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A Single Point Chiral Inversion that Selforganizes a Randomcoil Peptide. Apolar Solvent Conformation of Boc-(*L**D*)-Glu-Ala-Leu-LysNHMe

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Abstract: The tetrapeptide Boc-*L*-Glu-Ala-Leu-LysNHMe (1) reveals a random coil conformation, based on its Glu(γ) and Lys(ε) methylene proton aniosotropic shift, GluNH chemical shift, NOEs in chloroform-DMSO (6:1), and its amide proton temperature coefficients in DMSO, while on similar considerations, the diastereomer Boc-*D*-Glu-Ala-Leu-LysNHMe (2) is characterized as a highly ordered 3/10 type distorted protohelix with a remarkably stable intramolecular salt bridge under these solvent conditions.

INTRODUCTION

The *L* chiral nature of all asymmetric α-amino acids remains the fundamental consideration in protein folding process, determining its structural as well as energetic outcome. The underlying principles have, however, been difficult to decipher, given the complexity of globular proteins and the generally disordered nature of short peptides. As a possible approach to this essentially stereochemical problem, model peptides could be examined for effects of site specific chiral inversions. Critical comparisons between diastereomers of identical covalent structure but different chiralities at specified loci have the potential to illuminate peptide chain folding process for underlying stereochemical principles.

A transient type II turn in Boc-*L*-Glu-Ala-Gly-LysNHMe, with no discernible motional restriction of its oppositely charged side chain elements, was recently shown to collapse into a consecutive II' - 3_{10} - 3_{10} type turn, with a clearly discernible salt bridge of remarkable stability under apolar solvent conditions, following the inversion of its N terminal chirality.[1] The heterochiral peptide was also shown tc promote conformational order in the residues that were attached at its C-terminal.[2] As an extension of this study, we now report that Boc-*L*-Glu-Ala-Gly-LysNHMe loses its residual conformational order on replacing its Gly(3) with Leu(3), while on similar substitution, Boc-*D*-Glu-Ala-Gly-LysNHMe retains its essentially ordered conformation. Hence the largely random coil Boc-*L*-Glu-Ala-Leu-LysNHMe (1) becomes a globally ordered Boc-*D*-Glu-Ala-Leu-LysNHMe (2) due to the stereochemical consequence of chiral inversion. Based on comparisons involving simple diastereomeric models stereochemical principles in peptide chain folding process can thus be critically examined.

RESULTS

The ^{1}H NMR spectra of peptides 1 and 2 in $CDCl_3$-DMSO (6:1) are in Figs. 1 and 2, respectively. The observed chemical shifts and line widths were essentially invariant under this solvent condition as well as in DMSO, in the concentration range 10-40 mM. No perceptible intermolecular association was thus indicated for either one under either of the solvent conditions. Chemical shifts in $CDCl_3$-DMSO (6:1) as well as in DMSO, assigned on the basis of COSY and ROESY spectra, are in Table 1. $^3J_{NH\alpha}$ values in $CDCl_3$-DMSO mixture, obtained directly from 1D spectra in this solvent, are in Table 2. The corresponding Φ torsional angle, calculated using a Karplus type relationship,[3] are also in Table 2. In DMSO, the amide proton chemical shifts in both the peptides were linearly dependent on temperature. The derived temperature coefficients are in Table 2. In the ROESY spectrum of peptide 1 (not shown), only the $d_{\alpha N}(i,i+1)$ NOEs were observed. The ROESY spectrum of peptide 2, recorded in $CDCl_3$-DMSO, is in Figure 3. A rich pattern of NOEs is apparent in this case. The $d_{\alpha N}(i,i+1)$ NOEs are accompanied by the $d_{NN}(i,i+1)$ NOEs between Ala(2)-->Leu(3), Leu(3)-->Lys(4), and Lys(4)-->NHMe, the $d_{\alpha N}(i,i+2)$ NOEs between Glu(1)-->Leu(3), Ala(2)-->Lys(4) and Leu(3)-->NHMe, and the $d_{\alpha N}$ (i,i+3)-->NOE between Ala(2)-->NHMe.

