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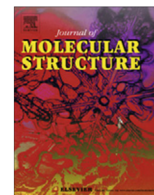

Conformational studies of γ-turn in pseudopeptides containing α-amino acid and conformationally constrained meta amino benzoic acid/meta nitro aniline

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HIGHLIGHTS

- ▶ Pseudopeptides **I** & **II** with appropriate N & C terminal protecting groups adopt γ-turn conformation.
- ▶ Pseudopeptides **III–V** displaying extended conformation in solid state forms γ-turn in solution.
- ▶ Importance of steric interactions amongst amino acid residues is crucial for γ-turn stabilization.
- ▶ The work may open a new avenue in introducing γ-turn within the bioactive conformation of peptides.

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ABSTRACT

Reverse turns (commonly β-turns and γ-turns), a common motif in proteins and peptides, have attracted attention due to their relevance in a wide variety of biological processes. In an attempt to artificially imitate and stabilize these turns in short acyclic peptides, a series of *N*-terminally protected pseudopeptides comprising of an α-amino acid and conformationally constrained meta amino benzoic acid (*m*ABA)/meta nitro aniline (*m*NA) (peptides **I–VI**) have been synthesized. The molecules were well characterized by various spectroscopic techniques and subjected to a systematic conformational analysis. Our experimental results reveal that only pseudopeptides **I** and **II** with methyl as the sidechain, tertiary butyloxy carbonyl as the *N*-terminal protecting group and (*m*ABA)/(*m*NA) at the C-terminus adopt γ-turn conformations in solid state as well as in solution. Even slight modification of any of the stated conditions donot support the formation of this γ-turn architecture in the solid state. Interestingly, the peptides **III–V** which displays extended conformation in solid state forms γ-turn structure in solution. Thus this result reflects the importance of co-operative steric interactions amongst various amino acid residues in stabilizing a particular conformation in peptides in different phases (solid and solution). This report may open a new avenue in introducing γ-turn motifs within the bioactive conformation of selected peptides.

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1. Introduction

In proteins, γ-turn consist of three amino acids, which may or may not be stabilized by an intramolecular hydrogen bond between the C=O of the first residue (i) and the NH of the third residue ($i+2$), forming a seven-membered ring [1,2]. Depending on whether the side chain of the residue $i+1$ is in an equatorial or axial orientation on the seven-membered ring, γ-turns are classified as inverse or classical, respectively giving rise to a kink in the chain or a direction change [3]. Although less recurrent than β-turn, γ-turns play important roles in various biological processes. Structural studies have revealed that naturally occurring peptides such as vasopressin and the related desmopressin, bradykinin, and angiotensin II, that function as hormones or neurotransmitters, or have other regulatory roles in organisms, adopt γ-turn conformations [4–7]. The γ-turn present in vitronectin has been reported to contribute to the specific recognition by integrin receptor $\alpha v\beta 3$ playing a role in tumor cell adhesion angiogenesis, and osteoporosis [8,9]. γ-Turn mimetic inhibitor forms complex with HIV-I protease which provides information about structure–activity relationship and helps in rational drug design [10]. It

Abbreviations: *m*ABA, *m*-amino benzoic acid; *m*NA, *m*-nitro aniline; Boc, tertiary butyloxy carbonyl; OMe, methoxy carbonyl; DCC, dicyclohexylcarbodiimide; HOBt, 1-hydroxy benzotriazole.

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is also proposed that in proteins, hydrated γ-turns promote helix-coil unfolding [11].

Various investigations suggest that γ-turns seldom exist in short, natural peptides. Initially it was believed that short acyclic peptides do not have the ability to adopt any preferential conformation. Spectroscopic studies indicate that the conformational space of tripeptides is more restricted than originally thought so that structures of limited stability can be formed [12]. Small peptides which are biomedically relevant as protease inhibitors, as taste receptors and for enzyme regulation, should maintain γ-turn conformation to show the activity [13–17]. Therefore designing and stabilizing γ-turn structure in small acyclic peptides is challenging and has been of great interest recently.

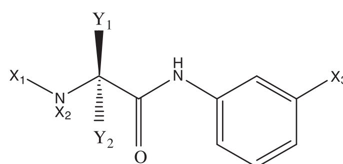
Jimenez et al. have demonstrated the use of D-c$_3$Dip (*N'*methyl-2,2-diphenyl-1-amino cyclopropane carboxamide), when coupled with acetyl L-Pro to constitute the first unequivocal evidence of the ability of a cyclopropane α-amino acid to adopt a γ-turn disposition in a non-propitious environment [18]. Y. D. Wu and his group has made a seminal contribution in inducing γ-turns in peptides comprising of alternating α-aminoxy acids and α-amino acids [19]. Martinez and his co-workers has made substantial inputs in designing γ-turn mimetics by incorporating 2-alkyl 2-carboxy-azetidine residue in the $i + 1$ position of simply modified peptides and showed that these non-protenogenic amino acids possess a tremendous ability to nucleate γ-turns [20,21]. Therefore, it is apparent that various governing factor dictating the overall nucleation of γ-turn formation remains still in its infancy.

