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# Topographically Constrained Aromatic α-Aza-Amino Acids. Part 2.[1] New AzaTic-containing Peptides: Synthesis, Conformation, and Intramolecular NH···N Interaction

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*Abstract*: The new pseudodipeptide Boc-azaTic-Leu-OMe (**1**), incorporating the conformationally and topographically constrained 3,4-dihydro-2(1*H*)-phthalazinecarboxylic acid (azaTic) residue, has been synthesized together with the three related models Boc-azaTic-NHMe (**3**), MeCO-azaTic-Gly-OMe (**4**), and azaTic-Leu-OMe (**6**). Both the epimers of **1**, generated by opposite absolute configuration at an azaTic nitrogen stereogenic centre, have been found in the asymmetric unit of the crystal. The conformational perturbations, deriving by the introduction of the azaTic residue into a peptide backbone, are described and the nature of the NH···N interaction, which gives rise to a typical backbone folding closing a 5-membered ring, has been studied. 

***Keywords***: aza-amino acids; chi space; hydrogen bonding; phthalazines

## INTRODUCTION

Incorporation of conformational constraint into small peptides is a common strategy in order to gain information on the nature of the bioactive conformations and to obtain more stable, selective, and potent ligands. Relevant approaches are focused on the reduction of the backbone flexibility (φ and ψ torsion angles) and more recently on the limitation of the rotameric distribution of the amino acid side chains (conformational constraint in chi space).[2] These latter efforts lead to topographically constrained peptide analogues which are valuable tools for relating side chain orientation with receptor/acceptor interactions. A well established example of a synthetic aromatic amino acid with biased side chain rotamers is the tetrahydroisoquinoline-3-carboxylic acid (Tic) which is a $N^{\alpha}$-$C^{\delta}$ cyclized phenylalanine analogue whose structure excludes the *trans* conformation of the benzylic side chain and confines the $\chi^1$ side chain rotamer population to either *gauche* (-) or *gauche* (+) orientations.[3]

By taking into account the influence that the replacement of the α-carbon with a nitrogen atom can exert on the orientation of the α-amino acid side chains in derived azapeptides, a new α-aza analogue of the Tic residue, namely 3,4-dihydro-2(1*H*)-phthalazinecarboxylic acid (azaTic), has been recently described.[4,5] In particular, the molecular and crystal structures of azaTic-$NH_2$ and MeCO-azaTic-NHMe have been examined and their properties compared with those of related Tic and azaPro containing models.[1] Initial results indicate

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that, in contrast with the azaPro residue, in which both the endocyclic hydrazino nitrogen atoms are pyramidal, in the case of the azaTic residue, only one of the two nitrogens is pyramidalized, the other being practically planar. Other relevant points concern the short contact between the first hydrazino nitrogen and the N-H of the residue following the azaTic as well as the low value of the ψ torsion angle which should strongly influence the backbone conformation.

Here we report synthesis and conformation of the azaTic containing dipeptide Boc-azaTic-Leu-OMe (**1**). This new compound should complement the information obtained with the previously studied two models; these are, in fact, representative of the aza-residue properties when this occupies an *N*-terminal or a central backbone position and possesses a free or an *N*-acylated amino group, respectively. In the new model **1** the azaTic is located at the *N*-terminal position but its nitrogen atom makes part of an urethane group; thus, the influence exerted by alkoxycarbonyl substituents on the nitrogen hybridization can be evaluated and compared with that exerted by acyl groups. During the course of this study the three related peptide models, Boc-azaTic-NHMe (**3**), MeCO-azaTic-Gly-OMe (**4**), and azaTic-Leu-OMe (**6**) have also been synthesized and examined in order to clarify factors influencing the azaTic-induced local folding and the spectral properties of the NH···N interaction.

