Factors governing 310-helix vs ?-helix formation in peptides: Percentage of C?-tetrasubstituted ?-amino acid residues and sequence dependence

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Factors Governing 3_{10}-Helix vs α-Helix Formation in Peptides: Percentage of C^{α}-Tetrasubstituted α-Amino Acid Residues and Sequence Dependence

Abstract: *As an additional step toward the dissection of the factors responsible for the onset of 3_{10}-helix vs α-helix in peptides, in this paper we describe the results of a three-dimensional (3D) structural analysis by x-ray diffraction of the N^{α}-acylated heptapeptide alkylamide mBrBz–L-Iva–L-(αMe)Val–L-Abu–L-(αMe)Val–L-(αMe)Phe–L-(αMe)Val–L-Iva–NHMe characterized by a single (L-Abu3) C^{α}-trisubstituted and six C^{α}-tetrasubstituted α-amino acids. We find that in the crystal state this peptide is folded in a mixed helical structure with short elements of 3_{10}-helix at either terminus and a central region of α-helix. This finding, taken together with the published NMR and x-ray diffraction data on the all C^{α}-methylated parent sequence and its L-Val2 analog (also the latter heptapeptide has a single C^{α}-trisubstituted α-amino acid) strongly supports the view that one C^{α}-trisubstituted α-amino acid inserted near the N-terminus of an N^{α}-acylated heptapeptide alkylamide sequence may be enough to switch a regular 3_{10}-helix into an essentially α-helical conformation. As a corollary of this work, the x-ray diffraction structure of the N^{α}-protected, C-terminal tetrapeptide alkylamide Z–L-(αMe)Val–L-(αMe)Phe–L-(αMe)Val–L-Iva–NHMe, also reported here, is clearly indicative of the preference of this fully C^{α}-methylated, short peptide for the 3_{10}-helix. As the same terminally blocked sequence is mixed 3_{10}/α-helical in the L-Abu3 heptapeptide amide but regular 3_{10}-helical in the tetrapeptide amide and in the parent heptapeptide amide, these results point to an evident plasticity even of a fully C^{α}-methylated short peptide.* © 2002 Wiley Periodicals, Inc. Biopolymers 64: 236–245, 2002

Keywords: *C^{α}-tetrasubstitution in amino acids; type of helical structure; x-ray diffraction analysis of oligopeptides; type of peptide helix; x-ray diffraction of peptides*

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INTRODUCTION

C^{α}-Tetrasubstituted α-amino acids are well known as the most effective peptide 3_{10}/ α-helix formers and stabilizers.[1–7] To this aim even Nature takes advantage of some of them (Aib, α-aminoisobutyric acid or $C^{\alpha,\alpha}$-dimethylglycine; Iva, isovaline or C^{α}-ethyl-C^{α}-methylglycine) in the family of membrane-active peptaibols (for terminology of peptaibols, see Ref. 8; for review articles, Refs. 9–11; for x-ray diffraction analyses of alamethicin, zervamicins, antiamoebins, and trichogin, Refs. 12, 13–16, 17 and 18, and 19, respectively). The Thorpe–Ingold effect[20,21] has been invoked to explain this structural tendency.[22] The question of the preferred helix type (3_{10}- vs α-helix) of peptides rich in C^{α}-tetrasubstituted α-amino acids has been experimentally addressed considering such factors as peptide main-chain length, percentages of C^{α}-tetrasubstituted α-amino acids, sequence dependence, solvent polarity, temperature, and intermolecular interactions.[3–6, 23–27]

This work is part of a program aiming at a deeper understanding of the factors mentioned above. In particular, in no peptide system based exclusively on C^{α}-tetrasubstituted α-amino acids explored so far (with one exception) has the α-helix ever been found, neither in the crystal state nor in solution [the single exception is given by N^{α}-acylated homo-octapeptide esters based on C^{α}-methyl-L-valine, L-(αMe)Val, which slowly undergo a 3_{10}-helix to α-helix conformational switch in solvents of high polarity under appropriate temperature and concentration conditions[28,29]]. In this connection we decided to better explore the effect of the number and positioning of C^{α}-trisubstituted α-amino acid residues needed to convert a 3_{10}-helix into an α-helix in a peptide of a given length. The peptide substrate for this study was chosen to be an N^{α}-acylated heptapeptide alkylamide (equivalent to an N^{α}-acylated octapeptide ester in terms of intramolecular H-bond potential) because peptides having this main-chain length are known to be at the interface between 3_{10}- and α-helices, and are presumably the most susceptible to this type of helix → helix conformational transformation.[3] The only published work on the preferred conformation of N^{α}-acylated octapeptide esters (or heptapeptide alkylamides) containing a single C^{α}-trisubstituted α-amino acid deals with a host –$(Aib)_8$– chain (**1**, Figure 1) with an Aib → L-Leu guest replacement at position 6 (**2**) (i.e., in an internal position, near the C-terminus of the chain). Results of x-ray diffraction and spectroscopic analyses clearly indicated that the –C(═O)–$(Aib)_5$–L-Leu–Aib– sequence **2** is folded in a regular 3_{10}-helical structure both in the crystal state and in solution,[30–35] as it is the –$(Aib)_8$– parent sequence.[36,37]

