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Preferred Conformation of Peptides Rich in Ac_8c, a Medium-ring Alicyclic $C^{\alpha,\alpha}$-disubstituted Glycine

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A complete series of terminally blocked, monodispersed homo-oligopeptides (to the pentamer level) from the sterically demanding, medium-ring alicyclic $C^{\alpha,\alpha}$-disubstituted glycine 1-aminocyclooctane-1-carboxylic acid (Ac_8c), and two Ala/Ac_8c tripeptides, were synthesized by solution methods and fully characterized. The preferred conformation of all the oligopeptides was determined in deuterochloroform solution by IR absorption and ^{1}H-NMR. The molecular structures of the amino acid derivative Z-Ac_8c-OH, the dipeptide *p*BrBz-(Ac_8c)$_2$-OH and the tripeptide *p*BrBz-(Ac_8c)$_3$-O*t*Bu were assessed in the crystal state by X-ray diffraction. Conformational energy computations were performed on the monopeptide Ac-Ac_8c-NHMe. Taken together, the results obtained strongly support the view that the Ac_8c residue is an effective β-turn and helix former. A comparison is also made with the conformational preferences of α-aminoisobutyric acid, the prototype of $C^{\alpha,\alpha}$-disubstituted glycines, and of the other members of the family of 1-aminocycloalkane-1-carboxylic acids (Ac_nc, with $n=3$, 5–7) investigated so far. The implications for the use of the Ac_8c residue in peptide conformational design are considered.

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

INTRODUCTION

The exploitation of $C^{\alpha,\alpha}$-disubstituted glycines in the synthesis of peptides with restricted conformational flexibility has acquired increasing importance in the design of analogues of bioactive compounds [1–5]. In this connection the family of Ac_nc ($n=3$, 5–7) cycloaliphatic residues has recently proven to be valuable in the construction of conformationally constrained peptide backbones [3–5].

More specifically, the three-membered ring Ac_3c residue exhibits a marked preference for the 'bridge' region of the conformational space [6], in particular for $\phi,\psi = \pm 90°$, 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 3_{10}-helices [10]. Interestingly, however, the preferred conformations (*regular* type III(III') β-turns and 3_{10}-helices) theoretically predicted and experimentally found for the Ac_5c, Ac_6c and Ac_7c residues, with the cyclic moieties significantly larger than that of Ac_3c, closely parallel those of Aib, the prototype of $C^{\alpha,\alpha}$-disubstituted glycines [1–5].

Abbreviations: Ac_nc, 1-aminocycloalkane-1-carboxylic acid; Ac_8c, 1-aminocyclooctane-1-carboxylic acid; Aib, α-aminoisobutyric acid; (αMe)Phe, C^{α}-methyl phenylalanine; *p*BrBz, para-bromobenzoyl; TEMPO, 2,2,6,6-tetramethylpiperidinyl-1-oxy.

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With the aim of further contributing to the picture of the structural preferences of the medium-ring residues of this family, we report here the synthesis, characterization, and a theoretical (conformational energy calculations) and experimental (FTIR, ^{1}H-NMR, and X-ray diffraction) structural analysis of a number of peptides rich in Ac_8c, the largest cycloaliphatic amino acid residue studied so far. The crystal structure of the amino acid hydrobomide (H-Ac_8c-OH·HBr) has been published [11]. A preliminary account of part of this work has been reported [12].

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_8c peptides were determined using a C. Erba model 3A 30 amino acid analyser (Rodano, Milan, Italy). Elution of Ac_8c was observed after the Phe peak and before the His peak. The Ac_8c colour yield with ninhydrin is about 30 times lower than those of protein amino acids.

Infrared Absorption

Infrared absorption spectra 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. 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). The band positions are accurate to ± 1 cm^{-1}.

^{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).

Conformational Energy Calculations

The geometries of the acetamido and methylamido N-and C-blocking groups were those proposed by Scheraga and coworkers [13, 14]. The geometrical parameters for the Ac_8c residue were derived from the crystal structure analyses reported in this work. The eight-membered ring conformation was fixed as a BC (boat-chair) with $\chi^{1,1}$ and $\chi^{1,2}$ side-chain torsion angles in the *t*, g^+ conformation. Conformational energy calculations were performed using the Insight/Discover [15] package with the Consistent Valence Force Field (CVFF) [16–18]. A dielectric constant of 1 was assumed in all calculations. The conformational space was mapped by calculating the conformational energy at 5° intervals for the ϕ,ψ angles. The ω angles were fixed at 180°. The terminal methyl groups were frozen into staggered conformations. Minimum energy conformations were obtained in all low-energy regions located in the above search, minimizing the energy with respect to all torsion angles using the Newton–Raphson algorithms [19]. Conformational energies are expressed as $\Delta E = E - E_0$, where E_0 is the energy of the most stable conformation. All computations were performed on a Silicon Graphics Personal Iris 4D35 GT Turbo of the Centro Interdipartimentale di Ricerca sui Peptidi Bioattivi at the University of Naples.

X-ray Diffraction

Single crystals of Z-Ac_8c-OH, *p*BrBz-(Ac_8c)$_2$-OH and *p*BrBz-(Ac_8c)$_3$-O*t*Bu were obtained by slow evaporation at room temperature from the solvents and with the crystal habits indicated in Table 1. Determination of the cell constants and X-ray intensity data collection were performed on a CAD4 Enraf-Nonius graphite monochromated, Ni filtered, single crystal X-ray diffractometer of the Centro di Biocristallografia, CNR, at the University of Naples 'Federico II', equipped with a Micro VAX II Digital

Computer. Unit cell parameters were obtained in all cases by a least-squares procedure on the angular parameters of 25 reflections in the range 17–22°, using the CuKα ($\lambda = 1.54184$ Å) radiation. Crystal data are reported in Table 1. Data were collected up to 70° in θ, at 295 K.

