Chemistry of Peptide Materials: Synthetic Aspects and 3D Structural Studies

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Acronyms

Aib	α-Aminoisobutyric acid or C^{α}-methylalanine
(αMe)Phe	C^{α}-methylphenylalanine
(αMe)Val	C^{α}-methylvaline
BOP	(Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium
CIP	2-Chloro-1,3-dimethylimidazolidium phosphate
Db_zg	$C^{\alpha,\alpha}$-dibenzylglycine
Deg	$C^{\alpha,\alpha}$-diethylglycine
Dϕg	$C^{\alpha,\alpha}$-diphenylglycine
Dp_ng	$C^{\alpha,\alpha}$-di-*n*-propylglycine
EDC	1-(3-Dimethylamino)propyl-3-ethylcarbodiimide
HATU	2-(1*H*-7-aza-1,2,3-benzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate
HBTU	2-(1*H*-1,2,3-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HOAt	7-Aza-1-hydroxy-1,2,3-benzotriazole
HOBt	1-Hydroxy-1,2,3-benzotriazole
HOSu	1-Hydroxysuccinimide
Iva	Isovaline or C^{α}-methyl-α-aminobutyric acid

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PyBOP	(Benzotriazol-1-yloxy)tripyrrolidinophosphonium
SPPS	Solid-phase peptide synthesis
TBTU	2-(1*H*-1,2,3-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate

1.1 Introduction

This chapter deals with α-amino acids and α-peptides only, as they are currently the most extensively exploited in the materials chemistry field. However, it is worth remembering that studies and applications of β-peptides (and γ- and δ- as well, although to a limited extent) are growing significantly.

In 1907 Emil Fischer published the chemical synthesis of the 18-mer peptide H–L-Leu–$(Gly)_3$–L-Leu–$(Gly)_3$–L-Leu–$(Gly)_8$–Gly–OH, which represents a milestone in the history of peptide chemistry [1]. After Fischer's remarkable achievement, it took almost 50 years for peptide chemists to synthesize a peptide longer than an octadecapeptide [2]. Only the introduction of the carbodiimide activating reagent in 1955 [3] and the SPPS technique in 1963 [4] ignited an impressive development. The chemical synthesis of peptides then became accessible even to researchers without a specific training in organic synthesis.

Currently, a variety of methods is available to accomplish the chemical synthesis of peptides. Among the numerous reference books [5–9] and review articles [10–16], it is worth recalling the comprehensive and detailed account contained in the five-volume Houben–Weyl series [17]; theoretical aspects and experimental procedures, both for protection and activation methods, are described in great detail. Therefore, in view of the ample literature and for the sake of brevity, Section 2.2 of this chapter will briefly describe only a few synthetic methods useful for the assembly of nonstandard peptides. Indeed, peptides designed for use in materials chemistry often contain sterically hindered, noncoded α-amino acids. The high number of protecting groups available will not be reviewed, as there is no need, in general, to use a specific protecting group when dealing with a difficult coupling.

Sections 2.3 to 2.5 will present the basic elements and parameters (amide bond and torsion angles) required to understand peptide conformations and an overview of the known, both common and unusual, peptide 3D structures.

With the aim at facilitating information retrieval, all sections are laid down in a schematic way.

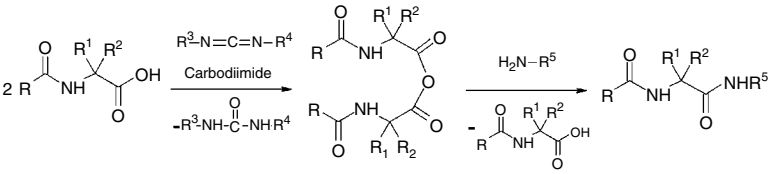
2.2 Synthesis of Difficult Peptide Sequences

Couplings involving noncoded, sterically hindered α-amino acids are often characterized by low yields even after long reaction times. This modest reactivity may lead to racemization of chiral C-activated α-amino acids possessing an H atom on their α-carbon. Racemization (or epimerization) is even easier when the activation involves a peptide segment, because of the high chance of 5(4*H*)-oxazolone formation [7, 9, 18]. Therefore, syntheses via segment condensation have to be planned carefully. In general, racemization-free activation methods should be employed also in the case of a stepwise main-chain elongation.

Among the variety of available coupling reagents, those reported hereafter were selected because of their effectiveness with sterically hindered α-amino acids. The procedures described can be applied both to solution and solid-supported syntheses.

Symmetrical anhydride

- The symmetrical anhydrides of α-amino acids are usually prepared *in situ* by treating an N^{α}-protected amino acid with a half-equivalent of carbodiimide. However, better results in the coupling reactions are obtained when the anhydride is isolated beforehand [16, 17]. A tertiary amine is added to neutralize the N^{α}-protected amino acid liberated during the anhydride aminolysis.

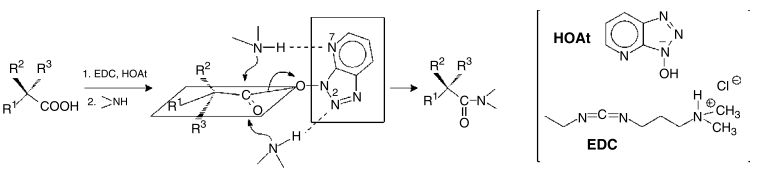


Symmetrical anhydride

- Peptide bond formation proceeds in satisfactory yields and low amounts of side products.
- When expensive amino acids are involved, the need for two equivalents of N^{α}-protected residues to form the symmetrical anhydride represents a serious drawback [14].

EDC/HOAt

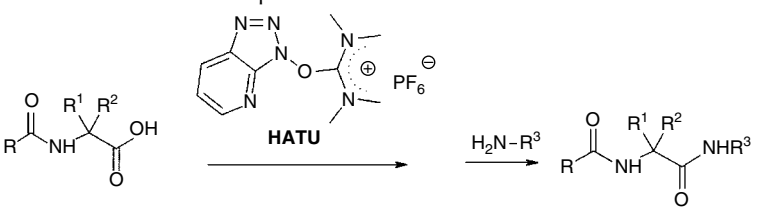
- The hydrosoluble carbodiimide EDC greatly facilitates the isolation and purification procedures when operating in solution. It mediates the formation of an active ester between the aminoacyl moiety and the HOAt [19] hydroxyl group.
- HOAt has a greater racemization suppression ability as compared to HOSu [20] and HOBt [21] and it is more effective in catalyzing peptide bond formation. For the latter reason, it is used even when racemization is not a concern. Through its N^2 and N^7 atoms, HOAt assists the (amine) nucleophilic attack on both faces of the planar active ester (this is the conformation that was observed in the crystal state [22, 23]) [19, 24, 25].



- An active ester with HOAt (or HOBt) forms also when the CIP/HOAt [26], BOP [27], PyBOP [28], HBTU [29], TBTU [30], and HATU [31, 32] activating reagents are used.

