Crystals **2011**, *1*, 163-170; doi:10.3390/cryst1030163

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crystals

ISSN 2073-4352

www.mdpi.com/journal/crystals

Article

Crystal Structure of the *N*-benzyloxycarbonyl-Alanyl-Phenylalanyl-methyl ester: the Importance of the H-bonding Pattern

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Received: 15 July 2011; in revised form: 15 August 2011 / Accepted: 19 August 2011
Published: 22 August 2011

Abstract: Large crystals of the methyl ester of the *N*-α-benzyloxycarbonyl protected Ala-Phe dipeptide (Z-AF-OMe) were obtained after the very slow evaporation of a solution of the corresponding carboxylic acid (Z-AF-OH) in methanol containing an excess of HCl. The structure was confirmed by single crystal X-ray diffraction data. It crystallizes in the orthorhombic space group $P2_12_12_1$ with unit cell dimensions a = 5.0655(6) Å, b = 8.4614(8) Å, c = 46.856(5) Å, V = 2008.3(4) Å^3, Z = 4. In the crystal, the molecules form hydrogen bonded chains running along the a axis of the unit cell. Other secondary interactions are also discussed.

Keywords: peptides; alanylphenylalanyl derivative; crystal structure; hydrogen bond

1. Introduction

The study of the assembly of small peptidic sequences is a very interesting research topic because it serves to create simple models of the non-covalent interactions found in more complicated peptides

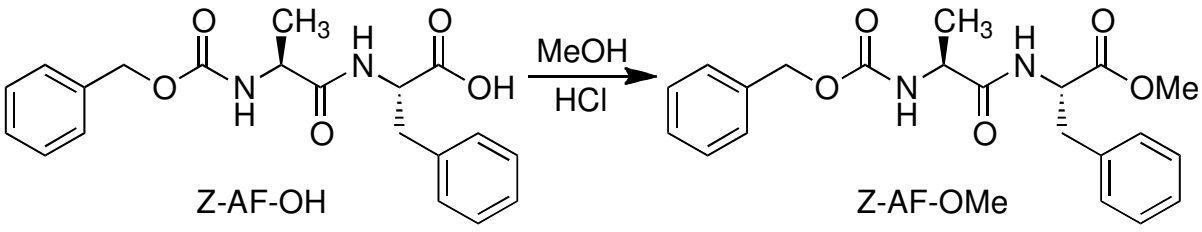
and proteins [1]. In this regard, crystallographic studies on simple short peptidic sequences have shown the formation of supramolecular structures in the solid state which resemble the interaction patterns found in helices [2-4], sheets [5-7] and turns [8,9]. These structural patterns are normally stabilized by the synergic action of weak, non-covalent interactions such as H-bonds, electrostatic, aromatic face to face or edge to face and van der Waals contacts [10,11]. The advantages of using small model peptides are the ease of preparation at large scale through well described synthetic methodologies [12], as well as the possibility of using a broader set of crystallization conditions, not suitable for more elaborate peptides and proteins [13,14]. Moreover, an understanding of the forces ruling the formation of supramolecular aggregates in the solid state is extremely useful for the controlled assembly of simple peptidic sequences at the nanometric scale, with important applications in the preparation of nanomaterials [15-17].

During our ongoing research program in the field of pseudopeptidic compounds [18,19], we became interested in the self-aggregation process of the molecules through non-covalent interactions [20-22]. Within this field, the peptidic sequence Z-AF-OH appeared as an interesting target for molecular recognition by synthetic pseudopeptidic macrocycles [23]. The deep study of the binding phenomena showed that this sequence is able to self-aggregate through H-bonding interactions both in solution and in the solid state [23]. Here we report on the solid state structure of a closely related dipeptide (Z-AF-OMe) where the possibility of one of the H-bonding contacts found in the crystal structure of Z-AF-OH has been eliminated.

