New Type of Helix and 2_7 Ribbon Structure Formation in Poly ΔLeu Peptides: Construction of a Single-Handed Template

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α,β-Dehydroamino acid residues due to the presence of $C_\alpha = C_\beta$ double bond influences the main chain and the side chain conformations. These residues have interesting chemical features including the increased resistance to enzymatic degradation. The chain length dependent conformational behavior of poly α,β-dehydroleucine (ΔLeu) peptides in both the pure forms *Z* and *E* and their various combinations like alternate *ZE*/*EZ* etc. have been investigated by using quantum mechanical method PCILO (perturbative configuration interaction of localized orbitals). The conformational states in alternate *Z* and *E* forms, with Φ, Ψ values of −10°, 105°/1°, −88° for *Z* form and 35°, 22°/−34°, −27° for the *E* form are found to be the most stable and degenerate than the states in pure *Z* and *E* forms and the *EZ* form etc. The repeated Φ, Ψ values give rise to altogether new types of left and right handed helices, and their stability increases with increasing chain length. These structures are stabilized by intramolecular hydrogen bonding, carbonyl−carbonyl interactions and hydrophobic interactions between the side chains of Δ^ZLeu and Δ^ELeu residues. The 2_7 ribbon structure (seven-membered hydrogen-bonded ring involving two consecutive amino acid residues) is found to be most stable and degenerate in the pentapeptide Ac-Δ^ELeu$_5$-NHMe, due to the formation of maximum hydrogen bonds. A right-handed template from achiral ΔLeu peptides has been achieved by incorporating L-Leu at the C-terminal or D-Leu at the N-terminal.

Introduction

Identification of a peptide backbone with new, well-defined regular secondary structural elements is of utmost importance in the design of predetermined, simple structural and functional motifs with potential applications in biological and material science.[1−3] The design of hydrophobic or amphipathic structure has led to the de-novo design of synthetic antimicrobial peptides incorporating the amphipathic α-helical motif.[4−9] Various amphipathic peptides with potent antimicrobial activities containing number of contiguous hydrophobic residues with branching at C_γ-position in their side chains appear to be essential requirement for significant hemolysis to occur. In the molecular design of peptides, it is essential to restrict the number of conformations to have a single-handed structure, so that it can serve as a pattern for generating a complementary molecule, i.e., a template. α,β-Dehydroamino acids are natural, noncoded amino acids in which both electronic and steric factors introduce conformational constraints in peptide backbone.[10−14] These furnish analogs with improved structure-bioactivity relationships and thus, have become important tools.[15,16] Dehydropeptides are also of particular interest because of their potential pharmacological and physiological usefulness, and they have been incorporated into various bioactive peptides including peptide opiods,[17−23] thyroliberin,[24] TRH,[25] bradykinin,[26] and inhibitors of the N-acetylated α-linked acidic dipeptides.[27] Number of studies have clearly recognized the strong tendency of conformationally restricted, C_β-substituted (Δ^ZPhe) residues, γ-branched (Δ^ZLeu) residues to stabilize β-turns in short model peptides[28,29] and to nucleate the 3_{10}-helical structure[2,29] in longer peptides. Dehydroleucine (Δ^ZLeu) and dehydrophenylalanine (Δ^ZPhe) are present in albonoursin,[30] while dehydrovaline (ΔVal) is found in penicillin and cephalosporin.[31]

α,β-dehydro residues in the *E* form are less common in nature than *Z*-analogs and are not easily available synthetically due to their lower thermodynamic stability. Thus, little is known about their conformational preferences. The *Z*- or *E*- orientation of the β-substituents of an α,β-dehydroamino acid often serve as a topographic probe[32,33] for local ligand−receptor interaction in which receptor proteins discriminate precisely between *Z*- or *E*-disposition of dehydropeptide double bond C_α=C_β in their ligands. For example, (i) (D-Ala,[2] Δ^EPhe,[4] Leu[5]) enkephalin exhibits very weak affinity for δ- and μ-opiod receptors compared to the *Z*- counterpart;[32] (ii) the Δ^ZPhe and Δ^EPhe isomers of a cyclic peptide Tyr-c[D-Cys-Phe-D-Pen]-OH, a high affinity δ-opiod receptor, selective agonist, display differential receptor binding affinity;[33] and (iii) the Δ^ZPhe litorin is an antagonist, whereas the corresponding Δ^EPhe litorin is an agonist.[34,35]

Only the conformational behavior of polypeptides of ΔAla and Δ^ZPhe has been investigated both experimentally and computationally. The conformational properties of Δ^ZPhe are very sensitive to the number, position, content and nature of natural amino acids in the peptide[10,16,29,32,33,36−44] and have been maximal explored to construct rigid folds.[41] Poly(α,β-dehydrophenylalanine) peptides Ac-(ΔPhe)$_n$-NHMe with $n > 4$ have been shown to adopt degenerate helical structures with Φ, Ψ values of ∼0°, ±90° and are stabilized by carbonyl−carbonyl and NH/π interactions.[45] The effect of ΔAla, Gly, Ala or Leu residues in Ac-(ΔPhe)$_n$-NHMe at the terminal positions has been investigated and it has been shown that chiral residues Ala and Leu lift the degeneracy.[46] Recently, we have also examined the effect of chiral residue on the conformational behavior of Ac-(Aib-ΔPhe)$_3$-NHMe and the type of interactions that lift the degeneracy.[47] The ambiguity regarding the structure adopted by poly ΔAla peptides has recently been solved by different techniques using solid state ^{13}C cross polarization magic angle spinning NMR,[48,49] computational,[40,47] and X-ray crystal-

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lographic studies.[50] It has been proved beyond doubt that it adopts an extended (flat) conformation stabilized by five-membered hydrogen bond formation and CH−O interactions.[40,47,50]

Until now there has been hardly any report on the conformations of α,β-dehydroamino acid residues in the pure *E* form, alternate *ZE* or *EZ* form, and various combinations of *Z* and *E* forms. There is even no systematic study on the sequential peptides containing achiral ΔLeu residues. Therefore, this study deals with (i) the chain length dependent conformational behavior of branched γ-carbon (ΔLeu) dehydro residue peptides in Ac-$(\Delta Leu)_n$-NHMe with $n = 1-8$ in the pure *Z* and *E* forms and their different combinations; (ii) since the ΔLeu residue does not contain any chiral center, therefore, the effect of the chiral residue, i.e., L-/D-Leu at both N and C terminal positions on the most stable and degenerate forms of Ac-$(\Delta^Z Leu\text{-}\Delta^E Leu)_3$-NHMe (i.e., peptide I to IV) has been investigated to construct a single handed template

I. Ac-L-Leu-ΔLeu_6-NHMe II. Ac-D-Leu-ΔLeu_6-NHMe

III. Ac-ΔLeu_6-L-Leu-NHMe IV. Ac-ΔLeu_6-D-Leu-NHMe

Graphical display of these hydrophobic peptides in the most stable states will reveal the type of structure (hydrophobic or amphipathic) and the adopted structure may be exploited in designing of antimicrobial and cell-signaling peptides.

