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N and N+1 cap effects of Gly, Ala, Leu, Val, Pro and Aib in Boc-(D)Glu_1-Xxx_2-Yyy_3-Lys_4-NHMe, a 3_{10} type protohelix endlocked by Boc-(D)Glu

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Gly, Ala, Leu, Val, Pro and Aib are introduced as N cap (Xxx_2) or N+1 cap (Yyy_3) residues in Boc-(D)Glu_1-Xxx_2-Yyy_3-Lys_4-NHMe, a 3_{10} like protohelix endcapped by Boc-(D)Glu. NOEs, NH temperature coefficients and $^3J_{NHC\alpha}$ coupling constants establish that all variants, except Boc-(D)Glu_1-Ala_2-Pro_3-Lys_4-NHMe, **1e** are distorted 3_{10} type protohelices, while the response of Glu_1 NH- a reporter of $Lys_4 \rightarrow Glu_1$ salt bridge-to solvent, salt and temperature induced perturbations, reveals that the protohelix variants differ appreciably in the degree of ordering and overall stability. The capping position variations are thus shown to affect the protohelix stability in a manner that is strongly reminiscent of the presumed effects in helix nucleation.

Keywords: 3_{10} Helix, II′ turn, reverse turn, D amino acids, NMR studies

The earlier observation of the effects of D amino acid in the stability and geometry of an ordered helical structure[1,2] prompted the exploitation of the model Boc-(D)Glu_1-Xxx_2-Yyy_3-Lys_4-NHMe to generally assess the position dependent effect of the D amino acid in a helical type fold. Based on this rationale, we describe here the studies of two series of tetra-peptides, one with Xxx = Ala and Yyy = Gly **1a**, Ala **1b**, Leu **1c**, Val **1d**, Pro **1e**, Aib **1f,** and the other with Yyy = Gly and Xxx = Gly **2b**, Leu **2c**, Val **2d**, Pro **2e** and Aib **2f**. Thus nine variants of the earlier described prototypes **1a** and **1c** are examined for solvent, salt and temperature induced perturbations. The structure variations are thus shown to influence the morphology of the protohelix and its overall stability in a manner that is strongly reminiscent of the presumed effects in helix nucleation[5].

Results and Discussion

Table I shows the ^{1}H chemical shifts of all the peptide variants under $CDCl_3$-DMSO-d_6 mixture (6:1 with every peptide and 3:1 in case of **1b**, on account of its solubility requirement) derived from 2D COSY and ROESY spectra. All the variants were examined for amide temperature coefficients, NOEs, $^3J_{NHC\alpha}$ coupling constants, and the response of Glu NH to solvent, salt and temperature induced perturbations.

Gross conformational characteristics

Solvent shielded amide protons in DMSO-d_6: The observed amide temperature coefficients in all the peptide variants under DMSO-d_6 are summarized in **Table II**. With the notable exception of **1e**, the last three amide NHs in all peptide variants display temperature coefficients that are always less than 3 ppb/K, diagnostic of solvent shielded amide protons[6]. Accordingly all variants display evidence for intramolecular H-bonds as required in the type II′ turn initiated 3_{10} like protohelix.

An appreciable increase in the coefficients occurs in **1d** or **1f** in which Val or Ala replaces Gly at N+1 cap position and in **2e** or **2f** in which Pro or Aib replace its Ala at N cap position suggest that these structure variations either distort the protohelix or cause partial unwinding.

NOE patterns in $CDCl_3$-DMSO-d_6 mixture: All diagnostic NOEs in specific peptide variants under this solvent condition are summarized in **Table III**. Many of the diagnostic NOEs[7] appear in all the peptide variants except in **1e**. With a single weak d_{NN} NOE between its Lys and NHMe, **1e** appears to be largely unordered in consonance with its largely solvent exposed NHs under DMSO-d_6. All the expected $d_{NN}(i, i+1)$ NOEs spanning the segment Xxx-NHMe are observed in every tetra-peptide variant (**Table III**), however, the long range

Table I — ^{1}H NMR chemical shifts (δ, ppm) in $CDCl_3$:DMSO-d_6 (6:1) mixture

Residue	NH	$C^{\alpha}H$	$C^{\beta}H$	$C^{\gamma}H$	$C^{\delta}H$	$C^{\varepsilon}H$
1b						
(D)Glu	8.95	4.01	1.05/1.82	2.39/2.22	—	—
(L)Ala	8.82	4.01	1.41	—	—	—
(L)Ala	7.56	4.18	1.46	—	—	—
(L)Lys	7.54	3.98	1.77	1.54/1.17	1.72	2.95/2.78
NHMe	7.01	2.67	—	—	—	—
1d						
(D)Glu	8.15	4.10	1.94	2.26/2.52	—	—
(L)Ala	8.30	3.92	1.35	—	—	—
(L)Val	7.38	3.60	2.05	1.00	—	—
(L)Lys	7.57	4.12	1.95	2.00/1.65	1.70	2.85/2.75
NHMe	7.03	2.70	—	—	—	—
1e						
(D)Glu	7.02	4.14	1.86	2.20	—	—
(L)Ala	8.46	4.49	1.31	—	—	—
(L)Pro	—	4.38	2.20/1.88	1.93	3.59	—
(L)Lys	7.99	4.36	1.79	1.88	1.79	2.88
NHMe	7.63	2.67	—	—	—	—
1f						
(D)Glu	8.32	3.91	2.06/2.05	2.32/2.25	—	—
(L)Ala	8.52	3.89	1.43	—	—	—
Aib	7.36	—	1.52/1.43	—	—	—
(L)Lys	7.58	3.95	1.96/1.66	1.96/1.66	1.96/1.66	2.94/2.80
NHMe	6.98	2.74	—	—	—	—
2b						
(D)Glu	8.65	4.09	1.94	2.26/2.52	—	—
Gly	8.94	3.70/3.92	—	—	—	—
Gly	8.33	3.58/4.12	—	—	—	—
(L)Lys	7.61	4.17	1.90	1.30	1.70	2.81/2.95
NHMe	6.98	2.74	—	—	—	—
2c						
(D)Glu	8.65	4.02	1.91	2.25/2.52	—	—
(L)Leu	8.47	4.07	1.66	1.81	0.96	—
Gly	8.23	3.54/4.07	—	—	—	—
(L)Lys	7.56	4.18	2.00	1.35	1.70	2.70
NHMe	7.02	2.75	—	—	—	—
2d						
(D)Glu	9.00	4.15	1.90	2.22/2.50	—	—
(L)Val	8.58	3.94	2.22	1.05	—	—
Gly	8.17	3.50/4.00	—	—	—	—
(L)Lys	7.64	4.08	1.90	1.40	1.70 2.78/2.96	
NHMe	7.03	2.72	—	—	—	—
2e						
(D)Glu	9.25	4.19	1.92	2.30/2.52	—	—
(L)Pro	—	—	4.37	2.12/2.40	2.15 3.70/4.08	

—Contd

Table I — ^{1}H NMR chemical shifts (δ, ppm) in $CDCl_3$:DMSO-d_6 (6:1) mixture—(*Contd*)

Residue	NH	$C^{\alpha}H$	$C^{\beta}H$	$C^{\gamma}H$	$C^{\delta}H$	$C^{\varepsilon}H$
Gly	7.90	3.46/4.03	—	—	—	—
(L)Lys	7.68	4.05	1.78	1.52	1.65 2.80/2.98	
NHMe	6.95	2.71	—	—	—	—
2f						
(D)Glu	9.05	3.95	1.90	2.28/2.50	—	—
(L)Aib	8.54	—	1.45/1.52	—	—	—
Gly	8.18	3.50/4.00	—	—	—	—
(L)Lys	7.92	4.15	2.00	1.40	1.75 2.80/3.00	
NHMe	7.08	2.75	—	—	—	—

