Max-Bergmann Lecture

Handedness preference and switching of peptide helices. Part II: Helices based on non-coded α-amino acids‡

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Short title: PEPTIDE HELIX HANDEDNESS (II)

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‡ This is the second part of a review article based upon a lecture presented by Prof. Claudio Toniolo at the Max-Bergmann Conference in Badenweiler, Germany, October 6-8, 2013, where he was awarded the Max-Bergmann Gold Medal 2013 (for the first part, see ref. [1]).

Abstract

In this second part of our review article on the preferred screw sense and interconversion of peptide helices, we discuss the most significant computational and experimental data published on helices formed by the most extensively investigated categories of non-coded α-amino acids. The are: (i) N-alkylated Gly residues (peptoids); (ii) C^{α}-alkylated α-amino acids; (iii) $C^{\alpha,\beta}$-sp^2 configurated α-amino acids; (iv) combinations of residues of type (ii) and (iii). Hopefully, the large body of interesting papers examined and classified in this editorial effort will stimulate the development of helical peptides in many diverse areas of bio- and nanosciences.

Keywords: chirality; handedness; helical structures; nuclear magnetic resonance; peptides; X-ray diffraction crystallography; spectroscopy; switches

Introduction

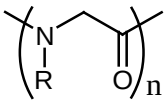
This article is the second part of an extensive review on the preferred screw sense and helix/helix equilibria in peptides from α-amino acids. The first part [1] dealt with helices based on protein amino acids [Asp (and its β-esters) and Asn; β-branched residues with a chiral β-carbon (Ile and Thr); and Pro (and its side-chain derivatives)]. For an updating of those topics, the reader should refer to the very recent, interesting papers [2-11].

This second part is focused on the same conformational phenomena but in peptides rich in non-coded α-amino acids, namely: (i) N-substituted Gly residues (peptoids), (ii) C^{α}-tetrasubstituted α-amino acids [in particular, the achiral Aib, α-aminoisobutyric acid and the chiral (αMe)Val, C^{α}-methyl valine], (iii) α,β-didehydro α-amino acids (more specifically, ΔPhe, α,β-didehydrophenylalanine), and (iv) both Aib and ΔPhe. However, it is worth pointing out that the screw sense preference of peptide helices formed by chiral C^{α}-tetrasubstituted α-amino acids (but not their switching) have been recently reviewed [12].

N-Substituted Gly Residues

Poly-(*N*-substituted) Gly residues or peptoids [13] are members of a family of backbone-modified peptidomimetic molecules which in the last 23 years [14] received enormous interest in the bio- and nanochemical literature (for excellent review articles on this general topic, the reader is referred to [15-27]). At variance with those of peptides, peptoid side chains are covalently linked to the main-chain *nitrogen* atom where they form tertiary amide bonds. In peptoids, this shift in the backbone position: (i) is responsible for their ability to resist protease degradation; (ii) removes chirality from their α-carbon atom; (iii) removes their H-bonding donor potentiality, leaving only their H-bonding acceptor property; and (iv) enhances their 3D- conformational flexibility by reducing the difference in stability between the amide *trans* (remarkably higher in the usual, secondary amide-bond containing peptides) and *cis* conformations.

As we discussed in the first part of this review-article [1] for peptides based exclusively on L-Pro or its side-chain derivatives, which also are characterized by all tertiary amide backbones, two types of helical structures are typically adopted, termed type-I and type-II. The sets of φ,ψ backbone torsion angles shown by these dimorphic, *semi*-extended structures are very close: -70°,160° for type-I and -70°,145° for type-II. It is relevant pointing out here that in these compounds the φ value at about -70° is dictated by the restriction generated by the Pro five-membered pyrrolidine cyclic structure. Clearly, this specific constraint is removed in peptoids in view of their acyclic backbone amino acid components. However, those two types of helices are structurally significantly different since type-I has all its tertiary amide bonds (ω torsion angles) in the *cis* (0°) disposition, whereas in type-II these bonds are all *trans* (ω = 180°). Therefore, the right-handed type-I helix, as compared to the left-handed type-II helix, is significantly less elongated (by about 2Å per turn, 7.33Å *versus* 9.36Å) and its number of amino acid residues per turn is slightly larger (3.3 *versus* 3.0). Since in all amide bonds of poly-$(Pro)_n$ the proton on the α-nitrogen is missing, these two helices are not stabilized by any intramolecular NH···O=C H-bond, typical of classical peptide 3D-structures, such as the α-helix, 3_{10}-helix [28-31], and β-hairpin. In this section, we exclusively summarize the conformational properties of the most extensively investigated linear α-peptoids (where the -NR- and C=O groups are separated by only one carbon atom). Therefore, those pertaining to β- or γ-peptoids, cyclic peptoids, extended peptoids, thiono-peptoids, arylopeptoids, and peptomers, although of increasing importance, will not be discussed.



oligo-(poly-) peptoids

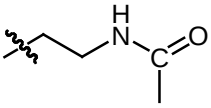
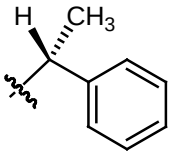
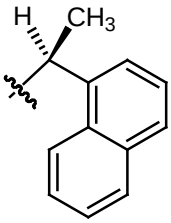
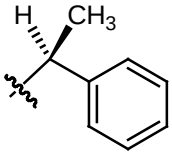
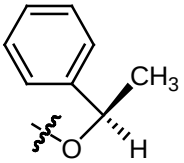
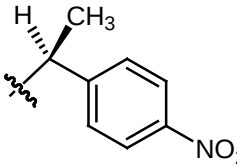
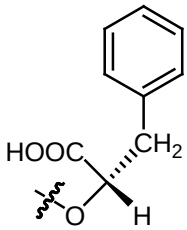
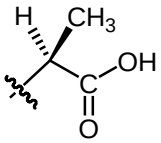
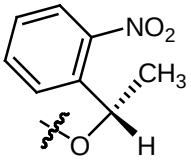
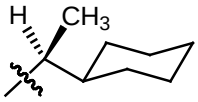
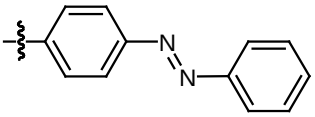
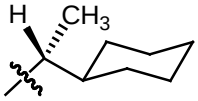
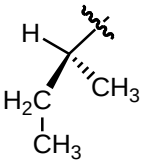
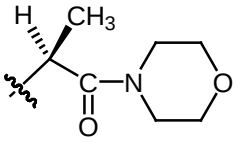
Entry	R–	Abbreviation = name	Entry	R–	Abbreviation = name
1		*N*Ala = *N*-methylGly (Sar, sarcosine)	**9**		Nam = *N*-(2-acetamidoethyl)Gly
2		*N*spe = (S)-*N*-(1-phenylethyl)Gly	**10**		Ns1npe = (*S*)-*N*-1(1-naphtyl)ethyl)Gly
3		*N*rpe = (*R*)-*N*-(1-phenylethyl)Gly	**11**		NOrpe = (*R*)-*NO*-(1-phenylethyl)Gly
4		Nsnp = (*S*)-*N*-[(1-(*p*-nitrophenyl)ethyl])Gly	**12**		*N*scp = (*S*)-*N*-(1-carboxy-2-phenylethyl)Gly
5		*N*sce = (*S*)-*N*-(1-carboxyethyl)Gly	**13**		*N*s2ne = (*S*)-*N*-[1-(2-nitrophenyl)ethyl]Gly
6		*N*sch = (*S*)-*N*-(1-cyclohexylethyl)Gly	**14**		*N*azb = *N*-(*p*-phenylazo-phenyl)Gly
7		*N*rch = (*R*)-*N*-(1-cyclohexylethyl)Gly	**15**		*N*ssb = (*S*)-*N*-(*sec*-butyl)Gly
8		Nsme = (*S*)-*N*-[1-((morpholino)carbonyl)-Ethyl]Gly			

Figure 1. The chemical formulas of the abbreviated peptoid building blocks mentioned in this article.

Sar (sarcosine), also termed *N*-methylglycine (MeGly) or *N*Ala according to the original abbreviation proposed by Simon *et al.* [14], has the simplest and one of the least hydrophobic [32] side-chain chemical structure (a methyl group) among the members of the extremely large family of peptoid building blocks investigated to date (Figure 1). Beginning in 1971, the results of a large body of computational studies on the preferred conformations of poly-$(Sar)_n$ and Sar derivatives and short homo-peptides have been reported [13,33-51]. Considerable conformational restrictions due to steric overlaps involving the *N*-methyl group, particularly severe for a Sar residue with the preceding *cis* amide bond, were found. Not surprisingly, the energetically most stable regular conformations of *cis* or *trans* poly-$(Sar)_n$ are highly dependent on the geometry (bond lengths and bond angles) of the molecule and on solvent effects. The conformational energy maps for *trans* Sar peptides change little when the main chain is elongated. However, the maps for *cis* Sar peptides are significantly modified on going from the "mono-peptide" Ac-Sar-NMe_2 (Ac, acetyl; NMe_2, dimethylamino) to the polypeptide. The conformational energies of the most stable *cis* poly-$(Sar)_n$ structures are remarkably lower than those of the *cis* model compounds. In $(Sar)_n$ with $n \geq 4$ it is the all-*cis* conformer that is energetically preferred. The most stable structure for poly-$(Sar)_n$ is *semi*-extended (φ= -90°, ψ= 150°), right-handed, with all-amide *cis*, and 3.3 residues per helix turn. It is compact and similar to the type-I poly-$(L\text{-}Pro)_n$ helix [52]. The major stabilizing forces include van der Waals contacts involving the *N*-methyl group with the carboxyl oxygen and the α-carbon atoms of adjacent residues. A structure for poly-$(Sar)_n$ of good stability is also *semi*-extended (φ= -60°, ψ= 150°), but *left*-handed, with all-amide *trans*. It is much more elongated and quite close to the type-II poly-$(L\text{-}Pro)_n$ helix [53]. The transitions between right- and left-handed helix conformations of poly-$(Sar)_n$ are found to be rare during the simulations. However, some of the most recent papers have questioned part of the aforementioned conclusions. In any case, all authors claim that substitution at the nitrogen atom makes β-pleated sheet formation more difficult. Also, it is generally agreed that the dipole···dipole attractions between consecutive C=O carbonyl groups (extensively discussed in our previous ref. [1]) are among the main stabilizing internal forces of the $(Sar)_n$ helices. Incidentally, it is rather curious to note that only in 2011 [49] the $(Sar)_n$ peptides were explicitely termed *peptoids*.

There are only two X-ray diffraction structures of Sar homo-peptides solved so far. Both compounds are very short (dipeptides). The unprotected, charged $^{+}H_2\text{-}(Sar)_2\text{-}O^{-}$ shows an "almost" extended conformation with the central tertiary amide bond in the *cis* disposition (cited in ref.[36]). Conversely, in the N-protected $–(Sar)_2-$ peptide the central tertiary amide bond is *trans* [54]. Not surprisingly, in view of its tertiary urethane nature [55] the N-terminal Boc (*tert*-butyloxycarbonyl)-Sar bond is *cis*. The N-terminal Sar residue is *semi*-extended. Clearly, the extremely limited number

of X-ray diffraction structures available does not allow any conclusive comment on the conformational bias of the Sar-Sar ω torsion angle in the crystalline state.

The most detailed information on the solution conformational tendency of poly-$(Sar)_n$ and the Sar homo-oligomers was obtained from the NMR spectroscopic results. Indeed, being achiral, the Sar homo-peptides exhibit a featureless CD spectrum [56]. Moreover, because in these molecules all nitrogen atoms do not bear any proton, one cannot take advantage of the usually conformationally quite informative N-H stretching mode in the IR absorption spectra either. According to the few IR investigations published [54,57,58], the position of the absorption maximum of the C=O stretching vibration in $(Sar)_n$ molecules (near 1665cm^{-1}) is dependent on the solvent nature and addition of H-bonding donor compounds. In any case, the H-bonding acceptor (basicity) character of these tertiary amide-based $(Sar)_n$ compounds was found to be weak. For poly-$(Sar)_n$, this finding was tentatively explained in terms of delocalization of the -CO-N(CH_3)- π-electrons along the macromolecular main chain. No conformational insight on these peptides was provided by this spectroscopy. Poly-$(Sar)_n$ bearing a terminal electron donor moiety and a terminal electron acceptor moiety were synthesized and their intrachain charge (or energy) transfer complexes evaluated by Vis absorption (or fluorescence) [59-61]. In these spectroscopic studies it was also demonstrated although indirectly, that these relatively weak intrachain forces play a relevant role in biasing the overall poly-$(Sar)_n$ conformation in solution. Comparable outcomes were seen in a study of intramolecularly catalyzed hydrolysis of a C-terminal ester by an N-terminal pyridyl moiety [62]. Several groups have studied the conformational preferences of $(Sar)_n$ peptides in different solvents using NMR [36,42,59,63-70]. In the ^1H NMR spectra the splittings of both the *N*-methyl and methylene signals were analyzed in detail and found to be informative on the local tertiary amide *cis*/*trans* situations. Poly-$(Sar)_n$ samples of different average molecular weight, Sar derivatives, and terminally protected Sar homo-peptides from dimer to heptamer were examined (an example is reported in Figure 2). The spectra of the highest oligomers and the polymer are quite complex because the peaks of the *N*-CH_3 and *N*-CH_2 protons reflect not only the state of that specific residue, but also those of the nearest neighboring residues. Eight peaks are expected in the fine structure of each region, assuming the *N*-CH_3 and *N*-CH_2 resonances exhibit both positional and conformational sensitivities. In ethanol and dimethylsulfoxide (DMSO) the *trans* and *cis* amide conformations of poly-$(Sar)_n$ are almost equally preferred (overall random conformation), but in chloroform and water the *trans* conformation appears to be more biased. From the temperature dependence of the spectra, a double coalescence phenomenon was observed in which the eight peaks first coalesce into two peaks (*cis* and *trans*) and then the two peaks collapse into a single peak. At -70°C the protected homo-tripeptide shows only one conformer (*cis* or *trans*), but under these

conditions the corresponding heptapeptide exhibits a complex spectrum. The fraction of overall *cis* amide bond (but not the fraction of the *cis*-*cis* dyad) reaches a maximum, 35-50%, as the main-chain length increases to $n \approx 90$, independently from the solvent. In summary, in partial contrast with the results from conformational energy calculations, the available experimental (NMR) data for poly-$(Sar)_n$ support the absence of a fully developed all-*trans* or all-*cis* conformation under any conditions verified, and suggest that $(Sar)_n$ stretches should not be used as "rigid" spacers acritically.

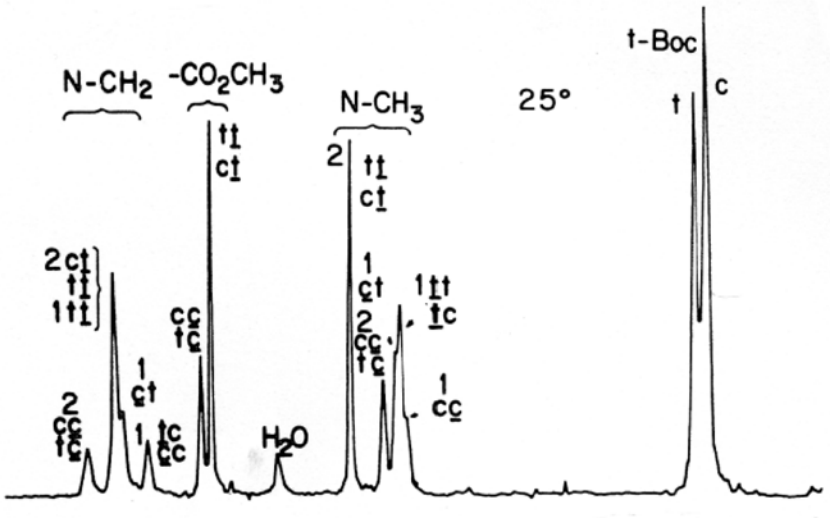


Figure 2. ^{1}H NMR spectrum of Boc-$(Sar)_2$-OMe in DMSO,d_6 solution at 25°C, showing the *cis*/*trans* assignments for all proton peaks. Adapted from ref. [68].

