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**PERSPECTIVE****Single and multiple peptide  $\gamma$ -turns: literature survey and recent progress**

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**Abstract**

In contrast to the extensively investigated  $\beta$ -turn conformation in peptides and proteins, single and multiple  $\gamma$ -turns have been much less commonly studied. Single and non-contiguous multiple  $\gamma$ -turns have been relatively often authenticated in small cyclic peptides, but these important peptide main-chain reversal motifs have been examined carefully neither in linear peptides nor in globular proteins. This Perspective article summarizes literature data on this aspect of peptide stereochemistry, expanding the discussion also to the rarely found, contiguous multiple  $\gamma$ -turns which generate incipient or fully-developed  $2.2_7$ -( $\gamma$ -) helices. Unpublished results of recent research activities on this topic ongoing in our laboratories are also briefly outlined.

## 1. Introduction

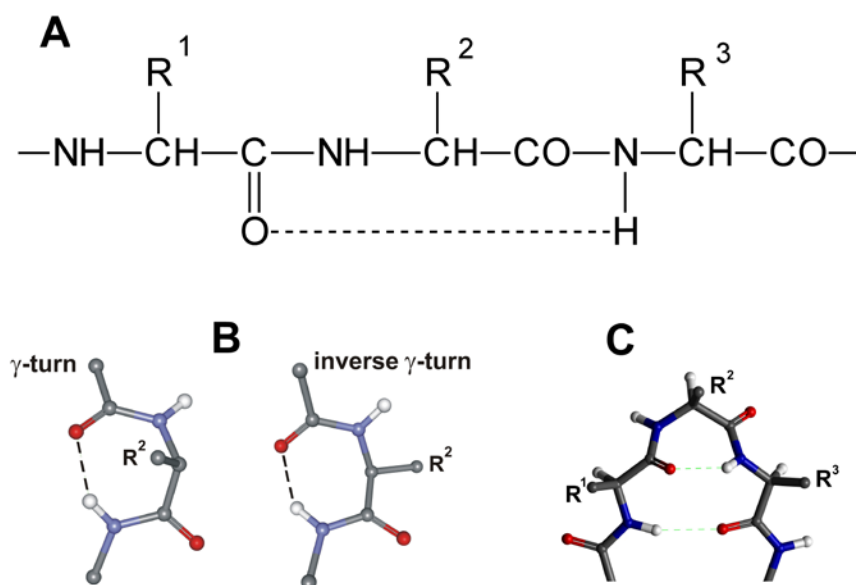
In the experimentally observed folded,  $\alpha$ -amino acid-based, peptides and proteins, the direction of the main chain often reverses over a very limited number of residues. The most frequently reported folding structure of this class is the  $\beta$ -turn (also termed  $1 \leftarrow 4$  intramolecularly C=O ... H-N H-bonded or  $C_{10}$  form), originally proposed independently by Geddes et al.<sup>1</sup> and Venkatachalam<sup>2</sup> in 1968 and followed by a more in-depth analysis by Scheraga and coworkers<sup>3</sup> a few years later. Numerous review articles on this fundamental 3D-structural motif, which consists of *four* amino acid residues, two of which entirely incorporated into the *pseudo*-ring form, were subsequently published.<sup>4-11</sup>

In addition to the  $\gamma$ -turn, which represents the main focus of this article and is discussed below, other types of peptide folding motifs have been mentioned and reported in the literature, albeit rarely. They are the  $\alpha$ -turn ( $1 \leftarrow 5$  intramolecularly H-bonded or  $C_{13}$  form),<sup>4,12-15</sup>  $\delta$ -turn ( $2 \rightarrow 3$  intramolecularly H-bonded or  $C_8$  form),<sup>4,16,17</sup>  $\epsilon$ -turn ( $2 \rightarrow 4$  intramolecularly H-bonded or  $C_{11}$  form),<sup>4</sup> and  $\pi$ -turn ( $1 \leftarrow 6$  intramolecularly H-bonded or  $C_{16}$  form).<sup>18,19</sup>

## 2. Single $\gamma$ -turns

$\gamma$ -Turns (also termed  $1 \leftarrow 3$  intramolecularly C=O ... H-N H-bonded or  $C_7$  forms) (Fig. 1), despite being less abundant in peptides and proteins than  $\beta$ -turns,<sup>4,8,9,11,20-26</sup> are by far more extensively investigated than any other type of turn. In  $\gamma$ -turns, which consist of *three* amino acid residues, a remarkable variation in direction takes place over two amide groups and a single  $C^\alpha$  atom.  $\gamma$ -Turns are *pseudo*-cyclic structures that are stabilized by an intramolecular H-bond between the N(3)-H(3) and O(1)=C(1) groups. The *trans* amide groups lie in two planes, which make an angle of about  $115^\circ$ . When  $R^2$  is not an H atom, two different isomers (with equatorial and axial side-chain dispositions) can exist, which are represented in the classical Ramachandran map by two centrosymmetric points, the backbone torsion angle coordinates of which for an (L)-residue are about  $\phi = -75^\circ$ ,  $\psi = 65^\circ$  (for the more stable, equatorial or inverse  $\gamma$ -turn form) and  $\phi = 75^\circ$ ,  $\psi = -65^\circ$  (for the less stable, axial or classical  $\gamma$ -turn). While the H-bond is strongly bent, it has a normal H(3) ... O(1) distance, still making a sizable contribution to the stabilization of the folded peptide structure. Some deviations from the usual *trans*-planarity of the two  $\omega$  amide torsion angles are often found. However, the small energy penalty associated with these torsions is more than compensated for by the energy of the H-bond. If the (guest)  $\gamma$ -turn containing peptide main chain is

further elongated, an additional (host) intramolecularly N-H .. O=C H-bonded form ( $\epsilon$ -turn)<sup>4</sup> may occur, involving the N(1)-H(1) and C(3)=O(3) groups of the same (first and third) residues (Fig. 1C). This latter 3D-structural motif is sometime found when the peptide chain folds back and forth to generate an antiparallel  $\beta$ -pleated sheet structure.<sup>4</sup>

