Design, construction and properties of peptide *N*-terminal cap templates devised to initiate α-helices. Part 3.† Caps derived from *N*-[(2*S*)-2-chloropropionyl]-(2*S*)-Pro-(2*R*)-Ala-(2*S*,4*S*)-4-thioPro-OMe

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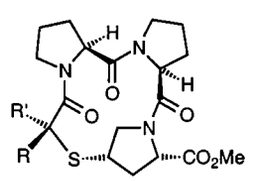
The construction of a 12-membered macrocyclic template capable of entraining attached peptides in helical conformations from acyclic *N*-[(2*S*)-2-chloropropionyl]-(2*S*)-Pro-(2*S*)-Pro-(2*S*,4*S*)-4-thioPro-OMe precursors has been severely hampered by the problem of simultaneously aligning carboxamide dipoles in the transition state for cyclisation. Previously we provided a detailed conformational analysis of the system and tested two methods for forcing the acyclic precursor into the macrocyclic conformation required for helix initiation. First, the destabilisation of unwanted conformations in the transition state for cyclisation, and second, the stabilisation of the favoured transition state structure through the introduction of a hydrogen-bonding interaction. Both strategies were unsuccessful. A third strategy based upon removing the requirement for all of the carbonyl dipoles to align in the transition state at the same time was also tested and the results are presented here. The relaxation of the highly restrained C^{α}–N bond torsion for Pro^{3} in the acyclic precursor, through its substitution for a (2*R*)-alanine residue, effectively decouples the motion of the second carboxamide group from the C^{α}–N bond torsion and allows the second carboxamide group to rotate. This rotation allows a helical conformation to develop in the transition state to the macrocycle without the need to align all of the carboxamide dipoles and results in successful cyclisation to give template structures of the all *trans* (*ttt*) form. Derivatives of the template were prepared by extending the *C*-terminus and these were characterised by NMR spectroscopy and restrained simulated annealing. In deuterochloroform solution at low temperature, separate sets of NMR signals were observed for two rapidly interconverting helical conformational isomers of the thioether macrocycle based on (2*R*)-*N*-propionyl-(2*S*)-Pro-(2*R*)-Ala-(2*S*)-Pro which possessed an appended trialkylammonium ion. The free energy of activation for the transition ($\Delta G_c^{\ddagger}$) was 48 kJ mol^{-1}. A similar time-averaged conformation was also observed in aqueous solution. At −80 °C in dichloromethane the rate of conformational exchange was slowed sufficiently to obtain resonance assignments and NOE data separately for each isomer. In the minor isomer (40%), the four carbonyl oxygen hydrogen-bond acceptors of the template are aligned in an α-helical conformation and in the major conformer the Pro^{2} carbonyl dipole was anti-aligned with the other three dipoles. Thus, the conformers differ in the orientation of one carbonyl group. Molecular modelling calculations showed that the minor isomer was stabilised by coulombic interactions between the trialkylammonium salt and the carbonyl group dipole moments.

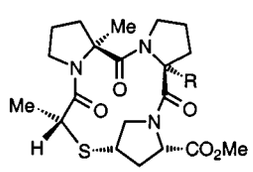
Introduction

It is well established that local electrostatic fields have a profound effect upon reactivity in proteins. However, it is not currently possible to quantify the influence of helix polarity upon the pK_a of side chain functionalities. Isolated short peptides tend towards random coils,[1] and in order to increase the probability of secondary structure initiation the folded peptides have required stabilisation in environments with an uncertain dielectric constant. Therefore, access to isolated solvated helices will be useful in constructing computational forcefields for modelling chemical reactivity in protein reaction centres.

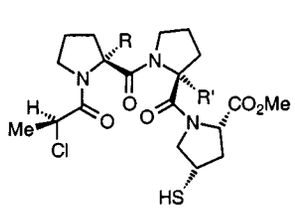
The design of stable folded polypeptides has been the focus of many research groups in recent years,[2,3] and much of the work has been reviewed in the previous articles.[4,5] Of particular relevance to our own studies directed towards the construction of an α-helical cap template was the report of a conformationally constrained triproline macrocycle **1** by Kemp.[6,7] This structure was extremely similar to one of our own targets **2**, and was designed to position four aligned carbonyl oxygen H-bond acceptors below the plane of the macrocycle pointing towards the *C*-terminal end of the helix. For nucleating α-helices the cap



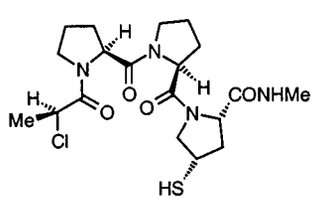
1; R = H, R' = H
2; R = Me, R' = H
3; R = H, R' = Me



4; R = H
5; R = Me



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



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† For Part 2, see ref. 5.

1 must cyclise in a conformation where the carbonyl dipoles are aligned and all three of the amide bonds exist as *trans* rotomers, the so called '*ttt*' state. Macrocycle **1**, however, adopts the *ctt* and *cct* conformers in aqueous solution and does not possess useful helix-initiating properties. We reasoned that in order to form stable isolated α-helices the cap design must incorporate factors to destabilise the potential alternative conformations or stabilise the required ones. In the previous article we undertook a detailed analysis of the 12-membered macrocyclic template system **1** and several of its derivatives (**2**–**5**) and tested two potentially useful methods for coaxing the acyclic precursors into an α-helical conformation. First, the destabilisation of unwanted conformations in the transition state for cyclisation was achieved through the introduction of steric bulk at the C-2 positions of selected residues in the precursors (**6**–**8**). This strategy was partially successful in preventing the formation of unwanted macrocyclic products containing *cis*-amide bonds, but was unsuccessful in that dimerisation rather than cyclisation to the 12-membered macrocycles (*e.g.* **2**) occurred. Second, the strategy of stabilising the favoured transition state structure was tested through the introduction of a potential 13-membered hydrogen-bonding interaction of the type commonly found in α-helices. Preparatively, this necessitated altering the fourth carbonyl group from a carboxylic ester to a carboxamide in order to provide a hydrogen bond donor at the *C*-terminus of the template **3**. This strategy also was unsuccessful because the acyclic precursor **9** was stabilised in a conformation in which the thiol group nucleophile was held away from the electrophilic α-chloroacylamide group. Thus, the hydrogen bond stabilised the ground state such that any beneficial hydrogen bonding interactions with the first carbonyl group in the transition state for cyclisation were largely cancelled.

A third strategy, based upon removing the requirement for all of the carbonyl dipoles to align in the transition state at the same time, was also tested and the results are presented here. The relaxation of the highly restrained C^{α}–N bond torsion for Pro[3] in the acyclic precursor **6**, through its substitution for a (2*R*)-alanine residue, effectively decouples the motion of the second carboxamide group from the C^{α}–N bond torsion and allows the second carboxamide group to rotate. This leads to the successful cyclisation of modified acyclic precursor **10** to template structures (*e.g.* **11**) possessing *ttt* amide bond stereochemistry. We now report on the synthesis and characterisation of these modified proline-based macrocycles, and on the properties of analogues extended at the *C*-terminus.

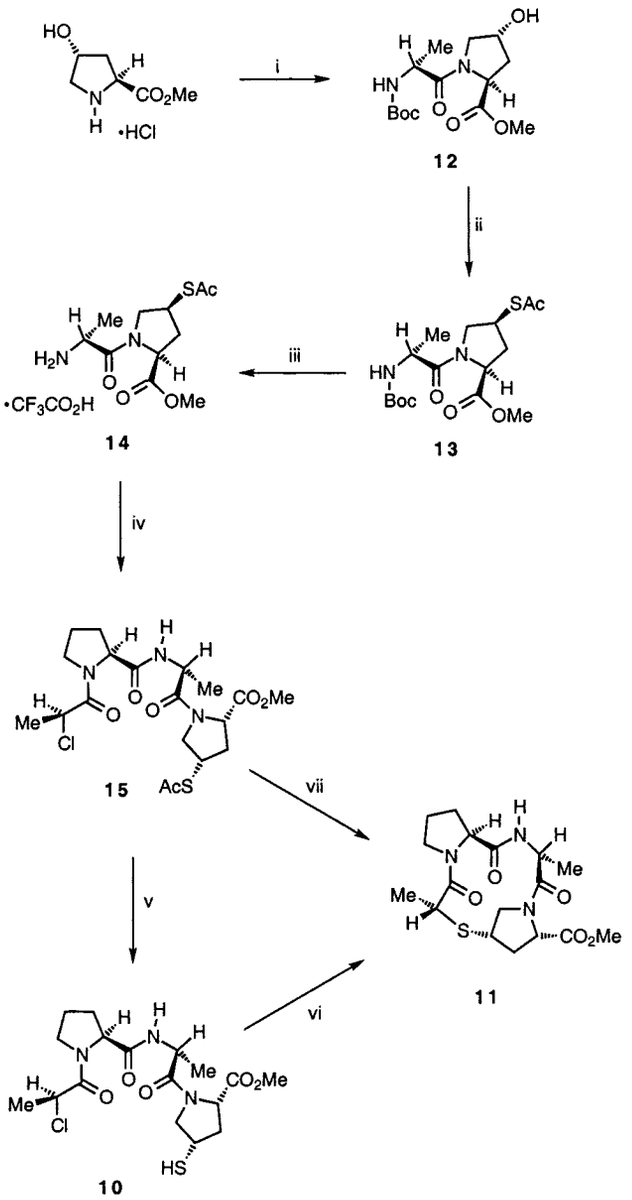
Results and discussion

The synthesis of compound **10** followed analogous routes to those developed for the preparation of the acyclic precursor **6**.[5] Thus, Boc-(2*R*)–Ala–OH was coupled with (2*S*,4*R*)-4-hydroxyproline methyl ester using BOP-Cl to give protected dipeptide **12** in 55% yield, Scheme 1. Mitsunobu inversion gave the thioacetate **13**, and removal of the Boc group gave amine trifluoroacetate salt **14** cleanly in 92% yield over the 2 steps.

All of these compounds displayed the expected analytical properties and NMR spectroscopy revealed the presence of small amounts (10–20%) of a conformation with a *cis* Ala–Pro amide bond. Such conformations were not observed in analogous Pro–Pro sequences in the absence of external stabilisation of the *cis*-form.[5]

Peptide bond formation between amine trifluoroacetate **14** and *N*-[(2*S*)-2-chloropropionyl]-(2*S*)-Pro, prepared as described previously,[4] was mediated by BOP-Cl and gave 68% of the Pro-(2*R*)–Ala–Pro peptide **15** which was fully characterised and displayed the expected properties.

Surprisingly, mild alkaline hydrolysis of the thioester **15** to the free thiol **10** did not give a pure product. It appeared that some other process had occurred in addition to cleavage of the thioester. Apparently, some thiolate alkylation had already



Scheme 1 *Reagents and conditions*: i, (2*R*)-*N*-(*tert*-butoxycarbonyl)-alanine, BOP-Cl, DIEA, CH_2Cl_2, 0–5 °C, 5 h then rt, 4 days, 55%; ii, DIAD, PPh_3, AcSH, THF, 0 °C; 2.5 h then rt, 15 h, 92%; iii, TFA, CH_2Cl_2, 0 °C, 90 min; iv, *N*-[(2*S*)-2-chloropropionyl]-(2*S*)-proline, BOP-Cl, DIEA, CH_2Cl_2, 0–5 °C, 3 h then rt, 2 days, 68% (2 steps); v, KOH, MeOH, H_2O, 2.5 h; vi, Cs_2CO_3, DMF, 4 days, 25%; vii, MeOH, KOH, H_2O, 65 °C, 2.5 h, 69%.

taken place. The impure material **10** was taken straight on and subjected to cyclisation using caesium carbonate in DMF under high dilution conditions at room temperature over 4 days, Scheme 1. Column chromatography of the crude product allowed the isolation of a compound displaying NMR spectra corresponding to those expected of the 12-membered macrocycle **11**. Gratifyingly, mass spectrometric analysis revealed this to be the desired *monomeric* cyclic compound **11** ($[M + H]^+$ = 384). Some of the cyclic dimer **12** was also formed, but was easily separated by chromatography. This methodology allowed a 25% conversion of thioacetate **15** to the macrocyclic compound **11**. Optimisation of the process established that compound **11** could be produced directly from the thioacetate **15** *via* action of a dilute solution of potassium hydroxide in methanol–water at 65 °C for 2.5 h in an excellent 69% yield.

