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Induced Conformational Preferences in a Non-chiral β-Ala Residue: X-ray Diffraction, ^{1}H NMR, FT-IR and CD Studies of Boc-β-Ala-D-Ala-NHCH$_3$ and Boc-β-Ala-L-Ala-NHCH$_3$

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Abstract: In order to demonstrate the conformational features that can be induced by a chiral residue in a β-Ala moiety, X-ray diffraction structure of Boc-β-Ala-D-Ala-NHCH$_3$ **1** have been determined and compared with Boc-β-Ala-L-Ala-NHCH$_3$ **2**. An analysis of the crystal molecular conformations reveals the establishment of non-superimposable stereogeometrical features across β-Ala residues. The supramolecular assembly of the highly ordered L-shaped molecules, generated by the parallel arrangements of the peptide molecules, is stabilised by a network of intermolecular H-bonds between the CO and NH groups. The chiroptical investigations, in solvents of varying polarities, provide experimental evidence which strongly supports that the stereochemical preferences of the β-Ala residue can restrict significant conformational averaging.

INTRODUCTION

The unsubstituted, non-coded beta-amino acid, β-Ala (3-amino propionic acid) though widely distributed in nature, is less frequently found in small peptides. Being an important constituent of several naturally occurring biomolecules and polypeptides implies that this non-chiral residue would be critically important for their conformational features, responsible for their specific performances.[1] Conformationally, the β-Ala residue introduces an additional torsional angle, μ in the peptide backbone which may exert dramatic structural effects, the potentials of which can be exploited for the design and development of appropriate molecular tools capable of stabilising unique conformations representing various *extended* or *folded* structures. High resolution X-ray structural analysis of short linear peptides containing a β-Ala residue has provided evidence that this amino acid is capable of accommodating a large number of well defined three dimensional structures *e.g.* from *fully extended* to *semi-folded* to compact *folded* conformations.[2] Analyses of a large number of cyclic peptides of smaller ring sizes ranging from di- to hexa-peptides[2a,3] and short linear peptides incorporating one or more β-Ala residue(s) has revealed the potential of this residue for stabilising a wide range of conformations mimicking fragments of various secondary structures *i.e.* α-helix, β- and γ-turns, commonly observed in proteins and polypeptides.[4]

Despite the ability of β-Ala to accommodate a wide spectrum of *novel* conformational features, relatively limited studies of short linear peptides incorporating this residue have been reported.[2] As a part of our continuing program aimed at defining systematically the conformational features that can be induced and accommodated by an unsubstituted non-chiral β-Ala, we have recently described the influence of

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various chiral and non-chiral constraints. In this report, we wish to describe the X-ray diffraction analysis of two suitably designed model peptides: Boc-β-Ala-D-Ala-$NHCH_3$, **1** and Boc-β-Ala-L-Ala-$NHCH_3$, **2** which permitted an unambiguous establishment of the novel non-superimposable stereogeometrical features across a β-Ala residue. In conjunction with the ^1H NMR, FT-IR and chiroptical spectroscopic investigations the data allowed us to draw correlations between the solution and solid state conformations. A major feature of the novel conformational features assigned to this unusual non-coded amino-acid for the design and development of the peptides, has been highlighted.

RESULTS AND DISCUSSION

Single Crystal X-ray Studies

In order to establish unambiguously the conformational characteristics accommodated in a non-chiral β-Ala residue, an X-ray diffraction study of **1** was performed and compared with the crystal structure of **2**. The schematic view and the numbering scheme of **1** along with bond distances and bond angles is shown in Figure 1. All the bond distances, bond angles, the geometry of the Boc group and the secondary amide bonds are in excellent agreement with the literature values and compare well with their corresponding values in **2**.[5a] All the peptide bonds are *trans* and no significant deviations are observed. The characteristic torsion angles θ_1 (C1-O-C-N1) and ω_0 (O-C-N1-C1$^\beta$) of the urethane moiety, are -178.5° and -176.2° respectively. The relative *trans-trans* arrangements allowed us to classify it as type *b*.[5a] The torsion angle θ_1' (C1-O-C-O') is 1.7° exhibiting *syn-planar* orientation of C1-O and C-O' bonds similar to those observed in **2**.

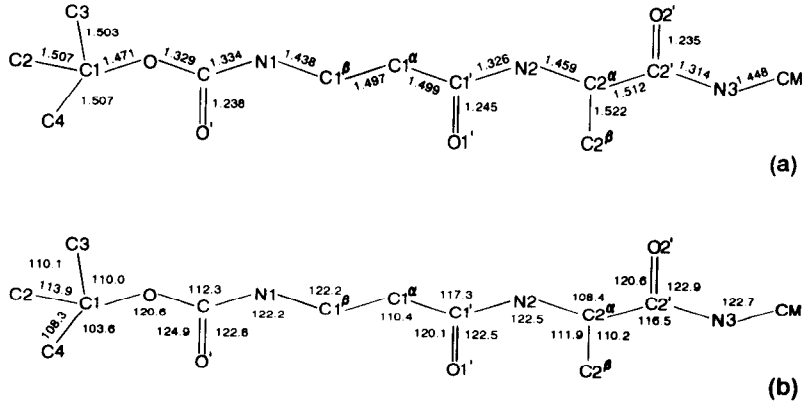

Figure 1: Atomic numbering scheme of **1** along with (a) bond length (Å) and (b) bond angle (°) values.

For describing the backbone torsional angles of the β-Ala residue, we followed the nomenclature proposed by Karle *et al* in 1975.[3a] All the backbone torsion angles fall within the conformationally allowed E-region of the Ramachandran map. The crystal molecular conformation of **1** is shown in Figure 2. The β-Ala residue adopts an *extended* conformation characterised by torsion angles: ϕ = -120.9°, μ = +167.2° and ψ = +117.7° which correspond to *skew* (-), *trans* (+) and *skew* (+) conformations, respectively. The D-Ala residue adopts a *semi-extended* conformation with dihedral angles: ϕ = +116.6° and ψ = -116.2° exhibiting a slight bend at the C^α atom. The terminal amide bond adopts a *syn-planar* conformation.[5b] The overall L-shape conformation of the peptide is devoid of any intramolecular hydrogen-bond.

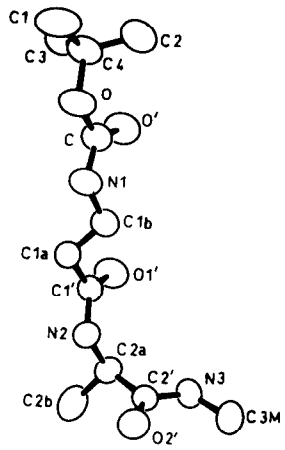

Figure 2: Molecular conformation of **1** generated from the crystal data.

