Acta Crystallographica Section C
Crystal Structure Communications
ISSN 0108-2701

The tetrapeptide Z-Leu-Aib-Pro-Val-OBg monohydrate[1]

Renate Geßmann,[a]* Norbert Schiemann,[b] Hans Brückner[b] and Kyriacos Petratos[a]

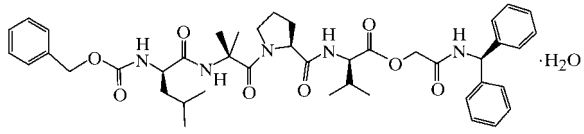
[a]IMBB/FORTH, PO Box 1527, 71110 Iraklion, Crete, Greece, and [b]Department of Food Sciences, Interdisciplinary Research Centre, Justus-Liebig University of Giessen, Heinrich-Buff-Ring 26, 35392 Giessen, Germany
Correspondence e-mail: rege777@yahoo.com

Received 12 February 2003
Accepted 12 May 2003
Online 12 July 2003

The intramolecular hydrogen-bonding pattern of Z-Leu-Aib-Pro-Val-OBg monohydrate [(*N*-benzhydrylamino)carbonylmethyl *N*-benzyloxycarbonyl-α-aminoisobutyrylprolylvalinate monohydrate], $C_{43}H_{55}N_5O_8\cdot H_2O$, is unusual for a tetrapeptide because, in addition to a 1←4 hydrogen bond, a second hydrogen bond of the type 1←5 is formed. This folding reflects the intramolecular hydrogen-bonding pattern that this amino acid sequence adopts in the naturally occurring peptaibol alamethicin.

Comment

The Leu-Aib-Pro-Val sequence represents a segment of the naturally occurring peptaibol antibiotics [peptides containing α-aminoisobutyric acid (Aib) and a C-terminal β-amino alcohol (Brückner & Graf, 1983; Benedetti *et al.*, 1982)], such as alamethicin (Pandey *et al.*, 1977; Brückner *et al.*, 1985), gliodeliquescin (Brückner & Przybylski, 1984), trichobrachin (Kripp, 1990), trichocellin (Wada *et al.*, 1994), trichokonin (Huang, Tezuka, Hatanaka *et al.*, 1995; Huang, Tezuka, Kikuchi *et al.*, 1995) and trichorzianin (Bodo *et al.*, 1985). Benzhydrylglycolamide (OBg) is a unique protecting group for the C-termini of synthetic peptides, since it can be cleaved under very mild alkaline conditions, such as treatment with aqueous 10% sodium carbonate (Amblard & Rodriguez, 1988). The backbone conformation that the tetrapeptide adopts (Fig. 1) is quite unusual. Leu1 lies with its torsion angles (Table 2) in the semi-extended region of a Ramachandran plot, which is unusual for an N-terminal-protected residue of an Aib-containing peptide. In most other cases, the C═O group of the protection group is involved in an intramolecular hydrogen bond with the third or fourth residue, thereby forcing the first residue to adopt a helical conformation [for examples, see Geßmann *et al.* (1991, 1997) and Geßmann (1999)]. In (I), the third residue is proline, which cannot act as a hydrogen-bond donor, and instead the Val4 N atom forms a 1←4 hydrogen bond with Leu1. Furthermore, the φ/ψ values of Leu1 are in an allowed, albeit not the most favoured, region of the Ramachandran plot. While Aib2 and Pro3 are in the most favoured helical region, Val4 is again just in the additional allowed helical region. The torsion angles for the standard amino acids Val and Leu in Aib-containing peptides vary more than the equivalent angles in proteins (Geßmann *et al.*, 1994, 1997; Geßmann, 1999), probably as a result of the smaller number of intramolecular hydrogen bonds in peptides.