To reduce the flexibility of the secondary structure of poly-$(Sar)_n$ peptoids, numerous theoretical and experimental studies were conducted on compounds of the same family which utilize an *N*-side chain more complex than a methyl. The first conformational energy map reported for this sub-class of compounds was that of $(Nspe)_8$ [43] (in this widely accepted nomenclature [19], the first small letter, "s", designates the stereochemistry of the α-chiral *N*-side chain and "pe" is the abbreviation for 1-phenylethyl) (Figure 1). It was found that this substituent has a dramatic effect on the overall main-chain conformation. This homo-oligomer forms a stable right-handed, *semi*-extended, threefold helix with all tertiary amide bonds in the *cis* conformation ($\omega = 0$) and the backbone torsion angles φ,ψ = -75°, 170°, strictly analogous to the type-I poly-$(L\text{-}Pro)_n$ helix [52]. These (-,+)values imply that the *N*-side chain α-chiral center can impart a relevant bias to a preferred (right-handed) backbone screw sense. Two important interactions play a role in this 3D-structural selection: (i) a steric interaction between the carbonyl oxygen of each residue and the two (*N*)CαH- substituents of different size, and (ii) an electronic repulsion between the same oxygen

atom and the π-electrons of the aromatic ring [clearly, this unfavorable interaction is operative only if an aromatic substituent is present at (*N*)CαH]. The carbonyl dipoles in this helix are nearly parallel to its long axis, thus generating a macrodipole with a partial negative charge at the *N*-terminus and a partial positive charge at the *C*-terminus (interestingly, the direction of this macrodipole is opposite to that observed for α-helical peptides). However, it should be kept in mind that the energy differences calculated for helices of different handedness and the estimated energy barriers as well, for example those which imply a change of ω from 0° to 180°, are rather small. The handedness of a given conformation is governed by the side-chain chirality. The lack of an enantiomeric preference for peptoids the side chains of which are achiral is expected to increase substantially the explorable conformational space. Using a statistical mechanical approach, it was found that, at variance with peptides where the two-helix bundles are not stable, in peptoids they are [71]. Quantum mechanics calculations and implicit-solvent simulations in AMBER indicate that *N*-aryl (anilide) side chains represent a valuable tool to enforce the occurrence of *trans* amide bonds [48,72]. Theoretical works allowed the identification of a (usually limited) number of low-energy local conformations even in *N*-alkyl peptoids [73]. Computational modeling shows that *N*-alkoxy peptoid oligomers, *e.g.* $(N\text{Orpe})_n$, possess a strong tendency to adopt an all-*trans*, type-II poly-$(\text{Pro})_n$ helical structure [74]. Non-planar anilides that are chiral (despite the absence of any chiral center) due to restricted rotation about the (aryl)carbon/nitrogen bond (atropoisomerism) were shown to promote conformational ordering in *N*-aryl peptoid [75]. *N*-Methoxyethylglycyl peptoid (PMP) oligomers with n= 20 and 50 exhibit Ramachandran plots similar to those of $(\text{Sar})_n$ discussed above, with all-*cis* amide, left-handed helices favored over all-*trans* amide, right-handed helices [49]. A computational study on the $(N\text{rch})_5$ peptoid suggests for the lowest free-energy state in solution an almost complete *cis*-amide (*c*+*c*+*c*+*t*), left-handed helix [48]. In an additional theoretical work on (tri)peptoids, *N*-aryl side chains were confirmed to restrict the main-chain amide conformation almost exclusively to the *trans* disposition [76]. Any bulky substituent on the aromatic group produces additional biasing to the same 3D-structural direction. Linear *N*-alkyl aide chains offer few local conformational constraints, but long-range interactions between them can have a structural impact. Other detailed studies clearly showed that, in addition to the well known n→π*(amide) interactions [1], n→π*(aromatic) interactions can further stabilize peptoid (and thiopeptoid as well) helices even *via* a newly characterized "bridge" mode of interaction mediated by σ orbitals [77-81].

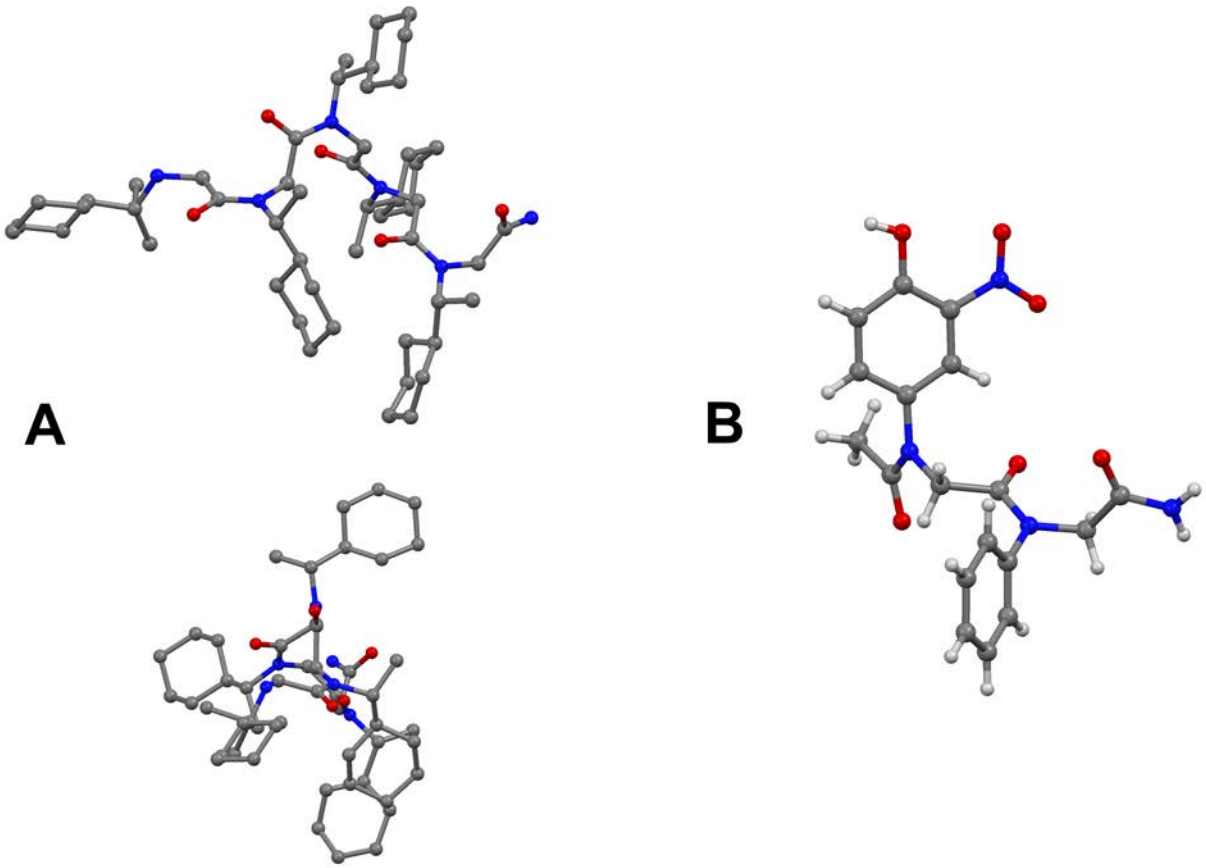


Figure 3. X-Ray diffraction structures of the all-*cis* amide H-(*N*rch)$_5$-NH$_2$ peptoid homo-pentamer (**A**) (the structure is seen perpendicular, top, and parallel, bottom, to the long axis of the helix) (adapted from ref. [82]) and one of the enantiomers of the all-*trans* amide *N*-(4-hydroxy-3-nitrophenyl)glycine-*N*-(phenyl)glycine peptoid hetero-dimer racemate (**B**) (adapted from ref. [72]).

Despite the relative peptoid flexibility, which does not help crystallization, numerous 3D-structures were solved by X-ray diffraction. The first published (2003) X-ray structure is that of the chiral, aliphatic (Nrch)$_5$ peptoid (Figure 3) which exhibits a left-handed helix with all-*cis* tertiary amide bonds, approximately three residue per helix/turn, and a pitch close to 6.7 Å [82], similar to that of type-I poly-(Pro)$_n$ [52]. The helical screw sense is governed by the selection of the side-chain enantiomer. A peptoid hetero-dimer racemate in crystal shows that the two *N*-aryl substituted main-chain tertiary amide bonds adopt the *trans* geometry [72]. In a homo-dipeptoid containing *N*-hydroxyglycyl building blocks the tertiary amides are both *trans* and the residues are *semi*-extended [83]. Individual molecules, arranged in an antiparallel fashion to one another, pack in the crystal through O-H···O intermolecular H-bonds. This is the first H-bonded, pleated sheet-like structure to be reported for peptoids. A branched, *N*-aryl trimeric peptoid was characterized by X-ray diffraction and found to adopt a unique acyclic reverse-turn conformation [79]. The crystal structures of the homo-di-, tri-, and tetrapeptides based on the naphthylethyl *N*s1npe [84] residue revealed a threefold, type-I poly-(Pro)$_n$-like helix similar to that of (Nrch)$_5$ discussed above. These latter examples are the first published for peptoids with an α-chiral aromatic side chain. Steric interaction between amide side chains could be responsible for the observed amide deviations from planarity. The X-ray diffraction structure of an atropoisomeric, chiral *N*-aryl/*N*-alkyl dipeptoid was also reported [85]. It is folded in an intramolecularly H-bonded *trans*/*cis* amide motif, forming a reverse turn reminiscent of the peptide β-turn with a central *cis* amide bond [86,87]. In the crystal state an N-terminally unprotected peptoid trimer with *N*-alkyl (phenylethyl) side chains has the two tertiary amide bonds in the *cis* disposition with the side-chain moieties of residues 1 and 3 on the same side of the backbone [76]. The crystal-state structure of a terminally protected, chiral tetrapeptoid with an alternating sequence [(*N*-aryl/*N*-alkyl)$_2$] shows that it is remarkably capable of adopting one full unit of a ribbon-like structure with an overall *trans*, *cis*, *trans*, *cis*, geometry [88]. The reverse turn shape of the molecule brings the *i*,*i*+2 methylene carbons of residues 1 and 3 in close proximity. Even *N*-alkyl peptoids can assume a type-I poly-(Pro)$_n$ structure with an all-*cis* amide geometry, as shown by a recent X-ray diffraction analysis of a protected *Nt*Bu dimer [89]. However, it is the very bulky *N*-*tert*-butyl side chain which is able to lock the tertiary amide bonds in that conformation [90]. In general, the X-ray diffraction data published to date on peptoid molecules fit nicely with those extracted from conformational energy calculations. Collectively, a variety of 3D-structures (left- and right-handed helices with all-*cis* and all-*trans* amide bonds, respectively; pleated sheet-like structures; reverse-turn conformations), reminiscent of similar crystalline structures exhibited by peptides, was unveiled.

For the characterization of conformationally flexible molecules, EPR and fluorescence spectroscopies are important additions to the most extensively utilized NMR and CD. EPR methods, *e.g.* double quantum coherence, give distances in the 10-75Å range directly. This technique was applied to a set of peptoids in which the relative positions of two *N*-side chain nitroxide (TEMPO) free-radical spin probes were systematically varied along the main chain [91]. The results obtained, *e.g.* the 17 Å separation in an *i*,*i*+6 dyad-containing heptapeptoid, with the probes eclipsed in this threefold helix, coincide with those extracted from consideration of X-ray crystal structures of similar helical peptoid sequences. Moreover, using this approach the conformational heterogeneity of chiral, well structured peptoids was determined and established to be lower than that of achiral, less structured similar sequences. Interestingly, mononitroxide-containing peptoid helical scaffolds are able to induce enantioselectivity on the oxidative resolution of a racemic alcohol, which was found to depend primarily on the handedness of the asymmetric catalysts [92]. Fluorescent peptoids reported so far are involved in: (i) a study using the intramolecular fluorescence resonance energy transfer technique to measure the change of the distance between the components of peptoid two-helix boundle superstructures when they bind zinc ions (the nitrophenol-based fluorescence quencher near the *C*-terminus is an *N*-substituted building block) [93]; (ii) an investigation with the 4-*N*,*N*-dimethylamino-1,8-naphthalimide fluorophore as the *N*-side chain of a mono-labeled peptoid to study its helix structure (the fluorescence emission intensity is maximized when the probe is located in the middle of the hydrophobic face of the amphiphilic helical peptoid) [94]; (iii) a structural work of water-soluble, threefold-helical peptoids, containing several *i*,*i*+3 spaced, chiral (*S*)-*N*-1-(naphthylethyl)glycine building blocks, with increasing number of helix turns (the longest peptoids self-associate *via* hydrophobic interactions as revealed by excimer emission from the probes) [95]; and (iv) a water-soluble, helical peptoid pH sensor based on the chiral (*S*)-*N*-(1-carboxy-2-phenylethyl)glycine building block, where the fluorescence intensity of the environmentally sensitive, single probe varies over the pH range studied [96].

The preferred conformations adopted by peptoids in solution were analyzed in detail in a large variety of NMR [64,70,72,74,75,77-80,82-84,88,89,97-106] and CD [12,43,74,75,78,82,84,88,91-96,98-103,107-130] studies. Curiously, the first ^{1}H-NMR spectra recorded for poly-peptoids based on higher (*N*-ethyl, *N*-*n*-propyl, and *N*-*n*-butyl) homologs of *N*-methyl-glycine (Sar) were published as early as in 1972 [64]. From inspection of the methylene proton peaks, it was concluded that the introduction of bulkier *N*-alkyl substituents tends to increase the *cis* content of the tertiary amide bonds. The results were explained by invoking an increase of the local steric hindrance. The major species of *N*-alkyl-α-chiral peptoids is a right-handed helix with *cis*-amide bonds, as shown by 2D-NMR spectroscopy [82,99]. The presence of additional

minor peaks for each proton points to the co-existence of minor conformers with different local amide bond geometries. Peptoid oligomers with multiple α-aromatic substituted, α-chiral residues show stable all-*cis*, right-handed helical structures [100]. NMR spectra of exceptional quality obtained for the α-aromatic, α-chiral -(*N*spe)$_9$- nonapeptoid, ^{13}C-enriched at the backbone C$^{\alpha}$-position, imply an extremely high conformational purity and are typical of a newly discovered 3D-structure, termed "threaded loop" [101]. It is characterized by a closed-loop backbone conformation, side chains exposed to solvent (reminiscent of a globular protein turned inside-out), a total of four *cis*- and four *trans*-amide bonds, and all sets of φ,ψ torsion angles *semi*-extended. It is also exceptionally thermal stable, backbone-length specific, and bears chain-terminating functional groups. The preferred conformation of a linear peptoid containing an 1,5-substituted triazole δ-amino acid was found to be compact and turn-forming with the *N*- and *C*-termini of the main chain in close proximity [102]. This finding expanded the already large repertoire of published peptoid 3D-structures. The rate of *cis*-*trans* isomerization of amides in peptoids is generally slower than that around amide bonds of Pro-based peptides [70]. The pattern of NOEs of oligo-(*N*-arylglycines) is dictated by 90% repeating *trans*-amide bonds [72,97]. This novel peptoid helix resembles that of left-handed, type-II poly-(Pro)$_n$ [53]. An -NO_2 aromatic moiety in a nonapeptoid can destabilize or stabilize the threaded loop conformation depending upon the position of the nitro group (either *ortho* or *para*, respectively) [103]. In peptoids containing (*S*)1-naphthylethyl side chains (*N*s1npe), *trans*-amide conformers are effectively eliminated [78,84]. This strategy generates a conformationally extremely homogeneous (all *cis*-amide) class of peptoids. Reasonably, steric interactions between the bulky naphthyl-containing side chains play an essential role. Incorporation of *N*-aryl side chains capable of H-bonding (*e.g.* with an *ortho*-OH group) may induce reverse turns in peptoids [79]. Oligo-(*N*-alkoxyglycines) exhibit NMR spectra representative of a single, all-*trans* amide conformation [74,83]. The existence of only one conformer is remarkable considering the number of rotatable bonds. The oligo-(*N*-hydroxyglycines), however, can either form intramolecularly H-bonded (with nearby C=O groups) or promote the onset of self-associated, sheet-like structures. Remarkable energy barriers to rotation about the stereogenic C-N bond in atropoisomeric peptoids with *ortho*-substituted *N*-aryl side chains were observed [75]. All of the peptoids examined by NMR with sterically hindered *N*-(*t*Bu) side chains show a single set of signals indicative of an all-*cis* amide arrangement [89]. A water-soluble hexamer peptoid with all *N*-(4-aminobutyl)glycine residues adopts a pseudo-helical structure with all-*cis* amide bonds according to a combined QM/MM force field-NMR strategy [106]. The protonated ammonium side chains are extended, a prerequisite for efficient cell-penetrating compounds. Most notably, NMR and X-ray diffraction crystallography coincide in assigning a well-defined ribbon secondary

structure to peptoids with alternating sequences of *cis*-amide *N*-naphthylalkyl and *trans*-amide *N*-aryl residues [88]. This result represents a level of 3D-structural characterization yet to be achieved for other peptoid secondary structures.

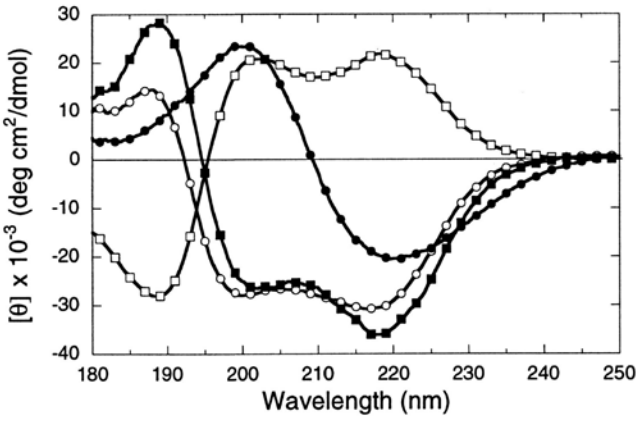


Figure 4. CD spectra of the oligopeptoids -[*N*spe-*N*spe-*N*sce]$_3$- -■- , -[*N*rpe-*N*rpe-*N*am]$_4$- -□-, -[*N*sce-*N*sce-*N*spe]$_{10}$- -○-, and -(-*N*sme)$_{12}$- -●- . Adapted from ref. [98].

