Peptides from chiral Calpha,alpha-disubstituted glycines : crystallographic characterization of conformation of Calpha-methyl, Calpha-isopropylglycine [(alphaMe)Val] in simple derivatives and model peptides

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Peptides from chiral $C^{\alpha,\alpha}$-disubstituted glycines

Crystallographic characterization of conformation of C^{α}-methyl, C^{α}-isopropylglycine [(αMe)Val] in simple derivatives and model peptides*

G. VALLE,[1] M. CRISMA[1], C. TONIOLO,[1] S. POLINELLI[2], W.H.J. BOESTEN[2], H.E. SCHOEMAKER[2], E.M. MEIJER, and J. KAMPHUIS[2]

[1]*Biopolymer Research Centre, C.N.R., Department of Organic Chemistry, University of Padova, Padova, Italy;*
[2]*DSM Research, Bioorganic Chemistry Section, Geleen, The Netherlands*

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The molecular and crystal structures of one derivative and three model peptides (to the pentapeptide level) of the chiral $C^{\alpha,\alpha}$-disubstituted glycine C^{α}-methyl, C^{α}-isopropylglycine [(αMe)Val] have been determined by X-ray diffraction. The derivative is mClAc-L-(αMe)Val-OH, and the peptides are Z-L-(αMe)Val-(L-Ala)$_2$-OMe monohydrate, Z-Aib-L-(αMe)Val-(Aib)$_2$-O*t*Bu, and Ac-(Aib)$_2$-L-(αMe)Val-(Aib)$_2$O*t*Bu acetonitrile solvate. The tripeptide adopts a type-I β-turn conformation stabilized by a 1 ← 4 N—H ... O=C intramolecular H-bond. The tetra- and pentapeptides are folded in regular right-handed 3_{10}-helices. All four L-(αMe)Val residues prefer ϕ, ψ angles in the right-handed helical region of the conformational map. The results indicate that: (i) the (αMe)Val residue is a strong type-I/III β-turn and helix former, and (ii) the relationship between (αMe)Val chirality and helix screw sense is the same as that of C^{α}-monosubstituted protein amino-acids. The implications for the use of the (αMe)Val residue in designing conformationally constrained analogues of bioactive peptides are briefly discussed.

Key words: conformational analysis; crystal state conformation; $C^{\alpha,\alpha}$-disubstitution glycines; 3_{10}-helix; α-methyl valine peptides; peptide conformation; X-ray diffraction; β-turns

α-Methyl amino-acids have been shown to impart well-defined conformational constraints to the peptide backbone. In particular, the *achiral* $C^{\alpha,\alpha}$-dimethyl glycine (α-aminoisobutyric acid, Aib) residue strongly prefers folded backbone conformations in the $3_{10}/\alpha$-helical region of the ϕ, ψ space ($\phi = +60 \pm 20°$, $\psi = \pm 30 \pm 20°$) (for recent surveys see refs. 2–5). The prototype of *chiral* amino acids of this family, C^{α}-methyl, C^{α}-ethylglycine (isovaline, Iva), appears to be more versatile than Aib, in the sense that it can be accommodate either in a fully-extended conformation (at least in simple derivatives) (6) or in β-turns/3_{10}-helices (in small peptides) (7–10). However, on the basis of the crystallographic analyses performed to date it proved to be impossible to establish a clear-cut correlation between Iva configuration and helix handedness.

As part of a program aimed at assessing the minimum length of the R side chain in the -NH-$C(CH_3)R$-CO- residue required to induce a preferential screw sense in helical peptides containing chiral amino acids of this family, we describe here the crystal-state structural characterization of the C^{α}-methyl, C^{α}-isopropylglycine [α-methyl valine, (αMe)Val] (L- or S-enantiomer) residue in one derivative and three suitably selected model peptides (to the pentapeptide level) by using X-ray diffraction. (αMe)Val is the next member of this amino-acid series, since it has only one carbon atom in the R side chain more than Iva. The X-ray diffraction analysis of the only (αMe)Val compound reported so far, Z-L-Ile-L-(αMe)Val-benzocaine (Z, benzyloxycarbonyl), shows the formation of a slightly distorted type-III β-turn with the L (or S-) (αMe)Val residue falling in the region of the conformational map where *right*-handed helices are found (11). The synthesis, characterization, and theoretical and solution conformational analyses of L-(αMe)Val derivatives and peptides have been described in the preceding paper (1).

