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ANALOG OF DOLASTATIN 3
SYNTHESIS, ^{1}H NMR STUDIES AND SPATIAL CONFORMATION

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Abstract - The total synthesis of the peptide cyclo [Pro-Leu-Val-(gly)Thz-(gly)Thz] a simplified analog of dolastatin 3 is reported here. Analysis of the 350 MHz ^{1}H NMR spectrum permits the design of a spatial molecular model. This conformational feature is of interest in the tentatives to assign the real structure of dolastatin 3 in the knowledge of intramolecular interaction of this drug with biological receptors.

Since antiquity, extracts of plants and animals have been recognized to possess biologically important properties. Recently, the Indian Ocean sea hare Dolabella auricularia has revealed to be an exceptionally productive source of anticancer biosynthetic products (1, 2, 3). One of its extracted substance, dolastatin 3 was found to exhibit an exceptional in vitro antineoplastic activity (P388 leukemia cells : ED_{50} = 1 x 10^{-4} to 1 x 10^{-7} µg/ml). On the basis of 1 mg amount from 100 kg of wet sea hare, Pettit et al (3) proposed a structure for dolastatin 3 (fig. 1) after studies by high-resolution EI mass spectroscopy, ^{1}H and ^{13}C NMR and amino acid composition.

The cyclic backbone included a tripeptide Pro-Leu-Val associated with two thiazole amino acids which are supposed to have been formed by biosynthetic condensations : glycine + cysteine giving the 2-aminomethyl-4-carboxythiazole designed as (gly)Thz and glutamine + cysteine conventionally called (gln)Thz (3).

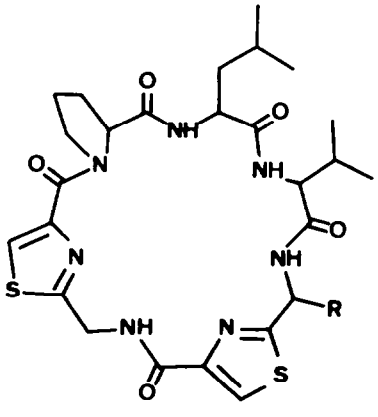

Figure 1

Proposed structure of dolastatin 3 (R = CH_2-CH_2-$CONH_2$) and structure of the simplified model (R = H)

Because of its large biological interest related to its high antineoplastic activity and owing to its low and tedious natural availability, this cyclic peptide has initiated attempts of total synthesis. Thus, different chemical ways to dolastatin 3 and to its isomers have been described (4,5,6) but, unfortunately, different biological and physical characteristics have been found, which were in discrepancy with the structure proposed by Pettit et al. To date, no new proposal has been made to rectify the formula of this cytotoxic drug which must be close to that proposed by Pettit in the light of the results described in his publication (3). This led us to undertake the synthesis of the simplified cyclic peptide [Pro-Leu-Val-(gly)Thz-(gly)Thz] which can be used as a reference for the structure of the cyclic backbone. Indeed, the assignments of the protons of the ^{1}H NMR spectrum can gain new information on the spatial structure and can be useful to the elucidation of the real formula of dolastatin 3 by comparison with previous works.

SYNTHESIS

The preparation of the protected tripeptide BOC-Pro-Leu-Val-OMe has been achieved using the procedure described previously (7) while the (gly)thiazole was prepared from BOC-Gly thioamide and ethyl bromopyruvate by an improvement of the classical Hantzsch condensation (8). The preparation of BOC-Gly thioamide was carried out by thionation of BOC-Glycinamide. That reaction was performed by a modification of the initial procedure of Lawesson (9) using 2,4-bis(4-phenoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide (PTPS) (10) in anhydrous THF at 20°C. This thiation was selective since t-BOC N-protecting group reacts with Lawesson's reagent at 110°C (9). The cyclization of the open chain model was obtained in diluted pyridine solution of the active ester. After purification of the cyclic peptide by column chromatography, its ^{1}H-NMR spectrum has been recorded in $(CD_3)_2SO$ using TMS as internal reference. The protons chemical shifts have been unambiguously assigned from spin decoupling experiments on the NH and the α-CH, β-CH and CH_3 protons. The study of the temperature dependence of amide proton chemical shifts and the knowledge of coupling constants permit to propose a three-dimensional structure for the peptide backbone.

The general pathway to the cyclic peptide is summarized in figure 2. BOC protecting groups were removed either by trifluoroacetic acid or by hydrogen bromide. DCC was used as coupling reagent in the presence of activating agents such as HOBt or HONSu.