CD spectroscopy provided rapid and useful signatures for the new peptoid structures. Obviously, this technique gives mostly qualitative conformational information and is limited to chiral compounds. In peptoids, chirality may arise from an asymmetric center (*e.g.* a carbon atom) or a dissymmetric (*e.g.* atropoisomeric) moiety, both located on nitrogen. Typically, the chiral carbon in peptoids is found in α-position of the nitrogen side chain of their building blocks. Most of these chiral carbons bears an aromatic (usually a phenyl, phenol or a nitrophenyl) moiety *directly* linked to them. This is a spectroscopically unfortunate situation because the contributions of the electronic transitions of the backbone tertiary amides in the far-UV region [131-133] (providing information on the peptoid secondary structures) are severely overlapped by the ^{1}La transitions originated from the α-aromatic chromophores [131,134-137]. The bands in the CD spectrum of the α-chiral aliphatic homo-pentapeptoid H-(*N*sch)$_5$-NH$_2$ [*N*sch, *N*-(*S*)-(1-cyclohexylethyl)glycine] are relatively weak, reflecting only a partial helical ordering in solution [82,98]. However, those of the longer oligomers investigated, for example, H-(*N*sch)$_{15}$-NH$_2$, show a distinct positive maximum at 200 nm, and two negative maxima at 190 nm and 220 nm, respectively. The CD bands are spectral characteristics that are typically associated with those of the type-I poly-(Pro)$_n$ helix [1]. Therefore, these CD findings are consistent with the results from the crystallographic analyses and other spectroscopies. The shapes of the CD spectra and their steady increase in intensity with lengthening

of the main chain are qualitatively similar for the *N*ssb and *N*sme (Figure 4) peptoid homo-oligomers. This latter observation points to a more ordered helical fold for the longest oligopeptoids. CD also demonstrated that this helical structure is quite stable upon heating. This result is consistent with a 3D-structure predominantly stabilized by steric repulsion rather than by intrachain H-bonding. Introduction of the achiral, water-solubilizing *N*-(4-aminobutyl)glycine and *N*-(3-guanidinopropyl)glycine guest residues into the host *N*ssb or *N*sch homo-peptoid chains allowed CD measurements to be performed in aqueous buffer solutions [111,114,117]. Under these conditions, the CD spectra are weak, reminiscent of that of a unordered peptide conformation. However, in organic solvents and in membrane mimetic vesicles the curves are more intense and resemble those of type-I poly-$(Pro)_n$. Not surprisingly, the intensity of the CD curves is proportional to the percentage of chiral *N*-substituted residues. In α-aromatic peptoids, however, this same helix can be recognized by a diagnostic CD spectrum, which, for the *S*-configured compounds features two negative maxima, near 220 nm and 200 nm, and a positive maximum at approximately 190 nm [98-101,107,109,113]. Curiously, despite the presumably strong influence of the α-aromatic chromophores, the overall shape of this spectrum closely resembles that of an α-helical peptide [131,132]. In the course of these CD studies it was noted that the α-aromatic 9-mer peptoids exhibit a unique spectrum where the negative band with the strongest ellipticity over a wide range of concentrations is that near 200 nm [101,107]. Using other physical and spectroscopic techniques, the authors were able to show that this CD spectrum is associated with a threaded loop conformation (see above). The characteristic CD features of α-aryl peptoid helices in the UV region are not perturbed by free-radical TEMPO side chains [91,92] despite the presence of an additional (nitroxyl) chromophore in the latter residues [138]. However, the potentially interesting, CD-active, transitions in the visible region of the spectrum were not investigated. A water-soluble series of -$(N\text{scp})_2$- homo-oligomers, studied extensively by CD, exhibits a dramatic, pH-dependent rearrangement between two well-defined conformational states, governed by the ionization of the -COOH containing side chains [120]. Unfortunately, the 3D-structural states properties of this new sub-class of peptoids were not examined in detail. CD analysis demonstrated that the peptoid type-I poly-$(Pro)_n$ helix is not significantly altered by insertion of an *N*azb residue and subsequent *trans*-to-*cis* photoisomerization of its azobenzene side chain [123]. According to the authors [103], incorporation of an *N*-terminal α-aromatic moiety containing an *ortho* (*N*s2ne) or a *para*- (*N*snp) nitro group into a nonapeptoid either destabilizes or stabilizes, respectively, the threaded loop conformation [103]. However, the CD spectra were discussed only in terms of steric and H-bonding interactions, and electron-withdrawing properties as well, but the potential contribution of the nitroaryl chromophores was neglected [134-137]. A recent, very stimulating addition to the CD

signatures of peptoids is represented by the spectra of the two enantiomers of a derivative of an atropoisomeric building block based on an *N*-aryl moiety bearing a *tert*-butyl substituent on the *ortho*-position [75]. Beside a CD band near 225 nm, for the first time a band (of the same sign) in the near-UV region (at approximately 265 nm, reasonably related to the *N*-acyl anilino chromophore [137]) was reported. Syntheses and CD characterizations of chirally pure, atropoisomeric homo-oligo peptoids, including those based on the *N*-naphthyl moiety [85], should represent the next step of the research in this field. The CD curves of all-*trans* amide peptoids was reported for the first time for oligo-(*N*-alkoxy glycines) [74]. They are dominated by two strong, but length-dependent, bands of the same sign at about 215 and 205 nm. However, the electronic transitions of the phenyl chromophore in α-position relative to the chiral center might have influenced the experimental results. Intriguingly, the CD spectra of all-*cis* amide peptoid helices generated by α-chiral *N*s1npe side chains show extremely intense band with an overall pattern which changes abruptly at the level of the homo-tetramer [84]. It was suggested that this spectral alteration might be associated with *i*, *i*+3 through-space interactions of the overlapping naphthyl side chains. If made water soluble by insertion of *N*sce building blocks, these peptoids tend to self-associate, as highlighted by a remarkable variation of their CD band near 230 nm [95]. The CD signature for the recently published, stable alternating *cis*-*trans* amide, *φ*,*ψ semi*-extended, peptoid ribbon secondary structure shows a CD band near 225 nm followed by a broad band of opposite sign in the 200 nm region [88]. Finally, the exciton-coupled CD spectra of peptoid all-*cis* amide type-I poly-$(Pro)_n$ [1] helices carrying at *i*, *i*+3 or *i*, *i*+6 relative positions two or three *N*Lys building blocks decorated with the tetraphenylporphyrin(TPP)-CO- chromophore were reported. The intense, split Cotton effect in the Soret (420 nm) region reflects the chirality of the orientations of the transition dipole moments of the through-space interacting porphyrins. From the observed positive couplet, the (right) handedness of the peptoid helix was extracted. Similar results were obtained for TPP-terminally substituted threefold helical peptides [139]. Taken together, the CD results discussed here underscore the difficulty of assigning secondary structures from the experimental data obtained for the novel peptoid oligomeric folds by use of this spectroscopy alone. This severe limitation is due to the absence of theoretical CD studies in this area, combined with the massive presence of overlapping aromatic chromophores in most of the compounds examined. Hopefully, the former issue will be soon overcome. Nevertheless, the correlation of CD data to the peptoid 3D-structures determined by independent techniques (NMR and X-ray diffraction) has proven to be extremely valuable for a rapid analysis of new peptoids. Remarkable advancements in biosynthetic and chemical ligation methodologies through native amide bonds are currently expanding the range of accessible peptoid oligomer-peptide (protein) conjugates [140,141]. The influence of each of the

two components on the overall macromolecular conformation, including helix handedness preference and switching, of the resulting hybrid compounds is an open avenue to structural biochemists.

C^{α}-Tetrasubstituted α-Amino Acids

Our original interest in the 3D-structures of peptides rich in C^{α}-tetrasubstituted α-amino acids, in particular in the simplest member of this class, the achiral Aib (Figure 5), was stimulated by their potential usefulness as a new type of conformational constraint in the synthesis of enzyme-resistant, bioactive peptide analogs, and by the occurrence of some of them in the naturally-occurring, membrane-modifying peptaibiotics [142]. Moreover, the high crystallinity of their derivatives and peptides was expected to allow one to carry out unambiguous conformational characterizations by X-ray diffraction.

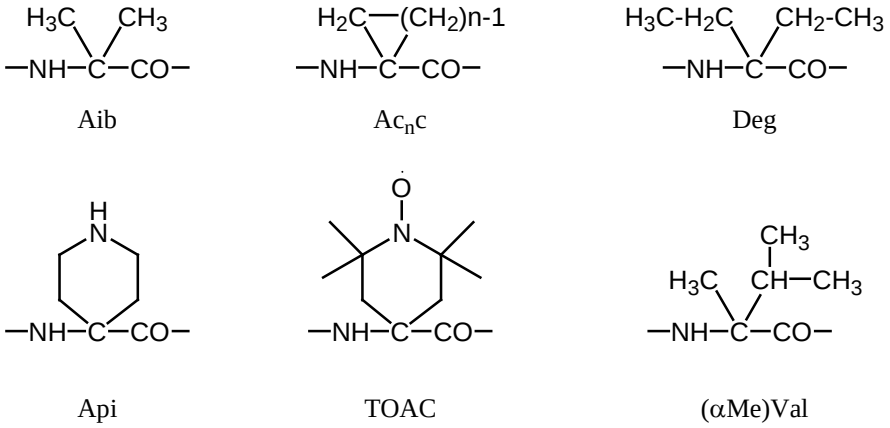


Figure 5. C^{α}-Tetrasubstituted α-amino acids, the abbreviations of which are mentioned in this paper.

In the initial part of this section, the discussion will be focused on the 3D-structures preferred by chemically well-characterized, monodisperse, terminally-protected homo-peptides from Aib and its achiral $C^{\alpha,\alpha}$-cyclized analogs (Ac_nc, Figure 5) of varying main-chain length (review articles on this subject have been already published elsewhere [30,31,143-146]). In particular, conformational energy computations of Ac-$(Aib)_n$-NHMe (NHMe, methylamino)

showed that the presence of two methyl groups on the C^{α} atom imposes a significant restriction on the available conformational space. The 3_{10}-helical ($\phi = \pm 60°$, $\psi = \pm 30°$) and α-helical ($\phi = \pm 55°$, $\psi = \pm 45°$) structures [28-31,147,148] are the most stable conformations observed. In addition, the energy difference and barrier between the 3_{10}- and α-helices are rather small. In this connection, it is worth pointing out that the slight (by 1 – 2°) distortion from the regular tetrahedral (109.5°) value for the critical τ (N-C^{α}-C′) bond angle extracted from the X-ray diffraction analyses favors the 3_{10}- over the α-helix. Elongation of the peptide main-chain length further increases the relative stabilities of these two helical conformations with respect to other peptide secondary structures.

The conformational preferences of terminally-protected $(Aib)_n$ homo-peptides from dimer (n = 2) through undecamer (n = 11) were analyzed in the solid / crystal state [149-167]. All of them were found to be folded into the incipient (n = 3 and 4) or fully-developed (n = 5-11) 3_{10}-helical conformation. The statistically-derived parameters characterizing this threefold helix were summarized in ref. [28]. The first clear observation at atomic resolution of this theoretically predicted, but then experimentally novel, peptide conformation was reported by Balaram as early as in 1978 for $(Aib)_5$ [150]. The longest regular 3_{10}-helix published to date is that of $(Aib)_{11}$ [152]. The centrosymmetric crystals of all these achiral homo-peptides contain molecules with both right- and left-handed twists of the peptide chain. These results well justify the assumption that, at least in this homo-peptide series, main-chain length is not an overriding factor in governing helical folding. Aib homo-dipeptides with different types of terminal protections may adopt non-helical, more extended structures [160,161,166]. More recently, Clayden and coworkers [162(a)] described the X-ray diffraction structure of two $(Aib)_{16}$ homo-oligopeptides with a single, flexible Gly residue positioned in the middle of the sequence. In both peptides, the two $(Aib)_8$ segments are broadly 3_{10}-helical, but in their central regions loosenings of the 3_{10}-helices were seen. A ^{13}C NMR study in the solid state on $(Aib)_n$ (n = 3-8) homo-oligomers, based on the characteristic splitting of their C^{β} peaks as well as the conformation-dependent displacements of their C^{α} and C′ peaks, essentially confirmed the crystallographic findings [167].

The conformational preferences of the alicyclic Ac_nc (1-amino-1-cycloalkane carboxylic acid) from n = 3 [168-171] to n = 12 [172] (Figure 5) residues were assessed in homo-peptides by X-ray diffraction. The results are strictly comparable among them and closely parallel those of their prototype Aib discussed above. It has been suggested to incorporate this set of cyclic residues, with different effective volumes and hydrophobicities but similar 3D-structural preferences, at selected positions of biologically relevant peptides (*Ac_nc scan approach*) to delineate the precise nature of peptide … protein receptor interactions [168,172]. However, it is fair mentioning that in the peptides formed by Ac_3c, at variance with those by all other alicyclic residues of this class, the

regularity of the 3_{10}-helix is significantly compromised as the result of the remarkable asymmetry of the average geometry of the small (cyclopropane) ring. Specifically, the N-C^{α}-C′ (τ) bond angle is markedly expanded from the regular tetrahedral value. This finding is based on the unambiguously determined propensity of the Ac_3c residue to adopt a set of ϕ,ψ torsion angles close to ±90°,0° (in the so-called "bridge" region of the Ramachandran map).

For $(Aib)_n$ homo-peptides in helix-supporting solvents (*e.g.*, $CDCl_3$) the same conformation (3_{10}-helix) is largely prevailing [173-176]. Using FT-IR absorption and ^1H NMR we established that populations close to 90 and 100% of 3_{10}-helical conformers are attained at the hexamer and octamer level, respectively. Interestingly, it was also reported that Aib-rich 3_{10}-helical peptides do not unfold even at 150° in DMSO [177]. Recently, it was shown that interaction of the achiral H-$(Aib)_8$-O*t*Bu (O*t*Bu, *tert*-butoxy) with a chiral (enantiomeric) micellar aggregate made of L- (or D-) N-dodecyl-Pro-O^- led to the deracemization of the 3_{10}-helical oligopeptide and allowed for the first time to detect the CD signature in aqueous solution of this ordered secondary structure lacking the contribution of any chiral residue [178]. For a related CD work on a chromophoric $(Ala)_{17}$ *racemate* twisted assembly exhibiting macroscopic chirality in the presence of traces of L- or D-amino acid, see [179].

Despite their remarkable stability, in the late 1980s it was shown that 3_{10}-helices made of achiral Aib residues easily interconvert between right- and left-handed screw senses [180]. This phenomenon was investigated first on a terminally-protected $(Aib)_{10}$ by ^{13}C NMR through the coalescence method, *i.e.* by exploiting the magnetic non-equivalence of the two C^{β} carbon atoms of Aib in a helical conformation and the temperature dependence of the corresponding signals (Figure 6). Specifically, in a right-handed 3_{10}-helix, the *pro*-(*R*) methyl group is *syn-periplanar* to the C=O bond of the same residue and is located at only 2.7 Å from the oxygen atom, whereas the *pro*-(*S*) methyl group points away from the backbone. Therefore, the two methyl groups experience different magnetic environments. If the helix is left-handed, the situation is reversed and it is the *pro*-(*S*) methyl group which comes closer to the oxygen atom. Experimentally, at 203 K in CD_2Cl_2, the twenty methyl groups of Z-$(Aib)_{10}$-O*t*Bu (Z, benzyloxycarbonyl) give rise to two ^{13}C NMR signals, separated by 5.3 ppm. By increasing the temperature, their chemical shifts become progressively closer, up to coalescence at 300 K. These findings provided strong evidence for a mono-molecular, all-or-nothing process between the enantiomeric *P*- and *M*-helical forms of this homo-decapeptide. Interestingly, the free energy of activation estimated from the NMR data is of the order of 11 kcal/mol, which indicates the occurrence of about 1200 screw-sense interconversions/second at room temperature.

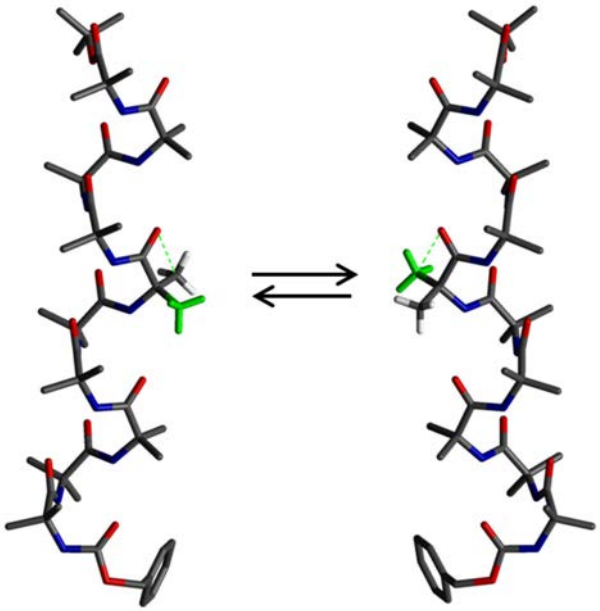


Figure 6. Model of the interconversion between left-handed (left) and right-handed (right) 3_{10}-helices of Z-$(Aib)_{10}$-O*t*Bu. The *pro*-(*R*) methyl group of one Aib residue is depicted in green. The short $C^{\beta}(i)$ … O(*i*) contacts involving either the *pro*-(*R*) C^{β} in the right-handed helix or the *pro*-(*S*) C^{β} in the left-handed helix are highlighted.

Much more recently, NMR work on hexa- and octa- Aib homo-peptides selectively ^{13}C-enriched at both methyl groups of a single, internal residue allowed a more detailed evaluation of the process of helix interconversion in CD_2Cl_2 [181]. The hexamer interconverts about three times faster than the octamer. Such a difference is related to the entropic term (negative for both peptides, but larger for the octapeptide), whereas the enthalpies of activation are identical for the two peptides despite the presence of two additional H-bonds in the octapeptide. These results are not consistent with a transition state in which all intramolecular hydrogen bonds are simultaneously broken. Conversely, the disruption of helical structure that leads to successful interconversion is likely limited to very few rotations at a time. It is reasonable to assume that unwinding of the helix should begin at the level of terminal residues, rather than in its central part where cooperativity effects are more pronounced. Rewinding of terminal residues in the opposite screw sense would then propagate and extend throughout the sequence.

It was also reported that for the Aib homo-octapeptide the rate of helix interconversion increases with increasing amounts of 2,2,2-trifluoroethanol (TFE) in CD_2Cl_2 solution, with a

temperature-dependent saturation effect [182]. However, the enthalpies of activation do not show a monotonic trend. This finding was interpreted as the result of a complex interplay between destabilization of the ground state helical conformation and stabilization of the transition state through H-bonding to the exposed N-H groups.

