Journal of Peptide Science, Vol. 3, 110–122 (1997)

Conformational Characterization of the 1-Aminocyclobutane-1-carboxylic Acid Residue in Model Peptides

MADDALENA GATOS[1], FERNANDO FORMAGGIO[1], MARCO CRISMA[1], CLAUDIO TONIOLO[1], GIAN MARIA BONORA, ZETTORE BENEDETTI[3], BENEDETTO DI BLASIO[3,4], ROSA IACOVINO[3] , ANTONELLO SANTINI[3], MICHELE SAVIANO[3] and JOHAN KAMPHUIS[5]

[1]Biopolymer Research Centre, University of Padova, Padova, Italy
[2]Pharmaco-, Chemico-, Technological Department, University of Cagliari, Cagliari, Italy
[3]Biocrystallography Research Centre, University of Naples "Federico II", Napoli, Italy
[4]Facoltà di Scienze Ambientali, Seconda Università di Napoli, Caserta, Italy
[5]DSM Research, Bioorganic Chemistry Section, 6160 MD Geleen, The Netherlands

Received 18 March 1996
Accepted 13 May 1996

Abstract: A series of N- and C-protected, monodispersed homo-oligopeptides (to the dodecamer level) from the small-ring alicyclic $C^{\alpha,\alpha}$-dialkylated glycine 1-aminocyclobutane-1-carboxylic acid (Ac_4c) and two Ala/Ac_4c tripeptides were synthesized by solution methods and fully characterized. The conformational preferences of all the model peptides were determined in deuterochloroform solution by FT-IR absorption and ^{1}H-NMR. The molecular structures of the amino acid derivatives Z-Ac_4c-OH and Z_2-Ac_4c-OH, the tripeptides Z-$(Ac_4c)_3$-O*t*Bu, Z-Ac_4c-(L-Ala)$_2$-OMe and Z-L-Ala-Ac_4c-L-Ala-OMe, and the tetrapeptide Z-$(Ac_4c)_4$-O*t*Bu were determined in the crystal state by X-ray diffraction. The average geometry of the cyclobutyl moiety of the Ac_4c residue was assessed and the τ(N–C^{α}–C′) bond angle was found to be significantly expanded from the regular tetrahedral value. The conformational data are strongly in favour of the conclusion that the Ac_4c residue is an effective β-turn and helix former. A comparison with the structural propensities of α-aminoisobutyric acid, the prototype of $C^{\alpha,\alpha}$-dialkylated glycines, and the other extensively investigated members of the family of 1-aminocycloalkane-1-carboxylic acids (Ac_nc, with $n = 3$, 5–8) is made and the implications for the use of the Ac_4c residue in conformationally constrained peptide analogues are briefly examined. © 1997 European Peptide Society and John Wiley & Sons, Ltd

Keywords: β-bend; cyclic amino acid; 3_{10}-helix; peptide conformation, X-ray diffraction

INTRODUCTION

Many experimental observations have provided a firm foundation for the incorporation into peptides of α-amino acids in which the C^{α}-proton has been replaced with an alkyl group as a means of restricting backbone conformations as well as a tool for inhibiting biodegration [1–5]. In this regard the family of the cycloaliphatic residues Ac_nc ($n = 3$, 5–8) is valuable in the design of conformationally constrained peptides [3–5].

More specifically, the three-membered ring Ac_3c residue shows significant preference for the 'bridge' region of the conformational space [6], in particular for the backbone torsion angles ϕ, $\psi = \pm 90°$, 0°, that is for the position $i + 2$ of type I(I′) and type II(II′) β-turns [7–9]. This small-ring residue can also be accommodated in distorted type III(III′) β-turns and

Abbreviations: Ac_nc, 1-aminocycloalkane-1-carboxylic acid; Ac_4c, 1-aminocyclobutane-1-carboxylic acid; Aib, α-aminoisobutyric acid or $C^{\alpha,\alpha}$-dimethylglycine; TEMPO, 2,2,6,6-tetramethylpiperidinyl-1-oxy.

Address for correspondence: Prof. Claudio Toniolo, Department of Organic Chemistry, University of Padova, Via Marzolo 1, 35131 Padova, Italy. Tel. (39) 49-827-5247; fax. (39) 49-827-5239.

CCC 1075-2617/97/020110-13 $17.50

right- and left-handed 3_{10}-helices [10]. The expansion of the conformationally sensitive N–C^{α}-C′(τ) bond angle (116–117°) from the regular tetrahedral value is a remarkable indication of the Bayer strain operative in the Ac_3c ring structure.

However, the conformational preference [regular type III(III′) β-turns, and right- and left-handed 3_{10}-helices] found for the Ac_5c, Ac_6c, Ac_7c and Ac_8c residues, with their cyclic moieties significantly larger than that of Ac_3c, is the same as that of Aib, the smallest open-chain $C^{\alpha,\alpha}$-dialkylated glycine [1–5]. In these latter alicyclic amino acids as well as in Aib the τ bond angle (110–111°) is normal for a tetrahedral carbon.

The present conformational analysis of Ac_4c in model peptides was performed to complete the picture of the geometrical and structural preferences of the family of Ac_nc ($n = 3$–8) residues. Here we report the synthesis, characterization and solution (FT-IR absorption and ^{1}H-NMR) conformational studies of the Ac_4c homo-oligomers Z-$(Ac_4c)_n$-O*t*Bu ($n = 1$–6 and 12) and the tripeptides Z-Ac_4c-(L-Ala)$_2$-OMe and Z-L-Ala-Ac_4c-L-Ala-OMe. The X-ray diffraction structures of the derivatives Z-Ac_4c-OH and Z-Ac_4c-OH the tripeptides Z-$(Ac_4c)_3$-O*t*Bu, Z-Ac_4c-(L-Ala)$_2$-OMe and Z-L-Ala-Ac_4c-L-Ala-OMe, and the tetrapeptide Z-$(Ac_4c)_4$-O*t*Bu are also described.

The crystal structure of the amino acid hydrochloride monohydrate (H-Ac_4c-OH · HC1 · H_2O) has already been reported [11]. According to a ^{1}H-NMR and CD study the terminally blocked dipeptide Z-L-Ile-Ac_4c-NHAr is partially folded in an intramolecularly H-bonded conformation in solution [12]. Conformational energy calculations have been presented on Ac_4c-containing model compounds using molecular mechanics methods [13]. The energetically most favoured secondary structures for this residue are the γ-turn [8, 14] and the $3_{10}/\alpha$-helical conformations. In previous literature the Ac_4c residue was abbreviated as either Acb or ACBC.

MATERIALS AND METHODS

Synthesis and Characterization of Peptides

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 D thermostat. Thin-layer chromatography was performed on Merck (Darmstadt, Germany) Kieselgel 60F_{254} precoated plates using the following solvent systems: 1 ($CHCl_3$-EtOH, 9 : 1), 2 (Bu^nOH–AcOH–H_2O, 3 : 1 : 1), 3 (toluene–EtOH 7 : 1). The chromatograms were examined by UV fluorescence or developed by chlorine–starch–potassium iodide or ninhydrin chromatic reaction as appropriate. All the compounds were obtained in a chromatographically homogeneous state. Amino acid analyses of the Ala/Ac_4c peptides were determined using a C. Erba model 3A 30 amino acid analyzer (Rodano, Milan, Italy). Elution of Ac_4c was observed after the Ile peak and before the Tyr peak.

Infrared Absorption

The solid-state infrared absorption spectra (KBr disk technique) were recorded with a Perkin-Elmer (Norwalk, CT) model 580 B spectrophotometer equipped with a Perkin-Elmer model 3600 IR data station and a model 660 printer. The solution spectra were obtained using a Perkin-Elmer model 1720 X FT-IR spectrophotometer, nitrogen flushed, equipped with a sample-shuttle device, 2 cm^{-1} nominal resolution, averaging 100 scans. 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 Merck (Darmstadt, Germany). Solvent (baseline) spectra were recorded under the same conditions.

^{1}H Nuclear Magnetic Resonance

The ^{1}H nuclear magnetic resonance spectra were recorded with a Bruker (Karlsruhe, Germany) model AM 400 spectrometer. Measurements were carried out in deuterochloroform (99.96% *d*; Aldrich, Milwaukee, WI) and deuterated dimethylsulphoxide (99.96% d_6; Stohler, Waltham, MA) with tetramethylsilane as the internal standard. The free radical TEMPO was purchased from Sigma (St. Louis, MO).

