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γ-Turn Conformation Induced by α,α-Disubstituted Amino Acids with a Cyclic Six-Membered Side Chain

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Abstract : 4-Aminotetrahydrothiopyran-4-carboxylic acid (Thp) is an unusual achiral cyclic α,α-disubstituted amino acid mimicking the natural Met residue. The conformational energy map computed for Ac-Thp-NHMe shows that the γ-turn is the lowest minimum. ^{1}H-NMR and IR studies performed on For-Thp-Leu-OMe (**2a**), a short peptide unable to give a 4···>1 H-bond, indicate that the γ-turn is adopted in $CDCl_3$ solution, whereas is not retained in $(CD_3)_2SO$. An analogous conformational behaviour in solution has been observed for the strictly related For-Ac$_6$c-Leu-OMe (**2b**), containing 1-aminocyclohexane carboxylic acid (Ac$_6$c).

Introduction

The incorporation of α,α-dialkylated amino acids into small bioactive peptides, which leads to conformationally restricted analogs, is a well established approach to provide information on the biologically active conformations and to develope stable, effective, and selective ligands.

Extensive studies on *N*-protected peptides containing a $C^{\alpha,\alpha}$-dialkylated glycine, namely α-aminoisobutyric acid (Aib), 1-aminocyclopropane carboxylic acid (Ac$_3$c), 1-aminocyclopentane carboxylic acid (Ac$_5$c), 1-aminocyclohexane carboxylic acid (Ac$_6$c), and 1-aminocycloheptane carboxylic acid (Ac$_7$c), have evidenced the marked tendency of these residues to induce β-bend or 3_{10} helical folded conformations, both in solid state and in solution.[1,2] However, in a recent paper concerning molecular mechanics calculations on model compounds containing the Ac$_3$c residue the authors report that the γ-turn (C_7 conformation) is the most favoured structure.[3] In this connection a molecular dynamics study on a peptidomimetic containing a cyclopropyl residue (cyclo-methionine) together with ^{1}H-nmr studies carried out in DMSO-d$_6$ solution indicate that this peptide prefers a γ-turn structure centered at the cyclopropyl residue.[4]

As part of our continuing interest in the field of chemical modification of chemotactic *N*-formylpeptides[5,6] we recently reported synthesis, conformation and biological activity of For-Thp-Leu-Δ^{z}Phe-OMe.[7] This analog of the prototypical chemotactic tripeptide For-Met-Leu-Phe-OMe contains 4-aminotetrahydrothiopyran-4-carboxylic acid (Thp) and (*Z*)-2,3-didehydrophenylalanine (Δ^{z}Phe) as achiral conformationally restricted mimics of Met and Phe, respectively. ^{1}H-nmr analysis and IR studies carried out in chloroform solution suggest that the β-turn, found for the formyltripeptide in the solid state, is not preferred in $CDCl_3$ solution where a γ-turn, centered at the Thp residue, prevails. However, the presence of a certain population of intramolecularly H-bonded β-bend conformers could not be completely ruled out.

In order to gain further information on the effects of the Thp residue on the secondary structures, a complete search of the allowed conformational space for this amino acid has been now carried out by using energy calculations performed on Ac-Thp-NHMe, with axial NH group, as model. At the same time we also report a study of the conformation adopted in $CDCl_3$ solution by For-Thp-Leu-OMe (**2a**), a new formyldipeptide unable to adopt β-bend structures; the results have been then compared with those obtained for the strictly related peptide For-Ac_6c-Leu-OMe (**2b**) which contains Ac_6c in place of the Thp residue.

Energy calculations

The geometry of Ac-Thp-NHMe (see Figure 1) was derived from the crystal structure of For-Thp-Leu-Ain-OMe[8] and used in the energy calculations. The C^β atom of the Thp residue occupying the same position of C^β in L-amino acids is designated as $C^\beta{}_L$ and the other atom as $C^\beta{}_D$. The atoms bound to $C^\beta{}_L$ and $C^\beta{}_D$ are indicated as $C^\gamma{}_L$ and $C^\gamma{}_D$, respectively.