More recently, we have expanded our investigation to the analysis of the effect of a single, internal C^{α}-trisubstituted α-amino acid positioned near the N-terminus of the peptide chain. To this purpose, a standard, fully C^{α}-methylated, N^{α}-acylated heptapeptide alkylamide sequence –C(═O)–L-Iva–L-(αMe)Val–L-Iva–L-(αMe)Val–L-(αMe)Phe–L-(αMe)Val–L-Iva–NH– (**3**) [L-(αMe)Phe, C^{α}-methyl phenylalanine] was first synthesized and conformationally characterized.[38] Both x-ray diffraction and bidimensional NMR/molecular dynamics simulation analyses clearly showed that the peptide adopts a fully developed, regular right-handed 3_{10}-helical structure under all experimental conditions tested. In the next step, for an NMR investigation purpose, we designed two singly modified peptide sequences (**4** and **5**) in which (a) L-(αMe)Val2 was replaced by the C^{α}-trisubstituted protein L-Val residue (peptide **4**), and (b) an L-Abu (Abu, α-aminobutyric acid) residue was substituted for the original L-Iva3 (peptide **5**). It is worth noting that in both modified peptides the longest (*iso*propyl and ethyl, respectively) side chain at the replacement site was preserved. The L-Val2 sequence **4**, more soluble in organic solvents, studied by bidimensional NMR/molecular dynamics simulations, was shown to exist in a right-handed 3_{10}/α-helical equilibrium with a slight preference for the α-helical conformation.[39] Thus, the missing C^{α}-methylation of Val2 seems to have introduced some flexibility, which had an impact on the overall structure. Conversely, the sparingly soluble *m*BrBz–L-Iva–L-(αMe)Val–L-Abu–L-(αMe)-Val–L-(αMe)Phe–L-(αMe)Val–L-Iva–NHMe **5** (*m*BrBz, *meta*-bromobenzoyl; NHMe, methylamino), nicely crystallized out of an acetone solution. Here, we describe details of the x-ray diffraction structure of this L-Abu3 N^{α}-acylated heptapeptide alkylamide analog. The *m*BrBz group was incorporated at the N-terminus to help solve the phase problem in the roentgenographic investigation by virtue of its heavy atom (Br). The results are analyzed in parallel with those of the C-terminal sequence Z–L-(αMe)Val–L-(αMe)Phe–L-(αMe)Val–L-Iva–NHMe (**6**) (Z, benzyloxycarbonyl), the x-ray diffraction structure of which is also described in the present article. This study allowed us to compare the crystal-state conformational preference of this N^{α}-acylated tetrapeptide alkylamide in isolation with that when it is inserted into a longer peptide sequence.

MATERIALS AND METHODS

Synthesis and Characterization of Peptides

Melting points were determined using a Leitz (Wetzlar, Germany) model Laborlux 12 apparatus and are not cor-

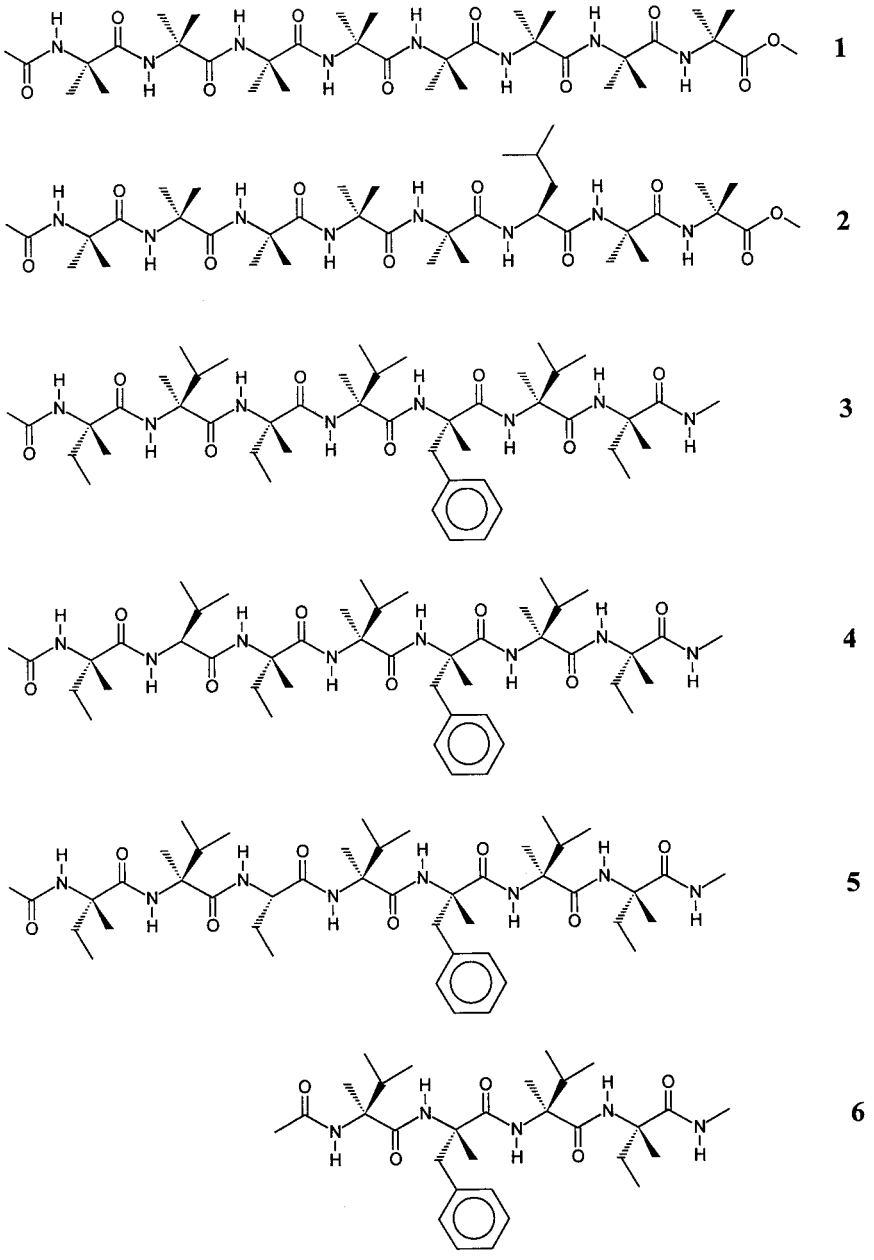

FIGURE 1 Chemical formulae of the peptide sequences discussed in this work.

rected. Optical rotations were measured using a Perkin-Elmer (Norwalk, CT) model 241 polarimeter equipped with a Haake (Karlsruhe, Germany) model D thermostat. Thin-layer chromatography was performed on Merck (Darmstadt, Germany) Kieselgel 60F254 precoated plates with the following solvent systems: (1) chloroform/ethanol, 9:1; (2) 1-butanol/acetic acid/water, 3:1:1; (3) toluene/ethanol, 7:1. The chromatograms were examined using ultraviolet fluorescence or developed by chlorine/starch/potassium iodide or ninhydrin chromatic reaction as appropriate. All compounds were obtained in a chromatographically homogeneous state. Their analytical data and physical properties are listed in Table I. All of the synthetic intermediates and the final compound were also characterized by ^{1}H-NMR (data not reported). The chemical structure of the final compound was confirmed by amino acid analysis and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (data not reported).

