Conformational restriction through $C^{\alpha}_{i} \longleftrightarrow C^{\alpha}_{i}$ cyclization: 1-aminocycloheptane-1-carboxylic acid (Ac_7c)

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A complete series of *N*- and *C*-blocked, monodispersed homo-oligopeptides to the pentamer level from 1-aminocycloheptane-1-carboxylic acid (Ac_7c), an α-amino acid conformationally restricted through $C^{\alpha}_{i} \longleftrightarrow C^{\alpha}_{i}$ cyclization, and three tripeptides with Ac_7c combined with Ala, Leu, and Val residues have been synthesized by solution methods and fully characterized. The solution conformational preferences have been determined by IR absorption and ^{1}H NMR spectroscopy. In addition, the molecular structures of three derivatives (Ac_7c hydantoin, $ClCH_2CO$-Ac_7c-OH, and Z-Ac_7c-OH; Z = benzyloxycarbonyl) and four peptides [the dipeptide Z-Ac_7c-L-Ala-OMe, the tripeptides Z-Ac_7c-(L-Ala)$_2$-OMe and Z-(Ac_7c)$_3$-OBu^t, the tetrapeptide Z-(Ac_7c)$_4$-OBu^t, and the pentapeptide Z-(Ac_7c)$_5$-OBu^t] have been assessed in the crystal state by X-ray diffraction. The results obtained confirm the tentative conclusions put forward on the basis of our previous preliminary study, namely that β-bends and 3_{10}-helices are preferentially adopted by Ac_7c-based peptides. A comparison with the structural tendencies extracted from published work on peptides from α-aminoisobutyric acid, the prototype of $C^{\alpha,\alpha}$-dialkylated glycines, and the other extensively investigated members of the class of 1-aminocycloalkane-1-carboxylic acids (Ac_nc, with n = 3–6, 8, 9) is made and the implications for the use of the Ac_7c residue in conformationally constrained analogues of bioactive peptides are briefly discussed.

Introduction

In recent years conformationally constrained analogues of bioactive peptides have acquired increasing popularity among medicinal chemists in an effort to firmly establish 3D structure–bioactivity relationships and to develop new pharmaceutical agents with prolonged action and/or more selective properties.[1–5] In particular, conformational restriction through $C^{\alpha}_{i} \longleftrightarrow C^{\alpha}_{i}$ cyclization generates the family of 1-aminocycloalkane-1-carboxylic acid (Ac_nc) residues.[6] Theoretical and experimental studies of the preferred conformations of peptides characterized by the Ac_nc (n = 3–6, 8, 9) residues have been the subject of recent review articles and papers.[7–11] In a close parallelism to the structural behaviour of Aib (α-aminoisobutyric acid or $C^{\alpha,\alpha}$-dimethylglycine),[7–9,12,13] the prototype of $C^{\alpha,\alpha}$-dialkylated glycines, it was shown that regular or slightly distorted β-bend forms[14–16] or 3_{10}-helical structures[17] are adopted as a function of main-chain length and side-chain size. The cyclopropyl-containing amino acid is the only residue of this family known to strongly prefer the 'bridge' region ($\varphi = \pm 90°$, $\psi = 0°$)[18] of the conformational space. This anomalous conformational propensity appears to be associated with a distorted geometry, more specifically with the observed widening of the exocyclic τ(N–C^{α}–C′) bond angle to 116–117°.

With the aim of further contributing to our knowledge of the geometrical and structural preferences of the medium-ring residues of this family, in this work we describe the synthesis, characterization and an extensive solution (IR absorption and ^{1}H NMR spectroscopy) conformational investigation of the homo-oligomeric series Z-(Ac_7c)$_n$-OBu^t (Ac_7c, 1-aminocycloheptane-1-carboxylic acid; Z, benzyloxycarbonyl; OBu^t, *tert*-butoxy) (n = 1–5) and three Ac_7c-containing tripeptides. The X-ray diffraction structures of three derivatives [Ac_7c hydantoin, ClAc-Ac_7c-OH (ClAc, monochloracetyl), and Z-Ac_7c-OH] and four peptides [the dipeptide Z-Ac_7c-L-Ala-OMe (OMe, methoxy), the tripeptides Z-Ac_7c-(L-Ala)$_2$-OMe and Z-(Ac_7c)$_3$-OBu^t, the tetrapeptide Z-(Ac_7c)$_4$-OBu^t, and the pentapeptide Z-(Ac_7c)$_5$-OBu^t] are also reported.

Only scant information is available in the literature on bioactivity and conformational preferences of Ac_7c, and its derivatives and peptides. The tripeptide HCO-L-Met-Ac_7c-L-Phe-OMe shows excellent activity in the release of histamine and lysosomal enzymes.[19,20] The free amino acid itself is bitter[21] as it is the aspartame analogue H-L-Asp-Ac_7c-OMe.[22] An Ac_7c-based dihydroimidazol-4-one is a potent nonpeptide AT_1 angiotensin II receptor antagonist.[23] The crystal structures of H-Ac_7c-OH hydrobromide monohydrate[24,25] and the symmetrical anhydride (Z-Ac_7c)$_2$O[26] have been described. The synthesis and β-bend forming tendency of the dipeptide amides and tripeptide esters Z-Ile-Ac_7c-NHAr,[27,28] Boc-L-Ala-Ac_7c-L-Ala-OMe (Boc, *tert*-butoxycarbonyl),[29] Boc-Aib-Ac_7c-NHMe (NHMe, methylamino),[30] Boc-L-Pro-Ac_7c-L-Ala-OMe,[30] and HCO-L-Met-Ac_7c-L-Phe-OMe[20] have been published. Preliminary accounts of a limited part of this work have been reported.[31,32]

Table 1 Physical properties and analytical data for the Ac_7c derivatives and peptides

Compound	Yield (%)	Mp[a] /°C	Recrystallisation solvent[b]	$[\alpha]_D^{20}$[c]	TLC[d] $R(I)_F$	$R(II)_F$	$R(III)_F$	ν/cm^{-1}[e]
(*a*) *Derivatives*								
Ac_7c hydantoin	38	215–216	MeOH/DE	—	0.60	—	—	3445, 3286, 1768, 1708
Z-Ac_7c-OH	40	117–118	AcOEt/LP	—	0.65	0.95	0.40	3304, 1704, 1526
5(4*H*)-oxazolone from Z-Ac_7c-OH	97	Oil	AcOEt/LP	—	0.95	—	0.85	1825, 1683
ClAc-Ac_7c-OH	54	167–170	AcOEt/LP	—	—	0.90	—	3392, 1734, 1632, 1532
Z-Ac_7c-OtBu	61	68–70	AcOEt/LP	—	0.95	0.95	0.85	3373, 1717, 1520
(*b*) *Peptides*								
Z-$(Ac_7c)_2$-OBu^t	62	154–156	AcOEt/LP	—	0.95	0.95	0.85	3401, 3291, 1719, 1650, 1529
Z-$(Ac_7c)_3$-OBu^t	67	158–159	AcOEt/LP	—	0.95	0.95	0.60	3422, 3358, 1701, 1649, 1522
Z-$(Ac_7c)_4$-OBu^t	76	223–224	AcOEt/LP	—	0.95	0.95	0.50	3429, 3352, 1705, 1675, 1527
Z-$(Ac_7c)_5$-OBu^t	98	251–252	Hot toluene	—	0.90	0.95	0.45	3430, 3341, 1699, 1666, 1524
Z-Ac_7c-L-Ala-OMe	66	100–101	AcOEt/LP	−23.8	0.95	0.95	0.55	3321, 1733, 1688, 1651, 1537, 1519
Z-L-Ala-Ac_7c-L-Ala-OMe	60	176–178	Hot AcOEt	−47.7	0.85	0.95	0.45	3386, 3294, 1742, 1703, 1678, 1638
Z-$(Ac_7c)_2$-L-Ala-OMe	62	99–101	AcOEt/DE	−21.4	0.90	0.95	0.45	3427, 3348, 1759, 1681, 1650, 1584
Z-Ac_7c-L-Val-OMe	72	97–98	AcOEt/LP	−14.4	0.95	0.95	0.70	3323, 1744, 1691, 1524
Boc-L-Leu-Ac_7c-L-Val-OMe	62	150–151	AcOEt/LP	−37.7	0.95	0.95	0.45	3308, 1728, 1702, 1664, 1518

[a] Determined on a Leitz Model Laborlux 12 apparatus (Wetzlar, Germany). [b] DE, diethyl ether; AcOEt, ethyl acetate; LP, light petroleum. [c] Determined on a Perkin-Elmer Model 241 polarimeter equipped with a Haake Model L thermostat (Karlsruhe, Germany); $c = 0.5$ (MeOH). [d] Silica gel plates 60F-254 (Merck) using the following solvent systems: (*I*) chloroform–ethanol 9:1; (*II*) butan-1-ol–acetic acid–water 6:2:2; (*III*) toluene–ethanol 7:1. The compounds were revealed either with the aid of a UV lamp or with the hypochloride–starch iodide chromatic reaction. A single spot was observed in each case. [e] Determined on a Perkin-Elmer Model 580 B spectrophotometer equipped with a Perkin-Elmer Model 3600 IR data station and a Model 660 printer. For the IR measurements the KBr disk technique was used.

Experimental

Materials

Relevant physical properties and analytical data for the newly synthesized Ac_7c derivatives and peptides are listed in Table 1. In addition, the results of the amino acid analyses [C. Erba (Rodano, Milan, Italy) Model 3A 30 amino acid analyser] are as follows: Z-Ac_7c-L-Ala-OMe (Ala 0.98, Ac_7c 1.01); Z-L-Ala-Ac_7c-L-Ala-OMe (Ala 1.95, Ac_7c 1.05); Z-Ac_7c-(L-Ala)$_2$-OMe (Ala 1.98, Ac_7c 1.02); Z-Ac_7c-L-Val-OMe (Val 0.99, Ac_7c 1.02); Boc-L-Leu-Ac_7c-L-Val-OMe (Val 0.98, Leu 1.00, Ac_7c 1.02).

