Pergamon

Bioorganic & Medicinal Chemistry, Vol. 3, No. 9, pp. 1211–1221, 1995

0968-0896/95 $9.50 + .00

0968-0896(95)00109-3

The Polypeptide 3_{10}-Helix as a Template for Molecular Recognition Studies. Structural Characterization of a Side-chain Functionalized Octapeptide

Claudio Toniolo,[a]* Alberto Bianco,[a] Fernando Formaggio,[a] Marco Crisma,[a] Gian Maria Bonora,[a] Ettore Benedetti,[b]* Valerio Del Duca,[b] Michele Saviano,[b] Benedetto Di Blasio,[b] Carlo Pedone[b] and André Aubry[c]

[a]*Biopolymer Research Center, C.N.R., Department of Organic Chemistry, University of Padova, 35131 Padova, Italy*
[b]*Biocrystallography Research Center, C.N.R., Department of Chemistry, University of Naples "Federico II", 80134 Naples, Italy*
[c]*Laboratory of Mineralogy–Crystallography and Infrared Physics, URA CNRS 809, University of Nancy I, 54506 Vandoeuvre-les-Nancy, France*

Abstract—A N^{α}-blocked, Aib-rich octapeptide methylamide containing two N^{ω}-benzoylated L-Lys residues at positions 3 and 6 was synthesized by solution methods and fully characterized. A solution and crystal-state conformational analysis, performed by using FT-IR, ^{1}H NMR, CD, and X-ray diffraction techniques, showed that the peptide is folded into a regular, right-handed 3_{10}-helix stabilized by seven consecutive N–H...O=C intramolecular H-bonds of the β-turn III type. The two benzamidobutyl L-Lys side chains, located on the same side of the helix after one complete turn, generate a cleft the minimal width of which was found to be 3.47 Å.

Introduction

A proper understanding of the mechanisms of molecular recognition depends heavily upon the ability to design and build conformationally constrained structures whose intercomponent geometry is well defined. In this connection we chose to focus on structurally restricted helical oligopeptides, which fold to bring into close proximity two partners positioned one turn apart, as appropriate templates.[1–7] More specifically, in the present study we synthesized and investigated the solution and crystal-state preferred conformation of a side-chain functionalized, terminally blocked octapeptide containing six Aib (α-aminoisobutyric acid or $C^{\alpha,\alpha}$-dimethylglycine) residues to induce a strong 3_{10}-helical bias.[8–10] Furthermore, two N^{ω}-benzoylated L-Lys residues are installed within the sequence at positions 3 and 6.

The 3_{10}-helix, first predicted as a reasonably stable polypeptide secondary structure more than fifty years ago,[11] has only recently attracted the attention of structural biochemists and protein crystallographers. It represents the third principal structure occurring in globular proteins (in addition to the α-helix and the β-pleated sheet structure) and has been described at atomic resolution in model peptides and in peptaibol antibiotics (for recent review articles see Refs 12–14).

Results and Discussion

The solution preferred conformation of the terminally blocked octapeptide Ac-$(Aib)_2$-L-Lys(Bz)-$(Aib)_2$-L-Lys(Bz)-$(Aib)_2$-NHMe (Ac, acetyl; Bz, benzoyl; NHMe, methylamino) was determined in DMSO (dimethylsulfoxide) and $CHCl_3$:DMSO solvent mixtures using FT-IR absorption and ^{1}H NMR (this peptide is not soluble in a 100% $CHCl_3$ solution), and in TFE (2,2,2-trifluoroethanol) using CD.

In the 3500–3250 cm^{-1} (amide A) region the IR absorption bands at 3426 cm^{-1} (weak), 3343 cm^{-1} (strong) and 3316 cm^{-1} (strong) are assigned, respectively, to free peptide and amide NH groups, to intramolecularly H-bonded NH groups, and to N–H...O=S DMSO-solvated[15] NH groups. In the 1750–1500 cm^{-1} (C=O stretching) region two intense IR absorption bands are visible, at 1657–1656 cm^{-1} (amide I) and 1540–1535 cm^{-1} (amide II), respectively (results not shown).[16–18] From these data we can safely exclude the occurrence to a significant extent of unordered and β-sheet structures in the conformational equilibrium mixture of the octapeptide; however, a discrimination between 3_{10}- and α-helices[12–14] is not possible on the basis of this spectroscopic technique alone, as both structures are known to exhibit their conformationally most significant bands in the spectral ranges 3350–3330, 1670–1655, and 1540–1530 cm^{-1}.[16–18]

In order to get more detailed information on the solution conformation of the Aib/Lys(Bz) host/guest octapeptide, the ^{1}H NMR NH chemical shifts were investigated as a function of: (i) addition of the strong H-bonding acceptor solvent DMSO[15] (beginning from 5%

DMSO) to the $CDCl_3$ solution;[19] (ii) addition of the paramagnetic free radical TEMPO[20] to a 95:5 $CDCl_3$:DMSO solution; and (iii) heating a 100% DMSO solution.[21,22]

The only NH resonance identified unambiguously is that of the C-terminal methylamido NH group by virtue of its multiplicity (quartet). The other NH resonances have been assigned to the type of NH group only, namely to Aib NH groups (singlets), Lys αNH groups (doublets) or Lys εNH groups (triplets).

In our ^1H NMR titration and perturbation study two classes of NH protons were observed: (i) two singlet (Aib) and two triplet (Lys side-chain) NH protons, whose chemical shifts are particularly sensitive to addition of DMSO (Fig. 1A) and heating (Fig. 1B), and whose resonances significantly broaden upon addition of TEMPO (not shown); and (ii) all other NH protons, whose resonances, in terms of chemical shifts and linewidth, are only marginally sensitive to environmental changes. These conclusions (four freely accessible NH protons and seven almost inaccessible, and therefore intramolecularly H-bonded, NH protons) are corroborated by the low- and high-field positions of accessible and inaccessible NH protons, respectively, in the ^1H NMR spectrum (Fig. 1B).

Although we did not make an unambiguous assignment for any NH proton resonance (except for the C-terminal methylamido NH proton) these ^1H NMR findings, taken together with those of the FT-IR absorption study discussed above, strongly support the view that the structure preferentially adopted in $CHCl_3$:DMSO solution by Ac-$(Aib)_2$-L-Lys(Bz)-$(Aib)_2$-L-Lys(Bz)-$(Aib)_2$-NHMe is the 3_{10}-helix[12-14] (a series of consecutive, type-III β-turns[23-25]) rather than the α-helix,[12-14] which would have required the backbone NH protons not involved in the intramolecular H-bonding to be three, including one (doublet) Lys αNH proton, instead of only the two NH protons (Aib singlets) expected for the 3_{10}-helix.

Also in the H-bonding donor solvent TFE the terminally blocked octapeptide appears to be predominantly folded in a 3_{10}-helix. In the CD spectrum a negative shoulder is seen near 220 nm (peptide $n\rightarrow\pi^*$ transition) followed by the exciton splitting of the peptide $\pi\rightarrow\pi^*$ transition (strong negative maximum at 205 nm and strong positive maximum in the vicinity of 190 nm) (Fig. 2). This dichroic pattern is suggestive of the onset of a significant amount of right-handed helical conformation.[26] The screw sense of the helix is dictated by the two internal Lys residues of the L-configuration. On the basis of the criterion proposed by Manning and Woody[27] for distinguishing 3_{10}- and α-helices by CD (3_{10}-helices are expected to have a weaker 222 nm band relative to their 208 nm band) we can safely con-clude that the helical structure adopted by the octa-peptide in TFE solution is of the 3_{10}-type.

