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An All-*Anti* Conformation of the β-Ala Moiety in the Crystalline State

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Abstract: In marked contrast to the most favourable *folded* conformation, predicted from *ab initio* quantum mechanical calculations, the crystal structure analysis of the model system incorporating -CONH-CH_2-CH_2-CONH- moiety, revealed the existence of an all-*anti* conformation, characterized by the backbone torsion angles: $\phi \approx -146°$, $\mu \approx 172°$ and $\psi \approx 155°$. Potential applications of the residue to form highly ordered, novel β-sheet like structure(s) with distinct faces have been suggested.

In recent years conformational analysis of short linear peptides incorporating the β-Ala residue, two methylene units flanked by amide bonds (-CONH-CH_2-CH_2-CONH-), has received considerable interest since, this residue has not only exhibited the potential of accommodating a wide range of well defined secondary structural features[1] but also showed excellent stability towards proteases.[2] However, a rational approach, incorporation and/or substitution of the β-Ala residue into the polypeptide chain, would require an understanding of the conformational behavior of this *flexible* residue. As an attempt to understand the conformational properties of this residue we, along with others, have recently demonstrated that the folding behaviour of the two methylene units can be significantly influenced and dictated by neighbouring constraints.[3]

The present investigation was motivated by the recent theoretical, an *ab initio* quantum mechanics calculation, study of a model system: Ac-β-Ala-NH_2, by Wu and Wang.[4] Using the HF/6-31 G** method these authors predicted that the most favorable conformation of the β-Ala residue would be significantly *folded* and the torsion angles: $\phi \approx 88°$, $\mu \approx 63°$ and $\psi \approx -179°$ should characterize the backbone conformation. The preference for the folded C6 structure with no or very weak intramolecular hydrogen bonding interaction was proposed. Surprisingly, none of the predicted structures exhibited an all-*anti* conformation for the methylene segments. Moreover, the critical μ torsion angle close to an extended *trans* conformation: $\mu \approx -174°$, in solution conditions, was found to be 1.3-1.6 kcal/mol less stable. From another systematic conformational investigation of two glutaramide analogues (R-NHCO-$(CH_2)_3$-CONH-R, R = C_3H_7 or CH_3), by theoretical *ab initio* quantum mechanical calculations *in vacuo* and experimental X-ray diffraction structure analysis in crystalline state, Navarro et al. hypothesised[5a] that "if a small number of methylene groups are present, the repulsive interactions between the amide groups can induce *folding* of the methylene units in order to give a more favourable orientation."

This work is not only an attempt to provide experimental evidence, but also tests the proposed hypothesis. The *Letter* describes the X-ray crystallographic characterization of $(CH_3)_3$C-O-CONH-CH_2-CH_2-CONH-CH_3, **1**.[6] In the model system the terminal secondary amide group provides a convenient evaluation of the N-H stretch bands of the IR spectral data. Fourier transform infra-red (FT-IR) study of the solid state provides strong support for the observed conformational behaviour of **1** in crystalline state. In contrast to the recent theoretical quantum mechanics calculations, the crystal structure and FT-IR analyses favour the existence of an all-*anti* conformation of the β-Ala residue.

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The molecular conformation with numbering scheme of **1** is shown in Figure 1 and the relevant torsion angles are summarised in Table 1.

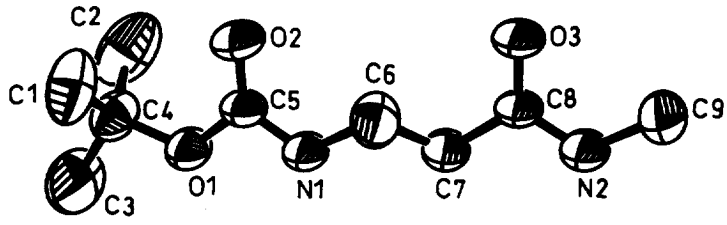

Figure 1: An ORTEP representation of the molecular structure of $(CH_3)_3C$-O-CONH-CH_2-CH_2-CONH-CH_3, **1** in solid state. The thermal ellipsoids are shown to the 40% probability level.

Table 1: A comparison of relevant backbone torsion angles (°) of the β-Ala residue.

