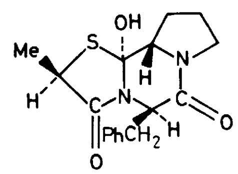
Peptidic Thiacyclols. Synthesis and Structural Studies[1]

Giancarlo Zanotti, Francesco Pinnen, and Gino Lucente *
Centro di Studio per la Chimica del Farmaco del CNR, and Istituto di Chimica Farmaceutica dell'Università di Roma, P. le A. Moro, 00185 Roma, Italy
Silvio Cerrini, Walter Fedeli, and Fernando Mazza
Istituto di Strutturistica Chimica 'G. Giacomello' CNR, C.P.n. 10 00016 Monterotondo Stazione, Roma, Italy

Deprotection with tri-n-butylphosphine in aqueous medium of 2-t-butyldithiopropionyl-L-phenylalanyl-L-proline *p*-nitrophenyl ester gives stable thiacyclols. These compounds are isomeric with nine-membered peptidic thiolactones and possess the same stereochemistry at the phenylalanine and proline chiral centres as found in natural oxacyclols (ergot alkaloids). Spectroscopic properties and an *X*-ray crystallographic analysis are reported. Crystals of the thiacyclol (**14**) are orthorhombic with $a = 16.462(7)$, $b = 15.763(7)$, $c = 6.487(4)$ Å, $Z = 4$, space group $P2_12_12_1$.

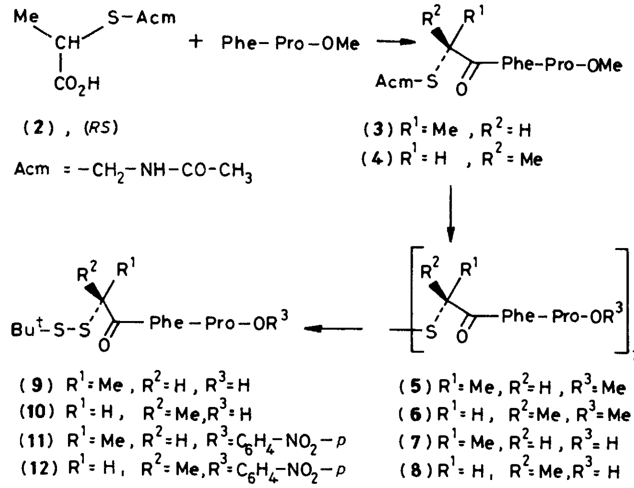
It is well established that the stability of peptidic cyclols, isomeric with homodetic and heterodetic nine-membered cyclotripeptides, is strongly dependent on the nature, configuration, and sequence of the residues of the three α-substituted acids involved.[2–8] In an earlier paper[9] we examined the synthesis of thiacyclolic peptides, isomeric with nine-membered cyclothiodepsipeptides (peptidic thiolactones). By following the route of mercaptoacyl incorporation into the ring amide bond of a diketopiperazine, activated by *N*-acylation with 2-mercaptopropionic acid, the stable thiacyclol (1) was isolated. Compound (1) is a sulphur-analogue of the natural peptidic oxacyclols



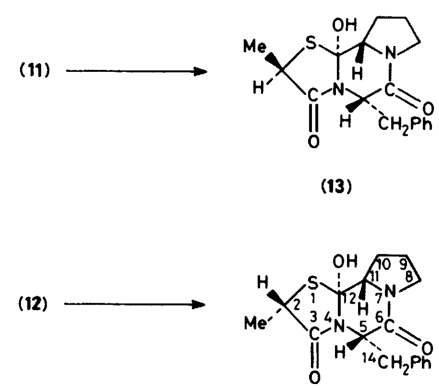
(1)

(ergot alkaloids) from which it differs since it contains proline and phenylalanine residues with opposite absolute configurations. The stereochemistry of the thiacyclol (1) reflects the properties of the key intermediate (namely an *N*-acyl-*trans*-diketopiperazine) used in the first synthetic approach.[9] It is well known in fact that the *cis*-isomers of proline containing diketopiperazines can easily epimerize to give the more stable *trans*-isomers and that *N*-acylation strongly favours this process.[2] The more-stable folded conformers (with the aromatic side chain bent over the diketopiperazine ring)[10] are formed by *trans*- and not by *cis*-isomers.[11] In thiacyclol (1) the folded conformation found in the parent *trans*-diketopiperazine and, presumably, the corresponding stabilization, are retained.

The synthesis of stable peptidic thiacyclols possessing the same absolute configuration at the phenylalanine and proline chiral centres, as found in the natural oxacyclols, has now been realized through direct cyclization, under mild conditions, of 2-mercaptopropionyl-L-phenylalanyl-L-proline *p*-nitrophenyl esters. The strategy for the synthesis of these linear precursors required the use of a thiol protecting group, selectively removable under mild non-oxidative conditions, so as to avoid any involvement of the activated carboxy function and of the cyclization products. The t-butylthio group, initially considered suitable, was later discarded owing to the difficulties encountered during the synthesis of 2-t-butyldithiopropionic acid. The acetamidomethyl group, because it could be removed by mercury(II) ions, was then considered and adopted



Scheme 1.