For the large-scale production of the optically pure L-Iva, L-(αMe)Val, and L-(αMe)Phe, we exploited an economically attractive, chemoenzymatic synthesis developed by DSM Research a few years ago.[40,41] It involves a combination of organic synthesis for the preparation of the racemic α-amino acids followed by the use of a broadly specific aminopeptidase to achieve optical resolution. The Z N^{α}-protected, C^{α}-methylated α-amino acids[42–44] were acti-

Table I Analytical Data and Physical Properties for the Newly Synthesized Peptides

Compound	Yield (%)	Melting Point (°C)	Crystalliz. Solvent[a]	$[\alpha]_D^{20}$ (deg)[b]	Thin Layer Chromatography			IR (cm^{-1})[c]
					R_{F1}	R_{F2}	R_{F3}	
Z–L-Abu–OH	55	66–68	DE/PE	−13.4	0.45	0.90	0.25	3418, 1743, 1727, 1662, 1546
Z–L-Abu–L-(αMe)Val–L-(αMe)Phe–L-(αMe)Val–L-Iva–NHMe	34	204–205	EtOAc/PE	−3.8[d]	0.70	0.95	0.35	3330, 1697, 1657, 1524
Z–L-(αMe)Val–L-Abu–L-(αMe)Val–L-(αMe)Phe–L-(αMe)Val–L-Iva–NHMe	48	196–198	$CHCl_3$/PE	−6.4	0.70	0.90	0.30	3327, 1702, 1659, 1524
Z–L-Iva–L-(αMe)Val–L-Abu–L-(αMe)Val–L-(αMe)Phe–L-(αMe)Val–L-Iva–NHMe	66	227–228	EtOAc/PE	15.4	0.65	0.90	0.30	3322, 1696, 1656, 1526
*m*BrBz–L-Iva–L-(αMe)Val–L-Abu–L-(αMe)Val–L-(αMe)Phe–L-(αMe)Val–L-Iva–NHMe	71	311–312	CH_2Cl_2/PE	14.0[e]	0.60	0.95	0.20	3362, 1660, 1522

[a] DE: diethyl ether; PE: petroleum ether; EtOAc: ethyl acetate.
[b] $c = 0.5$, MeOH.
[c] The ir absorption spectra were obtained in KBr pellets; only bands within the 3500–3200 and 1800–1500 cm^{-1} regions are reported.
[d] $[\alpha]_{436}^{20}$.
[e] $c = 0.2$, 2,2,2-trifluoroethanol.

vated by the acid fluoride method.[38,45,46] The Z group was removed by catalytic hydrogenation in methanol (MeOH) solution. The synthesis of the *m*BrBz-heptapeptide **5** was achieved by use of *m*BrBz-OAt[38,47] (OAt, 1-oxy-7-aza-1,2,3-benzotriazole) in a 9:1 CH_2Cl_2/CH_3CN solvent mixture in the presence of N-methylmorpholine.

X-Ray Diffraction

Colorless crystals of the heptapeptide alkylamide **5** and tetrapeptide alkylamide **6** were grown from methanol and from acetone, respectively, by slow evaporation. Data collection was performed by using a Philips PW 1100 four-circle diffractometer. The two structures were solved by direct methods (SHELXS 97 program[48]). In the heptapeptide alkylamide **5** refinement was carried out by full-matrix least-squares on F^2, using all data, with the SHELXL 97 program.[49] In the tetrapeptide alkylamide **6** the full-matrix block least-squares procedure was exploited for refinement. All non-H atoms were refined anisotropically. A planarity restraint was applied to all phenyl rings in both peptides. In the tetrapeptide alkylamide **6** restraints were also imposed to the anisotropic displacement parameters of the phenyl rings to approach isotropic behavior. In both peptides H-atoms were calculated at idealized positions and during the refinement they were allowed to ride on their carrying atom, with U_{iso} set equal to 1.2 (or 1.5 for methyl groups) times the U_{eq} of the parent atom. The two highest peaks on the final ΔF map of the heptapeptide alkylamide **5** (0.997 and 0.890 e · $Å^{-3}$) are located near the Br atom.

Details of the crystallographic data and diffraction parameters for the two structures are given in Table II. Further details of the crystal structures, including final atomic parameters for the non-H atoms, have been deposited with and are available on request from the Director of the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ (England), on quoting the full journal citation.

RESULTS AND DISCUSSION

We determined by x-ray diffraction the molecular and crystal structures of the N^{α}-acylated heptapeptide alkylamide *m*BrBz–L-Iva–L-(αMe)Val–L-Abu–L-(αMe)-Val–L-(αMe)Phe–L-(αMe)Val–L-Iva–NHMe (**5**) and its C-terminal tetrapeptide alkylamide sequence Z–L-(αMe)Val–L-(αMe)Phe–L-(αMe)Val–L-Iva–NHMe (**6**). The molecular structures with the atomic numbering schemes are illustrated in Figures 2 and 3, respectively. Selected backbone and side-chain torsion angles[50] are given in Table III. In Table IV the intra- and intermolecular H-bond parameters are listed. For comparison the two tables also report the corresponding data for the parent N^{α}-acylated heptapeptide alkylamide **3**.

Table II Crystal Data and Diffraction Parameters for the Heptapeptide Amide *m*BrBz–L-Iva–L-(αMe)Val–L-Abu–L-(αMe)Val–L-(αMe)Phe–L-(αMe)Val–L-Iva–NHMe (5) and the Tetrapeptide Amide Z–L-(αMe)Val–L-(αMe)Phe–L-(αMe)Val–L-(αMe)Val–L-Iva–NHMe (6)

Parameter	Heptapeptide Amide (**5**)	Tetrapeptide Amide (**6**)
Empirical formula	$C_{50}H_{77}BrN_8O_8$	$C_{36}H_{53}N_5O_6$
Formula weight (amu)	998.1	651.8
Temperature (K)	293(2)	293(2)
Wavelength (λ)	Cu Kα (1.54178 Å)	Cu Kα (1.54178 Å)
Crystal system	Orthorhombic	Monoclinic
Space group	$P2_12_12_1$	$P2_1$
a (Å)	9.658(2)	9.707(3)
b (Å)	18.973(3)	35.641(8)
c (Å)	28.904(4)	11.859(5)
β (deg)	90.00	113.61(3)
V (Å^3)	5296(2)	3759(2)
Z (molecules/unit cell)	4	4
Density (calc.) (g/cm^3)	1.252	1.152
Absorption coeff. (mm^{-1})	1.523	0.634
F(000)	2128	1408
Data collection method	θ–2θ	θ–2θ
Crystal size (mm)	0.30 × 0.20 × 0.20	0.40 × 0.30 × 0.10
θ range (deg)	2.79–60.02	2.48–60.02
Index ranges	$-1 \le h \le 10$; $-1 \le k \le 21$; $-32 \le 1 \le 0$	$-10 \le h \le 9$; $-1 \le k \le 40$; $0 \le l \le 13$
Reflections collected	5315	6208
Reflections unique	5151 [*R*(int) = 0.0549]	5886 [*R*(int) = 0.0342]
Data/restraints/parameters	5151/18/614	5886/151/829
Goodness-of-fit on F^2	0.948	0.868
Final *R* indices [$I>2(I)$]	$R_1 = 0.0564$, $wR_2 = 0.1353$	$R_1 = 0.0562$, $wR_2 = 0.1295$
R indices (all data)	$R_1 = 0.0806$, $wR_2 = 0.1480$	$R_1 = 0.1041$, $wR_2 = 0.1431$
Δρ (*e*/Å^3)	0.997/–0.388	0.272/–0.198