## RESULTS AND DISCUSSION

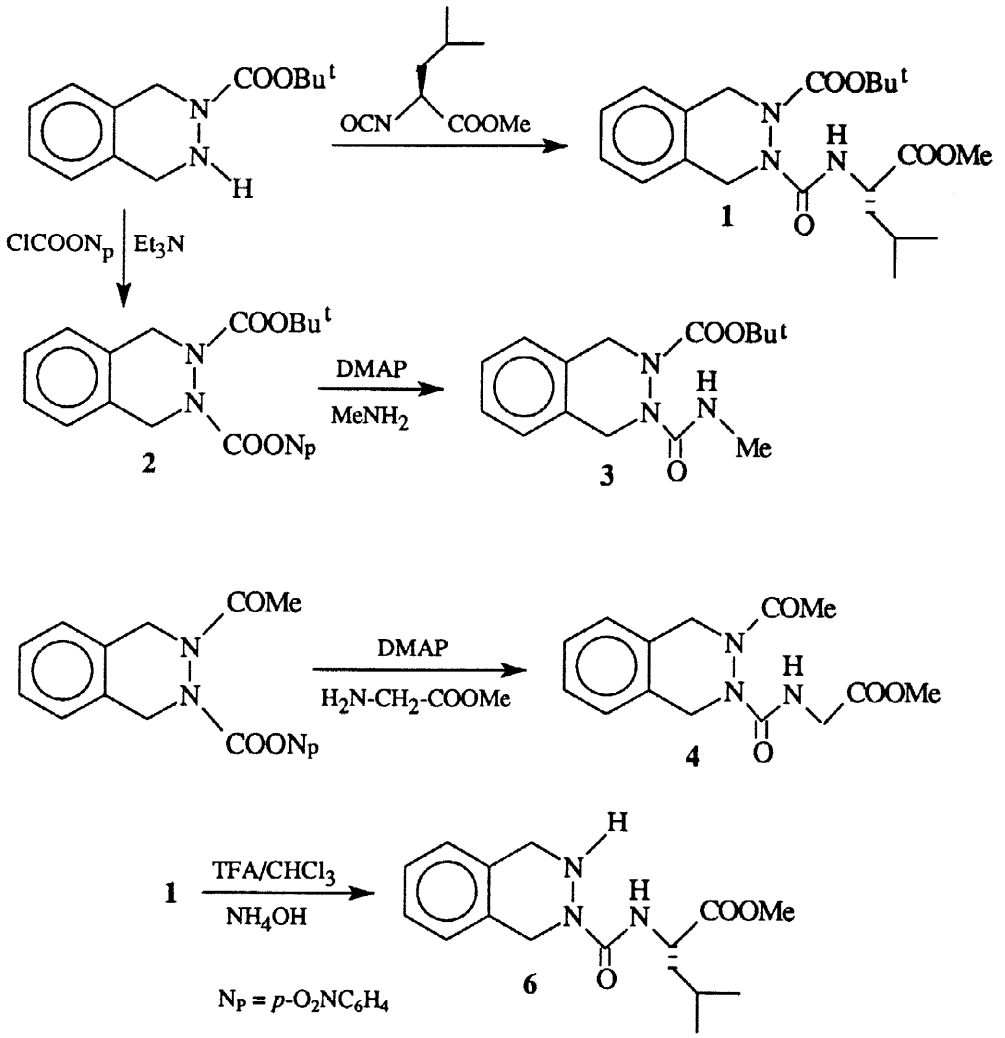
### *Chemistry*

The azaTic-containing derivatives **1**, **3**, and **4** were prepared according to Scheme 1. The aza-dipeptide **1** was obtained adding *t*-butyl 3,4-dihydro-2(1*H*)-phthalazinecarboxylate (azaTic-OBu$^t$)[4] to the isocyanate prepared by action of bis(trichloromethyl)carbonate (triphosgene)[6] on L-leucine methyl ester. Acylation of azaTic-OBu$^t$ with *p*-nitrophenyl chloroformate afforded the intermediate *p*-nitrophenyl ester **2**. Treatment of **2** with $MeNH_2$ in the presence of 4-(dimethylamino)pyridine (DMAP) gave the desired methylaminocarbonyl derivative **3**. The aza-dipeptide **4** was synthesized by coupling *p*-nitrophenyl 3-acetyl-3,4-dihydro-2(1*H*)-phthalazinecarboxylate[1] with glycine methyl ester in the presence of DMAP. Deprotection of the aza-dipeptide **1** by treatment with trifluoroacetic acid (TFA) afforded *N*-[(1,2,3,4-tetrahydro-2-phthalazinyl)carbonyl]-L-leucine methyl ester (**6**).

### *Crystal and Molecular Structure of 1*

Two independent molecules populate the asymmetric unit of the crystal of Boc-azaTic-Leu-OMe (**1**); these are the two diastereomers [Boc-(*S*-$N_1$)-azaTic-Leu-OMe (**1A**) and Boc-(*R*-$N_1$)-azaTic-Leu-OMe (**1B**)] possessing the same (*S*)-chirality at the Leu $C^\alpha$ and opposite chiralities at the Boc protected azaTic *N*-terminal $N_1$ nitrogen atom. The atomic numbering scheme of the two molecules **1A** and **1B** is given in Figure 1 where a perspective view of their conformations and relative orientation are also given; the most relevant torsion angles are reported in Table 1. It is clear from Figure 1 and from the data summarized in Table 2 that the $N_1{}^\alpha$, replacing the $C_1{}^\alpha$ atom, is practically planar ($sp^2$) whereas pronounced pyramidality affects the nitrogen $N_1$ making part of the urethane moiety. As a consequence of the reduced electronic conjugation of $N_1$ with the adjacent carbonyl, the $N_1$-$C_0'$ and $C_0'$-$O_0$ bonds are (see Table 2) larger and shorter, respectively, as compared with standard values; analogously, in the case of the planar ($sp^2$) $N_1{}^\alpha$, the $N_1{}^\alpha$-$C_1'$ and $C_1'$ -$O_1$ bonds are shorter and longer, respectively. Apart from the Boc group and the Leu side chain, the other torsion angles of

the two epimeric molecules **1A** and **1B** show very similar absolute values but opposite signs, indicating the presence of a pseudo-center of symmetry between the two molecules.




**Scheme 1**

The pseudopeptidic junction azaTic-Leu is, as expected, *trans*-planar (see $\omega_1$ in Table 1); the Boc-N group adopts a *trans-cis* conformation as usually found when tertiary nitrogen atoms are involved.[7] In this arrangement the two carbonyl groups, bound at the two azaTic nitrogen atoms, point in opposite direction as already found in the case of MeCO-azaTic-NHMe (**5**).[1] It is worth noting the high degree of $sp^3$ character exhibited by the $N_1$ atom despite the presence of a directly bound carbonyl group; this feature is in accordance with the large deviation from planarity shown by the $C'_0$-$N_1$ bond connecting the Boc group to the azaTic residue (see $\omega_0$ in Table 1). The ($\varphi$, $\psi$) sequence found in **1** (see Table 1) is characterized, as compared with azaPro[8] and previously studied azaTic derivatives,[1] by a very low value of the $\psi$ torsion angle (**1A**: -5.5°; **1B**: 5.6°). The amidic Leu $N_2$-H hydrogen points directly towards the preceding azaTic $sp^3$ $N_1$ atom closing a near planar 5-membered ring possessing three consecutive ($N_1^{\alpha}$, $C_1'$, $N_2$) $sp^2$ hybridized atoms. This structural motif is completed by a short contact which the azaTic-$N_1$ makes with the Leu $N_2$-H (Molecule **1A**: 2.17 Å; molecule **1B**: 2.28 Å); the angle at the hydrogen atom $N_1 \cdots H\text{-}N_2$, internal to the 5-membered ring, being 106 and 103° for molecule **1A** and **1B**, respectively. The Leu $N_2$-H amide proton is also involved in an

intermolecular interaction with the carbonyl oxygen $O_1$ on a neighbouring molecule (O···H contact of 2.42 and 2.25 Å for molecule **1A** and **1B**, respectively) giving rise to a three centered (or bifurcated) H-bond. It should be noted that a high number of intramolecular N-H···N interactions, so far evidenced in the X-ray crystal structures, participates to a three-centered system.[8b,9,10]

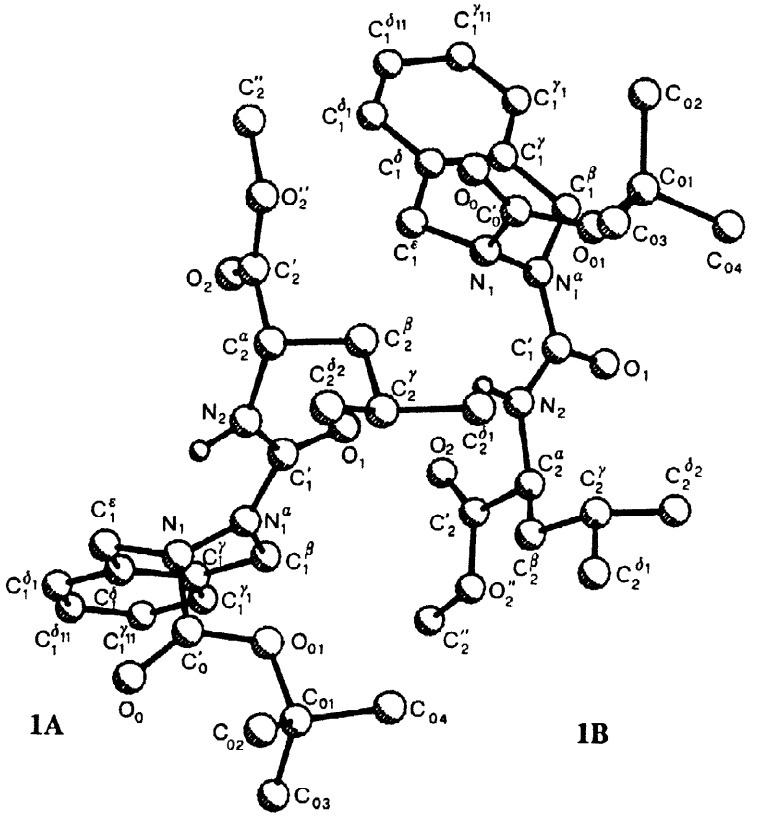


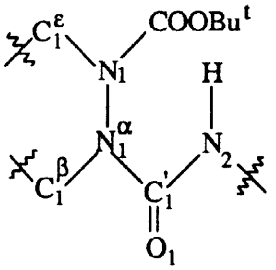

**Figure 1.** A perspective view of the crystal conformation of Boc-(*S*-$N_1$)-azaTic-Leu-OMe (**1A**) and Boc-(*R*-$N_1$)-azaTic-Leu-OMe (**1B**)

