Acta Cryst. (1998). B**54**, 300–307

Structure of *Z*-(Aib)$_9$OBu^t

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(*Received 14 August 1997; accepted 10 October 1997*)

Abstract

The structure of the synthetic protected oligopeptide *Z*-(Aib)$_9$OBu^t, *tert*-butoxynona(α-aminoisobutyric acid), which contains the unusual α-aminoisobutyric acid (Aib), was determined by X-ray crystallography. The two independent molecules in the asymmetric unit fold into 3_{10}-helices, each stabilized by seven intramolecular hydrogen bonds. The C terminus of one of the molecules is disordered and adopts a semi-extended conformation, which is rather unusual for Aib residues. This is the first observation of such a conformation involved in a disorder in Aib-containing oligopeptides. The existence of a second conformation for the C-terminal residue might explain the difficulties in crystallizing the title compound and a different behaviour of the title compound in thin layer chromatography compared with the other homopeptides.

1. Introduction

α-Aminoisobutyric acid (Aib) differs from the usual amino acids in that it has two methyl groups attached to the Cα atom. The presence of two methyl groups reduces the accessible region in the conformational space considerably (Paterson *et al.*, 1981; Prasad & Sasisekharan, 1979). Aib residues are known as strong helical formers in peptides, also in the presence of common amino acids, favouring α- or 3_{10}-helices, depending on chain length, peptide composition and position of the Aib residues. Peptides consisting of only Aib residues show a clear preference for left- and right-handed 3_{10}-helices in solution and crystal structures (Benedetti *et al.*, 1982); this is confirmed by recent theoretical studies (Zhang & Hermans, 1994; Hodgkin *et al.*, 1990). The two methyl groups attached to Cα are staggered with the methyl groups of residue $n + 3$ and N—H···O=C main-chain–main-chain hydrogen bonds are formed between residue $n + 3$ and n. The helical backbone shows the form of a regular triangular rod. In fact, all crystal structures of Aib homopeptides have common properties: centrosymmetric space group, one molecule in the asymmetric unit, a regular 3_{10}-helix with the maximum number of hydrogen bonds, including the N-terminal protection group, a reversal of the helical sense at the C-terminal residue and crystal packing in the form of head-to-tail hydrogen-bonded helical columns, most of which pack in an antiparallel fashion (Benedetti *et al.*, 1982; Vlassi *et al.*, 1992; Di Blasio *et al.*, 1991; Pavone *et al.*, 1991; Vlassi *et al.*, 1993; Bavoso *et al.*, 1986; Pavone *et al.*, 1990).

The title compound fills the gap between the largest available so far structures of homopeptides (including homopeptides consisting of standard amino acids), *i.e.* the structures of *p*BrBz-(Aib)$_8$-OBu^t and *p*BrBz-(Aib)$_{10}$-OBu^t.

2. Experimental

Z-(Aib)$_9$OBu^t was synthesized from *Z*-(Aib)$_5$-Ox and H-(Aib)$_4$-OBu^t (Brückner, 1989; *Z* represents benzyloxycarbonyl, Ox the oxazolone of C-terminal Aib and OBu^t the *tert*-butyl ester) by conventional solution-phase procedures and crystallized from a chloroform–methanol–*n*-hexane mixture and recrystallized from a hot methanol–water mixture.

Only one crystal suitable for X-ray diffraction could be obtained; the colourless crystal was grown by slow evaporation from a methanol/water solution over a period of several years. X-ray diffraction data were collected on an automated Enraf–Nonius CAD-4 diffractometer. The unit-cell parameters were determined from 25 reflections ($15 \leq \theta \leq 23°$) by a least-squares fitting. Crystal data are listed in Table 1.

The structure was solved by direct methods; one set from a *MULTAN*87 run (Debaerdemaeker *et al.*, 1987) in $P\bar{1}$ with the options *SAYTAN*, *RANTAN*3 and *MAXSET*500 revealed two helical fragments with 55 and 13 atoms, respectively; both formed left-handed helices.

The positions of an additional 36 non-H atoms in the two fragments were located by assigning phases to the 2712 largest normalized structure factors E ($|E| > 1.3$) and using the *TEXP* option of *SHELXS*86 (Sheldrick, 1985) in two consecutive runs; difference-Fourier syntheses located three other non-H-atom positions. At this stage none of the remaining 34 atoms could be

Table 1. *Experimental details*

Crystal data	
Chemical formula	$2(C_{48}H_{79}N9O_{12}).4(O_{0.5}).0.5(CO)$
Chemical formula weight	1994.41
Cell setting	Triclinic
Space group	$P\bar{1}$
a (Å)	11.253 (6)
b (Å)	19.657 (9)
c (Å)	28.546 (14)
α (°)	105.32 (4)
β (°)	98.03 (6)
γ (°)	100.92 (5)
V (Å^3)	5858 (5)
Z	2
D_x (Mg m^{-3})	1.131
Radiation type	Cu $K\alpha$
Wavelength (Å)	1.54184
No. of reflections for cell parameters	25
θ range (°)	15–23
μ (mm^{-1})	0.681
Temperature (K)	293 (2)
Crystal form	Plate
Crystal size (mm)	1.2 × 0.7 × 0.2
Crystal colour	Colourless
Data collection	
Diffractometer	Enraf–Nonius CAD-4
Data collection method	$2\omega/2\theta$ scans
Absorption correction	Empirical *via* ψ scans
T_{min}	0.698
T_{max}	0.996
No. of measured reflections	14 926
No. of independent reflections	14 172
No. of observed reflections	9643
Criterion for observed reflections	$I > 2\sigma(I)$
R_{int}	0.0469
θ_{max} (°)	59.96
Range of h, k, l	$0 \rightarrow h \rightarrow 12$ $-22 \rightarrow k \rightarrow 21$ $-32 \rightarrow l \rightarrow 31$
No. of standard reflections	5
Frequency of standard reflections (min)	10 000
Intensity decay (%)	0.27
Refinement	
Refinement on	F^2
$R[F^2 > 2\sigma(F^2)]$	0.1007
$wR(F^2)$	0.2606
S	1.175
No. of reflections used in refinement	14 172
No. of parameters used	1501
H-atom treatment	Riding model approach
Weighting scheme	$w = 1/[\sigma^2(F_o^2) + (0.2000P)^2 + 0.0000P]$, where $P = (F_o^2 + 2F_c^2)/3$
$(\Delta/\sigma)_{max}$	0.05
$\Delta\rho_{max}$ (e Å^{-3})	0.541
$\Delta\rho_{min}$ (e Å^{-3})	−0.443
Extinction method	None
Source of atomic scattering factors	*International Table for Crystallography* (1992, Vol. C, Tables 4.2.6.8 and 6.1.1.4)

