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MIXED INTRAMOLECULAR H-BONDS OF SECONDARY THIOAMIDES

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Abstract - IR and NMR studies on thioamide models containing one or more C=O acceptor groups revealed the existence of three types of -C=O...H-N-CS- mixed intramolecular H-bonds (MIH's) and the formation of thioamide turn conformations in nonpolar solvents. Thioacetyl alanine methyl ester (2) was found to adopt an extended or C_{5t} conformation fixed with a $2_t \rightarrow 2$ MIH. The dominant conformation of the N-protected dipeptide N'-methyl thioamide models 7a and 8 is a β_t-turn featuring a $1\leftarrow4_t$ MIH. ^{1}H NOE data suggest that its Φ and Ψ torsion angles are close to those found in β-turns of peptides. Mixed $1\leftarrow3_t$ intramolecular H-bonded conformations (γ_t or C_{7t}) seem to be less favoured as a result of the repulsion between the thiocarbonyl sulphur and the C_β group at Ψ angles required for these conformations.

As shown by X-ray diffraction studies on simple models,[1,2,3] the geometry of endothiopeptides is rather similar to that of peptides. However, due to the longer C=S bond and the larger van der Waals radius of the sulphur atom, the allowed ranges of the Φ and Ψ torsion angles are narrower in thiopeptides than in peptides.[1] As a consequence, the thioamide unit is not expected to replace an amid group in secondary structures like α-helix or β-pleated sheet without major distorsions of these conformations.

The above considerations, together with the enhanced enzyme resistency[4] of endothiopeptides explain the growing interest in the synthesis of thionated peptides of biological importance[4-9] by using Lawesson's reagent[10] which makes possible the facile and racemization free conversion of amides into thioamides.

Amides and thioamides differ also in their H-bond forming properties. Thioamides are stronger acids (pK_a = 11-13) but weaker bases than amides.[11] Accordingly, intra- and intermolecular H-bonds between the C=O and NH group of amides are expected to be weaker than the mixed ones formed with the participation of the amide C=O as acceptor and the thioamide NH as donor group.

The role of the mixed intramolecular H-bonds (MIH's) in determining the conformation and chiroptical properties of protected endothiodipeptides has been recognized in our previous work.[12] Prompted by the results of this study, we prepared a series of simple thionated methylamide derivatives of N-protected amino acids and dipeptides (Table 1). This paper reports the results of IR studies on the NH spectral region of these models in the nonpolar solvents CCl_4 and CH_2Cl_2 in comparison with their conformation inferred from NMR experiments.

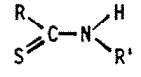
Table 1. Thioamide models. Synthesis and characterization.

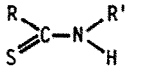
Nr	Compound	Isolation procedure[a]	Yield (%)	M.p.(°C)	R_F[a]	Formula	Calc. N%	Calc. S%	Found N%	Found S%
1	H-Pyrt-OBu^t	D+B_3	62	89-90	c: 0.60	$C_9H_{15}O_2NS$	6.96	15.93	6.66	16.02
2	Act-Ala-OMe	A_1	56	oil	b: 0.50	$C_6H_{11}O_2NS$	8.68	19.80	8.78	19.25
3a	Boc-Prot-$NHCH_3$	B_1	83	182-184	a: 0.35	$C_{11}H_{20}O_2N_2S$	11.46	13.12	11.22	13.42
3b	Z-Prot-$NHCH_3$	A_1+B_1	52	127-128	a: 0.40	$C_{14}H_{18}O_2N_2S$	10.06	11.51	9.88	11.39
3c	Ac-Prot-$NHCH_3$	B_2	55	119-121	d: 0.55	$C_8H_{14}ON_2S$	15.04	17.21	15.16	16.82
4a	Boc-Alat-$NHCH_3$	A_2	82	122-126	a: 0.25	$C_9H_{18}O_2N_2S$	12.83	14.68	12.76	14.65
4b	Z-Alat-$NHCH_3$	A_2	55	96-97	b: 0.70	$C_{12}H_{16}O_2N_2S$	11.10	12.70	11.13	12.74
4c	Ac-Alat-$NHCH_3$	A_3	75	153-156	c: 0.50	$C_6H_{12}ON_2S$	17.48	20.00	17.26	19.60
5	Boc-Glyt-$NHCH_3$	A_1	43	115-116	b: 0.35	$C_8H_{16}O_2N_2S$	13.71	15.69	13.35	15.30
6	Z-Phet-$NHCH_3$	A_1	63	116-117	a: 0.60	$C_{18}H_{20}O_2N_2S$	8.53	9.76	8.35	9.30
7a	Boc-Pro-Glyt-$NHCH_3$	C+B_1	88	138-142	c: 0.75	$C_{13}H_{23}O_3N_3S$	13.94	10.64	13.53	10.66
7b	Boc-Prot-Glyt-$NHCH_3$	A_1	39	oil	a: 0.35	$C_{13}H_{23}O_2N_3S_2$	13.24	20.20	13.05	19.63
8	Boc-Ala-Glyt-$NHCH_3$	C	70	oil	d: 0.70	$C_{11}H_{21}O_3N_3S$	15.26	11.64	14.96	11.50

