β-Alanine and β-Bends. X-Ray Diffraction Structures of Three Linear Oligopeptides

Vincenzo Pavone,[a] Benedetto Di Blasio,[a] Angela Lombardi,[a] Carla Isernia,[a] Carlo Pedone,[a] Ettore Benedetti,[a] Giovanni Valle,[b] Marco Crisma,[b] Claudio Toniolo*[,b] and Raghuvansh Kishore[c]

[a] *Biocrystallography Research Centre, C.N.R., Department of Chemistry, Uniersity of Naples, 80134 Naples, Italy*

[b] *Biopolymer Research Centre, C.N.R., Department of Organic Chemistry, University of Padova, 35131 Padova, Italy*

[c] *National Dairy Development Board, Goregaon (E), Bombay 400 063, India*

A crystal-state structural analysis of *t*-Boc-L-Ala-β-Ala-NHMe, *t*-Boc-Aib-β-Ala-NHMe, and *t*-Boc-Aib-Aib-β-Ala-NHMe has been performed by X-ray diffraction. While the conformation adopted by *t*-Boc-L-Ala-β-Ala-NHMe and *t*-Boc-Aib-β-Ala-NHMe is essentially extended, *t*-Boc-Aib-Aib-β-Ala-NHMe is folded into two consecutive intramolecularly hydrogen-bonded structures of the $i + 3 \longrightarrow i$ type (β-bends), with Aib(1)–Aib(2) and Aib(2)–β-Ala(3), respectively, as corner residues. Owing to the presence of the β-aminoacid, the latter β-bend is characterized by an unusual C_{11} hydrogen-bonded ring. These results indicate that: (i) a β-aminoacid may be incorporated into a β-bend without a major perturbation of the overall geometry of this folded conformation, and (ii) the propensity of the β-Ala residue for β-bend formation is rather low, unless other conformational constraints (*e.g.* a preceding β-bend) are present in the linear peptide molecule.

β-Alanine (β-Ala; 3-aminopropionic acid) is widely distributed in the animal and plant kingdoms. Peptides containing β-Ala include homo-glutathione, carnosine, anserine, ophidine, destruxin, efrapeptin and leucinostatins.[1–3]

The $i + 3 \longrightarrow i$ intramolecularly hydrogen-bonded conformation, also called β-bend or C_{10}-ring conformation [Fig. 1(*a*)], is the fundamental structural determinant of the regions where polypeptide chains fold.[4,5] If, however, a β-aminoacid is incorporated in either the $i + 1$ or the $i + 2$ position of the bend, the corresponding $i + 3 \longrightarrow i$ intramolecularly hydrogen-bonded conformation will contain one more tetrahedral carbon atom (C_{11}-ring conformation) [Fig. 1(*b*)].

Among the various X-ray structures of β-Ala-containing peptides reported in the literature,[6–19] the C_{11} β-bend conformation has not been found so far, neither in the cyclic nor in the short linear compounds having the minimal main chain length required for formation of the intramolecular hydrogen bond. The only example of a C_{11}-ring structure is seen in the X-ray structure of the nonapeptide antibiotic leucinostatin A,[20] at the level of Aib(8)–β-Ala(9) sequence (where Aib is α-aminoisobutyric acid). The C_{11} β-bend is preceded by an α-helical stretch, spanning from the N-terminus of the molecule to Aib(8), and it is followed by another intramolecularly hydrogen-bonded structure involving the protonated C-terminal, *N*,*N*-dimethylpropane-1,2-diamine as the hydrogen-bonding donor. The C_{11} β-bend, therefore, can be considered internal to, and induced by, an overall helical structure. The question arises whether β-Ala might be accommodated into a C_{11}-ring structure when the latter is not part of a helical segment and, in particular, when it is not followed by another intramolecularly hydrogen-bonded structure.

In the present contribution the synthesis and the structural analyses in the crystal state (by X-ray diffraction) of *t*-Boc-L-Ala-β-Ala-NHMe (**1**), *t*-Boc-Aib-β-Ala-NHMe (**2**), and *t*-Boc-Aib-Aib-β-Ala-NHMe (**3**) (*t*-Boc, *tert*-butyloxycarbonyl; NHMe, *N*-methylamino) are reported. Compounds **1** and **2** have the minimal chain length required for the formation of a C_{11}-ring conformation; in addition, the latter incorporates an Aib residue that is known to have a high tendency to promote a folded conformation.[21–25] Compound **3** can give rise in principle to two consecutive β-bends. Further interest in compounds **2** and **3** arises from the observation that their sequences correspond to the protected 8–9 and 7–9 segments, respectively, of leucinostatins,[26–33] thus allowing a direct comparison between their conformational preferences as isolated segments and as inserted into the helical leucinostatin A peptide molecule.[20]

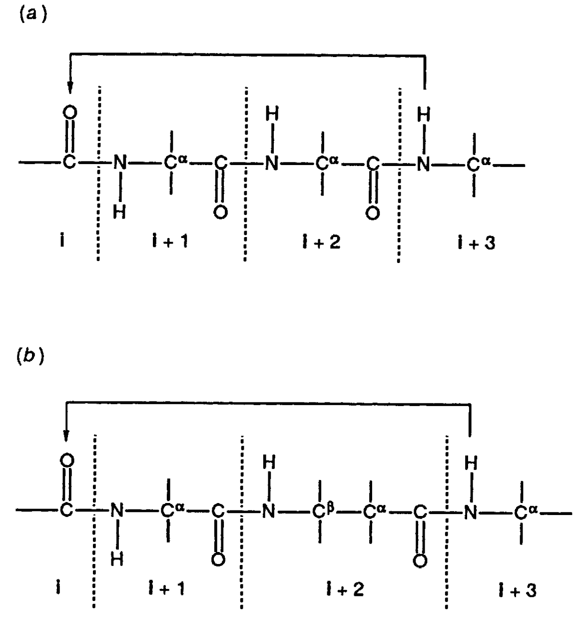

Fig. 1 The $i + 3 \longrightarrow i$ intramolecularly hydrogen-bonded structure in a sytem of four linked peptide units, containing: (*a*) two α-aminoacids as the central residues, and (*b*) one α-aminoacid followed by a β-aminoacid as the central residues