Table 1 (*cont.*)

Computer programs	
Data collection	*CAD-4* (Enraf–Nonius, 1989)
Cell refinement	*CAD-4* (Enraf–Nonius, 1989)
Data reduction	*SDP* (B. A. Frenz & Associates, 1985)
Structure solution	*MULTAN87* (Debaerdemaeker *et al.*, 1987); *SHELXS86* (Sheldrick, 1985); *SDP* (B. A. Frenz, 1985)
Structure refinement	*SDP* (B. A. Frenz & Associates, 1985), *SHELXL96* (Sheldrick & Schneider, 1997)
Preparation of material for publication	*SHELXL96* (Sheldrick & Schneider, 1997)

located. Furthermore, the correct N-to-C-terminal direction of the larger helix was not clearly detectable, because in the electron density maps the position of the −NH and −C=O groups revealed similar features. This could only be interpreted as a pattern resulting from the superposition of two helices, related by space-group symmetry by means of a wrong origin. The space group was changed to $P1$.

Subsequently, using coordinates of the smaller helical fragment in the left-handed conformation, substituting the coordinates of the larger helical fragment through application of the space-group symmetry (whereby an arbitrary decision about the N-to-C-terminal direction was taken) and expanding the structure by assigning phases to the 800 largest normalized structure factors E ($|E| > 2.0$), it was possible to locate the position of all 276 atoms in four helical fragments, two of them being left-handed and two right-handed. This procedure gave a better R value than using the coordinates of the two helical fragments (both left-handed) as they were located in $P\bar{1}$ (R = 0.242 for 261 atoms and 3263 E values *versus* R = 0.262 for 282 atoms and 3263 E values).

The space group was then changed again to $P\bar{1}$ with a shift to the new origin. The two molecules which form right-handed helices will be subsequently termed mol A and mol B. Difference-Fourier techniques located six solvent atoms, which were refined as one methanol and four water molecules. As the solvent molecules are located too close to their symmetry-equivalent counterparts to form favourable contacts, they were refined with 50% occupancy. After refinement of the thermal parameters of the disordered positions at the C-terminus of mol A, both conformations were assigned 50% occupancy and refined simultaneously. Isotropic refinement with the *SDP* package (B. A. Frenz & Associates Inc., 1985) converged at R = 0.214 for 8802 reflections with $I > 3\sigma(I)$ and unit weights.

At this stage an attempt was made to describe the disorder in mol A with two independent A molecules in $P1$. The C-terminal residues were omitted and difference-Fourier techniques clearly showed an identically

Table 2. *Fractional atomic coordinates and equivalent isotropic displacement parameters (Å^2)*

$U_{eq} = (1/3)\Sigma_i\Sigma_j U^{ij} a^i a^j \mathbf{a}_i.\mathbf{a}_j$.