The structure of Z-Ac$_8$c-OH was solved by direct methods, using the SIR 92 package [20]. The solution with the best figure of merit revealed the coordinates of most of the non-hydrogen atoms; the remaining ones were recovered using difference Fourier techniques. For the refinement, the SDP (Structure Determination Programs) package [21] and a full-matrix least-squares procedure were used, minimizing the quantity $\Sigma w(F_o^2 - F_c^2)^2$, with a weight w equal to $1/\sigma(F_o^2)$. The final R index was 0.058 ($R_w = 0.060$). The structures of *p*BrBz-(Ac$_8$c)$_2$-OH and of *p*BrBz-(Ac$_8$c)$_3$-O*t*Bu were solved by the Patterson technique by determining the position of the 'heavy' bromine atom, which allowed the phasing of all observed reflections in the data sets. The positions of the remaining atoms in both structures were recovered from subsequent difference Fourier maps. The final conventional R values were 0.058 ($R_w = 0.060$), 0.062 ($R_w = 0.060$) and 0.090 ($R_w = 0.094$) for Z-Ac$_8$c-OH, *p*BrBz-(Ac$_8$c)$_2$-OH and *p*BrBz-(Ac$_8$c)$_3$-O*t*Bu, respectively. In all cases the non-hydrogen atoms were refined with anisotropic temperature factors. Hydrogen atom positional parameters were stereochemically determined and introduced in the calculations with isotropic thermal factors equal to the Beq of the carrier atom. Their parameters were included in the structure factors calculations with an isotropic thermal parameter equal to the Beq of the corresponding carrier atom but not refined.

Final positional parameters and equivalent thermal factors for non-hydrogen atoms for the three molecules, together with their estimated standard deviations, have been deposited with the Cambridge Crystallographic Data Centre.

RESULTS

Synthesis of Ac$_8$c and its Derivatives and Peptides

Ac$_8$c amide hydrochloride was prepared by treatment of cyclooctanone with sodium cyanide, acetic acid, excess of ammonia and subsequent acid hydrolysis (HCl/HCOOH at 10–20 °C) of the amino

Table 1 Crystallographical Data for the Ac$_8$c Derivatives and Peptides

	Z-Ac$_8$c-OH	*p*BrBz-(Ac$_8$c)$_2$-OH	*p*BrBz-(Ac$_8$c)$_3$-O*t*Bu
Empirical formula	$C_{16}H_{21}NO_4$	$C_{25}H_{35}N_2O_4Br \cdot CH_3OH \cdot H_2O$	$C_{38}H_{58}N_3O_5Br \cdot 1/2C_4H_8O_2 \cdot 5/2H_2O$
Formula weight (a.m.u.)	291.35	557.53	805.91
Temperature (K)	293	293	293
Crystal system	Tetragonal	Monoclinic	Triclinic
Space group	$I4_1/a$	$P2_1/c$	$P\bar{1}$
a (Å)	19.887(5)	14.638(7)	11.436(1)
b (Å)	19.887(5)	15.455(5)	21.142(4)
c (Å)	16.981(6)	13.958(8)	18.230(1)
α (deg.)	90.00	90.00	93.85(3)
β (deg.)	90.00	117.39(4)	93.90(3)
γ (deg.)	90.00	90.00	101.03(1)
Volume (Å^3)	6715.3(4)	2803.5(6)	4301.9(3)
Z (molecules/cell)	16	4	4
Density (calc.), (g/cm^3)	1.153	1.220	1.247
Solvent	MeOH[a]	MeOH/H_2O (1 : 1)	AcOEt[a]
Crystal size (mm)	0.3 × 0.2 × 0.2	0.3 × 0.3 × 0.2	0.4 × 0.2 × 0.3
Independent reflections	3373	5529	16253
Reflections used	2983	4592	3937
Goodness-of-fit on F	1.042	0.998	2.350
R	0.058	0.062	0.090
R_w	0.060	0.060	0.094
ΔF (e/Å^3)	+0.251, −0.320	+0.320, −0.240	+0.530, −0.104

nitrile intermediate (Strecker synthesis). Acid hydrolysis (6N, HCl, under reflux) of Ac_8c amide hydrochloride afforded the free amino acid. The synthesis of this alicyclic $C^{\alpha,\alpha}$-disubstituted glycine was already reported [22–24] according to the Bücherer–Lieb hydantoin method [25].

The Ac_8c and L-Ala Z-protected derivatives were obtained by reacting the free amino acid with benzyloxycarbonylchloride. The 5(4*H*)-oxazolone from *p*BrBz-Ac_8c-OH was prepared by treating the free amino acid with an excess of para-bromobenzoylchloride in anhydrous pyridine. The symmetrical

Table 2 Physical Properties for Ac_8c, Its Derivatives and Peptides

Compound	Melting point (°C)	Recryst. solvent[a]	$[\alpha]_D^{20}$ (°)[b]	TLC			IR[c]	Amino acid analysis
				R_{F1}	R_{F2}	R_{F3}		
H-Ac_8c-OH	272–273	—	—	0.00	0.45	0.00	3432, 1588	—
HCl·H-Ac_8c-NH_2	262–263	—	—	0.05	0.50	0.00	3355, 3161, 1685, 1611	—
Z-Ac_8c-OH	144–145	AcOEt/PE	—	0.70	0.85	0.40	3342, 1705, 1688	—
(Z-Ac_8c)$_2$O	120–121	EE/PE	—	0.98	0.80	0.75	3413, 1814 1744, 1718	—
Z-Ac_8c-O*t*Bu	82–83	EE/PE	—	0.98	0.95	0.90	3385, 1712	—
Z-(Ac_8c)$_2$-O*t*Bu	160–161	AcOEt/PE	—	0.65	0.90	0.75	3397, 3305, 1719, 1652	—
Z-Ac_8c-L-Ala-OMe	115–116	AcOEt/PE	−27.3	0.95	0.90	0.45	3308, 1750, 1694, 1666, 1651	Ac_8c 1.10; Ala 0.90
Z-L-Ala-Ac_8c-L-Ala-OMe	178–179	MeOH/H_2O	−71.4	0.75	0.90	0.35	3377, 3287, 1741, 1702, 1675, 1642	Ac_8c 1.09; Ala 1.91
Z-Ac_8c-(L-Ala)$_2$-OMe	99–100	AcOEt/PE	−24.9	0.70	0.90	0.40	3310, 1742, 1700, 1652	Ac_8c 1.01; Ala 1.99
5(4*H*)-oxazolone from *p*BrBz-Ac_8c-OH	91–92	Toluene/PE	—	0.95	—	0.95	1812, 1648	—
*p*BrBz-(Ac_8c)$_2$O*t*Bu	197–198	$CHCl_3$/PE	—	0.98	0.90	0.70	3435, 3293, 1730, 1666	—
*p*BrBz(Ac_8c)$_2$-OH	251–252	AcOEt/PE	—	0.55	0.98	0.30	3294, 1733 1650, 1633	—
5(4*H*)-oxazolone from *p*BrBz-(Ac_8c)$_2$- OH	216–217	AcOEt/PE	—	0.95	—	0.90	3308, 1807, 1677, 1637	—
*p*BrBz-(Ac_8c)$_3$-O*t*Bu	235–236	$CHCl_3$/PE	—	0.95	0.98	0.45	3430, 3335, 1705, 1682, 1666, 1642	—
*p*BrBz-(Ac_8c)$_3$-OH	271–272	MeCN	—	0.50	0.98	0.35	3320, 1732 1648	—
5(4*H*)-oxazolone from *p*BrBz-(Ac_8c)$_3$- OH	199–200	Toluene/PE	—	0.95	—	0.75	3427, 3320, 1807, 1661	—
*p*BrBz-(Ac_8c)$_4$-O*t*Bu	305–307	$CHCl_3$/PE	—	0.90	0.98	0.35	3431, 3341, 1725, 1669	—
*p*BrBz-(Ac_8c)$_4$-OH	305–307	MeCN	—	0.50	0.98	0.20	3319, 1736, 1657	—
*p*BrBz-(Ac_8c)$_5$- O*t*Bu	>340	$CHCl_3$	—	0.90	0.98	0.35	3430, 3318, 1709, 1665	—
*p*BrBz-(Ac_8c)$_5$-OH	324–325	MeCN	—	0.50	0.98	0.20	3312, 1732, 1655	—