2. Results and Discussion

During our research on the supramolecular chemistry of simple pseudopeptidic compounds [18-23], we screened the crystallization conditions of several different *N*-protected dipeptide sequences. Thus, we observed that when the Z-AF-OH dipeptide was placed in a MeOH solution containing an excess of 12 N HCl, large crystals appeared after very long time (several months). Surprisingly, the X-ray diffraction analysis of these crystals unambiguously showed that they contained the corresponding methyl ester dipeptide (Z-AF-OMe) most likely produced by the acid-catalyzed esterification of the initial carboxylic acid-terminated compound (Scheme 1).

Scheme 1. Acid-catalyzed formation of Z-AF-OMe.



Crystal Structure Determination

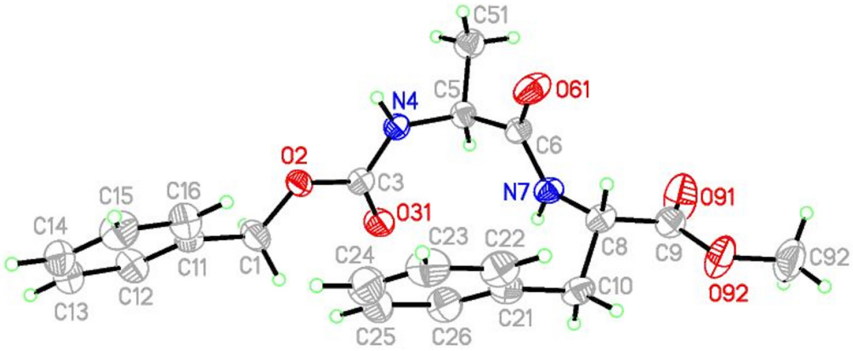
The molecular structure of the title compound, along with the atom-numbering scheme, is depicted in Figure 1. Bond lengths and angles are in the usual ranges. The uretane and amide moieties adopt a *trans* conformation with torsion angles O2-C3-N4-C5 = 178.9(3)° and C5-C6-N7-C8 = –174.8(3)°. The peptide units are essentially planar (r.m.s. deviation is 0.025Å for O2, C3, O31, N4, H4, C5 as

well as for C5, C6, O61, N7, H7, C8) and enclose a dihedral angle of 71.89(19)°. Further characteristic torsion angles describing the backbone conformation are given in the following Table 1.

Table 1. Selected torsion angles for (Z-AF-OMe) [°].

C3-N4-C5-C6	–104.6(4)°
N4-C5-C6-N7	101.0(4)°
C6-N7-C8-C9	–115.2(4)°
N7-C8-C9-O92	–172.1(3)°
N7-C8-C10-C21	–58.3(4)°
C8-C10-C21-C22	–83.5(5)°
C8-C10-C21-C26	96.5(5)°
C1-O2-C3-N4	172.7(3)°
Dihedral angle between the two aromatic rings	12.6(2)°

Figure 1. Perspective view of Z-AF-OMe with displacement ellipsoids at the 50% probability level.



The crystal packing is stabilized by intermolecular N-H···O=C hydrogen bonds (Table 2). Those H-bonds involve the interaction of the amide hydrogen of the peptide bond with the carbonyl oxygen of the same group in a second molecule and that of the uretane hydrogen with the carbonyl oxygen of the uretane group in another molecule. Two molecules form an $R^2_2(12)$ ring (Figure 2) [24]. These entities are further connected to chains running along the a axis (Figure 3). Additional aryl-aryl contacts of the edge-to-face type between the phenyl groups of the Phe side chains and those of the Z groups are established along the c axis (Figure 3). The C15-H15···cog(C11,C12,C13,C14,C15,C16) distance is 3.021 Å.

Table 2. Hydrogen bonds for (Z-AF-OMe) [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N(4)-H(4)...O(31)#1	0.85(3)	2.09(3)	2.904(4)	161(3)
N(7)-H(7)...O(61)#2	0.84(4)	2.05(4)	2.848(4)	159(3)

Symmetry transformations used to generate equivalent atoms: #1 x–1, y, z, #2 x+1, y, z.