To gain further insight regarding the design of γ-turns in small acyclic peptides and contribution of various residues in the induction of this motif, we have synthesized pseudopeptides, **I–VI** (Table 1, Entry a–f), comprising of an α-amino acid and conformationally biased meta amino benzoic acid (*m*ABA)/meta nitro aniline (*m*NA), with an all trans extended configuration. The idea was to introduce some conformational constraint on the adjacent peptide bond incorporating *m*ABA/*m*NA which may compel the carbonyl at the *i*th position and NH of the $i + 2$th amino acid to come closer in the same direction and attain the γ-turn conformation. Keeping this view in mind, our design aims in performing a

Table 1
List of pseudopeptides (Entry a–k) with torsion angles (°) of the residues at $i + 1$.

Entry	Peptides	φ_1 (°)	ψ_1 (°)	Ref.
	Idealized classical γ-turn	75.0	−64.0	[1–3]
	Idealized inverse γ-turn	−79.0	69.0	[1–3]
a.	Boc-L-Ala-*m*NA[a] (**I**)	−105.9(2)	95.7(2)	This work
b.	Boc-D-Ala-*m*NA[a] (**II**)	107.7(3)	−95.0(3)	This work
c.	Piv-L-Ala-*m*NA[a] (**III**)	−157.3(1)	160.1(1)	This work
d.	Fmoc-L-Ala-*m*ABA-OMe[b] (**IV**)	–	–	This work
e.	Boc-L-β-cyano-Ala-*m*ABA-OMe[b] (**V**)	145.3(2)	−79.5(2)	This work
f.	Boc-Me-Gly-*m*ABA-OMe[b] (**VI**)	−85.4(2)	−160.8(1)	This work
g.	Boc-L-Ala-*m*ABA-OMe[b]	101.66	−96.31	[28]
h.	Boc-Gly-*m*ABA-OMe[b] (Mol.A)	62.6	−130.6	[29]
	(Mol. B)	−65.2	−179.6	
i.	Boc-β-Ala-*m*ABA-OMe[b]	−139.2	142.8	[22]
j.	Boc-γ-Aba-*m*ABA-OMe[b]	155.8	177.6	[22]
k.	Boc-β-Ala-*m*NA[a]	136.4	140.2	[30]
l.	Boc-γ-Aba-*m*NA[a]	–	–	[30]
m.	Boc-Gly-*p*NA[c]	−60.70	143.11	[31]
n.	Boc-β-Ala-*p*NA[c]	95.24	−165.30	[31]
o.	Boc-Pro-*p*NA[c]	55.13	−165.27	[31]
p.	Boc-Pro-*m*NA[a]	−68.02	176.31	[32]
q.	Boc-Pro-*m*ABA-OMe[b]	76.58	−179.62	[32]

Aib = α aminoisobutyric acid, β-Ala = β-Alanine, γ-Aba = γ-amino butyric acid.
[a] *m*NA = meta nitro aniline.
[b] *m*ABA-OMe = meta amino benzoic acid.
[c] *p*NA = para nitro aniline.



Entry	Peptide	Protected group: X_1	X_2	Y_1	Y_2	X_3
1.	**I**	Boc	H	Me	H	NO_2
2.	**II**	Boc	H	H	Me	NO_2
3.	**III**	Piv	H	Me	H	NO_2
4.	**IV**	Fmoc	H	Me	H	COOMe
5.	**V**	Boc	H	Me	H	COOMe
6.	**VI**	Boc	Me	H	H	COOMe

Fig. 1. Schematic representations of Peptides **I–VI**.

systematic conformational analysis of γ-turn formation in pseudopeptides PRO-Xaa-Yaa (PRO; protecting group at the *N*-terminus, Xaa; amino acid, Yaa: *m*ABA/*m*NA) by replacement of various parameters in the sequences like configuration of the *N*-terminal amino acid, different protecting groups and different *N*-terminal amino acids. Importantly this investigation will provide more information about the creation of structural diversities in the peptide backbone depending upon the co-operative steric interactions amongst the side chains of amino acid residues (Fig. 1).

2. Experimental section

2.1. Synthesis of peptides

The dipeptides were synthesised by conventional solution phase methodology [22]. The *t*-butyloxycarbonyl and methyl ester groups were used for amino and carboxyl protections and dicyclohexylcarbodiimide (DCC), 1-hydroxybenzotriazole (HOBT) as coupling agents. Methyl ester hydrochloride of *m*-ABA were prepared by the thionyl chloride-methanol procedure [23]. All the intermediates obtained were checked for purity by thin layer chromatography (TLC) on silica gel and used without further purification. The final peptides were purified by column chromatography using silica gel (100–200 mesh) as the stationary phase and ethyl acetate and petroleum ether mixture as the eluent.

