The First Water-Soluble 3_{10}-Helical Peptides

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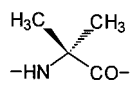
Abstract: Two water-soluble 3_{10}-helical peptides are synthesized and fully characterized for the first time. The sequence of these terminally blocked heptamers comprises two residues of the C^{α}-trisubstituted α-amino acid 2-amino-3-[1-(1,4,7-triazacyclononyl)]propanoic acid and five residues of a C^{α}-tetrasubstituted α-amino acid (either α-aminoisobutyric acid or isovaline). Using CD and NMR techniques we were able to show that both heptapeptides are well structured in water, and that the type of conformation adopted is indeed the ternary 3_{10}-helix.

Keywords: amino acids · conformation analysis · helical structures · oligopeptides · structure elucidation

Introduction

The 3_{10}-helix, first predicted as a reasonable polypeptide secondary structure 59 years ago,[1a] has only relatively recently attracted the attention of structural biochemists and protein crystallographers.[1b,c] Besides the classical α-helix, β-pleated sheet, and β-turn conformations, it represents the fourth principal structural element in globular proteins and has been described at atomic resolution by X-ray crystallography in model peptides and in peptaibol antibiotics.[1d–i] The average conformational parameters for the α-helix are close to those for the 3_{10}-helix, the latter being slightly tighter and more elongated.[1d] In particular, the backbone φ, ψ torsion angles for the two helices differ only by 6° and 12°, respectively. However, the C=O···H–N intramolecular H-bonding schemes are different, $i \leftarrow i+3$ for the 3_{10}-helix, and $i \leftarrow i+4$ for the α-helix.

Research from our as well as other laboratories have firmly established that most of the C^{α}-tetrasubstituted α-amino acids, in particular the prototype of this family α-aminoisobutyric acid (Aib), are extremely strong promoters of the $3_{10}/\alpha$-helical conformations, [1e–i] and are much more effective than the helicogenic C^{α}-trisubstituted (protein) amino acids. The main factors that govern the type of peptide helix were found to be the main-chain length, Aib fraction, and amino acid sequence. In the Karle–Balaram plot[1f] for peptides with Aib and protein amino acids the threshold between α- and 3_{10}-helices in *the crystal state* is given by 1 Aib/7 residues, 2 Aib/8 residues, 4 Aib/9 residues, 5 Aib/10 residues, 6 Aib/11 residues, 8 Aib/12 residues, and so on. For higher Aib fractions the 3_{10}-helix is preferred, whereas the α-helix is predominantly adopted for lower Aib fractions. Aib-containing peptides also adopt the 3_{10}-helical conformation *in solution* provided that the above factors are operative. Formation of fully developed, stable 3_{10}-helices in solvents of low polarity has been reported for Aib homooctapeptides and longer pleionomers.[2] Recently, our group[3] and McLaughlin and Hammer and their co-workers[4] have made attempts to design and synthesize water-soluble 3_{10}-helical peptides. To this end we exploited the positively charged, protein amino acid Lys, while McLaughlin and Hammer et al. took advantage of the very interesting, homemade, helix-inducing, positively charged, C^{α}-tetrasubstituted α-amino acid Api (4-aminopiperidine-4-carboxylic acid). However, all of the peptides that have been synthesized either proved to be soluble in aqueous/organic solvent mixtures only or, if fully water-soluble, they were found to fold in mixed $3_{10}/\alpha$-helices. Therefore, the observation of a stable peptide 3_{10}-helix in bulk water remained elusive.



Aib

Herein we describe for the first time the design, synthesis by solution methods, characterization, and conformational analysis by CD and ^{1}H NMR techniques under a variety of different experimental conditions of two water-soluble 3_{10}-helical peptides.

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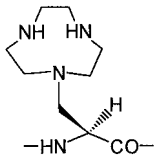
Results and Discussion

Design: Our aim was to combine in a single peptide two apparently contradictory properties: a high 3_{10}-helical propensity and a good water solubility. We decided to synthesize:

1) A peptide *N*-acylated at the N-terminus, in order to get an extra H-bond acceptor (the carbonyl of the blocking group) which allows us to reduce the number of residues in the backbone by one. The poorly hydrophobic acetyl (Ac) group was an obvious choice.
2) A peptide of seven residues with five (72%) C^{α}-tetrasubstituted α-amino acids, to maximize helical stability without biasing the conformation toward the α-helix.
3) An extremely water-solubilizing α-amino acid. In this connection, (L)-2-amino-3[1-(1,4,7-triazacyclononane)]propanoic acid (ATANP), an azacrown-functionalized α-amino acid, recently synthesized in our laboratory,[5] was a promising tool in our hands, due to its very favorable ratio of charged

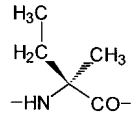


ATANP

heteroatoms to methylene groups. Thus, we planned to incorporate two nonconsecutive ATANP residues into the sequence to avoid formation of a potentially α-helix nucleating dipeptide stretch in the absence of a C^{α}-tetrasubstituted α-amino acid.