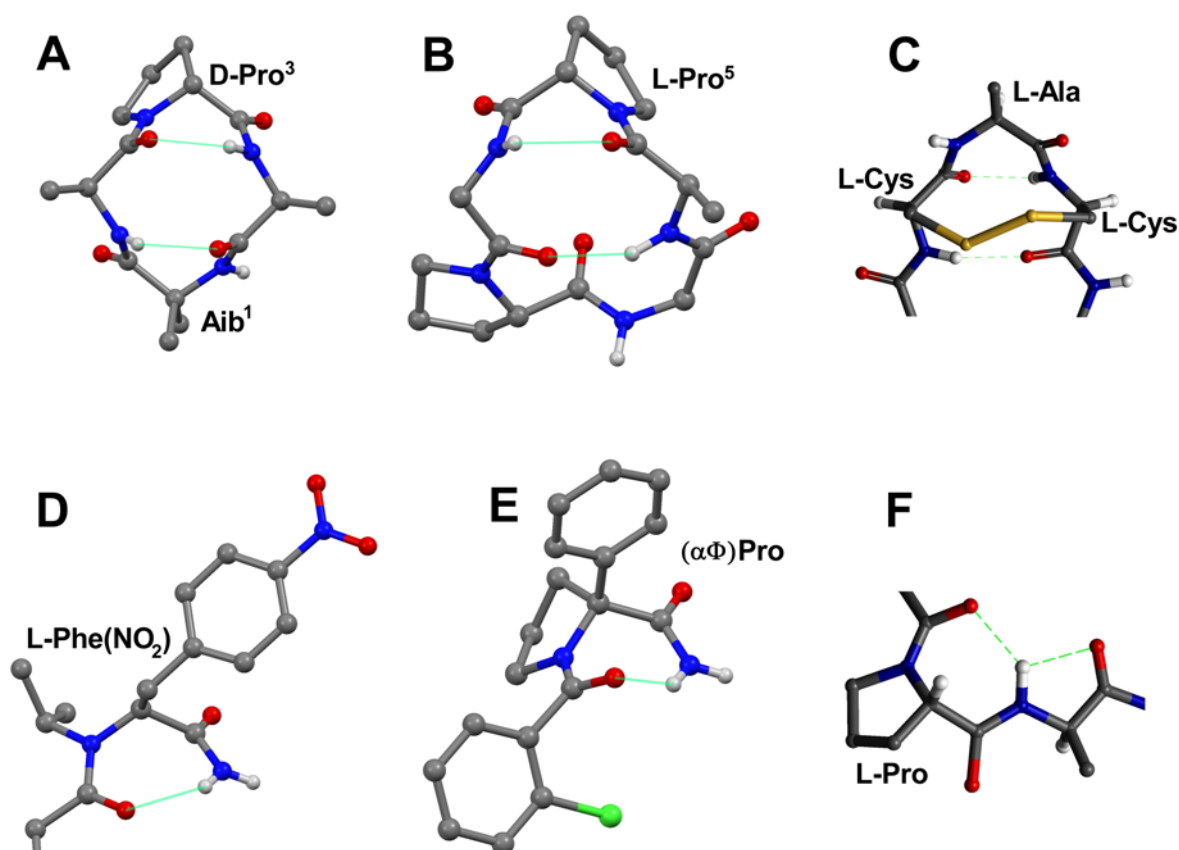


**Fig. 1** Three (A-C) different representations of a peptide main chain folded into and axial / classical  $\gamma$ - (or equatorial / inverse  $\gamma$ -) turn. The intramolecular H-bonds, which generate seven-membered *pseudo*-ring structures, are marked as dashed lines. The side-chain C <sup>$\beta$</sup>  atoms for the L-amino acids are labeled. In C, a  $\gamma$ -turn enclosed into a larger  $\epsilon$ -turn (C<sub>11</sub> form) is illustrated.

In this article, we will discuss only single and multiple  $\gamma$ -turns encompassing an  $\alpha$ -amino acid (therefore, C<sub>7</sub>-ring structures in *peptidomimetics* will not be treated). Emphasis will be given to  $\gamma$ -turns occurring in cyclic and linear peptides, and in globular proteins as well.  $\gamma$ -Turns, quite common (but not exclusive) in the conformationally forced, small-ring, *cyclo*-4- and *cyclo*-5-peptides, are presented first. In this connection, it is worth pointing out that two of the first unambiguous proofs for the existence of the intramolecularly H-bonded  $\gamma$ -turn were reported in the *crystal* state by Karle more than 35 years ago, for the *cyclo*-4-peptide dihydrochlamydocin and the *cyclo*-5-peptide *c*-(Gly-L-Pro-Gly-D-Ala-L-Pro), respectively. Interestingly, two, but *non-contiguous*,  $\gamma$ -turns are typically seen in *cyclo*-4-peptides, while only one (in addition to a  $\beta$ -turn) is

found in *cyclo-5*-peptides. In dihydrochlamydocin,<sup>27</sup> the four all-*transoid* peptide units are considerably less planar than those commonly observed in peptides (Fig. 2A). In particular, the  $\omega$  angle values for the  $\gamma$ -turn forming Aib<sup>1</sup> (Fig. 3) and D-Pro<sup>3</sup> residues are 162° and -156°, respectively. The Aib<sup>1</sup>  $\phi, \psi$  angles have values 72°, -65° and those for the inverse  $\gamma$ -turn forming D-Pro<sup>3</sup> of 82°, -73°. The N ... O distances are in the usual range, 2.82 and 2.94 Å, respectively. This *bis*  $\gamma$ -turn conformation was subsequently confirmed in solution by NMR investigations for chlamydocin.<sup>28,29</sup> The X-ray diffraction structure of the natural [Ala<sup>1</sup>] chlamydocin was recently published.<sup>30</sup> In the model *cyclo-5*-peptide,<sup>31</sup> the L-Pro<sup>5</sup> residue in the inverse  $\gamma$ -turn conformation exhibits -86°, 70° for its  $\phi, \psi$  angles ( $\omega = -160^\circ$ ) (Fig. 2B). The H ... O separation is 2.25 Å and the N-H ... O angle is near 121°. For this compound, the same 3D-structure was anticipated in a solution study by NMR spectroscopy.<sup>32</sup>