Computational Methodology

The geometry of Ac-ΔLeu-NHMe in both the *Z* and *E* forms was optimized by using a potential function of the type suggested by Vinter et al.[51] and the optimized bond lengths and bond angles were in agreement with crystallographic data on dehydroamino acid residues.[52] Standard bond lengths and bond angles have been used for the Leu residues.[53,54] The Φ, Ψ maps and χ potential energy curves for ΔLeu were constructed in model dipeptide Ac-ΔLeu-NHMe in both the *Z* and *E* forms, by systematic variation of two torsion angles in steps of 30° to have the knowledge of global, local and low-energy minima. The minima values for Leu residues in both the L and D forms have been taken from the previous work.[47] Energy calculations were carried out using the quantum mechanical method PCILO[55] (perturbative configuration interaction of localized orbitals) on a Sun W, Ultra 5-10; Sparc workstation. The minima obtained by PCILO calculations were also the minima at the *ab initio* level for the usual amino acids and for dehydro amino acids. In addition, the PCILO results[16,40] for peptides containing usual[56] and unusual amino acids[47,57,58] were in conformity with *ab initio* results[59,60] and knowledge-based crystallographic data.[42,46,61−63] Conformational states were generated from the global, local, and low-energy minima found in the Φ, Ψ maps and χ_i, χ_j curves/maps, and their energies were computed. Minimization was further refined by varying Φ, Ψ, and χ values in the neighborhood of the minima, first in steps of 5° and then in steps of 2°.

Results and Discussion

The conformational results for the peptide Ac-ΔLeu-NHMe in both the *Z*- and *E*- forms are summarized in Table 1. In the Ramachandaran map for Ac-Δ^ELeu-NHMe, the Φ, Ψ values of −9, 101° and 15, 58° belong to the same region and likewise, the Φ, Ψ values of 9, −97° and −9, −70° correspond to another similar region in the Φ, Ψ map. The low-energy minima with Φ, Ψ values of ∓60, ±30° correspond to the 2_7 ribbon structure region. In the *Z* form, the states with Φ, Ψ values of 0°, ±90° are found to be degenerate and the helical regions, both right and left handed are ∼ 3 kcal mol^{-1} higher in energy. These Φ, Ψ values have been used for the generation of conformational states for higher peptides. It is apparent from the results in Table 2a that the dipeptide Ac-ΔLeu_2-NHMe is predicted to be most stable in the mixed form, i.e., Ac-Δ^ZLeu-Δ^ELeu-NHMe rather than the pure *ZZ*/*EE* or *EZ* forms. The states with Φ, Ψ values of −1, −87°/−6, 88° for Δ^ZLeu and ∼−38, −22°/36, 22° for Δ^ELeu are found to be the most stable and degenerate. The molecular view of the peptide in one of these degenerate conformational states is shown in Figure 1, and it clearly reflects the formation of a 10-membered hydrogen-bonded ring. The carbonyl−carbonyl interactions ($d_{(C)Oi\cdots Ci+1(O)} \sim 2.4$ Å) and hydrophobic interactions between the side chains of the Δ^ZLeu and Δ^ELeu also contribute to the stability of these states. The hydrogen bond formation and hydrophobic interactions accounts for the increased stability of these states over the 0, 90°/0, −90° conformational states in the pure *ZZ*/*EE* forms or alternate *EZ* form. The results in Table 2b further confirm that the stability of poly ΔLeu with alternate *Z* and *E* forms of ΔLeu increases with chain length (except $n = 5$) over the alternate *E* and *Z* and pure *Z*/*E* forms.

Surprisingly, the pentapeptide Ac-ΔLeu_5-NHMe is found to be most stable in the *E* form and not in the pure *Z* or alternate *Z* and *E* or *E* and *Z* forms. The conformational states with Φ, Ψ values in the neighborhood of 60, −30° or −60, 30° for all the residues or with alternate Φ, Ψ values of 60, −30° and −60, 30° are found to be degenerate. The stability of these states arises due to the formation of five, seven membered hydrogen-bonded rings ($d_{O\cdots H} = 1.66-1.88$ Å, $d_{O\cdots N} = 2.52-2.77$ Å) resulting in the formation of a ribbonlike structure. A molecular view of the peptide with all the Φ, Ψ values in the neighborhood of −60, 30° is shown in Figure 2a and these states are characterized by rise per residue of ±1.68 Å, rotation per residue of ∓152° and number of residues per turn being ∼2.4. Possibly, this is the the first study where the 2_7 ribbon structure is found to be most stable in peptides constructed from unusual amino acids. With alternate Φ, Ψ values of ∼60, −30° and −60, 30°, the peptide adopts a semicircular type structure (Figure 2b) in which half the alternate C═O and side chains of Δ^ELeu residues lie above the plane of the backbone of the peptide and the rest half lie below the plane. The carbonyl−carbonyl interactions in all these states were found to be very weak. The stability of 2_7 ribbon structures arises due to the formation of maximum number of hydrogen bonds.

