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The effect of Glu→Asp substitution on a 3_{10} type fold in Boc-(D)-Glu_1-Ala_2-Gly_3-Lys_4-NHMe

V D Bobade*, P C Mhaske & N D Gaikwad

Department of Chemistry, HPT/RYK Science College Nashik 422 005, India

E-mail: v_bobade@rediffmail.com

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The solution conformation of the peptide Boc-(D)-Asp_1-Ala_2-Gly_3-Lys_4-NHMe based on NMR studies is compared with its (D)-Glu_1 analog reported earlier. Results establish that (D)-Asp analog is devoid of the salt bridge and is a complex ensemble of consecutive folds.

Keywords: D-Amino acid, 3_{10}-helical conformation, helix design, salt bridge, β-turns

IPC: Int.Cl.[8] C07C

A D-amino acid mediated conformational ordering was observed in Boc-(D)-Glu_1-Ala_2-Gly_3-Lys_4-NHMe and in its longer variant Boc-(D)-Glu_1-Ala_2-Gly_3-Lys_4-Ala_5-Leu_6-OMe. The parent and all L-peptides were random coils which on substitution of (D)-Glu_1 became II' turn initiated consecutive 3_{10} turns to the last C-terminal residue[1-3]. This stereochemically promoted ordering of specific main chain conformer apparently involves two independent but mutually cooperative phenomena. A type II' β-turn is stereochemically favored[4] and seems to template the remaining C-side residues in 3_{10} conformation. The Glu_1 γ-carboxylate to Lys_4 ε-ammonium interaction is promoted and locks the specific main chain conformer in a salt bridge interaction. As a means to assess the relative importance of these two essentially independent phenomenon, (D)-Glu_1 in the tetrapeptide Boc-(D) Glu_1-Ala_2-Gly_3-Lys_4-NHMe was substituted with (D)-Asp_1. The stereochemical effect ordering type II' turn element is thus preserved, while the carboxylate forming the salt bridge is drawn away from its counter ion and brought closer to the main chain due to the reduced methylene spacer. An NMR comparison in apolar solvents, which promote appreciable ordering of (D)-Glu_1 analog, reveals that while the (D)-Asp_1 analog is devoid of the salt bridge, its backbone is at least partially ordered and samples alternative conformational folds which Boc-(D)-Asp_1 could, in principle, nucleate by accepting a 3→1 or 4→1 type H-bond to its Boc C=O. This alternative hydrogen bonding pattern seems feasible because the required torsional angles of (D) Asp_1 are its stereochemically favored inverse β region of the Ramachandaran diagram[5,6]. A Boc protected D-amino acid may thus be intrinsically capable of nucleating alternative folds, and the specific ordering of (D)-Glu_1 analog as a 3_{10} type fold appears attributable to the Lys_4 → Glu_1 salt bridge. (D)-Asp_1 analog lacks the salt bridge and appears to thus primarily order as a pseudo α type fold, but only partially and with a severe distortion due to a specific stereochemical conflict.

Results and Discussion

The ^{1}H NMR spectrum of (D)-Asp_1 analog recorded in $CDCl_3$-DMSO-d_6 (6:1) mixture is shown in **Figure 1**. The solvent specific chemical shift assigned with the combined use of 2D COSY and ROESY spectra are in **Table I**.

The Gly_3 $C^{\alpha}H_2$ proton resonance is geminally resolved in $CDCl_3$-DMSO-d_6 mixture but is an ill resolved multiplet in DMSO-d_6. The Asp_1 NH is relatively up field in both the solvents, which is usual for Boc protected urethane type NH[1-3,7]. The $Lys_4C^{\varepsilon}H_2$ methylene proton resonance is an unresolved multiplet in both the solvents. Peptide thus appears to lack the saltbridge. Moreover, the amide temperature coefficient data in **Table II** imply that the Asp_1 β carboxylate could be in a transient H bond interaction with its own NH, and is thus unlikely to be freely