[a]For abbreviations, isolation procedures and eluent systems see Experimental.

RESULTS AND DISCUSSION

Secondary thioamides are known to exist in two forms:

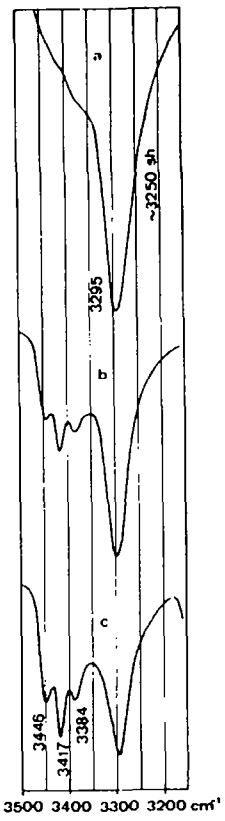

Z (trans)


E (cis)

In most cases, the energy barrier of the rotation around the C–N bond is greater than 100 kJ/mol which means that the two forms exist separately as rotational isomers at room temperature. Walter and Kubersky studied[14] the ratio of the Z-E isomers by IR spectroscopy in secondary thioamides. Except for derivatives having bulky phenyl, substituted phenyl of trityl R' groups, the proportion of the Z isomer was found to be above 95 per cent in nonpolar solution. The predominance of the Z form was explained, in addition to steric effects, by the marked dipol character of the thioamide group.[14] Intramolecular H-bonds of 1←3 and 1←4 types which cannot be formed from the E isomer may also stabilize the Z conformer.

As shown by our earlier studies[12] as well as X-ray data on the model Boc-Gly-Alat-Aib-OCH_3,[2] thiopeptides have a tendency to form folded conformations. Based on the similar geometry of the amide and thioamide unit, these folded conformations can be regarded as distorted variants of the turns formed by peptides. In the present study structures with three types of intramolecular H-bonds (2_t←2, 1←3_t and 1←4_t) are considered for Z thioamides (Fig. 1). It is kept in mind, however, that depending on the ranges of the Φ and Ψ torsion angles, both 1←3_t and 1←4_t conformations can be divided into subgroups as in the case of amides[13,15] (for definition of the torsion angles see Experimental).

NH stretching vibration data on models 1-8 are summarized in Table 2. Measurements were performed at 2-3 concentrations to differentiate between free, intra- and intermolecular H-bonded amide (NH_a) and thioamide (NH_t) groups. As shown in Fig. 2, the 3500-3100 cm^{-1} region of the spectra of models having both secondary amide and thioamide groups is rather rich in bands. Their relative intensity strongly depends on concentration. In agreement with the results of Walter and Kubersky[14] we found that the stretching vibration band of the free NH group of Z thioamides appears around 3420 cm^{-1} in dilute CCl_4 solution. The broad band in the 3280-3240 cm^{-1} range and a weaker one at ~3060 cm^{-1} which become dominant at higher concentrations were assigned to chain