2.1.1. Boc-L-Ala-m-NA (***I***)

Boc-L-Ala-OH 0.95 g (5 mmol) was dissolved in dimethylformamide (DMF; 3 mL). 0.68 g (5 mmol) of *m*NA was added followed by DCC (1.0 g, 5 mmol). The reaction mixture was stirred at room temperature for 3 days. The precipitated dicyclohexylurea (DCU) was filtered and diluted with ethyl acetate (80 mL). The organic layer was washed with excess of water, 1 N HCl (3 × 30 mL), 1 M Na_2CO_3 solution (3 × 30 mL) and again with water. The solvent was then dried over anhydrous Na_2SO_4 and evaporated in vacuo, giving a brown solid. Yield: 1.39 g (90%). Single crystals were grown from a methanol–water mixture by slow evaporation and were stable at room temperature. Mp = 162–163 °C; LR – MS: $C_{14}H_{19}N_3O_5$ $[M + H]^+ = 310.14$, $[M + Na]^+ = 332.12$, Mcalcd $C_{14}H_{19}N_3O_5$ $[M + H]^+ = 310$, $[M + Na]^+ = 332$; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.47 (C^{β}Hs of Ala, 3H, m); 1.49 (Boc-CH_3s, 9H, s); 4.46–4.48 (C^{α}Hs of Ala, 1H, m); 5.44–5.46 (Ala-NH, 1H, d, *J* = 8 Hz); 7.30–7.34 ((Hc (*m*-ABA), 1H, m); 7.70 (Hd (*m*-ABA), 1H, d, *J* = 8 Hz); 7.80 (Hb (*m*-ABA), 1H, d, *J* = 8 Hz); 8.38–8.39 (Ha (*m*-ABA), 1H, m); 9.46 (*m*-ABA NH, 1H, s); ^{13}C NMR (100 MHz $CDCl_3$,

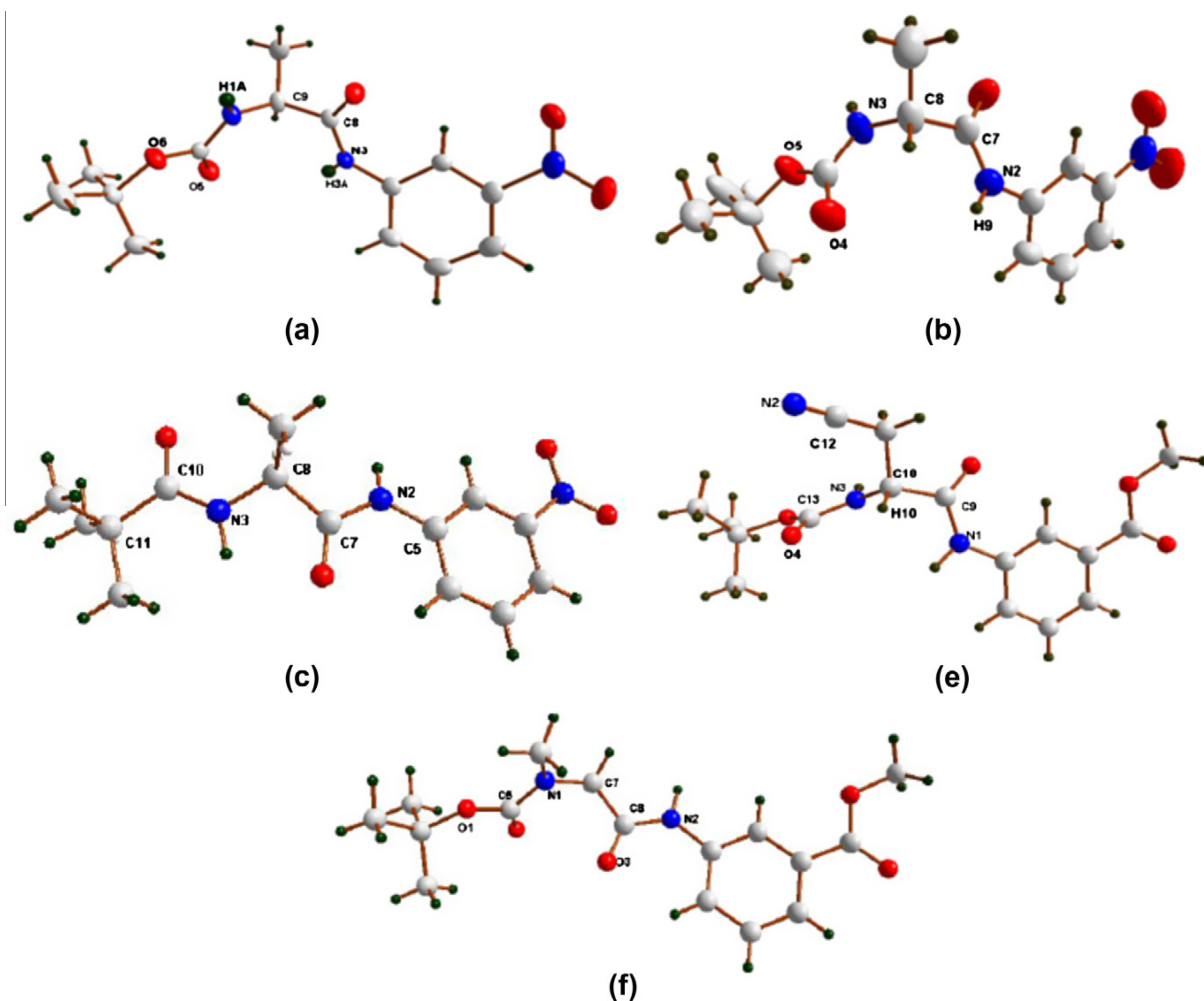


Fig. 2. Crystal structure of peptide (a) **I**, (b) **II**, (c) **III**, (e) **V**, (f) **VI** with atom numbering scheme.

