °C, 2 h then rt, 2 days, 69% (2 steps); v, 0.05 mol dm^{-3} KOH, MeOH, H_2O, 4 h, 66%.

3342 cm^{-1}, which correspond to non-hydrogen bonded and intramolecularly hydrogen bonded N–H groups respectively.

Alcohol **42** was converted to thioester **43**, and subsequent deprotection of the amino terminus gave amine trifluoroacetate salt **44** which showed only one conformation (Scheme 5). There is now only one isomerisable bond, and the removal of the urethane hydrogen bond acceptor site precludes any stabilising influence over the *cis*-configuration, so that only the *trans*-form is now stable. Amine trifluoroacetate salt **44** was subsequently

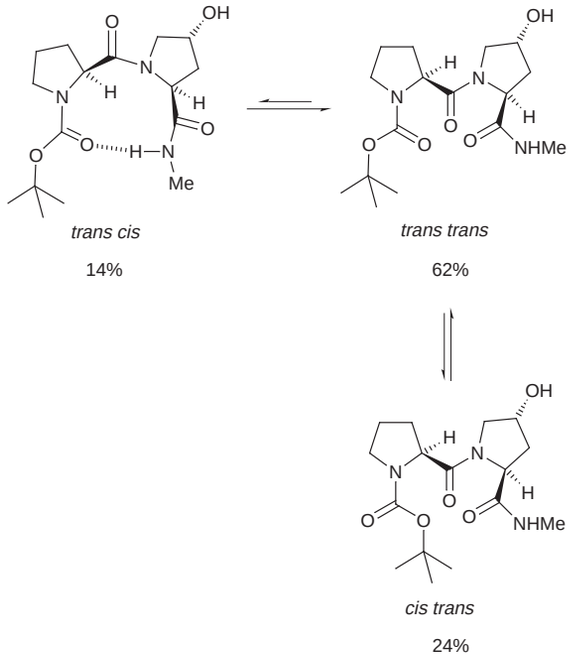


Fig. 7 Conformations adopted by methylamide **42**.

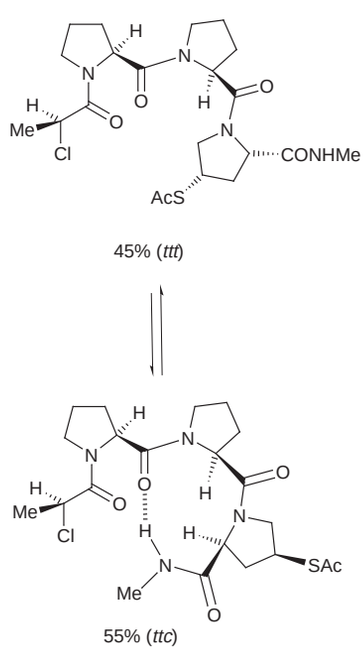


Fig. 8 Conformations adopted by thioester **45**.

coupled to *N*-[(2*S*)-2-chloropropionyl]-(2*S*)-Pro to afford the thioester **45** in 69% yield. NMR spectra of the thioester **45** revealed the presence of two conformations, corresponding to the *ttt* (45%) and *ttc* (55%) forms respectively. This finding accords with the re-establishment of *cis–trans* isomerisation about the Pro^3–Pro^4 amide bond (Fig. 8).

Conversion of thioester **45** to the free thiol **41** does not change the conformational equilibrium adopted in chloroform solution and the *ttt* and *ttc* forms exist in a ratio of approximately 1:1. NMR Spectra recorded in ^2H_2O show only the *ttt* state, presumably because the intramolecular hydrogen bond found in the *ttc* form in chloroform is replaced by intermolecular solvent–peptide hydrogen bonds. Thus, it appears that addition of a hydrogen bonding group at the *C*-terminus will not be able to assist the peptide thiol **41** to adopt the required conformations for close approach of the nucleophile, the thiol moiety itself, to the electrophile. A hydrogen bond is present in approximately 50% of the population of molecules in

chloroform solution and to some degree the hydrogen bond allows the molecule to overcome repulsive interactions which would otherwise prohibit the folded form. However, the stabilised conformation is one in which the thiol group is held away from the intended site of reaction, and as such is of no use in assisting the cyclisation process relative to the situation for the methyl ester **7**. Hence, the hydrogen bond stabilises the ground state such that any beneficial hydrogen bonding interactions between the methylamide proton and the first carbonyl group in the transition state for cyclisation would be largely cancelled.

In summary, it is evident that using methods to force the acyclic template **7** into a helical structure by destabilising undesirable conformations in the transition state are insufficient on their own to overcome the problems of aligning the carbonyl group dipoles. It is also evident that attempting to stabilise the conformation of an α-helical transition state by providing a mechanism to form the 13-membered hydrogen-bonding network typical of α-helices, is fraught with problems due to the stabilisation of usually rare ground-state conformations. The remaining strategy for the preparation of nearly natural α-helical cap templates, based on the notion that it is too difficult to align at least three carboxamide group dipoles simultaneously, is presented in the following article. In this strategy the relaxation of the highly restrained C^{α}–N bond torsion of Pro^3 in the structure **7** by its substitution for a (2*R*)-alanine residue, effectively decouples the motion of the second carbonyl group from the C^{α}–N bond torsion. This modification of structure **7** resulted in successful cyclisation to give macrocyclic template structures of the *ttt* form.

Experimental

NMR spectra were recorded on a Bruker AM-300 spectrometer (^{1}H, 300 MHz; ^{13}C, 75.44 MHz), a Varian Gemini spectrometer (^{1}H, 200 MHz; ^{13}C, 50.3 MHz), a Varian Gemini spectrometer (^{1}H, 300 MHz; ^{13}C, 74.76 MHz) and a Varian Unity Plus 500 spectrometer (^{1}H, 500 MHz; ^{13}C, 125.6 MHz). ^{1}H-NMR spectra were referenced internally to $(C^2H_3)_2SO$ (δ 2.47), ^{2}HOH (δ 4.68) or C^2HCl_3 (δ 7.27). ^{13}C-NMR were referenced to $(C^2H_3)_2SO$ (δ 39.70) or C^2HCl_3 (δ 77.5). *J* values are given in Hz. Carbon and proton resonances of amino acids in NMR spectra are assigned as α, β, γ and δ according to normal convention. Where more than one conformational isomer is present due to the presence of a tertiary amide bond, these are assigned as *c* (*cis*) or *t* (*trans*), according to the isomeric state of the amide bond. If the isomeric states of the amide bonds are not known, the conformations are assigned as *A*, *B*, *C etc*. For definitions of Chp and Thp see previous paper.

Mass spectra and accurate mass measurements were recorded on a VG 70-250 SE, a Kratos MS-50 or by the SERC service at Swansea using a VG AZB-E. Fast atom bombardment spectra were recorded using glycerol as a matrix. Major fragments were given as percentages of the base peak intensity (100%). Infrared spectra were recorded using a Perkin-Elmer 1710 FT-IR spectrometer. The samples were prepared as Nujol mulls or thin films between sodium chloride discs. Absorption maxima are given in wavenumbers (cm^{-1}) relative to a polystyrene standard. Melting points were measured using an electrothermal melting point apparatus and are uncorrected. Optical rotations were measured on an Optical Activity AA-1000 polarimeter using 10 cm path length cells at room temperature and are given in units of 10^{-1} deg cm^2 g^{-1}.

All experiments were performed at room temperature (20–25 °C) unless otherwise stated. Flash chromatography was performed according to the method of Still *et al*.[24] using Fluka C60 (40–60 mm mesh) silica gel. Analytical thin layer chromatography was carried out on 0.25 mm precoated silica gel plates (Macherey-Nagel SIL g/UV_{254}) and compounds were visualised using UV fluorescence, iodine vapour, ninhydrin, ethanolic phosphomolybdic acid or aqueous potassium permanganate.

Light petroleum refers to the fraction boiling at 40–60 °C. Solvents and common reagents were purified according to the method of Perrin and Armarego.[25] Thionyl chloride was distilled over sulfur and the initial fractions were always discarded. *N*-Methylmorpholine was distilled over ninhydrin. All other reagents were used without further purification.

Protected amino acid precursors were purchased from Calbiochem-Novabiochem (UK) Ltd (Beeston, Nottingham).

Abbreviations

Boc, *tert*-butoxycarbonyl; BOP-Cl, *N*,*N*-bis(2-oxooxazolidin-3-yl)phosphinic chloride; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DIEA, diisopropylethylamine; DMAP, 4-(*N*,*N*-dimethylamino)pyridine; DMF, *N*,*N*-dimethylformamide; HATU, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; LDA, lithium diisopropylamide; NMM, *N*-methylmorpholine; pyBroP, bromotripyrrolidinophosphonium hexafluorophosphate; ROESY, rotating frame NOE correlation spectroscopy; TFA, trifluoroacetic acid; THF, tetrahydrofuran.

Methyl (2*S*,4*R*)-4-hydroxyprolinate hydrochloride

Thionyl chloride (6.7 cm^3, 78.5 mmol) was added dropwise to methanol (100 cm^3) at 0 °C. (2*S*,4*R*)-4-Hydroxyproline (10.0 g, 76.3 mmol) was added and the resulting solution heated under reflux for 90 min. The solvents were removed under reduced pressure to afford the hydrochloride as a white solid (13.5 g, 98%). A small portion was recrystallised to afford white needles, mp 162–164 °C (lit.,[26] 162–164 °C); $[\alpha]_D$ −25.3 (*c* 1.0 in MeOH) [lit.,[26] −24.3 (*c* 1.05 in MeOH)]; ν_{max}(Nujol)/cm^{-1} 3325 (OH), 1742 (CO), 1246 (OH def). and 1180 (C–O); δ_H(200 MHz; ^2H_2O) 2.19 (1 H, ddd, J_1 14.8, J_2 8.8, J_3 4.2, $\frac{1}{2}\beta CH_2$), 2.40 (1 H, dd, J_1 14.8, J_2 8.8, $\frac{1}{2}\beta CH_2$), 3.25 (1 H, d, *J* 12.6, $\frac{1}{2}\delta CH_2$), 3.35 (1 H, dd, J_1 12.6, J_2 3.6, $\frac{1}{2}\delta CH_2$), 3.68 (3 H, s, CO_2CH_3) and 4.48–4.58 (2 H, m, αCH and γCH); δ_C(50.31 MHz; ^2H_2O) 37.07 (βCH_2), 53.87 (δCH_2), 54.23 (CO_2CH_3), 58.65 (αCH), 69.85 (γCH) and 170.62 (ester CO); *m*/*z* (EI) 146 (11%, $[M - HCl]^+$), 86 (100, $OC_4H_8N^+$), 68 (44, $C_4H_6N^+$) and 58 (21, $NHCH_2COH^+$).

Methyl (2*S*,4*R*)-*N*-[(2*S*)-*N*-{(2*S*)-*N*-[(2*S*)-2-chloropropionyl]-prolyl}prolyl]-4-hydroxyprolinate 11

A solution of (2*S*)-*N*-{(2*S*)-*N*-[(2*S*)-2-chloropropionyl]prolyl}-proline **10** (1.27 g, 4.2 mmol) prepared as described previously,[4] and pyridine (0.89 cm^3, 11.0 mmol) in dichloromethane (35 cm^3) was treated dropwise with thionyl chloride (0.7 cm^3, 9.5 mmol). After 5 min, a solution of methyl (2*S*,4*R*)-4-hydroxyprolinate hydrochloride (0.79 g, 4.4 mmol) and DMAP (1.04 g, 8.6 mmol) in dichloromethane (40 cm^3) was added in one portion. The solution was allowed to stir for 3 days, and was then washed with aqueous HCl (0.5 mol dm^{-3}, 2 × 15 cm^3), aqueous sodium hydrogen carbonate (5%, 2 × 15 cm^3) and brine (15 cm^3). The solution was dried ($MgSO_4$), and the solvent removed under reduced pressure to afford the product **11** as a brown oil (1.08 g, 60%) which defied further purification (HRMS: found M^+, 429.1657. $C_{19}H_{28}{}^{35}ClN_3O_6$ requires 429.1667); δ_H(300 MHz; C^2HCl_3) 1.56 [3 H, d, *J* 6.6, βCH_3(Chp)], 1.90–2.42 [10 H, m, 2 × βCH_2(Pro), βCH_2(Hyp) and 2 × γCH_2(Pro)], 3.54–3.82 [6 H, m, 2 × δCH_2(Pro) and δCH_2(Hyp)], 3.63 (3 H, s, CO_2CH_3) and 4.40–4.65 [5 H, m, αCH(Chp), 2 × αCH(Pro), αCH(Hyp) and γCH(Hyp)]; δ_C(74.76 MHz; C^2HCl_3) 20.52 [βCH_3(Chp)], 24.75 and 24.79 [2 × γCH_2(Pro)], 28.01 and 28.32 [2 × βCH_2(Pro)], 37.27 [βCH_2(Hyp)], 47.13 and 47.37 [2 × δCH_2(Pro)], 51.34 [αCH(Chp)], 52.22 (CO_2CH_3), 54.57 [δCH_2(Hyp)], 57.63, 58.01 and 58.55 [2 × αCH(Pro) and αCH(Hyp)], 69.91 [γCH(Hyp)], 167.85 [CO (Chp)], 170.52 and 170.89 (2 × amide CO) and 172.91 (ester CO); *m*/*z* (EI) 431 and 429 (2

and 7%, chlorine isotopes, M^+), 287 and 285 {2 and 9, chlorine isotopes, $[M - NCH_2CH(OH)CH_2CHCO_2CH_3 + H]^+$}, 259 and 257 {3 and 11, chlorine isotopes, $[M - CONCH_2CH(OH)CH_2CHCO_2CH_3 + H]^+$}, 190 and 188 (17 and 61, chlorine isotopes, $C_8H_{11}NO_2Cl^+$), 162 and 160 (24 and 79, chlorine isotopes, $C_7H_{11}NOCl^+$) and 70 (100, $C_4H_8N^+$).