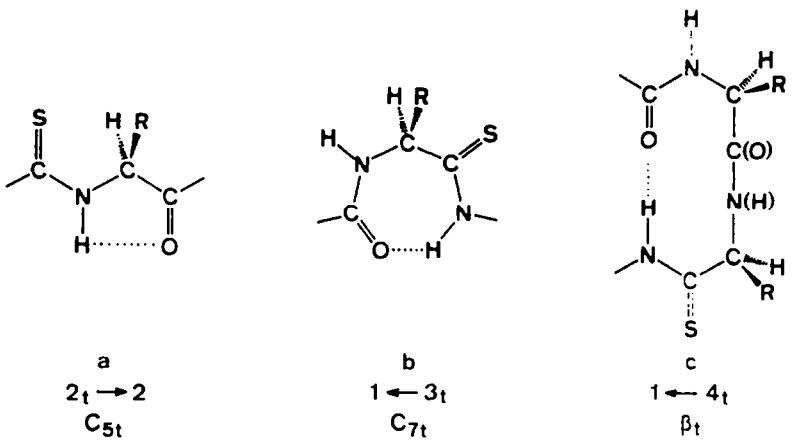


Fig.1. Schematic representation of the thioamide turn conformations.

Fig.2. (left) NH region of the IR spectra in CCl_4 of Boc-Alat-$NHCH_3$ (4a). (a) c = 50 mg/mL, (b) c = 1.5 mg/mL, (c) c = 0.15 mg/mL.

associates.[16,17] The free NH group of open-chain E thioamides gives an absorption below 3400 cm^{-1}, whereas in five-membered ring thiolactams the free NH band appears near 3430 cm^{-1}.[17] Thioamides of E conformation form both dimer and chain associates at higher concentrations. The dimer[16] gives a broad absorption near 3170 cm^{-1} usually accompanied by a weaker one at 3055 cm^{-1}. (See t-butyl 2-pyrrolidinethione-5-carboxylate (1) in Table 2. Due to steric reasons, there is no possibility in 1 for the formation of a mixed intramolecular H-bond between the thioamide NH and the C=O or C-O oxygen atoms of the ester group. Consequently, no NH band can be found in the 3430-3275 cm^{-1} region of its spectrum, see later.)

Model with a $2_t \rightarrow 2$ MIH (2)

The IR spectra of Act-Ala-OMe (2) in CCl_4 show three NH bands at about 3417 (free NH_t), 3390 (dominant in dilute solutions, probably due to a $2_t \rightarrow 2$ MIH of the C_{5t} conformation), and 3300 cm^{-1} (intermolecular: broad, with a shoulder near 3250 cm^{-1}).[17]

^1H NOE data in $CDCl_3$ (c = 1 mg/mL) are in agreement with the results of IR studies. At room temperature none of the signals of 2 is doubled. Irradiation of the NH resonance results in a 4% enhancement of the proton signal of CH_3 (acetyl) indicating the steric proximity of these protons in the Z conformation. The NOE's observed for the $C_\alpha H$ (5.6%) and $C_\beta H_3$ (1.5%) signals on irradiating the NH resonance are consistent with a Φ torsion angle near -180°. The 3 and 6.3% enhancements of the NH resonance on saturating the $C_\alpha H$ and $C_\beta H_3$ proton signals, respectively, also support this assumption.

In N-acetyl N'-methylamide derivatives of amino acids,[15] the frequency difference of the free and 2→2 H-bonded NH vibrations (see Fig. 1a with O instead of S) ranges between 28 and 40 cm^{-1}. In model 2 (c = 0.5 mg/mL), the corresponding frequency shift is 27 cm^{-1}. This indicates that the $2_t \rightarrow 2$ MIH in the C_{5t} conformer is somewhat weaker than the 2→2 H-bond of the C_5 conformation.

In the C_5 conformation the values of the Φ and Ψ angles are -170° and 170°, respectively.[15] It is likely that due to steric repulsion between the S atom and the $C_\beta H_3$ group, the adoption of a C_{5t} conformation requires a small decrease of the absolute value of the torsion angles.