4) A protected C-terminus (in the form of the poorly hydrophobic methyl ester (OMe)) to facilitate the synthesis by elimination of a final hydrolytic step.

Therefore, we have synthesized and studied two Ac/OMe blocked heptapeptides, each based on a different C^{α}-tetrasubstituted α-amino acid. Peptide **1**, Ac-Aib-L-ATANP-(Aib)$_2$-L-ATANP-(Aib)$_2$-OMe, is characterized by Aib, the simplest achiral member of this family. Conversely, peptide **2**, Ac-L-Iva-L-ATANP-(L-Iva)$_2$-L-ATANP-(L-Iva)$_2$-OMe, is rich in Iva (isovaline), the simplest chiral member of this family. Aib and Iva are known to strongly support peptide helical conformations.[6] In addition, peptides based on the chiral Iva are more suitable than Aib-rich peptides for a CD characterization. A preliminary account of the exploitation of peptide **1** complexed with two Zn^{II} ions as a transphosphorylation catalyst in water has already been reported.[7]



Iva

Synthesis and characterization: Z-L-ATANP(Boc)$_2$-OH was obtained by regiospecific ring-opening of N^{α}-Z-protected L-2-amino-β-lactone with Boc-diprotected 1,4,7-triazacyclononane as previously described.[5] The N^{α}-Z- protected Aib[8a,b] and L-Iva[6b] residues were synthesized from Z-OSu [*N*-(benzyloxycarbonyl)succinimide].[9a,b] Aib[8a] and Iva[6d] methylester hydrochlorides were prepared by treatment of the free amino acid with thionyl chloride in methanol. The two heptapeptides were synthesized by solution methods. Introduction of Aib and Iva residues was achieved either by the symmetrical anhydride[8] or by the EDC/HOAt (EDC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide; HOAt, 1-hydroxy-7-aza-1,2,3-benzotriazole)[9c] condensation method. The side-chain-protected L-ATANP residues were incorporated by the EDC/HOAt method. N^{α}-Acetylation was obtained by treatment of the N^{α}-deprotected peptide with acetic anhydride in methylene chloride. The side chain *N*-Boc protecting groups were removed by acidolysis. Removal of the N^{α}-Z-protection was achieved by catalytic hydrogenation.

The two heptapeptides and their synthetic intermediates were characterized by melting point determination, optical

Abstract in Italian: *Per la prima volta sono stati sintetizzati e caratterizzati in dettaglio due peptidi 3_{10}-elicoidali idrosolubili. La sequenza di questi eptameri N- e C- bloccati si basa su due residui di un α-amminoacido C^{α}-trisostituito, l'acido 2-ammino-3-[1-(1,4,7-triazaciclonano)] propionico, e cinque residui di un α-amminoacido C^{α}-tetrasostituito (acido α-amminoisobutirrico o isovalina). Utilizzando le tecniche CD e NMR si è dimostrato che entrambi gli eptapeptidi sono ben strutturati in soluzione acquosa e che il tipo di conformazione adottata è realmente l'elica ternaria 3_{10}.*

rotatory power, thin-layer chromatography in three different solvent systems, and solid-state IR absorption (Table 1), and by HPLC, mass determination and ^{1}H NMR spectroscopy (data not shown).

Conformational analysis: The preferred conformations of the Aib/L-ATANP peptide **1** and the L-Iva/L-ATANP peptide **2** were examined by far-UV CD in aqueous solution as a function of concentration, pH, and temperature, and in mixed aqueous/organic (TFE; 2,2,2-trifluoroethanol) solutions (Figure 1 and Table 2 and Table 3).