IR absorption spectra

Infrared absorption spectra were recorded with a Perkin-Elmer (Norwalk, CT, USA) Model 1720X FTIR spectrophotometer, nitrogen flushed, at 2 cm^{-1} nominal resolution, averaging 16 scans for 10 and 1.0×10^{-3} mol dm^{-3} sample concentrations or 64 scans for 0.1×10^{-3} mol dm^{-3} sample concentrations. Solvent (baseline) spectra were recorded under the same conditions. Cells with path lengths of 0.1, 1.0 and 10 mm (with CaF_2 windows) were used. Spectrograde deuteriochloroform (99.8% ^{2}H) was purchased from Merck (Darmstadt, Germany).

^{1}H NMR spectra

^{1}H NMR spectra were recorded with a Bruker (Karlsruhe, Germany) Model AM 400 spectrometer. Measurements were carried out in [^{2}H]chloroform (99.96% ^{2}H; Aldrich, Milwaukee, WI, USA) and in [$^2H_6$]DMSO ([$^2H_6$]dimethyl sulfoxide) (99.96% $^2H_6$; Stohler, Waltham, MA, USA) with tetramethylsilane as the internal standard. The free radical TEMPO (2,2,6,6-tetramethyl-1-piperidyloxyl) was purchased from Sigma (St Louis, MO, USA).

X-Ray diffraction analysis

Colourless single crystals of the Ac_7c hydantoin, ClAc-Ac_7c-OH, Z-Ac_7c-OH, Z-Ac_7c-L-Ala-OMe, Z-Ac_7c-(L-Ala)$_2$-OMe, Z-$(Ac_7c)_3$-OBu^t, Z-$(Ac_7c)_4$-OBu^t, and Z-$(Ac_7c)_5$-OBu^t were grown by slow evaporation at room temp. from the solvents reported in Tables 2 and 3. The X-ray data for the three Ac_7c derivatives were collected on a Philips PW1100 diffractometer (Eindhoven, The Netherlands), while the data for the five peptides were obtained using an Enraf-Nonius CAD4 diffractometer (Delft, The Netherlands) of the Biocrystallography Research Centre, at the University of Naples Federico II. During all data collection, three reflections were measured every 120 min in order to check the stability of the crystals and the electronics. The observed intensity decreases were within 3%. The intensities were corrected for Lorentz and polarization factors, but no absorption correction was applied. Unit cell determinations were carried out for all crystals by least-squares refinement of the setting angles of at least 25 high angle reflections accurately centred. Crystal data are listed in Tables 2 and 3.

The structures of the three Ac_7c derivatives [Ac_7c hydantoin, ClAc-Ac_7c-OH, and Z-Ac_7c-OH] were solved by direct methods and refined by the full-matrix least-squares procedure on F^2 (all data) with anisotropic thermal factors for all non-hydrogen atoms. Hydrogen atoms of Ac_7c hydantoin were calculated, and during the refinement were allowed to ride on the carrying atoms, with U_{iso} set equal to 1.2 times the U_{eq} of the attached atom. One carbon atom of the cycloheptane ring of ClAc-Ac_7c-OH ($C^{\gamma 2}_1$) is disordered over two sites (**A** and **B**), which refined with population parameters of 0.50. Hydrogen atoms of ClAc-Ac_7c-OH were calculated and treated as described above for those of Ac_7c hydantoin. The hydrogen atom of the two independent molecules of Z-Ac_7c-OH belonging to the carboxy group was located on a ΔF map, while the positions of all other hydrogen atoms were calculated. During the refinement all hydrogen atoms were allowed to ride on their carrying atoms, with U_{iso} set equal to 1.2 (or to 1.5 for the carboxylic acid hydrogen atom) multiplied by the U_{eq} of the attached atom.

The structures of the other Ac_7c compounds [Z-Ac_7c-L-Ala-OMe (with two crystallographically independent molecules, **A** and **B**, in the asymmetric unit), Z-Ac_7c-(L-Ala)$_2$-OMe, Z-$(Ac_7c)_3$-OBu^t, Z-$(Ac_7c)_4$-OBu^t, and Z-$(Ac_7c)_5$-OBu^t] were solved by direct methods and refined by full-matrix least-squares procedures on F^2 (all data) for the tri-, tetra- and penta-homopeptides and on F for the other compounds. As for Z-$(Ac_7c)_5$-OBu^t, difference Fourier techniques revealed one methanol molecule with statistical disorder on two sites, which were refined with population parameters of 0.5. Two of the carbon atoms of the cycloheptane ring in the **B** molecule of Z-Ac_7c-L-Ala-OMe ($C^{\gamma 1}_1$ and $C^{\gamma 2}_1$) are disordered each over two sites (**A** and **B**), both of which were refined with population parameters of 0.60 and 0.40. One of the carbon atoms of the cycloheptane ring of Z-Ac_7c-(L-Ala)$_2$-OMe ($C^{\gamma 1}_1$) is disordered over two sites

Table 2 Crystal data for the Ac_7c derivatives and the dipeptide

Parameter	Ac_7c hydantoin	ClAc-Ac_7c-OH	Z-Ac_7c-OH	Z-Ac_7c-L-Ala-OMe
Molecular formula	$C_9H_{14}N_2O_2$	$C_{10}H_{16}ClNO_3$	$C_{16}H_{21}N_2O_4$	$C_{20}H_{28}N_2O_5$
Formula mass	182.2	233.7	291.3	362.5
Crystallization solvent	Hot EtOH–H_2O [a] (1:1)	Acetone–LP [a]	AcOEt–LP [a]	$CHCl_3$–MeOH [a]
Crystal size/mm	0.6 × 0.5 × 0.4	0.5 × 0.3 × 0.1	0.6 × 0.3 × 0.3	0.3 × 0.5 × 0.4
Crystal system	Monoclinic	Monoclinic	Monoclinic	Monoclinic
Space group	$P2_1/c$	$P2_1/c$	I 1 2/a 1 (No. 15)	$P2_1$
Z, molecules/unit cell	4	4	16	4
a/Å	6.980(1)	9.827(1)	20.729(3)	9.553(2)
b/Å	6.969(1)	9.652(1)	14.375(2)	19.02(1)
c/Å	19.061(3)	12.553(2)	21.521(3)	11.587(5)
β/°	95.5(1)	107.4(1)	97.2(1)	103.32(2)
V/Å^3	992.9(2)	1136.2(7)	6362(2)	2049(2)
D_c/g cm^{-3}	1.31	1.37	1.22	1.22
Independent reflections	2219	2737	7465	4023
Observed reflections	1646 [$F > 4\sigma(F)$]	1148 [$F > 4\sigma(F)$]	1261 [$F > 4\sigma(F)$]	3474 [$I > 3\sigma(I)$]
Radiation/Å	Mo-Kα(0.71073)	Mo-Kα(0.71073)	Mo-Kα(0.71073)	Cu-Kα(1.54178)
Data collection method	θ–2θ	θ–2θ	θ–2θ	ω–2θ
θ range	2.2–28.0	2.2–28.0	2.2–28.0	1–70
Temperature	Ambient	Ambient	Ambient	Ambient
Solved by	SHELXS 86 [b]	SHELXS 86 [b]	SHELXS 86 [b]	SIR 92 [g]
Refined by	SHELXL 93 [c]	SHELXL 93 [c]	SHELXL 93 [c]	SDP [h]
R value	0.052 (R_1, on F)	0.042 (R_1, on F)	0.04261(R_1, on F)	0.072
R_w value	0.144 (wR_2, on F^2, all data)	0.128 (wR_2, on F^2, all data)	0.211 (wR_2, on F^2, all data)	0.064
w	d	e	f	$1/\sigma(F^2)$
S	0.997	0.835	0.637	0.891
$(\Delta\rho)_{max}$/e Å^{-3}	0.375	0.275	0.336	0.394
$(\Delta\rho)_{min}$/e Å^{-3}	−0.224	−0.225	−0.200	−0.086

[a] LP, light petroleum; AcOEt, ethyl acetate. [b] Ref. 33. [c] Ref. 34. [d] $w = 1/[\sigma^2(F_0^2) + (0.1143\,P)^2]$ where $P = (F_0^2 + 2F_c^2)/3$. [e] $w = 1/[\sigma^2(F_0^2) + (0.0577\,P)^2]$ where $P = (F_0^2 + 2F_c^2)/3$. [f] $w = 1/[\sigma^2(F_0^2) + (0.0878\,P)^2]$ where $P = (F_0^2 + 2F_c^2)/3$. [g] Ref. 35. [h] Ref. 36.