	x	y	z	U_{eq}
Mol *A*				
C01	1.4193 (5)	0.1044 (4)	0.4461 (2)	0.0778 (15)
C02	1.4237 (6)	0.0392 (4)	0.4172 (3)	0.0925 (18)
C03	1.3775 (7)	−0.0241 (5)	0.4278 (3)	0.107 (2)
C04	1.3292 (7)	−0.0210 (5)	0.4689 (3)	0.108 (2)
C05	1.3259 (7)	0.0420 (6)	0.4994 (3)	0.117 (3)
C06	1.3721 (6)	0.1070 (5)	0.4894 (3)	0.102 (2)
C07	1.4638 (5)	0.1713 (4)	0.4333 (3)	0.096 (2)
O01	1.3675 (3)	0.1815 (2)	0.39766 (15)	0.0880 (11)
C08	1.2887 (5)	0.2201 (3)	0.4169 (2)	0.0707 (14)
O08	1.2981 (4)	0.2486 (2)	0.46067 (14)	0.0936 (12)
N1	1.2001 (4)	0.2202 (2)	0.38049 (16)	0.0669 (11)
C1*A*	1.1096 (5)	0.2640 (3)	0.38934 (18)	0.0621 (12)
C1*L*	1.0246 (6)	0.2494 (4)	0.3397 (2)	0.0831 (17)
C1*R*	1.1716 (6)	0.3436 (3)	0.4107 (3)	0.0900 (18)
C1	1.0300 (4)	0.2392 (3)	0.42473 (17)	0.0622 (12)
O1	0.9857 (3)	0.2835 (2)	0.45139 (13)	0.0778 (10)
N2	1.0103 (4)	0.1705 (2)	0.42429 (14)	0.0628 (10)
C2*A*	0.9329 (4)	0.1405 (3)	0.45450 (17)	0.0642 (13)
C2*L*	0.9568 (6)	0.0667 (3)	0.4541 (2)	0.0804 (16)
C2*R*	0.7950 (5)	0.1335 (4)	0.4323 (2)	0.0859 (18)
C2	0.9666 (5)	0.1887 (3)	0.50729 (17)	0.0626 (12)
O2	0.8894 (3)	0.1964 (2)	0.53364 (12)	0.0736 (9)
N3	1.0882 (4)	0.2166 (2)	0.52629 (15)	0.0716 (12)
C3*A*	1.1368 (5)	0.2566 (4)	0.5787 (2)	0.092 (2)
C3*L*	1.2757 (5)	0.2841 (6)	0.5851 (3)	0.148 (4)
C3*R*	1.1113 (10)	0.2046 (5)	0.6108 (2)	0.128 (3)
C3	1.0822 (4)	0.3227 (3)	0.5953 (2)	0.0760 (16)
O3	1.0795 (3)	0.3475 (2)	0.63918 (13)	0.0932 (13)
N4	1.0396 (4)	0.3518 (2)	0.56129 (18)	0.0787 (13)
C4*A*	0.9838 (6)	0.4137 (3)	0.5723 (2)	0.0904 (19)
C4*L*	0.9238 (10)	0.4210 (5)	0.5234 (3)	0.140 (4)
C4*R*	1.0784 (9)	0.4831 (4)	0.6027 (4)	0.145 (4)
C4	0.8768 (5)	0.3982 (3)	0.60022 (19)	0.0693 (14)
O4	0.8516 (4)	0.4504 (2)	0.62813 (13)	0.0852 (11)
N5	0.8194 (3)	0.3307 (2)	0.59075 (15)	0.0645 (11)
C5*A*	0.7142 (5)	0.3082 (3)	0.6138 (2)	0.0787 (16)
C5*L*	0.6859 (8)	0.2277 (4)	0.6010 (3)	0.133 (3)
C5*R*	0.6026 (6)	0.3328 (6)	0.5951 (3)	0.133 (3)
C5	0.7492 (5)	0.3391 (3)	0.66998 (19)	0.0683 (14)
O5	0.6684 (3)	0.3500 (2)	0.69468 (14)	0.0865 (11)
N6	0.8686 (4)	0.3517 (2)	0.69200 (15)	0.0654 (11)
C6*A*	0.9128 (5)	0.3806 (3)	0.74517 (19)	0.0738 (15)
C6*L*	1.0548 (6)	0.4045 (5)	0.7547 (2)	0.102 (2)
C6*R*	0.8695 (9)	0.3261 (4)	0.7716 (3)	0.110 (2)
C6	0.8694 (5)	0.4510 (3)	0.76604 (18)	0.0675 (13)
O6	0.8502 (4)	0.4676 (2)	0.80851 (13)	0.0880 (11)
N7	0.8573 (4)	0.4921 (2)	0.73662 (15)	0.0638 (10)
C7*A*	0.8277 (5)	0.5627 (3)	0.7522 (2)	0.0720 (14)
C7*L*	0.8049 (7)	0.5881 (3)	0.7065 (2)	0.095 (2)
C7*R*	0.9317 (6)	0.6173 (4)	0.7919 (3)	0.109 (2)
C7	0.7052 (5)	0.5552 (3)	0.77180 (18)	0.0675 (13)
O7	0.6916 (3)	0.6071 (2)	0.80312 (13)	0.0851 (11)
N8	0.6201 (4)	0.4946 (2)	0.75131 (15)	0.0700 (11)
C8*A*	0.4965 (5)	0.4822 (3)	0.7648 (3)	0.0906 (19)
C8*L*	0.4303 (7)	0.4037 (4)	0.7359 (4)	0.138 (4)
C8*R*	0.4243 (7)	0.5337 (5)	0.7511 (3)	0.119 (3)
C8	0.5111 (6)	0.4886 (3)	0.8197 (3)	0.094 (2)
O8	0.4388 (5)	0.5126 (3)	0.8442 (2)	0.135 (2)
N9	0.5993 (5)	0.4630 (3)	0.8401 (2)	0.1028 (18)
C9*A*	0.627 (4)	0.461 (2)	0.8946 (16)	0.124 (14)
C9*R*	0.6935 (14)	0.3989 (7)	0.8892 (4)	0.173 (5)

Table 2 (*cont.*)