The analysis of the crystal structure of **1** reveals, in their torsion angles, a remarkable similarity with **2**. As expected, the chiral D- and L-Ala residues in **1** and **2** adopt the ϕ, ψ torsion angles in their respective conformationally allowed E-regions of the ϕ, ψ map. A comparison of the relevant backbone torsion angles of **1** and **2** are summarised in Table 1. The most significant point to be noted is that the signs as well as the magnitudes of the backbone torsional characteristics observed for β-Ala residues, in both peptides, seem to be dictated by the chirality of the neighbouring Ala residue. The backbone torsional angles of the β-Ala residue in **1**: ϕ = -120.9°, μ = +167.2°, ψ = +117.7° and in **2**: ϕ = +120.9°, μ = -167.1°, ψ = -118.2°, are close to *skew* (±), *trans* (±) and *skew* (±) conformations, respectively. Surprisingly, the chiral D-Ala residue in **1** induces the *flexible* non-chiral β-Ala residue to adopt the ϕ, ψ angles in the L-region of the Ramachandran map while in peptide **2**, the L-Ala residue induces the corresponding torsion angles in the D-region in a systematic manner.[2f] Similarly, the critical μ torsion angle also exhibits reverse trends for its conformational preferences. Moreover, from the results of X-ray analyses it is clear that in **1** and **2**, the magnitudes of all the torsional angles are comparable while their signs are opposite, consequently resulting in a non-superimposable mirror image relationship across a β-Ala residue, as depicted in

Table 1. Backbone Torsion Angles (°) of **1** and **2** in the Crystalline State [a]

Atoms in the sequence	Designation	Value [b]	
		1	2
C1 - O - C - O'	θ_1'	1.7	4.8
C1 - O - C - N1	θ_1	- 178.5	- 177.5
O - C - N1 - C1$^\beta$	ω_0	- 176.2	179.6
C - N1 - C1$^\beta$ - C1$^\alpha$	ϕ_1	- 120.9	120.9
N1 - C1$^\beta$ - C1$^\alpha$ - C1'	μ_1	167.2	- 167.1
C1$^\beta$ - C1$^\alpha$ - C1' - N2	ψ_1	117.7	- 118.2
C1$^\alpha$ - C1' - N2 - C2$^\alpha$	ω_2	- 177.1	176.3
C1' - N2 - C2$^\alpha$ - C2'	ϕ_2	116.6	- 116.4
N2 - C2$^\alpha$ - C2' - N3	ψ_2	- 116.2	115.8
C2$^\alpha$ - C2' - N3 - CM	ω_2	178.6	- 178.6

[a] For the atom numbering see Figure 1.
[b] The standard deviation in the least significant digits are omitted for the sake of clarity.

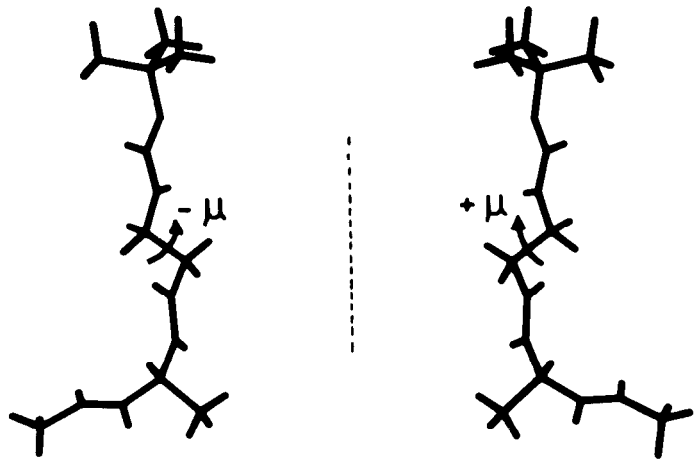

Figure 3: A comparison of molecular structure of **1** (right) and **2** (left) depicting a non-superimposable mirror image relationship. The central μ torsion angles of the β-Ala residue are indicated.

Figure 3. These findings indicate that despite the fact that the β-Ala is a non-chiral, a handed orientation may be enforced by the molecular torsion of the chiral moiety.

An observation of a *skew*, *trans* and *skew* conformation of the β-Ala moiety, induced by a chiral Ala residue in **1** and **2**, is indeed interesting since, theoretical *ab initio* quantum mechanical calculations of the model system: Ac-β-Ala-NH_2 has revealed significantly less stability of an extended *trans* conformation as compared to the folded one.[6] The energy profile for the optimised geometries reflected a strong preference for the most stable *folded* conformation characterised by the μ torsion angles close to *gauche* (Table 2). On the contrary, the crystal molecular structure of Boc-β-Ala-$NHCH_3$, in conjunction with an FT-IR study in the solid state, suggested that the β-Ala moiety favoured the accommodation of an extended all-*anti* conformation: the magnitudes of the torsion angles being $\phi \approx -146°$, $\mu \approx 172$ and $\psi \approx 155°$. These values are substantially deviated from those of **1** and **2**, clearly indicating the influence of the neighbouring chiral Ala residue(s).[2h] Recently, the role of *neighbouring constraints* on the conformational adaptability of the 'flexible' β-Ala residue have also been emphasised and demonstrated experimentally.[2h,i] Further, the observed conformational angles of the β-Ala residue in **1** are very close to an ideal parallel β-sheet structure ($\phi = -119°$; $\psi = 113°$) comprised of L-amino acids, proposed by Pauling *et al* from model building.[7] It may be worth underlining that the reported examples of parallel β-sheet structures: Gly-Phe-Gly and Val-Gly-Gly, on the other hand, exhibited significant deviations in their backbone torsion angles: $\phi \approx -126°$ and $-155°$ and $\psi \approx 132°$ and $155°$, respectively.[8]

Table 2. A comparison of relevant backbone torsion angles (°) of the β-Ala residue

Peptides	ϕ	μ	ψ	References
Boc-β-Ala-D-Ala-$NHCH_3$	-120.9	167.2	117.7	This paper
Boc-β-Ala-L-Ala-$NHCH_3$	120.9	-167.1	-118.2	This paper
Boc-β-Ala-$NHCH_3$	-145.5	171.6	154.5	2h
Ac-β-Ala-NH_2	88.5	62.8	-179.1	6

Another important conformational feature of β-Ala peptides that needs to be stressed is the central μ dihedral angle between the two tetrahedral carbon atoms (sp^2-CH_2-CH_2-sp^2) which plays a decisive role in dictating the overall three-dimensional structure of the molecules.[2,3] An analysis of available X-ray crystal structure of a number of short linear β-Ala peptides *i.e.* Boc-L-Ala-β-Ala-$NHCH_3$, Boc-Aib-β-Ala-$NHCH_3$, Boc-Pro-β-Ala-$NHCH_3$ and Boc-Aib-β-Ala-Aib-OCH_3, including those having high propensity for adopting *folded* conformations (μ ≈ ±60±20°), indicates an overwhelming preference for a fully extended *trans* conformations (μ ≈ ±180±20°).[2a,b] However, recently there have been a few exceptions to this usual viewpoint (unpublished data).[2g,i] The observed inclinations for the μ torsion angle close to ≈ +167° in **1** and ≈ -167° in **2**, clearly suggests that in the absence of any *significant* conformational constraint(s) the sign of the torsion angle can also be dictated by the neighbouring chiral residue(s) and presumably the complex chemical nature of the side chain(s).

