Chemistry - A European Journal

Peptide delta-Turn: Literature Survey and Recent Progress

--Manuscript Draft--

Manuscript Number:	
Article Type:	Minireview
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Keywords:	cis amide; conformational energy calculations; peptide turns; X-ray diffraction; statistical analysis
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Abstract:	Among the various types of α-peptide folding motifs, δ-turn, which requires a central cis-amide disposition, has been one of the least extensively investigated. In particular, this main-chain reversal topology has been studied in-depth neither in linear / cyclic peptides nor in proteins. This minireview article assembles and critically analyzes relevant data from a literature survey on the δ-turn conformation in those compounds. Unpublished results from recent conformational energy calculations and a preliminary solution-state analysis on a small model peptide, currently ongoing in our laboratories, are also briefly outlined.
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	In attachment, please find a copy of our MINIREVIEW entitled "PEPTIDE δ-TURN: LITERATURE SURVEY AND RECENT PROGRESS" (authors: C. Toniolo, M. Crisma, C. Peggion, A. Moretto, F. Formaggio, C. Aleman, C. Cativiela, C. Ramakrishnan, and P. Balaram) which we want to submit for your consideration for publication in CHEM. EUR. J. as the MINIREVIEW article representing our contribution to the Journal in the occasion of the celebration of its 20th VOLUME ANNIVERSARY. Biographical sketches and photos of seven co-author are enclosed in the manuscript, along with acknowledgements, keywords (just before the References), a graphical abstract for the table of contents, and a graphical suggestion for the first page of the Minireview frontispiece. The biographical sketches and photos of the two co-authors from India, both currently hospitalized, will be provided as soon as possible. Competent reviewers for our contribution are: 1. Prof. Lila M. Gierasch, Department of Biochemistry, University of Massachusetts, Amherst, MA, USA. E-mail: gierasch@biochem.umass.edu 2. Prof. Ernest Giralt, Institute for Research in Biomedicine, Parc Cientific de Barcelona, Barcelona, Spain. E-mail: ernest.giralt@irbbarcelona.org 3. Dr. Francisco Rodriguez, Department of Chemistry, Technical University of Darmstadt, Darmstadt, Germany. E-mail: rodriguez@csi.tu-darmstadt.de 4. Prof. H. Wennemers, Laboratory for Organic Chemistry, ETH Zürich, Zürich, Switzerland. E-mail: helma.wennemers@org.chem.ethz.ch 5. Prof. C.M. Deber, Research Institute, Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada. E-mail: deber@sickkids.ca With my best personal regards Prof. Claudio Toniolo
Section/Category:	
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Question	**Response**
Dedication	
Submitted solely to this journal?	Yes
Has there been a previous version?	No
Animal/tissue experiments?	No

Peptide δ-Turn: Literature Survey and Recent Progress

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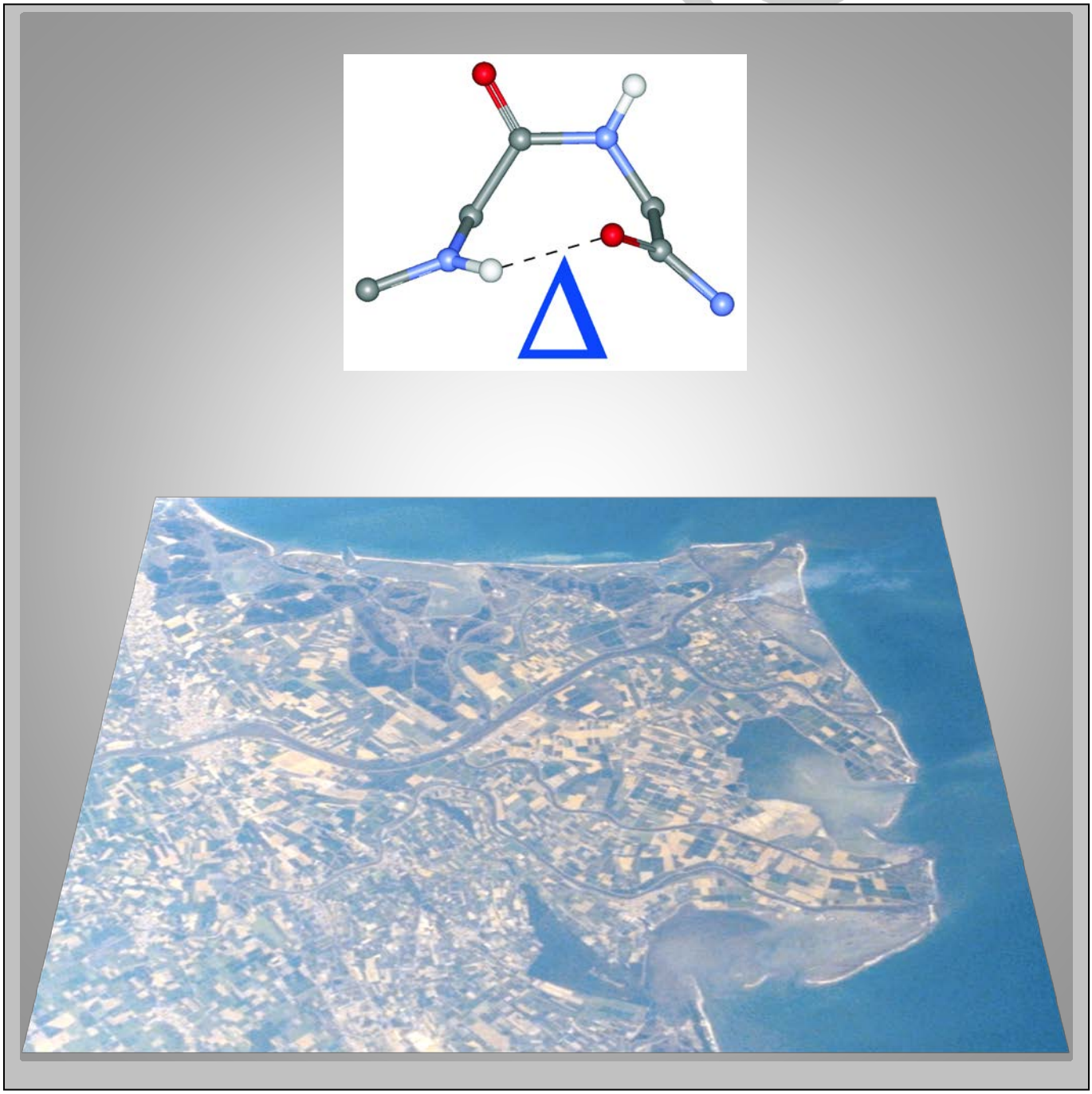

Abstract: Among the various types of α-peptide folding motifs, δ-turn, which requires a central *cis*-amide disposition, has been one of the least extensively investigated. In particular, this main-chain reversal topology has been studied in-depth neither in linear / cyclic peptides nor in proteins. This minireview article assembles and critically analyzes relevant data from a literature survey on the δ-turn conformation in those compounds. Unpublished results from recent conformational energy calculations and a preliminary solution-state analysis on a small model peptide, currently ongoing in our laboratories, are also briefly outlined.

1. Introduction

Folded conformations stabilized by intramolecular H-bonds between a main-chain amide N-H donor and a main-chain amide C=O acceptor are very common observations in solution studies and crystal-state analyses of peptides and proteins. Moreover, fully-extended, intramolecularly H-bonded, 3D-structural motifs were also reported, although to a limited extent.