Scheme 2.

in a synthetic sequence which led to 2-acetamidomethylthiopropionyl-Phe-Pro-ONp. Mercury(II) ions gave incomplete deprotection of this active ester and complex mixtures resulted. The problem was solved by using the acetamidomethyl group during the early steps of the synthesis and by changing to the t-butylthio group in the last steps. The synthesis of the two diastereoisomeric *S*-protected active esters (**11**) and (**12**) used as linear precursors for cyclization reactions, is reported in Scheme

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

Residue		^{1}H N.m.r.	^{13}C N.m.r.
2-Mercaptopropionic acid	Me	1.56d (7.0)	21.52q
	$C_\alpha H$	3.67q (7.0)	43.44d
	C=O		170.72s*
Phenylalanine	Ph	7.12—7.25m	139.92s
			130.30d
			128.72d
			126.96d
	$C_\alpha H$	4.69dd	57.68d
	$C_\beta H_A$	3.23dd (6.5; 13.5)	34.16t
	$C_\beta H_B$	3.47dd (3.5; 13.5)	
	C=O		166.00s*
Pyrrolidine	$C_\alpha H$	3.58m[c]	69.04d
	$C_\beta H_2$	1.60—2.05m	27.52t
	$C_\gamma H_2$		22.24t
	$C_\delta H_2$	3.41m	47.12t
	C–OH	1.29d (1.8)	91.68s

[a] Chemical shifts are δ values from $SiMe_4$; solvent $CDCl_3$; J/Hz in parentheses. [b] Chemical shifts in δ/p.p.m. downfield from $SiMe_4$; solvent $(CD_3)_2SO$; asterisked values may be interchanged. [c] This multiplet turns into a double doublet by irradiation at δ 1.29.

Table 2. Final fractional co-ordinates of the non-hydrogen atoms with e.s.d.s in parentheses for the thiacyclol (**14**)

Atom	x/a	y/b	z/c
S(1)	0.0669(2)	0.1985(2)	−0.0014(6)
C(2)	−0.0343(10)	0.2131(9)	0.0940(30)
C(3)	−0.0500(8)	0.1480(7)	0.2619(25)
N(4)	0.0158(6)	0.0969(6)	0.2867(17)
C(5)	0.0152(6)	0.0359(7)	0.4609(19)
C(6)	0.0928(7)	0.0421(9)	0.5977(19)
N(7)	0.1486(6)	0.0987(6)	0.5423(14)
C(8)	0.2303(8)	0.1037(10)	0.6435(24)
C(9)	0.2766(9)	0.1706(11)	0.5267(35)
C(10)	0.2367(8)	0.1734(9)	0.3187(26)
C(11)	0.1454(8)	0.1580(7)	0.3642(20)
C(12)	0.0930(6)	0.1181(6)	0.1963(20)
C(13)	−0.0974(13)	0.2041(24)	−0.0772(39)
C(14)	0.0107(8)	−0.0573(7)	0.3907(20)
C(15)	−0.0623(7)	−0.0716(7)	0.2424(20)
C(16)	−0.0483(8)	−0.0970(8)	0.0396(22)
C(17)	−0.1146(12)	−0.1139(11)	−0.0947(26)
C(18)	−0.1918(10)	−0.1010(10)	−0.0214(34)
C(19)	−0.2062(7)	−0.0776(10)	0.1772(32)
C(20)	−0.1400(9)	−0.0614(9)	0.3080(20)
O(1)	−0.1126(5)	0.1421(7)	0.3558(23)
O(2)	0.1001(6)	−0.0048(6)	0.7402(14)
O(3)	0.1327(5)	0.0498(5)	0.1170(14)

1. Since tri-n-butylphosphine was shown to be a powerful and specific agent for the cleavage of the disulphide bonds under mild conditions,[12] the active esters (**11**) and (**12**) were treated (Scheme 2) at room temperature with a small excess (1.5 mol) of tri-n-butylphosphine in a dilute (3.10^{-3} mol l^{-1}) water–n-propanol solution. T.l.c. examination of the reaction mixtures showed that, after 3 days at room temperature, the active esters disappeared. Removal of the n-propanol followed by fractionation, afforded a mixture from which thiacyclols (**13**) and (**14**) could be isolated by column chromatography in 11 and 20% yields respectively. Both cyclols are high-melting crystalline compounds which are cleaved by treatment at room temperature with methanolic hydrazine hydrate, affording *cyclo*-(Phe-Pro).

