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STUDIES ON AMINO ACIDS AND PEPTIDES VIII.[a]

SYNTHESIS AND CRYSTAL STRUCTURE OF TWO MONOTHIATED ANALOGUES OF Boc-Gly-S-Ala-Aib-OMe

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Abstract - The model tripeptide Boc-Gly-S-Ala-Aib-OMe (2b) and the two monothiated analogues Boc-Gly($^1\psi^2$CSNH)-S-Ala-Aib-OMe (2c) and Boc-Gly-S-Ala($^2\psi^3$CSNH)-Aib-OMe (2a) were synthesized.[b] Peptide 2a was obtained by thiation of 1a using 2,4-bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane 2,4-disulfide, Lawesson's Reagent (LR), followed by deprotection of the Boc group and coupling with Boc-Gly-OH. Thiation of 2b with LR regiospecifically transformed the protected tripeptide to the monothiated analogue 2c. X-Ray diffraction analysis showed that the type-III β-turn formed by the reference peptide 2b is preserved in the monothiated analogue 2a; conversely, the structure of the isomeric tripeptide 2c is partially extended.

INTRODUCTION

The replacement of the amide bond by a thioamide bond in physiologically active peptides is one of several backbone modifications introduced in search for compounds that would be more potent and/or selective than their parent structures.[c] The resistance afforded by the thioamide bond against enzymatic cleavage may play an important part in enhancing the potency of the pseudopeptide.[3,4] Unfortunately, sometimes reduced activity is observed.[1,5] This may be due to several factors, *e.g.* subtle changes in conformation as a consequence of the substitution of the softer sulfur for the harder oxygen at the amide linkage.

The number of different conformational states of a linear peptide is often reduced by incorporating sterically hindered amino acids such as Aib (an acronym for α-aminoisobutyric acid or 2-methylalanine) into the sequence.[6] Aib-containing peptides have drawn considerable attention since this residue was discovered to be naturally occurring in the antibiotic I.C.I. 13959.[7] Now, a special class of antibiotics termed "peptaibols",[8,9] is known to consist of peptides having several Aib residues.[d]

To study the effect of thioamide incorporation we choose to synthesise analogues of the model peptide 2b, the solid-state structure of which was thoroughly examined by Jung and coworkers.[13]

† Deceased, March 5, 1985.

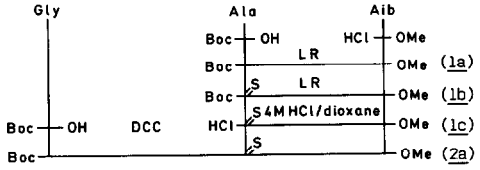
[a] For Part VII, see ref. 1.

[b] The nomenclature of the compounds is in accordance with the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature, *Pure Appl.Chem.* 5b, 595 (1984).

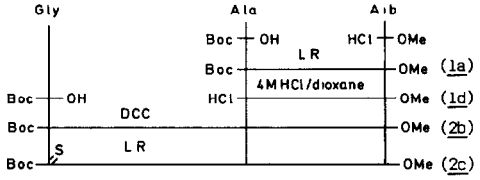
[c] For a review see ref. 2.
[d] For reviews see ref. 10-12.

RESULTS AND DISCUSSION

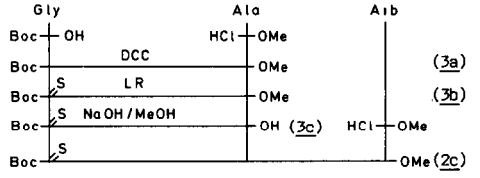
For the syntheses of 2a-c (Fig. 1 and Schemes 1-3), DCC as well as LR[14] coupling procedures were used. The general application of LR as a coupling reagent is outlined in Scheme 4. The Boc group was cleaved by treatment with 4M HCl/dioxane. Deprotection of the methyl ester group was achieved by saponification (NaOH/MeOH). The thiation of 3a with LR proceded smoothly at room temperature to give 3b (Scheme 5). The reaction time was 4 hrs. However, under comparable conditions thiation of 1a could barely be performed. This may be ascribed to the steric crowding caused by the *gem*-methyl groups of Aib about the amide bond region. This was confirmed by the successful thiation of 1a with LR in toluene at 100 °C for 1/2 hr to give 1b (Scheme 5). Selective thiation under these conditions is possible since urethanes[15] and esters[16] do not react with LR at 100 °C, but at 110 °C and 140 °C, respectively. Deprotection of the Boc group of 1b followed by coupling with Boc-Gly-OH gave 2a (Scheme 1). Coupling of 3c with H-Aib-OMe to give 2c was unsuccessful by either the DCC/HOBT or the mixed anhydride method (Scheme 3). The dramatic difference in the thiation conditions of 3a and 1a was therefore exploited for the synthesis of 2c. Thiation of 2b with LR proceeded regiospecifically at room temperature to give 2c in 85% yield (Scheme 5). The reac-



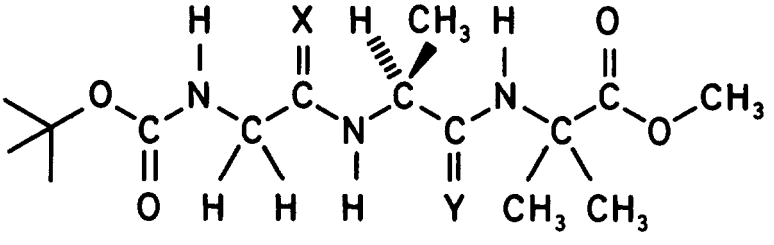
Scheme 1. Synthesis of 2a.



Scheme 2. Synthesis of 2c.

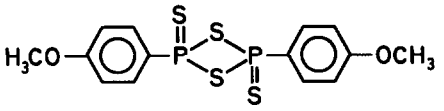


Scheme 3. Attempted synthesis of 2c.

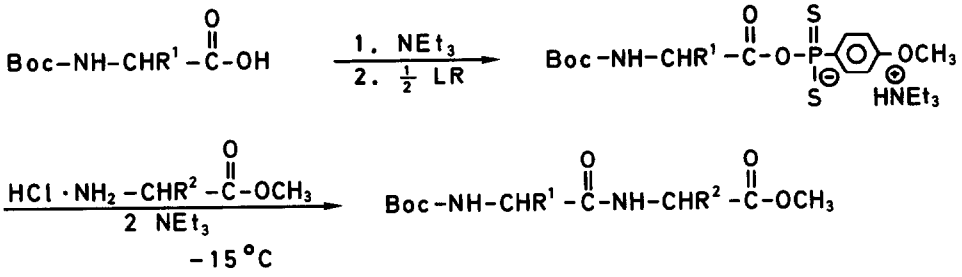


2a : X = O, Y = S
2b : X = O, Y = O
2c : X = S, Y = O

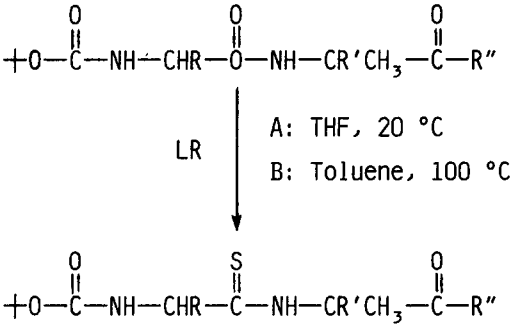
Fig. 1



LR



Scheme 4



B) R = R′ = CH_3, R″ = OCH_3 (1a → 1b)
A) R = R′ = H, R″ = OCH_3 (3a → 3b)
A) R = R′ = H, R″ = $NH-C(CH_3)_2-C(=O)-OCH_3$ (2b → 2c)

The different conditions (A and B) for the thiation of 1a and 3a providing regiospecific thiation of 2b to 2c.