Table 3 Crystal data for the Ac_7c tri-, tetra-, and penta-peptides

Parameter	Z-Ac_7c-(L-Ala)$_2$-OMe	Z-$(Ac_7c)_3$-OBu^t	Z-$(Ac_7c)_4$-OBu^t	Z-$(Ac_7c)_5$-OBu^t
Molecular formula	$C_{23}H_{33}N_3O_6$	$C_{36}H_{55}N_3O_6$	$C_{43}H_{68}N_4O_7$	$C_{52}H_{81}N_5O_8CH_3OH$
Formula mass	447.5	625.9	753.0	936.3
Crystallization solvent	AcOEt–LP [a]	$CHCl_3$	AcOEt [a]	Toluene
Crystal size/mm	0.5 × 0.3 × 0.3	0.4 × 0.5 × 0.4	0.4 × 0.5 × 0.3	0.3 × 0.5 × 0.5
Crystal system	Monoclinic	Monoclinic	Monoclinic	Monoclinic
Space group	$P2_1$	$P2_1/c$	$P2_1/n$	$P2_1/n$
Z, molecules/unit cell	2	4	4	4
a/Å	11.434(3)	11.904(4)	19.105(7)	12.722(2)
b/Å	11.624(2)	19.867(5)	22.663(9)	20.501(7)
c/Å	9.524(3)	16.355(6)	10.576(4)	21.937(7)
β/°	105.46(2)	111.2(1)	101.4(1)	104.9(1)
V/Å^3	1220.1(6)	3605(2)	4489(3)	5530(3)
D_c/g cm^{-3}	1.22	1.15	1.11	1.12
Independent reflections	2431	6823	8502	10468
Observed reflections	1994 [$I > 3\sigma(I)$]	2238 [$I > 3\sigma(I)$]	5497 [$I > 2\sigma(I)$]	7566 [$I > 2\sigma(I)$]
Radiation/Å	Cu-Kα(1.54178)	Cu-Kα(1.54178)	Cu-Kα(1.54178)	Cu-Kα(1.54178)
Data collection method	ω–2θ	ω–2θ	ω–2θ	ω–2θ
θ range	1–70	1–70	1–70	1–70
Temperature	Ambient	Ambient	Ambient	Ambient
Solved by	SIR 92 [b]	SIR 92 [b]	SIR 92 [b]	SIR 92 [b]
Refined by	SDP [c]	SDP [c]	SHELXL 93 [d]	SHELXL 93 [d]
R value	0.076	0.076	0.081 [e]	0.086 [e]
R_w value	0.070	0.076	0.161 [f]	0.260 [d]
w	$1/\sigma(F^2)$	$1/\sigma(F^2)$	g	g
S	0.768	2.394	1.693	0.919
$(\Delta\rho)_{max}$/e Å^{-3}	0.409	0.300	0.540	0.636
$(\Delta\rho)_{min}$/e Å^{-3}	−0.697	−0.14	−0.315	−0.225

[a] AcOEt, ethyl acetate; LP, light petroleum. [b] Ref. 35. [c] Ref. 36. [d] Ref. 34. [e] R_1 (on F). [f] wR_2 (on F^2, all data). [g] $w = [\sigma^2 F_0^2 + 0.3625\,P^2 + 3.0739\,P]$ where $P = (F_0^2 + 2F_c^2)/3$.

(**A** and **B**), which were refined with population parameters of 0.80 and 0.20. Statistical disorder over two sites (**A** and **B**) was also found for residue 1 of Z-$(Ac_7c)_3$-OBu^t-($C^{\gamma 2}{}_1$ with population parameters of 0.50), for residues 1 and 4 of Z-$(Ac_7c)_4$-OBu^t- ($C^{\gamma 1}{}_1$ and $C^{\gamma 2}{}_1$, both with population parameters of 0.60 and 0.40, and $C^{\gamma 2}{}_4$ with population parameters of 0.80 and 0.20), and for residues 1,2 and 4 of Z-$(Ac_7c)_5$-OBu^t-($C^{\gamma 2}{}_1$ with population parameters of 0.60 and 0.40, $C^{\gamma 1}{}_2$ with population parameters of 0.80 and 0.20, and $C^{\gamma 1}{}_4$ with population parameters of 0.50). In all cases the non-hydrogen atoms were refined with anisotropic temperature factors. Positional parameters of the hydrogen atoms for Z-Ac_7c-L-Ala-OMe and Z-Ac_7c-(L-Ala)$_2$-OMe 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. Hydrogen atoms of Ac_7c homo-tri-, tetra- and penta-peptides were calculated and during the refinement were allowed to ride on their carrying atoms, with U_{iso} set equal to 1.2 times the U_{eq} of the attached atom.

Complete lists of bond lengths, bond angles and torsion

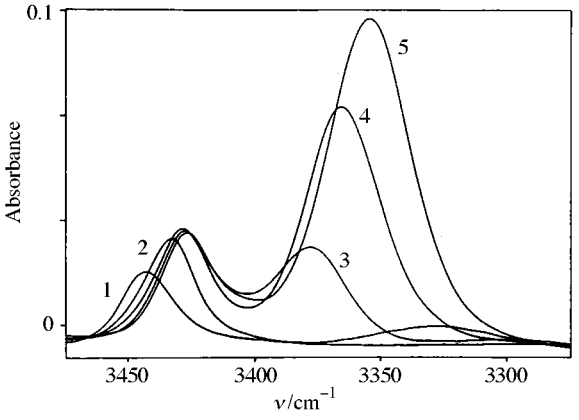


Fig. 1 IR absorption spectra (3500–3250 cm^{-1} region) of the Z-$(Ac_7c)_n$-OBu^t (n = 1–5) homopeptide series in $CDCl_3$ solution (conc.: 1×10^{-3} mol dm^{-3}). Numbers refer to peptide main-chain length.

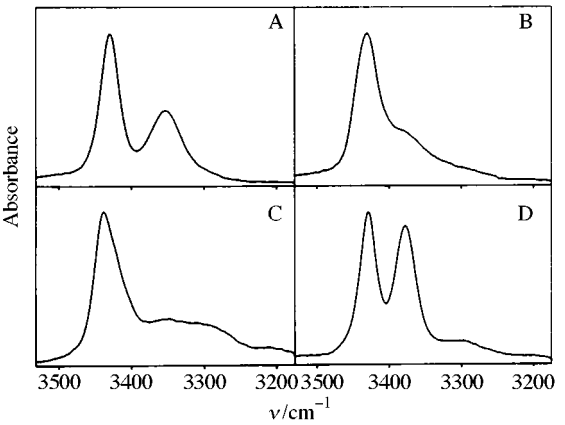


Fig. 2 IR absorption spectra (3500–3200 cm^{-1} region) of the tripeptides Z-Ac_7c-(L-Ala)$_2$-OMe (A), Z-L-Ala-Ac_7c-L-Ala-OMe (B), Boc-L-Leu-Ac_7c-L-Val-OMe (C), and Z-$(Ac_7c)_3$-OBu^t(D) in $CDCl_3$ solution (conc.: 1×10^{-3} mol dm^{-3})

angles, final positional parameters for all non-hydrogen atoms along with their thermal factors, have been deposited and are available from the Cambridge Crystallographic Data Centre (CCDC). See 'Instructions for Authors', *J. Chem. Soc., Perkin Trans. 2*, 1997, Issue 1. Any request to the CCDC for this material should quote the full literature citation and reference number 188/90.

Results and Discussion

Synthesis and characterization

Ac_7c hydantoin[22,29,37] was prepared by treatment of cycloheptanone with sodium cyanide and excess of ammonium carbonate in a 1:1 water–ethanol mixture under reflux for 6 h. Alkaline hydrolysis (with a 3 M NaOH solution) of the hydantoin, followed by acidification, afforded the free amino acid.[21,29,38–40]

The Z-protected Ac_7c derivative was obtained by reacting the free amino acid with *N*-(benzyloxycarbonyloxy)succinimide. Treatment of Z-Ac_7c-OH with one equivalent of *N*-ethyl,*N*′-(3-dimethylaminopropyl)carbodiimide (EDC) gave the 5(4*H*)-oxazolone from Z-Ac_7c-OH. The subsequent reaction of Z-Ac_7c-OH with the above mentioned oxazolone in an equimolar amount afforded the symmetrical anhydride (Z-Ac_7c)$_2$O.[26] Z-Ac_7c-OBu^t was obtained by esterification of the *N*-protected amino acid with isobutene in the presence of a catalytic amount of sulfuric acid. ClAc-Ac_7c-OH was synthesized by reacting ClAc-Cl with the free amino acid in an aqueous solution at alkaline pH.

Ac_7c-Ac_7c, L-Ala-Ac_7c and Ac_7c-L-Ala [the latter in the -Ac_7c-(L-Ala)$_2$-tripeptide] peptide bond formation was achieved by the symmetrical anhydride method. On the other hand, Ac_7c-L-Ala (in the dipeptide), Ac_7c-L-Val, and L-Leu-Ac_7c peptide bond formation was achieved using the EDC–HOBt (1-hydroxy-1,2,3-benzotriazole) method. Removal of the Z-group was performed by catalytic hydrogenation. The various peptides were characterized by melting point determination, optical rotatory power, TLC (in three solvent systems) and solid-state IR absorption spectroscopy (Table 1), amino acid analysis (Experimental section), and ^{1}H NMR spectroscopy (data not reported).

Solution conformational analysis

The conformational preferences adopted by the *N*- and *C*-protected Ac_7c-rich peptides were determined in the structure supporting solvent $CDCl_3$ by IR absorption spectroscopy and ^{1}H NMR spectroscopy as a function of concentration (over the range 10–0.1 $\times 10^{-3}$ mol dm^{-3}).