Methyl (2*S*,4*R*)-*N*-[(2*S*)-*N*-(*tert*-butoxycarbonyl)prolyl]-4-hydroxyprolinate 14

A solution of (2*S*)-*N*-(*tert*-butoxycarbonyl)proline (4.0 g, 18.6 mmol) and *N*,*N*-diisopropylethylamine (9.0 cm^3, 52.0 mmol) in dry dichloromethane (115 cm^3) was treated with BOP-Cl (4.92 g, 19.3 mmol) and the resulting suspension stirred under nitrogen at 0 °C for 20 min. A suspension of methyl (2*S*,4*R*)-4-hydroxyprolinate hydrochloride (2.93 g, 16.1 mmol) in dry dichloromethane (35 cm^3) was then added. The mixture was allowed to warm to room temperature and stirred for a further 6 days. The solution was washed with aqueous HCl (0.5 mol dm^{-3}, 2 × 100 cm^3), aqueous sodium hydrogen carbonate (5%, 2 × 100 cm^3) and brine (100 cm^3), then dried ($MgSO_4$). The solvent was removed under reduced pressure, and the residual oil purified by column chromatography using ethyl acetate as the eluent to afford the dipeptide **14** as colourless crystals (3.41 g, 62%), mp 92–94 °C; R_f 0.24 (Found: C, 55.95; H, 7.8; N, 8.15. $C_{16}H_{26}N_2O_6$ requires C, 56.15; H, 7.65; N, 8.2%); $[\alpha]_D$ −112.6 (*c* 0.8 in MeOH); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3458 (OH), 2981 (CH), 1748 (ester CO), 1690 (urethane CO), 1662 (amide CO), 1407 (alcohol C–O) and 1165 (ester C–O); δ_H(200 MHz; C^2HCl_3) (2 conformations, *ct* and *tt*) 1.17 [*ct*, 9 H, $C(CH_3)_3$], 1.21 [*tt*, 9 H, $C(CH_3)_3$], 1.61–2.06 [6 H, m, βCH$_2$(Pro), βCH$_2$(Hyp) and γCH$_2$(Pro)], 3.14–3.89 [4 H, m, δCH$_2$(Pro) and δCH$_2$(Hyp)], 3.47 (3 H, s, CO_2 CH_3) and 4.16–4.42 [3 H, m, αCH(Pro), αCH(Hyp) and γCH(Hyp)]; δ_C(50.31 MHz; C^2HCl_3) 23.47 [*ct*, γCH$_2$(Pro)], 23.98 [*tt*, γCH$_2$(Pro)], 28.26 [*ct*, $C(CH_3)_3$], 28.38 [*tt*, $C(CH_3)_3$], 29.00 [*tt*, βCH$_2$(Pro)], 29.73 [*ct*, βCH$_2$(Pro)], 36.95 [*tt*, βCH$_2$(Hyp)], 37.15 [*ct*, βCH$_2$(Hyp)], 46.51 [*ct*, δCH$_2$(Pro)], 46.86 [*tt*, δCH$_2$(Pro)], 52.08 (CO_2CH_3), 54.45 [δCH$_2$(Hyp)], 57.70 [αCH(Pro)], 57.93 [αCH(Hyp)], 69.68 [*ct*, γCH(Hyp)], 69.99 [*tt*, γCH(Hyp)], 79.71 [$C(CH_3)_3$], 153.96 (*ct*, urethane CO), 154.47 (*tt*, urethane CO), 171.36 (*tt*, amide CO), 171.78 (*ct*, amide CO), 172.65 (*ct*, ester CO) and 172.75 (*tt*, ester CO); *m*/*z* (CI) 343 (42%, $[M + H]^+$), 287 (48, $[M - C_4H_8 + H]^+$), 243 (100, $[M - CO_2 - C_4H_8 + H]^+$) and 57 (84, $[C_4H_8 + H]^+$).

Methyl (2*S*,4*R*)-*N*-[(2*S*)-prolyl]-4-(acetylthio)prolinate trifluoroacetate 13

Diisopropyl azodicarboxylate (0.46 g, 2.3 mmol) was added to a solution of triphenylphosphine (0.61 g, 2.3 mmol) in dry THF (15 cm^3) at 0 °C, and the resulting suspension was stirred under nitrogen at 0 °C for 30 min. A solution of alcohol **14** (0.69 g, 2.0 mmol) and thioacetic *S*-acid (0.17 cm^3, 2.5 mmol) in dry THF (10 cm^3) was then added dropwise to this solution. The mixture was stirred at 0 °C for 2 h, and then at room temperature for 18 h. The solvent was removed under reduced pressure to yield a pale orange oil which was partially purified by column chromatography, using ethyl acetate–light petroleum (2:1) as the eluent, to afford the thioester **15** as an oil (1.12 g). The above oil was redissolved in dichloromethane (20 cm^3), the solution cooled to 0 °C, and trifluoroacetic acid (10 cm^3) was added. the solution was stirred at 0 °C for 2 h, and the solvents were removed under reduced pressure. The residual oil was redissolved in water (20 cm^3) and the solution washed with ether (2 × 10 cm^3). The aqueous phase was concentrated under reduced pressure and lyophilised to yield the amine trifluoroacetate salt **13** as a colourless oil (0.58 g, 69%) (Found: C, 42.0; H, 5.3; N, 6.35. $C_{13}H_{20}N_2O_4S{\cdot}CF_3CO_2H{\cdot}H_2O$ requires C, 41.65; H, 5.35; N, 6.5%) (HRMS: found $[M - CF_3CO_2]^+$, 301.1222. $C_{13}H_{21}N_2O_4S$ requires 301.1222); $[\alpha]_D$ −93.8 (*c* 0.2 in MeOH); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 2956 (CH), 1750 (ester CO), 1696 (thioester CO), 1663 (amide CO) and 1200 (C–O); δ_H(300 MHz; C^2HCl_3) 1.90–2.15 [4 H, m, βCH$_2$(Pro^1) and γCH$_2$(Pro^1)], 2.31 (3 H, s, $SCOCH_3$), 2.44 [1 H, m, ½βCH$_2$(Pro^2)], 2.73 [1 H, dt, J_1 13.2, J_2 7.8, ½βCH$_2$(Pro^2)], 3.35 [1 H, dd, J_1 10.2, J_2 7.8, ½δCH$_2$(Pro^2)], 3.41–3.58 [2 H, m, δCH$_2$(Pro^1)], 3.71 (3 H, s, CO_2CH_3), 4.00 [1 H, quintet, *J* 7.8, γCH(Pro^2)], 4.12 [1 H, dd, J_1 10.2, J_2 7.2, ½δCH$_2$(Pro^2)] and 4.49–4.69 [2 H, m, 2 × αCH(Pro)], 7.71 (1 H, br s, NH) and 11.80 (1 H, br s, CO_2H); δ_C(50.51 MHz; C^2HCl_3) 24.70 [γCH$_2$(Pro^1)], 29.01 [βCH$_2$(Pro^1)], 30.77 ($SCOCH_3$), 34.45 [βCH$_2$(Pro^2)], 39.87 [γCH(Pro^2)], 47.27 [δCH$_2$(Pro^1)], 52.56 (CO_2CH_3), 53.09 [δCH$_2$(Pro^2)], 59.05 [αCH(Pro^1)], 60.15 [αCH(Pro^2)], 116.46 (q, J_{CF} 286, CF_3CO_2H), 161.93 (q, J_{CF} 38, CF_3CO_2H), 167.95 (amide CO), 171.49 (ester CO) and 195.04 (thioester CO); *m*/*z* (CI) 301 (70%, $[M - CF_3CO_2]^+$), 269 (100, $[M - CF_3CO_2 - OCH_3]^+$), 160 [30, $NC_4H_7(S)CO_2CH_3^+$] and 70 (17, $C_4H_8N^+$).

Methyl (2*S*,4*S*)-*N*-[(2*S*)-*N*-{(2*S*)-*N*-[(2*S*)-2-chloropropionyl]-prolyl}prolyl]-4-(acetylthio)prolinate 12

This compound was prepared in a manner identical with that for the dipeptide **14**, using (2*S*)-*N*-[(2*S*)-2-chloropropionyl]-proline (0.38 g, 1.85 mmol) and amine trifluoroacetate **13** (0.50 g, 1.21 mmol) to afford thioacetate **12** as a white foam (0.53 g, 90%) (Found: C, 49.95; H, 6.0; N, 8.65. $C_{21}H_{30}ClN_3O_6S$ requires C, 49.85; H, 6.35; N, 8.3%) (HRMS: found M^+, 487.1552. $C_{21}H_{30}{}^{35}ClN_3O_6S$ requires 487.1544); $[\alpha]_D$ −133.1 (*c* 0.3 in MeOH); ν_{max}(thin film)/cm^{-1} 1748 (ester CO), 1695 (thioacetate CO), 1652 (amide CO), 1435 (CH def.) and 1200 (C–O); δ_H(200 MHz; C^2HCl_3) 1.63 [3 H, d, *J* 6.6, βCH$_3$(Chp^1)], 1.85–2.20 [9 H, m, βCH$_2$(Pro^2), βCH$_2$(Pro^3), ½βCH$_2$(Pro^4), γCH$_2$(Pro^2) and γCH$_2$(Pro^3)], 2.31 (3 H, s, $SCOCH_3$), 2.60–2.80 [1 H, m, ½βCH$_2$(Pro^4)], 3.42 [1 H, dd, J_1 10.1, J_2 7.2, ½δCH$_2$(Pro^4)], 3.57–3.84 [4 H, m, δCH$_2$(Pro^2) and δCH$_2$(Pro^3)], 3.70 (3 H, s, CO_2CH_3), 4.01–4.10 [1 H, m, γCH(Pro^4)], 4.30 [1 H, dd, J_1 10.1, J_2 7.2, ½δCH$_2$(Pro^4)] and 4.48–4.71 [4 H, m, αCH(Chp^1) and 3 × αCH(Pro)]; δ_C(74.76 MHz; C^2HCl_3) 20.88 [βCH$_3$(Chp^1)], 25.19 and 25.26 [γCH$_2$(Pro^2) and γCH$_2$(Pro^3)], 28.39 and 28.66 [βCH$_2$(Pro^2) and βCH$_2$(Pro^3)], 30.87 ($SCOCH_3$), 35.13 [βCH$_2$(Pro^4)], 40.38 [γCH$_2$(Pro^4)], 47.35 and 47.71 [δCH$_2$(Pro^2) and δCH$_2$(Pro^3)], 51.68 [αCH(Chp^1)], 52.57 [δCH$_2$(Pro^4)], 52.76 (CO_2CH_3), 58.29, 58.54 and 58.84 [3 × αCH(Pro)], 168.16 [CO (Chp^1)], 170.71 and 171.14 (2 × amide CO), 172.36 (ester CO) and 194.84 (thioester CO); *m*/*z* (EI) 489 and 487 (7 and 16%, chlorine isotopes, M^+), 451 (7, $[M - HCl]^+$), 327 {14, $[M - ClCH(CH_3)CONC_4H_7]^+$}, 287 and 285 (6 and 21, chlorine isotopes, $[M - CH_3COSC_4H_6NCO_2CH_3]^+$), 190 and 188 [32 and 100, chlorine isotopes, $ClCH(CH_3)CONC_4H_7CO^+$], 162 and 160 [26 and 77, chlorine isotopes, $ClCH(CH_3)CONC_4H_7^+$] and 70 (53, $C_4H_8N^+$).