Crystal Packing

In the crystal packing of **2** the *extended* L-shaped molecules are associated in the parallel fashion along the *b* axis. The molecules pack with the identity period of the order of 4.967 Å and this repeat distance corresponds to the spacing between two parallel neighbouring molecules in a sheet structure. Between each pair of molecules all the potential H-bond donor and acceptor groups participate in the formation of three C=O···N-H intermolecular H-bonding interactions (Figure 4). The overall packing of the molecules in the crystals of **1** and **2** are quite similar. The geometric parameters of the H-bonds observed in the crystal lattice of both the peptides are summarised in Table 3. It is noteworthy that despite relatively less favourable parallel arrangements of the molecules, significant linear H-bonds are formed. Surprisingly, the characteristic parallel and antiparallel orientations of the carbonyl groups across the β-Ala and chiral Ala residues respectively, are retained in the molecules. Consequently, the two unidirectional H-bonds between the β-Ala moieties of adjacent molecules encompass a *unique* 14-membered ring motif while across the proteinogenic Ala residue they constitute a usual 12-membered ring motif since the directions of the amide dipoles across the chiral Ala moiety are typical of those found in parallel β-sheet structures comprised of α -amino acids. Taken together, the simultaneous existence of the two novel ring motifs described in short linear β-Ala peptides **1** and **2**, are not only fascinating but also somewhat reminiscent of the ones found in antiparallel and parallel β-sheet structures of proteins and polypeptide chains.[4,7] The detailed conformational comparisons of the present crystal structure with **2**, as depicted in Figure 3, serve as valuable models to further investigate and establish correlation between solution and solid state conformations.

Table 3. Intermolecular hydrogen bond parameters for **1** and **2**.

Donor N-H	Acceptor O	Symmetry Equivalence of O	Distances (Å) N···O	H···O	Angle (°) N-H···O
		Boc-β-Ala-D-Ala-$NHCH_3$, **1**			
N1-H	O′	x+1, y, z	2.852	1.867	164.82
N2-H	O1′	x+1, y, z	2.995	1.997	178.62
N3-H	O2′	x-1, y, z	2.903	1.927	172.58
		Boc-β-Ala-L-Ala-$NHCH_3$, **2**			
N1-H	O′	x, y, z-1	2.866	2.103	157.65
N2-H	O1′	x, y, z-1	3.010	2.230	168.64
N3-H	O2′	x, y, z+1	2.908	2.224	178.57

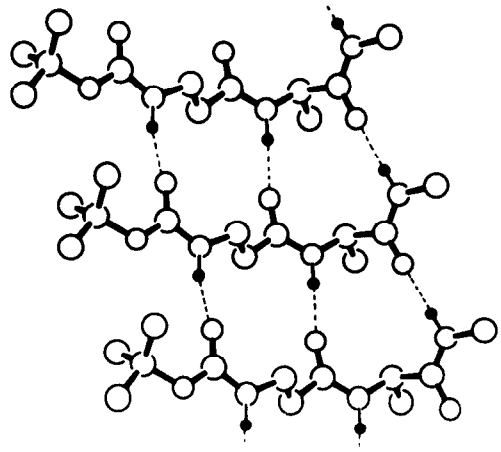

Figure 4: A view of the parallel arrangement of Boc-β-Ala-D-Ala-$NHCH_3$ along the *b* axis. Black circles indicate H-atoms. Note the parallel orientations of the two amide groups across β-Ala residues.

^1H NMR Studies

A comparison of the chemical shifts of the ^1H NMR resonances and $^3J_{HN\text{-}C^\alpha H}$ coupling constants reveal valuable information about peptide conformation in solution.[9] The observed NMR pattern of $C^\beta H_2$ and $C^\alpha H_2$ resonances of **1** and **2**, in $CDCl_3$ as well as DMSO-d_6, are characteristic of A_2M_2X spin system and have often been indicative of an all-*anti* conformation of the β-Ala moiety.[2f,h] An analysis of the proton chemical shifts of **1** and **2** reveal that all the resonances, except amide protons, experience an upfield shift (~ 0.06-0.29 ppm) on going from $CDCl_3$ to DMSO-d_6 which may be due to an increased degree of solvations in the more polar medium, a characteristic observation described earlier for peptides which tend to aggregate in an apolar non-interacting medium.[10] The $\Delta\delta_{NH}$ ($\delta_{DMSO\text{-}d_6}$-δ_{CDCl_3}) values of amide NHs, usually employed to distinguish between solvent shielded (or intramolecularly H-bonded) and solvent exposed (or non H-bonded) amide protons, exhibit significant down field shifts ($\Delta\delta_{NH} \geq 1.08$ ppm) of all the three amide NHs, indicative of the absence of any intramolecularly H-bonded structures.

Extensive use of the Karplus equation, for peptides with *trans* amide bonds has established that the $^3J_{HN\text{-}C^\alpha H}$ coupling constants are well correlated with the ϕ angle. The observed ^3J values ~ 7.3-7.6 Hz for chiral Ala residues in $CDCl_3$ and DMSO-d_6 are largely consistent with the observed ϕ angle ≈ ±116° in the crystal structure, assuming a single conformation is important.[9] The non-availability of the parameters of the Karplus type relationship for the β-Ala, prevented the evaluation of backbone ϕ value for this residue.