X-Ray Diffraction

Single crystals of the amino acid derivatives Z-Ac_4c-OH and Z_2-Ac_4c-OH, the tripeptides Z-$(Ac_4c)_3$-O*t*Bu, Z-Ac_4c-(L-Ala)$_2$-OMe and Z-L-Ala-Ac_4c-L-Ala-OMe, and the tetrapeptide Z-$(Ac_4c)_4$-O*t*Bu were obtained by slow evaporation at room temperature from the solvents reported in Tables 1 and 2. Data collections were performed on CAD4 Enraf-Nonius single X-ray diffractometers of the Centro di Studio di Biocristallografia, CNR, at the University of Naples 'Federico II', and of the Department of Chemistry, at University of Basilicata. Unit cell determination

Table 1 Crystallographic Data for the Ac_4c Derivatives and Peptides

	Z-Ac_4c-OH	Z_2-Ac_4c-OH	Z-Ac_4c-(*L*-Ala)$_2$-OMe
Empirical formula	$C_{13}H_{15}NO_4$	$C_{21}H_{21}NO_6$	$C_{20}H_{27}N_3O_6 \cdot 3H_2O$
Formula weight (a.m.u.)	249.3	383.4	459.5
Crystal system	Monoclinic	Triclinic	Orthorhombic
Space group	C2/c	$P\bar{1}$	$P2_12_12_1$
a(Å)	22.587(4)	9.076(1)	8.746(2)
b(Å)	6.487(5)	11.301(1)	15.132(2)
c(Å)	18.447(2)	11.602(1)	18.491(3)
α(°)	90	102.634(8)	90
β(°)	105.79(1)	110.17(1)	90
γ(°)	90	111.549(9)	90
V(Å^3)	2601(2)	954(9)	2447.0(7)
Z (molecules/unit cell)	8	2	4
Density (calc) (g/cm^3)	1.273	1.334	1.246
Independent reflections	2287	3601	2264
Observed reflections	1014[$I > 3.0\sigma(I)$]	3323[$I > 3.0\sigma(I)$]	1241[$I > 1.5\sigma(I)$]
Solved by	SIR92	SIR92	SIR92
Refined by	SDP	SDP	SDP
S	1.090	1.10	2.334
R (unweighted)	0.053	0.047	0.085
R (weighted)	0.054	0.046	0.085
w	$1/\sigma(F^2)$	$1/\sigma(F^2)$	$1/\sigma(F^2)$
Temperature	Ambient	Ambient	Ambient
Radiation (λ, Å)	MoKα (0.71073)	CuKα (1.54178)	MoKα (0.71073)
θ range	1–35°	1–70°	1–35°
Crystallization solvent [a]	AcOEt/PE	AcOEt	AcOEt/PE
$\Delta\rho_{max}$ and $\Delta\rho_{min}$	0.166/−0.089	0.434/−0.230	0.287/−0.171

[a]AcOEt, ethyl acetate; PE, petroleum ether.

was done for all crystals by least-squares refinement of teh setting angles of 25 high-angle reflections accurately centred, using MoKα radiation for Z-Ac_4c-OH and Z-Ac_4c-(L-Ala)$_2$-OMe, and CuKα radiation for Z_2-Ac_4c-OH, Z-L-Ala-Ac_4c-L-Ala-OMe, Z-(Ac_4c)$_3$-O*t*Bu, and Z-(Ac_4c)$_4$-O*t*Bu. Crystal data are listed in Tables 1 and 2. Data were collected up to 35° and 70° in θ at 295 K, using the MoKα and CuKα radiations, respectively.

All the structures were solved by direct methods, using the SIR 92 program [15]. The solution with the best figure of merit revealed the coordinates of most of the non-hydrogen atoms; the remaining ones and the co-crystallized water molecules for the Z-Ac_4c-(L-Ala)$_2$-OMe structure were recovered using difference Fourier techniques. As for the refinement, the SDP (Structure Determination Programs) package [16] and a full matrix least-squares procedure were used, minimizing the quantity $\Sigma w(F_0 - F_c)^2$, with a weight w equal to $1/\sigma(F_0^2)$. The final conventional R values were 0.053 ($R_w = 0.054$), 0.047 ($R_w = 0.046$), 0.085 ($R_w = 0.085$), 0.061 ($R_w = 0.055$), 0.055 ($R_w = 0.060$) and 0.060 ($R_w = 0.056$) for Z-Ac_4c-OH, Z_2-Ac_4c-OH, Z-Ac_4c-(L-Ala)$_2$-OMe, Z-L-Ala-Ac_4c-L-Ala-OMe, Z-(Ac_4c)$_3$-O*t*Bu and Z-(Ac_4c)$_4$-O*t*Bu, respectively. In all cases the non-hydrogen atoms were refined with anisotropic temperature factors. Positional parameters of the hydrogen atoms were stereochemically determined and introduced in the calculations with isotropic thermal parameters equal to B_{eq} of the corresponding carrier atom, but not refined.

Final positional parameters and equivalent thermal factors for non-hydrogen atoms for the six structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as a supplementary publication. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336-033 or e.mail: teched@chemcrys.cam.ac.uk).

RESULTS AND DISCUSSION

Synthesis of Ac_4c and Its Derivatives and Peptides

Ac_4c amide hydrochloride was prepared by treatment of cyclobutanone with sodium cyanide, acetic

Table 2 Crystallographic Data for the Ac_4c Peptides

	Z-L-Ala-Ac_4c-L-Ala-OMe	Z-(Ac_4c)$_3$-O*t*Bu	Z-(Ac_4c)$_4$-O*t*Bu
Empirical formula	$C_{20}H_{27}N_3O_6$	$C_{27}H_{37}N_3O_6$	$C_{32}H_{44}N_4O_7$
Formula weight (a.m.u.)	405.5	499.6	596.7
Crystal system	Monoclinic	Orthorhombic	Monoclinic
Space group	C2	Pbca	$P2_1/n$
a (Å)	19.065(7)	11.371(3)	14.454(5)
b (Å)	5.919(3)	19.200(8)	18.068(5)
c (Å)	20.959(7)	25.085(9)	12.854(4)
α (°)	90	90	90
β (°)	112.61(1)	90	96.81(3)
γ (°)	90	90	90
V (Å^3)	2184(1)	5477(3)	3333(2)
Z (molecules/unit cell)	4	8	4
Density (calc) g/cm^3)	1.233	1.211	1.190
Independent reflections	2286	5199	6309
Observed reflections	1694[$I > 3.0\sigma(I)$]	2509[$I > 3.0\sigma(I)$]	4365[$I > 3.0\sigma(I)$]
Solved by	SIR92	SIR92	SIR92
Refined by	SDP	SDP	SDP
S	1.548	1.030	1.416
R (unweighted)	0.061	0.055	0.060
R (weighted)	0.055	0.060	0.056
w	$1/\sigma(F^2)$	$1/\sigma(F^2)$	$1/\sigma(F^2)$
Temperature	Ambient	Ambient	Ambient
Radiation (λ, Å)	GuKα (1.54178)	CuKα (1.54178)	GuKα (1.54178)
θ range	1–70°	1–70°	1–70°
Crystallization solvent [a]	MeOH/H_2O	MeOH/H_2O	$CHC1_3$/MeOH
$\Delta\rho_{max}$ and $\Delta\rho_{min}$	0.231/−0.116	0.310/−0.350	0.328/−0.280

[a]MeOH, methanol.

acid, excess of ammonia and subsequent acid hydrolysis (HCl/HCOOH at 10–20 °C) of the amino nitrile intermediate (Strecker synthesis). Acid hydrolysis (6N HC1, under reflux) of Ac_4c amide hydrochloride afforded the free amino acid. The synthesis of this alicyclic $C^{\alpha,\alpha}$-dialkylated glycine (*via* the hydantoin) was already reported [17–22].