Conformational studies

Macrocycle **11** was crystallised as a hemihydrate from ethyl acetate–hexane for analysis by X-ray diffraction. In the solid-

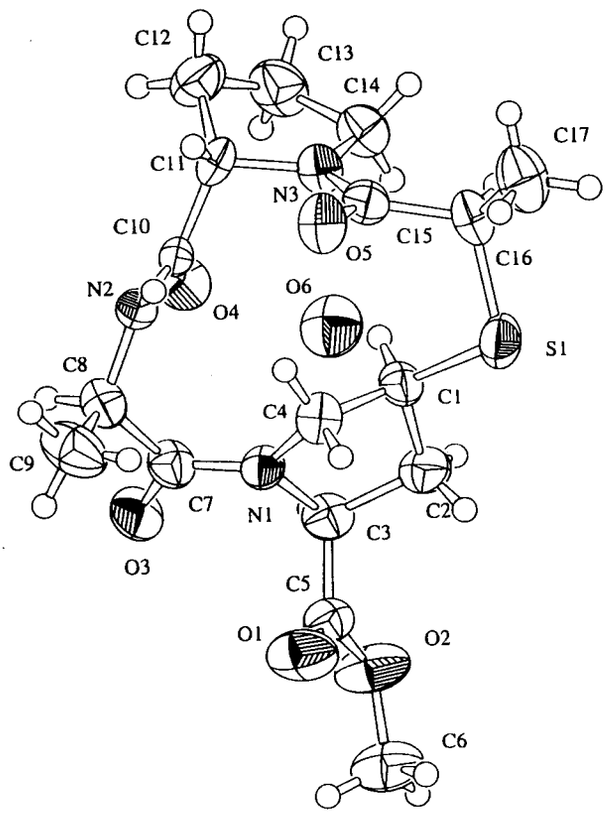


Fig. 1 X-Ray structure of compound **11**.

state structure **11** (see Fig. 1 and footnotes) all amide bonds exist in the *trans* configuration, fulfilling the first objective. However, the *ttt* sub-state adopted is apparently incapable of helix initiation, as the second and third carboxamide carbonyl group lack the required alignment. In fact, the molecule lacks any significant dipole and although the first carboxamide group (in the mercaptopropionyl bridge) possesses an approximately α-helical geometry, the Pro[2] carbonyl group has roughly opposite alignment, and the Ala[3] carbonyl is splayed out. Of course, this is the expected situation in the absence of any H-bond donors and the structure vindicates our original concerns regarding the alignment of carboxamides dipoles in synthesising the macrocycle.[4,5] Thus, it now appears certain that the reason the acyclic precursor can cyclise is because the Pro[2] carbonyl group can 'about turn' in the transition state and minimise the repulsive forces associated with bringing together aligned carbonyl dipoles. Note that in Kemp's system **1** a similar result was observed, but here, because the system was so rigid, one or more amide bonds needed to rotate to the *cis*-form in order to minimise the transition state energy for cyclisation.

Before elaborating the system it was necessary to verify that the conformer present in the solid-state was not merely a consequence of crystal packing. The solution state conformation was characterised by NMR spectroscopy and restrained simulated annealing. In [$^2H_6$]DMSO solution, the macrocycle **11** displayed only one conformation, which corresponded almost exactly to that found in the solid state. The observation of one set of resonance lines for the macrocycle indicated that there was no significant conformational exchange that was slow on the NMR timescale, and any minor conformation was below the limit of detection (~11% population). The diagnostic NOEs which indicate the "up" alignment (*i.e.* anti-alignment) of the Pro[2] carbonyl group all involve the NH proton of the Ala[3] residue. This proton shows NOE interactions with the α-CH hydrogen of Pro[2] and both of the δCH_2 protons of Pro[4] (Fig. 2), which also characterises a *trans* configuration for the amide bond. The absence of NOEs to either Pro[2] βCH_2 proton argues against any significant population of the α-helical target conformation with the Pro[2] carbonyl aligned "down".

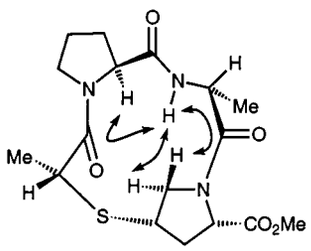


Fig. 2 NOEs observed for macrocycle **11** in [$^2H_6$]DMSO solution.

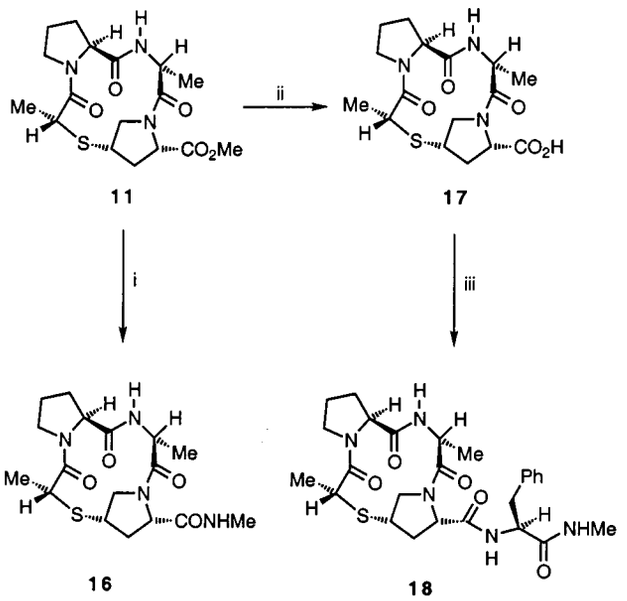
Evaluation of macrocycle 11 as an α-helix initiator

At first glance, the arrangement of carbonyl groups in macrocycle **11** may not appear to be favourable for initiating an α-helix. The Pro[2] carboxamide group has the opposite alignment to that required, and the Ala[3] carbonyl is also appreciably displaced from the position expected for an α-helix. However, the first carboxamide is in approximately the correct position to accept a hydrogen bond from the first amide NH group of an attached peptide and, thus, induce α-helical geometry in at least the first appended residue. Note that the formation of such a H-bond requires the movement of the Ala[3] carbonyl O-atom into the correct position and that, as a result, the entire cap structure would become α-helical, except the Pro[2] carbonyl group would stay anti-aligned in the absence of its own H-bond donor, to minimise the molecular dipole. The next (second) NH group of an attached peptide could be in the correct position to hydrogen bond to the Pro[2] carbonyl group, if this carbonyl group is able to flip alignment, and there is no steric reason why it should not do so. If it does align correctly, the Ala[3] and Pro[4] carbonyl groups would be in the desired position, as defined by the constrain in the pyrrolidine ring of Pro[4]. Indeed, subsequent H-bond formation would be to carbonyl groups that are already positioned correctly through serving as NH H-bond donors. Thus, after the formation of two H-bonds, the system should behave as a perfect template for α-helix propagation. This "conformational co-operativity" is favoured by the formation of hydrogen bonds, but disfavoured on entropic grounds and by repulsive dipolar interactions. For now then, we should take heed of the fact that peptide helices display "fraying" at their termini and, thus, for short added sequences we should expect to observe significant motion at the *C*-terminus.

From the analysis above, it is evident that a rotation of the Pro[2] carboxamide (about the dihedral angles ψ of Pro[2] and φ of Ala[3]) would be essential to bring the carbonyl group into the correct alignment for helix propagation. Therefore, it was an important objective to assess the magnitude of activation energy required for its re-alignment and how the template might be modified to stabilise the correctly aligned rotamer. In the study of the feasibility of α-helix induction by template **11**, which would require the synthesis of derivatives with appended peptide chains, it was decided to address the question of Pro[2] carboxamide dipole re-alignment first through the sequential introduction of H-bond donors and/or positive charges.

One hydrogen bond donor was introduced through conversion of the methyl ester **11** to the methylamide **16** *via* direct aminolysis, Scheme 2. The required amide product was recovered in quantitative yield and displayed the expected analytical and spectroscopic properties.

Conformational analysis of compound **16** in H_2O–^2H_2O (9 : 1) indicated that there was no change in the conformation of the macrocycle upon addition of the hydrogen bond donor. The Pro[2] carbonyl group did not flip to form a 3_{10}-helical hydrogen bonding network with the methylamide NH proton. Experiments to establish whether there was an α-helical 13-membered hydrogen bonding network connecting the methylamide NH to the mercaptopropionyl carbonyl group were inconclusive. Only weak NOE cross peaks were observed to support the notion that the methylamine group may spend some time folded



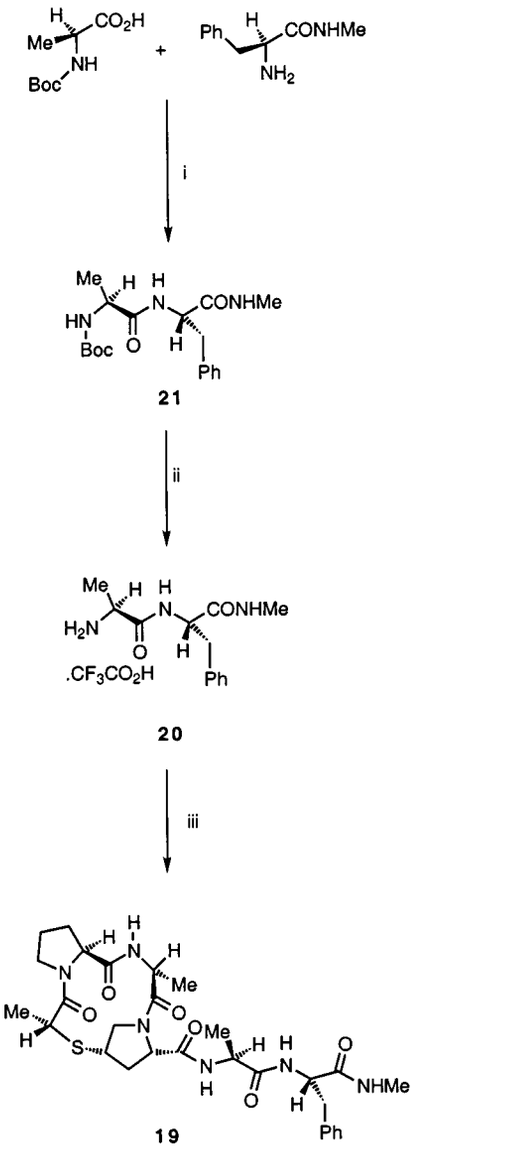
Scheme 2 *Reagents and conditions*: i, $MeNH_2$, MeOH, 0 °C, 2 days, 100%; ii, NaOH, MeOH, H_2O, 2 h, 83%; iii, (2*S*)-phenylalanine methylamide, BOP-Cl, DIEA, CH_2Cl_2, 0–5 °C, 2 h then rt, 20 h, 62%.

underneath the macrocycle. This was the expected result since a large movement of the Pro[4] carboxamide moiety and the proper alignment of the Ala[3] carbonyl dipole would be required to form a 13-membered H-bonding interaction.

In order to attach more than one hydrogen bond donor site to the template it was necessary to hydrolyse the methyl ester **11** and perform subsequent peptide coupling steps. The ester was saponified to afford the acid **17**, as colourless crystals in 83% yield. The acid displayed the expected analytical and spectroscopic properties and would be a precursor for several derivatives. The template cap acid **17** was coupled to (2*S*)-phenylalanine methylamide using BOP-Cl activation to give the template–peptide conjugate **18** in 62% yield (Scheme 2), which possessed the expected analytical properties.