	x	y	z	U_{eq}
Mol *A*-I				
C9*L*	0.7007 (18)	0.5266 (12)	0.9289 (8)	0.126 (6)
C9	0.5041 (18)	0.4303 (10)	0.9090 (8)	0.102 (6)
O9	0.4815 (11)	0.4574 (5)	0.9502 (3)	0.113 (3)
O*M*	0.426 (2)	0.3736 (13)	0.8798 (15)	0.143 (15)
Mol *A*-II				
C9*A*	0.598 (4)	0.4432 (15)	0.8833 (13)	0.113 (14)
C9*L*	0.597 (2)	0.5045 (13)	0.9269 (8)	0.125 (7)
C9	0.457 (4)	0.372 (2)	0.8681 (12)	0.128 (16)
O9	0.413 (2)	0.3480 (8)	0.8199 (6)	0.252 (11)
O*M*	0.407 (4)	0.372 (2)	0.8981 (12)	0.198 (19)
Mol *A*-I and mol *A*-II				
C*M*1	0.3049 (17)	0.3245 (11)	0.8889 (7)	0.221 (11)
C*M*2	0.3116 (16)	0.3134 (9)	0.9370 (6)	0.195 (5)
C*M*3	0.218 (2)	0.3831 (13)	0.8864 (8)	0.304 (13)
C*M*4	0.254 (2)	0.2772 (14)	0.8475 (8)	0.54 (3)
Solvent				
O*S*	0.3962 (8)	0.5155 (6)	0.4637 (5)	0.116 (4)
C*S*	0.480 (3)	0.5265 (13)	0.4810 (7)	0.162 (10)
O*W*2	0.967 (6)	0.5477 (16)	1.0056 (16)	0.56 (5)
O*W*1	0.6483 (12)	0.5406 (6)	0.4563 (5)	0.096 (4)
O*W*3	0.949 (2)	0.5759 (11)	0.9423 (16)	0.45 (3)
O*W*4	1.0381 (17)	0.4716 (10)	0.9521 (10)	0.250 (12)
Mol *B*				
C01	0.5961 (6)	0.2877 (4)	0.3023 (2)	0.0907 (18)
C02	0.6949 (9)	0.2900 (5)	0.3356 (4)	0.158 (4)
C03	0.7025 (12)	0.3175 (6)	0.3865 (4)	0.173 (5)
C04	0.6155 (11)	0.3445 (6)	0.4034 (3)	0.151 (4)
C05	0.5146 (10)	0.3443 (8)	0.3715 (4)	0.211 (7)
C06	0.5052 (8)	0.3164 (6)	0.3213 (3)	0.159 (4)
C07	0.5916 (8)	0.2555 (6)	0.2490 (2)	0.116 (3)
O01	0.4967 (3)	0.2752 (2)	0.22027 (12)	0.0767 (10)
C08	0.4857 (5)	0.2508 (3)	0.1707 (2)	0.0664 (13)
O08	0.5473 (4)	0.2104 (2)	0.15121 (14)	0.0845 (11)
N1	0.4025 (3)	0.2755 (2)	0.14718 (14)	0.0594 (10)
C1*A*	0.3678 (4)	0.2532 (3)	0.09358 (18)	0.0621 (12)
C1*L*	0.2827 (5)	0.2991 (4)	0.0795 (2)	0.0824 (17)
C1*R*	0.3015 (6)	0.1726 (3)	0.0739 (3)	0.0892 (18)
C1	0.4828 (4)	0.2675 (3)	0.07025 (17)	0.0576 (12)
O1	0.4883 (3)	0.2256 (2)	0.03057 (12)	0.0718 (10)
N2	0.5711 (3)	0.3260 (2)	0.09459 (15)	0.0597 (10)
C2*A*	0.6832 (4)	0.3485 (3)	0.07590 (19)	0.0632 (13)
C2*L*	0.7742 (5)	0.4067 (3)	0.1187 (2)	0.0847 (18)
C2*R*	0.6511 (6)	0.3768 (4)	0.0331 (3)	0.096 (2)
C2	0.7451 (4)	0.2850 (3)	0.06181 (18)	0.0607 (12)
O2	0.8024 (3)	0.28065 (19)	0.02733 (13)	0.0730 (9)
N3	0.7392 (3)	0.2385 (2)	0.08798 (14)	0.0564 (9)
C3*A*	0.8034 (4)	0.1793 (3)	0.08003 (17)	0.0578 (12)
C3*R*	0.9442 (4)	0.2100 (4)	0.0940 (2)	0.089 (2)
C3*L*	0.7593 (6)	0.1313 (3)	0.11127 (19)	0.0752 (15)
C3	0.7673 (4)	0.1323 (2)	0.02584 (16)	0.0546 (11)
O3	0.8397 (3)	0.10061 (18)	0.00561 (11)	0.0645 (9)
N4	0.6512 (3)	0.1246 (2)	0.00135 (13)	0.0565 (10)
C4*A*	0.6014 (4)	0.0781 (3)	−0.04956 (17)	0.0621 (13)
C4*R*	0.5975 (6)	−0.0013 (3)	−0.0528 (2)	0.0895 (19)
C4*L*	0.4731 (4)	0.0886 (4)	−0.0642 (2)	0.086 (2)
C4	0.6801 (4)	0.1006 (3)	−0.08559 (16)	0.0551 (11)
O4	0.6739 (3)	0.05646 (19)	−0.12566 (11)	0.0701 (9)
N5	0.7482 (3)	0.1675 (2)	−0.07255 (14)	0.0592 (10)
C5*A*	0.8278 (5)	0.1940 (3)	−0.10425 (19)	0.0680 (13)
C5*L*	0.9084 (7)	0.2671 (3)	−0.0740 (3)	0.099 (2)
C5*R*	0.7482 (7)	0.1975 (4)	−0.1508 (2)	0.100 (2)
C5	0.9149 (4)	0.1430 (3)	−0.11754 (17)	0.0587 (12)
O5	0.9555 (3)	0.13818 (19)	−0.15600 (12)	0.0719 (9)
N6	0.9459 (3)	0.1079 (2)	−0.08564 (13)	0.0531 (9)
C6*A*	1.0282 (4)	0.0583 (3)	−0.09341 (16)	0.0560 (11)

Table 2 (*cont.*)

	x	*y*	*z*	U_{eq}
C6*L*	1.0212 (6)	0.0185 (4)	−0.0548 (2)	0.0868 (19)
C6*R*	1.1593 (4)	0.0995 (4)	−0.0886 (2)	0.0893 (19)
C6	0.9856 (4)	0.0013 (3)	−0.14386 (17)	0.0597 (12)
O6	1.0617 (3)	−0.02643 (19)	−0.16538 (12)	0.0694 (9)
N7	0.8639 (3)	−0.0200 (2)	−0.16405 (14)	0.0619 (10)
C7*A*	0.8118 (5)	−0.0757 (3)	−0.2114 (2)	0.0773 (15)
C7*L*	0.6765 (5)	−0.0731 (5)	−0.2264 (3)	0.115 (3)
C7*R*	0.8228 (8)	−0.1491 (3)	−0.2085 (3)	0.115 (3)
C7	0.8785 (4)	−0.0573 (3)	−0.25241 (18)	0.0707 (14)
O7	0.8854 (4)	−0.1068 (2)	−0.28759 (13)	0.0901 (12)
N8	0.9182 (4)	0.0121 (3)	−0.24862 (14)	0.0653 (11)
C8*A*	0.9750 (5)	0.0362 (4)	−0.28639 (19)	0.0744 (15)
C8*L*	1.0211 (7)	0.1160 (4)	−0.2679 (3)	0.104 (2)
C8*R*	0.8834 (6)	0.0153 (5)	−0.3351 (2)	0.107 (3)
C8	1.0899 (5)	0.0048 (3)	−0.29375 (18)	0.0714 (14)
O8	1.1170 (4)	−0.0125 (3)	−0.33465 (12)	0.0977 (14)
N9	1.1616 (4)	0.0035 (2)	−0.25325 (14)	0.0693 (12)
C9*A*	1.2779 (4)	−0.0200 (3)	−0.25292 (18)	0.0675 (13)
C9*L*	1.2542 (6)	−0.1002 (3)	−0.2725 (3)	0.0947 (19)
C9*R*	1.3498 (5)	0.0073 (4)	−0.19983 (19)	0.0855 (18)
C9	1.3547 (4)	0.0152 (3)	−0.28443 (17)	0.0637 (13)
O9	1.4084 (3)	−0.0189 (2)	−0.31286 (14)	0.0851 (11)
O*M*	1.3624 (3)	0.0848 (2)	−0.27407 (13)	0.0792 (10)
C*M*1	1.4337 (6)	0.1290 (4)	−0.3003 (2)	0.0934 (18)
C*M*2	1.3781 (7)	0.1015 (4)	−0.3548 (2)	0.100 (2)
C*M*3	1.4102 (14)	0.2031 (4)	−0.2798 (4)	0.172 (5)
C*M*4	1.5717 (6)	0.1246 (6)	−0.2902 (3)	0.131 (3)