Figure 2. Representation of two H-bonded molecules of Z-AF-OMe forming a $R^2_2(12)$ ring, highlighted in green. Non-polar H-bonds are omitted for clarity.

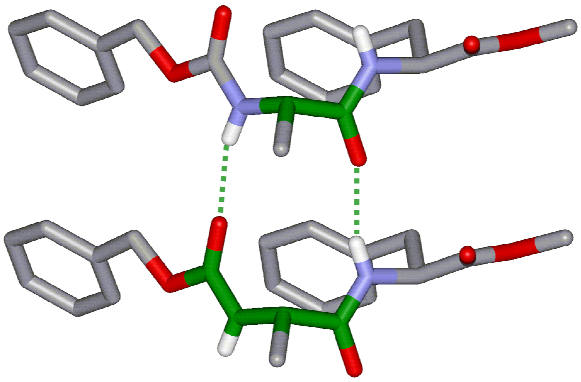
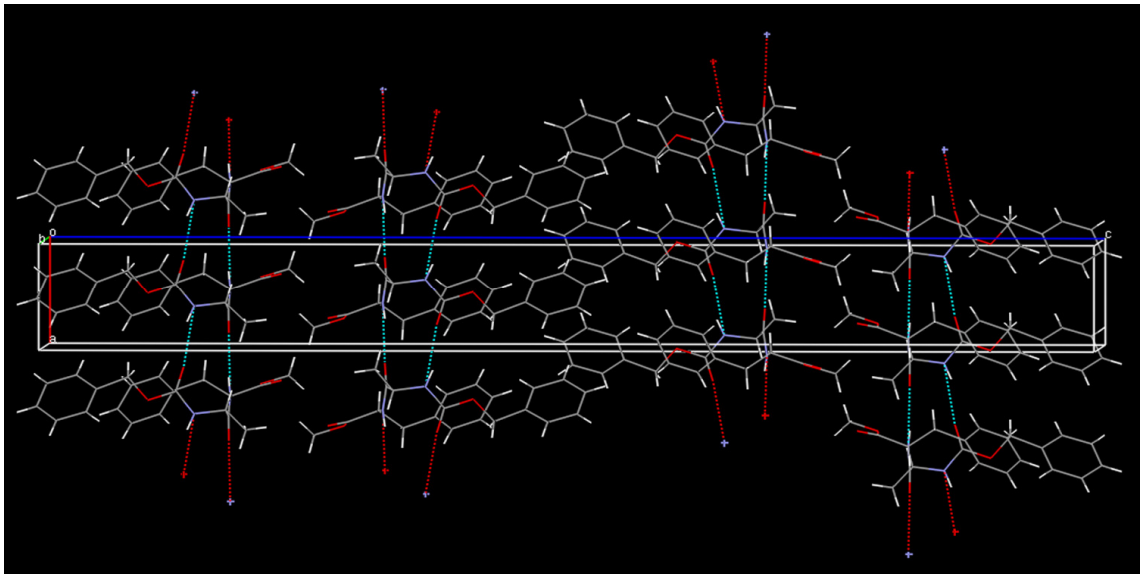


Figure 3. A packing diagram of Z-AF-OMe with view onto the ac plane. Hydrogen bonds are drawn as dashed lines.



As previously commented, it is interesting to note that a similar compound in which the ester methyl group of the title compound is substituted by an H atom (Z-AF-OH), has exactly the same molecular conformation [23]. A least-squares fit of the two molecules is shown in Figure 4. Besides, also in this case, a similar H-bonding network implicating the amide and uretane groups was found in the solid state. This H-bonding network is also responsible for the self-assembling of Z-AF-OH in solution [23] and resembles that observed in natural β-sheet peptidic motives [5-7]. In both cases—the Z-AF-OH and the Z-AF-OMe compounds—the assembly can be described as a straight parallel β-sheet [25], without any twist between the backbond strands along the H-bonding direction, often found in related *N*-protected dipeptides [26]. However, in the crystals of Z-AF-OH, we had observed additional intermolecular H bonding interactions between the carboxylic acids that are not possible in the methyl ester compound Z-AF-OMe. A further comparative study between the two crystals is shown in Figure 5. The relative disposition of the peptidic backbones is parallel in Z-AF-OMe while anti-parallel in Z-AF-OH, with respect to the N to C termini direction (see green arrows in Figure 5 A,B). This