The φ, ψ map of Ac-Thp-NHMe is reported in Figure 2: the energy contours, drawn at increments of 2 kcal/mole, are relative to the lowest energy regions. The conformations together with the torsion angles and the relative energies of the local minima, obtained after minimization, are reported in Table 1. The $C_7{}^{ax}$ conformation, also known as γ-turn (3--->1 hydrogen bonded), is the deepest minimum whereas the two helical conformations, α_L and α_R, have energies respectively 0.5 and 0.9 kcal/mole higher. Two other minima with energies 3.6 and 5.2 kcal/mole correspond to quasi-extended conformations ($\varphi \sim \pm 60$, $\psi \sim 180°$). Finally, the $C_7{}^{eq}$ or inverse γ-turn conformation (3--->1 hydrogen bonded) has an energy which is 8.4 kcal/mole higher than that of the absolute minimum; its region is not reported in the map. Regions with φ in the ranges 90°÷180° and -90°÷180° are strongly disallowed.

Repulsive contacts $C^\beta{}_L \cdots C'_{i-1}$ = 2.98 Å and $H^\beta{}_L \cdots H_{i+1}$ = 1.70 Å, significantly shorter than their van der Waals radii sum, render the intramolecular energy of the inverse γ-turn higher than that of the γ-turn.

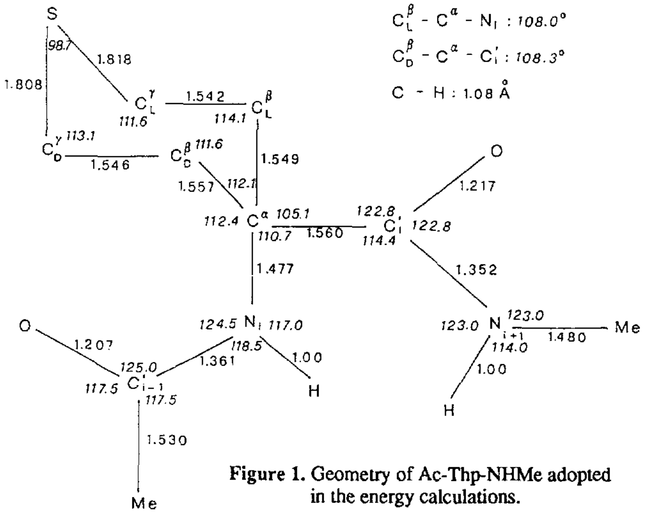


Figure 1. Geometry of Ac-Thp-NHMe adopted in the energy calculations.

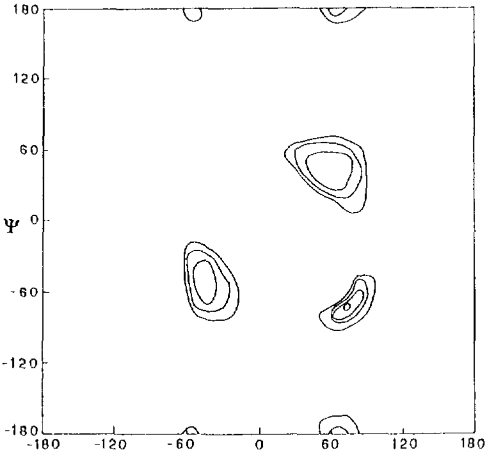


Figure 2. Energy map of Ac-Thp-NHMe.

Table 1.

Local minima found in the conformational energy map of Ac-Thp-NHMe assumed with the geometry reported in Fig. 1.

Minima	Φ	Ψ	E^a
C_7^{ax} (γt)	72	-70	0.0
α_L	56	46	0.5
α_R	-48	-48	0.9
q.e.$^+$	64	180	3.6
q.e.$^-$	-56	177	5.2
C_7^{eq} (iγt)	-62	76	8.4

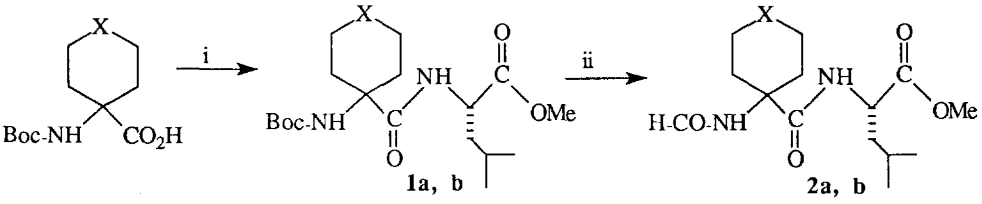
^{a} Energies in Kcal/mol.