The cyclolic structure assigned to (**13**) and (**14**) is based on spectroscopic data and for compound (**14**) is supported by an *X*-ray crystallographic analysis. The i.r. spectra ($CHCl_3$) show broad OH absorption centred at 3 250 cm $^{-1}$ and two carbonyl bands at 1 680 and 1 650 cm^{-1}; there was no absorption associated with amide II or a sulphur–hydrogen bond. ^{1}H N.m.r. spectra of the two cyclols are almost superimposable. The exchangeable proton of (**14**) appears as a doublet long-

Table 3. Bond angles (°) with e.s.d.s in parentheses for the thiacyclol (**14**)

C(2)–S(1)–C(12)	93.7(0.7)	C(8)–C(9)–C(10)	104.5(1.3)
S(1)–C(2)–C(3)	108.6(1.0)	C(9)–C(10)–C(11)	104.4(1.2)
S(1)–C(2)–C(13)	111.6(1.4)	N(7)–C(11)–C(10)	102.3(1.0)
C(3)–C(2)–C(13)	110.1(1.6)	N(7)–C(11)–C(12)	108.4(0.9)
C(2)–C(3)–N(4)	110.5(1.2)	C(10)–C(11)–C(12)	118.5(1.1)
C(2)–C(3)–O(1)	124.2(1.3)	S(1)–C(12)–N(4)	103.7(0.7)
N(4)–C(3)–O(1)	125.2(1.3)	S(1)–C(12)–C(11)	110.1(0.7)
C(3)–N(4)–C(5)	118.0(1.0)	S(1)–C(12)–O(3)	113.0(0.9)
C(3)–N(4)–C(12)	121.2(1.0)	C(11)–C(12)–N(4)	107.8(1.0)
C(5)–N(4)–C(12)	117.8(0.9)	C(11)–C(12)–O(3)	108.9(0.9)
N(4)–C(5)–C(6)	112.8(0.9)	O(3)–C(12)–N(4)	113.3(0.9)
N(4)–C(5)–C(14)	113.1(1.0)	C(5)–C(14)–C(15)	111.1(0.9)
C(6)–C(5)–C(14)	105.6(0.9)	C(14)–C(15)–C(16)	119.9(1.1)
C(5)–C(6)–N(7)	117.0(1.0)	C(14)–C(15)–C(20)	121.1(1.2)
C(5)–C(6)–O(2)	119.1(1.1)	C(16)–C(15)–C(20)	119.1(1.2)
N(7)–C(6)–O(2)	123.9(1.1)	C(15)–C(16)–C(17)	120.3(1.3)
C(6)–N(7)–C(8)	122.5(1.0)	C(16)–C(17)–C(18)	118.1(1.6)
C(6)–N(7)–C(11)	127.4(1.0)	C(17)–C(18)–C(19)	122.0(1.6)
C(8)–N(7)–C(11)	109.8(1.0)	C(18)–C(19)–C(20)	119.1(1.3)
N(7)–C(8)–C(9)	105.7(1.2)	C(19)–C(20)–C(15)	121.3(1.3)

Table 4. Relevant torsion angles (δ)* with e.s.d.s in parentheses for compound (**14**)

Ring A	
C(12)–S(1)–C(2)–C(3)	−6.7(1.1)
S(1)–C(2)–C(3)–N(4)	−1.6(1.5)
C(2)–C(3)–N(4)–C(12)	13.1(1.7)
C(3)–N(4)–C(12)–S(1)	−17.3(1.4)
N(4)–C(12)–S(1)–C(2)	12.4(0.9)
Ring B	
C(12)–N(4)–C(5)–C(6)	32.5(1.4)
N(4)–C(5)–C(6)–N(7)	−0.7(1.5)
C(5)–C(6)–N(7)–C(11)	0.6(1.7)
C(6)–N(7)–C(11)–C(12)	−27.9(1.5)
N(7)–C(11)–C(12)–N(4)	54.3(1.1)
C(11)–C(12)–N(4)–C(5)	−60.5(1.2)
Ring C	
N(7)–C(8)–C(9)–C(10)	−23.8(1.5)
C(8)–C(9)–C(10)–C(11)	36.0(1.4)
C(9)–C(10)–C(11)–N(7)	−34.0(1.3)
C(10)–C(11)–N(7)–C(8)	19.5(1.2)
C(11)–N(7)–C(8)–C(9)	2.1(1.5)
Phenyl residue	
N(4)–C(5)–C(14)–C(15)	−53.2(1.3)
C(6)–C(5)–C(14)–C(15)	−177.1(1.0)
C(5)–C(14)–C(15)–C(16)	116.8(1.2)
C(5)–C(14)–C(15)–C(20)	−64.4(1.5)
Peptidic groups	
C(12)–N(4)–C(3)–O(1)	−168.2(1.4)
C(5)–N(4)–C(3)–O(1)	−8.5(2.0)
C(5)–N(4)–C(3)–C(2)	172.9(1.1)
C(8)–N(7)–C(6)–O(2)	5.7(1.9)
C(11)–N(7)–C(6)–O(2)	178.3(1.2)
C(8)–N(7)–C(6)–C(5)	−172.0(1.1)

* Computed according to W. Klyne and V. Prelog, *Experientia*, 1960, **16**, 521.