Scheme 5

tion time was 2 hrs. Compound 2c may also be obtained by coupling the dithioester of Boc-Gly-OH with 1d. The preparation of the active ester, however, is a four-step process.[17] The site of thiation was verified by ^{1}H NMR.

The ^{1}H and ^{13}C NMR spectra show large downfield shifts for the thioamide protons and thiocarbonyl carbons with respect to the corresponding amide analogues (Tables 1, 2). The observed ranges are in accordance with previously reported values for thioamides,[17-19] 1.6-2.03 ppm for protons and 29.9-32.3 ppm for carbons. The α-carbons and α-hydrogens also exhibit downfield shifts, 7.70-11.3 ppm and 0.24-0.62 ppm, respectively. The chemical shifts of the N- and C-terminal protecting groups are left relatively unchanged. IR absorption spectroscopy showed the urethane carbonyl at 1690-1710 cm^{-1}, the amide carbonyl at 1670 and 1550 cm^{-1}, and the ester carbonyl at 1720-1750 cm^{-1}.[17] The thioamide C=S bands (1485-1525 cm^{-1}) are difficult to assign since they fall in a region where other vibrators strongly absorb. The UV absorption spectra of the thioamide-containing peptides showed the characteristic π-π* absorption at 265 nm with lg ε values in the range 3.8-4.1.[17] In MS only occasionally the molecular ion ($M^{+\cdot}$) was observed. Otherwise, the ($M^{+\cdot}$ + 1) and/or the characteristic fragmentation products (M->=) and ($M^{+\cdot}$ - +O) from the Boc protected peptides were found. Also loss of HS[17] was observed.

Table 1. ^{1}H NMR Chemical shifts of the peptides and fragments used in the syntheses

	Boc	Gly		Ala			Aib		OMe
	$3(CH_3)$	NH	HC_α	NH	HC_α	H_3C_β	NH	H_3C_β	OCH_3
1a	1.30			5.33	4.05	1.20	6.90	1.39	3.54
1b	1.35			5.26	4.29	1.30	8.49	1.59	3.58
2a	1.26	5.12	3.58	7.28	4.76	1.20	9.04	1.48	3.46
2b	1.32	5.31	3.65	6.81	4.38	1.21	7.01	1.39	3.55
2c	1.35	5.60	4.03	8.69	4.90	1.34	6.94	1.41	3.58
3a	1.38	5.41	3.71	6.90	4.52	1.31			3.64
3b	1.39	4.49	4.09	8.65	5.10	1.49			3.68

Table 2. ^{13}C NMR Chemical shifts of the peptides and fragments used in the syntheses

	Boc			Gly		Ala			Aib			OMe
	$-\overset{\vert}{C}-$	Me_3	C=O	C_α	C=X	C_α	C_β	C=Y	C_α	C_β	C=O	OCH_3
1a	79.9	28.2	155.5			50.0	18.0	172.1	56.1	24.7	174.7	52.4
1b	80.5	28.3	155.7			59.9	21.1	204.4	56.7	24.1	173.3	52.6
2a	80.5	28.3	155.8	44.5	169.0	60.0	22.4	203.3	54.1	24.2	173.2	52.5
2b	80.2	28.3	156.0	44.3	169.6	48.7	18.2	171.2	56.3	24.6	174.7	52.5
2c	80.6	28.2	156.1	52.1	199.5	53.9	17.0	170.2	56.6	24.7	174.6	52.6
3a	80.4	28.3	156.0	44.5	169.0	48.0	18.4	173.2				52.5
3b	80.7	28.2	156.2	52.2	199.9	53.1	16.9	172.2				52.6

The molecular structures of 2a and 2c with the atomic numbering schemes are shown in Fig. 2 and 3, respectively. Bond lengths and bond angles, and their estimated standard deviations, are given in Tables 3 and 4, respectively. The backbone torsion angles[20] are listed in Tables 5 and 6, respectively. The observed bond lengths and bond angles of the N-protecting group in both tripeptides are in agreement with previously described results for the geometry of the Boc urethane group.[21] In particular, unfavourable interactions between the bulky *tert*-butyl group and spatially proximate atoms, especially carbonyl oxygen O(2), result in alteration of several bond angles, relative to values observed in unhindered compounds. The C(4)-O(1)-C(5) bond angle is 121.1° for

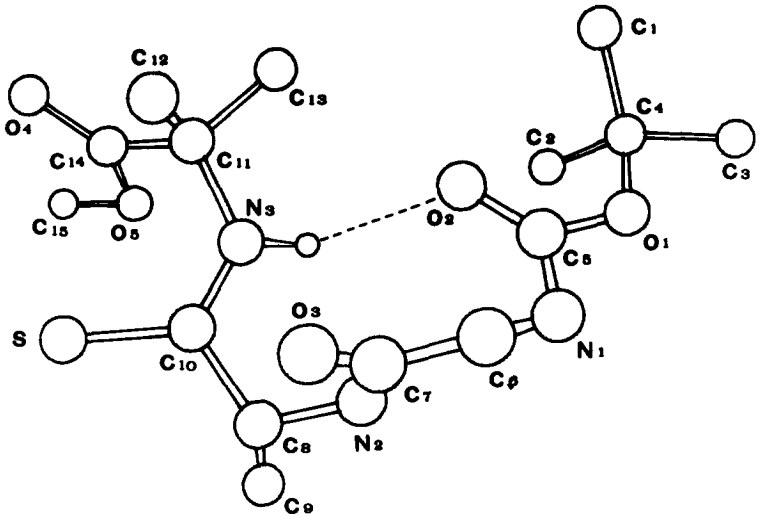


Fig. 2. Molecular structure of Boc-Gly-S-Ala($^{2}\psi^{3}$CSNH)-Aib-OMe (2a).