(I)

There are two intramolecular hydrogen bonds in (I). One is the 1←4 hydrogen bond between the Val4 NH group and the Leu1 C═O group, which forms a ring with the usual (for an incipient 3_{10}-helix) ten atoms. The second hydrogen bond is formed between the OBg NH group and the Aib2 C═O group. The number of ring atoms is 13, which is typical for an α-helical 1←5 hydrogen bond. A hydrogen bond of this type has, to our knowledge, never been observed in a tetrapeptide and is clearly due to the substitution of the amino group in a residue for an O atom in the OBg protection group, prohibiting a 1←4 interaction between C═O (Aib2) and O (OBg). The only crystal structure of a natural peptide that comprises the sequence Val-Aib-Pro-Leu is alamethicin (Fox & Richards, 1982). Interestingly, (I) and alamethicin show similar hydrogen-bond patterns, *viz*. no intramolecular hydrogen bond involving the C═O group of the residue preceding Leu12, a 1←4 hydrogen bond between the C═O group of

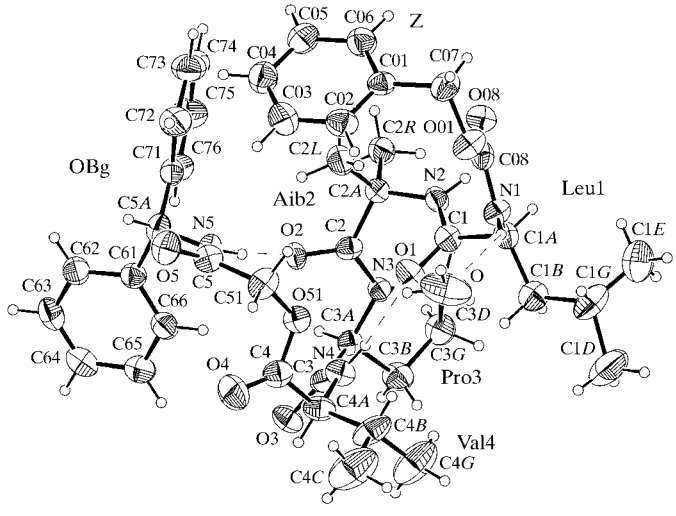


Figure 1
The molecular structure of (I), showing displacement ellipsoids at the 30% probability level.

[1] Nomenclature: Z is benzyloxycarbonyl, Leu is leucine, Aib is α-aminoisobutyric acid, Pro is proline, Val is valine and OBg is benzhydrylglycolamide ester.

 DOI: 10.1107/S0108270103010291

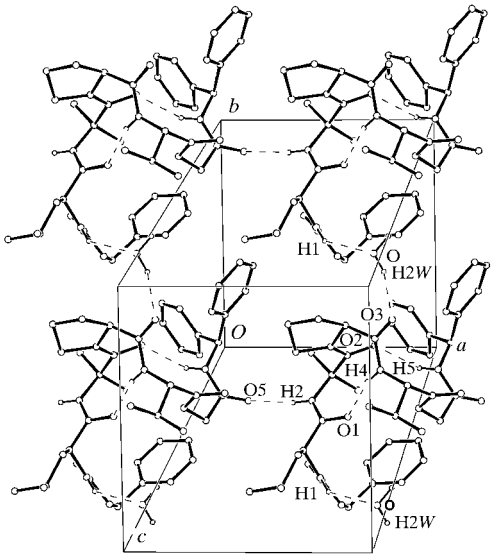


Figure 2
The crystal packing of (I), viewed approximately along [011]. Hydrogen bonds are indicated by broken lines and H atoms have been omitted for clarity.

Leu12 and the N—H group of Val15, and a 1←5 hydrogen bond between the C═O group of Aib13 and the N—H group of residue 17, which is in the place of the N—H group of the OBg protection group in alamethicin. The peptide unit in (I) adopts the usual *trans* planar conformation, with an average deviation of 6.1° from ideal geometry ($\omega = 180°$); this deviation is unusually large, especially for Leu1 (Table 2). The side chain of Leu1 adopts the most common conformation (Janin *et al.*, 1978), which belongs to the type g^+ for χ_1 and g^+t for χ_2. The pyrrolidine ring of Pro3 adopts the C_γ-*endo* (Ashida & Kakudo, 1974) conformation [the Cremer & Pople (1975) puckering parameters are $Q = 0.368$ Å and $\Phi = 281.9°$]. The angles between the N-terminal benzene plane and the two C-terminal benzene planes are 66.7 (4) and 79.6 (4)°, respectively, while the angle between the two C-terminal planes is 80.2 (4)°.