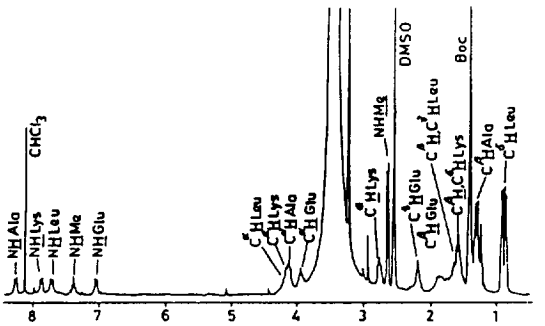


Figure 1: 300 MHz ^{1}H-NMR Spetrum of tetrapeptide 1 in $CDCl_3$ - $(CD_3)_2SO$ (6:1) mixture.

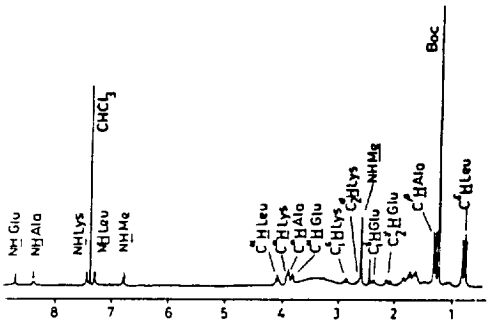


Figure 2: 300 MHz ^{1}H-NMR Spetrum of tetrapeptide 2 in $CDCl_3$ - $(CD_3)_2SO$ (6:1) mixture.

Interpeptide H-bonds are reflected in peptide **1**, in the partial burial of three of its C terminal amide protons in DMSO. However, the relative magnitudes of temperature coefficients imply that these H-bonds are much weaker than those operative in the diastereomeric peptide. Further, the non observation of any $d_{NN}(i,i+1)$ NOEs in $CHCl_3$-DMSO (6:1) mixture (results not shown), implies that no specific backbone fold is populated to any appreciable degree in peptide **1**, even under this appreciably apolar solvent condition. The calculated Φ torsional angles in the peptide (Ala = -83, Leu = -86 and Lys = -91) are somewhat larger than those reported earlier for Boc-*L*-Glu-Ala-Gly-LysNHMe (Ala = -67, Gly = -73/-79, and Lys = -85), as well as those now observed in peptide **2** (Ala = -77, Leu = -89 and Lys = -77). Accordingly, the substitution of Gly(3) with the stereochemically more constrained Leu(3) has the opposite effect of further randomizing the backbone in peptide **1**, while on chiral inversion the peptide is nearly as constrained as its Gly(3) analog, in which the corresponding Φ values were Ala = -66, Gly = -74/-81 and Lys = -90.

In peptides, the immobilization of side chain elements of oppositely charged residues in specific rotameric states is often accompanied by the enhanced shift dispersion of their diastereotopic protons. The effect is clearly noticeable in Gluγ(1) and Lysε(4) methylene protons in peptide **2** (Table 1), but is absent in peptide **1**, and was also absent in its conformationally more constrained Gly(3) analog.[1] Accordingly, Glu(1) and Lys(4) side chains in peptide **1** reveal no motional restriction, and hence a stable intramolecular salt bridge appears not to be operative in the molecule. Apparently, the electrostatic interaction between its charge groups is of a transient nature.

Table. 1 Proton chemical shifts (δ, ppm) of peptides **1** and **2** in $CDCl_3$-DMSO (6:1) at the concertration ~ 12mM.

