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Stable Type II' Reverse Turn - 3_{10} Helix Conformation of Boc-D-Glu-Ala-Gly-Lys-Ala-Leu-OMe in Apolar Solvents.

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Abstract: The solution conformation of the title compound, based on one and two dimensional NMR techniques in $CDCl_3$ and $(CD_3)_2SO$ at 500 MHz, has been deduced as a 3_{10} helix initiated by an electrostatically locked type II' reverse turn.

INTRODUCTION

The formation of a local fold featuring a 4→1 or 5→1 type intrapeptide H-bond represents a fundamentally important event in the protein folding process that can bring sequentially distant elements in a polypeptide into close stereochemical juxtaposition, to direct the course of events that culminate in the formation of a globular protein. Based on their morphologies, the elementary peptide folds can have distinctly different influences on the folding process by constraining the conformational choices of the residues that flank them. Thus, the type III β-turn or the α-helical turn may direct the helical conformation in the flanking residues, on account of template assisted conformational replication, while the type I and type II β-turns may direct the formation of an antiparallel β-hairpin, by positioning the flanking residues for the interstrand H-bonding. Proper appreciation of the protein folding process is thus critically dependent on the understanding of how local folds are formed and how they dictate its stereochemical course as well as the free energy profile.

β-Turns are commonly specified by the ϕ and ψ torsional angles of the middle two residue in a tetrapeptide unit[1], and in the H-bonded type II β-turn these angles are ϕ_2=-60, ψ_2 = 120 and ϕ_3=80, ψ_3=0. Artificial symmetry inverted type II' β-turns, predicted for heterochiral peptides and designated as the DL turns on this basis[2], have ϕ_2=60, ψ_2 =-120 favored for a D amino acid, and ϕ_3=-30, ψ_3=0 favored for an L amino acid. As these ϕ_3, ψ_3 torsional angles fall in the α_R region of the Ramachandran diagram (Fig. 1), the type II' β-turn could be expected to serve as a conformational template that may cause the residues that succeed it towards the C terminal to adopt the helical conformation. Here we present 500 MHz NMR evidence that demonstrates the type II' reverse turn initiated 3_{10} helix conformation in the hexapeptide Boc-*D*-Glu-Ala-Gly-Lys-Ala- Leu-OMe **1** in apolar solvents. The result is illustrative of the importance of residue level stereochemical effects in dictating the thermodynamic stabilities of elementary peptide folds, and of the influence that such folds can have in the propagation of protein folding process.

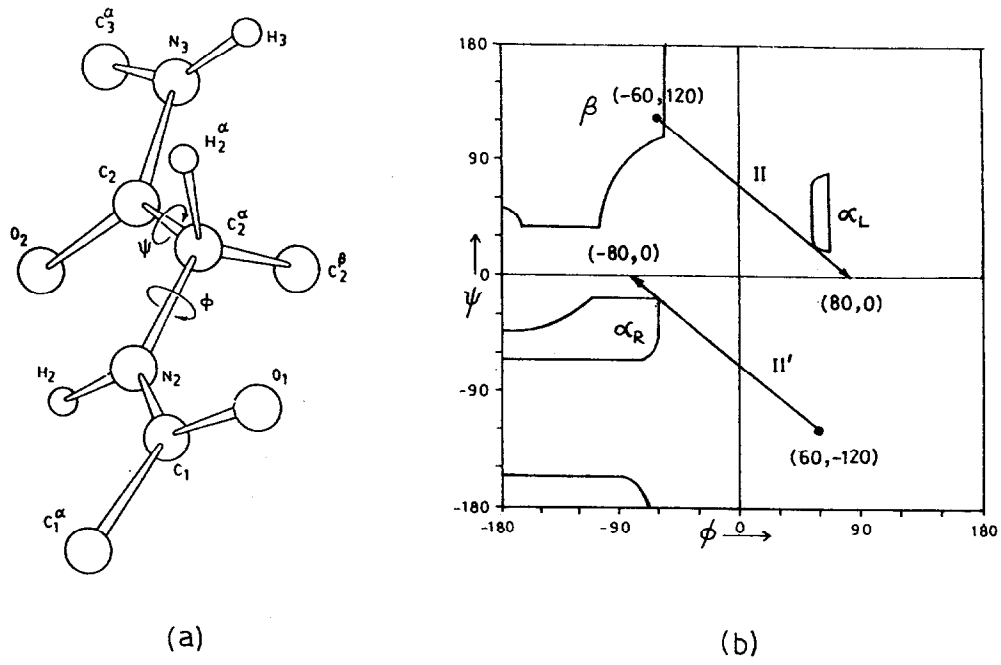

Fig.1. a) Torsional angles ϕ,ψ of the peptide backbone b) Ramachandran diagram representing allowed regions of the conformational space for the peptide backbone and the type II and II'β−turn conformations as proposed by Venkatachalam et. al[2].