In C^2HCl_3 solution, the macrocyclic portion of compound **18** showed no change in conformational preference relative to that in the methyl ester **11**. However, several NOEs indicate that there was some interaction between the lower face of the macrocycle and the attached peptide. This implied that the Phe[5] residue spent some time folded underneath the macrocycle in a position conducive to the formation of a hydrogen bond between the Phe NH and the mercaptopropionyl carbonyl group. The existence of such a hydrogen bonding interaction requires the Phe[5] residue to adopt an α-helical position. This notion is supported by a 0.2 ppm upfield shift for the methyl protons of the mercaptopropionyl residue, which is consistent with a ring current effect caused by the close proximity of the phenyl ring. There was also a 0.8 ppm downfield shift of the Phe[5] NH proton relative to its position in a model linear peptide in which it is known intramolecular hydrogen bonding is absent. While the folded conformation is evidently populated to some extent, other evidence indicates that the attached peptide is extremely mobile. For example, some NOE cross-peaks are present which are not possible for the folded conformation, and the $^3J_{NH\text{-}\alpha}$ value for the Phe[5] residue is 8.0 Hz, which is not consistent with α-helical geometry.[8] Thus, as expected, the *C*-terminus shows the properties of fraying[9] and a good deal of mobility.

In order to provide hydrogen bond donors for each acceptor carboxamide group in the macrocycle **11**, one further derivative containing an attached dipeptide amide was required, compound **19**. The peptide component (2*S*)-Ala-(2*S*)-Phe-NHMe **20** was synthesised by activating Boc-(2*S*)-Ala-OH using the mixed anhydride method and adding (2*S*)-phenylalanine methylamide. The protected dipeptide **21** was obtained in 67% yield as colourless needles and displayed the expected properties. Removal of the Boc protecting group using trifluoroacetic acid afforded the trifluoroacetate salt **20** which, without further purification, was coupled to the template cap acid **17** to give the required conjugate **19** in 33% yield (Scheme 3).



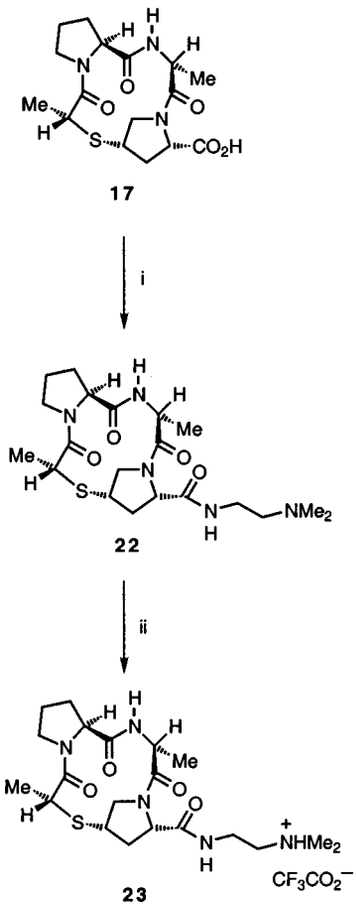
Scheme 3 *Reagents and conditions*: i, IBCF, NMM, THF, −15 °C→ rt, 2 h, 67%; ii, TFA, CH_2Cl_2, 0 °C, 2 h; iii, **17**, BOP-Cl, DIEA, CH_2Cl_2, 0–5 °C, 2 h then rt, 18 h, 33% (2 steps).

NMR studies performed on the template conjugate **19** in C^2HCl_3 solution provided convincing evidence for the presence of a 13-membered intramolecular hydrogen bond network between the NH of the appended (2*S*)-Ala[5] residue and the first carbonyl group. There was a relatively strong set of NOEs similar to those obtained for compound **18**, indicative of the peptide folding underneath the macrocyclic template. The $^3J_{NH\text{-}C\alpha}$ value for the attached alanine residue was 3.9 ± 0.4 Hz, from which we estimate a φ dihedral angle of −57°,[8] which is consistent with α-helical geometry. These data therefore indicate that the macrocycle possesses α-helical character and that the first attached amino acid residue, Ala[5], is part of the helical structure, but not the subsequent residues. The less mobile, tighter α-helical conformation obtained for this conjugate over derivative **18** could reflect the higher intrinsic α-helical propensity of Ala compared with Phe,[10,11] or the fact that the fraying *C*-terminus had been moved further away by one residue.

Whatever the cause, it was evident that the Pro[2] carbonyl group in conjugate **19** was still aligned antiparallel to the aligned mercaptopropionyl and Ala[3] carbonyl groups. From this analysis we must conclude that the enthalpic benefit of hydrogen bonding between short appended peptides and the template in the "helical" conformation appears unable to counterbalance the repulsive dipolar forces and entropic losses associated with this conformation.

Hydrogen bonds are not the only stabilising forces acting upon peptide and protein α-helices in nature. Another common stabilising feature is the presence of an associated counter charge near one of the helix termini which can interact favourably with the helix dipole.[12–14] The desired alignment of carbonyl groups in the parent macrocycle is destabilised by repulsive dipole–dipole interactions but this arrangement could be stabilised by a positively charged group located on the helix axis at the *C*-terminus. The latter effect was demonstrated by Gellman *et al.* who appended a tetraalkylammonium group at the *C*-terminus of a depsipeptide with the resulting induction of an α-helical conformation.[15] The introduction of an anionic group at the *N*-terminus should exert a similar effect, but this is synthetically challenging and an on-going objective in our laboratory. However, since the conjugates **18** and **19** were showing helical characteristics, it was clearly of interest to further investigate the properties of the parent macrocycle **11** by placing a positive charge at the *C*-terminus. The question of whether the Pro[3] carboxamide group in conjugates of **11** could be aligned with the other template derived carboxamide groups needed to be addressed.

We chose to attach dimethylethylenediamine at the *C*-terminus of the macrocycle **17** since this strategy would introduce a hydrogen bond donor, and, after protonation of the tertiary amine, a suitably disposed cationic group. The amide bond formation was achieved by reacting the mixed anhydride of template acid **17** directly with *N*,*N*-(dimethyl)ethylenediamine to afford the conjugate **22** as colourless crystals in 78% yield, Scheme 4. The compound displayed the expected analytical and spectroscopic properties and was directly protonated with trifluoroacetic acid in DCM to give the required trialkylammonium salt **23**.



Scheme 4 *Reagents and conditions*: i, *N*,*N*-(dimethyl)ethylenediamine, IBCF, NMM, THF, DMF, −15 °C→rt, 4 h, 78%; ii, TFA, CH_2Cl_2, 100%.

In deuterochloroform solution the ^1H and ^{13}C NMR spectra of conjugate **23** showed one set of resonances, but with significant broadening of the resonance lines for the Pro[2] Cα and CO ($\Delta\nu_{\frac{1}{2}}$ = 30 Hz for Cα at 30 °C). NOESY assignments for the Ala[3] amide proton indicated several short H–H distances that could not be realised simultaneously in a single conformation. On cooling, the sample exhibited two distinct sets of resonances (Fig. 3). The free energy of activation at coalescence ($\Delta G_c^{\ddagger}$) can be estimated from the following expression:[16]

$$\Delta G_c^{\ddagger} = 4.57\ T_c\ [9.97 + \log\ (T_c/\Delta\nu)]$$

where T_c is the coalescence temperature and $\Delta\nu$ is the difference in resonance frequency of the two spins in the slow exchange limit. For the Ala[3] NH, with a coalescence temperature of 243 ± 5 K and $\Delta\nu$ of 87 Hz, the calculated free energy of activation for the transition is 48 kJ mol^{-1}. The corresponding exchange rate at coalescence was 190 s^{-1}.

At −55 °C conformational exchange rates were sufficiently low to obtain full ^1H and ^{13}C resonance assignments for each of two conformational isomers, with a relative abundance of 3:2 (estimated from peak integrals of resolved amide proton resonances). For a two-site conformational exchange with relative populations in the ratio 3:2 the difference in free energy of the two sites is 1.0 kJ mol^{-1}. However, the conformational exchange was too rapid to separate the NOEs for each isomer.

In d_2-dichloromethane solution at −80 °C the full assignment of NOE spectra was still complicated by residual exchange between two conformers with almost degenerate chemical shifts (Table 1). Conformational exchange also precluded the separation of spin systems by NMR filtration techniques requiring more than a few milliseconds mixing time. Only one NOE between two non-exchangeable protons, the NOE between Ala[3] Hα and Pro[4] Hδ, differs significantly in the two conformers (Table 2). NOE assignments for conjugate **23** at −80 °C were accomplished only for the NOEs involving NH protons (Table 2), but both conformers could be characterised sufficiently from the assignment of amide proton NOEs alone. In the major conformation the NOE between Ala[3] NH and Pro[2] Hα was strong, whilst that between Ala[3] NH and Ala[3] Hα was weak. In the minor conformer these relative intensities were reversed, indicating that the conformational difference was the orientation of the Pro[2]–Ala[3] amide group. The variation was not, however, an amide bond isomerisation; a *cis*-amide bond would give no NOE between Ala[3] NH and any Pro[2] protons, whereas NOEs were observed from Ala[3] NH to Pro[2] Hα in the major isomer and to Pro[2] Hβ and Pro[2] Hδ in the minor isomer. Relative NOEs were quantified using cross-peak volumes from a NOESY experiment, with 200 ms mixing time, for use as modelling restraints during simulated annealing (*vide infra*).

It was hoped that the helix cap would be capable of templating helices in neat aqueous solution, without additional co-solvents. In aqueous solution (95% H_2O, 5% D_2O, 50 mM Tris-d_{11} buffer, pH 7.5) the ^{13}C spectra of compound **23** showed one set of resonance lines. Acetone-d_6 (15% v/v) was added to facilitate low temperature solution studies, and had an insignificant effect on ^{13}C chemical shifts, from which we infer that a low concentration of acetone did not alter the conformational preference. When cooled to −15 °C, some resonances of Pro[2] broadened appreciably; $\Delta\nu_{\frac{1}{2}}$ for Cβ and the carbonyl carbon were ~40 and >100 Hz, respectively, and the Cα resonance was similarly broadened (but not resolved). The line broadening was consistent with the observations in deuterochloroform

Table 1 ^{1}H Resonance assignments for macrocycle **23** in CD_2Cl_2 at −80 °C

	NH	Hα	Hα′	Hβ	Hβ′	Hγ	Hγ′	Hδ	Hδ′
Major isomer									
Thp[1]	—	3.631	—	1.259	—	—	—	—	—
Pro[2]	—	4.802	—	2.201	1.868	2.392	2.029	3.598	3.450
Ala[3]	8.609	4.494	—	1.098	—	—	—	—	—
Pro[4]	—	4.504	—	2.372	2.186	3.828	—	3.589	3.086
TAA[5,a]	8.029	3.811	3.489	3.374	3.241	9.319	—	2.873	2.804
Minor isomer									
Thp[1]	—	3.799	—	1.274	—	—	—	—	—
Pro[2]	—	4.801	—	2.184	1.864	2.335	2.009	4.027	3.519
Ala[3]	8.769	3.809	—	1.350	—	—	—	—	—
Pro[4]	—	4.270	—	2.402	1.861	3.924	—	3.594	3.476
TAA[5]	9.456	3.911	3.316	3.471	3.072	7.737	—	2.971	2.819

[a] TAA = trialkylammonium, where NH is the amide proton, and Hγ is the ammonium proton.