Methyl (2*S*,4*S*)-*N*-[(2*S*)-*N*-{(2*S*)-*N*-[(2*S*)-2-chloropropionyl]-prolyl}prolyl]-4-mercaptoprolinate 7

Thioacetate **12** (0.15 g, 0.31 mmol) was dissolved in methanol (10 cm^3) and aqueous potassium hydroxide (0.05 mol dm^{-3}, 10 cm^3) was added. The mixture was stirred for 4 h, after which time aqueous HCl (0.05 mol dm^{-3}, 10 cm^3) was added and the methanol was removed under reduced pressure. The solution was further acidified to pH 1–2 and then extracted with ethyl acetate (5 × 30 cm^3). The combined organic extracts were dried ($MgSO_4$) and the solvent was removed under reduced pressure to yield the thiol **7** as a colourless oil (0.13 g, 95%) (HRMS: found $[M + H]^+$, 446.1506. $C_{19}H_{28}{}^{35}ClN_3O_5S$ requires 446.1516); $[\alpha]_D$ −135.5 (*c* 0.4 in MeOH); ν_{max}(thin film)/cm^{-1} 2954 (CH), 2539 (SH), 1744 (ester CO), 1654 (amide CO), 1434 (CH def.) and 1199 (C–O); δ_H(200 MHz; C^2HCl_3) 1.60 [3 H, d, *J* 6.6, βCH$_3$(Chp^1)], 1.80–2.20 [9 H, m, βCH$_2$(Pro^2), βCH$_2$(Pro^3), ½βCH$_2$(Pro^4), γCH$_2$(Pro^2) and γCH$_2$(Pro^3)], 2.64–2.71 [1 H, m, ½βCH$_2$(Pro^4)], 3.32–3.45 [2 H, m, γCH$_2$(Pro^4) and ½δCH$_2$(Pro^4)], 3.58–3.86 [4 H, m, δCH$_2$(Pro^2) and δCH$_2$(Pro^3)], 3.69 (3 H, s,

CO_2CH_3), 4.27 [1 H, dd, J_1 8.4, J_2 5.7, $\frac{1}{2}\delta CH_2(Pro^4)$], 4.49 [2 H, m, αCH(Chp^1) and αCH(Pro)] and 4.67 [2 H, m, 2 × αCH-(Pro)]; δ_C(74.76 MHz; C^2HCl_3) 20.61 [$\beta CH_3(Chp^1)$], 24.97 and 25.04 [$\gamma CH_2(Pro^2)$ and $\gamma CH_2(Pro^3)$], 28.27 and 28.43 [βCH_2-(Pro^2) and $\beta CH_2(Pro^3)$], 35.82 [$\beta CH_2(Pro^4)$], 39.26 [γCH_2-(Pro^4)], 47.22 and 47.50 [$\delta CH_2(Pro^2)$ and $\delta CH_2(Pro^3)$], 51.43 [αCH(Chp^1)], 52.33 (CO_2CH_3), 56.20 [$\delta CH_2(Pro^4)$], 58.10, 58.63 and 58.74 [3 × αCH(Pro)], 167.96 [CO (Chp^1)], 170.67 and 170.83 (2 × amide CO) and 172.25 (ester CO); *m*/*z* (CI) 448 and 446 (21 and 60%, chlorine isotopes, $[M + H]^+$), 412 (100, $[M - Cl + H]^+$), 380 (32, $[M - Cl - OCH_3 + H]^+$), 227 {49, $[M - ClCH(CH_3)CONC_4H_7 - CO_2CH_3 + H]^+$}, 154 (31, $[CH_3CHCONC_4H_7CO + H]^+$), 126 (26, $OCNC_4H_8CO^+$) and 70 (24, $C_4H_8N^+$).

Dimethyl (3*S*,9*S*,15*R*,17*S*,19*S*,22*S*,28*S*,34*R*,36*S*,38*S*)-15,34-dimethyl-2,8,14,21,27,33-hexaoxo-16,35-dithia-1,7,13,20,26,32-hexaazaheptacyclo[34.2.1.1^{17,20}.0^{3,7}.0^{9,13}.0^{22,26}.0^{28,32}]tetracontane-19,38-dicarboxylate 16

Sodium hydride (60% dispersion in mineral oil, 11 mg, 0.29 mmol) was added to a stirred solution of thiol **7** (105 mg, 0.24 mmol) in dry THF (40 cm^3) and the mixture heated at reflux under nitrogen for 2 h. The suspension was cooled to room temperature, and the solvent was removed under reduced pressure. The residual oil was purified by gradient column chromatography on silica gel using dichloromethane–methanol as the eluent to yield the cyclic product **16** as a pale oil (21 mg, 22%); R_f 0.65 (dichloromethane–methanol, 9:1) (HRMS: found $[\frac{1}{2}M + H]^+$, 410.1742. $C_{19}H_{27}N_3O_5S$ requires 410.1750); $[\alpha]_D$ −64.1 (*c* 0.3 in MeOH); ν_{max}(thin film)/cm^{-1} 1742 (ester CO), 1634 (amide CO) and 1200 (C–O); δ_H(300 MHz; C^2HCl_3) 1.35 [6 H, d, *J* 6.6, $\beta CH_3(Thp^{1,1'})$], 1.85–2.35 [18 H, m, $\beta CH_2(Pro^{2,2'})$, $\beta CH_2(Pro^{3,3'})$, $\frac{1}{2}\beta CH_2(Pro^{4,4'})$, $\gamma CH_2(Pro^{2,2'})$ and $\gamma CH_2(Pro^{3,3'})$], 2.76 [2 H, dt, J_1 13.2, J_2 8.4, $\frac{1}{2}\beta CH_2(Pro^{4,4'})$], 3.25 [2 H, dd, J_1 10.2, J_2 7.5, $\frac{1}{2}\delta CH_2(Pro^{4,4'})$], 3.45–3.92 [10 H, m, γCH-($Pro^{4,4'}$), $\delta CH_2(Pro^{2,2'})$ and $\delta CH_2(Pro^{3,3'})$], 3.69 (6 H, s, 2 × CO_2-CH_3), 4.23 [2 H, dd, J_1 10.2, J_2 7.9, $\frac{1}{2}\delta CH_2(Pro^{4,4'})$] and 4.62–4.71 [8 H, m, αCH(Thp$^{1,1'}$) and 6 × αCH(Pro)]; δ_C(74.76 MHz; C^2HCl_3) 17.56 [$\beta CH_3(Thp^{1,1'})$], 25.11 and 25.54 [$\gamma CH_2(Pro^{2,2'})$ and $\gamma CH_2(Pro^{3,3'})$], 28.20 and 28.58 [$\beta CH_2(Pro^{2,2'})$ and βCH_2-($Pro^{3,3'}$)], 38.42 [$\beta CH_2(Pro^{4,4'})$], 40.45 [$\gamma CH_2(Pro^{4,4'})$], 40.90 [αCH(Thp$^{1,1'}$)], 47.35 and 48.36 [$\delta CH_2(Pro^{2,2'})$ and δCH_2-($Pro^{3,3'}$)], 52.39 (2 × CO_2CH_3), 52.68 [$\delta CH_2(Pro^{4,4'})$], 58.58, 58.82 and 59.06 [6 × αCH(Pro)], 170.63 and 170.78 (4 × amide CO), 172.08 [CO (Thp$^{1,1'}$)] and 172.67 (2 × ester CO); *m*/*z* (ES$^+$) 857 (8%, $[M + K]^+$), 841 (57, $[M + Na]^+$), 819 (16, M^+) and 364 (96, $[\frac{1}{2}M - 2CH_3 - OCH_3]^+$).

(3*S*,9*S*,15*R*,17*S*,19*S*,22*S*,28*S*,34*R*,36*S*,38*S*)-15,34-Dimethyl-2,8,14,21,27,33-hexaoxo-16,35-dithia-1,7,13,20,26,32-hexaazaheptacyclo[34.2.1.1^{17,20}.0^{3,7}.0^{9,13}.0^{22,26}.0^{28,32}]tetracontane-19,38-dicarboxylic acid 17

A solution of methyl ester **16** (11 mg, 27 μmol) in methanol (0.2 cm^3) was treated with aqueous sodium hydroxide (1.0 mol dm^{-3}, 0.2 cm^3) and the solution stirred for 2 h. The solvents were removed and the residue redissolved in water (5 cm^3). The aqueous solution was acidified to pH 1 with aqueous HCl (1 mol dm^{-3}) and washed with dichloromethane (2 × 4 cm^3). The solvent was removed from the aqueous phase and the residual solids extracted several times with hot dichloromethane. The combined extracts were filtered and the solvent removed under reduced pressure to afford the acid **17** as a colourless glass (7 mg, 65%); $[\alpha]_D$ −33.9 (*c* 0.4 in MeOH); $\nu_{max}(CH_2Cl_2)$/cm^{-1} 2978 (CH), 1754 (acid CO), 1642 (amide CO) and 1437 (CH def.); δ_H(300 MHz; ^2H_2O) 1.32 [6 H, d, *J* 7.2, $\beta CH_3(Thp^{1,1'})$], 1.86–2.01 [4 H, m, $\frac{1}{2}\beta CH_2(Pro^{2,2'})$ and $\frac{1}{2}\beta CH_2(Pro^{3,3'})$], 2.00–2.13 [10 H, m, $\frac{1}{2}\beta CH_2(Pro^{4,4'})$, $\gamma CH_2(Pro^{2,2'})$ and $\gamma CH_2(Pro^{3,3'})$] 2.31–2.44 [4 H, m, $\frac{1}{2}\beta CH_2(Pro^{2,2'})$ and $\frac{1}{2}\beta CH_2(Pro^{3,3'})$], 3.02 [2 H, dt, J_1 12.6, J_2 7.8, $\frac{1}{2}\beta CH_2(Pro^{4,4'})$], 3.35 [2 H, m, $\frac{1}{2}\delta CH_2(Pro^{4,4'})$], 3.60 [2 H, m, $\gamma CH_2(Pro^{4,4})$], 3.62–3.91 [8 H, m, $\delta CH_2(Pro^{2,2'})$ and $\delta CH_2(Pro^{3,3'})$], 4.17 [2 H, dd, J_1 9.0, J_2 6.0, $\frac{1}{2}\delta CH_2(Pro^{4,4'})$], 4.27 [2 H, q, *J* 7.2, αCH(Thp$^{1,1'}$)], 4.51 [2 H, t, *J* 7.8, $\alpha CH_2(Pro^{4,4'})$] and 4.71–4.78 [4 H, m, $\alpha CH_2(Pro^{2,2'})$ and αCH($Pro^{3,3'}$)]; δ_C(75.44 MHz; ^2H_2O) 16.13 [$\beta CH_3(Thp^{1,1'})$], 24.58 and 24.78 [$\gamma CH_2(Pro^{2,2'})$ and $\gamma CH_2(Pro^{3,3'})$], 27.71 and 28.25 [βCH_2-($Pro^{2,2'}$) and $\beta CH_2(Pro^{3,3'})$], 37.96 [$\beta CH_2(Pro^{4,4'})$], 40.30 [αCH-($Thp^{1,1'}$) and $\gamma CH_2(Pro^{4,4'})$], 47.83 and 48.94 [$\delta CH_2(Pro^{2,2'})$ and $\delta CH_2(Pro^{3,3'})$], 51.34 [$\delta CH_2(Pro^{4,4'})$], 58.73 and 59.51 [6 × αCH-(Pro)], 171.56 and 171.97 (4 × amide CO), 174.13 [CO ($Thp^{1,1'}$)] and 175.27 (2 × acid CO).

(2*R*,5*S*)-2-*tert*-Butyl-5-methyl-1-aza-3-oxabicyclo[3.3.0]octan-4-one 22

Pivalaldehyde (25 cm^3, 0.23 mol) was added to a suspension of (2*S*)-proline (14.0 g, 0.12 mol) in pentane (400 cm^3) and the mixture heated under reflux, using a Dean–Stark apparatus, for 7 days. During this time three further 25 cm^3 portions of pivalaldehyde were added at 2 day intervals. The solvent and excess pivalaldehyde were removed under reduced pressure to afford the bicyclooctanone **21** as a pale orange–brown oil (21.5 g). A portion of this oil (6.3 g, 34.4 mmol) was redissolved in dry THF (140 cm^3) and the solution cooled under nitrogen to −78 °C. A solution of lithium diisopropylamide in hexanes–THF (1 mol dm^{-3}, 36 cm^3) was added and the mixture was stirred for 1 h at −78 °C. Methyl iodide (2.4 cm^3, 38.6 mmol) was then added dropwise and the suspension allowed to warm to −30 °C over 2 h. The suspension was poured into a mixture of water (100 cm^3) and dichloromethane (250 cm^3). The organic phase was separated, dried ($MgSO_4$) and the solvents removed under reduced pressure to give a yellow–orange solid. the crude solid was redissolved in dichloromethane and the solution was filtered. The filtrate was concentrated under reduced pressure to yield a brown semi-solid mass; Kugelrohr distillation of this residue under reduced pressure afforded bicyclooctanone **22** as a colourless oil (3.9 g, 55%), bp 110 °C/~0.05 mmHg (lit.,[17] 85 °C/0.05 mmHg); $[\alpha]_D$ −28.7 (*c* 1.05 in $CHCl_3$) [lit.,[17] −29.8 (*c* 0.7 in $CHCl_3$)]; ν_{max}(thin film)/cm^{-1} 2973 (CH), 1784 (CO) and 1192 (C–O); δ_H(200 MHz; C^2HCl_3) 0.90 [9 H, s, $C(CH_3)_3$], 1.37 (3 H, s, βCH_3), 1.64–1.86 (3 H, m, $\frac{1}{2}\beta CH_2$ and γCH_2), 2.12–2.19 (1 H, m, $\frac{1}{2}\beta CH_2$), 2.76–2.87 (1 H, m, $\frac{1}{2}\delta CH_2$), 3.05–3.18 (1 H, m, $\frac{1}{2}\delta CH_2$) and 4.25 (1 H, s, OCHN); δ_C(75.44 MHz; C^2HCl_3) 24.54 [$C(CH_3)$], 25.84 and 25.96 (βCH_3 and γCH_2), 36.86 [$C(CH_3)_3$] 39.14 (βCH_2), 58.34 (δCH_2), 69.30 (αC), 106.36 (NCO) and 179.76 (CO); *m*/*z* (CI) 198 (82%, $[M + H]^+$), 140 (6, $[M - C_4H_9]^+$), 130 (100, $[M - C_5H_7 + H]^+$) and 84 (14, $NC_4H_7CH_3^+$).