RESULTS AND DISCUSSION

Assignment of Resonances:

Fig. 2 shows the 500 MHz ^{1}H NMR spectrum of hexapeptide **1** in $CDCl_3$-$(CD_3)_2SO$ (6:1). The proton chemical shifts of the hexapeptide, assigned on the basis of COSY[3] (Fig. 3) and ROESY[4] spectra, are shown in Table 1. The observed chemical shifts were concentration independent in the range 10-40 mM implying the absence of any noticeable self aggregation of the molecule under these solvation conditions.

The chemical shift assignments in $(CD_3)_2SO$ were similarly made using the COSY and ROESY spectra (not shown) and are listed in Table 1.

Backbone Hydrogen Bonding:

The possible involvement of NH groups in intramolecular hydrogen bonding was investigated using the temperature coefficients ($-d\delta/dT$) of amide proton shifts[5] of hexapeptide **1** in $(CD_3)_2SO$ (in the range 298-323K). The chemical shifts have a linear dependence on temperature for all the NH groups (Fig. 4). Four NH resonances belonging to Gly(3)NH [0.0], Lys(4)NH [0.0023], Ala(5)NH [0.0] and Leu(6)NH [0.0] exhibit $d\delta/dT$ values < 0.003 ppm/K, that are characteristic of solvent shielded (intramolecularly hydrogen-bonded) NH groups. The $d\delta/dT$ values for Glu(1)NH [0.0156] and Ala(2)NH [0.010] resonances,

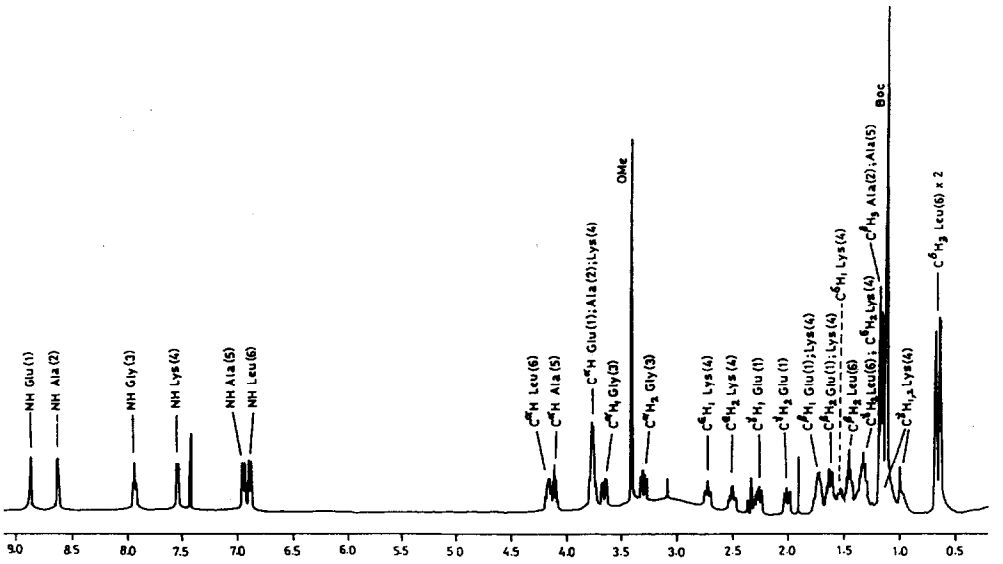

Fig.2. 500 MHz ^{1}H NMR spectrum of hexapeptide 1 in $CDCl_3$–$(CD_3)_2SO$ (6:1).

Table 1 : Proton chemical shift data (δ, ppm) of the hexapeptide measured in $CDCl_3(CD_3)_2SO$ (6:1) at concentration of ~ 10mM.

	Glu(1)	Ala(2)	Gly(3)	Lys(4)	Ala(5)	Leu(6)
NH	8.87 (8.92)	8.64 (8.86)	7.95 (8.16)	7.55 (7.84)	6.95 (7.59)	6.89 (7.54)
$C^{\alpha}H$	3.77 (3.95)	3.77 (4.04)	3.66/3.30 (3.70/3.47)	3.77 (4.26)	4.11 (4.23)	4.16 (4.00)
$C^{\beta}H$	1.72/1.63 (1.90/1.70)	1.18 (1.32)	--- (---)	1.70/1.60 (1.88/1.68)	1.16 (1.25)	1.46 (1.65)
$C^{\gamma}H$	2.25/2.01 (2.25/2.05)	--- (---)	--- (---)	1.20/1.00 (1.40/1.20)	--- (---)	1.35 (1.50)
$C^{\delta}H$	--- (---)	--- (---)	--- (---)	1.55/1.35 (1.60/1.50)	--- (---)	0.67 (0.88)
$C^{\varepsilon}H$	--- (---)	--- (---)	--- (---)	2.70/2.50 (2.80/2.70)	--- (---)	--- (---)

Figures in parentheses represent chemical shifts observed in $(CD_3)_2SO$.