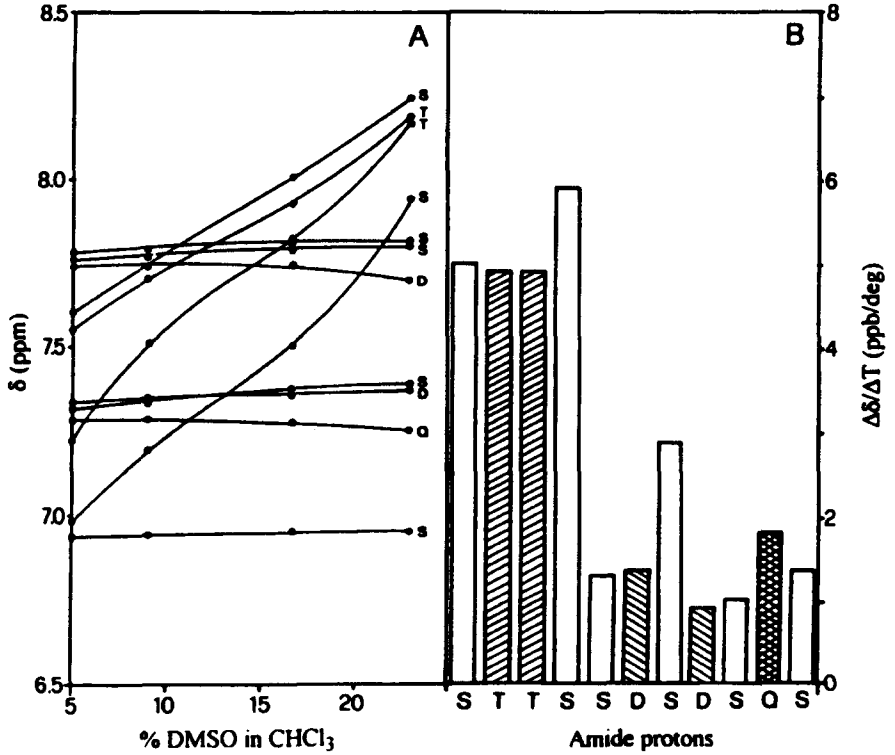

Figure 1. (A) Plot of the NH chemical shifts in the ^1H NMR spectrum of Ac-$(Aib)_2$-L-Lys(Bz)-$(Aib)_2$-L-Lys(Bz)-$(Aib)_2$-NHMe versus increasing percentages of DMSO added to the $CDCl_3$ solution (v/v). Peptide concentration is 1×10^{-3} M. Symbols *S*, *D*, *T*, and *Q* refer to singlet, doublet, triplet, and quartet, respectively. (B) Temperature coefficients of the NH protons of the same peptide in DMSO solution (peptide concentration 5×10^{-3} M) measured in the range 298–343 K. Symbols are as in part (A). Signals are listed according to their position in the ^1H NMR spectrum beginning from low fields.

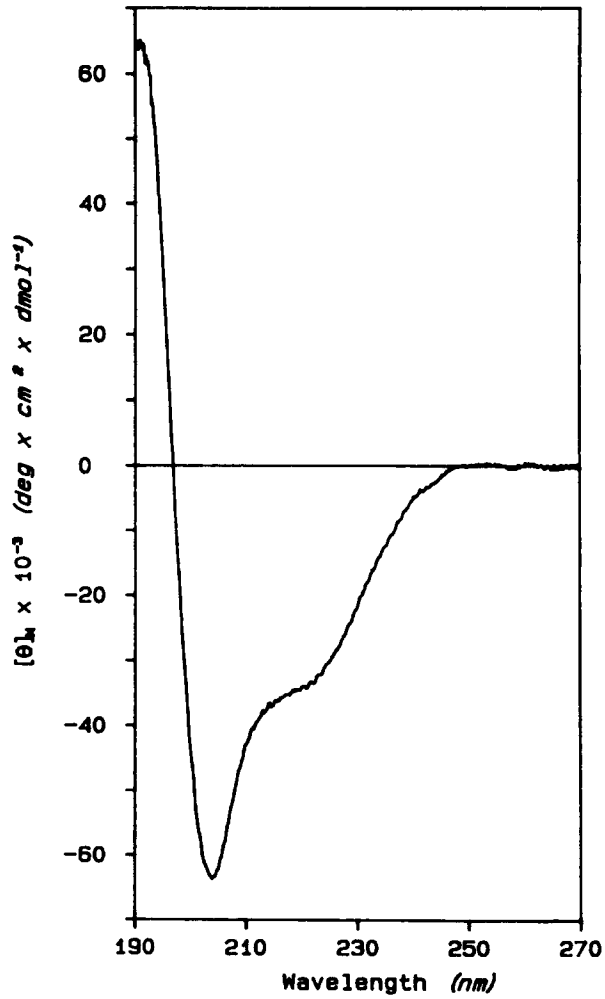

Figure 2. Circular dichroism spectrum of Ac-(Aib)$_2$-L-Lys(Bz)-(Aib)$_2$-L-Lys(Bz)-(Aib)$_2$-NHMe in TFE solution (peptide concentration 1 × 10^{-3} M).

The crystal-state structure of the octapeptide was determined in its monohydrated form by X-ray diffraction. Two perspective views of the molecule are shown in Figures 3 and 4, respectively. The atom numbering and the torsion angles notation follow the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature.[28] Relevant torsion angles are given in Table 1. In Table 2 the H-bond parameters are listed.

Bond lengths and bond angles (deposited) are in general agreement with previously reported values for the geometry of the acetamido[29] and benzamido[30] moieties, the methylamido group,[31] the peptide unit,[32,33] and the Aib[34,35] and Lys[36] residues. In particular, the geometry around the C^{α} atom is asymmetric for all six Aib residues: if one designates as $C^{\beta 1}$ and $C^{\beta 2}$ the atoms in Aib which correspond, respectively, to the C^{β} and the H^{α} atoms in protein L-amino acids, then the bond angles N_i-$C^{\alpha}{}_i$-$C^{\beta 1}{}_i$ and C'_i-$C^{\alpha}{}_i$-$C^{\beta 1}{}_i$ are significantly smaller than the tetrahedral value (109.5°), while the bond angles N_i-$C^{\alpha}{}_i$-$C^{\beta 2}{}_i$ and C'_i-$C^{\alpha}{}_i$-$C^{\beta 2}{}_i$ are larger. The mean values are 106.9°, 106.9°, and 110.6°, 110.8°, respectively. The asymmetry in the geometries around the Aib C^{α} atoms and the direction of the deviations of the ω torsion angles are in excellent agreement with conformational energy calculations on Aib homopeptides.[34]

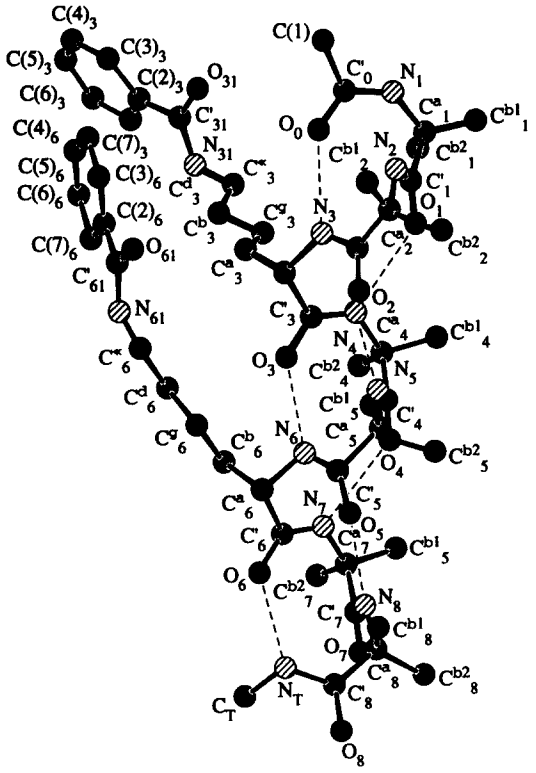

Figure 3. X-Ray diffraction structure of Ac-(Aib)$_2$-L-Lys(Bz)-(Aib)$_2$-L-Lys(Bz)-(Aib)$_2$-NHMe with atom numbering. The intramolecular H-bonds are indicated as dashed lines.