An additional, interesting example of an inverse  $\gamma$ -turn in a (much larger) *cyclic* peptide was found by X-ray diffraction in the bioactive *cyclo-11*-peptide cyclosporin A.<sup>33,34</sup> The residue involved is L-Ala<sup>7</sup> ( $\phi, \psi = -92^\circ, 64^\circ$ ;  $\omega = 166^\circ$ ). Other  $\gamma$ -turn conformations were established in model *cyclic* peptides of sizes ranging from 5 to 12 residues in the early days of NMR.<sup>35-38</sup> In *heterodetic cyclic* peptides, a stabilization of  $\gamma$ -turns was hypothesized (by NMR analysis) to be operative in solution by intramolecular (disulfide or carbon-carbon linkages) side-chain to side-chain bridging, *e.g.* in the -L-Cys-L-Ala-L-Cys- sequence<sup>39</sup> (Fig. 2C) or in the -L-Trp-L-Pro-L-Trp- sequence with the linker joining the indole C(2) atoms.<sup>40</sup>



**Fig. 2** X-Ray diffraction or proposed structures of  $\gamma$ -turn forming peptides or  $\alpha$ -amino acid derivatives. **(A)** The two  $\gamma$ -turns at the Aib<sup>1</sup> and D-Pro<sup>3</sup> residues of the *cyclo*-4-peptide dihydrochlamydocin. Most of the side-chain atoms of residues 2 and 4 are omitted for clarity (adapted from ref. 27). **(B)** The 3D-structure of the model *cyclo*-5-peptide (adapted from ref. 31), which shows the  $\gamma$ -turn (top) and the  $\beta$ -turn (bottom) forms. **(C)** The disulfide-stabilized  $\gamma$ -turn proposed for a cysteine-containing tripeptide (adapted from ref. 39). **(D)** The  $\gamma$ -turn exhibited by the N <sup>$\alpha$</sup> -alkylated derivative of Prp-L-Phe(NO<sub>2</sub>)-NH<sub>2</sub>. For clarity, only a part of the N <sup>$\alpha$</sup> -alkyl group is shown (adapted from ref. 51). **(E)** The  $\gamma$ -turn found in the  $\alpha$ -amino acid derivative (*o*-Cl)Bz-D,L-( $\alpha\Phi$ )Pro-NH<sub>2</sub> (adapted from ref. 52). **(F)** The intramolecularly bifurcated H-bonded (C<sub>7</sub>,C<sub>5</sub>) peptide conformation postulated for the -CO-L-Pro-D-Ala-sequence (adapted from refs. 57 and 58).

The first unambiguous evidence for a  $\gamma$ -turn was reported in a *globular protein* by Matthews as early as in 1972 using X-ray diffraction.<sup>23,24</sup> The sequence of interest in the thermostable enzyme

thermolysin is -Ser<sup>25</sup>-Thr<sup>26</sup>-Tyr<sup>27</sup>-. The side chains of the three residues are all exposed to solvent. Thr<sup>26</sup> is located at an apex of the molecule, at the end of its longest diagonal. Access of solvent to the classical  $\gamma$ -turn H-bond (N<sup>27</sup>-H<sup>27</sup> ... O<sup>25</sup>=C<sup>25</sup>) is partly protected by the Thr<sup>26</sup> and Tyr<sup>29</sup> side-chains. The Thr<sup>26</sup>  $\phi, \psi$  angles are 86°, -57°. In a careful statistical analysis of the occurrence of  $\gamma$ -turns in proteins, Milner-White and coworkers<sup>41,42</sup> unexpectedly found that a number of classic  $\gamma$ -turns do occur in proteins of known 3D-structure. Moreover, almost all  $\gamma$ -turns of this type are associated with main-chain reversal, at variance with inverse  $\gamma$ -turns which give rise predominantly to only a kink in the backbone.  $\gamma$ -Turns are often situated directly at either end of  $\alpha$ -helices or of  $\beta$ -sheet strands. They are well conserved during evolution and some are found at key positions. In globular proteins, the ratio  $\gamma$ -turns /  $\beta$ -turns is about 1:7. The H-bonds in  $\gamma$ -turns are generally weaker than most of other backbone ... backbone H-bonds. In a recent review article on sparsely populated peptide conformations in globular proteins, Balaram and coworkers<sup>43</sup> showed that in the completely “arid”, sterically allowed region in the top left quadrant of the Ramachandran map a few, distorted, inverse  $\gamma$ -turn examples occur in which the intramolecular H-bond is significantly lengthened. Energy compensations arise from local H-bond interactions involving either neighboring residues or water molecules of hydration. In addition, in the bottom right quadrant, although largely devoid of a sterically allowed region, a significant number of residues was found to populate the classical  $\gamma$ -turn conformation.

Due to the much higher conformational flexibility of *linear* peptides, the number of  $\gamma$ -turns authenticated in these compounds (and in the simpler  $\alpha$ -amino acid derivatives as well) is much more limited. A conformational search in the Cambridge Structural Database,<sup>44</sup> with the concomitant fulfillment of the criteria  $\phi = -50^\circ \div -90^\circ$ ,  $\psi = 50^\circ \div 90^\circ$  (or  $\phi = 50^\circ \div 90^\circ$ ,  $\psi = -50^\circ \div -90^\circ$ ), and N ... O distance 2.70  $\div$  3.30 Å, returned the X-ray diffraction structures of only two *short* and linear peptides based on *coded* residues, Boc-Gly-L-Ala-OH<sup>45,46</sup> (Boc, *tert*-butyloxycarbonyl) and Boc-L-Leu-D-Leu-Aib-L-Leu-D-Leu-OMe<sup>47</sup> (OMe, methoxy). In the 3D-structure of the V-shaped N <sup>$\alpha$</sup> -protected dipeptide, the Gly<sup>1</sup> residue shows  $\phi, \psi$  values of 85°, -55°. <sup>45,46</sup> The intramolecular H-bond is characterized by N ... O and H ... O separations of 3.03 and 2.29 Å, respectively. The N-H ... O angle is 138°. The terminally protected, heterochiral pentapeptide is folded into a distorted  $\beta$ -hairpin nucleated by a type-II'  $\beta$ -turn-like structure at the -D-Leu<sup>2</sup>-Aib<sup>3</sup>- sequence.<sup>47</sup> Interestingly, the L-Leu<sup>1</sup> residue exhibits  $\phi, \psi$  angles (-87.6°, 69.8°) typical for an inverse  $\gamma$ -turn. It is worth mentioning that in none of the two papers the  $\gamma$ -turn