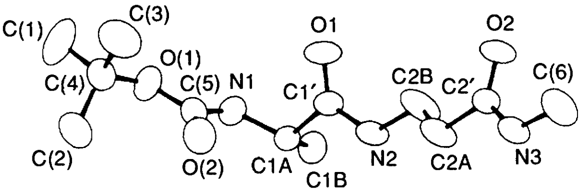

Fig. 2 Molecular structure of *t*-Boc-L-Ala-β-Ala-NHMe

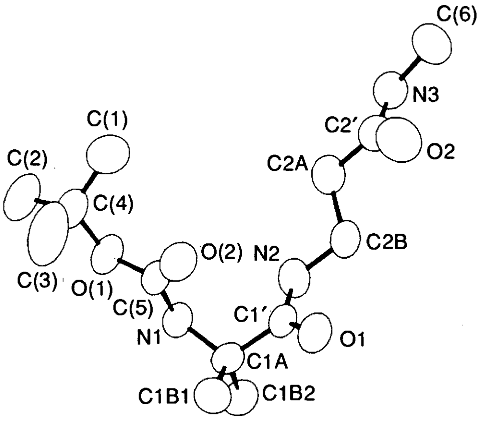

Fig. 3 Molecular structure of *t*-Boc-Aib-β-Ala-NHMe

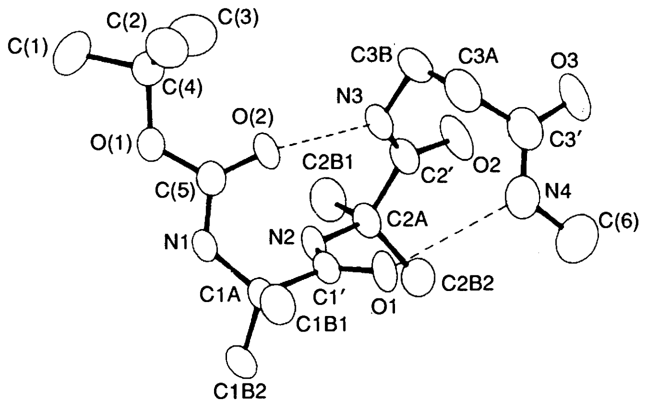

Fig. 4 Molecular structure of *t*-Boc-Aib-Aib-β-Ala-NHMe. The intramolecular hydrogen-bonds are indicated as dashed lines.

Experimental

Materials.—*t*-Boc-L-Ala-β-Ala-NHMe **1** was synthesized in 74% yield by reacting *t*-Boc-L-Ala-β-Ala-OMe (prepared, in turn, from *t*-Boc-L-Ala-OH and H-β-Ala-OMe hydrochloride in the presence of triethylamine and *N,N′*-dicyclohexylcarbodiimide) with gaseous methylamine in anhydrous methanol: m.p. 194 °C; TLC (silica gel plates) R_{f1} ($CHCl_3$–MeOH 94:6) 0.27.

t-Boc-Aib-β-Ala-NHMe **2** was obtained in a similar way, in 84% yield, starting from *t*-Boc-Aib-β-Ala-OMe (prepared, in turn, from *t*-Boc-Aib-OH and H-β-Ala-OMe hydrochloride in the presence of triethylamine and *N,N′*-dicyclohexylcarbodiimide): m.p. 152 °C; R_{f1} 0.20.

t-Boc-Aib-Aib-β-Ala-NHMe **3** was prepared in a similar way, in 84% yield, from *t*-Boc-Aib-Aib-β-Ala-OMe (synthesized, in turn by reacting *t*-Boc-Aib-Aib-OH and H-β-Ala-OMe hydrochloride in the presence of triethylamine and *N,N′*-dicyclohexylcarbodiimide): m.p. 162 °C; R_{f1} 0.23.

Crystal data for t-Boc-L-Ala-β-Ala-NHMe (**1**). $C_{12}H_{23}N_3O_4$, $M = 273.3$. Monoclinic, $a = 11.063(4)$, $b = 6.321(3)$, $c = 11.691(4)$ Å, $\beta = 111.68(9)°$, $V = 759.7(9)$ Å^3, space group $P2_1$, $Z = 2$, $D_c = 1.195$ g cm^{-3}, $D_m = 1.19$ g cm^{-3}, final R value 0.044, final R_w value 0.042.

Crystal data for t-Boc-Aib-β-Ala-NHMe **2**. $C_{13}H_{25}N_3O_4$, $M = 287.4$. Monoclinic, $a = 11.077(2)$, $b = 8.391(1)$, $c = 9.368(1)$ Å, $\beta = 94.0(2)°$, $V = 868.6(3)$ Å^3, space group P_c, $Z = 2$, $D_c = 1.099$ g cm^{-3}, $D_m = 1.10$ g cm^{-3}, final R value 0.040, final R_w value 0.047.

Crystal data for t-Boc-Aib-Aib-β-Ala-NHMe **3**. $C_{17}H_{32}N_4O_5$, $M = 372.5$. Orthorhombic, $a = 16.214(6)$, $b = 15.497(6)$, $c = 16.406(7)$ Å, $V = 4122.4(7)$ Å^3, space group *Pbca*, $Z = 8$, $D_c = 1.200$ g cm^{-3}, $D_m = 1.20$ g cm^{-3}, final R value 0.068, final R_w value 0.062.

