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MOLECULAR AND CRYSTAL STRUCTURES OF TWO β-BEND FORMING MONOTHIATED ANALOGUES OF MELANOSTATIN[a]

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Abstract - The molecular and crystal structures of two monothiated analogues of melanostatin, namely *t*-Boc-L-ProΨ(CSNH)-L-Leu-Gly-NH_2 (**1**)[c] and L-Top-L-Leu-Gly-NHEt (**2**), determined by X-ray diffraction analyses, are reported. In compound (**1**) the tertiary urethane bond adopts the *cis* conformation. Both tripeptide amides are folded in a type-II β-bend conformation at the -L-Leu-Gly-sequence. A weak intramolecular H bond, involving the thioamide sulfur atom as the acceptor, is a significant characteristic of compound **1**. The two structures are discussed, in particular, in comparison to those of H-L-Pro-L-Leu-Gly-NH_2 and *t*-Boc-L-Pro-L-Leu-Gly-NH_2, the latter recently solved in the Padova laboratory.

INTRODUCTION

In the field of drug design and molecular pharmacology much effort has recently been spent in a systematic search for analogues of small peptides with altered properties, *e.g* different solubility, higher potency or higher stability towards proteolytic enzymes. In this connection a promising approach is based on the use of the thioamide function as an isosteric amide replacement (for recent reviews see references 1 and 2).

As for the preferred conformation of thiated peptides, X-ray diffraction structures have been reported for two analogues of $Z(Gly)_nOBzl$ (Z, benzyloxycarbonyl; OBzl, benzyloxy; n = 2, 3), adopting either extended or partially bent forms.[3-5] On the other hand, we are currently investigating the structural effect of incorporating a sulfur atom into a peptide chain with a high tendency to fold into a β-bend conformation.[6-9] In this context, X-ray diffraction structures of two Aib containing, monothiated, blocked tripeptides have recently been described.[10] In this paper we report the molecular and crystal structures of two monothiated analogues of the β-bend forming tripeptide amide melanostatin (H-L-Pro-L-Leu-Gly-NH_2), namely *t*-Boc-L-ProΨ(CSNH)-L-Leu-Gly-NH_2 (**1**) (*t*-Boc, *tert*-butyloxycarbonyl) and L-Top-L-Leu-Gly-NHEt (**2**) (Top, 5-thioxoproline; NHEt, ethylamino), determined by X-ray diffraction analysis. The conformational preferences of the two compounds are compared, in particular, with those of melanostatin itself[11] and that of its N^{α}-*tert*-butyloxycarbonylated derivative (the latter structure has recently been solved in the Padova laboratory).[12]

a) This work is dedicated to the memory of Prof. S.-O. Lawesson, a pioneer in the synthesis of thiated peptides, on the occasion of the second anniversary of his untimely death.

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c) The nomenclature of this compound is in accord with the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature, *Eur. J. Biochem.* **138**, 9 (1984).

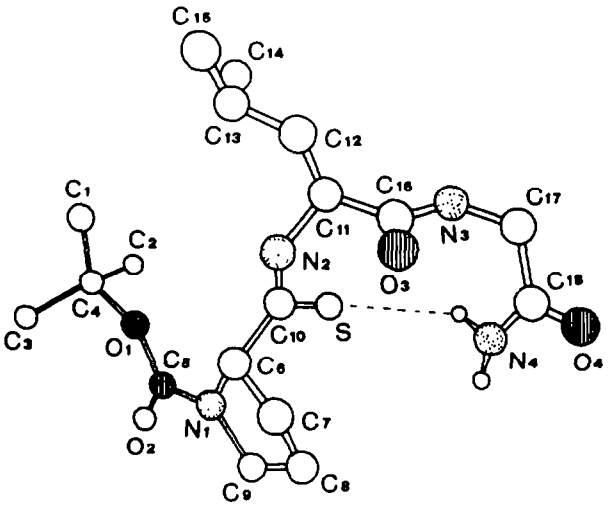

Fig. 1 - Molecular structure of *t*-Boc-L-ProΨ(CSNH) L-Leu-Gly-NH_2 (1). The N-H....S=C intramolecular H-bond is indicated as a dashed line.

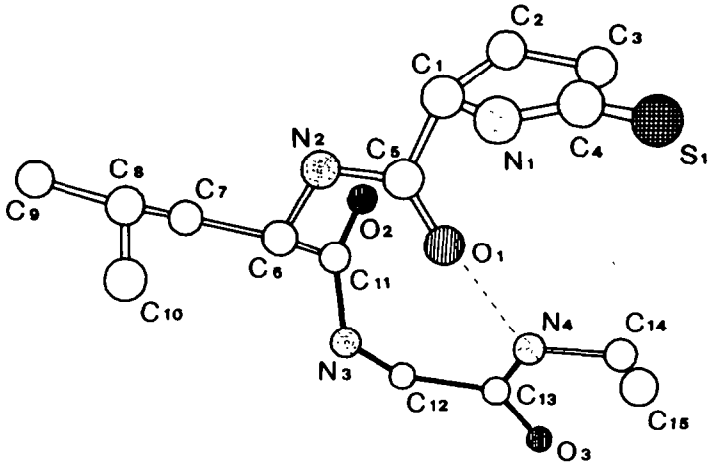

Fig. 2 - Molecular structure of L-Top-L-Leu-Gly-NHEt (2). The N-H....O=C intramolecular H-bond is indicated as a dashed line.