The Z-protected Ac_4c derivative was obtained by reacting the free amino acid with the benzyloxycarbonyl-1-hydroxy-succinimide ester. In addition to Z-Ac_4c-OH, treatment of the free amino acid with benzyloxycarbonylchloride gave the *bis*-benzyloxycarbonyl derivative (Z_2-Ac_4c-OH) [23] and the N^{α}-protected di- and tripeptide free acids. The symmetrical anhydrides from Z-Ac_4c-OH and Z-L-Ala-OH were prepared by dehydration of the N^{α}-protected amino acid with N-ethyl, N′-(3-dimethylaminopropyl)-carbodiimide. The L-Ala methylester hydrochloride was synthesized using the MeOH/$SOCl_2$ method. Z-Ac_4c-O*t*Bu was obtained by esterification of the N-protected amino acid with isobutene in the presence of a catalytic amount of sulphuric acid.

Ala-Ala, Ala-Ac_4c and Ac_4c-Ac_4c (the latter in the dimer, trimer and tetramer) peptide bond formation was achieved by the symmetrical anhydride method. On the other hand, Ac_4c-Ac_4c peptide bond formation in the pentamer, hexamer and dodecamer was obtained using the 5(4*H*)-oxazolone method. The N^{α}-protected dipeptide 5(4*H*)-oxazolone was synthesized from Z-(Ac_4c)$_2$-OH and N-ethyl, N′-(3-dimethylaminopropyl)-carbodiimide at 0 °C. The N^{α}-protected hexapeptide 5(4*H*)-oxazolone was prepared by treatment of Z-(Ac_4c)$_6$-OH with acetic anhydride at 120 °C. The N^{α}-protected peptide free acids were prepared from the corresponding *tert*-butyl esters by treatment with diluted trifluoroacetic acid. Removal of the Z-group was performed by catalytic hydrogenation. The physical properties and analytical data of Ac_4c, and its derivatives and peptides are listed in Table 3.

Interestingly, linear di- and tripeptides containing the Ac_4c residue were previously synthesized by Heimgartner and coworkers [24–26] using a 2*H*-azirine as the Ac_4c equivalent.

Table 3 Physical and Analytical Properties for Ac_4c Its Derivative and Peptides

Compound	Melting point (°C)	Recryst. solvent[a]	$[\alpha]_D^{20}$ (°)[b]	TLC R_{FI}	TLC R_{FII}	TLC R_{FIII}	IR (cm^{-1})[c]	Amino acid analysis
H-Ac_4c-OH	264–265	MeOH/EE	–	0.05	0.50	0.05	3403, 1622	–
HCl · H-Ac_4c-NH_2	239–240	MeOH/EE	–	0.10	0.50	0.05	3338, 1669, 1611	–
Z-Ac_4c-OH	89–90	EE/PE	–	0.75	0.90	0.45	3301, 1690	–
Z_2-Ac_4c-OH	127–129	AcOEt	–	0.90	0.95	0.45	1748, 1715	–
(Z-Ac_4c)$_2$O	124–125	AcOEt/PE	–	0.55	0.95	0.35	3293, 1822, 1739, 1708, 1683	–
Z-Ac_4c-O*t*Bu	71–72	EE/PE	–	0.95	0.95	0.70	3336, 1710	–
Z-(Ac_4c)$_2$-O*t*Bu	119–120	AcOEt/PE	–	0.95	0.95	0.50	3388, 3295, 1717, 1698, 1665, 1650	–
Z-(Ac_4c)$_2$OH	173–174	AcOEt	–	0.45	0.95	0.25	3335, 3249, 1695, 1648	–
5(4*H*)-oxazolone from Z-(Ac_4c)$_2$-OH	142–144	MeOH/H_2O	–	0.95	0.90	0.55	3246, 1841, 1715, 1654	–
Z-(Ac_4c)$_3$-O*t*Bu	171–172	AcOEt/PE	–	0.80	0.90	0.40	3388, 3295, 1717, 1698, 1665, 1650	–
Z-(Ac_4c)$_3$OH	150–151	AcOEt/PE	–	0.50	0.90	0.20	3355, 3309, 1725, 1695, 1651	–
Z-(Ac_4c)$_4$-O*t*Bu	184–185	AcOEt/PE	–	0.80	0.90	0.35	3394, 3350, 3334, 1698, 1671, 1644	–
Z-(Ac_4c)$_5$-O*t*Bu	218–219	MeCN	–	0.80	0.95	0.30	3324, 1726, 1696, 1654	–
Z-(Ac_4c)$_6$O*t*Bu	240–241	$CHC1_3$/PE	–	0.70	0.95	0.25	3316, 1726, 1695, 1652	–
Z-(Ac_4c)$_6$-OH	247–249	MeOH/EE	–	0.20	0.90	0.05	3317, 1697, 1655	–
5(4*H*)-oxazolone from Z-(Ac_4c)$_6$-OH	201–202	MeOH/H_2O	–	0.65	0.90	0.30	3316, 1814, 1681, 1651	–
Z-(Ac_4c)$_{12}$O*t*Bu	> 340	EtOH/EE	–	0.65	0.85	0.15	3323, 1726, 1658	–
Z-Ac_4c-L-Ala-OMe	84–85	AcOEt/PE	− 35.2	0.90	0.95	0.45	3304, 1739, 1681, 1654	–
Z-L-Ala-Ac_4c-*L*-Ala-OMe	149–150	AcOEt/PE	− 71.0	0.85	0.90	0.40	3373, 1741, 1732, 1704, 1678, 1628	Ac_4c 1.00; Ala 2.00
Z-Ac_4-(L-Ala)$_2$OMe	81–82	AcOEt/PE	− 36.5	0.80	0.90	0.40	3293, 1739, 1689, 1664, 1642	Ac_4c 1.03; Ala 1.97

[a]MeOH, methanol; AcOEt, ethyl acetate; PE, petroleum ether; EE, ethyl ether; MeCN, acetonitrile.
[b]c = 0.5, methanol;
[c]The IR absorption spectra were obtained in KBr pellets (only bands in the 3500–3200 and 1850–1600 cm^{-1} regions are reported).

Solution Conformational Analysis

The preferred conformation adopted by the Ac_4c-rich peptides in solution was determined in a solvent of low polarity ($CDC1_3$) by FT-IR absorption and ^{1}H-NMR as a function of concentration (over the range 10–0.1 mM).

Figure 1 shows the FT-IR absorption spectra (N–H stretching region) of the Ac_4c homo-peptide series (from monomer to hexamer, and the dodecamer). The curves of the tripeptide and the higher oligomers are characterized by two bands, at 3431–3425 cm^{-1} (free, solvated NH groups) and 3378–3322 cm^{-1} (H-bonded NH groups), respectively [27]. The intensity of the low-frequency band relative to the high-frequency band (A_H/A_F ratio) markedly increases as main-chain length increases. Concomitantly, the absorption maximum shifts significantly to lower wave numbers. An inspection of the spectrum of the homo-tripeptide, compared with those of the Ac_4c/Ala tripeptides Z-Ac_4c-(L-Ala)$_2$-OMe and Z-L-Ala-Ac_4c-L-Ala-OMe (the two latter spectra not shown), allows us to conclude that the 3378–3355 cm^1 band

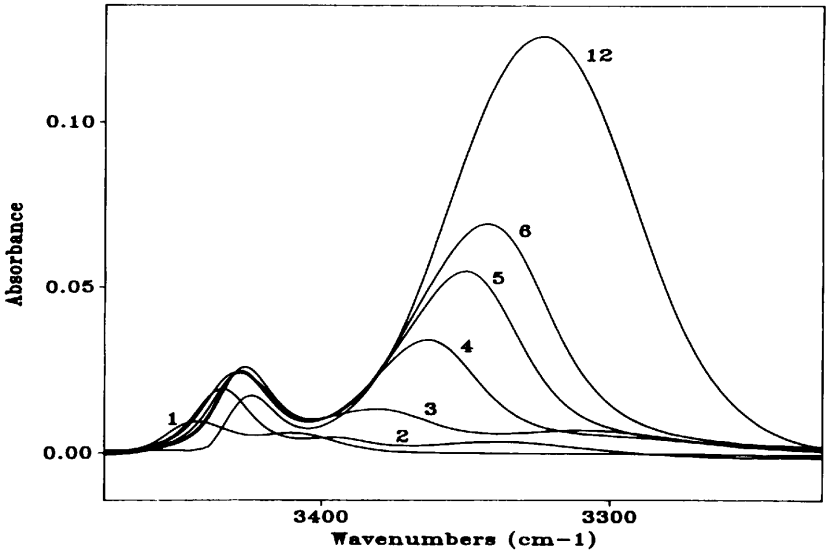

Figure 1FTIR absorption spectra (N–H stretching region) of Z-$(Ac_4c)_n$-O*t*Bu ($n = 1$–6, 12) in $CDC1_3$ solution (peptide concentration 0.1 mM).