Table 2
Selected torsion angles (°) of peptides **I–III**, and **V–VI**.

Residues	φ_1	ψ_1	ω_0	φ_2	ψ_2	ω_1	θ_1	θ_2
Peptide **I**	−105.9(2)	95.7(2)	178.3(1)	−34.7(3)	−0.5(3)	−175.4(2)	178.4(2)	−178.7(2)
Peptide **II**	107.7(3)	−95.0(3)	−179.3(3)	34.9(5)	1.2(5)	175.2(3)	−178.6(3)	179.3(3)
Peptide **III**	−157.3(1)	160.1(1)	−179.0(1)	172.1(1)	−4.1(2)	−178.0(1)	−179.2(1)	179.0(1)
Peptide **V**	145.3(2)	−79.5(2)	168.7(2)	14.2(4)	−0.3(4)	−177.4(2)	14.3(3)	−177.1(2)
Peptide **VI**	−85.4(2)	−160.8(1)	171.3(1)	−163.8(2)	11.1(2)	175.8(2)	−180.0(1)	178.3(2)

Table 3
Hydrogen bond parameters for peptides **I–III**, **V** and **VI**.

D-H—A	H—A(Å)	D—A(Å)	D-H—A(°)
Peptide I			
N3-H3A—O5[i]	2.16	2.98	160.73
N2-H1A—O5[ii]	2.11	2.93	171.86
Peptide II			
N3-H3—O4[i]	2.28	2.96	156.96
N2-H9—O3[ii]	2.18	2.99	161.00
Peptide III			
N2-H2A—O4[iii]	2.04	2.85	171.36
Peptide V			
N1-H1A—N2[iv]	2.11	3.06	162.86
N3-H2A—O4[ii]	2.04	2.85	171.36
Peptide VI			
N2-H1A—O2[v]	2.11	3.03	164.93

Symmetry codes:
[a] $x-1, y, z$.
[b] $x+1, y, z$.
[c] $-x+2, -y, -z+1$.
[d] $-x+1, y+1/2, -z+1$.
[e] $-x+1, y-1/2, -z+1/2$.

δ ppm): 17.43, 28.12, 51.52, 80.75, 99.01, 114.29, 117.59, 125.24, 128.88, 139.16, 148.04, 156.25,171.51.

2.1.2. Boc-D-Ala-m-NA (*II*)

The peptide **II** was synthesised following the similar procedure as that of peptide **I**. Yield: 1.24 g (80%). Single crystals were grown from a methanol water mixture by slow evaporation and were stable at room temperature. Mp = 157–158 °C; LR – MS: $C_{14}H_{19}N_3O_5$ $[M+H]^+ = 310.14$, $[M+Na]^+ = 332.12$, Mcalcd $C_{14}H_{19}N_3O_5$ $[M+H]^+ = 310$, $[M+Na]^+ = 332$; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.46–1.47 (C^{β}Hs of Ala, 3H, m); 1.49 (Boc-CH_3s, 9H, s); 4.44 (C^{α}Hs of D-Ala, 1H, m); 5.35 (D-Ala-NH, 1H, d, *J* = 8 Hz); 7.33–7.36 ((Hc (*m*-ABA), 1H, m); 7.71–7.72 (Hd (*m*-ABA), 1H, m); 7.83 (Hb (*m*-ABA), 1H, d, *J* = 8 Hz); 8.39 (Ha (*m*-ABA), 1H, s); 9.39 (*m*-ABA NH, 1H, s); ^{13}C NMR 100 MHz ($CDCl_3$, δ ppm): 16.86, 27.82, 50.30, 80.74, 113.96, 117.61, 124.59, 128.88, 138.87, 147.73, 155.92, 171.18.

2.1.3. Piv-L-Ala-m-NA (*III*)

The peptide **III** was synthesised following the similar procedure as that of peptide **I**. Yield: 1.44 g (85%). Single crystals were grown from a methanol water mixture by slow evaporation and were stable at room temperature. Mp = 158–160 °C; LR – MS: $C_{14}H_{19}N_3O_4$ $[M+H]^+ = 294.32$, Mcalcd $C_{14}H_{19}N_3O_4$ $[M+H]^+ = 294$; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.14 (Piv-CH_3s, 9H, s); 1.35–1.36 (C^{β}Hs of Ala, 3H, m); 4.54 (C^{α}H of Ala, 1H, br); 6.89–6.90 (Ala-NH, 1H, m); 7.38–7.84 (Hd, Hc, Hb (*m*-ABA), 3H, m); 8.53 (Ha (*m*-ABA), 1H, s); 10.21 (*m*-ABA NH, 1H, s); ^{13}C NMR 100 MHz ($CDCl_3$, δ ppm): 18.20, 27.61, 39.36, 49.54, 113.83, 117.12, 124.67, 129.49, 139.47, 147.92, 172.05, 177.75.