[a] AcOEt, ethyl acetate; PE, petroleum ether; EE, ethyl ether; MeOH, methanol; MeCN, acetonitrile.
[b] $c = 0.5$, methanol.
[c] The IR absorption spectra were obtained in KBr pellets (only bands in the 3500–3150 and 1850–1550 cm^{-1} regions are reported).

anhydrides from Z-Ac_8c-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_8c-OtBu 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_8c and Ac_8c-Ala peptide bond formation was achieved by the symmetrical anhydride method. On the other hand, Ac_8c–Ac_8c peptide bond formation was obtained using the appropriate 5(4*H*)-oxazolone. N^{α}-para-bromobenzoylated peptide 5(4*H*)-oxazolones were synthesized from the pertinent N^{α}-blocked peptides and acetic anhydride at 120 °C. The N^{α}-blocked, 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_8c, its derivatives and peptides are listed in Table 2.

Solution Conformational Analysis

The preferred conformation adopted by the Ac_8c-rich peptides in solution was determined in a solvent of low polarity ($CDCl_3$) by IR absorption and ^{1}H-NMR as a function of concentration (over the range 10–0.1 mM).

Figure 1 shows the IR absorption spectra (N–H stretching region) of the Ac_8c homo-peptide series,

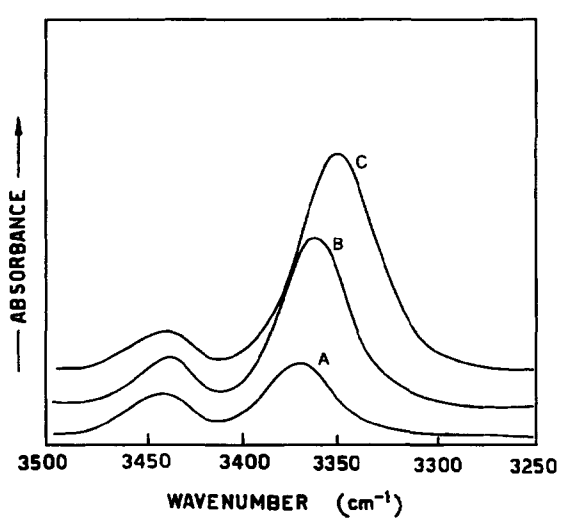

Figure 1 IR absorption spectra (N–H stretching region) of *p*BrBz-(Ac_8c)$_3$-OtBu (A), *p*BrBz-(Ac_8c)$_4$-OtBu (B) and *p*BrBz-(Ac_8c)$_5$-OtBu (C) in $CDCl_3$ solution (concentration 1 mM).

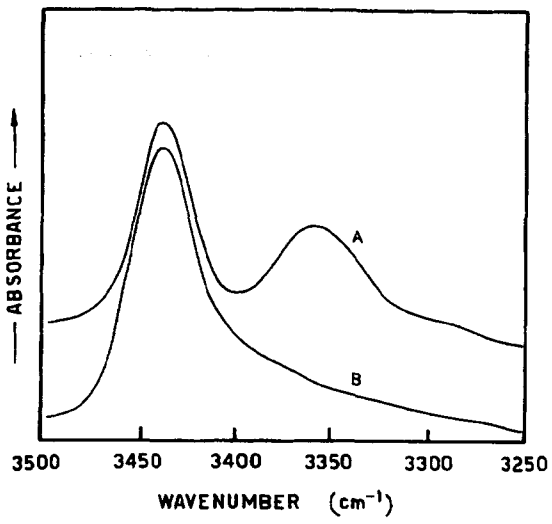

Figure 2 IR absorption spectra (N–H stretching region) of Z-Ac_8c-(L-Ala)$_2$-OMe (A) and Z-L-Ala- Ac_8c-L-Ala-OMe (B) in $CDCl_3$ solution (concentration 1 mM).

from tri-to pentapeptide. Figure 2 compares the IR absorption spectra of two Ac_8c/Ala tripeptides differing by the position of the Ac_8c residue in the sequence. The curves of the homo-tri, tetra- and pentapeptides are characterized by two bands, at 3442–3437 cm^{-1} (free, solvated NH groups) and 3370–3350 cm^{-1} (H-bonded NH groups), respectively [26]. 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 wavenumbers. In the spectrum of the homo-dipeptide (not shown) no absorption maximum is visible in the 3370–3350 cm^{-1} region. An inspection of the spectra of the tripeptides studied allows us to conclude that the A_H/A_F ratio is very high for the homo-tripeptide, still significant for the Ac_8c-(L-Ala)$_2$ sequence, but almost negligible for the L-Ala-Ac_8c-L-Ala sequence. We have also been able to demonstrate that even at 10 mM concentration there are only minor changes in the spectra of the various peptides in the 3500–3330 cm^{-1} region (not shown). Therefore, the observed H-bonding band at 3370–3350 cm^{-1} should be interpreted as arising almost exclusively from intramolecular N—H···O=C interactions.