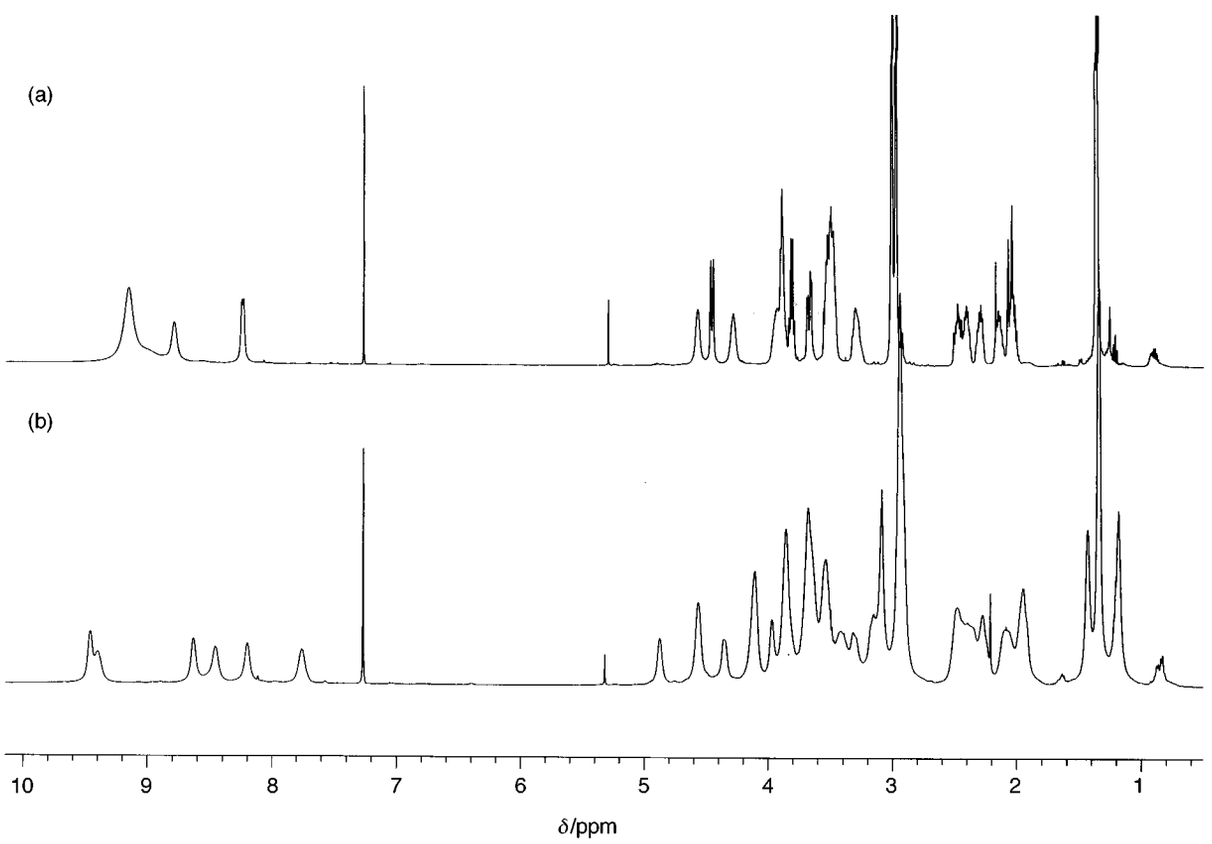


Fig. 3 ^{1}H NMR spectra of macrocycle **23**, cyclic[Pro–Ala–Pro(mercaptopropionyl)] $NHCN_2CH_2NHMe_2$, in deuterochloroform solution at (a) 30 °C and (b) −55 °C.

solution. Additionally, there was broadening of the *N*-methyl carbon resonances. Intensities of NOEs (measured from a NOESY spectrum with 200 ms mixing time) were characteristic of the two rapidly exchanging conformers observed previously in dichloromethane.

Models for each isomer were generated by restrained simulated annealing, using the distance restraints derived from the dichloromethane solution at −80 °C, as shown in Table 2. Two sets of simulations were performed, applying the constraints from the two isomers separately. AM1 *in vacuo* semi-empirical energy minimizations were performed on the lowest energy annealed model of each isomer, to give the optimised structures depicted in Fig. 4. In the AM1 optimised model of the target conformation [Fig. 4(b)] the ammonium proton is situated equidistant (2.2 Å) from the carbonyl oxygens of Pro[2] and the mercaptopropionyl moiety, suggestive of possible hydrogen bonding, but had no interactions with Ala[3].

Restrained and unrestrained molecular dynamics simulations for the minor (40%) conformation using a relative permittivity of four (approximating to a non-polar environment) showed no evidence of hydrogen bonding between the macrocycle and the pendant chain. Instead, the trialkylammonium cation was positioned beneath the centre of the macrocycle, allowing maximum charge–dipole interaction with the aligned carbonyl groups. When the relative permittivity was increased (to 80) to simulate an aqueous environment, the trialkylammonium group was found to drift away from the position beneath the macrocycle.

This striking change in macrocycle conformation shows that the generation of potential α-helix initiating templates is possible by judicious use of charge–dipole interactions. Modelling studies suggest that these initiating forms are stabilised solely by the charged group effect without contribution from hydrogen bonding.

Table 2 Modelling restraints, and comparison of experimental *vs.* modelled internuclear distances for amide proton signals of macrocycle **23** in CD_2Cl_2 solution at −80 °C

From	To	Major isomer			Minor isomer		
		Restraints[a]	r_{noe}[b]/Å	r_{model}[c]/Å	Restraints[a]	r_{noe}[b]/Å	r_{model}[c]/Å
Ala^3 NH	Ala^3 Hα	weak	2.7	3.0	strong	2.6	2.3
Ala^3 NH	Ala^3 Hβ	weak	2.7	3.1	weak	3.7	3.3
Ala^3 NH	Pro^2 Hα	strong	2.2	2.2	—	—	3.6
Ala^3 NH	Pro^2 Hβ	—	—	4.5	weak	3.4	2.4
Ala^3 NH	Pro^2 Hγ	—	—	5.9	weak	2.9	4.1
Ala^3 NH	Pro^2 Hδ	—	—	4.3	weak	3.3	2.7
Ala^3 NH	Pro^4 Hδ	weak	2.7	3.1	strong	2.7	2.4
TAA^5 NH	Pro^2 Hα	weak	3.4	4.4	—	—	6.2
TAA^5 NH	Pro^4 Hα	medium	2.7	3.4	weak	4.0	2.3
TAA^5 NH	Pro^4 Hδ	weak	3.1	3.6	weak	3.7	5.1
TAA^5 NH	TAA^5Hα	strong	2.4	2.9	medium	3.2	2.4
TAA^5 NH	TAA^5 Hα′	strong	2.2	3.0	medium	2.9	3.0
TAA^5 NH	TAA^5 Hβ	weak	2.6	2.7	weak	3.7	3.7
TAA^5 NH	TAA^5 Hδ	weak	2.9	3.3	—	—	3.1
TAA^5 NH	Ala^3 Hβ	weak	3.8	4.9	—	—	4.8
TAA^5 Hγ	TAA^5 Hα	medium	2.9	2.7	—	—	3.2
TAA^5 Hγ	TAA^5 Hα′	medium	2.7	3.5	—	—	3.8
TAA^5 Hγ	TAA^5 Hβ	medium	2.5	2.6	—	—	2.5
TAA^5 Hγ	TAA^5 Hβ′	medium	2.6	2.9	—	3.3	3.0
TAA^5 Hγ	TAA^5 Hδ	strong	2.2	2.4	medium	2.9	2.4
TAA^5 Hγ	TAA^5 Hδ′	strong	2.2	2.4	—	—	2.4
TAA^5 Hγ	Pro^4 Hδ	—	—	4.7	weak	3.0	5.0
TAA^5 Hγ	Ala^1 Hβ	weak	3.2	4.0	—		5.9

[a] Defined as weak (r = 1.8–5.0 Å), medium (r = 1.8–3.3 Å), strong (r = 1.8–2.7 Å); TAA = trialkylammonium. [b] Effective time-averaged internuclear distance calculated from relative volume of NOESY cross-peaks, using the isolated spin-pair approximation (*i.e.* assuming a rigid molecule with isotropic reorientation). [c] Internuclear distance in lowest energy model from simulated annealing.

Experimental

NMR spectra were recorded on a Bruker AM-300 spectrometer (^{1}H, 300 MHz; ^{13}C, 75.4 MHz), a Varian Gemini spectrometer (^{1}H, 200 MHz; ^{13}C, 50.3 MHz) a Varian Gemini spectrometer (^{1}H, 300 MHz; ^{13}C, 75.4 MHz) and a Varian Unity Plus 500 spectrometer (^{1}H, 500.3 MHz; ^{13}C, 125.7 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.

For conformational analysis of the trialkylammonium salt, **23**, 500 MHz ^{1}H NMR spectra were recorded with nominal probe temperatures of 30 and −80 °C. One-dimensional ^{1}H spectra were acquired with 0.5 Hz/point digital resolution and a recycle time of 11 s/transient to allow accurate quantification from peak integrals. TOCSY experiments were acquired with 80 ms MLEV-17 spin-lock (7.2 kHz field), 4 scans per FID, and a digital resolution of 2.6 and 10.4 Hz/point in f_2 and f_1, respectively. The NOESY spectra were acquired with 200 ms mixing time, 8 scans per FID and a digital resolution of 2.1 Hz/point in f_2 and 8.5 Hz/point in f_1. Spectra were processed with cosine-bell weighting in each dimension. For analysis in aqueous solution the corresponding pulse sequences were modified with double pulsed field gradient spin-echo solvent suppression,[17] with selective inversion of water magnetization in a 230 Hz field, and z-gradient pulse pairs of 0.1 and 2.0 ms at 9 G cm^{-1}.

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.*[18] 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 visualized 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 *et al.*[19] 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.

In the molecular modelling studies a wide conformational space was sampled by generating fifty pseudo-random starting structures using unrestrained MD simulations at high temperature (750 K). Each model was minimized by restrained simulated annealing (1 ps of MD at each temperature from 500–300 K, in steps of 50 K, then from 290–10 K in steps of 10 K, and finally at 5 K, followed by conjugate gradients minimization). The lowest energy model was taken as the starting structure for a 510 ps MD simulation at 303 K, storing the atomic coordinates after every 250 timesteps of 1 fs. Initial atom velocities were assigned from a Maxwell–Boltzmann distribution at 303 K, and the thermal energy was scaled by coupling to a thermal bath with a time constant of 0.6 ps. The cutoff distance for calculating non-bonded interactions was 15 Å. NOE restraints were

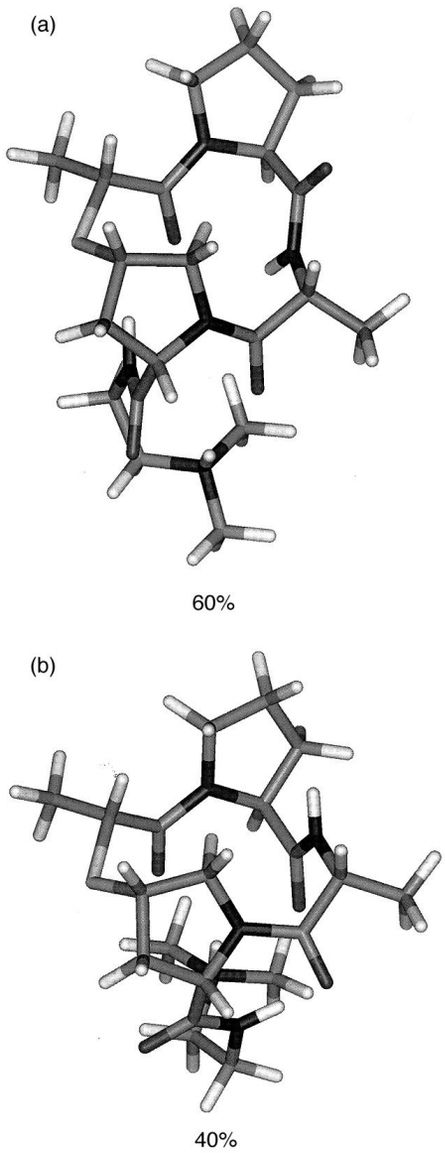


Fig. 4 AM1-optimised models of (a) the major isomer, and (b) the minor isomer of the cap macrocycle with attached trialkylammonium salt, **23**.

applied as symmetrical biharmonic potentials, with a force constant of 10.0 kcal mol^{-1} $Å^{-2}$ and an upper limit of 10.0 kcal mol^{-1} per restraint. No energy penalty was applied for restraints that were within the distance bounds; strong = 1.8–2.7 Å, medium = 1.8–3.3 Å, weak = 1.8–5.0 Å.