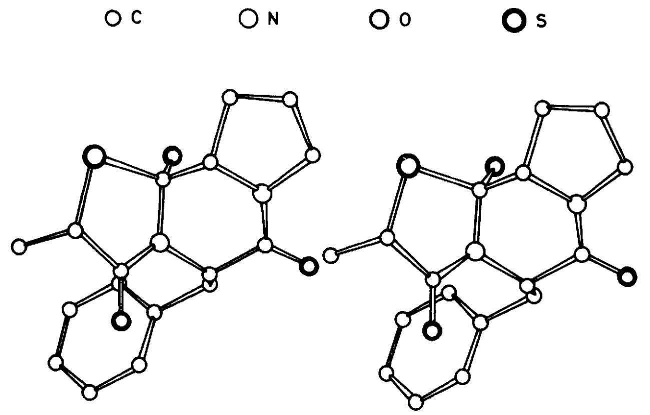


Figure 1. Stereoscopic diagram of the thiacyclol (**14**)

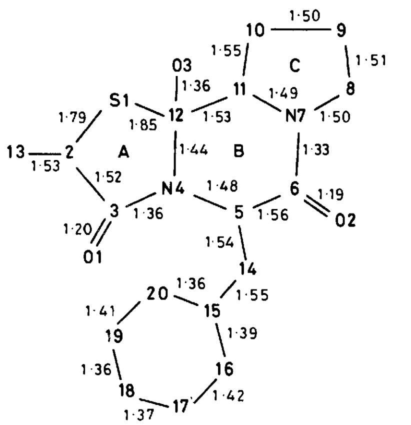


Figure 2. Atom numbering scheme and bond lengths (Å) for the thiacyclol (**14**). The standard deviations range from 0.01 to 0.03 Å

range coupled (J 1.8 Hz) to Pro $C_{\alpha}H$, which is found at δ 3.58 (Table 1). This latter value is comparable with the chemical shift found for Pro $C_{\alpha}H$ in natural oxacyclols and indicates that this proton is located *trans* to the benzylic side-chain at C-5; a *cis*-arrangement of these two substituents is revealed by the upfield shift induced by the aromatic ring on the cisoidal Pro $C_{\alpha}H$. Thus the signal of Pro $C_{\alpha}H$ in the thiacyclol (**1**) is found at δ 2.26 with a shielding effect by the aromatic ring of 1.3 p.p.m. relative to thiacyclol (**14**). The Phe H_{α}-H_{β} protons give rise to a typical ABX pattern; an analysis of the vicinal coupling constants (Table 1) by using Pachler's parameters,[13] indicates that in $CDCl_3$ solution the folded rotamer (both the H_{β}'s *gauche* to H_{α}) is preferred, with a fractional population of 0.56. A preponderance of the folded rotamer, although in higher proportion (*ca.* 0.70 mol fraction in $CDCl_3$) is also found in the case of the thiacyclol (**1**).[9]

The ^{13}C n.m.r. spectrum of (**13**) and (**14**) shows two carbonyl signals and a singlet centred at *ca.* δ 91, consistent with the presence of a non-protonated carbon atom bonded to three heteroatoms.[4] The mass spectrum shows significant peaks at m/z 332 (M^+), 314 (M^+ − 18) and a peak at m/z 245 corresponding to the *cyclo*-(Phe-Pro) fragment.

Crystallization of thiacyclol (**14**) from ethyl acetate afforded suitable crystals for *X*-ray crystallographic analysis. In Figure 1 a stereoscopic diagram of the molecule is reported; Figure 2 shows the adopted atom numbering scheme together with bond lengths. From the knowledge of the absolute configurations of proline and phenylalanine residues used in starting materials, the chiral centre at C-2 can be assigned an *S* configuration. Figure 1 shows also the *anti*-orientation of the hydrogen at C-11

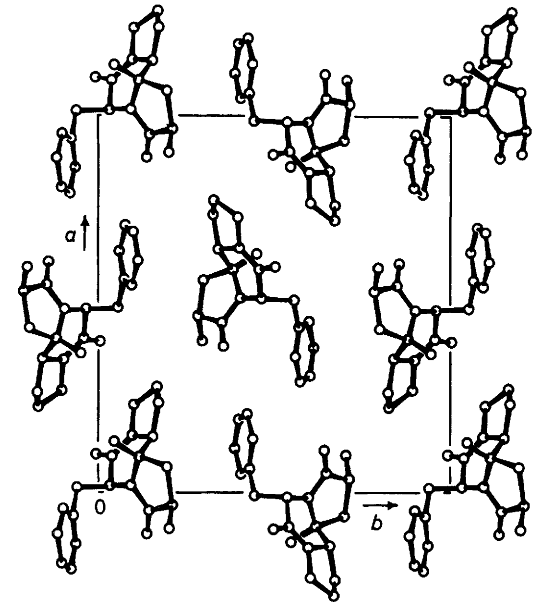


Figure 3. The crystal packing of the thiacyclol (**14**) viewed along the *c* axis

relative to the hydroxy group at C-12; the nucleophilic attack on the proline carbonyl follows then, as found in other cyclolization reactions,[2,6,14] a stereospecific course, in which 11,12 *syn*-isomers are not formed.

