

Peptide with Built-In Conformational Constraints: Structure of Z-(Aib)₇-O'Bu

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Abstract

C₄₀H₆₅N₇O₁₀.2H₂O, $M_r = 803.1$, triclinic, $P\bar{1}$, $a = 10.082$ (3), $b = 15.523$ (5), $c = 16.301$ (5) Å, $\alpha = 87.32$ (3), $\beta = 77.00$ (3), $\gamma = 79.70$ (2)°, $V = 2445.7$ (8) Å³, $Z = 2$, $D_x = 1.09$ g cm⁻³, $\lambda(\text{Cu } K\alpha) = 1.5418$ Å, $\mu = 6.1$ cm⁻¹, $F(000) = 868$, $T = 293$ K, final $R = 0.077$ for 5187 observed reflections. The peptide adopts a 3_{10} -helical conformation consisting of five consecutive 10-atom hydrogen-bonded β -turns of type III. Both helical senses (left- and right-handed) are present in the crystal. The molecules are hydrogen bonded head-to-tail in the crystal forming infinitely long helical columns along the direction $[1\bar{1}0]$.

Introduction

The design of peptides containing sterically constrained amino acids provides a powerful method for engineering synthetic protein mimics with well-defined conformations and novel functions (DeGrado, 1988). The conformationally constrained amino acid α -aminoisobutyric acid (Aib) has provoked a great deal of interest because the conformational space available to it comprises only a few percent of the space accessible to the standard amino acids and contains the particularly useful regions of left- and right-handed α - and 3_{10} -helices (DeGrado, 1988; Paterson, Rumsey, Benedetti, Nemethy & Sheraga, 1981). Furthermore, Aib is of particular biological interest because it occurs extensively in transmembrane channel-forming peptide antibiotics of the alamethicin family (peptaibols). So far, crystallographic investigations of Aib-containing peptides have clearly established the preference for

α -helical (Schmitt, Winter, Bosch & Jung, 1982; Fox & Richards, 1982) or 3_{10} -helical (Bosch, Jung, Schmitt & Winter, 1985; Bavoso, Benedetti, Di Blasio, Pavone, Pedone, Toniolo & Bonora, 1986; Vlasi, Brueckner & Kokkinidis, 1992) conformations. These investigations have yielded surprising insights into the nature of helix folding and conformational flexibility (Karle, Flippen-Anderson, Uma & Balaram, 1990; Gessmann, Brueckner & Kokkinidis, 1991). However, in order to achieve more accurate structure predictions and to improve peptide design, a larger database of structures is required. In the course of our investigation of Aib-containing peptides we have carried out the X-ray structure analysis of the terminally blocked homopeptide Z-(Aib)₇-O'Bu (where Z represents benzyl-oxycarbonyl and O'Bu the *tert*-butyl ester). Previously reported structures of Aib-containing homopeptides have clearly established a preference for the formation of 3_{10} -helices (Bavoso, Benedetti, Di Blasio, Pavone, Pedone, Toniolo & Bonora, 1986; Vlasi, Brueckner & Kokkinidis, 1992).

Experimental

The title compound was synthesized from Z-Aib₅-Ox (Brueckner, 1989; Jones, Kenner, Preston & Shepard, 1965; Ox represents the oxazolone of C-terminal Aib) and H-Aib₂-O'Bu (Brueckner & Jung, 1982) by conventional solution-phase procedures and crystallized from ethyl acetate/*n*-hexane. Colourless single crystals were obtained by evaporation from a methanol/H₂O (1:1) solution. X-ray intensity data were collected from a crystal with the dimensions 0.2 × 0.3 × 0.1 mm using an Enraf-Nonius CAD-4 automatic diffractometer (graphite-monochromated Cu $K\alpha$ radiation, ω - 2θ scans up to $2\theta = 130^\circ$). Unit-cell parameters were determined using 25 high-angle