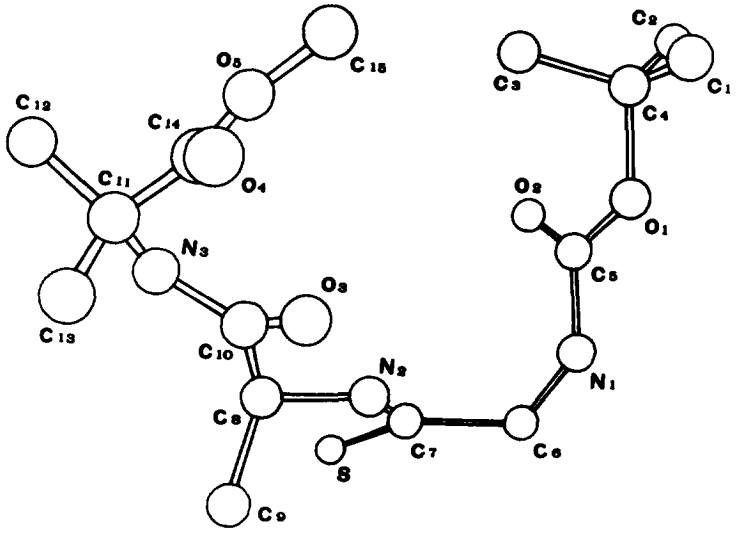


Fig. 3. Molecular structure of Boc-Gly($^{1}\psi^{2}$CSNH)-S-Ala-Aib-OMe (2c).

2a and 120.3° for 2c, *i.e.* about 2-3° larger than the value generally found in esters.[22] The C(1)-C(4)-C(2) bond angle is also opened up to 111.6° for 2a and 113.0° for 2c. This change is accompanied by a decrease of the O(1)-C(4)-C(3), (102.6° for 2a), O(1)-C(4)-C(2), (103.8° for 2c), and O(1)-C(5)-N(1) bond angles (110.1° for 2a and 108.6° for 2c, respectively). The repulsive interaction between O(2) and C(6) results in a widening of the O(2)-C(5)-N(1) bond angle to 125.0° for 2a as well as 2c. Bond lengths and bond angles of the Gly residue in 2a and the S-Ala residue in

Table 3

Bond lengths (Å) and bond angles (°) for Boc-Gly-S-Ala($^1\psi^2$CSNH)-Aib-OMe (2a). ESD's are given in parentheses.

Bond Lengths		Bond Angles	
S - C(10)	1.663(4)	C(4) - O(1) - C(5)	121.1(3)
O(1) - C(4)	1.469(4)	C(14)- O(5) - C(15)	116.3(4)
O(1) - C(5)	1.354(5)	C(5) - N(1) - C(6)	119.0(3)
O(2) - C(5)	1.212(4)	C(7) - N(2) - C(8)	120.6(3)
O(3) - C(7)	1.222(5)	C(10)- N(3) - C(11)	125.5(3)
O(4) - C(14)	1.212(4)	O(1) - C(4) - C(1)	109.2(3)
O(5) - C(14)	1.324(6)	O(1) - C(4) - C(2)	111.2(4)
O(5) - C(15)	1.435(7)	O(1) - C(4) - C(3)	102.6(3)
N(1) - C(5)	1.339(4)	C(1) - C(4) - C(2)	111.6(4)
N(1) - C(6)	1.473(5)	C(1) - C(4) - C(3)	110.9(5)
N(2) - C(7)	1.345(6)	C(2) - C(4) - C(3)	111.1(4)
N(2) - C(8)	1.441(5)	O(1) - C(5) - O(2)	124.9(3)
N(3) - C(10)	1.321(4)	O(1) - C(5) - N(1)	110.1(3)
N(3) - C(11)	1.481(4)	O(2) - C(5) - N(1)	125.0(3)
C(1) - C(4)	1.521(8)	N(1) - C(6) - C(7)	115.4(4)
C(2) - C(4)	1.524(7)	C(6) - C(7) - N(2)	119.1(3)
C(3) - C(4)	1.517(6)	C(6) - C(7) - O(3)	119.1(5)
C(6) - C(7)	1.508(6)	N(2) - C(7) - O(3)	121.7(4)
C(8) - C(9)	1.530(9)	C(9) - C(8) - N(2)	109.6(3)
C(8) - C(10)	1.532(4)	C(10)- C(8) - N(2)	112.8(3)
C(11)- C(12)	1.549(5)	C(9) - C(8) - C(10)	109.3(4)
C(11)- C(13)	1.530(8)	N(3) - C(10)- S	123.9(2)
C(11)- C(14)	1.516(7)	C(8) - C(10)- S	119.4(3)
		C(8) - C(10)- N(3)	116.5(3)
		C(12)- C(11)- N(3)	106.5(3)
		C(13)- C(11)- N(3)	110.1(3)
		C(12)- C(11)- C(13)	111.1(4)
		C(14)- C(11)- N(3)	111.4(4)
		C(14)- C(11)- C(12)	104.8(3)
		C(14)- C(11)- C(13)	112.6(3)
		C(11)- C(14)- O(4)	123.4(5)
		C(11)- C(14)- O(5)	112.8(3)
		O(4) - C(14)- O(5)	123.4(5)

Table 4

Bond lengths (Å) and bond angles (°) for Boc-Gly($^1\psi^2$CSNH)-S-Ala-Aib-OMe (2c). ESD's are given in parentheses.

Bond Lengths		Bond Angles	
S - C(7)	1.662(4)	C(4) - O(1) - C(5)	120.3(4)
O(1) - C(4)	1.469(6)	C(14)- O(5) - C(15)	114.7(4)
O(1) - C(5)	1.349(6)	C(5) - N(1) - C(6)	116.9(4)
O(2) - C(5)	1.192(6)	C(7) - N(2) - C(8)	124.7(3)
O(3) - C(10)	1.230(5)	C(10)- N(3) - C(11)	123.3(3)
O(4) - C(14)	1.193(5)	O(1) - C(4) - C(1)	112.1(5)
O(5) - C(14)	1.339(6)	O(1) - C(4) - C(2)	103.8(4)
O(5) - C(15)	1.455(7)	O(1) - C(4) - C(3)	107.5(5)
N(1) - C(5)	1.355(6)	C(1) - C(4) - C(2)	113.0(6)
N(1) - C(6)	1.457(6)	C(1) - C(4) - C(3)	110.9(7)
N(2) - C(7)	1.312(5)	C(2) - C(4) - C(3)	109.1(7)
N(2) - C(8)	1.447(5)	O(1) - C(5) - O(2)	126.5(5)
N(3) - C(10)	1.333(5)	O(1) - C(5) - N(1)	108.6(4)
N(3) - C(11)	1.468(5)	O(2) - C(5) - N(1)	125.0(5)
C(1) - C(4)	1.447(12)	N(1) - C(6) - C(7)	114.5(4)
C(2) - C(4)	1.496(8)	C(6) - C(7) - N(2)	116.7(3)
C(3) - C(4)	1.508(12)	C(6) - C(7) - S	119.4(3)
C(6) - C(7)	1.520(6)	N(2) - C(7) - S	123.9(3)
C(8) - C(9)	1.517(8)	C(9) - C(8) - N(2)	110.5(4)
C(8) - C(10)	1.519(6)	C(10)- C(8) - N(2)	109.5(3)
C(11)-C(12)	1.540(7)	C(9) - C(8) - C(10)	108.7(4)
C(11)-C(13)	1.516(8)	N(3) - C(10)- O(3)	123.0(4)
C(11)-C(14)	1.521(6)	C(8) - C(10)- O(3)	121.0(4)
		C(8) - C(10)- N(3)	115.9(4)
		C(12)- C(11)- N(3)	106.9(4)
		C(13)- C(11)- N(3)	109.4(4)
		C(12)- C(11)- C(13)	111.0(4)
		C(14)- C(11)- N(3)	110.1(3)
		C(14)- C(11)- C(12)	108.6(4)
		C(14)- C(11)- C(13)	110.7(4)
		C(11)- C(14)- O(4)	124.5(4)
		C(11)- C(14)- O(5)	111.6(4)
		O(4) - C(14)- O(5)	123.7(4)