difference is due to the H-bonding interactions observed between the COOH groups of Z-AF-OH, which are obviously absent in the corresponding methyl ester. However, the same N-H···O=C interactions within the backbones are present in both structures. The absence of the H-bonds implicating COOH in the title compound (Z-AF-OMe) produced a shift between the planes of the backbone strands (Figure 5C) which were perfectly aligned in the case of the original Z-AF-OH compound (Figure 5D). Thus, the presence or absence of interactions along the peptidic backbone direction is reflected in the alignment of the strands and in their relative senses (parallel or antiparallel), while retaining essentially the same conformation of the dipeptide.

Figure 4. A least-squares fit of the title compound (Z-AF-OMe, full bonds) with *N*-((Benzyloxy)carbonyl)alanylphenylalanine (Z-AF-OH, open bonds).

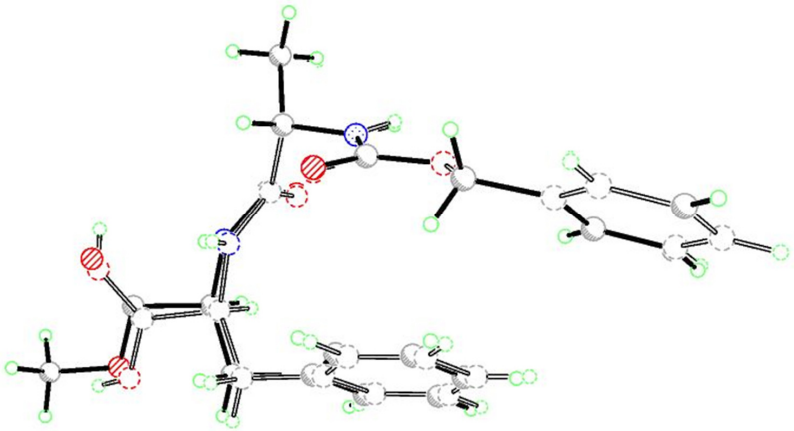
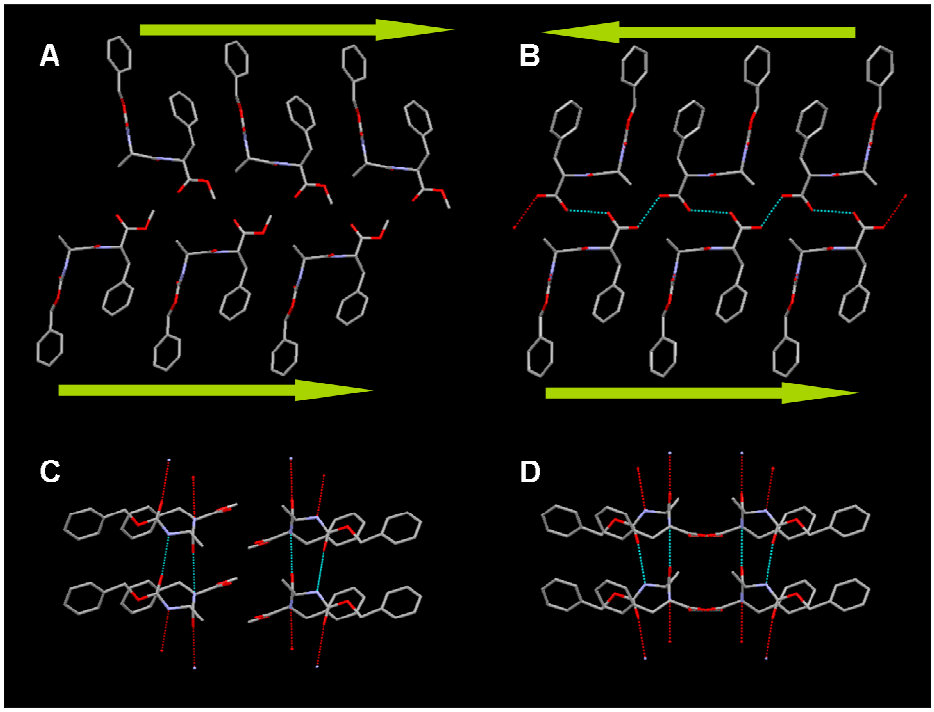