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reflections. Eight standard reflections were monitored periodically and showed no significant change of intensity during data collection. A total of 6354 unique reflections were measured at room temperature. 5210 reflections with $I \geq 2.5\sigma(I)$ were classified as observed and used for structure determination and refinement: $-11 \leq h \leq 11$, $-18 \leq k \leq 18$, $0 \leq l \leq 19$. All intensities were corrected for Lorentz and polarization factors but not for absorption ($\mu = 6.1 \text{ cm}^{-1}$ for Cu $K\alpha$ radiation); extinction was disregarded. The structure was solved by direct methods using *SAYTAN* in the *MULTAN87* (Debaerdmackeer, Germain, Main, Tate & Woolfson, 1987) package with the 471 largest E values ($|E| > 1.2$). An E map calculated from the phase set with the lowest ψ_0 figure of merit ($\psi_0 = 1.257$) revealed 30 out of 57 non-H atoms. The remaining atoms and the O atoms of co-crystallized water molecules were found from subsequent Fourier syntheses using *SHELXS86* (Sheldrick, 1986). The R value at the end of the isotropic refinement was 0.18, and reached the value of 0.11 after refinement of anisotropic thermal parameters. The H atoms of the NH groups were located by difference Fourier syntheses (except for Aib3 and Aib6) and refined isotropically. Other H atoms were placed in geometrically calculated positions using the riding model approach (C—H = 1.08 Å). No H atoms were calculated for the water molecules. Refinement was carried out by least squares on F using *SHELX76* (Sheldrick, 1976). 5187 observed reflections were used for the refinement of 575 parameters [23 reflections with $||F_o| - |F_c|| > 4\sigma(F)$ were excluded in the final stage of the refinement]. Final $R = 0.077$, $wR = 0.091$, $\sum w(\Delta F)^2$ minimized, $w = [\sigma^2(F) + 0.0274455F^2]^{-1}$, $S = 0.73$; in the final cycle of refinement $(\Delta/\sigma)_{\max} = 0.8$ (for non-H atoms). Maximum and minimum electron densities were 0.28 and -0.44 e \AA^{-3} , respectively, in the final difference Fourier synthesis. Atomic scattering factors were taken from the *International Tables for X-ray Crystallography* (1974, Vol. IV). Torsion angles and other geometrical parameters were calculated using *PARST* (Nardelli, 1983) and *XANADU* (Roberts & Sheldrick, 1975).

Results and discussion

Atomic coordinates and equivalent isotropic temperature factors with their estimated standard deviations are listed in Table 1.* A perspective view of the

* Lists of structure factors, anisotropic thermal parameters, full bond lengths and angles, torsion angles, intermolecular contacts, and H-atom parameters have been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 55666 (41 pp.). Copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England. [CIF reference: MU0298]

title compound with all atoms [named according to the definition by IUPAC-IUB Commission on Biochemical Nomenclature (1970)] is given in Fig. 1 (*PLUTO*; Motherwell & Clegg, 1987). The heptapeptide forms a regular 3_{10} -helix. Owing to the space-group symmetry both helical senses (left- and right-handed 3_{10} -helix) are present in the crystal. The helix is formed by five consecutive 10-atom hydrogen-bonded β -turns of type III (right-handed helix) or III' (left-handed helix). Selected bond lengths and angles are given in Table 2. The backbone torsion angles φ , ψ and ω (as defined by IUPAC-IUB) which describe the folding of the peptide are given in Table 3. The conformational angles φ , ψ of residues Aib1–Aib6 have the average values of $\langle\varphi\rangle = -54.2^\circ$, $\langle\psi\rangle = -28.9^\circ$ (for the right-handed helix) and are comparable to those observed for 3_{10} -helices in proteins (Barlow & Thornton, 1988) or in Aib-containing homopeptides (Bavoso, Benedetti, Di Blasio, Pavone, Pedone, Toniolo & Bonora, 1986; Vlassi, Brueckner & Kokkinidis, 1992). In residue Aib7, however, there is a change of signs for φ , ψ relative to the preceding residues, which is a common behaviour in Aib-containing peptides (Vlassi, Brueckner & Kokkinidis, 1992); the φ , ψ angles of Aib7 are close to the standard values for α -helices ($\varphi = -59^\circ$, $\psi = -44^\circ$ for a right-handed helix) given by Arnott & Wonacott (1966). The intramolecular hydrogen-bonding pattern of the molecule is typical for a 3_{10} -helix (Fig. 1; Table 4) consisting of five 4 \rightarrow 1 hydrogen bonds which are fairly short and linear. The remaining hydrogen-bonding capacity of the molecule is saturated either intermolecularly or with hydrogen bonds involving four water molecules (Table 4). The parameters of the hydrogen-bond geometries and the frequency of hydration of the C=O and NH groups are in excellent agreement with the values obtained from protein structures (Baker & Hubbard, 1984). The valence geometry around the C^α atom is asymmetric for all Aib residues: if one designates as C_1^β and C_R^β the atoms in Aib which occupy the same position as C^β and the α -H atom respectively in L-amino acids, then for a right-handed (negative φ , ψ values) helical conformation the bond angles $N_i-C^\alpha-C_R^\beta$ (where i is the identifier of the i th Aib residue) and $C_i-C^\alpha-C_R^\beta$ are significantly greater than the tetrahedral value (109.45°) while the angles $N_i-C^\alpha-C_L^\beta$ and $C_i-C^\alpha-C_L^\beta$ are smaller (Table 2, residues Aib1–Aib6). The opposite asymmetry is found for a left-handed (positive φ , ψ values) helical conformation (Table 2, residue Aib7). The peptide units adopt the usual *trans* conformation. Their deviation from the ideal planar geometry ($\omega = 180^\circ$) is on average approximately 3° , with the sign of the deviation being correlated in a consistent manner with the sign of φ , ψ , i.e. negative values of ω for negative φ , ψ and