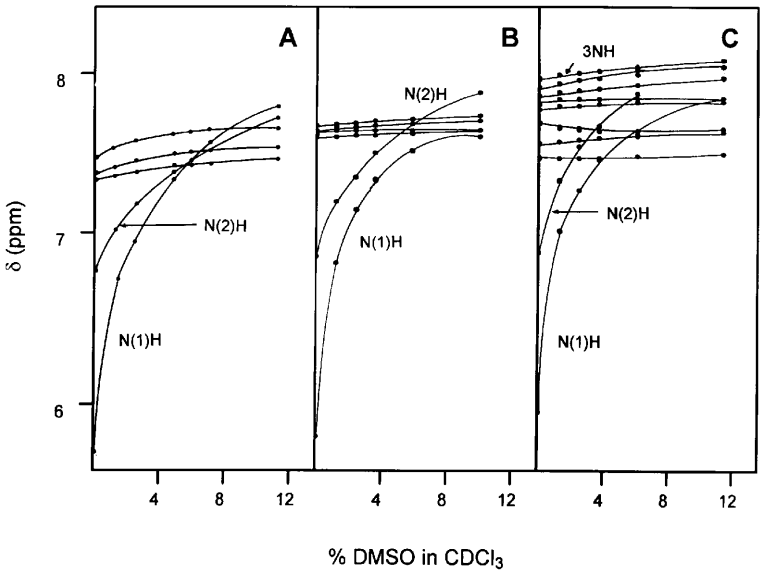

Figure 2Plots of NH chemical shifts in the ^{1}H-NMR spectra of Z(Ac_4c)ĥ-O*t*Bu ($n = 5$, A; $n = 6$, B; $n = 12$, C) as a function of increasing percentages of DMSO added to the $CDCl_3$ solution (v/v). Peptide concentration: 1mM for pentamer and hexamer, while 0.1 m*M* for dodecamer.

is higher (relative to the 3431–3425 cm^{-1} band) in the homo-tripeptide. We have also been able to demonstrate that, even at 10mM concentration, there are only minor changes in the spectra of the homo-peptides to the tetramer level in the 3500–3330 cm^{-1} region (not shown). Therefore, in those peptides the observed H-bonding band at 3377–3348 cm^{-1} should be interpreted as arising almost exclusively from intramolecular N–H$\cdots$O=C interactions. However, in the homo-pentamer and hexamer a remarkable variation in the A_H/A_F ratio is noted above 1 mM concentration. The homo-dodecamer is not soluble at concentrations higher than 0.1 mM.

The present FT-IR absorption investigation has provided convincing evidence that main-chain

length-dependent intramolecular H-bonding is an essential factor influencing the conformation of the terminally blocked Ac_4c homo-peptides in $CDCl_3$ solution. Our findings also support the view that Ac_4c is a better inducer of intramolecularly H-bonded structures than Ala.

The delineation of inaccessible (or intramolecularly H-bonded) NH groups of the Ac_4c homo-peptides by ^{1}H-NMR was carried out using: (i) solvent dependence of NH chemical shifts, by adding increasing amounts of the strong H-bonding acceptor solvent DMSO [28, 29] to the $CDC1_3$ solution and (ii) free-radical (TEMPO) induced line broadening of NH resonances [30]. As a typical example, Figure 2 illustrates the behaviour of the NH resonances of the pentamer, hexamer and dodecamer upon addition of DMSO. The upfield resonance in $CDC1_3$ solution is unequivocally assigned to the N(1)H urethane group. A tentative assignment has been performed for the second upfield resonance to the N(2)H proton, by analogy with the chemical shifts in the same halohydrocarbon and the spectroscopic behaviour upon addition of DMSO of other N^{α}-benzyloxycarbonylated homo-peptides from different types of $C^{\alpha,\alpha}$-dialkylated glycines [31, 32]. From an analysis of the spectra as a function of concentration (10–1 mM) in $CDC1_3$ solution (results not shown), we have been able to conclude that dilution induces a negligible shift to higher fields of the NH resonances of the shortest peptides. However, this effect becomes significant for the N(1)H and N(2)H resonances of the penta-and hexapeptides, where shifts of about 0.15–0.50 p.p.m. were observed. In the Ac_4c peptides examined in the $CDC1_3$–DMSO solvent mixtures and in the presence of the paramagnetic perturbing agent TEMPO (the latter results not shown) two classes of NH protons were observed. Class (i) [N(1)H and N(2)H protons] includes protons whose chemical shifts are extremely sensitive to the addition of DMSO and whose resonances broaden significantly upon addition of TEMPO. Class (ii) [N(3)H to N(12)H protons] includes those displaying a behaviour characteristic of shielded protons (relative insensitivity of chemical shifts to solvent composition and of line widths to the presence of TEMPO).

In summary, these ^{1}H-NMR results allow us to conclude that, in $CDC1_3$ solution at a concentration higher than 1 mM, the homo-pentapeptide and the higher oligomers have a tendency to self-associate and that in this process the urethane N(1)H and the peptide N(2)H protons play a major role as H-bonding donors. At lower concentrations, the N(3)H to N(12)H protons of the tripeptide and longer oligomers are almost inaccessible to perturbing agents and are, therefore, most probably, intramolecularly H-bonded. In view of these observations and by analogy with the conformational propensities of other cycloaliphatic $C^{\alpha,\alpha}$-dialkylated glycines [3–5], it is reasonable to conclude that the most populated structures adopted in $CDC1_3$ solution by the terminally blocked Ac_4c homo-tri- and tetrapeptides, and the higher oligomers are the β-turn, two consecutive β-turns, and the 3_{10}-helix, respectively. These conclusions are in agreement with those extracted from the FT-IR absorption study discussed above.

Crystal-state Conformational Analysis

We determined by X-ray diffraction the molecular and crystal structure of the following Ac_4c derivative and peptides: Z-Ac_4c-OH, Z_2-Ac_4c-OH, Z-Ac_4c-(L-Ala)$_2$-OMe, Z-L-Ala-Ac_4c-L-Ala-OMe, Z-(Ac_4c)$_3$-O*t*Bu and Z-(Ac_4c)$_4$-O*t*Bu. The molecular structures with the atomic numbering schemes are illustrated in Figures 3–8, respectively. The relevant N^{α}-protecting group, backbone and side-chain torsion angles [33] are given in Table 4. In Table 5 the intra- and intermolecular H-bond parameters are listed, while the average bond distances and bond angles characterizing the four-membered ring system of the Ac_4c residue are given in Table 6.

Bond lengths and bond angles are in general agreement with previously reported values for the geometry of the benzyloxycarbonylamino moiety

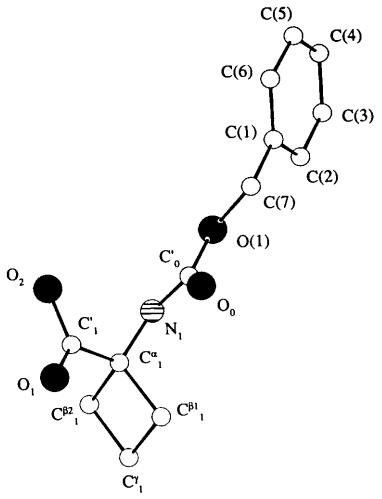

Figure 3X-ray diffraction structure of Z-Ac_4c-OH with numbering of atoms.

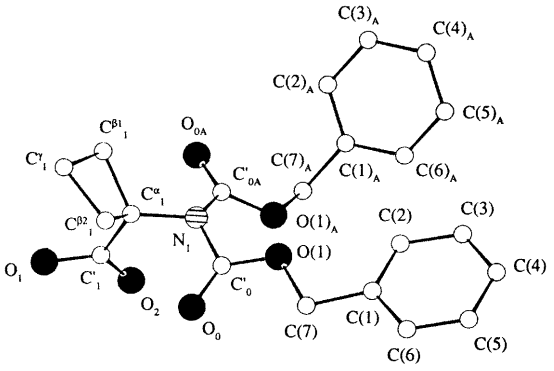

Figure 4X-ray diffraction structure of Z_2Ac_4c-OH with numbering of atoms.