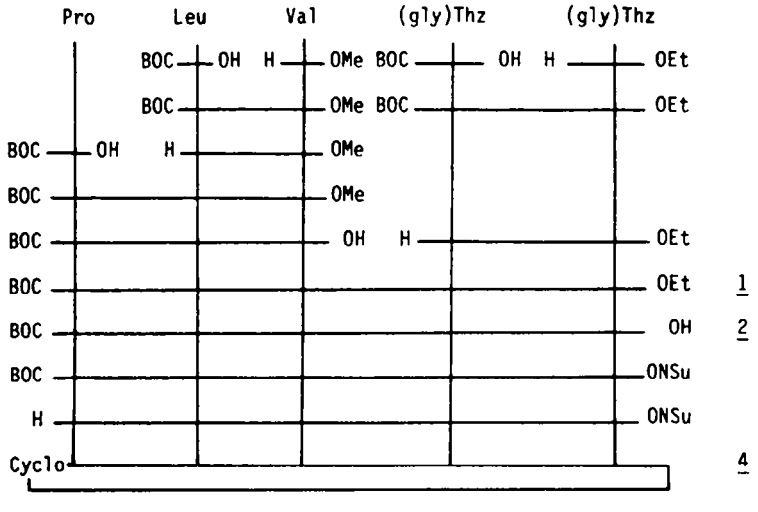

Figure 2

Synthesis of Cyclo [Pro-Leu-Val-(gly)Thz-(gly)Thz]

RESULTS AND DISCUSSION

In tables 1 and 2 are listed the chemical shifts (δ) of the protons in the low and high field regions. In figure 3, is shown the low field region of the 350 MHz ^{1}H NMR spectrum of the cyclic peptide, along with assignments and selective homospin decoupling. The assignments of all the protons have been established taking into account spin decoupling experiments and comparisons with shorter synthetic fragments.