The present IR absorption investigation has provided convincing evidence that main-chain length and sequence-dependent intramolecular H-bonding is an important factor influencing the conformation of the terminally blocked Ac_8c peptides in $CDCl_3$ solution. The observation of the 3370–3350 cm^{-1}

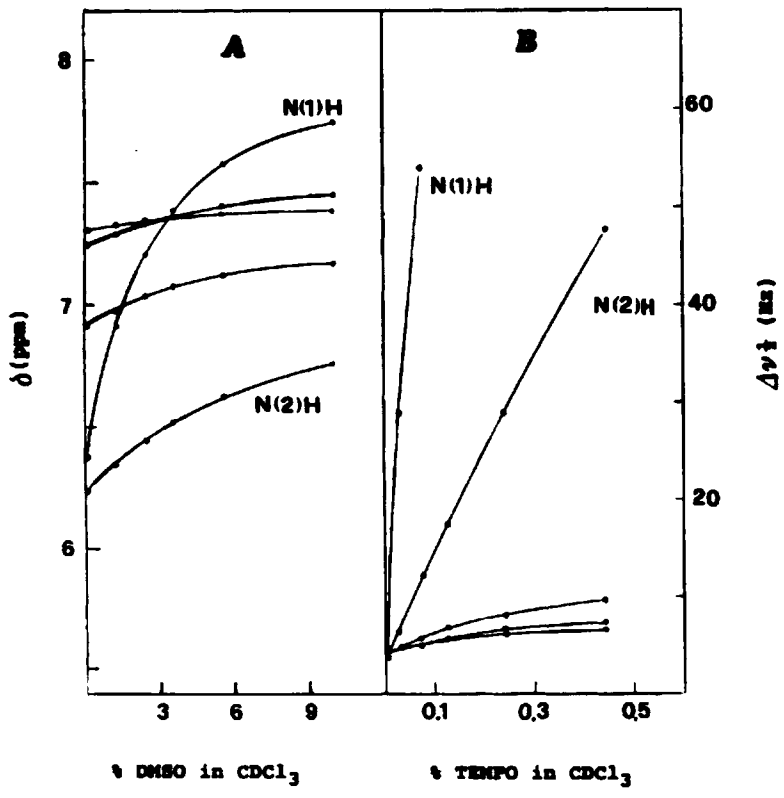

Figure 3 (A) Plot of NH chemical shifts in the ^{1}H-NMR spectrum of *p*BrBz-$(Ac_8c)_5$-OtBu as a function of increasing percentages of DMSO added to the $CDCl_3$ solution (v/v). (B) Plot of bandwidth of the NH signals of the same peptide as a function of increasing percentages of TEMPO (w/v) in $CDCl_3$ solution. Peptide concentration 2 mM.

band in the tri-, tetra-and pentapeptides, which is absent in the dipeptides, seems to indicate that Ac_8c peptides do not tend to fold into a γ-turn form [8, 27], and highlights the propensity of the tripeptides to adopt a β-turn conformation which may evolve in a series of consecutive β-turn (3_{10}-helices) in longer peptides. Our findings also support the view that Ac_8c is: (i) a better β-turn former than L-Ala, and (ii) a better β-turn former at the corner position $i+1$ than at the corner position $i+2$ of this folded structure.

The delineation of inaccessible (or intramolecularly H-bonded) NH groups of the Ac_8c homopeptides 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 $CDCl_3$ solution and (ii) free-radical (TEMPO) induced line broadening of NH resonances [30]. As a typical example, Figure 3 illustrates the behaviour of the NH resonances of *p*BrBz-$(Ac_8c)_5$-OtBu upon addition of the two perturbing agents. A partial, tentative assignment has been performed for the two upfield resonances in $CDCl_3$ to the N(1)H and N(2)H protons, by analogy with the chemical shifts in the same halohydrocarbon and the spectroscopic behaviour upon addition of perturbing agents of other N_α-para-bromobenzoylated homo-peptides from different types of $C^{\alpha,\alpha}$-disubstituted glycines [31–33]. In one case, the (αMe)Phe pentapeptide, a complete assignment of the NH protons was achieved by analysis of the COSY and ROESY spectra [31]. 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 (< 0.02 p.p.m.) shift to higher fields of all the NH resonances of the di- and tripeptides. However, this effect becomes significant for the N(1)H resonance of the tetra- and pentapeptides, where a shift of about 0.08 p.p.m. was observed. In the Ac_8c peptides examined in the $CDCl_3$–DMSO solvent mixtures and in the presence of the paramagnetic perturbing agent TEMPO at 1 mM peptide concentration two classes of NH protons were observed. Class (i) (N(1)H