The asymmetry of the energy map reflects the asymmetric geometry of the residue reported in Figure 1; particularly evident is the narrowing of the valence angle C'-C^{α}-C^{β}_L (105.1°) and the widening of the associated N-C^{α}-C^{β}_D (112.4°), in addition to the differences between the two C^{β} and the two C^{γ} intraring valence angles and the bond lengths departing from these atoms.

In order to check whether the potential functions used in the energy calculations overstimate the γ-turn conformation, the calculations have been repeated by assuming a completely symmetric Ac-Thp-NHMe model with all the N-C^{α}-C^{β} and C'-C^{α}-C^{β} valence angles equal to 108.5° and the other angles at C^{α} equal to those found in the experimental model. A centrosymmetric map, not reported here, has been obtained in this case, where the helical conformations α_L and α_R correspond to the deepest minima ($\varphi = \pm 54°$, $\psi = \pm 50°$) and the γ-turns, together with the quasi-extended conformations, are respectively at 1.7 and 2.7 kcal/mole (minima at $\varphi = \pm 66°$, $\psi = \pm 74°$ and $\varphi = \pm 62°$, $\psi = \pm 177°$, respectively). This result indicates that the adopted potential functions do not overstimate the γ-turn conformation.

Peptide Synthesis

The synthesis of formyldipeptides **2a,b** has been performed as reported in the Scheme 1.



a: X = S; b: X = CH_2

i: *i*-BuOCOCl, H-Leu-OMe·HCl, *N*- methylmorpholine; ii: HCOOH, EEDQ

Scheme 1

The intermediate Boc-dipeptides **1a** and **1b** were obtained by the reaction of the carboxy-activated *N*-*t*-butoxycarbonyl-4-aminotetrahydrothiopyran-4-carboxylic acid[8] or *N*-*t*-butoxycarbonyl-1-aminocyclohexane carboxylic acid[9] with L-leucine methyl ester hydrochloride (Leu-OMe·HCl). Treatment of **1a** or **1b** with formic acid followed by *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ)[10] afforded the *N*-formylderivatives **2a,b**.

^{1}H-NMR and IR studies

In $CDCl_3$ solution the characteristic H-CO proton resonance occurs as a singlet in both dipeptides **2a** and **2b** at 8.25 and 8.24 ppm, respectively. The assignment of the NH resonances is straightforward: the Thp and Ac$_6$c NH resonate as singlets at higher field than the Leu NH which appears in both **2a** and **2b** as a doublet (J = 8 Hz). The involvement of NH groups in intramolecular hydrogen bonds was evaluated on the basis of the chemical shift solvent dependence in $CDCl_3$-$(CD_3)_2SO$ mixtures, temperature-induced NH shift variation, and paramagnetic radical-induced line broadening. In the solvent titration experiment (Figure 3) the Thp and Ac$_6$c NH show substantial downfield shifts ($\Delta\delta$ = 2.01 and 1.72 ppm, respectively), while the Leu NH group is practically unaffected by the change of the solvent composition ($\Delta\delta$ = 0.12 in both dipeptides). In $CDCl_3$ the temperature coefficients ($d\delta/dT$) of Thp and Ac$_6$c NH groups (0.0051 and 0.0040 ppm/K, respectively) are significantly higher than those calculated for the Leu NH group (0.0021 ppm/K in **2a** and 0.0020 ppm/K in **2b**). Furthermore, addition of the paramagnetic radical probe 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) to the $CDCl_3$ solutions of **2a** and **2b** results in a dramatic broadening of Thp and Ac$_6$c NH signals whereas the Leu NH resonance is much less affected (Figure 4).