Molecular mechanics computations were performed using the DISCOVER package (Biosym, San Diego) with the CVFF force-field parameters. Calculations were performed *in vacuo*, with a fixed dielectric constant to partly simulate bulk solvent. Partial charges for the two conformers were generated from semi-empirical AM1 energy calculations.

Abbreviations

Boc, *tert*-butoxycarbonyl; BOP-Cl, *N*,*N*-bis(2-oxooxazolidin-3-yl)phosphinic chloride; DCM, dichloromethane; DIAD, diisopropyl azodicarboxylate; DIEA, *N*,*N*-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; IBCF, isobutyl chloroformate; NMM, *N*-methylmorpholine; NOE, nuclear Overhauser effect; NOESY, NOE correlation spectroscopy; TFA, trifluoroacetic acid; THF, tetrahydrofuran.

Methyl (2*S*,4*R*)-*N*-[(2*R*)-*N*-(*tert*-butoxycarbonyl)alanyl]-4-hydroxyprolinate 12

A solution of (2*R*)-(*tert*-butoxycarbonyl)alanine (8.00 g, 42.3 mmol) and *N*,*N*-diisopropylethylamine (10.1 cm^3, 58.4 mmol) in dry dichloromethane (250 cm^3) was treated with BOP-Cl (10.8 g, 42.4 mmol) and the resulting suspension stirred under nitrogen at 0 °C for 25 min. A suspension of methyl (2*S*,4*R*)-4-hydroxyprolinate hydrochloride (6.68 g, 36.8 mmol) in dry dichloromethane (50 cm^3) was then added. The mixture was stirred at 0 °C for 5 h, then allowed to warm to room temperature and stirred for a further 4 days. The solution was washed with aqueous HCl (0.5 mol dm^{-3}, 2 × 150 cm^3), aqueous sodium hydrogen carbonate (5%, 2 × 150 cm^3) and brine (150 cm^3), then dried ($MgSO_4$). The solvent was removed under reduced pressure to afford the dipeptide **12** as a white foam (6.34 g, 55%) (HRMS: Found $[M + H]^+$, 317.1721. $C_{14}H_{25}N_2O_6$ requires 317.1713); $[a]_D$ −16.3 (*c* 1.0 in MeOH); $\nu_{max}(CH_2Cl_2)$/cm^{-1} 3425 (NH), 2981 (CH), 1747 (ester CO), 1708 (urethane CO), 1653 (tertiary amide CO) and 1171 (C–O); δ_H(300 MHz; C^2HCl_3) 1.31 [3 H, d, *J* 6.9, βCH$_3$(Ala)], 1.42 [9 H, s, C(CH$_3$)$_3$], 2.00–2.11 [1 H, m, ½βCH$_2$(Hyp)], 2.25–2.37 [1 H, m, ½βCH$_2$(Hyp)], 3.56 [1 H, d, *J* 11.0, ½δCH$_2$(Hyp)], 3.73 (3 H, s, CO_2CH_3), 3.83 [1 H, dd, J_1 11.0, J_2 4.4, ½δCH$_2$(Hyp)], 4.38–4.63 [3 H, m, αCH(Hyp), αCH(Ala) and γCH(Hyp)] and 5.47 (1 H, d, *J* 8.0, NHCH$_3$); δ_C(50.31 MHz; C^2HCl_3) 18.70 [βCH$_3$(Ala)], 28.64 [C(*C*H$_3$)$_3$], 37.65 [βCH$_2$(Hyp)], 48.22 [αCH(Ala)], 52.65 (CO_2CH_3), 55.23 [δCH$_2$(Hyp)], 58.30 [αCH(Hyp)], 68.43 [γCH(Hyp)], 80.02 [*C*(CH$_3$)$_3$], 155.46 (urethane CO), 172.19 (amide CO) and 173.03 (ester CO); *m*/*z* (CI) 317 (40%, $[M + H]^+$), 261 (100, $[M - C_4H_8 + H]^+$), 217 (28, $[M - CO_2C_4H_8 + H]^+$) and 57 (10, $C_4H_9^+$).

Methyl (2*S*,4*S*)-*N*-[(2*R*)-*N*-(*tert*-butoxycarbonyl)alanyl]-4-(acetylthio)prolinate 13

Diisopropyl azodicarboxylate (7.30 g, 34.3 mmol) was added to a solution of triphenylphosphine (9.7 g, 37.0 mmol) in THF (100 cm^3) at 0 °C. The resulting suspension was stirred for 30 min, after which time a solution of alcohol **12** (6.10 g, 19.0 mmol) and thioacetic acid (2.75 cm^3, 37.3 mmol) in THF (50 cm^3) was added dropwise. The mixture was stirred at 0 °C for 2.5 h and then at room temperature overnight after which the solvent was removed under reduced pressure. Excess triphenylphosphine and its oxide were removed by crystallisation (from light petroleum–ethyl acetate) and filtration. The remaining oil was purified by column chromatography using light petroleum–ethyl acetate as the eluent to afford the thioacetate **13** as a clear colourless oil (6.64 g, 92%); R_f 0.49 (ethyl acetate) (HRMS: Found $[M + H]^+$, 375.1593. $C_{16}H_{27}N_2O_6S$ requires 375.1590); $[a]_D$ −24.1 (*c* 0.2 in MeOH); $\nu_{max}(CH_2Cl_2)$/cm^{-1} 3428 (NH), 2982 (CH), 1749 (ester CO), 1703 (urethane and thioester CO), 1655 (tertiary amide CO) and 1171 (C–O); δ_H(300 MHz; C^2HCl_3) (2 conformations, *tt* and *tc*) 1.26 [3 H, d, *J* 6.6, βCH$_3$(Ala)], 1.36 [*tc*, 9 H, s, C(CH$_3$)$_3$], 1.38 [*tt*, 9 H, s, C(CH$_3$)$_3$], 1.84–1.95 [1 H, m, ½βCH$_2$(Pro)], 2.26 (*tc*, 3 H, SCOCH$_3$), 2.30 (*tt*, 3 H, SCOCH$_3$), 2.60–2.72 [*tt*, 1 H, m, ½βCH$_2$(Pro)], 2.72–2.84 [*tc*, 1 H, m, ½βCH$_2$(Pro)], 3.36 [*tc*, 1 H, dd, J_1 12.9, J_2 4.7, ½δCH$_2$(Pro)], 3.52 [*tt*, 1 H, dd, J_1 8.8, J_2 7.0, ½δCH$_2$(Pro)], 3.69 (*tt*, 3 H, s, CO_2CH_3), 3.74 (*tc*, 3 H, s, CO_2CH_3), 3.92–4.19 [2 H, m, γCH(Pro) and ½δCH$_2$(Pro)], 4.35–4.44 [2 H, m, αCH(Ala) and *tc*, αCH(Pro)], 5.00–5.14 [*tt*, 1 H, m, αCH(Pro)] and 5.33 (1 H, d, *J* 8.2, NHCH$_3$); δ_C(75.44 MHz; C^2HCl_3) 18.27 [*tc*, βCH$_3$(Ala)], 18.86 [*tt*, βCH$_3$(Ala)], 28.60 [C(*C*H$_3$)$_3$], 30.79 (SCO*C*H$_3$), 34.83 [*tt*, βCH$_2$(Pro)], 37.67 [*tc*, βCH$_2$(Pro)], 39.04 [*tc*, γCH(Pro)], 39.83 [*tt*, γCH(Pro)] 47.55 [*tc*, αCH(Ala)], 48.15 [*tt*, αCH(Ala)], 52.38, 52.70, 52.76 and 53.03 [δCH$_2$(Pro) and CO_2CH_3], 58.77 [αCH(Pro)], 79.94 [*tt*, *C*(CH$_3$)$_3$], 80.18 [*tc*, *C*(CH$_3$)$_3$], 155.42 [*tt*, urethane CO), 155.82 (*tc*, urethane CO), 171.74 and 172.03 (*tt*, amide and ester CO), 172.75 and 173.32 (*tc*, amide and ester CO) and 195.09 (thioester CO); *m*/*z* (CI) 375 (41%, $[M + H]^+$), 319 (100, $[M - C_4H_8 + H]^+$) and 275 (31, $[M - CO_2C_4H_8 + H]^+$).

Methyl (2*S*,4*S*)-*N*-[(2*R*)-*N*-{(2*S*)-*N*-[(2*S*)-2-chloropropionyl]-prolyl}alanyl]-4-(acetylthio)prolinate 15

A solution of thioester **13** (3.25 g, 8.7 mmol) in dichloromethane (30 cm^3) at 0 °C was treated with trifluoroacetic acid (15 cm^3) and the solution stirred at 0 °C for 90 min. The solvents were removed under reduced pressure to give the trifluoroacetate salt **14** as a yellow oil.

A solution of (2*S*)-*N*-[(2*S*)-2-chloropropionyl]proline (2.37 g, 11.5 mmol) and *N*,*N*-diisopropylethylamine (6.0 cm^3, 34.5 mmol) in dichloromethane (120 cm^3) was treated with BOP-Cl (3.10 g, 12.2 mmol) at 0 °C. After 45 min, a solution of amine trifluoroacetate **14** in dichloromethane (40 cm^3) was added and the mixture was stirred at 0–5 °C for 3 h, then at room temperature for 2 days. 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 (80 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 as the eluent to afford the thioester **15** as a thick yellow oil (2.73 g, 68%), R_f 0.28 (Found: C, 47.85; H, 6.3; N, 8.65. $C_{19}H_{28}ClN_3O_6S{\cdot}H_2O$ requires: C, 47.55; H, 6.3; N, 8.75%) (HRMS: Found $(M + H)^+$, 462.1457. $C_{19}H_{29}{}^{35}ClN_3O_6S$ requires 462.1466); $[\alpha]_D$ −312.5 (*c* 0.9 in MeOH); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3404, 3310 (NH), 2955 (CH), 1748 (ester CO), 1693 (secondary amide and thioester CO) and 1658 (tertiary amide CO); δ_H(300 MHz; C^2HCl_3) (2 conformations, *ttt* and *ttc*) 1.28 [3 H, d, *J* 6.6, $\beta CH_3(Ala^3)$], 1.63 [*ttc*, 3 H, d, *J* 6.3, $\beta CH_3(Chp^1)$], 1.64 [*ttt*, 3 H, d, *J* 6.6, $\beta CH_3(Chp^1)$], 1.85–2.26 [5 H, m, $\beta CH_2(Pro^2)$, $\frac{1}{2}\beta CH_2(Pro^4)$ and $\gamma CH(Pro^2)$], 2.27 (*ttc*, 3 H, s, $SCOCH_3$), 2.31 (*ttt*, 3 H, s, $SCOCH_3$), 2.60–2.72 [*ttt*, 1 H, m, $\frac{1}{2}\beta CH_2(Pro^4)$], 2.74–3.08 [*ttc*, 1 H, m, $\frac{1}{2}\beta CH_2(Pro^4)$], 3.36 [*ttc*, 1 H, dd, J_1 12.9, J_2 4.7, $\frac{1}{2}\delta CH_2(Pro^4)$], 3.48–3.70 [3 H, m, $\delta CH_2(Pro^2)$ and *ttt*, $\frac{1}{2}\delta CH_2(Pro^4)$], 3.68 (*ttt*, 3 H, s, CO_2CH_3), 3.74 (*ttc*, 3 H, s, CO_2CH_3), 3.92–4.16 [2 H, m, $\gamma CH(Pro^4)$ and $\frac{1}{2}\delta CH_2(Pro^4)$], 4.37–4.57 [3 H, m, $\alpha CH(Chp^1)$, $\alpha CH(Pro^2)$ and $\alpha CH(Pro^4)$], 4.63 [1 H, quintet, *J* 6.9, $\alpha CH(Ala^3)$] and 7.15 (1 H, m, NH); δ_C(75.44 MHz; C^2HCl_3) 17.82 [*ttc*, $\beta CH_3(Ala^3)$], 18.04 [*ttt*, $\beta CH_3(Ala^3)$], 20.98 [$\beta CH_3(Chp^1)$], 25.06 [*ttt*, $\gamma CH_2(Pro^2)$], 25.34 [*ttc*, $\gamma CH_2(Pro^2)$], 28.44 [$\beta CH_2(Pro^2)$], 30.85 ($SCOCH_3$), 34.86 [*ttt*, $\beta CH_2(Pro^4)$], 37.56 [*ttc*, $\beta CH_2(Pro^4)$], 39.08 [*ttc*, $\gamma CH(Pro^4)$], 39.87 [*ttt*, $\gamma CH(Pro^4)$], 46.78, 47.05, 47.40 and 47.60 [$\alpha CH(Ala^3)$ and $\delta CH_2(Pro^2)$], 51.30 [*ttc*, $\alpha CH(Chp^1)$], 51.69 [*ttt*, $\alpha CH(Chp^1)$], 52.33, 52.46, 52.71 and 53.05 [$\delta CH_2(Pro^4)$ and CO_2CH_3], 58.75 [$\alpha CH(Pro^4)$], 60.12 [*ttc*, $\alpha CH(Pro^2)$], 60.74 [*ttc*, $\alpha CH(Pro^2)$], 169.10 [$CO(Chp^1)$], 170.83, 171.03 (2 × amide CO), 171.99 (*ttt*, ester CO), 172.88 (*ttc*, ester CO) and 195.13 (thioester CO); *m*/*z* (CI) 464 and 462 (21 and 48%, chlorine isotopes, $[M + H]^+$), 371 {26, $[M - ClCH(CH_3)CO + H]^+$}, 208 and 206 {56 and 100, chlorine isotopes, $[ClCH(CH_3)CONC_4H_7CO_2H]^+$} and 170 (48, $[CH_2{=}CHCONC_4H_7CO_2H + H]^+$).