Table 1. Fractional coordinates ($\times 10^4$) and equivalent isotropic temperature factors (\AA^2) of the non-H atoms with e.s.d.'s in parentheses

The coordinates correspond to the right-handed helical conformation of Z-(Aib)₇-O'Bu.

$$B_{\text{eq}} = (4/3) \sum_i \beta_i \mathbf{a}_i \cdot \mathbf{a}_i$$

Z	x	y	z	B_{eq}		x	y	z	B_{eq}
C(1)	7983 (4)	-2319 (3)	-4311 (2)	6.7 (1)	C_{R4}^{β}	-740 (5)	803 (3)	-2541 (3)	6.8 (1)
C(2)	8450 (6)	-2345 (5)	-3588 (4)	10.3 (2)	C_{L4}^{β}	-371 (3)	-99 (2)	-1273 (3)	5.9 (1)
C(3)	9349 (6)	-1768 (6)	-3463 (5)	11.3 (3)	C_4	44 (3)	1418 (2)	-1380 (2)	3.9 (1)
C(4)	9688 (8)	-1212 (6)	-4090 (5)	12.0 (3)	O_4	-1067 (2)	1924 (1)	-1197 (2)	4.6 (1)
C(5)	9308 (8)	-1157 (4)	-4826 (6)	11.6 (3)	Aib5				
C(6)	8408 (6)	-1697 (3)	-4920 (3)	8.0 (2)	N_5	1166 (2)	1549 (1)	-1129 (2)	3.8 (1)
C(7)	7071 (6)	-2913 (4)	-4537 (3)	8.7 (2)	C_5^{α}	1190 (3)	2333 (2)	-667 (2)	3.7 (1)
O(8)	6407 (4)	-3356 (2)	-3809 (2)	8.8 (1)	C_{R5}^{β}	423 (4)	2293 (2)	237 (2)	5.1 (1)
C(9)	5250 (4)	-2889 (2)	-3342 (2)	5.8 (1)	C_{L5}^{β}	2707 (3)	2373 (2)	-717 (2)	5.0 (1)
O(10)	4775 (3)	-2147 (2)	-3513 (2)	6.4 (1)	C_5	610 (3)	3170 (2)	-1104 (2)	3.9 (1)
					O_5	3 (2)	3822 (1)	-692 (1)	5.1 (1)
Aib1									
N_1	4737 (3)	-3352 (2)	-2665 (2)	5.3 (1)	Aib6				
C_1^{α}	3440 (3)	-3013 (2)	-2088 (2)	4.9 (1)	N_6	868 (2)	3161 (1)	-1953 (2)	3.8 (1)
C_{R1}^{β}	2217 (4)	-2976 (3)	-2495 (3)	7.0 (1)	C_6^{α}	466 (3)	3913 (2)	-2473 (2)	4.3 (1)
C_{L1}^{β}	3299 (4)	-3626 (2)	-1320 (3)	6.4 (1)	C_{R6}^{β}	1159 (4)	4692 (2)	-2347 (3)	6.3 (1)
C_1	3472 (3)	-2110 (2)	-1775 (2)	4.4 (1)	C_{L6}^{β}	926 (4)	3607 (3)	-3397 (2)	5.9 (1)
O_1	2430 (2)	-1545 (1)	-1634 (2)	5.0 (1)	C_6	-1119 (3)	4199 (2)	-2310 (2)	4.2 (1)
					O_6	-1608 (2)	4922 (1)	-2555 (2)	5.4 (1)
Aib2									
N_2	4691 (2)	-1970 (1)	-1653 (2)	4.2 (1)	Aib7				
C_2^{α}	4845 (3)	-1161 (2)	-1289 (2)	4.6 (1)	N_7	-1882 (2)	3601 (2)	-1994 (2)	4.9 (1)
C_{R2}^{β}	4121 (4)	-1097 (3)	-357 (3)	6.5 (1)	C_7^{α}	-3375 (3)	3764 (2)	-1891 (3)	5.8 (1)
C_{L2}^{β}	6398 (4)	-1168 (2)	-1393 (3)	6.1 (1)	C_{R7}^{β}	-3850 (4)	2869 (3)	-1745 (5)	9.4 (2)
C_2	4295 (3)	-359 (2)	-1769 (3)	4.6 (1)	C_{L7}^{β}	-4072 (4)	4407 (3)	-1180 (3)	7.8 (1)
O_2	3869 (2)	-359 (1)	-1430 (2)	6.1 (1)	C_7	-3731 (3)	4108 (2)	-2737 (3)	5.6 (1)
					O_7	-4657 (3)	4707 (2)	-2785 (2)	6.8 (1)
Aib3									
N_3	4364 (3)	-454 (2)	-2606 (2)	5.0 (1)	O'Bu				
C_3^{α}	3911 (4)	249 (2)	-3152 (3)	6.3 (1)	$O'(1)$	-2985 (3)	3615 (2)	-3368 (2)	6.7 (1)
C_{R3}^{β}	4906 (5)	899 (3)	-3329 (6)	11.2 (3)	$C'(1)$	-3201 (6)	3743 (3)	-4243 (4)	8.6 (2)
C_{L3}^{β}	3851 (7)	-173 (3)	-3984 (3)	10.0 (2)	$C'(2)$	-4641 (7)	3635 (5)	-4249 (6)	13.0 (3)
C_3	2426 (4)	721 (2)	-2793 (2)	4.9 (1)	$C'(3)$	-2148 (8)	2988 (5)	-4696 (5)	13.9 (3)
O_3	2067 (3)	1488 (1)	-2991 (2)	5.8 (1)	$C'(4)$	-2811 (8)	4605 (5)	-4574 (4)	11.3 (3)
Aib4									
N_4	1590 (3)	250 (2)	-2295 (2)	4.5 (1)	$O(w1)$	-2792 (4)	-3441 (2)	-1602 (3)	8.5 (1)
C_4^{α}	150 (3)	594 (2)	-1885 (2)	4.5 (1)	$O(w2)$	-1736 (5)	5535 (2)	-286 (3)	9.6 (2)