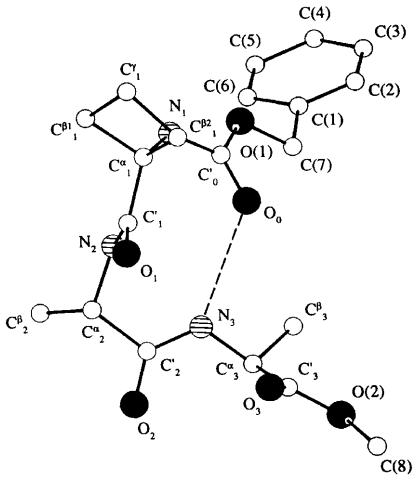

Figure 5X-ray diffraction structure of Z-Ac_4c-(*L*-Ala)$_2$-OMe with numbering of atoms. The intramolecular H-bond is represented by a dashed line.

[34], the ester group [35] and the peptide unit [36, 37]. We have also calculated the average geometry for the Ac_4c residue. All the parameters are close to those reported in the literature for the free amino acid [11]. In particular, the average C–C bond length of the cyclobutane ring is 1.521 Å (with average lengths of 1.546 Å for the C^{α}–C^{β} bonds and 1.507 Å for the C^{β}–C^{γ} bonds) in good agreement with the literature average value of 1.52 Å for the $-CH_2-CH_2-$ distance [38]. The values for the N–C^{α}, C^{α}–C′ and C′–O bond lengths fit nicely with the corresponding values for peptides from protein amino acids [36]. The bond angles internal to the four-membered ring are significantly strained, deviating markedly from the regular tetrahedral value (109.5°). The average bond angle in the ring is 89.5°, which is close to the mean value of 89.0° reported for other cyclobutane

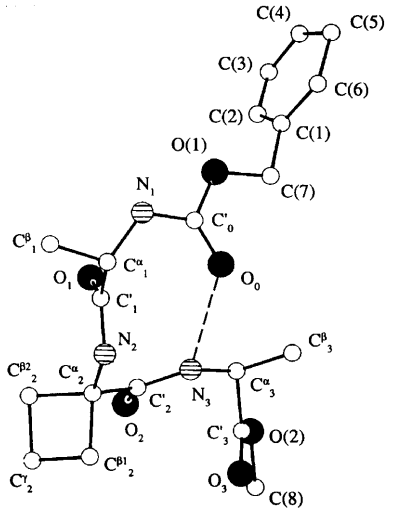

Figure 6X-ray diffraction structure of Z-L-Ala-Ac_4c-L-Ala-OMe with numbering of atoms. The intramolecular H-bond is represented by a dashed line.

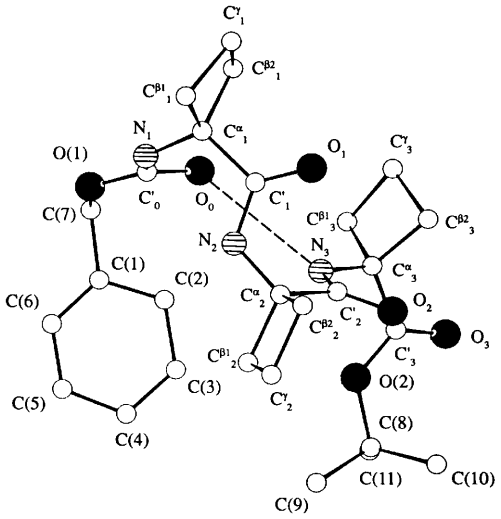

Figure 7X-ray diffraction structure of Z-$(Ac_4c)_3$-O*t*Bu with numbering of atoms. The intramolecular H-bond is represented by a dashed line.

structures [39]. The bond angles actually vary from 88.4(5)° (at C^{α}) to 91.4(7)° (at C^{γ}), the largest value being observed for the carbon atom most removed from the peptide chain. In addition, the bond angles indicate an asymmetric geometry for the C^{α} atom. More specifically, the bond angles involving the $C^{\beta 1}$ atom are narrower by about 2° than those involving the $C^{\beta 2}$ atom. This observation is common also to Aib- and Ac_nc-rich ($n = 3$, 5–8) peptides [3, 5]. The N–C^{α}–C′ (τ) bond angle, external to the cyclic system, is 114.7(5)°, remarkedly widened compared to the

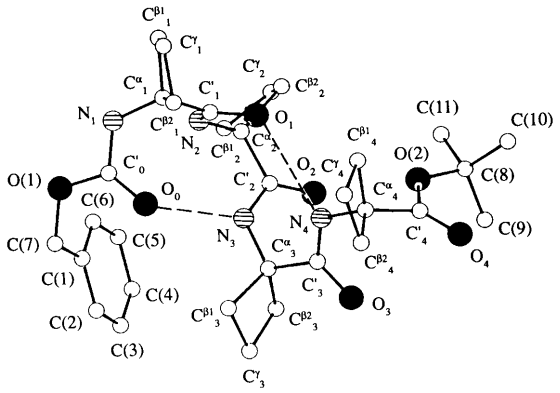

Figure 8X-ray diffraction structure of Z-$(Ac_4c)_4$-O*t*Bu with numbering of atoms. The two intramolecular H-bonds are represented by dashed lines.

value expected for $C^{\alpha,\alpha}$-dialkylated glycines forming regular helices (110–111°) [5, 40]. the cyclobutane ring is puckered with the C^{γ} atom deviating by 0.14 Å from the plane defined by $C^{\beta 1}$, C^{α} and $C^{\beta 2}$. In addition, the nitrogen atom deviates by 0.64 Å from the plane defined by the C^{α}, C′ and O atoms.

All the Ac_4c residues are found in the helical region A (A*) of the conformational map [6], with the exception of the unusual Z_2-Ac_4c-OH derivative. Each molecule of Z-Ac_4c-OH, Z_2-Ac_4-OH, Z-$(Ac_4c)_3$-O*t*Bu, and Z-$(Ac_4c)_4$-O*t*Bu, having no chiral atoms, crystallizes with retention of the centre of symmetry; thus, in each unit cell molecules of both handedness simultaneously occur (as asymmetric unit, we have chosen the right-handed helical molecule). The average values for the ϕ, ψ backbone torsion angles of the Ac_4c residue completely involved in a helical structure are $\pm 63.7°$, $\pm 26.9°$, close to those expected for a 3_{10} helix ($\pm 57°$, $\pm 30°$) [10]. Also the C-terminal Ac_4c residue of the homo-tripeptide adopts a conformation in the helical region, but it has an handedness opposite to that exhibited by the preceding residues, a common observation for Aib- and Ac_4c-rich ($n = 3$, 5–8) peptides [3, 5].

Table 4 Selected N^{α}-Protecting Group, Backbone and Side-chain Torsion Angles (°) for the Ac_4c Derivatives and Peptides