Methyl (2*S*)-2-methylprolinate hydrochloride 23

An ice-cooled solution of bicyclooctanone **22** (6.6 g, 33.5 mmol) in dry methanol (75 cm^3) was treated dropwise with thionyl chloride (4.0 cm^3, 54.6 mmol). The solution was heated under reflux for 2 h, then cooled and the solvents removed under reduced pressure to yield a dark brown solid. The crude solid was recrystallised from ethyl acetate–methanol to afford the amine hydrochloride **23** as colourless crystals (3.5 g, 58%), mp 106–108 °C (Found: C, 46.5; H, 8.15; N, 7.7. $C_7H_{14}ClNO_2$ requires C, 46.8; H, 7.85; N, 7.8%); $[\alpha]_D$ −31.6 (*c* 1.3 in MeOH); $\nu_{max}(CH_2Cl_2)$/cm^{-1} 2995 (CH) and 1747 (CO); δ_H(200 MHz; ^2H_2O) 1.68 (3 H, s, βCH_3), 1.99–2.21 (3 H, m, $\frac{1}{2}\beta CH_2$ and γCH_2), 2.36–2.46 (1 H, m, $\frac{1}{2}\beta CH_2$), 3.41–3.51 (2 H, m, δCH_2) and 3.84 (3 H, s, CO_2CH_3); δ_C(75.44 MHz; $C^2H_3O^2H$) 21.86 (βCH_3), 23.97 (γCH_2), 36.75 (βCH_2), 46.75 (δCH_2), 54.68 (CO_2CH_3), 70.50 (αC) and 173.17 (ester CO); *m*/*z* (CI) 144 (100%, $[M - Cl]^+$) and 84 (26, $[M - HCl - CO_2CH_3]^+$).

(2*S*)-*N*-[(2*S*)-2-Chloropropionyl]-2-methylproline 25

A solution of (2*S*)-2-chloropropionic acid (1.21 g, 11.1 mmol), methyl (2*S*)-2-methylprolinate hydrochloride **23** (1.00 g, 5.5

mmol) and *N*,*N*-diisopropylethylamine (3.3 cm^3, 18.9 mmol) in dry dichloromethane (100 cm^3) was treated with BOP-Cl (2.98 g, 11.7 mmol) and the resulting suspension was stirred under nitrogen at 0–5 °C for 4 h. The mixture was allowed to warm to room temperature and stirred for a further 15 h. The solution was washed with aqueous HCl (0.5 mol dm^{-3}, 2 × 50 cm^3), aqueous sodium hydrogen carbonate (5%, 2 × 50 cm^3) and brine (50 cm^3), then dried ($MgSO_4$). The solvent was removed under reduced pressure to yield the dipeptide **24** as a colourless oil (0.78 g).

The above oil was redissolved in methanol (7 cm^3), treated with aqueous sodium hydroxide (1.0 mol dm^{-3}, 9 cm^3), then stirred for 2 days. Following addition of aqueous HCl (1.0 mol dm^{-3}, 9 cm^3), the solution was concentrated to ~15 cm^3 under reduced pressure and then extracted with ethyl acetate (3 × 25 cm^3). The combined organic extracts were dried ($MgSO_4$) and the solvent removed under reduced pressure to afford the product as a tan solid (0.53 g, 43%); a small portion was recrystallised to yield the acid **25** as colourless crystals, mp 136–137 °C (Found: C, 49.5; H, 6.8; N, 6.45. $C_9H_{14}ClNO_3$ requires C, 49.2; H, 6.4; N, 6.4%); $[\alpha]_D$ −3.9 (*c* 0.3 in MeOH); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3000 (OH), 2989 (CH), 1758 (ester CO), 1674 (amide CO) and 1260 (C–O); δ_H(300 MHz; C^2HCl_3) 1.53 [3 H, s, βCH_3(Pro)], 1.57 [3 H, d, *J* 6.6, βCH_3(Chp)], 1.85–2.06 [3 H, m, $\frac{1}{2}\beta CH_2$(Pro) and γCH_2], 2.21–2.30 [1 H, m, $\frac{1}{2}\beta CH_2$-(Pro)], 3.57–3.69 (1 H, m, $\frac{1}{2}\delta CH_2$), 3.78–3.85 (1 H, m, $\frac{1}{2}\delta CH_2$), 4.42 [1 H, q, *J* 6.6, αCH(Chp)] and 7.13 (1 H, br s, CO_2H); δ_C(75.44 MHz; C^2HCl_3) 20.49 [βCH_3(Pro)], 20.90 [βCH_3(Chp)], 24.26 (γCH_2), 38.66 [βCH_2(Pro)], 48.46 (δCH_2), 51.80 [αCH(Chp)], 67.00 [αC(Pro)], 168.33 [CO (Chp)] and 177.61 (acid CO); *m*/*z* (EI) 222 and 220 (1 and 4%, chlorine isotopes, M^+), 176 and 174 (32 and 56, chlorine isotopes, $[M - CO_2H + H]^+$), 140 (45, $[M - CO_2H - HCl + H]^+$), 128 {21 $[M - ClCH(CH_3)CO]^+$} and 84 (100, $C_5H_{10}N^+$).

Methyl (2*S*,4*S*)-*N*-[(2*S*)-*N*-{(2*S*)-*N*-[(2*S*)-2-chloropropionyl]-2-methylprolyl}prolyl]-4-mercaptoprolinate 19

A solution of (2*S*)-*N*-[(2*S*)-2-chloropropionyl]-2-methylproline **25** (0.20 g, 0.91 mmol) and *N*,*N*-diisopropylethylamine (0.50 cm^3, 2.87 mmol) in dry dichloromethane (15 cm^3) was treated with BOP-Cl (0.25 g, 0.98 mmol) and the resulting suspension stirred under nitrogen at 0 °C for 20 min. A solution of amine trifluoroacetate **13** (0.28 g, 0.83 mmol) in dry dichloromethane (5 cm^3) was then added. The mixture was stirred at 0–5 °C for 5 h, then at room temperature for 15 h. The solution was washed with aqueous HCl (0.5 mol dm^{-3}, 2 × 5 cm^3), aqueous sodium hydrogen carbonate (5%, 2 × 5 cm^3) and brine (100 cm^3) and dried ($MgSO_4$). The solvent was removed under reduced pressure, and the residual oil was purified by column chromatography using ethyl acetate–methanol (19 : 1) as the eluent, to yield the thioacetate **26** as a colourless foam (0.13 g). The crude foam was redissolved in methanol (9 cm^3) and the solution treated with aqueous potassium hydroxide (0.05 mol dm^{-3}, 9 cm^3). The solution was stirred for 4 h, after which time aqueous HCl (0.05 mol dm^{-3}, 10 cm^3) was added. The methanol was removed under reduced pressure, then the solution was further acidified to pH 1 and extracted with ethyl acetate (3 × 25 cm^3). The combined organic extracts were dried ($MgSO_4$) and the solvent removed under reduced pressure to afford the thiol **19** as a colourless oil (0.11 g, 29%) (HRMS: found $[M + H]^+$, 460.1682. $C_{20}H_{31}{}^{35}ClN_3O_5S$ requires 460.1673); $[\alpha]_D$ −108.4 (*c* 1.1 in MeOH); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 2546 (SH), 1747 (ester CO), 1662 (amide CO), 1423 (CH def.) and 1200 (C–O); δ_H(300 MHz; C^2HCl_3) 1.57 [3 H, d, *J* 6.6, $\beta CH_3(Chp^1)$], 1.78 [3 H, s, $\beta CH_3(Pro^2)$], 1.80–2.10 [8 H, m, $\frac{1}{2}\beta CH_2(Pro^2)$, $\beta CH_2(Pro^3)$, $\frac{1}{2}\beta CH_2(Pro^4)$, $\gamma CH_2(Pro^2)$ and $\gamma CH_2(Pro^3)$], 2.35 [1 H, m, $\frac{1}{2}\beta CH_2(Pro^3)$], 2.63 [1 H, dt, J_1 13.3, J_2 8.0, $\frac{1}{2}\beta CH_2(Pro^4)$], 3.30–3.41 [2 H, m, $\gamma CH_2(Pro^4)$ and $\frac{1}{2}\delta CH_2(Pro^4)$], 3.52–3.80 [4 H, m, $\delta CH_2(Pro^2)$ and $\delta CH_2(Pro^3)$], 3.62 (3 H, s, CO_2CH_3), 4.20 [1 H, dd, J_1 10.0, J_2 7.6, $\frac{1}{2}\delta CH_2(Pro^4)$], 4.42 [2 H, m, $\alpha CH(Chp^1)$ and αCH(Pro)] and 4.71 [1 H, m, αCH(Pro)]; δ_C(75.44 MHz; C^2HCl_3) 20.62 [$\beta CH_3(Chp^1)$], 23.52 and 23.60 [$\beta CH_3(Pro^2)$ and $\gamma CH_2(Pro^3)$], 25.55 [$\gamma CH_2(Pro^2)$], 27.26 [$\beta CH_2(Pro^3)$], 36.00 [$\beta CH_2(Pro^4)$], 38.23 [$\beta CH_2(Pro^2)$], 39.36 [$\gamma CH_2(Pro^4)$], 48.36 [$\delta CH_2(Pro^3)$], 48.69 [$\delta CH_2(Pro^2)$], 52.22 [$\alpha CH(Chp^1)$], 52.65 (CO_2CH_3), 56.32 [$\delta CH_2(Pro^4)$], 58.75 [$\alpha CH(Pro^3)$], 60.51 [$\alpha CH(Pro^4)$], 68.58 [$\alpha C(Pro^2)$], 167.90 [CO (Chp^1)], 171.09 and 171.71 (2 × amide CO) and 172.58 (ester CO); *m*/*z* (CI) 460 (33 and 100%, chlorine isotopes, $[M + H]^+$), 426 (13, $[M HCl + H]^+$), 392 (13, $[M - Cl - SH + H]^+$), 301 and 299 {3 and 7, chlorine isotopes, $[M - NC_4H_6(SH)CO_2CH_3]^+$)}.

Dimethyl (3*S*,9*S*,15*R*,17*S*,19*S*,22*S*,28*S*,34*R*,36*S*,38*S*)-9,15,28,34-tetramethyl-2,8,14,21,27,33-hexaoxo-16,35-dithia-1,7,13,20,26,32-hexaazaheptacyclo[34.2.1.1^{17,20}.0^{3,7}.0^{9,13}.0^{22,26}.0^{28,32}]tetracontane-19,38-dicarboxylate 27

Caesium carbonate (210 mg, 0.65 mmol) was added to a solution of thiol **19** (150 mg, 0.33 mmol) in DMF (80 cm^3) and the mixture stirred with heating at 80–90 °C for 2 h. The suspension was cooled and the solvent removed under reduced pressure. The residual orange–brown oil was purified by column chromatography using dichloromethane–methanol (19 : 1) as the eluent to afford the macrocycle **27** as a white foam (41 mg, 30%); R_f 0.43 (Found: C, 54.4; H, 7.1; N, 9.5. $C_{40}H_{58}N_6O_{10}S_2{\cdot}2H_2O$ requires: C, 54.4; H, 7.0; N, 9.8%); $[\alpha]_D$ −58.4 (*c* 0.1 in MeOH); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 2983 (CH), 1747 (ester CO), 1658 (amide CO), 1626 (amide CO) and 1200 (C–O); δ_H(300 MHz; ^2H_2O) 1.31 [6 H, d, *J* 6.9, $\beta CH_3(Thp^{1,1'})$], 1.80 [6 H, s, $\beta CH_3(Pro^{2,2'})$], 1.93–2.21 [18 H, m, $\beta CH_2(Pro^{2,2'})$, $\beta CH_2(Pro^{3,3'})$, $\frac{1}{2}\beta CH_2(Pro^{4,4'})$, $\gamma CH_2(Pro^{2,2'})$ and $\gamma CH_2(Pro^{3,3'})$], 2.85–2.94 [2 H, m, $\frac{1}{2}\beta CH_2$-$(Pro^{2,2'})$], 3.44 [2 H, t, *J* 9.4, $\frac{1}{2}\delta CH_2(Pro^{4,4'})$], 3.58–3.69 [4 H, m, $\frac{1}{2}\delta CH_2(Pro^{2,2'})$ and $\gamma CH(Pro^{4,4'})$], 3.76 (6 H, s, 2 × CO_2CH_3), 3.76–3.85 [6 H, m, $\frac{1}{2}\delta CH_2(Pro^{2,2'})$ and $\delta CH_2(Pro^{3,3'})$], 4.11–4.22 [4 H, m, $\alpha CH(Thp^{1,1'})$ and $\frac{1}{2}\delta CH_2(Pro^{4,4'})$], 4.56 [2 H, t, *J* 7.3, $\alpha CH(Pro^{4,4'})$] and 4.70–4.79 [4 H, m, $\alpha CH(Pro^{2,2'})$ and αCH-$(Pro^{3,3'})$]; δ_H(75.44 MHz; ^2H_2O) 16.41 [$\beta CH_3(Thp^{1,1'})$], 23.01 and 23.24 [$\beta CH_3(Pro^{2,2'})$ and $\gamma CH_2(Pro^{2,2'})$], 25.20 [γCH_2-$(Pro^{3,3'})$], 26.66 [$\beta CH_2(Pro^{3,3'})$], 37.55 [$\beta CH_2(Pro^{4,4'})$], 38.00 [$\beta CH_2(Pro^{2,2'})$], 40.87 [$\gamma CH_2(Pro^{4,4'})$], 41.58 [$\alpha CH(Thp^{1,1'})$], 49.20 and 49.41 [$\delta CH_2(Pro^{2,2'})$ and $\delta CH_2(Pro^{3,3'})$], 52.08 (2 × CO_2CH_3), 53.17 [$\delta CH_2(Pro^{4,4'})$], 59.38 [$\alpha CH(Pro^{4,4'})$], 61.03 [$\alpha CH(Pro^{3,3'})$], 69.31 [$\alpha C(Pro^{2,2'})$], 172.55, 173.15 and 173.52 (6 × amide CO) and 174.45 (2 × ester CO); *m*/*z* (CI) 847 (8%, $[M + H]^+$), 452 {72, $[CONC_4H_6(CH_3)COCH(CH_3)SC_4H_6$-$(CO_2CH_3)NCOC_4H_7NCO + H]^+$}, 354 {80, $[CONC_4H_6(CH_3)$-$COCH(CH_3)SC_4H_6(CO_2CH_3)]^+$} and 145 {100, $[NC_4H_6(S)$-$CO_2H]^+$}.