Replacement of Aib residues with the achiral, C^{α}-tetrasubstituted α-amino acid 1-piperidine-4-amino-4-carboxylic acid (Api) (Figure 5) at positions 3 and 6 of the $(Aib)_8$ sequence allowed exploitation of the two Api side-chain piperidine amino functionalities, separated by one 3_{10}-helical turn, for either metal chelation [183] or intramolecular crosslinking through formation of two amide bonds with *p*-phenylenediacetic acid [184]. In both cases, variable temperature ^{1}H NMR experiments clearly indicate that the helix interconversion, although not totally suppressed in the temperature range investigated, occurs at a lower rate compared to the *bis* side-chain Boc-protected parent peptide, although quantification of the kinetics was not feasible. Interestingly, the X-ray diffraction structure of the *p*-phenylenediacetylamide-stapled octapeptide was reported as a single, right-handed helical enantiomer in the chiral space group *Pba*2 [184]. Although the authors did not comment on this finding, it should be taken as an indication that the enantiomeric helices of the stapled octapeptide preferentially crystallize separately (as a conglomerate), rather than as a racemate. To the best of our knowledge, only three other examples were reported thus far of 3_{10}-helical peptides exclusively composed of achiral C^{α}-tetrasubstituted α-amino acids (Aib or Deg, $C^{\alpha,\alpha}$-diethyl glycine, Figure 5) which undergo spontaneous resolution in the crystal state [163,164,185]. Finally, it was also demonstrated that an intramolecular chiral (L,L) cystine bridge between the two Api side chains of the achiral octapeptide was able to induce exclusively a single screw sense (*P*) in the 3_{10}-helical peptide and transforms the dynamic helix into a static one [186].

When the conformational preferences of C^{α}-tetrasubstituted α-amino acids with at least two carbon atoms in each side chain (provided that cyclization on the C^{α}-atom would be absent) (Figure 5) were investigated, a novel peptide conformation was unambiguously authenticated. The theoretical and experimental results supporting this 3D-structure (termed 2.0_5-helix or multiple, consecutive, fully-extended conformation), with the ϕ,ψ backbone torsion angles equal (or very close) to 180°,180°, are presented and discussed in detail in two review articles, the first published by Tanaka in 2007 [187] followed by our, more recent and entirely devoted to it, contribution in 2013 [188]. The 2.0_5-helix, generated by a succession of local C_5 (intramolecularly H-bonded, 5-membered pseudo-cyclic forms) is a common conformation for these amino acids. X-Ray diffraction studies on homo-oligopeptides of this class to the pentamer level allowed a detailed description of the geometrical and 3D-structural features of the 2.0_5-helix. Its characteristic spectroscopic (IR absorption and NMR) parameters were also reported. Conformational energy

calculations are in good agreement with experimental data. As the contribution per amino acid residue to the length of this uncommon, *all-trans* helix is by far the longest (3.85 Å) possible, its use as a molecular ruler is very promising. However, it is a fragile 3D-structure, particularly sensitive to an increase in solvent polarity. Interestingly, in such a case, it may reversibly transform into the about 50% shorter 3_{10}-helix (Figure 7), thus producing an attractive molecular spring. A development of the research in this intriguing area was recently reported by our group [189].

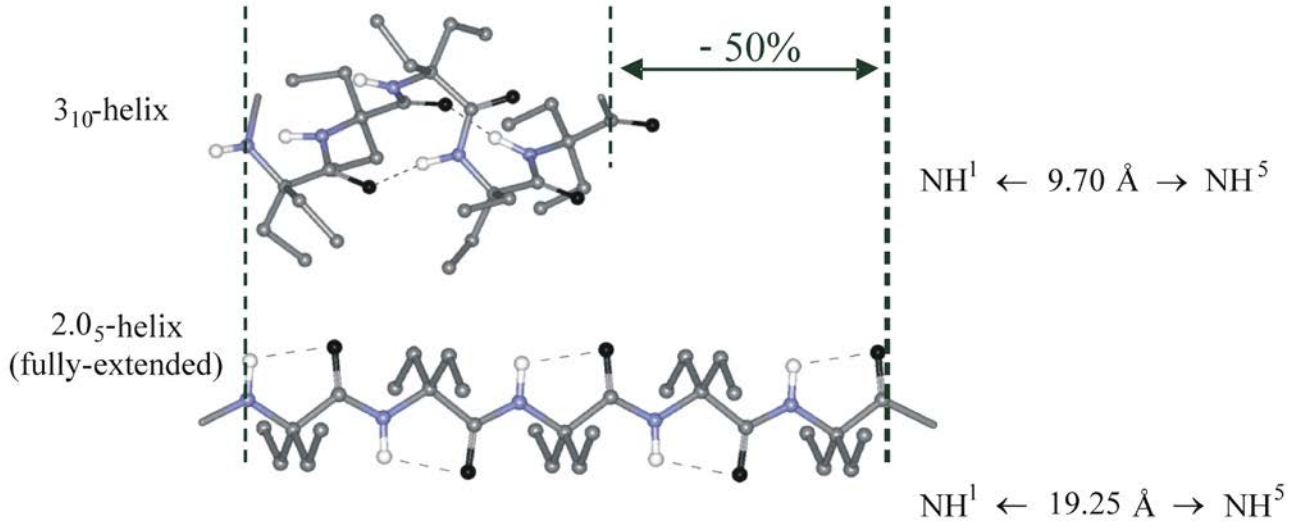


Figure 7. Comparison of the NH^1 to NH^5 separations for a Deg homo-oligopeptide segment in the 3_{10}-helix and 2.0_5-helix conformations. Adapted from ref. [188].

As stated in the *Introduction*, in ref. [12] we already reviewed the preferred helical screw sense of the: (i) chiral, C^{α}-methylated and C^{α}-ethylated α-amino acids; (ii) chiral side-chain substituted Ac_nc residues (including those possessing *exclusively* side-chain chirality), and (iii) $C^{\alpha,\alpha}$-cyclized residues endowed with *axial* chirality. In the following part of this section, we will discuss the most relevant examples of 3_{10}- / α-helix and turn / helical screw-sense switches exhibited by peptides rich in C^{α}-tetrasubstituted α-amino acids.

The two systems where the 3_{10}-helix to α-helix transition was initially investigated in detail are segments of peptaibiotics (in particular, emerimicin) and simple Ala/Aib model peptides. X-Ray diffraction, electronic CD, vibrational CD, and IR absorption analyses of the terminally protected 2-9 sequence of emerimicin -$(Aib)_3$-Val-Gly-Leu-$(Aib)_2$- clearly showed that this octapeptide, with a central non-Aib triplet, is folded in the 3_{10}-helical structure under different experimental conditions

[190-193]. Conversely, the corresponding nonapeptide, bearing the naturally-occurring Phe residue at position 1, exhibits essentially pure α-helical character [194].

A main-chain length dependent 3_{10}-helix to α-helix transition was also highlighted in a terminally blocked -(Aib-Ala)$_n$- sequential peptide series. The hexapeptide (n = 3) molecules are completely 3_{10}-helical in the crystal state, while the octapeptide (n = 4) was shown to adopt a largely predominant α-helix (but with an incipient 3_{10}-helix at the N-terminus) [195]. The longer (n = 5,6) oligomers are basically α-helical [196]. Interestingly, both terminate with a Schellman's motif [197], *inter alia* characterized by a 1 ← 6 intramolecularly H-bonded C_{16} *pseudo*-ring form. A series of peptides with the same overall composition, but starting with Ala, -(Ala-Aib)$_n$-, revealed that the octapeptide (n = 4) and hexadecapeptide (n = 8) adopt the 3_{10}-helix and the α-helix structures, respectively, in the crystal state, whereas in acetonitrile (according to an NMR study) both of them were found in an α-helix [198]. A critical solvent effect was also noted in Ala-(Aib-Ala)$_3$- [199]. In the more polar (deuterated) DMSO the heptapeptide adopts the α-helical conformation, while in the less polar $CDCl_3$ the 3_{10}-helix is preferred. Finally, using CD, Jung and coworkers [200] showed that Boc-protected -(Aib-Ala)$_n$- (n = 1-7) peptides C-terminally free or covalently linked to the solubilizing support poly-ethylene glycol fold into a helical conformation at n = 4 in ethanol. Taken together, all these results emphasize the subtle interplay of many different features (Aib percentage, main-chain length, precise amino acid sequence, and environmental conditions, such as crystalline state and solvent polarity) governing the 3_{10}- / α-helix preference [144]. In this connection, it is worth mentioning that, based on these (and other) data, Millhauser [201,202] proposed that the 3_{10}-helix would be a thermodynamic and/or kinetic intermediate in α-helix protein folding.

The 3_{10}- / α-helix preferences and equilibria were subsequently studied extensively by use of X-ray diffraction, CD, NMR, IR absorption, fluorescence, and electrochemistry on a number of Aib host peptides 6 to 15 residues long, containing different C^{α}-trisubstituted (mostly protein) contiguous and non-contiguous amino acids and, more rarely, some (αMe)Val, C^{α}-methyl valine, (Figure 5) residues as well [203-214]. The following most interesting information was extracted: (i) In selected decapeptides, solvent polarity is critical in directing type of helical structure [203]. (ii) Appropriate sequence permutation in a 75% Aib-rich peptide induces transition between the two alternative helical forms [204]. A theoretical model was proposed for the 3_{10}- / α-helix equilibrium constant, which takes into consideration main-chain length effect and the "extra" intrahelical H-bond, with respect to the α-helix, characterizing the 3_{10}-helix [205]. (iv) A 14-mer Aib/Ala-based peptide helix is as stable (to denaturants and heating) in water as a (26-mer) Ala-based peptide helix

almost double its length [206,207]. (v) Conformational heterogeneity in a water-soluble, 12-mer, mostly helical, Aib-based peptide was detected by NMR [208]. (vi) A reversible transition between an α- and a 3_{10}-helix in a 15-mer, fluorescent-labeled, Aib-containing peptide was reported [209,210]. (vii) An Aib/(αMe)Val/His heptapeptide behaves as a solvent [*iso*propanol / 1,1,1,3,3,3-hexafluoro*iso*propanol (HFIP)] -driven molecular spring thanks to an α- / 3_{10}-helix equilibrium [211,212]. In crystals obtained from a methanol (MeOH) -containing solvent mixture, the molecules are folded in the 3_{10}-helix structure. (viii) X-Ray diffraction and solid-state (KCl disk) CD measurements showed that a terminally-protected, 6-mer peptide characterized by two Aib residues (at positions 3 and 6) is 3_{10}-helical, while the same peptide, where two doubly side-chain substituted Ac_5c residues were inserted into the same positions, is α-helical [213]. It was therefore suggested that the steric properties of the multiply substituted Ac_5c cyclopentane ring may play a significant role in governing the type of helical structure assumed by a peptide. (ix) A 12-mer, helical -(Leu-Aib)$_6$- sequential peptide immobilized on an Au surface exhibits alternation between two (longer and shorter) states by changing the polarity of the applied electric field as a result of its interactions with the large and differently oriented dipole moments of the 3_{10}- and α-helices [214]. At this point, it should be mentioned that an additional spectroscopic technique, EPR, proved to be extremely useful in discriminating between 3_{10}- and α-helices in solution in peptides containing Aib and protein amino acids. In the compounds investigated, the nitroxide free-radical, strongly helicogenic, C^{α}-tetrasubstituted α-amino acid TOAC (2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid) was incorporated as an EPR-active probe [215].

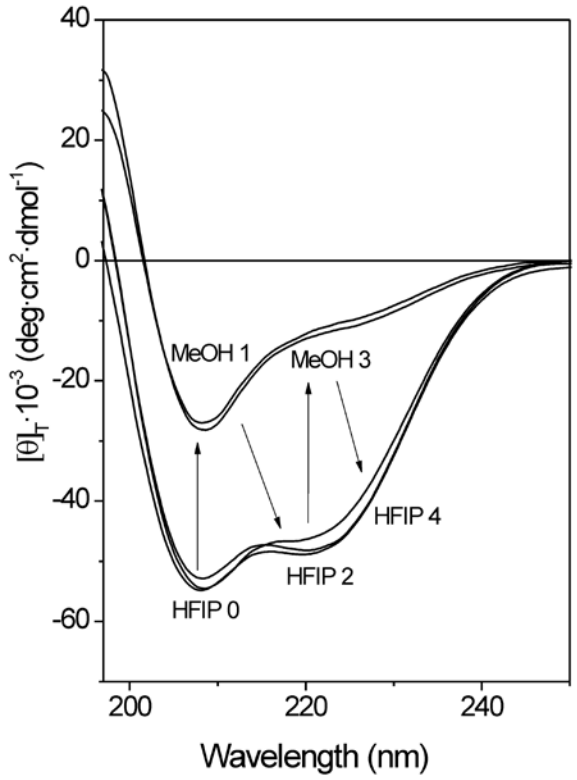


Figure 8. CD spectra of Ac-[L-(αMe)Val]$_7$-NH*i*Pr, where NH*i*Pr is *iso*propylamino, in MeOH and HFIP solutions. Repeated cycles of 3_{10}-helix / α-helix conversion were carried out, the order of solvent switching being HFIP 0, MeOH 0, HFIP 1, MeOH 1, and HFIP 2. Adapted from ref. [215].

A reversible switching of a 6-mer peptide, *entirely* made of C^{α}-tetrasubstituted [four Aib and two L-(αMe)Val] residues, between the two helical conformations was described following the change of polarity of the surrounding medium [212]. However, the set of peptides where the competition between 3_{10}- and α-helices was examined most carefully is that of the L-(αMe)Val homo-oligomers [216-218]. Using peptides of varying main-chain length (for the homo-heptamer, see Figure 8), we showed that a well-defined, polarity solvent (MeOH / HFIP) –controlled, reversible 3_{10}- / α-helix transition takes place even in a homo-oligomer as short as a terminally-blocked hexapeptide. Significantly, L-(αMe)Val sequences blocked as an amide or a urethane at the N-terminus, and as an ester or an N-alkylamide at the C-terminus, are all appropriate to detect this helical dimorphism. Interestingly, for an N^{α}-acylated homo-heptapeptide alkylamide this phenomenon was also described in the crystal state.

In the last part of this section we will treat and classify the large body of available literature data on the control of the screw-sense of achiral, Aib-rich, β-turn, incipient or fully-developed 3_{10}-

helical (depending on peptide main-chain length) host sequences induced by one (or two contiguous) chiral, either C^{α}-tri- or C^{α}-tetrasubstituted α-amino acid(s). The discussion will be first focused on the case where the interpretation of the results is the most straightforward, namely when the localization of the guest residue(s) is in an *internal* position of the sequence [160,190,213,219-238]. Here, a *single* C^{α}*-alkylated* α-amino acid guest [e.g. Xxx = isovaline; C^{α}-methyl, C^{α}-allylglycine; C^{α}-ethylleucine; *bis*-azido side-chain substituted Ac_5c] often generates two diastereomeric turns / helices in Aib/Xaa peptides, where Xxx exhibits negative ϕ,ψ values in one molecule and positive ϕ,ψ values in the other molecule. This phenomenon can be viewed as the result of the interplay and balance between the intrinsic difference in stability beween the two helical diastereomers and the energetics of intermolecular interactions involved in crystal packing. The differences in terms of direction and extent of screw-sense bias among chiral C^{α}-tetrasubstituted α-amino acids are analyzed in ref. [12].

On the other hand, if the *single* guest residue was a C^{α}*-tri*substituted α-amino acid, the relationship between α-carbon chirality and helical handedness was overwhelmingly that typically found in proteins, *i.e.* an *L*-amino acid has negative (right-handed) ϕ,ψ values [239]. Obviously, if the guest was a D-residue, the ϕ,ψ values were found to be positive. However, one exception, where right-/left-handed diastereomeric helices concomitantly occur in the same (one L-Ala / seven Aib) octapeptide crystal, was published [238(c)]. Interestingly, the same situation was even reported for (i) a heptapeptide characterized by two *non*-contiguous (L-Leu, L-Ala) protein residues [238(d)], and (ii) a sequence of two contiguous Leu residues of opposite chiralities (-D-Leu-L-Leu-) [236].

When the single *N-terminal* residue is the chiral guest, the major driving factor biasing the screw sense of the following helical structure is the type of β-turn (whether II / II′ or III / III′) formed at the beginning of the sequence [160,162(b),219,225,229,235,237,240-255]. Three laboratories (Tanaka and coworkers in Japan, Clayden and coworkers in Manchester, U.K., and our group in Padova) contributed to *ca*. 90% of the publications in this area. In all peptides of this series investigated, Aib is by far the achiral helicogenic residue most utilized, but Ac_6c, Api and ΔPhe ($C^{\alpha,\beta}$-didehydrophenylalanine), also helicogenic, occur in the reported sequences, although much more rarely. It should also be kept in mind that a large part of the literature papers are based on X-ray diffraction data, where the results may sometime be overturned by crystal packing forces. In any case, we believe that in general most of the results can be classified on the basis of the following considerations.

(1) If the N-terminal, chiral L-α-amino acid (even a chiral L-α-hydroxy acid was used as an α-amino acid replacement) is C^{α}-trisubstituted, in general two possibilities should be taken into

account. They are: (1a) This residue may prefer the *semi*-extended conformation ($\phi \approx -60°$, $\psi \approx 150°$) which, combined with the adjacent achiral residue, will easily produce a type-II β-turn and force the helix to be *left*-handed. (1b) Alternatively, this residue may adopt the 3_{10}-helical conformation ($\phi \approx -60°$, $\psi \approx -30°$), which will generate a type-III β-turn and induce the helix to be *right*-handed. Clearly, opposite helix handedness are seen when the chiral residue is D-configurated. However, a few exceptions were described: (i) peptides with an N-terminal -L-Xxx-Aib- (where Xxx is a proein amino acid) sequence, which are characterized by a type-III′ β-turn followed by a left-handed helix [240,248,249], and (ii) peptides with multiple independent molecules in the asymmetric unit of their crystals, 50% of them being right-handed helical and 50% left-handed helical [162(b),241(a),242,252].

