Endoannular Interactions in Cysteine-containing Cyclotripeptides: One-step Synthesis and Crystal Structure of a Tetracyclic Aza-cyclol [1]

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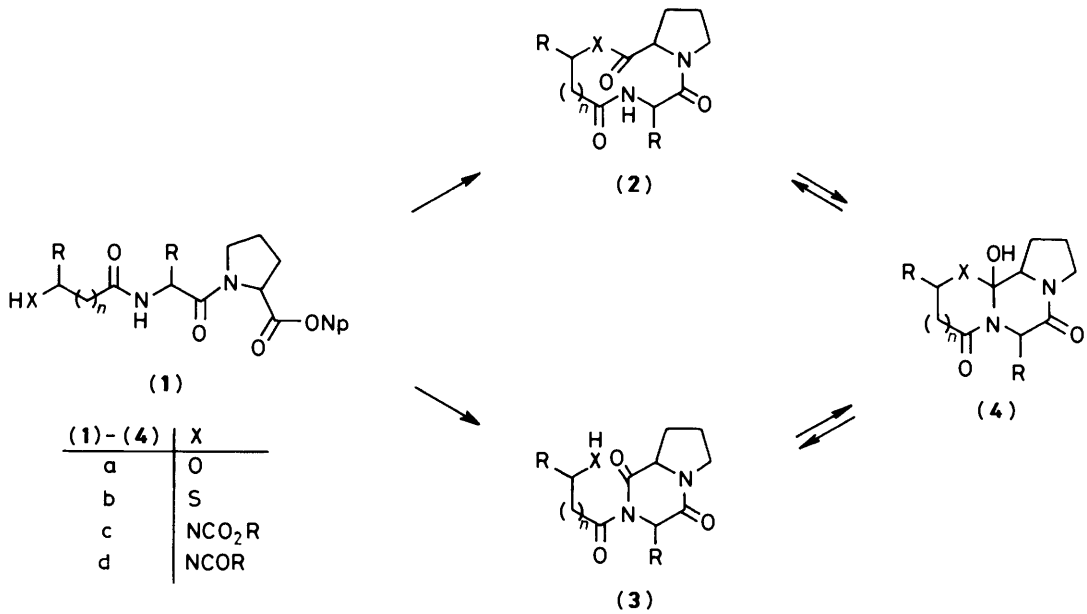
Mild unmasking of the thiol group of the linear tripeptide *N*-chloroacetyl-*S*-t-butylthio-L-cysteinyl-L-phenylalanyl-L-proline *p*-nitrophenyl ester (**8**) with tributylphosphine in aqueous solution at room temperature gave, in one step, the tetrahedral adduct (aza-cyclol) (**13**). The same adduct was obtained starting from (*R*)-5-oxothiomorpholin-3-ylcarbonyl-L-phenylalanyl-L-proline *p*-nitrophenyl ester (**12**). The conformationally rigid polycyclic skeleton stabilizes the structure of the aza-cyclol (**13**) which is an example of a tetrahedral adduct derived from amide–amide interaction. Relevant conformational details, as revealed by an *X*-ray crystallographic analysis, are: the proline ring adopts a C_s-C_β*endo* conformation; the phenylalanine side-chain is extended towards its nitrogen; the proline H_α and hydroxylic hydrogen atom are in the characteristic planar W conformation.

The potential of small cyclic peptides for studying conformation–spectral data and conformation–biological activity relationships is well known.[2] Unusual among small cyclic peptides in their chemistry, structure, and conformation, are cyclotripeptides.[3–6] These compounds, which are extensively used as molecular models, are stable and easily synthesized when the three junctions of the ring are tertiary amide (CONR) or lactone bonds. Homodetic and heterodetic nine-membered cyclotripeptides containing even a single secondary amide bond (CONH) in the ring represent, on the other hand, a special class of cyclopeptides. They can, in fact, undergo endoannular interactions leading to more stable tautomeric forms[4,7] or to dehydration products. This property offers a rare opportunity to isolate the biological relevant tetrahedral intermediates (cyclols)[8–10] derived from the addition of an amide NH to an amide or lactone carbonyl group.

Our previous research in the field of nine-membered homodetic cyclotripeptides (see Scheme 1) led to the first examples of tetrahedral intermediates (**4c**; $n = 0$)[7] characterized by chemical stability comparable to that shown by ergot peptides (**4a**; $n = 0$) and by their synthetic sulphur analogues (**4b**; $n = 0$).[11–12] These compounds (aza-cyclols) were obtained by cyclizing under mild conditions *N*-alkoxycarbonyltripeptides (**1c**; $n = 0$) containing carboxy-activated *C*-terminal proline. Investigations of the factors which stabilize the tetrahedral forms relative to the carbonyl forms revealed that, in addition to conformational and stereoelectronic effects,[13] the nature of the acylating group at the *N*-terminal nitrogen plays an important role. Thus, cyclization of linear tripeptides containing acylamino groups (**1d**; $n = 0$), instead of urethane protecting groups, affords tetrahedral adducts of lower stability which tend to equilibrate with the carbonyl tautomers.[3]