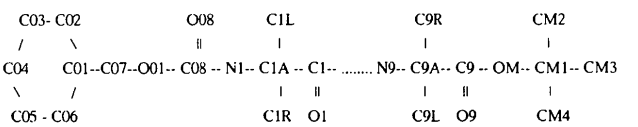
disordered C-terminal residue in both molecules *A*. It was then decided to continue with $P\bar{1}$.

Anisotropic temperature factor refinement was carried out with *SHELXL*96 (Sheldrick & Schneider, 1997). All 14 233 unique reflections were used for the least-square refinement of 1506 parameters against F_o^2, which was performed in four blocks. The disordered C-terminal residue of mol *A* and the solvent atoms were refined without connectivity to the alternative positions. All H atoms of the oligopeptides were placed in geometrically calculated positions [C—H (CH_3) = 0.96, C—H (CH_2) = 0.97, C—H (C_6H_5) = 0.93, N—H = 0.86 Å] and the riding model approach was used. No H atoms were calculated for the solvent molecules. For five H atoms the temperature factor was set to 1.5 times the equivalent isotropic one of the atom on which it is riding (C9*R*, C9*L* and C*M*3, in mol *A*) owing to unusually high values resulting from refinement; 61 reflections with $||F_o| - |F_c|| > 3.8\sigma(F)$ were excluded in the last stage of refinement. In a final difference-Fourier synthesis no electron density above 0.54 e Å^{-3} in the solvent region or above 0.53 e Å^{-3} in the region of the disordered C-terminus of mol *A* was found. Elsewhere, maximum and minimum electron density peaks are 0.39 and −0.44 e Å^{-3}, respectively. The relatively high *R* value obtained at the end of the refinement can be mainly explained by the poor quality of the crystal and the difficulty in adequately modelling the solvent structure of the title compound.

A final attempt was then undertaken to test the space group *P*1 in order to describe the disorder of the C-terminus and the water molecules in this space group in the presence of well refined parameters for all other atoms; the five disordered non-H atoms and all solvent molecules were omitted from refinement in *P*1 and subsequent calculation of a difference-Fourier synthesis. All the atoms in question were again found as the highest electron density peaks in positions which are consistent with $P\bar{1}$, which was then retained as the space group.

3. Results and discussion

The labelling scheme of the atoms in the individual molecules is as follows.



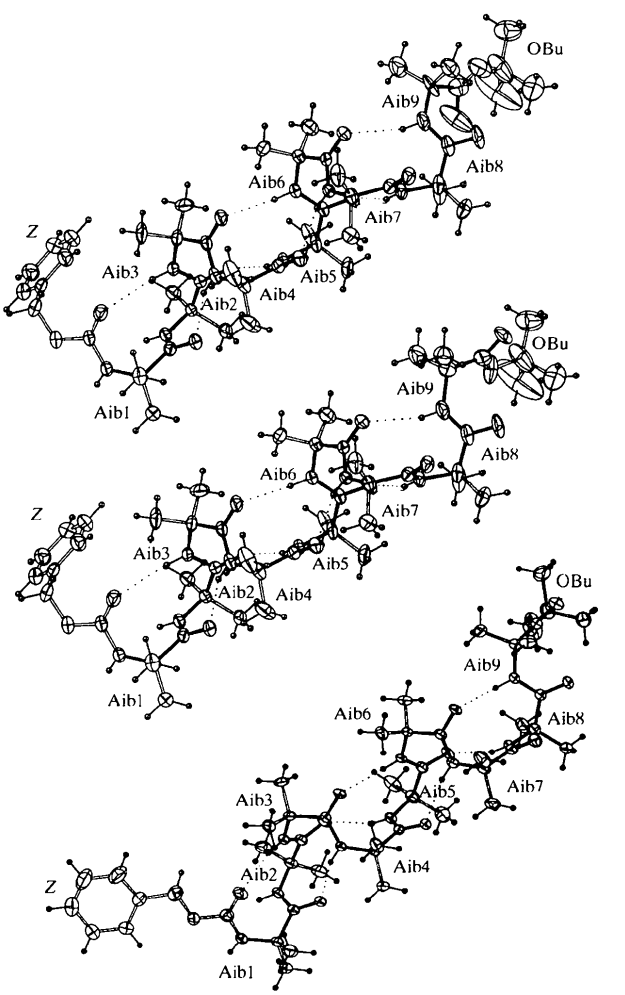


Fig. 1. Perspective drawing of the structure of *Z*-(Aib)$_9$OBu^t in the right-handed helical conformations using the program *ORTEP*III (Burnett & Johnson, 1996). The molecules are shown in the same orientation. The backbone is highlighted and intramolecular hydrogen bonds are indicated by broken lines. (*a*) Mol *A*-I; (*b*) mol *A*-II; (*c*) mol *B*.