**Table 1.** Relevant torsion angles (°) of Boc-(*S*-$N_1$)-azaTic-Leu-OMe (**1A**) and Boc-(*R*-$N_1$)-azaTic-Leu-OMe (**1B**). E.s.d.'s are in the range 0.4°-1.0°.

| | | 1A | 1B | | | 1A | 1B |
|---|---|---|---|---|---|---|---|
| $C_{02}$-$C_{01}$-$O_{01}$-$C_0'$ | | -62.0 | -60.1 | $N_1$-$N_1{}^{\alpha}$-$C_1{}^{\beta}$-$C_1{}^{\gamma}$ | ($\chi_1{}^1$) | 40.5 | -43.6 |
| $C_{01}$-$O_{01}$-$C_0'$-$N_1$ | ($\vartheta^1$)[a] | 178.8 | 178.2 | $N_1{}^{\alpha}$-$C_1{}^{\beta}$-$C_1{}^{\gamma}$-$C_1{}^{\delta}$ | ($\chi_1{}^2$) | -6.0 | 10.1 |
| $O_{01}$-$C_0'$-$N_1$-$C_1{}^{\varepsilon}$ | | 159.5 | -161.2 | $C_1{}^{\beta}$-$C_1{}^{\gamma}$-$C_1{}^{\delta}$-$C_1{}^{\varepsilon}$ | | -3.7 | 1.3 |
| $O_{01}$-$C_0'$-$N_1$-$N_1{}^{\alpha}$ | ($\omega_0$) | 21.2 | -22.0 | $C_1{}^{\gamma}$-$C_1{}^{\delta}$-$C_1{}^{\varepsilon}$-$N_1$ | | -17.6 | 17.6 |
| $C_0'$-$N_1$-$N_1{}^{\alpha}$-$C_1'$ | ($\varphi_1$) | -106.6 | 106.0 | $C_1{}^{\delta}$-$C_1{}^{\varepsilon}$-$N_1$-$N_1{}^{\alpha}$ | | 49.4 | -49.9 |
| $N_1$-$N_1{}^{\alpha}$-$C_1'$-$N_2$ | ($\psi_1$) | -5.5 | 5.6 | $C_1{}^{\varepsilon}$-$N_1$-$N_1{}^{\alpha}$-$C_1{}^{\beta}$ | | -64.6 | 66.5 |
| $N_1{}^{\alpha}$-$C_1'$-$N_2$-$C_2{}^{\alpha}$ | ($\omega_1$) | -173.6 | 174.8 | $N_2$-$C_2{}^{\alpha}$-$C_2{}^{\beta}$-$C_2{}^{\gamma}$ | | -55.9 | -61.1 |
| $C_1'$-$N_2$-$C_2{}^{\alpha}$-$C_2'$ | ($\varphi_2$) | 68.8 | -90.1 | $C_2{}^{\alpha}$-$C_2{}^{\beta}$-$C_2{}^{\gamma}$-$C_2{}^{\delta 1}$ | | 175.8 | -177.5 |
| $C_2{}^{\alpha}$-$C_2'$-$O_2''$-$C_2''$ | ($\omega_T$) | 179.6 | -171.7 | $C_2{}^{\alpha}$-$C_2{}^{\beta}$-$C_2{}^{\gamma}$-$C_2{}^{\delta 2}$ | | -60.3 | -51 |

[a]See ref. 7.

**Table 2.** Relevant geometrical and structural features of **1A** and **1B**



| Compound | | **1A** | **1B** |
|---|---|---|---|
| Bond lengths (Å) | $C_1^{\varepsilon}-N_1$ | 1.469 | 1.460 |
| | $N_1-C_0'$ | 1.381 | 1.389 |
| | $C_0'-O_0$ | 1.208 | 1.204 |
| | $N_1-N_1^{\alpha}$ | 1.402 | 1.395 |
| | $C_1^{\beta}-N_1^{\alpha}$ | 1.436 | 1.452 |
| | $N_1^{\alpha}-C_1'$ | 1.366 | 1.359 |
| | $C_1'-O_1$ | 1.222 | 1.236 |
| | $C_1'-N_2$ | 1.363 | 1.341 |
| AzaTic N atoms hybridization[a] | $\Sigma N_1$ (°) | 347.3 | 347.6 |
| | $\Delta N_1$ (Å) | 0.295 | -0.291 |
| | $\Sigma N_1^{\alpha}$ (°) | 359.9 | 360.0 |
| | $\Delta N_1^{\alpha}$ (Å) | 0.024 | 0.009 |
| Short contacts (Å) | $N_1\cdots N_2$ | 2.61 | 2.67 |
| | $N_1\cdots H(N_2)$ | 2.17 | 2.28 |

[a] $\Sigma$ N = Sum of the valence angles at the N atom; $\Delta$N = displacement of the N atom from the plane of its three substituents.

The conformation of the tetrahydrophthalazine structure of both the molecules **1A** and **1B** is characterized by a pseudo two-fold axis bisecting the opposite $N_1$-$N_1^{\alpha}$ and $C_1^{\gamma}$-$C_1^{\delta}$ bonds; this can be described by a half-chair leaving the $N_1$ and $N_1^{\alpha}$ atoms on opposite side of the other four ring atoms (0.476, -0.208 Å in molecule **1A**; 0.434, -0.264 Å in **1B**). In this conformation (see Figure 2) the Boc group occupies a quasi-axial orientation while the lone pair on $N_1$, together with the carbonyl group on $N_1^{\alpha}$, are quasi-equatorially oriented. The orientation of the benzylic side chain of **1** is better represented by the Newman projection of Figure 2 where the situation found in **1B** and in the previously studied MeCO-azaTic-NHMe (**5**) is reported. From these representations it can be seen that in both **1B** and **5** the aromatic side chain adopts a *g* (-) conformation [$\chi_1^1$ = -57° in MeCO-(*R*)-azaTic-NHMe (**5**) and -43.6° in **1B**]; however, although the benzylic side chain points toward the *N*-terminus in both compounds, the different hybridization state of the

$N_1^{\alpha}$ in the two models leads to quite different orientation of the aromatic side chain relative to the peptide backbone.