Methyl (1*S*,3*R*,9*S*,15*S*)-3,12-dimethyl-4,10,13-trioxo-2-thia-5,11,14-triazatricyclo[12.2.1.0^{5,9}]heptadecane-15-carboxylate 11

Thioester **15** (0.34 g, 0.74 mmol) was dissolved in a mixture of 0.05 mol dm^{-3} aqueous potassium hydroxide (25 cm^3) and methanol (25 cm^3). After stirring for 2.5 h, aqueous HCl (0.5 mmol dm^{-3}, 2.5 cm^3) was added and the methanol was removed under reduced pressure. The solution was then further acidified to pH 1 and extracted with ethyl acetate (6 × 40 cm^3). The combined organic extracts were dried ($MgSO_4$) and the solvent was removed under reduced pressure to afford thiol **10** in an impure state as a colourless oil (0.22 g).

The above oil was redissolved in DMF (150 cm^3) and the solution was treated with caesium carbonate (0.34 g, 1.04 mmol). The suspension was stirred under nitrogen for 4 days after which it was filtered through Celite, and the solvent removed under reduced pressure to give a brown oil. The crude oil was purified by column chromatography using dichloromethane–methanol (24 : 1) as the eluent to afford macrocycle **11** as colourless crystals (70 mg, 25%), mp 202–204 °C (Found: C, 51.9; H, 6.45; N, 10.55. $C_{17}H_{25}N_3O_5S{\cdot}\frac{1}{2}H_2O$ requires C, 52.05; H, 6.7; N, 10.7%); $[\alpha]_D$ −149.9 (*c* 0.5 in MeOH); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3308 (NH), 2953 (CH), 1763 (ester CO), 1682 (secondary amide CO), 1663 (tertiary amide CO) and 1450 (CH def.); δ_H(500 MHz; $[^2H_6]$DMSO) 1.27 [3 H, d, *J* 6.5, $\beta CH_3(Thp^1)$], 1.28 [3 H, d, *J* 6.6, $\beta CH_3(Ala^3)$], 1.57–1.62 [1 H, m, $\frac{1}{2}\beta CH_2(Pro^4)$], 1.80–1.88 [1 H, m, $\frac{1}{2}\beta CH_2(Pro^4)$], 1.96–2.00 [1 H, m, $\frac{1}{2}\gamma CH_2(Pro^2)$], 2.10–2.14 [1 H, m, $\frac{1}{2}\beta CH_2(Pro^2)$], 2.20–2.28 [1 H, m, $\frac{1}{2}\gamma CH_2(Pro^2)$], 2.48–2.55 [1 H, m, $\frac{1}{2}\gamma CH_2(Pro^2)$], 2.90 [1 H, dd, J_1 11.8, J_2 6.8, $\frac{1}{2}\delta CH_2(Pro^4)$], 3.12–3.17 [1 H, m, $\gamma CH(Pro^4)$], 3.61–3.67 [2 H, m, $\delta CH_2(Pro^2)$], 3.65 (3 H, s, CO_2CH_3), 4.09 [1 H, q, *J* 6.9, $\alpha CH(Thp^1)$], 4.09–4.16 [1 H, m, $\alpha CH(Ala^3)$], 4.31 [1 H, dd, J_1 11.7, J_2 4.8, $\frac{1}{2}\delta CH_2(Pro^4)$], 4.41 [1 H, dd, J_1 9.3, J_2 5.4, $\alpha CH(Pro^4)$], 4.88 [1 H, d, *J* 7.2, $\alpha CH(Pro^2)$] and 8.17 [1 H, d, *J* 4.3, NH]; δ_C(125.73 MHz; $[^2H_6]$DMSO) 16.45 [$\beta CH_3(Ala^3)$], 16.73 [$\beta CH_3(Thp^1)$], 24.41 [$\gamma CH_2(Pro^2)$], 25.62 [$\beta CH_2(Pro^2)$], 33.56 [$\beta CH_2(Pro^4)$], 40.26 [$\alpha CH(Thp^1)$], 41.56 [$\gamma CH(Pro^4)$], 46.38 [$\delta CH_2(Pro^2)$], 50.85 [$\delta CH_2(Pro^4)$], 51.08 [$\alpha CH(Ala^3)$], 51.79 (CO_2CH_3), 57.12 [$\alpha CH(Pro^4)$], 57.70 [$\alpha CH(Pro^2)$], 169.26 [$CO(Ala^3)$], 170.17 [$CO(Pro^2)$], 171.05 [$CO(Pro^4)$] and 171.48 (ester CO); *m*/*z* (FAB^+) 406 (5%, $[M + Na]^+$), 384 (64, $[M + H]^+$) and 128 {84, $[NC_4H_6(S)CO]^+$}.

Compound **11** could be obtained directly from thioester **15** by modification of the initial hydrolysis step. Thioester **15** (200 mg, 0.43 mmol) was dissolved in a mixture of 0.05 mol dm^{-3} aqueous potassium hydroxide (17 cm^3) and methanol (400 cm^3) and the mixture heated to reflux for 2.5 h. Aqueous HCl (1.0 mol dm^{-3}, 10 cm^3) was added, and after the removal of methanol, the aqueous phase was extracted with ethyl acetate (10 × 15 cm^3). The combined organic extracts were dried ($MgSO_4$) and the solvent was removed under reduced pressure to give thioester **15** as a clear oil, which was purified as above to yield the pure product as a colourless oil (115 mg, 69%).

Crystallographic data for macrocycle 11 ‡

$C_{17}H_{26}N_3O_5S$, M = 392.47, orthorhombic, space group $C222_1$ (#20), a = 9.371(10), b = 16.744(9), c = 25.705(7) Å, V = 4033(4) Å^3, Z = 8, D_{calc} = 1.293 g m^{-3}, T = 293 K. 1511 Unique reflections were collected on a Rigaku AFC7S, diffracting with graphite monochromated Mo-Kα radiation (λ = 0.71069 Å) of which 1020 [$I > 3\sigma(I)$] were used for refinement. Convergence at $R(F)$ = 4.9%, $R_w(F)$ = 4.6% for 240 variable parameters. The structure was solved and expanded using Fourier techniques and was refined using TEXSAN.[21]

***N*-Methyl-[(1*S*,3*R*,9*S*,12*R*,15*S*)-3,12-dimethyl-4,10,13-trioxo-2-thia-5,11,14-triazatricyclo[12.2.1.0^{5,9}]heptadecane]-15-carboxamide 16**

Methyl ester **11** (42 mg, 0.11 mmol) was dissolved in a saturated solution of methylamine in methanol (5 cm^3) at 0 °C. The flask was stoppered, allowed to warm to room temperature, and left standing for 2 days. The flask was cooled to 0 °C, and the stopper removed. The excess methylamine was driven off by bubbling nitrogen through the solution for 30 min. The solvent was removed under reduced pressure to afford methylamide **16** as white crystals in quantitative yield, mp 280 °C (decomp.) (Found: C, 53.85; H, 6.8; N, 14.5. $C_{17}H_{26}N_4O_4S$ requires C, 53.4; H, 6.85; N, 14.65%); $[\alpha]_D$ −46.3 (*c* 0.6 in MeOH); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3348 (NH), 2940 (CH), 1671 (secondary amide CO), 1621 (tertiary amide CO) and 1532 (NH def.);

‡ Full crystallographic details, excluding structure factor tables, have been deposited at the Cambridge Crystallographic Data Centre (CCDC). For details of the deposition scheme, see 'Instructions for Authors', *J. Chem. Soc.*, *Perkin Trans. 1*, available *via* the RSC Web page (http://www.rsc.org/authors). Any request to the CCDC for this material should quote the full literature citation and the reference number 207/268.

δ_H(300 MHz; C^2HCl_3) 1.34 [3 H, d, *J* 6.6, βCH$_3$(Thp^1)], 1.37 [1 H, d, *J* 7.1, βCH$_3$(Ala^3)], 1.88–2.01 [2 H, m, ½βCH$_2$(Pro^4) and ½γCH$_2$(Pro^2)], 2.02–2.16 [2 H, m, ½CH$_2$(Pro^2) and ½γCH$_2$(Pro^2)], 2.37–2.53 [2 H, m, ½βCH$_2$(Pro^2) and ½βCH$_2$(Pro^4)], 2.82 (3 H, d, *J* 4.9, NHC*H*$_3$), 3.22 [1 H, d, *J* 11.0, ½δCH$_2$(Pro^4)], 3.49–3.58 [1 H, m, γCH(Pro^4)], 3.61–3.72 [3 H, m, δCH$_2$(Pro^2) and ½δCH$_2$(Pro^4)], 3.81 [1 H, q, *J* 7.1, αCH(Thp^1)], 4.38–4.51 [2 H, m, αCH(Ala^3) and αCH(Pro^4)], 4.83 [1 H, d, *J* 7.1, αCH(Pro^2)], 7.51 [1 H, d, *J* 6.9, NH(Ala^3)] and 7.64 (1 H, m, N*H*CH$_3$); δ_C(75.44 MHz; C^2HCl_3) 17.10 and 17.15 [βCH$_3$(Thp^1) and βCH$_3$(Ala^3)], 25.55 [βCH$_2$(Pro^2) and γCH$_2$(Pro^2)], 26.71 (NHCH$_3$), 34.70 [βCH$_2$(Pro^4)], 43.08 and 43.15 [αCH(Thp^1) and γCH(Pro^4)], 47.49 [δCH$_2$(Pro^2)], 49.43 [CH(Ala^3)], 51.37 [δCH$_2$(Pro^4)], 59.73 [αCH(Pro^4)], 61.36 [αCH(Pro^2)], 169.66 [CO(Pro^2)], 172.19 [CO(Pro^4)], 172.69 [CO(Ala^3)] and 174.63 [CO(Thp^1)]; *m*/*z* (CI) 383 (100%, [M + H]$^+$) and 351 (23, [M − S + H]$^+$).