2.1.4. Fmoc-L-Ala-mABA-OMe (*IV*)

The peptide **IV** was synthesised following the similar procedure as that of peptide **I**. Yield: 1.07 g (75%). Mp = 165–167 °C; LR – MS: $C_{26}H_{24}N_2O_5$ $[M+H]^+ = 445.18$, Mcalcd $C_{26}H_{24}N_2O_5$ $[M+H]^+ = 445$; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.46–1.47 (C^{β}Hs of Ala, 3H, d, *J* = 8 Hz), 3.82 (Methoxy, 3H, s), 4.15 (Fmoc, Fulvene, 1H, m); 4.42 (Fmoc-benzylic H, 1H, m), 4.52 (C^{α}Hs of Ala, 1H, m), 5.84 (Ala-NH, 1H, m), 7.30–7.70 (Fmoc and *m*ABA aromatic H's, 7H, m); 8.09 (Ha (*m*-ABA), 1H, s); 8.92 (*m*-ABA NH, 1H, s).

2.1.5. Boc-β-cyano-Ala-mABA-OMe (*V*)

The peptide **V** was synthesised starting from Boc-Asn [24] following the similar procedure as that of peptide **I**. Yield: 1.51 g (90%). Single crystals were grown from a methanol water mixture by slow evaporation and were stable at room temperature. Mp = 150–153 °C; Yield: 1.2 g (80%). Mp = 160–162 °C; LR – MS: $C_{17}H_{21}N_3O_5$ $[M+H]^+ = 348.36$, Mcalcd $C_{17}H_{21}N_3O_5$ $[M+H]^+ = 348.36$; ^{1}H NMR 400 MHz ($CDCl_3$, δ ppm): 1.38 (Boc-MeHs, 9H, s), 2.19–2.21 (β-CN-Ala C^{β}Hs, 2H, m), 3.80 (Methoxy, 3H, s), 4.58 (β-CN-Ala C^{α}H, 1H, m), 4.89 (β-CN-Ala NH, 1H, m), 7.66–8.13 (*m*ABA aromatic H's, 4H, m)), 9.96 (*m*ABA NH, 1H, s).

2.1.6. Boc-MeGly-mABA-OMe (*VI*)

The peptide **VI** was synthesised following the similar procedure as that of peptide **I**. Yield: 1.40 g (90%). Single crystals were grown from a methanol water mixture by slow evaporation and were stable at room temperature. Mp = 123–124 °C; LR – MS: $C_{16}H_{22}N_2O_5$ $[M+H]^+ = 323.16$, $[M+Na]^+ = 345.14$, Mcalcd $C_{16}H_{22}N_2O_5$ $[M+H]^+ = 323$, $[M+Na]^+ = 345$; ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.49 (Boc-CH_3s, 9H, s), 3.02 (NMe's 3H, s), 3.89 (Methoxy, 3H, s), 4.0 (C^{α}Hs of Gly, 1H, s), 7.35–7.36 ((Hb (*m*-ABA), 1H, m), 7.74–7.75 (Hc (*m*-ABA), 1H, m), 7.80–7.81 (Hd (*m*-ABA), 1H, m); 8.05 (Ha (*m*-ABA), 1H, s), 8.72 (*m*-ABA NH, 1H, br); ^{13}C NMR (100 MHz $CDCl_3$, δ ppm): 28.31, 35.69, 51.85, 53.71, 81.06, 120.37, 124.01, 125.37, 128.02, 129.08, 130.11, 137.66, 165.99, 168.11.

3. Results and discussion

3.1. Solid state structures of peptides

Colorless crystal, suitable for X-ray diffraction analysis was obtained from methanol–water solution of peptides **I–III, V** and **VI** by slow evaporation method. The crystal structure of peptide **I**, Boc-Ala-*m*NA reveal that the molecule adopts a folded conformation corresponding to a slightly distorted inverse γ-turn structure with L-Ala occupying the *i* + 1 position (Fig. 2a). Interestingly, the crystal structure of peptide **II**, that contains D-Ala at the *N*-terminal position of peptide **I** exhibits a classical γ-turn almost unnoticed in acyclic peptides. Although there are some examples of constrained cyclic peptides by inserting *o*-substituted benzenes to mimic the turn regions of neurotrophin, a nerve growth factor, peptides **I** and **II** present two novel examples where conformationally restricted nitro gamma amino moieties has been incorporated in the γ-turn region of acyclic dipeptides [25]. The idealized torsion