Table 2. Amide and Thioamide NH Band Frequencies of Models 1-8 in Nonpolar Solution[a]

Nr	Compound	Solvent	c (g/L)	Free ν_a	Free ν_t	MIH ν_t cm^{-1}	Assoc. $\nu_{a,t}$ + $1\leftarrow4_t$ MIH ν_t	
1	Pyrt-OBu^t	CCl_4	25-0.25		3428-30		~3275sh	3156
2	Act-Ala-OMe	CCl_4	50-0.5		3410-17sh	3388-90	3317-3300[b]	
3a	Boc-Prot-$NHCH_3$	CCl_4	0.5-0.12		3413sh	3380	3265	3205
		CH_2Cl_2	50-5		3400sh	3370	3270	~3215sh
3b	Z-Prot-$NHCH_3$	CCl_4	15-0.3		3417sh	3387	3284-80	3220
3c	Ac-Prot-$NHCH_3$	CH_2Cl_2	50-5		3400sh	3377	3248	
4a	Boc-Alat-$NHCH_3$	CCl_4	50	3445sh	3417sh	3370sh	3295[b]	3085
			0.15	3446	3417	3384	3296[b]	3085
		CH_2Cl_2	15-5	3425	3398	3375	3288[b]	
4b	Z-Alat-$NHCH_3$	CCl_4	15-0.12	3434-42	3410-18	3387-94sh	3300-3303[b]	
4c	Ac-Alat-$NHCH_3$	CH_2CH_2	15-1	3450sh	3423-25sh	3392-95	3256-67	
5	Boc-Glyt-$NHCH_3$	CCl_4	0.6-0.06	3465	3415-18	3387	3298-3306[b]	
6	Z-Phet-$NHCH_3$	CCl_4	0.5-0.06	3458-55sh	3413	3394-90sh	3294[b]	
7a	Boc-Pro--Glyt-$NHCH_3$[c]	CH_2Cl_2	50-5	3435-36		3380sh	3283[b]	3095
7b	Boc-Prot--Glyt-$NHCH_3$	CCl_4	50-0.5		3400-5	3350sh	3270-85[b]	3105
8	Boc-Ala--Glyt-$NHCH_3$[c]	CH_2Cl_2	50-5	3433			3295-3300[b]	3090sh

[a]The assignment is based on the concentration dependence of ϵ_{max} values. [b]With a shoulder near or below 3250 cm^{-1}. [c]Not soluble in CCl_4.

Models with $1\leftarrow3_t$ MIH forming potential (3-6)

Proline-containing models (3a-c)

Proline-containing derivatives featuring one NH_t but no NH_a group (3a-c) are the simplest models for studying the possibility of the formation of a $1\leftarrow3_t$ MIH conformation. Based on the concentration dependence of the band intensities in CCl_4 of models Boc-Prot-$NHCH_3$ (3a) and Z-Prot-$NHCH_3$ (3b), the bands appearing above 3410 cm^{-1} and below 3300 cm^{-1} were assigned to the free and associated NH_t oscillators (Table 2). The band near 3380 cm^{-1} is attributable to a 1←3 H-bonded NH_t group. In the IR spectrum of 3c in CH_2Cl_2 the free, $1\leftarrow3_t$ and intermolecular H-bonded NH_t bands show 10-30 cm^{-1} down shifts (solvent effect).

In the ^1H NMR spectra of Boc-Prot-$NHCH_3$ (3a) and Boc-Alat-$NHCH_3$ (4a) the N-CH_3 protons give a sharp doublet at 3.19 and 3.16 ppm, respectively, which suggests the same Z isomeric state of the thioamide group. The NH_t signal of 3a is, however, smeared between 8-9 ppm and has the shape of a saddle, contrary to the broad ragged singlet or triplet of the amide or thioamide protons of the other models. The rate of deuteration with D_2O is low. The integrated spectrum shows that this signal arises from one proton and at 20°C the ratio of intensity of the broadened lines at 8.67 and 7.96 ppm is 2:3. The coalescence temperature is 27°C which corresponds to a low rate of exchange ($k \sim 310$ sec^{-1}). In the 101 MHz ^{13}C NMR spectrum of 3a all the resonance signals are broadened at room temperature. At -20°C there are two sharp signals for each carbon in the molecule. The ^{13}C spectrum of the oxoamide model Boc-Pro-$NHCH_3$ shows the same temperature dependence. The chemical shifts below -15°C of the C^γ (23.678, 24.518 ppm) and C^β carbons (28.194, 31.015 ppm) of proline are characteristic of the Z(trans) - E(cis) isomerisation of the urethane CO-N bond. (Values