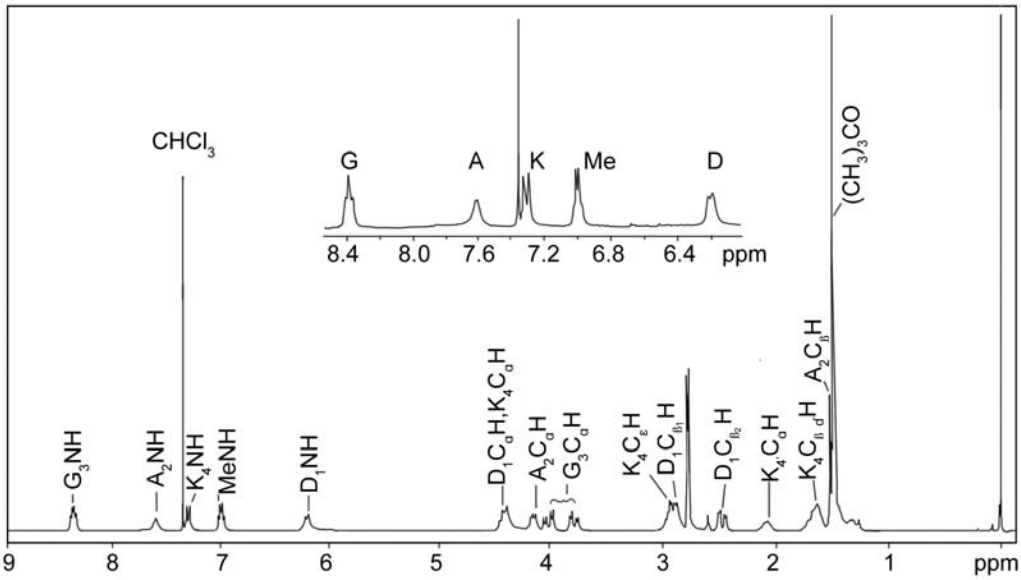

Figure 1 — ^{1}H NMR spectrum of Boc-(D)-Asp_1-Ala_2-Gly_3-Lys_4-NHMe in $CDCl_3$-DMSO-d_6 (6:1) mixture

Table I — ^{1}H NMR chemical shifts (δ, ppm) of Boc-(D)Asp_1-Ala_2-Gly_3-Lys_4-NHMe in $CDCl_3$:DMSO-d_6 (6:1) mixture and DMSO-d_6[a]

Residue	NH	$C^{\alpha}H$	$C^{\beta}H$	$C^{\gamma}H$	$C^{\delta}H$	$C^{\varepsilon}H$
(D)Asp	6.19	4.18	2.89/2.47	—	—	—
	(6.67)	(4.08)	(2.50/2.27)	(—)	(—)	(—)
Ala	7.61	4.41	1.52	—	—	—
	(8.37)	(4.04)	(1.29)	(—)	(—)	(—)
Gly	8.38	4.00/3.79	—	—	—	—
	(8.71)	(3.64)	(---)	(—)	(—)	(—)
Lys	7.30	4.21	1.70	2.11	1.70	2.90
	(7.52)	(4.13)	(1.52)	(1.73)	(1.52)	(2.73)
NHMe	7.00	2.79	----	----	----	----
	(7.54)	(2.56)	(---)	(---)	(---)	(---)

[a](Chemical shifts in parenthesis are in DMSO-d_6)

available for an ion pairing with Lys_4 NH_3^+. The partial ROESY spectrum in $CDCl_3$-DMSO-d_6 (6:1) mixture is in **Figure 2**. A summary of the observed connectivities between backbone protons in the peptide, solvent specific coupling constant and temperature coefficients of its amide resonances in DMSO-d_6 are in **Table II**.

The medium range NOEs do not appear. The peptide, however, is at least moderately folded, especially towards the C-terminal, because d_{NN}(i, i+1) type NOEs are observed between its Gly_3 and Lys_4, and C-terminal methyl amide, while relatively weaker d_{NN} (i, i+1) type NOE is also observed between its Asp_1 and Ala_2. (D)-Asp_1 appears to thus sample at least a partially folded conformation and hence a II' type turn in this peptide, seems to be weakly populated. The calculated Φ torsional angles[8,9] from its observed coupling constants in two solvents (**Table II**), are ≈+86° for Asp_1, -80° for Ala_2, -74°, -82° for Gly_3 and ≈ -100° for Lys_4. The torsional angles are generally larger and suggest that the peptide may sample at best partially ordered conformational folds.