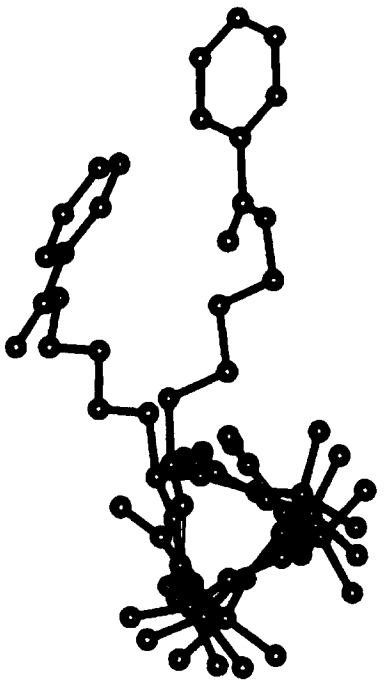

Figure 4. X-Ray diffraction structure of Ac-(Aib)$_2$-L-Lys(Bz)-(Aib)$_2$-L-Lys(Bz)-(Aib)$_2$-NHMe as viewed along the helix axis (nearly coincident with the $x+y$ and $x-y$ crystallographic directions).

Table 1. Relevant torsion angles (°) for Ac-(Aib)$_2$-L-Lys(Bz)-(Aib)$_2$-L-Lys(Bz)-(Aib)$_2$-NHMe monohydrate

	Aib[1]	Aib[2]	Lys(Bz)[3]	Aib[4]	Aib[5]	Lys(Bz)[6]	Aib[7]	Aib[8]
ϕ	-59.7(7)	-54.7(6)	-61.7(6)	-52.3(7)	-53.9(7)	-56.8(7)	-51.8(7)	-58.1(6)
ψ	-32.6(6)	-27.9(6)	-18.5(6)	-28.8(6)	-27.9(7)	-24.3(7)	-31.9(6)	-35.3(7)
ω	-172.2(4)	-178.0(4)	175.5(4)	-179.1(4)	180.0(5)	176.9(5)	-177.6(4)	-168.3(5)
χ^1			68.6(6)			-65.8(6)		
χ^2			179.1(5)			167.3(4)		
χ^3			169.4(5)			-179.0(5)		
χ^4			-177.5(5)			167.7(4)		
$C^{\delta}i$-$C^{\varepsilon}i$-Ni1-C'i1			90.0(6)			-142.1(5)		
$C^{\varepsilon}i$-Ni1-C'i1-C(2)i			176.7(4)			170.9(4)		
Ni1-C'i1-C(2)i-C(3)i			159.4(5)			176.5(5)		

C(1)-C'O-N1-C^{α}1 = −164.0(5)°

Table 2. Hydrogen bond parameters for Ac-(Aib)$_2$-L-Lys(Bz)-(Aib)$_2$-L-Lys(Bz)-(Aib)$_2$-NHMe monohydrate

Type	Donor D	Acceptor A	Distance (Å) D-A	Distance (Å) H-A	Angle(°) D-H--A	Symmetry operation
Intramolecular	N_3-H	O_0	3.26	2.41	167.5	x,y,z
	N_4-H	O_1	2.91	2.07	174.5	x,y,z
	N_5-H	O_2	3.00	2.08	163.5	x,y,z
	N_6-H	O_3	2.95	2.18	166.1	x,y,z
	N_7-H	O_4	2.93	1.96	158.2	x,y,z
	N_8-H	O_5	2.99	2.01	162.4	x,y,z
	N_T-H	O_6	2.97	2.17	160.6	x,y,z
Intermolecular	N_1-H	O_7	2.81	1.96	176.2	1+x,y-1,z
	N_2-H	O_8	2.97	2.01	160.1	1+x,y-1,z
	N_{31}-H	O_{61}	3.00	2.12	158.6	x-½,½-y,-z
Peptide-Solvent	N_{61}-H	O_w	2.89	1.89	160.7	½+x,½-y,-z
	O_w-H	O_0	2.85	2.02	157.6	x-1,y,z
	O_w-H	O_{31}	2.85	1.98	165.2	x-1,y,z

The octapeptide is folded in a regular, right-handed helix with average absolute values for the ϕ,ψ backbone torsion angles equal to 56.1° and 28.4°, very close to those expected for a 3_{10}-helix (ϕ = 57°, ψ = 30°)[12-14] and comparable to those observed in Aib homo-peptides.[37-44] The almost perfect triangular shape of the 3_{10}-helix stands out clearly in Figure 4. The amide and peptide units adopt the usual *trans* conformation. Their deviation from the ideal planar geometry is on average 5.4°. However, the amide ω_0 and ω_8 values differ by as much as 16.0° and 11.7°, respectively, from the *trans* planar value, indicating a more consistent deviation near the N- and C-terminal parts of the molecule.

The side-chain torsion angles of the two benzamidobutyl Lys side chains, facing the same side of the helix exactly one turn away from each other, show closely related values with the single exception of χ^1 (N_i-C_i^{α}-C_i^{β}-C_i^{γ}), *gauche*$^+$ for Lys^3 but *gauche*$^-$ for Lys^6. The intrahelical distances between atoms at the same position are the following: C^{α}...C^{α} 5.75 Å, C^{β}...C^{β} 5.67 Å, C^{γ}...C^{γ} 5.95 Å, C^{δ}...C^{δ} 5.52 Å, C^{ε}...C^{ε} 6.88 Å, N^{ω}...N^{ω} 6.34 Å, C'...C' 6.30 Å, O...O 6.79 Å, C(2)...C(2) 5.73 Å, C(3)...C(3) 5.48 Å, C(4)...C(4) 5.70 Å, C(5)...C(5) 5.05 Å, C(6)...C(6) 5.15 Å, and C(7)...C(7) 5.56 Å. The shortest intrahelical distance between atoms at different positions is 3.47 Å [$C(4)_6$...$C(7)_3$]. The angle between normals to the average planes of the two phenyl rings is 35.4°.

Additionally, the intramolecular H-bonding pattern is typical for a 3_{10}-helix, as it consists of seven $i \leftarrow i+3$ C=O...H–N H-bonds, which give rise to a series of consecutive type-III β-turns. The range of observed intramolecular N...O distances is 2.91–3.00 Å for six out of seven H-bonds, only the first H-bond, N_3...O_0, being rather long (3.26 Å).[45-47] Probably, this is to be ascribed to the interaction of the N-terminal part of the molecule with the co-crystallized water molecule by means of an O_w...O_0 (2.85 Å) H-bond.[48,49] The remaining H-bond capacity of the molecule is satisfied by peptide–water[50] and peptide–peptide intermolecular H-bonds. Two intermolecular H-bonds, N_1...O_7 and N_2...O_8, link the peptide molecules head-to-tail in the crystal, originating infinitely long helical columns approximately along the $x+y$ and $x-y$ directions (Fig. 5). The co-crystallized water molecules interconnect by H-bonds adjacent antiparallel columns of helical molecules, forming double layers in which the inner core is constituted