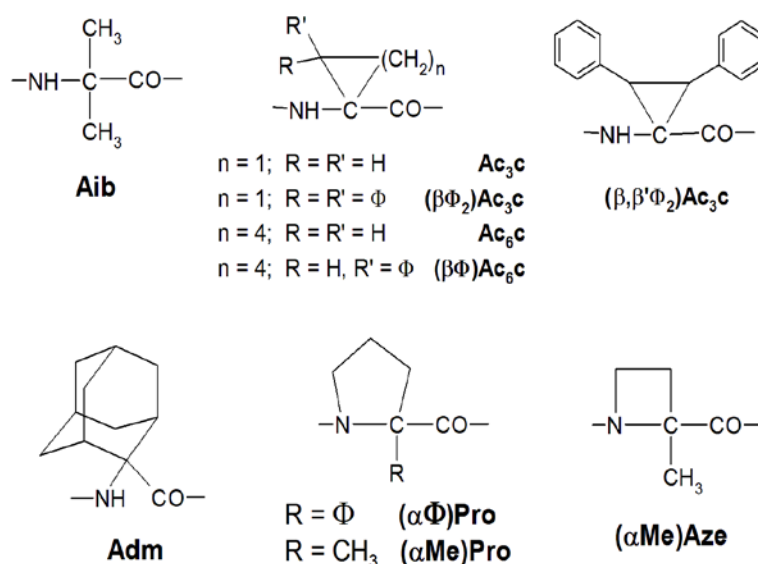


structural motif was explicitly discussed. Additional examples of *unreported*, likely candidates for the occurrence of an inverse  $\gamma$ -turn (identified through the same conformational search in the Cambridge Structural Database) in *longer* peptides are the Cys<sup>7</sup> residue of molecule II in the structure of octreotide,<sup>48</sup> a synthetic octapeptide, short analog of somatostatin, and the Asp<sup>2</sup> residue in six out of the eight independent molecules of the 14-residue, antimicrobial peptide mastoparan.<sup>49</sup> Moreover, the occurrence of a (distorted)  $\gamma$ -turn was emphasized in the X-ray diffraction structure of the terminally blocked dipeptide Piv-Pro-(2*R*,3*R*)-( $\beta,\beta'\Phi$ )Ac<sub>3</sub>C-NHiPr [Piv, pivaloyl; ( $\beta,\beta'\Phi$ )Ac<sub>3</sub>C, 1-amino-2,3-diphenylcyclopropane-1-carboxylic acid (Fig. 3); NHiPr, isopropylamino] at the level of the non-coded ( $\beta,\beta'\Phi$ )Ac<sub>3</sub>C residue.<sup>50</sup> The values of the N ... O and H ... O distances (3.25 Å and 2.47 Å, respectively) and of the N-H ... O angle (134°) are consistent with the occurrence of the intramolecularly H-bonded C<sub>7</sub> form despite the unusually low value of the  $\psi$  torsion angle ( $\phi,\psi = 86^\circ,-20^\circ$ ).

The  $\phi,\psi$  angles of a few  $\alpha$ -amino acid derivatives have values near those expected for a  $\gamma$ -turn conformation. In an N <sup>$\alpha$</sup> -alkylated derivative of Prp-L-Phe(NO<sub>2</sub>)-NH<sub>2</sub> [where Prp is propionyl and Phe(NO<sub>2</sub>) is *para*-nitrophenylalanine], an intramolecular H-bond forming a 7-membered *pseudo*-ring was reported which involves the primary amide N<sup>2</sup>-H as the donor and the Prp carbonyl oxygen as the acceptor (the N ... O distance is 2.74 Å and the N-H ... O angle is 139°)<sup>51</sup> (Fig. 2D). In three N <sup>$\alpha$</sup> -acylated C <sup>$\alpha$</sup> -phenylproline [( $\alpha\Phi$ )Pro] (Fig. 3) amide derivative racemates, a  $\gamma$ -turn conformation was observed.<sup>52,53</sup> They adopt a butterfly-like structure where the Pro residue is the “body” and the two aromatic moieties [of ( $\alpha\Phi$ )Pro and the benzoyl N <sup>$\alpha$</sup> -protecting group] are the “wings”. The 3D-structure of one of them, (*o*-Cl)Bz-D,L-( $\alpha\Phi$ )Pro-NH<sub>2</sub>, where (*o*-Cl)Bz is *ortho*-chlorobenzoyl, is shown in Fig. 2E. The intramolecular N ... O distances are in the range 2.73 ÷ 2.85 Å and the N-H ... O angle is between 146° and 155°. The ( $\alpha\Phi$ )Pro  $\phi,\psi$  angles are compatible with those expected for a  $\gamma$ -turn.

The occurrence of a  $\gamma$ -turn and its characteristic 1  $\leftarrow$  3 intramolecular C=O ... H-N H-bond *in solution* for linear peptides were proposed by several groups using mainly NMR, IR absorption, and CD measurements. For the earliest papers in this field, see refs. 4,54-62. However, because the general view among peptide spectroscopists is that, because of the severe competition with other turn conformations, at least in N <sup>$\alpha$</sup> -acylated dipeptide amides and longer compounds of this class, the  $\gamma$ -turn conformation “was invoked too readily to explain peptide spectra”.<sup>26</sup>  $\gamma$ -Turns were also considered in *conformational energy calculations* of model and naturally occurring peptides (see for example refs. 62-64). Again, however, they were often overemphasized, particularly in the

extensively investigated, simplest Ac-Xxx-NHMe (Ac, acetyl; Xxx, any  $\alpha$ -amino acid; NHMe, methylamino) compounds, where the only alternative intramolecularly H-bonded form is the fully-extended ( $C_5$ ) conformation.<sup>4,9,61,65</sup>



**Fig. 3** Chemical structures of the non-coded,  $C^\alpha$ -tetrasubstituted  $\alpha$ -amino acids discussed in this paper.