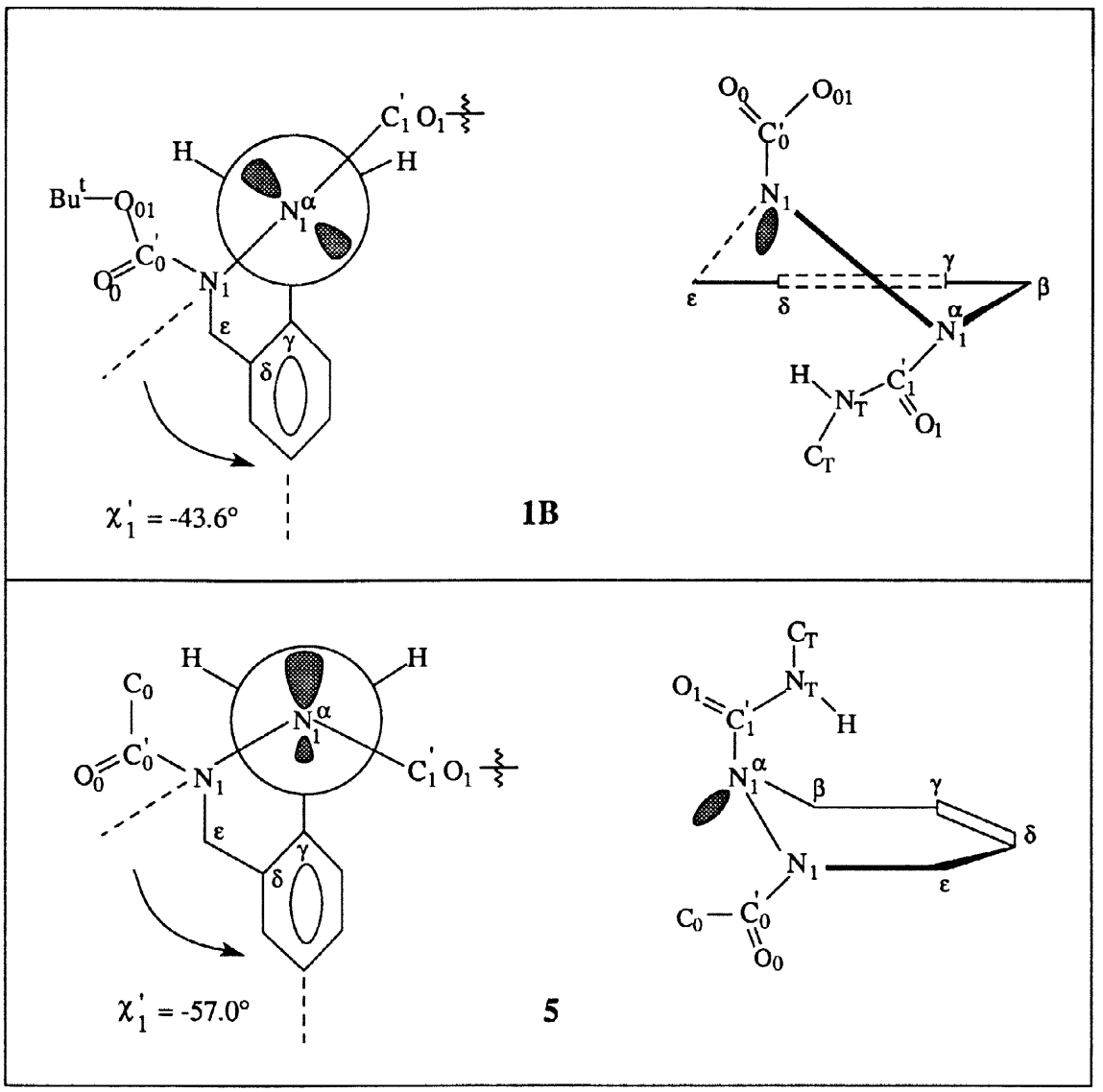



**Figure 2.** Newman projection along the $N_1^{\alpha}$-$C_1^{\beta}$ and tetrahydropyridazine ring conformation in Boc-(*R*-$N_1$)-azaTic-Leu-OMe (**1B**) and MeCO-(*R*)-azaTic-NHMe(**5**)[1].

### *Solution Conformation*

In order to gain information on the intramolecular H-bonds of the new compounds and in particular on the NH···N interaction observed in the crystal of the azaTic derivative **1**, an $^1$H NMR spectroscopic analysis based on the NH solvent accessibility in $CDCl_3$ was undertaken. The results obtained for the new compounds **1**, **3**, and **4** have been compared with those concerning the previously reported derivative MeCO-azaTic-NHMe (**5**).

In the solvent titration experiments (Figure 3) the NH group of the *t*-butoxycarbonyl derivatives **1** and **3** is scarcely affected by the change of the solvent composition, while the NH resonance of the *N*-acetyl derivatives **4** and **5** moves downfield with increasing concentration of $(CD_3)_2SO$.

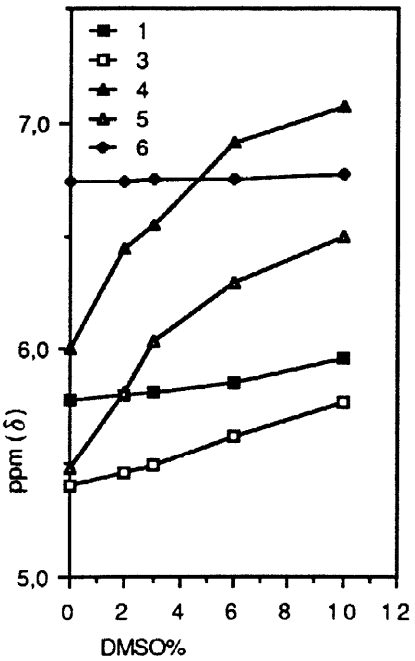



**Figure 3.** Delineation of hydrogen-bonded amide NH groups in the azaTic-containing derivatives **1**, **3**, **4**, **5**, and **6**. Chemical shift dependence of the NH resonances as a function of the DMSO-$d_6$ concentration (% v/v) in $CDCl_3$ solution. Compound concentration 10 mM.

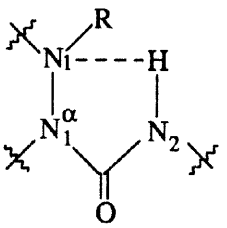
The temperature-induced NH chemical shift variation experiments[11] confirm the titration results. In particular, the temperature coefficients ($\Delta\delta/\Delta T$) of the NH group of **4** and **5** (-3.7 and -3.8 ppb/K, respectively) are significantly higher than those of the corresponding group in **1** and **3** (-0.9 and -1.2 ppb/K, respectively). These data indicate that the NH group is solvent exposed in the *N*-acetyl derivatives **4** and **5** while in the two *N*-alkoxycarbonyl derivatives **1** and **3** is not accessible. Thus, a H-bond of NH···N type could be present in **1** and **3**; however, on the basis of NMR spectroscopic data it cannot be excluded that the observed inaccessibility to the solvent could be determined by a 3-->1 NH···OC interaction ($\gamma$-turn or $C_7$ structure) involving the *t*-BuOCO carbonyl group or by steric interference exerted by this bulky group.

The IR absorptions in the NH stretching region of **1**, **3**, and **4** were then examined (Table 3). All these compounds exhibit a strong band in the 3430 and 3460 $cm^{-1}$ range; the same absorption is found in the spectrum of the previously studied MeCO-azaTic-NHMe (**5**); no significant lower absorptions are observed. The bands in this region pertain to vibrations of the NH groups in the free state, while absorptions of intramolecularly bonded groups are shifted to lower frequency ($\nu < 3400$ $cm^{-1}$). Thus, the IR results clearly indicate the absence in all the examined compounds of intramolecular H-bonds of the usual NH···OC type, ruling out any involvement of $\gamma$-turn structures.