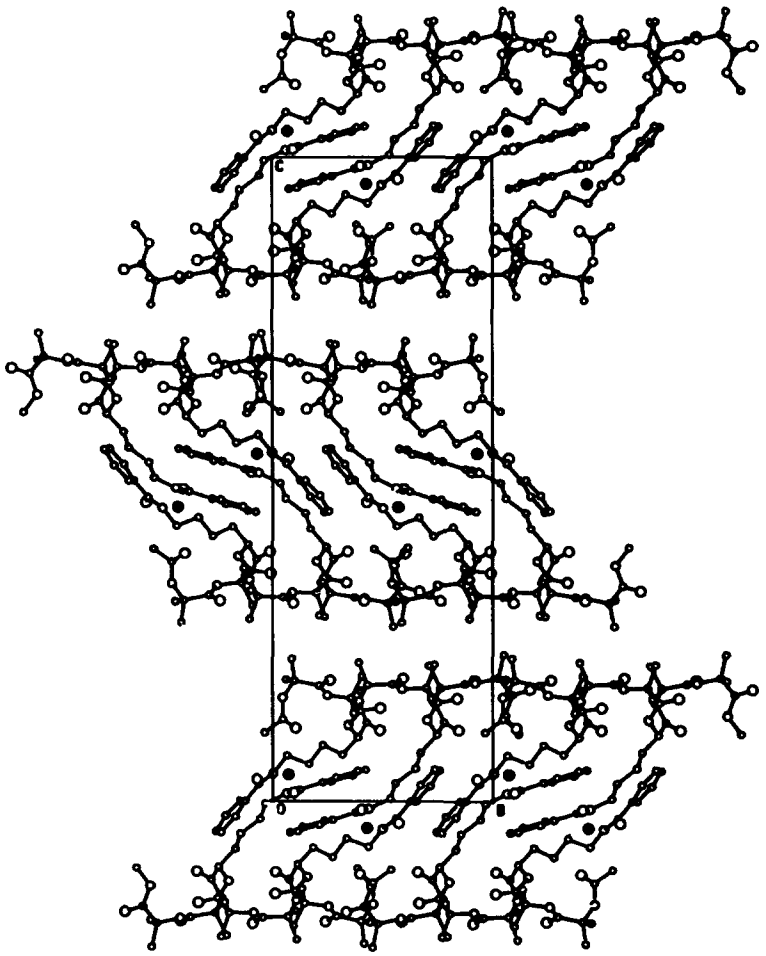

Figure 5. Crystal packing mode of the molecules of Ac-$(Aib)_2$-L-Lys(Bz)-$(Aib)_2$-L-Lys(Bz)-$(Aib)_2$-NHMe monohydrate projected down the *a* axis. The oxygen atoms of the water molecules are represented as isolated filled circles.

by the partially hydrophilic N^{ω}-benzoylated Lys side chains and the outer surface by the hydrophobic methyl groups. The N^{ω}-benzoylated Lys side chains lie on slightly different planes, so that the intermolecular contacts between side-chain atoms of adjacent columns have distances > 3.5 Å.

Conclusions

In recent years the 3_{10}-helical peptide architecture has been shown to provide access to the investigation of through-space interactions between side chains of two amino acid residues incorporated at the *i* and *i* + 3 relative positions.[51,52] By exploiting the conformationally restricted Aib and other members of the $C^{\alpha,\alpha}$-disubstituted glycine family as the main building blocks, stable 3_{10}-helicity and excellent structural rigidity can be achieved at peptide main-chain lengths as short as five to eight residues.[8-10]

In this work we confirmed that a terminally blocked, Aib-rich octapeptide containing two side-chain benzoylated L-Lys residues at positions 3 and 6 forms a stable, regular, right-handed 3_{10}-helical structure both in solution and in the crystal state. In addition, the two benzamidobutyl L-Lys side chains, facing the same side of the helix, generates a cavity that includes a bottom polar section characterized by peptide N–H and C=O groups. The minimal width of the cavity is 3.47 Å. It may be concluded that conformational constrained 3_{10}-helical peptide templates rich in $C^{\alpha,\alpha}$-disubstituted glycines are excellent potential tools for molecular recognition studies, provided that two amino acids with suitable side-chain functional groups are inserted in the sequence at the appropriate positions.

Experimental

Peptide synthesis

Melting points were determined using a Leitz (Wetzlar, Germany) model Laborlux 12 apparatus and are not corrected. Optical rotations were measured using a Perkin–Elmer (Norwalk, CT) model 241 polarimeter equipped with a Haake (Karlsruhe, Germany) model L thermostat. Thin-layer chromatography was performed on Merck (Darmstadt, Germany) Kieselgel $60F_{254}$ precoated plates using the following solvent systems: I ($CHCl_3$:EtOH, 9:1), II (1-BuOH:HOAc:H_2O, 3:1:1); III (toluene:EtOH, 7:1). The chromatograms were examined by UV fluorescence or developed by Cl_2:starch:KI or ninhydrin chromatic reaction as appropriate. All the

compounds were obtained in a chromatographically homogeneous state. Amino acid analyses were determined using a C. Erba (Rodano, Milan, Italy) model 3A27 amino acid analyzer. Elution of Aib was observed immediately after the Ala peak. The Aib color yield with ninhydrin is about 20 times lower than those of protein amino acids. Molecular weight determinations were performed by matrix-assisted laser desorption ionization mass spectrometry (MALDI-TOF MS) using a Bruker (Karlsruhe, Germany) reflex-time-of-flight mass spectrometer operating at nominal accelerating potential of 30 keV (in both linear and reflector modes). 2,5-Dihydroxybenzoic acid was used as matrix.

Z-Aib-NHMe (Z: benzyloxycarbonyl). This compound was synthesized from $(Z\text{-Aib})_2O$[53] and $MeNH_2$ in a $THF:H_2O$ mixture at 0 °C for 1 h and at room temperature for 2 h. Yield 77%; mp 106–107 °C (from EtOAc:PE) (PE, petroleum ether); TLC R_fI 0.60, R_fII 0.80, R_fIII 0.25; IR (KBr): 3416, 3350, 1719, 1690, 1613, 1530 cm^{-1}; ^1H NMR ($CDCl_3$): δ (ppm) 7.36 (*m*, 5H, Z CH), 6.31 (*q*, 1H, NHMe NH), 5.27 (*s*, 1H, Aib NH), 5.09 (*s*, 2H, Z CH_2), 2.80 (*d*, 3H, NHMe CH_3), 1.53 (*s*, 6H, Aib CH_3).

Z-(Aib)$_2$-NHMe. This compound was prepared from $(Z\text{-Aib})_2O$ and H-Aib-NHMe (the latter prepared, in turn, by catalytic hydrogenolysis in MeOH of the corresponding Z-derivative) in MeCN under reflux for 5 h and at room temperature overnight in the presence of *N*-methylmorpholine (NMM). Yield 56%; mp 143–144 °C (from EtOAc:PE); TLC R_fI 0.55, R_fII 0.80, R_fIII 0.15; IR (KBr): 3384, 3294, 1701, 1652, 1527 cm^{-1}; ^1H NMR ($CDCl_3$): δ (ppm) 7.34 (*m*, 5H, Z CH), 7.02 (*q*, 1H, NHMe NH), 6.26 (*s*, 1H, Aib^2 NH), 5.28 (*s*, 1H, Aib^1 NH), 5.11 (*s*, 2H, Z CH_2), 2.75 (*d*, 3H, NHMe CH_3), 1.47–1.43 (12H, Aib CH_3).

Z-L-Lys(Bz)-OH. This compound was prepared from Z-L-Lys-OH and $(Bz)_2O$ in CH_2Cl_2 in the presence of $(CH_3)_3SiCl$ and NMM according to the procedure described by Barlos *et al.*:[54] Yield 56%; oil; TLC R_fI 0.10, R_fII 0.85, R_fIII 0.05; $[\alpha]_D^{20}$ –4.7° (*c* 0.5, MeOH); IR (KBr): 3330, 1706, 1640, 1540 cm^{-1}; ^1H NMR ($CDCl_3$): δ (ppm) 7.76–7.72 (*m*, 2H, Bz CH), 7.49–7.35 (*m*, 3H, Bz CH), 7.30 (*m*, 5H, Z CH), 6.64 (*t*, 1H, Lys ωNH), 5.75 (*d*, 1H, Lys αNH), 5.04 (*s*, 2H, Z CH_2), 4.36 (*m*, 1H, Lys αCH), 3.99 (*m*, 2H, Lys εCH$_2$), 1.94–1.32 (*m*, 6H, 2Lys βCH$_2$, 2Lys γCH$_2$, 2Lys δCH$_2$).