Moreover, the  $\gamma$ -turn motif may be further stabilized if it is part of an intramolecularly *bifurcated* H-bonded peptide conformation. A number of examples were reported in the literature, among the earliest of which Ac-L-Pro-D-Ala-OMe in an inert solvent<sup>57,58</sup> is of particular interest (Fig. 2F). The strong, intramolecularly bifurcated H-bond O(1) ... H(3) ... O(3) generates a  $C_7, C_5$  form, where the  $\gamma$ -turn involving L-Pro<sup>1</sup> is juxtaposed to a fully-extended form involving D-Ala<sup>2</sup>. The perturbation experienced by the H(3) atom results in a large bathochromic frequency shift for the N-H stretching band in the amide A region of the IR absorption spectrum and in a marked shift to lower fields for the corresponding proton signal in the NMR spectrum.

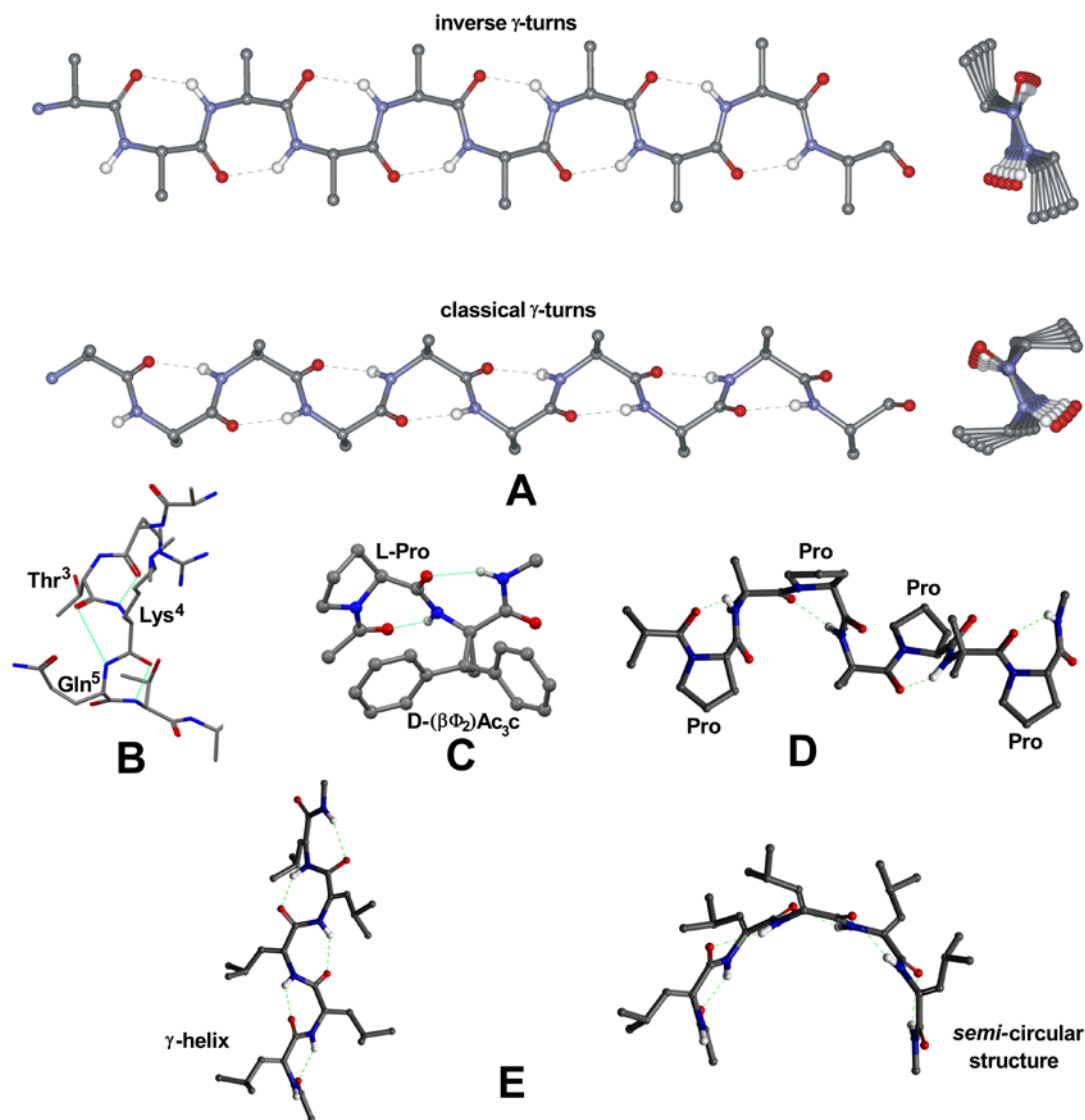
Finally, the *oxy analogs of the  $\gamma$ -turns*, which could represent an important feature for peptide chains at their C-end, the C-terminal -OH group of the carboxylic acid moiety in the uncommon *anti* disposition and the preceding amide carbonyl are involved in a seven-membered

*pseudo*-ring motif containing a somewhat bent C=O ... H-O H-bond.<sup>4</sup> As in the case of  $\gamma$ -turns, two oxy analogs (classical and inverse) could exist for all coded  $\alpha$ -amino acids except Gly. This 3D-structural motif was postulated by IR absorption experiments on simple derivatives, *e.g.* in Boc N $^{\alpha}$ -protected and Ac N $^{\alpha}$ -blocked  $\alpha$ -amino acids. However, despite a number of X-ray diffraction analyses performed so far on these compounds, no evidence for this oxy-analog form was validated.<sup>4,59</sup> Instead, the observed shift to lower wavenumbers of the urethane or amide carbonyl vibrator in the IR absorption spectrum should be related to the onset of an *intermolecular* H-bond.