To verify the chain length dependent conformational behavior of poly ΔLeu peptides, the conformations of highmer of ΔLeu in completely *Z* and *E* forms and with alternate forms of *Z* and *E* or *E* and *Z* for ΔLeu residues, i.e., Ac-$(\Delta Leu)_n$-NHMe with $n = 6-8$, have been carried out. It is obvious from the results in Table 2b, that the conformational states with alternate Φ, Ψ values of approximately 0, 90°/0, −90° for the Δ^ZLeu residues and with Φ, Ψ values in the left/right handed helical regions for the Δ^ELeu residues, are found to be most stable and

Table 1. Various Energy Minima in the Conformational Energy Maps of Ac-ΔLeu-NHMe

form of ΔLeu	Φ,Ψ (deg)	χ_2 (deg)	ΔE (kcal/mol)
Z	−3, 90	120	0.00
Z	5, −90	120	0.11
E	9, −97	152	1.34
E	−9, 101	93	1.43
E	−9, −70	90	1.56
E	−60, 30	108	1.80
E	60, −31	133	1.95
E	15, 58	150	2.06

Table 2. (a) Summary of the Stable Conformations of Ac-ΔLeu$_n$-NHMe, with $n = 2-4$, and (b) Conformational Results for the Peptides Ac-ΔLeu$_n$-NHMe, $n = 5-8$, with ϕ, Ψ, and χ Values in Degrees.

a. Summary of the Stable Conformations

$n = 2$				$n = 3$				$n = 4$			
form of ΔLeu	Φ,Ψ (deg)	χ_2 (deg)	Δ*E* (kcal/mol)	form of ΔLeu	Φ,Ψ (deg)	χ_2 (deg)	Δ*E* (kcal/mol)	form of ΔLeu	Φ,Ψ (deg)	χ_2 (deg)	Δ*E* (kcal/mol)
ZE	1, −87	119	0.00	*ZEZ*	1, −87	120	0.00	*ZEZE*	−6, 93	118	0.00
	−38, −22	100			−36, −22	100			36, 22	146	
					−2, −88	123			−3, 88	115	
ZE	−6, 88	113	0.38						36, 25	145	
	36, 22	146		*ZEZ*	−6, 88	118	0.19				
					36, 22	136					
ZZ	−2, 88	120	1.06		2, 83	120		*ZEZE*	6, −93	125	0.06
	0, 88	120							−40, −22	100	
				ZZZ	1, −88	120	0.61		8, −98	128	
ZZ	0, −85	115	1.17		0, −83	116			−36, −20	90	
	0, −85	120			0, −87	118					
								ZZZZ	0, −90	120	
EE	−10, 105	90	1.68	*ZZZ*	0, 85	120	1.38		0, −85	120	1.44
	35, 22	144			0, 85	117			−5, −75	115	
					0, 85	121			0, −90	120	
EE	1, −88	87	2.00								
	−34, −27	98		*EEE*	−6, −76	92	3.61	*EZEZ*	21, 50	147	
					−29, −36	99			−6, 93	118	
EZ	−5, −77	93	2.55		−29, −36	90			38, 23	144	2.10
	1, −88	117							−3, 88	123	
				EEE	−62, 41	109	6.92				
EZ	6, 85	152	2.56		−60, 30	114		*EZEZ*	−20, −47	93	
	−6, 93	118			−60, 30	108			6, −93	122	
									−41, −20	100	
EE	−16, −66	93	2.65						6, −94	124	2.20
	−44, −18	103									
								ZZZZ	−6, 91	121	
EE	27, 45	140	4.52						−6, 91	117	
	51, 7	135							5, 75	120	
									0, 85	118	
EE	−62, 41	115	5.19								2.39
	−60, 30	108						*EEEE*	−18, −66	95	
									−32, −38	97	
EE	−60, 32	108	5.23						−20, −61	86	
	62, −41	124							−46, −17	103	
EE	60, −35	126	5.30					*ZEEE*	0, −90	122	4.56
	60, −30	132							−16, −57	96	4.69
									−10, −70	84	
									−18, −57	92	

b. Conformational Results

	residue number								
form of ΔLeu	1	2	3	4	5	6	7	8	Δ***E*** (kcal/mol)
				Ac-ΔLeu$_5$-NHMe					
EEEEE	65, −35	−65, 35	67, −35	−60, 32	62, −38				0.00
	118	128	121	108	106				
EEEEE	−65, 40	−67, 45	−70, 40	−60, 40	−55, 15				0.16
	121	125	103	108	106				
EEEEE	65, −36	65, −35	70, −40	62, −45	60, −33				0.59
	116	118	122	129	106				
ZEZEZ	−21, 113	61, −10	−3, 88	41, 20	−3, 90				11.22
	113	111	120	140	130				
ZEZEZ	21, −113	−61, 3	8, −98	−41, −15	3, −95				11.81
	130	125	128	100	125				
ZZZZZ	−40, 113	−40, 117	33, 50	−2, 87	1, 84				15.94
	111	115	127	122	115				
ZZZZZ	33, −106	33, −108	−40, −57	2, −89	−6, −77				16.00
	118	118	113	116	106				

Table 2. Continued

b. Conformational Results

form of ΔLeu	residue number 1	2	3	4	5	6	7	8	Δ***E*** (kcal/mol)
				Ac-ΔLeu$_6$-NHMe					
ZEZEZE	−5, 95	36, 22	−13, 108	41, 15	−3, 95	32, 36			0.00
	118	136	120	140	118	135			
ZEZEZE	6, −93	−36, −22	13, −108	−41, −15	3, −95	−32, −26			0.13
	120	95	128	100	130	105			
EZEZEZ	−16, −52	6, −93	−41, −15	3, −93	−37, −26	3, −95			2.03
	95	123	100	126	80	121			
EZEZEZ	17, 55	−5, 95	37, 21	−1, 91	37, 20	−3, 90			2.12
	149	121	140	119	85	120			
ZZEZEE	6, −98	−4, −76	−19, −57	−21, −56	−45, −19	−6, −81			5.31
	122	109	96	113	80	95			
ZZZZZE	0, −88	1, −88	−6, −81	−2, −84	2, −93	−19, −57			5.53
	116	117	112	110	127	90			
ZZZZEE	−2, 89	0, 85	0, 85	0, 80	1, 81	21, 50			5.72
	121	122	120	110	80	146			
EEEEEE	14, 63	24, 51	19, 57	21, 56	40, 19	18, 56			8.94
	140	142	140	140	138	151			
				Ac-ΔLeu$_7$-NHMe					
ZEZEZEZ	6, −93	−36, −22	3, −88	−36, −20	3, −95	−32, −26	−3, −75		0.00
	130	100	118	100	130	105	110		
ZEZEZEZ	−6, 93	36, 22	−3, 88	36, 25	−3, 95	32, 26	−3, 95		0.75
	118	136	120	140	120	135	115		
ZZZZZZE	0, −90	4, −87	−2, −82	−5, −80	−6, −80	−4, −83	−38, −27		2.63
	116	115	120	115	116	112	110		
ZZZZZZZ	−2, 90	5, 78	2, 86	11, 68	2, 82	7, 74	8, 80		3.53
	123	128	120	128	120	127	127		
EEEEEEE	50, −1	−60, 32	63, −51	−60, 30	56, −32	−65, 35	57, −28		17.50
	130	113	130	108	128	110	133		
EEEEEEE	60, −33	58, −27	60, −33	62, −45	60, −33	60, −35	58, −27		18.94
	133	131	135	127	135	132	130		
				Ac-ΔLeu$_8$-NHMe					
ZEZEZEZE	−6, 93	36, 22	−3, 88	36, 25	−3, 95	32, 26	−3, 95	32, 26	0.00
	118	136	120	140	120	135	120	140	
ZEZEZEZE	6, −93	−36, −22	8, −98	−41, −15	3, −95	−32, −26	3, −95	−32, −26	0.81
	125	100	128	100	115	100	120	105	
ZZZZZZZZ	0, 88	−6, 87	0, 85	4, 78	5, 75	2, 80	0, 85	1, 82	4.94
	120	120	120	125	130	128	115	120	
ZZZZZZZZ	2, −87	0, −85	−4, −83	−2, −81	−5, −80	0, −85	−5, −75	3, −90	5.56
	116	120	120	120	115	120	110	120	