PEPTIDE	$N^{\alpha}H$	$C^{\alpha}H$	$C^{\beta}H$	$C^{\gamma}H$	$C^{\delta}H$	$C^{\epsilon}H$
1. L-Glu	7.05	3.95	1.88	2.20	--	--
	(7.34)	(3.88)	(1.78)	(2.06)	--	--
L-Ala	8.27	4.10	1.35	--	--	--
	(8.28)	(4.10)	(1.23)	--	--	--
L-Leu	7.70	4.18	1.60	1.80	0.90	--
	(7.90)	(4.18)	(1.58)	(1.60)	(0.85)	--
L-Lys	7.85	4.15	1.70	1.30	1.60	2.78
	(8.05)	(4.15)	(1.55)	(1.45)	(1.55)	(2.65)
NHMe	7.38	2.65	--	--	--	--
	(7.58)	(2.58)	--	--	--	--
2. D-Glu	8.65	3.75	1.75	2.05/2.30	--	--
	(9.08)	(3.95)	(1.86)	(2.02/2.30)	--	--
L-Ala	8.32	3.86	1.25	--	--	--
	(8.88)	(3.98)	(1.28)	--	--	--
L-Leu	7.25	4.02	1.55	1.65	0.70	--
	(7.52)	(4.05)	(1.75)	(1.63)	(0.84)	--
L-Lys	7.40	3.80	1.55	1.00	1.40	2.60/2.80
	(7.47)	(3.80)	(1.97)	(1.58)	(1.67)	(2.66/2.85)
NHMe	6.75	2.55	--	--	--	--
	(7.18)	(2.54)	--	--	--	--

Figures in parentheses are the chemical shifts in neat $(CD_3)_2SO$.

Table. 2. Coupling constants, Φ torsional angels, and the amide proton tempertature coefficient of peptides 1 and 2.

PEPTIDE	J (Hz)	(ϕ)	$d\delta/dT \times 10^{-3}$ (ppm/K)
	($CDCl_3$-DMSO; 6:1)		(DMSO-d_6)
1. L-Glu	6.80	67,53,-83,-157	7.2
L-Ala	6.80	67,53,-83,-157	6.0
L-Leu	7.20	--,--,-86,-154	1.3
L-Lys	7.80	--,--,-91,-149	0.4
NHMe	----	----	1.6
2. D-Glu	----	----	10.0
L-Ala	6.00	83,37,-77,-163	7.4
L-Leu	7.50	--,--,-89,-151	0.0
L-Lys	6.00	83,37,-77,-163	0.0
NHMe	----	----	0.8

The close to zero temperature coefficients of three C terminal amide protons of peptide 2 in DMSO, possibly due to consecutive 4-->1 type interpeptide H-bonds, is accompanied in this case by the observation of the short range $d_{NN}(i,i+1)$, the medium range $d_{\alpha N}(i,i+2)$ as well as the long range $d_{\alpha N}$ (i,i+3) NOEs in $CHCl_3$-DMSO (6:1), characterizing consecutive 3_{10} type turns for Ala-Leu-LysNHMe segment of the peptide. The long range NOEs, reflecting spatial proximity between sequentially remote protons, are a particular evidence for appreciable global order in the peptide under the given solvent condition. On transferring peptide 2 into DMSO, the $d_{\alpha N}(i, i+2)$ as well as $d_{\alpha N}(i,i+3)$ NOEs, but not the d_{NN} (i,i+1)NOEs, were found to vanish (results not shown), reflecting partial randomization of its backbone under this aggressive H-bond acceptor solvent. The Φ torsional angles calculated for the segment Ala-Leu-LysNHMe in $CHCl_3$-DMSO (Ala = -77, Leu = -89 and Lys = -77) are somewhat larger than those in the standard type III turn ($\Phi_2 = \Phi_3 = -60$) but approach more closely the average Φ value for 3_{10} helical segments in proteins ($\Phi = -71$).[4] Further, based on the absence of $d_{NN}(i,i+1)$ NOE between Glu(1) and Ala(2), and the appreciably more intense $d_{\alpha N}(i,i+1)$ NOE between these residues compared to other such NOEs in the peptide, a pseudo four residue type II' turn is implied for the segment Boc-Glu-Ala-Leu in peptide 2, with Glu(1) ($\Phi_2 = 60$, $\Psi_2 = -120$) and Ala(2) ($\Phi_3 = -80$ and $\Psi_3 = 0$) being its first and second corner residues, and with Boc-C=O serving as its i^{th} residue, accepting the H-bond from Leu(3)NH. It may be noted that Glu(1) is in β^{inv} conformation stereochemically favored for *D* chiral residues, while Ala(2) occupies a position that is common to the type II' turn (standard torsional angles[5]: $\Phi_3 = -80$ and $\Psi_3 = 0$) and the short 3_{10}-helical segment (standard torsional angles: $\Phi_i = -74$ and $\Psi_i = -4$)[6] of the peptide.