Peptides	ϕ	μ	ψ	**References**
*t*Boc-β-Ala-$NHCH_3$	-145.5	171.6	154.5	This paper
*t*Boc-β-Ala-OH	87.0	67.0	-	7
Ac-β-Ala-NH_2	88.5	62.8	-179.1	4

An attractive feature of the crystal structure of **1** is an observation of a significantly extended all-*anti* conformation of the β-Ala residue. The backbone torsion angles: ϕ = -145.5°, μ = 171.6° and ψ = 154.5° characterize the molecular conformation. The parallel orientation of the two carbonyl groups present on either sides of the methylene units is noteworthy. Such an all-*anti* conformation would preclude the proximation of the intraresidue C=O and the N-H groups, to form the *weak* intramolecular H-bonded C6 structure predicted theoretically. The existence of an intraresidue electrostatic attraction between the negatively charged nitrogen and the positively charged carbonyl carbon was hypothesised to explain the stability of the folded *gauche* conformation.[4] From these results it is apparent that the internal non-hydrogen-bonded electrostatic (or dipole) interactions alone may not be sufficient to force the methylene units of the β-Ala residue to adopt the folded conformation. Interestingly, on the other hand the crystal structure analysis of *t*Boc-β-Ala-COOH indicated a significantly folded backbone conformation with the critical μ torsion angle close to a *gauche* conformation.[7] The experimentally observed conformational features in **1** are distinctly different and inconsistent from those predicted from the quantum mechanical calculations in solution conditions.

Another interesting feature of the crystal structure analysis is the existence of a highly ordered, energetically more favorable antiparallel arrangement of the molecules (Figure 2). All potential H-bond acceptor and the donor groups are involved in the formation of four C=O···N-H *inter*molecular interactions. The geometrical parameters for the two parallel H-bonds observed in the crystal lattice of **1** are presented in Table 2. Here, it is worth stressing that such repetitive unidirectional arrangements of the 14-membered H-bonding ring motif, reminiscent of the one found in an antiparallel β-sheet comprised of α-amino acids, generate a novel β-sheet-like structure (Figure 2). In marked contrast to an α-amino acid residue in the β-sheet structures, the β-Ala residue in its extended form may have a tendency to exhibit such conformational characteristics (unpublished).[2d,3e] The formation of a two-dimensional supramolecular assembly, stabilized *via* amide-amide intermolecular H-bonding interactions, is suggested to be enthalpically favorable.[5b]

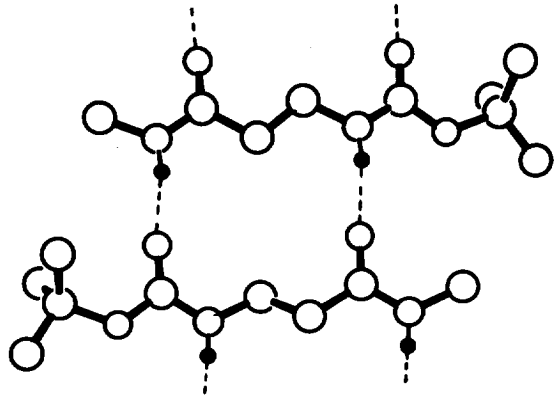

Figure 2: The two antiparallel strands of *t*Boc-β-Ala-$NHCH_3$, **1** along the a axis. Black circles indicate H-atoms. The parallel arrangements of the two H-bonding interactions are indicated by dotted lines.

Table 2: Intermolecular hydrogen bond parameters for *t*Boc-β-Ala-$NHCH_3$.