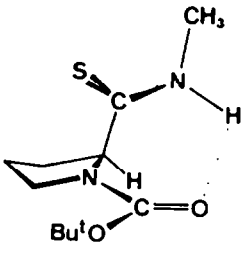


Fig.3. C_{7t} conformation of the Z(trans) rotameric form of Boc--Prot-$NHCH_3$ (3a) with a $1\leftarrow3_t$ MIH, $\Phi\sim100^{\circ}$.

assigned[18] to the E isomer are underlined). Due to the steric proximity of the C=S group, the chemical shifts of the C^{β} lines of 3a are different (32.02 and 34.353 ppm) but those of the C^{γ} lines at 23.448 and 24.123 ppm are very close to the values measured for the E and Z forms of the oxoamide model.

Based on the above features of the ^1H and ^{13}C spectra, it is likely that Boc-Prot-$NHCH_3$ (3a) in nonpolar solution is present as a mixture of two rotamers around the urethane CO-N bond. The NH_t proton of the Z(trans) conformer forms a $1\leftarrow3_t$ MIH with the urethane C=O (Fig. 3). This turn conformation (C_{7t}) is probably a hybrid of the 1←3 intramolecular H-bonded C_7 and γ conformations of amides.[15] (The Φ and Ψ angles of these conformations are -80°, 80° and -60°, 140°, respectively.) The E conformer allows the involvement of NH_t only in intermolecular associates.

Models with one thioamide and one amide NH group (4-6)

The NH region of the IR spectra of models 4-6 shows four well separated NH bands in dilute CCl_4 solution (Table 2). The free NH_a vibration, as expected,[15] appears in the 3465-3440 cm^{-1} range. The band showing up in the 3418-3413 cm^{-1} range belongs to the free NH_t vibration of the Z conformer[14] while that near 3390 cm^{-1} can be assigned to the NH_t group in a $1\leftarrow3_t$ MIH. The position of the latter band also depends on the character of the N-protecting and side chain groups. In Z-Phet-$NHCH_3$ (6) having two aromatic groups, this band appears as a shoulder even in dilute solution.

In the spectra measured in CH_2Cl_2 the NH_a and NH_t bands show characteristic solvent shifts. The two types of the free NH bands are less separated than in the spectra in CCl_4. The band which can be assigned to a $1\leftarrow3_t$ MIH appears between 3395 and 3375 cm^{-1} (Table 2).

200 MHz ^1H NOE studies were performed on Boc-Alat-$NHCH_3$ (4a) and Ac-Alat-$NHCH_3$ (4c) in $CDCl_3$. At room temperature only one set of resonance signals was observed for both models. The significant enhancement of the $C_{\alpha}H$ signals (14.5 and 9%, respectively) on irradiation of the NH_t protons and the ~7% NOE's of the NH_t signals when saturating the $C_{\alpha}H$ resonances indicate both the prevailing Z conformation of the thioamide group and the steric proximity of these two protons ($\Psi = 120\pm20^{\circ}$). (Irradiation of the amide NH proton also causes a definite (~1%) enhancement of the methyl signal of the acetyl group in 4c.) An important information on the stereochemistry of the N-terminal moiety of the molecules (Φ angle) can be drawn from the nonobservation of NOE's between the NH_a and $C_{\alpha}H$ protons. This, together with the lack of an NOE of the $C_{\beta}H_3$ signal on irradiating at the NH_a proton suggests a large negative value ($-120\pm20^{\circ}$) for the Φ angle.

Fig.4. Conformational movement of Ac-Alat-$NHCH_3$ (4c). (a) Extended state, $\Phi\sim-140^{\circ}$, $\Psi\leq140^{\circ}$, (b) Folded state, $\Phi\sim-100^{\circ}$, $\Psi\sim100^{\circ}$.