The CD patterns of peptides **1** and **2** are similar, and are characterized by a negative Cotton effect in the 201–206 nm region accompanied by a pronounced negative shoulder centered at approximately 222 nm. Whereas in the right-handed α-helix the intensities of the two negative maxima centered at 208 nm (parallel component of the $\pi \rightarrow \pi^*$ transition) and 222 nm ($n \rightarrow \pi^*$ transition) are very close,[10a] it is worth recalling that in the 3_{10}-helix the $n \rightarrow \pi^*$ transition exhibits a drastically reduced intensity with respect to that of the $\pi \rightarrow \pi^*$ transition, and tends to undergo a modest blue shift.[10b] More specifically, a value of 0.15–0.40 for the $[\theta]_{222}/[\theta]_{\text{neg. max.}(\cong 205\,\text{nm})}$ (R) ratio is considered diagnostic for the 3_{10}-helical conformation, while values close to unity are seen as typical of the α-helix. On this basis and with the CD spectrum of Ac-[L-(αMe)Val]$_8$-O*t*Bu (O*t*Bu, *tert*-butoxy) in TFE as the reference,[10c–e] the two peptides examined in this work adopt the right-handed 3_{10}-helical conformation to a substantial extent in aqueous and aqueous/organic environments.

In water the CD curves do not appreciably change with a decrease in peptide concentration (at least from 1.5 to

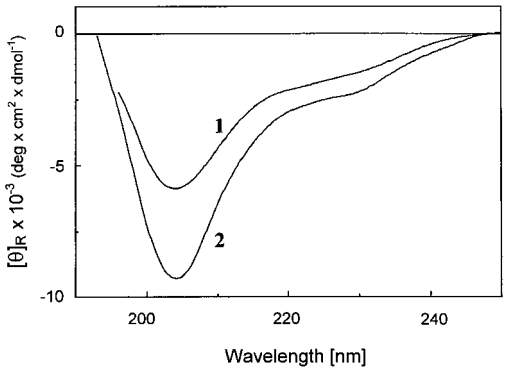


Figure 1. CD spectra of the terminally blocked heptapeptides **1** and **2** in water. Peptide concentration: 1 mM. The data are expressed in terms of $[\theta]_R$, the *residue* molar ellipticity (deg cm^2 dmol^{-1}).

Table 2. Wavelength maxima of CD bands, corresponding residue molar ellipticity values, and $[\theta]_{222}/[\theta]_{\text{neg.}}$ max. ($\cong$205 nm) (R) ratios for the terminally blocked heptapeptide **1**.

Experimental conditions	λ [nm] ($[\theta]_R \times 10^{-3}$)		R
H_2O (1.0 mM)[a]	202 (−4.3)	222 (−0.8)	0.18
H_2O (pH 6; 1.0 mM)	204 (−5.4)	222 (−1.8)	0.35
H_2O (pH 7; 1.0 mM)	204 (−6.0)	222 (−2.2)	0.36
H_2O (pH 8; 1.0 mM)	204 (−5.9)	222 (−2.2)	0.37
H_2O (pH 9; 1.0 mM)	205 (−5.7)	222 (−2.2)	0.39
H_2O (pH 8; 1.0 mM; 0 °C)	204 (−6.8)	222 (−2.7)	0.40
H_2O (pH 8; 1.0 mM; 15 °C)	204 (−6.8)	222 (−2.5)	0.37
H_2O (pH 8; 1.0 mM; 25 °C)	204 (−6.0)	222 (−2.3)	0.38
H_2O (pH 8; 1.0 mM; 40 °C)	204 (−5.8)	222 (−2.2)	0.39
H_2O (pH 8; 1.0 mM; 60 °C)	205 (−5.3)	222 (−1.9)	0.35

[a] Peptide concentration in parentheses.

Table 1. Physical properties and analytical data for the terminally blocked Aib/L-ATANP and L-Iva /L-ATANP peptides.