Table II — NH chemical shifts, coupling constants and the amide temperature coefficients. $CDCl_3$ refers to $CDCl_3$-DMSO-d_6 (6:1)

Residues	δ NH (ppm)		$^3J_{NH\alpha}$ (Hz)		dδ/dT (ppm/k)
	$CDCl_3$	DMSO-d_6	$CDCl_3$	DMSO-d_6	DMSO-d_6
1a					
(D)Glu	8.79	9.15	—	—	16.3
(L)Ala	8.74	8.95	4.7	4.9	8.0
Gly	8.20	8.14	*J* (AX) 5.6	5.6	0.5
			J (BX) 6.6	6.3	
(L)Lys	7.60	7.50	7.6	7.2	0.0
NHMe	6.99	7.20	—	—	0.0
1b					
(D)Glu	8.95	8.98	—	3.3	12.5
(L)Ala	8.82	8.83	4.3	4.5	6.6
(L)Ala	7.56	7.58	7.2	7.5	1.7
(L)Lys	7.54	7.48	6.6	6.6	0.2
NHMe	7.01	7.20	—	—	0.1
1c					
(D)Glu	8.65	9.08	—	—	10.0
(L)Ala	8.32	8.88	6.0	6.0	7.4
(L)Leu	7.25	7.52	7.5	8.1	0.0
(L)Lys	7.40	7.47	6.9	6.3	0.0
NHMe	6.75	7.18	—	—	0.8
1d					
(D)Glu	8.15	7.75	—	—	9.0
(L)Ala	8.30	8.38	—	5.4	5.9
(L)Val	7.38	7.72	6.6	8.1	1.0
(L)Lys	7.57	7.78	6.0	7.2	1.7
NHMe	7.03	7.55	—	—	1.8
1e					
(D)Glu	7.02	7.02	—	6.6	7.5
(L)Ala	8.46	8.32	6.0	7.2	4.6
(L)Pro	—	—	—	—	—
(L)Lys	7.99	7.97	7.5	8.4	5.4
NHMe	7.63	7.72	—	—	4.3

—*Contd*

Table II — NH chemical shifts, coupling constants and the amide temperature coefficients. $CDCl_3$ refers to $CDCl_3$-DMSO-d_6 (6:1)—*Contd*

Residues	δ NH (ppm)		$^3J_{NH\alpha}$ (Hz)		dδ/dT (ppm/k)
	$CDCl_3$	DMSO-d_6	$CDCl_3$	DMSO-d_6	DMSO-d_6
1f					
(D)Glu	8.32	8.46	3.9	4.8	10.7
(L)Ala	8.52	8.65	3.3	3.6	5.9
Aib	7.36	7.49	—	—	1.6
(L)Lys	7.58	7.47	6.9	6.9	2.8
NHMe	7.38	7.39	—	—	0.6
2b					
(D)Glu	8.65	8.23	—	5.3	15.0
Gly	8.94	8.76	*J* (AX) 4.1	4.7	8.0
			J (BX) 6.2	6.0	
Gly	6.9	—	*J* (AX) 5.8	5.9	0.5
			J (BX) 6.3	6.4	
(L)Lys	7.61	8.68	7.6	7.9	0.0
NHMe	6.98	7.51	—	—	0.0
2c					
(D)Glu	8.65	8.90	—	—	16.0
(L)Leu	8.47	8.72	3.7	5.9	8.4
Gly	8.23	8.19	*J* (AX) 5.5	6.0	0.1
			J (BX)5.9	5.6	
(L)Lys	7.56	7.48	7.7	7.3	0.0
NHMe	7.02	7.31	—	—	0.0
2d					
(D)Glu	9.00	8.43	—	4.2	12.0
(L) Val	8.58	8.39	4.5	6.2	7.5
Gly	8.17	8.23	*J* (AX) 5.9	5.6	0.6
			J (BX)6.4	6.0	
(L)Lys	7.64	7.64	7.2	7.7	0.5
NHMe	7.03	7.44	—	—	0.0
2e					
(D)Glu	9.25	9.35	—	—	6.6
(L)Pro	—	—	—	—	—
Gly	7.90	7.80	*J* (AX) 5.5	5.5	0.3
			J (BX)6.9	6.6	
(L)Lys	7.68	7.57	7.5	7.3	1.5
NHMe	6.95	7.14	—	—	1.2
2f					
(D)Glu	9.05	9.20	—	2.7	8.0
(L)Aib	8.54	8.99	—	—	6.8
Gly	8.18	8.13	*J* (AX) 5.7	6.3	0.0
			J (BX)6.0	—	
(L)Lys	7.92	7.74	7.8	7.5	1.7
NHMe	7.08	7.13	—	—	0.3

Table III — Observed NOE connectivities in peptides **1b** to **1f** in $CDCl_3$:DMSO-d_6 (6:1) mixture

NOE connectivities	Observed NOEs
1a (D)E_1-A_2-G_3-K_4-NHMe	
d_{NN}(i, i+1)	A_2-G_3, G_3-K_4, K_4-NHMe
$d_{\alpha N}$(i, i+1)	E_1-A_2, A_2-G_3, G_3-K_4, K_4-NHMe
$d_{\alpha N}$(i, i+2)	E_1-G_3, A_2-K_4, G_3-NHMe
$d_{\alpha N}$(i, i+3)	A_2-NHMe
1b (D)E_1-A_2-A_3-K_4-NHMe	
d_{NN}(i, i+1)	A_2-A_3, A_3-K_4, K_4-NHMe
$d_{\alpha N}$(i, i+1)	E_1-A_2, A_2-A_3, A_3-K_4, K_4-NHMe
$d_{\alpha N}$(i, i+2)	E_1-G_3, A_2-K_4, A_3-NHMe
$d_{\alpha N}$(i, i+3)	A_2-NHMe
1c (D)E_1-A_2-L_3-K_4-NHMe	
d_{NN}(i, i+1)	A_2-L_3, L_3-K_4, K_4-NHMe
$d_{\alpha N}$(i, i+1)	E_1-A_2, A_2-L_3, L_3-K_4, K_4-NHMe
$d_{\alpha N}$(i, i+2)	A_2-K_4, L_3-NHMe
$d_{\alpha N}$(i, i+3)	A_2-NHMe
1d (D)E_1-A_2-V_3-K_4-NHMe	
d_{NN}(i, i+1)	A_2-V_3, V_3-K_4, K_4-NHMe
$d_{\alpha N}$(i, i+1)	E_1-A_2, A_2-V_3, V_3-K_4, K_4-NHMe
$d_{\alpha N}$(i, i+2)	E_1-G_3
$d_{\alpha N}$(i, i+3)	—
1e (D)E_1-A_2-P_3-K_4-NHMe	
d_{NN}(i, i+1)	K_4-NHMe
$d_{\alpha N}$(i, i+1)	E_1-A_2, P_3-K_4, K_4-NHMe
$d_{\alpha N}$(i, i+2)	—
$d_{\alpha N}$(i, i+3)	—
1f (D)E_1-A_2-B_3-K_4-NHMe	
d_{NN}(i, i+1)	A_2-B_3, B_3-K_4, K_4-NHMe
$d_{\alpha N}$(i, i+1)	E_1-A_2, A_2-B_3, B_3-K_4, K_4-NHMe
$d_{\alpha N}$(i, i+2)	—
$d_{\alpha N}$(i, i+3)	—
2b (D)E_1-G_2-G_3-K_4-NHMe	
d_{NN}(i, i+1)	G_2-G_3, G_3-K_4, K_4-NHMe
$d_{\alpha N}$(i, i+1)	E_1-G_2, G_2-G_3, G_3-K_4, K_4-NHMe
$d_{\alpha N}$(i, i+2)	E_1-G_3
$d_{\alpha N}$(i, i+3)	—
2c (D)E_1-L_2-G_3-K_4-NHMe	
d_{NN}(i, i+1)	L_2-G_3, G_3-K_4, K_4-NHMe
$d_{\alpha N}$(i, i+1)	E_1-L_2, L_2-G_3, G_3-K_4, K_4-NHMe
$d_{\alpha N}$(i, i+2)	—
$d_{\alpha N}$(i, i+3)	—
2d (D)E_1-V_2-G_3-K_4-NHMe	
d_{NN}(i, i+1)	V_2-G_3, G_3-K_4, K_4-NHMe
$d_{\alpha N}$(i, i+1)	E_1-V_2, V_2-G_3, G_3-K_4, K_4-NHMe
$d_{\alpha N}$(i, i+2)	E_1-G_3, V_2-K_4, G_3- NHMe
$d_{\alpha N}$(i, i+3)	—
2e (D)E_1-P_2-G_3-K_4-NHMe	
d_{NN}(i, i+1)	G_3-K_4, K_4-NHMe
$d_{\alpha N}$(i, i+1)	P_2-G_3, G_3-K_4, K_4-NHMe
$d_{\alpha N}$(i, i+2)	P_2-K_4, G_3-NHMe
$d_{\alpha N}$(i, i+3)	P_2-NHMe
2f (D)E_1-B_2-G_3-K_4-NHMe	
d_{NN}(i, i+1)	B_2-G_3, G_3-K_4, K_4-NHMe
$d_{\alpha N}$(i, i+1)	E_1-B_2, B_2-G_3, G_3-K_4, K_4-NHMe
$d_{\alpha N}$(i, i+2)	—
$d_{\alpha N}$(i, i+3)	—