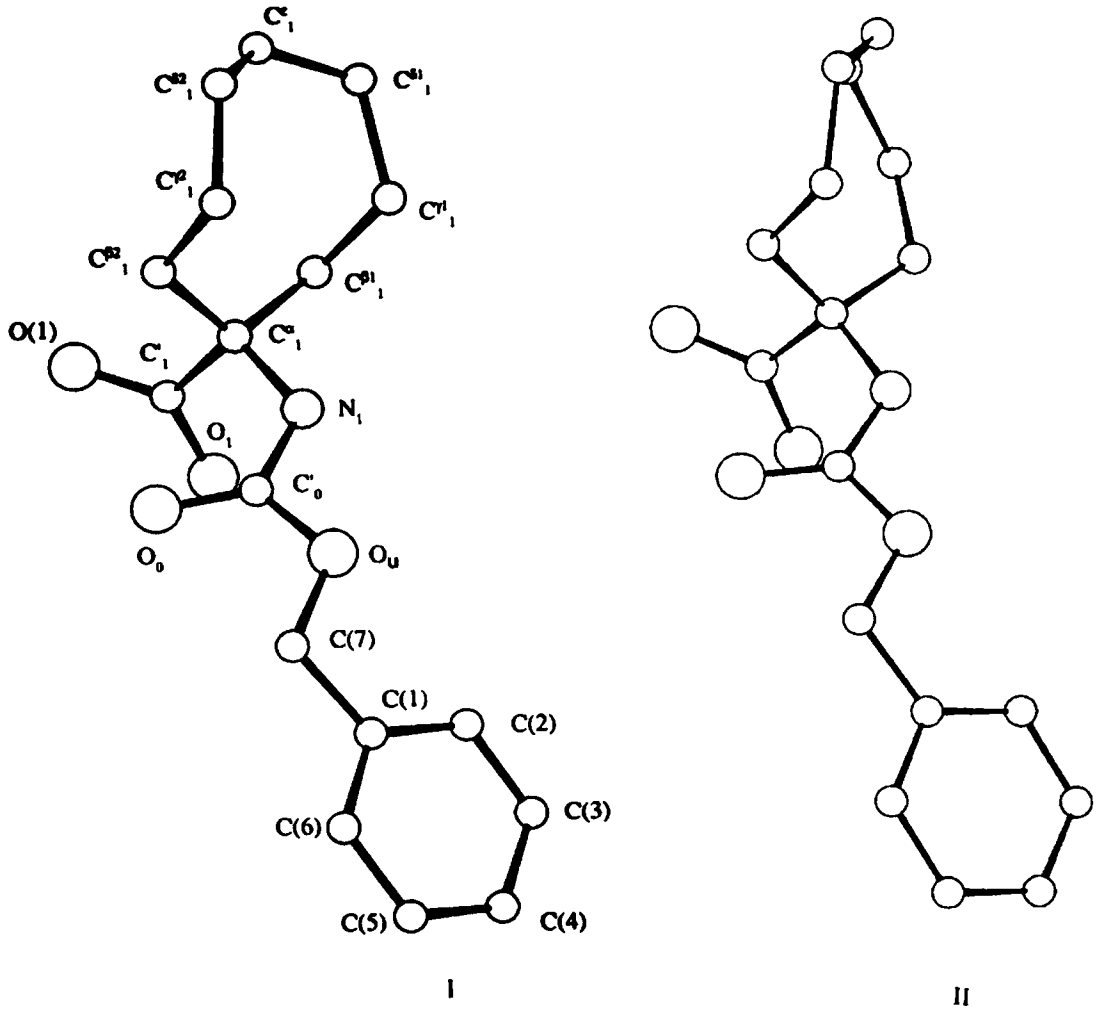

Figure 4 X-ray diffraction structure of conformations I and II (occupancy factor 50%) of Z-Ac_8c-OH (with numbering of atoms in conformation I).

and N(2)H protons) includes protons whose chemical shifts are 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 5 mM concentration, only the tetra- and pentapeptides have a tendency (although modest) to self-associate and that in this process the amide N(1)H proton plays a major role as H-bonding donor. At lower concentrations, the N(3)H to N(5)H protons of the tri-, tetra- and pentapeptides are almost inaccessible to perturbing agents and are, therefore, most probably, intramolecularly H-bonded. In view of these observations, it is reasonable to conclude that the most populated structures adopted in $CDCl_3$ solution by the terminally blocked Ac_8c homo-tri-, 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 IR absorption study discussed above.

Crystal-state Conformational Analysis

We determined by X-ray diffraction the molecular and crystal structures of the following Ac_8c derivatives and peptides: Z-Ac_8c-OH, *p*BrBz-$(Ac_8c)_2$-OH, and *p*BrBz-$(Ac_8c)_3$-O*t*Bu. The two conformations I and II of Z-Ac_8c-OH are illustrated in Figure 4, while the structure of *p*BrBz-$(Ac_8c)_2$-OH and the two independent molecules present in the asymmetric unit of *p*BrBz-$(Ac_8c)_3$-O*t*Bu are shown in Figures 5

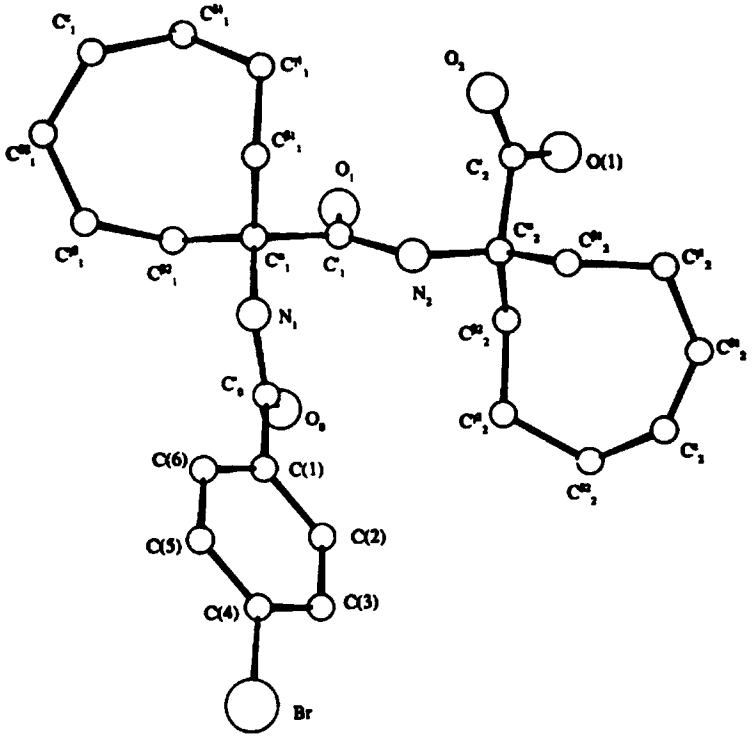

Figure 5 X-ray diffraction structure of *p*BrBz- $(Ac_8c)_2$-OH with numbering of the atoms.

and 6, respectively. The average bond angles for the Ac_8c residue, as obtained from a statistical analysis of the X-ray diffraction structures reported in this paper, are shown in Figure 7. Relevant backbone and side-chain torsion angles [34] are given in Table 3. In Table 4 the intra-and intermolecular H-bond parameters are listed, while the parameters characterizing the eight-membered ring system of the Ac_8c residue are given in Table 5.

Bond lengths and bond angles are in general agreement with previously reported values for the geometry of the benzyloxycarbonylamino [35] and para-bromobenzamido [36] moieties, the ester group [37] and the peptide unit [38, 39]. We have also calculated the average geometry for the Ac_8c residue. All bond lengths are close to those reported in the literature [11]. In particular, the average C–C bond length in the ring is 1.52 Å (with average lengths of 1.53 Å for both C^{α}–C^{β} and C^{β}–C^{γ} bonds, and of 1.51 and 1.49 Å for C^{γ}–C^{δ} and C^{δ}–C^{ε} bonds, respectively; these latter values are influenced by the higher thermal factors of C^{ε} and C^{δ} atoms), in good agreement with the mean value of 1.54 Å assigned by Bixon and Lifson [40] from theoretical studies on the conformation of the cyclooctane system. The literature average value for the —CH_2—CH_2— distance is 1.52 Å [41]. On the other hand, the bond angles internal to the eight-membered ring deviate markedly from the regular tetrahedral value (109.5°). The average C–C–C bond angle in the ring is 117.5°. The bond angles actually vary from 114.1° (at C^{α}) to 119.9° (at $C^{\delta 2}$), the largest values being observed for the C atoms most removed from the peptide chain. The theoretical value predicted by Bixon and Lifson is 115° [40]. 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 4–5° than those involving the $C^{\beta 2}$ atom. This observation is common also to Aib-and Ac_nc-($n = 3$, 5–7) rich peptides [4, 5]. The conformationally sensitive N–C^{α}–C′ (τ) bond angle is 109.3°, reasonably close to the value expected for helical $C^{\alpha,\alpha}$-disubstituted glycines ($\sim$110–111°) [5, 42].