RESULTS AND DISCUSSION

The molecular structures of compounds **1** and **2** with their atomic numbering schemes are shown in Figs. 1 and 2, respectively. Bond lengths and bond angles (together with their estimated standard deviations) are given in Tables 2 and 3. Torsion angles[13] are listed in Tables 4 and 5. The geometry of the intra- and intermolecular H bonds is given in Table 6.

Structural parameters

Bond lengths and bond angles observed for compounds **1** and **2** are in agreement with previously described results for the geometry of the *t*-Boc-urethane,[14] ester,[15] and amide[16] groups, Top,[17] Pro,[18] Leu,[19,20] and Gly residues, and the peptide unit.[21,22]

In particular: (i) In the *t*-Boc N^{α}-protecting group of compound **1** the *tert*-butyl C(1)-C(4), C(2)-C(4), and C(3)-C(4) bonds show some artificial shortening due to the treatment of the thermal motion with anisotropic temperature factors [the U_{eq} tensor components for the C(1), C(2), and C(3) atoms are in the range of 0.112 to 0.132 Å^2]. Unfavourable interactions between the bulky *tert*-butyl group and spatially proximate atoms, especially the carbonyl oxygen O(2), result in the alteration of several bond angles relative to values observed in unhindered compounds.[14] The C(4)-O(1)-C(5) bond angle is 123.4°, *i e* about 6° larger than the value generally found in esters.[15]

(ii) The C(10)-S(1) bond length (1.664 Å) of compound **1**, relatively long for an acyclic thiopeptide unit,[3-5,23] should probably be related to an increased delocalization of the electrons on the sulfur atom involved as acceptor in a weak H bond[10] (see below). In addition, the S(1)-C(10)-N(2) bond angle (124.9°) is significantly wider than the S(1)-C(10)-C(6) bond angle (121.8°), a typical feature of acyclic thiopeptide units.[3-5,10,23]

(iii) The C(4)-S(1) bond length (1.675 Å) and the wide S(1)-C(4)-C(3) and S(1)-C(4)-N(1) bond angles (125-126°) of the cyclic thioamide moiety of compound **2** are close to those observed in the related compounds H-L-Top-OH[17] and H-L-Top-OMe[17] (OMe, methoxy).

Table 1. Crystal data for t-Boc-L-ProΨ(CSNH)-L-Leu-Gly-NH_2 (**1**) and L-Top-L-Leu-Gly-NHEt (**2**)

Parameter	Compound **1**	Compound **2**
Molecular formula	$C_{18}H_{32}N_4O_4S$	$C_{15}H_{26}N_4O_3S$
M.W. (amu.)	400.5	342.4
Density (calcd.) g/cm^3	1.13	1.147
Density (exptl.) g/cm^3	1.13	1.14
Space group	$P2_12_12_1$	$P2_12_12_1$
Z	4	4
a (Å)	17.027(3)	25.672(3)
b (Å)	13.372(3)	12.108(3)
c (Å)	10.306(3)	6.376(3)
α (°)	90	90
β (°)	90	90
γ (°)	90	90
Reflections [$I \geq 3\sigma(I)$]	1930	1509
R value	0.052	0.058
R_w value	0.056	0.064

The pyrrolidine ring of compound **1** exhibits an approximate C_s (envelope) symmetry, the mirror plane passing through the C^{β} atom. This structure may be designated as C_s-C^{β} *exo*,[24] the C^{β} and C' atoms residing on opposite sides of the N-C^{α}-C^{δ} plane (conformation B according to the classification proposed by Balasubramanian *et al*).[25] The heterocyclic ring of compound **2** has also an approximate C_s symmetry, the mirror plane passing through the C^{γ} atom. This structure may be classified as C_s-C^{γ} *endo*[24] (the C^{γ} and C' atoms are on the same side of the N-C^{α}-C^{δ} plane) or B.[25]

The Leu side-chain conformation of both compounds **1** and **2** is the common g^- (t g^-) conformation.[19,20] Atoms in these side chains have high temperature factors; in particular the U_{eq} tensor components for the two C^{δ} atoms range from 0.092 to 0.145 Å^2.