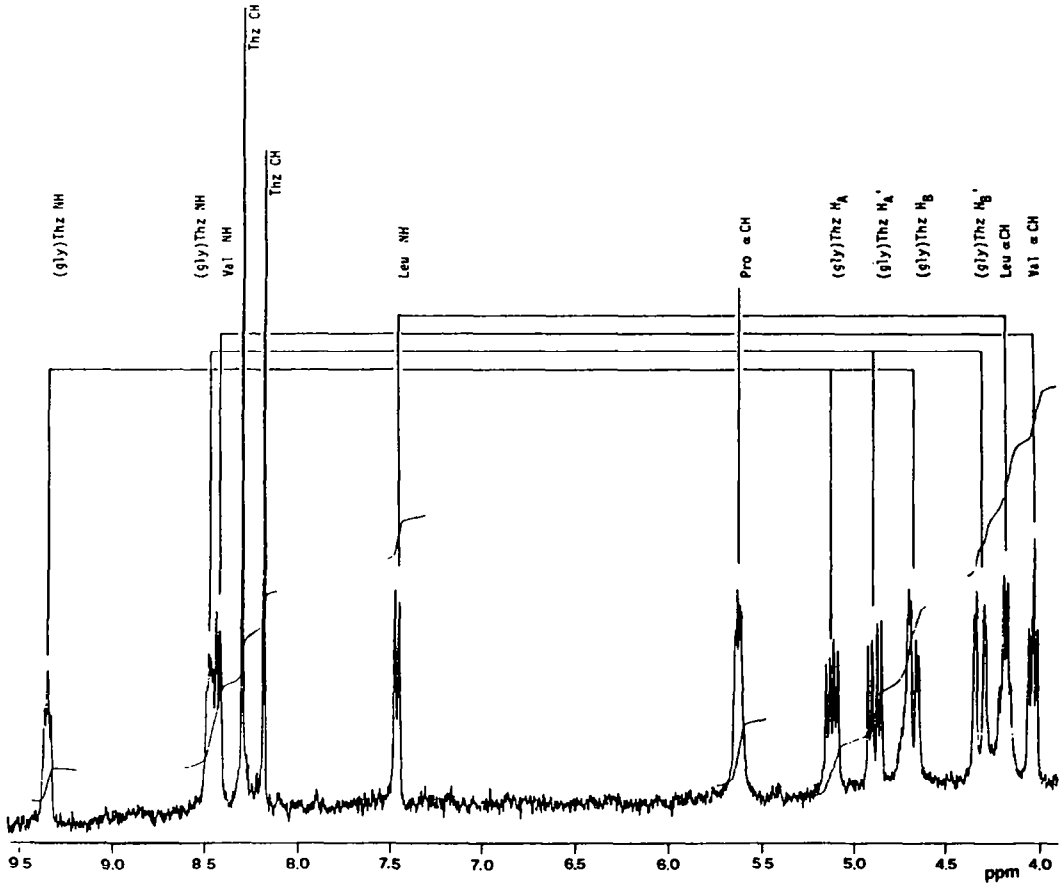

Figure 3

350-MHz ^{1}H NMR spectrum of Cyclo [Pro-Leu-Val-(gly)Thz-(gly)Thz] in $(CD_3)_2SO$. Connecting lines indicate coupled spin systems and assigments as determined by coupling experiments

The temperature dependence of amide chemical shifts (dδ/dt) (see figure 4) can be explained by the accessibility of the amide protons to the solvent, implying hydrogen bonding with the $(CD_3)_2$ SO solvent. Such is the case for (gly)Thz and Val NH which exhibit a mean value of $d\delta/dt = 5 \times 10^{-3}$ ppm/°C. The lack of effect of the temperature variations on the Leu NH ($d\delta/dt = 0.4 \times 10^{-3}$ ppm/°C) is consistent with the presence of an intramolecular hydrogen bond involving this proton and very likely the opposite (gly)Thz carbonyl on the cyclic backbone. That constraint yet gives a good assessment of the three-dimensional structure.

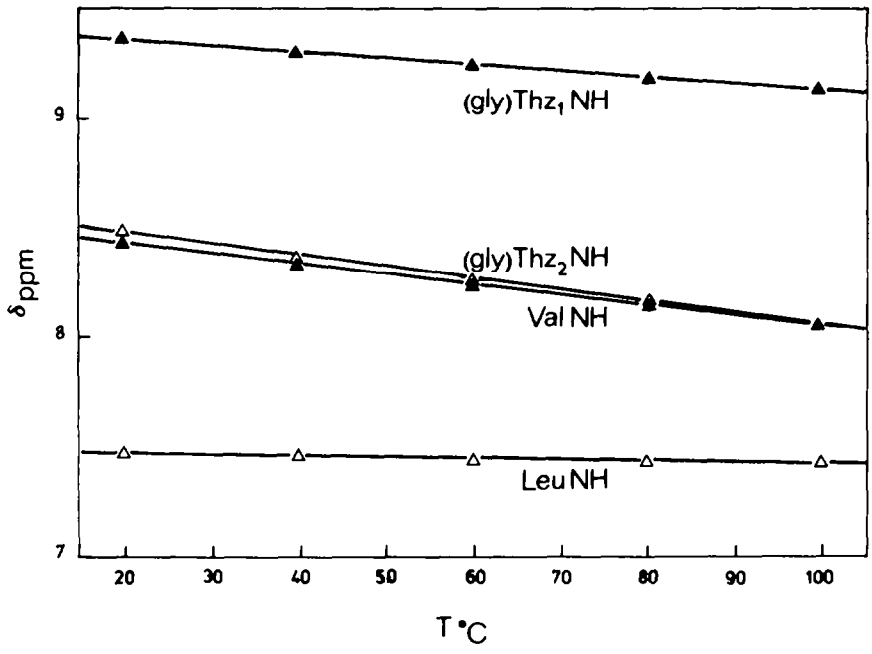

Figure 4

Temperature dependence of NH chemical shifts of cyclo [Pro-Leu-Val-(gly)Thz-(gly)Thz] in $(CD_3)_2SO$

A detailed study of the chemical shifts and coupling constants of protons in the low field region of the cyclic peptide spectrum brings some additional lights on its three-dimensional structure. The rigidity of the backbone is substantiated by a non-equivalence of the geminal methylene protons of the (gly)Thz (see figure 3) in accordance with previous data on dolastatin 3 (6). By spin-decoupling experiments the vicinal and geminal coupling constants are well defined = $-CH_AH_B-NH$: H_A, 5.10, dd, 1H, $J(H_A\ H_B)$ = 14.7 Hz (α = 107°), $J(NH-H_A)$ = 8.05 Hz (θ= 135°) ; H_B = 4.70 dd, 1H, $J(H_AH_B)$ = 14.7 Hz, $J(NH-H_B)$ = 4.9 Hz (θ= 25°) ; $-CH_{A'}H_{B'}-NH-$: $H_{A'}$, 4.91 dd, 1H, $J(H_{A'}H_{B'})$ = 17.5 Hz, (α = 108°), $J(NH-H_{A'})$ = 7.8 Hz (θ= 140°) ; $H_{B'}$, 4.33, dd, 1H, $J(H_{A'}H_{B'})$ = 17.5 Hz, $J(NH-H_{B'})$ = 3.85 Hz (θ = 30°) (see figure 5). A spatial molecular structure of the thiazole part of the molecule can be proposed, taking into account dihedral angles values calculated from coupling constants according to Neel's equation (11) for the vicinal correlation, and according to Karplus one for geminal correlation (12). The planarity of the thiazole-4-carboxamide moiety and the knowledge of the 2-aminomethylene conformation permits to define with precision the position of the thiazole rings (figure 5).