The amide temperature coefficients in DMSO-d_6 (**Table II**) indicate that every NH is at least partially sequestered from the solvent with the exception of Ala_2 NH, which apparently is fully solvent accessible[8]. Multiple H-bond interactions are thus implied and argued for multiple transient turn type folds in the peptide.

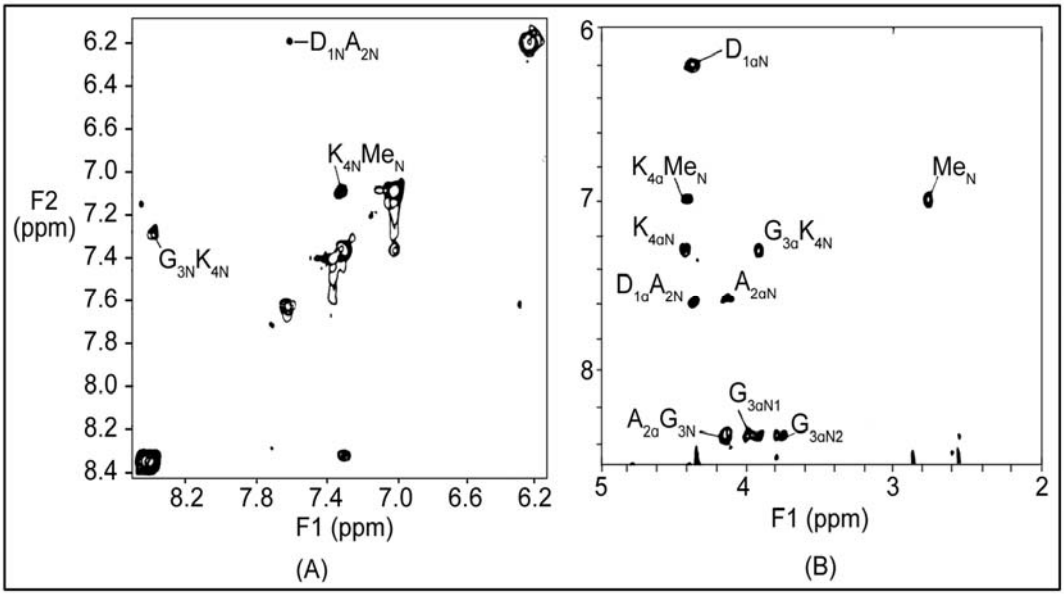

Figure 2 — NH-NH (A) and NH-C$^{\alpha}$H region (B) of the ROESY spectrum of Boc-(D)-Asp_1-Ala_2-Gly_3-Lys_4-NHMe in $CDCl_3$-DMSO-d_6 (6:1) mixture

Table II — Summary of NOEs, temperature coefficients dδ/dT (ppb/K) and coupling constants $^3J_{NH\alpha}$(Hz) of peptide Boc-(D)Asp_1-Ala_2-Gly_3-Lys_4-NHMe in $CDCl_3$:DMSO-d_6 (6:1) mixture and DMSO-d_6

	Boc	D	A	G	K	NHMe
$d_{\alpha N}$ d_{NN}						
$^3J_{NH\alpha}$	7.2	#	5.6/6.4	9.0	----	$CDCl_3$-DMSO-d_6 (6:1)
$^3J_{NH\alpha}$	8.4	6.4	5.7/6.3	8.4	----	DMSO-d_6
dδ/dT	1.60	4.74	2.57	0.00	0.00	DMSO-d_6

Not observed due to a broadened signal.

In fact marked temperature coefficients variation are noted which suggest that not all the solvent sequestered NH's may populate the H-bonded states equitably. Possibly rapidly equilibrating multiple folded conformational states are populated in which Asp_1 and Gly_3 NH's are fully H-bonded in light of diminished temperature coefficients.

A stereochemical model of the unique conformer proposed earlier for (D)-Glu_1 analog based on its NMR characteristics in apolar solvents[1] as **Figure 3(A)**. The H-bond between Boc-C=O and Gly_3 NH in the model is a consequence of its stereochemically favoured[4] pseudo type II' turn centered at (D)-Glu_1 and (L)-Ala_2.