Z-L-Lys(Bz)-(Aib)$_2$-NHMe. This compound was synthesized from Z-L-Lys(Bz)-OH and H-$(Aib)_2$NHMe (the latter prepared, in turn, by catalytic hydrogenolysis in MeOH of the corresponding Z-derivative) in a THF:CH_2Cl_2:DMF (DMF: *N*,*N*-dimethylformamide) mixture at –50 °C for 30 min and at room temperature overnight in the presence of isobutylchloroformate and NMM. Yield 38% (after purification by flash chromatography on a silica gel column using a 95:5 $CHCl_3$:EtOH mixture); mp 90–91 °C (from $CHCl_3$:MeOH); TLC R_fI 0.85, R_fII 0.80, R_fIII 0.20; $[\alpha]_D^{20}$ –16.3° (*c* 0.5, MeOH); IR (KBr): 3333, 1704, 1646, 1539 cm^{-1}; ^1H NMR ($CDCl_3$): δ (ppm) 7.84–7.79 (*m*, 2H, Bz CH), 7.54–7.36 (*m*, 3H, Bz CH), 7.34 (*m*, 5H, Z CH), 7.14 (*q*, 1H, NHMe NH), 6.84 (*s*, 1H, Aib NH), 6.81 (*t*, 1H, Lys ωNH), 6.78 (*s*, 1H, Aib NH), 6.48 (*d*, 1H, Lys αNH), 5.16–5.02 (2*d*, 2H, Z CH_2), 3.91 (*m*, 1H, Lys αCH), 3.64–3.36 (*m*, 2H, Lys εCH$_2$), 2.74 (*d*, 3H, NHMe CH_3), 1.94–1.76 (*m*, 2H, Lys βCH$_2$), 1.70–1.55 (*m*, 2H, Lys δCH$_2$), 1.49–1.32 (14H, 2 Lys γCH$_2$, 12 Aib CH_3); amino acid analysis: Lys 1.09, Aib 1.91.

Z-Aib-L-Lys(Bz)-(Aib)$_2$-NHMe. This compound was synthesized from $(Z\text{-Aib})_2O$ and H-L-Lys(Bz)$(Aib)_2$-NHMe (the latter prepared, in turn, by catalytic hydrogenolysis in MeOH of the corresponding Z-derivative) in MeCN at room temperature overnight in the presence of NMM. Yield 76% (after purification by flash chromatography on a silica gel column using a 92:8 $CHCl_3$:EtOH mixture); mp 139–140 °C (from EtOAc:PE); TLC R_fI 0.60, R_fII 0.85, R_fIII 0.20; $[\alpha]_D^{20}$ –12.0° (*c* 0.5, MeOH); IR (KBr): 3329, 1703, 1650, 1536 cm^{-1}; ^1H NMR ($CDCl_3$): δ (ppm) 7.74 (*m*, 2H, Bz CH), 7.54–7.36 (5H, 3Bz CH, Lys αNH, Aib NH), 7.35 (*m*, 5H, Z CH), 7.17 (*q*, 1H, NHMe NH), 6.87 (*s*, 1H, Aib NH), 6.55 (*t*, 1H, Lys ωNH), 5.41 (*s*, 1H, Aib^1 NH), 5.10 (2*d*, 2H, Z CH_2), 3.90 (*m*, 1H, Lys αCH), 3.50 (2*m*, 2H, Lys εCH$_2$), 2.75 (*d*, 3H, NHMe CH_3), 1.90–1.16 (24H, 2 Lys βCH$_2$, 2 Lys γCH$_2$, 2 Lys δCH$_2$, 18 Aib CH_3); amino acid analysis: Lys 0.98, Aib 3.02.

Z-(Aib)$_2$-L-Lys(Bz)-(Aib)$_2$-NHMe. This compound was prepared from $(Z\text{-Aib})_2O$ and H-Aib-L-Lys(Bz)-$(Aib)_2$-NHMe (the latter prepared, in turn, by catalytic hydrogenolysis in MeOH of the corresponding Z-derivative) in MeCN at room temperature overnight in the presence of NMM. Yield 70%; mp 196–197 °C (from EtOAc); TLC R_fI 0.60, R_fII 0.90, R_fIII 0.15; $[\alpha]_D^{20}$ 15.7° (*c* 0.5, MeOH); IR (KBr): 3297, 1697, 1658, 1536 cm^{-1}; ^1H NMR ($CDCl_3$): δ (ppm) 7.78–7.73 (*m*, 2H, Bz CH), 7.58 (*s*, 1H, Aib NH), 7.57 (*d*, 1H, Lys αNH), 7.49–7.35 (*m*, 3H, Bz CH), 7.34 (*m*, 5H, Z CH), 7.23 (*q*, 1H, NHMe NH), 6.86 (*s*, 1H, Aib NH), 6.60 (*s*, 1H, Aib NH), 6.40 (*t*, 1H, Lys ωNH), 5.50 (*s*, 1H, Aib^1 NH), 5.12 (2*d*, 2H, Z CH_2), 3.96 (*m*, 1H, Lys αCH), 3.44 (*m*, 2H, Lys εCH$_2$), 2.71 (*d*, 3H, NHMe CH_3), 1.86 (*m*, 2H, Lys βCH$_2$), 1.66–1.32 (28H, 2 Lys γCH$_2$, 2 Lys δCH$_2$, 24 Aib CH_3); amino acid analysis: Lys 1.00, Aib 4.00.

Z-L-Lys(Bz)-(Aib)$_2$-L-Lys(Bz)-(Aib)$_2$-NHMe. This compound was synthesized from Z-L-Lys(Bz)-OH and H-$(Aib)_2$-L-Lys(Bz)-$(Aib)_2$-NHMe (the latter prepared, in turn, by catalytic hydrogenolysis in MeOH of the corresponding Z-derivative) in a THF:$CHCl_3$:DMF mixture at –50 °C for 30 min and at room temperature overnight in the presence of isobutylchloroformate and NMM. Yield 53%; mp 127–128 °C (from EtOAc:PE); TLC R_fI 0.75, R_fII 0.90, R_fIII 0.25; $[\alpha]_D^{20}$ 5.5° (*c* 0.5, MeOH); IR (KBr): 3312, 1652, 1536 cm^{-1}; ^1H NMR ($CDCl_3$): δ (ppm) 7.83–7.72 (*m*, 5H, 4 Bz CH, Lys^4 αNH), 7.65 (*s*, 1H, Aib NH), 7.49–7.26 (m, 12H, 6 Bz CH, 5 Z CH, NHMe NH), 7.20 (s, 1H, Aib NH), 7.03 (m, 2H, Lys^1 αNH, 1 Aib NH), 6.95 (*s*, 1H, Aib NH), 6.74 (*t*, 1H, Lys ωNH), 6.67 (*t*, 1H, Lys ωNH), 5.21–5.01 (2*d*, 2H,

Z CH_2), 3.94 (*m*, 1H, Lys αCH), 3.79 (*m*, 1H, Lys αCH), 3.70–3.30 (*m*, 4H, 2 Lys εCH_2), 2.73 (*d*, 3NH, NHMe CH_3), 1.93–1.26 (36H, 4 Lys βCH_2, 4 Lys γCH_2, 4 Lys δCH_2, 24 Aib CH_3); amino acid analysis: Lys 2.15, Aib 3.85.