The deshielding of H_A and $H_{A'}$ protons can be explained by the proximity of the oxygen atom of the carbonyls (with a dihedral angle CH_A-CO and $CH_{A'}$-CO of -25° and -20° respectively). On the basis of the above information, the whole cyclic backbone is easily reconstituted (figure 5).

The molecule have been built with molecular Dreiding models and its spatial structure fits well with other features exhibited by the spectrum. Irradiation at 2.10 ppm transforms the multiplet at 5.60 ppm in a single peak which substantiates the assignments of the signal at 2.10 ppm as Pro βCH_2 and at 5.60 ppm as Pro αCH. The deshielding of the latter proton can be attributed to the ring current effect of the vicinal thiazole. Methyl groups of Leu are not equivalent. They appear as two doublets at 0.32 and 0.48 ppm with J = 7 Hz. Their irradiation led to a simplification of the peak at 0.80 ppm (Leu γCH). The high field shift of the Leu side chain protons is consistent with the placement of those hydrogens in position where they experience the ring current effect emanating from the thiazole (figure 5).

	NH-CH_2	NH-CH_2	Thz-CH	Thz-CH	Val NH	Leu NH	Pro NH	COOH	NH-CH_2	NH-CH_2	LeuαCH	ValαCH	ProαCH
1	9.35 (1H,t)	9.05 (1H,t)	8.48 (1H,s)	8.45 (1H,s)	8.05 (1H,d)	7.75 (1H,d)			4.75 (2H,m)	4.65 (2H,m)	4.45 (1H,m)	4.24 (1H,m)	4.20 (1H,m)
2	9.30 (1H,t)	9.15 (1H,t)	8.27 (1H,s)	7.95 (1H,s)	8.10 (1H,d)	7.85 (1H,d)		9.10 (1H,s)	4.75 (2H,m)	4.65 (2H,m)	4.45 (1H,m)	4.25 (1H,m)	4.20 (1H,m)
3	9.37 (1H,t)	9.06 (1H,t)	8.38 (1H,s)	8.32 (1H,s)	8.05 (1H,d)	8.67 (1H,d)	8.57 (1H,m)	9.32 (1H,s)	4.75 (2H,m)	4.65 (2H,m)	4.50 (1H,m)	4.23 (1H,m)	4.27 (1H,m)
4	9.35 (1H,m)	8.49 (1H,m)	8.30 (1H,s)	8.18 (1H,s)	8.46 (1H,d)	7.47 (1H,d)			5.10 (1H,dd) 4.70 (1H,dd)	4.91 (1H,dd) 4.33 (1H,dd)	4.17 (1H,m)	4.05 (1H,m)	5.60 (1H,m)

TABLE 1

Low field region of the 350 MHz specta in $(CD_3)_2SO$

	ProδCH$_2$	ProβCH$_2$	ProαCH$_2$	ValβCH	LeuβCH$_2$	LeuαCH	ValCH$_3$	LeuCH$_3$
1	3.24 (m, 2H)	2.12 (m, 2H)	1.80-1.60 (m, 2H)	2.05 (m,1H)	1.52 (m, 2H)	1.32 (m, H)	0.90 (d, 2x3H)	0.90 (d, 2x3H)
2	3.24 (m, 2H)	2.12 (m, 2H)	1.80-1.60 (m, 2H)	2.05 (m,1H)	1.50 (m, 2H)	1.31 (m, H)	0.90 (d, 2x3H)	0.90 (d, 2x3H)
3	3.25 (m, 2H)	2.10 (m, 2H)	1.80-1.60 (m, 2H)	2.05 (m,1H)	1.50 (m, 2H)	1.30 (m, H)	0.90 (d, 2x3H)	0.90 (d, 2x3H)
4	3.22 (m, 2H)	2.10 (m, 2H)	1.80-1.60 (m, 2H)	2.01 (m,1H)	0.86 (m, 2H)	0.80 (m, H)	0.90 (d, 2x3H)	0.32 (d,3H) 0.48 (d,3H)

TABLE 2

High field region of the 350 MHz ^{1}H NMR spectra in $(CD_3)_2SO$.