HATU

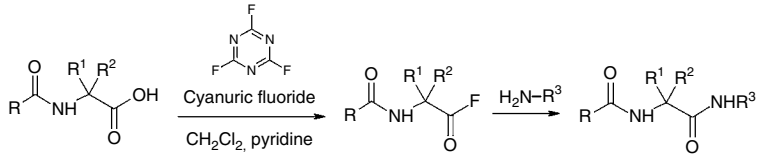
- Among the currently extensively used uronium salts, HATU is the reagent of choice in terms of coupling efficiency [33, 34] and amino acid derivative or peptide optical stability [35, 36]. It is often used in combination with one equivalent of HOAt.



- A major acylating species is believed be the active ester intermediate, as in the case of the EDC/HOAt activation procedure.
- In view of its simpler procedure, the use of HATU represents a good choice for the SPPS incorporation of sterically hindered residues.

α-Aminoacyl fluoride

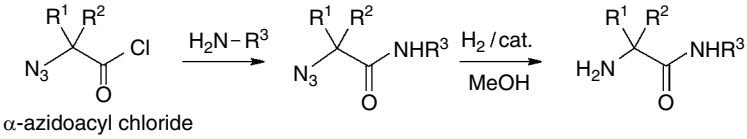
- The α-aminoacyl fluoride method [37, 38] is more effective than the EDC/HOAt and symmetrical anhydride procedures because of: (i) the small size of the leaving group (the fluoride ion), (ii) the activation of the carboxyl group induced by the high electronegativity of fluorine, and (iii) the slow rate of formation of the poorly reactive 5(4*H*)-oxazolone [38–40].
- N^{α}-protected α-aminoacyl fluorides are easily prepared by treating the corresponding carboxylic acids with cyanuric fluoride [37]. *In situ* preparation is also feasible [41]. However, better results are obtained with previously isolated α-aminoacyl fluorides.



- Remarkable achievements using this procedure are represented by the syntheses of the octapeptide –[(αMe)Val]$_8$– [42] and the heptapeptide –Iva-(αMe)Val–Iva–(αMe)Val–(αMe)Phe–(αMe)Val–Iva– [43].
- The α-aminoacyl fluoride activation method is also excellent for SPPS protocols: four Aib residues in a row were successfully linked [44], peptaibols were synthesized in high purity and yields [45], and unnatural, sterically hindered amino acids were easily incorporated [46]. Its coupling efficiency was shown to be superior to that of the easy-to-use HATU [39, 47]. In addition, couplings can be performed even in the absence of any base, thus avoiding premature deblocking of the Fmoc N-protecting group or the undesired 5(4*H*)-oxazolone formation [10].

α-Azidoacyl chlorides

- In recent years, the use of α-azidoacyl chlorides in the synthesis of difficult peptide sequences significantly increased [48–51]. These reagents are very efficient in peptide bond formation mainly because they cannot form the slowly reacting 5(4*H*)-oxazolone (because the azide group lacks a carbonyl moiety).
- The azide behaves as an N^{α}-protecting group: it can be easily converted into the free amine by catalytic hydrogenation.



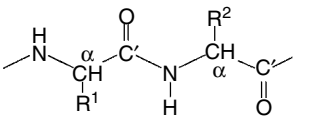
α-azidoacyl chloride

- This coupling procedure requires preparation of appropriate α-azido acid precursors and their subsequent conversion into α-azidoacyl chlorides. For this reason and for the care required in handling the potentially exploding azides, this activating method has not yet found a wider application. However, being probably the most effective among those illustrated here, it appears to be the method of choice for the formation of very difficult amide bonds.

1.3 Peptide (Amide) Bond

Where

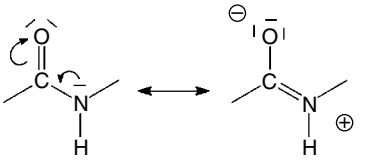
- Proteins and enzymes (and a variety of hormones and antibiotics as well) are naturally occurring polypeptide molecules.
- A dipeptide unit, formed by two α-amino acid building blocks, can be depicted as follows:



- N, $C^{\alpha}_{sp^3}$, C'_{sp^2}: three atoms per amino acid residue in the main chain (backbone).
- R^1, R^2 : side chains that differentiate the amino acids.
- According to a long-used convention [52, 53], the N-terminus of the main chain (residue 1) should be written on the far left side.

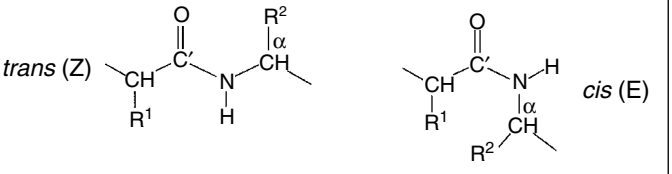
Electronic structure

Resonance of the *secondary* (–CO–NH–) peptide (amide) bond



Mean length of C'_{sp^2}–N bond in peptides: 1.34 Å (from X-ray diffraction analyses), intermediate between the lengths of C_{sp^3}–N (1.49 Å) and C_{sp^2}=N (1.27 Å) bonds. Therefore, the C'_{sp^2}–N bond in peptides has a partial double bond character.

Cis/trans isomerism

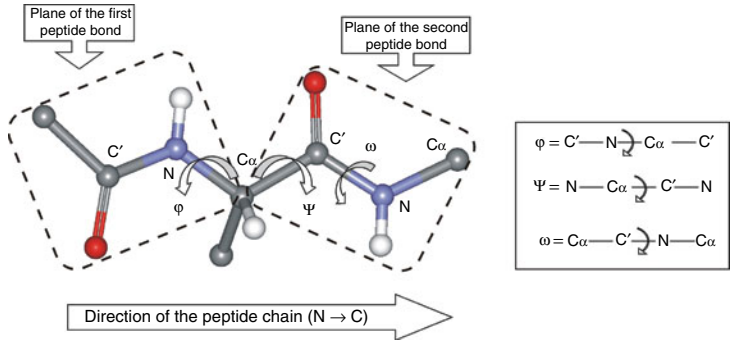


- In the *trans* isomer the $R^2 \cdots O$ repulsion plays a significant role.
- However, in the *cis* isomer the $R^1 \cdots R^2$ repulsion is even more severe.
- For secondary amides, the *trans* isomer is more stable than the *cis* isomer (by ~2 kcal/mole); the energy barrier between the two isomers (rotation about the C′–N bond) is ~18 kcal/mole.

1.4 Peptide Torsion Angles

Stereo-chemical definitions

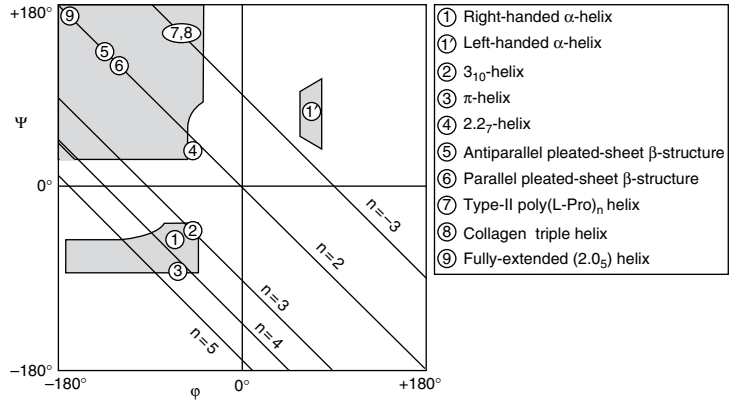
Peptide torsion angles (dihedral angles; angles between planes).