Ac-(Aib)$_2$-L-Lys(Bz)-(Aib)$_2$-L-Lys(Bz)-(Aib)$_2$-NHMe. This compound was prepared from the 5(4*H*)oxazolone from Ac-$(Aib)_2OH$[55] and H-L-Lys(Bz)-$(Aib)_2$-L-Lys(Bz)-$(Aib)_2$-NHMe (the latter prepared, in turn, by catalytic hydrogenolysis in MeOH of the corresponding Z-derivative) in MeCN under reflux for 4 h. Yield 15%; mp 240–241 °C (from MeOH:Et_2O); TLC R_fI 0.20, R_fII 0.80, R_fIII 0.05; $[\alpha]_D^{20}$ 14.8° (*c* 0.5, MeOH); IR (KBr): 3308, 1654, 1537 cm^{-1}; ^1H NMR (DMSO): δ (ppm) 8.45 (*s*, 1H, Aib NH), 8.38 (*t*, 2H, Lys εNH), 8.20 (*s*, 1H, Aib NH), 7.80–7.76 (*m*, 7H, 4H Bz CH, Lys αNH, 2 Aib NH), 7.53 (*d*, 1H, Lys αNH), 7.50–7.38 (*m*, 6H, Bz CH), 7.28 (*s*, 1H, Aib NH), 7.15 (*q*, 1H, NHMe NH), 6.91 (*s*, 1H, Aib NH), 3.80 (*m*, 2H, Lys αCH), 3.21 (*m*, 4H, Lys εCH_2), 2.54 (*d*, 3H, NHMe CH_3), 1.86 (*s*, 3H, Ac CH_3), 1.76 (*m*, 4H, Lys βCH_2), 1.51 (*m*, 4H, Lys γCH_2), 1.48–1.27 (40H, 4 Lys δCH_2, 36 Aib CH_3); amino acid analysis: Lys 2.06, Aib 5.94; MALDI-MS: m/z 1084 $(M + K)^+$ (theor. 1086), 1068 $(M + Na)^+$ (theor. 1070).

^1H nuclear magnetic resonance

The ^1H NMR spectra were recorded with Bruker Model AC-200 and Model AM-400 spectrometers. Measurements were carried out in deuterochloroform (99.96% *d*, Merck) and dimethyl-d_6 sulfoxide (99.96 d_6, Fluka) with tetramethylsilane as the internal standard. The free radical TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy) was purchased from Sigma (St. Louis, MO).

IR absorption

The solid-state IR absorption spectra (KBr disk technique) were recorded with a Perkin–Elmer (Norwalk, CT) model 580B spectrophotometer equipped with a Perkin–Elmer model 3600 data station. The solution spectra were obtained using a Perkin–Elmer model 1720X FT-IR spectrophotometer, nitrogen-flushed, equipped with a sample-shuttle device, at 2 cm^{-1} nominal resolution, averaging 100 scans. Solvent (baseline) spectra were recorded under the same conditions. Cells with path lengths of 0.1, 1.0 and 10 mm (with CaF_2 windows) were used. Spectrograde deuterochloroform (99.8% *d*) was purchased from Fluka (Büchs, Switzerland) and dimethylsulfoxide from Merck (Darmstadt, Germany).

Circular dichroism

The CD spectrum was recorded with a Jasco (Tokyo, Japan) J-600 spectropolarimeter, using a Hellma (Müllheim, Germany) quartz cell of 0.1 mm path length and TFE (Fluka) as the solvent. The values are given as $[\theta]_M$, the total molar ellipticity (deg cm^2 $dmol^{-1}$).

X-Ray diffraction

Colorless single crystals of Ac-$(Aib)_2$-L-Lys(Bz)-$(Aib)_2$-Lys(Bz)-$(Aib)_2$-NHMe were obtained by a vapour diffusion technique, using a w-shaped tube, sealed at the extremities, containing water and a peptide-methanol solution. A well-shaped crystal (dimensions 0.3 × 0.2 × 0.2 mm) was used for intensity data collection on a CAD4 Enraf–Nonius (Delft, The Netherlands) automated diffractometer, using graphite-monochromated CuKα radiation (λ = 1.54178 Å). The independent reflections were measured in the θ range 1°–70° at low temperature (115 K), using the Enraf–Nonius model FR558NH liquid-nitrogen cryostat.

Unit cell parameters were determined by least-squares refinement of the setting angles of 25 high angle reflections (18° < θ < 22°). Details of crystallographic data are reported in Table 3. Three standard reflections, monitored periodically, showed no significant change during data collection. A total of 6275 independent reflections were measured with a ω/0.33θ scan mode and a scan angle equal to (1.2 + 0.35 tanθ)°; background counts were taken in an additional area of Δω/4 on both sides of the main scan, with the same scan speed for each reflection. Using a prescan speed of 4.12° min^{-1}, reflections with a net intensity $I < 0.5\sigma(I)$ were flagged as 'weak'; those with $I \geq 0.5\sigma(I)$ were measured at lower speed depending on the value of $\sigma(I)/I$. A total of 4637 reflections with $I \geq 2.0\sigma(I)$ were classified as observed and used for structure determination and refinement. All intensities were corrected for Lorentz, polarization and extinction factors, but not for absorption ($\mu = 5.9$ cm^{-1} for CuKα radiation).

The structure was solved by Patterson search technique using PATSEE.[56] As search model, we used a fragment of the 3_{10}-helical structure of Ac-$(Aib)_5$-D-(αMe)Leu-$(Aib)_2$-O*t*Bu [(αMe)Leu, C^α-methyl leucine].[57] The fragment included the acetyl moiety and five Aib residues, with two C_β atoms for each residue 1, 2, 4 and 5. The best solution (CFOM = 0.945, RE = 0.194) was used as input for SHELXS-86[58] in order to expand the structure. Using 1754 *E* values ($E > 1.2$), the corresponding *E*-map revealed all the atoms of the molecule and the oxygen atom of the co-crystallized water molecule. At the end of isotropic refinement, 10 out of 11 NH hydrogen atoms were located on successive difference Fourier maps and the others calculated in their stereochemically expected positions. Refinement of the structure was performed using SHELXL-93[59] by a four blocks matrix least-square procedure on F^2 with overlapping blocks. The first block involved the positional parameters of all the atoms of the first five residues, the second one the thermal parameters of the same atoms, the third and the fourth blocks the positional and thermal parameters, respectively, of the atoms between Aib_4 and Aib_8. The final *R* factor was 0.0618 (calculated on *F*, with *F* set to zero for negative F^2), while the weighted *R* factor *wR* was 0.1540 (*wR*

Table 3. Crystal data and structure refinement for Ac-(Aib)$_2$-L-Lys(Bz)-(Aib)$_2$-L-Lys(Bz)-(Aib)$_2$-NHMe monohydrate

Empirical formula	$C_{53}H_{81}N_{11}O_{11}\cdot H_2O$
Formula weight (amu)	1066.3
Temperature	115(2) K
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a= 11.413(2) Å α= 90 deg.
	b= 13.485(2) Å β= 90 deg.
	c= 38.760(2) Å γ= 90 deg.
Volume	5965.3(14) Å^3
Z	4
Density (calculated)	1.187 Mg/m^3
Absorption coefficient	0.695 mm^{-1}
F(000)	2296
Crystal size	0.3 x 0.2 x 0.2 mm
Θ range for data collection	2.28 to 69.80 deg.
Index ranges	$0\leq h\leq 13, 0\leq k\leq 16, 0\leq l\leq 47$
Reflections collected	6545
Independent reflections	6275 [R(int)= 0.0176]
Refinement method	Full-matrix-block least-squares on F^2
Data / restraints / parameters	4637 / 0 / 802
Goodness-of-fit on F^2	1.113
Final R indices [$I>2\sigma(I)$]	R1= 0.0618, wR2= 0.1540
Absolute structure parameter	0.2(4)
Extinction coefficient	0.00077(11)
Largest diff. peak and hole	0.307 and -0.331 e.Å^{-3}

and goodness of fit S were calculated on F^2; statistically they are about twice as large as those based on F). In the final difference Fourier synthesis the maximum and minimum electron density were 0.307 and –0.331 e Å^{-3}, respectively. The scattering factors for all atomic species were calculated from Cromer and Waber.[60]

All calculations were performed on a IBM-RISC 6000 workstation at the laboratories of URA-CNRS 809, University of Nancy I, Vandoeuvre les Nancy, France. Final atomic parameters and equivalent thermal factors for non-H atoms with their standard deviations are reported in Table 4.