X-Ray Structure Determination of t-Boc-L-Ala-β-Ala-NHMe **1** *and t*-Boc-Aib-Aib-β-Ala-NHMe (**3**).—Colourless crystals of *t*-Boc-L-Ala-β-Ala-NHMe (**1**) and *t*-Boc-Aib-Aib-β-Ala-NHMe (**3**) were grown by slow evaporation from chloroform–ethanol and ethyl acetate–chloroform–methanol solutions, respectively. Crystal system and space group were determined by preliminary Weissemberg photographs. Enraf-Nonius CAD4 diffractometer, θ–2θ scan mode up to $2\theta = 140°$; graphite-monochromatized Cu-Kα radiation ($\lambda = 1.541\,78$ Å); 1655 and 4174 unique reflections for **1** and **3**, respectively, were corrected for Lorentz and polarization effects; 1542 and 3722 reflections with $I \geqslant 3\sigma(I)$ were considered observed and used for the refinement of **1** and **3**, respectively. Both structures were solved by MULTAN;[34] the E-maps corresponding to the highest combined figure of merit revealed the position of all heavy atoms. Hydrogen atoms were in part located on successive difference Fourier maps, and in part calculated. Refinement of both structures was performed by a full-matrix least-squares procedure minimizing the quantity $w(F_o - F_c)^2$, with $w = 1/\sigma(F_o)^2$. All non-hydrogen atoms were refined anisotropically, while hydrogen atoms were included in the calculations with isotropic thermal parameters equal to the B equivalent of the carrier atom and they were not refined. The atomic scattering factors for all atomic species were calculated according to Cromer and Waber.[35] All calculations were performed on a MicroVAX Digital Computer at the *Centro di Metodologie Chimico-Fisiche* at the University of Naples, using the SDP package.[36] Full details of the experimental procedure are given in ref. 37.

X-*Ray Structure Determination of t*-Boc-Aib-β-Ala-NHMe **2**.—Colourless crystals of **2** were grown by slow evaporation from a chloroform–ethanol solution. Philips PW 1100 four-circle diffractometer; θ–2θ scan mode up to $2\theta = 56°$; graphite-monochromatized Mo-Kα radiation ($\lambda = 0.7107$ Å); 2227 unique reflections were corrected for Lorentz and polarization effects; 1094 reflections with $F \geqslant 7\sigma(F)$ were considered observed. The structure was solved by direct methods using SHELXS-86[38] and refined by blocked least-squares with weight $w = 1/[\sigma^2(F) + 0.003\,95\ F^2]$. The thermal parameters were anisotropic for all non-hydrogen atoms. Hydrogen atoms were found on a difference Fourier map and refined isotropically. All calculations were performed on a MicroVAX 3400 Digital Computer with SHELX-76 software.[39]

Tables of hydrogen atom co-ordinates, bond lengths and angles and thermal parameters for compounds **1**–**3** have been deposited at the Cambridge Crystallographic Data Centre.* Fractional atomic co-ordinates are given in Table 1.

Results and Discussion

The molecular structures of *t*-Boc-L-Ala-β-Ala-NHMe **1**, *t*-Boc-Aib-β-Ala-NHMe **2**, and *t*-Boc-Aib-Aib-β-Ala-NHMe **3**, deter-

---

* For details of the deposition scheme see 'Instruction for Authors,' *J. Chem. Soc., Perkin Trans. 2*, 1992, issue 1.

Table 1 Fractional atomic co-ordinates with estimated standard deviations in parentheses