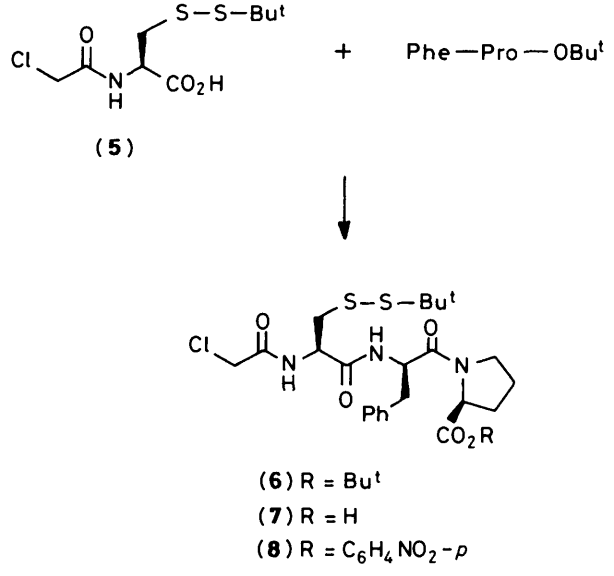
In order to investigate factors influencing formation and stability of (**4d**)-type adducts, intermediates of acyl-transfer reactions between amide NH, we started to examine the



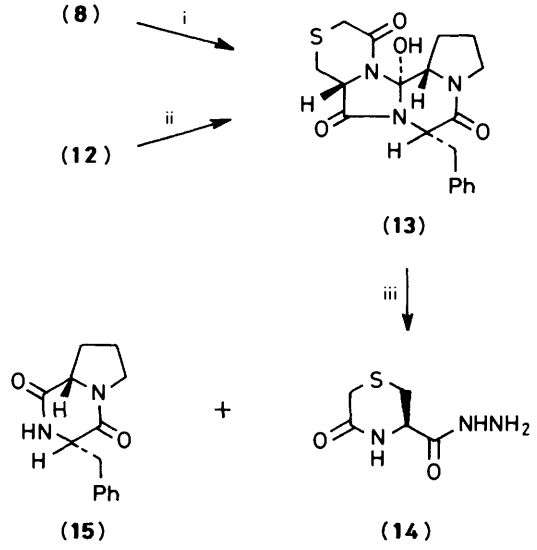
Scheme. 1

cyclization of β-substituted *N*-acyl dipeptides (**1**; $n = 1$), precursors of the less strained and conformationally more flexible 10-membered ring systems (**2**; $n = 1$).[14] In this context, linear precursors containing *N*-terminal α,β-disubstituted residues such as RCO-Cys-Xaa-Pro and RCO-Ser-Xaa-Pro, with different *N*-acylating groups, appeared of interest. Each of these models can give rise, in fact, to a variety of cyclization products derived from competing equilibria (Scheme 1) involving both 9- and 10-membered cyclotripeptides.[15] This should provide information on the preferred cyclization patterns as well as on the tendency of acylating groups to stabilize the adducts in different ring systems. In this paper we report on the synthesis and conformation of the tetracyclic tetrahedral intermediate (**13**) obtained by one-step cyclization of the polyfunctional linear precursor *N*-chloroacetyl-Cys(SBu^t)-Phe-Pro-ONp (**8**).

Compound (**8**) has been synthesized as shown in Scheme 2. In order to assure suitably mild reaction conditions the *S*-t-butylmercapto group was used to protect the cysteine thiol function. This protection can be selectively removed in an aqueous medium at room temperature without affecting the activation carboxylic acid. Cyclization and unmasking was thus performed at the same time by treating an aqueous propanol solution of (**8**) with $NaHCO_3$ and tributylphosphine[16] (Scheme 3). After 8 h at room temperature, a single non-halogenated cyclization product was isolated in 50% yield. On the basis of the spectral data, supported by an *X*-ray crystallographic analysis, structure (**13**) was assigned to this product. Particularly informative was the ^{13}C n.m.r. spectrum (Table 1) which showed only three amide carbonyl signals and a singlet centred at δ 96.11 p.p.m., characteristic of a quaternary carbon bonded to three heteroatoms. Compound (**13**) can be stored unchanged at room temperature for months; chromatographic analysis and n.m.r. spectra taken in $CDCl_3$ and $(CD_3)_2SO$ showed no evidence of tautomeric equilibria. Treatment of (**13**) with methanolic hydrazine hydrate gave 5-oxothiomorpholin-3-ylcarbohydrazide (**14**) and *cyclo*(-Phe-Pro-) (**15**) (Scheme 3).



Scheme 2.



Scheme 3.

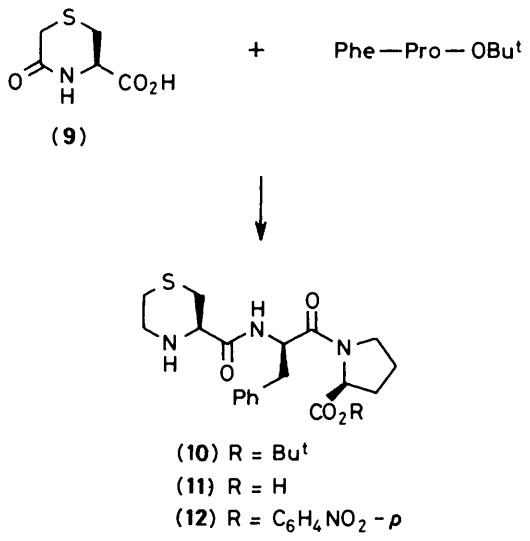
Table 1. ^1H N.m.r.[a] and ^{13}C n.m.r.[b] data for compounds (**13**) and (**14**)