FT-IR Studies

Further support for the presence of H-bonded backbone conformation in **1** and **2**, in the solid state, was obtained from FT-IR studies in KBr. The assessment of the bands in the amide A region of **2**, shown in Figure 5, exhibit intense bands at ~ 3333 and 3311 cm^{-1} corresponding to N-H groups involved in strong H-bonding interactions.[2h] From the observation it is safe to attribute these bands to intermolecular interactions similar to those observed in crystalline state. The amide I region exhibits three strong absorption bands at ~ 1684, 1655 and 1632 cm^{-1}. The bands at 1632 and 1655 cm^{-1} are characteristic of

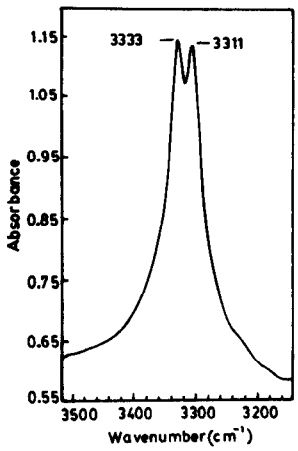

Figure 5: FT-IR spectrum, amide A region, of **1** in KBr disc.

strongly H-bonded amide C=O acceptor groups involved in an extended β-sheet conformation. The band at 1680 cm^{-1} may be assigned to the urethane carbonyl group participating in H-bond formation similar to those described in the literature which usually diminishes and/or disappears on dilution in a poorly interacting medium. Interestingly, an analysis of amide I bands of a large number of proteins and polypeptides secondary structures, involved in helical conformations exhibit characteristic IR bands at ~1655±5 cm^{-1}.[11] The absence of these conformational features in **1** and **2** suggest that in β-Ala peptides such characteristic IR bands may arise from secondary structural features other than helical conformations.

CD Studies

The chiroptical investigations of **1** and **2** reveal chiral distinctions with the assumption that the crystal structures are retained when CD measurements are made in solution.[12] The characteristic band positions and molar ellipticity values measured in MeOH, TFE, dioxane and TMP have been reported. The CD spectra of **1** and **2** recorded under similar conditions, were almost identical in band positions, shapes and magnitudes, except were of opposite signs reflecting a mirror image relationship. The results of CD studies corroborated well with those obtained from the X-ray diffraction studies which indicate the existence of the handedness conformational characteristics across β-Ala residue, when compared **1** and **2**.[2f]

The CD study of Ac-L-Ala-$NHCH_3$ provide a good model system for comparison with **2**, which on the contrary, exhibited strong solvent dependence. In methanol the CD spectrum exhibits a strong negative peak at ~ 200 nm, while in an aprotic non-polar solvent like dioxane, it has a distinct negative band at ~ 220 nm. The intensity of the long wavelength band gradually diminishes in the solvents of higher polarity and completely abolished in water. In light of ^1H NMR and IR studies, the presence of intramolecular H-bonded structures have been suggested in Ac-L-Ala-$NHCH_3$. The observed CD bands, in the solvents of different polarities, have been assigned to specific conformations and attributed to their shifts from C_5- to C_7-structures on decreasing polarity of the medium.[12a-c] It may be noted that such conformational features

in **1** and **2** are entirely absent. Moreover, it may be worth emphasising that the ^1H NMR spectroscopic signatures of an intramolecularly H-bonded C_5-structure across a $C^{\alpha}H$ bearing chiral proteinogenic residue, have been made available only recently.[4e]

Table 4: Comparative CD band positions (nm) and molar ellipticity, $[\theta]_M$ in different solvents.

Solvents	λ (nm)	1	2
MeOH	198	+ 18120	- 18660
TFE	200	+ 13500	- 13630
Dioxane	217	+ 13500	- 10840
TMP	200	+ 12690	- 12270
	225 (s)	+ 2910	- 2710

$[\theta]_M$ in deg. cm^2 $dmol^{-1}$ and (s) indicates shoulder

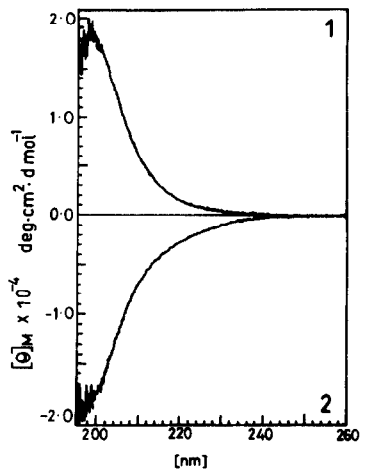

Figure 6: CD spectra of **1** and **2** in MeOH

The observation of the strong CD signals and their overall similarities in the range of solvents studied are supportive of the proposal that a well defined structure predominates in solution. The data does not reflect conformational averaging since significantly high ellipticity values have normally been attributed to the presence of a significant population of one conformation. Low band intensities are normally attributed to the equilibrium between two or more structures.[12] The CD studies on **1** and **2** in a range of solvents, provide unequivocal experimental evidence which strongly suggest that the β-Ala residue not only influenced the CD spectral pattern of the chiral Ala residue but also restricts conformational averaging. This particular conclusion may receive strong support from our on going CD investigations of various model peptides adopting diverse secondary structural features (unpublished data).

CONCLUSION

Conformational analysis of two suitably modelled linear peptides, **1** and **2** allowed us to establish an accommodation of chirality induced non-superimposable, stereogeometrical features across a non-chiral β-Ala residue. There exists a strong correlation between conformational features determined in solution with those established in crystalline state. The CD studies in a range of solvents provided conclusive evidence which strongly suggest that the preferred central μ rotation of the β-Ala residue is capable of significantly restricting the conformational averaging. From the results it appears to be possible to switch the conformational characteristics of β-Ala from right handed to left handed or *vice versa*, by chiral effects which can be exploited to construct peptide or non-peptide candidates of opposite handedness features. The unique aggregating tendencies of the β-Ala residue, by making use of directional noncovalent interactions, may play a critical role in architecturing complex supramolecular assemblies and crystal engineering.

Additionally, the β-Ala has frequently been substituted for an achiral α-amino acid and less commonly for a chiral α-amino acid. Such simple homologation of biologically active peptides generally did not improve the potency of the resulting analogue, which may be attributed to our limited understanding of the topography of conformational effects in peptides which precludes rational predictions. However, there have been a few exceptions.[14] The information on the conformational features across the β-Ala residue presented in this paper and those reported elsewhere may stimulate further interest in design and development of relatively unconstrained peptidomimetics using achiral, unconstrained, non-coded amino acids.

EXPERIMENTAL PROCEDURES

Peptide Synthesis: All amino-acids, tert-butylcarbazate, dicyclohexylcarbodiimide (DCCI), triethylamine (TEA) and deuterated solvents were from Sigma Chemical Company, USA. 25% methylamine solution and solvents chloroform, dichloromethane, ethyl acetate, methanol *etc.* were purchased locally from Ranbaxy Chemicals and distilled or purified before use. Silica gel with 13% $CaSO_4$ (BDH) and silica gel, 60-120 mesh (SRL) were used for thin layer and column chromatography, respectively. For the detection of the peptides, the iodine vapour staining method and/or ninhydrin reaction were employed. Solvent system 5% MeOH-$CHCl_3$ mixture was used for R_f determination. Melting points (m.p.) and optical rotations $[\alpha]_D^{25}$ were determined with a Electrothermal digital melting point apparatus and Autopol III automatic polarimeter, respectively. The peptide was synthesised using standard solution phase procedure.