2c are in agreement with the observed values in peptides.[23] The thiopeptide group is planar in 2a (ω_2 = 180°) or close to planarity in 2c (ω_1 = 175.5°), and its geometry is not affected by the longer and weaker C=S bond. The bond lengths and bond angles of the thiopeptide unit are within 0.01 Å and 0.5° of those values normally found in peptides.[23] The substitution of an oxygen with the less electronegative sulfur atom does not change the π-electron distribution in the peptide bond to such an extent that the length is changed. These results compare well with those already reported for the only two other thioamide-containing amino acid derivatives and peptides, the crystal structures of which have been solved by X-ray diffraction.[24,25] The bond angles about the C_α atom of the Aib residue in both 2a and 2c significantly deviate from the ideal tetrahedral value. This feature has already been found in other Aib-containing peptide structures.[26,27]

Table 5

Backbone torsion angles (°) for Boc-Gly-S-Ala($^2\psi^3$CSNH)-Aib-OMe (2a). ESD's are given in parentheses.

O(1) - C(5) - N(1) - C(6)	ω_0	-165.6
C(5) - N(1) - C(6) - C(7)	Φ_{Gly}	- 65.1
N(1) - C(6) - C(7) - N(2)	ψ_{Gly}	- 21.8
C(6) - C(7) - N(2) - C(8)	ω_1	177.9
C(7) - N(2) - C(8) - C(10)	Φ_{Ala}	- 69.8
N(2) - C(8) - C(10)- N(3)	ψ_{Ala}	- 26.2
C(8) - C(10)- N(3) - C(11)	ω_2	180.0
C(10)- N(3) - C(11)- C(14)	Φ_{Aib}	53.2
N(3) - C(11)- C(14)- O(5)	ψ_{Aib}	41.6

Table 6

Backbone torsion angles (°) for Boc-Gly($^1\psi^2$CSNH)-S-Ala-Aib-OMe (2c). ESD's are given in parentheses.

O(1) - C(5) - N(1) - C(6)	ω_0	-178.4
C(5) - N(1) - C(6) - C(7)	Φ_{Gly}	- 74.9
N(1) - C(6) - C(7) - N(2)	ψ_{Gly}	- 28.9
C(6) - C(7) - N(2) - C(8)	ω_1	-175.5
C(7) - N(3) - C(8) - C(10)	Φ_{Ala}	-156.0
N(2) - C(8) - C(10)- N(3)	ψ_{Ala}	142.1
C(8) - C(10)- N(3) - C(11)	ω_2	164.2
C(10)- N(3) - C(11)- C(14)	Φ_{Aib}	46.8
N(3) - C(11)- C(14)- O(5)	ψ_{Aib}	52.0

The C(4)-O(1) bond of the urethane moiety 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 - 172.6° for 2a and - 171.3° for 2c. This structural property, accompanied by the *trans* conformation of the CO-NH moiety (ω_0 = - 165.6° for 2a and - 178.4° for 2c), allows us to classify the urethane group of these two compounds as type b.[21,28]

Peptide 2a adopts a folded β-turn conformation stabilized by a 4-1 intramolecular H-bond between the NH group of the Aib residue and the carbonyl oxygen of the Boc group [N(3)...O(2) = 3.02 Å].[29] The Φ, ψ values for the Gly (Φ = -65.1°, ψ = - 21.8°) and S-Ala($^2\psi^3$CSNH) (Φ = - 69.8°, ψ = - 26.2°) residues lie close to the values expected for an ideal type-III β-turn conformation.[30] Conversely, in the crystal structure of peptide 2c, no intramolecular H-bond is present. In fact, in contrast to the folded conformation of the Gly($^1\psi^2$CSNH) residue (Φ = - 74.9°, ψ = -28.9°), the S-Ala residue adopts an extended conformation (Φ = - 156.0°, ψ = 142.1°).

The N(1)H and N(2)H groups of 2a act as intermolecular H-bonding donors to the sulfur atom and the O(3) carbonyl oxygen of two neighbouring molecules, respectively (Fig. 4). The N(1)....S and N(2)....O(3) distances are 3.33 Å[31] and 3.01 Å,[29] respectively. The three NH groups of 2c [N(1)H, N(2)H, and N(3)H] form intermolecular H-bonds with the O(3) and O(4) carbonyl oxygens and the sulfur atoms of two different neighbouring molecules, respectively (Fig. 5). The N(1)...O(3), N(2)...O(4), and N(3)...S distances are 3.09 Å,[29] 2.99 Å,[29] and 3.36 Å,[31] respectively. Details of the geometry of the various H-bonds present in the crystal structures of 2a and 2c are given in Table 7.

CONCLUSION

Recently, Jung and coworkers[13] described the formation of a type-III β-turn by the protected tripeptide Boc-Gly-S-Ala-Aib-OMe (2b) in the crystal state. This finding was somewhat surprising since the Gly-Ala sequence is not expected to favour such a folded conformation,[32] thereby raising the question of a possible "long-range" influence of the Aib residue (which is known to prefer strongly the type-III β-turn conformation in small peptides).[10-12,27]

In this work we have analysed by X-ray diffraction the crystal structures of two monothiated analogues of the above-mentioned tripeptide, namely Boc-Gly-S-Ala($^{2}\psi^{3}$CSNH)-Aib-OMe (2a) and Boc-Gly($^{1}\psi^{2}$CSNH)-S-Ala-Aib-OMe (2c). In contrast to the latter compound,which is partially extended, the structure of the Gly-Ala sequence in the former closely resembles that of the parent peptide. Interestingly, this is the first structure of a folded, β-turn forming peptide with a thiopeptide unit inside the ring closed by the intramolecular H-bond. In addition, these results again point to the possible role of the Aib residue in inducing the formation a β-turn conformation in an otherwise unfavourable sequence.[13] The next step in our research in this line, currently in progress in our laboratories, is the study of a monothiated analogue of a peptide sequence known to favour the onset of β-turn conformation in which the thiocarbonyl group is the candidate for accepting the intramolecular H-bond.