Donor	Acceptor	Symmetry	Distances (Å)		Angle (°)
N-H	O	Equivalence of O	N···O	H···O	N-H···O
N(1)-H	O(3)	x-1/2,-y+1/2,-z+1	2.856	2.00	170.9
N(2)-H	O(2)	x-1/2,-y+1/2,-z+1	2.859	2.02	166.5

Additionally, FT-IR absorption spectrum of **1** in KBr, in the informative amide A region, revealed two strong bands at ~3352 cm^{-1} and ~3310 cm^{-1}. From the IR results it is safe to conclude that both the amide N-H groups are involved in strong intermolecular H-bonding interactions.[8] Self association *via* C=O···N-H intermolecular interactions, presumably of different strengths, may be of major significance (unpublished data). The observed H-bonding interactions in the crystal structure compare well with the solid-state IR data. Unsurprisingly, FT-IR data reported for Ac-β-Ala-$NHCH_3$ in a 1mM CH_2Cl_2 solution, however, indicated almost complete absence of intramolecular as well as intermolecular H-bonding interactions *i.e.* the fully solvated monomeric species.[9] Further, the appearance of the ^1H NMR spectrum of $C^{\alpha}H_2$ and $C^{\beta}H_2$ resonances in **1** is typical of A_2M_2X spin system.[6] Such spectral pattern appears to be the diagnostic feature of a significantly extended all-*anti* conformation of the β-Ala residue.[3b-d]

Based on the results of *ab initio* quantum mechanical calculations, independent investigators have predicted that an all-*anti* conformation of the two or three methylene units flanked by amide groups, may be disfavored due to attractive or repulsive interactions, respectively.[4,5] However, the results of crystal structure analysis of **1** including those reported earlier,[3] are clearly at variance with the results of theoretical quantum mechanical calculations[4,5] and inclined us to suggest that the simple explanation provided by these authors are not sufficient to explain the complex folding-unfolding behaviour of the methylene units flanked by amide bonds. The disagreement between experimental data and computationally derived conclusions, even when solution conditions are taken into account, in any way may not be surprising.[5,10] We believe that in the case of short linear peptides, the *folded* and the fully extended all-*trans* conformations of the β-Ala residue have small energetic differences that can easily be overcome, influenced and stabilized by the subtle change in the solvent polarity and the nature of neighbouring constraints introduced (unpublished).[1-3]

In conclusion, an experimental conformational analysis of the model system reveals that the unsubstituted β-Ala residue can be exploited for incorporating *novel* β-sheet-like secondary structural features in peptides. The unique orientations of the potential H-bond donor and acceptor groups may result in an unusual network of the H-bond mediated molecular organization *i.e.* supramolecular assembly, and when such scaffolds are strategically positioned on the constituent molecules they may demonstrate their potential in engineering the structures in solid states.