NOEs $d_{\alpha N}$(i, i+2) and $d_{\alpha N}$(i, i+3) do not always appear. At the extreme, only the d_{NN}(i,i+1) NOEs appear in **1f**, **2e**, and **2f**, while nearly all the expected long range NOEs also appear in the **1b** and **2d**. The non sequential $d_{\alpha N}$ distances in the peptides could depend on the degree of ordering of their protohelical segments. All the long range NOE's in **1b** persist on introducing Gly in its position Yyy (**1a**), while most vanish on introducing Gly in its position Xxx as well (**2a**). A reasonable interpretation is that peptide **2a** with Gly-Gly segment is more disordered than **1a** with Ala-Gly segment, hence $d_{\alpha N}$ NOEs do not appear in **2a**. A corollary is that **1f**, with Aib-Gly segment, should be even more ordered than **1b**, and should therefore display all the $d_{\alpha N}$ NOEs; only the d_{NN} (i, i+1) NOEs are, however, observed in peptide **1f**. Similarly, with Ala-Gly as its central residue, **1a** not only reveals all the long range NOEs but it also has its amide coefficients close to zero. In contrast, the presumably more rigid **2e** and **2f**, with Pro-Gly and Aib-Gly segments, not only reveal lesser number of long range NOEs but also display amide coefficients that are appreciably larger than zero. Clearly, besides the rigidity of a peptide, specific stereochemical distortions appear to be also involved in determining the long range NOEs and the degree of solvent accessibility of the essentially H-bonded amide protons. In particular, Pro and Aib appear to either disrupt or distort the protohelix as N cap or N+1 cap residues.

$^3J_{NHC\alpha}$ coupling constants: Helical ϕ torsional biases were observed from Xxx onwards in all variants, except **1e**, in the $^3J_{NHC\alpha}$ coupling data summarized in **Table III**. The partial randomization of a peptide on its transfer from $CDCl_3$-DMSO-d_6 mixture into pure DMSO-d_6 is expected to manifest in a concomitant increase in most of its $^3J_{NHC\alpha}$ coupling constants. Generally increased coupling constants under DMSO-d_6 are, however, only observed in peptides **1d, 1e, 2b** and **2d**. Of these **1e** features Pro as N+1 cap residue, while **1d**, **2b** and **2d** feature Val or Gly as N cap and / or N+1 cap residue. All are thus associated with putative helix disruptures and could, therefore, partially unwind when challenged with the more polar solvent. Indeed, **1d**, **2b** and **2d** are partially unwound when transferred into pure DMSO-d_6. A relatively broad signal is generally observed for NH which broadens further on temperature increase in DMSO-d_6. The coupling constant for this signal in peptide **1e**, that lacks any discernible backbone fold, is 6.6 Hz under DMSO-d_6 but unobservable under $CDCl_3$-DMSO-d_6 mixture. The Glu_1 coupling constant in rest of the peptides, when observable spans the range 2.7-5.3 Hz, with an average of 4.1 Hz, which corresponds to the ϕ torsional angle 62° based on Karplus type relationship[8]. This value is close to first corner ϕ torsional angle of 60° in a standard type II′ turn[9] that has been earlier proposed[1,2] for the segment Boc-Glu-Xxx –Yyy in the peptides.

The *J* value at Xxx_2 under $CDCl_3$-DMSO-d_6 as well as pure DMSO-d_6 spans the range 3.3-6.0 Hz with the average of 4.6 Hz. This value corresponds to the ϕ torsional angle - 65°, in close approximation of the standard 3_{10} or type III torsional angle[10]. An appreciably smaller ϕ torsional angle is however implied in **1f**, which suggest a specific distortioning of the protohelix on placement of Aib as its N+1 cap residue. On the transfer into the more polar solvent the value at Xxx remains either unchanged (in **1c**), or increases marginally (0.2-0.3 Hz, in **1a**, **1b** and **1f)**, and shows an appreciable increase only in the variant **2c** (2.2 Hz) and **2d** (1.7 Hz). The relatively stronger perturbation at this position in **2d** is attributable to its partial unwinding since rest of its *J* values also increases, while in **2c** it may be the result of a specific protohelix distortioning since the Lys_4 coupling constant is concomitantly reduced. Clearly, the variants that appear to be appreciably ordered on other evidence reveal only minor ϕ torsional perturbation at N cap position on the solvent substitution.

At Yyy_3, the *J* values are in the range 5.5 to 7.5 Hz under $CDCl_3$-DMSO-d_6 mixture, but the average is 5.7 Hz corresponding to ϕ torsional angle of -75° when Yyy_3 is a Gly, and 7.1 Hz corresponding to ϕ torsional angle of -85° when Yyy is a non Gly. Clearly, the ϕ torsional angle approaches the standard 3_{10} value only when Yyy is Gly and is otherwise noticeably enlarged. A further enlargement in this value occurs in some of the variants under DMSO-d_6, presumably because the variants are partially unwound (**1d**, **2b** and **2d**) or are specifically distorted. At Lys_4 the *J* value are between 6.0 to 7.8 Hz but the average is 7.6 Hz (ϕ = 92°) when Yyy = Gly, and 6.4 Hz (ϕ = 80°) when Yyy = Ala, Leu, Val or Aib. Furthermore, the values are generally diminished when the variants are transferred into DMSO-d_6, except in **1d**, **1e**, **2b** and **2d**, which appear to become partially unwound. Thus the ϕ torsional angle at Lys_4 only approaches the 3_{10} value when Yyy is a non Gly and is otherwise appreciably enlarged. In summary, depending on the nature of Yyy position residue, the 3_{10} type protohelix reveals a relatively enlarged ϕ torsional angle at either Yyy_3 or Lys_4, and appears to be appreciably ordered under DMSO-d_6 as well, provided its N cap residue is an Ala, Leu, Pro or Aib, but not Gly or Val, and its N+1 cap residue is an Ala, Leu or Aib, but not Pro, Gly or Val.