Boc-β-Ala-D-Ala-$NHCH_3$ (1)

To 0.76 g. of Boc-β-Ala-OH (4.0 mmole) was added a mixture of 0.56 ml of TEA (4.0 mmole) and 0.56 g. of HCl·D-Ala-OMe (4.0 mmole) in dichloromethane. The reaction mixture was cooled to 0°C and after 10 minutes was added 0.84 g. of DCCI (4.0 mmole) under stirring. The reaction was continued at 0°C for 6 hrs and for another 8 hrs at room temperature. Dicyclohexylurea (DCU) precipitated was filtered off and the solution was concentrated. To this material was added about 100 ml of ethyl acetate and washed with 2N HCl and 0.5M $NaHCO_3$ solutions (3 × 25 ml each) and finally with saturated NaCl solution. Drying over anhydrous sodium sulphate and evaporation yielded the pure dipeptide ester as a gum. Yield 0.93 g. (3.4 mmole), 85%. $R_f = 0.32$. The dipeptide ester Boc-β-Ala-D-Ala-OMe 0.82 g. (3.0 mmole) was treated with anhydrous methanol saturated with dry methylamine gas for 48 hrs at room temperature. The completion of the reaction was monitored by tlc. Evaporation of solvent yielded yellowish product which was purified by silica-gel column chromatography using 1-2 % MeOH-$CHCl_3$ mixtures as eluants. Yield 0.74 g. (2.72 mmole); White solid; m.p. 141°C; [Found : C, 52.89; H, 9.13; N, 15.72, requires C, 52.73; H, 8.48; N, 15.37%]; 80 %, R_f (5% MeOH-$CHCl_3$) 0.25; $[\alpha]_D^{25}$ -26.8 (c 0.22, MeOH); ν_{max} (KBr) 3333, 3311, 1684, 1655, 1632, 1561, 1536 cm^{-1}; δ_H (300 MHz, $CDCl_3$, 25°C, 10 mg/ml, TMS) 1.37 (1H, d, *J* 7.0 Hz, Ala $C^{\beta}\underline{H}_3$), 1.43 (9H, s, $(C\underline{H}_3)_3$-O-), 2.44 (2H, t, *J* 5.9 Hz, β-Ala $C^{\alpha}\underline{H}_2$), 2.80 (3H, d, *J* 4.8, $NHC\underline{H}_3$), 3.40 (2H, q, *J* 6.1, β-Ala $C^{\beta}\underline{H}_2$), 4.48 (1H, m, Ala $C^{\alpha}\underline{H}$), 5.23 (1H, broad t, β-Ala N$\underline{H}$), 6.62 (1H, broad, N$\underline{H}CH_3$), 6.75 (1H, d, *J* 7.3 Hz, Ala N$\underline{H}$); δ_H (300 MHz, DMSO-d_6, 25°C, 10 mg/ml, TMS) 1.16 (1H, d, *J* 7.1 Hz, Ala $C^{\beta}\underline{H}_3$), 1.37 (9H, s, $(C\underline{H}_3)_3$-O-), 2.26 (2H, dt, *J* 2.2,7.3 Hz, β-Ala $C^{\alpha}\underline{H}_2$), 2.57 (3H, d, *J* 4.6 Hz, $NHC\underline{H}_3$), 3.11 (2H, q, *J* 5.9 Hz, β-Ala $C^{\beta}\underline{H}_2$), 4.19 (1H, m, Ala $C^{\alpha}\underline{H}$), 6.74 (1H, broad t, β-Ala N$\underline{H}$), 7.76 (1H, broad, N$\underline{H}CH_3$), 8.02 (1H, d, *J* 7.6 Hz, Ala N$\underline{H}$).

Boc-β-Ala-L-Ala-NHCH$_3$ (2)

This peptide was synthesised in the same manner as described for **1**. Briefly, a solution of Boc-β-Ala-OH (4.0 mmole), HCl·L-Ala-OMe (4.0 mmole) and an equivalent amount of TEA in dichloromethane was treated with DCC at 0°C for 4 hrs and overnight at room temperature. The dipeptide ester isolated was then treated with gaseous methylamine in anhydrous methanol. **2** was purified by silica gel column chromatography. Yield 0.7 g. (2.70 mmole); White solid; m.p. 140°C; [Found : C, 52.73; H, 9.13; N, 15.70, requires C, 52.73; H, 8.48; N, 15.37%]; R_f (5% MeOH-$CHCl_3$) 0.25; $[\alpha]_D^{25}$ +23.9 (c 0.23, MeOH); ν_{max} (KBr) 3333, 3311, 1684, 1655, 1632, 1561, 1537 cm^{-1}; δ_H (300 MHz, $CDCl_3$, 25°C, 10 mg/ml, TMS) 1.37 (1H, d, *J* 7.0 Hz, Ala C$^{\beta}$H$_3$), 1.43 (9H, s, (CH$_3$)$_3$-O-), 2.44 (2H, t, *J* 5.9 Hz, β-Ala C$^{\alpha}$H$_2$), 2.80 (3H, d, *J* 4.8, NHCH$_3$), 3.40 (2H, q, *J* 6.1, β-Ala C$^{\beta}$H$_2$), 4.48 (1H, m, Ala C$^{\alpha}$H), 5.25 (1H, broad t, β-Ala NH), 6.68 (1H, broad, NHCH$_3$), 6.80 (1H, d, *J* 7.3 Hz, Ala NH); δ_H (300 MHz, DMSO-d_6, 25°C, 10 mg/ml, TMS) 1.16 (1H, d, *J* 7.1 Hz, Ala C$^{\beta}$H$_3$), 1.37 (9H, s, (CH$_3$)$_3$-O-), 2.26 (2H, dt, *J* 2.2, 7.3 Hz, β-Ala C$^{\alpha}$H$_2$), 2.57 (3H, d, *J* 4.8 Hz, NHCH$_3$), 3.11 (2H, q, *J* 5.9 Hz, β-Ala C$^{\beta}$H$_2$), 4.19 (1H, m, Ala C$^{\alpha}$H), 6.74 (1H, broad t, β-Ala NH), 7.76 (1H, broad, NHCH$_3$), 8.02 (1H, d, *J* 7.6 Hz, Ala NH).

^{1}H NMR Studies: The one dimensional ^{1}H NMR spectra were recorded on a Brüker 300 MHz FT-NMR spectrometer equipped with Aspect 3000 computer. All experiments were performed at 25°C. The chemical shifts are reported in a reference to internal TMS at 0 ppm. Vicinal coupling constant were obtained directly from the 1D spectra without any correction for line width.