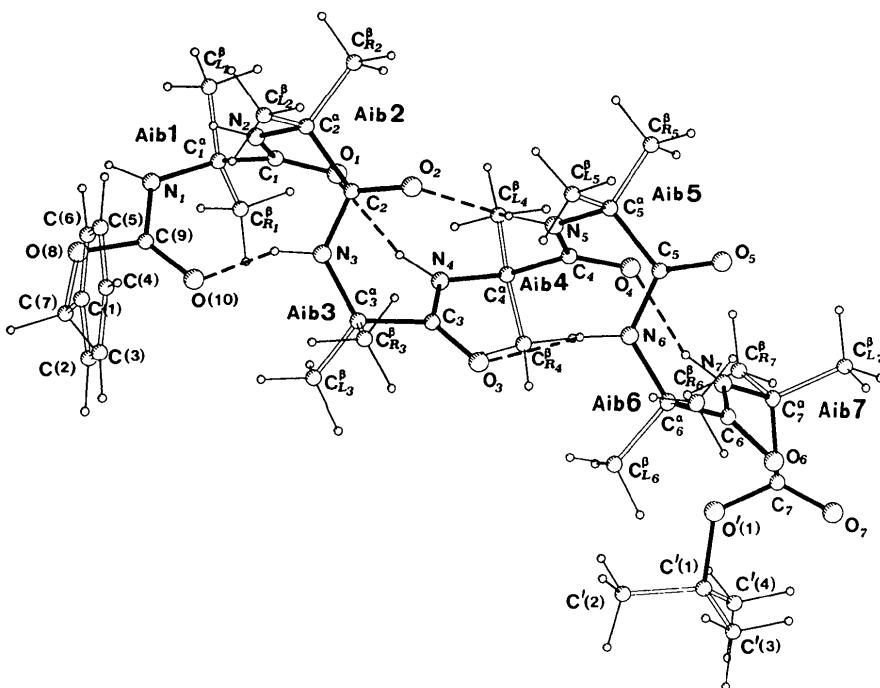


Fig. 1. Perspective drawing of the three-dimensional structure of Z-(Aib)₇-O'Bu (right-handed helix) showing the atom numbering scheme (as defined by IUPAC-IUB). The 'backbone' is highlighted and intra-molecular hydrogen bonds are indicated by broken lines.