The junction between the A and B rings is of a *quasi-cis* type (the torsion angles of junction[15] are −17.3 and −60.5° in the two rings respectively, as shown in Table 4) whereas the junction between the B and C rings is of a *quasi-trans* type (torsion angles of junction are −27.9 and 19.5° respectively). The conformation of the A ring can be described as an approximate C_s–C(12) envelope with C-12 displaced 0.246 Å out of the least-squares plane of the other ring atoms, on the opposite side of the hydroxy group. The B ring assumes a half-boat conformation with an approximate C_s symmetry through C-6 and C-12; the deviation of C-12 from the least-squares plane of the other five ring atoms is 0.672 Å. In the pyrrolidine C ring the C-10 atom is 0.548 Å out of the plane formed by the other four ring atoms and on the same side as the OH group. This feature corresponds to the C_s-C_{β} *endo*-conformation[16,17] usually found in proline-containing cyclic dipeptides; no correspondence is found in this case with the conformation of the related thiacyclol (**1**) which adopts for the C ring a C_2-C_{β}*endo*-C_{γ}*exo*[16] half-chair conformation. The sums of the bond angles around N-4 and N-7 are 357.0 and 359.7° respectively. The N-4 atom lies 0.145 Å out of the plane of its three substituents, whereas N-7 is only 0.05 Å out of the corresponding plane. Thus a more pyramidal character can be attributed to phenylalanine nitrogen in contrast to the proline nitrogen. The C(2)–C(3)–N(4)–C(5) torsion angle of the *trans*-amide bond joining the residue of the mercapto acid to the phenylalanine residue is 172.9° whereas the C(5)–C(6)–N(7)–C(11) torsion angle of the Phe-Pro *cis*-amide bond is −0.7°. The benzylic side group of (**14**) adopts in the crystal a conformation extended toward the phenylalanine nitrogen; this conformation differs from that preferred by (**14**) in $CDCl_3$ solution and from that adopted by the related thiacyclol (**1**) both in the solid and in solution.[9] The crystal packing is characterized by an intramolecular hydrogen bond of 2.65(1) Å between the O-2 of the phenylalanine residue of the reference molecule and the hydroxylic O-3 of another molecule, translated along the *c* axis; the angle C(6)–O(2) ··· O(3) is 122.3 (0.9)° (see Figure 3).

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 at 90 MHz were recorded on a Varian EM-390 spectrometer and at 360 MHz on a Bruker HX-360 *instrument*. ^{13}C N.m.r. spectra were determined with a Bruker WP 200 (50.28 MHz) instrument. Mass spectra were determined with a Hewlett-Packard 5982 A spectrometer operating at 70 eV. Optical rotations were taken at 25° with a Schmidt-Haensch 16065 polarimeter.

(RS)-2-*Acetamidomethylthiopropionic Acid* (**2**).—Acetamidomethanol (4.78 g, 5.37 mmol) was added to a solution of (*RS*)-2-mercaptopropionic acid (5.7 g, 5.37 mmol) in trifluoroacetic acid (50 ml). After 45 min at room temperature, the mixture was evaporated and the residue was taken up in ethyl acetate and extracted with saturated aqueous sodium hydrogen carbonate. The aqueous solution was acidified and extracted with ethyl acetate; the organic phase was washed with water, dried, and evaporated to give the *title acid* (**2**) (7.4 g, 78%) as a foam which could not be crystallized (Found: C, 40.2; H, 5.9; N, 7.5; S, 17.7. $C_6H_{11}NO_3S$ requires C, 40.7; H, 6.3; N, 7.9; S, 18.1%); δ_H [90 MHz; $(CD_3)_2SO$] 1.30 (3 H, d, *J* 7.0 Hz, CH*Me*), 1.85 (3 H, s, COMe), 3.53 (1 H, q, *J* 7.0 Hz, CH), 4.35 (2 H, d, *J* 6.0 Hz, CH_2), and 8.50 (1 H, t, *J* 6.0 Hz, D_2O exchangeable, NH); treatment with D_2O caused collapse of the CH_2 doublet to a singlet.