Atom	*x*	*y*	*z*
t-Boc-L-Ala-β-Ala-NHMe **1**			
C(1)	0.9522(3)	1.1487	0.5907(4)
C(2)	0.9464(4)	1.2724(8)	0.7905(5)
C(3)	0.9495(5)	0.8896(8)	0.7470(5)
C(4)	0.9043(3)	1.1027(6)	0.6934(3)
O(1)	0.7637(2)	1.1029(5)	0.6297(2)
C(5)	0.6827(3)	1.0695(6)	0.6902(2)
O(2)	0.7178(2)	1.0527(5)	0.8017(2)
N_1	0.5604(2)	1.0624(5)	0.6119(2)
C_1^{α}	0.4542(3)	1.0290(5)	0.6531(2)
C_1^{β}	0.3290(3)	1.1016(6)	0.5536(3)
C_1'	0.4404(3)	0.7955(6)	0.6834(2)
O_1	0.4864(2)	0.6520(4)	0.6429(2)
N_2	0.3714(3)	0.7648(5)	0.7548(2)
C_2^{β}	0.3309(4)	0.5544(7)	0.7761(3)
C_2^{α}	0.3487(4)	0.5125(6)	0.9020(3)
C_2'	0.3148(3)	0.2850(5)	0.9241(3)
O_2	0.3026(2)	0.1448(4)	0.8479(2)
N_3	0.2973(3)	0.2529(5)	1.0277(2)
C(6)	0.2660(4)	0.0502(7)	1.0649(3)
t-Boc-Aib-β-Ala-NHMe **2**			
C(1)	0.0679(8)	−0.8783(8)	0.141(1)
C(2)	−0.0763(5)	−0.7613(8)	0.3026(8)
C(3)	−0.0754(8)	−0.664(2)	0.050(1)
C(4)	−0.0010(4)	−0.7312(6)	0.1755(5)
O(1)	0.0845	−0.6093(4)	0.2310
C(5)	0.1697(3)	−0.5468(5)	0.1518(4)
O(2)	0.1912(3)	−0.5902(4)	0.0335(3)
N_1	0.2309(3)	−0.4334(4)	0.2281(4)
C_1^{α}	0.3220(4)	−0.3313(5)	0.1684(5)
$C_1^{\beta 1}$	0.2653(5)	−0.2310(6)	0.0466(6)
$C_1^{\beta 2}$	0.3738(5)	−0.2262(5)	0.2910(5)
C_1'	0.4263(4)	−0.4329(5)	0.1143(4)
O_1	0.4694(3)	−0.4009(4)	0.0003(3)
N_2	0.4681(3)	−0.5478(5)	0.1991(4)
C_2^{β}	0.5667(4)	−0.6530(6)	0.1656(6)
C_2^{α}	0.5373(5)	−0.8261(7)	0.1916(5)
C_2'	0.6211(4)	−0.9387(6)	0.1233(5)
O_2	0.6429(4)	−0.9216(5)	−0.0030(4)
N_3	0.6681(4)	−1.0546(4)	0.2028(4)
C(6)	0.7424(7)	−1.1800(8)	0.1471(8)
t-Boc-Aib-Aib-β-Ala-NHMe **3**			
C(1)	0.0113(2)	0.1763(3)	0.1935(2)
C(2)	0.0720(2)	0.2796(2)	0.0946(2)
C(3)	0.0299(2)	0.1303(3)	0.0495(3)
C(4)	0.0642(2)	0.1860(2)	0.1172(2)
O(1)	0.1443(1)	0.1512(1)	0.1441(1)
C(5)	0.2081(1)	0.1459(2)	0.0925(1)
O(2)	0.2072(1)	0.1734(1)	0.0217(1)
N_1	0.2727(1)	0.1057(1)	0.1262(1)
C_1^{α}	0.3521(1)	0.0990(2)	0.0848(1)
$C_1^{\beta 1}$	0.3936(2)	0.1867(2)	0.0784(2)
$C_1^{\beta 2}$	0.4059(2)	0.0353(2)	0.1315(1)
C_1'	0.3391(1)	0.0593(2)	−0.0017(1)
O_1	0.3764(1)	0.0882(1)	−0.0616(1)
N_2	0.2884(1)	−0.0083(1)	−0.0038(1)
C_2^{α}	0.2709(1)	−0.0612(2)	−0.0767(1)
$C_2^{\beta 1}$	0.2119(2)	−0.1322(2)	−0.0494(2)
$C_2^{\beta 2}$	0.3497(2)	−0.1001(2)	−0.1115(2)
C_2'	0.2268(2)	−0.0081(2)	−0.1428(1)
O_2	0.2194(1)	−0.0406(1)	−0.2114(1)
N_3	0.1954(1)	0.0677(1)	−0.1235(1)
C_3^{β}	0.1603(2)	0.1260(2)	−0.1838(2)
C_3^{α}	0.2257(2)	0.1855(2)	−0.2207(2)
C_3'	0.2841(2)	0.1377(2)	−0.2763(2)
O_3	0.2643(1)	0.1184(1)	−0.3472(1)
N_4	0.3574(2)	0.1181(2)	−0.2451(1)
C(6)	0.4162(3)	0.0666(3)	−0.2916(2)

Table 2 Torsion angles (°) (with estimated standard deviations in parentheses) for *t*-Boc-L-Ala-β-Ala-NHMe **1**

C(5)–O(1)–C(4)–C(1)	−179.3(5)
C(5)–O(1)–C(4)–C(2)	−60.5(5)
C(5)–O(1)–C(4)–C(3)	63.4(5)
O(2)–C(5)–O(1)–C(4)	5.8(5)
N_1–C(5)–O(1)–C(4)	−175.6(5)
C_1^{α}–N_1–C(5)–O(1)	−179.8(5)
C_1^{α}–N_1–C(5)–O(2)	−1.3(5)
C_1^{β}–C_1^{α}–N_1–C(5)	160.9(5)
C_1'–C_1^{α}–N_1–C(5)	−78.0(5)
O_1–C_1'–C_1^{α}–N_1	−20.5(5)
O_1–C_1'–C_1^{α}–C_1^{β}	100.6(5)
N_2–C_1'–C_1^{α}–N_1	161.2(5)
N_2–C_1'–C_1^{α}–C_1^{β}	−77.8(5)
C_2^{β}–N_2–C_1'–C_1^{α}	169.9(5)
C_2^{β}–N_2–C_1'–O_1	−8.5(5)
C_2^{α}–C_2^{β}–N_2–C_1'	135.9(5)
C_2'–C_2^{α}–C_2^{β}–N_2	−175.8(5)
O_2–C_2'–C_2^{α}–C_2^{β}	15.3(5)
N_3–C_2'–C_2^{α}–C_2^{β}	−163.4(5)
C(6)–N_3–C_2'–C_2^{α}	−179.5(5)
C(6)–N_3–C_2'–O_2	1.9(5)

Table 3 Torsion angles (°) (with estimated standard deviations in parentheses) for *t*-Boc-Aib-β-Ala-NHMe **2**