A H-bond joining the N-H group of the α-amino acid residue *m* of a peptide (protein) and a C=O group of the residue *n* of the same sequence is traditionally termed *m* → *n* (arrow goes from H-bonding donor to acceptor). Therefore, the possible conformations in a system of four linked peptide units are: (i) the 3 → 1 (or 4 → 2 or 5 → 3), the 4 → 1 (or 5 → 2), and the 5 → 1 (Figure 1**A**), and (ii) the 2 → 2 (or 3 → 3 or 4 → 4), the 2 → 3 (or 3 → 4), and the 2 → 4 (Figure 1**B**) intramolecularly H-bonded forms. The number of atoms in the *pseudo*-ring closed by the intramolecular H-bond is the basis of an additional, extensively used, terminology for those conformations: C_7, C_{10}, C_{13} (Figure 1**A**), and C_5, C_8, C_{11} (Figure 1**B**). Peptide main-chain reversal does take place in all those motifs, with the single exception of C_5, which is fully-extended.[1-4] Even more familiar to structural peptide chemists is a third type of nomenclature where the C_7, C_{10} and C_{13} intramolecularly H-bonded forms are called γ-,[1,2,5-15] β-[1,2,13-21] and α-turns,[1,22-25] respectively, while the C_5, C_8 and C_{11} forms are called fully-extended, δ- and ε-turns,[1] respectively. The C_8, C_{10}, C_{11} and C_{13} forms are either sterically forced to or may include a *cis*-amide disposition. The peptide δ-turn (or C_8 or 2 → 3 / 3 → 4 intramolecularly H-bonded motif) (Figure 2), only occasionally mentioned, rarely authenticated, and never highlighted in any exclusively devoted article so far, is the subject of the present Minireview.

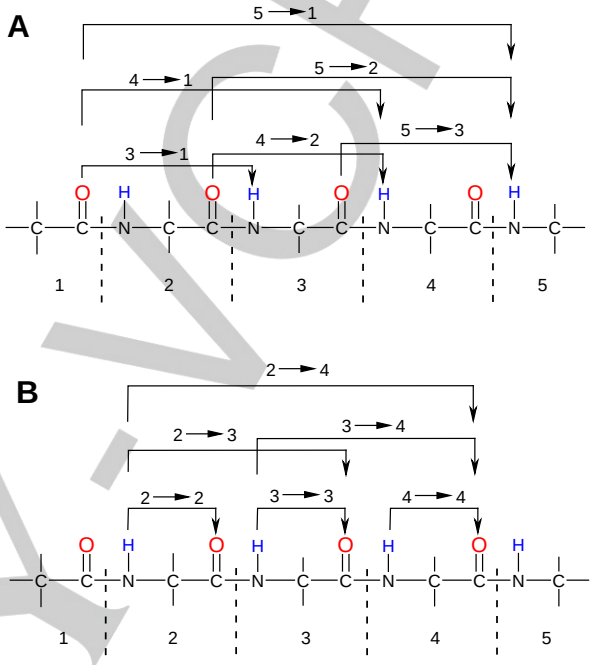

Figure 1. Possible intramolecularly H-bonded conformations in a system of four linked peptide units. H-Bond directionalities are: C=O … H-N in **A** and N-H … O=C in **B**.

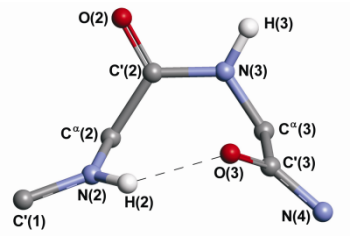

Figure 2. The 2 → 3 intramolecularly H-bonded (C_8) peptide structure (δ-turn). The central amide is in the *cis*-conformation.

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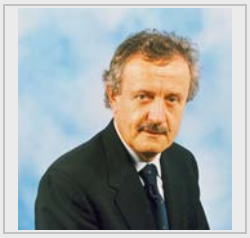


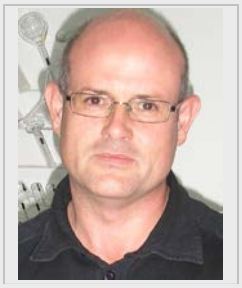
Claudio Toniolo received his Master Degree in Chemistry in 1965 at the Institute of Organic Chemistry, University of Padova (Italy) under the supervision of Prof. Ernesto Scoffone. In 1967–1968, he worked in the group headed by Prof. M. Goodman at the Polytechnic Institute of Brooklyn, New York (NY). Back to the University of Padova, in 1980 he was promoted Professor of Organic Chemistry. He retired in 2012 and is currently Senior Scientist at the University of Padova. He was co-Editor of *J Pept. Sci.* from 1995 to 2005. He is currently Associated Editor of *Biopolymers (Pept. Sci.)* and member of the Editorial Advisory Boards of *Chem. Eur. J.*, *ChemBioChem*, *Chem. Biodivers.*, and *Chemistry Open*. He is the author or co-author of over 900 review articles and research papers in peptide science.

Marco Crisma earned his Master Degree in Chemistry at the University of Padova (Italy) in 1980, with a thesis on Aib homo-peptides under the supervision of Prof. Claudio Toniolo. In 1981–1982, he worked as postdoctoral fellow with Prof. G.D. Fasman at Brandeis University (Waltham, MA, USA). Back to Italy, in 1985 he joined Toniolo's group in Padova, appointed by the National Research Council (CNR) of Italy, in which he currently holds the position of Senior Researcher in the Padova Unit of the Institute of Biomolecular Chemistry. His research interests are mainly focused on the conformational properties (both in solution and in the crystal state) of peptides from non-coded amino acids. He has co-authored over 350 publications.

Alessandro Moretto studied Chemistry at the University of Padova (Italy) and in the same University he completed his Ph.D. in 2002 under the supervision of Prof. Claudio Toniolo. He then started a postdoctoral work at the Scripps Institute Research Laboratories in San Diego (CA), followed by additional three years of a post-doctoral work in the DSM Life Science Company (Geleen, The Netherlands). Since 2006 he is Assistant Professor of Organic Chemistry at the University of Padova. He is co-author of more than 70 publications. His current research focuses on the design and investigation of peptide-based self-assembling systems, molecular machines, and nanoparticles.

Cristina Peggion achieved a Master Degree in Chemistry at the University of Padova (Italy) in 1993 under the guidance of Prof. Claudio Toniolo. She spent four years at the University of Lausanne, Switzerland, in the group of Prof. M. Mutter, where she received her Ph. D. in Chemistry in 1998. Then, she returned to Padova where she developed her academic career. She is currently Assistant Professor at the Department of Chemical Sciences, University of Padova. Her main research interests focus on peptide synthesis and conformational analysis of biologically active natural antibiotics and their tailor-made modified analogues, containing C^{α}-tetrasubstituted α-amino acids and spectroscopic probes for the exploration of their interaction with biological membranes. She is co-author of about 85 research papers.

Fernando Formaggio achieved a Master Degree in Chemistry at the University of Padova (Italy) in 1985 under the guidance of prof. Claudio Toniolo. After about two years as a research fellow at the University of Louisville (KY) in the laboratory of Prof. A.F. Spatola, he returned to the University of Padova where he developed his academic career. He is currently Professor of Organic Chemistry. His main research interests focus on peptide synthesis and bioactivity and on the exploitation of rigid peptide scaffolds for applications in spectroscopy, electrochemistry and material sciences. He has co-authored over 300 publications.



Carlos Alemán graduated in Chemistry from the University of Barcelona (Spain). He received his PhD from the Polytechnic University of Catalonia in 1994, where he was promoted to the positions of Assistant Professor, Associate Professor, and Professor of Physical Chemistry. He was postdoctoral researcher and Visiting Professor at the ETH (Zürich), and Visiting Professor at the University of Naples "Federico II", University of Twente, Universidade Federal do Rio Grande do Sul, among others. Since 2003 he is the leader of the Innovation in Materials and Molecular Engineering (IMEM) group. His research interests focus on conducting polymers with biomedical applications, peptides as materials, and peptide-polymer conjugates.