Table 2. Bond lengths (Å) and bond angles (°) for *t*-Boc-L-ProΨ(CSNH) L-Leu-Gly-NH_2 (1)
(ESD's are given in parentheses)

C(1) - C(4)	1.517(10)	C(1) -C(4) -C(2)	110.0(6)	C(6) -C(10)-S(1)	121.8(3)
C(2) - C(4)	1.518(10)	C(1) -C(4) -C(3)	112.4(5)	C(6) -C(10)-N(2)	113.2(3)
C(3) - C(4)	1.474(9)	C(2) -C(4) -C(3)	112.5(6)	S(1) -C(10)-N(2)	124.9(3)
C(4) - O(1)	1.477(6)	C(1) -C(4) -O(1)	108.4(5)	C(10)-N(2) -C(11)	123.4(3)
O(1) - C(5)	1.352(6)	C(2) -C(4) -O(1)	102.2(4)	N(2) -C(11)-C(12)	110.6(3)
C(5) - O(2)	1.217(6)	C(3) -C(4) -O(1)	110.9(5)	C(11)-C(12)-C(13)	114.1(3)
C(5) - N(1)	1.324(6)	C(4) -O(1) -C(5)	123.4(4)	C(12)-C(13)-C(14)	114.5(5)
N(1) - C(9)	1.461(6)	O(1) -C(5) -O(2)	124.7(4)	C(12)-C(13)-C(15)	111.0(4)
C(9) - C(8)	1.484(9)	O(1) -C(5) -N(1)	109.5(4)	C(14)-C(13)-C(15)	112.3(5)
C(8) - C(7)	1.505(9)	O(2) -C(5) -N(1)	125.8(4)	N(2) -C(11)-C(16)	108.0(3)
C(7) - C(6)	1.528(7)	C(5) -N(1) -C(9)	121.5(4)	C(12)-C(11)-C(16)	109.6(3)
N(1) - C(6)	1.459(6)	C(6) -N(1) -C(9)	113.1(4)	C(11)-C(16)-O(3)	121.0(3)
C(6) - C(10)	1.511(6)	N(1) -C(9) -C(8)	104.6(4)	O(3) -C(16)-N(3)	122.1(3)
C(10)- S(1)	1.664(4)	C(9) -C(8) -C(7)	106.5(5)	C(11)-C(16)-N(3)	116.9(3)
C(10)- N(2)	1.319(5)	C(8) -C(7) -C(6)	105.2(5)	C(16)-N(3) -C(17)	119.7(3)
N(2) - C(11)	1.448(5)	C(7) -C(6) -N(1)	101.6(4)	N(3) -C(17)-C(18)	115.8(3)
C(11)- C(12)	1.538(6)	C(6) -N(1) -C(5)	125.0(4)	C(17)-C(18)-O(4)	117.4(4)
C(12)- C(13)	1.503(6)	C(7) -C(6) -C(10)	111.2(4)	O(4) -C(18)-N(4)	125.1(4)
C(13)- C(14)	1.453(11)	N(1)- C(6) -C(10)	113.4(3)	C(17)-C(18)-N(4)	117.5(4)
C(13)- C(15)	1.541(7)				
C(11)- C(16)	1.536(5)				
C(16)- O(3)	1.220(5)				
C(16)- N(3)	1.334(5)				
N(3) - C(17)	1.442(5)				
C(17)- C(18)	1.522(6)				
C(18)- O(4)	1.215(5)				
C(18)- N(4)	1.313(5)				

Table 3. Bond lengths (Å) and bond angles (°) for L-Top-L-Leu-Gly-NHEt (2)
(ESD's are given in parentheses)

S(1) - C(4)	1.675(5)	S(1) -C(4) -C(3)	126.4(3)	C(7) -C(8) -C(9)	110.1(5)
C(4) - C(3)	1.501(7)	C(4) -C(3) -C(2)	105.6(4)	C(7) -C(8) -C(10)	114.4(6)
C(3) - C(2)	1.511(7)	C(3) -C(2) -C(1)	105.1(4)	C(10)-C(8) -C(9)	113.7(6)
C(2) - C(1)	1.534(7)	C(2) -C(1) -N(1)	103.5(4)	N(2) -C(6) -C(11)	109.3(3)
C(4) - N(1)	1.308(6)	C(1) -N(1) -C(4)	115.2(4)	C(7) -C(6) -C(11)	111.7(4)
N(1) - C(1)	1.458(6)	S(1) -C(4) -N(1)	124.7(4)	C(6) -C(11)-O(2)	120.9(4)
C(1) - C(5)	1.527(7)	N(1) -C(4) -C(3)	108.9(4)	C(6) -C(11)-N(3)	113.7(4)
C(5) - O(1)	1.215(6)	N(1) -C(1) -C(5)	110.3(4)	O(2) -C(11)-N(3)	125.4(4)
C(5) - N(2)	1.348(5)	C(5) -C(1) -C(2)	112.9(4)	C(11)-N(3) -C(12)	122.7(4)
N(2) - C(6)	1.436(6)	C(1) -C(5) -O(1)	122.1(4)	N(3) -C(12)-C(13)	115.9(4)
C(6) - C(7)	1.529(6)	N(2) -C(5) -O(1)	123.6(4)	C(12)-C(13)-O(3)	118.2(5)
C(7) - C(8)	1.540(8)	C(1) -C(5) -N(2)	114.3(4)	C(12)-C(13)-N(4)	118.8(4)
C(8) - C(9)	1.515(8)	C(5) -N(2) -C(6)	119.5(4)	O(3) -C(13)-N(4)	122.9(5)
C(8) - C(10)	1.484(12)	N(2) -C(6) -C(7)	111.8(4)	C(13)-N(4) -C(14)	122.7(5)
C(6) - C(11)	1.543(6)	C(6) -C(7) -C(8)	114.6(4)	N(4) -C(14)-C(15)	116.5(7)
C(11)- O(2)	1.216(6)				
C(11)- N(3)	1.336(6)				
N(3) - C(12)	1.467(7)				
C(12)- C(13)	1.498(7)				
C(13)- O(3)	1.230(6)				
C(13)- N(4)	1.324(7)				
N(4) - C(14)	1.454(8)				
C(14)- C(15)	1.401(17)				