Table 3. *Average and selected bond lengths (Å) and angles (°)*

	N_i-C_iA	C_iA-C_iL	C_iA-C_iR	C_iA-C_i
Mol *A*				
Aib1–Aib8†	1.464 (6)	1.520 (8)	1.524 (8)	1.537 (8)
Mol *A*-I				
Aib9	1.56 (4)	1.42 (5)	1.53 (5)	1.55 (4)
Mol *A*-II				
Aib9	1.39 (3)	1.49 (4)	1.52 (3)	1.82 (6)
Mol *B*				
Aib1–Aib8	1.465 (6)	1.514 (7)	1.518 (8)	1.541 (7)
Aib9	1.468 (6)	1.484 (8)	1.517 (7)	1.534 (7)
	$N_i-C_i-C_iL$	$C_i-C_iA-C_iL$	$N_i-C_i-C_iR$	$C_i-C_iA-C_iR$
Mol *A*				
Aib1–Aib8†	107.4 (4)	107.1 (5)	110.8 (5)	110.3 (5)
Mol *A*-I				
Aib9	114 (3)	115 (3)	101 (3)	104 (3)
Mol *A*-II				
Aib9	113.0 (19)	111 (2)	110.1 (16)	99.9 (18)
Mol *B*				
Aib1–Aib8†	107.6 (4)	106.9 (4)	110.6 (5)	110.2 (4)
Aib9	110.9 (5)	109.7 (5)	107.5 (4)	107.9 (4)

† Refers to the average values from residue Aib1–Aib8.

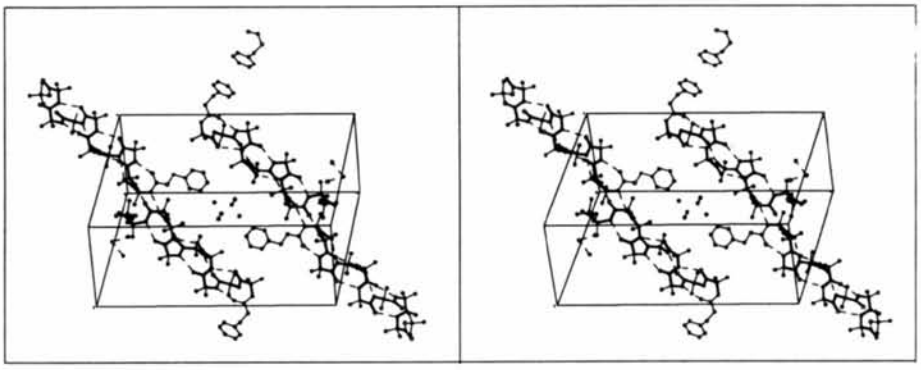
Aib residues are numbered Aib1–Aib9 in mol *A* and mol *B*. The conformations of the disordered mol *A* with two alternative positions for C9*A*, C9*L*, C9, O9 and O*M*, and their H atoms, are referred to as mol *A*-I and mol *A*-II. The disordered methanol molecule is named OS, CS, the water molecule nearby is O*W*1 and the three other water molecules O*W*2, O*W*3 and O*W*4, respectively. Positional and equivalent displacement parameters are listed in Table 2, and selected bonds and angles in Table 3.† They are in good agreement with findings from other Aib-containing peptides (Geßmann *et al.*, 1991). The valence geometry around the Cα atoms is asymmetric for all Aib residues, a common feature in 3_{10}-helices consisting of Aib residues. If one designates as C_iL and C_iR (*i* is the identifier of the Aib residue) the atoms in Aib which occupy the same position as Cβ and the α hydrogen, respectively, in L-amino acids, in right-handed helical conformations the bond angles $N_i-C_i-C_iL$ and $C_i-C_iA-C_iL$ are significantly smaller than the tetrahedral value (109.45°), and the angles $N_i-C_i-C_iR$ and $C_i-C_iA-C_iR$ are greater (Table 3, residues Aib1–Aib8). The average values are 107.5 (4)° for $N_i-C_i-C_iL$, 107.0 (5)° for $C_i-C_iA-C_iL$, 110.7 (5)° for $N_i-C_i-C_iR$ and 110.2 (4)° for $C_i-C_iA-C_iR$. The opposite asymmetry is found for the last residues and for the eight N-terminal residues of the two left-handed helices in the unit cell.

† Lists of atomic coordinates, anisotropic displacement parameters and structure factors have been deposited with the IUCr (Reference: SE0219). Copies may be obtained through The Managing Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

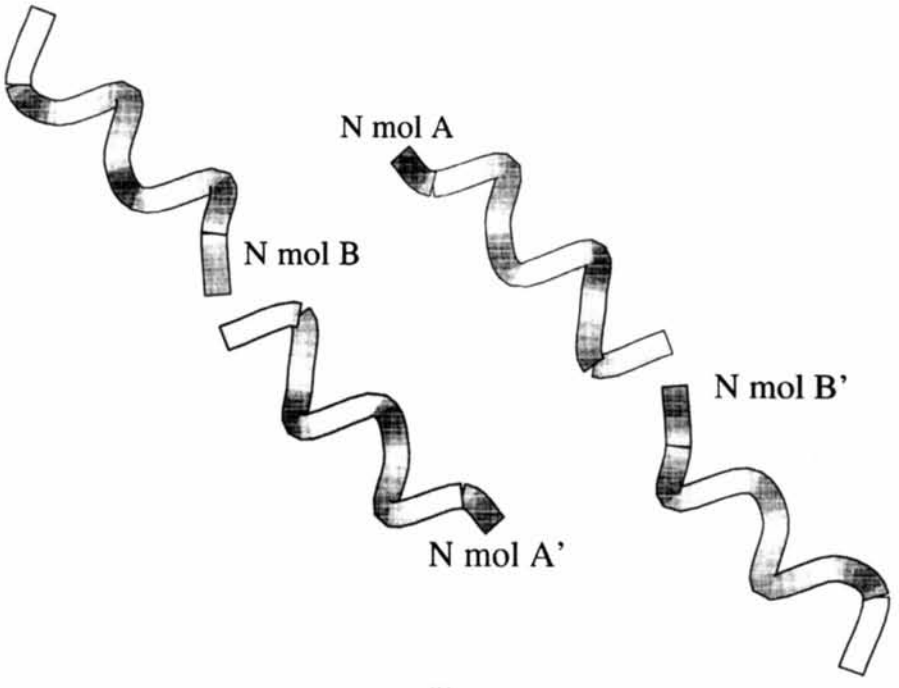
Table 4. *Backbone torsion angles (°) for the right-handed helical conformations*