Effect of capping position variations on the salt bridge strength

Shift anisotropies of side chain methylene protons: The diastereotopic Lys^{ε} and Glu^{γ} methylene protons normally appear as ill resolved multiplets, but often move further apart as the residues are immobilized on mutual salt bridge formation[1, 2, 11]. On titrating the peptide DMSO-d_6 solutions with $LiClO_4$, the diastereotopic proton resonances always moved progressively closer to each other and collapsed into ill resolved multiplets. Thus, with the notable exception of **1e**, the $Lys_4 \rightarrow Glu_1$ salt bridge is operative in all the variants, even under the relatively stronger dielectric and H-bond disruptive solvent DMSO-d_6.

Dependence of Glu NH shift on salt bridge integrity: A characteristic consequence of salt bridging was the movement of Glu NH to an abnormally downfield position. This effect is manifest in every peptide variant except **1e**. Under $CDCl_3$-

DMSO-d_6 mixture, the Glu NHs are in the range of δ 9.25 and 8.30, while in **1e** it is appreciably upfield at δ 7.02. That the deshielded Glu NH is the specific diagnostic of $Lys_1 \rightarrow Glu_1$ salt bridge is affirmed in the results of solvent substitution and salt titration. The Glu NH response in representative tetrapeptides to the added $LiClO_4$ under DMSO-d_6, to rupture their salt bridges. The Glu NHs shift progressively upfield, and are between δ 0.8 and 1.2 upfield at 2M $LiClO_4$. Clearly all the peptides feature a salt bridge which ruptures on the incremental addition of $LiClO_4$.

The Glu NH shifts in all peptide variants under $CDCl_3$-DMSO-d_6 mixture and pure DMSO-d_6 are summarized in **Table IV**. In **1e**, the variant with no observable salt bridge, the Glu NH shift is practically solvent insensitive. With rest of the variants, an upfield or downfield shift of Glu NH is observed under pure DMSO-d_6 (**Table IV**). The upfield shift, implying rupture of salt bridge, is only observable in peptides **1d**, **2b** and **2d**. Apparently, these ruptures are only partial since in two of the variants **2b** and **2d** the Glu NHs shift further upfield in the presence of $LiClO_4$. The partial rupture of the salt bridge is only evidenced in the variants that feature putative helix destabilizers as N cap and / N+1 cap residues [Val at Yyy (**1d**), Gly at Xxx as well as Yyy (**2b**) and Val at Xxx and Gly at Yyy (**2d**)]. The comparatively deshielded Glu NH under DMSO-d_6 would imply the salt bridges in **1a**, **1b, 1c, 1f, 2c, 2e** and **2f** remain fully integrated as the peptides are transferred from $CDCl_3$-DMSO-d_6 mixture (6:1) to pure DMSO-d_6.

The variants with fully integral salt bridges under DMSO-d_6 could manifest the relative salt bridge strengths in the Glu NH temperature coefficients. The Glu NHs coefficients in such variants, summarized in **Table IV**, vary widely and are often appreciably larger than the coefficient in **1e**, the variant with no salt bridge. The coefficients among peptides with a negative dδ (sol) value, which is taken as evidence for salt bridge integrity in DMSO-d_6 are however, of note. Among the variants in Xxx (**1a**, **2c**, **2e** and **2f**), the coefficients are notably small with Pro and Aib variants, and appreciably larger with Ala and Leu variants. These differences appear to be too large to be only accounted by conformation perturbations, and could manifest the relative salt bridge strengths. Therefore, as N cap residues, Pro and Aib appear to better stabilize the protohelix than Ala and Leu. Amongst the variants in Yyy (**1a**, **1b** and **1c**), the coefficients follow the order, Leu = Ala < Gly. The differences are relatively small; however, the implied order in protohelix stability is in accordance with the nature of Gly as a relative helix disrupter.

Table IV — Glu_1 NH chemical shifts (δ, ppm) in the presence of $LiClO_4$, dδ (solv) and dδ (salt)

Peptide	2M $LiClO_4$ (DMSO-d_6)	dδ (solv)	dδ (salt)
1b	7.36	+0.42	- 0.86
1c	7.73	- 0.25	- 1.17
1d	7.48	+0.57	- 0.95
1e	8.15	- 0.10	- 1.20
1f	8.43	- 0.15	- 0.77

Experimental Section

Melting points were determined on Veego melting point apparatus using the capillary method and are uncorrected. Peptide intermediates were achieved using reported procedures[15] and column purified over 100-200 mesh silica gel. Peptide synthesis was done in solution phase using mixed anhydride coupling method. BOC group was used for the amino protection. The side chain carboxy group of N-terminal glutamic acid was protected as benzyl ester while the side chain amino function of C-terminal lysine was protected by Z-group. The carboxy terminal of lysine was protected as methyl amide. The deprotection of the side chain functional groups was done by catalytic hydrogenation using 10% Pd over charcoal at RT and atmospheric pressure. Homogeneity of peptides and amino acid derivatives were established by TLC on silica gel-G plate using two solvent systems (i) $CHCl_3$-MeOH (9:1), (ii) *n*-BuOH-AcOH-H_2O (4:1:1). The purity of the final product was ascertained by HPLC on an analytical reverse phase column (Lichrosorb RP-18, 5 μm 250 multiply 4 mm) eluting with MeOH or 15% H_2O-MeOH, with the UV detector set at 220 nm. Structure of the peptide intermediates was confirmed by ^1H NMR spectra recorded on JEOL FX 90Q and Varian VXR 300 spectrometer with TMS as internal standard. The 1D and 2D ^1H NMR was obtained from peptides in $CDCl_3$-DMSO-d_6 (6:1) or neat DMSO-d_6. The observed chemical shifts and the line widths were essentially invariant under these solvent conditions at a concentration of 10 mM. No perceptible intermolecular association was thus indicated under these solvent conditions. Chemical shift assignments were largely based on two dimensional COSY and ROESY experiments[9]. Temperature coefficients for the amide resonances in DMSO-d_6 were obtained at six different temperatures over the range 298-323 K.

$^3J_{NH\alpha}$ coupling constants were obtained directly from 1D spectrum. The H, H-COSY spectra[9] were a total of 256 experiments, 16 scans each or more with relaxation delay of 1.5 s, size 1K and with shifted sine bell window multiplication for spectral processing. The ROESY spectra[16] were a total of 512 experiments, 64 scans each or more with relaxation delay of 1.5 s, 300 ms mixing time, size 2K and the spectral processing were with shifted sine bell window multiplication in both the dimensions.

Physical and ^{1}H NMR data

Boc-Lys (*Z*)-NHMe, 1: Yield 90%, R_f (i) 0.95, R_f (ii) 0.93, m.p. 99°C; ^{1}H NMR ($CDCl_3$, 90 MHz): δ 7.65(d, 1H, $N^{\alpha}H$), 7.35 (s, 5H, aromatic protons), 7.2 (t, 1H, $N^{\alpha}H$), 7.0 (broad, 1H, $N^{\alpha}H$), 5.1 (s, 2H, $CH_2C_6H_5$), 4.00 (bs, 1H, Lys $C^{\alpha}H$), 3.25 (broad, 2H, Lys $C^{\varepsilon}H_2$), 2.70 (d, 3H, NHMe), 1.86-1.66 (complex multiplet, 6H, Lys $C^{\beta}H_2$, $C^{\gamma}H_2$, $C^{\delta}H_2$), 1.37 (s, 9H, Boc CH_3).

Boc-Ala-Lys (*Z*)-NHMe, 2: Yield 81%, R_f (i) 0.76, R_f (ii) 0.91, m.p. 84-85°C; ^{1}H NMR ($CDCl_3$, 90 MHz): δ 7.3 (s, 5H, aromatic protons), 6.9-6.5 (broad, 3H, $N^{\alpha}H$), 5.1 (s, 2H, $CH_2C_6H_5$), 4.3 (bs, 2H, Lys $C^{\alpha}H$, Ala $C^{\alpha}H$), 3.2 (m, 2H, Lys $C^{\varepsilon}H_2$), 2.75 (d, 3H, NHMe), 1.86-1.66 (complex multiplet, 6H, Lys $C^{\beta}H_2$, $C^{\gamma}H_2$, $C^{\delta}H_2$), 1.45 (d, 3H, Ala $C^{\beta}H_2$, 1.3 (s, 9H, Boc CH_3).