		^1H N.m.r.		^{13}C N.m.r.	
Residue		(**13**)	(**14**)	(**13**)	(**14**)
Oxothiomorpholinecarboxylic acid	C*H*CO	4.15dd	4.29dd	59.8d	59.0d
	CH_2S	2.59dd (12,0, 12.0) 3.05dd (4.0, 12.0)	3.01dd (5.91, 13.7) 3.06dd (4.91, 13.7)	28.9t	28.6t
	C*H*$_2$CO	3.25, 3.35 ABq (15.0)	3.28, 3.35 ABq (17.1)	29.5t*	30.2t
	C*H*CO			163.8s*	169.6s*
	CH_2C*O*			164.4s*	171.1s*
Phenylalanine	C$_\alpha$H	4.80dd		54.8d	
	C$_\beta$H$_2$	3.05dd (11.2, 13.2) 3.55dd (5.0, 13.2)		39.0t	
	Ph	7.15—7.35m		137.0s 130.2d 128.1d 126.9d	
	CO			167.0s*	
Proline	C$_\alpha$H	3.70m		66.6d	
	C$_\beta$H$_2$ C$_\gamma$H$_2$	1.80—2.30m		29.5t* 22.0t	
	C$_\delta$H$_2$	3.58—3.65m		45.7t	
	C–OH	5.80s		96.1s	

[a] Chemical shifts in δ from $SiMe_4$ in $CDCl_3$ solution; *J*/Hz in parentheses. [b] Asterisked values may be interchanged.

The preferential formation of (**13**) appears to involve an intramolecular acylation of the Cys-Phe peptide bond by the activated proline carboxy group to give an intermediate *N*-(α-amidoacyl)oxopiperazine. The formation of a 6-membered ring and the presence of the *C*-terminal proline, which can readily form *cis*-amide bonds, favours the intermediacy of the *N*-acyldioxopiperazine. This compound may also result from an initially formed 10-membered thiolactone which, in turn, undergoes an intramolecular S to N acyl migration, possibly assisted by participation of the neighbouring chloroacetyl group. The subsequent step involves closure of the imidazolidinone 5-membered ring through stereospecific nucleophilic addition of the *N*-terminal amide NH to the proline carbonyl. The formation of the two above-described rings is accompanied by intramolecular alkylation of the SH by the chloroacetyl group, leading to the oxothiomorpholine entity.

In order to evaluate, at least in part, the sequence of reactions which converts (**8**) into the polycyclic adduct (**13**), we synthesized (Scheme 4) and subjected to cyclization the linear



Scheme 4.

precursor (**12**), containing the preformed oxothiomorpholine ring. By adopting reaction conditions analogous to those adopted for the active ester (**8**), compound (**13**) was isolated in 70% yield (Scheme 3). This result shows that the interaction of a lactam NH with an imide carbonyl is a highly efficient step and supports an early intramolecular alkylation of cysteine SH during the cyclization of (**8**).

Tetrahedral intermediates of *N*,*N*-acyl transfer reactions between amide groups find in the adduct (**13**) a model characterized by unusual stability. The features responsible for this property appear connected with the conformation imposed on the system by the annelation of the cysteine side-chain and with the low conformational flexibility of the resulting polycyclic system.

In order to acquire a detailed picture of the conformational features, an *X*-ray crystallographic analysis of (**13**) was undertaken. Figure 1 shows the molecular structure together with the numbering scheme. Table 2 reports the fractional atomic co-ordinates and Table 3 the bond lengths and valence angles for the non-hydrogen atoms. The mean values of bond distances involving hydrogen atoms are 0.972(1) Å, the mean values of angles are 100(3), 119.9(6), and 109.2(5)°, for C(sp^3), C(sp^2), and O carrier atoms, respectively. The conformation of the molecule is described by the torsion angles reported in Table 4.

Table 2. Atomic co-ordinates with their e.s.d.s in parentheses

	x	*y*	*z*
O(1)	0.516 4(3)	0.739 7(3)	0.153 9(1)
C(1)	0.587 0(4)	0.712 5(4)	0.126 3(1)
C(2)	0.750 2(4)	0.819 2(4)	0.116 2(1)
S(3)	0.858 7(1)	0.725 0(1)	0.122 11(4)
C(4)	0.756 5(4)	0.569 6(5)	0.084 8(1)
C(5)	0.616 2(3)	0.566 6(4)	0.067 2(1)
C(6)	0.507 5(4)	0.414 3(4)	0.048 0(1)
O(6)	0.529 1(3)	0.358 6(3)	0.015 5(1)
N(7)	0.382 9(3)	0.350 0(3)	0.074 0(1)
C(8)	0.249 3(4)	0.208 7(4)	0.062 9(1)
C(9)	0.104 7(4)	0.216 2(4)	0.069 8(1)
O(9)	−0.018 8(3)	0.101 8(3)	0.062 9(1)
N(10)	0.115 0(3)	0.343 0(3)	0.083 3(1)
C(11)	−0.018 2(4)	0.354 7(4)	0.093 8(1)
C(12)	0.046 5(4)	0.518 2(5)	0.105 0(1)
C(13)	0.204 5(4)	0.570 7(4)	0.121 5(1)
C(14)	0.256 3(3)	0.487 2(3)	0.091 1(1)
C(15)	0.376 5(3)	0.452 6(3)	0.106 3(1)
O(15)	0.343 6(2)	0.392 0(3)	0.148 4(1)
N(16)	0.528 8(3)	0.583 7(3)	0.102 0(1)
C(17)	0.237 7(4)	0.071 7(4)	0.087 9(1)
C(18)	0.334 8(4)	0.013 3(4)	0.068 9(1)
C(19)	0.481 3(5)	0.068 5(4)	0.082 8(1)
C(20)	0.569 7(5)	0.013 6(5)	0.064 8(1)
C(21)	0.506 2(6)	−0.102 1(6)	0.033 6(1)
C(22)	0.362 7(6)	−0.156 6(5)	0.020 1(1)
C(23)	0.275 4(5)	−0.100 6(4)	0.037 4(1)
O(40)	−0.134 3(4)	−0.134 3(4)	0.0000