(3*S*,9*S*,15*R*,17*S*,19*S*,22*S*,28*S*,34*R*,36*S*,38*S*)-9,15,28,34-Tetramethyl-2,8,14,21,27,33-hexaoxo-16,35-dithia-1,7,13,20,26,32-hexaazaheptacyclo[34.2.1.1^{17,20}.0^{3,7}.0^{9,13}.0^{22,26}.0^{28,32}]tetracontane-20,39-dicarboxylic acid 28

This compound was prepared in a manner identical with that for the acid **17**, using methyl ester **27** (20 mg, 4.7 μmol) to give the acid **28** as a colourless oil (15 mg, 78%) (HRMS: found $[M - H + 2Na]^+$, 863.3084. $C_{38}H_{53}N_6O_{10}S_2Na_2$ requires 863.3060); $[\alpha]_D$ −45.8 (*c* 0.1 in MeOH); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 2971 (CH), 1749 (acid CO), 1647 (amide CO) and 1456 (CH def.); δ_H(500 MHz; ^2H_2O) major conformation: 1.30 [6 H, d, *J* 7.2, $\beta CH_3(Thp^{1,1'})$], 1.79 [6 H, s, $\beta CH_3(Pro^{2,2'})$], 1.86–2.00 [4 H, m, $\frac{1}{2}\beta CH_2(Pro^{2,2'})$ and $\frac{1}{2}\gamma CH_2(Pro^{3,3'})$], 2.00–2.15 [8 H, m, $\frac{1}{2}\beta CH_2(Pro^{4,4'})$, $\gamma CH_2(Pro^{3,3'})$ and $\frac{1}{2}\gamma CH_2(Pro^{2,2'})$], 2.15–2.26 [4 H, m, $\frac{1}{2}\beta CH_2(Pro^{2,2'})$ and $\frac{1}{2}\beta CH_2(Pro^{3,3'})$], 2.88–2.97 [2 H, m, $\frac{1}{2}\beta CH_2(Pro^{4,4'})$], 3.37–3.42 [2 H, m, $\frac{1}{2}\delta CH_2(Pro^{4,4'})$], 3.49–3.68 [4 H, m, $\frac{1}{2}\delta CH_2(Pro^{4,4'})$ and $\gamma CH(Pro^{4,4'})$], 3.72–3.86 [6 H, m, $\delta CH_2(Pro^{2,2'})$ and $\frac{1}{2}\delta CH_2(Pro^{3,3'})$], 4.10–4.15 [2 H, m, $\frac{1}{2}\delta CH_2(Pro^{4,4'})$], 4.18 [2 H, q, *J* 7.2, $\alpha CH(Thp^{1,1'})$], 4.49 [2 H, t, *J* 7.8, $\alpha CH(Pro^{4,4'})$] and 4.70–4.75 [2 H, m, $\alpha CH(Pro^{3,3'})$]; minor

conformation: 1.42 [6 H, s, βCH$_3$(Pro$^{2,2'}$)], 1.48 [6 H, d, *J* 7.2, βCH$_3$(Thp$^{1,1'}$)], 1.68–1.73 [2 H, m, $\frac{1}{2}$γCH$_2$(Pro$^{3,3'}$)], 1.83–2.03 [6 H, m, γCH$_2$(Pro$^{2,2'}$) and $\frac{1}{2}$γCH$_2$(Pro$^{3,3'}$)], 2.04–2.15 [4 H, m, $\frac{1}{2}$βCH$_2$(Pro$^{2,2'}$) and $\frac{1}{2}$βCH$_2$(Pro$^{3,3'}$)], 2.15–2.33 [6 H, m, $\frac{1}{2}$βCH$_2$-(Pro$^{2,2'}$), $\frac{1}{2}$βCH$_2$(Pro$^{3,3'}$) and $\frac{1}{2}$βCH$_2$(Pro$^{4,4'}$)], 3.02–3.08 [2 H, m, $\frac{1}{2}$βCH$_2$(Pro$^{4,4'}$)], 3.37–3.42 [4 H, m, δCH$_2$(Pro$^{3,3'}$)], 3.49–3.56 [2 H, m, $\frac{1}{2}$δCH$_2$(Pro$^{4,4'}$)], 3.49–3.68 [2 H, m, δCH$_2$(Pro$^{2,2'}$)], 3.99–4.06 [2 H, m, γCH(Pro$^{4,4'}$)], 4.10–4.22 [4 H, m, αCH(Thp$^{1,1'}$) and $\frac{1}{2}$δCH$_2$(Pro$^{4,4'}$)], 4.48 [2 H, t, *J* 7.8, αCH(Pro$^{4,4'}$)] and 4.92 [2 H, d, *J* 7.8, αCH(Pro$^{2,2'}$)]; δ_C(75.44 MHz; ^2H_2O) 16.77 and 19.57 [βCH$_3$(Thp$^{1,1'}$)], 20.10 and 22.15 [βCH$_3$(Pro$^{2,2'}$)], 23.04, 23.28 and 25.23 [γCH$_2$(Pro$^{2,2'}$) and γCH$_2$(Pro$^{3,3'}$)], 26.80 and 30.84 [βCH$_2$(Pro$^{2,2'}$)], 34.96 and 38.10 [βCH$_2$(Pro$^{4,4'}$)], 38.86 and 39.86 [βCH$_2$(Pro$^{2,2'}$)], 40.41 and 41.30 [γCH(Pro$^{4,4'}$)], 43.61 and 43.95 [αCH(Thp$^{1,1'}$)], 47.62, 49.03, 49.30 and 49.63 [δCH$_2$-(Pro$^{2,2'}$) and δCH$_2$(Pro$^{3,3'}$)], 51.70 and 52.04 [δCH$_2$(Pro$^{4,4'}$)], 59.31, 61.22, 62.25 and 63.10 [αCH(Pro$^{3,3'}$) and αCH(Pro$^{4,4'}$)], 68.32 and 69.41 [αC(Pro$^{2,2'}$)], 171.40, 171.57, 172.78, 173.15 and 173.95 (6 × amide CO), 178.31 and 179.18 (2 × acid CO); *m*/*z* (FAB) 885 (53%, [M − 2H + 3Na]$^+$), 863 (100, [M − H + 2Na]$^+$) and 817 (18, [M − H]$^+$).

(2*S*)-*N*-(*tert*-Butoxycarbonyl)-2-methylproline 33

A suspension of (2*S*)-2-methylproline methyl ester hydrochloride **23** (1.00 g, 5.6 mmol) in dry dichloromethane (30 cm^3) at 0 °C was treated with *N*,*N*-diisopropylethylamine (2.40 cm^3, 13.8 mmol), DMAP (0.20 g, 1.9 mmol) and di-*tert*-butyl dicarbonate (1.85 g, 8.4 mmol). The mixture was allowed to reach room temperature and stirred for 3 days. The solution was washed with aqueous HCl (0.5 mol dm^{-3}, 2 × 20 cm^3), aqueous sodium hydrogen carbonate (5%, 2 × 20 cm^3) and brine (20 cm^3). The organic phase was dried ($MgSO_4$), and the solvent was removed under reduced pressure to yield the methyl ester **32** as a brown oil (1.24 g). The crude oil was redissolved in methanol (15 cm^3) and aqueous sodium hydroxide (1.0 mol dm^{-3}, 25 cm^3) was added. The solution was stirred for 2 days, concentrated to ~20 cm^3 and then acidified to pH 2 with aqueous HCl (0.5 mol dm^{-3}). The solution was extracted with ethyl acetate (3 × 25 cm^3) and the combined organic extracts dried ($MgSO_4$). The solvent was removed under reduced pressure to afford the acid **33** as pale yellow crystals (0.78 g, 61%), mp 129–130 °C [lit.,[27] 129–132 °C] (Found: C, 57.75; H, 8.4; N, 6.15. Calc. for $C_{11}H_{19}NO_4$: C, 57.6; H, 8.35; N, 6.1%); $[\alpha]_D$ −50.6 (*c* 1.4 in $CHCl_3$) [lit.,[27] −41.4 (*c* 1.45 in $CHCl_3$)]; $\nu_{max}(CH_2Cl_2)$/cm^{-1} 2982 (CH), 2664 (OH), 1749 (acid CO), 1692 (urethane CO) and 1165 (C–O); δ_H(200 MHz; C^{2}HCl$_3$) 1.40 [*t*, 9 H, s, C(CH$_3$)$_3$], 1.44 [*c*, 9 H, s, C(CH$_3$)$_3$], 1.50 (*t*, 3 H, s, βCH$_3$), 1.57 (*c*, 3 H, s, βCH$_3$), 1.84–2.00 (3 H, m, $\frac{1}{2}$βCH$_2$ and γCH$_2$), 2.18–2.43 (1 H, m, $\frac{1}{2}$βCH$_2$), 3.42–3.60 (2 H, m, δCH$_2$) and 10.05 (1 H, s, CO$_2$H); δ_C(75.44 MHz; C^{2}HCl$_3$) 22.45 (*c*, βCH$_3$), 23.09 (*t*, βCH$_3$), 23.14 (*t*, γCH$_2$), 23.39 (*c*, γCH$_2$), 28.61 [*t*, C(*C*H$_3$)$_3$], 28.75 [*c*, C(*C*H$_3$)$_3$], 39.35 (*c*, βCH$_2$), 40.67 (*t*, βCH$_2$), 48.14 (*t*, δCH$_2$), 48.63 (*c*, δCH$_2$), 65.17 (*t*, αC), 66.00 (*c*, αC), 81.06 [*C*(CH$_3$)$_3$], 154.26 (*t*, urethane CO), 155.50 (*c*, urethane CO), 179.34 (*c*, acid CO) and 181.11 (*t*, acid CO); *m*/*z* (EI) 229 (0.5%, M$^+$), 184 (27, [M − CO$_2$H]$^+$), 128 (100, [M − CO$_2$H − C$_4$H$_8$]$^+$), 84 (78, NC$_4$H$_7$CH$_3^+$) and 57 (79, C$_4$H$_9^+$).

Methyl (2*S*,4*R*)-*N*-(*tert*-butoxycarbonyl)-4-hydroxyprolinate 34

A stirred suspension of methyl (2*S*,4*R*)-4-hydroxyprolinate hydrochloride (7.0 g, 38.5 mmol) in dry THF (90 cm^3) was treated with *N*-methylmorpholine (8.8 cm^3, 78.7 mmol) and the mixture stirred for 1 h. Di-*tert*-butyl dicarbonate (9.3 g, 42.7 mmol) was added and the mixture stirred for a further 18 h. The hydrochloride salts were filtered off and the solvent removed from the filtrate under reduced pressure to give a brown oil which was redissolved in ethyl acetate (100 cm^3). The solution was washed with aqueous HCl (0.5 mol dm^{-3}, 2 × 70 cm^3), aqueous sodium hydrogen carbonate (5%, 2 × 70 cm^3), then dried ($MgSO_4$). The solution was then concentrated under pressure to yield compound **34** as a clear colourless oil (7.5 g, 79%) (Found: C, 53.6; H, 8.05; N, 5.8. $C_{11}H_{19}NO_5$ requires C, 53.85; H, 7.8; N, 5.75%) (HRMS: found [M + H]$^+$, 246.1341. $C_{11}H_{20}NO_5$ requires 246.1334); $[\alpha]_D$ −60.2 (*c* 1.0 in MeOH) [lit.,[26] −51.0 (*c* 1.0 in MeOH)]; ν_{max}(thin film)/cm^{-1} 3443 (OH), 1750 (ester CO), 1683 (urethane CO) and 1160 (C–O); δ_H(200 MHz; C^{2}HCl$_3$) 1.36 [*t*, 9 H, s, C(CH$_3$)$_3$], 1.41 [*c*, 9 H, s, C(CH$_3$)$_3$], 1.98–2.07 (1 H, m, $\frac{1}{2}$βCH$_2$), 2.22–2.32 (1 H, m, $\frac{1}{2}$βCH$_2$), 3.19–3.61 (2 H, m, δCH$_2$), 3.69 (3 H, s, CO$_2$CH$_3$) and 4.31–4.43 (2 H, m, αCH and γCH); δ_C(75.44 MHz; C^{2}HCl$_3$) 28.21 [*t*, C(*C*H$_3$)$_3$], 28.35 [*c*, C(*C*H$_3$)], 38.36 (*c*, βCH$_2$), 39.12 (*t*, βCH$_2$), 52.03 (*t*, CO$_2$*C*H$_3$), 52.18 (*c*, CO$_2$*C*H$_3$), 55.60 (*t*, δCH$_2$), 55.73 (*c*, δCH$_2$), 58.09 (*c*, αCH), 58.17 (*t*, αCH), 68.96 (*t*, γCH), 69.66 (*c*, γCH), 80.20 [*c*, *C*(CH$_3$)$_3$], 80.33 [*t*, *C*(CH$_3$)], 154.13 (*t*, urethane CO), 154.75 (*c*, urethane CO), 173.68 (*c*, ester CO) and 173.92 (*t*, ester CO); *m*/*z* (EI) 246 (6%, [M + H]$^+$), 186 (60, [M − CO$_2$CH$_3$]$^+$), 144 (54, [M − CO$_2$C$_4$H$_9$]$^+$), 130 (71, [M − CO$_2$-CH$_3$ − C$_4$H$_9$]$^+$), 86 (88, [M − CO$_2$CH$_3$ − CO$_2$C$_4$H$_9$]$^+$) and 57 (100, C$_4$H$_9^+$).