Table 4. Torsion angles (°) for *t*-Boc-L-ProΨ(CSNH) L-Leu-Gly-NH_2 (1)
(EDS's are given in parentheses)

C(1) -C(4) -O(1) -C(5)	-58.8(6)	N(1) -C(6) -C(10)-S(1)	-29.3(5)
C(2) -C(4) -O(1) -C(5)	-174.9(5)	C(7) -C(6) -C(10)-N(2)	-92.0(4)
C(3) -C(4) -O(1) -C(5)	65.0(6)	N(1) -C(6) -C(10)-N(2)	154.2(3)
C(4) -O(1) -C(5) -O(2)	-15.2(7)	C(6) -C(10)-N(2) -C(11)	169.0(3)
C(4) -O(1) -C(5) -N(1)	166.2(4)	S(1) -C(10)-N(2) -C(11)	-7.3(5)
O(1) -C(5) -N(1) -C(9)	-177.1(4)	C(10)-N(2) -C(11)-C(12)	162.9(3)
O(2) -C(5) -N(1) -C(9)	4.3(7)	C(10)-N(2) -C(11)-C(16)	-77.0(4)
O(1) -C(5) -N(1) -C(6)	-4.5(6)	N(2) -C(11)-C(12)-C(13)	-66.4(5)
O(2) -C(5) -N(1) -C(6)	176.9(4)	C(11)-C(12)-C(13)-C(14)	-60.1(6)
C(5) -N(1) -C(9) -C(8)	176.2(4)	C(11)-C(12)-C(13)-C(15)	171.4(4)
C(6) -N(1) -C(9) -C(8)	2.8(5)	C(13)-C(12)-C(11)-C(16)	176.5(4)
N(1) -C(9) -C(8) -C(7)	16.2(6)	N(2) -C(11)-C(16)-O(3)	-49.0(5)
C(9) -C(8) -C(7) -C(6)	-28.7(6)	C(12)-C(11)-C(16)-O(3)	71.7(5)
C(9) -N(1) -C(6) -C(7)	-19.9(5)	N(2) -C(11)-C(16)-N(3)	131.8(3)
C(10)-C(6) -C(7) -C(8)	-92.2(5)	C(12)-C(11)-C(16)-N(3)	-107.6(4)
C(8) -C(7) -C(6) -N(1)	28.9(5)	C(11)-C(16)-N(3) -C(17)	-178.0(3)
C(10)-C(6) -N(1) -C(9)	99.6(4)	O(3) -C(16)-N(3) -C(17)	2.7(6)
C(10)-C(6) -N(1) -C(5)	-73.6(5)	C(16)-N(3) -C(17)-C(18)	71.3(4)
C(7) -C(6) -N(1) -C(5)	166.9(4)	N(3) -C(17)-C(18)-O(4)	-164.3(4)
C(7) -C(6) -C(10)-S(1)	84.6(4)	N(3) -C(17)-C(18)-N(4)	15.6(5)

Table 5. Torsion angles (°) for L-Top-L-Leu-Gly-NHEt (2)
(EDS's are given in parentheses)