- In the fully extended (zig-zag) peptide conformation, depicted above, the torsion angles φ, ψ, ω are ±180° (all-*trans* conformation), according to the 1970 IUPAC-IUB Commission rules [54]. It is worth pointing out that all structural biochemistry textbooks are using this specific conformation for the illustration of a peptide chain, which, however, has been observed only extremely rarely in naturally occurring peptides and proteins (see below). Before 1970, the notation for φ, ψ, ω torsion angles was from 0° to 360°, as opposed to the current notation from –180° to 180°.
- The central α-amino acid is in the L-configuration (for almost all protein amino acids L ≅ *S*, according to the 1956 Cahn–Ingold–Prelog [55] rule and notation).
- As ≥ 95% of the (mostly secondary) peptide bonds are *trans* ($\omega = 180°$), the conformational problem can be satisfactorily simplified by describing exclusively the sets of φ, ψ torsion angles.
- If a set of φ, ψ torsion angles repeats itself almost identically along a peptide chain, then various types of helical structures may generate, the characteristic parameters of which are:
  - number of amino acid residues per helix turn (n);
  - axial translation (d), i.e. shift (in Å) along the helix axis per residue;
  - pitch (p), where $p = n \times d$, i.e. shift (in Å) along the helix axis per turn.

Ramachandran map

- The 1963 Ramachandran map [56, 57] was based on hard-sphere models.



- Subsequently, a variety of conformational energy computations largely confirmed this map.
- This (asymmetric) map is that typical of an L(*S*)-α-amino acid.
- The grey areas (15–20% of the total area) correspond to the *allowed* conformations, i.e. free from severe intramolecular steric interactions (based on van der Waals radii of atoms).
- The area of the allowed conformations for the only achiral protein amino acid (Gly, no R side chain) is much wider (~40% of the total area) and shows a symmetrical pattern with respect to φ, $\psi = 0°$.
- There are two φ, ψ maps for the only N-alkylated protein amino acid (Pro) as the Xxx–Pro *tertiary* peptide(amide) bond can rather easily accommodate in the unusual *cis* conformation ($\omega = 0°$) besides the common *trans* conformation ($\omega = 180°$).
- The largest population of allowed conformations is seen for φ values of about −60°, as this torsion angle permits the largest separation between the O atom (of the C=O group of the *i* residue) and the R side chain (in particular, the C^{β} atom) of the $i+1$ residue.
- Most of these helical structures are characterized by a *right*-handed screw sense, as they are positioned below and on the left side of the diagonal with $n = 2$ (where the 'flat', zig-zag helices are found). The only *left*-handed helices are the ($n = -3$) type-II poly-(L-Pro)$_n$ helix and the related collagen triple helix (see below), and the diastereomeric left-handed α-helix (7, 8 and 1′, respectively, in the figure).

1.5 Peptide Secondary Structures

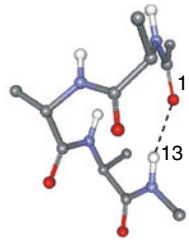
1.5.1 α-Helix

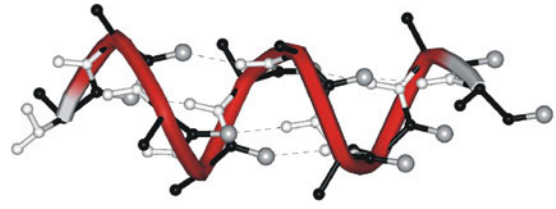
α-Helix: history

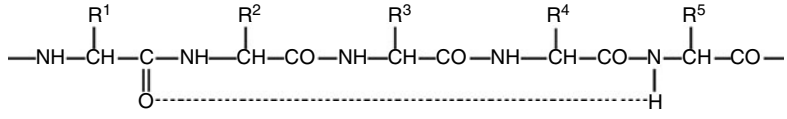
- The α-helix (3.6_{13}-helix in the 1950 Bragg–Kendrew–Perutz notation [58]) is the most abundant and stable ordered secondary structure in proteins.
- First proposed in 1950 by Pauling [59, 60], who used the 'bottom-up' approach.
- This helix is termed α because the Pauling proposal was in part based on the X-ray diffraction data of the fibrous protein α-keratin (from wool and hair) published by Atsbury in the 1930s [61], who, however, using the 'top-down' approach, failed to propose correct parameters for the α-helix.
- First experimentally authenticated using X-ray diffraction by Perutz in 1951 [62] and Kendrew in 1960 [63], who solved the crystal structures of the heme-containing, oxygen transporter, proteins hemoglobin and myoglobin, respectively.

α-Helix: structure

- α-Helices are stabilized by intramolecular, backbone···backbone C=O···H–N H-bonds involving 13 atoms (C_{13} form or α-turn). The helical-type α-turn is one of the various pseudo-cyclic forms, first studied in detail by Pavone [64]. It encompasses entirely three amino acid residues (those with the R^2, R^3, and R^4 side chains). The H bond is of the $1 \leftarrow 5$ type. All –CO–NH– bonds are in the *trans* conformation.



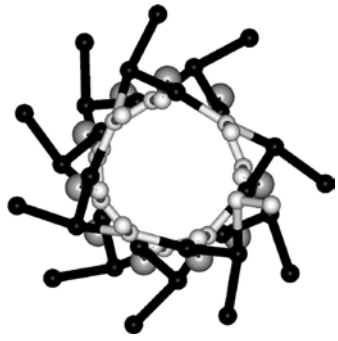




- The (right-handed) α-helical parameters (from the most recent statistical analysis of X-ray diffraction structures at atomic resolution of oligopeptides, published by Toniolo and Benedetti in 1991 [65]) are as follows:

$$\varphi = -63°,\ \psi = -42°,\ n = 3.63,\ d = 1.56\,\text{Å},\ p = 5.67\,\text{Å}$$

- It is worth pointing out that the α-helix is *not* characterized by an integer number of amino acids per turn (3.6). This is why Pauling had to fight against the general view held by structural biochemists in the early 1950s to make his proposal accepted by the scientific community (at that time, only polypeptide helices with an integer number of amino acids per turn were considered stable enough).
- A (right-handed) α-helical model of a decapeptide is viewed along the helix axis (the side chains are not eclipsed, but rather they are significantly staggered).