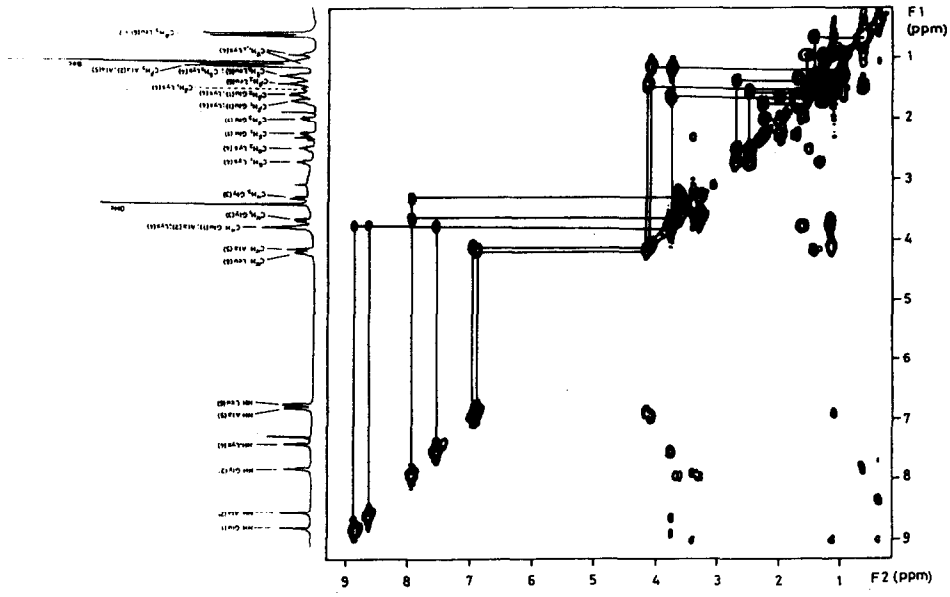

Fig.3. 500 MHz COSY spectrum of hexapeptide 1 in $CDCl_3$–$(CD_3)_2SO$ (6:1).

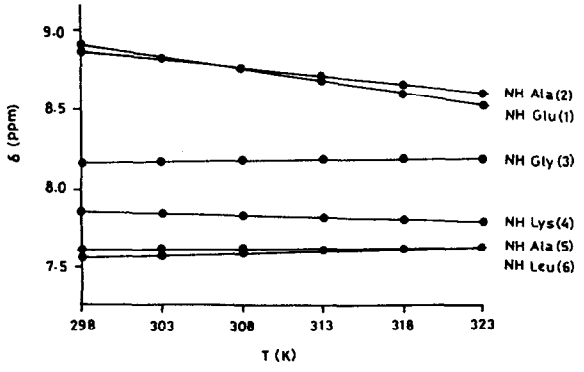

Fig.4. Temperature dependence of NH chemical shifts in $(CD_3)_2SO$.

being > 0.005 ppm/K, are indicative of the solvent exposed nature of these amide protons. Involvement of last four NH groups in intramolecular hydrogen bonding is consistent with 4 → 1 hydrogen bonding pattern of the hexapeptide, and with the 3_{10} helical conformation of its Ala(2)-Leu(6) segment.

Nuclear Overhauser Effects:

Consistent with helical conformation of its Ala(2)-Leu(6) segment, the ROESY spectrum of the hexapeptide in Fig. 5a reveals consecutive $d_{NN}(i,i+1)$ NOEs between all its backbone NH groups, except between D-Glu(1) and Ala(2), in which case an appreciably stronger $d_{\alpha N}(i,i+1)$ NOE appears, consistent with the type II' β-turn conformation[6] proposed for the Boc-Glu(1)-Ala(2)-Gly(3) segment. A number of medium range NOEs encompassing the Ala(2)-Leu(6) segment are also observed (Fig. 5b) and these include the $d_{\alpha N}(i,i+2)$ NOEs, characterizing the 3_{10} helix[6], between Ala(2)↔Lys(4), Gly(3)↔ Ala(5) and Lys(4)↔Leu(6) and the $d_{\alpha N}(i,i+3)$ NOEs between Glu(1)↔Lys(4) Ala(2)↔Ala(5) and Gly(3)↔Leu(6). Additional NOEs characteristic of helical peptides also observed in the molecule include Gly(3)$C^{\alpha}H$↔ $C^{\delta}H$ Leu(6); Gly(3)$C^{\alpha}H$↔ $C^{\beta}H$ Leu(6); Gly(3)$C^{\alpha}H$↔ $C^{\gamma}H$ Lys(4); Gly(3)NH↔ $C^{\gamma}H$ Lys(4); Gly(3)NH↔ $C^{\beta}H$ Ala(2); Leu(6)$C^{\alpha}H$↔ $C^{\beta}H$ Ala(5) and Leu(6)$C^{\beta}H$ ↔ $C^{\beta}H$ Ala(5), and all the expected $d_{\beta N}(i,i)$ NOEs except for that in Glu(1). One long range NOE of the $d_{\alpha\beta}(i,i+3)$ type also appears, between Gly(3)↔Leu(6), thus suggesting appreciable conformational order of C-terminal segment of the hexapeptide.