The two other H-bonds are the consequence of consecutive 3_{10} folds locked in by the $Lys_4 \rightarrow Glu_1$ salt bridge. On a reduction of the methylene spacer, to draw the carboxylate away from its counter ion, the salt bridge interaction in the (D)-Asp_1 peptide is found to become non-observable while the ordering of the single unique backbone conformer is also now no longer observed. Quite clearly, the specific ordering of (D)-Glu_1 peptide as a 3_{10} type fold is attributable to its salt bridge. On the loss of the salt bridge, however, the peptide does not completely relinquish its ordering, since multiple consecutive H-bonded turns clearly are evidenced in (D)-Asp_1 peptide and invite an attention to explain them.

The (D)-Asp_1, analog in fact reveals a most intriguing observation that while its coupling constant and its non observable NOE's demand conformational states with a largely extended backbone, the almost completely solvent sequestered nature of its two C-terminal NH's, on the other hand, demands frequently populated H-bonded states and therefore a backbone susceptible to facile folding. No unique conformational model can explain these apparently contradictory NMR observations. Either an ensemble of alternate or rapidly equilibrating H-bonded states or a major H-bonded state with unusual geometrical features needs to be considered. One may speculate on the H-bonds acceptors that may occlude the NH's and on possible stereochemical model(s) that may explain the postulated H-bonded state(s). The (D)-

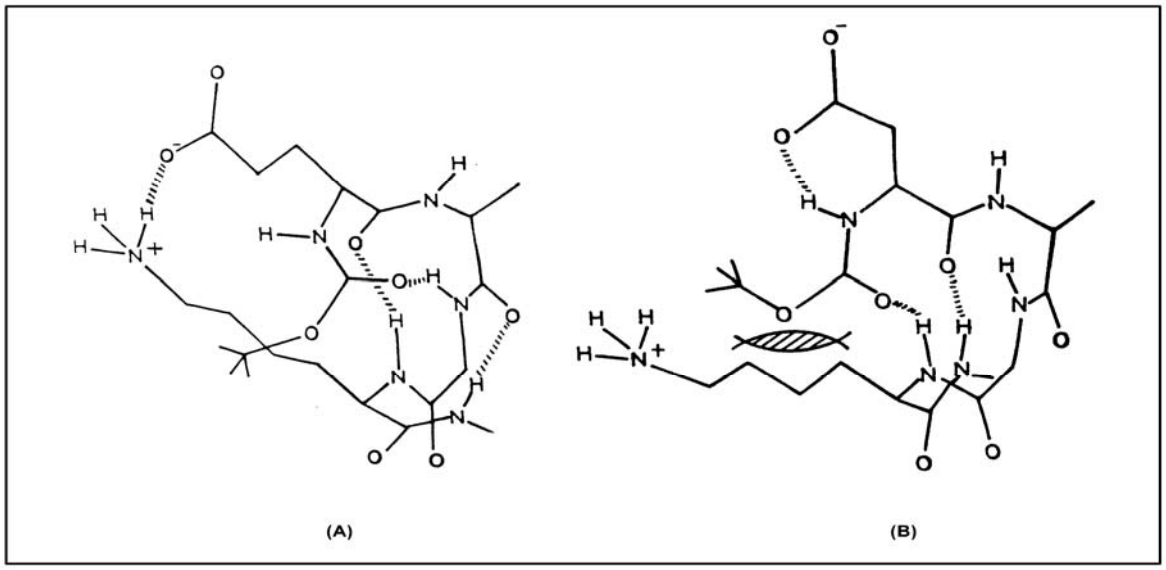

Figure 3 — Boc-(D)Glu_1-Ala_2-Gly_3-Lys_4-NHMe modeled as a type II' turn templeted 3_{10} helix (Panel A) Boc-(D)Asp_1-Ala_2-Gly_3-Lys_4-NHMe modeled as pseudo α type fold (Panel B) See the text for details.

Asp_1 carboxylate is an unlikely major partner for either Lys_4 NH or NHMe, since it is more likely to be transiently H-bonded with (D)-Asp_1 NH **Figure 3(B)** considering the partially occluded nature of the NH.

Experimental Section

Melting points were taken on Veego melting point apparatus using the capillary method and are uncorrected. Peptide intermediates were purified on 100-200 mesh silica gel. Homogeneity of peptides and amino acid derivatives were established by TLC on silica gel-G plate using two solvent system (A) $CHCl_3$-MeOH (9:1), (B) *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, 250×4 mm) eluting with MeOH or 15% H_2O-MeOH, with the UV detector set at 220 nm. Structure of all peptide intermediates were confirmed by ^{1}H NMR spectra recorded on JEOL FX 90Q and Varian VXR 300 spectrometers with TMS as internal standard. The NMR values are in δ scale. Synthesis of amino acid derivatives[10], tetrapeptides and their NMR studies[11] were done by the reported methods.