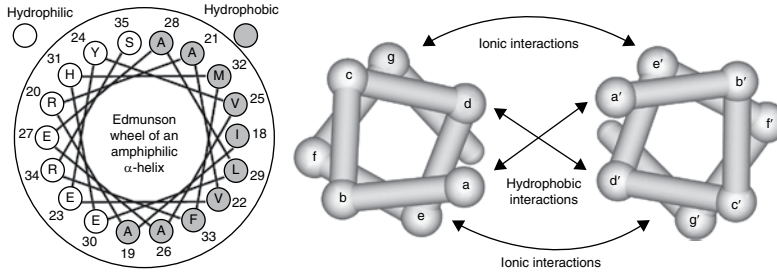


α-Helix: promoting residues

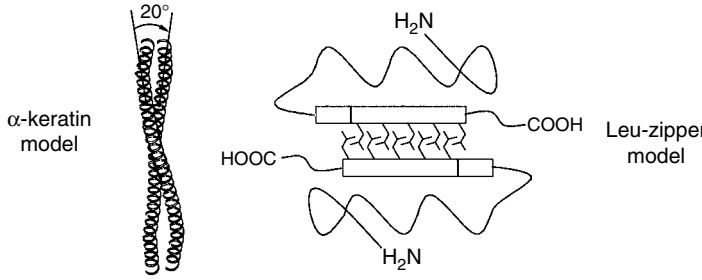
- Several α-amino acids are considered particularly effective α-helix promoters (Blout's classification, 1962 [66]): Ala, Leu, Glu, Lys, Met, Phe, Tyr (their side chains are either linear or γ-branched).
- L-Asp and L-Asn are also helicogenic, although moderately. However, due to their (intramolecular dipole···dipole and H-bonding) side-chain to main-chain interactions, they are frequently found in (*diastereomeric*) *left*-handed α-helical segments (helix 1′ in the Ramachandran map).

α-Helix: aggregates

- Since in the α-helix there are *~3.5 amino acids per turn*, the smallest integer number characterizing this helix is *7*, which requires two complete α-helical turns. This is the reason why the biologically relevant, amphiphilic (or amphipathic) helices (with one face hydrophobic and the other face hydrophilic) are characterized by *heptad* (*a*, *b*, *c*, *d*, *e*, *f*, *g*) repeats of amino acids, with analogous physicochemical properties at specific positions in the heptad (e.g. in aqueous solutions positions *a* and *d* require hydrophobic residues for antiparallel dimer formation; the hydrophilic positions *e* and *g*, immediately on the back, reinforce dimer stability via ionic interactions) [67].
- Membrane-active, antibacterial peptides typically fold into amphiphilic α-helices [68].



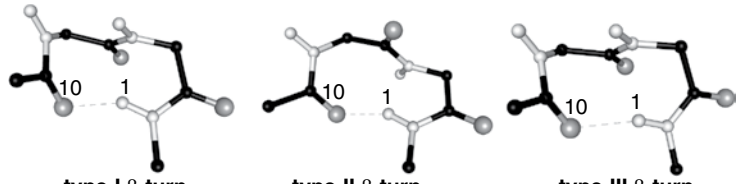
- In the case of α-keratin, in 1952 Crick [69] first suggested a self-association of two α-helices (termed a 'coiled coil' dimer) with an angle between their axes of about 20° and a 'knob-into-holes' packing mode of their side chains (for Leu-rich helices, in 1988 this motif was termed 'Leu zipper' by McNight [70]).



1.5.2 3_{10}-Helix

3_{10}-Helix: history

- First proposed by Taylor in 1941 [71], well before the classical α-helix (this is because the 3_{10}-helix is characterized by an *integer* number (3) of amino acids per turn).
- The (right-handed) type-III β-turn (C_{10} form), according to the 1968 Venkatachalam classification [72], is the building block for the right-handed 3_{10}-helix. Type-I and type-II β-turns, initially called β as they characterize the cross-β structure (see below), were first proposed by Geddes *et al.* in 1968 [73] who termed them type-A and type-B, respectively. A few months later, Venkatachalam [72] studied in detail type-I to type-III β-turns. Other types of β-turns were discussed later on [74].



	type-I β-turn	type-II β-turn	type-III β-turn
φ_2, Ψ_2	–60°, –30°	–60°, 120°	–60°, –30°
φ_3, Ψ_3	–90°, –0°	80°, 0°	–60°, –30°

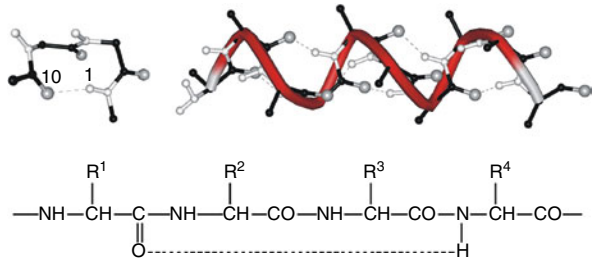
- The 3_{10}-helix was first experimentally authenticated by Balaram in 1978 [75] by X-ray diffraction analysis of a model, terminally protected, homo-pentapeptide from Aib (α-aminoisobutyric acid), namely Tos–$(Aib)_5$–OMe, where the acceptor of the N-terminal intramolecular H-bond is one of the two oxygen atoms of the *para*-toluenesulfonamide (Tos–NH–) group. The critical main-chain length for 3_{10}-helix formation for a terminally protected $(Aib)_n$ homo-peptide series was found to be $n = 3$ (Benedetti and Toniolo in 1982 [76]).
- 3_{10}-Helical residues represent about 10% of all helical residues in globular proteins [77]. The majority of the 3_{10}-helices are short (3–4 residues) and are mostly located either at the N-terminus or at the C-terminus ('extensions') of α-helices, but some of them have been identified with a length of 7–12 residues. 3_{10}-Helices have been proposed as intermediates in the process of folding of α-helices in globular proteins [78].

3_{10}-Helix: structure

- The (right-handed) 3_{10}-helical parameters (from the Toniolo and Benedetti statistical analysis [65]) are as follows:

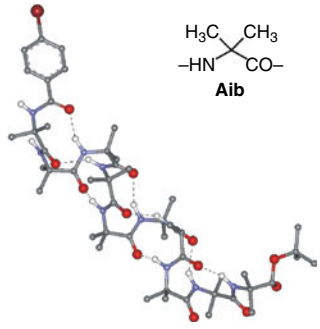
$$\varphi = -57°,\ \psi = -30°,\ \ n = 3.24,\ \ d = 1.94\,\text{Å},\ \ p = 6.29\,\text{Å}$$

- This helix is more elongated and less wide as compared to the α-helix. It is also less stable, since the lengths/angles of the intramolecular C=O···H–N H-bonds and the nonbonded steric interactions are less favorable. Remarkably, the experimentally found *n* value (3.24) is not an integer number. It is evident that this helix gains stability from a slight staggering of its side chains (almost one on top of the other after a complete helix turn).
- As mentioned above, 3_{10}-helices are stabilized by intramolecular, backbone···backbone, C=O···H–N H-bonds including 10 atoms (C_{10} form or β-turn). The helical-type (type-III) β-turn is one of the various pseudo-cyclic forms that encompasses entirely two amino acid residues (those with the R^2 and R^3 side chains). The H-bond is of the $1 \leftarrow 4$ type. All –CO–NH– amide bonds are in the *trans* conformation.