Compound	M. p. [°C][b]	$[\alpha]_D^{20}$ [c]	TLC[f]			IR $\tilde{\nu}$[cm^{-1}][g]
			R_f(I)	R_f(II)	R_f(III)	
Z-L-ATANP(Boc)$_2$-(Aib)$_2$-OMe	71–73		0.90	0.95	0.40	3361, 1741, 1684, 1520
Z-L-ATANP(Boc)$_2$-(L-Iva)$_2$-OMe	67–69	−28.3	0.95	0.90	0.35	3361, 1736, 1690, 1519
Z-Aib-L-ATANP(Boc)$_2$-(Aib)$_2$-OMe	98–100	−10.8	0.90	0.90	0.40	3342, 1739, 1686, 1521
Z-L-Iva-L-ATANP(Boc)$_2$-(L-Iva)$_2$-OMe	84–86	−14.2	0.95	0.90	0.35	3342, 1735, 1686, 1519
Z-(Aib)$_2$-L-ATANP(Boc)$_2$-(Aib)$_2$-OMe	113	−9.6	0.75	0.95	0.30	3329, 1739, 1686, 1528
Z-(L-Iva)$_2$-L-ATANP(Boc)$_2$-(L-Iva)$_2$-OMe	93–95	−13.1	0.95	0.90	0.30	3326, 1737, 1687, 1527
Z-L-ATANP(Boc)$_2$-(Aib)$_2$-L-ATANP(Boc)$_2$-(Aib)$_2$-OMe	133	−22.7	0.80	0.95	0.35	3326, 1739, 1687, 1526
Z-L-ATANP(Boc)$_2$-(L-Iva)$_2$-L-ATANP(Boc)$_2$-(L-Iva)$_2$-OMe	108–109	−26.4	0.95	0.90	0.30	3335, 1736, 1686, 1523
Z-Aib-L-ATANP(Boc)$_2$-(Aib)$_2$-L-ATANP(Boc)$_2$-(Aib)$_2$-OMe	150	−9.8	0.85	0.95	0.35	3322, 1740, 1688, 1663, 1528
Z-L-Iva-L-ATANP(Boc)$_2$-(L-Iva)$_2$-L-ATANP(Boc)$_2$-(L-Iva)$_2$-OMe	98–100	−10.9	0.90	0.95	0.35	3324, 1735, 1689, 1668, 1525
Ac-Aib-L-ATANP(Boc)$_2$-(Aib)$_2$-L-ATANP(Boc)$_2$-(Aib)$_2$-OMe	144	−12.9	0.70	0.80	0.25	3322, 1741, 1688, 1660, 1530
Ac-L-Iva-L-ATANP(Boc)$_2$-(L-Iva)$_2$-L-ATANP(Boc)$_2$-(L-Iva)$_2$-OMe	100–102		0.80	0.95	0.20	3303, 1731, 1659, 1533
Ac-Aib-L-ATANP-(Aib)$_2$-L-ATANP-(Aib)$_2$-OMe[a]	210–212	−4.1[d]				
		−12.2[e]	0.05	0.05	0.05	3425, 3310, 1726, 1657, 1538
Ac-L-Iva-L-ATANP-(L-Iva)$_2$-L-ATANP-(L-Iva)$_2$-OMe[a]	240–242	−32.9	0.05	0.05	0.05	3417, 3363, 1734, 1661, 1535

[a] Characterized as a salt (see Experimental Section). [b] Determined on a Leitz model Laborlux apparatus. [c] Determined on a Perkin Elmer model 241 polarimeter equipped with a Haake model L thermostat: $c = 0.5$ (methanol). [d] $c = 0.1$ (methanol). [e] $c = 0.1$ (methanol), $\lambda = 436$ nm. [f] Silica gel plates (60F-254 Merck), using the following solvent systems: (I) chloroform/ethanol 9:1; (II) butan-1-ol/water/acetic acid 3:1:1; (III) toluene/ethanol 7:1. The compounds were revealed either with the aid of a UV lamp or with the hypochlorite/starch/iodide chromatic reaction, as appropriate. A single spot was observed in each case. [g] Determined in KBr pellets on a Perkin-Elmer model 580 B spectrophotometer equipped with a Perkin Elmer model 3600 IR data station and a model 660 printer. Only bands in the 3500–3200 cm^{-1} and 1800–1500 cm^{-1} regions are listed.

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Table 3. Wavelength maxima of CD bands, corresponding residue molar ellipticity values, and $[\theta]_{222}/[\theta]_{\text{neg. max. (}\cong 205\text{ nm)}}$ (R) ratios for the terminally blocked heptapeptide **2**

Experimental conditions	λ [nm] ($[\theta]_R \times 10^{-3}$)		R
H_2O (1.5 mM)[a]	201 (−9.6)	222 (−1.8)	0.20
H_2O (1.0 mM)	201 (−10.0)	222 (−2.0)	0.20
H_2O (0.5 mM)	201 (−9.9)	222 (−2.7)	0.27
H_2O (pH 2; 1.0 mM)	201 (−9.8)	222 (−1.3)	0.15
H_2O (pH 4; 1.0 mM)	202 (−9.8)	222 (−1.8)	0.18
H_2O (pH 6; 1.0 mM)	204 (−9.6)	222 (−2.9)	0.29
H_2O (pH 7; 1.0 mM)	204 (−9.3)	222 (−2.8)	0.30
H_2O (pH 8; 1.0 mM)	204 (−9.3)	222 (−3.2)	0.34
H_2O (pH 10; 1.0 mM)	204 (−10.4)	222 (−3.6)	0.35
H_2O (pH 12; 1.0 mM)	206 (−10.3)	222 (−5.7)	0.55
H_2O/TFE (75:25)	203 (−9.6)	222 (−1.7)	0.17
H_2O/TFE (50:50)	203 (−10.5)	222 (−1.9)	0.18
H_2O/TFE (25:75)	203 (−10.5)	222 (−1.9)	0.18
H_2O (pH 8; 1.0 mM; 0 °C)	204 (−10.1)	222 (−3.6)	0.36
H_2O (pH 8; 1.0 mM; 20 °C)	203 (−9.9)	222 (−3.2)	0.32
H_2O (pH 8; 1.0 mM; 40 °C)	203 (−9.8)	222 (−2.9)	0.30
H_2O (pH 8; 1.0 mM; 60 °C)	204 (−9.5)	222 (−2.6)	0.27