Carlos Cativiela is Professor of Organic Chemistry at the University of Zaragoza (Spain) since 1996. His main scientific activity is related to the synthesis of non-proteinogenic amino acids in enantiomerically pure form. He has developed methodologies for the preparation of a wide variety of non-coded amino acids by applying enantio-/diastereoselective procedures or chromatographic resolution techniques. His current research interests also involve the use of such amino acids in different applications, mainly in the peptide field. He has co-authored more than 400 papers, including several review articles on the synthesis of amino acids.

2. Literature Survey

In the early days of polypeptide 3D-structural studies, the δ-type of folded forms was not considered energetically allowed.[17,26] Also, in an investigation on the classical pentapeptide model of the fibrous protein elastin -L-Val-L-Pro-Gly-L-Val-Gly- the existence of this 3D-structural motif was excluded.[27]

However, in 1977 from an analysis of molecular models by Scheraga,[28] the occurrence of the peptide C_8 conformation, although unlikely, was shown not to be impossible. In particular, if the disposition of the internal -CONH- group in Figure 2 is assumed to be *cis* ($\omega \approx 0°$), it is realistic to construct an *eight*-membered pseudo-ring form with the relevant N … O separation not far from the ideal value (≈ 3.0 Å).[29-31] For the simplest (terminally protected) sequence -Gly-L-Pro-, the most favorable backbone torsion angles *via* ECEEP calculations were found to be $\phi_2 = 173°$, $\psi_2 = 85°$, $\phi_3 = -75°$, $\psi_3 = 167°$[28] (this dipeptide was selected for this study because Gly is the least sterically hindered among coded amino acids, and the Xxx-Pro tertiary amide function is known to accommodate in the *cis*-conformation relatively often as compared to secondary amides).[1,19,21,26,32-37] Moreover, a stabilization of this turn structure was obtained when the amide torsion angle ω was allowed to fluctuate in the range ±10-15°. Interestingly, X-ray diffraction investigations on medium-sized lactams revealed that the eight-membered ring compound of this class (enantholactam) is too small to accommodate a *trans*-amide conformation ($\omega = 180°$).[38] In this case, the *cis*-amide is obligatorily disposed with both N-H and C=O functionalities pointing outward.

Good chances to experimentally observe a δ-turn were attributed to *cyclic* tetrapeptides with the amino acid sequence *cyclo*-(X-Y_3)-, where X is any coded residue except Pro and the three Y residues are any N-alkylated residues (Figure 3). Crystallographic investigations on the *cyclo*-[Gly-$(Sar)_3$]- where Sar is sarcosine (N-methylglycine) and *cyclo*-[D,L-Ala-$(Sar)_3$]-cyclo-tetrapeptides demonstrated that these molecules do adopt the classical (for cyclo-tetrapeptides) *trans-cis-trans-cis* amide conformation, with the single (Gly/Ala) secondary amide *trans*.[39] However, this N-H group does not participate in an intramolecular H-bond, but rather it is involved in an (intermolecular) H-bond with a neighboring molecule. Even solution studies on these cyclo-tetrapeptides did not reveal the presence of the intramolecular H-bond.[40] However, it is worth pointing out that only slight variations of the torsion angles of the annular system are required to provide the intramolecularly H-bonded C_8 conformation.

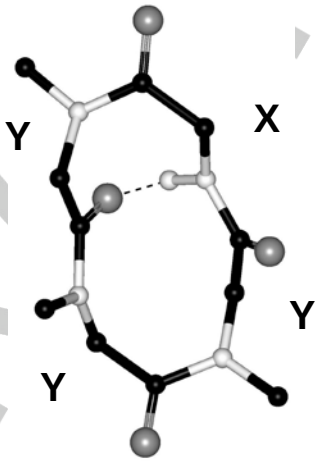


Figure 3. Model of a cyclo-tetrapeptide of the type *cyclo*-(X-Y_3)- with the sequence of amide conformations *trans-cis-trans-cis* and the potentially occurring, single (C_8-type) intramolecular H-bond. At variance with residue X, the three residues Y are N-alkylated α-amino acids.

The peptide C_8 turn might exist in *linear* peptides in solvents of low polarity at very low concentration (to avoid intermolecular amide … amide N-H … O=C H-bonds). In this connection, in 1977 Urry and coworkers carried out conformational energy calculations on Ac-L-Val-Gly-OMe (Ac, acetyl; OMe, methoxy) incorporating the effect of solvent and an NMR study on the same terminally blocked dipeptide.[41] Their results apparently suggest the co-existence of the C_8 conformation with other turn structures in the equilibrium mixtures in solvents such as CCl_4 and $CDCl_3$.

Experimental NMR data in aqueous solution of small linear peptides partially or fully deprotected at their N- / C-termini provided some support in favor of the occurrence of the C_8 form stabilized by interactions involving charged species, in which for example the H-bonding donor is either an ammonium or an amide moiety, and the H-bonding acceptor is the carboxylate moiety.[42-47]

Details of interesting eight-membered ring structures, which include a covalent -S-S- bond, were unraveled in X-ray diffraction studies of two cyclic disulfides from the terminally free or protected homo-dipeptide -L-Cys-L-Cys-.[48,49] Figure 4 illustrates the 3D-structure of the terminally protected, disulfide-containing, Boc-L-Cys-L-Cys-OMe (Boc, *tert*-butyloxycarbonyl) dipeptide. In both cases, the internal amide group was found in the *cis*-conformation with both the N-H and C=O groups pointing outward. However, the potentially occurring, intramolecularly H-bonded C_8 turn, further stabilized by an -S-S- bridge, was *not* observed.

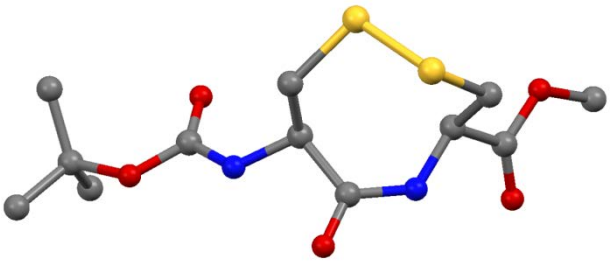


Figure 4. X-Ray diffraction structure of Boc-L-Cys-L-Cys-OMe (intramolecular disulphide). Adapted from ref. [49].

Also IR absorption spectroscopy was proposed by Balaram, Rao and coworkers in 1979 as a quick and reliable technique to probe the onset of intramolecularly H-bonded conformers in diluted solutions in $CDCl_3$.[50] The protected dipeptide Z-Aib-L-Pro-OMe (Z, benzyloxycarbonyl; Aib, α-aminoisobutyric acid) studied in this work is a very simplified model compound in that it carries a single NH donor group. Its interaction with the tertiary amide carbonyl oxygen generates a fully-extended C_5 conformation[1-4] (Figure 5**a**), while its interaction with the ester carbonyl oxygen produces a C_8 (δ-turn) conformation (Figure 5**b**). The IR absorption spectrum of this dipeptide shows two peaks, a significantly intense one at 3437 cm^{-1}, assigned to a free (solvated) N-H group, and a 50% less strong one at 3385 cm^{-1}, typical of an intramolecularly H-bonded N-H group.[4,13,51-54] The low intensity band at 3385 cm^{-1} may be ascribed to a relatively small percentage of molecules populating the H-bonded species. The high-frequency position of this maximum is compatible with the limited strengths of the C_5 and C_8 H-bonds. These results, however, did not allow the authors to discriminate between the contributions of the C_5 and/or C_8 conformation(s).