degenerate. The side chains of all the Δ^ZLeu residues are found to adopt similar χ_i, χ_j values of ∼ 180°, 120° and for the Δ^E-Leu side chains, the χ_i, χ_j values are ∼ 0°, 130°. The graphical representation of the peptide Ac-(Δ^ZLeu-Δ^ELeu)$_3$-NHMe in the left handed conformational state shown in Figure 3a depicts that the alternate peptide bonds are involved in the formation of ten membered hydrogen-bonded ring between the carbonyl-oxygen of *i*th Δ^ELeu residue and the NH moiety of *i*th+3 Δ^Z-Leu residue. The carbonyl oxygen of Δ^ELeu residues involved in the hydrogen bonding is also involved in the carbonyl−carbonyl interaction, with the carbonyl-carbon of the next Δ^Z-Leu residue ($d_{(C)Oi\cdots Ci+1(C)}$ ∼ 2.43 Å), which is not involved in hydrogen bond formation. Due to the adoption of regular and alternate Φ, Ψ values by the consecutive pair of Δ^ZLeu-Δ^ELeu residues near or in the helical regions, the resulted structures appear like the helices. Interestingly, a space filling model of the peptide in the right handed structure reveals the formation of a amphipathic structure as shown in Figure 3b. The amphipathic structure thus formed can be explored for the designing of antimicrobial peptides.

The peptide with alternate *E* and *Z* form, i.e., Ac-(Δ^ELeu-Δ^ZLeu)$_3$-NHMe adopts a degenerate helical structure with Φ, Ψ values in the left/right handed helical region for the Δ^ELeu residues and Φ, Ψ values of ∼0, 90°/0, −90° for the Δ^ZLeu residues, respectively. A molecular view of the peptide Ac-(Δ^ELeu-Δ^ZLeu)$_3$-NHMe in the left handed structure shown in Figure 4 clearly reveals the formation of two, 10-membered hydrogen-bonded rings between the carbonyl oxygen of the *i*th Δ^ELeu residue and NH moiety of the *i*th + 3 Δ^ZLeu residue, i.e., one less than the corresponding Ac-(Δ^ZLeu-Δ^ELeu)$_3$-NHMe peptide. On the basis of the distances, the magnitude of the carbonyl−carbonyl interactions were found to be almost the same in both the *ZE* and *EZ* peptides. Thus, the peptides in the *ZE* forms are 2 kcal mol^{-1} more stable than the corresponding *EZ* forms.

To characterize these helical structures, models have been constructed for Ac-(Δ^ZLeu-Δ^ELeu)$_8$-NHMe with the above set of Φ, Ψ values (i.e., corresponding to left and right handed structures). From the molecular view of the peptide shown in Figure 5 in the conformational state with Φ, Ψ values of ∼0,

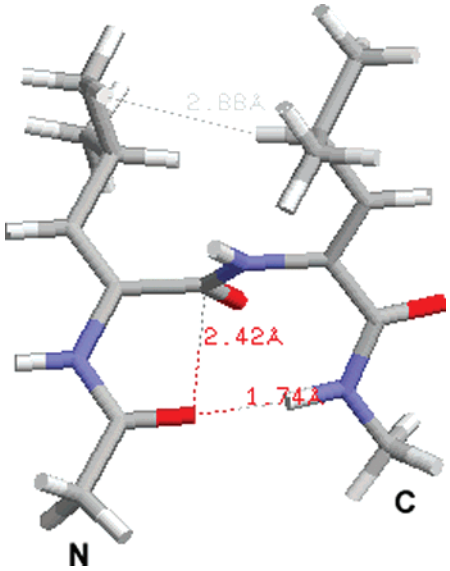

Figure 1. Molecular view of the dipeptide Ac-Δ^ZLeu-Δ^ELeu-NHMe in the most stable state with Φ, Ψ values of 1, −87°; −38, −22° showing the formation of a 10-membered hydrogen-bonded ring and hydrophobic interactions between side chains of ΔLeu residues.

90° for Δ^ZLeu residues and Φ, Ψ values in the left handed helical region for Δ^ELeu residues, it is obvious that the C_α of the *i*th residue is vertically above the C_α of *i*th +10 residue. In other words, this left handed helix is characterized by 5 pairs of Δ^ZLeu-Δ^ELeu per turn, rise per pair of 4.5 Å and rotation per pair of ∼72°. The interaction between the carbonyl oxygen of Δ^ZLeu residues not involved in hydrogen bonding with the carbonyl carbon of the next Δ^ELeu residue is found to be somewhat weaker on the basis of the distances ($d_{(C)Oi\cdots Ci+1(O)}$ = 2.64 Å). The hydrophobic interactions between the side chains of the alternate pairs of Δ^ZLeu and Δ^ELeu residues also contribute to the stability, as the distance between the (i) C_δ-H of Δ^ZLeu residues and Cγ-H of Δ^ELeu side chains is 2.64 Å, and (ii) Cγ-H of Δ^ZLeu residues and Δ^ELeu residues is 3.15 Å. The stability of these states in the alternate *ZE* form arises due to the hydrogen bond formation and hydrophobic interactions as mentioned above, over the other states in all *Z* or *E* and alternate *EZ* forms of ΔLeu residues. The carbonyl−carbonyl and hydrophobic interactions are not observed in the 2_7 ribbon structures, predicted for Ac-(Δ^ELeu)$_5$-NHMe. Thus, the conformational behavior of poly ΔLeu peptides is chain length dependent, and as expected for a peptide constructed from achiral amino acid residues, the most stable states are degenerate.