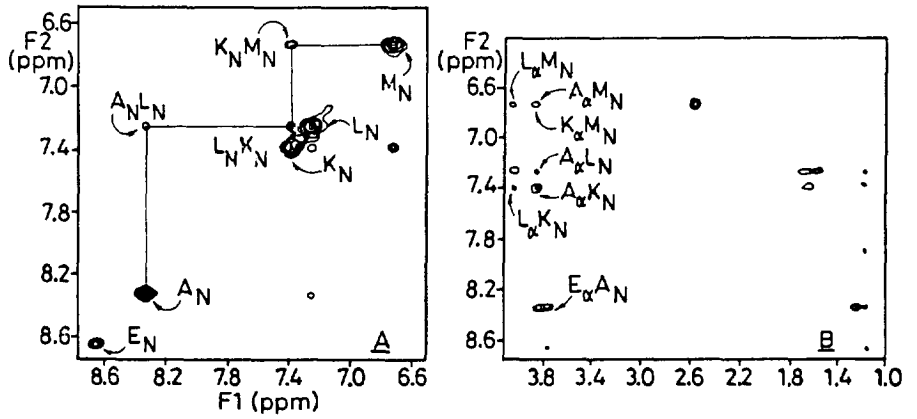


Figure 3: Portions of 300 MHz ROESY spectrum of tetrapeptide 2 in $CDCl_3$ - $(CD_3)_2SO$ (6:1) mixture. (A) NH-NH region; (B) $C^{\alpha}H$-NH region.

A remarkably stable intramolecular salt bridge is in evidence in peptide 2, from the appreciable attenuation of the shift anisotropy of its Gluγ(1) and Lysε(4) methylene protons, and from the appreciable down field position of its Glu(1)NH resonance in both $CHCl_3$-DMSO mixture and neat DMSO (Table 1). This signal in peptide 2, but not in peptide 1, was found to move up field on titrating the molecule with $LiClO_4$ in DMSO. The up field shift was 0.8 ppm at 2 M $LiClO_4$, and yet the saturation of the shift was not in evidence at even this appreciable salt concentration. Accordingly, an intramolecular salt bridge is operative in peptide 2, and the deshielding of its Glu(1)NH signal is attributable to this salt bridge. On modeling the peptide as a tandem II' - 3_{10} - 3_{10} turn, Glu(1) and Lys(4) side chains were seen to actually adopt a position that would favor the salt bridging, while Glu(1)NH was seen to adopt a position that would comprise the deshielding zone of Gluγ C=O. Unusual stability of the salt bridge in peptide 2 is further reflected in the response of its Glu(1)NH resonance to the solvent substitution. Glu(1)NH in peptide 2 resonates 0.33 ppm down field in neat DMSO than in $CDCl_3$-DMSO (6:1) mixture. This solvent induced shift in Glu(1)NH signal in peptide 1 is the almost identical 0.32 ppm. Accordingly, the observed shift in peptide 2 seems to reflect the intrinsic response of Glu(1)NH to the solvent substitution, and not the rupture of its salt bridge on the transfer into the solvent of higher dielectric strength, since the rupture would cause Glu(1)NH resonance to actually move up field in DMSO. The salt bridge thus appears to be essentially intact in DMSO. The weakening of the bridge on increasing the temperature in DMSO could be reflected in an up field shift of Glu(1)NH resonance in peptide 2. In conformity with this expectation, the temperature induced upfield shift of Glu(1)NH resonance in peptide 2 in DMSO is of somewhat larger magnitude than in peptide 1. The absolute magnitude of the temperature coefficient of Glu(1)NH in peptide 2 (0.01 ppm/K) is however rather small in relation to the shift difference of this resonance in peptide 1 and 2 in DMSO (1.74 ppm), as well as the shift induced in this signal in peptide 2 by the salt titration in DMSO (0.8 ppm at 2 M $LiClO_4$). Clearly, the salt bridge deshielding Glu(1)NH in peptide 2 is

remarkably stable even in DMSO, though the interamide H-bonds in the peptide appear to weaken perceptibly in this aggressively H-bond acceptor solvent.