Methyl (2*S*,4*S*)-4-(acetylthio)prolinate trifluoroacetate 36

Diisopropyl azodicarboxylate (3.75 g, 19.0 mmol) was added to an ice-cooled solution of triphenylphosphine (4.97 g, 19.0 mmol) in dry THF (70 cm^3) and the resulting suspension was stirred at 0 °C for 30 min. A solution of alcohol **34** (4.04 g, 16.5 mmol) and thioacetic acid (0.48 cm^3, 20.4 mmol) in dry THF (70 cm^3) was then added dropwise to this solution. The mixture was stirred with ice-cooling for 2 h, and at room temperature for 18 h. The solvent was removed under reduced pressure to yield a pale orange oil which was partially purified by column chromatography using light petroleum–ethyl acetate (6:1) as the eluent to yield the thioacetate **35** in an impure state as a colourless oil (4.05 g).

The above oil (1.1 g) was redissolved in dichloromethane (20 cm^3), the solution ice-cooled, and trifluoroacetic acid (7 cm^3) added. The solution was stirred with cooling for 90 min, and the solvents were removed under reduced pressure. The residual oil was redissolved in water (10 cm^3) and the solution washed with ether (2 × 7 cm^3). The aqueous phase was concentrated under reduced pressure and lyophilised to give the amine trifluoroacetate **36** as a colourless oil (1.03 g, 72%) (HRMS: found [M − CF$_3$CO$_2$]$^+$, 204.0691. $C_8H_{13}{}^{35}ClNO_3S$ requires 204.0694); $[\alpha]_D$ −25.9 (*c* 1.0 in MeOH); $\nu_{max}(CH_2Cl_2)$/cm^{-1} 2996 (CH), 1751 (ester CO), 1698 (thioacetate CO) and 1199 (C–O); δ_H(300 MHz; C^{2}HCl$_3$) 2.19 (1 H, dt, J_1 13.9, J_2 7.8, $\frac{1}{2}$βCH$_2$), 2.32 (3 H, s, SCOCH$_3$), 2.85 (1 H, dt, J_1 13.9, J_2 7.8, $\frac{1}{2}$βCH$_2$), 3.37 (1 H, dd, J_1 12.2, J_2 7.7, $\frac{1}{2}$δCH$_2$), 3.81 (3 H, s, CO$_2$CH$_3$), 3.86 (1 H, dd, J_1 12.2, J_2 7.7, $\frac{1}{2}$δCH$_2$), 4.08 (1 H, quintet, *J* 7.7, γCH), 4.53 (1 H, t, *J* 8.1, αCH) and 8.43 (2 H, br s, NH$_2$); δ_C(50.51 MHz; C^{2}HCl$_3$) 30.50 (SCO*C*H$_3$), 34.25 (βCH$_2$), 38.97 (γCH$_2$), 50.87 (CO$_2$*C*H$_3$), 53.84 (δCH$_2$), 59.06 (αCH), 116.40 (q, J_{CF} 290.8, *C*F$_3$CO$_2$H), 161.91 (q, J_{CF} 36.5, CF$_3$*C*O$_2$H), 169.06 (ester CO) and 194.85 (thioester CO); *m*/*z* (CI) 204 (100%, [M − CF$_3$CO$_2$]$^+$), 162 (26, [M − CF$_3$CO$_2$ − COCH$_3$ + H]$^+$) and 146 (19, [M − CF$_3$CO$_2$ − CO$_2$CH$_3$ + H]$^+$).

Methyl (2*S*,4*S*)-*N*-[(2*S*)-*N*-(*tert*-butoxycarbonyl)-2-methylprolyl]-4-(acetylthio)prolinate 37

This compound was prepared in a manner identical with that for the dipeptide **14**, using acid **33** (0.15 g, 0.65 mmol) and amine trifluoroacetate **36** (0.23 g, 0.73 mmol) to yield a yellow oil which was purified by column chromatography using ethyl acetate–light petroleum (1:1) as the eluent to afford the thioacetate **37** as colourless crystals (0.21 g, 55%); R_f 0.64 (ethyl acetate); mp 112–114 °C (Found: C, 55.05; H, 7.3; N, 6.75. $C_{19}H_{30}N_2O_6$ requires: C, 55.1; H, 7.55; N, 6.75%); $[\alpha]_D$ −109.8 (*c* 1.2 in MeOH); $\nu_{max}(CH_2Cl_2)$/cm^{-1} 2980 (CH), 1752 (ester CO), 1694 (urethane CO), 1640 (amide CO) and 1174 (C–O); δ_H(300

MHz; C^2HCl_3) 1.43 [9 H, s, $C(CH_3)_3$], 1.49 [3 H, s, $\beta CH_3(Pro^1)$], 1.72–2.07 [5 H, m, $\beta CH_2(Pro^1)$, $\frac{1}{2}\beta CH_2(Pro^2)$ and $\gamma CH_2(Pro^1)$], 2.29 (3 H, s, $SCOCH_3$), 2.51 [1 H, dt, J_1 13.7, J_2 7.4, $\frac{1}{2}\beta CH_2(Pro^2)$], 3.15 [1 H, t, J 10.3, $\frac{1}{2}\delta CH_2(Pro^2)$], 3.31 [1 H, m, $\frac{1}{2}\delta CH_2(Pro^1)$], 3.50 [1 H, m, $\frac{1}{2}\delta CH_2(Pro^1)$], 3.69 (3 H, s, CO_2CH_3), 3.80 [1 H, m, $\gamma CH(Pro^2)$], 4.04 [1 H, m, $\frac{1}{2}\delta CH_2(Pro^2)$] and 4.39 [1 H, t, J 8.1, $\alpha CH(Pro^2)$]; δ_C(75.44 MHz; C^2HCl_3) 22.46 [$\beta CH_3(Pro^1)$], 23.57 [$\gamma CH_2(Pro^1)$], 28.16 [*tt*, $C(CH_3)_3$], 28.69 [*ct*, $C(CH_3)_3$], 30.84 ($SCOCH_3$), 33.72 [$\beta CH_2(Pro^2)$], 39.79 and 40.21 [$\beta CH_2(Pro^1)$ and $\gamma CH(Pro^2)$], 47.61 [$\delta CH_2(Pro^1)$], 52.28 (CO_2CH_3), 52.51 [$\delta CH_2(Pro^2)$], 60.70 [$\alpha CH(Pro^2)$], 66.01 [$\alpha C(Pro^1)$], 81.25 [$C(CH_3)_3$], 154.06 (urethane CO), 172.57 (amide CO), 173.62 (ester CO) and 195.51 (thioester CO); *m*/*z* (EI) 415 (13%, M^+), 341 (14, $[M - SCOCH_3 + H]^+$), 315 (17, $[M - CO_2C_4H_9 + H]^+$), 184 [80, $C_4H_9OC(O)NC_4H_6CH_3^+$], 128 (100, $CH_3C_4H_7NCO_2^+$), 84 (65, $NC_4H_7CH_3^+$) and 57 (53, $C_4H_9^+$).

(2*S*,5a*S*,10a*S*)-2-Acetylthio-5a-methyl-5,10-dioxoperhydrodipyrrolo[1,2-*a*;1,2-*d*]pyrazine 38

A solution of thioester **37** (0.15 g, 0.36 mmol) in dichloromethane (3 cm^3) at 0 °C was treated with trifluoroacetic acid (1 cm^3). The mixture was stirred for a further 90 min and then the solvents were removed under reduced pressure to give the amine thioacetate **31** as a colourless oil.

A solution of (2*S*)-*N*-[(2*S*)-2-chloropropionyl]-2-methylproline **25** (0.16 g, 0.73 mmol) and *N*,*N*-diisopropylethylamine (0.22 cm^3, 1.26 mmol) in dry dichloromethane (6 cm^3) was treated with BOP-Cl (0.19 g, 0.75 mmol) and the resulting suspension stirred at 0 °C for 35 min. A solution of amine trifluoroacetate **31** (0.17 g, 0.36 mmol) in dry dichloromethane (2 cm^3) was then added. The mixture was stirred at 0–5 °C for 9 h, then at room temperature for 14 h. The solution was diluted with dichloromethane to ~20 cm^3 and washed with aqueous HCl (0.5 mol dm^{-3}, 2 × 5 cm^3), aqueous sodium hydrogen carbonate (5%, 2 × 5 cm^3) and brine (5 cm^3) then dried ($MgSO_4$). The solvent was removed under reduced pressure, and the residual oil purified by column chromatography using ethyl acetate as the eluent to afford the dioxopiperazine **38** as a white solid (50 mg, 49%); R_f 0.25 (ethyl acetate) (Found: C, 51.55; H, 6.55; N, 9.2. $C_{13}H_{18}N_2O_3S{\cdot}H_2O$ requires: C, 52.0; H, 6.7; N, 9.35%); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 2982 (CH), 1695 (thioester CO), 1664 (amide CO) and 1428 (CH def.); δ_H(300 MHz; C^2HCl_3) 1.41 [3 H, s, $\beta CH_3(Pro^1)$], 1.93–2.29 [5 H, m, $\beta CH_2(Pro^1)$, $\frac{1}{2}\beta CH_2(Pro^2)$ and $\gamma CH_2(Pro^1)$], 2.30 (3 H, s, $SCOCH_3$), 2.67 [1 H, dt, J_1 12.9, J_2 6.9, $\frac{1}{2}\beta CH_2(Pro^2)$], 3.42 [1 H, dd, J_1 12.0, J_2 8.1, $\frac{1}{2}\delta CH_2(Pro^2)$], 3.48–3.58 [2 H, m, $\delta CH_2(Pro^1)$], 3.84–3.89 [1 H, m, $\gamma CH(Pro^2)$], 3.99 [1 H, dd, J_1 10.2, J_2 6.6, $\frac{1}{2}\delta CH_2(Pro^2)$] and 4.30 [1 H, dd, J_1 9.3, J_2 7.2, $\alpha CH(Pro^2)$]; δ_C(75.44 MHz; C^2HCl_3) 21.56 [$\beta CH_3(Pro^1)$], 23.20 [$\gamma CH_2(Pro^1)$], 30.84 ($SCOCH_3$), 34.34 [$\beta CH_2(Pro^2)$], 35.57 [$\beta CH_2(Pro^1)$], 38.33 [$\gamma CH(Pro^2)$], 45.50 [$\delta CH_2(Pro^1)$], 51.22 [$\delta CH_2(Pro^2)$], 59.61 [$\alpha CH(Pro^2)$], 66.59 [$\alpha C(Pro^1)$], 165.60 and 169.89 (2 × amide CO) and 195.44 (thioacetate CO); *m*/*z* (CI) 283 (100%, $[M + H]^+$), 241 (8, $[M - COCH_3 + 2H]^+$) and 206 (9, $[M - COCH_3 - H_2S + H]^+$).

Methyl (2*S*)-*N*-{(2*S*)-*N*-[(2*S*)-2-chloropropionyl]-2-methylprolyl}-2-methylprolinate 39 and (2*S*)-*N*-[(2*S*)-2-chloropropionyl]-2-methylproline 1*H*-7-aza-1,2,3-benzotriazolyl ester 40

A solution of (2*S*)-*N*-[(2*S*)-2-chloropropionyl]-2-methylproline **25** (68 mg, 0.31 mmol), methyl (2*S*)-2-methylprolinate hydrochloride **23** (37 mg, 0.21 mmol) and *N*,*N*-diisopropylethylamine (110 mm^3, 0.63 mmol) in dry DMF (1 cm^3) under nitrogen at 0 °C was treated with HATU (120 mg, 0.32 mmol) and the mixture was stirred at 0 °C for 4 h and then at room temperature for 40 h. The solution was diluted to 30 cm^3 with ethyl acetate and then washed with aqueous HCl (0.5 mol dm^{-3}, 2 × 10 cm^3), aqueous sodium hydrogen carbonate (5%, 2 × 10 cm^3) and brine (10 cm^3). The organic phase was dried ($MgSO_4$), and the solvent was removed under reduced pressure. The residual oil was purified by column chromatography using ethyl acetate–light petroleum (3:2) as the eluent to give two products, the peptide **39** as a white solid (21 mg, 30%) and the 7-azabenzotriazolyl ester **40** as a colourless gum (38 mg, 37%).