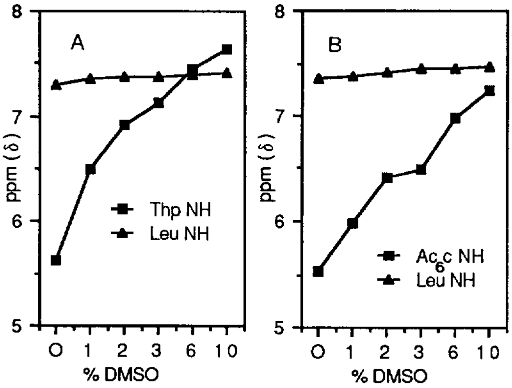


Figure 3. Chemical shift dependence of the NH resonances as a function of the DMSO-d_6 concentration (% v/v) in $CDCl_3$ solution. A: For-Thp-Leu-OMe (**2a**); B: For-Ac$_6$C-Leu-OMe (**2b**). Peptide concentration 20 mM.

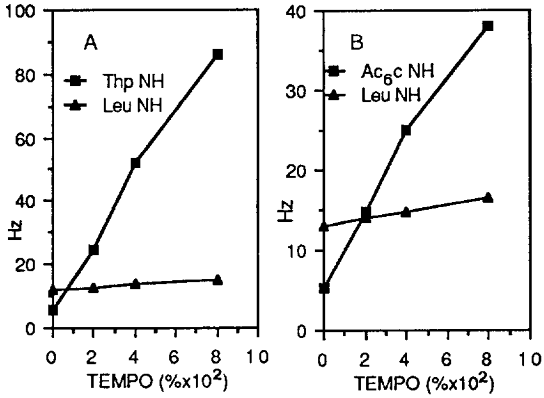


Figure. 4 Line broadening of NH resonances with increasing concentration of TEMPO radical in $CDCl_3$. A: **2a**; B: **2b**. Peptide concentration 17 mM.

All these results clearly indicate that the Leu NH is not accessible to the solvent in both formyldipeptides **2a** and **2b** and is presumably involved in intramolecular hydrogen bond, while the Thp and Ac$_6$c NH groups are solvent exposed.

Solution IR spectra have been examined in order to confirm that the Leu NH group is intramolecularly H-bonded. Figure 5A shows the NH stretching region of IR spectrum of **2a** in $CHCl_3$. Two bands are observed at 3306 and 3416 cm^{-1}, corresponding to the H-bonded and free NH groups, respectively. The ratio between the intensity of the two absorptions was found practically concentration independent over the range 10-0.3 mM. Thus, peptide self-association occurs, if any, at limited extent under these experimental conditions and the band at 3306 cm^{-1} can be assigned to intramolecularly H-bonded Leu NH group. IR specrum of **2b** (Figure 5B) shows a very close outcome in the NH stretching region: two bands are observed at 3304 and 3420 cm^{-1} , and the band at lower frequency can be assigned to intramolecularly bonded Leu NH .

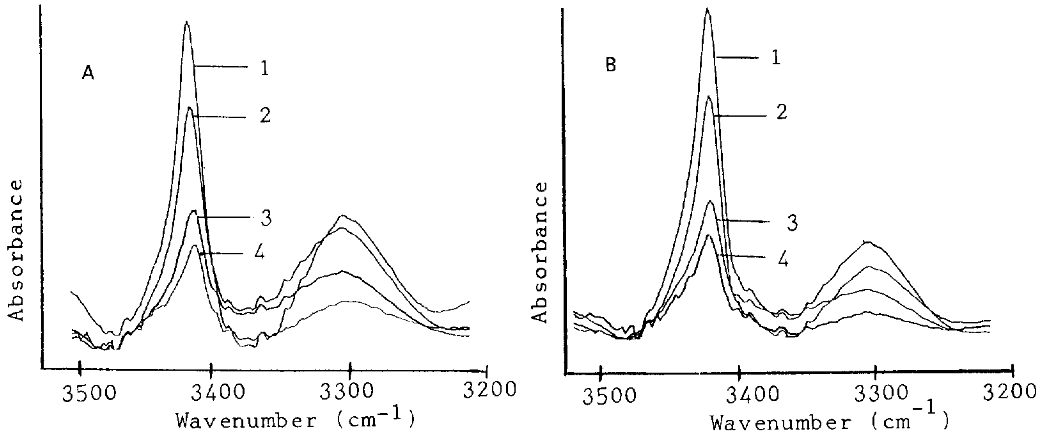


Figure 5. The IR spectra (NH stretching bands) of 2a (A) and 2b (B) in CHCl$_3$ at various concentrations: (1) 10 mM, (2) 5 mM, (3) 1 mM, and (4) 0.3 mM. The 0.5 mm cells were used for spectra 1 and 2, 2 mm and 3 mm cells for spectra 3 and 4, respectively.

