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Conformational Characterization of Peptides Rich in the Cycloaliphatic $C^{\alpha,\alpha}$-disubstituted Glycine 1-Amino-cyclononane-1-carboxylic Acid

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Abstract: A series of N- and C-protected, monodispersed homo-oligopeptides (to the pentamer level) from the cycloaliphatic $C^{\alpha,\alpha}$-dialkylated glycine 1-aminocyclononane-1-carboxylic acid (Ac_9c) and two Ala/Ac_9c tripeptides have been 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 mClAc-Ac_9c-OH and Z-Ac_9c-OtBu, the dipeptide *p*BrBz-$(Ac_9c)_2$-OtBu, the tetrapeptide Z-$(Ac_9c)_4$-OtBu, and the pentapeptide Z-($Ac_9c)_5$-OtBu were determined in the crystal state by X-ray diffraction. Based on this information, the average geometry and the preferred conformation for the cyclononyl moiety of the Ac_9c residue have been assessed. The backbone conformational data are strongly in favour of the conclusion that the Ac_9c residue is a strong β-turn and helix former. A comparison with the structural propensity 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 $n=3$–8) is made and the implications for the use of the Ac_9c residue in conformationally constrained analogues of bioactive peptides are briefly examined. © European Peptide Society and John Wiley & Sons, Ltd.

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INTRODUCTION

The exploitation of $C^{\alpha,\alpha}$-disubstituted glycines in the synthesis of peptides with restricted conformational flexibility has recently acquired increasing importance in the design of analogues of bioactive compounds [1–5]. Among these α-amino acids the cycloaliphatic Ac_nc ($n=3$–8) residues proved to be valuable in the preparation of conformationally constrained peptide backbones [3–7]. In particular, the preferred conformations, regular type III(III′)

Abbreviations: Ac_nc, 1-aminocycloalkane-1-carboxylic acid; Ac_9c, 1-aminocyclononane-1-carboxylic acid; Aib, α-aminoisobutyric acid or $C^{\alpha,\alpha}$-dimethylglycine; mClAc, monochloroacetyl; *p*BrBz, *para*-bromobenzoyl; TEMPO, 2,2,6,6-tetramethylpiperidinyl-l-oxy.

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β-turns [8–10] and 3_{10}-helices [11], theoretically predicted and experimentally found for the medium-ring Ac_5c, Ac_6c, Ac_7c and Ac_8c residues, closely parallel those of Aib, the prototype of $C^{\alpha,\alpha}$-disubstituted glycines.

The present conformational study of Ac_9c in model peptides was performed to expand the known picture of the geometrical and structural propensities of the family of Ac_nc residues. In this work we describe the synthesis, characterization and solution (FT-IR absorption and ^{1}H-NMR) conformational analysis of the Ac_9c homo-oligomers Z(Ac_9c)$_n$-OtBu (n=–5) and the tripeptides Z-Ac_9c-(L-Ala)$_2$-OMe and Z-L-Ala-Ac_9c-L-Ala-OMe. The X-ray diffraction structures of the derivatives mClAc-Ac_9c-OH and Z-Ac_9c-OtBu, the dipeptide *p*BrBz-(Ac_9c)$_2$-OtBu, the tetrapeptide Z-(Ac_9c)$_4$-OtBu and the pentapeptide Z-(Ac_9c)$_5$-OtBu are also discussed.

Only a very limited information is available on conformation and biological activity of Ac_9c, and its derivatives and peptides. The crystal structure of the symmetrical anhydride (Z-Ac_9c)$_2$O has been reported [12]. The tripeptide HCO-L-Met- Ac_9c-L-Phe-OMe exhibits a remarkable activity in human neutrophil chemotaxis, in the release of neutrophil granule enzymes, and in superoxide anion production [13]. The free amino acid itself is bitter [14]. Preliminary accounts of a limited part of this work have been reported [15, 16].

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_9c peptides were determined using a C. Erba model 3A 30 amino acid analyser (Rodano, Milan, Italy). Elution of Ac_9c was observed well after the Phe peak, its colour yield with ninhydrin being about 7% that of Ala.

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, at 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

Colourless single crystals of the amino acid derivatives mClAc-Ac_9c-OH and Z-Ac_9c-OtBu, the dipeptide *p*BrBz-(Ac_9c)$_2$-OtBu, the tetrapeptide Z-(Ac_9c)$_4$-OtBu and the pentapeptide Z-(Ac_9c)$_5$-OtBu were obtained by slow evaporation at room temperature from the solvents reported in Tables 1 and 2. Data collections were performed on a Philips PW1100 four circle diffractometer for the two amino acid derivatives and the dipeptide, while on a CAD4 Enraf-Nonius single X-ray diffractometer of the Centro di Studio di Biocristallografia, CNR, at the University of Naples 'Federico II', for the tetra- and pentapeptides. Unit cell determination was carried out for all crystals by least-square refinement of the setting angles of 25 high angle reflections accurately centred. No significant variation was observed in the intensities of the standard reflections monitored at regular intervals during data collection, thus

Table 1 Crystallographic Data for the Ac$_9$c Derivatives and the Dipeptide

	*m*ClAc-Ac$_9$c-OH	Z-Ac$_9$c-OtBu	*p*BrBz-(Ac$_9$c)$_2$-OtBu
Empirical formula	$C_{12}H_{20}NO_3Cl$	$C_{22}H_{33}NO_4$	$C_{31}H_{47}N_2O_4Br$
Formula weight (a.m.u.)	261.8	375.5	591.6
Crystal system	Orthorhombic	Monoclinic	Moniclinic
Space group	$P2_12_12_1$	$P2_1/a$	$P2_1/c$
a (Å)	31.755(3)	11.288(2)	11.452(2)
b (Å)	7.895(1)	17.595(2)	10.995(2)
c (Å)	5.684(1)	11.476(2)	25.266(2)
α (°)	90	90	90
β (°)	90	97.5(1)	94.3(1)
γ (°)	90	90	90
V (Å^3)	1425(1)	2260(1)	3172(1)
Z (molecules/unit cell)	4	4	4
Density (calc.) (g/cm^3)	1.220	1.104	1.239
Independent reflections	1280	5459	7587
Observed reflections	1193[$F > 3\sigma(F)$]	2070($F > 3\sigma(F)$]	3504[$F > 3\sigma(F)$]
Solved by	SHELX 86 [17]	SHELX 86	SHELX 86
Refined by	SHELX 76 [18]	SHELX 76	SHELX 76
S	1.094	1.496	1.265
R (unweighted)	0.055	0.057	0.055
R (weighted)	0.063	0.061	0.061
w	$1/[\sigma^2(F)+0.0051\ F^2]$	$1/[\sigma^2(F)+0.0075\ F^2]$	$1/[\sigma^2(F)+0.0013\ F^2]$
Temperature (K)	293	293	293
Radiation (λ)	Cu Kα (1.54178 Å)	Mo Kα (0.71073 Å)	Mo Kα (0.71073 Å)
Scan method	$\theta/2\theta$	$\theta/2\theta$	$\theta/2\theta$
θ range (°)	1–60	1–28	1–28
Crystallization solvent	Methanol	Ethyl acetate–petroleum ether	Ethyl acetate–methanol-water
Crystal size (mm)	2.0 × 0.4 × 0.2	0.6 × 0.4 × 0.2	0.5 × 0.3 × 0.2
$\Delta\rho_{max}$ and $\Delta\rho_{min}$	0.535/−0.325	0.16/−0.17	0.45/−0.39

implying electronic and crystal stability. Lorentz and polarization corrections were applied to the intensities, but no absorption corrections were made. Crystal data are listed in Tables 1 and 2.