Table 4. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (Å^2 x 10^3) for Ac-(Aib)$_2$-L-Lys(Bz)-(Aib)$_2$-L-Lys(Bz)-(Aib)$_2$-NHMe monohydrate. U(eq) is defined as one third of the trace of the orthogonalized Uij tensor

Atom	x	y	z	U(eq)
C(1)	13178(6)	–363(5)	1069(2)	37(2)
C'_0	12595(5)	454(4)	1273(1)	25(1)
O_0	11927(4)	1049(3)	1140(1)	32(1)
N_1	12866(4)	450(3)	1609(1)	23(1)
$C^{\alpha}{}_1$	12186(5)	1002(4)	1872(1)	21(1)
$C^{\beta 1}{}_1$	12823(5)	871(4)	2217(1)	26(1)
$C^{\beta 2}{}_1$	10944(5)	608(4)	1892(2)	32(1)
C'_1	12175(5)	2123(4)	1794(1)	20(1)
O_1	11327(3)	2630(3)	1879(1)	25(1)
N_2	13139(4)	2488(3)	1643(1)	21(1)
$C^{\alpha}{}_2$	13300(5)	3576(4)	1595(1)	22(1)
$C^{\beta 1}{}_2$	14399(4)	3701(3)	1367(1)	30(1)
$C^{\beta 2}{}_2$	13465(4)	4078(3)	1943(1)	33(1)
C'_2	12262(5)	4021(4)	1403(1)	25(1)
O_2	12027(4)	4910(3)	1444(1)	27(1)
N_3	11654(4)	3449(3)	1183(1)	22(1)
$C^{\alpha}{}_3$	10684(5)	3888(4)	991(1)	22(1)
$C^{\beta}{}_3$	10172(5)	3180(4)	724(1)	21(1)
C'_3	9520(5)	2285(4)	871(1)	23(1)

Table 4. *Continued*

$C^{\delta}{}_3$	9031(5)	1642(4)	578(1)	25(1)
$C^{\varepsilon}{}_3$	8574(5)	651(4)	709(1)	27(1)
N_{31}	8153(5)	15(3)	431(1)	27(1)
C'_{31}	8859(5)	−629(4)	266(1)	25(1)
O_{31}	9905(4)	−720(3)	340(1)	36(1)
$C(2)_3$	8293(5)	−1269(4)	1(1)	26(1)
$C(3)_3$	9008(6)	−1700(4)	−250(1)	34(2)
$C(4)_3$	8494(8)	−2344(5)	−493(2)	52(2)
$C(5)_3$	7324(7)	−2568(5)	−478(2)	49(2)
$C(6)_3$	6646(8)	−2147(5)	−235(2)	52(2)
$C(7)_3$	7107(6)	−1487(4)	4(2)	38(2)
C'_3	9707(5)	4275(4)	1223(1)	20(1)
O_3	8997(3)	4887(3)	1104(1)	24(1)
N_4	9655(4)	3947(3)	1546(1)	21(1)
$C^{\alpha}{}_4$	8796(5)	4328(4)	1801(1)	22(1)
$C^{\beta 1}{}_4$	9167(5)	3937(4)	2156(1)	30(1)
$C^{\beta 2}{}_4$	7568(5)	3995(4)	1712(2)	32(1)
C'_4	8855(5)	5471(4)	1816(1)	19(1)
O_4	7990(3)	5944(3)	1908(1)	26(1)
N_5	9892(4)	5896(3)	1741(1)	22(1)
$C^{\alpha}{}_5$	10055(5)	6988(4)	1752(1)	26(1)
$C^{\beta 1}{}_5$	11251(5)	7198(5)	1591(2)	46(2)
$C^{\beta 2}{}_5$	10003(7)	7357(4)	2122(2)	41(2)
C'_5	9136(5)	7506(4)	1528(1)	22(1)
O_5	8864(3)	8378(3)	1594(1)	25(1)
N_6	8696(4)	7016(3)	1259(1)	21(1)
$C^{\alpha}{}_6$	7822(5)	7456(4)	1034(1)	22(1)
$C^{\beta}{}_6$	7484(5)	6712(4)	751(1)	26(1)
$C^{\gamma}{}_6$	8469(5)	6480(4)	506(1)	25(1)
$C^{\delta}{}_6$	8214(5)	5584(4)	278(1)	29(1)
$C^{\varepsilon}{}_6$	9209(5)	5348(4)	39(1)	29(1)
N_{61}	9057(4)	4372(3)	−121(1)	24(1)
C'_{61}	9968(5)	3769(4)	−177(1)	22(1)
O_{61}	10999(3)	4035(3)	−134(1)	29(1)
$C(2)_6$	9689(5)	2727(4)	−292(1)	20(1)
$C(3)_6$	10623(6)	2116(4)	−368(1)	29(1)
$C(4)_6$	10463(6)	1131(4)	−468(2)	37(2)
$C(5)^6$	9329(6)	772(4)	−485(2)	34(1)
$C(6)_6$	8380(6)	1351(4)	−399(1)	29(1)
$C(7)_6$	8568(5)	2343(4)	−305(1)	25(1)
C'_6	6733(5)	7787(4)	1230(1)	20(1)
O_6	6091(4)	8437(3)	1107(1)	28(1)
N_7	6498(4)	7339(3)	1531(1)	21(1)
$C^{\alpha}{}_7$	5517(5)	7621(4)	1752(2)	28(1)
$C^{\beta 1}{}_7$	5688(6)	7116(5)	2105(2)	42(2)
$C^{\beta 2}{}_7$	4378(5)	7291(5)	1591(2)	43(2)
C'_7	5522(5)	8740(4)	1822(1)	20(1)
O_7	4587(3)	9173(3)	1877(1)	28(1)
N_8	6568(4)	9194(3)	1836(1)	23(1)
$C^{\alpha}{}_8$	6664(5)	10252(4)	1915(1)	23(1)
$C^{\beta 1}{}_8$	7956(5)	10526(5)	1866(2)	33(1)
$C^{\beta 2}{}_8$	6287(6)	10486(5)	2281(1)	35(2)
C'_8	5960(5)	10880(4)	1650(1)	22(1)
O_8	5535(4)	11677(3)	1733(1)	29(1)
N_T	5953(5)	10542(4)	1329(1)	27(1)
C_T	5516(6)	11160(5)	1048(1)	37(2)
O_W	1730(4)	712(3)	415(1)	31(1)

Acknowledgements

This work was supported by the Italian National Research Council (C.N.R.) 'Progetto Finalizzato Chimica Fine II' and the Human Capital and Mobility Program of the European Community (contract ERBCHRXCT 930286). The skillful technical assistance of Mr Vittorio Moretto is gratefully acknowledged.

References

1. Mutter, M.; Vuilleumier, S. *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 535.

2. Karle, I. L.; Balaram, P. *Biochemistry* **1990**, *29*, 6747.

3. Balaram, P. *Pure Appl. Chem.* **1992**, *64*, 1061.

4. Toniolo, C.; Formaggio, F.; Crisma, M.; Valle, G.; Boesten, W. H. J.; Schoemaker, H. E.; Kamphuis, J.; Temussi, P. A.; Becker, E. L.; Précigoux, G. *Tetrahedron* **1993**, *49*, 3641.

5. Shoemaker, K. R.; Fairman, R.; Schultz, D. A.; Robertson, A. D.; York, E. J.; Stewart, J. M.; Baldwin, R. L. *Biopolymers* **1990**, *29*, 1.

6. Voyer, N.; Guérin, B. *J. Chem. Soc., Chem. Commun.* **1992**, 1253.

7. Akerfeldt, K. S.; Kim, R. M.; Camac, D.; Groves, J. T.; Lear, J. D.; De Grado, W. F. *J. Am. Chem. Soc.* **1992**, *114*, 9656.

8. Venkataram Prasad, B. V.; Balaram, P. *C.R.C. Crit. Rev. Biochem.* **1984**, *16*, 307.

9. Toniolo, C.; Benedetti, E. *Macromolecules* **1991**, *24*, 4004.

10. Toniolo, C.; Crisma, M.; Formaggio, F.; Valle, G.; Cavicchioni, G.; Précigoux, G.; Aubry, A.; Kamphuis, J. *Biopolymers* **1993**, *33*, 1061.