The molecules of the Abu3 heptapeptide amide **5** are fully-developed right-handed helices, stabilized by a large set of intramolecular, consecutive, or bifurcated, C═O · · · H—N H-bonds. The right-handed helical screw sense is in particular dictated by the known conformational bias of the L-Iva (with a linear side chain)[7,38,44] and L-(αMe)Val (with a β-branched side chain)[7,38,46] residues. The average values for the seven sets of backbone φ,ψ torsion angles are −56.3°, −45.2°. The average φ value is closer to that typical of a 3_{10}-helix (−57°) than to that of an α-helix (−63°).[23] However, the opposite holds true for the average ψ value (−30° for a typical 3_{10}-helix and −42° for a typical α-helix). This mixed 3_{10}/α-helical structure, suggested by the backbone torsion angles, is confirmed by the analysis of the intramolecular H-bonds, although some of them are weak,[51–53] particularly if part of the bifurcation sites (in any case, intramolecular H-bonds with an N · · · O distance > 3.0 Å are a common observation in 3_{10}- and α-helices). At the N-terminus the molecular conformation displays a short 3_{10}-helix with two consecutive type III β-turn (C_{10}) structures.[54–56] The C═O group of the *m*BrBz group forms a H-bond with the L-Abu3 N–H group, while the L-Iva1 C═O group is involved in a H-bond with the L-(αMe)Val4 N—H group. However, the L-Iva1 C═O group is also involved in a weak interaction with the L-(αMe)Phe5 N—H group giving rise to an α-turn (C_{13}) conformation.[57–59] Then, this region of bifurcation is followed by two weakly stabilized, consecutive α-turn structures involving carbonyl oxygens of L-(αMe)Val2 and L-Abu3 and amino groups of L-(αMe)Val6 and L-Iva7. A second site of bifurcated arrangement is seen at the C-terminus, as the L-Iva7 amino group is also involved in the formation of a weakly stabilized β-turn with the L-(αMe)Val4 C═O group. The short C-terminal 3_{10}-helical segment ends up with a H-bond between the methylamido N—H and the L-(αMe)Phe5 carbonyl groups.

In the tetrapeptide amide **6** each independent molecule **A** and **B** is folded in a right-handed 3_{10}-helix stabilized by three intramolecular C═O · · · H—N H-bonds. Two regular type III β-turns are followed by a

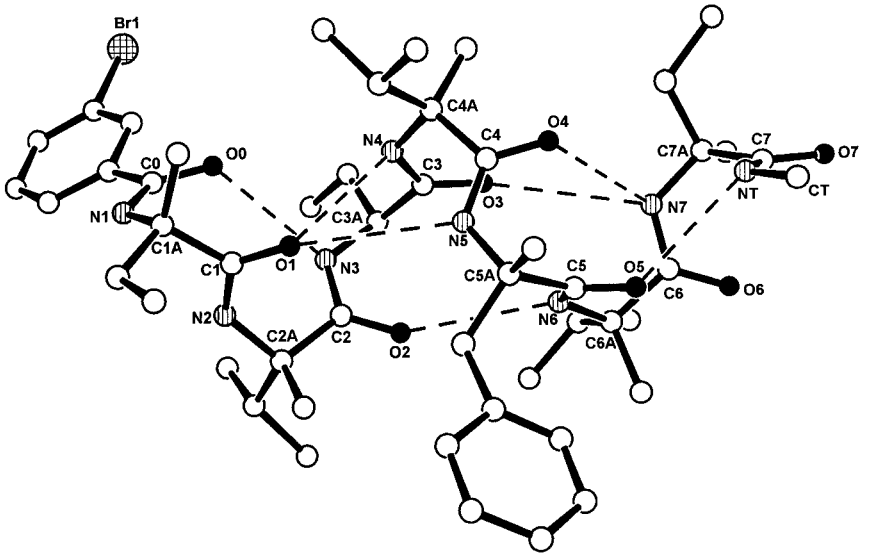

FIGURE 2 The x-ray diffraction structure of *m*BrBz–L-Iva–L-(αMe)Val–L-Abu–L-(αMe)Val–L-(αMe)Phe–L-(αMe)Val–L-Iva–NHMe (**5**) with numbering of the backbone atoms. Intramolecular H-bonds are represented by dashed lines.

C-terminal, distorted β-turn in which the values of the ϕ,ψ torsion angles of the L-(αMe)Val and L-Iva residues are intermediate between those of a type I and a type III β-turn (position $i+2$). The conformational differences, including side-chain dispositions (χ torsion angles),[60] between molecules **A** and **B** of the tetrapeptide amide are only of minor significance.