Data for **39**: mp 124–126 °C; R_f 0.45 (ethyl acetate) (HRMS: found $[M + H]^+$, 345.1586. $C_{16}H_{25}{}^{35}ClN_2O_4$ requires 345.1581); $[\alpha]_D$ −42.5 (*c* 0.8 in MeOH); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 2985 (CH), 1737 (ester CO), 1666 (amide CO), 1636 (amide CO), 1402 (CH def.) and 1193 (C–O); δ_H(300 MHz; C^2HCl_3) (2 conformations, *A* and *B*) 1.51 [*A*, 3 H, d, *J* 6.6, $\beta CH_3(Chp)$], 1.53 [3 H, s, $\beta CH_3(Pro)$], 1.61 [*B*, 3 H, d, *J* 6.6, $\beta CH_3(Chp)$], 1.64 [3 H, s, $\beta CH_3(Pro)$], 1.75–2.21 [8 H, m, 2 × $\beta CH_2(Pro)$ and 2 × γCH_2], 3.38–3.49 (1 H, m, $\frac{1}{2}\delta CH_2$), 3.60–3.68 (2 H, m, δCH_2), 3.70 (*B*, 3 H, s, CO_2CH_3), 3.74 (*A*, 3 H, s, CO_2CH_3), 3.87–3.95 (1 H, m, $\frac{1}{2}\delta CH_2$), 4.35 [*A*, 1 H, q, *J* 6.6, $\alpha CH(Chp)$] and 4.45 [*B*, 1 H, q, *J* 6.6, $\alpha CH(Chp)$]; δ_C(75.44 MHz; C^2HCl_3) 20.68, 21.79 and 22.70 [$\beta CH_3(Chp)$ and 2 × $\beta CH_3(Pro)$], 23.67 and 25.28 (2 × γCH_2), 37.39 and 37.69 [2 × $\beta CH_2(Pro)$], 47.89 and 49.06 (2 × δCH_2), 52.18 [$\alpha CH(Chp)$], 52.82 (CO_2CH_3), 68.46 [2 × $\alpha C(Pro)$], 167.55 [CO(Chp)], 170.83 (amide CO) and 175.29 (ester CO); *m*/*z* (CI) 347 and 345 (23 and 70%, chlorine isotopes, $[M + H]^+$), 315 and 313 (33 and 97, chlorine isotopes, $[M - 2CH_3 + H]^+$), 204 and 202 [32 and 100, chlorine isotopes, $ClCH(CH_3)CONC_4H_6(CH_3)CO^+$], 176 and 174 [26 and 82, chlorine isotopes, $ClCH(CH_3)CONC_4H_6CH_3^+$].

Data for **40** (Found: C, 47.45; H, 5.1; N, 19.55. $C_{14}H_{16}ClNO_3{\cdot}H_2O$ requires C, 47.25; H, 5.1; N, 19.7%); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 2987 (CH), 1750 (ester CO) and 1661 (amide CO); δ_H(300 MHz; C^2HCl_3) 1.73 [3 H, d, *J* 6.6, $\beta CH_3(Chp)$], 1.81 [3 H, s, $\beta CH_3(Pro)$], 2.20–2.35 [3 H, m, $\frac{1}{2}\beta CH_2(Pro)$ and γCH_2], 2.71–2.81 [1 H, m, $\frac{1}{2}\beta CH_2(Pro)$], 3.75–3.83 (1 H, m, $\frac{1}{2}\delta CH_2$), 3.95–4.03 (1 H, m, $\frac{1}{2}\delta CH_2$), 4.52 [1 H, q, *J* 6.6, $\alpha CH(Chp)$], 7.40 (1 H, dd, J_1 6.5, J_2 4.4, Ar-CH 5), 8.39 (1 H, dd, J_1 8.3, J_2 1.4, Ar-CH 6) and 8.71 (1 H, dd, J_1 4.4, J_2 1.4, Ar-CH 4); δ_C(75.44 MHz; C^2HCl_3) 20.64 [$\beta CH_3(Chp)$], 21.34 [$\beta CH_3(Pro)$], 24.86 (γCH_2), 38.80 [$\beta CH_2(Pro)$], 48.15 (δCH_2), 51.60 [$\alpha CH(Chp)$], 66.00 [$\alpha C(Pro)$], 121.32 (Ar-C 5), 129.90 (Ar-C 4), 135.51 and 141.18 (2 × quaternary Ar-C), 152.39 (Ar-C 6), 168.14 (amide CO) and 170.02 (ester CO).

(2*S*,4*R*)-*N*-[(2*S*)-*N*-(*tert*-Butoxycarbonyl)prolyl]-4-hydroxyproline methylamide 42

Methyl ester **14** (0.63 g, 1.84 mmol) was dissolved in a saturated solution of methylamine in methanol (35 cm^3) at 0 °C. The flask was stoppered and allowed to warm to room temperature, and then the reaction was allowed to proceed for 24 h. The flask was cooled to 0 °C before the stopper was removed, and then the excess methylamine was removed in a stream of nitrogen over 30 min. The solvent was removed under reduced pressure to afford the methylamide **42** as a white foam (0.63 g, 100%), mp 187–188 °C (Found: C, 56.6; H, 8.25; N, 12.45. $C_{16}H_{27}N_3O_5$ requires C, 56.3; H, 7.95; N, 12.3%); $[\alpha]_D$ −99.4 (*c* 1.1 in MeOH); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3448 (NH), 3342 (NH), 2981 (CH), 1677 (CO) and 1164 (C–O); δ_H(200 MHz; C^2HCl_3) (3 conformations, *ct*, *tt* and *tc*) 1.20 [*ct*, 9 H, $C(CH_3)_3$], 1.24 [*tt* and *tc*, 9 H, $C(CH_3)_3$], 1.63–1.91 [4 H, m, $\frac{1}{2}\beta CH_2(Pro)$, $\frac{1}{2}\beta CH_2(Hyp)$ and $\gamma CH_2(Pro)$], 1.95–2.09 [1 H, m, $\frac{1}{2}\beta CH_2(Hyp)$], 2.54 (3 H, d, *J* 4.4, $NHCH_3$), 3.20–3.62 [4 H, m, $\delta CH_2(Pro)$ and $\delta CH_2(Hyp)$], 4.22–4.43 [3 H, m, $\alpha CH(Pro)$, $\alpha CH(Hyp)$ and $\gamma CH(Hyp)$], 7.36 (*tt* and *ct*, 1 H, q, *J* 4.8, $NHCH_3$) and 7.76 (*tc*, 1 H, q, *J* 4.8, $NHCH_3$); δ_C(50.31 MHz; C^2HCl_3) 23.75 [*ct*, $\gamma CH_2(Pro)$], 24.43 [*tt*, $\gamma CH_2(Pro)$], 24.76 [*tc*, $\gamma CH_2(Pro)$], 26.40 (*tt*, $NHCH_3$), 26.47 (*ct*, $NHCH_3$), 28.31 [*tc*, $C(CH_3)_3$], 28.36 [*ct*, $C(CH_3)_3$], 28.57 [*tt*, $C(CH_3)_3$], 29.34 [*tt*, $\beta CH_2(Pro)$], 29.52 [*tc*, $\beta CH_2(Pro)$], 30.24 [*ct*, $\beta CH_2(Pro)$], 36.64 [*tt* and *ct*, $\beta CH_2(Hyp)$], 37.50 [*tc*, $\beta CH_2(Hyp)$], 46.79 [*ct*, $\delta CH_2(Pro)$], 47.17 [*tt*, $\delta CH_2(Pro)$], 47.29

[*tc*, δCH$_2$(Pro)], 54.26 [*tc*, δCH$_2$(Hyp)], 54.57 [*ct*, δCH$_2$(Hyp)], 55.33 [*tt*, δCH$_2$(Hyp)], 57.82 (*tc*, αCH), 58.31 (*ct*, αCH), 58.60 (*tt*, αCH), 59.26 (*tt*, αCH), 60.00 (*ct*, αCH), 67.87 [*tc*, γCH-(Hyp)], 69.97 [*ct*, γCH(Hyp)], 70.56 [*tt*, γCH(Hyp)], 79.88 [*ct*, *C*(CH$_3$)$_3$], 80.26 [*tt* and *tc*, *C*(CH$_3$)$_3$], 154.00 (*ct*, urethane CO), 155.02 (*tt* and *tc*, urethane CO), 172.04 (*tt*, amide CO), 172.10 (*ct*, amide CO), 172.40 (*ct*, amide CO), 172.71 (*tt*, amide CO) and 172.92 (*tc*, amide CO); *m*/*z* (CI) 342 (98%, [M + H]$^+$), 286 (100, [M − C$_4$H$_8$ + H]$^+$) and 242 (83, [M − CO$_2$C$_4$H$_8$ + H]$^+$).

(2*S*,4*S*)-*N*-[(2*S*)-*N*-(*tert*-Butoxycarbonyl)prolyl]-4-(acetylthio)-proline methylamide 43

Diisopropyl azodicarboxylate (0.33 g, 1.6 mmol) was added to a solution of triphenylphosphine (0.44 g, 1.7 mmol) in THF (5 cm^3) at 0 °C. The resulting suspension was stirred at 0 °C for 30 min, after which a solution of alcohol **42** (0.50 g, 1.5 mmol) and thioacetic acid (0.13 cm^3, 1.8 mmol) in THF (5 cm^3) was added dropwise. The mixture was stirred at 0 °C for 2.5 h, then at room temperature overnight. The solvent was removed under reduced pressure and the residual oil purified using column chromatography with light petroleum–ethyl acetate (2 : 1) as the eluent to afford the thioacetate **43** as a colourless oil (0.29 g, 50%); R_f 0.10 (ethyl acetate) (HRMS: found [M + H]$^+$, 400.1898. C$_{18}$H$_{30}$N$_3$O$_5$S requires 400.1906); ν_{max}(CH$_2$Cl$_2$)/cm^{-1} 3346 (NH), 2980 (CH), 1688 (urethane and thioester CO), 1666 (amide CO) and 1166 (C–O); δ_H(300 MHz; C^{2}HCl$_3$) (3 conformations, *ct*, *tt* and *tc*) 1.31 [*ct*, 9 H, s, C(CH$_3$)$_3$], 1.34 [*tt* and *tc*, 9 H, s, C(CH$_3$)$_3$], 1.71–2.52 [6 H, m, βCH$_2$(Pro^1), βCH$_2$(Pro^2) and γCH$_2$(Pro^1)], 2.20 (*tc*, 3 H, s, SCOCH$_3$), 2.22 (*tt*, 3 H, s, SCOCH$_3$), 2.24 (*ct*, 3 H, s, SCOCH$_3$), 2.65 (*tt* and *ct*, 3 H, d, *J* 4.7, NHC*H*$_3$), 2.71 (*tc*, 3 H, *J* 4.7, NHC*H*$_3$), 3.18–3.46 [3 H, m, δCH$_2$(Pro^1) and $\frac{1}{2}$δCH$_2$(Pro^2)], 3.81–4.17 [2 H, m, γCH(Pro^2) and $\frac{1}{2}$δCH$_2$(Pro^2)], 4.21–4.53 [2 H, m, 2 × αCH(Pro)], 7.10 (1 H, *tt* and *ct*, q, *J* 4.4, N*H*CH$_3$) and 8.02 (1 H, *tc*, q, *J* 4.4, N*H*CH$_3$); δ_C(75.44 MHz; C^{2}HCl$_3$) 23.75, 24.50 and 24.80 [γCH$_2$(Pro^1)], 26.45 and 26.65 (NHCH$_3$), 28.57 and 29.31 [C(*C*H$_3$)$_3$], 29.55, 30.62 and 30.65 [βCH$_2$(Pro^1)], 32.37 and 32.86 (SCO*C*H$_3$), 37.57 [*tt* and *ct*, βCH$_2$(Pro^2)], 38.84 [*tc*, βCH$_2$(Pro^2)], 40.09 [*tc*, γCH(Pro^2)], 40.28 [*tt* and *ct*, γCH(Pro^2)], 46.79, 46.96 and 42.48 [δCH$_2$(Pro^1)], 53.57 and 53.56 [δCH$_2$(Pro^2)], 58.11, 58.17, 59.23, 59.67, 60.02 and 60.48 [2 × αCH(Pro)], 79.87, 80.13 and 80.40 [*C*(CH$_3$)$_3$], 153.99, 154.96 and 155.13 (urethane CO), 171.00, 171.50, 171.74, 172.12, 172.16 and 173.12 (2 × amide CO), 195.27, 195.77 and 196.03 (thioester CO); *m*/*z* (CI) 400 (82%, [M + H]$^+$), 344 (63, [M − C$_4$H$_8$ + H]$^+$), 300 (100, [M − CO$_2$-C$_4$H$_8$ + H]$^+$), 170 (21, C$_4$H$_9$OCONC$_4$H$_7^+$), 114 (21, [NC$_4$H$_8$-CONH + H]$^+$) and 70 (22, C$_4$H$_8$N$^+$).

(2*S*,4*S*)-*N*-[(2*S*)-*N*-{(2*S*)-*N*-[(2*S*)-2-Chloropropionyl]prolyl}-prolyl]-4-(acetylthio)proline methylamide 45

A solution of thioester **43** (0.38 g, 0.75 mmol) in dichloromethane (7 cm^3) at 0 °C was treated with trifluoroacetic acid (3.5 cm^3) and the solution stirred at 0 °C for 2.5 h. The solvents were removed under reduced pressure to yield the trifluoroacetate salt **44** as a colourless oil.