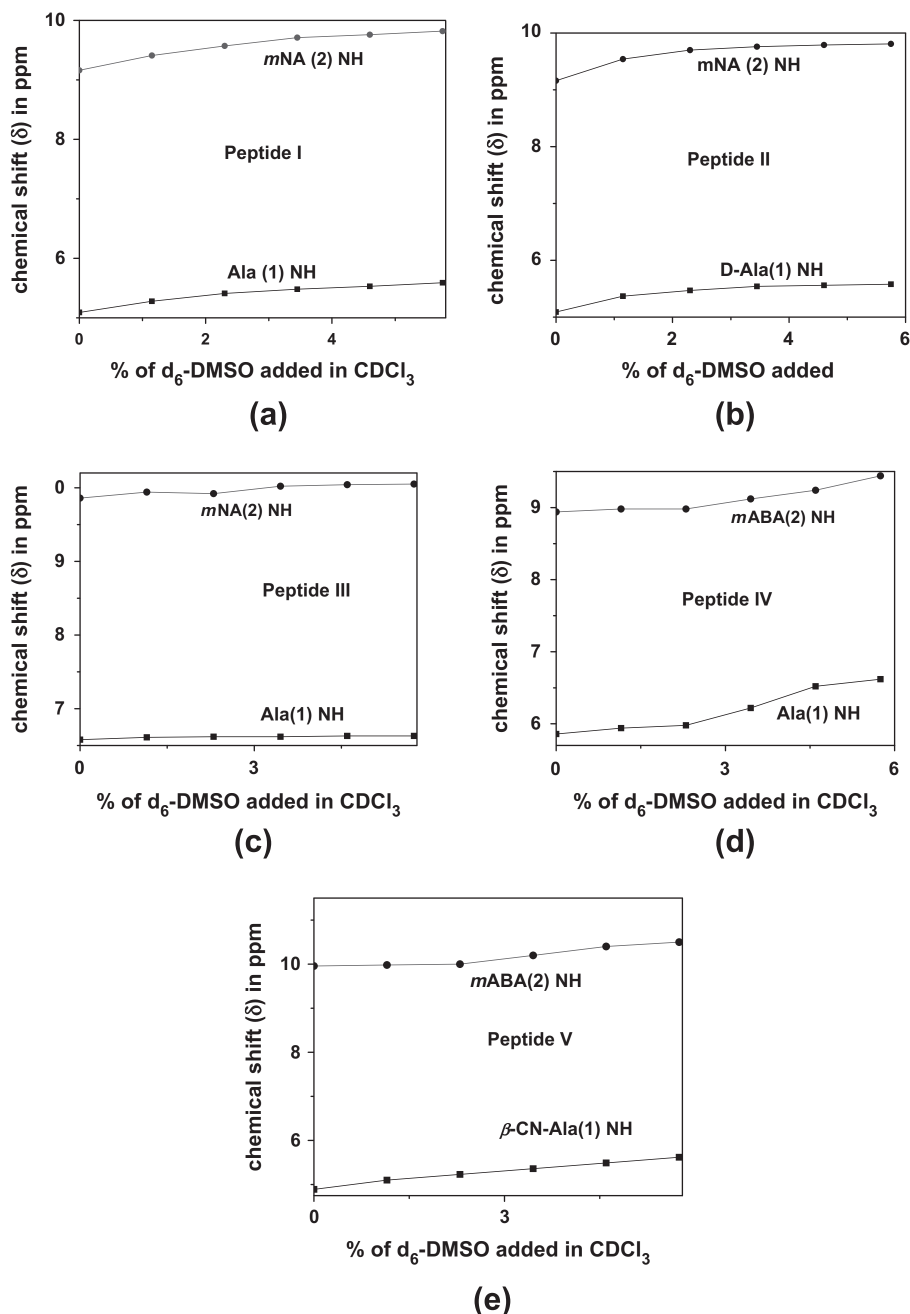


Fig. 3. NMR solvent titration curve for NH protons in peptide **I–V (a–e)** respectively.

angle values for an inverse γ-turn are $\varphi_i = -79.0°$, $\psi_i = 69.0°$ and for classical γ-turn are $\varphi_i = 75.0°$, $\psi_i = -64.0°$ respectively [1–3]. In peptides **I** and **II**, these torsion angles are found to be deviated as φ_i (−105.9(2)/107.7(3)°), ψ_i (95.7(2)/−95.0(3)°) (Table 2) from the idealized values. The crystal data of both the peptides are reported in Table 5 [26,27]. Nevertheless, peptide **I** and **II** produces novel γ-turn structures (inverse and classical) rarely observed in the literature.

Peptides **III** and **IV** was synthesized by varying the *N*-terminal protecting group of peptide **I**, from Boc to Piv in peptide **III** and Fmoc in peptide **IV**, to study the effects of protecting groups on γ-turn nucleation. The solid state structure of peptide **III** is presented in Fig. 2c. The torsion angles at Ala ($\varphi_1 = -157.3(1)$, $\psi_1 = 160.1(1)$, (Table 3) residue indicate a fully extended conformation. Thus, it is observed that peptide **III**, with Piv as *N*-terminal protecting groups fails to display the conformational preference for γ-turn. It was not possible to grow single crystals of peptide **IV**. Therefore we are unable to comment on the solid state structure and conformational preference of the molecule.

In an effort to examine whether change of the sidechain from electron donating methyl (peptide **I**) to electron withdrawing β-cyano methyl, produces similar conformational resemblance to that of peptide **I**, keeping the *N*-terminal protecting group unaltered, we designed and synthesized peptide **V**. The crystal structure of peptide **V** is presented in Fig. 3e which reveals a completely extended structure as is indicated by the backbone torsions at β-cyano-Ala residue ($\varphi_1 = 145.3(2)$, $\psi_1 = -79.5(2)$ (Table 3).