In the crystal packing, the unique molecule is hydrogen bonded to a symmetry-related molecule at $(x - 1, y, z)$, thus forming infinite columns in the $[\bar{1}00]$ direction. Each molecule is also hydrogen bonded along $[0\bar{1}0]$ *via* the cocrystallized water molecule (Table 1 and Fig. 2). Thus, planes of hydrogen-bonded molecules are formed parallel to the *ab* plane. These planes stack *via* apolar molecular contacts along the *c* axis.

Experimental

For the synthesis of Z-Leu-Aib-Pro-Val-OBg, (benzotriazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate (260 mg, 0.5 mmol; Novabiochem, Läufelfingen, Switzerland), H-Val-OBg trifluoroacetic acid (TFA; 250 mg, 0.5 mmol; Fluka, Buchs, Switzerland) and *N*-methylmorpholine (112 µl, 1.0 mmol; Fluka, Buchs, Switzerland) were added to Z-Leu-Aib-Pro-OH (225 mg, 0.5 mmol) in *N*,*N*-dimethylformamide (10 ml). After 16 h at room temperature, the solvent was removed *in vacuo*. The remaining residue was dissolved in ethyl acetate (100 ml) and washed successively (3 × 50 ml) with $KHSO_4$ (5%), $KHCO_3$ (5%) and water. The organic phase was dried overnight over anhydrous Na_2SO_4 and evaporated to dryness. To the remaining oil, a small amount of methanol was added, and the peptide was precipitated by addition of diethyl ether and *n*-hexane [yield: 385 mg (73%); m.p. 368 K]. Analysis calculated for $C_{43}H_{55}N_5O_8{\cdot}H_2O$ (M_r = 787.94): C 65.83, H 7.29, N 8.89%; found: C 65.80, H. 7.21, N 8.75%. Enantioselective gas chromatography on Chirasil-Val of a total hydrolysate of the title compound revealed less than 0.7% racemization of the L-amino acids. The tripeptide Z-Leu-Aib-Pro-OH was prepared by conventional stepwise solution phase synthesis, as described by Brückner & Koza (2003). H-Val-OBg·TFA was obtained by treatment of Boc-Val-OBg (Novabiochem, Läufelfingen, Switzerland) with TFA. The tetrapeptide was crystallized by cooling a hot (343 K) methanol–water mixture (70:30) to room temperature. Rod-shaped crystals suitable for X-ray analysis were obtained after a few days.

Crystal data

$C_{43}H_{55}N_5O_8{\cdot}H_2O$
M_r = 787.94
Orthorhombic, $P2_12_12_1$
a = 9.882 (11) Å
b = 10.705 (6) Å
c = 41.553 (17) Å
V = 4396 (6) Å^3
Z = 4
D_x = 1.191 Mg m^{-3}
Cu $K\alpha$ radiation
Cell parameters from 25 reflections
θ = 13.5–18.9°
μ = 0.68 mm^{-1}
T = 293 (2) K
Rod, colourless
0.8 × 0.1 × 0.1 mm

Table 1
Hydrogen-bonding geometry (Å, °).

D—H···A	D—H	H···A	D···A	D—H···A
N4—H4···O1	0.86	2.11	2.944 (7)	164
N5—H5···O2	0.86	2.13	2.932 (7)	154
N1—H1···O	0.86	2.29	3.002 (7)	140
N2—H2···O5^{i}	0.86	2.13	2.979 (7)	170
O—H2W···O3^{ii}	0.96	2.04	2.853 (6)	142 (6)

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

Table 2
Backbone and side-chain torsion angles (°).