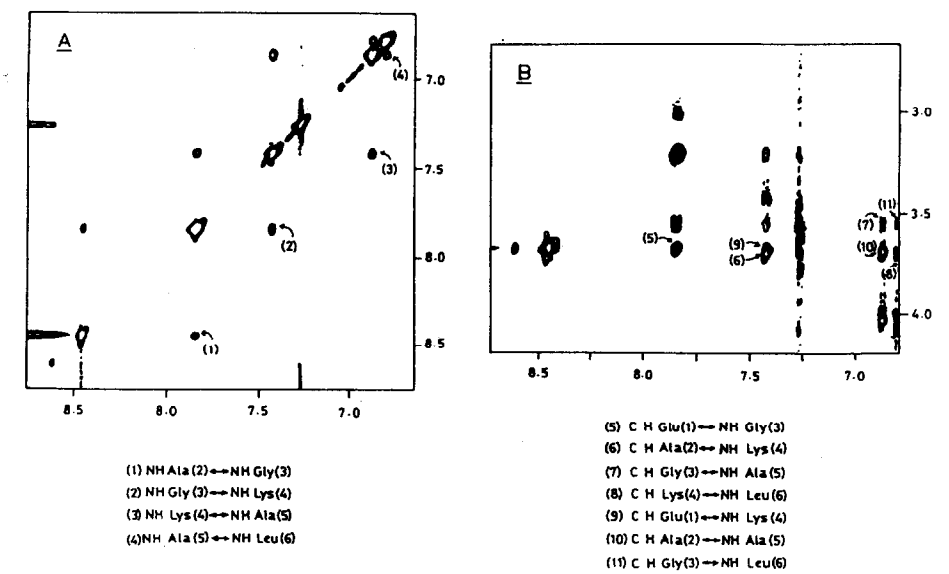

Fig.5. Portions of 500 MHz ROESY spectrum of hexapeptide 1 in $CDCl_3$–$(CD_3)_2SO$ (6 : 1), (a) NH – NH NOE region and (b) $C^{\alpha}H$ – NH NOE region.

Spin-spin coupling constants:

The observed $^3J_{NH\alpha}$ values in $CDCl_3$-$(CD_3)_2SO$ (6:1) were 3.62 [Ala(2)], 5.97 and 5.64 [Gly(3)], 6.25 [Lys(3)], 7.95 [Ala(4)] and 7.98 [Leu(5)] and the corresponding ϕ angles, obtained with a Karplus like relationship[7], were : ϕ [Ala(2)] = 12, 108, -58, -178; ϕ [Gly(3)] = 37, 83, -76, -164 and 33, 87, -74, -166; ϕ [Lys(4)] = 41, 79, -79, -161; ϕ [Ala(5)] = -93, -147; and ϕ [Leu(6)] = -93, -147. The torsional angles -58 [Ala(2)], -76/-74 [Gly(3)], -79 [Lys(4)], -93 [Ala(5)] and -93 [Leu(6)] agree well with the conformation proposed for the hexapeptide; however, the value -58 for Ala(2) implies a more acute local folding of its type II' reverse turn element, while the values -93 for Ala(5) and Leu(6) suggest partial fraying of this part of the helix - a phenomenon commonly observed with isolated helices[8] - despite the occurrence of several long range NOEs that span this segment.