The structures of the two amino acid derivatives and the dipeptide were solved by direct methods (SHELX 86) [17] and refined by the full-matrix blocked least-square procedure (SHELX 76) [18] with all non-hydrogen atoms anisotropic. Most of the hydrogen atoms of the two derivatives were located on a ΔF map and the remaining ones were calculated (they were all isotropically refined for mCl-Ac-Ac$_9$c-OH, whereas they were not refined for Z-Ac$_9$c-OtBu). The hydrogen atoms of the two cyclononane rings of the dipeptide were calculated and allowed to ride during the refinement on their carrying atoms with a fixed isotropic thermal factor. The remaining hydrogen atoms were in part located on a ΔF map and in part calculated, and not refined.

The structures of the tetra- and pentapeptides were solved by direct methods, using the SIR 92 program [19]. The solution with the best figure of merit revealed the coordinates of most of the non-hydrogen atoms; the remaining ones and the statistical atoms for the first ring of the tetrapeptide molecule were recovered using ΔF techniques. As for the refinement, the SDP (structure determination programs) package [20] and a full-matrix least-square procedure were used, minimizing the quantity $\Sigma w\ (F_o - F_c)^2$, with a weight w equal to $1/\sigma(F_o^2)$, and also refining the occupancy factors of ring atoms in the tetrapeptide molecule. 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 the isotropic thermal factor of the corresponding carrier atom, but not refined.

Table 2 Crystallographic Data for the Ac_9c Tetra- and Pentapeptides

	Z-$(Ac_9c)_4$-OtBu	Z-$(Ac_9c)_5$-OtBu
Empirical formula	$C_{52}H_{85}N_4O_7$	$C_{62}H_{101}N_5O_8$
Formula weight (a.m.u.)	878.3	1044.5
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/c$	$P2_1/c$
a (Å)	12.444(2)	11.589(2)
b Å)	21.946(4)	24.186(7)
c Å)	19.681(3)	21.958(7)
β (°)	104.2(2)	90.53(1)
V (Å^3)	5210(1)	6154(3)
Z (molecules/unit cell)	4	4
Density (calc.)(g/cm^3)	1.120	1.127
Independent reflections	9856	11686
Observed reflections	4809[$I > 4\sigma(I)$]	5147[$I > 4\sigma(I)$]
Solved by	SIR92 [19]	SIR92
Refined by	SDP [20]	SDP
S	2.331	2.688
R (unweighted)	0.080	0.081
R (weighted)	0.081	0.080
w	$1/\sigma(F^2)$	$1/\sigma(F^2)$
Temperature (K)	293	293
Radiation (λ, Å)	Cu Kα(1.54178)	Cu Kα(1.54178)
Scan method	$\omega/2\theta$	$\omega/2\theta$
θ range (°)	1–70	1–70
Crystallization solvent	Chloroform–ethanol	Chloroform–ethanol
Crystal size (mm)	0.3 × 0.4 × 0.2	0.3 × 0.4 × 0.5
$\Delta\rho_{max}$ and $\Delta\rho_{min}$	0.517/−0.067	0.534/−0.630

RESULTS

Synthesis of Ac_9c and its Derivatives and Peptides

Ac_9c amide hydrochloride was prepared by treatment of cyclononanone with sodium cyanide, acetic acid, excess of ammonia and subsequent acid hydrolysis (HCl/HCOOH at 0–20 °C) of the α-amino nitrile intermediate (Strecker synthesis). Acid hydrolysis (6N HCl, under reflux) of Ac_9c amide hydrochloride afforded the free amino acid [14].

The Z-protected Ac_9c derivative was obtained by reacting the free amino acid with N-(benzyloxycarbonyloxy)-succinimide. In addition to Z-Ac_9c-OH, treatment of the free amino acid with benzyloxycarbonylchloride gave the 5(4*H*)-oxazolone from Z-Ac_9c-OH. This latter compound was prepared in a higher yield by dehydration of the N^α-protected amino acid with *N*-ethyl, *N*′-(3-dimethylaminopropyl)-carbodiimide (1:1 ratio) in acetonitrile. The same method [but in a 2:1 ratio of N^α-protected amino acid: *N*-ethyl, *N*′-(3-dimethylaminopropyl)-carbodiimide] was used in the synthesis of the symmetrical anhydride from Z-L–Ala-OH. Equimolar amounts of Z-Ac_9c-OH and the 5(4*H*)-oxazolone from Z-Ac_9c-OH in acetonitrile gave the symmetrical anhydride from Z-Ac_9c-OH [12]. The L-Ala methylester hydrochloride was synthesized using the methanol/$SOCl_2$ method. Z-Ac_9c-OtBu was obtained by esterification of the N-protected amino acid with isobutene in the presence of a catalytic amount of sulphuric acid. The monochloro-acetyl-protected Ac_9c derivative was synthesized by treatment of the free amino acid with monochloro-acetylchloride in aqueous solution at alkaline pH. The 5(4*H*)-oxazolone from *p*BrBz-Ac_9c-OH was prepared by reacting the free amino acid with *para*-bromobenzoylchloride in pyridine.

L-Ala-L-Ala, L-Ala-Ac_9c and Ac_9c-Ac_9c (the latter in the Z-protected dimer, trimer, tetramer and pentamer) peptide bond formation was achieved by the symmetrical anhydride method. On the other hand, Ac_9c-L-Ala and Ac_9c-Ac_9 (the latter in the *p*BrBz-blocked dimer) peptide bond was obtained using the 5(4*H*)-oxazolone method. Removal of the Z-group was performed by catalytic hydrogenation. The physical properties and analytical data of Ac_9c, and its derivatives and peptides are listed in Table 3.

Table 3 Physical and Analytical Properties for Ac_9c, its Derivatives and Peptides

Compound	Melting point (°C)	Recryst. solvent[a]	$[\alpha]_D^{20}$ (deg)[b]	TLC R_{FI}	TLC R_{FII}	TLC R_{FIII}	IR(cm^{-1})[c]	Amino acid analysis
H-Ac_9c-OH	285–287	Hot H_2O	–	0.10	0.70	0.05	3463, 1647	–
HCl · H-Ac_9c-NH_2	272–273	MeOH/DE	–	0.20	0.65	0.10	3397,3360,1685,1587	–
*m*ClAc-Ac_9c-OH	199–200	AcOEt/PE	–	0.95	0.80	0.35	3318,1704,1650,1552	–
Z-Ac_9c-OH	152–154	AcOEt/PE	–	0.80	0.90	0.40	3355,1721,1699,1533	–
(Z-Ac_9c)$_2$O[d]	146–147	AcOEt/PE	–	0.95	–	0.80	3407,3355,1813,1749,1715, 1700	–
5(4*H*)-oxazolone from Z-Ac_9c-OH	Oil	AcOEt/PE	–	0.95	–	0.95	1827,1684	–
5(4*H*)-oxazolone from *p*BrBz-Ac_9c-OH	207–209	AcOEt/PE	–	0.95	–	0.95	1808,1649	–
Z-Ac_9c-OtBu	121–122	AcOEt/PE	–	0.95	0.95	0.85	3358,1714,1521	–
*p*BrBz-(Ac_9c)$_2$-OtBu	219–220	AcOEt/PE	–	0.95	0.95	0.65	3439,3298,1729,1667,1589, 1531	–
Z-(Ac_9c))$_2$-OtBu	181–182	AcOEt/PE	–	0.95	0.95	0.70	3401,3303,1718,1653,1531	–
Z-(Ac_9c)$_3$-OtBu	217–218	Hot AcOEt	–	0.95	0.95	0.55	3413,3317,1703,1638,1524	–
Z-(Ac_9c)$_4$-OtBu	262–263	CH_2Cl_2/PE	–	0.95	0.95	0.45	3423,3350,1725,1704,1672, 1522	–
Z(Ac_9c)$_5$-OtBu	298–300	$CHCl_3$/EtOH/PE	–	0.95	0.90	0.35	3443,3243,1716,1696,1666, 1642,1535	–
Z-Ac_9c-L-Ala-OMe	135–136	AcOEt/PE	−27.7	0.95	0.95	0.50	3314,1745,1693,1652,1530	–
Z-L-Ala-Ac_9c-L-Ala-OMe	168-169	AcOEt/PE	−50.0	0.90	0.90	0.40	3378,3286,1746,1702,1676, 1644,1537	Ala 1.92; Ac_9c 1.10
Z-Ac_9c-(L-Ala)$_2$-OMe	127–128	AcOEt/PE	−29.6	0.85	0.90	0.40	3320,1742,1703,1656,1530	Ala 1.91; Ac_9c 1.08