Based on these complementary considerations, peptide **1** appears to be a random coil sampling equiprobable conformational energy states that may include folded and open chain conformers, while peptide **2** is an N-capped 3_{10} type protohelix with an appreciable global order and a remarkably stable salt bridge.

DISCUSSION

The analysis of protein folding process for thermodynamic principles, based on a variety of complex experimental models,[7-11] has led to distinctions being made between the process of initiation of chain folding and its propagation. The initiation of folding, characterized by the ordering of an elementary H-bonded chain fold in a random coil peptide, is thought to be a thermodynamically uphill process, because the attendant loss of entropy does not appear to be compensated by the concomitant gain in enthalpy. Hence peptides capable of only populating elementary chain folds are usually disordered, as the stabilizing interactions realized on the propagation of folding are absent. With the conformationally ordered - and hence the necessarily more complex - linear peptides, complication can arise, because the considerations underlying initiation and propagation of chain folding may not be readily distinguished. Chiral inversions in peptide models only capable of populating elementary chain folds can therefore provide the opportunity to isolate and examine the impact of stereochemistry on the initiation of chain folding in proteins.

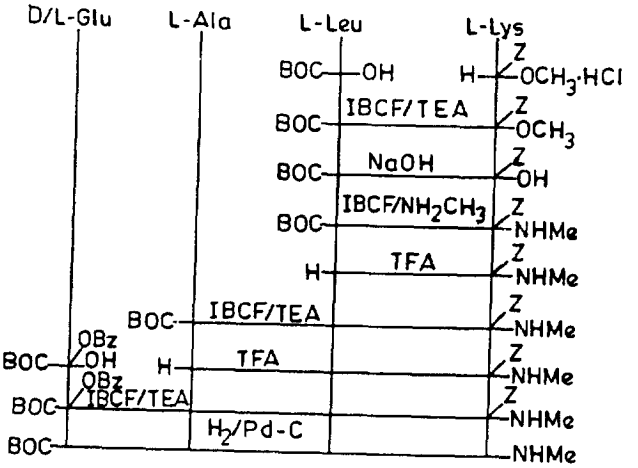
Accessory interactions, either between side chain and main chain elements or between side chain elements, can reinforce an elementary H-bonded chain fold, and make it experimentally observable.[12] A range of elementary chain folds are stereochemically feasible in peptide **1** and can place its charged functionalities in close spatial proximity. The resultant interaction could acquire an appreciable strength under the apolar and low dielectric solvent conditions used in this study. Yet, thermodynamic requirements for the observation of a discrete chain fold and a stable salt bridge are not met in peptide **1**. Alternative chain folds of insufficient stability appear to be populated, causing the peptide to be the random coil. Either, the loss of entropy on its ordering into a specific chain fold is intrinsically large, or the compensatory gain in enthalpy on salt bridge formation can not be met for stereochemical reasons. Considering the results with peptide **2**, the entropy loss on ordering of peptide **1** can not be so large as to be uncompensated by the possible formation of an interamide H-bond and a salt bridge under the given solvent conditions. Thus the stereochemical requirement for the optimal interaction between its charge groups appears not to be met in any of the stereochemically feasible chain folds in peptide **1**. In particular the β type I and III and the α helical turns are feasible in the peptide, however, none appears to position its Glu(1) and Lys(4) in the requisite geometry for an optimal charge group interaction. Indeed, the associative strength between Glu and Lys side chains in helical peptides is critically position dependent, as Lys-->Glu ion pairs are more helix stabilizing in 5-->1 spacing than in 4-->1 spacing.[13] The relatively greater degree of ordering observed earlier in Boc-*D*-Glu-Ala-Gly-LysNHMe would imply that the charge group interaction may be better realized when Glu(1) and Lsy(4) occupy the first and fourth position in a β

type II turn. Such a turn is unfeasible in peptide **1**, because specific stereochemical requirement for its second corner position is not met in this molecule.