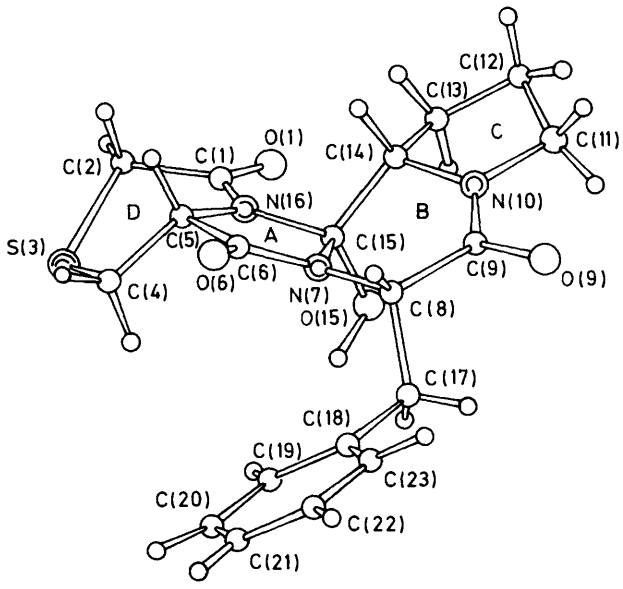


Figure. Molecular structure and atomic numbering scheme of the tetrahedral adduct (**13**)

The oxopiperazine B ring is characterized by a C_s symmetry corresponding to a sofa[17] conformation; the pseudo-mirror plane of the C_s symmetry passes through the carbonylic group and the cyclolic carbon atom C(15). This latter atom is 0.65 Å out of the best plane of the other ring atoms, on the same side of the benzylic side-chain. In the pyrrolidine C ring the C_β-Pro C(13) is 0.58 Å out of the plane formed by the other four ring atoms and on the same side as the OH group. This arrangement corresponds to the C_s-C_β endo envelope conformation usually found in the proline-containing cyclic dipeptides.[18,19] In the B

Table 3. (a) Bond lengths (Å) with e.s.d.s in parentheses

O(1)–C(1)	1.229(4)	N(10)–C(14)	1.488(6)
C(1)–C(2)	1.509(7)	C(11)–C(12)	1.506(8)
C(1)–N(16)	1.367(5)	C(12)–C(13)	1.520(7)
C(4)–C(5)	1.525(5)	C(13)–C(14)	1.529(4)
C(5)–C(6)	1.515(7)	C(14)–C(15)	1.521(4)
C(5)–N(16)	1.455(4)	C(15)–O(15)	1.390(4)
C(6)–O(6)	1.218(4)	C(15)–N(16)	1.476(6)
C(6)–N(7)	1.363(5)	C(17)–C(18)	1.515(5)
N(7)–C(8)	1.456(6)	C(18)–C(19)	1.384(7)
N(7)–C(15)	1.467(4)	C(18)–C(23)	1.397(7)
C(8)–C(9)	1.541(5)	C(19)–C(20)	1.399(6)
C(8)–C(17)	1.552(5)	C(20)–C(21)	1.402(9)
C(9)–O(9)	1.244(7)	C(21)–C(22)	1.35(1)
C(9)–N(10)	1.321(5)	C(22)–C(23)	1.389(6)
N(10)–C(11)	1.470(5)		

(b) Bond angles (°) with e.s.d.s in parentheses

O(1)–C(1)–C(2)	122.9(4)	N(10)–C(14)–C(13)	102.4(3)
O(1)–C(1)–N(16)	124.6(5)	N(10)–C(14)–C(15)	108.6(3)
C(2)–C(1)–N(16)	112.5(3)	C(13)–C(14)–C(15)	119.9(3)
C(5)–C(6)–O(6)	125.8(4)	N(7)–C(15)–C(14)	107.3(3)
C(5)–C(6)–N(7)	107.9(3)	N(7)–C(15)–O(15)	112.6(3)
O(6)–C(6)–N(7)	126.3(5)	N(7)–C(15)–N(16)	100.9(3)
C(6)–N(7)–C(8)	122.6(3)	C(14)–C(15)–O(15)	110.0(3)
C(6)–N(7)–C(15)	114.2(4)	C(14)–C(15)–N(16)	111.9(3)
C(8)–N(7)–C(15)	121.0(3)	O(15)–C(15)–N(16)	113.7(3)
N(7)–C(8)–C(9)	111.8(3)	C(1)–N(16)–C(5)	120.9(4)
N(7)–C(8)–C(17)	113.4(3)	C(1)–N(16)–C(15)	126.0(3)
C(9)–C(8)–C(17)	109.9(4)	C(5)–N(16)–C(15)	113.1(3)
C(8)–C(9)–O(9)	119.0(3)	C(8)–C(17)–C(18)	114.0(3)
C(8)–C(9)–N(10)	119.3(4)	C(17)–C(18)–C(19)	121.3(4)
O(9)–C(9)–N(10)	121.8(3)	C(17)–C(18)–C(23)	119.8(4)
C(9)–N(10)–C(11)	122.1(4)	C(19)–C(18)–C(23)	118.9(4)
C(9)–N(10)–C(14)	126.2(3)	C(18)–C(19)–C(20)	120.6(5)
C(11)–N(10)–C(14)	111.7(3)	C(19)–C(20)–C(21)	119.1(6)
N(10)–C(11)–C(12)	103.4(4)	C(20)–C(21)–C(22)	120.3(5)
C(11)–C(12)–C(13)	105.6(3)	C(21)–C(22)–C(23)	120.7(5)
C(12)–C(13)–C(14)	102.2(3)	C(18)–C(23)–C(22)	120.3(5)