[a] MeOH, methanol; DE, diethyl ether; AcOEt, ethyl acetate; PE, petroleum ether; EtOH, ethanol.
[b] $c = 0.5$, methanol.
[c] The IR absorption spectra were obtained in KBr pellets (only significant bands in the 3500–3200 and 1850–1520 cm^{-1} regions are reported).
[d] Ref. [12].

Solution Conformational Analysis

The preferred conformation adopted by the Ac_9c-rich peptides in solution was determined in a solvent of low polarity ($CDCl_3$) by FT-IR absorption and ^1H-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 Z-protected Ac_9c homo-peptide series (from monomer to pentamer) at 1 mM concentration. The curves of the tripeptide and the higher oligomers are characterized by two bands, at about 3425 cm^{-1} (free, solvated NH groups) and 3371–3348 cm^{-1} (H-bonded NH groups), respectively [21]. 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 of the low-frequency band shifts significantly to lower wavenumbers. An inspection of the spectrum of the homo-tripeptide, compared with those of the Ac_9c/Ala tripeptides Z-Ac_9c-(L-Ala)$_2$-OMe and Z-L-Ala-Ac_9c-L-Ala-OMe (Figure 2), leads to the conclusion that the 3375–3351 cm^{-1} band is much higher (relative to the 3431–3423 cm^{-1} band) in the homo-tripeptide. In addition, the low frequency band is higher when Ac_9c is located at position 1 than at position 2 (in the Ac_9c/Ala tripeptides). We have also been able to demonstrate that, even at 10 mM concentration, there are only minor changes in the spectra of the peptides to the tetramer level in the 3500–3350 cm^{-1} region (not shown). Therefore, in those peptides the observed H-bonding band at 3375–3351 cm^{-1} should be interpreted as arising almost exclusively from intramolecular N–H···O=C interactions. However, in the homo-pentamer a remarkable variation in the spectrum is noted at 10 mM concentration (Figure 3). Bands at 3302, 3250 and 3230 cm^{-1}, related to intermolecular N–H···O=C H-bonds, stand out clearly.

The present FT-IR absorption investigation has provided convincing evidence that intramolecular H-bonding that is dependent on main-chain length is an essential factor influencing the conformation of the terminally blocked, non-associated Ac_9c homo-peptides in $CDCl_3$ solution. The findings also support the view that Ac_9c is a better inducer of intramolecularly H-bonded structures than Ala.

The delineation of inaccessible (or intramolecularly H-bonded) NH groups of the Ac_9c peptides by ^1H-NMR was carried out using: (i) solvent dependence of NH chemical shifts, by adding increasing amounts of the strong H-bonding acceptor solvent

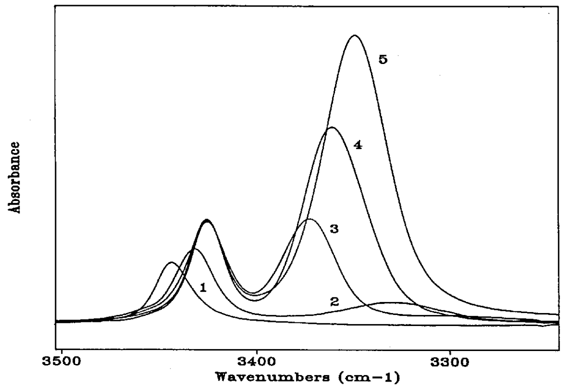

Figure 1 FT-IR absorption spectra (N–H stretching region) of the homo-peptide series Z-$(Ac_9c)_n$-OtBu (n=1–5) in $CDCl_3$ solution (peptide concentration 1 mM).

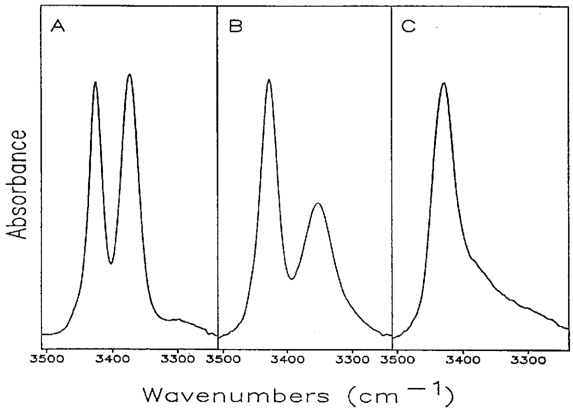

Figure 2 FT-IR absorption spectra (N–H stretching region) of Z-$(Ac_9c)_3$-OtBu (A), Z-Ac_9c-(L-Ala)$_2$-OMe (B) and Z-L-Ala-Ac_9c-L-Ala-OMe (C) in $CDCl_3$ solution (peptide concentration 1 mM).

DMSO ([22, 23] to the $CDCl_3$ solution and (ii) free-radical (TEMPO) induced line broadening of NH resonances [24]. As a typical example, Figure 4 illustrates the behaviour of the NH resonances of the pentamer upon addition of DMSO and TEMPO. The upfield resonance in $CDCl_3$ solution is unequivocally assigned to the N(1)H *urethane* group [21]. 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 peptides from different types of $C^{\alpha,\alpha}$-dialkylated glycines [21, 25, 26]. From an analysis of the spectra as a function of concentration (5–1 mM) in $CDCl_3$ solution (results not shown), we have been able to conclude that dilution induces a negligible

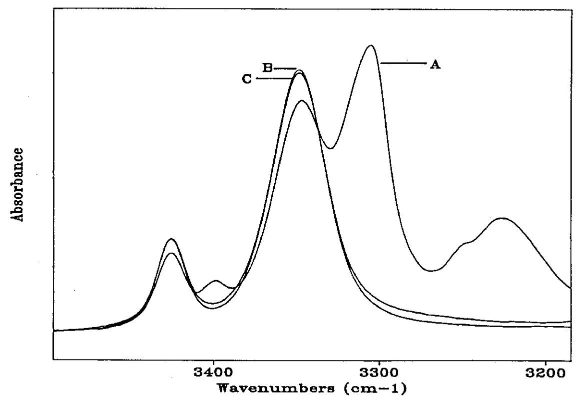

Figure 3 Peptide concentration effect on the FT-IR absorption spectrum (N–H stretching region) of the homo-pentapeptide Z-(Ac_9c)-OtBu in $CDCl_3$ solution: 5 mM (A), 1 mM (B) and 0.1 mM (C).