FT-IR Studies: The FT-IR absorption spectra of the solid samples were recorded using KBr disc on Nicholet Impact 400D spectrometer, equipped with CTX CMS-1561 multiscan, at 2 cm^{-1} resolution.

CD Studies: CD spectra were recorded on a Jasco J-720 spectropolarimeter equipped with a computer. The spectra were run in the range of 195-250 nm at the sample concentration of 0.5 mg / ml. The optics chamber was flushed continuously with nitrogen gas throughout each experiment. Cylindrical cell with an optical path length of 1 mm was used and the spectrum presented as an average of two scans. The data are expressed in terms of molar ellipticity, $[\theta]_M$, in units of deg. cm^2 dmol^{-1}. All measurements were done at 25° C.

X-ray Diffraction Studies: The crystals of **1**, for X-ray diffraction study, were obtained from methanol-chloroform mixtures by slow evaporation at room temperature.

Crystal data: Molecular Formula : $C_{12}N_3O_4H_{23}$; Molecular Weight : 273.33. A colourless, needle shaped crystal of the size 0.2 × 0.3 × 0.5 mm were used for data collections. The crystals was stable to air at room temperature. The peptide crystallised in orthorhombic space group P $2_12_12_1$ with a = 4.967 (Å), b = 11.316 (Å), c = 26.475 (Å); α = β = γ = 90°; F(000) = 592; V = 1488.1 (3) Å^3; D = 1.22 gm. cm^{-3} and Z = 4. The collections of X-ray diffraction intensity were performed on an automated four circle CAD4 Enraf Nonius Diffractometer using CuKα radiation (λ = 1.5418 Å) and graphite monochromator. The unit cell parameter were determined by a least-squares fit of the angular settings of 22 reflections in the 2θ range of 18-35°. The analysis of the peak profiles suggested an ω - 2θ scan mode. Three standard reflections were measured periodically and these showed no systematic variation in their intensities during the course of data collection. Reflections with a net intensity I < 2σ (I) were flagged as weak ; those having I > 2σ (I) were measured at lower speed (1.0°-3°/min) depending on the value of σ (I) / I. The observed intensities were corrected for lorentz and polarization factors, but not for absorption. A total of 2768 reflections were collected, 2343 of which having I > 2σ (I), were considered observed and used as such in subsequent calculations.

Table 5. Fractional Atomic Coordinates and Equivalent Isotropic Temperature Factors * of **1**.

ATOM	x	y	z	Beq (Å^2)	ATOM	x	y	z	Beq (Å^2)
C2	-0.8216 (14)	-0.1431 (7)	0.7900 (3)	6.7 (1)	C1'	-0.4392 (8)	-0.1042 (3)	0.5156 (2)	4.0 (1)
C3	-0.8303 (12)	-0.3489 (6)	0.7534 (3)	6.2 (1)	O1'	-0.6874 (6)	-0.0996 (4)	0.5094 (2)	5.9 (1)
C4	-0.4779 (12)	-0.2873 (10)	0.8135 (3)	9.0 (1)	N2	-0.2662 (7)	-0.0633 (3)	0.4817 (2)	4.4 (1)
C1	-0.6607 (9)	-0.2473 (5)	0.7714 (2)	5.6 (1)	C2$^{\alpha}$	-0.3527 (9)	-0.0070 (4)	0.4348 (2)	4.4 (1)
O	-0.4731 (6)	-0.2106 (4)	0.7314 (1)	5.9 (1)	C2$^{\beta}$	-0.2427 (19)	-0.0707 (5)	0.3886 (3)	7.1 (2)
C	-0.5649 (8)	-0.1659 (5)	0.6882 (2)	4.8 (1)	C2'	-0.2578 (7)	0.1199 (3)	0.4353 (2)	4.0 (1)
O'	-0.8054 (6)	-0.1489 (4)	0.6783 (2)	6.5 (1)	O2'	-0.0151 (6)	0.1434 (3)	0.4353 (2)	6.0 (1)
N1	-0.3669 (7)	-0.1404 (4)	0.6560 (2)	5.1 (1)	N3	-0.4459 (7)	0.2016 (3)	0.4353 (2)	5.0 (1)
C1$^{\beta}$	-0.4167 (9)	-0.0846 (4)	0.6081 (2)	5.0 (1)	CM	-0.3854 (13)	0.3267 (4)	0.4368 (3)	6.2 (1)
C1$^{\alpha}$	-0.3249 (8)	-0.1548 (4)	0.5634 (2)	4.4 (1)					

* Anisotropically refined atoms are given in the form of the isotropic equivalent displacement parameter defined as
$(4/3)\cdot [a^2\cdot B(1,1) + b^2\cdot B(2,2) + c^2\cdot B(3,3) + ab(\cos\gamma)\cdot B(1,2) + ac(\cos\beta)\cdot B(1,3) + bc(\cos\alpha)\cdot B(2,3)]$

Table 6. Anisotropic Thermal Parameters for **1**.

ATOM	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
C2	0.068 (4)	0.098 (4)	0.094 (4)	-0.014 (4)	0.021 (3)	-0.014 (3)
C3	0.061 (3)	0.086 (3)	0.091 (4)	-0.003 (4)	0.011 (3)	-0.004 (3)
C4	0.053 (3)	0.178 (8)	0.075 (4)	0.033 (5)	0.003 (3)	-0.007 (4)
C1	0.033 (2)	0.108 (4)	0.073 (3)	0.006 (3)	0.009 (2)	-0.005 (2)
O	0.026 (1)	0.129 (3)	0.069 (2)	0.020 (2)	0.002 (1)	0.002 (2)
C	0.029 (2)	0.086 (3)	0.066 (2)	0.010 (2)	-0.003 (2)	0.003 (2)
O'	0.024 (2)	0.129 (3)	0.092 (3)	0.018 (2)	-0.005 (1)	0.005 (2)
N1	0.027 (2)	0.101 (3)	0.066 (2)	0.014 (2)	-0.002 (1)	0.002 (2)
C1$^{\beta}$	0.053 (3)	0.072 (2)	0.065 (3)	0.003 (2)	0.001 (2)	0.006 (2)
C1$^{\alpha}$	0.039 (2)	0.062 (2)	0.068 (2)	0.011 (2)	0.001 (2)	0.005 (2)
C1'	0.029 (2)	0.056 (2)	0.068 (2)	0.002 (2)	-0.002 (2)	-0.001 (1)
O1'	0.027 (2)	0.113 (3)	0.086 (2)	0.013 (2)	-0.007 (1)	-0.002 (1)
N2	0.033 (2)	0.065 (2)	0.068 (2)	0.014 (2)	-0.004 (1)	-0.001 (1)
C2$^{\alpha}$	0.046 (2)	0.060 (2)	0.066 (2)	0.011 (2)	-0.009 (2)	-0.003 (2)
C2$^{\beta}$	0.144 (7)	0.068 (3)	0.072 (3)	0.002 (3)	-0.011 (4)	0.009 (4)
C2'	0.028 (2)	0.059 (2)	0.065 (2)	0.008 (2)	0.001 (2)	-0.001 (1)
O2'	0.028 (1)	0.084 (2)	0.114 (3)	0.019 (2)	-0.003 (2)	-0.006 (1)
N3	0.037 (2)	0.062 (2)	0.084 (3)	0.003 (2)	0.004 (2)	0.006 (1)
CM	0.076 (4)	0.058 (2)	0.101 (4)	-0.002 (3)	0.002 (3)	0.006 (2)