All the Ac_8c residues are found in the helical region $A(A^*)$ of the conformational map [6]. Each molecule, having no chiral atoms, crystallizes with retention of the centre of symmetry; thus, in each unit cell molecules of both handedness simultaneously occur (as an asymmetric unit, we have chosen the right-handed helical molecule). The mean values for the ϕ, ψ backbone torsion angles of the Ac_8 residue are $\pm$53.9°, $\pm$38.1°. Also the C-terminal Ac_8c residues of the dipeptide and of both molecules A and B of the tripeptide adopt a conformation in the

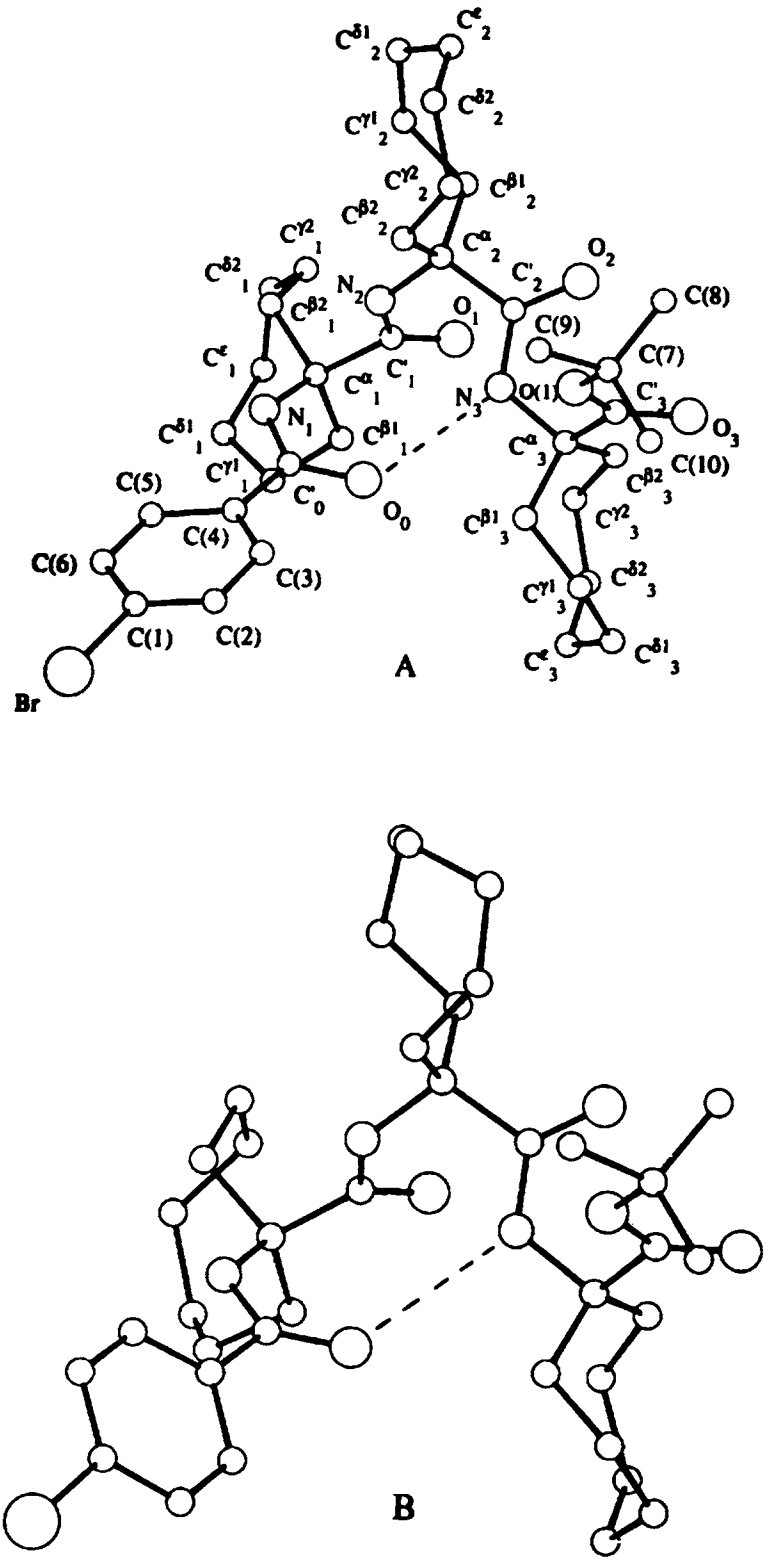

Figure 6 X-ray diffraction structure of the two independent molecules (A and B) in the asymmetric unit of *p*BrBz- $(Ac_8c)_3$-OtBu with numbering of the atoms. In each molecule the intramolecular H-bond is represented by a dashed line.

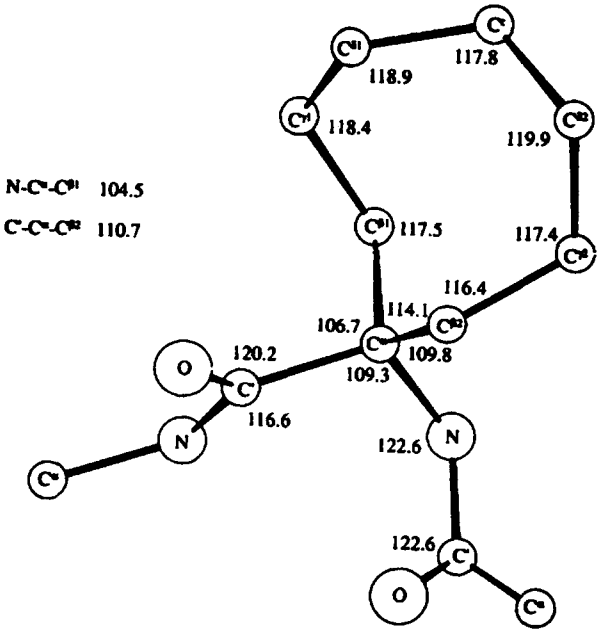

Figure 7 Average bond angles for the Ac_8c residue derived from a statistical analysis of the X-ray diffraction structures discussed in this work. The e.s.d. values are in the range 0.3–3°.