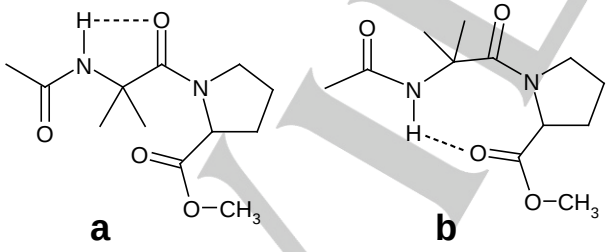


Figure 5. Possible intramolecularly H-bonded conformations for N^{α}-acyl-Aib-L-Pro-OMe: C_5 (**a**) and C_8 (**b**).

In 1987 Van Binst and coworkers[55] synthesized and investigated by 500 MHz 2D-NMR the preferred conformation of a variant of the highly active cyclopeptide analog of somatostatin, an effective inhibitor of growth hormone release, namely *cyclo*[Xxx-L-Phe-D-Trp-L-Lys-L-Thr-Yyy], known to include a *cis* amide bond between the Yyy and Xxx residues.[56] Their variant cyclopeptide is characterized by an *ortho*-AMPA [where AMPA is an (aminomethyl)phenylacetyl] spacer, which mimics a -Gly-Gly- unit with a *cis* pseudopeptide bond as a replacement for the Yyy-Xxx dipeptide sequence (Figure 6). In addition to the well known type-II′ β-turn encompassing the -D-Trp-L-Lys- sequence,[56] the authors proposed the predominant occurrence of an intramolecular H-bond connecting the N-H and C=O groups of the *ortho*-AMPA bridge, generating a *pseudo*-C_8 (δ-turn) local conformer.

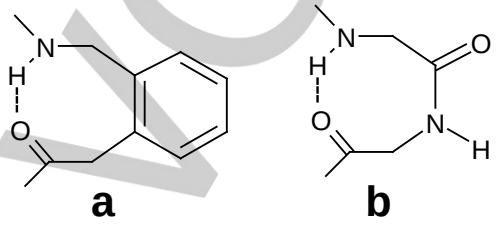


Figure 6. The *ortho*-AMPA spacer unit (**a**) mimics a -Gly-Gly- dipeptide sequence with a *cis pseudo*-peptide bond (*pseudo* C_8-turn).

Two unequivocal proofs for the presence of the intramolecularly H-bonded C_8 conformation were obtained in the crystal state by X-ray diffraction analyses. The first evidence, reported in 1980 by Duax *et al*.,[57] was found in the cyclo-octadepsipeptide *cyclo*[-(D-Ile-L-Lac-L-Ile-D-Hyi)$_2$-], where Lac and Hyi are two α-hydroxy acids, lactic acid and 2-hydroxy-3-methyl-valeric acid, respectively. In this compound, formed by cyclization of dimers of -D-Ile-L-Lac-L-Ile-D-Hyi- and synthesized as part of a study of ion-transport antibiotics analogs of valinomicin, the stereosequence of the heterochiral building blocks is -(DLLD)$_2$- and the alternating sequence of the bond joining the eight units is -(ester-amide-ester-amide)$_2$-. Despite these rather unusual features, this cyclic compound exhibits a pair of intramolecular H-bonds, both encompassing the -D-Ile-L-Lac- sequences and forming C_8 conformations associated with kinks in the depsipeptide backbone (Figure 7). The torsion angles are listed in Table 1. Some of the features in each C_8 turn of this 3D-structure are unexpected because: (i) the acceptor of the intramolecular H-bond is an *ester* carbonyl and (ii) the covalent bond, joining the two residues involved, is part of an *ester* function and exhibits a *trans* conformation. In this 3D-structural situation, the observed N … O separations are rather long (Table 2),[29-31] but the H-bonds are still strong enough to compensate for the highly distorted from planarity (by approximately 25°) ω torsion angles. Interestingly, the ϕ,ψ backbone torsion angles of the L-Lac residues (at position 3 in Figure 2) are quite close to those predicted by Scheraga,[28] whereas those of the D-Ile residues (at position 2 in Figure 2) differ substantially [however, it should be stressed that in this cyclo-depsipeptide the stereosequence (D-L) is *not homochiral*].

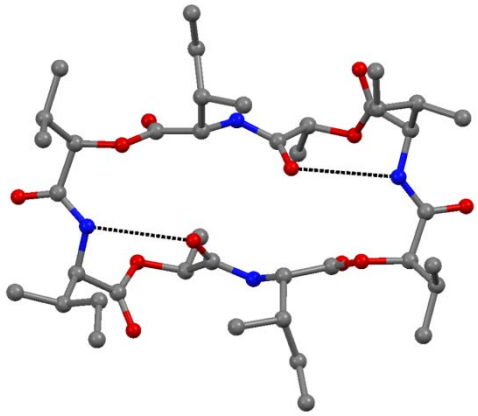


Figure 7. X-Ray diffraction structure of *cyclo*[-(D-Ile-L-Lac-L-Ile-D-Hyi)$_2$-]. The two (C_8) intramolecular H-bonds are highlighted by dashed lines. Adapted from ref. [57].

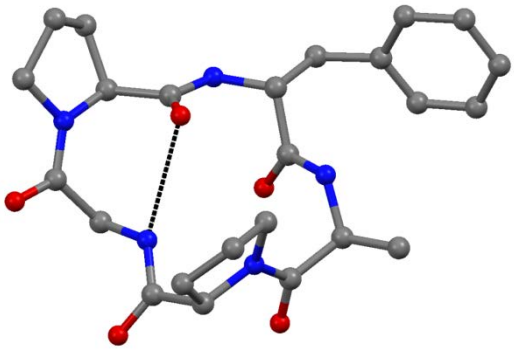


Figure 8. X-Ray diffraction structure of *cyclo*[Gly-L-Pro-D-Phe-L-Ala-L-Pro]. The C_8 intramolecular H-bond is represented by a dashed line. Adapted from ref. [58].

In the second, unambiguous example of a C_8-turn, the chemical features of the cyclopentapeptide *cyclo*[Gly-L-Pro-D-Phe-L-Ala-L-Pro], published by Gierasch and coworkers in 1988,[58] are much less unusual: (i) no α-hydroxy acid in the sequence and therefore no ester bond in the backbone, and (ii) only one D-residue. A *cis* peptide bond is seen between Gly and L-Pro (Figure 8 and Table 1). All other ω peptide bonds adopt the *trans* conformation, close to planarity (180°). A δ-turn is observed across the -Gly-L-Pro- sequence, with the Gly amide NH group as donor and the L-Pro carbonyl oxygen as acceptor of an intramolecular C_8 H-bond of normal strength[29,31] (Table 2). Except for the ϕ torsion angle of the flexible Gly, all other backbone torsion angles of the dipeptide sequence are quite close to those predicted by Scheraga.[28] Interestingly, the L-Pro amide carbonyl oxygen is also H-bonded to a co-crystallized water molecule. Preliminary NMR conformational results favored the conclusion that for the Gly-L-Pro bond both the *cis* and *trans* conformations exist in solution, the relative amount of which depends on the solvent properties.[58] Interestingly, in a previous study in the crystal state by the same group,[59] it was shown that the sequentially related cyclopentapeptide *cyclo*[L-Ala-L-Pro-Gly-D-Phe-L-Pro], although possessing a conformation at the -L-Ala-L-Pro- dipeptide unit close to that of -Gly-L-Pro-,[58] appears *not* to form the intramolecularly H-bonded δ-turn. The authors attributed the absence (or the extremely weak character[32]) of the H-bond to the steric requirements of the L-residue at the left corner position of the putative C_8 conformer. In particular, the ϕ,ψ torsion angles of the Ala residue are found in the region of the *left*-handed helix of the Ramachandran map, known to be unfavorable for an L-configured residue.

Therefore, experimental observations of the intramolecularly H-bonded δ-turn conformation in the crystal state for two cyclopeptides with entirely different characteristics[57,58] point to its intrinsic stability. Also, a common 3D-structural feature extracted from the energy calculations[28] and the X-ray diffraction investigations[57,58] is the *semi*-extended conformation of the residue at the right corner of the δ-turn (Figure 2), whereas the set of ϕ,ψ torsion angles of the residue at the left corner is more variable.