Torsion angle	Z-Ac_4c-OH	Z_2-Ac_4c-OH	Z-Ac_4c-(*L*-Ala)$_2$-OMe	Z-*L*-Ala-Ac_4c-*L*-Ala-OMe	Z-$(Ac_4c)_3$-O*t*Bu	Z-$(Ac_4c)_4$-O*t*Bu
θ^3	48.8(6)	−8.9(3)[−95.6(2)]	−56(2)	−76.8(12)	−88.8(8)	−158.8(3)
θ^2	−178.8(4)	174.9(2)[93.6(2)]	171(1)	166.0(7)	86.8(7)	73.2(4)
θ^1	−176.1(4)	−179.7(2)[−179.5(2)]	−172(1)	179.2(8)	−172.2(5)	−170.5(3)
ω_0	178.0(4)	−157.2(2)[−155.9(1)]	−172(1)	165.5(6)	−174.3(6)	−178.1(3)
ϕ_1	−64.5(6)	−81.5(2)[−72.3(2)]	−59.(2)	−53.7(8)	−71.4(6)	−60.4(4)
ψ_1	−28.3(6)[a]	−8.9(3)[171.5(2)][b]	−29(2)	138.9(5)	−20.6(7)	−31.2(4)
ω_1	–	–	−174(1)	172.9(5)	−177.7(5)	−175.9(3)
ϕ_2	–	–	−98(1)	66.2(7)	−58.3(7)	−64.0(4)
ψ_2	–	–	−0(2)	27.8(8)	−35.8(7)	−19.2(4)
ω_2	–	–	−175(1)	171.1(6)	−179.2(5)	179.2(3)
ϕ_3	–	–	−93(1)	−54.7(8)	70.5(7)	−66.5(4)
ψ_3	–	–	−172(1)[c]	138.3(6)[c]	21.0(7)[c]	−24.8(4)
ω_3	–	–	−173(1)[d]	−179.2(6)[d]	178.7(5)[d]	−175.9(3)
ϕ_4	–	–	–	-	-	−59.3(4)
ψ_4	–	–	–	-	-	−48.7(3)[e]
ω_4	–	–	–	–	–	−176.7(3)[f]
$\chi 1^{1,1}$	−121.7(4)	−133.3(2)	−107(1)	–	−127.1(6)	−114.4(4)
$\chi 1^{1,2}$	119.2(4)	134.6(2)	102(1)	–	124.5(7)	108.9(4)
$\chi 2^{1,1}$	–	–	–	131.7(7)	−122.8(6)	−128.5(3)
$\chi 2^{1,2}$	–	–	–	−129.6(7)	120.9(6)	127.4(3)
$\chi 3^{1,1}$	–	–	–	–	101.0(6)	−133.2(3)
$\chi 3^{1,2}$	–	–	–	—	−99.4(6)	130.0(3)
$\chi 4^{1,1}$	–	–	–	—	–	−105.9(4)
$\chi 4^{1,2}$	–	–	–	—	–	104.2(4)

[a] N_1-$C^{\alpha}{}_1$-C'_1-O_2. [b] N_1-$C^{\alpha}{}_1$-C'_1-O_1[N_1-$C^{\alpha}{}_1$-C'_1-O_2]
[c] N_3-$C^{\alpha}{}_3$-C'_3-O(2) [d] $C^{\alpha}{}_3$-C'_3-O(2)-C(8)
[e] N_4-$C^{\alpha}{}_4$-C'_4-O(2) [f] $C^{\alpha}{}_4$-C'_4-O(2)-C(8)

Table 5 Intra- and Intermolecular H-bond Parameters for the Ac_4c Derivatives and Peptides

Compound	Donor (D)	Acceptor (A)	Symmetry operation	Distance (A) D–A	Angle (°) N–O=C
Z-Ac_4c-OH	N_1	O_2	$1-x-y,-1-z$	3.002(5)	99.8(2)
	O_2	O_0	$1-x1-y,-1-z$	2.625(5)	122.0(3)
Z_2-Ac_4c-OH	O_2	O_1	$-x-y,1-z$	2.607(2)	120.9(1)
Z-Ac_4c-(L-Ala)$_2$-OMe	N_3	O_0	x,y,z	3.04(1)	124.6(9)
trihydrate	N_1	O_2	$1+x,y,z$	3.06(1)	137.7(8)
	N_2	Ow_1	x,y,z	2.88(1)	–
	Ow_3	O_0	x,y,z	2.90(2)	127.2(9)
	Ow_1	O_1	$-x,y-1/2,3/2-z$	2.77(1)	154.7(9)
	Ow_2	O_2	$1+x,y,z$	2.83(2)	142.6(9)
	Ow_3	O_3	$1/2+x,1/2-y2-z$	2.82(2)	145(1)
	Ow_1	Ow_2	x,y,z	2.82(2)	–
	Ow_2	Ow_3	$1/2-x,-y,z-1/2$	2.83(2)	–
Z-*L*-Ala-Ac_4c-*L*-Ala-OMe	N_3	O_0	x,y,z	3.314(7)	122.7(3)
	N_1	O_3	$1/2+x,3/2+y,z$	3.044(1)	144.0(5)
	N_2	O_2	$x,1+y,z$	2.872(7)	138.2(4)
Z-(Ac_4c)$_3$-O*t*Bu	N_3	O_0	x,y,z	3.367(7)	121.4(4)
	N_1	O_1	$-x-3/2,y,-z-1/2$	2.911(7)	151.9(4)
	N_2	O_2	$-x-3/2,y,-z-1/2$	3.364(7)	130.0(4)
Z-(Ac_4c)$_4$-O*t*Bu	N_3	O_0	x,y,z	3.044(3)	123.3(2)
	N_4	O_1	x,y,z	3.082(4)	125.1(2)
	N_1	O_3	$x-3/2,1/2-y,z-1/2$	2.897(4)	169.5(4)
	N_2	O_4	$x-3/2,1/2-y,z-1/2$	3.483(4)	139.0(2)

The Ac_4c-L-Ala–, –L-Ala-Ac_4c-, and -Ac_4c-Ac_4c sequences of the three tripeptides are all folded in a $1\leftarrow 4$ C=O···H-N– intramolecularly H-bonded β-turn conformation. Interestingly, however, the type of β-turn that is formed is different in the three cases, type I for -Ac_4c-L-Ala-, type II for -L-Ala-Ac_4c-, while type - III (III′) for -Ac_4c-Ac_4c-. Two of the three intramolecular $N_3\cdots O_0$ separations [3.314(7) and 3.367(7)Å] are at the upper limit expected for such H-bonds [41–43]. The -(Ac_4c)$_3$- sequence of the homo-tetramer forms an incipient 3_{10}-helix [two consecutive type III(III′) β-turn conformations] stabilized by two $1\leftarrow 4$ C=O...H–N intramolecular H-bonds of normal length.

The three-branched Z_2-Ac_4c-OH molecule has the imide N_1 atom at the centre. The N_1 atom is slightly out-of-plane, the sum of bond angles at N_1 being 354.0(2)°. The conformation of the imide moiety is a significantly distorted *cis–cis* type, the values of the torsion angles O(1)-C′$_0$-N_1-C′$_0$A and O(1)A-C′$_0$A-N_1-C′$_0$ being 51.3(2)° and −4.9(2)°, respectively. The best planes calculated for the two benzyloxycarbonyl substitutents from a angle of 39.0(2)°. The two ϕ_1 torsion angles have similar values but opposite signs. This is the first X-ray diffraction structure of an *N, N-bis*-benzyloxycarbonyl- amino acid reported to date. Recently, the X-ray structures of *N, N-bis-tert*-butyloxycarbonyl derivatives of Gly and L-Ala have been described [44, 45].

In the six compounds, few significant deviations of the ω torsion angles ($|\Delta\omega| > 8°$) from the ideal value of the *trans* planar urethane, peptide and ester units (180°) are observed. In particular, the ω_0 (urethane) torsion angles for Z_2-Ac_4c-OH and Z-L-Ala-Ac_4c-L-Ala-O*t*Bu differ by 14–24° from the *trans* planar value. The $\chi^{1,1}$ and $\chi^{1,2}$ torsion angles, relating the cyclobutyl ring to the peptide chain, have absolute values in the range $117\pm18°$. The *trans*-arrangement of the θ^1 torsion angle of the benzyloxycarbonyl moiety, found for all the Ac_4c derivatives and peptides examined, is that commonly observed for Z-amino acids and peptides [34]. As expected [34], the values of θ^2 are concentrated in the regions of 90° and 180°.

In the unit cell the Z-Ac_4c-OH molecules are held together by (carboxylic acid) O−H···O=C (urethane) intermolecular H-bonds, giving rise to dimers of molecules. In addition, the C–O–H group of the carboxylic acid moiety acts also as a H-bonding acceptor from the urethane N_1–H group, further

Table 6 Average Bond Distances and Bond Angles for the Ac_4c Residue

Bond distance (Å)		Bond angle (°)	
N–C^{α}	1.448(8)	N–C^{α}–C'	114.7(5)
C^{α}–C'	1.519(9)	$C^{\beta 1}$–C^{α}-$C^{\beta 2}$	88.4(5)
C'–O	1.234(9)	C^{γ}–$C^{\beta 1}$–C^{α}	89.1(6)
C^{α}–$C^{\beta 1}$	1.544(8)	C^{γ}–$C^{\beta 2}C^{\alpha}$	89.1(6)
C^{α}–$C^{\beta 2}$	1.547(9)	$C^{\beta 1}$–C^{γ}-$C^{\beta 2}$	91.4(7)
$C^{\beta 1}$–C^{γ}	1.509(8)	$C^{\beta 1}$-C^{α}-N	113.8(5)
$C^{\beta 2}$–C^{γ}	1.504(9)	$C^{\beta 2}$-C^{α}-N	115.4(6)
		$C^{\beta 1}$-C^{α}-C'	111.7(5)
		$C^{\beta 2}$-C^{α}-C'	113.9(6)

stabilizing the structure. Thus, along the *b* direction, rows of molecules stabilized by these H-bonds are formed. These rows pack with each other in the *ac* plane by van der Waals interactions between the hydrophobic groups.