shift to higher fields of the NH resonances of all the peptides investigated. In the Ac_9c peptides examined in the $CDCl_3$–DMSO solvent mixtures and in the presence of the paramagnetic perturbing agent TEMPO 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(5)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 $CDCl_3$ solution at a concentration lower than 5 mM, the N(3)H to N(5)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 [3–7], it is reasonable to conclude that the most populated structures adopted in $CDCl_3$ solution by the Ac_9c-containing terminally blocked tripeptides, and the Ac_9c homo-tetra- and pentapeptides 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

The molecular and crystal structures of the following Ac_9c derivatives and peptides were determined by

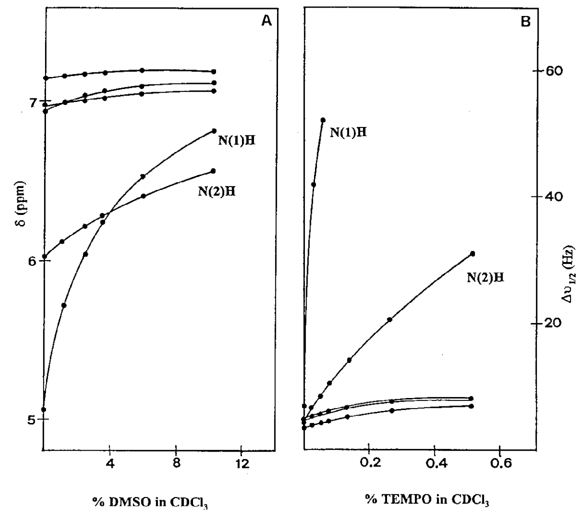

Figure 4 (A) Plot of NH chemical shifts in the ^{1}H-NMR spectrum of Z-(Ac_9c)$_5$-OtBu as a function of increasing percentages of DMSO added to the $CDCl_3$ solution (v/v). (B) Plot of bandwidth of the NH signals of the same peptide as a function of increasing percentages of TEMPO (w/v) in $CDCl_3$. Peptide concentration 1 mM.

X-ray diffraction: mClAc-Ac_9c-OH, Z-Ac_9c-OtBu, *p*BrBz-(Ac_9c)$_2$-OtBu, Z-(Ac_9c)$_4$-OtBu and Z-(Ac_9c)$_5$-OtBu. The molecular structures with the atomic numbering schemes are illustrated in Figures 5–9, respectively. Relevant N^{α}-protecting group, backbone and side-chain torsion angles [27] 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 nine-membered ring system of the Ac_9c 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 [28], monochloroacetamido [29], *para*-bromobenzamido [30] and ester [31] groups, and the peptide unit [32, 33]. The average geometry for the Ac_9c residue has also been calculated. All the parameters are close to those reported in the literature for cyclononylamine hydrobromide [34–37]. In particular, the average C–C bond length for the cyclononane ring is 1.53 Å (with the longest average length of 1.54 Å for the C^{α}–C^{β} bonds and the shortest average length of 1.51 Å for the $C^{\varepsilon 1}$–$C^{\varepsilon 2}$ bond), 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 based on protein amino acids [32]. The average value for the bond angles

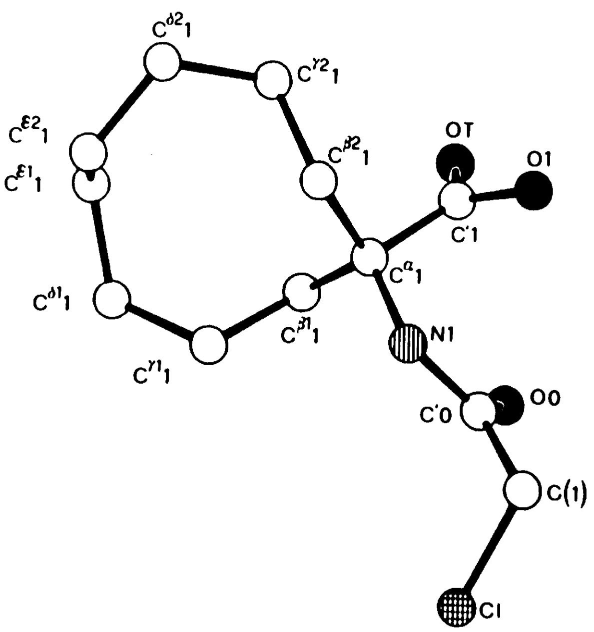

Figure 5 X-ray diffraction structure of mClAc-Ac$_9$c-OH with the atoms numbered.

internal to the nine-membered ring is 115.2°, definitely larger than the regular tetrahedral value (109.5°). However, seven of such bond angles are in the range 114.0–115.1°, while the bond angles at the two C^{δ} atoms are more significantly expanded (117.4° and 117.8°). In addition, the bond angles indicate an asymmetric geometry for the C^{α} atom. More specifically, the bond angles involving the $C^{\beta1}$ atom are narrower than those involving the $C^{\beta2}$ atom. This observation is common also to Aib- and Ac$_n$c-rich (n = 3–8) peptides [3, 5–7]. The value for the conformationally sensitive N–C^{α}–C′ (τ) bond angle, external to the cyclic system, is 110.0(4)°, comparable to that exhibited by the $C^{\alpha,\alpha}$-dialkylated glycines forming regular helices (110–111°) [3, 5, 6, 39].

All the Ac$_9$c residues are found in the helical region A (A*) of the conformational map [40], with the exception of the C-terminal residue of the dipeptide which is semi-extended. Each of the five compounds, having no chiral atoms, crystallizes with retention of the centre of symmetry; thus, in each unit cell, molecules of both handedness simultaneously occur. The average values for the ϕ, ψ backbone torsion angles of the Ac$_9$c residue completely involved in a helical structure are ±53.9°, ±32.4°, close to those expected for a 3_{10} helix (±57°, ±30°) [11]. Also the C-terminal Ac$_9$c residues of the homo-tetra- and pentapeptides adopt a conformation in the helical region, but they have an handedness opposite to that exhibited by the preceding residues, a common observation for Aib- and Ac$_n$c-rich (n = 3–8) peptides [3, 5].

The 1–3 sequence of the Ac$_9$c 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 [41–43]. The backbone of the homo-pentamer is folded in a regular right(left)-handed 3_{10}-helix.

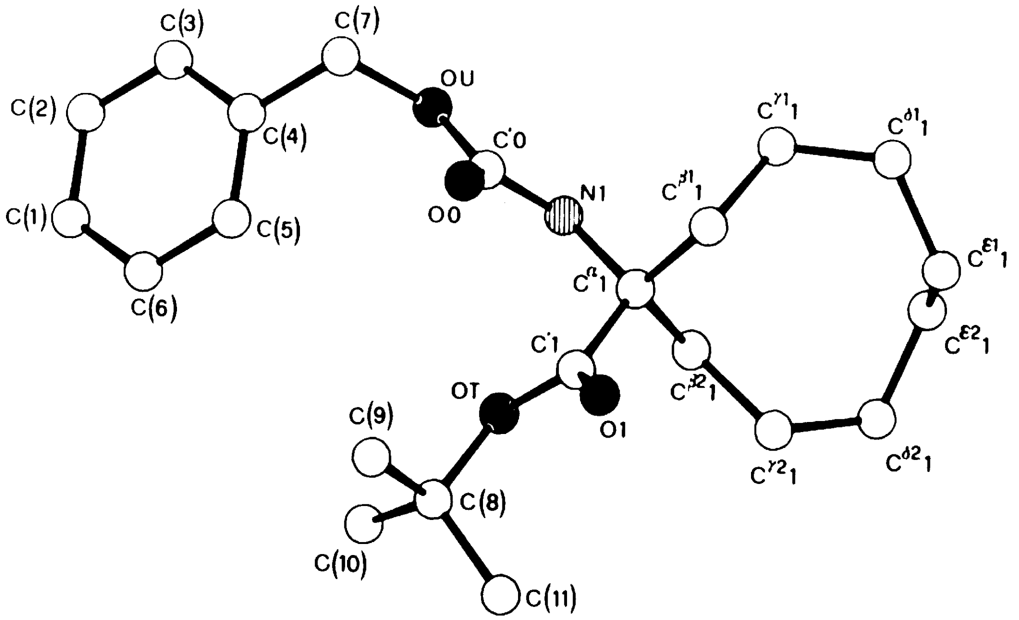

Figure 6 X-ray diffraction structure of Z-Ac$_9$c-OtBu with the atoms numbered.