For reason of comparison, in Tables III and IV the backbone and side-chain torsion angles, and the intra- and intermolecular H-bond parameters, respectively, for the regular 3_{10}-helical, parent L-Iva3 heptapeptide amide (**3**) (independent molecules **A** and **B**)[38] are also listed. Significant backbone conformational differences among the five molecules are observed: (a) in

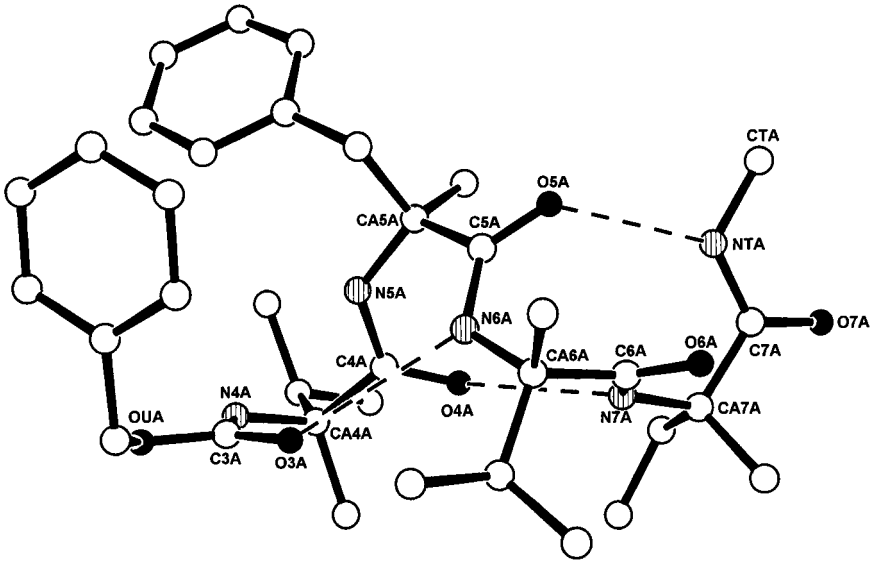

FIGURE 3 The x-ray diffraction structures of one (molecule **A**) of the two independent molecules in the asymmetric unit of Z–L-(αMe)Val–L-(αMe)Phe–L-(αMe)Val–L-Iva–NHMe (**6**) with numbering of the backbone atoms. Intramolecular H-bonds are represented by dashed lines. In order to align the atom numbering of this tetrapeptide amide with the corresponding atom numbering of the heptapeptide amides, its residues 1–4 have been renumbered 4–7.

Table III Relevant Backbone and Side-Chain Torsion Angles (°) for *m*BrBz–L-Iva–L-(αMe)Val–L-Iva–L-(αMe)Val–L-(αMe)Phe–L-(αMe)Val–L-Iva–NHMe (Heptapeptide Amide 3),[a] *m*BrBz–L-Iva–L-(αMe)Val–L-Abu–L-(αMe)Val–L-(αMe)Phe–L-(αMe)Val–L-Iva–NHMe (Heptapeptide Amide 5), and Z–L-(αMe)Val–L-(αMe)Phe–L-(αMe)Val–L-Iva–NHMe (Tetrapeptide Amide 6)

	Heptapeptide Amide (**3**)			Tetrapeptide Amide (**6**)	
Torsion Angle	Mol. **A**	Mol. **B**	Heptapeptide Amide (**5**)	Mol. **A**	Mol. **B**
ϕ_1	−61.5 (10)	−55.4 (15)	−52.0 (7)		
ψ_1	−29.9 (10)	−32.1 (13)	−44.5 (7)		
ω_1	−171.4 (7)	−170.8 (10)	−172.7 (5)		
ϕ_2	−52.8 (9)	−57.0 (15)	−53.8 (7)		
ψ_2	−32.3 (8)	−29.6 (13)	−40.7 (7)		
ω_2	−175.3 (6)	−179.5 (9)	−172.5 (5)		
ϕ_3	−51.0 (9)	−48.4 (14)	−67.6 (7)		
ψ_3	−41.6 (9)	−33.1 (12)	−43.5 (7)		
ω_3	−171.2 (7)	−170.9 (8)	−179.6 (5)		
ϕ_4	−54.7 (9)	−54.0 (10)	−57.3 (6)	−55.1 (6)	−57.3 (6)
ψ_4	−32.7 (9)	−26.3 (9)	−45.4 (6)	−31.6 (6)	−28.6 (6)
ω_4	−175.0 (7)	−176.8 (6)	−175.8 (4)	−172.9 (4)	−174.6 (4)
ϕ_5	−53.1 (9)	−51.2 (8)	−58.5 (6)	−58.1 (6)	−53.7 (6)
ψ_5	−32.9 (9)	−37.9 (8)	−49.0 (6)	−20.4 (6)	−28.0 (6)
ω_5	−178.1 (7)	−178.2 (6)	−173.9 (5)	174.2 (4)	−179.1 (4)
ϕ_6	−51.6 (9)	−51.2 (9)	−53.8 (7)	−47.5 (6)	−49.9 (6)
ψ_6	−45.1 (9)	−41.6 (9)	−44.2 (8)	−40.1 (6)	−35.7 (6)
ω_6	−175.5 (8)	−176.5 (7)	−174.1 (7)	−175.5 (5)	−178.1 (5)
ϕ_7	−78.012)	−56.2 (10)	−51.1 (10)	−67.1 (6)	−63.7 (6)
ψ_7	−8.0 (15)	−42.8 (10)	−49.0 (10)	−12.0 (7)	−17.8 (7)
ω_7	−178.1 (12)	−176.6 (9)	−175.5 (9)	178.5 (4)	179.1 (5)
Side chains					
χ_1^1	−74.0 (10)	176.6 (21)	177.2 (7)		
$\chi_2^{1,1}$	60.9 (9)	168.1 (16)	−177.0 (7)		
$\chi_2^{1,2}$	−171.9 (8)	−75.2 (28); 42.1 (19)	56.3 (8)		
χ_3^1	170.9 (9)	165.6 (13)	−61.9 (7)		
$\chi_4^{1,1}$	169.2 (9)	64.7 (9)	−67.6 (6)	59.5 (6)	62.7 (6)
$\chi_4^{1,2}$	−64.0 (9)	−171.2 (9)	167.8 (5)	−174.8 (5)	−172.1 (5)
χ_5^1	−178.8 (8)	176.8 (6)	−172.9 (2)	−54.6 (3)	−52.5 (4)
$\chi_5^{2,1}$	92.3 (8)	89.9 (7)	131.5 (3)	88.7 (2)	95.5 (3)
$\chi_5^{2,2}$	−88.0 (9)	−88.0 (7)	−53.2 (5)	−88.8 (5)	−86.7 (6)
$\chi_6^{1,1}$	166.6 (8)	171.4 (9)	−70.3 (7)	−70.6 (5)	−71.7 (5)
$\chi_6^{1,2}$	−70.6 (9)	−61.5 (9)	162.5 (6)	162.4 (5)	163.7 (5)
χ_7^1	−61.6 (10)	−178.9 (15); −78.8 (21)	175.5 (10)	−67.7 (8)	−69.3 (7)

[a] Taken from Ref. 38.