and C rings the root mean square deviation from the ideal C_s symmetry is $\Delta C_s = 4.1$ and 6.4°, respectively. The imidazolidinone A ring adopts a rather flattened envelope conformation with the mirror plane of the C_s symmetry passing through the phenylalanine N(7) and the middle point of cysteine N(16)–C(5) bond. The distance of N(7) from the best plane of the other ring atoms is only 0.14 Å, puckered towards the OH group. The thiomorpholine D ring has a slightly distorted boat conformation with the Cys-H_α in an axial orientation. The angle between the best planes formed by C(5), N(16), C(1), C(2) and C(5), C(4), S(3), C(2) is 124.1°.

The benzylic side-chain adopts a conformation extended towards the Phe nitrogen N(7). An extended conformation is also preferred in chloroform solution, as shown by measured values of the $^3J(C_\alpha H\text{-}C_\beta H_2)$ coupling constants (11.2 and 5.0 Hz) relative to the Phe residue (Table 1). The four bonds connecting the Pro-H_α to the hydroxylic hydrogen, through C(14), C(15), and O(15) atoms, lie approximately on the same plane in a characteristic W conformation.[20]

In the crystal the atoms O(15) and O(1) of different molecules, related by two-fold screw axes, are involved in hydrogen bonds (2.91 Å), which form infinite helices perpendicular to the three-fold screw axes. The oxygen of the water solvent molecule lies on a binary axis and links, by hydrogen bonds (2.84 Å), the O(9) atoms of the molecules related by this symmetry.

Experimental

M.p.s were determined on a Kofler hot-stage and are uncorrected. I.r. spectra were recorded with a Perkin-Elmer 521 spectrophotometer. ^{1}H N.m.r. spectra were recorded on a Bruker WP-200 instrument for compounds (**13**) and (**14**); on a Varian EM-390 for the other compounds. ^{13}C N.m.r. spectra were recorded on a Bruker WP-200 operating at 50.28 MHz. The mass spectrum of (**13**) was determined with a Hewlett-Packard 5980 A spectrometer operating at 70 eV. Optical rotations were taken at 25 °C with a Schmidt-Haensch 16065 polarimeter.

Table 4. Selected torsion angles (°) with e.s.d.s in parentheses

Within the rings	
N(16)–C(5)–C(6)–N(7)	−7.3(4)
C(5)–C(6)–N(7)–C(15)	11.1(4)
C(6)–N(7)–C(15)–N(16)	−9.6(4)
N(7)–C(15)–N(16)–C(5)	4.4(4)
C(15)–N(16)–C(5)–C(6)	1.4(4)
N(7)–C(8)–C(9)–N(10)	1.5(4)
C(8)–C(9)–N(10)–C(14)	3.0(4)
C(9)–N(10)–C(14)–C(15)	−31.7(4)
N(10)–C(14)–C(15)–N(7)	52.9(3)
C(14)–C(15)–N(7)–C(8)	−55.6(3)
C(15)–N(7)–C(8)–C(9)	27.0(4)
N(10)–C(14)–C(13)–C(12)	−34.1(3)
C(14)–C(13)–C(12)–C(11)	38.5(3)
C(13)–C(12)–C(11)–N(10)	−26.8(3)
C(12)–C(11)–N(10)–C(14)	4.6(3)
C(11)–N(10)–C(14)–C(13)	18.9(3)
N(16)–C(5)–C(4)–S(3)	−47.7(3)
C(5)–C(4)–S(3)–C(2)	−5.8(3)
C(4)–S(3)–C(2)–C(1)	56.0(3)
S(3)–C(2)–C(1)–N(16)	−54.1(3)
C(2)–C(1)–N(16)–C(5)	−6.4(5)
C(1)–N(16)–C(5)–C(4)	63.4(4)
Between the rings	
C(6)–N(7)–C(15)–C(14)	107.6(3)
C(8)–N(7)–C(15)–N(16)	−172.8(3)
C(9)–N(10)–C(14)–C(13)	−159.4(3)
C(11)–N(10)–C(14)–C(15)	146.6(3)
C(4)–C(5)–N(16)–C(15)	−117.9(3)
C(6)–C(5)–N(16)–C(1)	−177.2(3)
Side-chain	
C(6)–N(7)–C(8)–C(17)	100.3(4)
N(7)–C(8)–C(17)–C(18)	−79.2(3)
C(8)–C(17)–C(18)–C(19)	89.4(4)
N(10)–C(9)–C(8)–C(17)	128.4(3)
C(9)–C(8)–C(17)–C(18)	154.8(2)
C(8)–C(17)–C(18)–C(23)	−91.4(4)
Others	
C(5)–C(6)–N(7)–C(8)	174.0(3)
C(6)–N(7)–C(8)–C(9)	−134.8(3)
N(10)–C(14)–C(15)–N(16)	162.7(2)
C(14)–C(15)–N(16)–C(5)	−109.4(3)