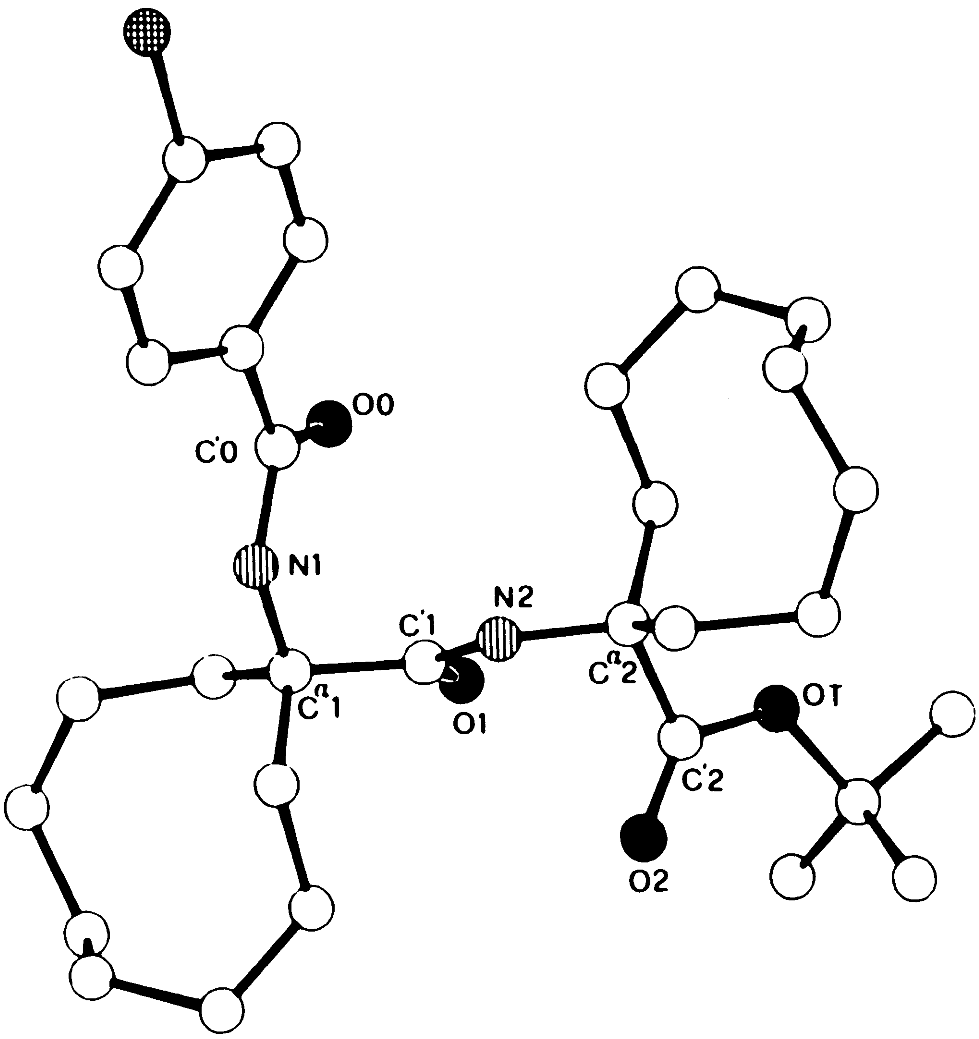

Figure 7 X-ray diffraction structure of *p*BrBz-$(Ac_9c)_2$-OtBu with the atoms numbered (for clarity only the backbone atoms are labelled).

Peptide groups N_3–H to N_5–H and $C'_0{=}O_0$ to $C'_2{=}O_2$ participate in three consecutive $1 \leftarrow 4$ $C{=}O \cdots H$-N intramolecular H-bonds.

In the five compounds, few significant deviations of the ω torsion angles ($|\Delta\omega| > 10°$) from the ideal value of the *trans* planar urethane, amide, peptide and ester units (180°) are observed. In particular, the C-terminal ester ω torsion angles for the homo-di- and tetrapeptides differ by about 10.5° and 13.5°, respectively, from the *trans* planar value. The *trans*-arrangement of the θ^1 torsion angle of the benzyloxycarbonylamino moiety, found for all the three Z-protected-Ac_9c derivatives and peptides investigated, is that commonly observed for Z-amino acids and peptides [28]. Not surprisingly [28], the values of θ^2 are concentrated in the regions of $\pm 90°$. The concomitant electrostatic repulsions of the chlorine atom with the O_0 and N_1 atoms of mClAc-Ac_9c-OH preclude the formation of a favourable $Cl \cdots H$–N_1 interaction (a 'C5' form) [9, 29], the resulting θ^1 torsion angle being close to 100°. In the *p*BrBz-blocked dipeptide the deviation of the plane of the *para*-bromophenyl moiety from that of the neighbouring amide group is about 27° The *tert*-butyl ester conformation with respect to the preceding C^α–N bond is intermediate between the *synplanar* and *synclinal* conformations in the homo-dimer, while intermediate between the *anticlinal* and *antiplanar* conformations in the monomer, homo-tetramer and homo-pentamer [44].

In each Ac_9c residue the side-chain χ torsion angles have values of about $\pm 60°$ (five angles), $+120°$ (three angles) and 180° (one angle). In a right-handed residue (with negative ϕ, ψ torsion