*Part 235 of the series "Linear Oligopeptides". For part 234 see ref. 1.

TABLE 1

Crystal data for the four L-*(αMe)Val derivatives and peptides*

Parameter	mClAc-L-(αMe)Val-OH	Z-L-(αMe)Val-(L-Ala)$_2$-OMe ·H_2O	Z-Aib-L-(αMe)Val-(Aib)$_2$-O*t*Bu	Ac-(Aib)$_2$-L-(αMe)Val-(Aib)$_2$-O*t*Bu ·CH_3CN
Molecular formula	$C_8H_{14}NO_3Cl$	$C_{21}H_{31}N_3O_6 \cdot H_2O$	$C_{30}H_{48}N_4O_7$	$C_{28}H_{51}N_5O_7 \cdot CH_3CN$
M.w.(a.m.u.)	580.5	439.3	576.7	610.8
Solvent of crystalliz.	ethyl acetate	acetone/H_2O	methanol	acetonitrile
Density (calc.) (g. cm^{-3})	1.28	1.22	1.10	1.14
Crystal system	monoclinic	monoclinic	monoclinic	orthorhombic
Space group	$P2_1$	$P2_1$	$P2_1$	$P2_12_12_1$
Z(mol/unit cell)	2	2	2	4
a(Å)	11.292(2)	10.885(2)	10.752(2)	36.548(3)
b(Å)	8.020(2)	12.618(2)	15.512(2)	10.772(2)
c(Å)	5.951(2)	8.845(1)	10.454(2)	8.976(2)
V(Å^3)	537.3	1200.9	1742.4	3533.8
α(°)	90.0	90.0	90.0	90.0
β(°)	94.44(2)	98.7(2)	92.1(2)	90.0
γ(°)	90.0	90.0	90.0	90.0
Solved by	SHELX S-86[a]	SHELX S-86	SHELX S-86	MULTAN 80[b]
No. of independent reflections	1386	2956	4436	4811
Reflections ($I \geqslant 2.56(I)$)	657	1973	1732	2384
S	0.70	0.77	0.90	0.89
Max. and min. heights	0.28	0.21	0.29	0.16
in final ΔF synthesis (e.Å^{-3})	−0.29	−0.22	−0.29	−0.20
w	1	1	$1/[\sigma^2(F) + 0.0085F^2]$	$1/\sigma^2(F) + 0.004F^2]$
R value	5.7	5.6	6.5	4.7
R_w value	5.7	5.6	6.8	5.3

[a]Ref. 12. [b]Ref. 13.

EXPERIMENTAL PROCEDURES

Peptide synthesis

The synthesis and characterization of mClAc-L-(αMe)Val-OH (mClAc, monochloroacetyl), Z-L-(αMe)Val-(L-Ala)$_2$-OMe (OMe, methoxy), Z-Aib-L-(αMe)Val-(Aib)$_2$-O*t*Bu(O*t*Bu, *tert*-butoxy), and Ac-(Aib)$_2$-L-(αMe)Val-(Aib)$_2$-O*t*Bu (Ac, acetyl) have been reported in the preceding paper (1).

X-ray diffraction

Reflections were collected on a Phillips PW 1100 four-circle diffractometer, using graphite-monochromatized MoKα radiation (λ = 0.7107 Å) with the θ-2θ scan mode to 2θ = 56°. During data collection three standard reflections were measured every 180 min to check the stability of the crystal and the electronics. Intensities were corrected for Lorentz and polarization effects and put on an absolute scale. No absorption corrections were applied. All non-hydrogen atoms for the four structures were refined with anisotropic thermal parameters by blocked least squares. Hydrogen atoms for mClAc-L-(αMe)Val-OH, Z-L-(αMe) Val-(L-Ala)$_2$-OMe monohydrate, and Ac-(Aib)$_2$-L-(αMe)Val-(Aib)$_2$-O*t*Bu acetonitrile solvate were in part located on a difference Fourier map and refined, and in part calculated. Hydrogen atoms for Z-Aib-L-(αMe)Val-(Aib)$_2$-O*t*Bu were in part located on a difference Fourier map, but not refined, and in part calculated. Crystallographic data for the four structures are summarized in Table 1. Complete lists of bond lengths, bond angles, torsion angles, and the final positional parameters of the non-hydrogen atoms along with equivalent and anisotropic thermal factors have been deposited at the Cambridge Crystallographic Data Centre.