(R)-2-*Acetamidomethylthiopropionyl- and* (S)-*2-Acetamidomethylthiopropionyl-*L-*phenylalanyl-*L-*proline Methyl Ester* (**3**) *and* (**4**).—Isobutyl chloroformate (9.98 g, 73 mmol) and *N*-methylmorpholine (8.14 g, 80 mmol) were added at −12 °C to a solution of (*RS*)-acetamidomethylthiopropionic acid (13.0 g, 73.4 mmol) in dry tetrahydrofuran (100 ml); after 10 min at −10 °C, a solution of Phe-Pro-OMe hydrochloride (22.9 g, 73.4 mmol) in methylene chloride (200 ml) containing *N*-methylmorpholine (8 ml), was added. The mixture was stirred for 12 h at room temperature after which the solvent was removed under reduced pressure and the residue taken up in ethyl acetate. The solution was washed with 2M-hydrochloric acid, saturated aqueous sodium carbonate, and water. Drying and evaporation gave an oily residue (24 g). Separation of the two epimeric peptides was achieved by column chromatography on silica gel (1.0 kg) with ethyl acetate–methanol (9:1) as eluant (20 ml fractions); the *tripeptide methyl ester* (**3**) was isolated as a viscous oil (6.0 g) by collecting fractions from 151 to 176, R_F 0.35, ethyl acetate–methanol (95:5) (Found: C, 55.7; H, 6.6; N, 9.0; S, 6.8. $C_{21}H_{29}N_3O_5S{\cdot}H_2O$ requires C, 55.6; H, 6.9; N, 9.3; S, 7.1%); δ_H (90 MHz; $CDCl_3$) 1.30 (3 H, d, *J* 7.5 Hz, *Me*CH), 1.6—2.2 (4 H, m, Pro $C_\beta H_2$ and $C_\gamma H_2$), 1.97 (3 H, s, MeCO), 2.8—3.3 (2 H, m, Phe $C_\beta H_2$), 3.51 (1 H, q, J 7.5 Hz, Me*CH*), 3.4—3.7 (2 H, m, Pro $C_\delta H_2$), 3.72 (3 H, s, OMe), 4.15 and 4.80 (2 H, m, SCH_2), 4.53 (1 H, m, Pro $C_\alpha H$), 4.90 (1 H, m, Phe $C_\alpha H$), 7.32 (5 H, m, ArH), 7.50 (1 H, m, CH_2N*H*), and 8.10 (1 H, d, *J* 7.5 Hz, Phe NH); the *tripeptide methyl ester* (**4**) was isolated as a viscous oil (5.0 g) by collecting fractions from 181 to 230; R_F 0.30, ethyl acetate–methanol (95.5) (Found: C, 55.5; H, 6.7; N, 9.15; S, 6.7. $C_{21}H_{29}N_3O_5S{\cdot}H_2O$ requires C, 55.6; H, 6.9; N, 9.3; S, 7.1%); δ_H (90 MHz; $CDCl_3$) 1.30 (3 H, d, *J* 7.5 Hz, *Me*CH), 1.6—2.2 (4 H, m, Pro $C_\beta H_2$ and $C_\gamma H_2$), 1.90 (3 H, s, MeCO) 2.9—3.4 (2 H, m, Phe $C_\beta H_2$), 3.49 (1 H, q, *J* 7.5 Hz, Me*CH*), 3.5 (2 H, m, Pro $C_\delta H_2$), 3.72 (3 H, s, OMe), 4.1 and 4.7 (2 H, m, SCH_2) 4.5 (1 H, m, Pro $C_\alpha H$), 4.9 (1 H, m, Phe $C_\alpha H$), 7.30 (5 H, m, ArH) and 7.80 (2 H, m, CH_2N*H* and Phe-N*H*).

(R)-2-*t-Butyldithiopropionyl-*L-*phenylalanyl-*L-*proline* (**9**).—A solution of iodine (6.95 g, 27.4 mmol) in methanol (200 ml) was added dropwise at room temperature during 45 min to a stirred solution of (**3**) (6.0 g, 13.8 mmol) in methanol (200 ml). After stirring had been continued for 3 h at room temperature, the reaction mixture was cooled at 0 °C and decolourized by the addition of aqueous 2M-sodium thiosulphate. Methanol was evaporated under reduced pressure and the aqueous solution was extracted with ethyl acetate. The organic layer was washed with 1M-aqueous sodium thiosulphate and water, dried (Na_2SO_4), and evaporated to give the symmetrical disulphide methyl ester (**5**) (5.04 g) which was dissolved in a mixture of dioxane (40 ml) and tetrahydrofuran (20 ml). Water was then added until incipient turbidity occurred and the mixture, cooled at 0 °C, was stirred and treated with 1M-sodium hydroxide (15.5 ml). After 12 h at 0 °C, the solution was neutralized with 1M-hydrochloric acid and concentrated under reduced pressure. The residue was taken up in ethyl acetate and extracted with aqueous saturated sodium carbonate. Acidification with citric acid and extraction with ethyl acetate gave the symmetrical disulphide acid (**7**) (3.3 g); R_F 0.8, ethyl acetate–methanol (9:1). 1M-Aqueous sodium hydroxide was added to a solution of (**4**) (3.3 g, 4.7 mmol) in dioxane (220 ml), until pH 8—9 was reached. Oxygen was bubbled through the solution over a 30 min period and 1,1-dimethylethanethiol (6.6 ml, 58.54 mmol) was added. The mixture was set aside for 3 days at room temperature under oxygen after which the solvent was evaporated under reduced pressure and the residue partitioned between ethyl acetate and 0.5M-aqueous potassium hydrogen sulphate. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated to give an oily residue; crystallization from ethyl acetate afforded the *title compound* (**9**) (1.62 g, 35.5%), m.p. 178—181 °C, $[\alpha]_D$ +32° (*c* 2.0 in $CHCl_3$); R_F 0.5, ethyl acetate–acetic acid (9:1) (Found: C, 57.5; H, 7.2; N, 5.9; S, 14.65; $C_{21}H_{30}N_2O_4S_2$ requires C, 57.5; H, 6.9; N, 6.4; S, 14.6%); δ_H [90 MHz; $(CD_3)_2SO$] 1.2 (12 H, m, 4 × Me), 1.6—2.1 (4 H, m, Pro $C_\beta H_2$ and Pro $C_\gamma H_2$), 2.65—3.15 (2 H, m, Phe $C_\beta H_2$), 3.3—3.8 (3 H, m, Pro $C_\delta H_2$ and *CH*Me), 4.3 (1 H, m, Pro $C_\alpha H$), 4.65 (1 H, m, Phe $C_\alpha H$), 7.3 (5 H, m, ArH), and 8.5 (1 H, d, *J* 8.5 Hz, NH).