Table 1. Backbone torsion angles [°] for the C_8-turn forming dipeptide sequences of the two cyclopeptides[57,58] discussed in this Section

	ϕ	ψ	ω
cyclo[-(D-Ile-L-Lac-L-Ile-D-Hyi)$_2$-]			
D-Ile	133	-41	156
L-Lac	-68	163	
D-Ile	125	-34	153
L-Lac	-76	157	
cyclo[Gly-L-Pro-D-Phe-L-Ala-L-Pro]			
Gly	109	94	9
L-Pro	-74	170	

Table 2. Intramolecular H-bond parameters observed for the C_8 turns occurring in the two cyclopeptides[57,58] discussed in this Section

Donor (D)	Acceptor (A)	D … A [Å]	H … A [Å]	< D-H…A [°]
cyclo[-(D-Ile-L-Lac-L-Ile-D-Hyi)$_2$-]				
N (Ile)	O (Lac)	3.13	2.21	141
N (Ile)	O (Lac)	3.15	2.26	138
cyclo[Gly-L-Pro-D-Phe-L-Ala-L-Pro]				
N (Gly)	O (Pro)	2.92	2.33	139

In 1992, a statistical analysis from available crystal data specifically devoted to the conformation of the *cis*-amide containing -Xxx-Pro- dipeptide segment in peptides and proteins was conducted by Ramakrishnan and collaborators.[32] In both type of compounds, it was shown in particular that, when Xxx is different from Gly, the preferred conformation at Pro is *semi*-extended, termed *trans′*. In proteins, when Xxx is different from Gly, the Xxx conformation is nearly extended. It is worth noting that in peptides the bulk of the *cis*-amide examples found and analyzed is associated to cyclic compounds where the overall conformation is largely dictated by the restrictions imposed by the ring closure. Obviously, among them the two cyclopentapeptides mentioned earlier in this text[58,59] were listed and discussed. From their data, the authors concluded that there is no direct explanation for the pertinent question why the intramolecularly H-bonded C_8-turn is not commonly observed in peptides.

To conclude this Section, it is interesting to mention two theoretical contributions,[32,60] which, although focused on the much more widespread β-turn motif, treated the δ-turn as well, also called 1 → 2 H-bonded conformer in reference [32] (Figures 9 and 10) and $\delta_L\varepsilon_D$ conformer (or its enantiomer $\delta_D\varepsilon_L$) in reference [60] (Figure 11), respectively. Ramakrishnan and collaborators[32] used grid-search energy calculations followed by an energy minimization procedure. In particular, they studied the -Gly-(*cis*)-L-Pro- sequence and the related sequences where Gly is replaced by L-Ala or D-Ala. Remarkably, among the variety of energy minima identified, they found that some minima contain the eight-membered *pseudo*-ring H-bond in a bent conformation. In each of the cases examined, two minima of this type were found (termed in particular G2 and G7, see the "arrow diagrams" in Figure 9 and their 3D-representations in Figure 10, for the -Gly-L-Pro-sequence). The conformer G2 implies a *cis′* disposition for L-Pro, at variance with the G7 conformer which requires a *trans′* disposition. Analogous results were reported for the dipeptides with L-Ala or D-Ala at the N-terminus.

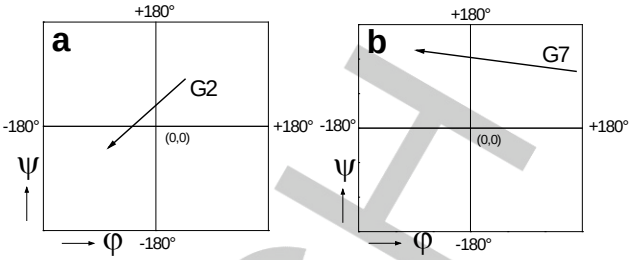


Figure 9. Arrow diagrams for the two intramolecularly H-bonded C_8 minimum energy conformations G2 (**a**) and G7 (**b**) of the -Gly-(*cis*)-L-Pro- peptide system. The base of the arrow corresponds to the conformation of Gly and the head to that of Pro. Adapted from ref. [32].

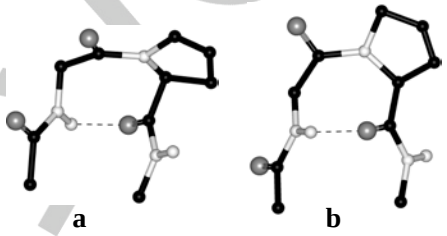


Figure 10. 3D-Representation of the intramolecularly H-bonded C_8 minimum energy conformations G2 (**a**) and G7 (**b**) of the -Gly-(*cis*)-L-Pro- peptide system. Adapted from ref. [32].

Perczel, Csizmadia and coworkers[60] computed by the HF/3-21G method a set of minimum energy conformations for HCO-$(Ala)_2$-NH_2, including, in addition to 30 different β-turns, a conformer characterized by an eight-membered *pseudo*-ring (Figure 11). Notably, all amide bonds of this structural motif are *trans*. Not unexpectedly, however, the intramolecular H-bond is weak, with a bent N-H … O system and a remarkable pyramidalization of the nitrogen atom of the Ala-Ala peptide bond.

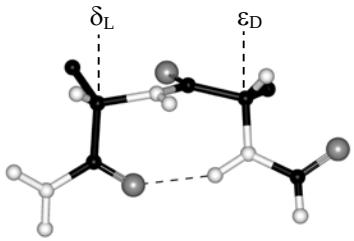


Figure 11. The C_8 conformation (called $\delta_L\varepsilon_D$) for the dipeptide HCO-$(Ala)_2$-NH_2. Adapted from ref. [60].

3. Statistical Analysis on Proteins

In the present work, Ramakrishnan and Balaram surveyed well-refined X-ray diffraction structures of globular proteins for the occurrence of dipeptide units Xxx-Yyy with a *cis*-peptide bond.

Not surprisingly,[1,19,21,26,32-37] almost all *cis*-peptide bonds have Yyy = L-Pro. The total number of *cis* Xxx-L-Pro bonds found is 1,276 (5.3%), while that of *trans* Xxx-L-Pro bonds is 22,659 (94.7%). Among the *cis* Xxx-L-Pro dipeptide units, L-Pro residues in the *semi*-extended (*trans′*) conformation are markedly preferred (824; 64.6%), while those in the right-handed helical (*cis′*) / "bridge"[61] conformation sum up to 452 (35.4%). Figure 12 graphically shows these data.

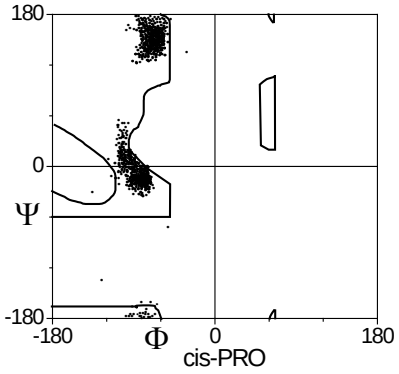


Figure 12. Ramachandran plot showing the distribution of the Pro conformations within cis-amide Xxx-L-Pro sequences in proteins (this work).