N-*Chloroacetyl*-S-*t-butylthio*-L-*cysteine* (**5**).—*N*-Methylmorpholine (2.0 g, 20 mmol) and chloroacetyl chloride (2.25 g, 20 mmol) were added to a stirred solution of *S*-t-butylthio-L-cysteine (4.2 g, 20 mmol) in dry tetrahydrofuran (25 ml) at 0 °C. After 2 h at room temperature, the reaction mixture was evaporated under reduced pressure and the residue taken up in saturated aqueous sodium carbonate. The aqueous solution was washed with ethyl acetate, acidified, and extracted with ethyl acetate. The extract was then dried and evaporated, and the residue (5.1 g) crystallized from ether to give the *title compound* (**5**) (4.0 g, 70%), m.p. 119—120 °C (Found: C, 37.9; H, 5.55; Cl, 12.25; N, 4.9; S, 22.55. $C_9H_{16}ClNO_3S_2$ requires C, 37.8; H, 5.6; Cl, 12.4; N, 4.9; S, 22.5%); $[\alpha]_D$ −58.0° (*c* 1.00 in $CHCl_3$); $\nu_{max.}$($CHCl_3$) 3 390br, 1 735, 1 675, and 1 520 cm^{-1}; δ_H[$(CD_3)_2SO$] 1.25 (9 H, s, 3 × Me), 3.1 (2 H, m, Cys-$C_\beta H_2$), 4.1 (2 H, s, CH_2Cl), 4.5 (1 H, m, Cys-$C_\alpha H$), and 8.65 (1 H, d, *J* 7.5 Hz, NH).

N-*Chloroacetyl*-S-*t-butylthio*-L-*cysteinyl*-L-*phenylalanyl*-L-*proline t-Butyl Ester* (**6**).—Isobutyl chloroformate (2.1 g, 15.3 mmol) and *N*-methylmorpholine (1.9 g, 19 mmol) were added with stirring to a solution cooled to −15 °C of compound (**5**) (4.4 g, 15.3 mmol) in tetrahydrofuran (15 ml) and methylene dichloride (15 ml). Stirring was continued at −10 °C for 10 min when a cold solution containing L-phenylalanyl-L-proline t-butyl ester trifluoroacetate (6.6 g, 15.4 mmol) and *N*-methylmorpholine (1.55 g, 15.4 mmol) in methylene dichloride (400 ml) was added. After 5 h at 0 °C, the reaction mixture was evaporated under reduced pressure and the residue taken up in ethyl acetate. Work-up gave an oily residue which was purified by column chromatography on silica gel using ether as eluant, to give the *t-butyl ester* (**6**) (3.5 g, 39%), m.p. 141—142 °C (from ether) (Found: C, 55.25; H, 6.8; Cl, 6.0; N, 7.1; S, 10.95. $C_{27}H_{40}ClN_3O_5S_2$ requires C, 55.3; H, 6.9; Cl, 6.05; N, 7.2; S, 10.9%); $[\alpha]_D$ −84.0° (*c* 1.00 in $CHCl_3$); $\nu_{max.}$($CHCl_3$) 3 380, 1 730, 1 670, 1 645, and 1 495 cm^{-1}; δ_H($CDCl_3$) 1.3 (9 H, s, S-Bu^t), 1.45 (9 H, s, O-Bu^t), 1.8—2.3 (4 H, m, Pro-$C_\beta H_2$ and Pro-$C_\gamma H_2$), 2.8—3.2 (4 H, m, Phe-$C_\beta H_2$ and Cys-$C_\beta H_2$), 3.6—3.9 (2 H, m, Pro-$C_\delta H_2$), 4.1 (2 H, s, CH_2Cl), 4.5—5.1 (3 H, m, Cys-$C_\alpha H$, Pro-$C_\alpha H$, and Phe-$C_\alpha H$), and 7.1—7.4 (7 H, m, ArH and 2 × NH).

N-*Chloroacetyl*-S-*t-butylthio*-L-*cysteinyl*-L-*phenylalanyl*-L-*proline* p-*Nitrophenyl Ester* (**8**).—The foregoing t-butyl ester (**6**) (2.4 g, 4.1 mmol) was treated with trifluoroacetic acid (35 ml) for 1 h at room temperature. The mixture was then evaporated under reduced pressure and the resulting tripeptide acid (**7**) (1.95 g, 3.7 mmol) was dissolved in methylene dichloride (50 ml). *p*-Nitrophenol (0.5 g, 3.7 mmol) and dicyclohexylcarbodi-imide (0.8 g, 3.7 mmol) were added at 0 °C with stirring to the solution. After 1 h at 0 °C and 12 h at room temperature, the reaction mixture was filtered and the filtrate repeatedly washed with saturated aqueous sodium carbonate and water, dried and evaporated. The residue was chromatographed on silica gel using ether as eluant to give pure *active ester* (**8**) as pale yellow oil (1.4 g, 58%) (Found: C, 53.7; H, 5.5; Cl, 5.2; N, 8.6; S, 10.1. $C_{29}H_{35}ClN_4O_7S_2$ requires C, 53.5; H, 5.4; Cl, 5.4; N, 8.6; S, 9.85%); $[\alpha]_D$ −90.0° (*c* 1.00 in $CHCl_3$); $\nu_{max.}$($CHCl_3$) 3 380br, 1 770, 1 650, and 1 500 cm^{-1}; δ_H($CDCl_3$) 1.3 (9 H, s, 3 × Me), 1.8—2.3 (4 H, m, Pro-$C_\beta H_2$ and Pro-$C_\gamma H_2$), 2.9—3.3 (4 H, m, Cys-$C_\beta H_2$ and Phe-$C_\beta H_2$), 3.6—3.9 (2 H, m, Pro-$C_\delta H_2$), 4.05 (2 H, s, CH_2Cl), 4.6—5.1 (3 H, m, Cys-$C_\alpha H$, Phe-$C_\alpha H$, and Pro-$C_\alpha H$), 7.1—7.5 (9 H, m, ArH, Phe-NH, and Cys-NH), and 8.3 (2 H, two lines, ArH).