S(1) -C(4) -C(3) -C(2)	-169.8(4)	C(6) -C(7) -C(8) -C(9)	176.3(5)
S(1) -C(4) -N(1) -C(1)	177.8(4)	C(6) -C(7) -C(8) -C(10)	-54.3(7)
C(4) -C(3) -C(2) -C(1)	-12.8(5)	C(5) -N(2) -C(6) -C(11)	-58.8(5)
N(1) -C(4) -C(3) -C(2)	9.1(6)	C(8) -C(7) -C(6) -C(11)	174.5(4)
C(3) -C(2) -C(1) -N(1)	12.0(5)	N(2) -C(6) -C(11)-O(2)	-45.6(6)
C(2) -C(1) -N(1) -C(4)	-7.1(5)	C(7) -C(6) -C(11)-O(2)	78.7(5)
C(4) -N(1) -C(1) -C(5)	113.9(5)	N(2) -C(6) -C(11)-N(3)	136.3(4)
C(1) -N(1) -C(4) -C(3)	-1.1(6)	C(7) -C(6) -C(11)-N(3)	-99.3(5)
C(3) -C(2) -C(1) -C(5)	107.3(4)	C(6) -C(11)-N(3) -C(12)	-174.7(4)
N(1) -C(1) -C(5) -N(2)	143.0(4)	O(2) -C(11)-N(3) -C(12)	7.3(8)
C(2) -C(1) -C(5) -O(1)	76.3(6)	C(11)-N(3) -C(12)-C(13)	89.4(6)
N(1) -C(1) -C(5) -O(1)	-38.9(6)	N(3) -C(12)-C(13)-O(3)	159.6(5)
C(2) -C(1) -C(5) -N(2)	-101.7(5)	C(14)-N(4) -C(13)-O(3)	3.0(9)
C(1) -C(5) -N(2) -C(6)	172.8(4)	N(3) -C(12)-C(13)-N(4)	-21.5(7)
O(1) -C(5) -N(2) -C(6)	-5.1(7)	C(12)-C(13)-N(4) -C(14)	-175.7(5)
C(5) -N(2) -C(6) -C(7)	176.9(4)	C(13)-N(4) -C(14)-C(15)	-120.7(8)
N(2) -C(6) -C(7) -C(8)	-62.6(5)		

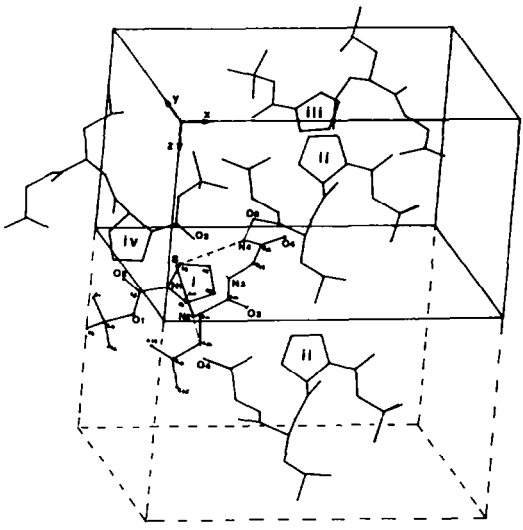

Fig. 3 - Packing mode of the *t*-Boc-L-ProΨ(CSNH) L-Leu-Gly-NH_2 (1) molecules viewed down the *b* axis. The intra- and intermolecular H-bonds are indicated as dashed and dotted lines, respectively.

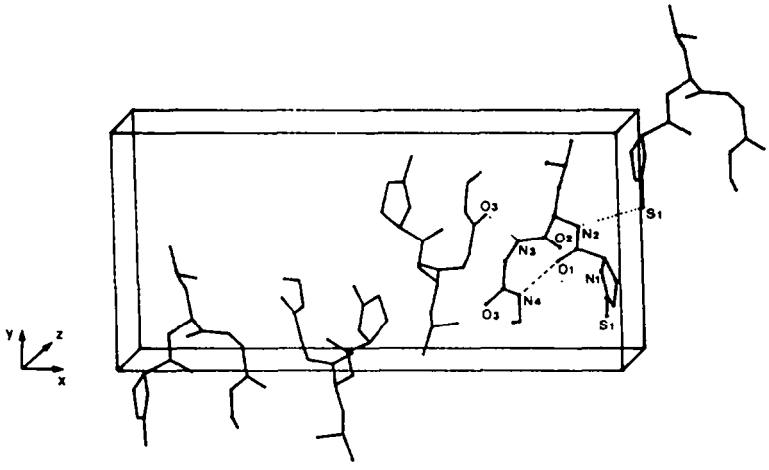

Fig. 4 - Packing mode of the L-Top-L-Leu-Gly-NHEt (2) molecules viewed down the *c* axis. The intra- and intermolecules H-bonds are indicated as dashed and dotted lines, respectively. For clarity, the intermolecular H-bond between N1-H and O2 (x,y,z+1) is not shown.

Backbone conformation

The tertiary urethane bond of the *t*-Boc-Pro moiety of compound **1** adopts the relatively common *cis* conformation, the O(1)-C(5)-N(1)-C(9) torsion angle being - 4.5°.[18,26] In the N^{α}-protecting *t*-Boc group, the C(4)-O(1) bond is in the usual *trans* arrangement relative to the C(5)-N(1) bond, the C(4)-O(1)-C(5)-N(1) torsion angle being 166.2°. This feature, accompanied by the *cis* arrangement of the O(1)-C(5) bond relative to the N(1)-C(6) bond, allows us to classify the urethane moiety of this compound as type *α*.[14,27]

The C(=S)-NH group is *trans* and close to planarity (ω = 169.0°), a common observation for an acyclic thiopeptide unit.[3-5,10,23]

The backbone conformation of compound **1** is folded at the Leu-Gly sequence. The Φ,Ψ values for the L-Leu (Φ = - 77°, Ψ = 131.8°) and Gly (Φ = 71.3°, Ψ = 15.6°) residues lie close to the values expected for an ideal type-II β-bend.[6-9] A weak 4 → 1 intramolecular H bond is seen between the carboxamido nitrogen of the Gly residue and the thiocarbonyl sulfur of the ProΨ(CSNH)-Leu sequence. The N....S separation, 3.56 Å, is less than the outer limit of 3.7 Å.[28-30] To our knowledge, this is the first observation of a (backbone) N-H....S=C (backbone) intramolecular H bond in pseudopeptides. The Pro residue is semi-extended (Φ = - 73.6°, Ψ = 154.2°).