[b] In order to align the torsion angles of this tetrapeptide amide with the corresponding torsion angles of the heptapeptide amides, its residues 1–4 have been renumbered 4–7.

the ψ_4 and ψ_5 torsion angles (the absolute values of which are consistently higher in the L-Abu3 heptapeptide amide **5**) and (b) in the related, α-turn type, O2 · · · H6—N6 and O3 · · · H7—N7 intramolecular H-bonds (seen only in the L-Abu3 heptapeptide amide **5**) and β-turn type O3 · · · H6—N6 intramolecular H-bond (seen only in the L-Iva3 heptapeptide amide **3** and in the tetrapeptide amide **6**). From this comparison, it may be concluded that this N^{α}-acylated tetrapeptide amide segment may adopt either a mixed $\alpha/3_{10}$-helical structure (when incorporated at the C-terminus of the L-Abu3 heptapeptide amide **5**) or an essentially 3_{10}-helical structure (either when in isolation or when incorporated at the C-terminus of the fully C^{α}-methylated L-Iva3 heptapeptide amide **3**).

The packing mode of the heptapeptide amide **5** is characterized by an intermolecular H-bond between the N1 group and the O6 carbonyl oxygen atom of a

Table IV Intra- and Intermolecular H-Bond Parameters for *m*BrBz–L-Iva–L-(αMe)Val–L-Iva–L-(αMe)Val–L-(αMe)Phe–L-(αMe)Val–L-Iva–NHMe (Heptapeptide Amide 3),[a] *m*BrBz–L-Iva–L-(αMe)Val–L-Abu–L–(αMe)Val–L-(αMe)Phe–L-(αMe)Val–L-Iva-NHMe (Heptapeptide Amide 5), and Z–L-(αMe)Val–L-(αMe)Phe–L-(αMe)Val–L-Iva–NHMe (Tetrapeptide Amide 6)

	Donor	Acceptor	Length (Å) (N · · · O)	Length (Å) (H · · · A)	Angle (°) (N—H · · · O)	Symmetry Operation
Heptapeptide amide (**3**)						
Intramolecular	N3A	O0A[b]	3.335 (10)	2.50	165	*x*, *y*, *z*
	N4A	O1A	3.084 (8)	2.23	155	*x*, *y*, *z*
	N5A	O2A	3.068 (8)	2.28	152	*x*, *y*, *z*
	N6A	O3A	3.185 (8)	2.37	158	*x*, *y*, *z*
	N7A	O4A	2.962 (9)	2.26	138	*x*, *y*, *z*
	NTA[b]	O5A	2.864 (13)	2.07	152	*x*, *y*, *z*
	N3B	O0B[b]	3.246 (11)	2.43	159	*x*, *y*, *z*
	N4B	O1B	3.004 (9)	2.15	171	*x*, *y*, *z*
	N5B	O2B	3.136 (10)	2.28	174	*x*, *y*, *z*
	N6B	O3B	3.132 (8)	2.32	158	*x*, *y*, *z*
	N7B	O4B	2.995 (8)	2.27	142	*x*, *y*, *z*
	NTB[b]	O5B	2.985 (9)	2.36	130	*x*, *y*, *z*
Head-to-tail	N1A	O6B	2.892 (8)	2.04	171	1+*x*, *y*, 1+*z*
	N2A	O7B	3.232 (9)	2.61	130	1+*x*, *y*, 1+*z*
	N1B	O7A	2.949 (11)	2.33	129	*x*, *y*, *z*
Heptapeptide amide (**5**)						
Intramolecular	N3	O0[b]	2.969 (6)	2.27	139	*x*, *y*, *z*
	N4	O1	3.199 (6)	2.69	120	*x*, *y*, *z*
	N5[c]	**O1**	3.340 (5)	2.50	166	*x*, *y*, *z*
	N6	**O2**	3.270 (6)	2.45	159	*x*, *y*, *z*
	N7	**O3**	3.429 (6)	2.63	156	*x*, *y*, *z*
	N7	O4	3.258 (7)	2.68	126	*x*, *y*, *z*
	NT[b]	O5	3.089 (8)	2.51	126	*x*, *y*, *z*
Head-to-tail	N1	O6	2.892 (6)	2.06	164	−*x*−1/2, −*y*, *z*−1/2
Tetrapeptide amide (**6**)						
Intramolecular	N6A[d]	O3A[b]	3.110 (6)	2.26	173	*x*, *y*, *z*
	N7A	O4A	3.063 (5)	2.23	162	*x*, *y*, *z*
	NTA[b]	O5A	2.898 (7)	2.07	161	*x*, *y*, *z*
	N6B	O3B[b]	3.142 (5)	2.29	172	*x*, *y*, *z*
	N7B	O4B	3.088 (6)	2.26	161	*x*, *y*, *z*
	NTB[b]	O5B	2.891 (6)	2.07	160	*x*, *y*, *z*
Head-to-tail	N4A	O7A	2.858 (6)	2.16	139	*x*, *y*, *z*+1
	N4B	O7B	2.891 (5)	2.20	137	*x*+1, *y*, *z*+1

[a] Taken from Ref. 38.

[b] O0 in the heptapeptide amides (and O3 in the tetrapeptide amide) is the carbonyl oxygen atom preceding the N-terminal residue; in all peptides NT is the amide nitrogen atom following the C-terminal residue.

[c] The atoms involved in an intramolecular H-bond forming an $\alpha(C_{13})$-turn, instead of a $\beta(C_{10})$-turn, are in bold.

[d] In order to align the atom numbering of this tetrapeptide amide with the corresponding atom numbering of the heptapeptide amides, its residues 1–4 have been renumbered 4–7.

($-x - ½$, $-y$, $z - ½$) symmetry related molecule. Rows of molecules, head-to-tail H-bonded and related through a crystallographic twofold screw axis, are thus formed through the *c* direction.

In the packing mode of the tetrapeptide amide **6** head-to-tail intermolecular H-bonds are observed between molecules of the same type. More specifically, the N4A—H group is H-bonded to the O7A carbonyl oxygen atom of a ($x + 1$, y, $z + 1$) translational equivalent of molecule **A**, while the N4B—H group is H-bonded to the O7B carbonyl oxygen atom of a ($x + 1$, y, $-z + 1$) translational equivalent of molecule **B**. As a consequence, rows of molecules **A** are formed along the *c* direction, while rows of molecules **B** are observed along the *ac* direction. Rows of the two kinds alternate along the *b* direction.