Conformation of Glu(1) and Lys(4) side chains:

The Gly(3) $C^{\alpha}H_2$ resonance in hexapeptide **1** is geminally resolved, a feature that is often indicative of restricted conformation of this residue in short peptides[9]. That this restriction may be the consequence of charge group interaction in the hexapeptide was reflected in the NMR spectrum of its side chain protected analog, in which both the Gly(3) $C^{\alpha}H_2$ protons were found to appear as a single unresolved multiplet in $(CD_3)_2SO$ (data not shown). As described by Sahal and Balaram[10], the chemical shifts for $C^{\epsilon}H_2$ Lys(4) protons of the peptide in $(CD_3)_2SO$ (Table 1) [2.7 and 2.8 δ] are in accordance with full protonation of its NH_2 function. The observed shift differences between the diastereotropic protons in Glu(1) and Lys(4) side chains implies a restriction of the rotameric freedom around all the sigma bonds in these side chains. The effect could arise from the immobilization of the side chains due to an intramolecular ion pair formation in the molecule. Accordingly, this feature was found to be absent in its side chain protected analog, in which all the side chain methylenes were found to resonate as ill resolved multiplets. The effect of side chain immobilization is also manifests in the ROESY spectrum of hexapeptide **1**, as only selected NOE cross peaks appear between the $C^{\alpha}H$ and $C^{\gamma}H_2$ protons in its Glu and Lys side chains and between the $C^{\gamma}H_2$ and $C^{\epsilon}H_2$ protons in its Lys side chain.

Fig. 6 presents the stereodiagram of the hexapeptide modeled as a tandem type II' reverse turn - 3_{10} helix in conformity with the preceding NMR evidences. The torsional angles used in modeling the hexapeptide are $\phi = 60, \psi = -120$ for *D*-Glu(1), $\phi = -80, \psi = 0$ for Ala(2) and $\phi = -74, \psi = -4$ for the remaining residues. The Boc-*D*-Glu-Ala-Gly segment with 4→ 1 type H-bond between Boc-C=O and Gly(3)NH thus describes the type II' reverse turn element in the hexapeptide, while its C-terminal residues from Ala(2) onwards describe the partially overlapping 3_{10} helix, with Boc-*D*-Glu serving as its N-cap element which also engages the Lys side chain to constrain a part of the molecule in an intramolecular salt bridge. The ϕ torsional angles in the Gly(3)-Lys(4) segment of the model are close to the 3_{10} helical torsional angles described by Pauling et al.[11], but are appreciably larger than the values -49[12] and -54[13] that are also quoted for such helices. Apparently, the juxtaposition of type II' and 3_{10} helical turns is a consideration that dictates the helical torsional angles in the hexapeptide.

Fixation of Glu(1) amide proton in the deshielding zone of the conformationally fixed Glu(1) carboxylate in the proposed model correlates with the appreciable down field position of this resonance in $CDCl_3$ as well as $(CD_3)_2SO$. Accordingly, titration of the molecule with $LiClO_4$ in $(CD_3)_2SO$, to disrupt

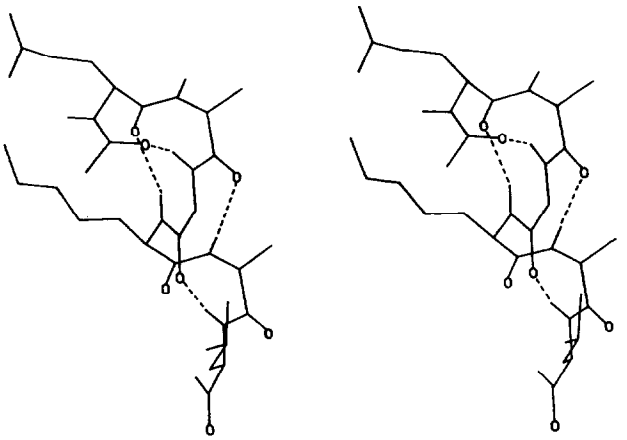

Fig.6. Stereodiagram of hexapeptide 1 modeled as tandem type II' turn -3_{10} helix. Boc group is represented as an acetyl function.

the salt bridge that fixes its carboxylate group, caused an appreciable upfield shift of its Glu(1) amide resonance (1.20 ppm at 2M $LiClO_4$) while leaving other amide proton resonances in the molecule relatively unaffected (the respective Δppm values at 2M $LiClO_4$ were 0.30 [Ala(2)]; 0.0 [Gly(3)]; -0.10 [Lys(4); -0.15 [Ala (5)] and -0.05 [Leu(6)]). The negligible shift difference of Glu(1) amide proton resonance in $(CD_3)_2SO$ and $CDCl_3(\Delta\delta = 0.05$ ppm) is of note and would suggest almost complete integrity of the salt bridge in the hexapeptide in these solvents despite the large difference in their dielectric strengths.

The conformational stability revealed by the title compound is, to our knowledge, unprecedented for a linear peptide of this length devoid of a covalent lock or a stereochemically constrained residue. The facile initiation of type II' reverse turn and the propagation of 3_{10} helical conformation in the molecule is thus illustrative of the critical importance of residue level stereochemical effects in dictating the thermodynamic stabilities of elementary peptide folds and of the effects that such folds can have on the succeeding events in protein folding process. The precise role of the D amino acid, of the charge group interactions and of the solvation effects in dictating the conformational attributes of the hexapeptide are however unclear for the present and need to be further examined. Such an inquiry is likely to be of value in better appreciating the stereochemical principles in protein folding initiation and propagation.