$\omega(Z)$	O01—C08—N1—C1A	173.0 (5)
$\varphi(1)$	C08—N1—C1A—C1	−69.6 (7)
$\psi(1)$	N1—C1A—C1—N2	106.2 (6)
$\omega(1)$	C1A—C1—N2—C2A	−166.8 (5)
$\varphi(2)$	C1—N2—C2A—C2	−50.0 (7)
$\psi(2)$	N2—C2A—C2—N3	−41.6 (7)
$\omega(2)$	C2A—C2—N3—C3A	−175.5 (5)
$\varphi(3)$	C2—N3—C3A—C3	−62.6 (6)
$\psi(3)$	N3—C3A—C3—N4	−17.2 (7)
$\omega(3)$	C3A—C3—N4—C4A	−177.4 (5)
$\varphi(4)$	C3—N4—C4A—C4	−107.6 (7)
$\psi(4)$	N4—C4A—C4—O51	−42.2 (7)
$\omega(4)$	C4A—C4—O51—C51	−175.5 (5)
$\varphi(5)$	C4—O51—C51—C5	−78.0 (7)
$\psi(5)$	O51—C51—C5—N5	−29.2 (9)
$\omega(5)$	C51—C5—N5—C5A	176.0 (6)
	C5—N5—C5A—C61	−108.8 (6)
	C5—N5—C5A—C71	122.8 (6)
$\chi(1)$	N1—C1A—C1B—C1G	−53.4 (8)
$\chi21$ (1)	C1A—C1B—C1G—C1E	−56.5 (10)
$\chi22$ (1)	C1A—C1B—C1G—C1D	175.4 (8)
$\chi1$ (3)	N3–C3A—C3B—C3G	−25.4 (6)
$\chi2$ (3)	C3A—C3B—C3G—C3D	38.0 (7)
$\chi3$ (3)	C3B—C3G—C3D—N3	−35.0 (7)
$\chi4$ (3)	C3A—N3–C3D—C3G	19.2 (7)
$\theta(3)$	C3D—N3—C3A—C3B	3.9 (6)
$\chi11$ (4)	N4—C4A—C4B—C4C	−177.3 (8)
$\chi12$ (4)	N4—C4A—C4B—C4G	−55.8 (9)

Data collection

Enraf–Nonius CAD-4 diffractometer
ω/θ scans
Absorption correction: analytical (*ABSORB*; Bouley & Hall, 2000)
T_{min} = 0.888, T_{max} = 0.953
7588 measured reflections
3701 independent reflections
2471 reflections with $I > 2\sigma(I)$

R_{int} = 0.110
θ_{max} = 59.9°
$h = -11 \rightarrow 11$
$k = -11 \rightarrow 12$
$l = -46 \rightarrow 46$
5 standard reflections
frequency: 120 min
intensity decay: 2%

Refinement

Refinement on F^2
$R[F^2 > 2\sigma(F^2)]$ = 0.061
$wR(F^2)$ = 0.184
S = 1.08
3701 reflections
521 parameters
H atoms: see below

$w = 1/[\sigma^2(F_o^2) + (0.0824P)^2]$ where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$ = 0.010
$\Delta\rho_{max}$ = 0.52 e Å^{-3}
$\Delta\rho_{min}$ = −0.24 e Å^{-3}
Extinction correction: *SHELXL*97
Extinction coefficient: 0.00064 (15)

Data were collected over a θ range of 1–60°, using a collimator with a diameter of 1 mm, resulting in 6444 unique reflections. Refinement against these reflections resulted in an unreasonable Flack (1983) parameter of −0.6 (4), and so the 2743 Friedel reflections were merged before the final refinement. Peptide H atoms were placed at calculated positions and refined as riding. The highest peak in the final difference Fourier synthesis was associated with a water H atom, although this H atom has no acceptor. All U_{eq}(H) values were fixed to $1.5U_{eq}$ of the parent atom, except for water H atoms, for which U_{eq}(H) values were fixed at $1.2U_{eq}$(O). These water H atoms were refined with distance restraints (0.96 Å) and a restrained H—O—H angle. The largest positive residual electron density is close (0.24 Å) to the water H atom without an acceptor.

Data collection: *CAD*-4 *Software* (Enraf–Nonius, 1989); cell refinement: *CAD*-4 *Software*; data reduction: *DIFDAT*, *ABSORB* and *ADDREF* in *Xtal*3.7 (du Boulay & Hall, 2000); program(s) used to solve structure: *CRISP* in *Xtal*3.7; program(s) used to refine structure: *SHELXL*97 (Sheldrick, 1997); molecular graphics: *PIG* in *Xtal*3.7, *ORTEP* (Davenport *et al.*, 2000) in *Xtal*3.7, and *SETOR* (Evans, 1993); software used to prepare material for publication: *SHELXL*97, *CIFIO* and *BONDLA* in *Xtal*3.7, and *PLATON* (Spek, 2003).