(2) If the N-terminal, chiral L-residue is C^{α}-*tetra*substituted, the above (1b) choice (helical residue conformation, type-III β-turn, right-handed helix) is much more frequently authenticated experimentally. In any case, co-existence of diastereomeric molecules (with L-configuration for the single chiral residue and right- or left-handed helices) was demonstrated in about 20% of the crystalline peptides of this type studied.

In our view, to date there is not enough experimental information to anticipate the role of the steric and electronic properties of the N^{α}-amino protecting / blocking group (urethane *versus* amide), such as bulkiness and/or basicity, in biasing the handedness of the helix that is formed [254], although this role cannot be excluded *a priori*. Also, the thus far available picture on the control of the N-terminal conformation (*semi*-extended *versus* helical) associated with its more or less pronounced bulkiness is not at all clear-cut. To probe the influence of the two above mentioned, potential biasing factors (protecting / blocking group and type of C^{α}-trisubstituted residue), more extensive, appropriately planned selections of the peptide substrates are needed. At this stage of the research in this area, a undisputable stereochemical conclusion is that one or, even better, two N-terminal C^{α}-tetrasubstituted L-(αMe)Val residue(s) induce(s) almost exclusively a right-handed helix by assuming itself (themselves) helical ϕ,ψ torsion angles.

Finally, two contributions [256,257] dealt with $-(Aib)_4-$ helices preceded by homo- or heterochiral -Pro-Xxx- (Xxx = Ala or Pro) dipeptide sequences. All four N-terminal Pro residues adopt the *semi*-extended conformation. Only one (L-Pro-D-Ala) heterochiral sequence is folded in the common type-II β-turn. As expected, this 3D-motif is followed by a left-handed helix. In the D-Pro-L-Pro sequence, formation of the type-II β-turn is prevented by interaction of the peptide carbonyls with a co-crystallized water molecule. As a result, the ϕ,ψ angles for the L-Pro residue are markedly distorted from those typical of position 2 for such a turn conformation. Nevertheless,

the -(Aib)$_4$- sequence is still left-handed helical. As for the two homo-chiral sequences, -D-Pro-D-Ala- unexpectedly forms a type-II′ β-turn which is regularly followed by a right-handed helix, while -L-Pro-L-Pro- is in the fully *semi*-extended, type-II poly-(Pro)$_n$ conformation followed by a left-handed helix. From these results on N-terminal chiral dipeptides it is quite clear that it is too early to draw any definitive conclusion on their role in governing the handedness of an attached helical sequence.

The number of published examples where the chiral guest residue is located at the *C-terminus* of a helix is significantly more limited [190, 219,225,238(b),244,258-261]. In analyzing these data, it should be kept in mind the important point, already stressed more than 30 years ago [143], that incipient and fully-developed, Aib/Ac$_6$c/ΔPhe-based 3$_{10}$-helical peptide esters (but not primary nor secondary amides, Figure 9) typically show at their C-terminal residue an opposite handedness as compared to that of the preceding residues. Considering that L-α-amino acids largely prefer negative ϕ,ψ angles, typical of right-handed folding, it is not surprising to detect very frequently left-handed helices for peptide ester segments containing a single, C-terminal L-residue. We have originally explained [225] this "inverse" relationship on the basis of an unfavorable O … O interaction taking place between the carbonyl oxygen atom of the *i*-2 amino acid from the C-terminus and either oxygen atom of the ester functionality if the sign of the ϕ torsion angle of the C-terminal (*i*) residue is the same as that of the preceding 3$_{10}$-helical residues, and that this interaction can be removed by changing the sign of ϕ, *i.e.* by rotating it by 180° as shown in Figure 9.

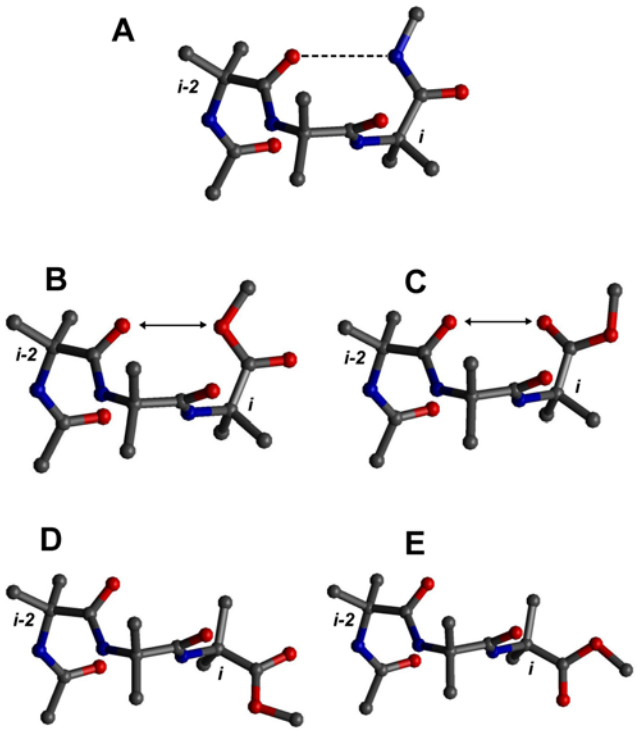


Figure 9. (A) Model of a right-handed, 3_{10}-helical peptide *N-alkyl amide*, showing the C=O…H-N intramolecular hydrogen bond typical of the type-III β-turn conformation at the C-terminus (in this model the torsion angles ϕ_i, ψ_i are -60°, -30°, where *i* is the C-terminal residue). (B) Model of a right-handed 3_{10}-helical peptide *ester*, showing the unfavorable C-terminal interaction between the C=O oxygen of the *i*-2 residue and the C-OR oxygen of the *i* residue taking place if ϕ_i, ψ_i are -60°, -30°. (C) Model of a right-handed 3_{10}-helical peptide *ester*, showing the unfavorable C-terminal interaction between the C=O oxygen of the *i*-2 residue and the C=O oxygen of the *i* residue taking place if ϕ_i, ψ_i are -60°, +120°. (D) Model of a right-handed 3_{10}-helical peptide *ester*, where ϕ_i, ψ_i are +60°, +30°. (E) Model of a right-handed 3_{10}-helical peptide *ester*, where ϕ_i, ψ_i are +60°, -120°. In models (D) and (E) the unfavorable O … O interaction is removed by rotation of the ϕ_i torsion angle by 180°.

However, the issue of the helical screw sense of peptide molecules with the only chiral residue positioned at the C-terminus is more complex than that expected, at least in the crystal state. Indeed, by X-ray diffraction we found that the full -(Aib)$_4$-L-Val- sequence, *including* the C-terminal chiral residue, is right-handed 3_{10}-helical, whereas the -(Aib)$_4$- sequence of the L-(αMe)Val based compound adopts both helical screw senses [261]. In each diastereomeric helix of

the latter pentapeptide the C-terminal chiral residue is characterized by an opposite screw sense with respect to that of the preceding -(Aib)$_4$- sequence. Clearly, this result implies that in the right-handed helix the helical L-(αMe)Val residue is forced to fall in the unfavorable 1,1 quadrant of the ϕ,ψ map. At this point, it is reasonable to assume that all four possible conformations [based on the right- or left-handed -(Aib)$_4$- sequence, and on the positive or negative ϕ torsion angle for the C-terminal chiral residue] would populate the equilibrium mixtures in *solution*, although to a different extent, and that the net results of this phenomenon would be unraveled by the CD spectra. On the other hand, we believe that in the *crystal* state additional (packing) forces would often overcome those predominant in solution and would drive the preferred structure to an energetically unfavorable situation for the isolated molecule. In any case, the observation of two molecules with opposite screw sense in the L-(αMe)Val peptides and only one molecule in the L-Val peptides indicates that in the crystal state the difference in stability among the various conformers is lower for the former compounds compared to their C$^\alpha$-unmethylated counterparts.

C$^{\alpha,\beta}$-Didehydro-α-Amino Acid Containing Peptides

α,β-Unsaturated (didehydro)-α-amino acids (Figure 10), typically represented by the notation ΔAAs, are often reported to occur in natural peptides of microbial origin and, to a limited extent, in proteins (*e.g.* in the bioluminescent proteins) as well [262-268]. In particular, they characterize the well-studied family of lantibiotics, *i.e.* polycyclic peptides with antibiotic activity. ΔAA-containing peptides exhibit peculiar lipophilic properties and an increased resistance to proteolytic attacks. Incorporation of ΔAAs into peptides by chemical synthesis is a useful method to analyze sequence-function relationships in bioactive peptide analogs.

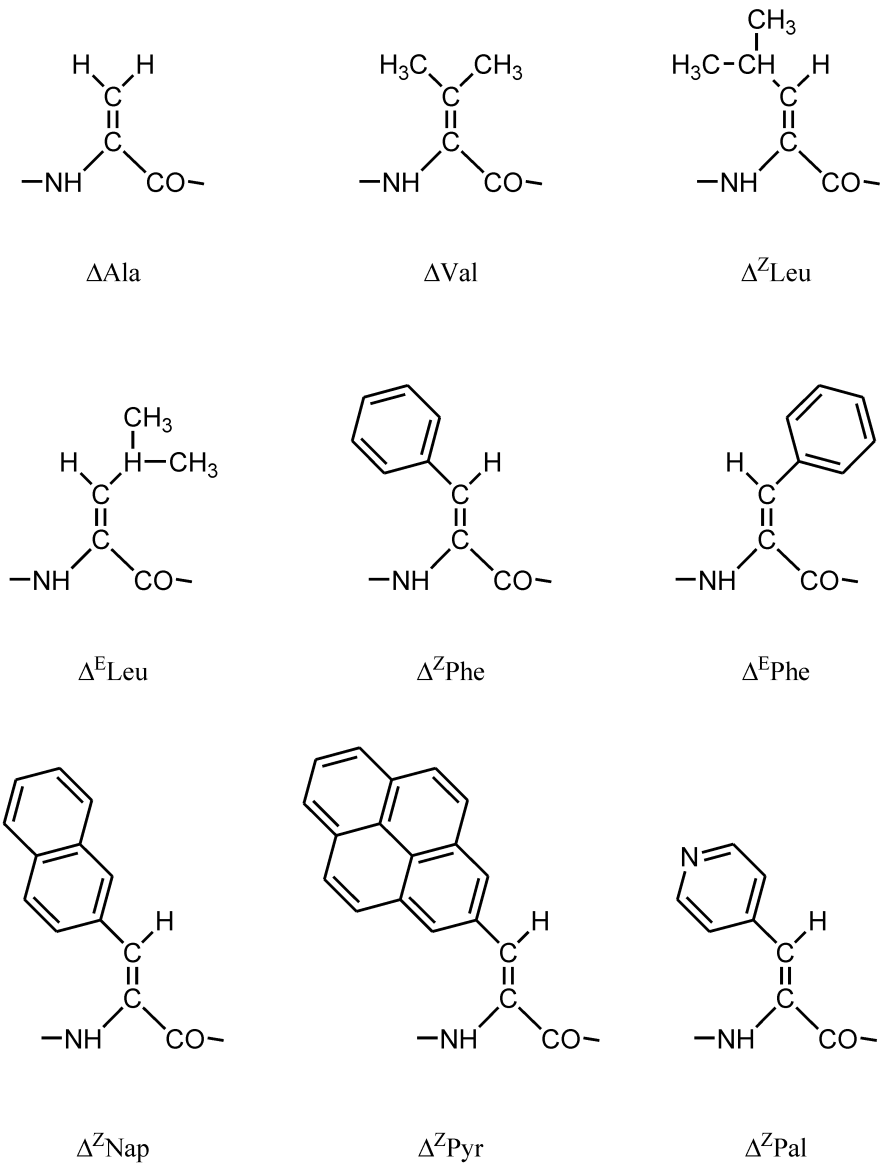


Figure 10. Chemical formulas of the α,β-didehydro (Δ) α-amino acid residues, the peptide conformations of which have been investigated.

The stereochemical effects of the three functionalities (the $C^{\alpha}=C^{\beta}$ double bond, in addition to the usual α-NH and α-CO groups) at the α-carbon atom are remarkable [269-274]. More specifically, the presence of an sp^2 hybridized α-carbon in the main chain along with the modified electron distribution associated with the α-β π-system, and the change in the side-chain rotamer population contribute markedly to the conformational preference of the peptide backbone. The double bond linking the C^{α} and C^{β} atoms significantly reduces the flexibility of both the peptide main chain and the specific amino acid side chain. Electron conjugation is typically associated with

an extended conformation, but in specific ΔAAs their overall conformation is also governed by side-chain steric requirements. All ΔAAs related to protein amino acids show *cis* (*Z*) – *trans* (*E*) geometrical isomerism about the C=C double bond, except ΔAla (Figure 10), without substituents at C^{β}, and ΔVal (Figure 10), with two substituents at C^{β}. An example of this type of isomerism is reported in Figure 10 for ΔPhe. In the *Z*-diastereomer the carbonyl group is in the *trans* disposition with respect to the phenyl moiety, while in the *E*-diastereomer it is in the *cis* disposition.

The three most widely examined conformational preferences are those of ΔAla-, ΔLeu- (Figure 10), and ΔPhe-containing peptides. In particular, the published computational and experimental 3D-structural analyses provide clear evidence that the fully-extended (C_5) conformation is that largely preferred by ΔAla homo-peptides to the octamer level [275-281]. Multiple, consecutive C_5 structures, which generate the 2.0_5-helix [188], predominate in solvents of low polarity and occur in the crystal state. These peptide molecules are completely flat, including the amino acid side chains, and form planar, isolated (non self-associated) sheets. This 3D-structure is stabilized by two types of intramolecular H-bonds, N1-H … O1=C′1 (typical of the 2.0_5-helix) and C^{β}1-H … O0=C′0 (characteristic of ΔAla peptides) (Figure 11). The former produces a 5-membered pseudo-ring (C_5), while the latter gives a 6-membered pseudo-ring (C_6). Not surprisingly, since this structure does not participate in any intermolecular H-bonding motif, a peptide containing the -$(\Delta Ala)_3$- sequence was reported to disrupt the nucleation of the Aβ amyloid peptide aggregation [282]. Clearly, the homo$(\Delta Ala)_n$ 2.0_5-helix, although 3D-structurally unique and of potential biomedical / nanotechnological interest, being completely flat and stable, is not amenable to undergo any screw sense preference or switching. Interestingly, however, peptides of the (X-$\Delta Ala)_n$ type, with X = any chiral (L) protein amino acid, containing ΔAla in alternate position adopt a β-turn ribbon structure in which the C=O moiety of the *i* residue is intramolecularly H-bonded to the N-H moiety of the *i*+3 residue, resulting in a repetitive, distorted type-II 10-membered pseudo-ring (C_{10}) form or β-turn in which only alternate peptide groups are involved in the H-bonding scheme [277]. Here, the ϕ,ψ torsion angles of the ΔAla residues are those typical of a *helical* amino acid. Therefore, not surprisingly, in the achiral (Aib-$\Delta Ala)_n$ sequential peptides the (right- and left-handed) 3_{10}-helical structures are the most stable. Unfortunately, all these calculated peptide 3D-structures are still awaiting for an experimental validation.

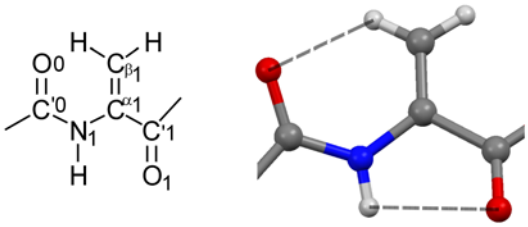


Figure 11. The flat 3D-structure (right) of the ΔAla building block (left), the repetition of which generates a unique fully-extended 2.0_5-helix.

On the basis of the experimental conformational observations of their linear peptides, ΔAAs were classified into a limited number of broad categories to derive useful design strategies [271]. In particular, the ΔAAs with a *single* substituent at the C^{β} atom, such as ΔLeu and ΔPhe, tend to exhibit a folded backbone induced by unfavorable steric effects between the atoms of the side chain on one side and those of the main chain or neighboring side chains on the other side. Specifically, the 3D-structures of only a few ΔLeu-containing di- and tripeptides were analyzed in solution or by X-ray diffraction in the crystal state (for review articles, see refs. [270-272]). Unfortunately, neither homo- nor longer peptides were examined. These examples, although quite limited in number to allow generalization, suggest that in peptides long enough to form an intramolecularly H-bonded β-turn conformation, *e.g.* N-acyl dipeptide amides or tripeptide esters, the Δ^{Z}Leu residue is found to nucleate such a turn, itself occupying the (*i*+2) corner position. In any case, it is evident that the field of the conformational preferences of Δ^{Z}Leu (and Δ^{E}Leu as well) homo-peptides deserves to be investigated by use of appropriate model compounds and modern physico-chemical techniques. However, it is worth pointing out that in 2007 the main-chain length dependent conformational behavior of $(\Delta Leu)_n$ homo-oligomers in both the pure *Z* and *E* diastereomeric forms and their various combinations was examined by quantum mechanical calculations [283]. New types of intramolecularly H-bonded, left- and right-handed helices (further stabilized by carbonyl … carbonyl interactions), including the unusual 2_7-helix structure, generated by multiple consecutive γ-turns [284,285], were found to be the most stable. Moreover, a right-handed template was obtained by incorporating a chiral L-Leu residue at the C-terminus or a D-Leu at the N-terminus of

a -(Δ^{Z}Leu-Δ^{E}Leu)$_n$- sequential repeat. So far, no attempt has been made to authenticate experimentally any of these new 3D-structures.