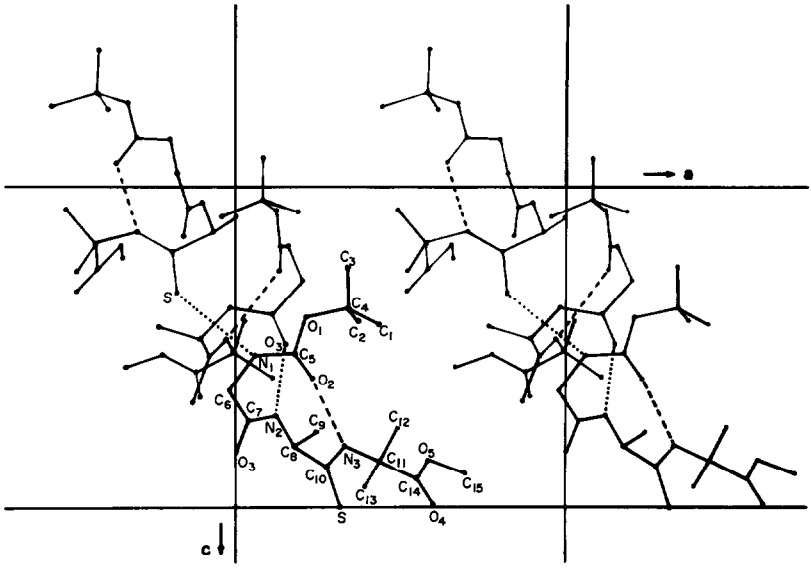


Fig. 4. Molecular packing of the Boc-Gly-S-Ala($^{2}\psi^{3}$CSNH)-Aib-OMe (2a) molecules viewed down the b axis. Intra- and intermolecular H-bonds are shown as dashed and dotted lines, respectively.

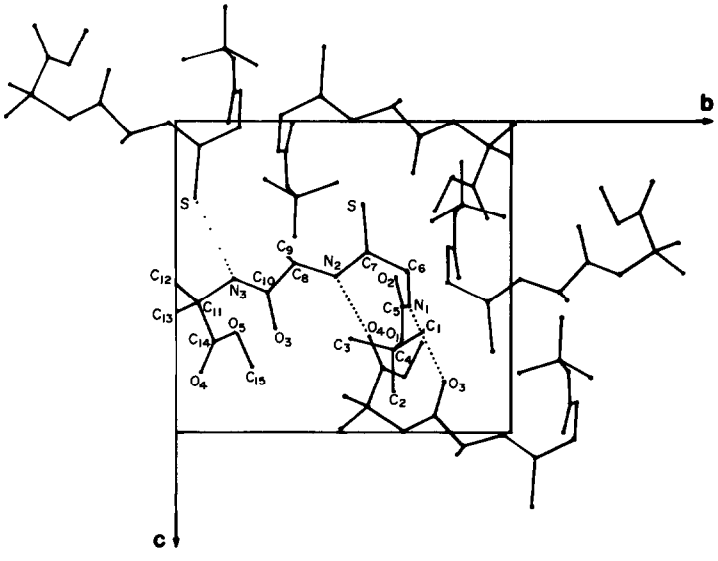


Fig. 5. Molecular packing of the Boc-Gly($^{1}\psi^{2}$CSNH)-S-Ala-Aib-OMe (2c) molecules viewed down the a axis. Intermolecular H-bonds are shown as dotted lines.

Table 7. Geometry of the H-bonds in the crystals of Boc-Gly-S-Ala($^2\psi^3$CSNH)-Aib-OMe (2a) and Boc-Gly($^1\psi^2$CSNH)-S-Ala-Aib-OMe (2c)

Donor (D)	Acceptor (A)	Symmetry Equiv. of Acceptor	Distances (Å)		Angle (°)
			D....A	H....A	D - H....A
(i) Boc-Gly-S-Ala($^2\psi^3$CSNH)-Aib-OMe (2a)					
N(3) - H	O(2)	x , y , z	3.02	2.24	150.5
N(2) - H	O(3)	y-x, -x , z-1/3	3.01	2.21	168.8
N(1) - H	S	-y, x-y , z-2/3	3.33	2.66	140.0
(ii) Boc-Gly($^1\psi^2$CSNH)-S-Ala-Aib-OMe (2c)					
N(1) - H	O(3)	1-x, y+1/2, 1/2-z+1	3.09	2.24	158.2
N(2) - H	O(4)	1-x, y+1/2, 1/2-z+1	2.99	2.35	130.9
N(3) - H	S	1-x, y-1/2, 1/2-z	3.36	2.60	147.3

EXPERIMENTAL

X-Ray diffraction analysis

Single crystals of Boc-Gly-S-Ala($^2\psi^3$CSNH)-Aib-OMe (2a) and Boc-Gly($^1\psi^2$CSNH)-S-Ala-Aib-OMe (2c) were grown by slow evaporation from ethyl acetate solutions.

X-Ray diffraction data were collected on a Philips PW 1100 four-circle diffractometer, using MoK_α 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 for the two peptides are summarized in Table 8.

Table 8. Crystal data for Boc-Gly-S-Ala($^2\psi^3$CSNH)-Aib-OMe (2a) and Boc-Gly($^1\psi^2$CSNH)-S-Ala-Aib-OMe (2c)

Molecular formula	$C_{15}H_{27}N_3O_5S$	$C_{15}H_{27}N_3O_5S$
MW (a.m.u.)	341.45	341.45
Density (calcd.)	1.15	1.129
Density (exptl.)	1.15	1.13
Space group	$P3_1$	$P2_12_12_1$
Z	3	4
a(Å)	12.052(3)	16.431(3)
b(Å)	12.052(3)	11.376(3)
c(Å)	11.756(3)	10.739(3)
α(°)	90	90
β(°)	90	90
γ(°)	120	90
Reflections ($I \geqslant 3\sigma(I)$)	1677	1423
R value	0.040	0.049
R_w value	0.042	0.045

The structures were solved by application of the direct methods and the program MULTAN 80.[33] The E maps of the set of phases with the best combined figure of merit revealed the position of 16 non-hydrogen atoms. The positions of the remaining non-hydrogen atoms were derived from subsequent difference Fourier maps. The structures were refined by the block-matrix least-squares procedure. The function minimized was $\Sigma w\Delta^2$, ($\Delta = |F_o|-|F_c|$), and w was $[\sigma(F_o) + 0.000571\ F_o^2]^{-1}$ for 2a and $[\sigma(F_o) + 0.000264\ F_o^2]^{-1}$ for 2c. Weighting-scheme analysis showed no serious dependence of the mean $w\Delta^2$ as a function of either $|F_o|$ or $\lambda^{-1}\sin V$. The scattering factors were taken from ref. 34. The correction for the real and imaginary parts of the anomalous dispersion was applied to sulfur.[34] Refinements were carried out allowing all non-hydrogen atoms to vibrate anisotropically, while hydrogen atoms were put in the calculated idealized positions (C-H, N-H = 1.0 Å) and were included in the last cycle.

Calculations were carried out using the SHELX-76 program.[35] The final conventional R value for the 1677 observed reflections of 2a with $[I \geqslant 3\sigma(I)]$ was 0.040 (R_w = 0.042), while that of the 1423 corresponding reflections of 2c was 0.049 (R_w = 0.045). In order to establish the absolute configurations we carried out the same refinements with inverse atomic coordinates. The resulting values of R = 0.041 and R_w = 0.043 for 2a, and R = 0.050 and R_w = 0.046 for 2c showed at a probability level greater than 99.5 % that the earlier models represented the absolute configurations.