Supplementary data for this paper are available from the IUCr electronic archives (Reference: TR1055). Services for accessing these data are described at the back of the journal.

References

Amblard, M. & Rodriguez, M. (1988). *Tetrahedron*, **44**, 5101–5108.
Ashida, T. & Kakudo, M. (1974). *Bull. Chem. Soc. Jpn*, pp. 1129–1133.
Benedetti, E., Bavoso, A., Di Blasio, B., Pavone, V., Toniolo, C. & Bonora, G. M. (1982). *Proc. Natl Acad. Sci. USA*, pp. 7951–7954.
Bodo, B., Rebuffat, S., El Hajji, M. & Davoust, D. (1985). *J. Am. Chem. Soc.* **107**, 6011–6017.
Boulay, D. J. du & Hall, S. R. (2000) *BONDLA*, *CRISP*, *DIFDAT*, *ABSORB*, *ADDREF*, *PIG* and *CIFIO* in *Xtal*3.7, edited by S. R. Hall, D. J. du Boulay & R. Olthof-Hazekamp. University of Western Australia, Perth: Lamb.
Brückner, H., Bokel, H. & Przybylski, M. (1985). *Peptides: Structure and Function*, editied by C. M. Deber, V. J. Hruby & K. D. Kopple, *Proceedings of the 9th American Peptide Symposium*, pp. 109–112. Rockford, USA: Pierce.
Brückner, H. & Graf, H. (1983). *Experientia*, **39**, 528–530.
Brückner, H. & Koza, A. (2003). *Amino Acids.* **24**, 311–323.
Brückner, H. & Przybylski, M. (1984). *Chromatographia*, **19**, 188–199.
Cremer, D. & Pople, J. A. (1975). *J. Am. Chem. Soc.* **97**, 1354–1367.
Davenport, G., Hall, S. R. & Dreissig, W. (2000). *ORTEP* in *Xtal*3.7, edited by S. R. Hall, D. J. du Boulay & R. Olthof-Hazekamp. University of Western Australia, Perth: Lamb.
Enraf–Nonius (1989). *CAD*-4 *Software.* Version 5.0. Enraf–Nonius, Delft, The Netherlands.
Evans, S. V. (1993). *J. Mol. Graphics*, **11**, 134–138.
Flack, H. D. (1983). *Acta Cryst.* A**39**, 876–881.
Fox, R. O. & Richards, F. M. (1982). *Nature* (*London*), **300**, 325–330.
Geßmann, R. (1999). PhD thesis, University of Hohenheim, Germany.
Geßmann, R., Brückner, H. & Kokkinidis, M. (1991). *Pept. Res.* **4**, 239–244.
Geßmann, R., Brückner, H. & Kokkinidis, M. (1994). *Z. Kristallogr.* **209**, 597–603.
Geßmann, R., Brückner, H. & Kokkinidis, M. (1997). *Z. Kristallogr.* **212**, 819–825.
Huang, Q., Tezuka, Y., Hatanaka, Y., Kikuchi, T., Nishi, A. & Tubaki, K. (1995). *Chem. Pharm. Bull.* **43**, 1663–1667.
Huang, Q., Tezuka, Y., Kikuchi, T., Nishi, A., Tubaki, K. & Tanaka, K. (1995). *Chem. Pharm. Bull.* **43**, 223–229.
Janin, J., Wodak, S., Levitt, M. & Maigret, B. (1978). *J. Mol. Biol.* **125**, 357–386.
Kripp, T. C. (1990). PhD thesis, University of Hohenheim, Germany.
Pandey, R. C., Cook, J. C. & Rinehart, K. L. (1977). *J. Am. Chem. Soc.* **99**, 5205–5206.
Sheldrick, G. M. (1997). *SHELXL*97. University of Göttingen, Germany.
Spek, A. L. (2003). *J. Appl. Cryst.* **36**, 7–13.
Wada, S. I., Nishimura, T., Iida, A., Toyama, N. & Fujita, T. (1994). *Tetrahedron Lett.* **35**, 3095–3098.