Table 2. Selected bond lengths (Å) and angles (°) defining the valency geometry around the C^α atoms for the right-handed helix of Z-(Aib)₇-O^tBu

	N _i -C ^α _i	C ^α _i -C _L ^β _i	C ^α _i -C _R ^β _i	C ^α _i -C _O _i
Aib1	1.456 (4)	1.533 (5)	1.518 (6)	1.522 (4)
Aib2	1.462 (4)	1.534 (5)	1.529 (5)	1.526 (4)
Aib3	1.451 (5)	1.551 (7)	1.520 (6)	1.541 (5)
Aib4	1.470 (3)	1.515 (5)	1.532 (6)	1.528 (4)
Aib5	1.468 (4)	1.526 (4)	1.507 (4)	1.539 (4)
Aib6	1.463 (4)	1.544 (5)	1.543 (5)	1.545 (4)
Aib7	1.453 (4)	1.525 (6)	1.539 (5)	1.549 (7)

	N _i -C ^α _i -C _L ^β _i	C _i -C ^α _i -C _L ^β _i	N _i -C ^α _i -C _R ^β _i	C _i -C ^α _i -C _R ^β _i
Aib1	107.3 (2)	107.3 (3)	111.5 (3)	110.1 (3)
Aib2	107.4 (2)	106.5 (3)	111.0 (3)	110.3 (3)
Aib3	107.3 (3)	105.3 (4)	110.8 (4)	110.6 (3)
Aib4	107.5 (2)	107.6 (3)	110.5 (3)	109.3 (2)
Aib5	106.6 (2)	106.1 (2)	111.3 (2)	111.0 (2)
Aib6	106.7 (2)	105.8 (3)	111.4 (3)	110.4 (2)
Aib7	111.3 (4)	111.2 (3)	107.1 (2)	105.3 (4)

Table 3. Backbone torsion angles (°) for the right-handed helical conformation

	φ	ψ	ω
Aib1	-53.7 (5)	-35.5 (4)	-174.8 (3)
Aib2	-53.8 (4)	-29.8 (4)	-178.7 (3)
Aib3	-52.0 (5)	-28.0 (5)	178.9 (3)
Aib4	-53.7 (4)	-22.4 (4)	177.7 (3)
Aib5	-48.9 (4)	-34.6 (4)	-176.4 (3)
Aib6	-63.1 (4)	-23.3 (4)	-173.7 (3)
Aib7	51.4 (5)	48.5 (4)	174.2 (4)

Table 4. Parameters of the hydrogen bonds (Å, °)

Donor N	Acceptor O	N...O	O...H	N-H...O
Intramolecular				
Aib3	Z	2.993 (4)	2.09 (7)	152.2 (5.8)
Aib4	Aib1	2.988 (3)	2.10 (3)	163.3 (2.8)
Aib5	Aib2	2.960 (3)	2.04 (3)	168.4 (2.5)
Aib6	Aib3	3.072 (3)	2.08 (5)	168.0 (4.1)
Aib7	Aib4	2.922 (3)	2.08 (4)	156.5 (3.3)
Intermolecular				
Aib1	Aib7 [†]	2.972 (4)	2.12 (4)	161.5 (3.5)
Hydrogen bonds involving water molecules				
	Donor D*	Acceptor A [†]	D...A	
	Aib2	O(w1) ^{††}	3.109 (4)	
	O(w2)	Aib5(C=O)	2.926 (4)	
	O(w2) ^m	Aib5(C=O)	2.935 (6)	
	O(w1) ^v	Aib6(C=O)	2.958 (4)	
	O(w2)	O(w1) ^v	2.893 (7)	

Symmetry codes: (i) $x + 1, y - 1, z$; (ii) $x + 1, y, z$; (iii) $-x, -y + 1, -z$; (iv) $x, y + 1, z$.

* NH group or water O.