Fig. 1 shows the IR absorption spectra (N–H stretching region) of the Ac_7c homo-peptides series (from monomer through to pentamer) at 1×10^{-3} mol dm^{-3} concentration. The curves of the tripeptide and the higher oligomers are characterized by two bands, at about 3428 cm^{-1} (free, solvated NH groups) and at 3377–3354 cm^{-1} (strongly H-bonded NH groups), respectively.[41,42] The intensity of the low-frequency band relative to the high-frequency band increases as main-chain length increases. Concomitantly, the absorption maximum of the low-frequency band shifts markedly to lower wavenumbers. An inspection of the spectrum of the homo-tripeptide, compared to those of the Ac_7c-containing tripeptides Z-Ac_7c-(L-Ala)$_2$-OMe, Z-L-Ala-Ac_7c-L-Ala-OMe and Boc-L-Leu-Ac_7c-L-Val-OMe (Fig. 2) allows us to conclude that the 3377–3351 cm^{-1} band is much higher (relative to the 3438–3429 cm^{-1} band) in the homo-tripeptide. Furthermore, in the co-peptides the low-frequency band is more intense when the Ac_7c is incorporated at position 1 than at position 2. We have also been able to demonstrate that, even at 10^{-2} mol dm^{-3} concentration, there are only marginal changes in the various peptides (not shown). Therefore, the observed band at 3377–3351 cm^{-1} should be interpreted as arising almost exclusively from intramolecular N–H···O=C interactions. The present IR absorption study has provided convincing evidence that main-chain length dependent intramolecular H-bonding is an essential factor influencing the conformation of the *N*- and *C*-protected Ac_7c-rich peptides in $CDCl_3$ solution. Our results also support the view that Ac_7c is a stronger inducer of intramolecularly H-bonded structures than the protein amino acids Ala and Leu.

To get more detailed information on the preferred conformation of these peptides in $CDCl_3$ solution we carried out a 400 MHz ^{1}H NMR study. The delineation of inaccessible (or intramolecularly H-bonded) NH groups 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[43,44] to the $CDCl_3$ solution and (*ii*) free-radical (TEMPO) induced line broadening of NH resonances.[45] As a typical example, Fig. 3 illustrates the behaviour of the NH resonances of the homo-pentamer upon addition of DMSO and TEMPO. The upfield resonance in $CDCl_3$ solution is unequivocally assigned to the N(1)H *urethane* group.[46] The second upfield resonance is assigned to the N(2)H proton by analogy with the chemical shifts in the same halohydrocarbon and the spectroscopic behaviour upon addition of the same perturbing agents of peptides from different types of $C^{\alpha,\alpha}$-dialkylated glycines.[46–48] In one case a complete assignment of the NH protons was achieved from the COSY and ROESY spectra.[48] From an analysis of the spectra as a function of concentration (10–1 $\times 10^{-3}$ mol dm^{-3}) in $CDCl_3$ solution (results not shown), we have been able to conclude that dilution induces a negligible shift to higher fields of the NH resonances of all the

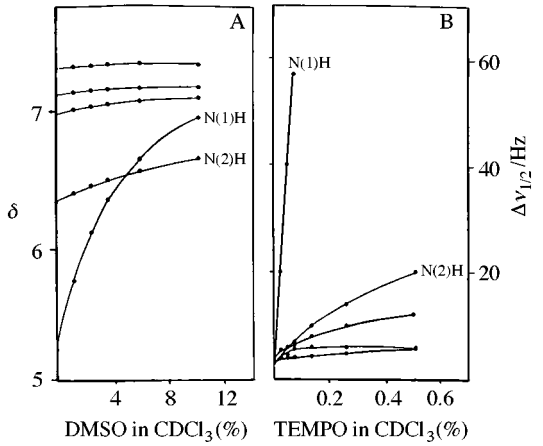


Fig. 3 (A) Plot of NH chemical shifts in the ^{1}H NMR spectrum of Z-$(Ac_7c)_5$-OBu^t as a function of increasing percentages of DMSO added to the $CDCl_3$ solution (v/v). (B) Plot of the bandwidth of the NH protons of the same peptide as a function of increasing percentages of TEMPO (mass/vol) in $CDCl_3$. Peptide concentration: 1 mol dm^3.

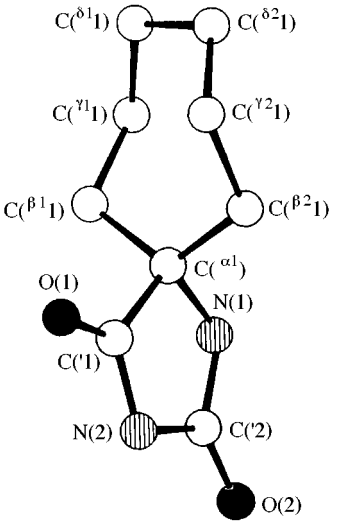


Fig. 4 X-Ray diffraction structure of Ac_7c hydantoin with numbering of the atoms

peptides investigated. In particular, the most sensitive N(1)H proton of the homo-pentapeptide shifts only by 0.05 ppm.

In the Ac_7c peptides examined in the $CDCl_3$–DMSO solvent mixtures and in the presence of the paramagnetic perturbing agent TEMPO at 1×10^{-3} mol dm^{-3} peptide concentration, 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. Interestingly, the sensitivity of the N(1)H proton is higher than that of the N(2)H proton. Class (*ii*) [N(3)H to N(5)H protons] includes those displaying behaviour characteristic of shielded protons (relative insensitivity of chemical shifts to solvent composition and of linewidths 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 10^{-2} mol dm^{-3}, the N(3)H to N(5)H protons of all the Ac_7c peptides studied are almost inaccessible to perturbing agents and are, therefore, most probably, intramolecularly H-bonded. In view of these IR absorption and ^{1}H NMR observations, it is reasonable to conclude that the most populated structures adopted in $CDCl_3$ solution by the *N*- and *C*-protected tri-, tetra- and penta-peptides are the β-turn, two consecutive β-turns, and the 3_{10}-helix, respectively.

Crystal-state conformational analysis

The structure of the Ac_7c hydantoin is represented in Fig. 4. As

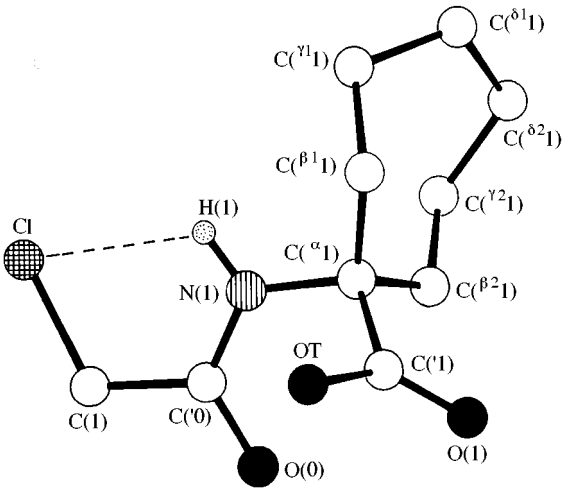


Fig. 5 X-Ray diffraction structure of ClAc-Ac_7c-OH with numbering of the atoms. The intramolecular H-bond is represented by a dashed line.

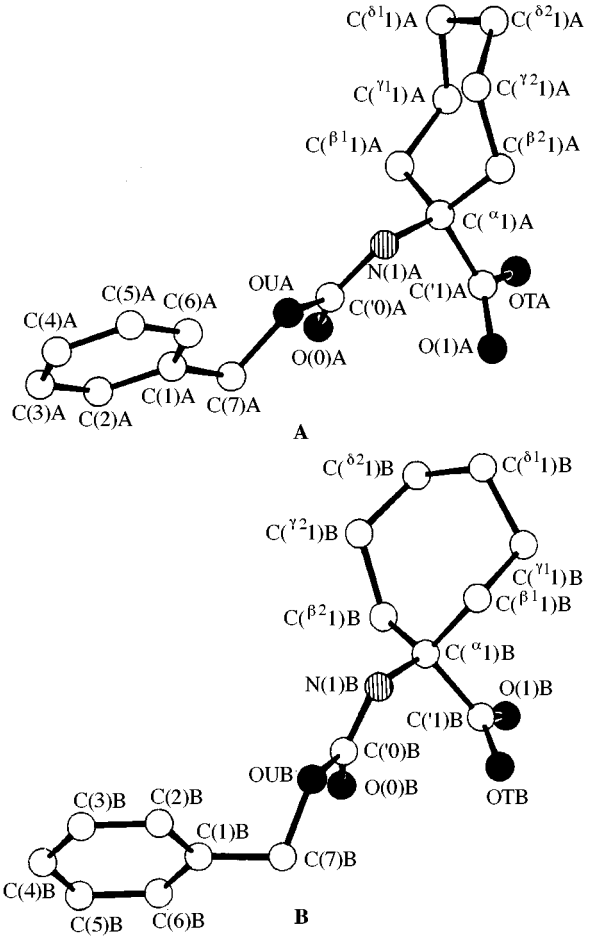
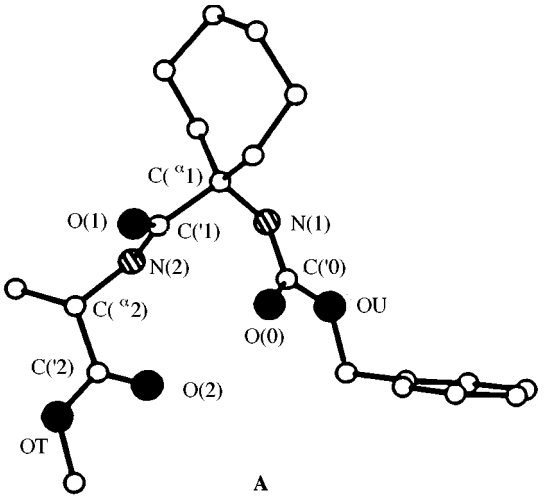