A solution of (2*S*)-*N*-[(2*S*)-2-chloropropionyl]proline (0.23 g, 1.1 mmol) and diisopropylethylamine (0.50 cm^3, 2.9 mmol) in dichloromethane (15 cm^3) was treated with BOP-Cl (0.29 g, 1.1 mmol) at 0 °C under nitrogen. After 20 min, a solution of amine trifluoroacetate **44** in dichloromethane (5 cm^3) was added and the mixture was stirred at 0–5 °C for 2 h and then at room temperature for 2 days. The solution was diluted to ~40 cm^3 with dichloromethane and was then washed with aqueous HCl (0.5 mol dm^{-3}, 2 × 25 cm^3), aqueous sodium hydrogen carbonate (5%, 2 × 25 cm^3) and brine (25 cm^3). The organic phase was dried (MgSO$_4$), and the solvent was removed under reduced pressure to afford the thioester **45** as a colourless oil (0.31 g, 69%) (HRMS: found [M + H]$^+$, 487.1788. C$_{21}$H$_{32}$^{35}ClN$_4$O$_5$S requires 487.1782); ν_{max}(CH$_2$Cl$_2$)/cm^{-1} 3331 (NH), 1690 (thioester and secondary amide CO), 1662 (tertiary amide CO) and 1433 (CH def.); δ_H(300 MHz; C^{2}HCl$_3$) (2 conformations, *ttt* and *ttc*) 1.51 and 1.53 [3 H, d, *J* 6.3, βCH$_3$(Chp^1)], 1.72–2.18 [9 H, m, βCH$_2$(Pro^2), βCH$_2$(Pro^3), $\frac{1}{2}$βCH$_2$(Pro^4), γCH$_2$(Pro^2) and γCH$_2$(Pro^3)], 2.19 and 2.21 (3 H, s, SCOCH$_3$), 2.33–2.45 [1 H, m, $\frac{1}{2}$βCH$_2$(Pro^4)], 2.63 and 2.65 (3 H, d, *J* 5.5, NHC*H*$_3$), 3.25–3.35 [1 H, m, $\frac{1}{2}$δCH$_2$(Pro^4)], 3.46–3.60, 3.61–3.81 [4 H, m, δCH$_2$-(Pro^2) and δCH$_2$(Pro^3)], 3.89–3.97 [*ttc*, 1H, m, γCH(Pro^4)], 4.05 [*ttt*, 1 H, dd, J_1 7.2, J_2 13.2, γCH(Pro^4)], 4.21–4.51 and 4.52–4.62 [4 H, m, 3 × αCH(Pro) and $\frac{1}{2}$δCH$_2$(Pro^4)], 6.81 (*ttt*, N*H*CH$_3$) and 8.11 (*ttc*, N*H*CH$_3$); δ_C(75.44 MHz; C^{2}HCl$_3$) 20.62 and 20.77 [βCH$_3$(Chp^1)], 24.96, 25.11 and 25.43 [γCH$_2$(Pro^2) and γCH$_2$(Pro^3)], 26.48 and 26.65 (NHCH$_3$), 28.05, 28.46 and 28.51 [βCH$_2$(Pro^2) and βCH$_2$(Pro^3)], 30.62 and 30.66 (SCOCH$_3$), 33.15 [*ttt*, βCH$_2$(Pro^4)], 37.25 [*ttc*, βCH$_2$(Pro^4)], 38.84 [*ttc*, γCH(Pro^4)], 40.10 [*ttt*, γCH(Pro^4)], 47.19, 47.43, 47.46 and 47.55 [δCH$_2$(Pro^2) and δCH$_2$(Pro^3)], 51.31 and 51.44 [αCH(Chp^1)], 53.44 and 53.52 [δCH$_2$(Pro^4)], 58.26, 58.52, 58.74, 58.87, 59.78 and 60.46 [3 × αCH(Pro)], 167.84 and 167.91 [CO(Chp^1)], 170.58, 170.95, 171.60 and 171.91 (3 × amide CO), 195.27 and 195.58 (thioester CO); *m*/*z* (CI) 489 and 487 (12 and 29%, chlorine isotopes, [M + H]$^+$), 396 {77, [M − ClCH-(CH$_3$)CO + H]$^+$}, 354 {11, [M − ClCH(CH$_3$)CO − CH$_3$CO + 2H]$^+$}, 322 {11, [M − ClCH(CH$_3$)CO − CH$_3$CO − S + 2H]$^+$} and 279 (100, [CH$_3$CHCONC$_4$H$_7$CONC$_4$H$_6$CONHCH$_3$]$^+$).

(2*S*,4*S*)-*N*-[(2*S*)-*N*-{(2*S*)-*N*-[(2*S*)-2-Chloropropionyl]prolyl}-prolyl]-4-mercaptoproline methylamide 41

This compound was prepared in a manner identical with that for the thiol **7**, using thioester **45** (0.25 g, 0.51 mmol) to afford thiol **41** as a colourless oil (0.15 g, 66%) (HRMS: found [M + H]$^+$, 445.1676. C$_{19}$H$_{30}$^{35}ClN$_4$O$_4$S requires 445.1676); [*a*]$_D$ −103.7 (*c* 0.6 in MeOH); ν_{max}(CH$_2$Cl$_2$)/cm^{-1} 3323 (NH), 2985 (CH), 1657 (CO) and 1435 (CH def.); δ_H(300 MHz; C^{2}HCl$_3$) (2 conformations, *ttt* and *ttc*) 1.63 [*ttc*, 3 H, d, *J* 6.6, βCH$_3$(Chp^1)], 1.65 [*ttt*, 3 H, d, *J* 6.6, βCH$_3$(Chp^1)], 1.80 (1 H, d, *J* 5.8, SH), 1.91–2.32 [9 H, m, βCH$_2$(Pro^2), βCH$_2$(Pro^3), γCH$_2$(Pro^2), γCH$_2$-(Pro^3) and *ttt*, $\frac{1}{2}$βCH$_2$(Pro^4)], 2.47–2.57 [1 H, m, $\frac{1}{2}$βCH$_2$(Pro^4)], 2.67–2.77 [*ttt*, 1 H, m, $\frac{1}{2}$βCH$_2$(Pro^4)], 2.74 (*ttc*, 3 H, d, *J* 4.7, NHC*H*$_3$), 2.79 (*ttt*, 3 H, d, *J* 4.7, NHC*H*$_3$), 3.32–3.41 [2 H, m, $\frac{1}{2}$δCH$_2$(Pro^4) and *ttc*, γCH(Pro^4)], 3.43–3.51 [*ttc*, 1 H, γCH-(Pro^4)], 3.59–3.70 [2 H, m, $\frac{1}{2}$δCH$_2$(Pro^2) and $\frac{1}{2}$δCH$_2$(Pro^3)], 3.77–3.94 [2 H, m, $\frac{1}{2}$δCH$_2$(Pro^2) and $\frac{1}{2}$δCH$_2$(Pro^3)], 4.16 [*ttc*, 1 H, dd, J_1 12.7, J_2 6.7, $\frac{1}{2}$δCH$_2$(Pro^4)], 4.25–4.35 [2 H, m, *ttt*, $\frac{1}{2}$δCH$_2$(Pro^4) and *ttc*, αCH(Pro^4)], 4.38–4.57 [2 H, αCH(Chp^1) and *ttt*, αCH(Pro^4)], 4.64–4.72 [2 H, m, αCH(Pro^2) and αCH(Pro^3)], 6.50 (*ttt*, 1 H, d, *J* 4.1, N*H*CH$_3$) and 8.13 (*ttc*, N*H*CH$_3$); δ_C(75.44 MHz; C^{2}HCl$_3$) 20.75 and 20.91 [βCH$_3$(Chp^1)], 25.12, 25.33, 25.40 and 25.61 [γCH$_2$(Pro^2) and γCH$_2$(Pro^3)], 26.79 (NHCH$_3$), 28.28, 28.64, 28.71 and 28.80 [βCH$_2$(Pro^2) and βCH$_2$(Pro^3)], 34.26 [*ttt*, βCH$_2$(Pro^4)], 35.94 [*ttc*, βCH$_2$(Pro^4)], 38.49 [*ttc*, γCH(Pro^4)], 41.52 [*ttt*, γCH(Pro^4)], 47.55 and 47.69 [δCH$_2$(Pro^2) and δCH$_2$(Pro^3)], 51.48 [*ttt*, αCH(Chp^1)], 51.59 [*ttc*, αCH(Chp^1)], 56.21 [*ttc*, δCH$_2$(Pro^4)], 57.26 [*ttt*, δCH$_2$-(Pro^4)], 58.55, 58.81, 59.02, 59.11, 60.60 and 60.73 [3 × αCH-(Pro)], 168.33 [CO(Chp^1)], 171.11, 171.54, 171.70 and 172.17 (3 × amide CO); δ_H(300 MHz; ^{2}H$_2$O) 1.45 [3 H, d, *J* 6.6, βCH$_3$-(Chp^1)], 1.61–1.98 [9 H, m, βCH$_2$(Pro^2), βCH$_2$(Pro^3), $\frac{1}{2}$βCH$_2$-(Pro^4), γCH$_2$(Pro^2) and γCH$_2$(Pro^3)], 2.17–2.30 [1 H, m, $\frac{1}{2}$βCH$_2$(Pro^4)], 2.60 (3 H, s, NHC*H*$_3$), 3.30–3.41 [2 H, m, γCH(Pro^4) and $\frac{1}{2}$δCH$_2$(Pro^4)], 3.47–3.57 and 3.64–3.78 [4 H, m, δCH$_2$(Pro^2) and δCH$_2$(Pro^3)], 4.16–4.25 [2 H, m, αCH(Pro) and $\frac{1}{2}$δCH$_2$(Pro^4)], 4.57–4.63 [2 H, m, 2 × αCH(Pro)] and 4.74 [1 H, q, *J* 6.6, αCH(Chp^1)]; δ_C(75.44 MHz; ^{2}H$_2$O) 19.86 [βCH$_3$-(Chp^1)], 24.35, 24.43 [γCH$_2$(Pro^2) and γCH$_2$(Pro^3)], 25.73 (NHCH$_3$), 27.95, 28.05 [βCH$_2$(Pro^2) and βCH$_2$(Pro^3)], 34.40 [βCH$_2$(Pro^4)], 39.09 [γCH(Pro^4)], 47.54, 47.92 [δCH$_2$(Pro^2) and δCH$_2$(Pro^3)], 51.49 [αCH(Chp^1)], 56.05 [δCH$_2$(Pro^4)], 58.59, 59.05, 60.78 [3 × αCH(Pro)], 170.43 [CO(Chp^1)], 171.70, 172.00

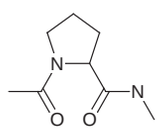
and 173.67 (3 × amide CO); *m/z* (CI) 447 and 445 (8 and 18%, chlorine isotopes, $[M + H]^+$), 278 (82, $CH_2CHCONC_4H_7CONC_4H_6CONHCH_3^+$), 234 (17, $CHCONC_4H_7CONC_4H_6CO^+$), 208 and 206 {33 and 100, chlorine isotopes, $[ClCH(CH_3)CONC_4H_7CO_2H + H]^+$}.

Methods for computation

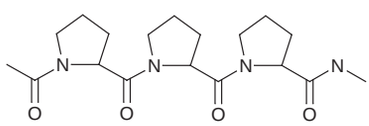
Conformational search and determination of heats of formation for cyclic, proline-containing, putative helix-caps. All optimisations were carried out using the AMBER forcefield and the Discover program from MSI. A distance dependent relative permittivity of 4*r* was chosen and atomic charges were determined by Discover.

Conformational search. To be sure of determining all possible minima for a molecule, it is necessary to identify all possible minima and use these as starting points for geometry optimisation. For linear molecules, this is a simple procedure as all available rotamers for the rotatable bonds can be identified by inspection (staggered conformations for single bonds, eclipsed for double bonds *etc*.) but can be problematic for cyclic systems.

One-dimensional Ramachandran plots for MeCO-Pro-NMe, **46**, were determined for both *cis*- and *trans*-amide bonds and



46



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showed ψ-angle minima at 165 and −39° for *cis*-amide ($\omega = 0°$) and at 66 and −23° for *trans*-amide ($\omega = 180°$).

Starting points for a linear triproline, MeCO-Pro-Pro-Pro-NMe **47**, were generated for each possible combination of ψ and ω angles (64 in all) and minimised. 60 distinct minima (final ψ and ω values varying by >1°) were retained and used as the starting points for optimisation of compound **4**. A sulfur atom was added to the C4 of the *C*-terminal Pro and a loop closing bond formed between this sulfur and the *N*-terminal methyl carbon. Inevitably this bond is extremely strained in most starting conformations, but relaxes rapidly on optimisation. As the loop closing bond is formed between two achiral centres, there is no question of stereochemical inversion at the ends of the loop closing bond. Minimisation of these 60 cyclic starting points yielded 34 distinct (>1° variation in backbone torsion angles) conformations, though these could be clearly grouped into a smaller number of families based on visible similarity. At typical experimental temperatures, closely related minima such as conformers ***O****, ***S**** and ***U**** would form a single population as the molecule would possess sufficient energy to cross the rather small barrier between these related minima.

The 34 distinct minima, labelled *A* through *Z* and *AA* through *AH*, for compound **4** were used as starting points for determination of the conformations of the two methylated derivatives **6** and **8**. Starting points for the α-methylproline derivatives, **19** and **29**, were obtained by replacing the α-proton of the appropriate proline residues and retaining the macrocycle conformation from the optimised compound **6**. In this latter case, we were only concerned with the relative energies of the nucleating and non-nucleating forms of the all-*trans* isomer, so optimisations based on conformations ***M*** and ***S**** only were considered.

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