Figure 5. Intermolecular contacts found in the solid state for Z-AF-OMe (A,C) and Z-AF-OH (B, D). H atoms have been omitted for clarity and H bonds are shown as dashed lines.



3. Experimental Section

X-ray Data Collection and Structure Refinement

Crystallographic data were recorded on a STOE IPDS-II diffractometer [27] using Mo Kα radiation (λ = 0.71073 Å) at T = 173 K. The structure was solved by direct methods [28] and refined by full-matrix least-squares using SHELXL-97 against F^2 using all data [28]. All non-H atoms were refined anisotropically. H atoms were positioned geometrically at distances of 0.95 Å (aromatic CH), 0.98 Å (methyl groups), 0.99 Å (methylene group) and 1.00 Å (tertiary CH) from the parent C atoms; a riding model was used during the refinement process and the U_{iso}(H) values were constrained to be 1.2 Ueq(C) or 1.5 Ueq(methyl C). The H atoms bonded to N were freely refined. Due to the absence of anomalous scatterers, the absolute structure could not be determined and was set according to the absolute configuration of the starting materials.

CCDC reference number: CCDC 832036. Copies of the data can be obtained, free of charge, on application to CHGC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

Crystal data. $C_{21}H_{24}N_2O_5$, 384.42 g mol^{-1}. Orthorhombic, $P2_12_12_1$ (no. 19), a = 5.0655(6) Å, b = 8.4614(8) Å, c = 46.856(5) Å, V = 2008.3 Å^3, Z = 4. Diffractometer IPDS-II, Stoe Darmstadt; Mo-K$_\alpha$ (graphite monochromator, λ = 0.71.073 Å); T = 173(2) K; 3.48° ≤ 2θ ≤ 50.48°; −6 ≤ h ≤ 6, −9 ≤ k ≤ 10, −55 ≤ l ≤ 56; ρ_{calc} = 1.271 g cm^{-3}; 14035 reflections measured of which 2159 were symmetrically independent; R_{int} = 0.1054; F(000) = 816; μ = 0.091 mm^{-1}. 261 refined parameters; R values: R_1/wR_2 for 1182 reflections with [$I_0 > 2\sigma(I_0)$]: 0.0416 / 0.0603, for all data: 0.0910 / 0.0697; S_{all} = 0.730; Δρ(min/max): –0.178 eÅ^{-3} / 0.156 eÅ^{-3}.

4. Conclusions

The crystal structure of an *N*-benzyloxycarbonyl-protected dipeptide derivative bearing a methyl ester on its carboxylic terminus (Z-AF-OMe) has been determined. The compound showed a very similar structure to that of its parent carboxylic dipeptide Z-AF-OH, in spite of lacking the favorable H bonding interactions between COOH groups. The results highlight the main role played by the H bonds of the type N-H···O=C implicating the peptidic backbone as responsible for the conformation and the self-assembly of the molecules. Other secondary contacts could be acting in the alignment of the strands and in their final disposition into parallel or antiparallel fashion.

Acknowledgements

The authors gratefully acknowledge the financial support from the Spanish Ministry of Science and Innovation, MICINN (CTQ2009-14366-C02 project).

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