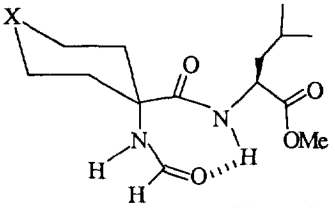
All these findings indicate that in CDCl$_3$ solution the formyldipeptides **2a** and **2b** adopt a γ-turn conformation stabilized by a 3-->1 intramolecular hydrogen bond between the formylic oxygen and the Leu NH. When (CD$_3$)$_2$SO solutions were examined, both the Thp and Leu NH groups of **2a** show relatively high temperature coefficients (0.0040 ppm/K and 0.0055 ppm/K, respectively) and similar values are exhibited by Ac$_6$c NH and Leu NH of **2b** (0.0041 ppm/K and 0.0050 ppm/K, respectively). These data suggest that the folded γ-turn structure adopted by **2a** and **2b** in CDCl$_3$ is not retained in the polar H-bonding acceptor solvent where an extended conformation probably prevails.

Discussion and Conclusions

The above reported potential energy calculations performed on the Ac-Thp-NHMe model, adopting the geometry observed in the crystal of the tripeptide For-Thp-Leu-Ain-OMe[8], show that three conformations are in the range of about one Kcal/mole; among these the γ-turn represents the deepest minimum. Since it is well known that geometrical factors and conformations are strictly related, it seems probable that the here observed preference for the γ-turn can be related to the asymmetric geometry of the Thp residue, mainly due to the narrowing of the valence angle C'-C$^{\alpha}$-C$^{\beta}_{L}$ and the widening of the N-C$^{\alpha}$-C$^{\beta}_{D}$. As far as other peptides containing α,α-disubstituted cyclic residues are concerned, it is interesting to note that in models containing the 1-aminocyclopropane carboxylic acid residue (cyclopropylpeptides), right and left handed γ-turn structures result as the energetically most favoured conformations.[3,11] Here, however, the widening of the N-C$^{\alpha}$-C' (τ) angle ($\sim$ 117°), caused by the strain introduced by the cyclopropyl ring at C$^{\alpha}$, has been considered as the geometrical factor causing the observed conformational preference.

Conformational energy calculations on Ac-Ac$_6$c-NHMe with axial NH group[12], strictly related to the here studied model Ac-Thp-NHMe, have been previously reported by other authors. In this case an average geometry derived from 13 crystal structures was adopted and the corresponding energy map was found to be centrosymmetric; right- and left-handed helical conformations populate the lowest minima while the quasi-extended and γ-turn structures correspond to higher energy minima.

NMR and IR data of For-Thp-Leu-OMe (**2a**) and For-Ac$_6$c-Leu-OMe (**2b**) dipeptides reported in the present paper (Figures 3-5) show that, for both the models, the γ-turn structure is present in chloroform (Figure 6), whereas is not found in a H-bonding acceptor polar solvent as dimethylsulphoxide; here intermolecular H-bonds with the solvent prevent the formation of the γ-turn structure. Thus, dilute solutions and apolar surroundings or hydrophobic receptor sites constitute additional factors favouring the γ-turn formation. It should be noted, however, that recent experimental findings indicate that the γ-turn conformation can be also found in polar solvents. This is the case of the tetrapeptide amide Phe-cyclo-Met-Arg-Phe-NH$_2$ which, in DMSO solution, adopts a γ-turn conformation centered at the residue containing the cyclopropyl ring at C$^{\alpha}$ (cyclo-methionine).[4] The tendency of the Thp residue to induce γ-turn structures confirms our previous observations on the conformation adopted in CDCl$_3$ by the tripeptide For-Thp-Leu-Δ^{Z}Phe-OMe.[7]