EXPERIMENTAL

The peptide synthesis was by solution phase methodology. All reactions were monitored by thin layer chromatography (TLC) in two solvent systems namely, A) $CHCl_3$:MeOH (9:1), B) BuOH: CH_3COOH: H_2O (4:1:1). Purification of hexapeptide **1** was carried out by reverse phase high performance liquid chro-

matography (HPLC) on Merck's LiChrosorb RP-18 column (10mm x 250mm) on a Hitachi HPLC system. The peptide was eluted with 15% water:MeOH (flow rate 3ml/min , detection 220nm). ^{1}H NMR spectra were recorded on a Bruker 500 MHz FT-NMR spectrometer fitted with Aspect 3000 computer.

Peptide Synthesis:

Hexapeptide **1** was synthesized according to the scheme outlined in Fig. 7. All amino acid derivatives were synthesized using the reported procedures[14].

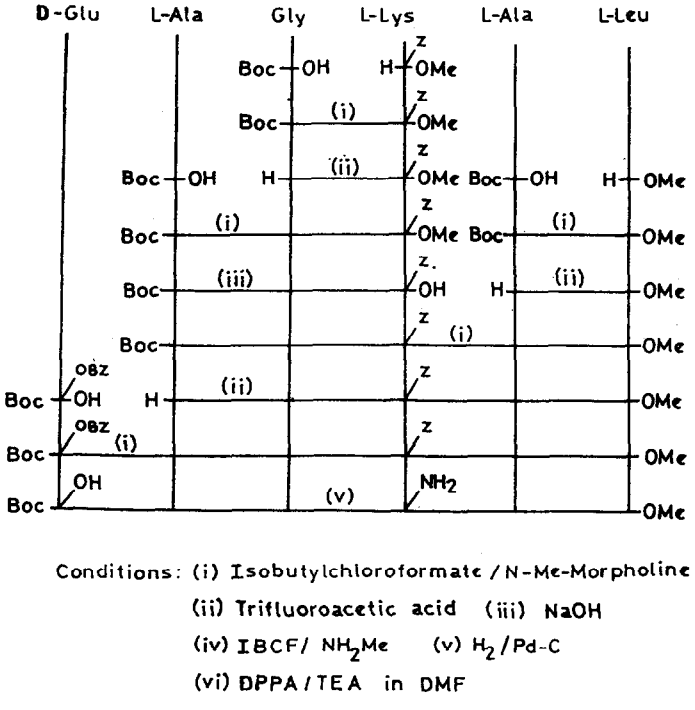

Fig.7. Synthetic scheme for hexapeptide 1.

Boc-Ala-Leu-OMe **2**

A mixture of Boc-L-Ala.OH (3.8gm, 20mM), NMM (2.2ml, 20mM) in dry THF (50ml) was cooled to -15°C and stirred with IBCF (2.6ml, 20mM) for 5min. To this was added a mixture of L-Leu-OMe.HCl (3.6gm, 20mM), NMM (2.2ml, 20mM) in THF and the resultant mixture stirred for 2hr. The solvent was removed and the residue extracted with ethylacetate. The organic layer was washed with saturated $NaHCO_3$, 10% citric acid, and water. The organic solution was dried (Na_2SO_4) and evaporated. Yield 5.6gm (88%), m.pt. 72-74°C, R_f(A) 0.67; R_f(B) 0.92. pmr ($CDCl_3$, 90 MHz) δ : 6.50 (broad, 1H, Leu

$N^{\alpha}\underline{H}$); 4.95 (broad, 1H, Ala $N^{\alpha}\underline{H}$); 4.60 (multiplet, 1H, Leu $C^{\alpha}\underline{H}$); 4.15 (multiplet, 1H, Ala $C^{\alpha}\underline{H}$); 3.70 (s, 3H, $OC\underline{H}_3$); 1.70-1.60 (multiplet, 3H, Leu $C^{\beta}\underline{H}_2$, Leu $C^{\gamma}\underline{H}$); 1.42 (s, 9H, $\{C\underline{H}_3\}_3$); 1.30 (d, 3H, Ala $C^{\beta}\underline{H}_3$); 0.90 (d, 6H, Leu 2 x $C^{\delta}\underline{H}_3$).