The C(=S)-NH group of the γ-thiolactam moiety of compound **2** is *cis* and planar, as expected, the C(1)-N(1)-C(4)-C(3) torsion angle being - 1.1°.[17] The backbone conformation of this peptide resembles that of compound **1** discussed above, partially extended at the Top residue and folded (type-II β-bend)[6-9] at the L-Leu-Gly sequence. The torsion angles are 143.0° (Ψ L-Top), - 58.8° and 136.3° (Φ, Ψ L-Leu), and 89.4° and - 21.5° (Φ, Ψ Gly). A weak 4 → 1 intramolecular H bond is observed between the carboxamido nitrogen of the Gly residue and the carbonyl oxygen of the Top residue. The N....O distance is 3.04 Å.[31,32]

Crystal packing

The crystal packing mode of compound **1** is characterized by a network of intermolecular N-H....O H bonds involving the three remaining NH donors (the Leu, Gly, and carboxamido NH protons, where the latter is the one not part of the intramolecular H bond) and the three remaining C=O acceptors (the *t*-Boc, Leu, and Gly carbonyls). The N....O distances of 2.79, 2.90, and 2.97 Å are within or close to the average range observed for a large number of intermolecular N-H....O=C H bonds in peptide structures (between 2.9 and 3.0 Å).[31,32]

In the crystal packing mode of compound **2**, in addition to two intermolecular N-H....O=C H-bonds (involving the Top and Gly NH donors and the Leu and Gly C=O acceptors), an intermolecular N-H....S=C H bond is seen (involving the Leu NH donor). The N....O separations are 2.80 and 2.81 Å,[31,32] while the N....S separation is 3.47 Å.[28-30] An intermolecular N-H....S=C H bond was also observed in the crystal structure of H-L-Top-OH, but not in that of its methyl ester.[17]

CONCLUSIONS

This X-ray diffraction study substantiates previous indications pointing to the high tendency of the X-L-Pro-L-Leu-Gly-NH- sequence to fold into β-bend conformations.[6-9] If an H-bonding acceptor is available on X, then two consecutive, intramolecularly H-bonded type-III β-bends might form at the L-Pro-L-Leu and L-Leu-Gly sequences, eventually giving rise to an incipient 3_{10}-helix, as observed for H-L-Cys(Bzl)-L-Pro-L-Leu-Gly-NH_2 (Bzl, benzyl).[33] It is worth noting that in this tetrapeptide amide the critical Cys(Bzl)-Pro amide group is *trans*. If, however, an H-bonding acceptor is not available before the Pro residue in the chain, then only one intramolecularly H-bonded β-bend might form. In this connection the first observation was the type-II β-bend formed by the L-Leu-Gly sequence of melanostatin, H-L-Pro-L-Leu-Gly-NH_2.[11] More recently, we showed that the same conformation is adopted also by *t*-Boc-L-Pro-L-Leu-Gly-NH_2 (in this N^{α}-protected tripeptide amide the tertiary urethane group is *cis* and therefore its C=O group cannot take part in the intramolecular H-bonding).[12]

In neither the structure of compound **1** nor in that of compound **2** presented here is an acceptor in the X-Pro sequence available for an intramolecular H-bond. In fact, the *N*-terminal tertiary

Table 6. Geometry of H bonds in the crystals of *t*-Boc-L-ProΨ(CSNH)-L-Leu-Gly-NH_2 (**1**) and L-Top-L-Leu-Gly-NHEt (**2**)

Donor (D)	Acceptor (A)	Symmetry Equiv. of Acceptor	Distances (Å) D....H	H....A	Angle (°) D - H....A
(i) *t*-Boc-L-ProΨ(CSNH)-L-Leu-Gly-NH_2 (**1**)					
N(4)-H	S(1)	x , y , z	3.56(1)	2.75(1)	147(1)
N(2)-H	O(4)	1/2-x, -y ,1/2+z	2.79(1)	1.85(1)	175(1)
N(3)-H	O(2)	-x ,1/2+y,3/2-z	2.90(1)	2.04(1)	147(1)
N(4)-H'	O(3)	1/2-x, -y ,z-1/2	2.97(1)	2.14(1)	157(1)
(ii) L-Top-L-Leu-Gly-NHEt (**2**)					
N(4)-H	O(1)	x , y , z	3.04(1)	2.20(1)	146(1)
N(1)-H	O(2)	x , y , z+1	2.80(1)	2.05(1)	148(1)
N(2)-H	S(1)	-x+2,y+1/2,-z+1/2	3.47(1)	2.62(2)	172(1)
N(3)-H	O(3)	-x+3/2, -y+1, z+1/2	2.81(1)	1.86(2)	176(1)

urethane group of compound 1 and the thioamide group of compound **2** are both *cis*. Therefore, it is not surprising that the conformational feature characterizing both compounds **1** and **2** is a single (type-II) β-bend.