(S)-2-*t-Butyldithiopropionyl-*L-*phenylalanyl-*L-*proline* (**10**).—Starting from the acetamidomethyl tripeptide (**4**) (5.0 g, 11.5 mmol), compounds (**6**) (4.2 g) and (**8**) (3.0 g) were prepared as described for (**5**) and (**7**), respectively. By treating (**8**) (3.0 g) with 1,1-dimethylethanethiol under the same conditions adopted for compound (**7**), the *title t-butyldithiopeptide* (**10**) (1.4 g, 37%) was obtained, m.p. 181—184 °C (from ethyl acetate) $[\alpha]_D$ −10.8° (*c* 5 in $CHCl_3$); R_F 0.5, ethyl acetate–acetic acid (9:1) (Found: C, 57.6; H, 6.6; N, 6.0; S, 14.6. $C_{21}H_{30}N_2O_4S_2$ requires C, 57.5; H, 6.9; N, 6.4; S, 14.6%); δ_H [90 MHz; $(CD_3)_2SO$] 1.2 (12 H, m, 4 × Me), 1.6—2.1 (4 H, m, Pro $C_\beta H_2$ and Pro $C_\gamma H_2$), 2.65—3.15 (2 H, m, Phe $C_\beta H_2$), 3.3—3.8 (3 H, m, Pro $C_\delta H_2$ and *CH*Me), 4.3 (1 H, m, Pro $C_\alpha H$), 4.65 (1 H, m, Phe $C_\alpha H$), 7.3 (5 H, m, ArH), and 8.5 (1 H, d, *J* 8.5 Hz, NH).

(R)-2-*t-Butyldithiopropionyl-*L-*phenylalanyl-*L-*proline* p-*Nitrophenyl Ester* (**11**).—*p*-Nitrophenol (514 mg, 3.7 mmol) and dicyclohexylcarbodi-imide (720 mg, 3.7 mmol) were added at 0 °C to a stirred solution of (**9**) (1.62 g, 3.7 mmol) in tetrahydrofuran (150 ml). Stirring at 0 °C was continued for 1.0 h and the mixture was then left overnight at room temperature. The solvent was evaporated under reduced pressure and the residue was taken up in ethyl acetate. The dicyclohexylurea was filtered off and the filtrate washed with aqueous sodium carbonate and brine, dried (Na_2SO_4), and evaporated to give the *active ester* (**11**) (2.0 g) as a viscous oil, R_F 0.85 (ether) (Found: C, 56.7; H, 6.3; N, 7.4; S, 11.3. $C_{27}H_{33}N_3O_6S_2$ requires C, 57.0; H, 6.1; N, 7.7; S, 11.7%).

(S)-2-*t-Butyldithiopropionyl-*L-*phenylalanyl-*L-*proline* p-*Nitrophenyl Ester* (**12**).—Compound (**12**) was prepared in the same manner as described for (**11**). Starting from compound (**10**) (1.41 g, 3.22 mmol), the *title active ester* (**12**) (1.7 g) was obtained as a viscous oil, R_F 0.85 (ether) (Found: C, 56.85; H, 6.3; N, 7.3; S, 11.6 $C_{27}H_{33}N_3O_6S_2$ requires C, 57.0; H, 6.1; N, 7.7; S, 11.7%).