In order to exclude possible steric interference by the bulky $COOBu^t$ group during NMR spectroscopic titration experiments, the azaTic derivative **6**, obtained by removing the *t*-butoxycarbonyl of **1**, has been prepared. The titration data indicate that the Leu NH of **6** is less affected ($\Delta\delta$ = 0.03 ppm) than the corresponding groups of **1** and **3** ($\Delta\delta$ = 0.18 and 0.35 ppm, respectively) by the change of the solvent composition; thus, this group must be engaged in an efficient intramolecular H-bond with the lone pair of azaTic NH. The IR spectrum of **6**, performed in $CHCl_3$, shows, in the NH stretching region, a strong band at

3405 $cm^{-1}$, a frequency which, although lower than that observed in the case of the related compounds (Table 3), still pertains to the region where the free NH stretching usually occurs.

**Table 3.** $N_2H\cdots N_1$ interaction in azaTic derivatives **1, 3-6**: solvent accessibility and infrared NH stretching.



| Compound | R | Δδ[a] (ppm) | Δδ/ΔT (ppb/K) | ν ($cm^{-1}$)[b] |
|---|---|---|---|---|
| **6** | -H | 0.03 | 1.1 | 3405 |
| **1** | -COOBu$^t$ | 0.18 | 0.9 | 3430 |
| **3** | -COOBu$^t$ | 0.35 | 1.2 | 3457 |
| **5** | -COMe | 1.03[c] | 3.8 | 3460[c] |
| **4** | -COMe | 1.07 | 3.7 | 3447 |

[a]Differences between NH chemical shifts observed in $CDCl_3$ containing $(CD_3)_2SO$ (10%) and those in $CDCl_3$; see also Figure 3. [b]Compound concentration 10 mM in $CHCl_3$. [c]See Ref. 1

The above NMR spectroscopic data indicate that the N-H···Nsp$^3$ interaction evidenced in compounds **1** and **3** causes inaccessibility of the NH to the solvent which is not reflected by the IR spectra in the expected stretching region. A related unusual behaviour has recently been noted by Marraud and coworkers while studying the semicarbazone moiety as dipeptide isostere.[12] A final observation concerns the importance of the IR data in order to distinguish N-H···N intramolecular H-bonds from γ-turn structures in compounds such as **1** and **3**; conclusions based solely on the solvent accessibility (NMR spectroscopy) and temperature coefficients should lead in fact to erroneously consider γ-turn structures as responsible for the observed results.

## CONCLUSION

The crystal structure of Boc-azaTic-Leu-OMe (**1**) reported in Figure 1 allows a clear-cut albeit unusual observation of both the epimeric forms of a pseudopeptide which differ in their absolute configuration at a stereogenic centre constituted by a pyramidal nitrogen atom. Furthermore, the local folding found in **1**, which involves a 5-membered ring H-bond and is typical of azapeptides, exhibits the most compact structure so far encountered, with a ψ torsion angle near zero and three consecutive sp$^2$ hybridized pseudo-ring atoms. This

means that the NH···N contact is minimal ($\psi = 0°$; see formula in Table 3), the Leu N-H bond points directly towards the lone-pair of the preceding acceptor nitrogen ($\phi = 90°$)[13] and the peptide backbone undergoes, as recently observed by Kline *et al.*, a unique atypical kink.[5a]

As shown in Figure 2 the orientation of the aromatic side chain relative to the backbone is strongly dependent upon the conformation adopted by the tetrahydrophthalazine system of the azaTic residue. An analysis of the data so far obtained suggests that this heterocyclic 6-membered ring adopts a conformation in which the two hydrazinic nitrogen atoms ($N_i$ and $N_i^{\alpha}$) are alternatively planar ($sp^2$) and pyramidal ($sp^3$), respectively (see Figure 2); as a consequence, the substituents at these atoms are maintained in a reciprocal pseudo-axial / pseudo-equatorial orientation, thus avoiding 1,2-diequatorial steric conflict. The factor which controls the nitrogen hybridization state is the substitution at $N_i$: $N_i$ is pyramidal and $N_i^{\alpha}$ planar when $N_i$ is free or protected by an alkoxycarbonyl group; conversely, when $N_i$ is acylated by a carboxylic acid carbonyl, it is $N_i$ which is planar and $N_i^{\alpha}$ pyramidal. Thus, pseudopeptides containing an internal or *C*-terminal azaTic residue should be characterized by an equatorial *N*-terminus and axial *C*-terminus; an *N*-terminal azaTic residue, with free or *N*-Boc protected amino group, on the other hand, will tend to maintain the backbone in a pseudo-equatorial orientation as it is the case of Boc-azaTic-Leu-OMe (**1**).

By taking into account the key role of topographical properties of peptides for the recognition and binding to receptor - as it was shown by Hruby *et al.*[3a] by studying ligands incorporating the Tic residue- the above reported results may represent a fundamental information for the induction of proper topography into bioactive peptide models containing this new constrained aza-analogue of phenylalanine.

There is considerable interest in studying nature, role and spectral properties of the 5-membered NH···N intramolecular interaction originally detected and studied by Gieren *et al.*[13] This structural feature is typical of the local folding found in α-azapeptides and related pseudopeptides, whose structural and conformational aspects have been extensively studied by the group of Aubry and Marraud.[10,14] Recently, intramolecular interactions of this type have also been observed in conventional peptides[15] and cyclopeptide alkaloids[16] and are recognized as the main factor in some cyclization reactions.[17] It should be noted, however, that the discrimination between free and H-bonded groups, based on the IR frequency of the NH stretching bands, refers to the NH···O=C interaction and few data regarding the influence of the NH···N interaction on the position of the NH absorptions are, at the present, available.[12] The results here obtained with the azaTic-containing peptides put in evidence the IR behaviour of the NH···N interaction and the influence exerted on it by the hybridization state of the accepting nitrogen. In particular, the two models Boc-azaTic-Leu-OMe (**1**) and Boc-azaTic-NHMe (**3**), both possessing $sp^3$ acceptor nitrogen atoms whose lone pair does not interact conjugatively with the adjacent urethane carbonyl groups, exhibit NH groups inaccessible to the solvent (see Figure 3); the same effect is more evident in the case of azaTic-Leu-OMe (**6**) in which the $sp^3$ acceptor nitrogen is not bonded to a carbonyl group. Compounds **4** and **5**, on the other hand, both possessing carbonyl conjugated $sp^2$ acceptor nitrogens, show freely solvent accessible NH protons. It is worth noting, however, that whereas the amide NH infrared absorptions in $CHCl_3$ exhibited by compounds **1**, **3**, and **6** appear in the region of free NH, their frequency steadly increases together with the accessibility to the solvent (Table 3), thus indicating that lone pair availability is responsible for the observed frequency shift.

These results indicate that N-H···N$sp^2$ contacts found in the solid state are too weak bonding interactions to be detected in solution. On the other hand, N-H···N$sp^3$ contacts give rise to bonding interactions which are strong enough to persist in $CHCl_3$ solution as shown by the titration results and

temperature coefficients; these H-bonds, however, are not revealed by IR in the pertinent NH stretching region. The reasons which determine this distinct behaviour, relative to related intramolecular N-H···OC hydrogen bonds appearing in secondary structures such as $C_5$[18] or γ-turns ($C_7$)[19], deserve further investigation; it could be argued, however, that the highly distorted character of the formers together with the different nature of the accepting atom, although not decisively influencing the bond strength, may sensibly alter the IR stretching frequency.