In addition, the present results stress the following points:

(i) Replacement of an oxygen by a sulfur atom in a peptide (amide) unit near the *N*-terminus does not produce any significant change in the propensity of the X-L-Pro-L-Leu-Gly-NH-sequence to fold.

(ii) A thioamide sulfur atom can act as an H-bonding acceptor in a β-bend conformation.

Finally, the bond angles and bond lengths not directly involving the sulfur atom of thiated peptides are generally very close to those of the unsubstituted peptides (this work and references 3-5, 10, 23), further enhancing the attractiveness of this type of isosteric amide bond replacement.[1] As for the solution conformation, it has recently been demonstrated by ^{1}H NMR that the *trans* disposition of the C(=S)-NH group is that preferred by acyclic thiated peptides in organic solvents,[34] as already well established for their oxygenated counterparts.

EXPERIMENTAL

Materials

The synthesis and characterization of *t*-Boc-L-ProΨ(CSNH)-L-Leu-Gly-NH_2 (**1**) and L-Top-L-Leu-Gly--NHEt (**2**) have already been reported.[35,36]

X-Ray Diffraction Analysis

Single crystals of *t*-Boc-L-ProΨ(CSNH)-L-Leu-Gly-NH_2 (**1**) were grown by slow evaporation from an acetone solution.

X-Ray diffraction data were collected on a Philips PW 1100 four-circle diffractometer, using MoKα radiation monochromatized by a graphite crystal (λ = 0.71069 Å). Intensities were corrected for Lorentz and polarization effects and put on an absolute scale by Wilson's method. No absorption correction was applied. The crystallographic data are summarized in Table 1.

The structure was solved by application of the direct methods program MULTAN 80.[37] The E-maps of the set of phases with best combined figure of merit revealed the positions of 22 non-hydrogen atoms. The positions of the remaining non-hydrogen atoms were derived from subsequent difference Fourier maps. The structure was refined by the block matrix least-squares procedure. The function minimized was $\Sigma w\Delta^2$, ($\Delta = |F_o| - |F_c|$) and w was $[\sigma(F_o) + 0.0020\ F_o^2]^{-1}$. Weighting-scheme analysis showed no serious dependence of the mean $w\Delta^2$ as a function of either $|F_o|$ and $\lambda^{-1}\sin\theta$. The scattering factors were taken from the International Tables for X-Ray Crystallography.[38]

The correction for the real and imaginary parts of the anomalous dispersion was applied to sulfur. The refinement was carried out allowing all non-hydrogen atoms to vibrate anisotropically. The hydrogen atoms were located from difference Fourier maps and included in the last cycle. Calculations were carried out using the SHELX-76 program.[39] The final conventional R value for the 1930 observed reflections [$I \geq 3\sigma(I)$] was 0.052 (R_w = 0.056).

Single crystals of L-Top-L-Leu-Gly-NHEt (**2**) were grown by slow evaporation from a methanol/diethyl ether/*n*-hexane solution. All data and methods were identical to those reported above for t-Boc-L-Pro$^{\Psi}$(CSNH)—L-Leu-Gly-NH_2 (**1**) with the following exceptions: (i) $\underline{w}$ was $[\sigma(F_0) + 00.34\ F_0^2]^{-1}$; (ii) the final conventional R value for the 1509 observed reflections [$I \geq 3\sigma(I)$] was 0.058 (R_w = 0.064).

The crystallographic data for compounds **1** and **2** are listed in Table 1.

The final positional parameters of the non-hydrogen atoms, along with the equivalent isotropic thermal factors and hydrogen positional parameters have been deposited at the Cambridge Crystallographic Data Centre. Anisotropic temperature factors and structure factor tables are available on request from Dr. R. Bardi.