† Water O or C=O group.

positive ω for positive φ, ψ . The asymmetry in the valence geometries and the deviations from peptide-unit planarity are in excellent agreement with conformational energy calculations (Paterson, Rumsey, Benedetti, Nemethy & Sheraga, 1981). In the crystal the molecules are linked head-to-tail (Fig. 2) *via* an intermolecular hydrogen bond between the NH group of Aib1 and the C=O group of Aib7 (Table 4) and *via* a water molecule which links the N- and C-termini of successive molecules by being hydrogen

bonded with residue Aib2 (NH group) of the first molecule and residue Aib6 (C=O group) of the second molecule. The resulting pattern of intra- and intermolecular hydrogen bonds corresponds to an infinitely long helical column along [1 $\bar{1}$ 0]. Neighbouring columns associate, *via* hydrogen bonds involving water molecules (Table 4), so that the helical columns are arranged antiparallel to each other. The antiparallel helix packing is presumably preferred due to energetically favourable helix dipole-dipole interactions (Hol, Halie & Sander, 1981). Pairs of antiparallel helical columns combine forming layers parallel to the plane defined by the directions [1 $\bar{1}$ 0] and [101]. The layer-forming interactions (they involve the groups Z and O^tBu) and the interactions between neighbouring layers are hydrophobic.

In summary, it may be concluded that Z-(Aib)₇-O^tBu confirms the preference of Aib-containing homopeptides for the formation of rigid 3_{10} -helices. Although the φ, ψ angles of residues Aib1-Aib6 deviate from the ideal values of a 3_{10} -helix ($\varphi = -60^\circ, \psi = -30^\circ$), the hydrogen-bonding pattern is clearly 3_{10} -helical. This behaviour has been also observed in other cases (Bosch, Jung, Schmitt & Winter, 1985; Kokkinidis, Tsernoglou & Brueckner, 1986; Kokkinidis, Banner, Tsernoglou & Brueckner, 1986) and indicates that the backbone dihedral angles alone are insufficient for the assignment of a particular helix type in Aib-containing peptides. Despite extensive hydration in the crystal (Fig. 2), the 3_{10} -helix shows no distortions due to interactions with water (Blundell, Barlow, Borkakoti & Thornton, 1983) or disruptions of the intramolecular hydrogen-bonding pattern (Karle, Flippen-Anderson, Uma & Balaram, 1990). This rigid helix

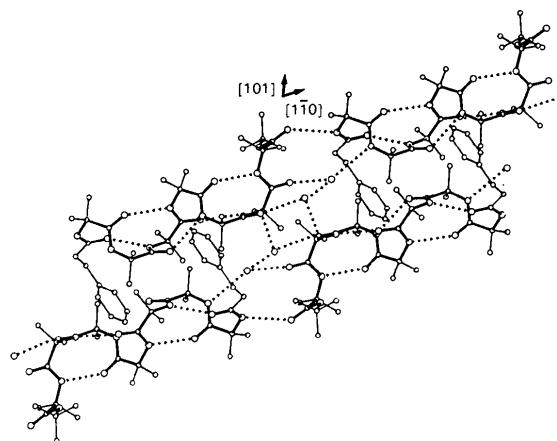


Fig. 2. Crystal packing of Z-(Aib)₇-O^tBu. The network of intra- and intermolecular hydrogen bonds is indicated by dotted lines.

remains basically unaltered in the context of different protection groups or in different environments. This can be concluded from the recently determined structure of Z-(Aib)₇-OMe (Pavone, Di Blasio, Pedone, Santini, Benedetti, Formaggio, Crisma & Toniolo, 1991). Despite very different crystalline environments, hydrations and differences in the orientation of the protection groups, the parameters of the helical parts of Z(Aib)₇-O'Bu and Z-(Aib)₇-OMe are in excellent agreement, with average deviations of only 4–5° in the conformational angles φ , ψ , and of 0.08 Å in the hydrogen-bond lengths.

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Resonance-Assisted Hydrogen Bonding. III. Formation of Intermolecular Hydrogen-Bonded Chains in Crystals of β -Diketone Enols and its Relevance to Molecular Association*

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Abstract

The β -diketone enol (or enolone) HO—C=C—C=O fragment produced by enolization of

β -diketones is known to form strong intramolecular O—H \cdots O hydrogen bonds where the decrease of the O \cdots O contact distance (up to 2.40 Å) is correlated with the increased π -delocalization of the O=C=C—C=O heteroconjugated system; the phenomenon has been interpreted by the resonance-assisted hydrogen-bonding (RAHB) model [Gilli,

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