## EXPERIMENTAL

### Synthesis

Melting points were obtained using a Büchi oil bath apparatus and are uncorrected. Optical rotations were taken at 20 °C with a Schmidt-Haensch Polartronic D polarimeter in a 1 dm cell. IR spectra were recorded on 983 and 16FPC FT-IR Perkin-Elmer spectrophotometers. $^1$H NMR spectra were determined in $CDCl_3$ solution with Bruker AM 200 (200 MHz) and Varian XL-300 (300 MHz) spectrometers using tetramethylsilane as internal standard. *J* values are in Hz. Column chromatographies were carried out using Merck silica gel 60 (230-400 mesh). TLC and PLC were performed on silica gel Merck 60 $F_{254}$ plates. The drying agent was sodium sulphate. All the reactions were carried out under nitrogen atmosphere.

### *N*-[(2-*t*-Butoxycarbonyl-1,2,3,4-tetrahydro-3-phthalazinyl)-carbonyl]-L-leucine methyl ester (1)

*N*-methylmorpholine (NMM) (0.15 ml, 1.38 mmol) was added to a suspension of H-Leu-OMe·HCl (251 mg, 1.38 mmol) in dry dichloromethane (5.5 ml) and the mixture was stirred at room temperature for 15 min. Bis(trichloromethyl)carbonate (136 mg, 0.46 mmol) and NMM (0.3 ml, 2.76 mmol) were then added at 0 °C, and stirring was continued for 2 h. A solution of *t*-butyl 3,4-dihydro-2(1*H*)-phthalazinecarboxylate[4] (324 mg, 1.38 mmol) in dry dichloromethane (3.2 ml) was added and the reaction mixture was stirred at room temperature for 20 h. Some water was added and the solvents were evaporated under vacuum. The residue was dissolved in ethyl acetate and the organic phase was washed with 5% aq. $KHSO_4$, water, saturated aq. $NaHCO_3$, brine, and dried. After removal of the solvent, the residue (534 mg) was purified by PLC (*n*-hexane-ether, 1:1, 3 runs) to give the title compound **1** (463 mg, 83%) as a white solid, mp 111-111.5 °C (from EtOAc-*n*-hexane); $[\alpha]_D$ = -11 ($CHCl_3$, *c* 1.0); $\nu_{max}$ (KBr)/cm$^{-1}$ 3375, 1753, 1744, 1721, 1671, and 1657; $\delta_H$ 0.91-1.00 [6H, m $CH(CH_3)_2$], 1.44 [9H, s, $C(CH_3)_3$], 1.47-1.73 [3H, m, $CH_2$-$CH(CH_3)_2$], 3.70 (3H, s, $COOCH_3$), 4.29 and 5.20 (2H, A and X of an AX, *J* = 16.5, $CH_2$-N-COO), 4.42 and 5.05 (2H, A and X of an AX, *J* = 16.5, $CH_2$-N-CON), 4.57 (1H, m, Leu α-CH), 5.77 (1H, d, *J* = 9.1, NH), 7.10-7.24 (4H, m, aromatic). Two isomers have been detected and the signals of the major component are reported.
Anal. Calcd for $C_{21}H_{31}N_3O_5$: C, 62.20; H, 7.71; N, 10.36. Found: C, 62.20; H, 7.97; N, 10.17.

### *t*-Butyl *p*-nitrophenyl 1,4-dihydro-2,3-phthalazinedicarboxylate (2)

To a chilled solution of *t*-butyl 3,4-dihydro-2(1*H*)-phthalazinecarboxylate[4] (313 mg, 1.33 mmol) in dry EtOAc (5.3 ml) and triethylamine (0.22 ml, 1.6 mmol), a solution of *p*-nitrophenyl chloroformate (323 mg, 1.6 mmol) in EtOAc (2.6 ml) was added during 15 min. The mixture was stirred at 0 °C for 15 min and at 45 °C for 4 h. The solvent was evaporated under vacuum, dichloromethane was added in excess, and the organic phase was washed with 5% aq. $KHSO_4$, water, saturated aq. $Na_2CO_3$, and brine. The organic layers were dried and evaporated to give a residue (536 mg) which was chromatographed on a silica column (1:40),

eluting with *n*-hexane-ether (8:2). Further purification by PLC (*n*-hexane-ether, 4:6) of nearly homogeneous chromatographic fractions afforded the ester **2** (368 mg, 69%) as an amorphous solid, homogeneous by TLC, $\nu_{max}$ ($CHCl_3$)/$cm^{-1}$ 1736, 1720, 1525, 1348, and 1160; $\delta_H$ 1.46 [9H, s, $C(CH_3)_3$], 4.52 (2H, A part of two AB systems, $J$= 16.5, 1H of each $CH_2$-N-COO), 5.04 and 5.09 (2H, B part of two AB systems, $J$= 16.5, 1H of each $CH_2$-N-COO), 7.09-8.30 (8H, m, aromatic).

Anal. Calcd for $C_{20}H_{21}N_3O_6$: C, 60.14 H, 5.30; N, 10.52. Found: C, 59.93; H, 5.15; N, 10.47.

***t*-Butyl 3,4-dihydro-3-[(methylamino)carbonyl]-2(1*H*)-phthalazinecarboxylate (3)**

To a stirred solution of the active ester **2** (200 mg, 0.5 mmol) and DMAP (15.3 mg, 0.125 mmol) in dry *N*,*N*-dimethylformamide (DMF) (3.2 ml) methylamine hydrochloride (33.8 mg, 0.5 mmol), neutralized with NaOH dissolved in the minimum amount of water, was added dropwise at room temperature. After 7 and 25 h the same amount of methylamine was added and the stirring was continued for 27 h. The solvent was evaporated under reduced pressure and the residue partitioned between EtOAc and water. The organic phase was washed with 5% aq. $KHSO_4$, water, saturated aq. $Na_2CO_3$, and brine. After drying and evaporation the residue (185 mg) was purified by PLC (*n*-hexane-$Et_2O$, 4:6) to give the derivative **3** (116 mg, 79%) as white solid, mp 149-150 °C (from EtOAc-*n*-hexane); $\nu_{max}$ (KBr)/$cm^{-1}$ 3394, 1703, and 1675; $\delta_H$ 1.43 [9H, s, $C(CH_3)_3$], 2.85 (3H, d, $J$ = 4.1 , $CH_3$-NH), 4.28 and 5.22 (2H, A and X of an AX, $J$ = 16.5, $CH_2$-N-COO), 4.31 and 4.99 (2H, A and X of an AX, $J$ = 16.5, $CH_2$-N-CON), 5.41 (1H, m, NH), 7.00-7.22 (4H, m, aromatic).

Anal. Calcd for $C_{15}H_{21}N_3O_3$: C, 61.84; H, 7.27; N, 14.42. Found: C, 61.85; H, 7.35; N, 14.30.