[a] Peptide concentration in parentheses.

0.5 mM). This finding, which reflects the absence of extensive self-association, is not surprising as the high percentage of the helicogenic Aib/Iva residues in the amino acid sequence is known to prevent significant β-sheet formation.[11] Also, the far-UV CD spectra are not affected by coordination of metal ions such as Zn^{II} or Cu^{II}.

In view of the presence of the three amino groups in the side chain of each of the two ATANP residues we have carefully examined the influence of pH on the preferred conformation of peptides **1** and **2**. Indeed, if the secondary structure adopted is 3_{10}-helical, then the two ATANP residues will be positioned on the same side of the ternary helix (Figure 2). Thus, the two heterocyclic moieties, despite the rotation allowed by the $C^{\beta}H_2$ group, should approximately face each other after one complete helical turn. The electrostatic repulsion between these two protonated side chains might somehow influence the peptide helical conformation. The pK_a values reported in the literature for 1,4,7-triazacyclononane are 2.41, 6.82, and 10.42,[12] but it is reasonable to assume that the proximity of the two heterocycles combined with the N-monoalkylation would introduce some changes in those values. Our CD results support the view that upon complete neutralization of the ATANP side chains the $\pi \rightarrow \pi^*$ negative maximum undergoes a 5-nm red-shift, while the intensity of the $n \rightarrow \pi^*$ negative shoulder increases from acidic pH values to neutrality, remains approximately constant in the $6 < pH < 9$ region and eventually increases again from neutral to alkaline pH values. The R ratio is pH-dependent, in the sense that it roughly parallels the observed intensity of the $n \rightarrow \pi^*$ transition. It is our contention that the CD pattern at pH 12, at which the triazacyclononane moieties are fully deprotonated, would reflect a significant population of both 3_{10}- and α-helical conformations.

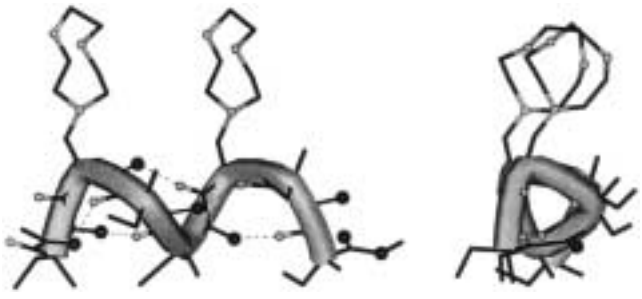

Figure 2. Two orthogonal views of the proposed molecular model for heptapeptide **2**. The peptide backbone is in the canonical 3_{10}-helical conformation. The five C=O···H–N intramolecular hydrogen bonds are indicated by dashed lines. The Iva and ATANP side chain torsion angles are arbitrary.

Although the molecular mechanisms by which TFE influences the peptide conformation are not well understood despite a number of investigations,[13] the helix-stabilizing properties of TFE have been widely demonstrated since their discovery by Goodman and co-workers.[14] However, in the case of our peptides rich in the helicogenic C^{α}-tetrasubstituted amino acids it seems that TFE would not induce any major variation, neither in the helix content nor in the relative amount of $3_{10}/\alpha$-helices. We interpret these results as clear evidence for the occurrence of a remarkable helix stabilization even in aqueous solution. This conclusion is supported by a modest decrease of the intensities of both negative maxima upon heating the aqueous solution (pH 8) from 0 to 60 °C.

A simple visual comparison of the CD spectra of peptides **1** and **2** under the same experimental conditions clearly shows that the general shape of the curves and the R values are comparable, whereas the negative ellipticity values are almost doubled for the L-Iva/L-ATANP peptide **2**. We believe that this observation is strictly related to the presence in peptide **2** of five chiral L-Iva residues as opposed to five achiral Aib residues in the Aib/L-ATANP peptide **1**. More specifically, it is our contention that this experimental finding is mainly associated with the concomitant occurrence of a nonnegligible amount of *left*-handed 3_{10}-helix in the conformational equilibrium mixtures of peptide **1**, characterized by less than 30% of chiral (L) residues. Indeed, there is no evidence in the literature for a different ability of the structurally related Aib and Iva residues to support a helical conformation.