In the crystal packing the Z_2-Ac_4c-OH molecules are linked by (carboxylic acid) O–H···O=C (carboxylic acid) intermolecular H-bonds across centres of symmetry along the *c* direction. This arrangement gives dimers of molecules along the *c* direction, which pack together in the *ab* plane by van der Waals interactions between benzyloxycarbonyl groups of symmetry related molecules.

Each Z-Ac_4c-(L-Ala)$_2$-OMe molecule co-crystallizes with three water molecules. The urethane N_1–H group forms a H-bond with the peptide $C_2' = O_2$ group of a molecule translated along the *a* direction, giving rise to rows of molecules. The donor N_2–H group forms an H-bond with the oxygen atom of the Ow_1 water molecule. The three peptide and the urethane $C' = O$ groups act as H-bonding acceptors for the O–H groups of the three water molecules. In addition, the water molecules, interconnected through an H-bond network, bridge together rows of peptide molecules in the *bc* plane, Van der Waals interactions between hydrophobic groups further stabilize the crystal structure.

In the crystal packing of Z-L-Ala-Ac_4c-L-Ala-OMe structure infinite rows of molecules are piled up along the *b* direction being held together by an intermolecular H-bond (N_2–H···O_2=C_2'). In addition, these rows are bridged together by an intermolecular H-bond along the *a* direction between the urethane N_1–H group and the ester $C_3' = O_3$ group of a symmetry-related molecule, giving rise to layers of molecules nearly parallel to the *ab* plane. Van der Waals interactions between hydrophobic groups of adjacent layers further stabilize the structure.

The Z-(Ac_4c)$_3$-O*t*Bu molecules pack together along the *a* direction, producing rows of molecule stabilized by (amide or urethane) N–H···O=C (peptide) intermolecular H-bonds [N_1–H···O_1=C'_1 and N_2–H···O_2=C_2']. Then, hydrophobic interactions link together rows of peptide molecules running in the *b* and *c* directions.

In the unit cell the Z-(Ac_4c)$_4$-O*t*Bu molecules are held together along the *a* direction in rows stabilized by (amide or urethane) N–H···O=C' (peptide or ester) intermolecular H-bonds. In addition, the crystal structure is stabilized by van der Waals interactions between the hydrophobic groups in the *bc* plane.

CONCLUSIONS

The result of the present solution and crystal-state conformational analysis strongly suggest that the small-ring alicyclic Ac_4c residue imparts considerable restriction to the peptide backbone and is forced to adopt conformations in the $3_{10}/\alpha$-helical region of the ϕ, ψ space. Therefore, the Ac_4c residue can be easily accomodated in either position $i+1$ or $i+2$ of type III(III′) β-turn and at the position $i+1$ of type I(I′) β-turn. It may also be located, although with some distortion from the preferred conformation, at the position $i+2$ of either type I(I′) or type II(II′) β-turn. ϕ, ψ Angles corresponding to position $i+1$ of type II(II′) β-turn are not available to Ac_4c. Interestingly, the widening of the $Ac_4c\tau$(N–C^{α}-C′) bond angle [to 114.7(5)°] does not seem to be correlated to any significant distortion of the 3_{10}-helical structure, at variance with published observations on Ac_3c-rich peptides [3, 5], which, however, have a larger average τ (116.7°).

Recently, considerable interest has been focused on the design of conformationally constrained bioactive peptides [46–52]. In this connection it is of interest that the (Ac_4c)^2-analogue of the formyl-methionyl tripeptide chemoattractant is highly active in the release of lysosomal enzymes [53] and the L-aspartic acid-based dipeptide derivative of H-Ac_4c-OMe is sweet [21], as is the free amino acid H-Ac_4c-OH itself [20]. It seems reasonable to foresee that future investigations on analogues of other bioactive peptides, incorporating the turn- and helix-former Ac_4c residue at selected positions, will be rewarding. It is also worth noting that Ac_4c has been site-specifically inserted into proteins using a recently developed general biosynthetic method [54].

Acknowledgments

We gratefully acknowledge MURST, the Ministry of University and of the Scientific and Technological Research, and the National Council of Research, CNR, for their continuous and generous support to this research. We thank Dr Gabriella De Vita for technical assistance.

REFERENCES

1. G. R. Marshall in: *Intra-Science Chemistry Report*, N. Kharasch, Ed., p. 305–316, Gordon and Breach, New York 1971.
2. I. L. Karle and P. Balaram (1990). Structural characteristics of α-helical peptide molecules containing Aib residues. *Biochemistry 29*, 6747–6756.
3. C. Toniolo and E. Benedetti (1991). Structures of polypeptides from α-amino acids disubstituted at the α-carbon. *Macromolecules 24*, 4004–4009.
4. P. Balaram (1992). Non-standard amino acids in peptide design and protein engineering. *Current Opin. Struct. Biol. 2*, 845–851.
5. C. Toniolo (1993). $C^{\alpha,\alpha}$-Symmetrically disubstituted glycines: useful building blocks in the design of conformationally restricted peptides. *Janssen Chim. Acta 11*, 10–16.
6. S. S. Zimmerman, M. S. Pottle, G. Nemethy and H. A. Scheraga (1977). Conformational analysis of the 20 naturally occurring amino acid residues using ECEPP. *Macromolecules 10*, 1–9.
7. C. M. Venkatachalam (1968). Stereochemical criteria for polypeptides and proteins. V. Conformation of a system of three-linked peptide units. *Biopolymers 6*, 1425-1436.
8. C. Toniolo (1980). Intramolecularly hydrogen-bonded peptide conformations. *CRC Crit. Rev. Biochem. 9*, 1–44.
9. G. D. Rose, L. M. Gierasch and J. P. Smith (1985). Turns in peptides and proteins. *Adv. Protein Chem. 37*, 1–109.
10. C. Toniolo and E. Benedetti (1991). The polypeptide 3_{10}-helix. *TIBS 16*, 350–353.
11. K. K. Chacko and R. Zand (1975). 1-Aminocyclobutane carboxylic acid hydrochloride monohydrate. *Cryst. Struct. Commun. 4*, 17–19.
12. P. Wipf and H. Heimgartner (1988). Konformationsanalysen von Modell-Tripeptides: Der Einfluss von α,α,-disubstituierten α-Aminosäuren auf die Sekundarstruktur. Teil I. NMR-und CD-Untersuchungen. *Helv. Chim. Acta 71*, 258–267.
13. V. N. Balaji, K. Ramnarayan, M. F. Chan and S. N. Rao (1995). Conformational studies on model peptides with 1-aminocyclobutane-1-carboxylic acid residues. *Pept. Res. 8*, 178–186.
14. G. Nemethy and M. P. Printz (1972). The γ-turn, a possible folded conformation of the polypeptide chain. Comparison with the β-turn. *Macromolecules 5*, 755–758.
15. A. Altomare, G. Cascarano, G. Giacovazzo, A. Guagliardi, M. C. Burla, G. Polidori and M. Camalli (1992). SIR 92. A program for automatic solution of crystal structures by direct methods. *J. Appl. Crystallogr. 27*, 435.
16. B. A. Frenz and Associates, Inc. SDP (Structure Determination Package), College Station, Texas, and Enraf-Nonius, Delft, The Netherlands 1985.
17. T. A. Connors and W. C. J. Ross (1960). Some derivatives of 1-aminocyclopentanecarboxylic acid and related compounds. *J. Chem. Soc.*, 2119–2132.
18. R. Sudo and S. Ichihara (1963). Synthesis of 1-aminocyclobutanecarboxylic acid. *Bull. Chem. Soc. Jpn 36*, 145–147.
19. W. Dvonch, H. Fletcher III and H. E. Alburn (1964). Preparation of new amino acids and 2,5-oxazolidinediones. *J. Org. Chem. 29*, 2764–2765.
20. R. Treleano, H. D. Belitz, H. Jugel and H. Wieser (1978). Beziehungen zwisches Struktur and Geschmack bei Aminosäuren mit cyclischen Seitenketten. *Z. Lebensm. Unters. Forsch. 167*, 320–323.
21. J. W. Tsang, B. Schmied, R. Nyfeler and M. Goodman (1984). Peptide sweeteners. Structural studies on the C-terminal amino acid of *L*-aspartyl peptide sweeteners. *J. Med. Chem. 27*, 1663–1668.
22. F. Avotins (1993). Aminoacids of the cyclobutane series. *Russian Chem. Rev. 62*, 897–906.
23. N. L. Benoiton, D. Akyurekli and F. M. F. Chen (1995). *N, N*-Dialkoxycarbonylamino acids from the sodium hydride-mediated reaction of alkyl chloroformates with mixed anhydride of *N*-alkoxycarbonylamino acids. *Int. J. Pept. Protein Res. 45*, 466–470.
24. P. Wipf and H. Heimgartner (1987). Selective Amidspaltung bei Peptiden mit α,α-disubstituierten α-Aminosäuren. *Helv. Chim. Acta 70*, 354–368.
25. P. Wipf and H. Heimgartner (1988). Anwendung der Azirin-Oxazolon-Methode in der Peptid-Chemie: Synthese von Modell-Tripeptiden. *Helv. Chim. Acta 71*, 140–154.
26. J. M. Villagordo and H. Heimgartner (1993). Synthesis of a novel heterospirocyclic 3-(*N*-methyl-*N*-phenylamino)2*H*-azirine and its use as an amino acid equivalent in the preparation of a model tripeptide. *Tetrahedron 49*, 7215–7222.
27. G. M. Bonora, C. Mapelli, C. Toniolo, R. R. Wilkening and E. S. Stevens (1984). Conformation analysis of linear peptides: 5. Spectroscopic characterization of β-turns in Aib-containing oligopeptides in chloroform. *Int. J. Biol. Macromol. 6*, 179–188.
28. K. D. Kopple and M. Ohnishi (1969). Conformations of cyclic peptides. IV. Nuclear magnetic resonance studies of cyclopentaglycyl-*L*-leucyl and cyclodigycyl-*L*-histidyldiglycyl-*L*-tyrosyl. *Biochemistry 8*, 4087–4095.