The next step was to identify the intramolecularly H-bonded C_8 (δ)-turns within the 1,276 cases of *cis* Xxx-L-Pro bonds. Although most of the 57 examples found (without X-ray resolution cutoff and tetrapeptide sequence identity cutoff filters) would clearly be from homologous entries, at least six of them can be considered prototypes. All exhibit acceptable parameters for both the ϕ,ψ,ω torsion angles (Table 3 and Figure 13) and the intramolecular H-bonds (Figure 13). They were recognized in the following proteins: (i) cytochrome P450 105P1 from *Streptomyces avermitilis*;[62] (ii) pyridoxamine-pyruvate aminotransferase from *Mesorhizobium loti* MAFF303099;[63] (iii) phosphoserine aminotransferase from *Mycobacterium tuberculosis*;[64] (iv) Io1I protein from *Bacillus subtilis*;[65] (v) calf spleen purine nucleoside phosphatase;[66] and (vi) subunit B of A_1A_0 ATP synthase.[67] Interestingly, upon binding the bisubstrate analog pyridoxyl-L-Ala-OH (PLA), the region of the *cis* peptide bond in the enzyme pyridoxamine-pyruvate aminotransferase approaches the active site by 1.7 Å, thus plugging it and shielding the small PLA molecule from the solvent environment.[63] It is worth pointing out that as many as four out of the six *cis* Xxx-L-Pro bonds are in fact Gly-L-Pro bonds.[62-65] This finding agrees well with the experimental observation on the cyclopentapeptide model compound[58] and the Scheraga's predictions.[28] The same conclusions apply to the dipeptide ϕ,ψ torsion angles. In all four examples, the L-Pro residue is *semi*-extended (*trans′*), while the set of *positive* torsion angles for the residue at the left corner are compatible with an achiral and flexible amino acid such as Gly. The additional two examples, involving an L-Ile-L-Pro[66] and an L-Asp-L-Pro[67] sequence, respectively, are, at least in part, 3D-structurally more unusual. The signs of the ϕ,ψ torsion angles for L-Ile-L-Pro are all negative, typical of L-residues, (right-handed helical for L-Ile, but outside any allowed region for L-Pro).[66] On the other hand, the signs and absolute values for the L-Asp residue in the L-Asp-L-Pro dipeptide sequence are both positive (typical of D-residues) and similar to those of the Gly residues in the four Gly-L-Pro dipeptide sequences, while those of L-Pro are right-handed helical (*cis′* conformation).

Table 3. Backbone torsion angles [°] for the C_8-turn dipeptide sequences in proteins

Protein[a]	Dipeptide sequence	ϕ_2	ψ_2	ω_2	ϕ_3	ψ_3	Ref.
3E5J	Gly-Pro	111	76	3	-82	158	[62]
2Z9X	Gly-Pro	130	113	-9	-62	169	[63]
2FYF	Gly-Pro	126	114	-9	-51	161	[64]
1NJH	Gly-Pro	115	110	0	-64	177	[65]
1A9T	Ile-Pro	-93	-31	0	-82	-104	[66]
2BMH	Asp-Pro	134	80	-1	-84	-77	[67]

[a] PDB code.

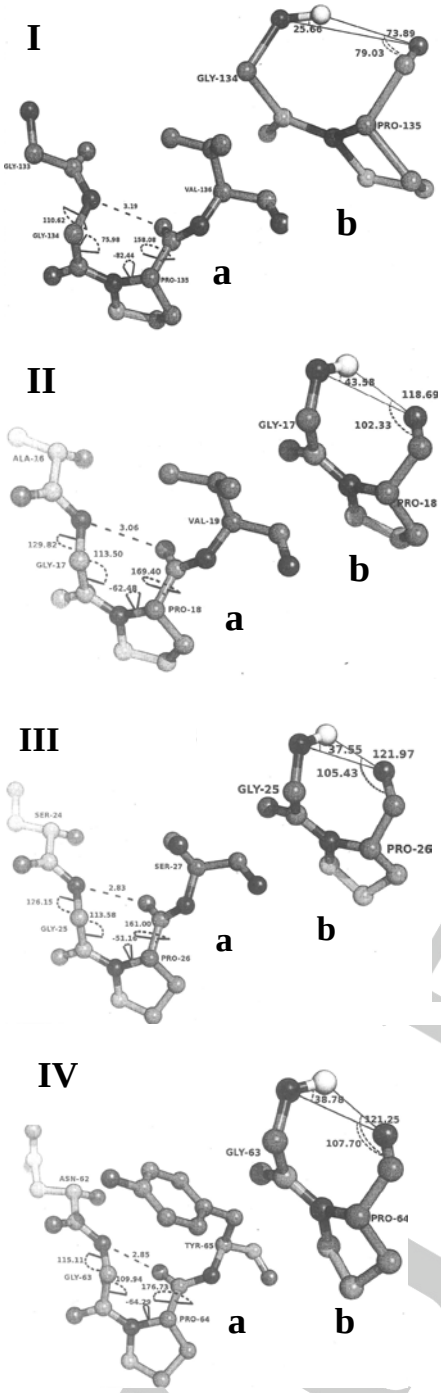


Figure 13. X-Ray diffraction structures of the four tetrapeptide sequences in proteins (I-IV) containing a central *cis*-amide Gly-L-Pro bond (**a**) and 3D-structural details of the related intramolecularly H-bonded δ-turn motifs (**b**) (this work).

4. Conformational Energy Calculations on Model Dipeptides

Based on the data from our literature survey (statistical analysis) described earlier in the text, we decided to investigate the conformational preferences of the terminally blocked dipeptide sequence -Gly-L-Pro- (compound **1** in Figure 14) using conformational energy calculations *in vacuo* and to compare them to those obtained in solution (*via* FTIR absorption and NMR spectroscopies). Moreover, this study was expanded to include the corresponding results for the related dipeptide -Gly-L-Dmp- (compound **2** in Figure 14), where Dmp is 5,5-dimethylproline. This residue,[68-74] as well as its 3D-structurally related 2,2-dimethyl-thiazolidine-4-carboxylic acid,[75-77] is known (from preliminary energy calculations and NMR analyses in solution) to provide an excellent "conformational lock"[69] for the *cis*-amide disposition in peptides generated by the severe steric hindrance associated with the presence of the *gem*-dimethyl substituents on the carbon atom close to the nitrogen of the five-membered heterocyclic moiety. More specifically, we analyzed N^{α}-acylated dipeptide *esters* to minimize the number of H-bonding donor groups and consequently to reduce to only two (C_5 or fully-extended[1-4] and C_8) the number of competing intramolecularly H-bonded conformers, as clearly shown in Figure 5.

In this work, density functional theory (DFT) calculations were performed by Alemán on two model peptides with the sequence Ac-Gly-L-Pro-OMe (**1**) and Ac-Gly-L-Dmp-OMe (**2**) (Figure 14) at the B3LYP/6-31+G(d,p) level *in vacuo*.

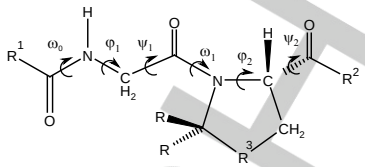
The conformational potential energy surfaces of **1** and **2** were systematically explored using a procedure inspired by the build-up method developed by Scheraga and coworkers in 1987.[78] According to this approach, which is based on the assumption that short-range interactions play a dominant role in determining the conformation of a given peptide, accessible starting geometries for **1** and **2** were constructed by combining all of the minima identified for each of the two residues involved (*i.e.* Gly and L-Pro or L-Dmp) after an exhaustive search. Geometry optimization *in vacuo* of all of the starting 3D-structures built for **1** and **2** resulted in 19 and 10 different conformations, respectively, which were characterized as minima by computing the frequencies. The relative free energies (ΔG_{gp}) were obtained by adding the zero-point vibrational energies and both thermal and entropic corrections to the energies. The following analysis of our results is specifically focused on the relative stability of the C_8- (δ-) turn conformations. Figures 15(**a**) and 15(**b**) display representative minima obtained for **1** and **2**, respectively, while the corresponding torsion angles (Figure 14) and ΔG_{gp} values are listed in Table 4.