CONCLUSIONS

This paper clearly shows that in the crystal state an N^{α}-acylated heptapeptide alkylamide, containing six C^{α}-tetrasubstituted α-amino acids and a single C^{α}-trisubstituted residue near the N-terminus (L-Abu3) (**5**), tends to fold in a well-developed α-helical structure in the central region, although this segment is preceded and followed by two short 3_{10}-helical segments. This result is in good agreement with the published NMR data on the L-Val2 analog **4**[39] of the same, fully 3_{10}-helical, parent peptide **3**,[38] and beautifully parallels that of an NMR study on an Ala-rich peptide, where an α-helical sequence was identified in the middle and short 3_{10}-helical stretches at both termini.[61]

A previous detailed structural investigation on an N^{α}-acylated octapeptide ester (equivalent to a heptapeptide alkylamide as far as its capability of intramolecular H-bond formation is concerned), characterized by seven Aib residues and a single C^{α}-trisubstituted α-amino acid (L-Leu6) near the C-terminus (**2**), unequivocally established the onset of a fully developed, rigid, regular 3_{10}-helical conformation,[30-35] as observed in the $-(Aib)_8-$ homopeptide sequence (**1**).[36,37] Taken together, these findings support the view that, even in the case of a very high percentage of C^{α}-tetrasubstituted α-amino acids discussed here, the precise positioning of the single C^{α}-trisubstituted residue in the sequence may have a dramatic effect on the helical structure of the peptide backbone. More specifically, incorporation of the guest residue near the N-terminus seems critical for the observation of the conformational transition. Even if in a single, specific case (an N^{α}-*tert*-butyloxycarbonylated or N^{α}-acetylated heptapeptide methyl ester characterized by as many as six C^{α}-trisubstituted amino acids) it has been reported that in the crystal state the nature of the N^{α}-blocking may play a role on the type of helical structure adopted,[62] it is evident that in our heptapeptide methylamides **3**–**5** such an effect cannot be operative as all three compounds are N^{α}-acylated with the same moiety (*m*BrBz).

Other interesting information extracted from this work is that the preferred type of helical structure of even a fully C^{α}-tetrasubstituted, N^{α}-acylated tetrapeptide alkylamide, as **6**, may be governed by the presence/absence of additional residues in the sequence.

REFERENCES

1. Marshall, G. R. In Intra-Science Chemistry Report; Kharasch, N., Ed.; Gordon &Breach: New York, 1971; Vol 5, pp 305–316.
2. Paterson, Y.; Rumsey, E.; Benedetti, E.; Némethy, G.; Scheraga, H. A. J Am Chem Soc 1981, 103, 2947–2955.
3. Karle, I. L.; Balaram, P. Biochemistry 1990, 29, 6747–6756.
4. Toniolo, C.; Bonora, G. M.; Bavoso, A.; Benedetti, E.; Di Blasio, B.; Pavone, V.; Pedone, C. Biopolymers 1983, 22, 205–215.
5. Toniolo, C.; Benedetti, E. Macromolecules 1991, 24, 4004–4009.
6. Benedetti, E.; Toniolo, C In Polymeric Materials Encyclopedia; Salamone, J. C., Ed.; CRC Press: Boca Raton, FL, 1996; Vol 8P, pp 6472–6481.
7. Toniolo, C.; Crisma, M.; Formaggio, F.; Valle, G.; Cavicchioni, G.; Précigoux, G.; Aubry, A.; Kamphuis, J. Biopolymers 1993, 33, 1061–1072.
8. Benedetti, E.; Bavoso, A.; Di Blasio, B.; Pavone, V.; Pedone, C.; Toniolo, C.; Bonora, G. M. Proc Natl Acad Sci USA 1982, 79, 7951–7954.
9. Nagaraj, R.; Balaram, P. Acc Chem Res 1981, 14, 356–362.
10. Samson, M. S. P. Progr Biophys Mol Biol 1991, 55, 139–235.
11. Toniolo, C., Crisma, M.; Formaggio, F.; Peggion, C.; Epand, R. F.; Epand R. M. Cell Mol Life Sci 2001, 58, 1179–1188.
12. Fox, R. O., Jr.; Richards, F. M. Nature 1982, 300, 325–330.
13. Karle, I. L.; Flippen-Anderson, J.; Sukumar, M.; Balaram, P. Proc Natl Acad Sci USA 1987, 84, 5087–5091.
14. Karle, I. L.; Sukumar, M.; Balaram, P. Proc Natl Acad Sci USA 1986, 83, 9284–9288.
15. Karle, I. L.; Flippen-Anderson, J. L.; Agarwalla, S.; Balaram, P. Proc Natl Acad Sci USA 1991, 88, 5307–5311.
16. Karle, I. L.; Flippen-Anderson, J. L.; Agarwalla, S.; Balaram, P. Biopolymers 1994, 34, 721–735.
17. Karle, I. L.; Perozzo, M. A.; Mishra, V. K.; Balaram, P. Proc Natl Acad Sci USA 1998, 95, 5501–5504.
18. Snook, C. F.; Woolley, G. A.; Oliva, G.; Pattabhi Vasantha; Wood, S. P.; Blundell, T. L.; Wallace, B. A. Structure 1998, 6, 783–792.
19. Toniolo, C.; Peggion, C.; Crisma, M.; Formaggio, F.; Shui, X.; Eggleston, D. S. Nature Struct Biol 1994, 1, 908–914.
20. Beesley, R. M.; Ingold, C. K.; Thorpe, J. F. J Chem Soc 1915, 105, 1080–1087.
21. Sammes, P. G.; Weller, D. J. Synthesis 1995, 1205–1222.
22. Toniolo, C.; Crisma, M.; Formaggio, F.; Peggion, C. Biopolymers (Peptide Sci) 2002, in press.
23. Toniolo, C.; Benedetti, E. Trends Biochem Sci 1991, 16, 350–353.
24. Benedetti, E.; Di Blasio, B.; Pavone, V.; Pedone, C.; Santini, A.; Crisma, M.; Toniolo, C. In Molecular Conformation and Biological Interactions; Balaram, P., Ramaseshan, S., Eds.; Indian Academy of Sciences: Bangalore, 1991; pp 497–502.