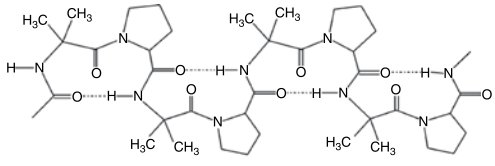
3_{10}-Helix promoting residues

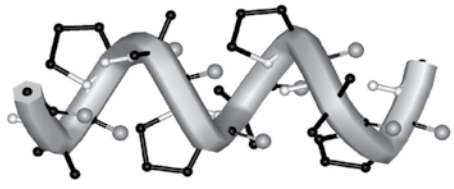
- Because of the Thorpe–Ingold (*gem*-dimethyl) effect [79], Aib is strongly helicogenic and imparts an extremely high crystallinity to its peptides. It also characterizes a family of naturally occurring, membrane-active, peptide antibiotics called 'peptaibols' (Benedetti and Toniolo [80]) or 'peptaibiotics' (Toniolo and Brückner [81]). Upon self-association, some of them form ion-conducting channels in the membranes.
- The right-/left-handed 3_{10}-helical structures of the achiral $(Aib)_n$ ($n = 10, 11$) homo-oligomers have been reported (Benedetti and Toniolo [82], Gessman *et al*. [83]). These are the longest 3_{10}-helices and the longest homo-peptides from any amino acid, the 3D structures of which have been solved by X-ray diffraction (at atomic resolution). All of the C^{α}-methylated α-amino acids investigated tend to support the 3_{10}-helical structure [79].



β-Bend ribbon spiral

- A variant of the 3_{10}-helix is the β-bend ribbon spiral, generated by an alternation of a Pro residue (lacking the H-bonding donor NH group) and a strongly helicogenic residue (Aib).
- First observed by Karle and Balaram in 1987 [84] in their X-ray diffraction analysis of the peptaibol zervamycin, it was characterized in detail by X-ray diffraction in several $(Aib–Pro)_n$ model peptides in 1992 (Benedetti and Toniolo [85]).

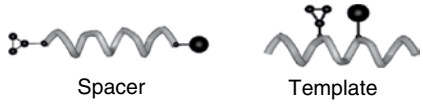




- All Aib–Pro bonds are *trans*. The Pro–Aib bonds deviate markedly ($|\Delta\omega| > 10°$) from the planar *trans* value (180°).

3_{10}-Helix: spacer and template

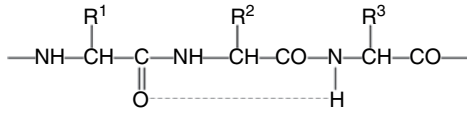
- Amphiphilic 3_{10}-helices can be easily prepared by taking advantage of appropriate amino acid side chains. In view of their almost threefold repeating units, they are even more suitable than α-helices as spacers (bridges) [86] or templates [87] for studies in various areas of chemistry.



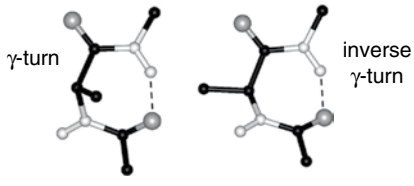
1.5.3 2.2_7-Helix

γ-Turns

- There are two γ-turn (C_7) conformations for an L-amino acid residue. Both pseudo-cyclic forms include 7 atoms and encompass entirely one amino acid residue (that with the R^2 side chain). The intramolecular H-bond is of the 1←3 type. The internal –CO–NH– amide bond is in the *trans* conformation. The intramolecular H-bond is strongly bent. The ω torsion angles deviate somewhat from the planar *trans* (180°) value. The first proposal and conformational energy computations were published by Némethy and Printz in 1972 [88].
- These types of turns are rare in linear peptides. However, they are quite common in the conformationally forced, small ring, *cyclo*-4- and *cyclo*-5-peptides. In globular proteins the ratio γ-turns/β-turns is ~ 1:7 (the first γ-turn in a globular protein, thermolysin, where the central (R^2) residue is Thr, was reported by Matthews in 1972 [89]).

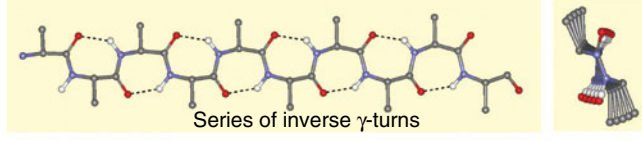


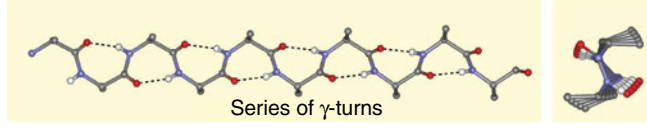
- For an L-residue, the two types of turns are called:
  (I) γ-turn (φ, ψ = 70°, –70°) less stable; side-chain R: *axial*,
  (II) inverse γ-turn (φ, ψ = –70°, 70°) more stable; side-chain R: *equatorial*.



2.2_7-Helices (γ-helices)

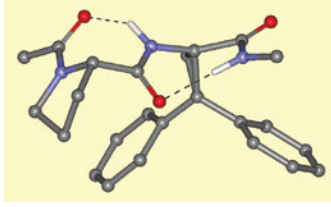
- Two or more consecutive γ-turns generate a 2.2_7(γ)-helix. This helix is tighter and more elongated than the 3_{10}-helix. Its rise per residue (the *d* parameter) is ~ 2.80 Å.

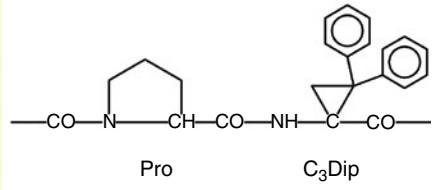




2.2$_7$-Helix: examples

- Two consecutive inverse γ-turns (the incipient 2.2$_7$-helix) have been reported in model peptides for the first time by Cativiela in 2005 [90] for the dipeptide heterochiral sequence –L-Pro–D–c_3Dip–. This sequence will only form a γ-bend ribbon spiral (because Pro lacks the H-bonding donor NH group), not an ideal 2.2$_7$-helix. In any case, this heavily side-chain substituted C^{α}-cyclopropyl amino acid is a promising tool for the construction of the 2.2$_7$-helix.





- In globular proteins two examples are known: (i) a repetive γ-turn segment formed by the two residues –Gly–Ile– (a distorted γ-turn followed by a regular inverse γ-turn) [91] and (ii) the serpentine shape of three consecutive, inverse γ-turns formed by the –Thr–Lys–Gln– stretch [92]. In any case, it is quite evident that much work remains to be performed in this specific area of ordered peptide secondary structures.