***N*-[(2-Acetyl-1,2,3,4-tetrahydro-3-phthalazinyl)-carbonyl]-glycine methyl ester (4)**

To a stirred mixture of *p*-nitrophenyl 3-acetyl-3,4-dihydro-2-(1*H*)phthalazinecarboxylate[1] (235 mg, 0.69 mmol) and DMAP (21 mg, 0.17 mmol) in dry DMF (3.6 ml), a solution of glycine methyl ester hydrochloride (87 mg, 0.69 mmol) in dry DMF (2.8 ml), neutralized with NaOH dissolved in the minimum amount of water, was added dropwise at room temperature. After 22 h the same amount of glycine methyl ester was added and the stirring was continued at room temperature for 7 days. The solvent was evaporated under reduced pressure and the residue partitioned between EtOAc and water. The organic phase was dried and evaporated to give a residue (274 mg) which was chromatographed on a silica column (1:40). Elution with dichloromethane-EtOAc (8:2) afforded the azapeptide **4** (114 mg, 57%) as white solid, mp 182-183 °C (from EtOAc- *n*-hexane); $\nu_{max}$ (KBr)/$cm^{-1}$ 3315, 1744, 1687, and 1654; $\delta_H$ 2.25 (3H, s, CO-$CH_3$), 3.74 (3H, s, $COOCH_3$), 4.05 (2H, m, Gly $CH_2$), 4.26 and 5.47 (2H, A and X of an AX, $J$ = 16.5, $CH_2$-N-$COCH_3$), 4.28 and 5.44 (2H, A and X of an AX, $J$ = 16.5, $CH_2$-N-CON), 6.00 (1H, m, $J$ = 9.1, NH), 7.09-7.27 (4H, m, aromatic).

Anal. Calcd for $C_{14}H_{17}N_3O_4$: C, 57.72; H, 5.88; N, 14.42. Found: C, 57.85; H, 5.98; N, 14.25.

The assignments to two tetrahydrophthalazine $CH_2$ groups of compounds **1**, **3**, and **4** can be inverted.

***N*-[(1,2,3,4-Tetrahydro-2-phthalazinyl)carbonyl]-L-leucine methyl ester (6)**

The aza-dipeptide **1** (113 mg, 0.279 mmol) was dissolved in a mixture of TFA (0.17 ml) and dry $CHCl_3$ (0.5 ml) and stirred at room temperature for 19 h. The solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate. The solution was basified with 12% $NH_4OH$ and the organic phase was washed with water, dried and evaporated. The residue was purified by PLC (*n*-hexane-ethyl acetate, 1:1) to give the title oily azaTic derivative **6** (61 mg, 71%), $[\alpha]_D$ = +19 ($CHCl_3$, *c* 1.0); $\nu_{max}$

($CHCl_3$)/$cm^{-1}$ 3405, 1735, and 1657; $\delta_H$ 0.93[6H, d, $J$ = 5.8, CH(C$H_3$)$_2$], 1.47-1.80 [3H, m, C$H_2$-C$H$(CH$_3$)$_2$], 3.58-3.74[4H, m, azaTic NH and $COOCH_3$ (s at 3.68)], 4.02 (2H, d, $J$ =7.0, C$H_2$-NH), 4.50 (1H, m, Leu α-CH), 4.68 (2H, s, $CH_2$-N-CO), 6.71 (1H, d, $J$ = 8.8, Leu NH), 6.98-7.29 (4H, m, aromatic).
Anal. Calcd for $C_{16}H_{23}N_3O_3$: C, 62.93; H, 7.59; N, 13.76. Found: C, 62.98; H, 7.65; N, 13.76.

**X-ray Data Collection and Reduction**

Crystals of the aza-dipeptide **1** were obtained from EtOAc-*n*-hexane by slow evaporation. X-ray data were collected at room temperature on a Rigaku AFC5R diffractometer with graphite monochromated Cu-kα radiation and a 12 KW rotating anode generator. Cell constants and an orientation matrix for data collection were obtained from a least-squares fit of the angular settings of 22 carefully centered reflections in the range $62°<2\theta<70°$. The cell parameters, refined on higher angle reflections, are reported on Table 4. Intensity data were collected by the $\omega/2\theta$ scan technique, with a scan width of $(1.5 + 0.3tg\theta)°$at a variable and appropriate speed to a maximum $2\theta$ of 124°. Stationary background counts were recorded on each side of the reflection. The peak counting time was twice that of the background. Reflections with $I<25\sigma(I)$ were rescanned with an accumulation of counts to improve counting statistics. Out of 3953 collected reflections, 3725 were unique ($R_{int}$ = 0.037), 2951 had $I>3\sigma(I)$ and were used in the refinement. The intensities of three standard reflections, measured after every 147 reflections, remained constant throughout data collection indicating crystal and electronic stability. An empirical absorption correction, based on azimuthal scans of several reflections, was applied resulting in transmission factors ranging from from 0.86 to 1.00. The data were corrected for Lorentz and polarization effects.

**Table 4.** Crystal data for compound **1**.

| | | | |
|---|---|---|---|
| Empirical formula | $C_{21}H_{31}N_3O_5$ | F(000) | 872 |
| Formula weight | 405.5 | λ (Cu-kα) (Å) | 1.5418 |
| Crystal system | monoclinic | μ (Cu-kα) ($mm^{-1}$) | 0.7 |
| a (Å) | 10.231 (1) | Crystal size (mm) | 0.3x0.4x0.2 |
| b (Å) | 11.480 (5) | $2\theta_{max}$ (°) | 124 |
| c (Å) | 19.154 (2) | Refl. with $I>3\sigma(I)$ | 2951 |
| β (°) | 97.17 (1) | R, $R_w$ | 0.045, 0.055 |
| V ($Å^3$) | 2232 (1) | Weighting scheme | $4 F_0^2/\sigma(F_0^2)$ |
| Space group | $P 2_1$ | S | 2.1 |
| $d_c$ ($g/cm^3$) | 1.21 | Reflections/parameter | 5.7 |
| Z | 4 | max., min. ($e.Å^{-3}$) | 0.24, -0.18 |

**Structure Solution and Refinement**

The structure was solved by direct methods using the program SIR92[20] and successive Fourier maps. All the non-H atoms were refined anisotropically by the full matrix least-squares method; the function minimized was $\Sigma w (|F_O| - |F_C|)^2$ where $w = 4F_O^2/\sigma^2(F_O^2)$.The H atoms, located at the expected positions, were included in the last structure factor calculation with isotropic thermal parameters deduced from the carrier atoms. The final R and Rw are 0.045 and 0.055, respectively. The atomic scattering factors were those of Cromer and Mann.[21] Anomalous dispersion effects were taken into account adopting Δf' and Δf" values of Cromer.[22] The final fractional coordinates of the non-H atoms together with their Beq and individual e.s.d.'s are deposited at the Cambridge Crystallographic Data Centre. All the calculations were performed using TEXSAN[23] crystallographic software package.