Finally, if the CD spectrum of peptide **2** in water is compared with that of the (αMe)Val homooctapeptide in TFE solution:[10c–e] 1) The shapes of the curves are very similar, despite an approximately 5-nm blue-shift for peptide **2** in water. 2) Although both R values would be in the range expected for a 3_{10}-helix, that of peptide **2** is appreciably reduced as a consequence of a concomitant higher intensity of the negative $\pi \rightarrow \pi^*$ band and a lower intensity of the negative $n \rightarrow \pi^*$ band. Thus, on the basis of this chirospectroscopic analysis, despite a range of opinion on the nature of the CD spectra of 3_{10}-/α-helices,[15] we are confident that peptides **1** and **2** would be folded in the 3_{10}-helical structure under the above discussed experimental conditions.

We have expanded the conformational analysis by examination of peptide **2** in a 9:1 D_2O/H_2O mixture using the NMR technique. By combining the information extracted from the two-dimensional TOCSY spectrum[16a] with the through-space connectivities of the $C^{\alpha}H(i) \rightarrow NH(i+1)$ and $NH(i) \rightarrow NH(i+1)$ types, as obtained from the ROESY experi-

ments,[16b,c] we have been able to assign all of the proton resonances, with the exception of those related to the methylene protons of the triazacyclononane moieties (Table 4). In particular, the N^{α}-acetyl group was easily recognized from the isolated methyl resonance at $\delta = 1.95$. The strong cross-peak between the acetyl protons and one NH proton has allowed us to assign this latter resonance to Iva[1]. By a successive analysis of the ROESY spectrum in the amide proton region (Figure 3) we have identified without ambiguity the doublet at $\delta = 7.72$ to be associated with the ATANP[5] NH proton and, by exclusion, the doublet at $\delta = 8.20$ with the ATANP[2] NH proton. By following the sequential NH(i) → NH($i+1$) connectivities a complete assignment of all of the amide protons was easily achieved.

Table 4. Assignment of ^{1}H resonances for the terminally blocked heptapeptide **2** in a 9:1 D_2O/H_2O solvent mixture (peptide concentration: 10 mM)

Residue	^{1}H	δ (ppm)	Residue	^{1}H	δ (ppm)
Iva[1]	NH	8.22	ATANP[5]	NH	7.72
	βCH_3	1.32		αCH	4.41
	βCH_2	1.65, 1.82		βCH_2	2.93, 3.14
	γCH_3	0.76			
ATANP[2]	NH	8.20	Iva[6]	NH	7.83
	αCH	4.50		βCH_3	1.35
	βCH_2	3.02, 3.09		βCH_2	1.77, 1.85
				γCH_3	0.78
Iva[3]	NH	7.98			
	βCH_3	1.32			
	βCH_2	1.72, 1.84	Iva[7]	NH	7.45
	γCH_3	0.77		βCH_3	1.34
				βCH_2	1.78
Iva[4]	NH	7.53		γCH_3	0.76
	βCH_3	1.33			
	βCH_2	1.71, 1.81			
	γCH_3	0.75			

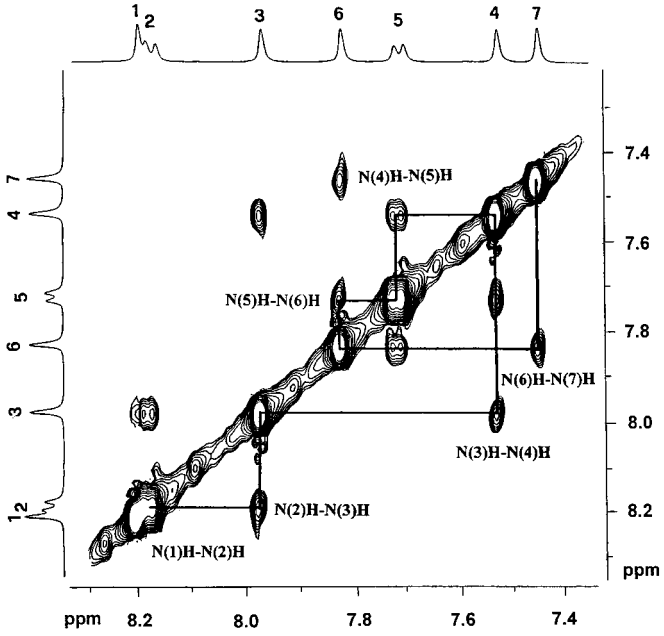

Figure 3. Section of the ROESY spectrum of the terminally blocked heptapeptide **2** in a 9:1 D_2O/H_2O mixture (peptide concentration: 10 mM). The NH(i → NH($i+1$) sequential connectivities are indicated.