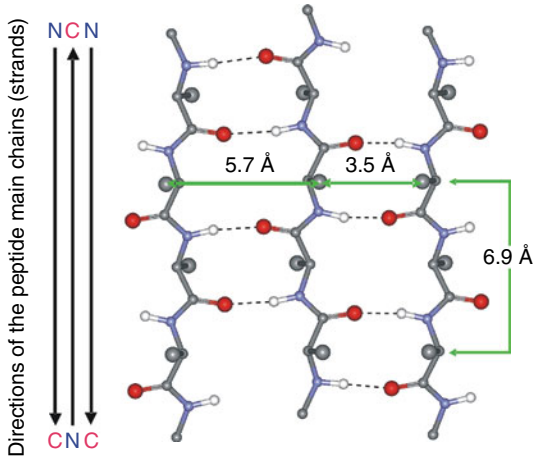
1.5.4 Pleated-Sheet β-Structures

β-Sheets: history

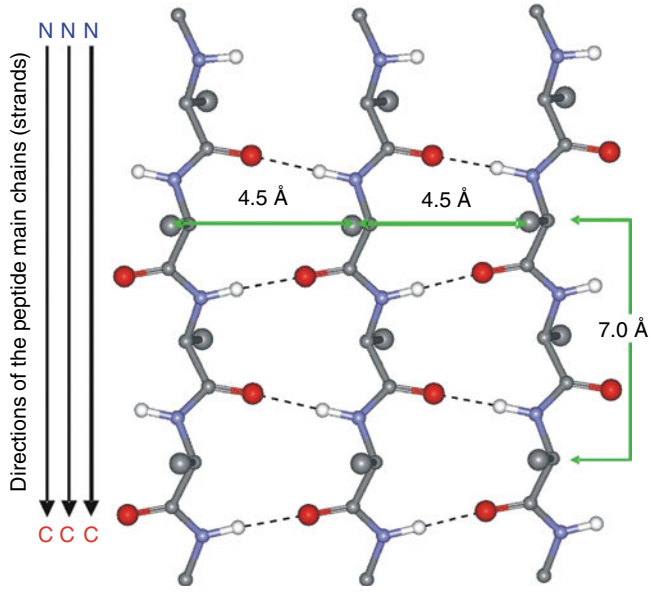
- The pleated-sheet β-structure is the second most common type of ordered secondary structure in *globular* proteins.
- Both types [*parallel* (β||)- or *antiparallel* (β⊥)-chains] of pleated-sheet β-structures have been proposed by Pauling and Corey in 1951 [93].
- The *pleated*-sheet β-structures (where each residue is extended) are more stable than the *flat*-sheet β-structures (where each residue is *fully* extended, $\varphi = \psi = 180°$) (see below), because in the former less unfavorable intra- and interresidue(s) nonbonded interactions are operative.

β-Sheets: structures

- The *antiparallel*-chain β-sheet structure is more stable (and common) than its parallel-chain counterpart since the directionality of its interchain H-bonds is optimal.



- In the *parallel*-chain β-sheet structure the distances between the C^{β} atoms of the side chains (R) of the residues in register and in adjacent strands repeat themselves identically (~ 4.5 Å). In contrast, in the antiparallel-chain β-sheet structure these same distances strictly alternate between a longer distance (~ 5.7 Å) and a shorter distance (~ 3.5 Å). The short (~ 3.5 Å) distance in the antiparallel-chain β-sheet structure well explains the observation that severely sterically hindered amino acids (e.g. the β-branched Val and Ile) strongly prefer the parallel-chain β-sheet structure (Toniolo in 1978 [94]).

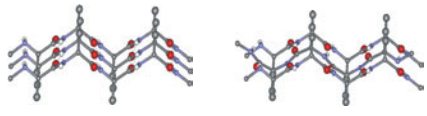


β-Sheets: parameters

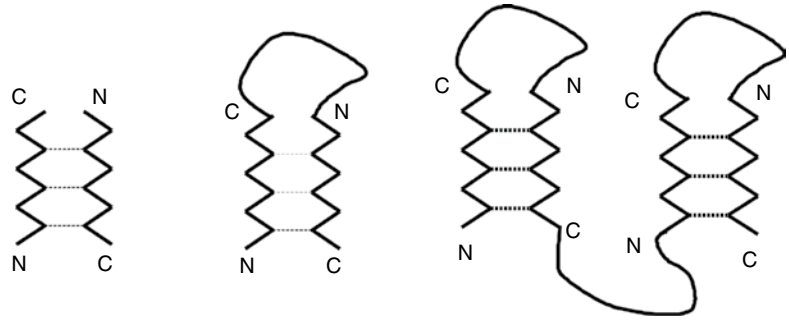
- In both types of β-sheet structures the side chains of successive residues alternate (up/down) with respect to the average plane of the main chain. Thus, if the polar/apolar characters of the side chains alternate as well, then *amphiphilic* β-sheet structures may originate [95].
- The helical parameters for the two types of pleated-sheet β-structures are close [95]:

  Antiparallel: $\varphi = -139°$, $\psi = 135°$, $n = 2.00$, $d = 3.47$ Å, $p = 6.94$ Å
  Parallel: $\varphi = -119°$, $\psi = 113°$, $n = 2.00$, $d = 3.50$ Å, $p = 7.00$ Å

- Due to their integer number of amino acids per turn ($n = 2$), both types of β-sheet structures lie on the corresponding diagonal of the Ramachandran map. The differences of their φ, ψ torsion angles from those of the fully extended structure (see below) explain their slightly wavy appearance.



- The pleated-sheet β-structures can be either of the *intra*- or *inter*-molecular type. For example, they can be of the antiparallel-chain type.



Two strands β-hairpin "Cross-β" or "β-meander"

β-Sheets: promoting residues

- According to the Blout classification (1962) [66], non α-helix forming and, as a consequence, effective β-sheet structure-forming amino acid residues are those with sterically demanding, β-branched, side chains (i.e. Val, Ile, and Thr) and those that can form side-chain to main-chain H-bonds (i.e. Ser, Thr, Cys). While Pro is a β-sheet structure breaker, one of the preferred structures for Gly is the antiparallel-chain β-sheet structure.
- The β-sheet structure does also occur in *fibrous* proteins. A well-known case is that of fibroin [96], the protein characterizing the *Bombix mori* silk, which is rich not only in Gly and Ala but in Ser as well, and largely adopts an amphiphilic antiparallel-chain β-sheet structure (the word *serine* comes from the Greek term 'seros', which means *silk*).

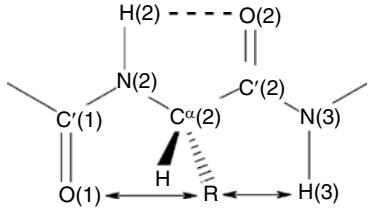
β-Sheets: effects

- Due to their extremely poor solubility, β-sheet structures exhibit low reactivity in peptide bond formation ('difficult sequences'; Mutter and Toniolo [97]).
- More importantly, the β-sheet structures are responsible for the onset of a variety of neurodegenerative 'conformational diseases', such as those characterized by fibril formation and amyloid deposits (Alzheimer, prion, Parkinson, Huntington, Machado ataxia, dementia with Lewy bodies, kuru, Creutzfeld–Jakob, bovine spongiform encephalopathy or 'mad-cow' diseases).