### 3. Multiple $\gamma$ -turns

A series of two (or more) contiguous  $\gamma$ -turns generates a  $\gamma$ - (or 2.2 $\gamma$ -) helix.<sup>66,67</sup> The  $\gamma$ -helix is tighter and more elongated than the 3 $_{10}$ - and  $\alpha$ -helices.<sup>66,68</sup> Its number of amino acids per turn is 2.2 and its rise per residue is 2.75 Å. It is very important to note that this  $\gamma$ -helix should not be confused with the  $\gamma$ -helix proposed by Pauling and coworkers as early as in 1951 as a potential alternative to their classical  $\alpha$ - (or 3.6 $_{13}$ -) helix formulated in the same paper<sup>69</sup> but never experimentally authenticated.<sup>66,70</sup>

The presence of an incipient  $\gamma$ -helix was assessed in two globular proteins. In the stoichiometric complex with the enzyme porcine pancreatic elastase, its 57-amino acid residue long, specific inhibitor elafin exhibits in the crystal state a spiral shape which includes a severely distorted  $\gamma$ -turn ( $\phi, \psi = 50^\circ, -112^\circ$ ) centered at Gly<sup>40</sup>, immediately followed by an inverse  $\gamma$ -turn ( $\phi, \psi = -86^\circ, 69^\circ$ ) at Ile<sup>41</sup>.<sup>71</sup> An NMR study of a peptide segment from the MUC1 protein core showed an S-shaped backbone which encompasses two consecutive inverse  $\gamma$ -turn conformations (located in the -Asp-Thr- sequence) in the conformational equilibrium ensemble.<sup>72</sup> To our knowledge, the only  $\gamma$ -helix (with three consecutive  $\gamma$ -turns) published to date is that found by X-ray diffraction in the 3D-structure of the enzyme histone-H3, Lys-specific, demethylase in complex with a derivatized (21-mer) H3 peptide.<sup>73</sup> The -Thr<sup>3</sup>-Lys<sup>4</sup>-Gln<sup>5</sup>- residues of the H3 peptide bound to the enzyme adopt two classical  $\gamma$ -turns at Thr<sup>3</sup> ( $\phi, \psi = 65^\circ, -50^\circ$ ) and Lys<sup>4</sup> ( $\phi, \psi = 85^\circ, -69^\circ$ ), followed by an inverse  $\gamma$ -turn at Gln<sup>5</sup> ( $\phi, \psi = -64^\circ, 76^\circ$ ) (Fig. 4B), enabling an ideal side-chain spacing for its N-terminus into the enzyme anionic pocket. The enzyme-bound histone-H3 peptide is severely compressed and shows a compact, serpentine shape.



**Fig. 4** (A) Two views each for the series of consecutive inverse  $\gamma$ -turns (above) and classical  $\gamma$ -turns (below) which generate 2.2<sub>7</sub>-helices. (B) The 3D-structure of part of the histone derivatized H3 peptide (-Thr<sup>3</sup>-Lys<sup>4</sup>-Gln<sup>5</sup>-) bound to the Lys-specific demethylase enzyme (adapted from ref. 73). (C) The two consecutive  $\gamma$ -turns adopted by Ac-L-Pro-D-( $\beta\Phi_2$ )Ac<sub>3</sub>C-NHMe (adapted from ref. 74). (D) Perspective diagram of the lowest-energy conformation calculated for the sequential polypeptide -(Aib-L-Pro)<sub>n</sub>- with only the L-Pro residues folded in the  $\gamma$ -turns (adapted from ref. 79). (E) Molecular views of two stable conformations calculated for the Ac-( $\Delta^E$ Leu)<sub>5</sub>-NHMe homo-didehydro-pentapeptide: (left) the  $\gamma$ -helix (all residues adopt the inverse  $\gamma$ -turn conformation); (right): the *semi-circular* structure (the residues adopt alternate classic / inverse  $\gamma$ -turns) (adapted from ref. 83).

The only unequivocal example available in the literature of a multiple (double), consecutive  $\gamma$ -turn conformation in linear peptides encompasses the terminally-blocked -L-Pro-D-( $\beta\Phi_2$ )Ac<sub>3</sub>C-sequence (where the C <sup>$\alpha$</sup> -tetrasubstituted  $\alpha$ -amino acid, shown in Fig. 3, is 2,2-diphenyl-1-aminocyclopropane-1-carboxylic acid) (Fig. 4C).<sup>74</sup> In this heterochiral dipeptide the L-Pro<sup>1</sup>  $\phi, \psi$  angles are  $-82^\circ, 61^\circ$  (inverse  $\gamma$ -turn), while those of D-( $\beta\Phi_2$ )Ac<sub>3</sub>C are  $-72^\circ, 47^\circ$  (values typical for a slightly distorted classical  $\gamma$ -turn formed by a D-residue). Since this is the first report where a Pro residue is accommodated in a  $\gamma$ -turn in the crystal state in linear peptides,<sup>75</sup> it is evident that the observed turn disposition can be mostly ascribed to the presence of the heavily side-chain substituted Ac<sub>3</sub>C residue. This finding is not entirely surprising because many years ago it was clearly established that peptides rich in the ring unsubstituted Ac<sub>3</sub>C residue have a marked propensity for the “bridge” region of the  $\phi, \psi$  map ( $\psi \approx 0^\circ$ ),<sup>76,77</sup> not much separated from the region where the  $\gamma$ -turn conformation is found. In any case, as Pro lacks the H-bonding donor N-H group, main-chain elongation by repetition of this specific sequence will only result in a  $\gamma$ -turn ribbon spiral, not in an ideal, fully H-bonded, 2.2<sub>7</sub>-helix.