29. D. Martin and H. G. Hauthal, *Dimethyl Sulphoxide*, Van Nostrand-Reinhold, Wokingham, 1975.
30. K. D. Kopple and T. J. Schamper (1972). Proton magnetic resonance line broadening produced by association with a nitroxide radical in studies of amide and peptide conformation. *J. Am. Chem. Soc. 94*, 3644–3646.
31. C. Toniolo, G. M. Bonora, V. Barone, A. Bavoso, E. Benedetti, B. Di Blasio, P. Grimaldi, F. Lelj, V. Pavone and C. Pedone (1985). Conformation of pleionomers of α-aminoisobutyric acid. *Macromolecules 18*, 895–902.
32. M. Crisma, G. M. Bonora, C. Toniolo, V. Barone, E. Benedetti, B. Di Blasio, V. Pavone, C. Pedone, A. Santini, F. Fraternali, A. Bavoso and F. Lelj (1989). Structural versatility of peptides containing $C^{\alpha,\alpha}$-dialkylated glycines: conformational energy computations, i.r. absorption and ^{1}H n.m.r. analysis of 1-aminocyclopropane-1-carboxylic acid homo-peptides. *Int. J. Biol. Macromol. 11*, 345–352.
33. IUPAC-IUB Commission of Biochemical Nomenclature (1970). Abbreviations and symbols for the description of the conformation of polypeptide chains. *Biochemistry 9*, 3471–3479.
34. E. Benedetti, C. Pedone, C. Toniolo, M. Dudek, G. Nemethy and H. A. Scheraga (1983). Preferred conformation of the benzyloxycarbonyl-amino group in peptides. *Int. J. Pept. Protein Res. 21*, 163–181.
35. W. B. Schweizer and J. D. Dunitz (1982). Structural characteristics of the carboxylic ester group. *Helv. Chim. Acta 65*, 1547–1554.
36. E. Benedetti in: *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins*, Vol. 6, B. Weinstein, Ed., p. 105–184, Dekker, New York 1982.
37. T. Ashida, Y. Tsunogae, I. Tanaka and T. Yamane (1987). Peptide chain structure parameters, bond angles and conformational angles from the Cambridge structural database. *Acta Crystallogr. B43*, 212–218.
38. F. H. Allen, O. Kennard, D. G. Watson, L. Brammer, A. G. Orpen and R. Taylor (1987). Tables of bonds lengths determined by X-ray and neutron diffraction. Part 1. Bond lengths in organic compounds. *J. Chem. Soc., Perkin Trans. 2*, S1–S19.
39. C. D. Shirrell and D. E. Williams (1973). The crystal structure of 2,2,4,4-tetramethyl-1,3-cyclobutanedithione. *Acta Crystallogr. B29*, 1648–1653.
40. Y. Paterson, S. M. Rumsey, E. Benedetti, G. Nemethy and H. A. Scheraga (1981). Sensitivity of polypeptide conformation to geometry. Theoretical conformational analysis of oligomers of α-aminoisobutyric acid. *J. Am. Chem. Soc. 103*, 2947–2955.
41. C. Ramakrishnan and N. Prasad (1971). Study of hydrogen bonds in amino acids and peptides. *Int. J. Protein Res. 3*, 209–231.
42. R. Taylor, O. Kennard and W. Versichel (1984). The geometry of the N-H...O=C hydrogen bond. 3. Hydrogen-bond distances and angles. *Acta Crystallogr. B40*, 280–288.
43. C. H. Gorbitz (1989). Hydrogen-bond distances and angles in the structures of amino acids and peptides. *Acta Crystallogr. B45*, 390–395.
44. J. Lubkowski, L. Lankiewicz and Z. Kosturkiewicz (1992). Structure of *N*,*N*-di-*tert*-butyloxycarbonyl-glycine *N*'-methoxy-*N*'-methylamide. *Acta Crystallogr. C48*, 1616–1619.
45. T. Gustafsson and K. Gunnarsson (1993). Crystal structure of N^{α}-di-*tert*-butyloxycarbonyl-*L*-alanine. *Acta Chem. Scand. 47*, 33–37.
46. V. J. Hruby, F. Al-Obeidi and W. Kazmierski (1990). Emerging approaches in the molecular design of receptor-selective peptide ligands: conformational, topographical and dynamic considerations. *Biochem. J. 268*, 249–262.
47. C. Toniolo (1990). Conformationally restricted peptides through short-range cyclizations. *Int. J. Pept. Protein Res. 35*, 287–300.
48. G. Holzemann (1991). Peptide conformation mimetics. *Kontakte (Darmstadt)*, 3–12 (part 1) and 55–63 (part 2).
49. A. Giannis and T. Kolter (1993). Peptidomimetics for receptor ligands. Discovery, development, and medical perspectives. *Angew. Chem. Int. Ed. Engl. 32*, 1244–1267.
50. A. E. P. Adang, P. H. H. Hermkens, J. T. M. Linders, H. C. J. Ottenheijm and C. J. van Staveren (1994). Case histories of peptidomimetics: progression from peptides to drugs. *Recl. Trav. Chim. Pays-Bas 113*, 63–78.
51. J. Gante (1994). Peptidomimetics: taylored enzyme inhibitors. *Angew. Chem. Int. Ed. Engl. 33*, 1699–1720.
52. R. M. J. Liskamp (1994). Conformationally restricted amino acids and dipeptides, (non)peptidomimetics and secondary structure mimetics. *Recl. Trav. Chim. Pays-Bas 113*, 1–19.
53. E. L. Becker, F. Formaggio, M. Crisma and C. Toniolo, to be published.
54. V. W. Cornish, D. Mendel and P. G. Schultz (1995). Probing protein structure and function with an expanded genetic code. *Angew. Chem. Int. Ed. Engl. 34*, 621–633.