To make the degenerate state to be nondegenerate, Scheraga et al.[64] gave the concept of identifying sets of residues from the trans-membrane spanning segment of melittin in which there is a deviation from regular α-helical character. Rigoustsos et al.[65] have now incorporated this idea in Bioinformatic tools to create a nondegenerate motif. It is worth mentioning here that this study deals with the peptides constructed from unusual, achiral and hydrophobic residues. Therefore, to construct a template from these peptides is a formidable task.

Choice of L-/D-Leu Residue. Gramicidin-A is a linear hydrophobic pentadecapeptide, with alternating D- and L-amino acid residues with a formyl group at the N-terminus and a characteristic C-terminal ethanolamine moiety.[66−68] It contains amino acid residues in the D-form, which have the branching at the β or γ positions. The N-terminus contains L-amino acid residues with smaller side chains and the C-terminus has the repeat sequence D-Leu-Trp. In the conformational analysis of the peptides Ac-(X-D-Leu)$_3$-NHMe with *X* = Gly, Ala, Aib,

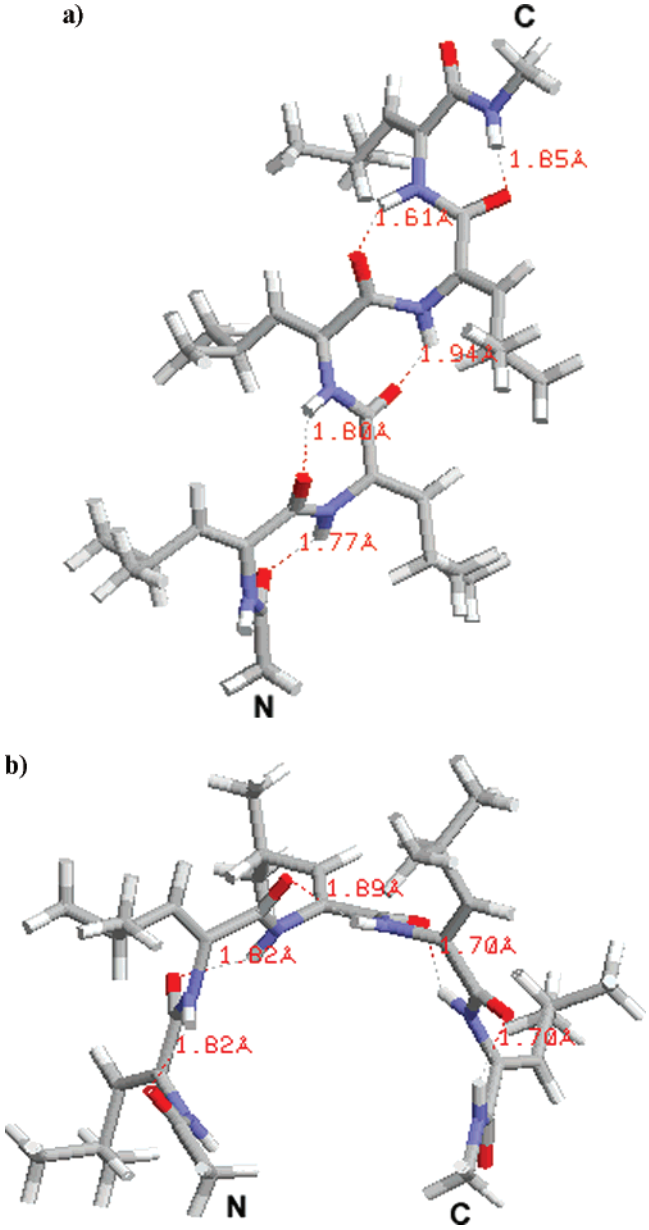

Figure 2. Molecular view of the peptide Ac-(Δ^ELeu)$_5$-NHMe depicting the formation of seven membered hydrogen-bonded rings (a) with Φ, Ψ values of approximately −60, 30° (b) with alternate Φ, Ψ values of ∼60, −30° and −60, 30° adopting a semicircular type structure.

Abu, Val, Ile, Leu, Phe, Tyr, Trp, we have seen that the 3_{10} left handed helical structure is populated in all those cases where the preceding residue to D-Leu is an amino acid residue with smaller side chain, i.e., Gly, Ala or an aromatic amino acid residue.[69,70] The Aib residue is the promoter of the helix and the peptide Ac-(Aib-L-Leu)$_3$-NHMe adopts a right handed helical structure. These results form the basis of designing a template with a single-handed structure from achiral amino acid residues by incorporating the Leu residue at terminal positions.

Terminal effect of the L-/D-Leu Residue. From the conformational results for the peptides I to IV containing the Leu residue in both the L/D forms at either of the terminal positions given in Table 3, it is obvious that the L-Leu residue at the N-terminal in Ac-(Δ^ZLeu-Δ^ELeu)$_3$-NHMe, with alternate *Z* and *E* forms of ΔLeu, has no effect on the conformations, i.e., the degeneracy is not lifted. D-Leu at the N-terminal (peptide II) and L-Leu at the C-terminal (peptide III) are found to have similar effects, and in both cases, a right handed helical structure