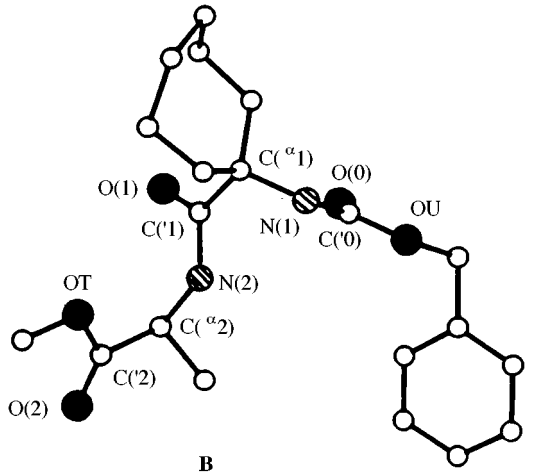
Fig. 6 X-Ray diffraction structure of the two independent molecules (**A** and **B**) in the asymmetric unit of Z-Ac_7c-OH

expected, the imidazolidine-2,5-dione ring is nearly planar with mean deviations of 0.002 Å from the average plane. All geometrical and conformational parameters (bond lengths and bond angles, torsion angles) are in good agreement with the corresponding average values obtained from a statistical analysis of the crystal-state hydantoin-containing compounds reported in the Cambridge Crystallographic Data Bank. Intermolecular H-bonding involves all available donors and acceptors (Table 6), with N···O distances within the average values for N–H···O=C separations.[49–51]

The molecular structures of ClAc-Ac_7c-OH, Z-Ac_7c-OH,



A



B

Fig. 7 X-Ray diffraction structure of the two independent molecules (**A** and **B**) in the asymmetric unit of Z-Ac_7c-L-Ala-OMe with numbering of the atoms

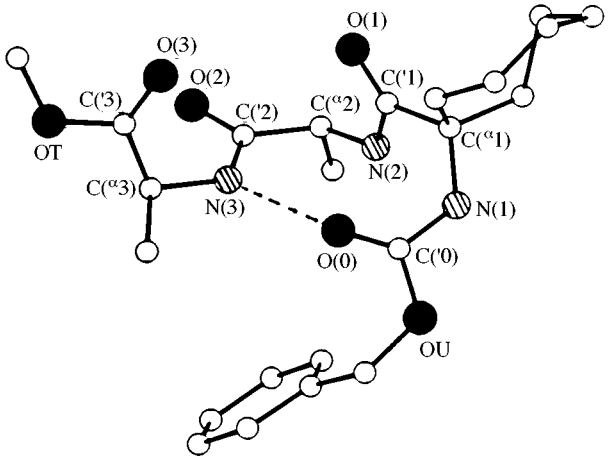


Fig. 8 X-Ray diffraction structure of Z-Ac_7c-(L-Ala)$_2$-OMe with numbering of the atoms. The intramolecular H-bond is represented by a dashed line.

Z-Ac_7c-L-Ala-OMe, Z-Ac_7c-(L-Ala)$_2$-OMe, Z-(Ac$_7$c)$_3$-OBu^t, Z-(Ac$_7$c)$_4$-OBu^t, and Z-(Ac$_7$c)$_5$-OBu^t with the atomic numbering schemes are shown in Figs. 5–11. Relevant backbone and side-chain torsion angles[52] are presented in Tables 4 and 5. In Table 6 the intra- and inter-molecular H-bond parameters are listed, while the average bond distances and bond angles characterizing the seven-membered ring system of the Ac_7c residue are given in Table 7.

Bond lengths and bond angles are in general agreement with

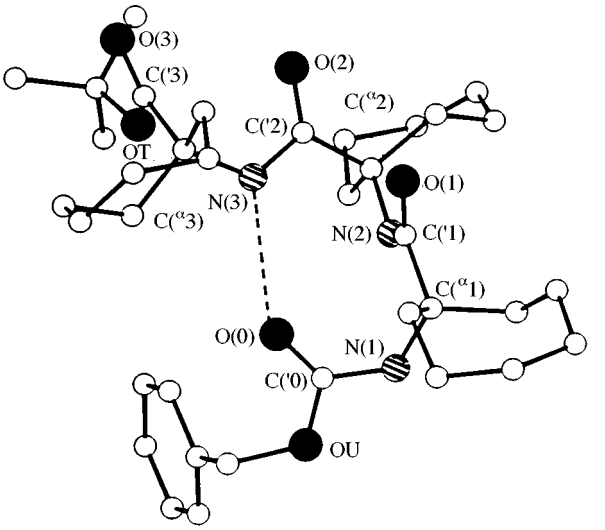


Fig. 9 X-Ray diffraction structure of Z-(Ac$_7$c)$_3$-OBu^t with numbering of the atoms. The intramolecular H-bond is represented by a dashed line.

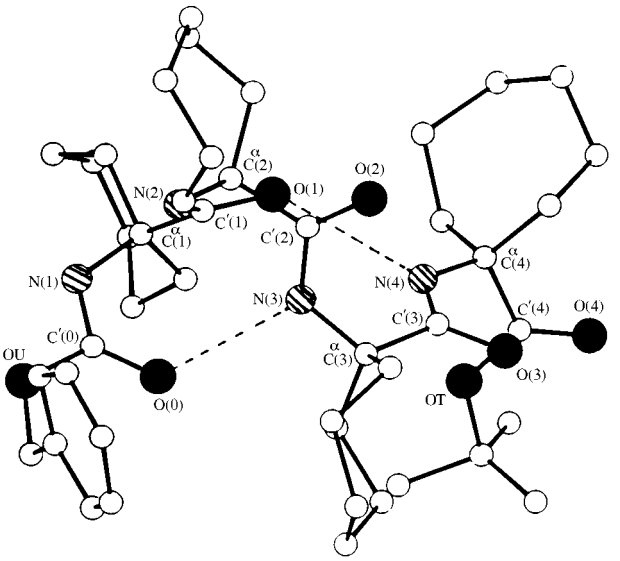


Fig. 10 X-Ray diffraction structure of Z-(Ac$_7$c)$_4$-OBu^t with numbering of the atoms. The two intramolecular H-bonds are represented by dashed lines.

previously reported values for the geometry of the benzyloxycarbonylamino[53] and monochloroacetamido[54] moieties, the ester groups,[55] and the peptide unit.[56,57] We have also calculated the average geometry for the Ac_7c residue. The average C–C bond length for the cycloheptane ring is 1.52 Å (with average lengths of 1.53 and 1.54 Å for the C^{α}–C^{β} bonds, 1.50 and 1.51 Å for the C^{β}–C^{γ} bonds, 1.52 and 1.55 Å for the C^{γ}–C^{δ} bonds, and 1.49 Å for the $C^{\delta 1}$–$C^{\delta 2}$ bond), in excellent agreement with the literature average value of 1.52 Å for the $-CH_2-CH_2-$ distance.[58] 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.[56] The average value for the bond angles internal to the seven-membered ring is 115.1°, definitely larger than the regular tetrahedral value (109.5°). The bond angles actually vary from 112.1(5)° at $C^{\gamma 2}$ to 117.6(5)° at $C^{\beta 2}$. The average geometrical parameters for the seven-membered ring of Ac_7c reported here compare well with those published for other Ac_7c residues and cycloheptane-containing compounds.[24–26,30,59] In addition, the bond angles indicate an asymmetric geometry for the C^{α} atom. This observation is common also to Aib- and Ac_nc- (n = 3–6, 8, 9) rich peptides.[7–11] The value for the conformationally sensitive N–C^{α}–C′ (τ) bond angle, external to the cyclic system, is 110.1(3)°, comparable to

Table 4 Selected torsion angles (°) for the Ac_7c derivatives and the dipeptide

Torsion angle	Ac_7c hydantoin	ClAc-Ac_7c-OH	Z-Ac_7c-OH		Z-Ac_7c-L-Ala-OMe	
			Mol. **A**	Mol. **B**	Mol. **A**	Mol. **B**
ω_0 [O_u–C'_0–N_1–C^{α}_1]	0.3(2)[a]	178.4(2)[c]	178.3(4)	169.3(4)	179.9(5)	178.4(5)
φ_1 [C'_0–N_1–C^{α}_1–C'_1]	−0.2(2)[b]	−50.9(3)	−54.5(6)	−49.1(6)	−50.7(7)	56.7(7)
ψ_1 [N_1–C^{α}_1–C'_1–N_2]	−0.1(1)	−44.4(3)[d]	159.3(4)[d]	−31.8(6)[d]	−45.7(7)	44.0(7)
ω_1 [C^{α}_1–C'_1–N_2–C^{α}_2]	0.3(2)				177.0(5)	175.3(5)
φ_2 [C'_1–N_2–C^{α}_2–C'_2]					−119.6(7)	−107.2(7)
ψ_2 [N_2–C^{α}_2–C'_2–O_T]					162.0(6)	28.3(8)
ω_2 [C^{α}_2–C'_2–O_T–C_T]					175.0(8)	−179.5(6)
$\chi_1^{1,2}$ [N_1–C^{α}_1–$C^{\beta 2}_1$–$C^{\gamma 2}_1$]	161.2(1)	74.9(4) [144.7(3)][e]	74.8(5)	85.7(5)	82.9(7)	−81.9(8) [−152(1)][e]
$\chi_1^{2,2}$ [C^{α}_1–$C^{\beta 2}_1$–$C^{\gamma 2}_1$–$C^{\delta 2}_1$]	−85.7(2)	77.3(5) [−81.4(4)][e]	−90.5(6)	88.3(6)	88.7(9)	−88(1) [86(2)][e]
$\chi_1^{3,2}$ [$C^{\beta 2}_1$–$C^{\gamma 2}_1$–$C^{\delta 2}_1$–$C^{\delta 1}_1$]	72.7(2)	−74.5(5) [75.8(5)][e]	72.8(7)	−74.2(7)	−75.6(9)	77(1) [−86(2)][e]
χ_1^{4} [$C^{\gamma 1}_1$–$C^{\delta 1}_1$–$C^{\delta 2}_1$–$C^{\gamma 2}_1$]	53.5(2)	9.2(5) [−53.7(4)][e]	60.0(8)	53.3(7)	55(1)	−59.1(8) [11(1)][e] [76(1)][e] [61(1)][e]
$\chi_1^{3,1}$ [$C^{\beta 1}_1$–$C^{\gamma 1}_1$–$C^{\delta 1}_1$–$C^{\delta 2}_1$]	69.3(2)	65.3(4)	79.8(7)	−69.8(6)	−73.2(9)	73.2(9) [−89(1)][e]
$\chi_1^{2,1}$ [C^{α}_1–$C^{\beta 1}_1$–$C^{\gamma 1}_1$–$C^{\delta 1}_1$]	−88.3(2)	−86.8(3)	−86.4(6)	89.9(5)	89.1(8)	−86.1(5) [91(1)][e]
$\chi_1^{1,1}$ [N_1–C^{α}_1–$C^{\beta 1}_1$–$C^{\gamma 1}_1$]	−82.0(2)	−70.1(3)	149.5(4)	−168.0(4)	−163.9(6)	−164.1(5) [80.9(9)][e]

[a] [N_2–C'_2–N_1–C^{α}_1]. [b] [C'_2–N_1–C^{α}_1–C'_1]. [c] [Cl–C'_0–N_1–C^{α}_1]. [d] [N_1–C^{α}_1–C'_1–O_T]. [e] Values in parentheses refer to atoms which show statistical occupancy.

