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The crystal structure of Z-Gly-Aib-Gly-Aib-OtBu

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The synthetic peptide Z-Gly-Aib-Gly-Aib-OtBu was dissolved in methanol and crystallized in a mixture of ethyl acetate and petroleum ether. The crystals belong to the centrosymmetric space group *P4/n* that is observed less than 0.3% in the Cambridge Structural Database. The first Gly residue assumes a semi-extended conformation (φ ±62°, ψ ∓131°). The right-handed peptide folds in two consecutive β-turns of type II′ and type I or an incipient 3_{10}-helix, and the left-handed counterpart folds accordingly in the opposite configuration. In the crystal lattice, one molecule is linked to four neighbors in the *ab*-plane via hydrogen bonds. These bonds form a continuous network of left- and right-handed molecules. The successive *ab*-planes stack via apolar contacts in the *c*-direction. An ethyl acetate molecule is situated on and close to the fourfold axis. Copyright © 2015 European Peptide Society and John Wiley & Sons, Ltd.

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Keywords: crystal structure; α-aminoisobutyric acid; Gly-Aib peptides; centrosymmetry; achiral peptides; semi-extended conformation

Introduction

The presence of Aib and Gly combines the residue with the greatest conformational flexibility (Gly) with a residue, the conformational space of which is severely restricted by the second methyl group attached to the C^{α} atom (Aib). This space available for Aib comprises the left-handed and right-handed helical region of the Ramachandran plot and only in few cases semi-extended conformation for a C-terminal Aib was observed [1,2]. Because of the absent side chain atoms Gly can adopt almost all conformations, and therefore torsion angles that are forbidden in other residues. This makes Gly a conserved residue in peptides and proteins because a mutation would change the secondary structure and/or the flexibility necessary for function. Gly is incorporated in roughly half of all known peptaibol sequences [3] and frequently as -Aib-Gly- dipeptide or as -Aib-Gly-Aib- tripeptide unit. On the contrary, the motifs -Gly-Aib-Gly-Aib- or -Gly-Aib-Gly- do not occur in any of the >1300 known-to-date peptaibiotics. However, repetitive sequences -Leu-Aib-Gly-Leu-Aib-Gly-Leu-Aib-Gly- constitute the C-terminus of the recently described stilboflavin C peptaibols produced by the filamentous fungus Stilbella flavipes CBS 146.81 [4]. Peptides composed of Gly and Aib only show an enormous structural flexibility [5–7] and therefore normally resist the generation of suitable sized crystals for structure analysis with X-rays. Furthermore, their structure determination is often tricky and challenging. The reported structure in the present work (Z-Gly-Aib-Gly-Aib-OtBu) is the first X-ray structure of a series of longer $(Gly\text{-}Aib)_n$ peptides for which crystallization experiments are in progress.

Materials and Methods

Synthesis and Crystallization

The fully protected tetrapeptide Z-Gly-Aib-Gly-Aib-OtBu (**4**) was synthesized in DMF at 40 °C by coupling Z-Gly-Aib-OH (**1**) and H-Gly-Aib-OtBu (**2**) using 1-hydroxybenzotriazole and water-soluble carbodiimide hydrochloride as coupling reagents. After removal of the solvent *in vacuo*, the remaining residue was dissolved in ethyl acetate, and the organic phase was washed successively with 5% $KHSO_4$, 5% $NaHCO_3$, and water. The organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness. Then the peptide (**4**) was dissolved in a small amount of methanol and crystallized by addition of ethyl acetate and petroleum ether (bp 50–70 °C) at 0 °C. The dipeptide Z-Gly-Aib-OtBu (**3**), serving as the starting material for dipeptides (**1**) and (**2**), was synthesized in DMF from Z-Gly-OH (purchased from Bachem) and H-Aib-OtBu [8] using HOBt and DCC as coupling reagents. The peptide (**1**) was obtained from (**3**) by trifluoroacetolytic cleavage of the tert-butyl group using a mixture of DCM/TFA (1 : 1) and peptide (**2**) from (**3**) by hydrogenolytic removal of the Z-group in methanol using 10% Pd on charcoal as catalyst. One single, fragile plate of (**4**) was used for data collection at our in-house diffractometer (Bruker AXS, Inc., [9]).