Boc-Ala-Ala-Lys (*Z*)-NHMe, 3: Yield 72%, R_f (i) 0.58, R_f (ii) 0.83, m.p. 162°C; ^{1}H NMR ($CDCl_3$, 90 MHz): δ 8.0 (broad, 1H, $N^{\alpha}H$), 7.7 (broad, 1H, $N^{\alpha}H$), 7.3 (s, 5H, aromatic protons), 6.4 (broad, 3H, $N^{\alpha}H$), 5.1 (s, 2H, $CH_2C_6H_5$), 4.2-3.8 (m, 3H, Lys $C^{\alpha}H$, Ala $C^{\alpha}H$), 3.25 (m, 2H, Lys $C^{\varepsilon}H_2$), 2.70 (d, 3H, NHMe), 1.86-1.66 (complex multiplet, 6H, Lys $C^{\beta}H_2$, $C^{\gamma}H_2$, $C^{\delta}H_2$), 1.5 (s, 9H, Boc CH_3), 1.4 (dd, 6H, Ala $C^{\beta}H_2$).

Boc-(D)Glu(OBz)-Ala-Ala-Lys(*Z*)-NHMe, 4: Yield 59%, R_f (i) 0.48, R_f (ii) 0.77, m.p. 205°C; ^{1}H NMR (DMSO-d_6, 90 MHz): δ 8.2 (broad, 1H, $N^{\alpha}H$), 7.9 (broad, 1H, $N^{\alpha}H$), 7.3 (s, 10H, aromatic protons), 7.2-7.0 (broad, 3H, $N^{\alpha}H$), 6.5 (broad, 1H, $N^{\alpha}H$), 5.1 (s, 4H, $CH_2C_6H_5$), 4.2-3.8 (m, 4H, Lys $C^{\alpha}H$, Ala $C^{\alpha}H$, Glu $C^{\alpha}H$), 3.1 (m, 2H, Lys $C^{\varepsilon}H_2$), 2.70 (d, 3H, NHMe), 2.3 (m, 2H, Glu $C^{\gamma}H_2$), 1.86-1.66 (complex multiplet, 8H, Glu $C^{\beta}H_2$, Lys $C^{\beta}H_2$, $C^{\gamma}H_2$, $C^{\delta}H_2$), 1.4 (s, 9H, Boc CH_3), 1.3 (dd, 6H, Ala $C^{\beta}H_2$).

Boc-(D)Glu-Ala-Ala-Lys-NHMe, 1b: Yield 94%, R_f (i) 0.40. The ^{1}H NMR data are shown in **Table I**.

Boc-Val-Lys(*Z*)-NHMe, 5: Yield 79%, R_f (i) 0.74, R_f (ii) 0.91, m.p. 98°C; ^{1}H NMR ($CDCl_3$, 90 MHz): δ 7.7(broad, 1H, $N^{\alpha}H$), 7.3 (s, 5H, aromatic protons), 7.2 (broad, 1H, $N^{\alpha}H$), 6.8 (broad, 1H, $N^{\alpha}H$), 5.1 (s, 2H, $CH_2C_6H_5$), 4.00 (m, 2H, Lys $C^{\alpha}H$, Val $C^{\alpha}H$), 3.25 (m, 2H, Lys $C^{\varepsilon}H_2$), 2.65 (d, 3H, NHMe), 1.86-1.66 (complex multiplet, 7H, Val $C^{\beta}H$, Lys $C^{\beta}H_2$, $C^{\gamma}H_2$, $C^{\delta}H_2$), 1.4 (s, 9H, Boc CH_3), 0.9 (dd, 6H, Val $C^{\gamma}H_3$).

Boc-Ala-Val-Lys(*Z*)-NHMe, 6: Yield 59%, R_f (i) 0.56, R_f (ii) 0.81, m.p. 174°C; ^{1}H NMR ($CDCl_3$, 90 MHz): δ 8.0(broad, 1H, $N^{\alpha}H$), 7.3 (s, 5H, aromatic protons), 7.2 (broad, 3H, $N^{\alpha}H$), 6.9 (broad, 1H, $N^{\alpha}H$), 5.1 (s, 2H, $CH_2C_6H_5$), 4.2-3.8(m, 3H, Ala $C^{\alpha}H$, Lys $C^{\alpha}H$, Val $C^{\alpha}H$), 3.25 (m, 2H, Lys $C^{\varepsilon}H_2$), 2.65 (d, 3H, NHMe), 1.86-1.66 (complex multiplet, 7H, Val $C^{\beta}H$, Lys $C^{\beta}H_2$, $C^{\gamma}H_2$, $C^{\delta}H_2$), 1.4 (s, 9H, Boc CH_3), 1.3 (d, 3H, Ala $C^{\beta}H_3$), 0.9 (dd, 6H, Val $C^{\gamma}H_3$).

Boc-(D)Glu(OBz)-Ala-Val-Lys(*Z*)-NHMe, 7: Yield 55%, R_f (i) 0.50, R_f (ii) 0.78, m.p. 188°C; ^{1}H NMR (DMSO-d_6, 90 MHz): δ 8.1(broad, 1H, $N^{\alpha}H$), 7.3 (s, 10H, aromatic protons), 7.2-7.0 (broad, 4H, $N^{\alpha}H$), 6.9 (broad, 1H, $N^{\alpha}H$), 5.1 (s, 4H, $CH_2C_6H_5$), 4.2-3.8(m, 4H, Glu $C^{\alpha}H$, Ala $C^{\alpha}H$, Lys $C^{\alpha}H$, Val $C^{\alpha}H$), 3.25 (m, 2H, Lys $C^{\varepsilon}H_2$), 2.7 (d, 3H, NHMe), 2.5(m, 2H, Glu $C^{\gamma}H_2$), 1.8-1.6 (complex multiplet, 9H, Val $C^{\beta}H$, Glu $C^{\beta}H_2$, Lys $C^{\beta}H_2$, $C^{\gamma}H_2$, $C^{\delta}H_2$), 1.4 (s, 9H, Boc CH_3), 1.3 (d, 3H, Ala $C^{\beta}H_3$), 0.9 (dd, 6H, Val $C^{\gamma}H_3$).

Boc-(D)Glu-Ala-Val-Lys-NHMe, 1d: Yield 91%, R_f (ii) 0.39. The ^{1}H NMR data are shown in **Table I**.

Boc-Pro-Lys(*Z*)-NHMe, 8: Yield 70%, R_f (i) 0.70, R_f (ii) 0.89, m.p. 88°C; ^{1}H NMR ($CDCl_3$, 90 MHz): δ 8.0 (broad, 1H, $N^{\alpha}H$), 7.3 (s, 5H, aromatic protons), 6.9 (broad, 2H, $N^{\alpha}H$), 5.1 (s, 2H, $CH_2C_6H_5$), 4.3 (m, 1H, Pro $C^{\alpha}H$), 3.5 (m, 3H, Lys $C^{\alpha}H$, Pro $C^{\delta}H_2$), 3.2 (m, 2H, Lys $C^{\varepsilon}H_2$), 2.7 (d, 3H, NHMe), 2.1-1.8 (complex multiplet, 10H, Pro $C^{\beta}H_2$, $C^{\gamma}H_2$, Lys $C^{\beta}H_2$, $C^{\gamma}H_2$, $C^{\delta}H_2$), 1.4 (s, 9H, Boc CH_3).