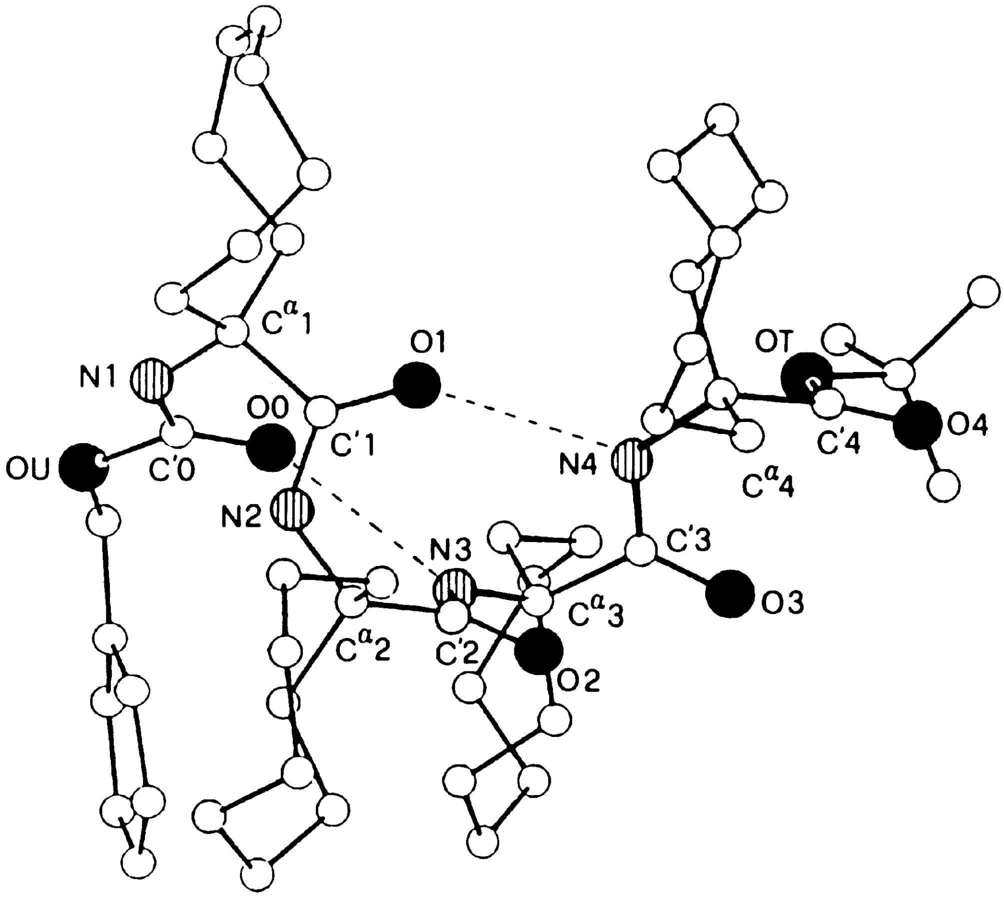

Figure 8 X-ray diffraction structure of Z-$(Ac_9c)_4$-OtBu with the atoms numbered (for clarity only the backbone atoms are labelled). The two intramolecular H-bonds are represented by dashed lines.

angles) four out of the five torsion angles with $\chi = 60°$ have negative values and all the three torsion angles with $\chi = 120°$ have positive values. The opposite is true for a left-handed residue. In particular, the side-chain $\chi^{1,1}$ and $\chi^{1,2}$ torsion angles, giving the disposition of the backbone nitrogen atom relative to the ring C^{γ} atoms, are about 180°, +60° for a right-handed helical residue, and 180°, −60° for a left-handed helical residue. This situation closely resembles that of one of the two independent molecules of cyclononylamine hydrobromide and is at variance with either the equatorial (180, 180°) or the axial (+60, −60°) disposition of cyclohexane [34–37]. An additional point of interest is the double occurrence in each Ac_9c moiety of two consecutive χ torsion angles with 60° and the *same* absolute value, again at variance with cyclohexane where the χ torsion angles of 60° about consecutive bonds always exhibit *alternate* signs [34–37]. In the cyclononane ring this arrangement is responsible for the larger separation between carbon atoms at relative positions 1:5, concomitantly offering enough space to the four additional carbon atoms to complete the cyclic structure. The low-energy forms of nine-membered rings have been analysed by several authors [45–53].

Each of the 13 nine-membered rings is found in approximately the twist-boat-chair (TBC) conformation, although a substantial degree of distortion from this conformation is observed. The TBC conformation, with D_3 symmetry, is that theoretically predicted as the minimum energy conformation for a cyclononane ring [50, 51]. The only exception is found for the first residue of the Z-$(Ac_9c)_4$-OtBu molecule in which a statistical population in the positions of the $C^{\delta 1}$ and $C^{\varepsilon 1}$ atoms occurs. For this residue the first conformation with an occupancy factor of 60% is of the TBC type, while the second conformation with an occupancy factor of 40% cannot be classified in any of the symmetrical conformations reported for cyclononane [50,51]. From an analysis of the experimental data it appears

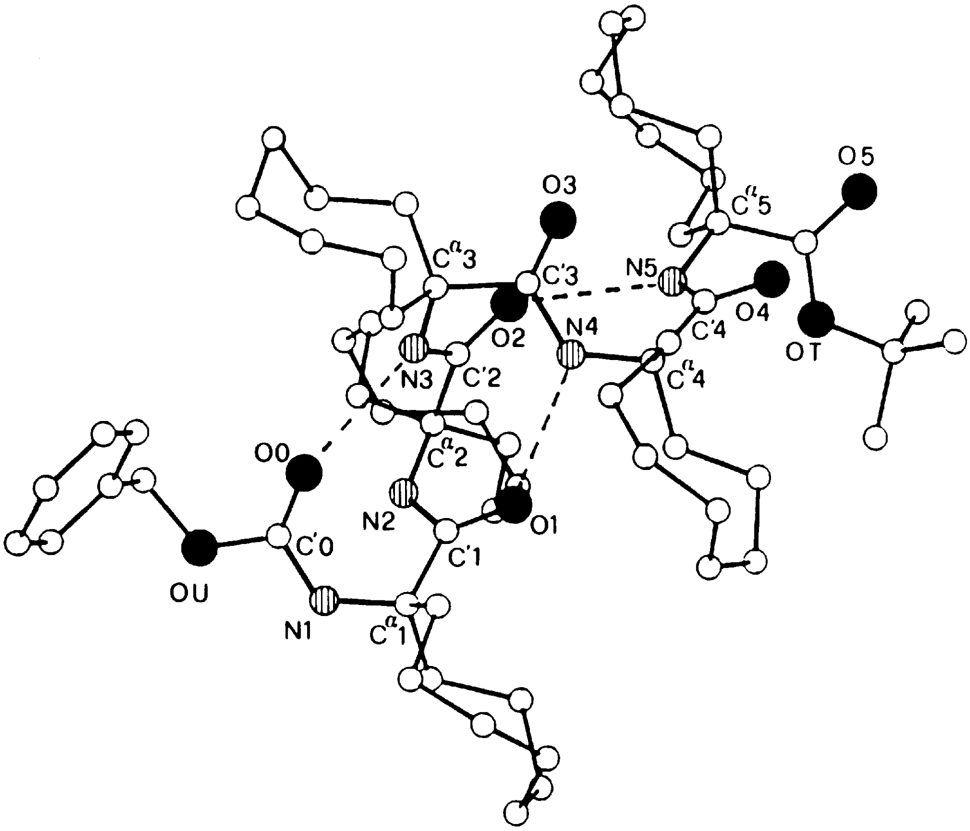

Figure 9 X-ray diffraction structure of Z-$(Ac_9c)_5$-OtBu with the atoms numbered (for clarity only the backbone atoms are labelled). The three intramolecular H-bonds are represented by dashed lines.

that the residues in the TBC conformation on the average present the torsion angles $\chi^5 = 123.7°$, $\chi^{4,1} = -56.3°$, $\chi^{4,2} = -57.3°$, $\chi^{3,1} = -55.1°$, $\chi^{3,2} = -53.5°$, $\chi^{2,1} = 126.3°$, $\chi^{2,2} = 123.3°$, $\delta^{1,1} = -56.2°$ and $\delta^{1,2} = -57.6°$ if occurring in the right-handed backbone conformation (or the oppositely signed values for a left-handed residue). These values are in good agreement with those calculated for a TBC conformation: $\chi^5 = 123.4°$, $\chi^{4,1} = -56.2°$, $\chi^{4,2} = -56.1°$, $\chi^{3,1} = -56.1$, $\chi^{3,2} = -56.1$, $\chi^{2,1} = 125.3°$, $\chi^{2,2} = 125.4°$, $\delta^{1,1} = -56.1°$ and $\delta^{1,2} = -56.2°$ [51]. In addition, it is worth noting that for all residues the $\chi^{1,1}$ and $\chi^{1,2}$ side-chain torsion angles are in the (*t*, g^+) and (*t*, g^-) conformations for right-handed and left-handed Ac_9c residues, respectively.