Boc-Gly-Lys(Z)-OMe **3**

17.5gm (100mM) Boc-Gly-OH and 11.0ml (100mM) NMM were suspended in 250ml dry THF. After cooling (-10°C), 13.0ml (100mM) IBCF was added and stirred for 15 min. To this was added a mixture of 33.8gm (100mM) H-Lys(Z)-OMe.HCl and 14.0ml (100mM) TEA in 100ml THF and the resultant mixture was stirred for 12 hr. Workup of the reaction afforded **3** as a oil. The oily residue was purified on a silica gel column (100-200 mesh) using a chloroform-methanol gradient. Appropriate fractions were pooled and evaporated to obtain **3** as a gum. Yield 38.7 gm (86%), R_f(A) 0.35; R_f(B) 0.83. pmr($CDCl_3$, 90 MHz) δ : 7.8 (d, 1H, $N^{\alpha}\underline{H}$); 7.6 (d, 1H, $N^{\alpha}\underline{H}$); 7.35 (s, 5H, aromatic protons); 7.2 (t, 1H, N^{ϵ} -$COCH_2C_6\underline{H}_5$); 5.1 (s, 2H, $C\underline{H}_2$-C_6H_5); 4.7-4.3 (broad, 1H, Lys $C^{\alpha}\underline{H}$); 3.8 (d, 2H, Gly $C^{\alpha}\underline{H}_2$); 3.7 (s, 3H, $OC\underline{H}_3$); 3.25-3.0 (broad, 2H, Lys $C^{\epsilon}\underline{H}_2$); 1.9-1.5 (complex multiplet, 4H, Lys $C^{\beta}\underline{H}_2$, $C^{\delta}\underline{H}_2$); 1.4 (s, 9H, $\{C\underline{H}_3\}_3C$); 1.2-1.0 (multiplet, 2H, Lys $C^{\gamma}\underline{H}_2$).

Boc-Ala-Gly-Lys(Z)-OMe **4**

A mixture of Boc-L-Ala.OH (2.83gm, 15mM), NMM (1.65ml, 20mM) and dry THF (100 ml) was cooled to -15°C and stirred with (IBCF) (1.95ml, 15mM) for 10 min. The dipeptide **3** (6.76gm, 15mM) was deprotected at the N-terminal using TFA:DCM (10ml, 1:1 v/v) and the TFA salt alongwith NMM (1.65ml, 15mM) was added and the resultant mixture was stirred at ice bath temperature for one hour and at room temperature for another one hour. The solvent was evaporated and the reaction was worked up as usual. The resultant tripeptide **4** was purified over silica gel column with chloroform-methanol gradient as eluent. Yield 7.1gm (91%) m.pt.102-105°C, R_f(A) 0.35; R_f(B) 0.80. pmr($CDCl_3$, 90 MHz) δ :8.8 (s, 1H, $N^{\alpha}\underline{H}$); 7.8 (s, 1H, $N^{\alpha}\underline{H}$); 7.5 (s, 1H, $N^{\alpha}\underline{H}$) 7.3 (s, 5H, aromatic protons); 7.2 (s, 1H, N^{ϵ} -$COCH_2C_6H_5$); 5.1 (s, 2H, $C\underline{H}_2-C_6H_5$); 4.5-3.8 (complex, 3H, Lys $C^{\alpha}\underline{H}$, Ala $C^{\alpha}\underline{H}$, Leu $C^{\alpha}\underline{H}$); 3.6 (s, 3H, $OC\underline{H}_3$); 3.2-3.0 (broad, 2H, Lys $C^{\epsilon}\underline{H}_2$); 1.9-1.5 (complex multiplet, 9H, Lys $C^{\beta}\underline{H}_2$, $C^{\gamma}\underline{H}_2$, $C^{\delta}\underline{H}_2$, Leu $C^{\beta}\underline{H}_2$,$C^{\gamma}\underline{H}$); 1.45 (d, 3H, Ala $C^{\beta}\underline{H}_3$); 1.4 (s, 9H $\{C\underline{H}_3\}_3C$); 0.85 (6H, dd, Leu 2 x $C^{\delta}\underline{H}_3$).