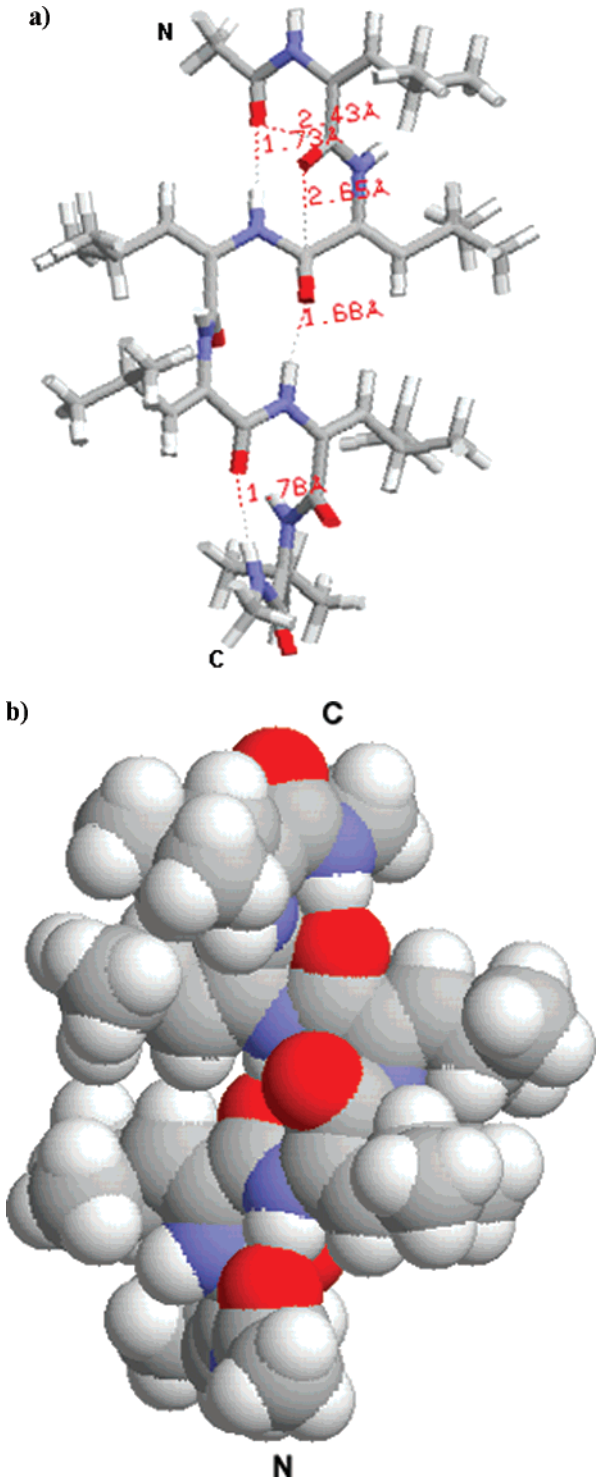

Figure 3. (a) Graphical view of the peptide Ac-(Δ^ZLeu-Δ^ELeu)$_3$-NHMe in the conformational state with alternate Φ, Ψ values of ~0, 90° and with Φ, Ψ values in the left handed helical region showing the formation of ten membered hydrogen-bonded ring in which alternate peptide bonds participate, carbonyl−carbonyl interactions and hydrophobic interactions between side chains of Δ^ZLeu and Δ^ELeu residues. (b) Space filling model of the peptide Ac-(Δ^ZLeu-Δ^ELeu)$_3$-NHMe with alternate Φ, Ψ values of ~0, −90° and with Φ, Ψ values in the right handed helical region showing the formation of amphipathic right handed helical β-sheet structure.

with alternate Φ, Ψ values of approximately 0, −90° for Δ^Z-Leu residues and with Φ, Ψ values in the right handed helical region for the Δ^ELeu residues is populated. The D-Leu residue in peptide II at the N-terminal adopts Φ, Ψ values of 25, −115° and this results in additional carbonyl−carbonyl interactions in

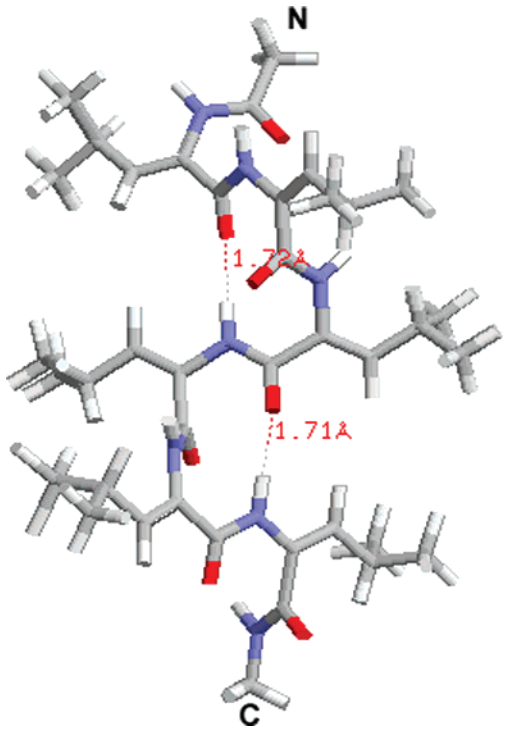

Figure 4. View of the peptide Ac-(Δ^ELeu-Δ^ZLeu)$_3$-NHMe in left handed helical β-sheet type structure in which a 10-membered hydrogen-bonded ring is one less than in the corresponding Ac-(Δ^Z-Leu-Δ^ELeu)$_3$-NHMe peptide.

the right handed structure as compared to the left handed structure. Due to the adoption of Φ, Ψ values of −55, −25° by the L-Leu residue at the C-terminal in peptide III, an additional 10-membered hydrogen-bonded ring is formed (Figure 6) between the carbonyl oxygen of the fifth Δ^ZLeu residue and the amide hydrogen $d_{(C)O\cdots HN}$ = 1.72 Å. Thus, a right-handed template can be constructed from poly ΔLeu peptides by incorporating either L-Leu at the C-terminal or D-Leu at the N-terminal.

The peptide IV is predicted to adopt a left handed helical structure with Φ, Ψ values of ~0, 90° for both the Δ^ZLeu and Δ^ELeu residues and 25, 55° for the D-Leu residue. The repeated Φ, Ψ values of ~0, 90° gives rise to a helical structure without hydrogen bonds, stabilized by carbonyl−carbonyl interactions between carbonyl oxygen of the *i*th residue and carbonyl carbon of *i*th + 1 residue and should be populated in the solvents of low dielectric constant, incapable of hydrogen bonding with the carbonyl moieties.[45,47,54] A 10-membered hydrogen-bonded ring formation is also seen between the carbonyl oxygen of the fifth Δ^ZLeu residue and the NH moiety of the amide bond. A graphical representation of the molecule showing these interactions is shown in Figure 7. Thus, a left handed helical structure without hydrogen bonds can be realized by keeping D-Leu at the C-terminal in poly Δ^ZLeu-Δ^ELeu peptides.