(1*S*,3*R*,9*S*,12*R*,15*S*)-3,12-Dimethyl-4,10,13-trioxo-2-thia-5,11,14-triazatricyclo[12.2.1.0^{5,9}]heptadecane-15-carboxylic acid 17

A solution of methyl ester **11** (0.30 g, 0.79 mmol) in methanol (3 cm^3) was treated with aqueous sodium hydroxide (1.0 mol dm^{-3}, 3.5 cm^3), and the mixture allowed to stir for 2 h. After addition of aqueous HCl (1.0 mol dm^{-3}, 5 cm^3), the methanol was removed under reduced pressure. The solution was acidified with aqueous HCl, saturated with NaCl, and exhaustively extracted with ethyl acetate (9 × 20 cm^3). The combined organic extracts were dried (MgSO$_4$), and the solvent was removed under reduced pressure to afford the macrocycle acid **17** as colourless crystals (0.24 g, 83%), mp 225–226 °C (decomp.) (Found: C, 52.15; H, 6.5; N, 11.0. $C_{16}H_{23}N_3O_5S$ requires C, 52.0; H, 6.3; N, 11.35%); $[\alpha]_D$ −134.4 (*c* 0.2 in MeOH); ν_{max}(CH$_2$Cl$_2$)/cm^{-1} 3317 (NH), 1751 (ester CO), 1686 (secondary amide CO) and 1672 (tertiary amide CO); δ_H(300 MHz; C^2HCl_3) 1.32 [6 H, m, βCH$_3$(Thp^1) and βCH$_3$(Ala^3)], 1.90–2.10 [3 H, m, ½βCH$_2$(Pro^2), ½βCH$_2$(Pro^4) and ½γCH$_2$(Pro^2)], 2.38–3.43 [3 H, m, ½βCH$_2$(Pro^2), ½βCH$_2$(Pro^4) and ½γCH$_2$(Pro^2)], 3.11 [1 H, d, *J* 11.8, ½δCH$_2$(Pro^4)], 3.50–3.66 [3 H, m, γCH(Pro^4) and δCH$_2$(Pro^2)], 3.83–3.91 [2 H, m, αCH(Thp^1) and ½δCH$_2$(Pro^4)], 4.46–4.50 [1 H, m, αCH(Ala^3)], 4.54–4.58 [1 H, m, αCH(Pro^4)], 4.81 [1 H, d, *J* 7.7, αCH(Pro^2)], 7.63 (1 H, d, *J* 6.6, NH) and 9.77 (1 H, br s, CO$_2$H); δ_C(75.44 MHz; C^2HCl_3) 16.94 and 17.00 [βCH$_3$(Thp^1) and βCH$_3$(Ala^3)], 25.37 and 25.73 [βCH$_2$(Pro^2) and γCH$_2$(Pro^2)], 34.25 [βCH$_2$(Pro^4)], 42.62 and 42.75 [αCH(Thp^1) and γCH(Pro^4)], 47.48 [δCH$_2$(Pro^2)], 49.47 [αCH(Ala^3)], 51.54 [δCH$_2$(Pro^4)], 59.82 and 60.13 [αCH(Pro^2) and αCH(Pro^4)], 169.98 [CO(Pro^2)], 172.56, 173.02 and 174.63 [CO(Thp^1), CO(Ala^3) and CO(Pro^4)]; *m*/*z* (CI) 370 (77%, [M + H]$^+$), 326 (14, [M − CO$_2$ + H]$^+$), 257 (59, [M − NC$_4$H$_7$CO$_2$ + H]$^+$) and 170 (100, SCHCONC$_4$H$_7$CO$^+$).

(2*S*)-*N*^{2}-{(1*S*,3*R*,9*S*,12*R*,15*S*)-3,12-Dimethyl-4,10,13-trioxo-2-thia-5,11,14-triazatricyclo[12.2.1.0^{5,9}]heptadecan-15-ylcarbonyl}phenylalanine methylamide 18

A solution of acid **17** (54 mg, 0.15 mmol) and *N*,*N*-diisopropylethylamine (80 cm^3, 0.46 mmol) in dry dichloromethane (2 cm^3) was treated with BOP-Cl (40 mg, 0.16 mmol) and the resulting suspension stirred under nitrogen at 0 °C for 1 h. A solution of (2*S*)-phenylalanine methylamide (30 mg, 0.17 mmol) in dry dichloromethane (1 cm^3) was then added. The mixture was stirred at 0–5 °C for 2 h, then allowed to warm to room temperature and stirred for a further 20 h. The solution was diluted to ~15 cm^3 with dichloromethane, 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) and then dried (MgSO$_4$). The solvent was removed under reduced pressure to afford the peptide **18** as a white solid (48 mg, 62%). A small portion of this was recrystallised from ethyl acetate to afford colourless needles, mp 258–259 °C (Found: C, 58.95; H, 6.65; N, 13.05. $C_{26}H_{35}N_5O_5S$ requires C, 58.95; H, 6.65; N, 13.2%); $[\alpha]_D$ −62.7 (*c* 0.15 in MeOH); ν_{max}(CH$_2$Cl$_2$)/cm^{-1} 3364 (NH), 2937 (CH), 1679 (secondary amide CO), 1642 (tertiary amide CO) and 1531 (NH def.); δ_H(500 MHz; C^2HCl_3) 1.14 [3 H, d, *J* 7.2, βCH$_3$(Thp^1)], 1.31 [3 H, d, *J* 6.6, βCH$_3$(Ala^3)], 1.84–1.96 [2 H, m, ½βCH$_2$(Pro^2) and ½βCH$_2$(Pro^4)], 2.04–2.11 [1 H, m, ½γCH$_2$(Pro^2)], 2.37–2.54 [3 H, m, ½βCH$_2$(Pro^2), ½βCH$_2$(Pro^4) and ½γCH$_2$(Pro^2)], 2.81 (3 H, d, *J* 4.7, NHC*H*$_3$), 3.12 [1 H, dd, J_1 15.9, J_2 10.0, ½βCH$_2$(Phe^5)], 3.24 [1 H, d, *J* 11.9, ½δCH$_2$(Pro^4)], 3.48 [1 H, q, *J* 7.9, αCH(Thp^1)], 3.58–3.67 [5 H, m, ½βCH$_2$(Phe^5), γCH(Pro^4), δCH$_2$(Pro^2) and ½δCH$_2$(Pro^4)], 4.45–4.49 [2 H, m, αCH(Ala^3) and αCH(Pro^4)], 4.84 [1 H, dd, J_1 1.5, αCH(Pro^2)], 5.02 [1 H, m, αCH(Phe^5)], 6.90 (1 H, q, *J* 4.3, N*H*CH$_3$), 7.15 (1 H, t, *J* 7.1, Ar-H *para*), 7.23–7.28 (4 H, m, Ar-H *ortho* and *meta*), 7.60 [1 H, d, *J* 7.2, NH(Ala^3)] and 7.86 [1 H, d, *J* 8.0, NH(Phe^5)]; δ_C(75.44 MHz; C^2HCl_3) 17.01 [βCH$_3$(Thp^1)], 17.46 [βCH$_3$(Ala^3)], 25.52 [βCH$_2$(Pro^2)], 25.71 [γCH$_2$(Pro^2)], 26.98 (NHCH$_3$), 34.48 [βCH$_2$(Pro^4)], 36.59 [βCH$_2$(Phe^5)], 43.34 [γCH(Pro^4)], 43.61 [αCH(Thp^1)], 47.49 [δCH$_2$(Pro^2)], 48.85 [αCH(Ala^3)], 51.19 [δCH$_2$(Pro^4)], 53.60 [αCH(Phe^5)], 59.98 [αCH(Pro^2)], 62.36 [αCH(Pro^4)], 126.59 (Ar-CH *para*), 128.70 (Ar-CH *meta*), 129.11 (Ar-CH *ortho*), 138.78 (Ar-C quaternary), 169.65 [CO(Pro^2)], 172.07 [CO(Pro^4)], 172.76 [CO(Phe^5)], 173.00 [CO(Ala^3)] and 174.47 [CO(Thp^1)]; *m*/*z* (CI) 530 (22%, [M + H]$^+$), 243 {100, [NC$_4$H$_6$CONHCH(CH$_2$Ph)CO]$^+$} and 201 {37, [SCH(CH$_3$)CONC$_4$H$_7$CONH + H]$^+$}.

(2*S*)-*N*^{2}-[(2*S*)-*N*-(*tert*-Butoxycarbonyl)alanyl]phenylalanine methylamide 21

A stirred solution of (2*S*)-*N*-(*tert*-butoxycarbonyl)alanine (0.64 g, 3.4 mmol) and *N*-methylmorpholine (0.38 cm^3, 3.4 mmol) in dry THF (15 cm^3) at −15 °C was treated dropwise with isobutyl chloroformate (0.46 cm^3, 3.4 mmol), followed after 2 min by a solution of (2*S*)-phenylalanine methylamide (0.60 g, 3.4 mmol) and *N*-methylmorpholine (0.38 cm^3, 3.4 mmol) in dry THF (5 cm^3).

The suspension was diluted with additional THF to allow efficient stirring, allowed to reach room temperature, then stirred for 2 h. The precipitated hydrochloride salts were filtered off and the solvents were removed under reduced pressure to yield a solid which was dissolved in ethyl acetate (50 cm^3). This solution was washed with aqueous HCl (0.5 mol dm^{-3}, 2 × 30 cm^3), aqueous sodium hydrogen carbonate (5%, 2 × 30 cm^3), and brine (30 cm^3). The solvent was removed under reduced pressure to yield the dipeptide methylamide **21** as a white solid (0.78 g, 67%). A small portion of this was recrystallised from ethyl acetate to afford colourless needles, mp 157–158 °C (lit.,[20] 157–158 °C); $[\alpha]_D$ −38.7 (*c* 1.1 in MeOH) [lit.,[20] −37.7 (*c* 1.0 in MeOH]; ν_{max}(CH$_2$Cl$_2$)/cm^{-1} 3411 (NH), 2981 (CH), 1701 (urethane CO), 1670 (amide CO) and 1505 (NH def.); δ_H(200 MHz; C^2HCl_3) 1.27 [3 H, d, *J* 7.0, βCH$_3$(Ala)], 1.37 [9 H, s, C(CH$_3$)$_3$], 2.70 (3 H, d, *J* 4.8, NHC*H*$_3$), 3.01–3.25 [2 H, m, βCH$_2$(Phe)], 4.05 [1 H, m, CH(Ala)], 4.66 [1 H, m, αCH(Phe)], 5.10 (1 H, m, NH), 6.74 (1 H, m, NH) and 7.14–7.32 (5 H, m, Ar-H); δ_C(50.31 MHz; C^2HCl_3) 18.57 [βCH(Ala)], 26.82 (NHCH$_3$), 28.69 [C(*C*H$_3$)$_3$], 38.36 [βCH$_2$(Phe)], 51.56 [αCH(Ala)], 54.42 [αCH(Phe)], 80.93 [*C*(CH$_3$)$_3$], 127.39 (Ar-CH *para*), 129.04 (Ar-CH *meta*), 129.70 (Ar-CH *ortho*), 137.11 (Ar-C quaternary), 156.10 (urethane CO), 171.70 and 173.15 (2 × amide CO); *m*/*z* (CI) 351 (100%, [M + H]$^+$), 294 (93, [M − C$_4$H$_8$]$^+$) and 250 (12, [M − CO$_2$C$_4$H$_8$]$^+$).

(2*S*)-*N*^{2}-[(2*S*)-*N*-{(1*S*,3*R*,9*S*,12*R*,15*S*)-3,12-Dimethyl-4,10,13-trioxo-2-thia-5,11,14-triazatricyclo[12.2.1.0^{5,9}]heptadecan-15-ylcarbonyl}alanyl]phenylalanine methylamide 19

A solution of methylamide **21** (0.50 g, 1.4 mmol) in dichloro-

methane (15 cm^3) at 0 °C was treated with trifluoroacetic acid (8 cm^3) and the solution stirred for 2 h. The solvents were removed under reduced pressure to yield the trifluoroacetate salt **20** as a white solid.