The final positional parameters of the non-hydrogen atoms and the equivalent isotropic thermal factors of 2a and 2c are listed in Tables 9 and 10, respectively. Anisotropic temperature factors, hydrogen positional parameters, and structure factor tables are available on request from R. Bardi.

Chromatography

The following solvent mixtures were used: CMA = chloroform/methanol/acetic acid (85:10:5). BAW = 1-butanol/acetic acid/water (4:1:1). TLC plates for reaction monitoring and R_F value measurements were prepared from Merck 60 HF (254+366) silica gel. A suspension (magnetic stirring) of 25 g

Table 9

Fractional coordinates (x 10^4) and equivalent isotropic temperature factors ($Å^2$ x 10^3) for Boc-Gly-S-Ala($^2\psi^3$CSNH)-Aib-OMe (2a). ESD's are given in parentheses.

Atom	x	y	z	U_{eq}
S	3136(1)	1806(1)	9999	60(1)
O(1)	2104(2)	3309(3)	4045(2)	51(1)
O(2)	2351(3)	3669(3)	5953(2)	53(1)
O(3)	60(3)	1590(3)	8238(2)	59(2)
O(4)	5971(3)	4851(3)	9877(3)	69(2)
O(5)	5751(3)	3487(3)	8492(2)	56(2)
N(1)	536(3)	2040(3)	5203(3)	44(1)
N(2)	1170(3)	1003(3)	7098(2)	38(1)
N(3)	3315(3)	2993(3)	8063(2)	40(1)
C(1)	4345(4)	3895(5)	4246(4)	62(2)
C(2)	3730(5)	5607(4)	4163(5)	68(2)
C(3)	3413(5)	4222(5)	2462(4)	77(3)
C(4)	3423(4)	4284(4)	3751(3)	49(2)
C(5)	1717(3)	3061(4)	5143(3)	43(2)
C(6)	-191(4)	1784(4)	6271(3)	49(2)
C(7)	365(3)	1454(3)	7279(3)	41(2)
C(8)	1753(4)	719(3)	8040(3)	42(2)
C(9)	2386(5)	-31(5)	7613(4)	69(3)
C(10)	2750(4)	1924(3)	8664(3)	42(2)
C(11)	4294(4)	4265(3)	8498(3)	46(2)
C(12)	4897(4)	5133(3)	7443(4)	56(2)
C(13)	3670(5)	4807(5)	9285(4)	65(2)
C(14)	5394(4)	4205(3)	9061(3)	48(2)
C(15)	6877(5)	3494(5)	8898(5)	74(3)

Table 10

Fractional coordinates (x 10^4) and equivalent isotropic temperature factors ($Å^2$ x 10^3) for Boc-Gly($^1\psi^2$CSNH)-S-Ala-Aib-OMe (2c). ESD's are given in parentheses

Atom	x	y	z	U_{eq}
S	5005(1)	5544(1)	2654(1)	75(1)
O(1)	3297(2)	6748(3)	7100(3)	58(2)
O(2)	3210(2)	6574(4)	4999(3)	73(2)
O(3)	4824(2)	2892(3)	6638(3)	53(2)
O(4)	3974(2)	722(3)	8039(3)	65(2)
O(5)	3159(2)	1777(3)	6804(3)	58(2)
N(1)	4413(2)	6842(3)	5979(3)	43(2)
N(2)	4967(2)	4738(3)	4949(3)	37(2)
N(3)	4404(2)	1733(3)	5064(3)	42(2)
C(1)	1890(5)	7281(12)	6765(8)	205(9)
C(2)	2384(4)	6452(8)	8734(6)	117(6)
C(3)	2296(6)	5227(9)	6866(11)	206(10)
C(4)	2442(3)	6455(5)	7344(5)	68(3)
C(5)	3595(3)	6700(4)	5930(5)	46(3)
C(6)	4848(3)	6848(4)	4796(4)	49(3)
C(7)	4941(3)	5645(4)	4196(4)	40(3)
C(8)	5109(3)	3538(4)	4558(4)	46(3)
C(9)	6014(4)	3300(5)	444(6)	87(4)
C(10)	4753(3)	2700(4)	5516(4)	39(3)
C(11)	4213(3)	699(4)	5828(4)	44(3)
C(12)	3616(3)	-62(4)	5071(5)	66(3)
C(13)	4991(4)	31(4)	6105(5)	66(3)
C(14)	3797(3)	1075(4)	7028(4)	46(3)
C(15)	2722(4)	2159(6)	7911(5)	87(4)

silica gel and 65 ml distilled water was spread on 130 glass plates (25 x 75 mm). The plates were dried at room temperature for 10-20 min. and 1 hr. at 120 °C. The silica gel layer produced in this was is 0.3 mm thick. Preparative plates were prepared from Merck 60 PF (254+366) silica gel. A suspension (mechanical shaking) of 150 g silica gel and 350 ml distilled water was spread on 5 glass plates (200 x 200 mm). The plates were dried for 24 hrs. at room temperature, and 1 hr. at 120 °C. This procedure gives a silica gel layer of 1.8 mm thickness. The ninhydrin spray solution consisted of 0.25 % ninhydrin in 1-butanol.

Instruments

^{1}H NMR spectra were recorded at 60 MHz with a Varian EM-360 spectrometer. ^{13}C NMR spectra were recorded at 25.16 MHz with a Varian XL-100-15 spectrometer with proton noise decoupling and a deuterium internal lock. The spinning sample tube had an external diameter of 12 mm; standard conditions were: Pulse with 10 sec, sweep with 5000 Hz and 6000 Hz (thioamide bonds), 8 K of data acquisition points and ca. 4000 scans. TMS was used as the internal standard and chemical shifts are expressed in δ values. $CDCl_3$ was used as solvent. UV absorption spectra were recorded on a Perkin Elmer 402 spectrophotometer. IR absorption spectra were obtained from a Beckman IR-18 spectrophotometer. Mass spectra and precise mass measurements were recorded on a Micromass 7070 F spectrometer operating at 70 eV with direct inlet. Optical rotations were recorded in a 1 dm cell on a Perkin-Elmer 241 polarimeter.