The role of stereochemistry in the thermodynamic trade off during peptide chain folding is remarkably exemplified by the impact of chiral inversion on peptide **1**. The magnitude of enthalpy gain on salt bridging appears to be appreciably attenuated on the chiral inversion, and thus a specific chain fold is thermodynamically favored in peptide **2** over alternative folds that may be equally feasible on stereochemical grounds. Apparently, the type II' turn element characterized in peptide **2** is in the position to better satisfy the charge group interaction while presumably allowing Glu(1) and Lys(4) side chain elements to retain their favored rotameric states. The elementary chain fold in peptide **2** also propagates; thus additional main chain H-bonds are established and the peptide achieves a remarkable degree of ordering at the global level.

EXPERIMENTAL

Peptide synthesis was by conventional solution phase methodology.[14] All the reactions were monitored by thin layer chromatography in two solvent systems; A) $CHCl_3$:MeOH (9:1), and B) BuOH:CH_3COOH:H_2O (4:1:1). The synthetic plan for peptides **1** and **2** is outlined in Scheme 1. Both were purified on a C_{18} column eluting with 15% H_2O-MeOH on a Hitachi HPLC. All NMR spectra



Scheme 1: Synthetic scheme for tetrapeptides **1** and **2**.

were acquired on a Varian VXR 300 spectrometer, in $CDCl_3$-DMSO (6:1), or in neat DMSO, using TMS or DMSO as the internal standard. The spin system identifications were with the 2-dimensional correlated spectroscopies COSY[15] and ROESY,[16] following the standard methodology. ROESY spectra were recorded with the spin locking radiofrequency field strength of 1.5 kHz and with the mixing time 300 ms. Both positive and negative peaks are plotted without discrimination in the ROESY spectra. The 2-dimensional spectra were processed on a SUN workstation with a Varian Associates software. Variable temperature experiments were in DMSO in the range 298-323 K.

Peptide synthesis:

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

2.3g (10mM) Boc-Leu-OH and 1.4ml (10mM) TEA in 50ml dry THF were cooled to -15° and treated with 1.36ml (10mM) IBCF. To this mixture, a solution of 3.37g (10mM) H-Lys(Z)-OMe.HCl and 1.4ml (10mM) TEA in 25ml THF was added after 15 min, and the reaction mixture was stirred for 1hr, followed by further stirring for 2hrs at room temperature. The solvent was removed under reduced pressure and the residue was extracted with ethyl acetate. The organic layer was washed successively with saturated $NaHCO_3$, 10% citric acid solution and water, and dried over anhydrous Na_2SO_4. The solvent was evaporated to yield compound **3**, which was purified on silica gel column (100-200 mesh) eluting with a chloroform-methanol gradient. Yield 4.6g (92%) M.p. 85-86°; R_f (A) 0.60; R_f (B) 0.80; pmr ($CDCl_3$ 90MHz) δ : 7.8 (s, 1H, $N^{\alpha}H$); 7.5(s, 1H, $N^{\alpha}H$); 7.35(s, 5H, aromatic protons); 7.0 (s, 1H, $N^{\varepsilon}H$-$COCH_2C_6H_5$); 5.1 (s, 2H, CH_2-C_6H_5); 4.5-4.3 (broad, 1H, $C^{\alpha}H$); 4.0 (broad, 1H, $C^{\alpha}H$); 3.7 (s, 3H, OCH_3); 3.25-3.0 (broad, 2H, $C^{\varepsilon}H_2$); 1.95-1.5 (complex multiplet, 9H, Lys $C^{\beta}H_2$, $C^{\gamma}H_2$, $C^{\delta}H_2$, Leu $C^{\beta}H_2$, $C^{\gamma}H$); 1.4 (s, 9H, $\{CH_3\}_3C$); 0.85 (dd, 6H, Leu 2x$CH^{\delta}{}_3$).