The thermal parameter is of the form:
$\exp [-2\pi^2 (U_{11}h^2a^{*2} + U_{22}k^2b^{*2} + U_{33}l^2c^{*2} + 2U_{12}hka^*b^* + 2U_{23}klb^*c^* + 2U_{13}hla^*c^*)]$

The structure was solved by means of direct methods using SHELXS-86 and E-map fourier cycling program and refined by SHELXL-93 (Sheldrick, 1993).[13] The non - hydrogen atoms C, N and O were refined by the full matrix anisotropic temperature factor least squares method minimising the quantity $\Sigma w (|F_o| - |F_c|)^2$ with weights w equal to $1/\sigma (F_o)^2$ where F_o is the standard deviation of the respected intensities and hydrogen atoms were introduced stereochemically at their expected positions with isotropic temperature factors equal to the B_{eq} factor of the atom to which each of them is linked. Refinements were ended when the atomic coordinates converge fully (R = 0.079). The goodness of fit was 1.0 and final density map showed no peaks outside the range between 0.26 and -0.35 e / Å^3. All calculations, using the SDP program package were carried out on a MicroVax II computer. Refined atomic parameters and equivalent B_{eq} factors for non-hydrogen atoms are listed in Table 5. The preliminary X-ray diffraction data of **2** have already been reported.[2e]

Table 7. Hydrogen Atom Coordinates for **1**.

ATOM	X/A	Y/B	Z/C	U_{iso} (Å^2)
H1 (C2)	-0.943 (11)	-0.167 (4)	0.823 (2)	0.09 (1)
H2 (C2)	-0.919 (10)	-0.110 (4)	0.759 (2)	0.10 (1)
H3 (C2)	-0.703 (10)	-0.073 (5)	0.803 (2)	0.09 (1)
H1 (C3)	-0.958 (11)	-0.325 (4)	0.726 (2)	0.09 (1)
H2 (C3)	-0.937 (10)	-0.386 (4)	0.785 (2)	0.10 (1)
H3 (C3)	-0.726 (10)	-0.419 (4)	0.741 (2)	0.09 (1)
H1 (C4)	-0.591 (9)	-0.311 (4)	0.842 (2)	0.10 (1)
H2 (C4)	-0.355 (11)	-0.218 (4)	0.824 (2)	0.11 (1)
H3 (C4)	-0.396 (11)	-0.357 (4)	0.799 (2)	0.11 (1)
H (N1)	-0.178 (11)	-0.152 (4)	0.669 (2)	0.09 (1)
H1 ($C1^{\beta}$)	-0.654 (10)	-0.067 (4)	0.603 (2)	0.11 (1)
H2 ($C1^{\beta}$)	-0.329 (11)	-0.002 (4)	0.606 (2)	0.09 (1)
H1 ($C1^{\alpha}$)	-0.097 (10)	-0.149 (4)	0.560 (2)	0.10 (1)
H2 ($C1^{\alpha}$)	-0.382 (10)	-0.237 (4)	0.565 (2)	0.08 (1)
H (N2)	-0.073 (11)	-0.074 (4)	0.491 (2)	0.09 (1)
H ($C2^{\alpha}$)	-0.574 (11)	-0.012 (4)	0.432 (2)	0.07 (1)
H1($C2^{\beta}$)	-0.323 (10)	-0.028 (4)	0.357 (2)	0.09 (1)
H2($C2^{\beta}$)	-0.311 (10)	-0.151 (4)	0.389 (2)	0.10 (1)
H3($C2^{\beta}$)	-0.018 (12)	-0.074 (4)	0.391 (2)	0.11 (1)
H (N3)	-0.635 (10)	0.177 (4)	0.433 (2)	0.09 (1)
H1(CM)	-0.556 (10)	-0.376 (4)	0.438 (2)	0.12 (1)
H2(CM)	-0.273 (10)	0.351 (4)	0.403 (2)	0.11 (1)
H3(CM)	-0.264 (10)	0.346 (4)	0.466 (2)	0.12 (1)

The U_{eq} of non-hydrogens are equivalent to U_{iso} of the corresponding non-hydrogens.