RESULTS AND DISCUSSION

We have determined by X-ray diffraction the molecular and crystal structures of the following L-(αMe)Val derivatives and peptides: mClAc-L-(αMe)Val-OH, Z-L-(αMe)Val-(L-Ala)$_2$-OMe monohydrate, Z-Aib-L-(αMe)Val-(Aib)$_2$-O*t*Bu, and Ac-(Aib)$_2$-L-(αMe)Val-(Aib)$_2$-O*t*Bu acetonitrile solvate. The four molecular structures with the atomic numbering schemes are illustrated in Figs. 1–4, respectively. Relevant torsion angles (14) are given in Table 2. In Table 3 the intra- and intermolecular H-bond parameters are listed.

Bond lengths and bond angles (deposited) are in general agreement with previously reported values for the geometry of the Z-urethane (15), acetamido (16), and monochloroacetamido (6, 17) moieties, the ester

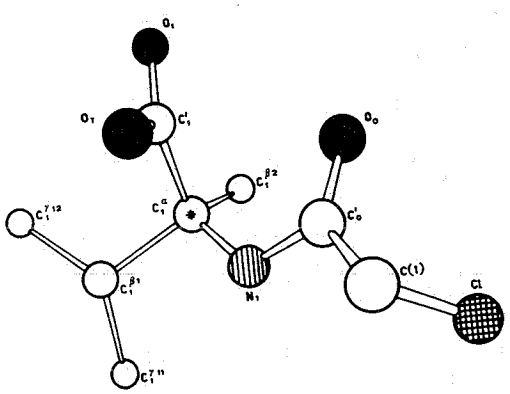

FIGURE 1

Molecular structure of mClAc-L-(αMe)Val-OH with numbering of the atoms.

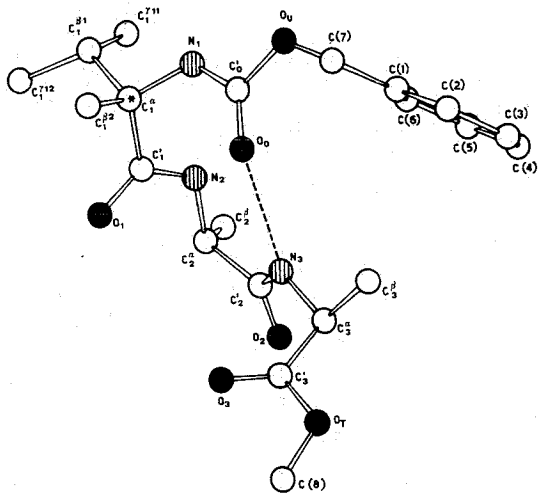

FIGURE 2

Molecular structure of Z-L-(αMe)Val-(L-Ala)$_2$-OMe with numbering of the atoms. The intramolecular H-bond is indicated as a dashed line.

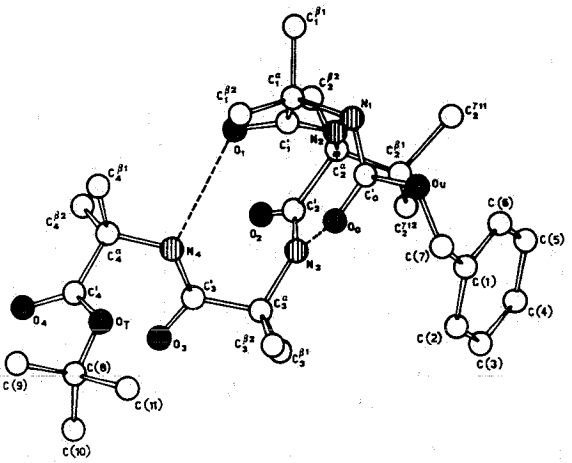

FIGURE 3

Molecular structure of Z-Aib-L-(αMe)Val-(Aib)$_2$-O*t*Bu with numbering of the atoms. The two intramolecular H-bonds are indicated as dashed lines.