At variance with all other ΔAAs, an extremely large body of publications deals with ΔPhe- (in particular, Δ^{Z}Phe-) containing peptides because of their relative easiness of synthesis and the presence of the exceptionally useful >C=CH-C_6H_5 (styryl) moiety. Notably, it generates the *E,Z* geometrical isomerism, but, more important, when conjugated to the peptide group, its chromophore exhibits a *near*-UV absorption spectrum characterized by a very strong, conformationally quite useful, absorption band with a maximum near 280 nm, which has been assigned to a charge-transfer transition from the electron-donating styryl group to the electron-accepting carbonyl group in the ΔPhe residue [286,287]. The chromophoric system of this residue, therefore, is essentially the cinnamoyl moiety, O=C-C=C-C_6H_5. None of the ΔAAs monosubstituted with an aliphatic moiety at the C^{β}-atom shows an UV absorption maximum above 250 nm (the most interesting peptides of this class, namely those based on ΔAla, afford a single sharp peak at 240 nm of medium intensity). The reasons for the spectral differences observed between the diastereomeric Δ^{Z}- *vs.* Δ^{E}-peptides are not yet well understood. An additional, strong absorption at about 220 nm dominates the far-UV electronic spectrum of ΔPhe peptides. It is evident that this latter spectroscopic property, unfortunately, is potentially confusing in terms of a correct peptide conformational assignment which is heavily based on the far-UV amide electronic transitions. In the near-UV region the absorption spectra of sequential peptides based on the related (*Z*)-β-(1-naphthyl)-didehydroalanine (Δ^{Z}Nap) (Figure 10) residue show an intense band at 310 nm, corresponding to that of Δ^{Z}Phe peptides at 280 nm [288]. These spectra do not change in the peptide series studied with (*i*, *i*+3) separated Δ^{Z}Nap residues, which indicates that no strong ground-state interactions take place between the Δ^{Z}Nap … Δ^{Z}Nap pairs. The transition moment of the 310 nm band lies approximately perpendicular to the long axis of naphthalene, suggesting that the transition has the typical character of the ^1L_a band of the 1-naphthyl chromophore. Moreover, the position of the λ_{max} depends markedly on the variation of the amino acid side-chain χ^2 torsion angle, which favors the conclusion that the transition has a less polarized character with λ_{max} sensitive to the side-chain conformation. This character will result in a broad band, as experimentally observed. A tripeptide based in an additional aromatic ΔAA, namely (*Z*)-β-(1-pyrenyl)-didehydroalanine, Δ^{Z}Pyr (Figure 10), was also examined by UV absorption [289]. In its spectrum two intense bands were found in the near-UV region (at 360 and 280 nm). Interestingly, in both bands of this compound the marked vibronic patterns characteristic of the pyrenyl chromophore are almost absent, which suggest that the presence of the C^{α}=C^{β} double bond influences significantly its excited state. In the

solid state, the two bands are remarkably broadened, indicating that this phenomenon is associated to the occurrence of ground-state interactions between pyrenyl groups that are arranged in a well specific manner under these experimental conditions.

The first theoretical conformational studies of ΔPhe derivatives and very short peptides were conducted by two Italian groups in the 1981-1991 decade [290-292] (Figure 12). Numerous minima were found, determined by *Z*- (and *E*-) side-chain / main-chain electronic conjugation effects and repulsive interactions between the phenyl and adjacent oxygen carbonyl groups. Steric hindrance can be relieved by twisting the styryl group (χ^2 side-chain torsion angle $\neq 0°$). After this optimization, the agreement with the X-ray diffraction data becomes reasonable. More recently, additional investigations were performed on those model peptides [293-296] (Figure 12). In general, it may be concluded that the achiral Δ^ZPhe residues can be easily accommodated in the regions of the ϕ,ψ map where right- and left-handed 3_{10}- / α-helices and types I (I′) and II (II′) (for the latter, at position *i*+2) β-turns are found. Fully-extended and pleated β-sheet conformations correspond to high energy regions. A different arrangement of the side chain in the *Z* and *E* isomers forces them to adopt different conformations. Moreover, both *Z* / *E* isomers of the β-methyl ΔPhe residue results in an increase of the conformational freedom [297]. The ϕ,ψ maps of the related Δ^ZNap and Δ^ZPyr [288,289,297,298] essentially reflect those of Δ^ZPhe. Thus, all β-aryl-didehydroalanines theoretically investigated tend to exhibit the same conformational preferences, irrespective of the size of the aromatic β-substituent. Sequential oligopeptides (with doublets or triplets containing a single Δ^ZPhe residue) were also examined computationally [299,300]. Different types of helices were found. Peptides with a chiral Ala or Leu residue preceding or following a -$(\Delta^Z\text{Phe})_6$- block were studied with the aim at designing a helical peptide with a desired screw sense [301]. The two calculated most stable conformations for the homo-peptide Ac-$(\Delta^Z\text{Phe})_6$-NHMe are the degenerated, isoenergetic states with ϕ,ψ values of 0°,±90° without intramolecular H-bonds and the 3 kcal/mol less stable ±30°,±30° helix structures (the screw sense of this helix is dictated by the the χ^2 torsion angle, *i.e.* with $\chi^2 = -60°$ the screw sense is left-handed and with $\chi^2 = 60°$ the screw sense is right-handed) [302]. The former structure is stabilized by carbonyl … carbonyl and the (*i*)N-H … π(*i*) interactions (where π represents one C-C edge of its own phenyl ring). The conformational results for the terminally-blocked, isomeric Δ^EPhe homo-hexapeptide are similar, except for the χ^2 values. In these latter peptides the N-H … π interactions are not operative.

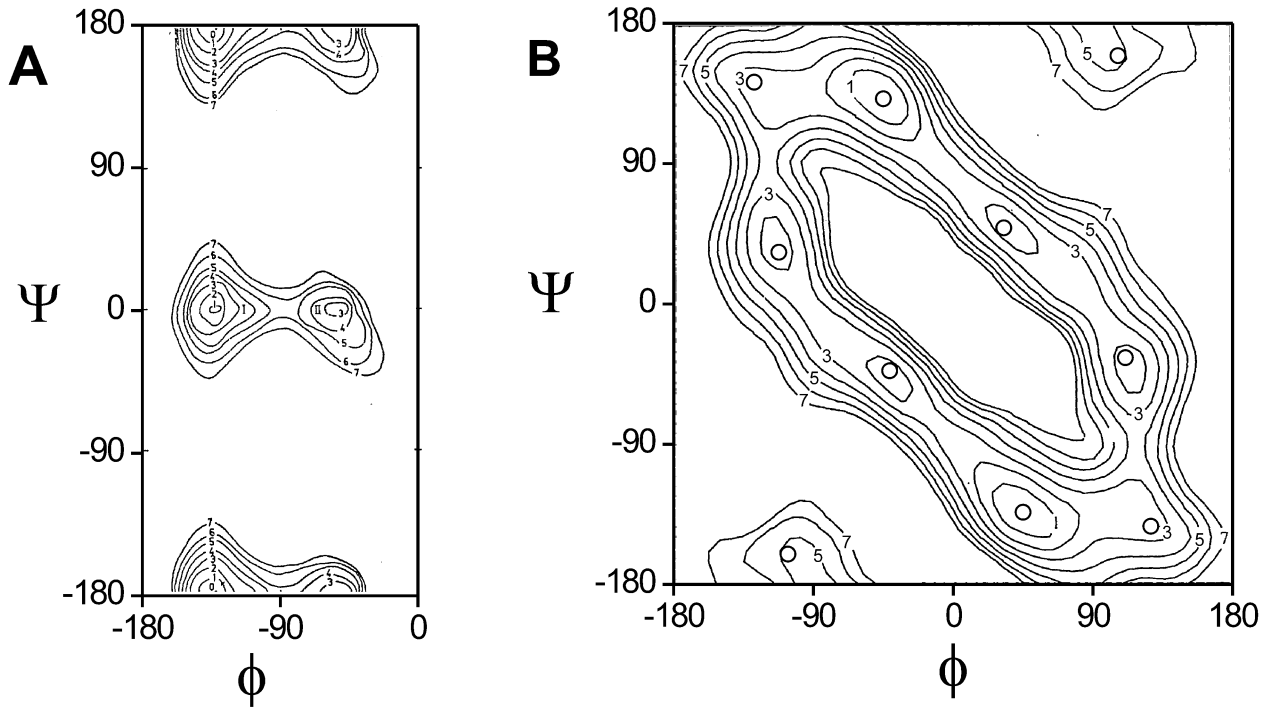


Figure 12. The ϕ,ψ conformational energy map for: (**A**) Ac-Δ^{Z}Phe-OH (adapted from [290]). Energy values are in kcal · mol^{-1} from the absolute minimum. As the molecule is achiral, only the region -180° ≤ ϕ ≤ 0° is shown. (**B**) Ac-Δ^{Z}Phe-NMe_2 at the *ab initio* HF/3-21G level of theory (adapted from [296]). The isopotential lines are spaced by 1 kcal · mol^{-1}.

Spectroscopic measurements were extensively employed for the assessment of the local (NMR) and overall (IR absorption) conformational properties of ΔPhe-rich peptides in solution, although with these compounds CD spectroscopy (see below) proved by far to be the technique of choice. In particular, IR absorption studies were not only conducted in solvents of low polarity, but extended to the solid state (KBr film, ZnSe crystal surface, Ag nanocolloids and electrodes) as well, where related spectroscopies (Raman, surface-enhanced Raman scattering, and vibrational CD) were also utilized [303-316]. These analyses were expanded to N^{α}-methylated ΔPhe residues where the *cis* tertiary amide conformation was shown to prevail. In solution, the conformationally useful amide A, I, and II absorption bands were assigned. Free (solvated) or inter-/intramolecular H-bonded bands were identified by recording the spectra at different solute concentrations. In this

connection, it should be kept in mind that: (i) the positions of the amide A and I vibrations in the IR absorption spectra are typically affected by the π-electron conjugation with the C=C bond; (ii) in the 1610-1690 cm^{-1} region a combined IR-Raman study, supported by DFT calculations, was needed to distinguish unambiguously the amide I band from that attributed to the $C^{\alpha}=C^{\beta}$ stretching mode. On the basis of the results obtained by use of these vibrational spectroscopies, β-/γ-turns and 3_{10}-/α-helical folded conformations were validated for the peptides investigated. Some IR absorption spectral differences between peptides containing Δ^{Z}Phe *vs.* Δ^{E}Phe residue were found. A study on two 5-mer peptides upon heating to 144°C showed that the 3_{10}-helix intramolecular H-bonds are more resistant to thermal denaturation than the corresponding H-bonds of the α-helix. Interestingly, a non-marginal degree of residual secondary structural elements were observed even above 104°C.

Privileged solution conformations of Δ^{Z}Phe-based peptides were satisfactorily determined by NMR analysis [269-273,281,289,294,297,298,305,307,308,310,311,315,317-337]. Short peptides containing Δ^{Z}Phe residues were generally shown to fold into conformations which may be perturbed by the H-bonding capabilities of the solvent. In the poorly solvating $CDCl_3$, intramolecularly H-bonded β-turns are usually preferred, while in more strongly solvating media, *e.g.* deuterated DMSO, an appreciable conformational heterogeneity, which includes extended species, is sometime observed. Longer peptides tend to adopt the 3_{10}-/α-helical structures. In general, these studies complemented well the IR absorption analyses in the same hydrocarbon. In these peptides, inaccessibility of one or more NH groups (beginning at the *i*+3 residue) to the solvent was established by NMR experiments, leading to the proposal that these groups are involved in the intramolecular H-bonding scheme. In N^{α}-protected tetrapeptide esters, two consecutive 1 ← 4 intramolecularly H-bonded β-turn structures (incipient 3_{10}-helices) were proposed to be compatible with the presence of the solvent shielded N(3)H and N(4)H groups. Fully developed helical structures are typical of longer peptides characterized by two (or more) Δ^{Z}Phe residues. One of the shortest examples of α-helix (succession of 1 ← 5 intramolecular H-bonds) is a heptapeptide with two Δ^{Z}Phe separated by three spacer residues. The choice between the 3_{10}-/α-helices depends on an appropriate combination of peptide main-chain length, number and relative positioning of the Δ^{Z}Phe residues, and solvent polarity. Two Δ^{Z}Phe residues in a hexapeptide separated by two residues are not enough to provide compelling conformational constraints in DMSO. Three Δ^{Z}Phe residues (at positions *i*, *i*+2, *i*+4) in an heptapeptide force it to adopt a 3_{10}-helix conformation. Interestingly, a pentapeptide containing three consecutive, central Δ^{Z}Phe (and two terminal L-Val residues) is folded in a *left*-handed 3_{10}-helix generated by a type-II β-turn at the

N-terminus. A 13-mer peptide consisting of six Δ^ZPhe residues at alternate positions is 3_{10}-helical in $CDCl_3$ solution. A terminally-blocked dipeptide with the -Pro-Δ^ZPhe- sequence largely prefers the type-II β-turn type of folding. In the *E*-diastereomer of the N^{α}-methylated Ac-Δ(Me)Phe-NHMe only the *cis* tertiary amide isomer is present, regardless of the solvent. This result is at variance with that of its *Z*-diastereomer, where the *trans* tertiary amide isomer largely prevails in polar solvents. However, this isomer disappears in solvents of low polarity. NMR studies allow distinguishing the *E*- and *Z*-isomers of Ac-ΔPhe-NMe_2. Conformational comparisons were made in 3-, 4-, and 6-meric peptides containing either one (or two) Δ^ZPhe or Δ^EPhe residues. In the hexamers, only the *bis*-Δ^EPhe peptide is able to adopt the 3_{10}-helix structure in DMSO solution. It was also found that the position of a single chiral protein amino acid in the main chain controls the screw sense of the 3_{10}-helix of a 5-mer peptide characterized by four achiral residues. As in the related Δ^ZPhe peptides, tripeptides based on a central Δ^ZNap or Δ^ZPyr residue are folded in a type-II β-turn conformation (in these compounds the protein amino acid at position 1 has the L-configuration). Longer (6-, 9-, and 12-mer) peptides containing 2, 3, and 4 Δ^ZNap residues, respectively, along with L-Ala / L-Leu amino acids, were found to accommodate predominantly in right-handed 3_{10}-helices. All these conformational conclusions were extracted from 1D- (^{1}H) NMR experiments as a function of peptide concentration, temperature, and solvent polarity, and from 2D-NMR NOESY spectra.

Induced CD proved to be an excellent tool to determine the solution conformation of peptides based on the achiral ΔPhe residue, especially in detecting the screw sense of the helices formed. It is principally for this reason that the number of publications on this topic is huge [272-274,287-289,299,303,316-319,323-333,338-352]. The Δ^ZPhe-containing peptides show different CD curves in the near-UV region depending on the main-chain length, and on the position in the sequence and number of the Δ^ZPhe residues. CD spectra of very short peptides with a single Δ^ZPhe residue exhibit only low-intensity bands. In general, the shape and sign of these absorptions are strongly affected by the nature of the nearby chiral α-amino acid. CD spectra of Δ^ZPhe tri- and longer peptides of this type show a broad, relatively intense band at about 280 nm, corresponding to the main absorption of the cinnamoyl chromophore. The varying intensities of this band are indicative of the different propensities of the peptides to fold into β-turns. This conclusion is confirmed by the solvent dependence of the intensity of this absorption.

Peptides containing two or more (up to eight, of which 2-4 are consecutive) Δ^ZPhe residues show a couplet of intense bands with opposite signs at 300 and 260 nm and a cross-over point near 280 nm (Figures 13 and 14). This CD pattern is typical of exciton splitting due to the dipole-dipole interactions between the Δ^ZPhe chromophores and is a strong indication that the two Δ^ZPhe residues

are placed in a mutual, fixed disposition within the 3D-structure of the molecule, generally a 3_{10}-helix. The (– +) signs (in the direction of decreasing wavelengths) of the couplet correspond to a 3_{10}-helix with a right-handed screw sense, while the (+ –) couplet is assigned to the helix with the left-handed screw sense. For a 3_{10}-helix characterized by an integral (3.0) number of amino acid residues per turn, the transition moments would have a parallel arrangement along the helix and would not show any exciton coupling. However, since these peptides do exhibit remarkable exciton couplets, one may conclude that they are folded in 3_{10}-helices with a non-integral number of residues per turn (as observed experimentally by X-ray diffraction analyses [28]). However, if a pentapeptide with two (*i*, *i*+2) Δ^ZPhe residues has an S-shaped conformation instead of a 3_{10}-helix, then the aromatic side chains are not interacting since they lie on opposite sides if the backbone. In this case, the exciton coupling phenomenon is not observed. Interestingly, two *quasi*-diastereomeric, 3_{10}-helical hexapeptides, one (carboxylic ester) containing two Δ^ZPhe residues at positions 2 and 5 and the other (free carboxylic acid) with two Δ^ZPhe residues at the same positions in the sequence show *quasi*-enantiomeric CD exciton couplets in the 250-320 nm region in CH_3CN solution (Figure 14).