Structure Solution and Refinement

The structure was solved by direct methods with SHELXS86 [10]. All 35 non-hydrogen peptide atoms could be located in the first

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Abbreviations: *Z, benzyloxycarbonyl; OtBu, tert butoxy; Aib, α-aminoisobutyric acid; DCC, N,N′-dicylohexylcarbodiimide; DCM, dichloromethane; TFA, trifluoroacetic acid; HOBt, 1-hydroxybenzotriazole; ESI-MS, electrospray ionization mass spectrometry.*

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electron density map and in addition six atoms (peaks 20, 30, 38, 39, 40, and 47) on or close to the fourfold rotation axis. This molecule was interpreted as ethyl acetate, and at a later stage of refinement, a disorder of the ethyl moiety was modeled. The existence of ethyl acetate in dried crystals was confirmed by high-resolution ESI-MS (Figure S1 of the Supplementary Material) on a LTQ-Orbitrap XL ETD mass spectrometer (Thermo Scientific, Bremen, Germany) [11]. The structure was refined using the program SHELXL [10]. Anisotropic refinement of the peptide was performed without any constraints or restraints. All peptide hydrogen atoms could be detected in a difference Fourier map, and each one of their four parameters was freely refined. The atoms of ethyl acetate were refined with restraints (7 DFIX, 4 DANG, 1 CHIV, 8 DELU, 43 SIMU), with two atoms on the fourfold axis with symmetry restraint and therefore 0.25 occupancy (they exist in every cell but only a quarter belongs to each of the four symmetry-related surrounding peptides), while the other four atoms were refined as existing statistically in every fourth cell with 0.25 occupancy factor. The five not disordered ethyl acetate atoms were refined anisotropically and the disordered ethyl acetate atoms isotropically. The isotropic displacement parameter of these disordered methyl groups had to be prevented from becoming negative. Hydrogen atoms were not added to the ethyl acetate. The refinement was performed with 506 parameters, with 63 restraints exclusively for the ethyl acetate and against 5122 unique reflections. The refined anisotropic atomic displacement parameters are shown in Figure 1. Data collection and refinement statistics are listed in Table 1; the final CIF file is provided as Supporting Information.

SPDBVIEWER [13] (http://www.expasy.org/spdbv/), XTALVIEW [14], COOT [15], POVRAY [16], and PyMOL [17] were used for geometric analysis, visualization and for the production of the figures.

Accession Number

The coordinates and structure factors have been deposited with the Cambridge Data Bank [18] under accession code CCDC 1038685. These data can be obtained from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

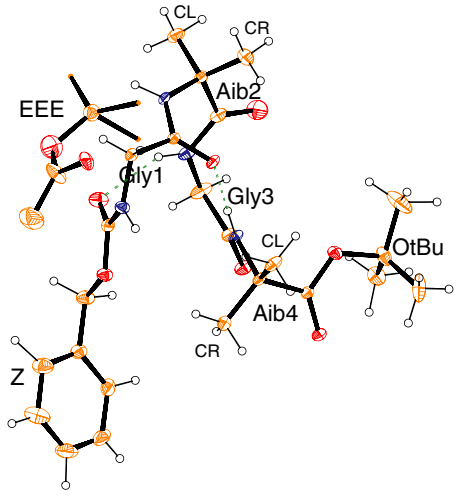

Figure 1. The molecular structure of Z-Gly-Aib-Gly-Aib-OtBu showing 40% probability displacement ellipsoids [11]. The two intramolecular hydrogen bonds are shown as green dashed lines. The disordered ethyl acetate on the symmetry axis is shown in the symmetry belonging to the shown asymmetric unit.

Results and Discussion

The peptide adopts a rather unusual conformation. The first Gly residue is in semi-extended conformation (Figure 1 and Table 2), while the other residues lie in the region of a left-handed 3_{10}-helix for the molecule chosen as asymmetric unit. To the authors' knowledge, a semi-extended conformation at a protected N-terminus of Aib-containing peptides is not very usual but can be found even with Gly-Gly [19] or Aib-Gly [20] or Gly-Aib [21] at the protected N-terminus. Here, there are two intramolecular hydrogen bonds formed (Table 3), both of type $4 \rightarrow 1$, one between the carbonyl group of the Z-protecting group and the N–H group

Table 1. Data collection and processing

Chemical formula	$(C_{24}H_{36}N_4O_7) \cdot 0.25(C_4O_2)$
M_r	512.58
Crystal system, space group	Tetragonal, *P4/n*
Temperature (K)	100
a, c (Å)	20.251(2), 13.933 (1)
V (Å^3)	5714 (1)
Z	8
Radiation type	Cu Kα
μ (mm^{-1})	0.73
Crystal size (mm)	$0.3 \times 0.3 \times 0.01$
Data collection diffractometer	Bruker AXS D8 Venture
Absorption correction	Multi-scan with SADABS (Bruker, 2008)
Transmission min, max	0.54, 1.0
Number of measured, independent and observed [$I > 2\sigma(I)$] reflections	84737, 5122, 4891
R_{int}	0.076
Resolution (Å)	0.82
$(\sin \theta/\lambda)_{max}$ (Å^{-1})	0.610
Refinement	
Final R_{cryst}, R_{crys} $F_o > 4\sigma F_o$	0.069, 0.067
Final R_{free}, R_{free} $F_o > 4\sigma F_o$	0.082, 0.080
Number of reflections	5122
Number of parameters	506
Number of restraints	63