Materials

Boc protected amino acids[36] as well as amino acid methyl esters[37] were prepared by standard procedures from the corresponding amino acids. LR was prepared as described earlier.[38]

tert-*Butyloxycarbonyl-S-alanyl-2-methylalanine methyl ester (Boc-S-Ala-Aib-OMe,* 1a*)*.[39,40] By DCC coupling. Scale: 0.01735 M. After usual work-up the product still contained *N,N'*-dicyclohexylurea which was removed by column chromatography in 40 % diethyl ether/CH_2Cl_2 as eluent. The yield of 1a as an oil (lit. m.p. 82-84 °C,[39] oil[40]) was 3.81 g (76 %). $[\alpha]_D^{22}$ = -34.3° (c = 0.2 in methanol) lit.[39,41] $[\alpha]_D^{25}$ = -30.7° (c = 0.2 in methanol), $[\alpha]_D^{25}$ = -39.4 (c = 0.2 in methanol) . R_F = 0.59 in ethyl acetate. R_F = 0.42 in 30 % diethyl ether/CH_2Cl_2. IR (film): 1670/1530 (amide), 1710 (urethane), 1750 cm^{-1} (ester). MS: *m/e* = 290 (M^+ + 2), 289 (M^+ + 1), 234 (M^+ + 2 - $Me_2C{=}CH_2$), 233 (M^+ + 1 - $Me_2C{=}CH_2$), 189 (M^+ + 1 - $Me_3COC{=}O$), 174 (M^+ + 2 - H-Aib-OMe), 173 (M^+ + 1 - H-Aib-OMe). 1a by the LR coupling procedure: 1.89 g (0.01 mol) of Boc-S-Ala-OH was dissolved in 10 ml CH_2Cl_2 and 1.01 g (0.01 mol) of triethylamine was added, followed by 2.02 g (0.005 mol) of LR. The mixture was stirred until it became a clear solution, then cooled to -15 °C. A mixture of 1.54 g (0.01 mol) of HCl-H-Aib-OMe and 2.02 g (0.02 mol) of triethylamine was added. After stirring for 1 hr at - 15 °C the temperature was allowed to rise to room temperature and stirring was continued for 4 hrs. After evaporation on silica gel the residue was chromatographed twice on a silica gel column (50 x 200mm) with 30 % diethyl ether/CH_2Cl_2 as eluent, giving 1a as an oil. Yield 1.9 g (66 %).

tert-*Butyloxycarbonyl-S-thioalanyl-2-methylalanine methyl ester* [*Boc-S-Ala($^1\psi^2$CSNH)-Aib-OMe*, 1b]. Boc-S-Ala-Aib-OMe (1a), 0.95 g (0.0033 mol), was dissolved in 4 ml of toluene and 0.67 g (0.0017 mol) of LR was added. The reaction mixture was stirred at 100 °C under anhydrous conditions. After 1/2 hr the solvent was removed by evaporation under reduced pressure on silica gel, the temperature being kept below 50 °C. The residue was chromatographed on a silica gel column (20 x 200 mm) in the following eluent systems: 5 % diethyl ether/CH_2Cl_2, 10 % diethyl ether/CH_2Cl_2, 20 % diethyl ether/CH_2Cl_2, yielding 0.16 g of pure 1b as an oil. R_F = 0.52 in 30 % diethyl ether/CH_2Cl_2. $[\alpha]_D^{20}$ = - 32.9° (c = 2.8 in ethyl acetate). IR (film): 1670/1510 (amide) 1705-1740 cm^{-1} (urethane, ester). UV (ethanol): λ_{max} (log ε) = 265 nm (3.8). MS: *m/e* = 306 (M^+ + 2), 305 (M^+ + 1), 304 (M^+), 250 (M^+ + 2 - $Me_2C{=}CH_2$), 249 (M^+ + 1 - $Me_2C{=}CH_2$), 248 (M^+ - $Me_2C{=}CH_2$), 233 (M^+ + 2 - Me_3CO), 232 (M^+ + 1 - Me_3CO), 231 (M^+ + 1 - $Me_2C{=}CH_2$). Precise mass measurement: Calc. *m/e* = 304.145, found *m/e* = 304.145.

S-Thioalanyl-2-methylalanine methyl ester hydrochloride [*HCl·H-S-Ala($^1\psi^2$CSNH)-Aib-OMe*, 1c]: Boc-S-Ala($^1\psi^2$CSNH)-Aib-OMe (1b), 0.95 g (0.003 mol) was dissolved in a mixture of 5.5 ml of dioxane, 1.5 ml anisole, 0.5 ml 2-mercaptoethanol, and 7.5 ml 4 M HCl/dioxane. The reaction, monitored by TLC (30 % diethyl ether/CH_2Cl_2), was completed after 1/2 hr. The solvent was evaporated under reduced pressure at 40-50 °C. The residue was washed with 3 x 50 ml of diethyl ether and again dried under vacuum. The product showed a single ninhydrin positive spot. R_F = 0.57 in BAW. Yield = 0.4 g (53 %) as a foam.

tert-*Butyloxycarbonyl-glycyl-S-thioalanyl-2-methylalanine methyl ester* [*Boc-Gly-S-Ala($^2\psi^3$CSNH)-Aib-OMe*, 2a]. HCl·H-S-Ala($^1\psi^2$CSNH)-Aib-OMe (1c), 0.4 g (0.0016 mol), and Boc-Gly-OH, 0.31 g (0.0018 mol) were dissolved in a mixture of 0.16 g (0.0016 mol) of *N*-methylmorpholine and 3 ml of CH_2Cl_2, precooled to 0 °C. Then 0.38 g (0.0018 mol) of DCC was added at - 15 °C. The mixture was stirred at room temperature overnight. After the usual washing procedure, the product was further purified on a preparative plate (2 mm), yielding 0.44 g (77 %) of 2a, which was recrystallized from ethyl acetate/petroleum ether. R_F = 0.41 in ethyl acetate. M.p. = 116 °C. $[\alpha]_D^{20}$ = - 24.1° (c = 0.8 in ethyl acetate). IR (KBr): 1655/1530 (amide), 1690-1705 (urethane), 1730 cm^{-1} (ester). UV (ethanol): λ_{max} (log ε) = 203 (3.8), 265 nm (3.9). MS: *m/e* = 363 (M^+ + 2), 362 (M^+ + 1), 361 (M^+), 307 (M^+ + 2 - $Me_2C{=}CH_2$), 306 (M^+ + 1 - $Me_2C{=}CH_2$), 305 (M^+ - $Me_2C{=}CH_2$), 290 (M^+ + 2 - Me_3CO), 289 (M^+ + 1 - Me_3CO), 288 (M^+ - Me_3CO). Precise mass measurement: Calc. *m/e* = 361.167, found *m/e* = 361.167.