Cyclization of the Active Ester (**8**).—Tributylphosphine (0.4 g, 2.0 mmol) and aqueous 0.1M sodium hydrogen carbonate (50 ml) were added under nitrogen to a solution of the active ester (**8**) (1.35 g, 2.1 mmol) in a mixture of the active ester (**8**) (1.35 g, 2.1 mmol) in a mixture of propanol (500 ml) and water (250 ml). After 8 h at room temperature, the solution was evaporated under reduced pressure and the residue, taken up in chloroform, was washed with saturated aqueous sodium carbonate and water, dried and evaporated to afford an oily residue (1.7 g). Purification of this by column chromatography on silica gel with ethyl acetate–methanol (9:1) as eluant followed by crystallization from ethyl acetate afforded *compound* (**13**) (0.4 g, 50%), m.p. 211—214 °C (Found: C, 57.5; H, 5.7; N, 10.55; S, 7.9. $C_{19}H_{21}N_3O_4S$·0.5H_2O requires C, 57.55; H, 5.6; N, 10.6; S, 8.1%); $[\alpha]_D$ −118.0° (*c* 0.70 in $CHCl_3$); $\nu_{max.}$($CHCl_3$) 3 430br, 1 730, and 1 650 cm^{-1}; *m/z* 387 (M^+, 19%), 369 (47), 250 (73), 244 (14), 153 (10), 125 (25), and 91 (100). Treatment with methanolic hydrazine followed by purification by preparative t.l.c. afforded 5-oxothiomorpholin-3-ylcarbohydrazide (**14**) (see Table 1) and *cyclo*(-Phe-Pro-) (**15**).[21]

(R)-5-*Oxothiomorpholine*-3-*carboxylic Acid* (**9**).—Bromoacetyl bromide (6.2 g, 40 mmol) and saturated aqueous sodium carbonate (10 ml) were added under nitrogen to a stirred solution of L-cysteine hydrochloride monohydrate (3.5 g, 20 mmol) in saturated aqueous sodium carbonate (10 ml). After 12 h at room temperature, the reaction mixture was acidified with 6M hydrochloric acid and evaporated under reduced pressure; the residue was then repeatedly taken up in methanol and the solution filtered. Evaporation of the filtrate provided a residue which was subjected to column chromatography [silica gel; ethyl acetate–methanol (9:1) as eluant] to give the title acid (**9**) (0.5 g, 15%) as an oil. This was characterized as its methyl ester after treatment with diazomethane; m.p. 70—71 °C (from ether) (Found: C, 41.1; H, 5.1; N, 8.0; S, 18.3. $C_6H_9NO_3S$ requires C, 41.1; H, 5.15; N, 8.0; S, 18.3%); $[\alpha]_D$ −4.0° (*c* 1.00 in $CHCl_3$); $\nu_{max.}$($CHCl_3$) 3 380, 1 745, and 1 670 cm^{-1}; δ_H[$(CD_3)_2SO$] 2.8—3.3 (4 H, m, CH_2O and CH_2CH), 3.7 (3 H, s, OMe), 4.4 (1 H, dd, CH), and 8.0 (1 H, d, *J* 3.5 Hz, NH).

(R)-5-*Oxothiomorpholin*-3-*ylcarbonyl*-L-*phenylalanyl*-L-*proline-t-Butyl Ester* (**10**).—Compound (**9**) (0.9 g, 5.5 mmol) was dissolved in a mixture of dimethylformamide (5 ml) and methylene dichloride (10 ml) and treated at 0 °C with 1-hydroxybenzotriazole (1.5 g, 10.7 mmol) and dicyclohexylcarbodi-imide (1.1 g, 5.5 mmol) with stirring. After addition of a cold solution containing L-phenylalanyl-L-proline t-butyl ester trifluoroacetate (2.35 g, 5.5 mmol) and *N*-methylmorpholine (0.55 g, 5.5 mmol) in methylene dichloride (150 ml), the mixture was stirred for 18 h at room temperature. It was then filtered and the filtrate evaporated under reduced pressure. Work-up gave a residue which, after purification by column chromatography [silica gel, ethyl acetate–methanol (95:5) as eluant], gave the *t-butyl ester* (**10**) (1.2 g, 47%) as an oil (Found: C, 59.7; H, 6.8; N, 9.0; S, 6.4. $C_{23}H_{31}N_3O_5S$ requires C, 59.85; H, 6.75; N, 9.1; S, 6.5%); $[\alpha]_D$ −81.0° (*c* 1.00 in $CHCl_3$); $\nu_{max.}$($CHCl_3$) 3 380, 1 730, 1 670, and 1 510 cm^{-1}; δ_H($CDCl_3$) 1.45 (9 H, s, 3 × Me), 1.8—2.3 (4 H, m, Pro-$C_\beta H_2$ and Pro-$C_\gamma H_2$), 2.8—3.2 (4 H, m, Phe-$C_\beta H_2$ and $CHCH_2S$), 3.3 (2 H, ABq, CH_2S), 3.4—3.8 (2 H, m, Pro-$C_\delta H_2$), 4.25 (1 H, m, $CHCH_2S$), 4.5 (1 H, m, Pro-$C_\alpha H$), 5.1 (1 H, m, Phe-$C_\alpha H$), 7.3 (5 H, m, ArH), 8.2 (1 H, d, *J* 7.5 Hz, Phe-NH), and 8.3 (1 H, d, *J* 6.0 Hz, Cys-NH).