C(5)–O(1)–C(4)–C(1)	63.2(6)
C(5)–O(1)–C(4)–C(2)	−180.0(4)
C(5)–O(1)–C(4)–C(3)	−61.4(6)
O(2)–C(5)–O(1)–C(4)	−5.7(6)
N_1–C(5)–O(1)–C(4)	177.3(4)
C_1^{α}–N_1–C(5)–O(1)	−173.9(4)
C_1^{α}–N_1–C(5)–O(2)	9.0(7)
$C_1^{\beta 1}$–C_1^{α}–N_1–C(5)	62.1(6)
$C_1^{\beta 2}$–C_1^{α}–N_1–C(5)	−177.1(4)
C_1'–C_1^{α}–N_1–C(5)	−59.7(5)
O_1–C_1'–C_1^{α}–N_1	138.1(4)
O_1–C_1'–C_1^{α}–$C_1^{\beta 1}$	15.8(6)
O_1–C_1'–C_1^{α}–$C_1^{\beta 2}$	−105.6(5)
N_2–C_1'–C_1^{α}–N_1	−44.8(5)
N_2–C_1'–C_1^{α}–$C_1^{\beta 1}$	−167.1(4)
N_2–C_1'–C_1^{α}–$C_1^{\beta 2}$	71.5(5)
C_2^{β}–N_2–C_1'–C_1^{α}	−179.2(4)
C_2^{β}–N_2–C_1'–O_1	−2.2(7)
C_2^{α}–C_2^{β}–N_2–C_1'	−132.8(5)
C_2'–C_2^{α}–C_2^{β}–N_2	165.0(4)
O_2–C_2'–C_2^{α}–C_2^{β}	−48.9(7)
N_3–C_2'–C_2^{α}–C_2^{β}	131.7(5)
C(6)–N_3–C_2'–C_2^{α}	174.6(5)
C(6)–N_3–C_2'–O_2	−4.8(8)

mined by X-ray diffraction, are illustrated in Figs. 2–4, respectively. Torsion angles[40] are reported in Tables 2–4. The backbone conformation of the β-Ala residue can be described by the φ, μ and ψ torsion angles,[9,15] corresponding to the rotation around N–C^{β}, C^{β}–C^{α} and C^{α}–C′ bonds, respectively. The chirality of L-Ala dictates the signs of torsion angles of *t*-Boc-L-Ala-β-Ala-NHMe **1**. In the unit cells of *t*-Boc-Aib-β-Ala-NHMe **2** and *t*-Boc-Aib-Aib-β-Ala-NHMe **3** molecules of both handedness occur simultaneously: in Tables 1, 3 and 4 only the positional parameters and the torsion angles, respectively, of the right-handed molecules are listed.

Bond distances and bond angles of structures **1**–**3** (deposited) are in good agreement with literature values of the *t*-Boc,[41] the secondary amide[42,43] and peptide[44] groups, and of the β-Ala[6–20] and Aib[45,46] residues.

In all three compounds the secondary amide bond of the urethane moiety is found in the *Z* (*trans*) conformation. Some distortion from the *trans*-planarity is observed for **2** and **3** ($\omega_0 = -173.9°$ and $-174.2°$, respectively). For the three

Table 4 Torsion angles (°) (with estimated standard deviations in parentheses) for *t*-Boc-Aib-Aib-β-Ala-NHMe **3**

C(5)–O(1)–C(4)–C(1)	177.9(6)
C(5)–O(1)–C(4)–C(2)	−64.7(5)
C(5)–O(1)–C(4)–C(3)	61.4(6)
O(2)–C(5)–O(1)–C(4)	4.4(5)
N_1–C(5)–O(1)–C(4)	−174.5(6)
C_1^{α}–N_1–C(5)–O(1)	−174.2(5)
C_1^{α}–N_1–C(5)–O(2)	7.0(5)
$C_1^{\beta 1}$–C_1^{α}–N_1–C(5)	68.5(5)
$C_1^{\beta 2}$–C_1^{α}–N_1–C(5)	−169.0(5)
C_1'–C_1^{α}–N_1–C(5)	−53.6(4)
O_1–C_1'–C_1^{α}–N_1	138.3(6)
O_1–C_1'–C_1^{α}–$C_1^{\beta 1}$	15.8(5)
O_1–C_1'–C_1^{α}–$C_1^{\beta 2}$	−104.8(6)
N_2–C_1'–C_1^{α}–N_1	−44.2(4)
N_2–C_1'–C_1^{α}–$C_1^{\beta 1}$	−166.7(6)
N_2–C_1'–C_1^{α}–$C_1^{\beta 2}$	72.7(5)
C_2^{α}–N_2–C_1'–C_1^{α}	−175.2(6)
C_2^{α}–N_2–C_1'–O_1	2.3(5)
$C_2^{\beta 1}$–C_2^{α}–N_2–C_1'	178.3(5)
$C_2^{\beta 2}$–C_2^{α}–N_2–C_1'	57.9(5)
C_2'–C_2^{α}–N_2–C_1'	−64.7(5)
O_2–C_2'–C_2^{α}–N_2	169.5(6)
O_2–C_2'–C_2^{α}–$C_2^{\beta 1}$	−74.2(6)
O_2–C_2'–C_2^{α}–$C_2^{\beta 2}$	46.1(5)
N_3–C_2'–C_2^{α}–N_2	−13.6(5)
N_3–C_2'–C_2^{α}–$C_2^{\beta 1}$	102.7(6)
N_3–C_2'–C_2^{α}–$C_2^{\beta 2}$	−137.1(6)
C_3^{β}–N_3–C_2'–C_2^{α}	173.5(6)
C_3^{β}–N_3–C_2'–O_2	−9.8(6)
C_3^{α}–C_3^{β}–N_3–C_2'	−88.0(6)
C_3'–C_3^{α}–C_3^{β}–N_3	71.0(5)
N_4–C_3'–C_3^{α}–C_3^{β}	−101.3(6)
O_3–C_3'–C_3^{α}–C_3^{β}	78.9(7)
C(6)–N_4–C_3'–C_3^{α}	175.5(8)
C(6)–N_4–C_3'–O_3	−4.7(6)

compounds the C(4)–O(1) bond of the urethane moieties is in the usual *trans* arrangement relative to the C(5)–N(1) bond, the C(4)–O(1)–C(5)–N(1) torsion angles being comprised between −174.5° and 177.3°. This structural property, accompanied by the *trans* conformation of the –CO–NH– group, allows us to classify the urethane moiety of all three compounds as type *b*.[40]

The secondary peptide and amide units are *trans*, the most significant deviation from planarity being exhibited by ω_1 of **1** (169.9°).