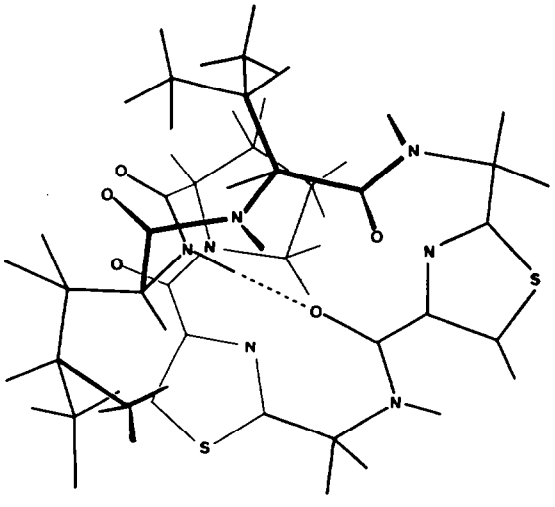

Figure 5

Proposed spatial molecular conformation of Cyclo [Pro-Leu-Val(gly)-Thz(gly)-Thz(gly)Thz] based on ^{1}H NMR analyses

CONCLUSION

The synthesis and the ^1H NMR spectrum study of a simpler analog of dolastatin 3 bring interesting information about the structure of such a cyclic depsipeptide. In particular the two plane thiazole rings planes are well defined and the Leu NH proton is involved in a strong intramolecular hydrogen bond while the Leu side chain is in the shielding conical zone of a thiazole. These conformational features can be of interest in the knowledge of the mode of interaction of such a drug with a biological receptor, and perhaps, the ^1H NMR spectrum can be useful to the finding of the exact structure of dolastatin 3 which remains an enigm.

EXPERIMENTAL PROCEDURES

Materials and Methods

General methods

Capillary melting points were determined on a Kofler hot-stage and are uncorrected. Thin layer chromatography was performed on 5 cm x 10 cm glass plates precoated with silica gel (0.25 mm thick, F_{254} Merck) using $CHCl_3$/MeOH (4:1) as solvent system in a saturated ammonia atmosphere. Detection was made by iodine vapors or ninhydrin. Infrared spectra were carried out on a Beckman Acculab I spectrometer using KBr pellets. Fast atom bombardment mass spectra were obtained on a VG ZAB HF low resolution spectrometer. Electron impact mass spectra were recorded on a Ribermag R10-10 quadrupole mass spectrometer combined with a Riber R 400 data system using direct insertion of the samples. Amino acids analyses were obtained from a Beckman 120 C amino acid analyzer following hydrolysis for 24 h at 105°C in 6 N HCl. All peptides were shown to contain the appropriate amino acids in equivalent amounts. The protected amino acids were purchased from Fluka (Switzerland).

BOC-Pro-Leu-Val-OMe
This tripeptide was prepared as previously described (6) ; white crystals ; mp : 110°C ; R_F = 0.875 ; EIMS : M^+ = 441 ; IR = 3330 cm^{-1} (NH BOC), 1735 cm^{-1} (CO ester), 1700 cm^{-1} (CO BOC) ; ^1H NMR (90 MHz, $(CD_3)_2SO$/TMS), δ= 8.05 (d, 1H, NH Val, J = 8.5 Hz) ; 7.90 (d, 1H, NH Leu, J = 8 Hz) ; 4.45 (m, 1H, αCH Leu) ; 4.30 (m, 1H, αCH Pro) ; 4.25 (m, 1H, αCH Val) ; 3.55 (s, 3H, O-CH_3) ; 3.40 (m, 2H, δCH_2 Pro) ; 2.15 (m, 1H, βCH Val) ; 2-1.3 (m, βCH_2 Leu, γCH Leu, β, γCH_2 Pro) ; 1.25 (s, 9H, BOC CH_3) ; 0.95 (d, 12 H, Leu and Val CH_3).