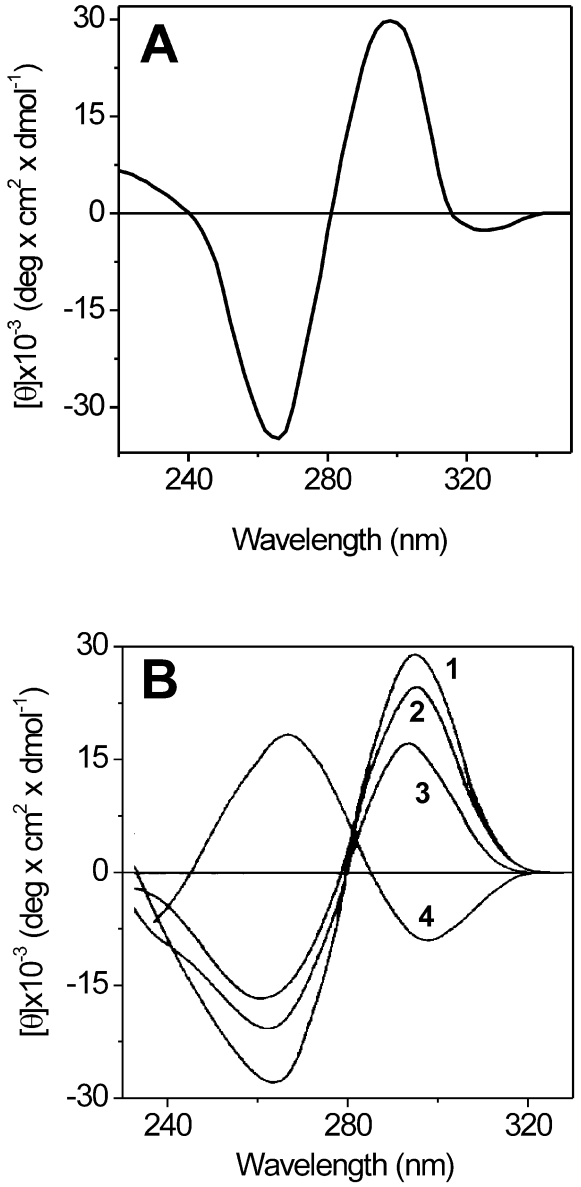


Figure 13. CD spectra of: (**A**) The tetrapeptide with three consecutive Δ^{Z}Phe residues Ac-(Δ^{Z}Phe)$_3$-L-Ala-OH in MeOH solution (adapted from [287]). (**B**) The pentapeptide Boc-L-Ala-Δ^{Z}Phe-Gly-Δ^{Z}Phe-L-Ala-OMe in CH_3CN (**1**), CH_2Cl_2 (**2**), MeOH (**3**), and 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) (**4**) solutions (adapted from[340]).

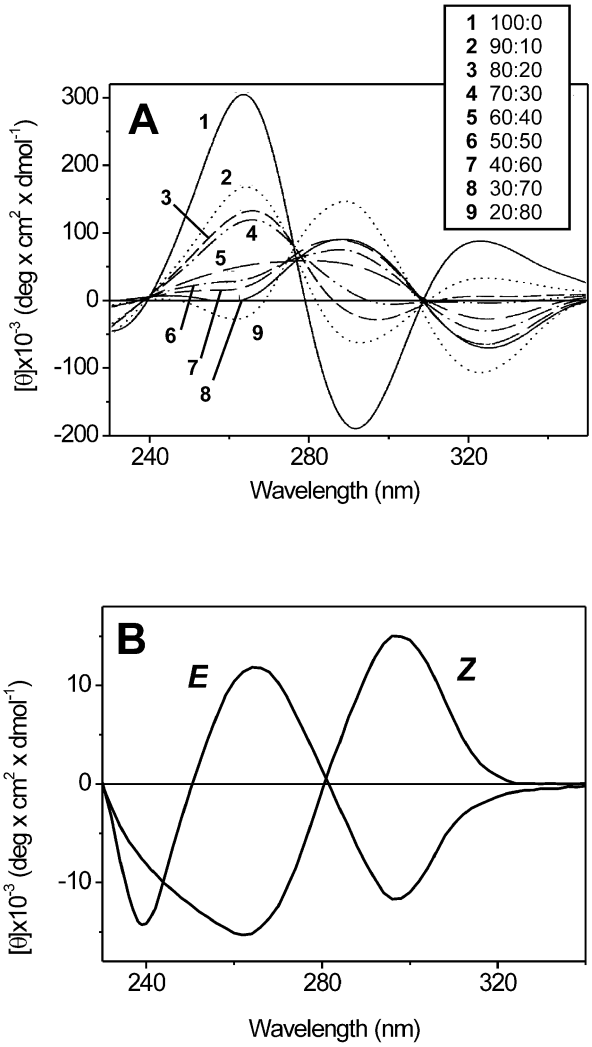


Figure 14. CD spectra of: (**A**) The decapeptide Boc-L-Ala-(Δ^{Z}Phe)$_4$-L-Ala-(Δ^{Z}Phe)$_3$-Gly-OMe in $CHCl_3$ – MeOH solvent mixtures (curve **1**: 100% $CHCl_3$) (adapted from [343]). (**B**) The hexapeptides Boc-Gly-Δ^{E}Phe-L-Phe-Gly-Δ^{E}Phe-L-Phe-OH (*E*) and Boc-Gly-Δ^{Z}Phe-L-Phe-Gly-Δ^{Z}Phe-L-Phe-OMe (*Z*) in CH_3CN solution (adapted from [332]).

Comparison of ellipticity of the peptides containing two and three Δ^{Z}Phe residues show similar values, in spite of the presence of an extra Δ^{Z}Phe in the latter case. This should actually be the case if the 3_{10}-helical conformation of the peptide would terminate with a type-I, instead of a

type-III, β-turn. It may also be a result of the change of the helix sense of the last residue or unwinding of the helix, a common observation at the helix C-terminus [28]. In the peptides where the *chiral* residue(s) are located in internal helix position(s) of the sequence, the helix is right-handed, while peptide esters (but not peptide primary / secondary amides) where the *single* chiral residue is at the C-terminus are found to predominantly adopt the left-handed helical sense. If the *single* chiral residue is, however, at the N-terminus, the CD results are consistent with the concomitant presence of right- and left-handed conformers also in solution, with a prevalence of the more stable right-handed helix (but only if the first β-turn is not type-II). Interestingly, in a few peptides a spectacular *reversible screw sense inversion* of the 3_{10}-helix was detected, depending on solvent (Figures 13 and 14) and temperature conditions. The authors used the signs of the CD couplets to unambiguously monitor the inversion of the helix sense. A CD band was also observed at 320 nm in some of these compounds that also changes sign with changing solvent polarity (Figure 14). One reason for the occurrence of this band may be the weak electronic transition polarized along the short axis of the benzene ring. This contribution to the CD spectrum suggests that the benzene ring is not free to rotate, as expected, owing to the presence of the $C^{\alpha}=C^{\beta}$ double bond. Its strong intensity also indicates that the phenyl ring is restricted to a preferred chiral disposition. The exciton coupled of an Ac-(Δ^{Z}Phe)$_2$- moiety covalently attached to the side-chain ε-amino function of Lys residues in a random (Glu_{92}, Lys_8)$_n$ co-polypeptide was found to be an excellent chirospectroscopic probe to detect the α-helix / unordered conformation transition in a pH-dependent investigation. It should be also pointed out an additional advantage of the ΔPhe chromophore as a CD probe, namely that spectra can be recorded even in solvents (*e.g.*, DMSO) that do not allow measurements in the far-UV (peptide) region, thus permitting a usually impossible comparison of the conformational preference of a given peptide in the *same* solvent by taking advantage of the two most informative spectroscopies (NMR and CD). The CD technique was also demonstrated to be quite effective and useful for readily obtaining preliminary conformational data in the solid state (KBr disk) on Δ^{Z}Phe-rich peptides, particularly on those difficult to crystallize, by observation of the 280 nm exciton couplet. Other α,β-didehydroaromatic residues the CD properties of which were studied both experimentally (in solution and in the solid state) and theoretically, include Δ^{Z}Nap and Δ^{Z}Pyr [288,289]. The observed CD curves were interpreted on the basis of the exciton chirality method.

As in the case of the Aib-peptides discussed above, many ΔPhe-containing, linear peptides are highly crystalline materials. This property allows X-ray diffraction analyses to be performed [269-274,287,289,298,303,304,320-327,330,334,342,343,345,346,352-392]. Almost all of them are characterized by the Δ^{Z}Phe configurational isomer. Clusters of crystallographically determined ϕ,ψ

backbone torsion angles values correspond to the regular (±60°,±30°) [and distorted (±80°,0°) as well] helical region or to the *semi*-extended conformation (-60°,140° or 60°,-140°). The ΔPhe achiral nature well justifies adoption of both isoenergetic, enantiomeric dispositions. Therefore, this residue can easily accommodate at either *i*+1 or *i*+2 corner position of type-I (I′) and type-II (II′) β-turns in terminally protected, short (tri-) peptides. These turns are typically stabilized by an 1 ← 4 intramolecular H-bond. Fully-extended (180°,180°) and β-sheet (-130°,125° or 130°,-125°) conformations, and the tighter γ-turns (-70°,70° or 70°,-70°) are unobserved yet. In tripeptides where the β-turn is not formed, an S-shaped conformation is occasionally seen. For example, this is the case for the -Δ^{Z}Phe-Δ^{Z}Phe-Ala- tripeptide sequence where the two consecutive Δ^{Z}Phe residues populate the left- and right-handed helical regions, respectively, and the aromatic lateral chains are disposed on opposite sides of the backbone.

In the Δ^{Z}Phe peptides longer than tripeptide, the fully developed (Figure 15) or incipient (Figure 16) 3_{10}-helix is the most common 3D-structure recorded in the crystalline state. Peptide X-ray diffraction structures were also solved with sequences characterized by two, three, and four consecutive Δ^{Z}Phe residues. The longest sequences are 10-, 11-, and even 21-amino acid long. This last peptide is based on as many as eight Δ^{Z}Phe residues. In peptides with multiple Δ^{Z}Phe residues, these unsaturated amino acids are spaced by one, two, three or four protein amino acids. At the C-terminus, some peptides with three or four residue spacers and the achiral ΔPhe as the penultimate amino acid display a π-turn (with 16 atoms in the *pseudo*-ring closed by the intramolecular C=O … H-N H-bond [393,394]) which cooperates in generating a Schellman motif [197]. In this structural element, the achiral residue (ΔPhe) is appropriately located at this strategic position where a *left*-handed helical amino acid is required. It facilitates formation of the 2 ← 5 intramolecular H-bond (in addition to the 1 ← 6 H-bond typical of the π-turn). In proteins, generally the achiral Gly [395] and less often the chiral L-Asn (known to fold easily in a left-handed helix [1]) are found in this position.

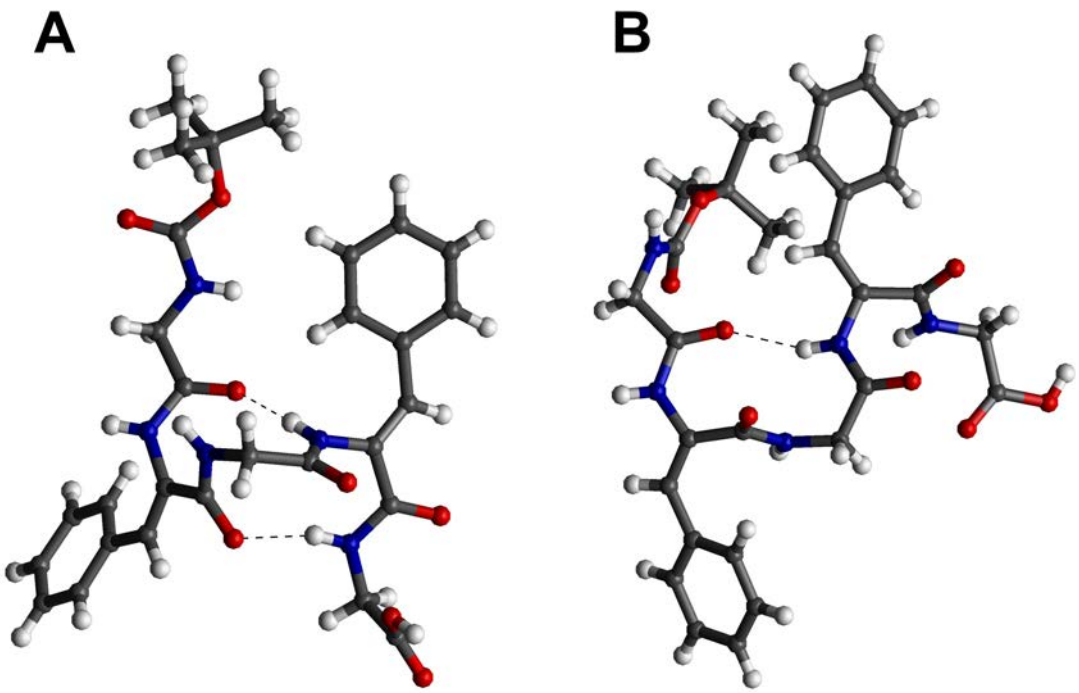


Figure 15. X-Ray diffraction structures of: (**A**) The pentapeptide Boc-(Gly-Δ^{Z}Phe)$_2$-Gly-OH with two consecutive type-III′ β-turns (incipient, left-handed 3_{10}-helix). (**B**) Its configurational isomeric peptide Boc-(Gly-Δ^{E}Phe)$_2$-Gly-OH with one type-II β-turn. The Δ^{Z}Phe residues at positions 2 and 4 in **A** and Δ^{E}Phe at position 4 in **B** are left-handed helical, in contrast to the *semi*-extended Δ^{E}Phe at position 2 in **B**. The 1 ← 4 intramolecular H-bonds are shown as dashed lines (adapted from [381]).

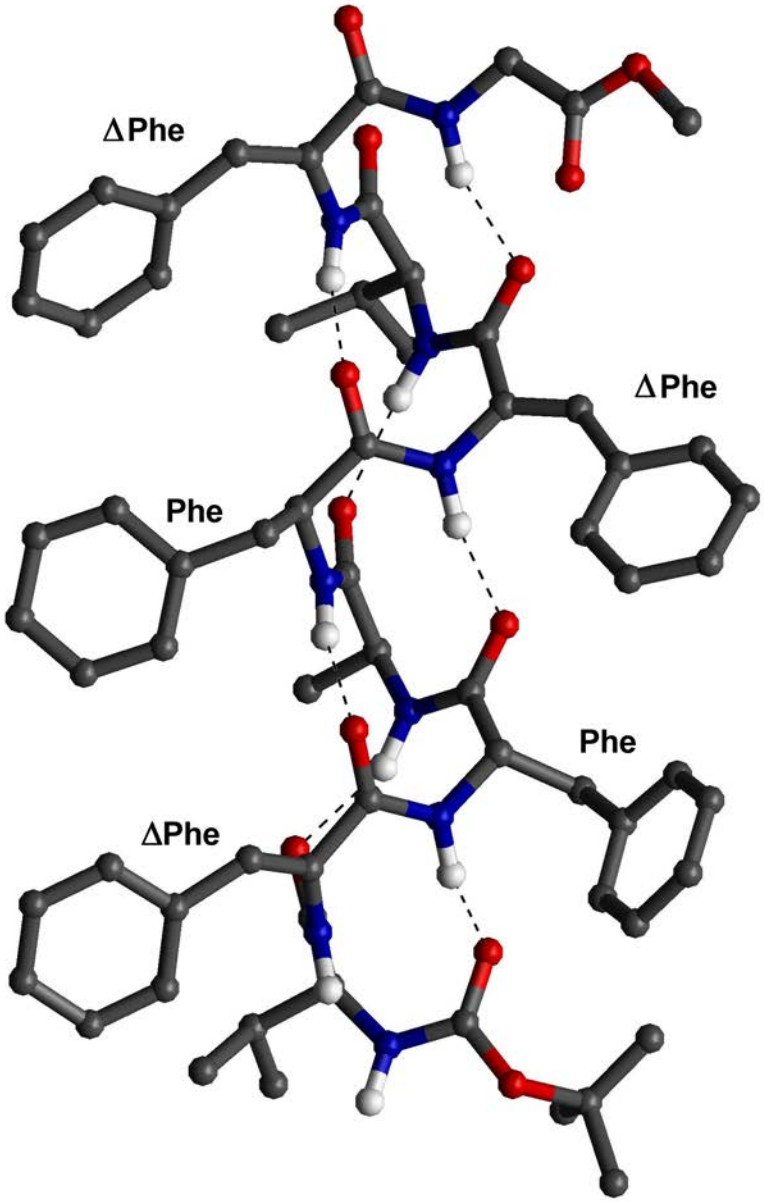


Figure 16. The X-ray diffraction structure of the nonapeptide Boc-Val-Δ^{Z}Phe-Phe-Ala-Phe-Δ^{Z}Phe-Val-Δ^{Z}Phe-Gly-OMe viewed perpendicularly to the right-handed 3_{10}-helix axis. The seven, consecutive 1 ← 4 intramolecular H-bonds are shown as dashed lines. The five benzyl side chains assume an energetically favorable, staggered arrangement, which slightly deviates from the ideal 3_{10}-helical arrangement where the side chains are completely eclipsed (adapted from [373]).

There are a few exceptions to the general rule of 3_{10}-helix formation in long Δ^{Z}Phe peptides. Interestingly, the shortest (two α-turns) α-helical structure validated to date in model compounds

was found for the terminally-protected pentapeptide sequence -Val-Δ^{Z}Phe-Ala-Leu-Gly-. In this connection, the length of the spacer segment between two Δ^{Z}Phe and the overall peptide length are both crucial features in governing the type (whether α- or 3_{10}) of the helix adopted. Another pentapeptide, with two Δ^{Z}Phe residues at positions 2 and 4, exhibited a flat β-turn ribbon instead of the expected 3_{10}-helix. All long peptides containing two, three, or four *consecutive* Δ^{Z}Phe residues exhibit 3_{10}-helical structures. These helices are right- or left-handed depending on the conformation of the N-terminal L-residue. If it is *semi*-extended, then the 3D-structure starts with a type-II β-turn which is obligatorily followed (for steric reasons) by a left-handed helix. Alternatively, if it is helical, then the 3D-structure starts with a type-III β-turn which is followed by a right-handed helix. It is also worth noting that in the -CO-L-Ala-(ΔPhe)$_2$-NHMe sequence both diastereomeric (right- and left-handed) helices occur in the same asymmetric unit. A pentapeptide provided the first example of a short β-sheet stretch that is terminated by a ΔPhe residue. This result will stimulate a new strategy for the development of amyloid formation inhibitors by use of ΔPhe as a β-breaker residue. The X-ray diffraction structures of two pentapeptides containing the *Δ^{E}Phe* configurational isomer were also reported: one has a Δ^{Z}Phe at position 2 and a Δ^{E}Phe at position 4, while the other has two Δ^{E}Phe residues at those positions (Figure 15). These results, although clearly limited in number, indicate that the Δ^{E}Phe residue is markedly more inclined than its stereoisomer to locate at the *i*+1 corner of a type-II (II′) β-turn where a *semi*-extended conformation is required, *i.e.* it is much less effective as a 3_{10}-helix former.