Table 3 Selected Backbone and Side-chain Torsion Angles (°) for the Ac_8c Derivatives and Peptides

	Z-Ac_8c-OH	*p*BrBz-(Ac_8c)$_2$-OH	*p*BrBz-(Ac_8c)$_3$-OtBu	
			mol. A	mol. B
ω_0	172.6	−179.7	−174.0	−167.9
ϕ_1	−53.4	−53.5	−53.3	−55.6
ψ_1	−34.7[a]	−41.7	−40.6	−38.4
ω_1	—	−176.0	−170.3	−174.5
ϕ_2	—	57.1	−61.6	−56.1
ψ_2	—	37.3[b]	−27.0	−32.9
ω_2	—	—	−174.6	−179.6
ϕ_3	—	—	50.9	52.8
ψ_3	—	—	49.4	46.3
ω_3	—	—	166.2	167.9
$\chi_1^{1,1}$	−67.8[−165.8]	177.0	174.6	−169.4
$\chi_1^{1,2}$	59.3	71.9	71.9	−61.5
$\chi_2^{1,1}$	—	176.5	−179.0	−174.4
$\chi_2^{1,2}$	—	−65.4	65.2	64.9
$\chi_3^{1,1}$	—	—	−176.1	179.3
$\chi_3^{1,2}$	—	—	−71.9	−67.5

[a] N_1-C_1^{α}-C_1'-O(1).
[b] N_2-C_2^{α}-C_2'-O(1).

helical region, but they have a handedness opposite to that exhibited by the preceding residues, a common observation for Aib- and Ac_nc- ($n=3$, 5–7) rich peptides [4, 5]. The Ac_8c–Ac_8c sequence of both molecules A and B of the tripeptide is folded in a $1\leftarrow 4$ C=O···H—N intramolecularly H-bonded type-III(III') β-turn conformation. The $N_3\cdots O_0$ separation is 2.99 Å for molecule A, and 2.97 Å for molecule B, in the range expected for such H-bonds [43–45]. The only relevant difference between conformations A and B of the tripeptide is seen in the rotation of the para-bromobenzoyl N^{α}-blocking group out of the adjacent amide plane (30.3° for molecule A, −19.6° for molecule B). In the dipeptide the para-bromobenzoyl group is rotated by 38.8° out of the adjacent amide plane. In the three compounds, few significant deviations of the ω torsion angles ($|\Delta\omega| > 8°$) from the ideal value of the *trans* planar amide, peptide and ester units (180°) are observed. In particular, the ω_1 (peptide) torsion angle for molecule A of the tripeptide, and the ω_3 (ester) torsion angle for both molecules A and B of the tripeptide differ by more than 9.5° from the *trans* planar value.

Each of the eight-membered rings belongs to the boat-chair (BC) conformational family, although the degree of distortion away from this symmetrical conformation differs from one ring to the next. The BC conformation is that experimentally found in the crystal structure of H-Ac_8c-OH·HBr [11] and theoretically predicted as that of minimum energy for a cyclooctane ring [40, 46–50]. The puckering parameters [48], aside from torsion angles, have been calculated and these aid a more detailed classification of each ring conformation. From the analysis of the available data it would appear that half of the rings are distorted versions of the BC conformer, lying on the BC/TBC (twisted boat-chair) pseudorotation pathway. The side-chain $\chi^{1,1}$ and $\chi^{1,2}$ torsion angles are predominantly (t, g^+) and (t, g^-) for right-handed and left-handed Ac_8c residues, respectively. Conformations I and II of Z-Ac_8c-OH (occupancy factor 50%) differ by the $\chi^{1,1}$ and $\chi^{1,2}$ values, (t, g^+) for conformation I, and the usual (g^-, g^+) for conformation II.

In the unit cell the Z-Ac_8c-OH molecules are held together by (urethane) N—H···O=C (urethane) intermolecular H-bonds along the *c*-direction and by (carboxylic acid) O—H···O=C (carboxylic acid) intermolecular H-bonds about centres of symmetry. The N···O and O···O distances are 2.87 Å [43–45] and 2.62 Å [51, 52], respectively. Thus, along the *c*-direction helical rows of molecules, stabilized

Table 4 Intra- and Intermolecular H-bond Parameters for the Ac_8c Derivatives and Peptides

Compound	Donor (D)	Acceptor (A)	Symmetry operation	Distance (Å) D···A	Angle (°) N···O=C′
Z-Ac_8c-OH	N_1	O_0	$x-\frac{3}{4}, \frac{3}{4}-y, \frac{1}{4}+z$	2.87	143.8
	O(1)	O_1	$^{3/2}-x, -y-^{1/2}, ^{1/2}-z$	2.62	121.8
*p*BrBz-(Ac_8c)$_2$- OH	N_1	O_w	$1-x, 1-y, 1-z$	3.08	[a]
	N_1	O_M	$x-^{1/2}, -y-^{1/2}, ^{1/2}+z$	3.17	134.9
	N_2	O_M	$x-^{1/2}, -y-^{1/2}, ^{1/2}+z$	3.13	134.2
	O_w	O_1	$-x-^{1/2}, ^{1/2}+y, ^{1/2}-z$	2.82	124.0
	O_w	O_2	$x, y, 1+z$	2.90	151.5
	O_M	O_w	$^{1/2}-x, ^{1/2}+y, -z-^{1/2}$	2.82	118.1
	O(1)	O_0	$^{1/2}-x, ^{1/2}+y, -z-^{1/2}$	2.62	115.1
*p*BrBz-(Ac_8c)$_3$- O*t*Bu	N_{3A}	O_{0A}	x, y, z	2.99	131.7
	N_{3B}	O_{0B}	x, y, z	2.97	132.1
	N_{1A}	O_{3B}	$x, y, 1+z$	3.02	162.7
	N_{1B}	O_{2A}	x, y, z	3.05	149.6
	N_{2A}	O_{1S}	$x-1, y, z$	3.23	129.2
	O_{w2}	O_{1B}	$1+x, y, 1+z$	3.08	147.9

[a] Hydrogen atom positions of the water molecule were not determined.

by N—H···O=C H-bonds, are generated, which pack together along the *b*- and *c*-directions through O—H···O=C H-bonds.