Table 2. Backbone torsion angles for the left-handed molecule

ξ3(Z)	C7-O1-C8-N_1*	174.8 (2)
ω(Z)	O1-C8-N_1-CA_1	175.3 (2)
φ(1)	C8_0-N_1-CA_1-C_1	−62.1 (2)
ψ(1)	N_1-CA_1-C_1-N_2	131.0 (2)
ω(1)	CA_1-C_1-N_2-CA_2	179.6 (2)
φ(2)	C_1-N_2-CA_2-C_2	57.8 (2)
ψ(2)	N_2-CA_2-C_2-N_3	24.5 (2)
ω(2)	CA_2-C_2-N_3-CA_3	−178.6 (2)
φ(3)	C_2-N_3-CA_3-C_3	88.8 (3)
ψ(3)	N_3-CA_3-C_3-N_4	3.5 (3)
ω(3)	CA_3-C_3-N_4-CA_4	176.1 (2)
φ(4)	C_3-N_4-CA_4-C_4	48.6 (2)
ψ(4)	N_4-CA_4-C_4-O_5	48.7 (2)
ω(4)	CA_4-C_4-O_5-C1–5	179.5 (2)

* The numbers after the underscore denote the residue number.

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of Gly 3 and the other between the C = O group of Gly 1 and the N–H group of Aib 4. The left-handed peptide folds in two consecutive β-turns of type II and type I′ or an incipient left-handed 3_{10}-helix; the right handed molecule folds in the opposite configuration and handedness, i.e. in two consecutive β-turns of type II′ and type I or an incipient right-handed 3_{10}-helix. Aib, Gly, both protecting groups and ethyl acetate consist only of achiral entities, a fact that results in the observed centrosymmetric space group and therefore in the equal populations of conformers of opposite torsion angles and helical sense. The peptide bonds adopt the usual trans-planar conformation, with small deviations from planarity ($\omega = 180°$, Table 2). The valence geometry around the C^{α} atom is asymmetric for the Aib residues (Table 4). If one designates as C^{L} and C^{R} the atoms that occupy the same position as C^{β} and the α-hydrogen in L-amino acids, respectively, the bond angles N-C^{α}-C^{L} and C-C^{α}-C^{L} are significantly smaller than the N-C^{α}-C^{R} and the C-C^{α}-C^{R} for the right-handed molecule. This observation is in excellent agreement with the theoretical calculations and with the bond angles of other right-handed 3_{10}-helical Aib peptides [22,23]. The ethyl acetate molecule sits with two atoms on the fourfold axis, and the other four atoms are close to this axis (Figure 2). In the crystal, the unit cell contains four right-handed and four left-handed molecules (Figure 3). Each molecule is hydrogen-bonded with four bonds to four different symmetry-related molecules (Table 3 and Figure 4). There are two hydrogen bonds in the middle of the molecule to two symmetry-related ones with the same handedness involving the groups N–H of Aib 2 and C = O of Gly 3 and in addition two more terminal ones to molecules of the opposite handedness involving the N–H group of Gly 1 and the C = O group of Aib 4. Thus, a network of hydrogen-bonded molecules is formed, building layers of hydrogen-bonded molecules in the *ab*-plane. These layers pack via apolar contacts along the short *c*-axis with a minimal C-C distance of 3.52 Å between layers. This unfavoured distance is formed between the only polar peptide group not involved in hydrogen bonding, namely the C = O group of Aib 2 and a methyl group of the C-terminal protecting group. The distance between the acetate oxygen and the C = O group of the Z-protecting group is only 2.79 Å, which is an unusually close van der Waals contact.

Table 3. Hydrogen-bond geometry (Å,°)

D[a]–H···A[a]	D–H	H···A	D···A	D–H···A
N_3–H_3···O8	0.95 (3)	2.25 (3)	3.137 (3)	155 (2)
N_4–H_4···O_1	0.92 (3)	2.01 (3)	2.912 (2)	171 (2)
N_1–H_1···O_4[b]	0.87 (3)	1.99 (3)	2.854 (2)	171 (3)
N_2–H_2···O_3[c]	0.88 (3)	1.99 (3)	2.853 (2)	167 (2)

[a]D is hydrogen bond donor and A is acceptor
Symmetry codes:
[b]$-y+1, x-1/2, -z$
[c]$y, -x+1/2, z$