The X-ray diffraction structures of the three longest Δ^{Z}Phe-based peptides provided interesting information on the supersecondary 3D-structural modules of globular proteins, particularly on helix handedness preference and role of weak forces on protein … protein interactions. The decapeptide illustrated in Figure 17 is characterized by two Δ^{Z}Phe stretches, -(Δ^{Z}Phe)$_4$- and -(Δ^{Z}Phe)$_3$- [343]. Quite unexpectedly, despite the presence of two chiral L-Ala residues in the sequence, this peptide does not exhibit a preferred screw sense. One conformer in the asymmetric unit is right-handed 3_{10}-helical while the other, arranged in an antiparallel fashion, is left-handed 3_{10}-helical. The two molecules interact through an extensive network of weak forces (C-H … O H-bonds and π … π interactions). "Extended phenyl embrace" motifs (Figure 17) are formed at the helix … helix interface. A groove between molecules makes space for the aromatic side chains from adjacent helices to interdigitate ("ΔPhe zipper"). This mutual recognition may help our understanding of the specific folding of the protein helix … helix hydrophobic core.

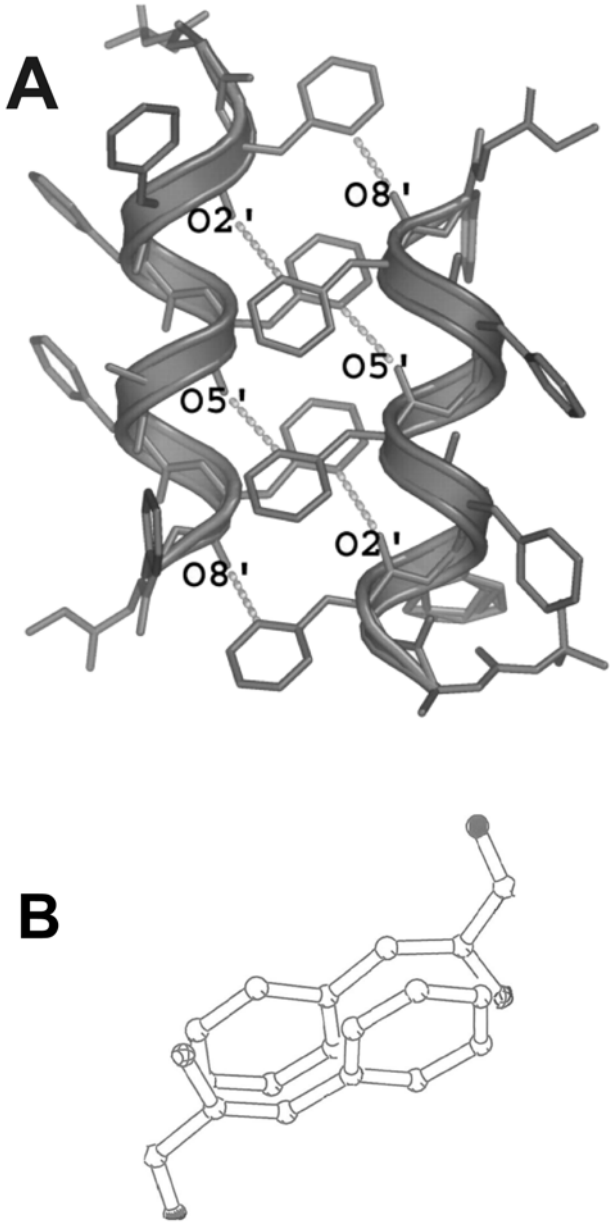


Figure 17. (**A**) The X-ray diffraction structure of the decapeptide Boc-L-Ala-(Δ^{Z}Phe)$_4$-L-Ala-(Δ^{Z}Phe)$_3$-Gly-OMe. The two 3_{10}-helical molecules of opposite screw sense are antiparallel and interact with each other through interdigitation of the Δ^{Z}Phe side chains. Intermolecular C-H … O=C H-bonds, but not intramolecular 1 ← 4 N-H … O=C H-bonds, are shown. (**B**) The intermolecular "extended phenyl embrace" motif between two Δ^{Z}Phe residues (adapted from [343]).

The crystal structures of three 11-mer peptides with the sequence Ac-Gly-L-Ala-Δ^ZPhe-(L-Leu-Xxx-Δ^ZPhe)$_2$-L-Ala-Gly-NH_2, where the Xxx dyads are formed by L-Val or L-Ala or Gly, differ substantially. While those with the "L-Val" or "L-Ala" central residues reveal the expected right-handed 3_{10}-helical conformations [345], that with the two "Gly" residues in the middle of the sequence adopt a 3_{10}-helix of ambidextrous screw sense with two molecules arranged antiparallely [392]. Interestingly, in the packing mode of the "L-Val" and "L-Ala" peptides, a given helix is surrounded by six other helices. This supersecondary module is reminiscent of those described by Karle [396] in a review article. Helix … helix interactions are achieved through N-H … O=C, C-H … O=C, and C-H … π H-bonds. An important feature of the "Gly" peptide is the occurrence of a "Gly zipper" motif at the Gly … Gly interface.

In the crystal state, a 21-residue long, Δ^ZPhe-based, heterochiral peptide folds into a helix-turn-helix ("helical hairpin") supersecondary structure in which a -(Gly)$_4$- sequence connects two 3_{10}-helices of opposite handedness [380]. The left-handed sequence is characterized by two D-Ala and five Δ^ZPhe residues, while the right-handed sequence is based on four L-Ala / L-Leu residues and three Δ^ZPhe. A similar fold, but with two right-handed 3_{10}-helices, was subsequently reported by the same group for a related 21-mer peptide formed by Δ^ZPhe and all-L Ala/Leu residues [352]. A remarkable property of this peptide is the unanticipated occurrence of an anion (acetate) binding motif in the linear region, strikingly similar to the "nest" motif observed in a number of proteins and synthetic model compounds [397].

Finally, only initial crystallographic studies on peptides based on unsaturated, aromatic ΔAA residues different from ΔPhe were reported. Both Boc-L-Ala-Xxx-L-Val-OMe (Xxx = Δ^ZNap or Δ^ZPyr) peptides are folded in a distorted type-II β-turn conformation with a helical ΔAA residue [289,298], as those with Δ^ZPhe in the second position of various trimers. The orientations of the aromatic moieties are nonplanar relative to the C^α=C^β-C^γ plane. This low-energy nonplanarity weakens the π-conjugation effect of the aromatic moieties on the conformational preferences of these bulky unsaturated residues. Also the Δ^ZPal [(*Z*)-β-(3-pyridyl)-α,β-didehydroalanine] residue is helical when located in the central position of an -L-Leu-Xxx-L-Leu- tripeptide [391], in analogy with the behavior of its Δ^ZPhe counterpart in the same sequence. In any case, it is evident that many more conformational studies should be performed on peptides of different length and sequence based on aromatic ΔAA residues different from ΔPhe with *Z* (and *E* as well) configurations and with either all-carbon ring side chains (*e.g.*, ΔTyr) or heterocyclic side chains (*e.g.*, ΔTrp) prior that any general conformational conclusion could be safely extracted.

Aib/Δ^{Z}Phe-Containing Peptides

In this section, we will analyze available literature data on helical peptide molecules each containing both C^{α}-tetrasubstituted α-amino acids (Aib and Api) and $C^{\alpha,\beta}$-didehydro-α-amino acids (Δ^{Z}Phe). As stated above, all these residues are strongly helicogenic and one of them (ΔPhe) is an excellent CD probe. Contributions in this area are much less numerous [refs. 245,252,259,300,302,398-422] than those in each separated area (for the only available review article comparing Aib and ΔPhe, see ref. [269]. Therefore, it is easily feasible to group most of them in two major thematic issues.

The X-ray diffraction structures of two (strictly sequential) achiral peptides, one penta- and one nonapeptide, containing exclusively Aib and ΔPhe were solved [405,410]. Not surprisingly, in view of the effective 3_{10}-helix inducing propensity of their building blocks, each peptide adopts a typical, enantiomeric 3_{10}-helical conformation. All L-configurated C^{α}-trisubstituted α-amino acids tested at the N-terminus of an Aib/ΔPhe stretch afforded largely prevailing, left-handed 3_{10}-helices (a similar situation was described above for Aib-rich peptides). The peptide with L-Ala shows the smallest amplitude for the styryl exciton couplet in each solvent and, consequently, the least effective induced chirality. Obviously, the opposite helix screw sense is preferentially adopted when the same residues at the N-terminus are D-configurated. The results obtained do not change when the number of -Aib-ΔPhe- couplets is increased from two to four. When an L-aminoacyl *methylamide* residue is positioned at the C-terminus of either an Aib/ΔPhe tetra- or pentapeptide sequence, the right-handed screw sense was preferentially adopted both in the crystal state and in solution. However, if an L-aminoacyl *methyl ester* is present at the C-terminus, the Aib/ΔPhe helix is left-handed. Finally, when a C^{α}-trisubstituted amino acid is inserted in the non-terminal position 2 of an Aib/ΔPhe hexapeptide, the type of preferred screw sense is largely solvent dependent (right-handed in $CHCl_3$, left-handed in MeOH). Thus, an L-residue at the position second from the N-terminus plays a unique role for energetically permitting both helical screw senses. The protein amino acid rank order for induction of the right handedness is L-Val > L-Leu ≈ L-Ala > L-Phe. Tentative explanations for these intriguing spectral data were provided [407]. However, if a couplet of L-Leu residues (one penultimate and one C-terminal) occurs in the sequence, the expected right-handed screw sense is that strongly predominant.

There is little doubt that the major novelty in the studies of Aib/ΔPhe-containing peptides is the so-called “noncovalent domino effect” (NCDE) introduced by Inai and coworkers in 2000 and

extensively developed by these researchers in the following years [404,406,408,409,411-413,415-417,419]. This phenomenon was defined as induction of one-handed helical screw sense in an achiral peptide through a domino effect based on the electrostatic interaction of its N-terminal free and protonated amino group with a chiral carboxylic acid. The original achiral compounds were sequential Aib/ΔPhe peptides. Sequences of at least four or five Aib-ΔPhe repeats were found to be optimal. The excess of one-handed screw sense was related to the chiral environment created by the acid … base interaction. This work provided a viable strategy for the control of a helical handedness of an achiral host peptide. It was found that Boc-α-amino acids provide an excellent type of chiral additives. The N-terminal segment of a right-handed 3_{10}-helix binds more favorably to a Boc-L-α-amino acid than to the corresponding Boc-D enantiomer [similar results were obtained with Boc N^{α}-protected $(L\text{-}Leu)_n$ (n = 2-4) peptides]. Control of the peptide helix screw sense through temperature tuning of the external Boc-D-amino acid interaction was also achieved. The two N-terminal free amide moieties of the achiral peptide, along with its N-terminal ammonium group, appear to be responsible for capturing effectively the Boc-α-amino acid molecule through three-point interactions [one (urethane) C=O … H-N (second amide) H-bond and two interactions involving the carboxylate anion of the chiral additive] (Figure 18). More complex studies of this type (external chirality-triggered control) were conducted on Aib/ΔPhe-based peptides containing a chiral protein residue. Replacement of the N-terminal Aib residue by the equally achiral β-Ala (β-alanine) or Gly was frequently demonstrated to markedly improve the NCDE, resulting in a more effective helicity control. A right-handed helix induced in an optically inactive 17-mer peptide by the Boc-L-Pro-OH stimulus was able to transfer the dynamic chiral information even to the 16^{th} position (about 30 Å away). This phenomenon was regarded as a primitive long-range allosteric effect in a synthetic polypeptide. An external chiral stimulus (Boc-L-Leu-OH) was shown to generate a privileged helical handedness in a dynamically optically inactive Aib/ΔPhe/Api helical peptide and this induced polymeric helicity was preserved in solution for a relatively long period of time ("chiral memory effect") after the complete replacement of the chiral additive with an achiral one (Boc-Aib-OH). The slow process and high barrier to inversion originate from the severe strain produced by the intramolecular *i*, *i*+3 cross-linking between two Api residues located one on top of the other on the same side of the 3_{10}-helix. Formation of a heterochiral helix (where both screw senses occur in the *same* molecule) was reported by increasing the main-chain length of single chirality [from L-Leu to $-(L\text{-}Leu)_5-$] at the N-terminus without the use of any L/D heterochiral sequence (covalent chiral domino effect). As mentioned above, the single L-Leu residue at the N-terminus favors a type-II β-turn and the following left-handed Aib/ΔPhe helix; however, the $(L\text{-}Leu)_{2\text{-}4}$ sequences exhibit the reasonable tendencies to promote a right-handed

helicity to a high extent. Surprisingly, when the $(L\text{-}Leu)_n$ sequence is long enough (n = 5) to adopt itself a right-handed helicity, it is able to shift the dynamic equilibrium of the two Aib/ΔPhe helical populations towards a left-handed helicity, thus generating a heterochiral helix.

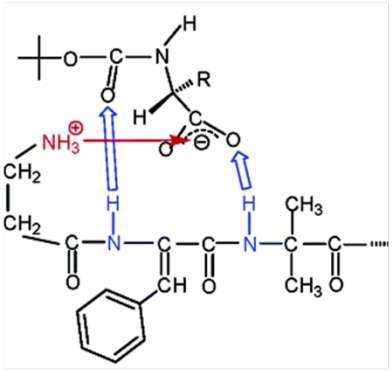


Figure 18. Proposed model for complexation of Boc-L-α-amino acid and the N-terminal segment of a $^{+}H_2$-β-Ala-Δ^{Z}Phe-Aib- peptide which generates a non-covalent chiral domino effect. Adapted from ref. [409].

A similar phenomenon was observed with two Aib/ΔPhe helices linked together *via* a synthetic, chiral Co(II) metal complex. Initially, the two helices were predominantly right-handed. However, when the achiral NO_3^- anion was added, the new coordination to Co(II) caused inversion of helicity of the metal center (from the Λ form to Δ form). This latter metal center form was shown to induce a sequential chirality transfer to the peptide helices. Other published, relevant investigation on Aib/ΔPhe-containing peptides include: (i) The stable right-handed screw sense of a L-(αMe)Val-$(Aib)_8$- helix with a remote ΔPhe in the middle of the homo-peptide stretch permitted a diastereoselective alkene hydrogenation to take place (long-range asymmetric induction). (ii) The same host helical peptide, -$(Aib)_8$-, with an N-terminal L-Phe (resulting in a left-handed helix) was shown to tolerate a central ΔPhe residue (good conductor of conformational preference). (iii) The first example of significant optical resolution of a racemic Aib/ΔPhe/D,L-Leu peptide helix was reported through stereoselective helix complexation by use of a cyclodimeric Zn-porphyrin host,

bearing a guest-binding chiral cavity, with two single-handed Aib/ΔPhe/ L- (or D-) Leu helical peptide units. Clearly, the host and guest molecules stereochemically recognized their helical structures rather than the point chiralities (Leu). (iv) According to an experimental and theoretical analysis, sequential -(L-Ala-Δ^ZPhe-Aib)$_n$- (*n* = 2-4) triplets form right-handed 3_{10}-helical structures with their aromatic side chains arranged regularly (with the stable conformation of 140° for the Δ^ZPhe χ^2 torsion angle [291]) along the helix axis, where the nearest phenyl pairs are separated by about 5.5 Å.

Summary and Outlook

In this article, we critically examined the results reported in the literature on properties (such as type, screw sense, and interconversion thereof) of helical peptides rich in *backbone*-modified amino acids. Due to the very large number of papers published in this area, the present study was restricted to α-amino acid surrogates only. Three types of modifications were thoroughly examined: (i) N-substitutions in Gly residues (peptoids), (ii) C^α-substitutions, and (iii) C^α=C^β bond-modified residues. Substitutions in types (i) and (ii) were limited to the most extensively investigated group (alkylations). Interestingly, various helical structures were authenticated, and equilibria and transitions among them were reported. Not only these studies unraveled changes in the backbone ϕ,ψ torsion angles, but variations in their amide ω angle values as well. To the aim of precisely assessing peptide 3D-structures and their variations in the crystal state and in solution, the X-ray diffraction technique, along with NMR and CD spectroscopies, proved to be most useful. Since the most common folded conformation exhibited by the peptides rich in C^α-tetrasubstituted and α,β-didehydro α-amino acids discussed in this article is the 3_{10}-helix, a deep knowledge of its electronic CD signature and how to differentiate it from that of the related α-helix [423-425] turned out to be particularly appropriate.

Studies on helical peptoids and their applications to bioactive compounds and nanomaterials began relatively recently (early 1990's). Therefore, it is not surprising that currently this field of research would still be in its infancy and exciting developments would be expected. Conversely, the first detailed theoretical and experimental investigations on the preferred helical conformations of peptides rich in Aib and ΔPhe, the prototypes of C^α-tetrasubstituted and $C^{\alpha,\beta}$-unsaturated α-amino acids, respectively, were published much earlier (more than 40 years ago). Nevertheless, it is gratifying to note that recent papers on these compounds highlighted that ample space is still available to interested peptide chemists. In this connection, it is worth mentioning that examples of these helical peptides with intriguing applications and implications to such diverse areas as

communication of stereochemical information, including remote asymmetric induction, over long distances (reviewed in ref. [426]), supramolecular organic chemistry, and prebiotic chemistry were reported.

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