Boc-Leu-Lys(Z)NHMe **4**

4.5g dipeptide **3** was dissolved in 100 ml methanol and 66ml 1N NaOH, for C-terminal ester deprotection. After 2hrs at room temperature, the solvent was romoved and the residue was taken up in water; acidified to pH 3 with citric acid and extracted with ethyl acetate. The organic layer was washed with water, dried over Na_2SO_4 and concentrated in vacuo. 4.0g (8.2mM) partially deprotected dipeptide and 1.15ml (8.2mM) TEA were taken up in 50ml dry THF, the solution was cooled to -15° and was treated with 1.06ml (8.2mM) IBCF. To this, a solution of 1.7g (24.6mM) methylamine hydrochloride and 3.43ml (24.6mM) TEA in 25ml THF:water (3:1) was added after 15 mir, and the reaction mixture was stirred for 1hr, followed by further stirring for 2hrs at room temperature. The solvent was removed under reduced pressure and the residue was extracted with ethyl acetate. The organic layer was washed successively with saturated $NaHCO_3$, 10% citric acid solution and water, and dried over anhydrous Na_2SO_4. The solvent was evaporated to yield compound **4**, which was purified on a silica gel column (100-200 mesh) eluting with chloroform-methanol gradient. Yield 3.9g (95%) M.p. 94-96°; R_f (A) 0.62; R_f (B) 0.80; pmr ($CDCl_3$ 90MHz) δ : 7.8 (s, 1H, $N^{\alpha}H$); 7.5(s, 1H, $N^{\alpha}H$); 7.35(s, 5H, aromatic protons);

7.0 (s, 1H, $N^{\varepsilon}H$-$COCH_2$ C_6H_5); 5.1 (s, 2H, CH_2-C_6H_5); 4.5-4.3 (broad, 1H, $C^{\alpha}H$); 4.0 (broad, 1H, C^{α} H); 3.25-3.0 (broad, 2H, $C^{\varepsilon}H_2$); 2.80 (d, 3H, $NHCH_3$);1.95-1.5 (complex multiplet, 9H, Lys $C^{\beta}H_2$, $C^{\gamma}H_2$, $C^{\delta}H_2$, Leu $C^{\beta}H_2$, $C^{\gamma}H$); 1.4 (s, 9H, $\{CH_3\}_3C$); 0.85 (dd, 6H, Leu 2x$CH^{\delta}{}_3$).

Boc-Ala-Leu-Lys(Z)NHMe **5**

1.3g (6.9mM) Boc-Ala-OH and 0.96ml (6.9mM) TEA in 50ml dry THF were cooled to -15° and stirred with 0.9ml (6.9mM) IBCF. 3.5g (6.9mM) dipeptide **4** was treated with 1.0ml TFA at 0° for 45min, concentrated and triturated several times with dry ether, to furnish a white solid. The solid, along with 0.96ml (6.9mM) TEA in 25ml THF, was added to the above stirred solution, and the reaction mixture was stirred for 1hr at -15° followed by further stirring for 2hrs at room temperature. The solvent was removed under reduced pressure and the residue was extracted with ethyl acetate. The organic layer was washed successively with saturated $NaHCO_3$, 10% citric acid solution and water, and dried over anhydrous Na_2SO_4. The solvent was evaporated to yield compound **5**, which was purified on a silica gel column (100-200 mesh) eluting with chloroform-methanol gradient. Yield 3.6g (92%) M.p. 153°; R_f (A) 0.53; R_f (B) 0.76; pmr($CDCl_3$ 90MHz) δ : 8.8 (s, 1H, $N^{\alpha}H$); 7.8 (s, 1H, $N^{\alpha}H$); 7.5(s, 1H, $N^{\alpha}H$); 7.3(s, 5H, aromatic protons); 7.0 (s, 1H, $N^{\varepsilon}H$-$COCH_2C_6H_5$); 5.1 (s, 2H, CH_2-C_6H_5); 4.5-3.8 (complex, 3H, Lys C^{α} H, Ala $C^{\alpha}H$, Leu $C^{\alpha}H$); 3.25-3.0 (broad, 2H, $C^{\varepsilon}H_2$); 2.7 (d, 3H, $NHCH_3$);1.95-1.5 (complex multiplet, 9H, Lys $C^{\beta}H_2$, $C^{\gamma}H_2$, $C^{\delta}H_2$, Leu $C^{\beta}H_2$, $C^{\gamma}H$); 1.4 (d, 3H, Ala $C^{\beta}H_3$); 1.4 (s, 9H, $\{CH_3\}_3C$); 0.85 (dd, 6H, Leu 2x$CH^{\delta}{}_3$).