The strong intensities of the NH(i) → NH($i+1$) cross-peaks (Figure 3) are consistent with the hypothesis that peptide **2** would largely adopt a helical structure in aqueous solution. Moreover, thanks to the presence of the two strategically positioned C^{α}-trisubstituted α-amino acids ATANP in the sequence, the ROESY spectrum (Figure 4) clearly shows two

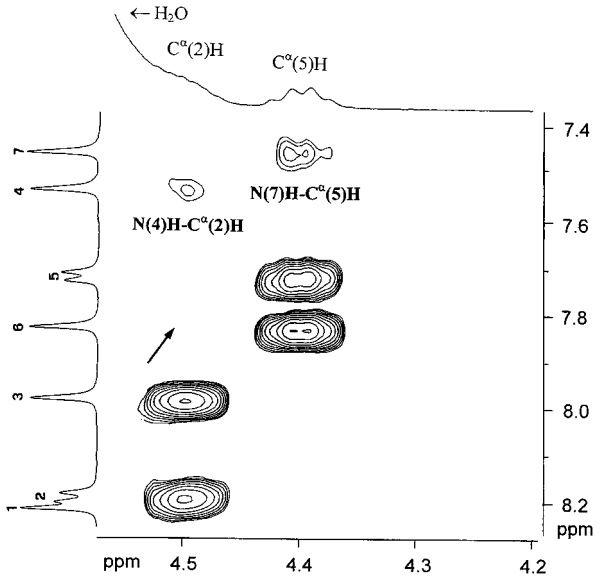

Figure 4. Section of the ROESY spectrum of the terminally blocked heptapeptide **2** in a 9:1 D_2O/H_2O mixture (peptide concentration: 10 mM). The two [$d_{\alpha N}(i, i+2)$] cross-peaks typical of a 3_{10}-helix are indicated. The arrow shows the position in the spectrum where the [$d_{\alpha N}(i, i+4)$] cross-peak, typical of an α-helix, is expected.

long-range connectivities, between the $C^{\alpha}H$ proton of ATANP[2] and the NH proton of Iva[4], and between the $C^{\alpha}H$ proton of ATANP[5] and the NH proton of Iva[7], both corresponding to the $d_{\alpha N}$ (i, $i+2$) interaction diagnostic of the 3_{10}-helix. In the α-helix, $d_{\alpha N}$ (i, $i+2$) would be approximately 4.5 Å, whereas in the 3_{10}-helix the same distance may vary from about 3.5 to 3.9 Å depending on whether the torsion angle ψ is closer to -20° or -30°, respectively. Therefore, only in a 3_{10}-helix with $\psi \cong -20^{\circ}$ one expects to detect the αN(i, $i+2$) cross-peak, although it would be of rather low intensity.[16d] In this peptide the only αN(i, $i+3$) detectable NOE is that relating the ATANP[2] $C^{\alpha}H$ proton to the ATANP[5] NH proton. Since this interaction is not observed, it is reasonable to assume that the ψ angles in the central part of the 3_{10}-helix are rather close to -20°. Indeed, with such a ψ value the $d_{\alpha N}(i, i+3)$ would be rather long (3.9 Å), while it is relatively short (3.5 Å) when $\psi = -30^{\circ}$.

The methyl protons of the N^{α}-acetyl group are positioned where the $C^{\alpha}H$ proton of an extra N-terminal residue would be located. Therefore, the distances acetyl $\cdots$ N(i, $i+j$) ($j = 2-4$) are equivalent to $d_{\alpha N}(i, i+j)$ ($j = 2-4$). Interestingly, the ROESY spectrum shows an acetyl $\cdots$ N(i, $i+2$) cross-peak, of comparable intensity to those of the αN(i, $i+2$) type described above, indicative of a 3_{10}-helical structure. However, the acetyl $\cdots$ N(i, $i+3$) NOE is also observed, but its intensity is very low despite the contribution of three protons. Finally, the α-helix characterizing, potentially visible NOEs

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acetyl $\cdots$ N(i, i+4) and $d_{\alpha N}$(i, i+4) between the $C^{\alpha}H$ proton of ATANP[2] and the NH proton of IVA[6] are absent altogether in the ROESY spectrum.