2a: X=S
2b: X=CH$_2$

Figure 6

The results of the present work are not expected on the basis of several previous conformational studies in solution which show the lack of the C$_7$ structure and the presence of the β-turn conformation in peptides containing Ac$_6$c and other α,α-disubstituted cyclic residues.[1,2,13] Actually, the occurrence of the strong NOE between NH$_{i+2}$ and NH$_{i+3}$ protons, which is diagnostic for the 10-membered folded structure[14], is not observed[12] or generally not reported in the ^{1}H NMR spectra of peptides containing the Ac$_6$c residue; thus, the observed solvent shielding of the NH$_{i+3}$ could be related to the presence of a certain population of γ-turn structure centered at the Ac$_6$c residue. In the case of peptides containing consecutive Ac$_6$c residues, such as Boc-(Ac$_6$c)$_3$-OMe[12], the solvent shielding of the NH groups of both the central and *C*-terminal residue, together with the very small NOE between these protons, could also be interpreted in terms of two consecutive γ-turns in addition to the β-turn structure.

EXPERIMENTAL SECTION

Energy Calculations

Semiempirical potential functions were used to compute conformational energies. The total energy is the sum of non-bonded, electrostatic and torsional strain energy. The generalized form $a \cdot \exp(-br) \cdot r^{-d} - c \cdot r^{-6}$ with the coefficients of ref. 15 was used to calculate the non-bonded energy. The electrostatic energy was calculated in the monopole-monopole approximation including the residual charges, estimated by the method of ref. 16, only for backbone atoms. A threefold potential was used to evaluate the torsional term. Hydrogen bond contribution in the form of the potential proposed by Stockmayer[17] was added to the other energy terms. Methyl groups were treated as single bulky atoms. The conformational space was mapped computing the energy at 10° intervals for the torsion angles. Minimum energy conformations were obtained in all the low-energy regions by minimizing the energy using the Metropolis Montecarlo procedure described in ref. 18. Conformational energies are expressed as $\Delta E = E\text{-}E_0$ where E_0 is the energy of the most stable conformation. All the calculations were performed on DEC 3500 AXP computer.

Peptide Synthesis

Melting points were determined with 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. Infrared spectra were recorded with a Perkin-Elmer 983 spectrophotometer (KBr disks) and with a Perkin-Elmer 16FPC FT-IR spectrophotometer using 0.5, 2, and 3 mm CsI cells. ^{1}H-NMR spectra were measured with a Varian XL-300 spectrometer in $CDCl_3$ (tetramethylsilane as internal standard). Thin-layer chromatographies were performed on silica gel Merck 60 F_{254} plates. Analytical data: Servizio Microanalisi del CNR, Area della Ricerca di Roma, Montelibretti, Italy.

Boc-Thp-Leu-OMe (1a)

Isobutyl chloroformate (0.082 mL, 0.6 mmol) was added at -15 °C to a stirred solution of Boc-Thp-OH (0.157 g, 0.6 mmol) in dry dichloromethane (5 mL) containing *N*-methylmorpholine (0.079 mL, 0.72 mmol). The temperature was kept at -15 °C for 15 min, then a precooled solution of H-Leu-OMe·HCl (0.109 g, 0.6 mmol) and *N*-methylmorpholine (0.066 mL, 0.6 mmol) in dry DMF-CH_2Cl_2 (1:1, 2 mL) was added. The mixture was stirred at room temperature for 24 h, and then evaporated under *vacuum*. The residue was dissolved in ethyl acetate and washed with 5% aqueous $KHSO_4$, water, saturated aqueous $NaHCO_3$ and brine. The organic phase was dried (Na_2SO_4) and evaporated to give 0.209 g (90%) of pure title compound **1a** which was crystallized from ethyl acetate-light petroleum 40-60 °C bp, mp 141.5-142 °C; $[\alpha]_D$ = -15.8° ($CHCl_3$, *c* 0.7); IR (KBr) ν_{max} 3345, 3312, 1749, 1686, 1649, and 1513 cm^{-1}; ^{1}H-NMR: δ0.91 (6H, d, J = 6 Hz, $CH(CH_3)_2$), 1.41 (9H, s, $C(CH_3)_3$), 1.47-1.77 (3H, m, $CH_2\text{-}CH(CH_3)_2$), 2.12-2.88 (8H, m, Thp β- and γ-CH_2), 3.70 (3H, s, $COOCH_3$), 4.61 (1H, m, Leu α-CH), 4.77 (1H, s, NH-COO), 7.04 (1H, d, J = 8.5 Hz, Leu NH). Anal. calcd for $C_{18}H_{32}N_2O_5S$: C, 55.64; H, 8.30; N, 7.21%. Found: C, 55.90; H, 8.53; N, 7.00%.