TABLE 2

Relevant torsion angles (°) for the four L-(αMe)Val *derivatives and peptides (e.s.d.'s are given in parentheses)*

Torsion angle	mClAc-L-(αMe)Val-OH	Z-L-(αMe)Val-(L-Ala)$_2$-OMe ·H_2O	Z-Aib-L-(αMe)Val-(Aib)$_2$-O*t*Bu	Ac-(Aib)$_2$-L-(αMe)Val-(Aib)$_2$-O*t*Bu ·CH_3CN
θ^3	———	−1.9(11)	−40.6(17)	———
θ^2	———	91.9(8)	71.3(12)	———
θ^1	105.7(8)	173.7(7)	−171.4(9)	———
ω_0	176.3(7)	−163.7(6)	−170.1(8)	−173.0(6)
ϕ_1	−51.7(10)	−61.0(9)	−60.3(11)	−55.9(6)
ψ_1	−41.9(9)[a]	−30.7(9)	−28.8(11)	−34.6(6)
ω_1	———	−174.2(6)	−174.8(8)	−166.1(4)
ϕ_2	———	−104.1(8)	−51.4(11)	−58.1(6)
ψ_2	———	10.5(10)	−40.1(11)	−30.9(6)
ω_2	———	170.0(7)	−176.5(8)	−177.8(4)
ϕ_3	———	−79.9(9)	−60.7(11)	−50.6(5)
ψ_3	———	174.7(7)[b]	−32.0(11)	−41.8(5)
ω_3	———	−178.7(7)[c]	−174.2(8)	−168.4(4)
ϕ_4	———	———	46.4(11)	−75.0(5)
ψ_4	———	———	47.3(10)[d]	−10.4(5)
ω_4	———	———	177.1(7)[e]	−165.7(4)
ϕ_5	———	———	———	49.7(5)
ψ_5	———	———	———	43.7(5)[f]
ω_5	———	———	———	178.5(4)[g]
$\chi^{1,1}$[L-(αMe)Val]	164.2(8)	53.7(9)	167.6(10)	166.4(4)
$\chi^{1,2}$[L-(αMe)Val]	−68.0(10)	−176.9(7)	−66.6(11)	−66.6(5)

[a] $\psi_1 = \psi_\tau(N_1\text{-}C_1^\alpha\text{-}C'_1\text{-}O_\tau)$. [b] $\psi_3 = \psi_\tau(N_3\text{-}C_3^\alpha\text{-}C'_3\text{-}O_\tau)$. [c] $\omega_3 = \omega_\tau[C_3^\alpha\text{-}C'_3\text{-}O_\tau\text{-}C(8)]$. [d] $\psi_4 = \psi_\tau(N_4\text{-}C_4^\alpha\text{-}C'_4\text{-}O_\tau)$. [e] $\omega_4 = \omega_\tau[C_4^\alpha\text{-}C'_4\text{-}O_\tau\text{-}C(8)]$. [f] $\psi_5 = \psi_\tau(N_5\text{-}C_5^\alpha\text{-}C'_5\text{-}O_\tau)$. [g] $\omega_5 = \omega_\tau[C_5^\alpha\text{-}C'_5\text{-}O_\tau\text{-}(2)]$.