Cyclization of the Active Ester (**11**).—The active ester (**11**) (1.96 g, 3.5 mmol) was dissolved in a mixture of propanol (700 ml) and water (450 ml). Atmospheric oxygen was removed with nitrogen and tri-n-butylphosphine (1.06 g, 5.23 mmol) was added with stirring. After 3 days at room temperature under nitrogen, the reaction mixture was evaporated under reduced pressure and the residue, taken up in ethyl acetate, was washed in turn with saturated aqueous sodium carbonate, 0.5M-hydrogen potassium sulphate, and brine. Drying (Na_2SO_4) and evaporation afforded an oil residue (1.8 g). Column chromatography on silica gel (100 g) with ethyl acetate as eluant afforded the *thiacyclol* (**13**) (130 mg, 11%), m.p. 218—220 °C (decomp.) (from ethyl acetate) (Found: C, 61.45; H, 6.2; N, 8.5; S, 9.6. $C_{17}H_{20}N_2O_3S$ requires C, 61.4; H, 6.1; N, 8.4; S, 9.6%); $[\alpha]_D$ +35° (*c* 3.0 in $CHCl_3$); $\nu_{max.}$($CHCl_3$) 3 520, 3 250br, 1 680, 1 650, and 1 440 cm^{-1}; δ_H (360 MHz; $CDCl_3$) 1.56 (3 H, d, *J* 7.0 Hz, Me), 1.21 (1 H, d, 2.0 Hz, OH), 1.6—2.0 (4 H, m, Pro $C_\beta H_2$ and $C_\gamma H_2$), 3.33 (1 H, dd, J_{AX} 6.65 Hz, J_{AB} 13.7 Hz, Phe $C_\beta H_A$), 3.50 (2 H, m, Pro $C_\delta H_2$), 3.57 (1 H, dd, J_{BX} 3.52 Hz, J_{AB} 13.7 Hz, Phe $C_\beta H_B$), 3.68 (1 H, ddd, Pro C_αH), 3.76 (1 H, q, *J* 7.0 Hz, Me*CH*), 4.68 (1 H, dd, Phe C_αH), 7.12—7.25 (5 H, m, ArH), the signal at δ 3.68 turns into a double doublet by irradiation at δ 1.21; *m/z* 332 (M^+, 15%), 314 (1.5), 245 (2), 241 (6), 131 (35), 125 (29), 91 (34), and 70 (pyrrolinium, 100).

Cyclization of the Active Ester (**12**).—The same procedure described for the thiacyclol (**13**) was followed. Starting from active ester (**12**) (1.69 g, 3.0 mmol) a crude residue (2.0 g) was obtained. Column chromatography on silica gel (100 g) with ethyl acetate as eluant afforded the *thiacyclol* (**14**) (200 mg, 20%), m.p. 226—228 °C (decomp.) (from ethyl acetate) (Found: C, 61.3; H, 6.1; N, 8.3; S, 9.5. $C_{17}H_{20}N_2O_3S$ requires C, 61.4; H, 6.1; N, 8.4; S, 9.6%); $[\alpha]_D$ −3° (*c* 6 in MeOH); $\nu_{max.}$ 3 520, 3 250 broad, 1 680, 1 655, and 1 445 cm^{-1}; *m/z* 332 (M^+, 17%), 314 (1), 245 (3.5), 241 (5), 131 (35), 125 (33), 91 (33), and 70 (pyrrolinium, 100).

Crystal Data.—The thiacyclol (**14**), $C_{17}H_{20}N_2O_3S$, M = 332.4, orthorhombic, a = 16.462(7), b = 15.763(7), c = 6.487(4) Å, U = 1 683(1) Å^3, D_c = 1.31 g cm^{-3}, Z = 4, Mo-K_α radiation, λ = 0.7107 Å, μ(Mo-K_α) = 0.21 mm^{-1}. Space group $P2_12_12_1$ from systematic absences. Intensities were recorded up to a maximum value 2θ of 56.0° by the θ—2θ technique. The quality of the crystals was very poor: in fact of the 2 356 independent reflections recorded, only 1 371 with $I > 1.0\sigma(I)$ were considered observed and used for the refinement.

Crystallographic Analysis.—Single crystals of the thiacyclol were obtained by slow evaporation of an ethyl acetate solution. Approximate unit-cell parameters and space group were determined from oscillation and Weissenberg photographs. Intensity data were recorded on an automatic four circle SYNTEX $P2_1$ diffractometer equipped with graphite monochromator using Mo-K_α radiation. Refined unit-cell parameters were obtained by a least-squares fit of the angular settings of 15 reflections. Lorentz and polarization corrections were applied, but intensities were not corrected for extinction and absorption.

Structure Solution and Refinement.—The sulphur atomic coordinates were obtained by interpretation of a sharpened[18] Patterson synthesis. By successive structure factor and electron density calculations the complete structure was determined. The structure was isotropically then anisotropically refined by block-diagonal least-squares. The function minimized was $\Sigma w(|F_o| - |F_c|)^2$ where $w = (a + |F_o| + c|F_o|^2)^{-1}$ with a and c of the order of $2F_{o(min)}$ and $2/F_{o(max)}$ respectively. Since most of the hydrogen atoms could not be located from the difference electron density map, their positional parameters except those of the methyl group and of the cyclolic hydroxy group, were calculated and introduced in the last stages of refinement together with thermal values deduced from the carrier atoms, keeping them fixed. The final R is 0.11 for all the observed reflections. Scattering factors were taken from International Tables for *X*-ray Crystallography (1974). All the calculations were carried out on the HP 21MX minicomputer[19] of the CNR Research Area of Rome. Observed and calculated structure factors, anisotropic thermal parameters for the non-hydrogen atoms, and the displacements from least-squares planes are listed in Supplementary Publication No. SUP 23890 (13 pp).*

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* For details of the Supplementary publications scheme see Instructions for Authors (1984), *J. Chem. Soc., Perkin Trans.* 1, 1984, Issue 1.

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