Boc-L/D-Glu(OBz)-Ala-Leu-Lys(Z)NHMe **6/7**

3.0g of tripeptide **5** was deprotected with TFA as described above, and the TFA salt, along with 0.73ml (5.2mM) TEA, was suspended in 50ml THF. To a precooled (-15°) solution of 0.5g (2.6mM) Boc-*L* or *D*-Glu(OBz)-OH and 0.37ml (2.6mM) TEA in dry THF (50ml), was added 0.34ml (2.6mM) of IBCF, and the mixture was stirred for 15 min. The solution containing deprotected tripeptide **5** was divided into two portions and added to the above stirred solutions and the mixtures were further stirred overnight. The products were isolated as described in previous steps, and were purified over silica gel columns eluting with chloroform-methanol gradients to furnish **6** and **7**.

6: Yield 1.6g (78%) M.p. 180-182°; R_f (A) 0.55; R_f (B) 0.84; pmr($CDCl_3$ 300MHz) δ : 8.9 (d, 1H, $N^{\alpha}H$); 8.7 (d, 1H, $N^{\alpha}H$); 8.4 (q, 1H, NHMe); 7.8 (d, 1H, $N^{\alpha}H$); 7.5 (d, 1H, $N^{\alpha}H$); 7.3(s, 10H, aromatic protons); 7.1 (q, 1H, $N^{\alpha}H$-$COCH_2C_6H_5$); 5.1 (s, 4H, CH_2-C_6H_5); 4.5-3.8 (complex, 4H, Lys $C^{\alpha}H$, Ala C $^{\alpha}H$, Glu $C^{\alpha}H$, Leu $C^{\alpha}H$); 3.25-3.0 (broad, 2H, $C^{\varepsilon}H_2$); 2.8 (d, 3H, $NHCH_3$); 2.6-1.6 (complex multiplet, 13H, Lys $C^{\beta}H_2$, $C^{\gamma}H_2$, $C^{\delta}H_2$, Glu $C^{\beta}H_2$, $C^{\gamma}H_2$, Leu $C^{\beta}H_2$, $C^{\gamma}H$); 1.4 (s, 9H, $\{CH_3\}_3C$); 1.3 (d, 3H, Ala C $^{\beta}H_3$);0.85 (dd, 6H, Leu 2x$CH^{\delta}{}_3$).

7: Yield 1.65g (80%) M.p. 174°; R_f (A) 0.55; R_f (B) 0.84; pmr($CDCl_3$ 300MHz) δ : 8.9 (d, 1H, $N^{\alpha}H$); 8.7 (d, 1H, $N^{\alpha}H$); 8.4 (q, 1H, NHMe); 7.8 (d, 1H, $N^{\alpha}H$); 7.5 (d, 1H, $N^{\alpha}H$); 7.3(s, 10H, aromatic protons);

7.1 (s, 1H, $N^{\varepsilon}H$-$COCH_2C_6H_5$); 5.1 (s, 4H, CH_2-C_6H_5); 4.5-3.8 (complex, 4H, Lys $C^{\alpha}H$, Ala $C^{\alpha}H$, Glu C $^{\alpha}H$, Leu $C^{\alpha}H$); 3.25-3.0 (broad, 2H, $C^{\varepsilon}H_2$); 2.8 (d, 3H, $NHCH_3$); 2.6-1.6 (complex multiplet, 13H, Lys C $^{\beta}H_2$, $C^{\gamma}H_2$, $C^{\delta}H_2$, Glu $C^{\beta}H_2$, $C^{\gamma}H_2$, Leu $C^{\beta}H_2$, $C^{\gamma}H$); 1.4 (s, 9H, $\{CH_3\}_3C$); 1.3 (d, 3H, Ala $C^{\beta}H_3$);0.85 (dd, 6H, Leu 2x$CH^{\delta}{}_3$).

Boc-L/D-Glu-Ala-Leu-LysNHMe **1/2**

1.0g (1.25mM) each of **6** and **7** were dissolved in 25ml methanol and hydrogenated over 100mg 10% Pd/C for 1hr. The catalyst was filtered through a celite bed, washed repeatedly with methanol, and the combined filterate was concentrated in vacuo. The residues were finally purified by HPLC. R_f (B) 0.40. PMR data for the peptides **1** and **2** are in Table. 1.

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