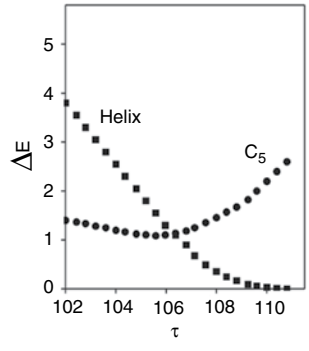
1.5.5 2.0_5-Helix

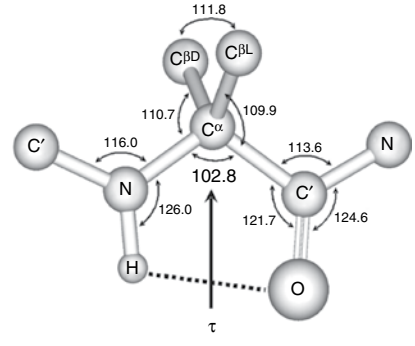
2.0_5-Helix: structure

- The fully extended peptide conformation, or 2.0_5-helix, with $\varphi = \psi = 180°$, was proposed at an early stage in structural studies of proteins [98]. The repeating motif of the fully extended peptide conformation is the intramolecularly H-bonded form depicted in the figure. The relative disposition of the two dipoles, N–H and C=O, is such that there is obviously some interaction between them. Since these four atoms, together with the central C^{α}-atom, are involved in a pentagonal pseudo-cyclic structure, this conformation is also called the C_5 structure [99].



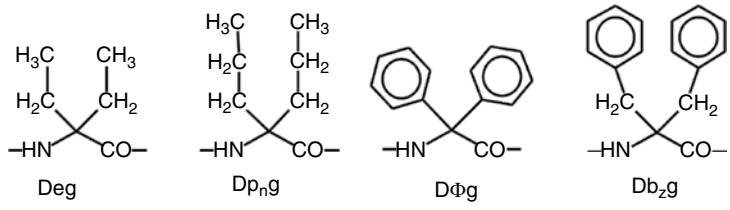
- This type ($i \rightarrow i$) of intramolecular H-bond is the only one among those mentioned in this chapter where the N–H donor group *precedes* the C=O acceptor group in the sequence. All other intramolecular H-bonds are of the $i \leftarrow i+n$ type. In other words, in this helix the usual C=O···H–N H-bond direction has become N–H···O=C.
- The influence of the bulkiness of the lateral substituent can easily be explained by considering the intramolecular nonbonded interactions between the side-chain group R and the preceding C=O and following N–H groups, which induce a warping of this nonsymmetrical structure.
- The bond angles internal to the pentagonal ring are smaller, while those including atoms of the main chain (external to the ring system) are larger than the corresponding average bond angles observed in peptides.
- The intramolecular $N_i \cdots O_i$ distance (2.54 Å) in the H-bonded, fully extended, peptides is much shorter than the corresponding distance usually observed in helical peptides (2.8–3.0 Å) [100, 101].





- Interestingly, the critical sp^3 N–C^{α}–C′ bond angle (τ) is dramatically narrowed (from 109.5° to less than 103°).
- From calculations it turns out that the energy ΔE (kcal/mol) of the C_5 conformation of the Ac–Deg–NHMe (Ac, acetyl; NHMe, methylamino) derivative becomes lower than that of the helical conformation when the bond angle τ is < 107° [102].

2.0$_5$-Helix: promoting residues



- The highly crystalline nature of peptides rich in the *achiral* C^{α}-tetrasubstituted residues shown above (with two side chains identical and longer than methyls) was exploited for extensive X-ray diffraction analyses [100, 101]. Multiple C_5 conformations are a common observation for these achiral peptides in the crystal state.
  Interestingly:
  (i) The Dp$_n$g and Deg homo-peptides represent the first examples (Toniolo in 1984 [103] and 1988 [102], respectively) in which consecutive C_5 forms (2.0$_5$-helices) have been experimentally observed.

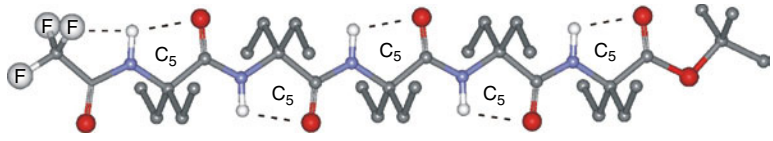
(ii) The N–H and C=O groups characterizing this intramolecularly H-bonded structure are not involved in the intermolecular H-bonding scheme.
(iii) The amino acid side chains of Deg and Dp$_n$g tend to be fully extended to relieve the unfavorable intramolecular side chain-to-main chain and side chain-to-side chain interactions.
(iv) The axial translation per residue in this helix is ≈ 3.70 Å, the longest possible for a single amino acid, which makes this conformation extremely attractive for its use as a spacer [86, 104].

2.0$_5$-Helix: natural occurrence

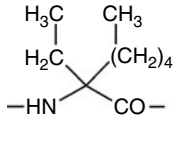
- For coded amino acids, unequivocal verification of the occurrence of the C_5 form has been obtained in the crystal state by X-ray diffraction analyses of a few, favorable compounds, i.e. Gly- and Ala-rich peptides with short side chains [98, 105].
- In globular proteins a repeating C_5 motif has been so far authenticated only in the X-ray diffraction analysis of the –(Gly)$_4$– sequence of His–*t*RNA–synthetase [106].

2.0$_5$-Helix: examples

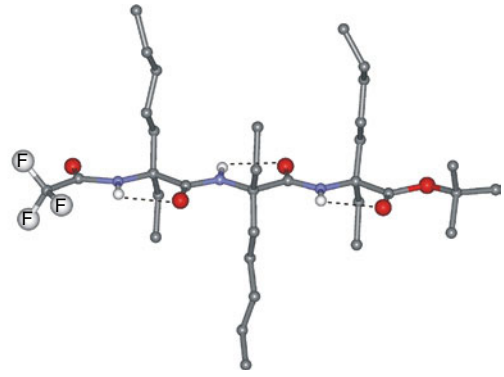
- The X-ray diffraction structure of the 2.0$_5$-helical, achiral, homo-pentapeptide Tfa–(Deg)$_5$–O*t*Bu (Tfa, trifluoroacetyl) shows five consecutive N–H···O=C intramolecular H-bonds, each giving rise to a C_5 form [102]. This is the longest 2.0$_5$-helix published to date.



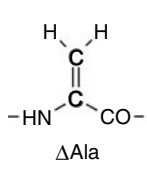
- In recent years, Imawaka, Tanaka, and Suemune (2000) [107] and Crisma *et al.* (2011) [108] clearly demonstrated that even for homo-peptides based on C$^\alpha$-tetrasubstituted *chiral* α-amino acids the 2.0$_5$-helix is a common observation, provided that both amino acid side chains are longer than a methyl group (e.g. any C$^\alpha$-ethylated protein amino acid, those from except Gly and Ala). As an example, the X-ray diffraction structure of the 2.0$_5$-helical, Tfa/O*t*Bu protected, chiral homo-tripeptide based on C$^\alpha$-ethyl, C$^\alpha$-*n*-pentylglycine (Epg) has been reported [108]. Three, consecutive N–H···O=C intramolecular H-bonds, each generating a C_5 form, are observed.