In *t*-Boc-L-Ala-β-Ala-NHMe **1** the L-Ala residue adopts a semi-extended conformation characterized by $\varphi_1 = -78.0°$, $\psi_1 = 161.2°$, while the β-Ala residue, with torsion angles $\varphi_2 = 135.9°$, $\mu_2 = -175.8°$, and $\psi_2 = -163.4°$, is essentially extended. As a result, the overall shape of the molecule is linear, except for a slight bend at the C^{α} atom of the L-Ala residue.

A sharp kink at the C^{α} atom of Aib determines the V-shaped backbone of *t*-Boc-Aib-β-Ala-NHMe **2**. This feature has to be ascribed to the helical conformation adopted by the Aib residue, ($\varphi_1 = -59.7°$, $\psi_1 = -44.8°$) a usual observation for this $C^{\alpha,\alpha}$-disubstituted α-aminoacid.[21–25] The φ_2, μ_2 and ψ_2 torsion angles of β-Ala are −132.8°, 165.0° and 131.7°, respectively. Therefore the molecule, although containing an Aib residue (a strong β-bend/helix inducer),[21–25] and having a main chain long enough for β-bend formation, does not adopt such an intramolecularly hydrogen-bonded conformation. This finding is in agreement with the results of the X-ray structural investigation of *t*-Boc-Aib-β-Ala-Aib-OMe,[6] which also does not form a β-bend, owing to the semi-extended conformation adopted by the β-Ala residue ($\varphi = -87°$, $\mu = -177°$, $\psi = 91°$).

Conversely, *t*-Boc-Aib-Aib-β-Ala-NHMe **3** is folded into two consecutive β-bends stabilized by two intramolecular hydrogen bonds, between the NH of β-Ala(3) and the carbonyl oxygen of the urethane moiety, and between the NH of the C-terminal *N*-methylamino group and the carbonyl of Aib(1), respectively. The $N_3 \cdots O(2)$ and $N_4 \cdots O_1$ distances are 2.90 Å and 3.07 Å, respectively.[47–49] The sets of φ, ψ torsion angles of Aib(1) and Aib(2), which define the geometry of the first β-bend ($\varphi_1 = -53.6°$, $\psi_1 = -44.2°$; $\varphi_2 = -64.7°$, $\psi_2 = -13.6°$) are close to those of an ideal type-III β-bend[4,5,50] ($\varphi_1 = \varphi_2 = -60°$; $\psi_1 = \psi_2 = -30°$). The second β-bend, having Aib(2) and the β-aminoacid β-Ala(3) as corner residues, is characterized by the presence of an intramolecularly hydrogen-bonded C_{11}-ring structure. Aib(2) shows φ,ψ torsion angles compatible with either a type-I or a type-III C_{10}-structure;[50] the conformation of the β-Ala(3) residue is defined by $\varphi_3 = -88.8°$, $\mu_3 = 71.0°$ and $\psi_3 = -101.3°$.

As already mentioned, the only other example of a C_{11} β-bend is found at the Aib(8)–β-Ala(9) sequence of leucinostatin A,[20] and it is characterized by the following conformational parameters: $\varphi_8 = -54°$, $\psi_8 = -53°$; $\varphi_9 = -103°$, $\mu_9 = 80°$, $\psi_9 = -78°$. A comparison of the backbone torsion angles of the C_{11} β-bends formed by *t*-Boc-Aib-Aib-β-Ala-NHMe **3** and leucinostatin A leads to the following conclusions: in both structures the orientation of the central peptide bond is the same as in type-I/III C_{10} β-bends,[50] and the β-Ala torsion angle around the C^{β}–C^{α} bond (μ) is close to the *gauche*$^+$ conformation; in addition, φ and ψ of β-Ala are both negative, but in the former structure φ is halfway between *gauche* and skew and ψ is close to skew, while in the latter φ and ψ are closer to skew and *gauche*, respectively. Overall, in the two structures the different φ and ψ values of β-Ala compensate each other, leading to a similar orientation of the NH that follows in the chain, appropriate for the formation of the intramolecular hydrogen bond. Interestingly, in the crystal structures of β-Ala derivatives and linear peptides the μ torsion angle is *trans* in *t*-Boc-Aib-β-Ala-Aib-OMe[6] and carnosine,[16,17] but *gauche* in *t*-Boc-β-Ala-OH,[7] β-Ala-ciliatine,[18] and in the L-Lys:D-pantothenate complex.[13]

In the crystals of *t*-Boc-L-Ala-β-Ala-NHMe **1** all potential hydrogen-bonding donors and acceptors are involved in intermolecular hydrogen bonds. N_1 and N_3 are bonded to O_1 and O(2), respectively, of a ($1 - x$, $-\frac{1}{2} - y$, $1 - z$) symmetry-related molecule, the $N_1 \cdots O_1$ and $N_3 \cdots O(2)$ distances being 2.89 Å and 2.80 Å.[47–49] The third hydrogen bond is observed between N_2 and O_2 (x, $-1 + y$, z), with a $N \cdots O$ distance of 2.85 Å. Such a hydrogen-bonding scheme gives rise to layers of molecules parallel to the *bc* plane; packing in the *a* direction is then achieved through van der Waals interactions between the hydrophobic moieties of the N-terminal *t*-Boc group.

In the packing mode of *t*-Boc-Aib-β-Ala-NHMe **2** N_1 and N_2 are hydrogen-bonded to O(2) and O_1, respectively, of a (x, $-1 - y$, $\frac{1}{2} + z$) symmetry-related molecule. The $N_1 \cdots O(2)$ and $N_2 \cdots O_1$ distances are 2.93 Å and 2.85 Å, respectively. Thus, along the *c* direction rows of molecules are generated, which are also linked in the *b* direction through $N_3 \cdots O_2$ (x, $-2 - y$, $\frac{1}{2} + z$) hydrogen bonds (the pertinent $N \cdots O$ distance is 2.80 Å). Packing is then completed along *a* through hydrophobic interactions.