Boc-Ac$_6$c-Leu-OMe (1b)

The dipeptide **1b** was synthesized in 83% yield starting from Boc-Ac$_6$c-OH (2 mmol) following the procedure previously described for **1a**, mp 138-138.5 °C (from ethyl acetate-*n*-hexane); $[\alpha]_D$ = -10° ($CHCl_3$, *c* 1.0); IR (KBr) ν_{max} : 3343, 3316, 1753, 1687, 1647, and 1513 cm^{-1}; ^{1}H-NMR: δ 0.92 (6H, m, $CH(CH_3)_2$), 1.20-2.10 (13H, m, $CH_2\text{-}CH(CH_3)_2$ and Ac$_6$c β-,γ-, δ-CH_2), 1.45 (9H, s, $C(CH_3)_3$), 3.71 (3H, s, $COOCH_3$), 4.58 (1H, m, Leu α-CH), 4.71 (1H, s, NH-COO), 7.11 (1H, br signal, Leu NH). Anal. calcd for $C_{19}H_{34}N_2O_5$: C, 61.59; H, 9.25; N, 7.56%. Found: C, 61.72; H, 9.58; N, 7.44%.

For-Thp-Leu-OMe (2a) and For-Ac$_6$c-Leu-OMe (2b)

Treatment of Boc-protected dipeptide **1a** (0.8 mmol) or **1b** (0.6 mmol) with formic acid and EEDQ as described in a previous paper[7] afforded the corresponding *N*-formyl derivatives **2a** (81%) and **2b** (85%).
Analytical and spectral data for **2a** : mp 139-139.5 °C (from ethyl acetate); $[\alpha]_D$ = -40° (MeOH, *c* 1.0); IR (KBr) ν_{max} : 3317, 1728, 1666, and 1514 cm^{-1}; ^{1}H-NMR: δ 0.94 (6H, m, CH(CH_3)$_2$), 1.50-1.70 (3H, m, CH_2-CH(CH$_3$)$_2$), 2.30-2.85 (8H, m, Thp β- and γ-CH$_2$), 3.73 (3H, s, COOCH$_3$), 4.55 (1H, m, Leu α-CH), 5.63 (1H, s, Thp NH), 7.30 (1H, d, J = 8 Hz, Leu NH), 8.25 (1H, s, HCO). Anal. calcd for $C_{14}H_{24}N_2O_4S$: C, 53.14; H, 7.65; N, 8.85%. Found: C, 52.82; H, 7.95; N, 8.72%.
Analytical and spectral data for **2b** : mp 132-132.5 °C (from ethyl acetate); $[\alpha]_D$ = -30° (MeOH, *c* 1.0); IR (KBr) ν_{max} : 3366, 3340, 1748, 1672, 1542, and 1519 cm^{-1}; ^{1}H-NMR: δ 0.93 (6H, m, CH(CH_3)$_2$), 1.20-2.22 (13H, m, CH_2-CH(CH$_3$)$_2$ and Ac$_6$c β-,γ-, δ-CH$_2$) , 3.72 (3H, s, COOCH$_3$), 4.54 (1H, m, Leu α-CH), 5.53 (1H, s, Ac$_6$c NH), 7.35 (1H, d, J = 8 Hz, Leu NH), 8.24 (1H, s, HCO). Anal. calcd for $C_{15}H_{26}N_2O_4$: C, 60.38; H, 8.78; N, 9.39%. Found: C, 60.42; H, 9.08; N, 9.49%.

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