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REFERENCES AND NOTES

1. a) Griffith, O. W. *Annu. Rev. Biochem.* **1986,** *55,* 855; b) Edmundson, A. B.; Harris, D. L.; Fan, Z. C.; Guddat, L. W.; Schley, B. T.; Hanson, B. L.; Tribbick, G.; Geysen, H. M. *Proteins: Struct., Funct. and Genet.* **1993,** *16,* 246; c) Saviano, G.; Temussi, P. A.; Motta, A.; Maggi, C. A.; Rovero, P. *Biochemistry* **1991,** *30,* 10176; d) Wrighton, N. C.; Balasubramanian, P.; Barbone, F. P.; Kashyap, A. K.; Farrell, F. X.; Jlliffe, L. K.; Barrett, R. W.; Dower, W. J. *Nature Biotech.* **1997,** *15,* 1261; e) Benedetti, E. *Biopolymers (Peptide Science)* **1996,** *40,* 3; f) Gellman, S. H. *Acc. Chem. Res.* **1998,** *31,* 173; g) Appella, D. H.; Christianson, L. A.; Klein, D. A.; Powell, D. R.; Huang, X.; Barchi, J. J. Jr.; Gellman, S. H. *Nature* **1997,** *387,* 381.
2. a) 1a; b) Rodriguez, M.; Fulcrand, P.; Laur, J.; Aumelas, A.; Bali, J. P.; Martinez, J. *J. Med. Chem.* **1989,** *32,* 522; c) Rane, D. F.; Girijavallabhan, V. M.; Ganguly, A. K.; Pike, R. E.; Saksena, A. K.; Mackphail. A. T. *Tetrahedron Lett.* **1993,** *34,* 3201; d) Seebach, D.; Overhand, M.; Kuhnle, N. M.; Martinoni, B. *Helv. Chim. Acta* **1996,** *79,* 913.
3. a) Benedetti, E.; Bavoso, A.; Di Blasio, B.; Grimaldi, P.; Pavone, V.; Pedone, C.; Toniolo, C.; Bonora, G. M. *Int. J. Biol. Macromol.* **1985,** *7,* 81; b) Aubry, A.; Marraud, M. *Biopolymers* **1989,** *28,* 109; c) Pavone, V.; Di Blasio, B.; Lombardi, A.; Isernia, C.; Pedone, C.; Benedetti, E.; Valle, G.; Crisma, M.; Toniolo, C.; Kishore, R. *J. Chem. Soc. Perkin. Trans. 2* **1992,** 1233; d) Bardi, R.; Piazzesi, A. M.; Crisma, M.; Toniolo, C.; Kishore, R. *Zeit. Krystallogr.* **1993,** *207,* 290; e) Kishore, R. *Tetrahedron Lett.* **1996,** *37,* 5747; f) 1e; g) Thakur, A. K.; Kishore, R. *Tetrahedron Lett.* **1998,** *39,* 9553.
4. Wu, Y.-D.; Wang, D.-P. *J. Am. Chem. Soc.* **1998,** *120,* 13485.
5. a) Navarro, E.; Alemán, C.; Puiggali, J. *J. Am. Chem. Soc.* **1995,** *117,* 7307; b) Navarro, E.; Aleman, C.; Puiggali, J. *Macromol.* **1998,** *31,* 408 and references therein.
6. The title compound **1,** ($(CH_3)_3C$-O-CONH-CH_2-CH_2-CONH-CH_3) was synthesized from *t*Boc-β-Ala-OH using mixed anhydride coupling method and purified by silica-gel (60-120 mesh) column chromatography. White solid, m.p. = 116°C, R_f = 0.4 (in 5% MeOH-$CHCl_3$ mixture). ^1H NMR (300 MHz, $CDCl_3$, 25°C, 10 mg/ml, TMS) **1:** δ = 1.43 (s, 9H, $(CH_3)_3$), 2.39 (t, *J* = 6.1 Hz, 2H, $C^{\beta}H_2$), 2.81 (d, *J* = 4.8 Hz, 3H, N-CH_3), 3.40 (q, *J* = 6.1 Hz, 2H, $C^{\alpha}H_2$), 5.18 (broad, 1H, NH), 5.80 (broad, 1H, NH-CH_3). Suitable single crystals for X-ray dffraction study were obtained from EtOAc solution by slow evaporation at room temperature. The diffraction data were collected on CAD4 Enraf-Nonius 4-circle automatic diffractometer using CuKα radiation (λ = 1.5418 Å) and graphite monochromator. Crystal data of **1:** Molecular Formula $C_{15}H_{27}N_3O_4$, Molecular weight = 313.39, Orthorhombic, Space group Pbca, Cell constants : a = 9.544 Å, b = 9.566 Å, c = 26.098 Å, α = β = γ = 90°, V = 2383 $Å^3$, Z = 8, Dc = 1.038 mg m^{-3}, T = 293 K, Final R = 0.0757, Final Rw = 0.2465. Details of crystal structure determination by application of direct method programmes SHELX-97, will be reported elsewhere.
7. Valle, G.; Bonora, G. M.; Toniolo,C. *Gazz. Chim. Ital.* **1984,** *114,* 341.
8. a) Pavone, V.; Benedetti, E.; Di Blasio, B; Lombardi, A.; Pedone, C.; Lorenzi, G. P. *Biopolymers* **1993,** *28,* 215; b) Sun, X. C.; Lorenzi, G. P. *Helv. Chim. Acta* **1994,** *77,* 1520; c) Bandekar, J. *Biochim. Biophys. Acta* **1992,** *1120,* 123.
9. a) Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1994,** *116,* 1054. IR spectrum of CH_3-CONH-CH_2-CH_2-CONH-CH_3 in the N-H stretch region exhibited a strong absorbance at ~3450±3 cm^{-1}. In conjunction with NMR data the results indicated that intramolecular H-bonded ring structures are disfavoured.
10. a) Smith, D. A.; Vijaykumar, S. *Tetrahedron Lett.* **1991,** *32,* 3616; b) Gellman, S. H.; Dado, G. P. *Tetrahedron Lett.* **1991,** *32,* 7377 and references therein.