The packing mode of the mClAc-Ac_9c-OH molecules is characterized by (carboxylic acid) O_T-H···O_0=C'_0 (amide) intermolecular H-bonds, forming rows along the *b* direction and by (amide) N_1–H···O_1=C'_1 (carboxylic acid) intermolecular H-bonds forming rows along the *c* direction. The geometrical parameters for the N–H···O and O–H···O intermolecular H-bonds observed in the examined structures are in the ranges expected for such interactions [41–43, 54, 55]. In the crystal packing the Z–Ac_9c–OtBu molecules are linked through (urethane) N_1-H···O_1=C'_1 (ester) intermolecular H-bonds producing rows of molecules along the *a* direction. The *p*BrBz-$(Ac_9c)_2$-OtBu molecules pack together in the unit cell via (amide) N_1–H···O_1=C'_1 (peptide) intermolecular H-bonds, running in the *b* direction.

The Z–$(Ac_9c)_4$–OtBu molecules pack together along the *c* direction, producing rows of molecules stabilized by (urethane) N–H···O=C (peptide) intermolecular H-bonds [N_1–H···O_3=C'_3]. Then, hydrophobic interactions link together rows of peptide molecules running in the *a* and *b* directions. In the unit cell the Z–$(Ac_9c)_5$–OtBu molecules are held together along the *a* direction in rows stabilized by (urethane) N–H···O=C (peptide) intermolecular H-bonds [N_1–H···O_4=C'_4]. Figure 10 shows the triangular shape of the 3_{10}-helix and the overlapping of the cyclononyl rings of residues 1 to 4, and of residues 2 to 5, each pair of residues being separated by a complete turn of the helical structure. In addition, the crystal structure is stabilized by van der Waals interactions between the hydrophobic groups in the *bc* plane.

CONCLUSIONS

The solution and crystal-state data reported in this work clearly indicate that the medium-ring cycloaliphatic Ac_9c residue can explore only a limited region of the conformational space and has a relevant intrinsic propensity to adopt ϕ, ψ backbone torsion angles typical of $3_{10}/\alpha$-helices. Therefore, the Ac_9c residue can be easily accommodated 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. However, ϕ, ψ torsion angles corresponding to position $i+1$ of type II(II′) β-turn are not available to Ac_9c.

Recently, considerable attention has been focused on the design of conformationally restricted biologically active peptides [56–62]. In this connection, the cycloaliphatic $C^{\alpha,\alpha}$-disubstituted glycines Ac_nc (with $n=3$–9) examined to date have increasing effective volume and hydrophobicity, but they possess a strictly comparable conformational preference. It seems reasonable to foresee that future studies on analogues of biologi-

Table 4 Selected N^{α}-Protecting Group, Backbone and Side-chain Torsion Angles (°) for the Ac_9c Derivatives and Peptides

Torsion angle	mClAc-Ac_9c-OH	Z-Ac_9c-OtBu	*p*BrBz-$(Ac_9c)_2$-OtBu	Z-$(Ac_9c)_4$-OtBu	Z-$(Ac_9c)_5$-OtBu
θ^1	99.2(3)	−175.8(3)		179.8(5)	−172.2(5)
θ^2		102.1(4)		93.9(7)	−92.6(7)
$\theta^{3,1}$		120.0(5)		167.8(7)	106.4(8)
$\theta^{3,2}$		−59.7(6)		−20.2(10)	−77.1(8)
ω_0	175.6(3)	179.7(3)	178.7(4)	−174.4(4)	176.4(5)
ϕ_1	−49.4(4)	−47.2(5)	−51.7(6)	−57.9(6)	−52.6(7)
ψ_1	−42.1(3)	−48.0(4)	−48.6(5)	−35.4(6)	−29.5(7)
ω_1		−176.0(3)	179.2(4)	−174.1(4)	178.5(5)
ϕ_2			54.2(5)	−52.2(7)	−50.3(7)
ψ_2			−149.6(4)	−31.9(6)	−30.1(7)
ω_2			−169.5(4)	−175.0(4)	179.9(5)
ϕ_3				−51.3(6)	−52.8(9)
ψ_3				−43.5(6)	−28.9(7)
ω_3				179.1(4)	−176.4(5)
ϕ_4				40.6(6)	−60.3(7)
ψ_4				53.4(5)	−27.2(7)
ω_4				177.1(4)	178.3(5)
ϕ_5					46.7(8)
ψ_5					50.9(8)
ω_5					166.4(6)
$\chi1^{1,1}$	−178.9(3)	−178.3(3)	178.6(4)	176.3(5)	−178.7(5)
$\chi1^{2,1}$	126.2(5)	128.1(5)	132.7(4)	122.1(6) [48.4(16)][a]	127.6(7)
$\chi1^{3,1}$	−57.9(8)	−54.5(7)	−60.1(6)	−48.9(10) [80.8(19)][a]	−58.1(9)
$\chi1^{4,1}$	−53.8(9)	−57.3(9)	−51.6(7)	−62.9(12) [−106.8(17)][a]	−54.8(9)
$\chi1^{5}$	123.0(7)	125.5(7)	122.6(6)	128.2(9) [65.6(23)][a]	125.6(8)
$\chi1^{4,2}$	−52.2(8)	−58.6(9)	−57.3(8)	−68.5(12) [−26.7(21)][a]	−54.5(11)
$\chi1^{3,2}$	−59.3(6)	−52.9(8)	−56.1(7)	−41.4(11)	−58.7(10)
$\chi1^{2,2}$	128.4(4)	124.7(5)	124.8(5)	116.1(7)	125.5(7)
$\chi1^{1,2}$	63.4(3)	61.0(5)	65.7(5)	63.1(6)	63.2(7)
$\chi2^{1,1}$			177.8(4)	−177.1(5)	−176.1(5)
$\chi2^{2,1}$			−117.8(6)	125.6(8)	127.6(6)
$\chi2^{3,1}$			41.5(9)	−53.7(12)	−58.5(8)
$\chi2^{4,1}$			68.6(10)	−58.4(12)	−54.2(9)

continued

Table 4 (*continued*) Selected N$^{\alpha}$-Protecting Group, Backbone and Side-chain Torsion Angles (°) for the Ac$_9$c Derivatives and Peptides