BOC-Pro-Leu-Val-OH
To a solution of BOC-Pro-Leu-Val-OMe (4.4 g, 10 mmol) in methanol (50 ml) was added NaOH (1.6 g, 40 mmol) diluted in 10 ml of water. The mixture was stirred at room temperature for 2 h and then neutralized with 2 N HCl. The solvent was evaporated under reduced pressure and ethanol was added (50 ml). The precipitate of NaCl was collected and the organic solution was evaporated again to dryness. The residue was crystallized from diethyl-ether giving 3.5 g of BOC-Pro-Leu-Val-OH as white crystals (81 %) ; mp : 98°C ; EIMS : M^+ = 427 ; R_F = 0.12 ; IR = 3340 cm^{-1} (NH BOC), 1700 cm^{-1} (CO acid), 1690 cm^{-1} (CO BOC).

BOC-glycinamide (BOC-Gly NH_2)
To a solution of 2.18 g of ditertiobutyl dicarbonate (10 mmol) in CH_2Cl_2 (50 ml) were added 1.1 g of Gly-NH_2, HCl (10 mmol) and 2.1 g of $N(Et)_3$. The reaction mixture was refluxed for 3 h. Triethylamine salts were extracted twice by 10 ml of water and the organic phase, dried over Na_2SO_4, was evaporated to give an oily residue which crystallized upon standing at room temperature (72 %). White crystals ; mp : 94°C ; R_F = 0.45 ; EIMS : M^+ = 174 ; IR = 3400 cm^{-1} (NH_2 amide), 3340 cm^{-1} (NH BOC), 1700 cm^{-1} (CO amide), 1690 cm^{-1} (CO BOC).

BOC-glycine-thioamide (BOC-Gly(S)NH_2)
To a solution of BOC-Gly NH_2 (1.7 g, 10 mmol) in dry THF (20 ml) was added PTPS (4.0 g, 7.4 mmol) under a nitrogen atmosphere. The reaction mixture was stirred at room temperature until the starting material was consumed (6 h) as monitored by t.l.c. The solvent was evaporated and the thick residue chromatographed on a silica gel column (eluted with CH_2Cl_2/ethyl acetate, 9:1). The resulting oil BOC-Gly(S) NH_2 crystallized on standing (88 %). White crystals ; mp : 124°C ; EIMS : M^+ = 190 ; R_F = 0.65 ; IR = 3420 cm^{-1} (NH_2 thioamide), 3300 cm^{-1} (NH BOC), 1680 cm^{-1} (CO BOC), 1650 cm^{-1} (CS thioamide).

Ethyl 2-(N-BOC-aminomethyl)-thiazole-4-carboxylate (BOC-(gly)Thz-OEt)
A mixture of 1.9 g (10 mmol) of the thioamide and 1.95 g (10 mmol) of ethyl bromopyruvate in 20 ml of dry diethyl-ether was stirred for 3 h at room temperature. The precipitate of BOC-(gly) Thz-OEt is collected and washed with ether to give white crystals (75 %) ; mp : 133°C ; EIMS : M^+ = 286 ; R_F = 0.68 ; IR = 3330 cm^{-1} (NH BOC), 1750 cm^{-1} (CO ester), 1690 cm^{-1} (CO BOC). ^1H NMR (90 MHz, $(CD_3)_2SO$/TMS) ; δ= 8.35 (s, 1H, CH Thz), 7.85 (t, 1H, NH), 4.45 (d, 2H, CH_2), 4.35 (q, 2H, O-$\underline{CH_2}$-CH_3), 1.5 (t, 3H, O-CH_2-$\underline{CH_3}$), 1.45 (s, 9H, BOC CH_3).

(Gly)Thz-OEt, HBr

BOC(gly)Thz-OEt (2.86 g, 10 mmol) (in acetic acid solution) was treated by HBr. After one hour at room temperature, the acidic solution was evaporated to dryness in vacuo and acetone was added to the residue, affording a precipitate of (gly)Thz-OEt, HBr, which was collected (94 %). White crystals ; mp : 190°C ; EIMS : M^+ = 186 ; R_F = 0.40 ; IR : 2400-2600 cm^{-1} (NH_2^+ salt), 1740 cm^{-1} (CO ester).

BOC-(gly)Thz-OH

To a solution of BOC(gly)Thz-OEt in methanol (2.86 g, 10 mmol in 50 ml) was added NaOH (1.6 g, 40 mmol in 10 ml of water). The mixture was stirred for 3 h at room temperature and then neutralized by 1 N HCl. After evaporation to dryness in vacuo, water (10 ml) was added to the residue affording a precipitate of BOC-(gly)Thz-OH on standing. White crystals (43 %) ; mp = 184°C ; EIMS : M^+ = 258 ; R_F = 0.10 ; IR = 3440 cm^{-1} (OH), 3360 cm^{-1} (NH BOC), 1720 (CO acid), 1690 cm^{-1} (CO BOC).