Synthesis of Boc-Gly-Lys (*Z*)-OMe, 1: Boc-Gly-OH (1.75 g, 10 m*M*) and *N*-methyl morpholine (1.1 mL, 10 m*M*) were suspended in 25 mL dry THF. After cooling (-10°C) isobutyl chloroformate (1.3 mL, 10 m*M*) was added and the reaction mixture was stirred for 15 min. To this was added a mixture of H-Lys(Z)-OMe.HCl (3.38 g, 10 m*M*) and 1.4 mL (10 m*M*) triethyl amine in 10 mL THF and the resultant mixture was stirred for 12 hr. The solvent was removed and the residue extracted with ethyl acetate. The organic layer was washed with saturated $NaHCO_3$, 10% citric acid and water. The organic solvent was dried with Na_2SO_4 and evaporated. The oily residue was purified by column chromatography. Yield 3.87 g (86%). R_f(A) 0.35, R_f(B) 0.83, ^{1}H NMR ($CDCl_3$): 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, $N^{\varepsilon}H$-$COCH_2C_6H_5$), 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 $C^{\gamma}H_2$).

Synthesis of Boc-Gly-Lys (*Z*)-NHMe, 2: 3.0 g (6.65 m*M*) of dipeptide **1** was dissolved in 10 mL MeOH and 6.6 mL 1N NaOH for C-terminal ester deprotection. After 2 h at RT the solvent was removed and residue was taken up in water, acidified to *p*H 3 with citric acid and extracted with ethyl acetate. The organic phase was washed with water, dried (Na_2SO_4) and evaporated under reduced pressure. The partially deprotected 2.62 g (6 m*M*) dipeptide was taken in 15 mL dry THF along with 0.66 mL (6 m*M*) N-methyl morpholine, cooled to –15°C and stirred with 0.78 mL (6 m*M*) isobutyl chloroformate for 15 min. To this was added a mixture of 1.22 g (18 m*M*) methylamine hydrochloride and 2.51 mL (18 m*M*) triethylamine in

15 mL (3:1) THF:water and the resultant mixture was stirred for 12 hr. The product was isolated and purified as in the previous step. Yield: 2.43 g (90%) m.p. 78-80°C, R_f(A) 0.42, R_f(B) 0.80, ^{1}H NMR ($CDCl_3$): 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, C*H*$_2C_6H_5$), 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, NHC*H*$_3$), 1.9-1.5 (complex multiplet, 4H, Lys C$^{\beta}$*H*$_2$, C$^{\delta}$*H*$_2$), 1.4 (s, 9H, Boc C*H*$_3$), 1.2-1.0 (multiplet, 2H, Lys C$^{\gamma}$*H*$_2$).

Synthesis of Boc-Ala-Gly-Lys (*Z*)-NHMe, 3: 0.9 g (5 m*M*) of Boc-Ala-OH and 0.54 mL (5 m*M*) of *N*-methyl morpholine, were suspended in 20 mL dry THF and the mixture was cooled to –15°C and stirred with 0.66 mL (5 m*M*) isobutyl chloroformate for 15 min. 2.25 g (5 m*M*) dipeptide **2** was treated with 2 mL trifluoroacetic acid at 0°C for 45 min., concentrated and triturated several times with dry ether to furnish a white solid. The solid, along with 0.7 mL (5 m*M*) triethylamine was added to the above stirred solution and the mixture was further stirred overnight. Workup of the reaction, as described in **1** afforded a white solid, which was purified by recrystallization from EtOAc-petroleum ether. Yield 2.36 g (91%), m.p. 107-110°C, R_f (A) 0.46, R_f (B) 0.86, ^{1}H NMR ($CDCl_3$): δ 8.8(s, 1H, N$^{\alpha}$*H*), 8.1 (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, C*H*$_2C_6H_5$), 4.2 – 3.8 (complex, 4H, Lys C$^{\alpha}$*H*, Ala C$^{\alpha}$*H*, Gly C$^{\alpha}$*H*$_2$), 3.25-3.0 (bs, 2H, Lys C$^{\varepsilon}$*H*$_2$), 2.7 (d, 3H, NHC*H*$_3$), 1.9-1.5 (complex multiplet, 4H, Lys C$^{\beta}$*H*$_2$, C$^{\delta}$*H*$_2$), 1.45 (d, 3H, Ala C$^{\beta}$*H*$_3$), 1.4 (s, 9H, Boc C*H*$_3$), 1.2-1.0 (multiplet, 2H, Lys C$^{\gamma}$*H*$_2$).