The above ranges of Φ and Ψ torsion angles are consistent with the rapid equilibrium of a folded and a slightly more extended conformation. In the extended state ($\Phi\sim-140^{\circ}$, $\Psi\leq140^{\circ}$) the thioamide NH is free or involved in intermolecular associates (NH_t bands near 3420 cm^{-1} and below 3300 cm^{-1}, respectively; Table 2). The amide NH is rather close to the thioamide sulphur atom and

likely forms a weak O=C-N-H-...S=C- H-bond with it. This interaction which cannot exist in the proline models due to the lack of an amide NH group, may also stabilize the extended form. In the other, folded state ($\Phi \sim -100^0$, $\Psi \sim 100^0$) the amide NH group is expected to be free (band near 3450 cm^{-1}). The enhanced steric repulsion between the thioamide sulphur and the methyl group is compensated by the formation of a -C=O...H-N-C=S $1\leftarrow3_t$ MIH. The corresponding NH_t band appears at 3384 and 3395 cm^{-1} in the spectra of Boc-Alat-$NHCH_3$ (4a) and Ac-Alat-$NHCH_3$ (4c), respectively. The alanine-models are likely to feature a butterfly with fluttering wings (Fig. 4).

Protected dipeptide models with $1\leftarrow4_t$ MIH forming potential (7, 8)

In the dipeptide models Boc-Pro-Glyt-$NHCH_3$ (7a), Boc-Prot-Glyt-$NHCH_3$ (7b) and Boc-Ala-Glyt-$NHCH_3$ (8) the relative position of the amide C=O and thioamide NH_t group(s) allows the formation of both $1\leftarrow3_t$ and $1\leftarrow4_t$ MIH's. In dilute CCl_4 or CH_2Cl_2 solution the NH region of the IR spectra of these models is characterized by a strong band in the 3300-3270 cm^{-1} region (Table 2). (Models 7a and 8 with one NH_t group are not soluble in CCl_4.) Contrary to the expressed concentration dependence of the band appearing around or below 3300 cm^{-1} in the spectra of all the other thioamide models, the relative intensity of this band decreases only slightly on dilution and it remains the predominant NH band even in $c \leq 5$ mg/mL solution. This behaviour suggests that this broad band is composed of at least two types of NH bands and one of its components remains unchanged (or even increases) on dilution. This component can therefore be assigned to the stretching vibration of the C-terminal thioamide NH group involved in a rather strong $1\leftarrow4_t$ MIH.

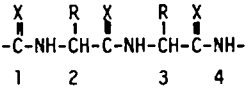
The simplest spectrum is shown by Boc-Ala-Glyt-$NHCH_3$ (8) in CH_2Cl_2. It has two bands at 3433 cm^{-1} (free NH_a and NH_t oscillators[15]) and around 3300 cm^{-1} ($1\leftarrow4_t$ H-bonded NH_t and associated NH_a and NH_t groups). Taking into consideration that the spectra of 8 in CH_2Cl_2 show no bands attributable to other types of intramolecular H-bonds, this model is expected to assume one major conformation with a $1\leftarrow4_t$ MIH (Fig. 1c).

200 MHz ^1H NOE experiments on Boc-Ala-Glyt-$NHCH_3$ (8) strongly support the dominance of this type of conformation in $CDCl_3$ solution. The strong positive NOE's measured between the N^3H and C_α^2H protons (Table 3) are in agreement with the steric proximity of these hydrogens ($\Phi^2 \sim 120^0$). The weak but definite NOE's observed between N^4H and N^3H in both direction suggest that N^4H points towards the interior of the bend. Similarly, the nonexistent NOE at N^2H on irradiation of the C_α^2H signal shows that these protons have a transoid conformation and it is the urethane carbonyl which assumes an inside position allowing the formation of a $1\leftarrow4_t$ H-bond.