BOC-(gly)Thz-(gly)Thz-OEt

To a chilled solution of BOC-(gly)Thz-OH (1.25 g, 5 mmol) and (gly)Thz-OEt, HBr (1.35 g, 5 mmol) in 50 ml of CH_2Cl_2 were added DCC (1.2 g, 6 mmol), HOBt (1.35 g, 10 mmol) and $N(Et)_3$ (0.5 g, 5 mmol). The mixture was stirred at 0°C for 4 h and at 20°C overnight. After the removal of the DCU, the solution was washed successively by 1 N HCl, water and 1 M $NaHCO_3$. The organic phase was dried over Na_2SO_4 and evaporated under reduced pressure. Diethyl-ether was added to the residue to afford white crystals which were collected (80 %) ; mp = 120°C ; EIMS : M^+ = 426 ; R_F = 0.85 ; IR = 3450 cm^{-1} (NH BOC), 1720 cm^{-1} (CO ester), 1780 cm^{-1} (NH BOC). ^1H NMR ($(CD_3)_2SO$/TMS) : δ = 9.35 (t, 1H, CH_2-NH), 8.45 (s, 1H, CH Thz), 8.25 (s, 1H, CH Thz), 7.90 (t, 1H, NH BOC), 4.75 (d, 2H, $\underline{CH_2}$-NH), 4.50 (d, 2H, $\underline{CH_2}$-NH), 4.45 (q, 2H, O-$\underline{CH_2}$-CH_3), 1.45 (s, 9H, BOC CH_3), 1.40 (t, 3H, O-CH_2-$\underline{CH_3}$).

(gly)Thz-(gly)Thz OEt, HBr

BOC (gly)Thz-(gly)Thz-OEt (1 g, 2.5 mmol) was dissolved in acetic acid and treated by HBr. The solution was stored for 1 h at room temperature and then evaporated in vacuo to dryness. Diethyl-ether was added to the residue to afford crystals which were further crystallized from ethanol (62 %) : mp = 205°C ; EIMS : M^+ = 326 ; R_F = 0.75 ; IR = 3380 cm^{-1} (NH BOC) ; 2640-2600 cm^{-1} (NH^+), 1730 cm^{-1} (CO ester), 1660 cm^{-1} (CO amide).

BOC-Pro-Leu-Val-(gly)Thz-(gly)Thz-OEt (1)

To a chilled solution of BOC-Pro-Leu-Val-OH (1.06 g, 2.5 mmol) and (gly)Thz-(gly)Thz-OEt, HBr (1 g, 2.5 mmol) in dry CH_2Cl_2 were added 0.55 g of DCC, 0.5 g of HOBt and 0.35 ml of $N(Et)_3$ (2.5 mmol). The reaction mixture was stirred at 0°C for 4 h and at 20°C overnight. The precipitate of DCU was collected and the solution was extracted successively by 1 N HCl, water, 1 M $NaHCO_3$. The organic phase was dried over Na_2SO_4 and evaporated in vacuo to dryness. Diethylether was added to the residue, affording white crystals (59 %) ; mp = 148°C ; EIMS : M^+ = 735 ; R_F = 0.84 ; ^1H NMR ($(CD_3)_2SO$/TMS) δ = 4.35 (q, 2H, O-$\underline{CH_2}$-CH_3), 1.35 (s, 9H, BOC $\underline{CH_3}$), 1.40 (t, 3H, O-CH_2-$\underline{CH_3}$), for other protons see table 1 and table 2.

BOC-Pro-Leu-Val-(gly)Thz-(gly)Thz-OH (2)

To a solution of 1 (0.735 g, 1 mmol) in methanol (20 ml) was added sodium hydroxide (0.16 g, 4 mmol) in 5 ml of water. The reaction mixture was stirred for 3 h at room temperature and then neutralized by 1 N HCl. After evaporation to dryness, 20 ml of water was added to the residue. BOC-Pro-Leu-Val-(gly)Thz-(gly)Thz-OH (2) precipitated (47 %) ; white crystals ; mp = 190°C ; FABMS : M^+ = 707 ; R_F = 0.15 ; IR = 3340 cm^{-1} (NH BOC), 1700 cm^{-1} (CO acid), 1680 cm^{-1} (CO BOC) ; ^1H NMR ($(CD_3)_2SO$/TMS) δ= 1.35 (s, 9H, BOC CH_3), for other protons see tables 1 and 2.
Amino acid analysis : Pro, 1.1 ; Leu, 1.0 ; Val, 1.0.