Boc-Ala-Pro-Lys(*Z*)-NHMe, 9: Yield 84%, R_f (i) 0.55, R_f (ii) 0.83, m.p. 91°C; ^{1}H NMR ($CDCl_3$, 90 MHz): δ 8.05 (broad, 1H, $N^{\alpha}H$), 7.35 (s, 5H, aromatic protons), 7.1 (broad, 1H, $N^{\alpha}H$), 6.7 (broad, 2H, $N^{\alpha}H$), 5.1 (s, 2H, $CH_2C_6H_5$), 4.2 (m, 2H, Ala $C^{\alpha}H$, Pro $C^{\alpha}H$), 3.6-3.3 (m, 3H, Lys $C^{\alpha}H$, Pro $C^{\delta}H_2$), 3.2 (m, 2H, Lys $C^{\varepsilon}H_2$), 2.8 (d, 3H, NHMe), 2.1-1.8 (complex multiplet, 10H, Pro $C^{\beta}H_2$, $C^{\gamma}H_2$, Lys $C^{\beta}H_2$, $C^{\gamma}H_2$, $C^{\delta}H_2$), 1.4 (s, 9H, Boc CH_3), 1.3 (d, 3H, Ala $C^{\beta}H_3$).

Boc-(D)Glu(OBz)-Ala-Pro-Lys(*Z*)-NHMe, 10: Yield 55%, R_f (i) 0.46, R_f (ii) 0.60, m.p. 142°C; ^{1}H NMR ($CDCl_3$, 90 MHz): δ 8.2 (broad, 1H, $N^{\alpha}H$), 7.3 (s, 10H, aromatic protons), 7.1 (broad, 1H, $N^{\alpha}H$), 6.7 (broad, 3H, $N^{\alpha}H$), 5.1 (s, 4H, $CH_2C_6H_5$), 4.2 (m, 3H, Glu $C^{\alpha}H$, Ala $C^{\alpha}H$, Pro $C^{\alpha}H$), 3.6-3.3 (m, 3H, Lys $C^{\alpha}H$, Pro $C^{\delta}H_2$), 3.2 (m, 2H, Lys $C^{\varepsilon}H_2$), 2.8 (d, 3H,

NHMe), 2.4 (m, 2H, Glu $C^{\gamma}H_2$), 2.1-1.8 (complex multiplet, 12H, Glu $C^{\beta}H_2$, Pro $C^{\beta}H_2$, $C^{\gamma}H_2$, Lys $C^{\beta}H_2$, $C^{\gamma}H_2$, $C^{\delta}H_2$), 1.4 (s, 9H, Boc CH_3), 1.3 (d, 3H, Ala $C^{\beta}H_3$).

Boc-(D)Glu-Ala-Pro-Lys-NHMe, 1e: Yield 89%, R_f (ii) 0.40. The ^{1}H NMR data are shown in **Table I**.

Boc-Aib-Lys(*Z*)-NHMe, 11: Yield 2.88 g 79%, R_f (i) 0.73, R_f (ii) 0.90; ^{1}H NMR ($CDCl_3$, 90 MHz): δ 8.0(broad, 1H, $N^{\alpha}H$), 7.3 (s, 5H, aromatic protons), 6.9 (s, 1H, $N^{\alpha}H$), 6.5 (broad, 1H, $N^{\alpha}H$), 5.1 (s, 2H, $CH_2C_6H_5$), 3.7 (m, 1H, Lys $C^{\alpha}H$), 3.2 (m, 2H, Lys $C^{\varepsilon}H_2$), 2.7 (d, 3H, NHMe), 2.1-1.8 (complex multiplet, 6H, Lys $C^{\beta}H_2$, $C^{\gamma}H_2$, $C^{\delta}H_2$), 1.4 (s, 15H, Boc CH_3, Aib $C^{\beta}H_3$).

Boc-Ala-Aib-Lys(*Z*)-NHMe, 12: Yield 77%, R_f (i) 0.52, R_f (ii) 0.75; ^{1}H NMR ($CDCl_3$, 90 MHz): δ 7.9 (broad, 1H, $N^{\alpha}H$), 7.25 (s, 5H, aromatic protons), 7.0-6.8 (broad, 4H, $N^{\alpha}H$), 5.0 (s, 2H, $CH_2C_6H_5$), 3.8 (m, 2H, Ala $C^{\alpha}H$, Lys $C^{\alpha}H$), 3.2 (m, 2H, Lys $C^{\varepsilon}H_2$), 2.7 (d, 3H, NHMe), 2.1-1.8 (complex multiplet, 6H, Lys $C^{\beta}H_2$, $C^{\gamma}H_2$, $C^{\delta}H_2$), 1.35 (s, 15H, Boc CH_3, Aib $C^{\beta}H_3$), 1.25 (d, 3H, Ala $C^{\beta}H_3$).

Boc-(D)Glu(OBz)-Ala-Aib-Lys(*Z*)-NHMe, 13: Yield 50%, R_f (i) 0.47, R_f (ii) 0.62, m.p. 135°C; ^{1}H NMR ($CDCl_3$, 90 MHz): δ 8.2 (broad, 2H, $N^{\alpha}H$), 7.4 (s, 10H, aromatic protons), 7.0 (broad, 3H, $N^{\alpha}H$), 6.5 (broad, 1H, $N^{\alpha}H$), 5.1 (s, 4H, $CH_2C_6H_5$), 4.2-4.0 (m, 3H, Glu $C^{\alpha}H$, Ala $C^{\alpha}H$, Lys $C^{\alpha}H$), 3.1 (m, 2H, Lys $C^{\varepsilon}H_2$), 2.6 (d, 3H, NHMe), 2.3 (m, 2H, Glu $C^{\gamma}H_2$), 2.1-1.6 (complex multiplet, 8H, Glu $C^{\beta}H_2$, Lys $C^{\beta}H_2$, $C^{\gamma}H_2$, $C^{\delta}H_2$), 1.4 (s, 15H, Boc CH_3, Aib $C^{\beta}H_3$), 1.3 (d, 3H, Ala $C^{\beta}H_3$).

Boc-(D)Glu-Ala-Aib-Lys-NHMe, 1f: Yield 90%, R_f(ii) 0.40. The ^{1}H NMR data are shown in **Table I**.

Boc-Gly-Lys (*Z*)-OMe, 14: Yield 86%, R_f (i) 0.35, R_f (ii) 0.83; ^{1}H NMR ($CDCl_3$, 90 MHz): δ 7.8(d, 1H, $N^{\alpha}H$), 7.6 (d, 1H, $N^{\alpha}H$), 7.35 (s, 5H, aromatic protons), 7.2 (t, 1H, $N^{\varepsilon}H$-CO- $CH_2C_6H_5$), 5.1 (s, 2H, $CH_2C_6H_5$), 4.7-4.3 (bs, 1H, $C^{\alpha}H$), 3.8 (d, 2H, Gly $C^{\alpha}H_2$), 3.7 (s, 3H, OCH_3), 3.25-3.0 (bs, 2H, Lys $C^{\varepsilon}H_2$), 1.9-1.5 (complex multiplet, 4H, Lys $C^{\beta}H_2$, $C^{\delta}H_2$), 1.4 (s, 9H, Boc CH_3), 1.2-1.0 (multiplet, 2H, Lys $C^{\gamma}H_2$).