A solution of acid **17** (80 mg, 0.22 mmol) and *N*,*N*-diisopropylethylamine (0.12 cm^3, 0.69 mmol) in dichloromethane (5 cm^3) was treated with BOP-Cl (60 mg, 0.24 mmol) at 0 °C. After 50 min, amine trifluoroacetate **20** (90 mg, 0.25 mmol) was added and the mixture was stirred at 0–5 °C for 2 h, then at room temperature for 18 h. The solution was diluted to ~15 cm^3 with dichloromethane, then washed with aqueous HCl (0.5 mol dm^{-3}, 2 × 6 cm^3), aqueous sodium hydrogen carbonate (5%, 2 × 6 cm^3) and brine (6 cm^3). The organic phase was dried ($MgSO_4$), and the solvent removed under reduced pressure. The crude residue was purified by column chromatography using dichloromethane–methanol (20 : 1) as the eluent to afford the peptide **19** as a white solid (43 mg, 33%), mp 129–131 °C (HRMS: Found (M + H)$^+$, 601.2798. $C_{29}H_{41}N_6O_6S$ requires 601.2808); $[\alpha]_D$ −25.7 (*c* 0.3 in MeOH); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3338 (NH), 2935 (CH), 1680 (secondary amide CO), 1662 (tertiary amide CO) and 1536 (NH def.); δ_H(300 MHz; C^2HCl_3) 1.16 [3 H, d, *J* 7.4, βCH$_3$(Ala^5)], 1.27 [3 H, d, *J* 6.6, βCH$_3$(Ala^3)], 1.30 [3 H, d, *J* 7.4, βCH$_3$(Thp^1)], 1.89–1.98 [2 H, m, ½βCH$_2$(Pro^2) and ½βCH$_2$(Pro^4)], 2.02–2.09 [1 H, m, ½γCH$_2$(Pro^2)], 2.33–2.40 [1 H, m, ½γCH$_2$(Pro^2)], 2.43–2.55 [2 H, m, ½βCH$_2$(Pro^2) and ½βCH$_2$(Pro^4)], 2.74 (3 H, d, *J* 4.7, NHC*H*$_3$), 2.86 [1 H, dd, J_1 14.4, J_2 11.7, ½βCH$_2$(Phe^6)], 3.22 [1 H, d, *J* 11.8, ½δCH$_2$(Pro^4)], 3.44–3.69 [4 H, m, ½βCH$_2$(Phe^6), δCH$_2$(Pro^2) and ½δCH$_2$(Pro^4)], 3.74–3.85 [2 H, m, αCH(Thp^1) and γCH(Pro^4)], 4.15–4.19 [1 H, m, αCH(Ala^5)], 4.37 [1 H, d, *J* 10.4, αCH(Pro^4)], 4.41–4.46 [1 H, m, αCH(Ala^3)], 4.60–4.68 [1 H, m, αCH(Phe^6)], 4.75 [1 H, d, *J* 6.6, αCH(Pro^2)], 6.87 (1 H, q, *J* 4.7, N*H*CH$_3$), 7.06 [1 H, d, *J* 9.1, NH(Phe^6)], 7.11–7.22 (5 H, m, Ar-H), 7.58 [1 H, d, *J* 7.1, NH(Ala^3)] and 8.33 [1 H, d, *J* 3.9, NH(Ala^5)]; δ_C(125.7 MHz; C^2HCl_3) 16.31 [βCH$_3$(Ala^5)], 16.79 [βCH$_3$(Thp^1)], 17.03 [βCH$_3$(Ala^3)], 25.16 [βCH$_2$(Pro^s)], 25.34 [γCH$_2$(Pro^2)], 26.47 (NHCH$_3$), 34.39 [βCH$_2$(Pro^4)], 37.31 [βCH$_2$(Phe^6)], 43.30 [γCH(Pro^4)], 43.57 [αCH(Thp^1)], 47.31 [δCH$_2$(Pro^2)], 48.24 [αCH(Ala^3)], 50.74 [δCH$_2$(Pro^4)], 51.01 [αCH(Ala^5)], 54.24 [αCH(Phe^6)], 59.88 [αCH(Pro^2)], 62.11 [αCH(Pro^4)], 126.32 (Ar-CH *para*), 128.22 (Ar-CH *meta*), 129.14 (Ar-CH *ortho*), 138.68 (Ar-C quaternary), 169.20 [CO(Pro^2)], 171.90, 172.18, 172.48 and 172.60 [CO(Ala^3), CO(Pro^4), CO(Ala^5) and CO(Phe^6)] and 174.24 [CO(Thp^1)]; *m*/*z* (CI) (100%, [M + H]$^+$), 569 (31, [M − NH$_2$CH$_3$]$^+$), 439 (10, [M − CH$_3$NHCOCH-CH$_2$Ph + H]$^+$) and 372 {100, [CONC$_4$H$_6$CONHCH(CH$_3$)-CONHCH(CH$_2$Ph)CONHCH$_3$]$^+$}.

N-{(1*S*,3*R*,9*S*,12*R*,15*S*)-3,12-Dimethyl-4,10,13-trioxo-2-thia-5,11,14-triazatricyclo[12.2.1.0^{5,9}]heptadecan-15-ylcarbonyl}-*N*′,*N*′-(dimethyl)ethylenediamine 22 and amine trifluoroacetate salt 23

A stirred solution of acid **17** (0.15 g, 0.41 mmol) and *N*-methylmorpholine (0.10 cm^3, 0.91 mmol) in dry THF (3 cm^3) and DMF (1 cm^3) under nitrogen at −15 °C was treated dropwise with isobutyl chloroformate (55 μl, 0.42 mmol), followed after 10 min by a solution of *N*,*N*-dimethylethylenediamine (60 μl, 0.55 mmol) in dry THF (1 cm^3). The solution was allowed to reach room temperature and stirred for 4 h. The solvents were removed under reduced pressure, and the residual oil redissolved in ethyl acetate (20 cm^3). The solution was washed with aqueous sodium hydrogen carbonate (5%, 2 × 5 cm^3), brine (5 cm^3) and then dried ($MgSO_4$). The solvent was removed under reduced pressure to afford the amine **22** as colourless crystals (0.14 g, 78%), mp 174–176 °C (Found: C, 53.95; H, 7.6; N, 15.25. $C_{20}H_{33}N_5O_4S\cdot\frac{1}{2}H_2O$ requires C, 53.55; H, 7.65; N, 15.6%); $[\alpha]_D$ −48.0 (*c* 0.85 in MeOH); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3337 (NH), 2983 (CH), 1668 (secondary amide CO), 1634 (tertiary amide CO), 1531 (NH def.) and 1269 (C–N); δ_H(300 MHz; C^2HCl_3) 1.23 [3 H, d, *J* 6.9, βCH$_3$(Thp^1)], 1.29 [3 H, d, *J* 7.1, βCH$_3$-(Ala^3)], 1.81–2.04 [3 H, m, ½βCH$_2$(Pro^2), ½βCH$_2$(Pro^4) and ½γCH$_2$(Pro^2)], 2.18 [6 H, s, N(CH$_3$)$_2$], 2.31–2.43 [4 H, m, C*H*$_2$N(CH$_3$)$_2$, ½βCH$_2$(Pro^2) and ½γCH$_2$(Pro^2)], 2.51–2.60 [1 H, m, ½βCH$_2$(Pro^4)], 3.15–3.22 [2 H, m, γCH(Pro^4) and ½δCH$_2$(Pro^4)], 3.35–3.62 [5 H, m, δCH$_2$(Pro^2), ½δCH$_2$(Pro^4) and C*H*$_2$HNCO], 3.71 [1 H, q, *J* 7.1, αCH(Thp^1)], 4.37–4.43 [2 H, m, αCH(Ala^3) and αCH(Pro^4)], 4.76 [1 H, d, *J* 8.2, αCH(Pro^2)], 7.70 (1 H, t, *J* 7.7, N*H*CH$_2$) and 7.90 [1 H, d, *J* 8.0, NH(Ala^3)]; δ_C(75.44 MHz; C^2HCl_3) 16.93 and 17.10 [βCH$_3$(Thp^1) and βCH$_3$(Ala^3)], 25.47 [βCH$_2$(Pro^2) and γCH$_2$(Pro^2)], 34.52 [βCH$_2$(Pro^4)], 37.63 (*C*H$_2$NHCO), 43.02 and 43.10 [αCH(Thp^1) and γCH(Pro^4)], 45.49 [N(CH$_3$)$_2$], 46.97 [δCH$_2$(Pro^2)], 48.79 [αCH(Ala^3)], 51.16 [δCH$_2$(Pro^4)], 57.96 [*C*H$_2$N(CH$_3$)$_2$], 59.59 [αCH(Pro^2)], 61.41 [αCH(Pro^4)], 169.53 [CO(Pro^2)], 171.92 and 172.30 [CO(Ala^3) and CO(Pro^4)] and 173.87 [CO(Thp^1)]; *m*/*z* (CI) 440 (100%, [M + H]$^+$), 408 (23, [M − 2CH$_3$ + H]$^+$), 369 {19, [M − CH-CH$_2$N(CH$_3$)$_2$ + H]$^+$} and 241 {82, [CH$_3$CHCONC$_4$H$_7$CONH-CH$_2$CH$_2$N(CH$_3$)$_2$ + H]$^+$}.

A solution of amine **22** in dichloromethane was treated with a slight excess of trifluoroacetic acid, followed by removal of the solvent under reduced pressure to afford the amine trifluoroacetate **23** as a colourless oil.

δ_H(500 MHz; C^2HCl_3) 1.36 [6 H, d, *J* 7.1, βCH$_3$(Thp^1) and βCH$_3$(Ala^3)], 2.01–2.08 [2 H, m, ½βCH$_2$(Pro^4) and ½γCH$_2$(Pro^2)], 2.11–2.18 (1 H, m, ½βCH$_2$(Pro^2)], 2.28–2.33 [1 H, m, ½βCH$_2$-(Pro^2)], 2.39–2.45 [1 H, m, ½γCH$_2$(Pro^2)], 2.45–2.51 [1 H, m, ½βCH$_2$(Pro^4)], 2.98 (3 H, s, NCH$_3$), 3.02 (3 H, s, NCH$_3$), 3.25–3.35 [1 H, m, ½C*H*$_2$N(CH$_3$)$_2$], 3.45–3.57 [4 H, m, ½C*H*$_2$NHCO, ½C*H*$_2$N(CH$_3$)$_2$, ½δCH$_2$(Pro^2) and ½δCH$_2$(Pro^4)], 3.68 [1 H, dd, J_1 12.5, J_2 4.5, ½δCH$_2$(Pro^4)], 3.82 [1 H, q, *J* 7.3, αCH(Thp^1)], 3.89–3.98 [3 H, m, ½C*H*$_2$NHCO, γCH(Pro^4) and ½δCH$_2$(Pro^2)], 4.23–4.33 [1 H, m, αCH(Ala^3)], 4.46 [1 H, d, *J* 10.5, αCH(Pro^4)], 4.55–4.62 [1 H, m, αCH(Pro^2)], 8.24 [1 H, d, *J* 6.8, NH(Ala^3)], 8.79 (1 H, br s, N*H*CH$_2$) and 9.16 [1 H, br s, N*H*(CH$_3$)$_2$]; δ_C(125.7 MHz; C^2HCl_3) 16.10 and 17.07 [βCH$_3$(Thp^1) and βCH$_3$(Ala^3)], 25.93 [γCH$_2$(Pro^2)], 26.84 [βCH$_2$(Pro^2)], 34.39 [βCH$_2$(Pro^4)], 35.00 (*C*H$_2$NHCO), 43.16 [γCH(Pro^4)], 43.82 [αCH(Thp^1)], 44.02 [NCH$_3$], 45.38 [NCH$_3$], 47.24 [δCH$_2$-(Pro^2)], 49.00 [αCH(Ala^3)], 51.81 [δCH$_2$(Pro^4)], 57.92 [*C*H$_2$-N(CH$_3$)$_2$], 62.37 [αCH(Pro^2) and αCH(Pro^4)], 116.12 (q, J_{CF} 289, *C*F$_3$CO$_2$), 160.88 (q, J_{CF} 38.5, CF$_3$*C*O$_2$), 173.43, 174.06 and 174.32 (4 × amide CO).

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