As a means of expanding our NOE data base and tightening the structural evidence, we have also looked for βN(i, i+2) and βN(i, i+3) NOEs which involve the C^{α}-methyl protons of the five Iva residues. As these resonances extensively overlap, the βN(i, i+2) and βN(i, i+3) NOEs are difficult to track. Indeed, under these experimental conditions the resolution is not sufficient to distinguish the contributions of these NOEs from those of the strong, overlapping intraresidue βN cross-peaks.

Taken together, these CD and NMR experimental observations point to the conclusion that peptide **2** tends to fold into a 3_{10}-helix in aqueous solution.

Conclusion

We have synthesized by solution methods, fully characterized, and investigated by CD and ^{1}H NMR techniques the preferred conformation of the two water-soluble, terminally blocked peptides Ac-Aib-L-ATANP-$(Aib)_2$-L-ATANP-$(Aib)_2$-OMe (**1**) and Ac-L-Iva-L-ATANP-$(L\text{-}Iva)_2$-L-ATANP-$(L\text{-}Iva)_2$-OMe (**2**). In aqueous solution the two heptamers are folded in the 3_{10}-helical conformation to a large extent. This is the first observation of such a peptide helix in a pure aqueous milieu and will open the door to this attractive system as a short and rigid molecular ruler or template to explore some fundamental principles of spectroscopic interactions and supramolecular chemistry under physiological conditions.

Experimental Section

Materials: The physical properties and analytical data for the terminally blocked Aib/L-ATANP and L-Iva/L-ATANP peptides are listed in Table 1. All Aib/L-ATANP peptides with side chain Boc-protection were purified by flash chromatography (Silica gel 60, 230–400 mesh, Merck) with $CHCl_3$/EtOH as eluents. The final deprotected heptapeptide was obtained as a salt in a chromatographically homogeneous form upon addition of diethyl ether to the HBr/CH_3COOH deprotecting solvent mixture. Additional experimental details on the Aib/L-ATANP peptide series may be found in the Supplementary Material in reference [7]. Also for the L-Iva/L-ATANP series a flash chromatography step (under conditions analogous to those used for the Aib/L-ATANP series) was required to purify the side-chain Boc-protected peptides. A 30 min treatment of the N^{α}-acetylated, side-chain-protected, heptapeptide with HCl (2 N) in methanol gave the final deprotected heptapeptide as a salt, which was purified by HPLC on a C_{18} column (Vydac 218TP510, 250 × 10 mm) by elution with a 0.05% HCl-containing H_2O/CH_3CN mixture (20% to 40% CH_3CN over 20 min at a flow rate of 3 mL min^{-1}).

Circular dichroism: The CD spectra of the terminally blocked heptapeptides **1** and **2** were recorded immediately after dissolution on a Jasco model J-715 dichrograph. Cylindrical, fused quartz cells of 1 and 0.1 mm path lengths were employed. The data are expressed in terms of $[\theta]_R$, the *residue* molar ellipticity (deg cm^2 dmol^{-1}). The length of *both* peptides **1** and **2** was calculated as seven residues, that is independent of the number of chiral residues. The concentration of each solution of peptide was determined by spectrophotometric titration with a Cu^{II} solution of known concentration by formation of a triazacyclononyl–Cu^{II} complex the absorption maximum of which is found at about 600 nm. 2,2,2-Trifluoroethanol is an ACROS product. The following buffer solutions have been used: pH 2, pH 4, pH 7, pH 8, pH 10, pH 12: 5 mM phosphate buffer; pH 6: 2 mM MES (4-morpholino-ethanesulfonic acid) monohydrated buffer; pH 9: 2 mM CHES [2-(cyclohexylamino)ethanesulfonic acid] buffer.

Nuclear magnetic resonance: The ^{1}H NMR spectra of the terminally blocked heptapeptide **2** were recorded with a Bruker model AM400 spectrometer. Measurements were carried out in a 9:1 D_2O (99.8% D; Fluka)/H_2O solvent mixture. Peptide concentration: 10 mM.

Mass spectrometry: Mass spectra were recorded using a time-of-flight matrix-assisted laser desorption ionization (MALDI) mass spectrometer (Kompact MALDI-1, Kratos-Shimadzu). α-Cyano-4-hydroxycinnamic acid, dissolved in acetonitrile and 0.1% aqueous TFA (2:3 ratio, by volume), was used as matrix, while bovine insulin and synthetic peptides of known molecular masses were used as standards for instrument calibration. Raw data were analyzed and stored by the software Kompact provided by Kratos.

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