Boc-Gly-Lys (*Z*)-NHMe, 15: Yield 90%, R_f (i) 0.42, R_f (ii) 0.80, m.p. 78-80°C; ^{1}H NMR ($CDCl_3$, 90 MHz): δ 8.1(s, 1H, $N^{\alpha}H$), 7.5 (s, 1H, $N^{\alpha}H$), 7.35 (s, 5H, aromatic protons), 7.15 (s, 1H, $N^{\varepsilon}H$-$COCH_2C_6H_5$), 5.1 (s, 2H, $CH_2C_6H_5$), 4.3 (bs, 1H, $C^{\alpha}H$), 3.8 (d, 2H, Gly $C^{\alpha}H_2$), 3.25-3.0 (bs, 2H, Lys $C^{\varepsilon}H_2$), 2.65 (d, 3H, $NHCH_3$), 1.9-1.5 (complex multiplet, 4H, Lys $C^{\beta}H_2$, $C^{\delta}H_2$), 1.4 (s, 9H, Boc CH_3), 1.2-1.0 (multiplet, 2H, Lys $C^{\gamma}H_2$).

Boc-Gly-Gly-Lys (*Z*)-NHMe, 16: Yield 87%, R_f (i) 0.50, R_f (ii) 0.80, m.p. 112-14°C; ^{1}H NMR ($CDCl_3$, 90 MHz): δ 8.0-7.8(b, 2H, $N^{\alpha}H$), 7.6 (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, $CH_2C_6H_5$), 4.2-4.0 (complex, 3H, Lys$C^{\alpha}H$, Gly $C^{\alpha}H$), 3.8 (m, 2H, Gly $C^{\alpha}H_2$), 2.95 (b, 2H, Lys $C^{\varepsilon}H_2$), 2.7 (d, 3H, $NHCH_3$), 1.9-1.5 (complex multiplet, 4H, Lys $C^{\beta}H_2$, $C^{\delta}H_2$), 1.4 (s, 9H, Boc CH_3), 1.2-1.0 (multiplet, 2H, Lys $C^{\gamma}H_2$).

Boc-(D)Glu(OBz)-Gly-Gly-Lys (*Z*)-NHMe, 17: Yield 87%, R_f (i) 0.48, R_f (ii) 0.84, m.p. 130-32°C; ^{1}H NMR ($CDCl_3$, 90MHz): δ 8.0-7.8(b, 2H, $N^{\alpha}H$), 7.7-7.5 (b, 1H, $N^{\alpha}H$), 7.3 (s, 5H, aromatic protons), 7.0 (s, 1H, $N^{\varepsilon}H$-$COCH_2C_6H_5$), 6.8 (d, 1H, $N^{\alpha}H$), 5.1 (s, 2H, $CH_2C_6H_5$), 4.2-4.0 (complex, 4H, Glu $C^{\alpha}H$, Lys $C^{\alpha}H$, Gly $C^{\alpha}H$), 3.8 (m, 2H, Gly $C^{\alpha}H_2$), 2.95 (b, 2H, Lys $C^{\varepsilon}H_2$), 2.7 (d, 3H, $NHCH_3$), 2.4(t, 2H, Glu $C^{\gamma}H$), 1.9-1.5 (complex multiplet, 6H, Glu $C^{\beta}H_2$, Lys $C^{\beta}H_2$, $C^{\delta}H_2$), 1.4 (s, 9H, Boc CH_3), 1.2-1.0 (multiplet, 2H, Lys $C^{\gamma}H_2$).

Boc-(D)Glu-Gly-Gly-Lys-NHMe, 2a: Yield 90%, R_f (ii) 0.42, The ^{1}H NMR data are shown in **Table I**.

Boc-Leu-Gly-Lys (*Z*)-NHMe, 18: Yield 90%, R_f (i) 0.50, R_f (ii) 0.86, m.p. 112-14°C; ^{1}H NMR ($CDCl_3$, 90 MHz): δ 8.2(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), 7.2 (s, 1H, $N^{\varepsilon}H$-$COCH_2C_6H_5$), 5.1 (s, 2H, $CH_2C_6H_5$), 4.5-3.8 (complex, 4H, Lys $C^{\alpha}H$, Gly $C^{\alpha}H$, Leu $C^{\alpha}H$), 3.2-3.0 (b, 2H, Lys $C^{\varepsilon}H_2$), 2.7 (d, 3H, $NHCH_3$), 1.9-1.5 (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, Boc CH_3), 0.85 (dd, 6H, Leu 2 × $C^{\delta}H_3$).

Boc-(D)Glu(OBz)-Leu-Gly-Lys (*Z*)-NHMe, 19: Yield 87%, R_f (i) 0.60, R_f (ii) 0.90, m.p. 62-163°C; ^{1}H NMR ($CDCl_3$, 90 MHz): δ 8.2(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), 7.2 (s, 1H, $N^{\varepsilon}H$-$COCH_2C_6H_5$), 7.0 (d, 1H, $N^{\alpha}H$), 5.1 (s, 2H, $CH_2C_6H_5$), 4.5-3.8 (complex, 5H, Glu $C^{\alpha}H$, Lys $C^{\alpha}H$, Gly $C^{\alpha}H$, Leu $C^{\alpha}H$), 3.2-3.0 (b, 2H, Lys $C^{\varepsilon}H_2$), 2.7 (d, 3H, $NHCH_3$), 2.5 (t, 2H, Glu $C^{\gamma}H_2$), 1.9-1.5 (complex multiplet, 11H, Glu $C^{\beta}H_2$, 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, Boc CH_3), 0.85 (dd, 6H, Leu 2 × $C^{\delta}H_3$).

Boc-(D)Glu-Leu-Gly-Lys-NHMe, 2c: Yield 90%, R_f (ii) 0.40, The ^{1}H NMR data are shown in **Table I**.

Boc-Val-Gly-Lys (*Z*)-NHMe, 20: Yield 93%, R_f (i) 0.52, R_f (ii) 0.84, m.p. 166-168°C; ^{1}H NMR ($CDCl_3$, 90 MHz): δ 8.2-8.0 (b, 2H, $N^{\alpha}H$), 7.6 (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, $CH_2C_6H_5$), 4.2-4.0 (complex, 2H, Lys $C^{\alpha}H$, Val $C^{\alpha}H$), 3.8 (m, 2H, Gly

$C^{\alpha}H$), 2.95 (b, 2H, Lys $C^{\varepsilon}H_2$), 2.7 (d, 3H, $NHCH_3$), 2.2 (m, 1H, Val $C^{\beta}H$), 1.9-1.5 (complex multiplet, 4H, Lys $C^{\beta}H_2$, $C^{\delta}H_2$), 1.4 (s, 9H, Boc CH_3), 1.2-1.0 (m, 2H, Lys $C^{\gamma}H_2$), 0.9 (dd, 6H, Val 2 × $C^{\gamma}H_3$).

Boc-(D)Glu(OBz)-Val-Gly-Lys (*Z*)-NHMe, 21: Yield 82%, R_f (i) 0.60, R_f (ii) 0.90, m.p. 178-80°C; ^{1}H NMR ($CDCl_3$, 90 MHz): δ 8.2-8.0 (b, 2H, $N^{\alpha}H$), 7.6 (s, 1H, $N^{\alpha}H$), 7.3 (s, 5H, aromatic protons), 7.1 (s, 1H, $N^{\varepsilon}H$-$COCH_2C_6H_5$), 6.8 (d, 1H, $N^{\alpha}H$), 5.1 (s, 2H, $CH_2C_6H_5$), 4.2-4.0 (complex, 3H, Glu $C^{\alpha}H$, Lys $C^{\alpha}H$, Val $C^{\alpha}H$), 3.8 (m, 2H, Gly $C^{\alpha}H_2$), 2.95 (b, 2H, Lys $C^{\varepsilon}H_2$), 2.7 (d, 3H, $NHCH_3$), 2.4 (t, 2H, Glu $C^{\gamma}H_2$), 2.2 (m, 1H, Val $C^{\beta}H_2$), 1.9-1.5 (complex multiplet, 6H, Glu $C^{\beta}H_2$, Lys $C^{\beta}H_2$, $C^{\delta}H_2$), 1.38 (s, 9H, Boc CH_3), 1.2-1.0 (m, 2H, Lys $C^{\gamma}H_2$), 0.9 (dd, 6H, Val 2 × $C^{\gamma}H_3$).