Next our attempt was to shift the steric bulk from the α-carbon to amino nitrogen using Methyl Glycine (Sarcosine) as the *N*-terminal aminoacid in peptide **VI**, keeping the protecting groups

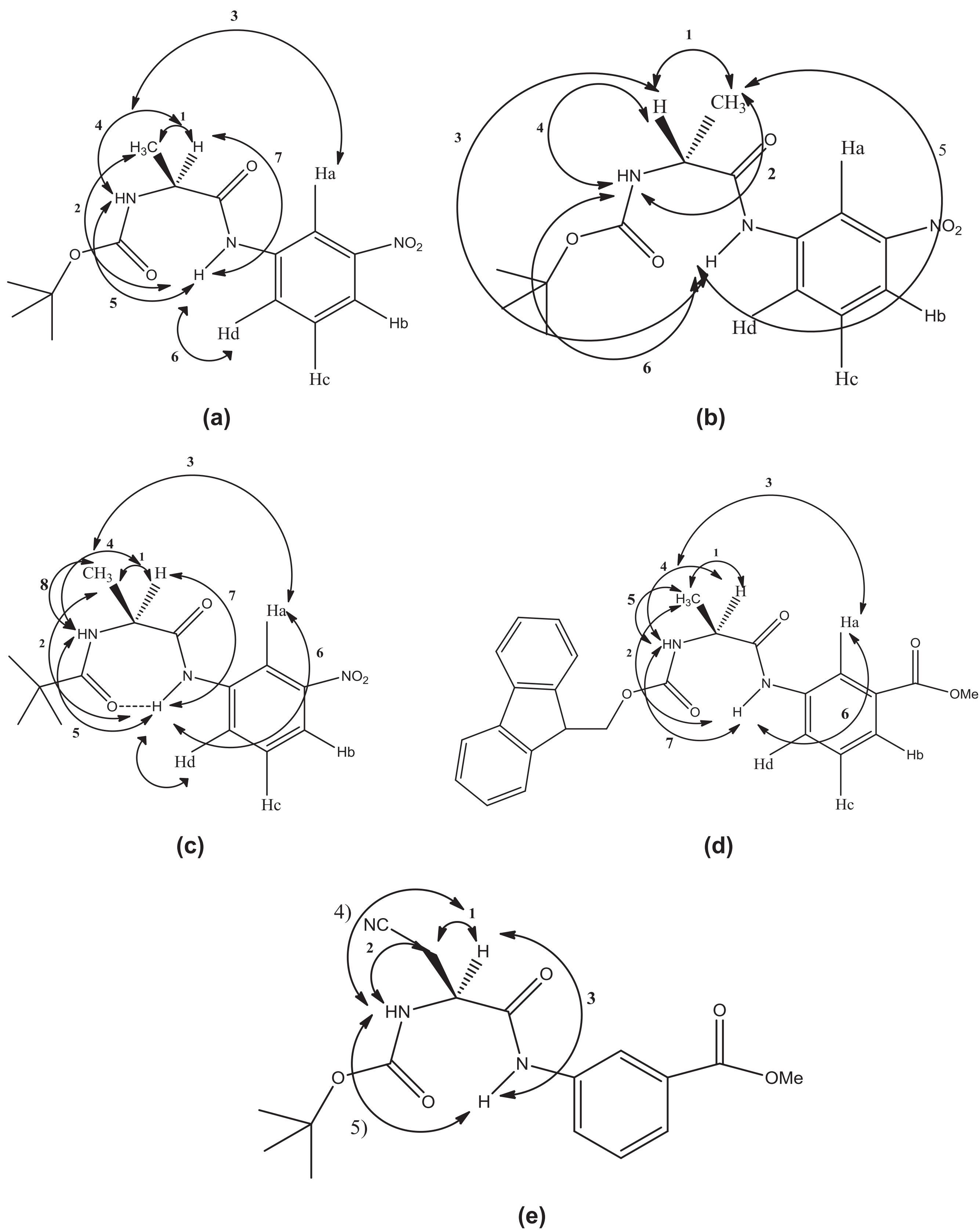


Fig. 4. Schematic representation of the proposed γ-turn in peptide **I–V**. The hydrogen bonds are shown as dotted lines. Observed NOEs are highlighted by double edged arrows in $CDCl_3$ (500 MHz).