Synthesis of Boc-D-Asp (OBz)-Ala-Gly-Lys (Z)-NHMe, 4: 2.08 g (4 m*M*) of the tripeptide **3** was deprotected with TFA as described above, and the TFA salt, along with 0.56 mL (4 m*M*) TEA, was suspended in 20 mL THF. To a precooled (-15°C) solution of 0.67 g (4 m*M*) Boc-D-Asp (OBz)-OH and 0.22 mL (4 m*M*) of *N*-methyl morpholine, were suspended in 10 mL dry THF, was added 0.26 mL (4 m*M*) isobutyl chloroformate and the mixture was stirred for 15 min. The deprotected tripeptide (**B**), was added to the above stirred solution and the mixture was further stirred overnight. Workup of the reaction, as described in **1** afforded a white solid, which was purified by recrystallization from EtOAc-petroleum ether. Yield 1.76 g (60%), m.p. 174-176°C, R_f(A) 0.60, R_f(B) 0.78, ^{1}H NMR ($CDCl_3$): δ 8.13(m, 2H, 2×N$^{\alpha}$*H*), 7.83 (d, 1H, N$^{\alpha}$*H*), 7.75 (q, 1H, N*H*Me), 7.35 (s, 10H, aromatic protons), 7.22 (t, 1H, Lys N$^{\varepsilon}$*H*-COCH$_2C_6H_5$), 7.03 (d, 1H, Glu N$^{\alpha}$*H*) 5.1 (s, 2H, C*H*-$_2C_6H_5$), 5.0 (s, 2H, C*H*$_2C_6H_5$), 4.23 (t, 1H, C$^{\alpha}$*H*), 4.10 (m, 1H, C$^{\alpha}$*H*), 3.95 (m, 1H, C$^{\alpha}$*H*), 3.72(m, 2H, C$^{\alpha}$*H*$_2$), 2.93 (m, 2H, Lys C$^{\varepsilon}$*H*$_2$), 2.55 (d, 3H, NHC*H*$_3$), 2.73 (t, 2H, Glu C$^{\gamma}$*H*$_2$), 2.0-1.4 (complex multiplet, 8H, Lys C$^{\gamma}$*H*$_2$, C$^{\beta}$*H*$_2$, C$^{\delta}$*H*$_2$, Glu C$^{\beta}$*H*$_2$), 1.35 (s, 9H, Boc C*H*$_3$), 1.20 (d, 3H, Ala C$^{\beta}$*H*$_3$).

Synthesis of Boc-D-Asp-Ala-Gly-Lys–NHMe, 5: 1.0 g (1.25 m*M*) of the tetrapeptide **4** was dissolved in 10 mL MeOH and hydrogenated in the presence of 100 mg 10% Pd-C for 1 h at RT. The catalyst was filtered through celite, washed repeatedly with MeOH, and the combined filtrate concentrated. The residue was purified by HPLC. Yield 0.6 g (90%), R_f(B) 0.42, ^{1}H NMR data is presented in **Table I**.

Conclusion

The alternative conformational model implies that a Boc protected D amino acid, by assuming either one of its two well recognized conformational choices in the inverse β region of Ramachandran diagram[5,6], may nucleate either the 3_{10} fold or pseudo α type fold in its C-side residues depending upon possible collateral effects. The folding mode adopted in (D)-Glu_1 peptide appears to reflect such a conformational selection imposed by the salt bridge interaction. In the absence of the salt bridge, these folding mode may be only partially realized in (D)-Asp_1 peptide and appears to be accompanied by pseudo α-type folding mode. The observed conformational polymorphism of (D)-Asp_1 peptide may thus be at least partially rationalized but would require a further definitive investigation.

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