The basic unit of a foldameric compound, which incorporates an inverse  $\gamma$ -turn, was identified by crystallographic analysis of a terminally-protected dipeptide characterized by an L-Pro residue at position 1 ( $\phi, \psi = -87^\circ, 53^\circ$ ).<sup>78</sup> In the immediately longer sequential compound, the protected tetrapeptide, a periodic (non-contiguous)  $\gamma$ -turn motif was unveiled by an NMR investigation. Conformational energy calculations on an -(Aib-L-Pro)<sub>n</sub>- sequential oligopeptide show that one of the two stereochemically feasible, regular structure is a  $\gamma$ -turn ribbon spiral (non-contiguous  $\gamma$ -turns) with only the L-Pro residues adopting  $\phi, \psi$  angles ( $-85^\circ, 55^\circ$ ) compatible with an (inverse)  $\gamma$ -turn conformation (Fig. 4D).<sup>79</sup> Only C <sup>$\gamma$</sup> -endo puckered Pro residues can be accommodated in this structure. The Aib residues are folded in their privileged, helical conformation.<sup>9,68</sup> However, solution and crystal-state investigations on a variety of -(Aib-L-Pro)<sub>n</sub>- model oligomers clearly demonstrated that the alternative 3D-structure ( $\beta$ -turn ribbon spiral) is that preferentially adopted.<sup>80-82</sup>

A theoretical analysis of poly- $\alpha, \beta$ -didehydroleucine oligopeptides produced intriguing results.<sup>83</sup> Surprisingly, the homo-pentapeptide Ac-( $\Delta$ Leu)<sub>5</sub>-NHMe was found to be most stable in the side-chain *E*-configured isomer. Conformations with  $\phi, \psi$  values near  $-60^\circ, 30^\circ$  (or the enantiomeric  $60^\circ, -30^\circ$ ) for all residues achieve their stability from five consecutive C<sub>7</sub>-ring motifs, resulting in the formation of a  $\gamma$ -helix structure (Fig. 4E). Its rise per residue is 1.68 Å and the

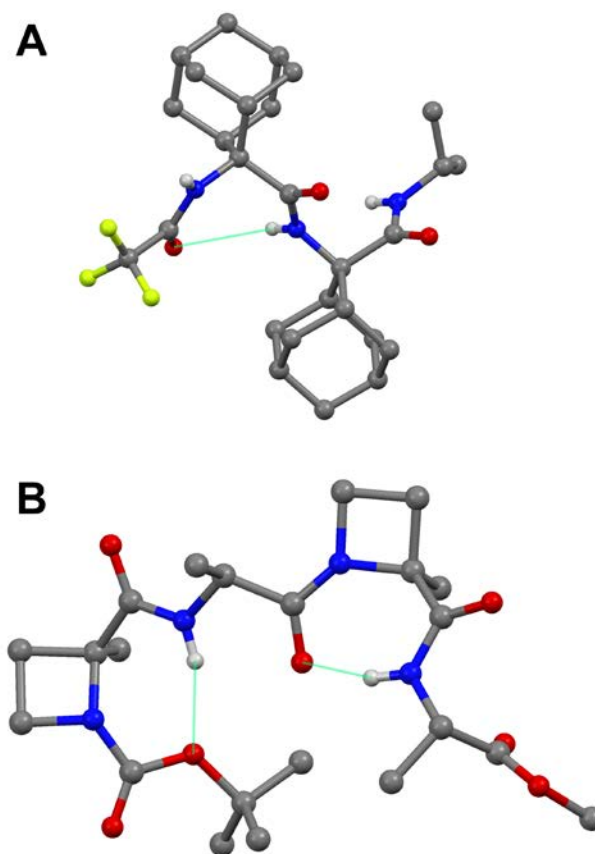
number of residues per turn is about 2.4. All  $\gamma$ -turns are either classical or inverse. Conversely, with alternate  $\phi, \psi$  values ( $60^\circ, -30^\circ$  followed by  $-60^\circ, 30^\circ$ ) the oligopeptide adopts a *semi*-circular disposition in which half of the alternate  $\Delta$ Leu carbonyls and side chains lie above the plane of the peptide backbone and the remaining half lie below the plane (Fig. 4E). In this 3D-structure each classical  $\gamma$ -turn is immediately followed by an inverse  $\gamma$ -turn, and *vice versa*. The stability of these conformations arise from the onset of the maximum number of intramolecular H-bonds.

#### 4. Ongoing $\gamma$ -helix research in our laboratories

In our groups we are currently actively working on linear peptides heavily based on a  $C^\alpha$ -cyclized,  $C^\alpha$ -tetrasubstituted  $\alpha$ -amino acid with a very bulky side chain and on the  $C^\alpha$ -methylated lower homolog of Pro. Our aim is to unambiguously demonstrate the onset of a fully developed (2.2<sub>7</sub>-)  $\gamma$ -helix peptide structure, which requires the occurrence of at least three contiguous  $\gamma$ -turns, and of the  $\gamma$ -turn ribbon spiral (with non-contiguous  $\gamma$ -turns), respectively. As stated above, only the former type was reported in the literature so far, but it was found in a globular protein,<sup>73</sup> not in a synthetic peptide.

The preferred conformations of the aforementioned  $C^\alpha$ -cyclized residue investigated in Padova are those of 2-aminoadamantane-2-carboxylic acid (Adm) (Fig. 3). The adamantane molecule consists of four fused cyclohexane rings. While theoretical and experimental studies on 1-aminocyclohexane-1-carboxylic acid (Ac<sub>6</sub>C) (Fig. 3) derivatives and dipeptides indicated that backbone torsion angles compatible with either the  $\gamma$ -turn or the  $\alpha$ -/<sub>3</sub>10-helical forms are privileged for this residue,<sup>84-86</sup> our solution and crystal-state studies on longer Ac<sub>6</sub>C homo-peptides clearly showed that the <sub>3</sub>10-helix is the best choice for this residue.<sup>86</sup> However, it was claimed that enhancing the bulkiness of the cyclohexane moiety [e.g., by introduction of a phenyl substituent at  $\beta$ -position, as in ( $\beta\Phi$ )Ac<sub>6</sub>C, Fig. 3], results in a significant stabilization of the  $\gamma$ -turn form.<sup>87</sup> Also, the Adm residue was already invoked as a  $\gamma$ -turn inducer on the basis of a theoretical analysis and NMR / IR absorption solution data on an amino acid derivative and a very short (di-) peptide.<sup>88</sup> Moreover, X-ray diffraction data on three RCO-Adm-OX (X = H, CH<sub>3</sub>) reported  $\phi$  values in the  $50^\circ \div 57^\circ$  range, characteristic of various turns (including  $\gamma$ -turn) and helical structures.<sup>89-91</sup> To date, in collaboration with C. Alemán (Polytechnic University of Catalunya, Barcelona, Spain), we theoretically examined, synthesized and solved the X-ray diffraction structures of a few amino acid derivatives and terminally protected dipeptides, including the homo-dipeptide Tfa-(Adm)<sub>2</sub>-NH<sub>i</sub>Pr