It is difficult to structurally characterize the hydrophobic peptides. ^{13}C cross polarization magic angle spinning (CPMAS) NMR spectroscopy has emerged as an excellent tool to determine the backbone conformation of hydrophobic polypeptides. Thus, it may be interesting to examine and explore the conformations of studied peptides by using CPMAS NMR spectroscopy.[48,49,71]

The Φ, Ψ values 0°, ±90° appears to be somewhat unusual but have been predicted to be minima at PCILO and INDO level calculations for amino acids.[47] *Ab initio* calculations at the HF/3.21G and HF/6.31 + G levels for the dipeptides of

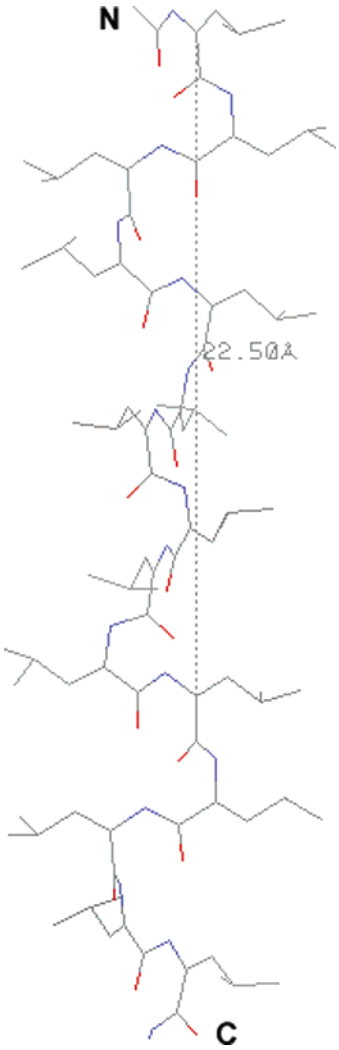

Figure 5. Molecular view of the peptide Ac-(Δ^ZLeu-Δ^ELeu)$_8$-NHMe with alternate Φ, Ψ values of approximately 0, 90° and with Φ, Ψ values of 36, 22° in the left handed helical region showing five pairs of Δ^ZLeu-Δ^ELeu residues involved per turn.

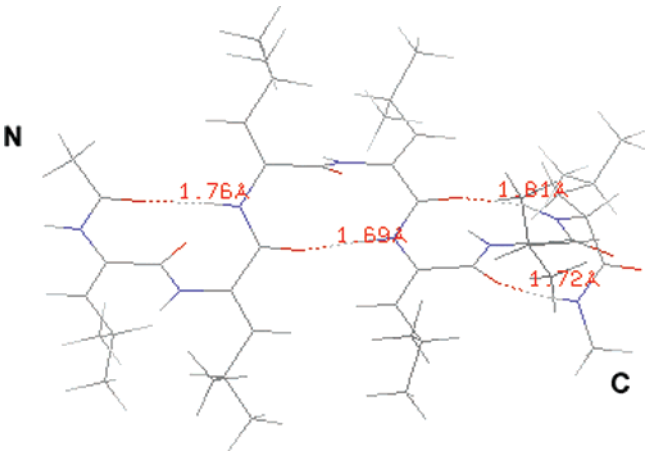

Figure 6. Molecular view of the single handed template constructed by incorporating L-Leu at C-terminal in Ac-(Δ^ZLeu-Δ^ELeu)$_3$-NHMe with Φ, Ψ values of 6, −93°; −36, −22°; 13, −108°; −41, −15°; 3, −95°; −32, −31°; and −55, −25° depicting the formation of an additional 10-membered hydrogen-bonded ring between carbonyl oxygen of the fifth residue and the amide hydrogen.

Gly and Ala[72] have predicted stationary point near $\Phi = 0°$ and $\Psi = 90°$. The importance of coloumbic interactions between backbone carbonyls in proteins as a stabilizing factor in

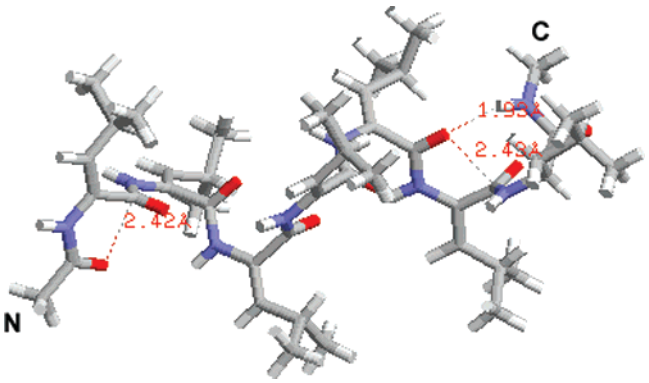

Figure 7. Molecular view of the peptide Ac-(Δ^ZLeu)$_6$-D-Leu-NHMe in left handed helical structure without hydrogen bond with Φ, Ψ values of ~0, 90° for the Δ^ZLeu and Δ^ELeu residues and 25, 55° for D-Leu stabilized by carbonyl−carbonyl interactions.

α-helices, β-sheets, and right handed twist often observed in β-strands is well documented.[73,74] Carbonyl−carbonyl interactions also stabilize the partially allowed Ramachandaran conformations of aspartic acid and asparagines.[75]

It is worth mentioning here that in the Ramachandran plots, based on the NMR-derived structure for 113 proteins, 84719 total residues plotted (Pro and Gly excluded), appreciable data points have been shown between left-handed helical and collagen-type structural regions[76,77] and between the right-handed helical and inverse collagen-type structural regions.[72,78,79] This supports that the minima corresponding to the Φ, Ψ values in neighborhood of 0, ±90° are not an overestimation.

Summary

The conformations of the poly Δ^ZLeu-Δ^ELeu peptides containing up to eight ΔLeu residues have been investigated and found to be chain length dependent and the adopted conformations are degenerate. Poly Δ^ZLeu-Δ^ELeu peptides were found to adopt new kind of regular secondary degenerate structures. On the energy scale, the structures adopted by poly Δ^ZLeu-Δ^ELeu peptides are found to be more stable than the most stable structures adopted by poly Δ^ZLeu or poly Δ^ELeu or poly Δ^ELeu-Δ^ZLeu peptides containing same number of ΔLeu residues. The stability of the degenerate conformational states with alternate and regular Φ, Ψ values of −10, 105° and 35, 22° for Δ^ZLeu and Δ^ELeu residues, respectively, or 1, −88° for Δ^ZLeu residues and −35, −27° for Δ^ELeu residues, increases over the other states with increasing chain length and are stabilized by (i) intramolecular hydrogen bonding in which alternate peptide bonds participate, (ii) carbonyl−carbonyl interactions, and (iii) hydrophobic interactions between the side chains of the alternate pairs of ΔLeu residues, i.e., between side chains of Δ^ZLeu and Δ^ELeu residues, and surprisingly, the structures are amphipathic in nature. Hence, these structures have been named as left/right handed helical β-sheet type structure, respectively, and they are characterized by five pairs of Δ^Z-Leu-Δ^ELeu residues per turn. Interestingly, the 2_7 ribbon structure is found to be most stable for the peptide Ac-(Δ^ELeu)$_5$-NHMe with Φ, Ψ values of approximately −60, 30° all, 60, −30° all and −60, 30° and 60, −30°, alternatively. This is the first study where a stable 2_7 ribbon structure has been reported.