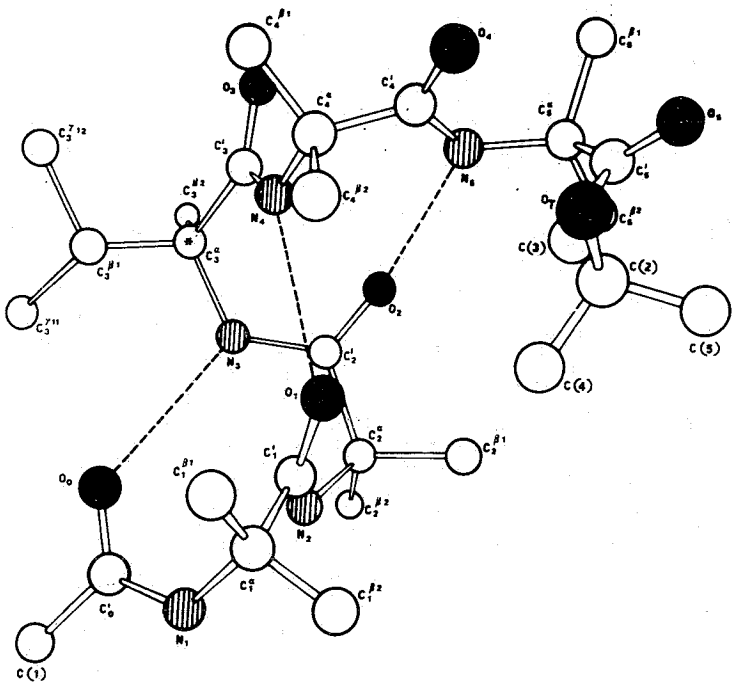

FIGURE 4
Molecular structure of Ac-$(Aib)_2$-L-(αMe)Val-$(Aib)_2$-O*t*Bu with numbering of the atoms. The three intramolecular H-bonds are indicated as dashed lines.

TABLE 3

Intra- and intermolecular H-bond parameters for the four L-*(αMe)Val derivatives and peptides*

Compound	Donor D-H	Acceptor A	Symmetry equivalence of A	Distance (Å) D ... A
mClAc-L-(αMe)Val-OH	N_1-H_1	O_1	x, y, 1 + z	2.943(8)
Z-L-(αMe)Val-(L-Ala)$_2$-OMe·H_2O	N_3-H_3	O_0	x, y, z	3.077(7)
	N_1-H_1	O_2	x, y, z-1	2.875(6)
	N_2-H_2	O_w	1-x, y-1/2, 1-z	2.955(7)
Z(Aib)-L-(αMe)Val-$(Aib)_2$-O*t*Bu	N_3-H_3	O_0	x, y, z	3.187(9)
	N_4-H_4	O_1	x, y, z	2.941(7)
	N_1-H_1	O_3	x, y, 1 + z	2.963(6)
Ac-$(Aib)_2$-L-(αMe)Val-$(Aib)_2$-O*t*Bu ·CH_3CN	N_3-H_3	O_0	x, y, z	3.328(5)
	N_4-H_4	O_1	x, y, z,	3.017(4)
	N_5-H_5	O_2	x, y, z,	3.179(5)
	N_1-H_1	O_3	x, y-1, z	3.061(5)
	N_1-H_1	N(CH_3CN)	1/2-x, − y, z-1/2	3.170(9)

group (18), the peptide unit (19), and the Aib (20, 21) and Ala residues.

The -L-(αMe)Val-L-Ala- sequence of the tripeptide is folded in a type-I β-turn conformation (22–24) stabilized by a $1 \leftarrow 4$ N–H ... O=C intramolecular H-bond. The (peptide) $N_3 \ldots O_0$ (urethane) separation is 3.077(7) Å (25,26). The tetra- and pentapeptides form regular right-handed 3_{10}-helices (27) in their -$(Aib)_n$-L-(αMe)Val-$(Aib)_2$- (n = 1,2) sequences with two and three consecutive intramolecular H-bonds, respectively. The pertinent N...O distances range from 2.941(7) Å to 3.328(5) Å. The latter H-bond, characterizing the *N*-terminal -Aib-Aib- sequence of the pentapeptide, is extremely weak (25, 26).

All four L-(αMe)Val residues, including that of the monochloroacetyl derivative, prefer ϕ, ψ backbone torsion angles in the right-handed helical region of the conformational map. The average ϕ, ψ values are

$-53.7°$, $-38.6°$. The critical $\tau(N-C^{\alpha}-C')$ bond angle for the L-(αMe)Val residues deviates only slightly ($\pm 1.2°$) from the regular tetrahedral value (109.5°), an additional indication of the helical preference of this amino acid (3, 28). The conformation of the L-(αMe)Val isopropyl side chain ($\chi^{1,1}$ and $\chi^{1,2}$ torsion angles) is the common (t, g^-) conformation (29, 30) in the monochloroacetylated derivative and in the tetra- and pentapeptides; however, this disposition is (g^+, t) in the tripeptide.