(Tfa, trifluoroacetyl) (Fig. 5A).<sup>92</sup> In all compounds (except in residue 2 of the homo-dipeptide), the Adm residue is folded in a (sometime slightly distorted)  $\gamma$ -turn motif. In conclusion, we confirmed on more sound bases the results of the previous report<sup>88</sup> that Adm is an extremely promising candidate for  $\gamma$ -turn formation, although an experimental evidence for  $\gamma$ -helix formation is still lacking. To this end, we are currently synthesizing longer, (hopefully crystalline) Adm homo-peptides.



**Fig. 5** X-Ray diffraction structures of (A) Tfa-(Adm)<sub>2</sub>-NH*i*Pr<sup>92</sup> and (B) Boc-[L-( $\alpha$ Me)Aze-L-Ala]<sub>2</sub>-OMe.<sup>102</sup>

On the other hand, in our ongoing search for the elusive  $\gamma$ -turn ribbon spiral, our two groups are also investigating the conformational tendency of 2-methyl-azetidine-2-carboxylic acid, ( $\alpha$ Me)Aze (Fig. 3), an additional C <sup>$\alpha$</sup> -tetrasubstituted  $\alpha$ -amino acid claimed to be an efficient  $\gamma$ -turn promoter on the basis of theoretical and solution spectroscopy studies.<sup>93-97</sup> Conversely, Madison *et*



*al.*'s<sup>98,99</sup> and our own<sup>100,101</sup> investigations in the crystalline state of ( $\alpha$ Me)Pro (Fig. 3) derivatives and terminally blocked dipeptide amides highlighted a well-defined propensity of this residue for the  $\alpha$ - $3_{10}$ -helix region of the  $\phi, \psi$  map (however, also in this case theory and solution measurements on the Ac- / -NHMe derivative were in favor of the  $\gamma$ -turn motif). Fig. 5B shows that the terminally-protected, sequential, homo-chiral tetrapeptide Boc-[L-( $\alpha$ Me)Aze-L-Ala]<sub>2</sub>-OMe is folded in a  $\gamma$ -turn ribbon spiral-like conformation.<sup>102</sup> Indeed, two intramolecularly H-bonded, non-contiguous  $\gamma$ -turn motifs are seen at the level of the ( $\alpha$ Me)Aze residues. However, two non-classical features characterize this structure: (i) the *cis* configuration of the Boc-L-( $\alpha$ Me)Aze urethane bond and (ii) the unusual acceptor of the N-terminal H-bond, namely the oxygen atom of the urethane *single* C-O bond instead of the expected C=O bond. In view of the non-canonical features of this structure, we are currently expanding our ( $\alpha$ Me)Aze peptide project to strictly related compounds where the Boc-urethane moiety is replaced by an amide bond. In Table 1 we summarized the  $\phi, \psi$  backbone torsion angles for the model peptides<sup>74,102</sup> known to adopt two  $\gamma$ -turn motifs in the crystal state.

## 5. Conclusions and perspectives

Our present literature survey has conclusively demonstrated that single and non-consecutive multiple  $\gamma$ -turns are common in *cyclo*-4- and *cyclo*-5-peptides. However, not enough attention has been given by structural biochemists to the occurrence of  $\gamma$ -turns in globular proteins, nor by synthetic peptide specialists, physical chemists, and crystallographers to the design, preparation, and spectroscopic / X-ray diffraction validation of appropriately designed linear peptides. In solution, peptide single and multiple  $\gamma$ -turn conformations have been thus far investigated almost exclusively in media of low polarity and at room temperature. To check the stability and possible cooperativity of these folding motifs, extension of these spectroscopic studies to different solvents and higher temperatures could be rewarding. We noted even less interest than that shown for this relevant class of peptide main-chain reversal to the discovery of very efficient, non-coded  $\alpha$ -amino acid  $\gamma$ -turn promoters which, when used for the synthesis of the related tri- or longer homo- and sequential oligopeptides, could provide compounds folded in the so far extremely poorly characterized 2.2<sub>7</sub>- ( $\gamma$ -) helix and  $\gamma$ -turn ribbon spiral motifs. It is quite clear that much experimental work remains to be performed in this specific area before a sufficiently deep knowledge can be achieved. Recent, promising developments in our laboratories point to that direction. In a short-time



perspective, this review article will hopefully stimulate peptide colleagues worldwide to devote their efforts to the identification and detailed characterization of these ordered secondary structures. If so, the “history of wild swings between over-use and complete obscurity” of the  $\gamma$ -turn turn conformation (quoted from J. S. Richardson et al.<sup>26</sup>) might come to an end.

**Table 1**  $\phi, \psi$  Backbone torsion angles ( $^\circ$ ) for the model peptides forming two  $\gamma$ -turns in the crystal state<sup>a</sup>

Peptide	Backbone torsion angles				Ref.
Ac-L-Pro-D-( $\beta\Phi_2$ )Ac <sub>3</sub> c-NHMe					74
	$\phi^1$	-82	$\phi^2$	-72	
	$\psi^1$	61	$\psi^2$	47	
Boc-[L-( $\alpha$ Me)Aze-L-Ala] <sub>2</sub> -OMe					102
	$\phi^1$	-97	$\phi^3$	-70	
	$\psi^1$	38	$\psi^3$	43	

<sup>a</sup>Amino acid residues are numbered from the N-terminus of the sequence.

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