The folded molecules of *t*-Boc-Aib-Aib-β-Ala-NHMe **3** are held together in the crystal by intermolecular hydrogen bonds between N_1 and N_2 (which do not take part in the intramolecular hydrogen-bonding scheme) with O_2 and O_3 of ($1 - x$, $-\frac{1}{2} - y$, $1 - z$) and (x, $-1 + y$, z) symmetry-related molecules, respectively. The latter interaction is rather weak (the $N_2 \cdots O_3$ distance is 3.20 Å), while in the former the $N_1 \cdots O_2$ distance has a value of 2.85 Å. The intermolecular hydrogen bonds give rise to layers of molecules parallel to the *ac* plane, which, in turn, pack in the *b* direction through van der Waals interactions.

Conclusions

In this paper we have reported the structural analysis in the crystal-state (by X-ray diffraction) of *t*-Boc-L-Ala-β-Ala-NHMe **1**, *t*-Boc-Aib-β-Ala-NHMe **2**, and *t*-Boc-Aib-Aib-β-Ala-NHMe **3**. The conformation adopted by *t*-Boc-L-Ala-β-Ala-NHMe is essentially extended. Replacement of an Aib residue (a strong β-bend/helix inducer) for L-Ala is not sufficient to fold the *t*-Boc-Aib-β-Ala-NHMe **2** molecule into a C_{11} β-bend. The latter structural motif is observed, however, at the Aib(2)–β-Ala(3) sequence of *t*-Boc-Aib-Aib-β-Ala-NHMe **3**, where a preceding C_{10} β-bend, with Aib(1) and Aib(2) as corner residues, is also present. Compounds **2** and **3** correspond to the terminally-blocked 8–9 and 7–9 sequences, respectively, of leucinostatin. The only other example of a C_{11} β-bend reported so far is indeed observed at the Aib(8)–β-Ala(9) sequence of leucinostatin A,[20] where the C_{11}-ring, being internal to a helical segment, is sandwiched between other intramolecularly hydrogen-bonded forms. These results, taken together, indicate that an -Aib-β-Ala- sequence can fold into a C_{11} β-bend even if it is not stabilized by the contribution of another hydrogen-bonded form at the C-terminus. However, a β-bend at the N-terminus of the -Aib-β-Ala-sequence seems to be the minimal requirement for the adoption of the C_{11} β-bend conformation.

Therefore, a β-aminoacid may be incorporated at the $i + 2$ position of a β-bend without a dramatic perturbation of its overall geometry. In both *t*-Boc-Aib-Aib-β-Ala-NHMe and leucinostatin A the α-aminoacid at position $i + 1$ of the C_{11} β-bend adopts φ, ψ torsion angles close to those typical of type-I/III C_{10} β-bends ($\varphi = -60°$, $\psi = -30°$), while the β-Ala torsion angle about the C^{β}-C^{α} bond (μ) is *gauche*$^{+}$. An inspection of simple molecular models does indeed suggest that the latter structural property is a prerequisite for the formation of the C_{11} β-bend. By conformational energy computations we are currently exploring the possible sets of backbone torsion angles that allow formation of this folded structure.

Acknowledgements

We wish to acknowledge the financial support from MURST (40% and 60% to E. B. and C. P.) and from CNR of Italy (Grants CNR 90.00847 and CNR 90.1943). This work has been supported in part by the Department of Biotechnology, India. R. K. wishes to acknowledge Prof. G. P. Talwar of National Institute of Immunology, India, for his encouragement.