Table 3. 200 MHz ^1H NMR and difference NOE data[a] on Boc-Pro-Glyt-$NHCH_3$ (7a) and Boc-Ala-Glyt-$NHCH_3$ (8)

Nr	Compound	Irradiated proton δppm[b]	Enhancements observed at proton (%)[c]								
			C^1H_3	C_α^2H	C^2H_3	N^2H	N^3H	C^3H_3	$C_\alpha^3H_2$	N^4H	C^4H_3
7a	Boc^1-Pro^2-Glyt^3-$NHCH_3^4$	C_α^2H 4.13 (5.7)	-		-	-	8.7	-		1-2	
		N^3H 6.84 (5.5)	-	14.1	-	-		-	3.2	4.8	
		N^4H 9.18	-		-	-	4	-	4		5.3
8	Boc^1-Ala^2-Glyt^3-$NHCH_3^4$	C_α^2H 4.038	-				6.4	-			
		N^3H 6.87	-	9.2				-	3	<1	
		N^4H 8.83	-				<1	-	1.5		4

[a]Performed as described in Experimental. For numbering see the formula below. [b]In $CDCl_3$, downfield from internal TMS. In parentheses: $J(NHC_\alpha H)$ values. [c]Largest NOE's observed. Enhancements of non-significant protons are not listed.



The NH region of Boc-Pro-Glyt-$NHCH_3$ (7a) in CH_2Cl_2 is characterized by two well defined bands at 3436 cm^{-1} (free NH_a) and 3283 cm^{-1} (1←4_t H-bonded NH_t as well as amide and thioamide NH's involved in intermolecular interaction). Due to the lack of a free NH_t band around 3410 cm^{-1}, the dominant conformer of this model is expected to be a 1←4_t H-bonded one, as suggested for model 8. The shoulder near 3380 cm^{-1} (Table 2) may, however, be the sign of the participation of the NH_t group in a 1←3_t intramolecular H-bond. The presence of this conformation is likely due to the Z⇌E isomerization of the urethane CO-N bond as observed for 3a.

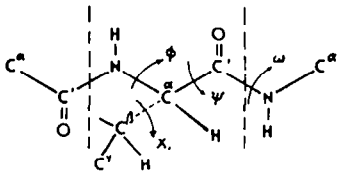
Boc-Pro-Glyt-$NHCH_3$ (7a) and Boc-Ala-Glyt-$NHCH_3$ (8) show similar ^1H NOE patterns (Table 3). However, irradiation of the N^3H and N^4H protons of 7a causes more expressed enhancements than for the alanine-model 8. The Ψ_2 torsion angle is probably fixed at $\sim 120^0$ in both models. In the dominant conformation, the hydrogen of N^4H points towards the interior forming a 1←4_t MIH with the urethane carbonyl. This is a type II β-turn-like conformation with two pairs of Φ and Ψ angles which may be very close to those found in ideal type II amide turns (-60^0, 120^0 and 80^0, 0^0). X-ray crystallographic data on Z-Glyt-Gly-OBzl[1] and Boc-Gly-Alat-Aib-OCH_3[2] show that the Ψ angle of a thioamino acid residue (Aaat) prefers to assume negative values around -30^0. This value of Ψ_2 may be favoured also in the β-turns of 7a and 8.

Boc-Prot-Glyt-$NHCH_3$ (7b) has no amide NH group. In its IR spectrum in dilute CCl_4 solution (Table 2), the band at 3405 cm^{-1} belongs to the free NH_t group[14] while the strong one at 3285 cm^{-1} with a shoulder reflects the occurrence of 1←4_t H-bonded and associated NH_t groups. Similarly to the other proline-model (7a), the major conformer is expected to be a 1←4_t H-bonded β_t-turn. However, the shoulder near 3350 cm^{-1} instead of 3380 cm^{-1} (see the IR spectrum of 7a) suggests an additional H-bond within the β_t-turn or the presence of a minor conformer.

The differences in the stability and geometry of turn conformations fixed by -C=O...H-N-CO- and C=O...H-N-CS- intramolecular H-bonds, respectively, may help to design models for studying the relationship between structure and biological activity, especially in cases, where intramolecular H-bonds are supposed to play an important role.