Table 4. Selected bond angles (°) for the right-handed molecule

N_2-CA_2-CL_2	107.8 (2)	N_2-CA_2-CR_2	111.1 (2)
C_2-CA_2-CL_2	106.6 (2)	C_2-CA_2-CR_2	109.7 (2)
N_4-CA_4-CL_4	107.9 (2)	N_4-CA_4-CR_4	110.4 (2)
C_4-CA_4-CL_4	107.4 (2)	C_4-CA_4-CR_4	110.4 (2)

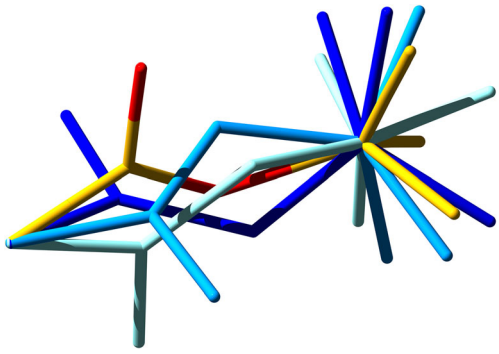

Figure 2. The ethyl acetate molecule on the fourfold axis shown in all four symmetry equivalents. The two atoms that are common in the four symmetry equivalents lie on the fourfold axis.

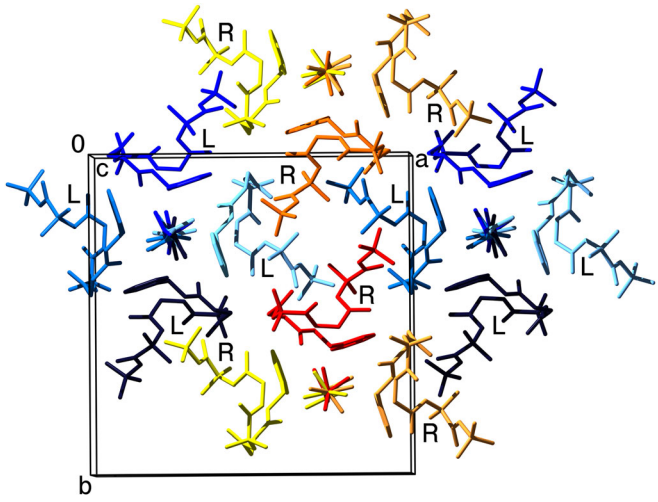

Figure 3. Crystal packing of the eight space group symmetry-related molecules in the unit cell. Two inner molecules are shown one time each, while the others are also shown translated along unit cell axis *a* or *b*; R and L mean right-handed and left-handed molecules and the ethyl acetate molecules, which do not coexist in one cell, are also shown.

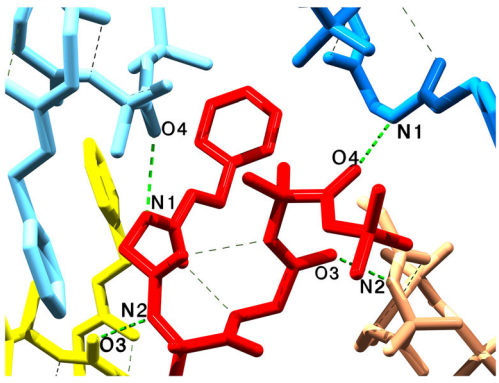

Figure 4. Hydrogen bonding patterns. Intramolecular hydrogen bonds are shown as thin gray dashed lines, and thick green dashed lines denote intermolecular hydrogen bonds. Atoms of the central red molecule that are involved in intermolecular hydrogen bonds are labeled together with the donor or acceptor atoms of the symmetry-related molecules. Hydrogen atoms have been omitted for clarity.

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This peptide structure by adopting a semi-extended conformation at the first residue confirms the notion that protected Gly-Aib peptides possess structures that are rather unusual and not yet predictable. The Aib homopeptides form 3_{10}-helices in crystals, as has been shown for the Z-$(Aib)_n$-OtBu peptides up to n = 11 [22,24]. Furthermore, even longer Aib homopeptides (up to n = 15) with a different C-terminal protecting group form very stable structures [25]. Unprotected Gly homopeptides with n = 3–5 assume fully extended conformation and form antiparallel β-sheets in crystals [26]. One could speculate that, in contrast to Aib or Gly homopeptides, the longer Gly-Aib peptides do not adopt regular helices or strands but some unusual structure, perhaps a combination of helices and strands or even a structure with alternating handedness. The difficulty of Gly-Aib peptides to crystallize signifies high flexibility that is not normally expected for pure right-handed and left-handed helical molecules or for β-sheets comprising both enantiomorphs.

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Supporting Information

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