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REFERENCES

1. a) Drey, C.N.C. *Chem. and Biochem. of the Amino Acids* **1985**, Barrett, G.C., Ed.; Chapman and Hall, London, pp.25. b) Kleinkauf, H.; Von Dohren, H. *Ann. Rev. Microbiol.* **1987**, 41, 259-289. c) Griffith, O.W. *Ann. Rev. Biochem.* **1986**, 55, 855-878.
2. a) Benedetti, E.; Bavoso, A.; Blasio, B.D.; Grimaldi, P.; Pavone, V.; Pedone, C.; Toniolo, C.; Bonora, G.M. *Int. J. Biol. Macromol.* **1985**, 7, 81-88. b) Aubry, A.; Marraud, M. *Biopolymers* **1989**, 28, 109. c) Tormo, J.; Puiggali, J.; Vives, J.; Fita, I.; Lloveras, J.; Bella, J.; Aymami, J.; Subirana, J.A. *Biopolymers* **1992**, 32, 643-648. d) Pavone, V.; Blasio, B.D.; Lombardi, A.; Isernia, C.; Pedone, C.; Benedetti, E.; Valle, G.; Crisma, M.; Toniolo, C.; Kishore, R. *J. Chem. Soc. Perkin Trans.* **1992**, 2, 1233-1237. e) Bardi, R.; Piazzesi, A.M.; Crisma, M.; Toniolo, C.; Kishore, R. *Z. Kristallogr.* **1993**, 207, 290-292. f) Kishore, R. *Tetrahedron Lett.* **1996**, 37, 5747-5750. g) Thakur, A.K.; Kishore, R. *Tetrahedron Letters* **1998**, 39, 9553-9556. h) Thakur, A.K.; Kishore, R. *Tetrahedron Letters* **1999**, in press. i) Thakur, A.K.; Kishore, R. *Biopolymers* **1999**, communicated.
3. a) Karle, I.L.; Handa, B.K.; Hassal, C.H. *Acta. Cryst.* **1975**, B 31, 555-560. b) White, D.J.; Morrow, C.; Cox, P.J.; Drey, C.N.C.; Lowbridge J. *J. Chem. Soc. Perkin II* **1982**, 8, 239-243. c) Cerrini, S.; Gavuzzo, E.; Lucente, B.; Pinnen, F.; Zanotti, G. *Int. J. Peptide Protein Res.* **1989**, 33, 6-13. d) Lombardi, A.; Saviano, M.; Nastri, F.; Maglio, O.; Mazzeo, M.; Isernia, C.; Paollilo, L.; Pavone, V. *Biopolymers* **1996**, 38, 693-703. e) Tamaki, M.; Akabori, S.; Muramatsu, I. *Biopolymers* **1997**, 39, 129-132. f) Benedetti, E. *Biopolymers (Peptide Science)* **1996**, 40, 1-44 and references in these.
4. a) Richardson, J.S. *Adv. Protein Chem.* **1981**, 34, 167-339. b) Rose, G.D.; Gierasch, L.M.; Smith, J.A. *Adv. Protein Chem.* **1985**, 37, 1-109. c) Richardson, J.S.; Richardson, D.C. *Prediction of Protein Structure and the Principles of Protein Conformation.* Fasman, G.D.; Ed. Plenum Press. New York, **1989**, 1-98. d) Rizo, J.; Gierasch, L.M. *Ann. Rev. Biochem.* **1992**, 61, 387-418 and references therein. e) Ashish; Kishore, R. *Tetrahedron Lett.* **1997**, 38, 2767-2770.
5. a) Benedetti, N.J.; Pedone, C.; Toniolo, C.; Nemethy, G.; Pottle, M.S.; Scheraga, H.A. *Int. J. Peptide Protein Res.* **1980**, 16, 156-172. b) Chakrabati, P.; Dunitz, J.D. *Helvetica Chemica Acta.* **1982**, 65, 1555-1562.
6. Wu. Y. -D.; Wang, D. -P. *J. Am. Chem. Soc.* **1998**, *120*, 13845.
7. Pauling, L.; Corey, R.B.; Branson, H.R. *Proc. Natl. Acad. Sci. USA.* **1951**, 37, 205-211.
8. a) Marsh, R.E.; Gluskar, J.P. *Acta Crystallogr.* **1961**, 14, 1110-1116. b) Lalitha, V.; Murali, R.; Subramanian, E. *Int. J. Peptide Protein Res.* **1986**, 27, 472-477.
9. a) Karplus, M. *J. Phys. Chem.* **1959**, 30, 11-15. b) Wuthrich, K. "*NMR in Biological Research : Peptides and Proteins*", **1976**, North-Holland: Amsterdam. c) Evans, J.N.S. "*Biomolecular NMR Spectroscopy*", **1995**, Oxford University Press, Oxford.

10. a) Kishore, R.; Kumar, A.; Balaram, P. *J. Am. Chem. Soc.* **1985**, 107, 8019-8023. b) Kishore, R.; Raghothama, S.; Balaram, P. *Biopolymers* **1987**, 29, 873 - 891.
11. a) Avignon, M.; Huong, P.V.; Lascombe, J.; Marraud, M.; Neel, J. *Biopolymers* **1969**, 8, 69-89. b) Toniolo, C.; Palumbo, M. *Biopolymers* **1977**, 16, 219-224. c) Narita, M.; Doi, M.; Kudo, K.; Terauchi, Y. *Bull. Chem. Soc. Jpn.* **1986**, 59, 3553-3557. d) Dong, A.; Huang, P.; Caughey, W.S. *Biochemistry.* **1990**, 29, 3303-3308. e) Haris, P.I.; Chapman, D. *Trends Biochem. Sci.* **1992**, 17, 328 - 333. f) Garcia-Quintana, D.; Garriga, P.; Manyosa, J. *J. Biol. Chem.* **1993**, 268, 2403-2409. g) Haris, P.I.; Chapman, D. Analysis of polypeptide and protein structures using fourier transform spectroscopy. In "*Microscopy, Optical Spectroscopy and Macroscopic Techniques*" (Jones, C.; Mulloy, B.; Thomas, A.H.; Eds.) *Methods in Mol. Biol.* **1996**, 22, Chapter 14, pp. 173-202.
12. a) Johnson, W.C. Jr.; Tinoco, I. Jr. *J. Am. Chem. Soc.* **1972**, 94, 4389. b) Crippen, G.M.; Yang, J.T. *J. Phys. Chem.* **1980**, 78, 1127. c) Madison, V.; Kopple, K.D. *J. Am. Chem. Soc.* **1980**, 102, 4855. d) *Circular Dichroism of Peptides* by Woody, R.W. *The Peptides, Analysis, Synthesis, Biology*, Udenfried S., Meinhofer, J., Eds. **Vol. 7**, *Conformation in Biology and Drug Design*, Hruby, V.J. Ed. **1985** Chapter 2, pp. 15-144. e) Johnson, W.C. Jr. *Methods of Biochemical Analysis.* **1987**, 31, 62-163. f) Woody, R.W. Circular Dichroism of Peptides. In *The Peptides, Analysis, Synthesis, Biology* (Udenfried S.; Meinhofer, J.; Eds.) Vol. 7, *Conformation in Biology and Drug Design*, (Hruby, V.J.; Ed.) **1985**, Chapter 2, pp. 15-114.
13. a) Sheldrick, G.M. "*Crystallographic Computing 3*" (Sheldrick, G.M.; Kruger, C.; Goddard, R. Eds.) Oxford University Press, Oxford, **1985**, pp. 175.
14. a) Saviano, G.; Temussi, P.A.; Motta, A.; Maggi, C.A.; Rovero, P. *Biochemistry* **1991**, 30, 10175-10181. b) Wrighton, N.C.; Balasubramanian, P.; Barbone, F.B.; Kashyap, A.K.; Farrell, F.X.; Jollife, L.K.; Barrett, R.W.; Dower, W.J. *Nature Biotechnol.***1997**, 15, 1261-1265. c) Bhatnagar, P.K; Alberts, D.; Callahan, J.F.; Heerding, D.; Huffman, W.F.; King, A.G.; LoCastro, S.; Pelus, L.M.; Takata, J.S. *J. Am. Chem. Soc.* **1996**, *118*, 12862-12863.