Mol *A*	φ	ψ	ω
Aib1	−62.8 (6)	−29.7 (6)	−177.5 (4)
Aib2	−48.2 (6)	−41.0 (6)	−171.9 (5)
Aib3	−57.7 (8)	−24.4 (7)	179.0 (5)
Aib4	−53.5 (7)	−29.0 (7)	−178.7 (5)
Aib5	−53.4 (7)	−28.7 (7)	−179.6 (5)
Aib6	−52.1 (7)	−31.8 (7)	−174.6 (5)
Aib7	−56.5 (6)	−32.8 (6)	−176.7 (5)
Aib8	−59.6 (7)	−37.7 (8)	
Mol *A*-I			
Aib8			−177 (2)
Aib9	47 (4)	46 (4)	169 (3)
Mol *A*-II			
Aib8			−157.6 (17)
Aib9	59.6 (19)	−143 (3)	178 (3)
Mol *B*			
Aib1	−55.4 (6)	−35.4 (6)	−177.1 (4)
Aib2	−51.9 (6)	−33.7 (6)	−175.2 (4)
Aib3	−55.0 (6)	−32.6 (6)	−176.4 (4)
Aib4	−58.4 (6)	−23.1 (6)	179.3 (4)
Aib5	−54.1 (6)	−28.2 (6)	−179.6 (4)
Aib6	−52.4 (6)	−29.9 (6)	−178.1 (5)
Aib7	−53.1 (7)	−32.8 (6)	−175.9 (4)
Aib8	−57.9 (6)	−40.0 (7)	−176.0 (5)
Aib9	46.1 (7)	47.9 (6)	179.5 (5)

The title compound is the only Aib homopeptide known to crystallize with two molecules in the asymmetric unit. Both conformers mol *A* and mol *B* differ slightly in the backbone conformations and, apart from the disorder at the C-terminus of mol *A*, they differ in the orientation of the *Z* protection group (Fig. 1); the rotation of the *Z* protection ring around the C01–C07–O01–C08 bond adopts values of 90.0 (7)° for



(*a*)



(*b*)

Fig. 2. Crystal packing of *Z*-(Aib)$_9$OBu^t. (*a*) *PLUTO* (Motherwell & Clegg, 1987) drawing indicating the formation of intermolecular hydrogen bonds (broken lines). Filled circles indicate solvent atoms. H atoms and intramolecular hydrogen bonds are omitted for clarity. The *c* axis runs horizontally, while the *a* axis runs vertically. (*b*) *MOLSCRIPT* (Kraulis, 1991) drawing (with the same molecules as *A*) to clarify the relative position of mol *A* and mol *B*. The primed molecules are related to the unprimed ones by a centre of symmetry. N indicates the N-terminus of the oligopeptides.

mol *A* and 178.0 (6)° for mol *B*. With these values the *Z* ring of mol *A* and its symmetry-related *A'* are staggered in the crystal; the same is true for the rings of mol *B* and *B'* (Fig. 2, top). The distance C03(mol *A*)—C05(mol *A'*) is 3.8 Å and C04(mol *B*)—C05(mol *B'*) is 8.1 Å; the angle between each pair of planes is 0°; the distance between the planes on which the rings are located is in the first case 3.7 Å and in the second case 2.6 Å.

Both nonapeptides mol *A* and mol *B* form regular 3_{10}-helices. Owing to the space-group symmetry, both helical senses (left- and right-handed) are present in the crystal. The backbone torsion angles are given in Table 4. The average values for φ/ψ in residues Aib1–Aib8 in the right-handed helices are −55.1 (6)/−31.9 (6)° and are comparable to the values given for 3_{10}-helices in proteins (Barlow & Thornton, 1988). The helices are formed by seven consecutive ten-atom hydrogen-bonded β-turns of type III (right-handed helices). The hydrogen-bonding parameters are given in Table 5. The semi-extended conformation of Aib9 in mol *A*-II with φ/ψ ±(60/−143)° is very rare for Aib residues in Aib-containing peptides. These peptides are usually conformationally constrained to the helical regions of the Ramachandran diagram (Paterson *et al.*, 1981) and only very rarely semi-extended Aib residues have been observed in crystal structures of Aib-containing linear peptides (Yamada *et al.*, 1993; Karle *et al.*, 1989; Di Blasio *et al.*, 1991).

Thin layer chromatography experiments for the series *Z*-(Aib)$_n$OBu^t (n = 2–12) peptides (Brückner, 1989) reveal that *Z*-(Aib)$_9$OBu^t does not form a single spot as with most of the other peptides, but rather a 'tail'. This property is consistent with a transition between two conformations. In this series only *Z*-

(Aib)$_5$OBu^t shows similar behaviour. Interestingly, this effect disappears by dilution for Z-(Aib)$_5$OBu^t, but not for Z-(Aib)$_9$OBu^t (Brückner, unpublished). The crystal structure (Benedetti *et al.*, 1982) of the pentapeptide shows the typical features of Aib homopeptides (Vlassi *et al.*, 1993, Toniolo *et al.*, 1991).