Boc-(D)Glu-Val-Gly-Lys-NHMe, 2d: Yield 86%, R_f (ii) 0.36, The ^{1}H NMR data are shown in **Table I**.

Boc-Pro-Gly-Lys (*Z*)-NHMe, 22: Yield 80%, R_f (i) 0.50, R_f (ii) 0.84, m.p. 112-14°C; ^{1}H NMR ($CDCl_3$, 90 MHz): δ 8.1(s, 1H, $N^{\alpha}H$), 7.5 (s, 1H, $N^{\alpha}H$), 7.3 (s, 5H, aromatic protons), 7.1 (s, 1H, $N^{\varepsilon}H$-$COCH_2C_6H_5$), 5.1 (s, 2H, $CH_2C_6H_5$), 4.7-4.0 (complex, 4H, Lys $C^{\alpha}H$, Gly $C^{\alpha}H$, Pro $C^{\alpha}H$), 3.8 (m, 2H, Pro $C^{\delta}H_2$), 3.0 (b, 2H, Lys $C^{\varepsilon}H_2$), 2.7 (d, 3H, $NHCH_3$), 2.4 (t, 2H, Glu $C^{\gamma}H_2$), 1.9-1.5 (complex multiplet, 10H, Glu $C^{\beta}H_2$, Lys $C^{\beta}H_2$, $C^{\delta}H_2$, Pro $C^{\beta}H_2$, $C^{\gamma}H$), 1.4 (s, 9H, Boc CH_3), 1.2-1.0 (m, 2H, Lys $C^{\gamma}H_2$).

Boc-(D)Glu(OBz)-Pro-Gly-Lys (*Z*)-NHMe, 23: Yield 85%, R_f (i) 0.56, R_f (ii) 0.88, ^{1}H NMR ($CDCl_3$, 90 MHz): δ 8.1(s, 1H, $N^{\alpha}H$), 7.5 (s, 1H, $N^{\alpha}H$), 7.3 (s, 5H, aromatic protons), 7.1 (s, 1H, $N^{\varepsilon}H$-$COCH_2C_6H_5$), 6.9 (s, 1H, $N^{\alpha}H$), 5.1 (s, 2H, $CH_2C_6H_5$), 4.7-4.0 (complex, 5H, Glu $C^{\alpha}H$, Lys $C^{\alpha}H$, Gly $C^{\alpha}H$, Leu $C^{\alpha}H$), 3.8 (m, 2H, Pro $C^{\delta}H_2$), 3.0 (b, 2H, Lys $C^{\varepsilon}H_2$), 2.7 (d, 3H, $NHCH_3$), 2.4 (t, 2H, Glu $C^{\gamma}H_2$), 1.9-1.5 (complex multiplet, 10H, Glu $C^{\beta}H_2$, Lys $C^{\beta}H_2$, $C^{\delta}H_2$, Pro $C^{\beta}H_2$, $C^{\gamma}H$), 1.4 (s, 9H, Boc CH_3), 1.2-1.0 (m, 2H, Lys $C^{\gamma}H_2$).

Boc-(D)Glu-Pro-Gly-Lys -NHMe, 2e: Yield 90%, R_f (ii) 0.45, The ^{1}H NMR data are shown in **Table I**.

Boc-Aib-Gly-Lys (*Z*)-NHMe, 24: Yield 85%, R_f (i) 0.48, R_f (ii) 0.80, m.p. 95-96°C; ^{1}H NMR ($CDCl_3$, 90 MHz): δ 7.8 (b, 1H, $N^{\alpha}H$), 7.6 (b, 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, $CH_2C_6H_5$), 4.2-4.0 (complex, 3H, Lys $C^{\alpha}H$, Gly $C^{\alpha}H$), 2.95 (b, 2H, Lys $C^{\varepsilon}H_2$), 2.7 (d, 3H, $NHCH_3$), 1.9-1.5 (complex multiplet, 4H, Lys $C^{\beta}H_2$, $C^{\delta}H_2$), 1.4 (s, 9H, Boc CH_3), 1.2-1.0 (singlet overlapped over multiplet, 8H, Aib 2 × $C^{\beta}H_3$, Lys $C^{\gamma}H_2$).

Boc-(D)Glu(OBz)-Aib-Gly-Lys (*Z*)-NHMe, 25: Yield 90%, R_f (i) 0.59, R_f (ii) 0.89; ^{1}H NMR ($CDCl_3$, 90 MHz): δ 7.8 (b, 1H, $N^{\alpha}H$), 7.6 (b, 1H, $N^{\alpha}H$), 7.3 (s, 5H, aromatic protons), 7.0 (s, 1H, $N^{\varepsilon}H$-$COCH_2C_6H_5$), 6.7 (d, 1H, $N^{\alpha}H$), 5.1 (s, 2H, $CH_2C_6H_5$), 4.2-4.0 (complex, 4H, Glu $C^{\alpha}H$, Lys $C^{\alpha}H$, Gly $C^{\alpha}H_2$), 2.95 (b, 2H, Lys $C^{\varepsilon}H_2$), 2.7 (d, 3H, $NHCH_3$), 2.4 (t, 2H, Glu $C^{\gamma}H_2$), 1.9-1.5 (complex multiplet, 6H, Glu $C^{\beta}H_2$, Lys $C^{\beta}H_2$, $C^{\delta}H_2$), 1.4 (s, 9H, Boc CH_3), 1.2-1.0 (singlet overlapped over multiplet, 8H, Aib 2 × $C^{\beta}H_3$, Lys $C^{\gamma}H_2$).

Boc-(D)Glu-Aib-Gly-Lys -NHMe, 2f: Yield 90%, R_f (ii) 0.47, The ^{1}H NMR data are shown in **Table I**.

Conclusion

A 3_{10} type protohelix[1], described in Boc-(D)Glu_1-Xxx_2-Yyy_3-Lys_4-NHMe **1a,** has been varied in what characterizes its N-cap (Xxx) and N+1 cap (Yyy) positions[3b]. The structure variation evoked an interest for possible implications in the phenomenon of helix nucleation[4a,5]. All the variants, except **1e**, feature 4→1 type H-bonds and salt bridge between Lys_4 and Glu_1 and are thus 3_{10} type protohelices endlocked by Boc-D-Glu. Specific deviations from standard 3_{10} like geometry are noted in the relatively enlarged Yyy_3 or Lys_4 ϕ torsional angles in the protohelix depending on the presence or absence of α substituents in its N+1 cap residue.

Evidence gathered from the complimentary results suggest the ranking Pro = Aib > Ala = Leu > Val = Gly for N cap position, and the ranking Aib > Ala = Leu > Val = Gly > Pro for N+1 cap position in the protohelix. Pro, a helix destabilizer as an internal helix residue[3c], disrupts the protohelix when placed as N+1 cap residue, and stabilizes it more favorably than any residue when placed as N cap residue. Aib, a helix stabilizer equally compatible with N cap and internal helix positions[12] also stabilizes the protohelix irrespective of its placement at N cap or N+1 cap position. Clearly, proline and valine with generally poor helix propensity[3] also weakens the protohelix compared to Ala or Leu as N cap as well as N+1 cap residue. Entropy loss on account of diminished rotameric freedom of its β-branched side chain is thought to be the effect that makes Val incompatible as its N cap or N+1 cap residue[3,13]. Gly, a general helix destabilizer[3c,14], ostensibly on account of its appreciable conformational freedom, also destabilizes the protohelix relative to Ala and Leu as N cap residue, and possibly as N+1 cap residue as well.

Thus, broad parallels are noted between the positional helix propensities of the residues examined and their effects on the protohelix stability.

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