Torsion angle	mClAc-Ac$_9$c-OH	Z-Ac$_9$c-OtBu	*p*BrBz-(Ac$_9$c)$_2$-OtBu	Z-(Ac$_9$c)$_4$-OtBu	Z-(Ac$_9$c)$_5$-OtBu
$\chi 2^{5}$			−129.7(8)	126.7(9)	123.9(7)
$\chi 2^{4,2}$			60.6(10)	−57.7(12)	−53.0(9)
$\chi 2^{3,2}$			50.4(9)	−54.7(11)	−58.7(8)
$\chi 2^{2,2}$			−123.6(6)	124.9(7)	126.7(6)
$\chi 2^{1,2}$			−60.8(5)	60.4(7)	60.9(6)
$\chi 3^{1,1}$				−179.1(4)	−174.8(5)
$\chi 3^{2,1}$				124.7(5)	127.5(6)
$\chi 3^{3,1}$				−51.0(8)	−57.5(10)
$\chi 3^{4,1}$				−59.4(9)	−57.0(11)
$\chi 3^{5}$				125.9(7)	125.5(9)
$\chi 3^{4,2}$				−58.3(9)	−55.5(11)
$\chi 3^{3,2}$				−53.7(8)	−57.7(10)
$\chi 3^{2,2}$				125.7(6)	124.6(7)
$\chi 3^{1,2}$				60.6(6)	59.6(7)
$\chi 4^{1,1}$				179.1(4)	−173.0(6)
$\chi 4^{2,1}$				−129.9(6)	125.5(8)
$\chi 4^{3,1}$				57.0(9)	−57.8(12)
$\chi 4^{4,1}$				53.2(12)	−48.9(16)
$\chi 4^{5}$				−116.2(12)	113.2(13)
$\chi 4^{4,2}$				48.8(16)	−67.3(20)
$\chi 4^{3,2}$				59.7(11)	−33.8(20)
$\chi 4^{2,2}$				−116.9(6)	113.1(10)
$\chi 4^{1,2}$				−65.5(6)	52.6(8)
$\chi 5^{1,1}$					178.2(6)
$\chi 5^{2,1}$					−128.1(7)
$\chi 5^{3,1}$					57.4(11)
$\chi 5^{4,1}$					53.6(11)
$\chi 5^{5}$					−122.9(9)
$\chi 5^{4,2}$					53.6(11)
$\chi 5^{3,2}$					59.6(10)
$\chi 5^{2,2}$					−126.9(7)
$\chi 5^{1,2}$					−64.5(7)

[a] The values in parentheses refer to statistically positioned atoms.

Table 5 Intra- and Intermolecular H-bond Parameters for the Ac$_9$c Derivatives and Peptides

Compound	Donor (D)	Acceptor (A)	Symmetry operation	Distance(Å) D···A	Angle (°) D–H···A
mClAc–Ac$_9$c-OH	N_1	O_1	$x, y, 1+z$	2.900(3)	146.3(27)
	O_T	O_0	$-x+1/2, 1/2+y, -z$	2.593(4)	149.2(11)
Z-Ac$_9$c-OtBu	N_1	O_1	$-1/2+x, -3/2-y, -z$	2.962(3)	152.8(2)
*p*BrBz-(Ac$_9$c)$_2$-OtBu	N_1	O_1	$-1-x, y+1/2, -1/2-z$	2.943(4)	160.5(3)
Z-(Ac$_9$c)$_4$-OtBu	N_3	O_0	x, y, z	3.085(6)	159.2(3)
	N_4	O_1	x, y, z	3.125(5)	140.7(2)
	N_1	O_3	$x, -y+1/3, z+1/2$	2.854(5)	170.3(3)
Z-(Ac$_9$c)$_5$-OtBu	N_3	O_0	x, y, z	2.987(6)	165.6(3)
	N_4	O_1	x, y, z	2.924(6)	163.8(3)
	N_5	O_2	x, y, z	3.005(6)	153.7(3)
	N_1	O_4	$x+1, y, z$	2.798(6)	154.5(3)

Table 6 Average Bond Distances and Bond Angles for the Ac_9c Residue

Bond distance (Å)		Bond angle (°)	
N–C^{α}	1.472(5)	N–C^{α}–C′	110.0(4)
C^{α}–C′	1.539(5)	$C^{\beta 1}$–C^{α}–$C^{\beta 2}$	114.0(4)
C′–O	1.230(5)	C^{α}–$C^{\beta 1}$–$C^{\gamma 1}$	114.5(5)
C^{α}–$C^{\beta 1}$	1.545(5)	$C^{\beta 1}$–$C^{\gamma 1}$–$C^{\delta 1}$	114.1(5)
$C^{\beta 1}$–$C^{\gamma 1}$	1.542(6)	$C^{\gamma 1}$–$C^{\delta 1}$–$C^{\varepsilon 1}$	117.4(7)
$C^{\gamma 1}$–$C^{\delta 1}$	1.519(8)	$C^{\delta 1}$–$C^{\varepsilon 1}$–$C^{\varepsilon 2}$	114.0(7)
$C^{\delta 1}$–$C^{\varepsilon 1}$	1.52(1)	$C^{\varepsilon 1}$–$C^{\varepsilon 2}$–$C^{\delta 2}$	114.8(8)
$C^{\varepsilon 1}$–$C^{\varepsilon 2}$	1.51(1)	$C^{\varepsilon 2}$–$C^{\delta 2}$–$C^{\gamma 2}$	117.8(6)
$C^{\varepsilon 2}$–$C^{\delta 2}$	1.52(1)	$C^{\delta 2}$–$C^{\gamma 2}$–$C^{\beta 2}$	114.8(6)
$C^{\delta 2}$–$C^{\gamma 2}$	1.534(9)	$C^{\gamma 2}$–$C^{\beta 2}$–C^{α}	115.1(5)
$C^{\gamma 2}$–$C^{\beta 2}$	1.532(6)	N–C^{α}–$C^{\beta 1}$	106.7(4)
$C^{\beta 2}$–C^{α}	1.543(6)	N–C^{α}–$C^{\beta 2}$	109.7(4)
		C′–C^{α}–$C^{\beta 1}$	108.0(4)
		C′–C^{α}–$C^{\beta 2}$	108.9(4)

cally active peptides, incorporating this family of residues at carefully selected positions, will be rewarding.

Acknowledgements

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Final positional parameters and equivalent thermal factors for non-hydrogen atoms for the five 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).

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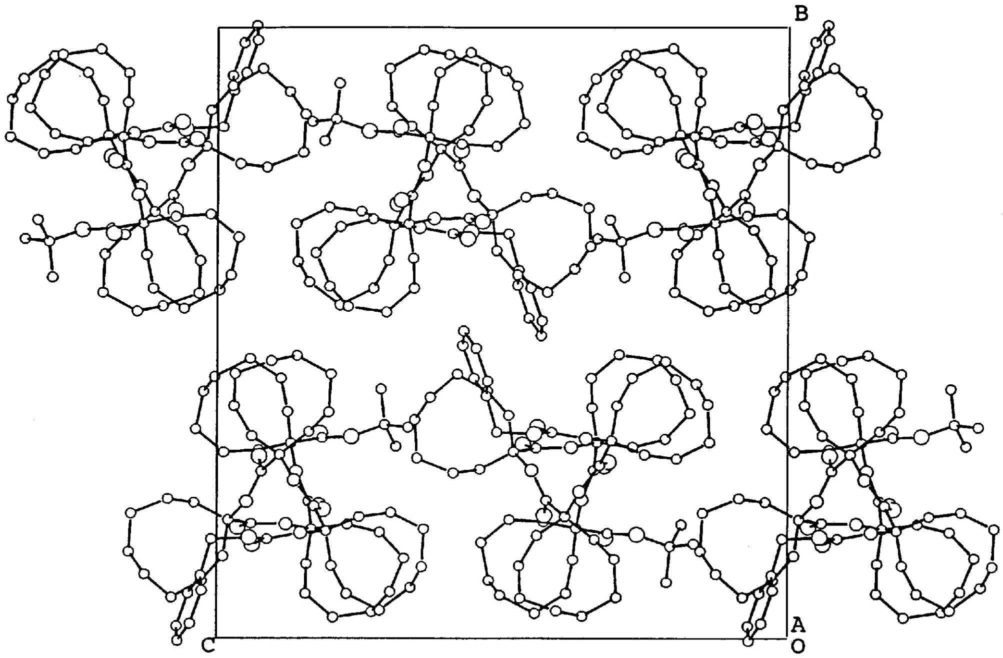

Figure 10 Crystal packing mode of the Z-$(Ac_9c)_5$-OtBu molecules projected down the *a* axis.

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