A characteristic property of the lowest energy conformation of **1** (**1a**) is the presence of the fully-extended (C_5) structure (ϕ_1,ψ_1 torsion angles in Figure 14; see also Figure 15) adopted byt the Gly residue and the *trans* arrangement of the peptide bond preceding Pro (ω_1 in Figure 14). This result is not surprising as Gly is a frequently found residue in the C_5 structure.[4] Four C_8 conformers (**1b**-**1e**) were identified among the 18 remaining minima. These 3D-structures, which are characterized by the *cis*

disposition of the ω_1 torsion angle, exhibit ΔG_{gp} values ranging from 5.6 (**1b**) to 8.5 kcal/mol (**1e**). The C_8-type intramolecular H-bond involves the ester C=O and C–O oxygen atoms in three conformers (**1b**, **1c** and **1e**) and one conformer (**1d**), respectively. It is well known that the strength of N–H···O(–C=O) hydrogen bonds is weaker than that of N–H···O=(C–O).[79] In general, this finding is consistent with the H-bond parameters (*i.e.* H···O distance and ∠N–H···O angle) displayed in Figure 15(**a**) in all cases with exception of **1e**. It is worth noting that this last conformer exhibits the most favorable H-bond parameters (*i.e.* the shortest H···O distance and the ∠N–H···O angle closer to 180º), even though it is destabilized by 8.5 kcal/mol. This apparent contradiction should be attributed to the strain accumulated in the Pro ϕ_2 torsion angle, which adopts a value of -91º rather than the typical value around -55º.[33,80] On the other hand, it is worth pointing out that the torsion angles of the most stable C_8 conformer, **1b**, are in excellent agreement with those obtained in the statistical analysis from X-ray diffraction structures mentioned earlier in the text.

As for **2**, the gem-dimethyls at the pyrrolidine ring not only in general reduce the conformational freedom of the dipeptide but, in particular, also produce some changes in the conformer preferences [Figure 15(**b**)]. Thus, the minimum with the lowest free energy (**2a**) corresponds to a 3D-structure in which the torsion angle ω_1 adopts a *cis* arrangement, whereas the ΔG_{gp} of the minimum with ω_1 arranged in *trans* (**2a′**) is 1.1 kcal/mol. Interestingly, the torsion angles of the most stable C_8 conformation (**2b**), which is destabilized by $\Delta G_{gp=}$ 4.3 kcal/mol with respect to **2a** (3.2 kcal/mol with respect to **2a′**), resemble those obtained for **1b** that was unfavored by $\Delta G_{gp=}$ 5.6 kcal/mol with respect to **1a**. The second and third C_8 minima identified for **2** (**2c** and **2d**) exhibit interactions and torsion angles similar to those of **1c** and **1d**, even though the former conformations are ~ 1 kcal/mol more stable with respect to **2a** than the latter with respect to **1a**. Obviously, this stabilizing effect is more pronounced when the energies are relative to **2a′** rather than to **2a**. Finally, the ΔG_{gp} of **2e** is 9.0 kcal/mol. Despite the favorable H-bonding geometry, the severe distortions observed in this 3D-structure (*i.e.* the torsion angle ω_1, which adopts a value of -26.6º) are responsible for such a large free-energy penalty.

From the results of our conformational energy calculations *in vacuo* on the model dipeptides -Gly-L-Pro- and -Gly-L-Dmp-, the following conclusions can be extracted: (i) In both cases, the most stable conformations (**1a**, **2a**, and **2a′**) appear to be fully-extended at Gly with an intramolecular H-bond of the C_5 type. Both -Gly-L-Pro- and -Gly-L-Dmp- systems may accommodate either a *cis* or a *trans* -Gly-Xxx- amide bond. In any case, it is quite evident that the preference for the C_5 conformer, which precludes the onset of the C_8-type intramolecular H-bond in the same molecule, is dictated by the presence of the Gly residue at position 1.[4] (ii) Immediately following **1a**, **2a**, and **2a′** in the list of the most stable conformers, a set of C_8-forming structures is found, all bearing the central amide in the *cis* disposition.



1: R = R = H, $R^1 = CH_3$, $R^2 = OCH_3$, $R^3 = CH_2$ Ac-Gly-L-Pro-OMe

2: R = R = CH_3, $R^1 = CH_3$, $R^2 = OCH_3$, $R^3 = CH_2$ Ac-Gly-L-Dmp-OMe

3: R = R = CH_3, R^1 =  , $R^2 = OCH_3$, $R^3 = CH_2$ I_3Bz-Gly-L-Dmp-OMe

Figure 14. Chemical structures and backbone torsion angles for the terminally-blocked dipeptides (**1** – **4**) discussed in this work.

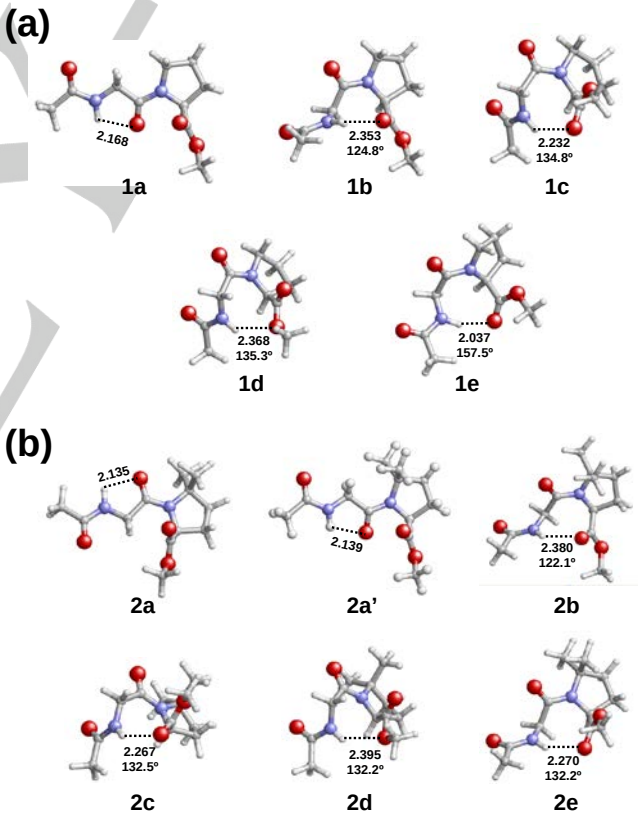


Figure 15. 3D-Structural representations of selected, calculated energy minima (i.e., the most stable C_5 conformers and those with the C_8-turn characterized at the B3LYP/6-31+G(d,p) level for the terminally-blocked dipeptides **1** (**a**) and **2** (**b**). Dotted lines represent intramolecular H-bonds. In each of the 3D-structures of the C_8-turn conformers, the H … O distance [Å] and the <N-H … O angle [°] are indicated. In the C_5 conformers, only the H … O distance [Å] is reported.