11. Taylor, H. S. *Proc. Am. Phyl. Soc.* **1941**, *85*, 1.

12. Toniolo, C.; Benedetti, E. *TIBS* **1991**, *16*, 350.

13. 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 Institute of Sciences: Bangalore, 1991; pp. 497–502.

14. Benedetti, E.; Di Blasio, B.; Pavone, V.; Pedone, C.; Toniolo, C.; Crisma, M. *Biopolymers* **1992**, *32*, 453.

15. Martin, D.; Hauthal, H. G. *Dimethyl Sulphoxide;* van Nostrand-Reinhold: Wokingham, U.K., 1975.

16. Palumbo, M.; Da Rin, S.; Bonora, G. M.; Toniolo, C. *Makromol. Chem.* **1976**, *177*, 1477.

17. Iqbal, M.; Balaram, P. *Biopolymers* **1982**, *21*, 1427.

18. Kennedy, D. F.; Crisma, M.; Toniolo, C.; Chapman, D. *Biochemistry* **1991**, *30*, 6541.

19. Pitner, T. P.; Urry, D. W. *J. Am. Chem. Soc.* **1972**, *94*, 1399.

20. Kopple, K. D.; Schamper, T. J. *J. Am. Chem. Soc.* **1972**, *94*, 3644.

21. Ohnishi, M.; Urry, D. W. *Biochem. Biophys. Res. Commun.* **1969**, *36*, 194.

22. Kopple, K. D.; Ohnishi, M.; Go, A. *Biochemistry* **1969**, *8*, 4087.

23. Venkatachalam, C. M. *Biopolymers* **1968**, *6*, 1425.

24. Toniolo, C. *C.R.C. Crit. Rev. Biochem.* **1980**, *9*, 1.

25. Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein Chem.* **1985**, *37*, 1.

26. Beychok, S. In *Poly-α-Amino Acids*; Fasman, G. D., Ed.; Dekker: New York, 1967; pp. 293–337.

27. Manning, M. C.; Woody, R. W. *Biopolymers* **1991**, *31*, 569.

28. IUPAC-IUB Commission on Biochemical Nomenclature, *Biochemistry* **1970**, *9*, 3471.

29. Chakrabarti, R.; Dunitz, J. D. *Helv. Chim. Acta* **1982**, *65*, 1555.

30. Bavoso, A.; Benedetti, E.; Di Blasio, B.; Pavone, V.; Pedone, C.; Toniolo, C.; Bonora, G. M.; Formaggio, F.; Crisma, M. *J. Biomol. Struct. Dyn.* **1988**, *5*, 803.

31. Leiserowitz, L.; Tuval, M. *Acta Crystallogr.* **1978**, *B34*, 1230.

32. Benedetti, E. In *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*; Weinstein, B., Ed.; Dekker: New York, 1982; pp. 105–184.

33. Ashida, T.; Tsunogae, Y.; Tanaka, I.; Yamane, T. *Acta Crystallogr.* **1987**, *B43*, 212.

34. Paterson, Y.; Rumsey, S. M.; Benedetti, E.; Némethy, G.; Scheraga, H. A. *J. Am. Chem. Soc.* **1981**, *103*, 2947.

35. Valle, G.; Crisma, M.; Formaggio, F.; Toniolo, C.; Jung, G. *Liebigs Ann. Chem.* **1987**, 1055.

36. Benedetti, E.; Morelli, G.; Némethy, G.; Scheraga, H. A. *Int. J. Pept. Protein Res.* **1983**, *22*, 1.

37. Shamala, N.; Nagaraj, R.; Balaram, P. *J. Chem. Soc., Chem. Commun.* **1978**, 996.

38. Benedetti, E.; Bavoso, A.; Di Blasio, B.; Pavone, V.; Pedone, C.; Crisma, M.; Bonora, G. M.; Toniolo, C. *J. Am. Chem. Soc.* **1982**, *104*, 2437.

39. Bavoso, A.; Benedetti, E.; Di Blasio, B.; Pavone, V.; Pedone, C.; Toniolo, C.; Bonora, G. M. *Proc. Natl Acad. Sci. U.S.A.* **1986**, *83*, 1988.

40. Di Blasio, B.; Santini, A.; Pavone, V.; Pedone, C.; Benedetti, E.; Moretto, V.; Crisma, M.; Toniolo, C. *Struct. Chem.* **1990**, *2*, 523.

41. Pavone, V.; Di Blasio, B.; Santini, A.; Benedetti, E.; Pedone, C.; Toniolo, C.; Crisma, M. *J. Mol. Biol.* **1990**, *214*, 633.

42. Pavone, V.; Di Blasio, B.; Pedone, C.; Santini, A.; Benedetti, E.; Formaggio, F.; Crisma, M.; Toniolo, C. *Gazz. Chim. Ital.* **1991**, *121*, 21.

43. Vlassi, M.; Brückner, M.; Kokkinidis, M. *Zeit. Kristallogr.* **1992**, *202*, 89.

44. Vlassi, M.; Brückner, M.; Kokkinidis, M. *Acta Crystallogr.* **1993**, *B49*, 560.

45. Ramakrishnan, C.; Prasad, N. *Int. J. Protein Res.* **1971**, *3*, 209.

46. Taylor, R.; Kennard, O.; Versichel, W. *Acta Crystallogr.* **1984**, *B40*, 280.

47. Görbitz, C. H. *Acta Crystallogr.* **1989**, *B45*, 390.

48. Mitra, J.; Ramakrishnan, C. *Int. J. Pept. Protein Res.* **1977**, *9*, 27.

49. Brown, I. D. *Acta Crystallogr.* **1976**, *A32*, 24.

50. Yang, C. H.; Brown, J. N.; Kopple, K. D. *Int. J. Pept. Protein Chem.* **1979**, *14*, 12.

51. Augspurger, J. D.; Bindra, V. A.; Scheraga, H. A.; Kuki, A. *Biochemistry* **1995**, *34*, 2566.

52. Toniolo, C.; Valente, E.; Formaggio, F.; Crisma, M.; Pilloni, G.; Corvaja, C.; Toffoletti, A.; Martinez, G. V.; Hanson, M. P.; Millhauser, G. L.; George, C.; Flippen-Anderson, J. *J. Pept. Sci.* **1995**, *1*, 45.

53. Valle, G.; Formaggio, F.; Crisma, M.; Bonora, G. M.; Toniolo, C.; Bavoso, A.; Benedetti, E.; Di Blasio, B.; Pavone, V.; Pedone, C. *J. Chem. Soc. Perkin Trans. 2* **1986**, 1371.

54. Barlos, K.; Papaioannou, D.; Theodoropoulos, D. *J. Org. Chem.* **1982**, *47*, 1324.

55. Levene, P. A.; Steiger, R. E. *J. Biol. Chem.* **1931**, *93*, 581.

56. Egert, E.; Sheldrick, G. M. *Acta Crystallogr.* **1985**, *A41*, 262.

57. Pantano, M.; Formaggio, F.; Crisma, M.; Bonora, G. M.; Toniolo, C.; Aubry, A.; Bayeul, D.; Dautant, A.; Précigoux, G.; Boesten, W. H. J.; Schoemaker, H. E.; Kamphuis, J. *Pept. Res.* **1993**, *6*, 196.

58. Sheldrick, G. M. *SHELXS-86. Program for the Solution of Crystal Structures*, University of Göttingen, Germany, 1986.

59. Sheldrick, G. M. *SHELXL93. Program for Crystal Structure Refinement*, University of Göttingen, Germany, 1993.

60. Cromer, D. T.; Waber, J. T. *International Tables for X-Ray Crystallography*; Kynoch Press: Birmingham, U.K., 1974; Vol. 4, Table 2.2 B.

(*Received in U.S.A.* 4 *January* 1995; *accepted* 5 *May* 1995)