(R)-5-*Oxothiomorpholin*-3-*ylcarbonyl*-L-*phenylalanyl*-L-*proline* p-*Nitrophenyl Ester* (**12**).—This compound was prepared by following the procedure reported for the preparation of the active ester (**8**). Deprotection of the t-butyl ester (**10**) (1.1 g, 2.4 mmol) with trifluoroacetic acid (20 ml) afforded the peptide acid (**11**) in 92% yield. Treatment of compound (**11**) (0.9 g, 2.2 mmol) with *p*-nitrophenol (0.3 g, 2.2 mmol) and dicyclohexylcarbodi-imide (0.45 g, 2.2 mmol) in tetrahydrofuran (20 ml) gave an oily residue which was

chromatographed on silica gel [ethyl acetate–methanol (95:5) as eluant] to give the *title compound* (**12**) as a pale yellow oil (0.7 g, 60%) (Found: C, 57.0; H, 5.1; N, 10.45; S, 6.0. $C_{25}H_{26}N_4O_7S$ requires C, 57.0; H, 4.95; N, 10.6; S, 6.1%); $[\alpha]_D$ −88.0° (*c* 1.00 in $CHCl_3$); $\nu_{max.}(CHCl_3)$ 3 380, 1 770, 1 670, and 1 515 cm^{-1}; $\delta_H(CDCl_3)$ 1.8—2.3 (4 H, m, Pro-$C_\beta H_2$ and Pro-$C_\gamma H_2$), 2.8—3.2 (4 H, m, Phe-$C_\beta H_2$ and CHCH_2S), 3.3 (2 H, ABq, CH_2S), 3.6—3.9 (2 H, m, Pro-$C_\delta H_2$), 4.2 (1 H, m, CHCH_2S), 4.6 (1 H, m, Pro-C_αH), 5.0 (1 H, m, Phe-C_αH), 7.25 (7 H, m, ArH), and 8.1—8.5 (4 H, m, ArH and 2 × NH).

Cyclization of the Active Ester (**12**).—Aqueous 0.1M sodium hydrogen carbonate (10 ml) was added to a stirred solution of the active ester (**12**) (0.35 g, 0.65 mmol) in a mixture of propanol (100 ml) and water (50 ml). After 18 h at room temperature, the reaction mixture was worked up as described for the cyclization of the active ester (**8**) to give, after crystallization from ethyl acetate, *compound* (**13**) (0.18 g, 70%), m.p. 211—213 °C (Found: C, 57.5; H, 5.65; N, 10.5; S, 8.0. $C_{19}H_{21}N_3O_4S{\cdot}0.5H_2O$ requires C, 57.55; H, 5.6; N, 10.6; S, 8.1%); $[\alpha]_D$ −121.0° (*c* 0.7 in $CHCl_3$).

X-*Ray Structure Determination of the Tetrahedral Adduct* (**13**).—Suitable single crystals of compound (**13**) were grown from ethyl acetate. The intensity data were measured at room temperature with a Nicolet P3 automatic diffractometer equipped with graphite monochromator and Mo-K_α radiation in ω-scan mode.

Crystal data. $C_{19}H_{21}N_3O_4S{\cdot}0.5H_2O$, M = 396.46, trigonal, a = 10.287(3), c = 30.432(8) Å, V = 2 789 Å^3, Mo-K_α, λ = 0.710 69 Å, space group $P3_121$, Z = 6, D_x = 1.42 g cm^{-3}, μ(Mo-K_α) = 2.1 cm^{-1}, colourless crystal of dimension 0.5 × 0.3 × 0.2 mm. Three standard reflections, measured every 100, revealed no significant *X*-ray damage of the crystal. The data collections were carried out with the following experimental conditions: Δω = ±0.7°, $(\sin\theta/\lambda)_{max.}$ = 0.63° Å^{-1}, scan speed 0.7—29.3° min^{-1} according to the intensity, counting time for background equal to a half of the scanning time. Of the 2 361 unique reflections measured, 2 016 had $I \geqslant 2\sigma(I)$, and these were considered observed and used for the structure elucidation. Lorentz and polarization corrections were applied to the data, but intensities were not corrected for extinction or absorption effects.

The structure was solved by multisolution direct methods with SIR-CAOS,[22] using the 300 *E*'s with largest values. The atomic parameters were refined by block-diagonal (9 × 9) least-squares calculations, minimizing the quantity $w(|F_o| - |F_c|)^2$ with $w = (a + |F_o| + b|F_o|^2)^{-1}$. Parameters a and b were given values of $2F_o$(min.) and $2/F_o$(max.), respectively, in order to obtain $< w(|F_o| - |F_c|)^2 >$ nearly constant in the ranges of F_o and $\sin\theta/\lambda$. Difference Fourier synthesis of electron density, computed at the end of the anisotropic refinement, showed all the hydrogen atoms in stereochemically feasible positions. The co-ordinates of the hydrogen atoms, with isotropic thermal parameters equal to those of the carrier atoms, were included and kept fixed in the last few cycles of least-squares calculations. The refinement converged to R = 0.039 and R_w = 0.056.* At the convergence the shift-to-error ratios were less than 0.1 for all refined parameters. All the calculations were performed on a Data General MW 8000, using the crystallographic package SIR-CAOS; atomic scattering factors were taken from the literature.[23]

* Tables of isotropic and anisotropic thermal parameters for non-hydrogen atoms and of atomic co-ordinates for the hydrogen atoms are available on request from the Cambridge Crystallographic Data Centre.†

† See 'Instructions for Authors (1988),' *J. Chem. Soc., Perkin Trans. 1*, 1988, Issue 1.

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