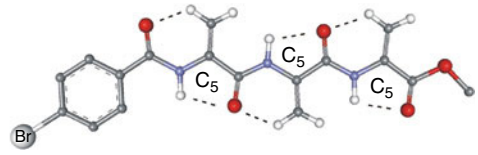


Epg



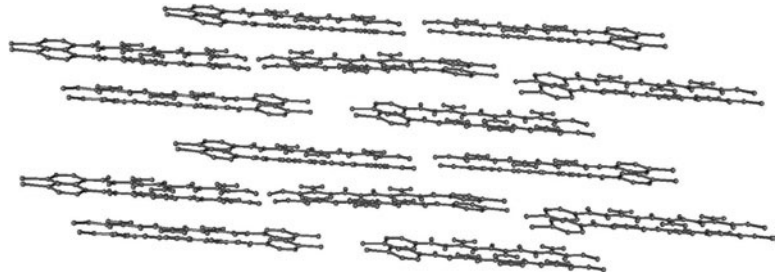
- Moreover, homo-peptides from C$^{\alpha,\beta}$-didehydro-alanine (ΔAla), characterized by sp^2 α- and β-carbon atoms, adopt a 2.0$_5$-helical structure in solution and in the crystalline state [109]. They are stabilized by two types of intramolecular H-bonds:
(i) N_i–H···O_i=C_i (forming a five-membered ring, typical of the 2.0$_5$-helix);
(ii) Cβ_{i+1}–H···O_i=C_i (forming a six-membered ring, typical of ΔAla peptides).





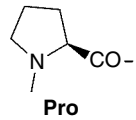
X-ray diffraction structure of *p*BrBz-(ΔAla)$_3$-OMe

- These completely flat molecules form planar sheets. They exist in isolation and pack in layers without any significant contribution from intermolecular C=O···H–N H-bonds. These graphene-like molecules may represent potentially active bridges in electron transfer reactions.

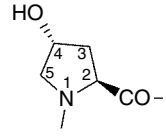


1.5.6 Poly-(l-Pro)$_n$ Helices and Collagen Triple Helix

Poly(L-Pro)$_n$ helices

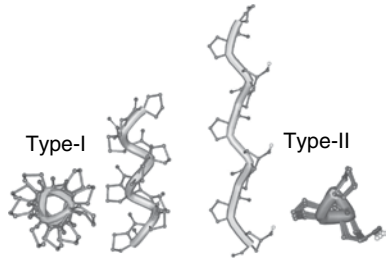


Pro



(4R)-Hyp

- The φ torsion angle of Pro and Hyp (4-hydroxyproline) is fixed by the pyrrolidine cyclic structure (to about –70° for the L-enantiomer).



- Type-I and type-II poly(L-Pro)$_n$ helix models [110–112]. Appropriate side-chain replacements may induce an amphiphilic character to type-II poly-(L-Pro)$_n$, a strictly threefold helix. This is the case of the [L-Pro–L-Pro–4(R)–L–Hyp]$_3$ type-II helix represented above (right). The view down the helix axis emphasizes its amphiphilic character.
- Type-I and type-II poly (L-Pro)$_n$ helices are *right*- and *left*-handed, respectively.

- Their sets of φ, ψ torsion angles are close, but they differ substantially because form I has all tertiary peptide bonds in the *cis* conformation ($\omega = 0°$), while in the form II these bonds are all in the *trans* conformation ($\omega = 180°$). Therefore, the less stable form I is remarkably more compact than the more stable form II. The kinetics of the folding and unfolding processes in globular proteins are often governed by the *cis*/*trans* Xxx–Pro conformational transition [113].
- Because all peptide bonds are tertiary (no NH groups), these two helices are not stabilized by any intramolecular H-bond. Their interconversions (called 'mutarotations' because they were originally followed by looking at variations of the polarimetric values with time) are solvent driven. Type-II poly-(L-Pro)$_n$ is the dimorph largely prevailing in polar solvents.

Poly (L-Pro)$_n$ helices: parameters

The parameters characterizing these two helices are as follows:

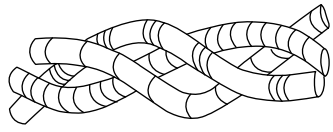
	Form I	Form II	
φ (°)	–83	–80	The φ, ψ values are very close.
ψ (°)	158	150	
ω (°)	0	180	Major conformational difference.
n	3.3	–3.0	The sign '-' indicates a *left*-handed helix for the L-enantiomer.
d (Å)	1.9	3.1	Form II is more elongated than form I.
p (Å)	6.3	9.3	

- In L-Pro-containing peptides the value of the L-Pro ψ torsion angles may be either ~ 150° (the preferred *trans*' conformation, *semi*-extended Pro) or ≅ –30° (the less common *cis*' conformation, helical Pro). In globular and transmembrane proteins [113, 114], Pro has indeed been found in 3_{10}-/α-helices, but only at the first two/three positions, because it lacks the H-bonding donor peptide NH group (the NH groups at these positions are not involved in the intramolecular H-bonding schemes of the 3_{10}-/α-helices). If inserted in an internal position of a helix, Pro has been shown to induce a kink of ~ 20°.
- Homo-(L-Pro)$_n$ stretches should be used with caution as spacers or bridges (often termed 'rigid rods'), because their rigidity is questionable in view of the variety of the possible conformations discussed above.

Collagen triple helix

- The most widely distributed fibrous protein in human body is *collagen*. It is characterized by a repeating triplet of amino acids, (L-Pro–Xxx–Gly)$_n$, where Xxx is almost any α-amino acid ('consensus' sequence). Its 3D structure is a 'triple helix coiled coil'. It was first proposed by Ramachandran and Kartha in 1955 [115]. The model was improved by Rich and Crick in the same year [116] (see also Ramachandran in 1956 [117]).
- Each helix is closely related to the left-handed type-II poly(L-Pro)$_n$ conformation. This is why collagen is very rich in Pro. Gly is always present at position 2 of the triplet because it is the least sterically demanding amino acid (in collagen, Gly occurs at each extremely hindered intersection of the three strands of the triple helix). Gly 'point mutations' induce severe 'molecular diseases' in bones [118]. The three helices are parallel to each other and in register.

- The most abundant type of collagen has two α_1 chains (formed by 338 consecutive triplets, about 1050 Å long) and one α_2 chain [119]. During the biosynthesis, the triple helix folds from the C-terminus (the rate determining step is the *cis*→*trans* Xxx–Pro conversion) [120]. There are two interchain H-bonds per triplet (one strong, (Gly)N–H···O=C(Pro), and one water-mediated and weak). About 10% of the Pro residues in collagen are replaced by posttranslationally generated (4R)Hyp residues [121, 122]. This enzyme (Pro hydroxylase)-mediated process is regio- and stereospecific. Their secondary alcohol side-chain groups form additional H-bonds (with peptide C=O groups and water molecules, and between each other), further stabilizing the triple helix structure.



Collagen triple helix

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