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REFERENCES

1. A. F. Spatola, in *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*, B. Weinstein, Ed., Dekker, New York, Vol. 7, pp. 267-357 (1983).
2. M. P. Cava and M. I. Levinson, *Tetrahedron* **41**, 5061 (1985).
3. T. F. M. La Cour, H. A. S. Hansen, K. Clausen and S.-O. Lawesson, *Int J Peptide Protein Res* **22**, 509 (1983).
4. H. A. S. Hansen, K. Clausen and T. F. M. La Cour, *Acta Cryst.*, **C43**, 519 (1987).
5. H. A. S. Hansen, K. Clausen and T. F. M. La Cour, *Acta Cryst* , **C43**, 522 (1987).
6. C. M. Venkatachalam, *Biopolymers* **6**, 1425 (1968).
7. P. Y. Chou and G. D. Fasman, *Biophys J* **26**, 367 (1979).
8. C. Toniolo, *CRC Crit Rev Biochem* **9**, 1 (1980).
9. G. D. Rose, L. M. Gierasch and J. A. Smith, *Adv Protein Chem.* **37**, 1 (1985).
10. O. E. Jensen, S.-O. Lawesson, R. Bardi, A. M. Piazzesi and C. Toniolo, *Tetrahedron* **41**, 5595 (1985).
11. L. L. Reed and P. L. Johnson, *J Am Chem Soc.* **95**, 7523 (1973).
12. C. Toniolo, G. Valle and R. L. Johnson, to be submitted.
13. IUPAC-IUB Commission on Biochemical Nomenclature, *Biochemistry* **9**, 3471 (1970).
14. E. Benedetti, C. Pedone, C. Toniolo, G. Némethy, M. S. Pottle and H. A. Scheraga, *Int. J Peptide Protein Res.* **16**, 156 (1980).
15. W. B. Schweizer and J. D. Dunitz, *Helv. Chim. Acta* **65**, 1547 (1982).
16. P. Chakrabarati and J. D. Dunitz, *Helv. Chim Acta* **65**, 1555 (1982).
17. T. P. Andersen, P. B. Rasmussen, I. Thomsen, S.-O. Lawesson, P. Jørgensen and P. Lindhardt, *Liebigs Ann Chem.* 269 (1986).
18. E. Benedetti, A. Bavoso, B. Di Blasio, V. Pavone, C. Pedone, C. Toniolo and G. M. Bonora, *Biopolymers* **22**, 305 (1983).
19. E. Benedetti, G. Morelli, G. Némethy and H. A. Scheraga, *Int. J Peptide Protein Res.* **22**, 1 (1983).
20. R. O. Gould, A. M. Gray, P. Taylor and M. D. Walkinshaw, *J Am. Chem. Soc.* **107**, 5921 (1985).
21. E. Benedetti, in *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*, B. Weinstein, Ed., Marcel Dekker, New York, Vol. 6, pp. 105-184 (1982).
22. I. L. Karle, in *The Peptides· Analysis, Synthesis, Biology*, E. Gross and J. Meienhofer, Eds., Academic Press, New York, Vol. 4, pp. 1-54 (1981).
23. K. I. Varughese, M. Przybylska, K. Sestanj, F. Bellini and L. G. Humber, *Can. J. Chem* **61**, 2137 (1983).
24. T. Ashida and M. Kakudo, *Bull Chem. Soc. Japan* **47**, 1129 (1974).
25. R. Balasubramanian, A. V. Lakshminarayanan, M. N. Sabesan, G. Tegoni, K. Venkatesan and G. N. Ramachandran, *Int. J. Protein Res.* **3**, 25 (1971).
26. C. M. K. Nair and M. Vijayan, *J. Indian Inst. Sci* **63 C**, 81 (1981).
27. E. Benedetti, A. Ciajolo, B. Di Blasio, V. Pavone, C. Pedone, C. Toniolo and G. M. Bonora, *Int J. Peptide Protein Res.* **14**, 130 (1979).
28. R. Srinivasan and K. K. Chacko, in *Conformation of Biopolymers*, G. N. Ramachandran, Ed., Academic Press, New York, Vol. 2, pp. 607-615 (1967).
29. J. Donohue, *J Mol Biol* **45**, 231 (1969).
30. V. K. Pogorelyi, *Russian Chem. Rev.* **46**, 316 (1977).
31. C. Ramakrishnan and N. Prasad, *Int. J Protein Res* **3**, 209 (1971).
32. R. Taylor and O. Kennard, *Acta Cryst.* **B39**, 133 (1983).
33. A. D. Rudko and B. W. Low, *Acta Cryst.* **B31**, 713 (1975).
34. D. J. S. Guthrie, C. H. Williams and D. T. Elmore, *Int. J. Peptide Protein Res* **28**, 208 (1986).
35. M. Thorsen, B. Yde, U. Pedersen, K. Clausen and S.-O. Lawesson, *Tetrahedron* **39**, 3429 (1983).
36. T. P. Andersen and A. Senning, *Liebigs Ann. Chem.*, 59 (1987).
37. P. Main, S. J. Fiske, S. E. Hull, L. Lessinger, G. Germain, J. P. Declercq and M. M. Woolfson, *MULTAN-80, A System of Computer Programs for the Automatic Solution of Crystal Structures from X-Ray Diffraction Data*, Univ. of York, England and Univ. of Louvain, Belgium, 1980.
38. *International Tables for X-Ray Crystallography*, Vol. IV, 2nd Ed., Kynoch Press, Birmingham, England, 1974.
39. G. M. Sheldrick, *SHELX-76, Program for Crystal Structure Determination*, Univ. of Cambridge, England, 1976.