## REFERENCES


1. Torrini, I.; Pagani Zecchini, G.; Paglialunga Paradisi, M.; Lucente , G.; Mastropietro, G.; Gavuzzo, E.; Mazza, F.; Pochetti, G. *Tetrahedron* **1998**, *54*, 165-178, part 1.
2. Hruby, V. J.; Li, G.; Haskell-Luevano, C.; Shenderovich, M. *Biopolymers (Peptide Sci.)* **1997**, *43*, 219-266.
3. (a) Kazmierski, W. M.; Yamamura, H. I.; Hruby, V. J. *J. Am. Chem. Soc.* **1991**, *113*, 2275-2283; (b) Kazmierski, W.; Hruby, V. J. *Tetrahedron* **1988**, *44*, 697-710; (c) Kazmierski, W.; Wire, W. S.; Lui, G. K.; Knapp, R. J.; Shook, J. E.; Burks, T. F.; Yamamura, H. I.; Hruby, V. J. *J. Med. Chem.* **1988**, *31*, 2170-2177; (d) Valle, G.; Kazmierski, W. M.; Crisma, M.; Bonora, G. M.; Toniolo, C.; Hruby, V. J. *Int. J. Peptide Protein Res.* **1992**, *40*, 222-232; (e) Salvadori, S.; Bryant, S. D.; Bianchi, C.; Balboni, G.; Scaranari, V.; Attila, M.; Lazarus, L. H. *J. Med. Chem.* **1993**, *36*, 3748-3756; (f) Josien, H.; Lavielle, S.; Brunissen, A.; Saffroy, M.; Torrens, Y.; Beaujouan, J.-C.; Glowinski, J.; Chassaing, G. *J. Med. Chem.* **1994**, *37*, 1586-1601; (g) Sawutz, D. G.; Salvino, J. M.; Seoane, P. R.; Douty, B. D.; Houck, W. T.; Bobko, M. A.; Doleman, M. S.; Dolle, R. E.; Wolfe, H. R. *Biochemistry* **1994**, *33*, 2373-2379; (h) Vitagliano, L.; Zagart, A.; Capasso, S.; Salvadori, S.; Balboni, G. *Acta Cryst.* **1994**, *C50*, 1135-1138; (i) Déry, O.; Josien, H.; Grassi, J.; Chassaing, G.; Couraud, J. Y.; Lavielle, S. *Biopolymers* **1996**, *39*, 67-74.
4. Grobelny, D. W. PCT Int. Appl. WO 93/ 18006 (16/09/93), (*Chem. Abstr.* **1994**, *120*: 324202v).
5. (a) Kline, T.; Mueller, L.; Sieber-McMaster, E.; Lau, W. F.; Meyers, C. A. *Int. J. Peptide Protein Res.* **1996**, *47*, 142-147; (b) Grobelny, D. W. U.S. US 5679688 (21/10/97), (*Chem. Abstr.* **1997**, *127*: 346660q); (c) Pagani Zecchini, G.; Torrini, I.; Paglialunga Paradisi, M.; Lucente, G.; Mastropietro, G.; Spisani, S. *Amino Acids* **1998**, *14*, 301-309, and references cited therein .
6. (a) Majer, P.; Randad, R. S. *J. Org. Chem.* **1994**, *59*, 1937-1938; (b) Cotarca, L.; Delogu, P.; Nardelli, A.; Sunjic, V. *Synthesis* **1996**, 553-576; (c) André, F.; Marraud, M.; Boussard, G.; Didierjean, C.; Aubry, A. *Tetrahedron Lett.* **1996**, *37*, 183-186; (d) André, F.; Marraud, M.; Tsouloufis, T.; Tzartos, S. J.; Boussard, G. *J. Peptide Sci.* **1997**, *3*, 429-441.
7. Benedetti, E.; Pedone, C.; Toniolo, C.; Némethy, G.; Pottle, M. S.; Scheraga, H. A. *Int. J. Peptide Protein Res.* **1980**, *16* , 156-172.
8. (a) Lecoq, A.; Boussard, G.; Marraud, M.; Aubry, A. *Biopolymers* **1993**, *33*, 1051-1059; (b) Didierjean, C.; Del Duca, V.; Benedetti, E.; Aubry, A.; Zouikri, M.; Marraud, M.; Boussard, G. *J. Peptide Res.* **1997**,

*50*, 451-457.

9. Jeffrey, G. A. *An Introduction to Hydrogen Bonding*; Oxford University Press, New York, 1997, chapter 1.
10. (a) Aubry, A.; Mangeot, J.-P.; Vidal, J.; Collet, A.; Zerkout, S.; Marraud, M. *Int. J. Peptide Protein Res.* **1994**, *43*, 305-311; (b) André, F.; Boussard, G.; Bayeul, D.; Didierjean, C.; Aubry, A.; Marraud, M. *J. Peptide Res.* **1997**, *49*, 556-562.
11. Rose, G. D.; Gierasch, L. M. Smith, J. A. *Adv. Protein Chem.* **1985**, *37*, 1-109.
12. Limal, D.; Grand, V.; Vanderesse, R.; Marraud, M.; Aubry, A. *Tetrahedron Lett.* **1994**, *35*, 3711-3714.
13. Gieren, A.; Dederer, B.; Schanda, F. *Z. Naturforsch.* **1980**, *35 c*, 741-746.
14. Zouikri, M.; Vicherat, A.; Aubry, A.; Marraud, M.; Boussard, G. *J. Peptide Res.* **1998**, *52*, 19-26, and references cited therein.
15. (a) Gellman, S. H.; Powell, D. R.; Desper, J. M. *Tetrahedron Lett.* **1992**, *33*, 1963-1964; (b) Raabe, G.; Sudeikat, A.; Woody, R. W. *Z. Naturforsch.* **1998**, *53 a*, 61-66.
16. Wipf, P.; Fritch, P. C.; Geib, S. J.; Sefler, A. M. *J. Am. Chem. Soc.* **1998**, *120*, 4105-4112.
17. Carret, S.; Baudy-Floc'h, M.; Robert, A.; Le Grel, P. *Chem. Commun.* **1997**, 1441-1442.
18. (a) Dentino, A. R.; Raj, P. A.; Bhandary, K. K.;Wilson, M. E.; Levine, M. J. *J. Biol. Chem.* **1991**, *266*, 18460-18468; (b) Torrini, I.; Paglialunga Paradisi, M.; Pagani Zecchini, G.; Lucente, G.; Gavuzzo, E.; Mazza, F.; Pochetti, G.; Traniello, S.; Spisani, S. *Biopolymers* **1997**, *42*, 415-426.
19. (a) Paglialunga Paradisi, M.; Torrini, I.; Pagani Zecchini, G.; Lucente, G.; Gavuzzo, E.; Mazza, F.; Pochetti, G. *Tetrahedron* **1995**, *51*, 2379-2386; (b) Tamaki, M.; Akabori, S.; Muramatsu, I. *Biopolymers* **1996**, *39*, 129-132.
20. Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A.; Burla, M. C.; Polidori, G.; Camalli, M. *J. Appl. Crystallogr.* **1994**, *27*, 435.
21. Cromer, D. T.; Mann, J. B. *Acta Cryst.* **1968**, *A24*, 321-324.
22. Cromer, D. T. *International Tables for X-ray Crystallography*, Vol. IV, The Kynoch Press, Birmingham, England, Tab. 2.3.1, 1974.
23. TEXSAN: TEXRAY *Structure Analysis Package*, Molecular Structure Corporation, 1985.