25. Benedetti, E.; Di Blasio, B.; Pavone, V.; Pedone, C.; Toniolo, C. Biopolymers 1992, 32, 453–456.
26. Bolin, K. A.; Millhauser, G. L. Acc Chem Res 1999, 32, 1027–1033.
27. Pal, L.; Basu, G. Protein Eng 1999, 12, 811–814.
28. Yoder, G.; Polese, A.; Silva, R. A. G. D.; Formaggio, F.; Crisma, M.; Broxterman, Q. B.; Kamphuis, J.; Toniolo, C.; Keiderling, T. A. J Am Chem Soc 1997, 119, 10278–10285.
29. Mammi, S.; Rainaldi, M.; Bellanda, M.; Schievano, E.; Peggion, E.; Broxterman, Q. B.; Formaggio, F.; Crisma, M.; Toniolo, C. J Am Chem Soc 2000, 122, 11735–11736.
30. Yasui, S. C.; Keiderling, T. A.; Formaggio, F.; Bonora, G. M.; Toniolo, C. J Am Chem Soc 1986, 108, 4988–4993.
31. Yasui, S. C.; Keiderling, T. A.; Bonora, G. M.; Toniolo, C. Biopolymers 1986, 25, 79–89.
32. Bavoso, A.; Benedetti, E.; Di Blasio, B.; Pavone, V.; Pedone, C.; Toniolo, C.; Bonora, G. M.; Formaggio, F., Crisma, M. J Biomol Struct Dynam 1988, 5, 803–817.
33. Toniolo, C.; Formaggio, F.; Crisma, M.; Schoemaker, H. E.; Kamphuis, J. Tetrahedron: Asymmetry 1994, 5, 507–510.
34. Formaggio, F.; Crisma, M.; Toniolo, C.; Kamphuis, J. Biopolymers 1996, 38, 301–304.
35. Bellanda, M.; Peggion, E.; Bürgi, R.; van Gusteren, W.; Mammi, S. J Pept Res 2001, 57, 97–106.
36. Bavoso, A.; Benedetti, E.; Di Blasio, B.; Pavone, V.; Pedone, C.; Toniolo, C.; Bonora, G. M. Proc Natl Acad Sci USA 1986, 83, 1988–1992.
37. Toniolo, C.; Bonora, G. M.; Bavoso, A.; Benedetti, E.; Di Blasio, B.; Pavone, V.; Pedone, C. Macromolecules 1986, 19, 472–479.
38. Gratias, R.; Konat, R.; Kessler, H.; Crisma, M.; Valle, G.; Polese, A.; Formaggio, F.; Toniolo, C.; Broxterman, Q. B.; Kamphuis, J. J Am Chem Soc 1998, 120, 4763–4770.
39. Dehner, A.; Plancker, E.; Gemmecker, G.; Broxterman, Q. B.; Bisson, W.; Formaggio, F.; Crisma, M.; Toniolo, C.; Kessler, H. J Am Chem Soc 2001, 123, 6678–6686.
40. Kruizinga, W. H.; Bolster, J.; Kellogg, R. M.; Kamphuis, J.; Boesten, W. H. J.; Meijer, E. M.; Schoemaker, H. E. J Org Chem 1988, 53, 1826–1827.
41. Kaptein, B.; Boesten, W. H. J.; Broxterman, Q. B.; Peters, P. J. H.; Schoemaker, H. E.; Kamphuis, J. Tetrahedron: Asymmetry 1993, 4, 1113–1116.
42. Toniolo, C.; Crisma, M.; Bonora, G. M.; Klajc, B.; Lelj, F.; Grimaldi, P.; Rosa, A.; Polinelli, S.; Boesten, W. H. J.; Meijer, E. M.; Schoemaker, H. E.; Kamphuis, J. Int J Pept Protein Res 1991, 38, 242–252.
43. Toniolo, C.; Formaggio, F.; Crisma, M.; Bonora, G. M.; Pegoraro, S.; Polinelli, S.; Boesten, W. H. J.; Schoemaker, H. E.; Broxterman, Q. B.; Kamphuis, J. Pept Res 1992, 5, 56–61.
44. Formaggio, F.; Crisma, M.; Bonora, G. M.; Pantano, M.; Valle, G.; Toniolo, C.; Aubry, A.; Bayeul, D.; Kamphuis, J. Pept Res 1995, 8, 6–15.
45. Carpino, L. A.; Mansour, E. S. M. E.; Sadat-Aalaee, D. J Org Chem 1991, 56, 2611–2614.
46. Polese, A.; Formaggio, F.; Crisma, M.; Valle, G.; Toniolo, C.; Bonora, G. M.; Broxterman, Q. B.; Kamphuis, J. Chem Eur J 1996, 2, 1104–1111.
47. Carpino, L. A. J Am Chem Soc 1993, 115, 4397–4398.
48. Sheldrick, G. M. SHELXS 97. Program for the Solution of Crystal Structures; University of Göttingen: Göttingen, Germany, 1997.
49. Sheldrick, G. M. SHELXL 97. Program for Crystal Structure Refinement; University of Göttingen: Göttingen, Germany, 1997.
50. IUPAC-IUB Commission on Biochemical Nomenclature, Biochemistry 1970, 9, 3471–3479.
51. Ramakrishnan, C.; Prasad, N. Int J Protein Res 1971, 3, 209–231.
52. Taylor, R.; Kennard, O.; Versichel, W. Acta Crystallogr 1984, B40, 280–288.
53. Görbitz, C. H. Acta Crystallogr 1989, B45, 390–395.
54. Venkatachalam, C. M. Biopolymers 1968, 6, 1425–1436.
55. Toniolo, C. CRC Crit Rev Biochem 1980, 9, 1–44.
56. Rose, G. D.; Gierasch, L. M.; Smith, J. P. Adv Protein Chem 1985, 37, 1–109.
57. Pavone, V.; Gaeta, G.; Lombardi, A.; Saviano, M.; Nastri, F.; Maglio, O.; Isernia, C.; Saviano, M. Biopolymers 1996, 38, 705–721.
58. Chou, K.-C. Biopolymers 1997, 42, 837–853.
59. Ramakrishnan, C.; Nataraj, D. V. J Pept Sci 1998, 4, 239–252.
60. Benedetti, E.; Morelli, G.; Némethy, G.; Scheraga, H. A. Int J Pept Protein Res 1983, 22, 1–15.
61. Millhauser, G. L.; Stenland, C. J.; Hanson, P.; Bolin, K. A.; van der Ven, F. J. M. J Mol Biol 1997, 267, 963-974.
62. Vijayalakshmi, S.; Balaji Rao, R.; Karle, I. L.; Balaram, P. Biopolymers 2000, 53, 84–98.