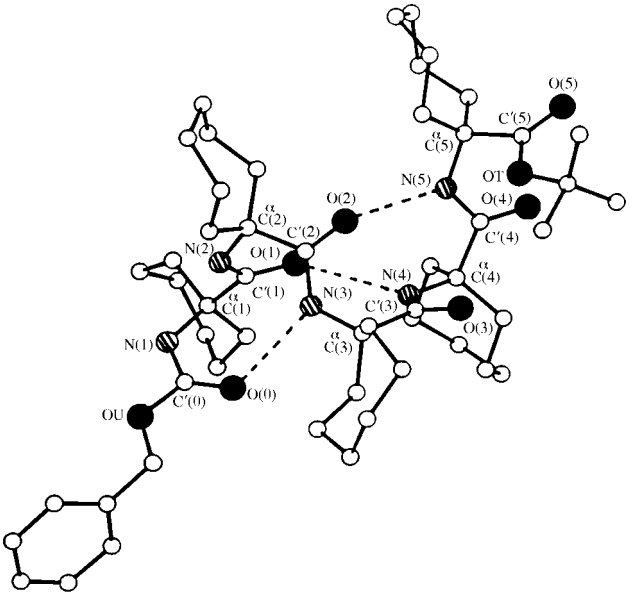


Fig. 11 X-Ray diffraction structure of Z-$(Ac_7c)_5$-OBu^t with numbering of the atoms. The three intramolecular H-bonds are represented by dashed lines.

that exhibited by the $C^{\alpha,\alpha}$-dialkylated glycines forming regular helices (110–111°).[7–11,60]

All of the Ac_7c residues are found in the helical region A(A*) of the conformational map,[18] with the exception of that of molecule **A** of Z-Ac_7c-OH which is *semi*-extended. Each of the Ac_7c derivatives and homo-peptides, having no chiral atoms, crystallizes in a centrosymmetric space group; thus, in each unit cell molecules of both handedness occur simultaneously. The average values for the φ, ψ backbone torsion angles of the Ac_7c residue completely involved in a bend or helical structure are ±58.8°, ±31.3°, close to those expected for a 3_{10} helix (±57°, ±30°).[17] Also the C-terminal Ac_7c residues of the homo- tri- and tetra-peptides adopt a conformation in the helical region, but they have opposite handedness to that shown by the preceding residues, a common observation for Aib- and Ac_nc- (n = 3–6, 8, 9) rich peptides.[7–11,13] However, the same helical handedness is maintained by the *C*-terminal residue of the homo-pentapeptide. The major conformational difference between molecules **A** and **B** of Z-Ac_7c-OH is seen in the ψ backbone torsion angle, extended for **A** while helical for **B**. Among the various conformational differences distinguishing molecules **A** and **B** of the chiral dipeptide Z-Ac_7c-L-Ala-OMe the most relevant is the opposite handedness of the helical Ac_7c residue. The Ac_7c-L-Ala and the *N*-terminal Ac_7c-Ac_7c sequences of the two tripeptides are folded in a 1 ⟵ 4 C=O ··· H–N intramolecularly H-bonded β-bend conformation. The β-bend is intermediate between type I ($\varphi_1 = -60°$, $\psi_1 = -30°$; $\varphi_2 = -90°$, $\psi_2 = 0°$) and type III ($\varphi_1 = -60°$, $\psi_1 = -30°$; $\varphi_2 = -60°$, $\psi_2 = -30°$)[14–16] in the Ac_7c/Ala tripeptides, whereas it is regular type III in the homo-trimer. The 1–3 sequence of the Ac_7c homo-tetramer forms an incipient 3_{10}-helix (two consecutive type-III β-turn conformations) stabilized by two 1 ⟵ 4 C=O ··· H–N intramolecular H-bonds. The backbone of the homo-pentamer is folded in a regular right(left)-handed 3_{10}-helix. Peptide groups N_3–H to N_5–H and C'_0=O_0 to C'_2=O_2 participate in three consecutive 1 ⟵ 4 C=O ··· H–N intramolecular H-bonds. In all bent and helical peptides the *N*-terminal intramolecular H-bond is weak.[49–51]

In the seven Ac_7c linear derivatives and peptides few significant deviations of the ω torsion angles from the ideal value of the *trans* planar urethane, amide, peptide and ester units (180°) are observed. In particular, the ω_0 torsion angles of molecule **B** of Z-Ac_7c-OH and of the homo-tri- and homo-penta-mer, and the ω_3 and ω_4 torsion angles of Z-$(Ac_7c)_4$-OBu^t differ by little more than 10° from 180°. In ClAc-Ac_7c-OH an intramolecular Cl ··· H–N_1 interaction is seen, producing a C_5 form,[15,54] the relevant θ^1[Cl–C(1)–C'_0–N_1] torsion angle being 13.8(4)°. The methyl and *tert*-butyl ester conformation with respect to the preceding C^{α}–N bond is intermediate between the *synperiplanar* and *synclinal* conformations in molecule **A** of Z-Ac_7c-L-Ala-OMe and in Z-Ac_7c-$(L\text{-Ala})_2$-OMe, while intermediate between the *anticlinal* and *antiperiplanar* conformations in molecule **B** of Z-Ac_7c-L-Ala-OMe and in the three Ac_7c homo-oligomers.[61]

Each of the ten seven-membered rings, in which statistically occupied positions are not present, is found in the twist-chair (TC) conformation,[62–70] although a substantial degree of distortion from this conformation is observed. The TC conformation, with C_2 symmetry, is that theoretically predicted as the minimum energy conformation for a cycloheptane ring.[70] A different behaviour is found for the nine Ac_7c residues of the molecules in which the statistically occupied positions for the seven-membered ring atoms occur. For these residues the most populated conformation is still the TC conformation, but a second conformation, the chair conformation (C), with C_s symmetry is found.[70] From the analysis of the experimental data it appears that the residues in the TC conformation have average dihedral angles ($\chi^4 = 51°$, $\chi^{3,1} = -73°$, $\chi^{3,2} = -68°$, $\chi^{2,1} = 87°$, $\chi^{2,2} = 86°$, $\delta^{1,1} = -32°$ and $\delta^{1,2} = -46°$) similar to those calculated for this conformation ($\chi^4 = 54.8°$, $\chi^3 = -72.5°$, $\chi^2 = 87.8°$ and $\delta^1 = -39.0°$).[70] Also the analysis of the residues in the C conformation shows average dihedral angles ($\chi^4 = -14°$, $\chi^{3,1} = 73°$, $\chi^{3,2} = -63°$, $\chi^{2,1} = -78°$, $\chi^{2,2} = 84°$, $\delta^{1,1} = 49°$ and

Table 5 Selected torsion angles (°) for the Ac_7c tri-, tetra-, and penta-peptides