Boc-Ala-Gly-Lys(Z)-Ala-Leu-OMe **5**

The tripeptide **4** (5.0gm, 9.6mM) was converted to its free acid by the usual alkaline hydrolysis with 1N NaOH (20ml) in methanol (50ml). The tripeptide free acid alongwith NMM (0.87ml, 7.9mM) in dry THF (50ml) was stirred with IBCF (1.0ml, 7.9mM) at -10°C for 10 min. The TFA salt of the dipeptide **2** (2.5gm, 7.9mM) (prepared as described earlier) along with NMM (0.87ml, 7.9mM) in THF (50ml) were added and the reaction was continued for 1hour at 0°C and overnight at room temperature. The pentapetide **5** was recovered and purified as in the previous step. Yield 4.2gm (75%) m.pt.175-176°C, R_f(A) 0.53; R_f(B) 0.86. pmr($CDCl_3$, 90 MHz) δ : 8.11(broad, 3H, $N^{\alpha}\underline{H}$); 8.02 (d, 1H, $N^{\alpha}\underline{H}$); 7.84 (d, 1H, $N\underline{H}Me$); 7.2 (t, 1H, $N^{\alpha}\underline{H}$); 7.35 (s, 5H, aromatic protons); 7.33 (s, 5H, aromatic protons); 5.1 (s, 2H, $C\underline{H}_2$-C_6H_5); 5.0 (s, 2H, $C\underline{H}_2$-C_6H_5); 4.3-4.2 (complex, 3H, Lys $C^{\alpha}\underline{H}$, Ala^1 $C^{\alpha}\underline{H}$, $Ala^2C^{\alpha}\underline{H}$, Leu $C^{\alpha}\underline{H}$); 3.95 (multiplet, 2.0-1.4 (complex multiplet, 9H, Lys $C^{\gamma}\underline{H}_2$, $C^{\delta}\underline{H}_2$, $C^{\beta}\underline{H}_2$, Leu $C^{\beta}\underline{H}_2$, $C^{\gamma}\underline{H}$); 1.38 (s, 9H, $\{C\underline{H}_3\}_3C$); 1.22 (d, 3H, Ala $C^{\beta}\underline{H}_3$); 0.85 (6H, dd, Leu 2 x $C^{\delta}\underline{H}_3$).

Boc–D-Glu(OBz)-Ala-Gly-Lys(Z)-Ala-Leu-OMe **6**

To a precooled (-15°C) solution of Boc-D-Glu(OBz).OH (0.95gm, 2.8mM) and NMM (0.31ml, 2.8mM) in dry THF (30 ml), IBCF (0.37ml, 2.8mM) was added, and the mixture was stirred for 10 min. The TFA salt of the pentapeptide **5** (2.0gm, 2.8mM) (as obtained in previous step) alongwith NMM (0.31ml, 2.8mM) was added to the above stirred solutions. After 2hr the solvent was evaporated and the reaction was worked-up as in the previous steps. Yield 1.5gm (60%) m.pt. 185-187°C, R_f(A) 0.60; R_f(B) 0.88. pmr(DMSO-d_6, 300 MHz) δ : 8.11(broad, 3H, $N^{\alpha}\underline{H}$); 8.02 (d, 1H, $N^{\alpha}\underline{H}$); 7.84 (d, 1H, $N\underline{H}Me$); 7.2 (t, 1H, $N^{\alpha}\underline{H}$); 7.04 (d, 1H, $N^{\alpha}\underline{H}$); 7.35 (s, 5H, aromatic protons); 7.33 (s, 5H, aromatic protons); 5.1 (s, 2H, $C\underline{H}_2$-C_6H_5); 5.0 (s, 2H, $C\underline{H}_2$-C_6H_5); 4.3-4.2 (complex, 4H, Lys $C^{\alpha}\underline{H}$, Ala^1 $C^{\alpha}\underline{H}$, Ala^2 $C^{\alpha}\underline{H}$, Leu $C^{\alpha}\underline{H}$); 3.95 (multiplet, 2H, Gly $C^{\alpha}\underline{H}_2$); 3.7 (d, 1H, Glu $C^{\alpha}\underline{H}$); 3.6 (s, 3H, $OC\underline{H}_3$); 2.95 (multiplet, 2H, Lys $C^{\epsilon}\underline{H}_2$); 2.4 (t, 2H, Glu $C^{\gamma}\underline{H}_2$); 2.0-1.4 (complex multiplet, 11H, Lys $C^{\gamma}\underline{H}_2$, $C^{\delta}\underline{H}_2$, $C^{\beta}\underline{H}_2$, Glu $C^{\beta}\underline{H}_2$, Leu $C^{\beta}\underline{H}_2$, $C^{\gamma}\underline{H}$); 1.38 (s, 9H, $\{C\underline{H}_3\}_3C$); 1.22 (d, 3H, Ala $C^{\beta}\underline{H}_3$); 0.85 (6H, dd, Leu 2 x $C^{\delta}\underline{H}_3$).

Boc–D-Glu-Ala-Gly-Lys-Ala-Leu-OMe **1**

The side chain protected hexapeptide **6** (2.0 g, 2.56 mM) was dissolved in methanol (20 ml) and hydrogenated using 10%Pd-C catalyst (100 mg) and the product was further purified by HPLC on a semipreparative reverse phase column (Lichrosorb RP-18, 7 μ m, 250 × 10 mm) eluting with 15% water-methanol, with the UV detector set at 220 nm; R_f(B) 0.50. PMR data for the side chain deprotected hexapeptide **1** in $CDCl_3$-$(CD_3)_2SO$ (6:1) and $(CD_3)_2SO$ is presented in Table 1.

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