Pro-Leu-Val-(gly)Thz-(gly)Thz-OH, HBr (3)

A solution of 2 (700 mg, 1 mmol) in acetic acid (20 ml) was treated by HBr and stored for 1 h at room temperature. After evaporation to dryness in vacuo, acetone was added to the residue to afford white crystals which were collected and washed with acetone (58 %) ; mp = 200°C ; FABMS : M^+ = 607 ; R_F = 0 ; IR = 3400 cm^{-1} (NH), 1720 cm^{-1} (CO acid), 1660 cm^{-1} (CO amide) ; ^1H NMR ($(CD_3)_2SO$/TMS) see tables 1 and 2.

Cyclo [Pro-Leu-Val-(gly)Thz-(gly)Thz] (4)

To a chilled solution of 2 (750 mg, 1.1 mmol) in 20 ml of dry DMF were added DCC (250 mg, 1.2 mmol) HONSu (250 mg, 2.2 mmol). The reaction mixture was stirred at 0°C for 4 h and at room temperature overnight. After evaporation to dryness in vacuo, CH_2Cl_2 was added to the residue, DCU was collected and the solution was evaporated to give white crystals of BOC Pro-Leu-Val-(gly)Thz-(gly)Thz ONSu which was used for the next step without purification (72 %) ; R_F = 0.75 ; IR = 3340 cm^{-1} (NH BOC), 1750 cm^{-1} (CO ester), 1690 cm^{-1} (CO BOC). The active ester (500 mg) was dissolved in acetic acid, treated with HBr and stirred for 1 h at room temperature. The acidic solution was evaporated in vacuo and the active HBr.Pro-Leu-Val-(gly)Thz-(gly)Thz ONSu, was dissolved in 20 ml of CH_2Cl_2. That solution was then added dropwise to 250 ml of pyridine and stirred for 48 h at room temperature. The pyridine solution was evaporated in vacuo to dryness, the residue was chromatographed on a silica gel column (2 cm x 12 cm) with $CHCl_3$/MeOH (4:1) as eluent solvent. The purified cyclic peptide was obtained as white crystals (22 %) ; mp = 202°C ; R_F = 0.70 ; FABMS : M^+ = 589 ; IR = 3330 cm^{-1} (NH amide), 2940 and 2860 cm^{-1} (CH_2), 1660 cm^{-1} (CO amide). Anal. calc. for $C_{28}H_{35}N_6O_5S_2$; C, 52.95 ; H, 5.98 ; N, 16.62 ; found : C, 52.47 ; H, 6.15 ; N, 16.43. ^1H NMR ($(CD_3)_2SO$/TMS) see tables 1 and 2.

^{1}H NMR measurements

^{1}H NMR spectra were recorded on a CAMECA 350 (CNRS Vernaison - France). Spectra were obtained at concentration of 10 mg peptide/ml by using a sweep width of 3521 136 Hz with 16 K data points. Approximatively 200 pulses were accumulated for each experiment. Decoupling experiments were performed on amide protons, αCH protons, and CH_3. The correlations with respectively the αCH protons and βCH protons were established through a modification of the peaks due to disappearance of a vicinal coupling factor. Variable temperature measurements were made over the range of 25-100°C. Chemical shifts are expressed in ppm from internal TMS.

Abbreviations : The customary L-configuration for amino acid residues has been omitted. Standard abbreviations for amino acids and derivatives are those recommended by the IUPAC-IUB commission on Biochemical Nomenclature (1972) Biochemistry, 11, 1726-1732. Other abbreviations used are : BOC, tert-butyloxycarbonyl ; HOBt, hydroxybenzotriazole ; HONSu, N-hydroxysuccinimide ; DCC, N,N'-dicyclohexylcarbodiimide ; DCU, N,N'-dicyclohexylurea, THF, tetrahydrofuran ; DMF, dimethylformamide, $N(Et)_3$, triethylamine, AcOH, acetic acid ; NMR, nuclear magnetic resonance ; $(CD_3)_2SO$, hexadeuterated dimethylsulphoxide ; TMS, tetramethylsilane, PTPS, 2,4-bis(4-phenoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide ; t.l.c., thin layer chromatography ; EIMS, electronic impact mass spectrometry ; FABMS, fast atom bombardment mass spectrometry ; (gly)Thz, 2-aminoethyl-thiazole-4-carboxylic acid.

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