H-S-Alanyl-2-methylalanine-methyl ester hydrochloride (HCl·H-S-Ala-Aib-OMe, 1d). Boc-S-Ala-Aib-OMe (1a), 2.58 g (0.009 mol), was deprotected as described for 1c, yielding 1d quantitatively as a foam. R_F = 0.52 in BAW, single ninhydrin positive spot.

tert-*Butyloxycarbonyl-glycyl-S-alanyl-2-methylalanine methyl ester (Boc-Gly-S-Ala-Aib-OMe*, 2b).[13] HCl·H-S-Ala-Aib-OMe (1d), 2.0 g (0.009 mol), and Boc-Gly-OH, 1.57 g (0.009 mol) were coupled as described for 2a, yielding 2.3 g (76 %) of 2b, m.p. = 110 °C (lit.[10] m.p. 112 °C). R_F = 0.38 in ethyl acetate, R_F = 0.12 in 30 % diethyl ether/CH_2Cl_2. $[\alpha]_D^{22}$ = - 33.1° (c = 0.2 in methanol). IR (KBr): 1650, 1670/1520, 1550 (amide), 1720 (urethane), 1740 cm^{-1} (ester). UV (ethanol): λ_{max} (log ε) = 202 nm (3.7). MS: *m/e* = 348 (M^+ + 3), 347 (M^+ + 2), 291 (M^+ + 1 - $Me_2C{=}CH_2$), 290 (M^+ + 1 - $Me_2C{=}CH_2$).

tert-*Butyloxycarbonyl-thioglycyl-S-alanyl-2-methylalanine methyl ester* [*Boc-Gly($^1\psi^2$CSNH)-S-Ala-Aib-OMe*, 2c]. Boc-Gly-S-Ala-Aib-OMe (2b), 0.8 g (0.0023 mol), was dissolved in 5 ml of tetrahydrofuran. LR, 0.47 g (0.0012 mol), was added and the mixture stirred for 2 hrs at room temperature. The solvent was removed on silica gel under reduced pressure and chromatographed on a silica gel column (50 x 260 mm) in the following eluent systems: CH_2Cl_2, 10 % diethyl ether/CH_2Cl_2, 20 % diethyl ether/CH_2Cl_2. Yield of 2c: 0.74 g (85 %). R_F = 0.67 in ethyl acetate. M.p. = 88 °C from ethyl acetate/petroleum ether. $[\alpha]_D^{22}$ = - 28.6° (c = 0.8 in ethyl acetate). IR (KBr): 1670/1530 (amide), 1690-1705 (urethane), 1720-1740 cm^{-1} (ester). UV (ethanol): λ_{max} (log ε) = 203 (4.0), 265 nm. MS: *m/e* = 362 (M^+ + 1), 361 (M^+), 331 (M^+ + 1 - HS), 330 (M^+ - HS), 306 (M^+ + 1 - $Me_2C{=}CH_2$), 305 (M^+ - $Me_2C{=}CH_2$), 289 (M^+ + 1 - Me_3CO), 288 (M^+ - Me_3CO). Precise mass measurement: Calc. *m/e* = 361.167, found *m/e* = 361.167.

tert-*Butyloxycarbonyl-glycyl-S-alanine methyl ester (Boc-Gly-S-Ala-OMe*, 3a). Boc-Gly-OH, 4.375 g (0.025 mol) was dissolved in 30 ml tetrahydrofuran. After cooling to -15 °C, *N*-methylmorpholine, 2.529 g (0.025 mol), and ethyl chloroformate, 2.605 g (0.024 mol), were added. The mixed anhydride was allowed to form in 1 min. of activation time; then a mixture of HCl·H-S-Ala-OMe, 3.438 g (0.025 mol), and *N*-methylmorpholine, 2.529 g (0.025 mol), in 10 ml of tetrahydrofuran were added. The reaction was allowed to proceed for 1/2 hr at - 15 °C and 2 hrs at room temperature; then, 25 ml of 50 % K_2CO_3 were added. The organic solvent was removed under vacuum. The aqueous solution was extracted with 2 x 50 ml of ethyl acetate. The combined organic phases were washed with 5 % $NaHCO_3$ (3 x 25 ml), water (1 x 25 ml), 1 N HCl (3 x 25 ml), and water (1 x 25 ml). After drying over anhydrous $MgSO_4$, the solvent was removed under vacuum to yield 4.18 g (64 %) of 3a as an oily product. R_F = 0.50 in ethyl acetate, R_F = 0.79 in BAW, R_F = 0.43 in ethyl acetate/CH_2Cl_2 1:1. $[\alpha]_D^{22}$ = - 4.9° (c = 0.84 in ethanol). IR (film): 1670/1530 (amide), 1700-1750 cm^{-1} (urethane, ester). MS: *m/e* = 262 (M^+ + 2), 261 (M^+ + 1), 206 (M^+ + 2 - $Me_2C{=}CH_2$), 205 (M^+ + 1 - $Me_2C{=}CH_2$), 204 (M^+ - $Me_2C{=}CH_2$), 183 (M^+ - Me_3CO), 161 (M^+ - $Me_2C{=}CH_2$-CO_2). Precise mass measurement: Calc. *m/e* = 260.292, found *m/e* = 260.292.

tert-*Butyloxycarbonyl-thioglycyl-S-alanine methyl ester* [*Boc-Gly($^1\psi^2$CSNH)-S-Ala-OMe*, 3b]. Boc-Gly-S-Ala-OMe (3a), 3.09 g (0.0119 mol), and LR, 2.40 g (0.0059 mol) were suspended in 20 ml of tetrahydrofuran and stirred at room temperature for 4 hrs. The reaction mixture became a clear solution, and TLC in ethyl acetate indicated completed thiation. The reaction mixture was evaporated to dryness on 4 g of silica gel and chromatographed on a silica gel column (50 x 300 mm) with CH_2Cl_2 (200 ml), and 20 % diethyl ether/CH_2Cl_2 as eluent systems. The yield was 3.05 g (93 % of pure 3b as an oil. R_F = 0.57 in 30 % diethyl ether/CH_2Cl_2. $[\alpha]_D^{22}$ = + 4.8° (c = 2.8 in ethyl acetate). IR (film): 1500-1540 (amide), 1700-1750 cm^{-1} (urethane, ester). UV (ethanol): λ_{max} (log ε) = 265 nm (4.0). MS: *m/e* = 278 (M^+ + 2), 277 (M^+ + 1), 276 (M^+). Precise mass measurement: Calc. *m/e* = 276.114, found *m/e* = 276.114.

tert-Butyloxycarbonyl-thioglycyl-S-alanine [*Boc-Gly($^1\psi^2$CSNH)-S-Ala-OH*, 3c]. Boc-Gly($^1\psi^2$CSNH)-S-Ala-OMe (3b), 1.57 g (0.00568 mol) was dissolved in 10 ml methanol and 6 ml of 1 N NaOH solution were dropwise added at room temperature. The reaction mixture was stirred for 1 hr, after which TLC (CMA) indicated complete saponification. After evaporation of methanol under vacuum the residue was acidified with 1 N HCl to pH 2 and, after saturation of the aqueous phase with NaCl, it was extracted with 2 x 25 ml ethyl acetate. The organic phase was dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to yield 1.20 g (81 %) of 3c as a foamy, hygroscopic solid. No m.p. could be measured because of the nature of the compound. R_F = 0.64 in CMA. $[\alpha]_D^{20}$ = + 26.8° (c = 3.9 in ethyl acetate). IR (film): 1500-1540 (amide), 1680-1740 cm (urethane, acid). UV (ethanol): λ_{max} (log ε) = 205 (3.7), 265 nm (3.9). MS: *m/e* = 262 (M^+), 206 (M^+ - $Me_2C{=}CH_2$), 189 (M^+ - Me_3CO), 173 (M^+ - $Me_2C{=}CH_2$- HS). Precise mass measurement: Calc. *m/e* = 262.098, found *m/e* = 262.098.

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