Torsion angle	Z-Ac_7c-(L-Ala)$_2$-OBu^t	Z-(Ac_7c)$_3$-OBu^t	Z-(Ac_7c)$_4$-OBu^t	Z-(Ac_7c)$_5$-OBu^t
ω_0 [O_u–C′$_0$–N$_1$–C$^\alpha_1$]	178.4(5)	−164.9(7)	−174.0(3)	−166.9(3)
φ_1 [C′$_0$–N$_1$C$^\alpha_1$–C′$_1$]	− 58.6(7)	−62(1)	−62.9(4)	−59.4(4)
ψ_1 [N$_1$–C$^\alpha_1$–C′$_1$–N$_2$]	− 29.9(7)	−33(1)	−26.8(3)	−36.1(3)
ω_1 [C$^\alpha_1$–C′$_1$–N$_2$–C$^\alpha_2$]	− 176.3(5)	−174.4(7)	−176.5(2)	−171.6(2)
φ_2 [C′$_1$–N$_2$–C$^\alpha_2$–C′$_2$]	− 72.6(8)	−57(1)	−52.7(3)	−54.5(3)
ψ_2 [N$_2$–C$^\alpha_2$–C′$_2$–N$_3$]	− 20.8(9)	−32.7(9)	−31.9(3)	−32.4(3)
ω_2 [C$^\alpha_2$–C′$_2$–N$_3$–C$^\alpha_3$]	179.8(6)	179.1(6)	−173.9(2)	−174.6(2)
φ_3 [C′$_2$–N$_3$–C$^\alpha_3$–C′$_3$]	− 69.6(8)	49.8(9)	−59.2(3)	−53.6(3)
ψ_3 [N$_3$–C$^\alpha_3$–C′$_3$–N$_4$]	157.8(5)[a]	47.9(8)[a]	−28.2(3)	−36.3(3)
ω_3 [C$^\alpha_3$–C′$_3$–N$_4$–C$^\alpha_4$]	179.7(6)[b]	174.8(6)[b]	−169.4(2)	−173.9(2)
φ_4 [C′$_3$–N$_4$–C$^\alpha_4$–C′$_4$]			54.7(4)	−67.6(3)
ψ_4 [N$_4$–C$^\alpha_4$–C′$_4$–N$_5$]			45.4(4)[d]	−25.7(3)
ω_4 [C$^\alpha_4$–C′$_4$–N$_5$–C$^\alpha_5$]			167.0(4)[e]	−174.9(2)
φ_5 [C′$_4$–N$_5$–C$^\alpha_5$–C′$_5$]				−50.2(3)
ψ_5 [N$_5$–C$^\alpha_5$–C′$_5$–O$_T$]				−49.8(3)
ω_5 [C$^\alpha_5$–C′$_5$–O$_T$–C$_T$]				−176.2(3)
$\chi_1^{1,1}$ [N$_1$–C$^\alpha_1$–C$^{\beta1}_1$–C$^{\gamma1}_1$]	158.1(7) [−78.2(7)][c]	65(1)	62.0(5) [140.9(6)][c]	60.0(4)
$\chi_1^{2,1}$ [C$^\alpha_1$–C$^{\beta1}_1$–C$^{\gamma1}_1$–C$^{\delta1}_1$]	94(1) [−84.7(7)][c]	84(1)	89.1(7) [−87(1)][c]	86.6(5)
$\chi_1^{3,1}$ [C$^{\beta1}_1$–C$^{\gamma1}_1$–C$^{\delta1}_1$–C$^{\delta2}_1$]	− 81(1) [80.4(8)][c]	−54(2)	−66(1) [96(1)][c]	−49.9(8)
χ_1^{4} [C$^{\gamma1}_1$–C$^{\delta1}_1$–C$^{\delta2}_1$–C$^{\gamma2}_1$]	47(1) [−22(1)][c]	−21(2) [41(2)][c]	−6(1) [−15(1)][c] [−75(1)][c] [54(1)][c]	−24.2(9) [35(1)][c]
$\chi_1^{3,2}$ [C$^{\beta2}_1$–C$^{\gamma2}_1$–C$^{\delta2}_1$–C$^{\delta1}_1$]	− 53(2)	79(1) [−78(2)][c]	68.(1) [−80(1)][c]	76.6(7) [−69(1)][c]
$\chi_1^{2,2}$ [C$^\alpha_1$–C$^{\beta2}_1$–C$^{\gamma2}_1$–C$^{\delta2}_1$]	71(2)	−75(2) [90(2)][c]	−71.3(7) [79(1)][c]	−70.2(6) [79(1)][c]
$\chi_1^{1,2}$ [N$_1$–C$^\alpha_1$–C$^{\beta2}_1$–C$^{\gamma2}_1$]	86(1)	−75(1) [−145(1)][c]	−74.6(5) [−136.1(7)][c]	−75.5(4) [−142.9(8)][c]
$\chi_2^{1,1}$ [N$_2$–C$^\alpha_2$–C$^{\beta1}_2$–C$^{\gamma1}_2$]		87.6(9)	77.8(4)	80.9(5) [153(1)][c]
$\chi_2^{2,1}$ [C$^\alpha_2$–C$^{\beta1}_2$–C$^{\gamma1}_2$–C$^{\delta1}_2$]		81(1)	86.3(5)	84.7(6) [−85(2)][c]
$\chi_2^{3,1}$ [C$^{\beta1}_2$–C$^{\gamma1}_2$–C$^{\delta1}_2$–C$^{\delta2}_2$]		−70(2)	−68.9(6)	−67.2(8) [92(2)][c]
χ_2^{4} [C$^{\gamma1}_2$–C$^{\delta1}_2$–C$^{\delta2}_2$–C$^{\gamma2}_2$]		51(1)	53.8(8)	48(1) [−16(1)][c]
$\chi_2^{3,2}$ [C$^{\beta2}_2$–C$^{\gamma2}_2$–C$^{\delta2}_2$–C$^{\delta1}_2$]		−67(1)	−72.5(6)	−65.4(8)
$\chi_2^{2,2}$ [C$^\alpha_2$–C$^{\beta2}_2$–C$^{\gamma2}_2$–C$^{\delta2}_2$]		87(1)	89.1(5)	87.5(5)
$\chi_2^{1,2}$ [N$_2$–C$^\alpha_2$–C$^{\beta2}_2$–C$^{\gamma2}_2$]		−165.1(7)	−161.4(3)	−166.3(3)
$\chi_3^{1,1}$ [N$_3$–C$^\alpha_3$–C$^{\beta1}_3$–C$^{\gamma1}_3$]		−86.0(8)	77.3(3)	155.2(3)
$\chi_3^{2,1}$ [C$^\alpha_3$–C$^{\beta1}_3$–C$^{\gamma1}_3$–C$^{\delta1}_3$]		−84.6(9)	91.1(4)	−85.5(4)
$\chi_3^{3,1}$ [C$^{\beta1}_3$–C$^{\gamma1}_3$–C$^{\delta1}_3$–C$^{\delta2}_3$]		71(1)	−70.1(5)	72.7(5)
χ_3^{4} [C$^{\gamma1}_3$–C$^{\delta1}_3$–C$^{\delta2}_3$–C$^{\gamma2}_3$]		−53(1)	49.3(5)	−56.3(6)
$\chi_3^{3,2}$ [C$^{\beta2}_3$–C$^{\gamma2}_3$–C$^{\delta2}_3$–C$^{\delta1}_3$]		67(1)	−68.7(4)	73.0(5)
$\chi_3^{2,2}$ [C$^\alpha_3$–C$^{\beta2}_3$–C$^{\gamma2}_3$–C$^{\delta2}_3$]		−88.2(8)	88.1(4)	−90.4(4)
$\chi_3^{1,2}$ [N$_3$–C$^\alpha_3$–C$^{\beta2}_3$–C$^{\gamma2}_3$]		167.7(6)	−159.4(2)	−78.1(3)
$\chi_4^{1,1}$ [N$_4$–C$^\alpha_4$–C$^{\beta1}_4$–C$^{\gamma1}_4$]		−157.6(3)	64.6(5) [140.2(4)][c]	
$\chi_4^{2,1}$ [C$^\alpha_4$–C$^{\beta1}_4$–C$^{\gamma1}_4$–C$^{\delta1}_4$]			84.9(5)	93.7(7) [−77.6(7)][c]
$\chi_4^{3,1}$ [C$^{\beta1}_4$–C$^{\gamma1}_4$–C$^{\delta1}_4$–C$^{\delta2}_4$]			−71.3(6)	−78.7(9) [79.0(8)][c]
χ_4^{4} [C$^{\gamma1}_4$–C$^{\delta1}_4$–C$^{\delta2}_4$–C$^{\gamma2}_4$]			57.0(7) [−13.7(7)][c]	−0.8(9) [−66.0(8)][c]
$\chi_4^{3,2}$ [C$^{\beta2}_4$–C$^{\gamma2}_4$–C$^{\delta2}_4$–C$^{\delta1}_4$]			−77.0(6) [88(1)][c]	72.2(8)
$\chi_4^{2,2}$ [C$^\alpha_4$–C$^{\beta2}_4$–C$^{\gamma2}_4$–C$^{\delta2}_4$]			90.5(4) [−86(1)][c]	−82.1(6)
$\chi_4^{1,2}$ [N$_4$–C$^\alpha_4$–C$^{\beta2}_4$–C$^{\gamma2}_4$]			83.7(3) [159.6(6)][c]	−71.5(3)
$\chi_5^{1,1}$ [N$_5$–C$^\alpha_5$–C$^{\beta1}_5$–C$^{\gamma1}_5$]				92.9(4)
$\chi_5^{2,1}$ [C$^\alpha_5$–C$^{\beta1}_5$–C$^{\gamma1}_5$–C$^{\delta1}_5$]				81.0(5)
$\chi_5^{3,1}$ [C$^{\beta1}_5$–C$^{\gamma1}_5$–C$^{\delta1}_5$–C$^{\delta2}_5$]				−71.3(7)
χ_5^{4} [C$^{\gamma1}_5$–C$^{\delta1}_5$–C$^{\delta2}_5$–C$^{\gamma2}_5$]				49.4(8)
$\chi_5^{3,2}$ [C$^{\beta2}_5$–C$^{\gamma2}_5$–C$^{\delta2}_5$–C$^{\delta1}_5$]				−64.2(6)
$\chi_5^{2,2}$ [C$^\alpha_5$–C$^{\beta2}_5$–C$^{\gamma2}_5$–C$^{\delta2}_5$]				91.0(4)
$\chi_5^{1,2}$ [N$_5$–C$^\alpha_5$–C$^{\beta2}_5$–C$^{\gamma2}_5$]				−174.4(3)

[a] [N$_3$–C$^\alpha_3$–C′$_3$–O$_T$]. [b] [C$^\alpha_3$–C′$_3$–O$_T$–C$_T$]. [c] Values in parentheses refer to atoms which show statistical occupancy. [d] [N$_4$–C$^\alpha_4$–C′$_4$–O$_T$]. [e] [C$^\alpha_4$–C′$_4$–O$_T$–C$_T$].