The right-handed helical molecules *A* and the left-handed helical molecules *B* are alternating in a head-to-tail fashion and form double hydrogen-bonded columns along the [$\bar{1}$11] direction (Fig. 2); antiparallel columns are formed by molecules with opposite helical handedness; within the same column the helical axes from molecules of type *A* are shifted by ~1.8 Å from the helical axes of molecules of type *B*. Each helical column is surrounded by eight others (Fig. 3); the helical columns are packed *via* apolar crystal contacts, with neighbouring columns related to the central one by translation in the direction of the *a* axis being oriented parallel, all others antiparallel. Antiparallel packing of helical columns is considered to be energetically more favourable (Hol, Halie & Sander, 1981) and most of the crystals of Aib-containing peptides show this preference.

No hydrogen bonds exist from the peptides to the methanol molecule and the nearby water or to the water cluster. The carboxyl groups of Aib9 in mol *A* and mol *B* are not involved in any hydrogen bond; the carboxyl oxygen of mol *A*-I is within 2.822 (16) Å of its symmetry-related counterpart (symmetry code $-x+1, -x+1, -z+2$), which is too close to form a favourable contact; carboxyl O atoms are negatively charged and they do not possess a hydrogen. However, this unfavourable contact is avoided in the mol *A*-I and mol *A*-II pair owing to the larger distance of the two O atoms. In fact, compared with mol *A*-I the oxygen of Aib9 in mol *A*-II is flipped arround the backbone (Fig. 1). This contact occurs, for example, in Fig. 3 between the central helical column and that below it.

In summary, the structure of this large homopeptide, which is built of an unusual amino acid, confirms the preference of Aib residues to form regular 3_{10}-helices.

Table 5. *Parameters of the hydrogen bonds (Å, °)*

D (N)	*A* (O)	N···O	O···H	N—H···O	C—O···H
Intramolecular					
Mol *A*					
Aib3	*Z*	3.283 (6)	2.478 (6)	156.25 (14)	122.03 (39)
Aib4	Aib1	2.991 (6)	2.175 (6)	158.26 (14)	126.61 (35)
Aib5	Aib2	3.019 (6)	2.186 (6)	163.14 (14)	127.25 (36)
Aib6	Aib3	2.986 (6)	2.149 (5)	164.45 (15)	127.97 (35)
Aib7	Aib4	2.975 (4)	2.138 (4)	164.33 (10)	127.77 (36)
Aib8	Aib5	3.066 (4)	2.256 (4)	156.94 (11)	124.14 (35)
Aib9	Aib6	3.074 (7)	2.348 (7)	142.35 (16)	119.53 (39)
Mol *B*					
Aib3	*Z*	3.064 (6)	2.253 (6)	157.29 (14)	124.13 (38)
Aib4	Aib1	2.989 (5)	2.177 (5)	157.28 (14)	128.66 (32)
Aib5	Aib2	3.016 (6)	2.185 (6)	162.38 (13)	125.27 (33)
Aib6	Aib3	3.039 (4)	2.202 (4)	164.41 (9)	128.03 (31)
Aib7	Aib4	3.006 (4)	2.165 (4)	165.48 (10)	126.90 (28)
Aib8	Aib5	3.024 (6)	2.197 (6)	161.25 (14)	127.55 (35)
Aib9	Aib6	3.036 (5)	2.310 (5)	142.29 (14)	120.25 (32)
Intermolecular					
Aib1(*A*)	Aib7(*B*)^{i}	2.877 (5)	2.080 (5)	153.84 (11)	151.28 (11)
Aib2(*A*)	Aib8(*B*)^{i}	3.367 (5)	2.892 (5)	116.64 (9)	115.32 (9)
Aib1 (*B*)	Aib7(*A*)^{ii}	2.842 (4)	1.999 (4)	166.49 (12)	161.49 (11)
Aib2(*B*)	Aib8(*A*)^{ii}	3.224 (3)	2.578 (3)	132.69 (7)	125.62 (7)
Solvent–solvent				*OW*3···*OW*2···*OW*4	
*OW*2	*OW*3	2.020 (53)		72.9 (24)	
*OW*2	*OW*4	2.193 (61)			

Symmetry codes: (i) $2-x, -y, -z$; (ii) $1-x, 1-y, 1-z$.

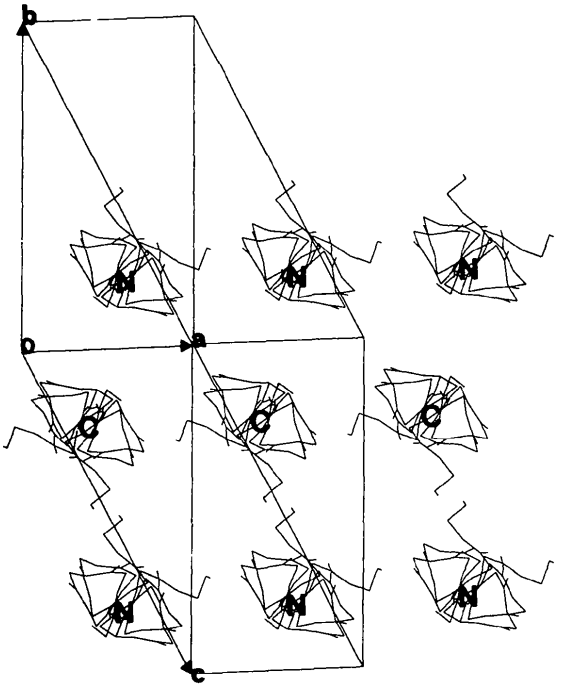


Fig. 3. Crystal packing of Z-(Aib)$_9$OBu^t viewed down the helical axes. Molecule *A* is shown in the conformation *A*-I. Head-to-tail hydrogen-bonded molecules of the types *A* and *B*′ (or *A*′ and *B*) form helical columns in a zigzag manner, which occur in the projection as two close helices. H atoms, protection groups, side chain and solvent atoms are omitted. N and C indicates the N- or C-terminal part of the helix, which points toward the viewer.

We thank Professor Dr G. M. Sheldrick for generous permission to use *SHELXL*96. We thank Dr P. Benos for assistance in data collection and software development work.

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