EXPERIMENTAL

Abbreviations used for amino acids and protecting groups are those generally accepted.[19] Aaat means the thiocarbonyl analogue (-NH-CHR-CS-) of an L amino acid residue.[5] Torsion angles (see below) are given as suggested in Ref. 20.



N-Protected amino acids and amino acid esters were prepared by standard procedures of the peptide synthesis.[21] Starting from N-protected amino acids, N'-methyl (oxo)amides were synthesized by the mixed anhydride method using isobutyl chloroformate and N-methyl morpholine in tetrahydrofuran.[22] Methylamine was liberated from its hydrochloride salt by N-methyl morpholine or given in the form of a solution in tetrahydrofuran. This method yielded Z-Ala-$NHCH_3$ (m.p. 130-131°C, $[\alpha]_D$ = -12.7° (c = 1.05, MeOH), in optically pure form (literature data[23] are 127-8°C and -13°, respectively). Urethane protected amino acid N'-methylamides as well as t-butyl L-pyroglutamate[25] and Ac-Ala-OMe were converted into thioamides by using Lawesson's reagent[10] (Method I). Hydrochloride salts of N'-methylamides of amino acids and thioamino acids (Aaat) were prepared from N-t-butyloxycarbonyl derivatives by using hydrogen chloride in dioxane.[21] Thioamino acid N'-methylamide hydrochlorides were acetylated with acetic anhydride in the presence of sodium acetate (Method II for preparation of 3c and 4c) or coupled[22] with N-Boc-amino acids (preparation of 7a and 8). Double-thionation of Boc-Pro-Gly-$NHCH_3$ yielded 7b. Urethane protected amino acid and dipeptide derivatives have been found[10,12,24] to resist racemization under the conditions of thionation.

Method I - 10 mmol of N-protected amino acid N'-methylamide in toluene (or N-protected amino acid ester) and Lawesson's reagent (5 mmol) was warmed at 60-70°C for 2 hrs. (10 mmol reagent was used for double-thionation.) After the solvent was distilled off at reduced pressure, the product was isolated and characterized as given in Table 1.

Method II - 10 mmol hydrochloride salt of thionated amino acid N'-methylamide was dissolved in water (10 mL). 20 mmol of sodium acetate and 40 mmol of acetic anhydride were added and the mixture was stirred at 20°C for 12 hrs. Evaporation of the solution at reduced pressure gave an oily solid which was dissolved in a small amount of water (5 mL). The aqueous solution was extracted with chloroform (6x5 mL). The chloroform solution was concentrated in vacuo and the crude product was purified as given in Table 1.

Microanalyses were carried out in the Microanalytical Laboratory of the Institute of Organic Chemistry, L. Eötvös University. Precoated silica gel foils (Kieselgel 60, Art 5748, E. Merck) were used for thin layer chromatography. Eluent systems: (a) dichloromethane - ethyl acetate (9:1), (b) dichloromethane - ethyl acetate (9:2), (c) ethyl acetate - pyridine - acetic acid - water (54:10:3:5), (d) butanol - acetic acid - water (2:1:1).

Isolation procedures - A Column chromatography on Kieselgel 60 (E. Merck) using the following eluents: dichloromethane and eluent a (A_1); eluent b (A_2); eluent c (A_3). - B Crystallization from ethyl acetate - petroleum ether (B_1); ethanol - ether (B_2) and cyclohexane (B_3). - C Washing of the chloroform solution of the crude product with 10% $NaHCO_3$ (2x), 10% citric acid (2x) and water. D Extraction with warm dry ether and cyclohexane.

IR data were recorded in 4-0.5 cm infrasil and in 0.1-0.01 cm KBr cells on a Specord IR 75 instrument (Carl Zeiss, Jena). 200 MHz ^1H NMR experiments were performed on a Bruker WP 200 SY spectrometer at ambient temperature. ^1H-{^1H} NOE spectra were recorded in the difference mode. Assignments were made by inspection and by comparison with published spectra of thioamides. Concentrations of the $CDCl_3$ solutions ranged between 1-5 mg/mL. 101 MHz ^{13}C NMR spectra were measured on a VARIAN XL-400 spectrometer.

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