$\delta^{1,2} = -52°$) similar to those calculated for this conformation ($\chi^4 = 1.2°$, $\chi^{3,1} = 67.2°$, $\chi^{3,2} = -67.2°$, $\chi^{2,1} = -85.6°$, $\chi^{2,2} = 85.6°$, $\delta^{1,1} = 64.6°$ and $\delta^{1,2} = -64.6°$).[70]

In addition, it is noteworthy that for residues in the TC conformation the $\chi^{1,1}$ and $\chi^{1,2}$ side-chain torsion angles are in the (t, g^+) and (g^-, t) conformations for right-handed and left-handed Ac_7c residues, respectively, while for residues in the C conformation they are in the (g^-, g^+) and (g^+, g^-) conformations for right-handed and left-handed Ac_7c residues, respectively.

The packing mode of the ClAc-Ac_7c-OH molecules is characterized by (carboxylic acid) O_T–H···O_0=C′$_0$ (amide) intermolecular H-bonds, forming rows along the b direction.[54] The geometrical parameters of this O–H···O H-bond are in the ranges expected for such interactions.[71,72]

In the crystal of Z-Ac_7c-OH the molecules are connected through a complex network of intermolecular H-bonds, in which O_T–H···O_1 H-bonds are formed between molecules of the same type (**A** or **B**), whereas N_1–H···O_0 H-bonds link **A** to **B** molecules.

The two independent molecules (**A** and **B**) of Z-Ac_7c-L-Ala-OMe pack together along the a direction, producing rows of molecules stabilized by four N–H···O=C intermolecular H-bonds [N_{1A}–H···O_{0B}=C′$_{0B}$, N_{2A}–H···O_{1B}=C′$_{1B}$, N_{1B}–H···O_{0A}=C′$_{0A}$ and N_{2B}–H···O_{1A}=C′$_{1A}$]. Then, van der Waals interactions link together rows of peptide molecules running in the b and c directions.

The Z-Ac_7c-(L-Ala)$_2$-OMe 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_2=C′$_2$). In addition, the crystal structure is stabilized by van der Waals interactions in the ab plane.

The packing modes of Z-(Ac_7c)$_3$-OBu^t, Z-(Ac_7c)$_4$-OBu^t and Z-(Ac_7c)$_5$-OBu^t molecules are similar and characterized by one intermolecular H-bond between the (urethane) N–H of the first residue and the C′=O (peptide) of the $n-1$ residue (N_1–H···O_{n-1}=C′$_{n-1}$). These intermolecular H-bonds are established along the a direction for Z-(Ac_7c)$_3$-OBu^t and Z-(Ac_7c)$_4$-OBu^t and the c direction for Z-(Ac_7c)$_5$-OBu^t molecules,

Table 6 Intra- and inter-molecular H-bond parameters for the Ac_7c derivatives and peptides

Peptide	Donor D–H	Acceptor A	Symmetry equiv. of A	Distance/Å D ··· A	Distance/Å H ··· A	Angle (°) D–H ··· A
Ac_7c hydantoin	N_1–H	O_2	$-x, \frac{1}{2}+y, \frac{1}{2}-z$	2.905(2)	2.082(2)	159.1(1)
	N_2–H	O_1	$-x, -y, 1-z$	2.896(2)	2.068(2)	161.9(1)
ClAc-Ac_7c-OH	N_1–H	Cl	x, y, z	2.973(3)	2.513(9)	114.3(2)
	O_T–H	O_0	$2-x, y-\frac{1}{2}, -\frac{1}{2}-z$	2.630(3)	1.840(3)	161.3(2)
Z-Ac_7c-OH	N_1B–H	O_0A	x, y, z	2.944(5)	2.239(5)	139.2(5)
	O_TA–H	O_1A	$\frac{3}{2}-x, \frac{3}{2}-y, \frac{3}{2}-z$	2.726(5)	1.912(5)	171.8(4)
	N_1A–H	O_0B	$2-x, y-\frac{1}{2}, \frac{3}{2}-z$	2.890(5)	2.069(5)	159.3(4)
	O_TB–H	O_1B	$2-x, 2-y, 2-z$	2.606(5)	1.794(5)	170.6(4)
Z-Ac_7c-L-Ala-OMe	N_1B–H	O_0A	x, y, z	2.905(6)	1.941(4)	165.9(3)
	N_2B–H	O_1A	x, y, z	2.891(6)	2.065(4)	141.1(3)
	N_1A–H	O_0B	$x+1, y, z$	3.018(6)	2.050(4)	168.5(3)
	N_2A–H	O_1B	$x+1, y, z$	2.973(6)	2.099(4)	147.9(4)
Z-Ac_7c-(L-Ala)$_2$-OMe	N_3–H	O_0	x, y, z	3.117(8)	2.220(5)	154.8(4)
	N_1–H	O_2	$-x, \frac{1}{2}+y, 1-z$	2.861(7)	2.066(6)	139.6(4)
Z-(Ac_7c)$_3$-OBu^t	N_3–H	O_0	x, y, z	3.144(8)	2.270(5)	150.9(4)
	N_1–H	O_2	$1-x, -y-\frac{1}{2}, -z+\frac{1}{2}$	2.930(9)	1.982(6)	172.4(4)
Z-(Ac_7c)$_4$-OBu^t	N_3–H	O_0	x, y, z	3.204(3)	2.368(3)	164.08(8)
	N_4–H	O_1	x, y, z	3.032(3)	2.187(3)	167.43(8)
	N_1–H	O_3	$x+\frac{1}{2}, -y+\frac{1}{2}, z+\frac{1}{2}$	2.885(3)	2.066(3)	158.87(9)
Z-(Ac_7c)$_5$-OBu^t	N_3–H	O_0	x, y, z	3.205(3)	2.338(3)	158.89(8)
	N_4–H	O_1	x, y, z	3.070(3)	2.261(3)	156.68(7)
	N_5–H	O_2	x, y, z	3.021(3)	2.226(3)	153.85(7)
	N_1–H	O_4	$x-\frac{1}{2}, -y+\frac{3}{2}, z-\frac{1}{2}$	2.888(3)	2.035(3)	167.02(7)

Table 7 Average bond distances and bond angles for the Ac_7c residue

Bond distance/Å		Bond angle (°)	
N–C^α	1.465(5)	N–C^α–C′	110.1(3)
C^α–C′	1.537(6)	$C^{\beta1}$–C^α–$C^{\beta2}$	113.7(4)
C′–O	1.220(6)	C^α–$C^{\beta1}$–$C^{\gamma1}$	116.6(5)
C^α–$C^{\beta1}$	1.534(4)	$C^{\beta1}$–$C^{\gamma1}$–$C^{\delta1}$	113.7(6)
$C^{\beta1}$–$C^{\gamma1}$	1.496(8)	$C^{\gamma1}$–$C^{\delta1}$–$C^{\delta2}$	116.7(6)
$C^{\gamma1}$–$C^{\delta1}$	1.518(9)	$C^{\delta1}$–$C^{\delta2}$–$C^{\gamma2}$	115.5(6)
$C^{\delta1}$–$C^{\delta2}$	1.49(1)	$C^{\delta2}$–$C^{\gamma2}$–$C^{\beta2}$	112.1(5)
$C^{\delta2}$–$C^{\gamma2}$	1.55(1)	$C^{\gamma2}$–$C^{\beta2}$–C^α	117.6(5)
$C^{\gamma2}$–$C^{\beta2}$	1.508(8)	N–C^α–$C^{\beta1}$	110.6(4)
$C^{\beta2}$–C^α	1.536(6)	N–C^α–$C^{\beta2}$	109.3(4)
		C′–C^α–$C^{\beta1}$	106.3(4)
		C′–C^α–$C^{\beta2}$	108.9(4)

producing long rows of H-bonded peptide molecules. The crystal structures are further stabilized by van der Waals interactions along the other crystallographic directions. The statistical methanol molecule in the Z-(Ac_7c)$_5$-OBu^t structure is not intermolecularly H-bonded, and is located in a solvophobic region in the crystallographic packing.

Conclusions

The experimental results described in this paper conclusively reconfirm the few and scattered data reported in previous studies.[20,27–30] strongly supporting the view that the medium-ring alicyclic Ac_7c residue imparts considerable restriction to the peptide backbone and is forced to adopt conformations in the 3_{10}/α-helical region of the φ, ψ space. Thus, the Ac_7c residue can be easily accommodated in either position $i + 1$ or $i + 2$ of type III(III′) β-bend and at the position $i + 1$ of type I(I′) β-bend. It may also be accommodated, although with some deviation from the expected φ, ψ values, at the position $i + 2$ of type I(I′) or type II(II′) β-bends. In summary, Ac_7c has a different effective volume and hydrophobicity to Aib and Ac_nc (with n = 4–6, 8, 9) residues, but all of these $C^{\alpha,\alpha}$-dialkylated glycines exhibit strictly comparable, strong conformational bias to bending and helix formation.[7–13] This remarkable lack of flexibility of Ac_7c is also reflected in the strictly comparable conformations found for its *N*- and *C*-protected tri-, tetra-, and penta-peptides in solution and in the crystal state.

Considerable recent interest has been focused on the development of conformationally constrained analogues of bioactive peptides.[1–5] The availability of highly active, structurally restricted agonists and antagonists is of great value in delineating the nature of receptor-bound conformations. It seems reasonable to foresee that future investigations on analogues of biologically relevant peptides, incorporating Aib and Ac_nc (with n = 4–9) residues at selected positions, will be rewarding.

Interestingly, $C^{\alpha,\alpha}$-di-*n*-propylglycine (Dpg),[20,28,29,73–77] with the same number of side-chain carbon atoms as Ac_7c, has been shown to favour fully extended (C_5) conformations (φ, $\psi \cong 180$, 180°).[15,78] Comparison of the conformational preferences of Dpg and Ac_7c serves to highlight the effect of side-chain cyclization on conformation.

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