Table 4. Backbone torsion angles [°] and related free energies (ΔG, kcal/mol) of the lowest energy conformations and the local minima with the C_8-turn obtained from DFT calculations i*n vacuo* at the B3LYP/6-31+G(d,p) level for the terminally-blocked dipeptides **1** and **2** (for the torsion angle nomenclature, see Figure 14)

Conformer	ω_0	ϕ_1	ψ_1	ω_1	ϕ_2	ψ_2	ΔG
			Ac-Gly-L-Pro-OMe (**1**)				
1a	179.4	-176.6	-178.3	178.6	-59.9	145.8	0.0
1b	-175.8	126.9	115.4	-9.3	-55.5	169.4	5.6
1c	-175.8	-84.7	-35.7	-7.7	-53.7	-57.8	7.6
1d	-176.3	-83.1	-40.0	-8.1	-54.5	134.8	8.3
1e	177.3	123.6	78.8	-0.2	-90.9	-77.5	8.5
			Ac-Gly-L-Dmp-OMe (**2**)				
2a	177.5	177.5	-171.1	-8.2	-56.7	151.2	0.0
2a′	179.1	178.8	-169.6	178.4	-56.1	144.0	1.1
2b	-174.6	125.5	122.8	-17.9	-48.5	166.1	4.3
2c	-176.2	-80.2	-35.6	-14.2	-46.4	-53.0	6.5
2d	-176.5	-79.4	-38.5	-14.3	-48.9	137.5	7.3
2e	176.4	115.0	115.7	-26.6	-54.0	-73.9	9.0

5. Experimental Solution Conformational Analysis on a Model Dipeptide

Our preliminary conformational investigation in solution focused on the promising terminally-blocked dipeptide -Gly-Dmp-. Recently, in the group of one of us (C.C.)[81] a versatile methodology for the synthesis of racemic Dmp derivatives in good amounts was developed. It represents a significant advancement with respect to the old procedure published by Todd and coworkers.[82,83] In Padova, we therefore synthesized a set of racemic N^{α}-acylated -Gly-D,L-Dmp-OMe compounds. However, in our hands all of them were found to be oily and extremely hygroscopic materials, except the solid compound **3** in Figure 14 (where the acyl moiety is 2,3,5-triiodobenzoyl), possibly because of its markedly higher molecular weight and the related higher melting point.

Infrared absorption spectroscopy in the N-H stretching (3500 – 3200 cm^{-1}) region in $CDCl_3$ solution at high dilution is useful to highlight the presence and to quantitate the extent of intramolecularly H-bonded species in peptides.[13,50-54] As mentioned in Section 2, Balaram, Rao and coworkers[50] published the IR absorption spectrum in $CDCl_3$ at 10^{-4} M concentration for the related Z-Aib-L-Pro-OMe dipeptide. It is characterized by a significantly intense band at 3437 cm^{-1} (free N-H groups), and a broad absorption of lower intensity at 3385 cm^{-1}, attributed to a relatively small percentage of molecules populating the C_5 and/or C_8 H-bonded states. In the present work, we recorded the FTIR absorption spectrum for compound **3** in Figure 14 under the same experimental conditions as those exploited in reference [50]. An intense, rather broad, band centered at 3383 cm^{-1} is seen in the spectrum, accompanied by a tiny (less than 10% of the total area) band near 3440 cm^{-1} (Figure 16). The positions of these two bands are strictly comparable to those published for Z-Aib-L-Pro-OMe,[50] but the intensity ratios diverge substantially, *i.e.* the intramolecularly H-bonded conformers are much more extensively adopted by the -Gly-Dmp- sequence.

In the 400 MHz ^{1}H NMR spectrum of compound **3** in $CDCl_3$ two sets of signals stand out clearly for each proton of the -Gly-Dmp- dipeptide segment. The experimental *trans* to *cis* ratio is approximately 1 : 4. From an analysis of the model built by using the calculated backbone torsion angles for the third most stable (C_8) conformer, the *cis*-amide **2b** (Table 4 and Figure 15), a strong NOE cross-peak [84] is expected between αCH(Gly) and αCH(Dmp). An analogous NOE effect was reported for each of the peptide sequences closely related to -Gly-Dmp- and attributed to the *cis*-amide conformer.[68,69,75] In contrast, in the model with the calculated backbone torsion angles for the second most stable (C_5) conformer, the *trans*-amide **2a′** (Table 4), the expected NOE is between αCH(Gly) and δCH_3(Dmp). As the former NOE was observed only for the more abundant conformer, and the latter NOE only for the less abundant conformer, the peak assignments (the more abundant conformer is *cis*-amide and the less abundant is *trans*-amide) were straightforwardly derived.

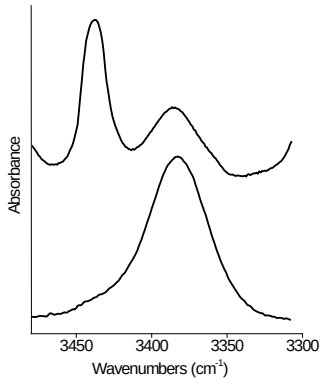


Figure 16. N-H stretching region of the FTIR absorption spectra of Z-Aib-L-Pro-OMe (top; adapted from ref. [50]) and 2,3,5-triiodobenzoyl-Gly-D,L-Dmp-OMe (bottom) in $CDCl_3$. Peptide concentration: 0.1 mM.

6. Summary and Outlook

The results of our detailed literature survey reported in this Minireview validate the conclusion that intramolecularly H-bonded δ-turns do occur, although not frequently, in *cyclic* peptides and in proteins. One reason for this rather limited presence is certainly associated with their strict 3D-structural requirements implying a rare *cis*-amide bond as an unescapable marker. Because of the well-established observation that the *cis* -Xxx-Yyy- bond in polypeptide molecules is much more common when this amide is tertiary, namely when Yyy is (among the coded amino acids) Pro, it is not surprising that the by far most usual dipeptide sequence involved would be of the -Xxx-Pro- type. The stringent 3D-structural properties of small cyclic peptides and the medium- and long-range interactions operative in proteins might have contributed to favorably bias δ-turn formation. The absence of any unambiguous report in the literature so far for the occurrence of a δ-turn in a *linear* peptide might be indirectly correlated to such requirements.

The overwhelming presence of Gly, the most flexible coded amino acid, as the Xxx residue in the δ-turn forming dipeptide sequences authenticated to date is the reason for our choice to investigate (both theoretically and experimentally) the -Gly-Pro- sequence, and its variant -Gly-Dmp- as well where the *cis* tertiary amide disposition is even more biased. Our results, although still at a preliminary stage, support our view that the -Gly-Dmp- sequence is a likely candidate for the first unambiguous experimental proof for the occurrence of the δ-turn even in a linear peptide, although our current data do not allow us to rule out the contribution of intramolecularly H-bonded C_5 forms to the conformational equilibrium. In this connection, work is in progress in the Padova laboratory to deepen our understanding of the conformational behavior in solution of the -Gly-Dmp- sequence, and, hopefully, to expand its analysis to the crystal state (by X-ray diffraction). To this aim, the design and study of additional, more appropriate -Xxx-Dmp- model compounds, potentially able to reduce the impact of the C_5 form, are also under scrutiny.

Moreover, we are confident that in future investigations greater attention will be paid by structural biochemists to the presence of δ-turns in globular proteins and by synthetic peptide chemists, spectroscopists, and X-ray diffraction specialists to the preparation and conformational investigations of linear peptides appropriately designed to this purpose. Hopefully, this Minireview will stimulate peptide and protein experts to specifically address the issue of the validation and in-depth characterization of this, still neglected, folded polypeptide secondary structure.

Acknowledgements

C. C is grateful to Ministerio de Economía y Competitividad (grant CTQ2013-40855-R) and Gobierno de Aragón–FSE (research group E40), and C. A. acknowledges support from MICINN-FEDER funds (MAT2012-34498) and CESCA.

Keywords: *cis* amide • conformational energy calculations • peptide turns • X-ray diffraction • statistical analysis

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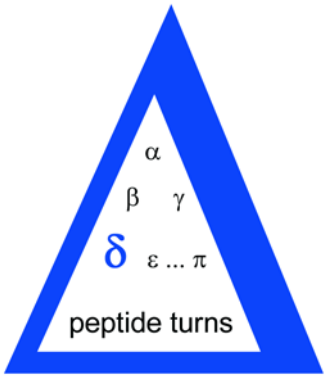
MINIREVIEW

***cis*-Peptide turn:** Published results on peptide δ-turn conformation, characterized by a *cis*-amide bond and an intramolecularly H-bonded, eight-membered *pseudo*-cyclic structure, are reviewed. Recent advancements in our laboratories on this rarely investigated, local folded structure are also presented.



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