References

1 C. N. C. Drey, in *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*, ed. B. Weinstein, Dekker, New York, 1977, vol. 4, p. 241.
2 C. N. C. Drey, in *Chemistry and Biochemistry of the Amino Acids*, ed. G. C. Barrett, Chapman and Hall, London, 1985, p. 25.
3 O. W. Griffith, *Annu. Rev. Biochem.*, 1986, **55**, 855.
4 C. Toniolo, *CRC Crit. Rev. Biochem.*, 1980, **9**, 1.
5 G. D. Rose, L. M. Gierasch and J. A. Smith, *Adv. Protein Chem.*, 1985, **37**, 1.
6 E. Benedetti, A. Bavoso, B. Di Blasio, P. Grimaldi, V. Pavone, C. Pedone, C. Toniolo and G. M. Bonora, *Int. J. Biol. Macromol.*, 1985, **7**, 81.
7 G. Valle, G. M. Bonora and C. Toniolo, *Gazz. Chim. Ital.*, 1984, **114**, 341.
8 V. Pavone, A. Lombardi, X. Yang, C. Pedone and B. Di Blasio, *Biopolymers*, 1990, **30**, 189.
9 B. Di Blasio, A. Lombardi, X. Yang, C. Pedone and V. Pavone, *Biopolymers*, 1991, **31**, 1181.
10 V. Pavone, A. Lombardi, G. D'Auria, M. Saviano, F. Nastri, L. Paolillo, B. Di Blasio and C. Pedone, *Biopolymers*, 1992, **32**, 173.
11 S. Cerrini, E. Gavuzzo, G. Lucente, G. Luisi, F. Pinnen and L. Radics, *Int. J. Pept. Protein Res.*, 1991, **38**, 289.
12 E. Papavinasam, S. Natarajan and N. C. Shivaprakash, *Int. J. Pept. Protein Res.*, 1986, **28**, 525.
13 D. M. Salunke and M. Vijayan, *Biochim. Biophys. Acta*, 1984, **798**, 175.
14 J. P. Springer, R. J. Cole, J. W. Dorner, R. J. Cox, J. L. Richard, C. L. Barnes and D. van der Helm, *J. Am. Chem. Soc.*, 1984, **106**, 2388.
15 I. L. Karle, B. K. Handa and C. H. Hassall, *Acta Crystallogr., Sect. B*, 1975, **31**, 555.
16 Y. Barrans, A. M. Bellocq, M. Cotrait and H. Richard, *J. Mol. Struct.*, 1976, **30**, 225.
17 H. Itoh, T. Yamane, T. Ashida and M. Kakudo, *Acta Crystallogr., Sect. B*, 1977, **33**, 2959.
18 M. Cotrait, J. Prigent and C. Garrigou-Lagrange, *J. Mol. Struct.*, 1977, **39**, 175.
19 D. N. J. White and J. D. Dunitz, *Isr. J. Chem.*, 1972, **10**, 249.
20 S. Cerrini, D. Lamba, A. Scatturin, C. Rossi and G. Ughetto, *Biopolymers*, 1989, **28**, 409.
21 C. Toniolo, G. M. Bonora, A. Bavoso, E. Benedetti, B. Di Blasio, V. Pavone and C. Pedone, *Biopolymers*, 1983, **22**, 205.
22 B. V. V. Prasad and P. Balaram, *CRC Crit. Rev. Biochem.*, 1984, **16**, 307.
23 C. Toniolo, E. Benedetti and C. Pedone, *Gazz. Chim. Ital.*, 1986, **116**, 355.
24 C. Toniolo and E. Benedetti, *ISI Atlas of Science: Biochemistry*, 1988, **1**, 225.
25 C. Toniolo and E. Benedetti, *Macromolecules*, 1991, **24**, 4004.
26 Y. Mori, M. Suzuki, K. Fukushima and T. Arai, *J. Antibiot.*, 1983, **36**, 1084.
27 K. Fukushima, T. Arai, Y. Mori, M. Tsuboi and M. Suzuki, *J. Antibiot.*, 1983, **36**, 1613.
28 C. G. Casinovi, L. Tuttobello, C. Rossi and Z. Benciari, *Phytopat. Medit.*, 1983, **22**, 103.
29 A. Isogai, A. Suzuki, S. Tamura, S. Higashikawa and S. Kuyama, *J. Chem. Soc., Perkin Trans. 1*, 1984, 1405.
30 J. G. Stroh, K. L. Rinehart, Jr., J. C. Cook, T. Kihara, M. Suzuki and T. Arai, *J. Am. Chem. Soc.*, 1986, **108**, 858.
31 C. G. Casinovi, C. Rossi, L. Tuttobello and M. Ricci, *Eur. J. Med. Chem. Chim. Ther.*, 1986, **21**, 527.
32 C. Rossi, L. Tuttobello, M. Ricci, C. G. Casinovi and L. Radics, *J. Antibiot.*, 1987, **40**, 130.
33 L. Radics, M. Kajtar-Peredy, C. G. Casinovi, C. Rossi, M. Ricci and L. Tuttobello, *J. Antibiot.*, 1987, **40**, 714.
34 G. Germain, P. Main and M. M. Woolfson, *Acta Crystallogr., Sect. A*, 1971, **27**, 368.
35 D. T. Cromer and J. T. Waber, in *International Tables for X-Ray Crystallography*, The Kynock Press, Birmingham, 1974, vol. 4, Table 2.2.B.
36 Enraf-Nonius (1979). Structure Determination Package, Enraf-Nonius, Delft, The Netherlands.
37 V. Pavone, E. Benedetti, B. Di Blasio, C. Pedone, A. Santini, A. Bavoso, C. Toniolo, M. Crisma and L. Sartore, *J. Biomol. Struct. Dyn.*, 1990, **7**, 1321.
38 G. M. Sheldrick, in *Crystallographic Computing 3*, eds. G. M. Sheldrick, C. Kruger and R. Goddard, Oxford University Press, Oxford, 1985, p. 175.
39 G. M. Sheldrick, SHELX-76, Program for Crystal Structure Determinations, University of Cambridge, 1976.
40 IUPAC–IUB Commission on Biochemical Nomenclature, *Biochemistry*, 1970, **9**, 3471.
41 E. Benedetti, C. Pedone, C. Toniolo, G. Némethy, M. S. Pottle and H. A. Scheraga, *Int. J. Pept. Protein Res.*, 1980, **16**, 156.
42 L. Leiserowitz and M. Tuval, *Acta Crystallogr., Sect. B*, 1978, **34**, 1230.
43 P. Chakrabarti and J. D. Dunitz, *Helv. Chim. Acta*, 1982, **65**, 1555.
44 E. Benedetti, in *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*, ed. B. Weinstein, Dekker, New York, 1982, vol. 6, p. 105.
45 Y. Paterson, S. M. Rumsey, E. Benedetti, G. Némethy and H. A. Scheraga, *J. Am. Chem. Soc.*, 1981, **103**, 2947.
46 G. Valle, M. Crisma, F. Formaggio, C. Toniolo and G. Jung, *Liebigs Ann. Chem.*, 1987, 1055.
47 C. Ramakrishnan and N. Prasad, *Int. J. Protein Res.*, 1971, **3**, 209.
48 R. Taylor, O. Kennard and W. Versichel, *Acta Crystallogr., Sect. B*, 1984, **40**, 280.
49 C. H. Görbitz, *Acta Crystallogr., Sect. B*, 1989, **45**, 390.
50 C. M. Venkatachalam, *Biopolymers*, 1968, **6**, 1425.

Paper 2/01108E
Received 2nd *March* 1992
Accepted 1st *April* 1992