Designing of single handed template from achiral ΔLeu peptides has been achieved by incorporating L/D-Leu in poly ΔLeu peptide Ac-(Δ^ZLeu-Δ^ELeu)$_3$-NHMe, at either of the terminal positions. By incorporating the L-Leu residue at the C-terminal or the D-Leu residue at N-terminal in Ac-(Δ^ZLeu-Δ^ELeu)$_3$-NHMe, the degeneracy is lifted and peptide can be

Table 3. Conformational Results (ϕ, Ψ, and χ Values in Degrees) for the Effect of L/D-Leu Residues on Ac-ΔLeu$_6$-NHMe

	residue number							
form of **ΔLeu**	1	2	3	4	5	6	Leu	ΔE (kcal/mol)
				I. Ac-L-Leu-ΔLeu$_6$-NHMe				
ZEZEZE	6, −93	−36, −22	13, −108	−41, - 15	3, −95	−32, −26	−25, −60	0.00
	125	95	128	100	130	105	178, 78	
ZEZEZE	−5, 95	36, 22	−13, 108	41, 15	−3, 95	32, 26	−30, 120	0.09
	123	136	120	140	118	135	175, 70	
ZEZEZE	6, −95	−36, −22	13, −108	−41, −15	3, −95	−32, −26	−25, 115	0.22
	130	95	128	100	130	105	175, 75	
ZEZEZE	6, −93	−36, −22	13, −108	−41, −15	3, −95	−32, −26	−75, 45	1.16
	130	95	128	100	130	105	−60, 170	
ZEZEZE	−5, 95	36, 22	−13, 108	41, 15	−3, 95	32, 26	−75, 45	1.28
	118	136	120	140	118	135	−60, 170	
ZEZEZE	−5, 95	36, 22	−13, 108	41, 15	−3, 95	32, 26	−25, −60	3.09
	118	136	120	140	118	135	173, 73	
				II. Ac-D-Leu-ΔLeu$_6$-NHMe				
ZEZEZE	1, −88	−36, −27	13, −108	−41, −15	3, −95	−37, −26	25, −115	0.00
	120	100	128	100	130	110	180, 170	
ZEZEZE	6, −93	−41, −17	13, −108	−41, −15	3, −95	−32, −26	73, −52	0.91
	125	100	128	100	130	105	62, 70	
ZEZEZE	−9, 93	36, 22	−8, 98	36, 20	−3, 95	32, 26	25, −110	1.19
	115	140	115	135	115	145	180, 160	
ZEZEZE	−5, 95	36, 22	−13, 108	41, 15	−3, 95	32, 26	21, 60	1.59
	123	136	120	140	118	135	−178, 163	
ZEZEZE	−5, 95	36, 22	−13, 108	41, 15	−3, 95	32, 26	73, −57	1.63
	123	136	120	140	118	135	61, 75	
ZEZEZE	6, −93	−36, −22	13, −108	−41, −15	3, −95	−32, −26	20, 65	3.53
	120	*95*	*128*	*100*	*130*	*105*	*−178, 163*	
				III. Ac-ΔLeu$_6$-L-Leu-NHMe				
ZEZEZE	6, −93	−36, −22	13, −108	−41, −15	3, −95	−32, −31	−55, −25	0.00
	130	95	128	100	130	100	−171, 75	
ZEZEZE	−5, 95	36, 22	−13, 108	41, 15	−3, 95	32, 26	−20, 111	2.25
	118	136	120	140	118	140	175, 77	
ZEZEZE	−5, 95	36, 22	−13, 108	41, 15	−3, 95	32, 26	−35, −50	3.59
	118	136	120	140	113	140	174, 70	
ZEZEZE	6, −93	−36, −22	13, −108	−41, −15	3, −95	−32, −26	−20, 115	3.63
	130	95	128	100	130	100	180, 70	
				IV. Ac-ΔLeu$_6$-D-Leu-NHMe				
ZEZEZE	−5, 95	36, 22	−13, 108	41, 15	2, 90	32, 26	50, 30	0.00
	118	136	120	140	123	140	178, 169	
ZEZEZE	6, −93	−36, −22	13, −108	−41, −15	3, −95	−32, −26	80, −60	0.10
	120	95	128	100	130	100	63, 80	
ZEZEZE	−5, 95	36, 22	−13, 108	41, 15	2, 90	32, 26	125, −25	1.31
	118	136	120	140	118	140	−65,127	
ZEZEZE	6, −93	−36, −22	13, −108	−41, −15	3, −95	−32, −26	15, −105	1.41
	120	95	128	100	130	100	180, 170	
ZEZEZE	6, −93	−36, −22	13, −108	−41, −15	3, −95	−32, −26	20, 65	2.66
	120	95	128	100	130	100	−177, 169	
ZEZEZE	−5, 95	36, 22	−13, 108	41, 15	2, 90	32, 26	21, −113	2.69
	118	136	120	140	118	140	180, 170	
ZZZZZZ	0, 90	0, 85	0, 85	5, 80	8, 72	−6, 88	25, 55	3.50
	123	120	120	125	128	126	−177, 174	

realized only in a right handed structure with Φ, Ψ values for the Δ^ZLeu of ~0, −90° and with Φ, Ψ values in the right handed helical region for Δ^ELeu residues. The peptide Ac-(Δ^Z-Leu-Δ^ELeu)$_3$-D-Leu-NHMe, i.e., with D-Leu at the C-terminal, adopts a left handed helical structure without hydrogen bonds, with Φ, Ψ values of ~0, 90° for both Δ^ZLeu and Δ^ELeu residues. The stability of this state over the right handed one is due to the adoption of $\Phi = 25°$, $\Psi = 55°$ by the D-Leu, resulting in the formation of a hydrogen bond between the carbonyl oxygen of the fifth Δ^ZLeu residue and the amide hydrogen.

Antimicrobial peptides adopt amphipathic structure. The poly Δ^ZLeu-Δ^ELeu peptides can serve as a template for the design of antimicrobial peptides of interest. The conformations of these hydrophobic peptides can be explored by using recent ^{13}CPMAS NMR spectroscopy[48,49,71,80] and can be exploited to transport nuclear localization sequences in order to probe intracellular signaling.[81]

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