Each *p*BrBz-(Ac_8c)$_2$-OH molecule co-crystallizes with one water and one methanol molecule. The amide N_1–H group forms a three-centre (or bifurcated) H-bond [53] with two acceptors, the oxygen atoms of symmetry related water [54] and methanol molecules. The peptide N_2–H group forms a single H-bond with a symmetry-related methanol molecule. These three N—H···O (solvent) H-bonds are very weak. The carboxylic acid O–H group is linked via a strong intermolecular H-bond to the urethane C=O group of a symmetry-related molecule. The peptide C'_1=O_1 and carboxylic acid C'_2=O_2 groups play the role of H-bonding acceptors from the O–H groups of symmetry-related water molecules.

The two independent molecules A and B in the asymmetric unit of *p*BrBz-(Ac_8c)$_3$-O*t*Bu pack together along the *c*-axis in a head-to-tail arrangement, producing rows of molecules stabilized by (amide) N—H···O=C (peptide or ester) intermolecular H-bonds. Molecules of co-crystallized solvents (water and ethyl acetate), in particular those of water, play a major role in the crystal stabilization by linking together rows of peptide molecules running in the other directions.

Conformational Energy Calculations

From an inspection of the energy map of Ac-Ac_8c-NHMe (Figure 8) the following conformational features emerge:

Table 5 Puckering Amplitudes (q_m), Phase Angles (ϕ_m) and Polar Angle (θ) for the Ac_8c Derivatives and Peptides[a]

Compound			q_2 (Å)	q_3 (Å)	q_4 (Å)	ϕ_2 (°)	ϕ_3 (°)	θ (°)
Z-Ac_8c-OH	(conf. I)		0.99	0.46	−0.38	262.4	137.2	108.9
	(conf. II)		0.98	0.41	−0.30	184.4	255.1	106.0
*p*BrBz-(Ac_8c)$_2$-OH	(residue 1)		1.02	0.59	−0.33	90.4	48.6	105.4
	(residue 2)		0.92	0.52	0.15	271.1	253.2	82.0
*p*BrBz-(Ac_8c)$_3$-O*t*Bu	(residue 1)	mol. A	1.07	0.52	0.26	90.0	129.0	77.9
		mol. B	0.89	0.64	−0.26	110.9	60.4	103.5
	(residue 2)	mol. A	1.00	0.57	−0.28	259.5	307.6	103.5
		mol. B	1.04	0.58	−0.30	90.4	48.8	104.0
	(residue 3)	mol. A	0.93	0.61	0.34	86.3	128.5	72.9
		mol. B	0.96	0.60	−0.28	266.0	310.6	104.0

[a] For a definition of these parameters, see [39].

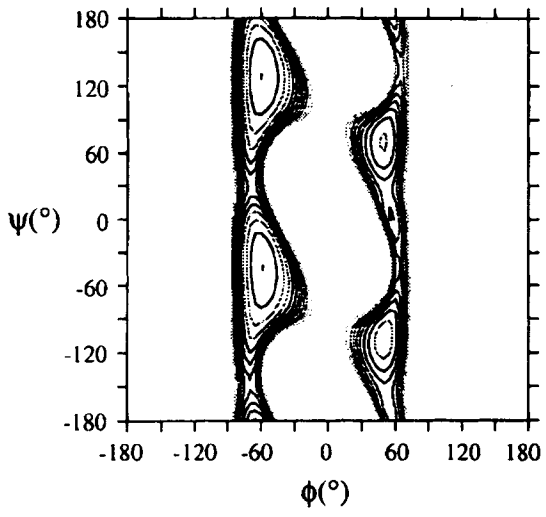

Figure 8 Rigid rotor (ϕ,ψ) map for Ac-Ac_8c-NHMe. Contour lines are drawn every 10 kcal/mol.

(1) Only a limited region of the ϕ,ψ space is accessible to the Ac_8c residue. In particular, the *BC* ring conformation and the $\chi^{1,1}$ and $\chi^{1,2}$ values (t, g^+) adopted give rise to an asymmetry of the rigid rotor map.

(2) The map also shows an almost blocked value for the ϕ angle, and a larger conformational freedom for the ψ angle.

(3) The two most stable conformations are detected with almost the same energy: the first in the right-handed helical region ($\phi = -60°$, $\psi = -45°$), the second in a semi-extended conformation ($\phi = -60°$, $\psi = 130°$).

(4) Additional minima are observed for the left-handed helical region with $\phi = 50°$ and $\psi = 70°$ (≈ 15 kcal/mol higher in energy) and for a semi-extended conformation with $\phi = 50°$, $\psi = -110°$ (≈ 15 kcal/mol higher in energy). The conformational energy computations are in good agreement with the experimental data. Actually, all experimental ϕ,ψ angles fall in the region of the deepest minimum.

Energy optimization of the geometrical parameters of the Ac_8c residue gave result in agreement with those obtained from X-ray analysis. The energy difference between the different minima remains practically unchanged after geometry optimization, with no significant change in the ϕ,ψ values.

DISCUSSION

The experimental and theoretical results obtained in this work demonstrate that the medium-ring alicyclic Ac_8c residue imparts considerable conformational restriction to the peptide backbone and is constrained to adopt conformations in the $3_{10}/\alpha$-helical region of the ϕ,ψ space. Thus, the Ac_8c residue can be easily accommodated in either position $i+1$ or $i+2$ of type III(III') β-turn and at position $i+1$ of type I(I') β-turn. It may also be located, although with some deviation from the preferred conformation, at position $i+2$ of types I(I') and II(II') β-turns. Furthermore, this new $C^{\alpha,\alpha}$-disubstituted glycine has increased effective volume and hydrophobicity compared to Aib and Ac_nc ($n=5$–7) residues, but it exhibits strictly comparable conformational preferences [1–5].

Considerable recent interest has been focused on the development of conformationally constrained analogues of bioactive peptides [3, 55–61]. The availability of highly active, conformationally constrained agonists is of value in delineating the nature of receptor-bound conformations. The only published study in the area of alicyclic $C^{\alpha,\alpha}$-disubstituted glycines (including Ac_8c) has described the development of position-2 analogues of the dipeptide sweetener aspartame, which highlighted the close relationship between the effective volume of the amino acid side chain and the taste properties [24]. In this homologous series, the H-L-Asp-Ac_nc-OMe dipeptides containing amino acids with small cycloalkane rings ($n=3$–5) are sweet (like H-L-Asp-Aib-OMe), whereas those with larger rings ($n=6$–8) are not. More specifically, the Ac_8c cyclic side chain is too large to affect the taste receptor. It seems reasonable to foresee that future investigations on analogues of other bioactive peptides, incorporating Aib and Ac_nc ($n=3$–8) residues at selected positions, will be rewarding.

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