The conformation of the two Z-urethane groups is the usual *trans*, *trans* (θ^1 and ω_0 torsion angle) or type-*b* conformation (15). A very large deviation from planarity is seen for the urethane amide (ω_0) torsion angle of the tripeptide. Also the values of the θ^2 and θ^3 torsion angles are typical for the Z–urethane group (15). The θ^1 torsion angle of mClAc-L-(αMe)Val-OH, characterizing the disposition of the Cl-C(1) bond with respect to the C'_0-N_1 bond, is 105.7 (8)°, thus precluding the onset of the intramolecular Cl ... H-N H-bond found in mClAc-D-Iva-OH (6). The amide, peptide, and ester groups (ω torsion angles) are *trans*, as expected, in all the four compounds, but three peptide bonds (ω_1, ω_3, and ω_4 torsion angles) of the pentapeptide show significant distorsions from planarity. The θ, ψ torsion angles of the *C*-terminal L-Ala residue of the tripeptide suggest that it is *semi*-extended. On the other hand, the *C*-terminal Aib residues of the tetra- and pentapeptides are helical, but the signs of their ϕ, ψ torsion angles are reversed with respect to those of the preceding residues, a common observation for 3_{10}-helix-forming peptides (31). In mClAc-L-(αMe)Val-OH the carboxylic acid group adopts a conformation with respect to the $C^{\alpha}_1 - N_1$ bond between the *antiplanar* and *anticlinal* conformations (32), the N_1-C^{α}_1-C'_1-O_1 torsion angle being 139.9(8)°.

In the crystals of mClAc-L-(αMe)Val-OH rows of molecules are generated in the *z*-direction through N–H ... O=C (acid) intermolecular H-bonds. The O-H function and the carbonyl group of the amide moiety do not participate in a H-bond. This is an unusual packing motif of N^{α}-acylated α-amino acids (33–35).

In the packing mode of Z-(L-αMe)Val-(L-Ala)$_2$-O*t*Bu monohydrate we find a linear array of molecules in the *z*-direction linked together by a (urethane) N_1-H_1 ... O_2=C_2 (peptide) H-bond. The water molecule plays the role of the acceptor of the H-bond from (peptide) N_2-H_2 (36).

The Z-Aib-L-(αMe)Val-(Aib)$_2$-O*t*Bu molecules pack into the unit cell in rows parallel to the *z*-direction *via* (urethane) N_1-H_1 ... O_3=C'_3 (peptide) H-bonds. The (peptide) N_2-H_2 does not seem to be involved in the H-bonding scheme.

The molecules of Ac-(Aib)$_2$-L-(αMe)Val-(Aib)$_2$-O*t*Bu acetonitrile solvate form rows along the *y*-direction with H-bonds of the (amide) N_1-H_1 ... O_3=C'_3 (peptide) type. A three-centre (bifurcated) H-bond (37) is generated by the N_1-H_1 group, in which the proton forms an additional weak interaction with the acetonitrile nitrogen atom (38, 39). As in the tetrapeptide, the (peptide) N_2-H_2 is free.

CONCLUSIONS

The results of the present X-ray diffraction analysis, together with those reported in the literature (11), indicate that the L-(αMe)Val residues of the five derivatives and peptides examined adopt in the crystal state ϕ, ψ backbone torsion angles in the right-handed helical region of the conformational space. It may be concluded that this $C^{\alpha,\alpha}$-disubstituted glycine: (i) is a type I/III β-turn and helix former much stronger than L-Val (40–44), and (ii) forms helices of the same handedness as that exhibited by the helix former C^{α}-monosubstituted protein amino acids. In addition, a comparison with the results of Iva (7–10), the lowest member of this family of chiral -NH-C(CH_3)R-CO- amino acids, suggests that a side chain R of three carbon atoms is the minimal requirement to induce a preferential screw sense in the helical structures.

Therefore, the incorporation of an (αMe)Val residue into a bioactive peptide might result in a significant stabilization of a β-turn and/or a 3_{10}/α-helix. In this connection, it is worth noting that the aspartame analogue H-L-Asp-D-(αMe)Val-O*i*Pr (O*i*Pr, isopropyloxy) is sweet (45) and the L-(αMe)Val replacement at position 4 of vasopressin is compatible with potent biological activity (46, 47).

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Address:

Prof. *C. Toniolo*
Biopolymer Research Centre, CNR
Department of Organic Chemistry
University of Padova
Via Marzolo 1
35131 Padova
Italy