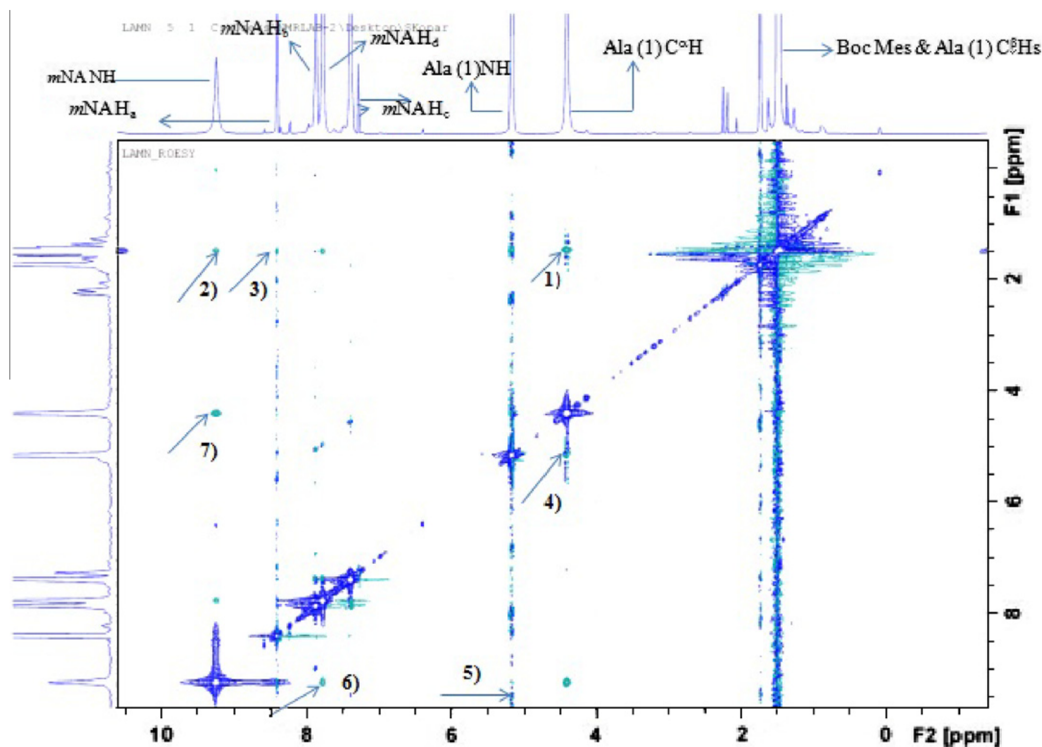


Fig. 5. ROESY spectrum of the proposed γ-turn in peptide **I** in $CDCl_3$.

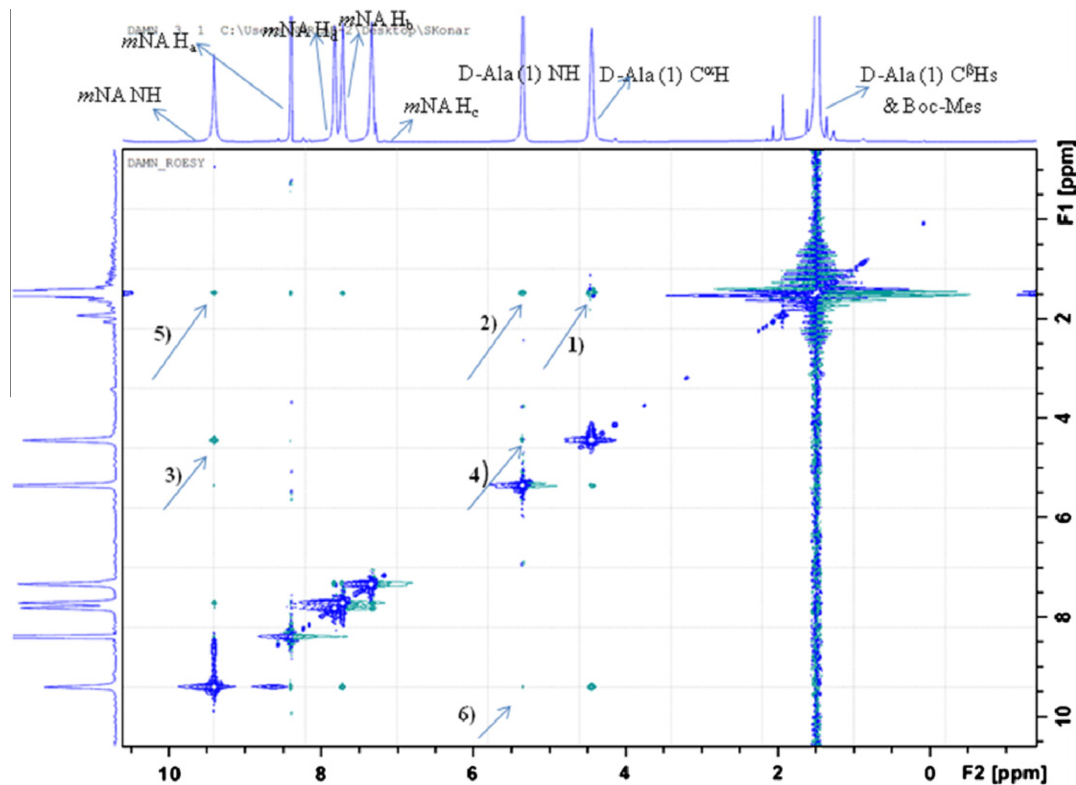


Fig. 6. ROESY spectrum of the proposed γ-turn in peptide **II** in $CDCl_3$.

unchanged, and study the conformational preference of the molecule. The crystal structure of peptide **VI** is presented in Fig. 3f. The torsion angles at Sar residue are $\varphi_1 = -85.4(2)$, $\psi_1 = -160.8(1)$ (Table 3) which also indicates a fully extended conformation.

A pseudopeptide Boc-Ala-*m*ABA-OMe, similar to peptide **I** was reported by Pramanik and his co-workers in the design of β-sheet structures [28] (Table 1, Entry g). From the work of the same group it was demonstrated that changing the *N*-terminal residue from Ala

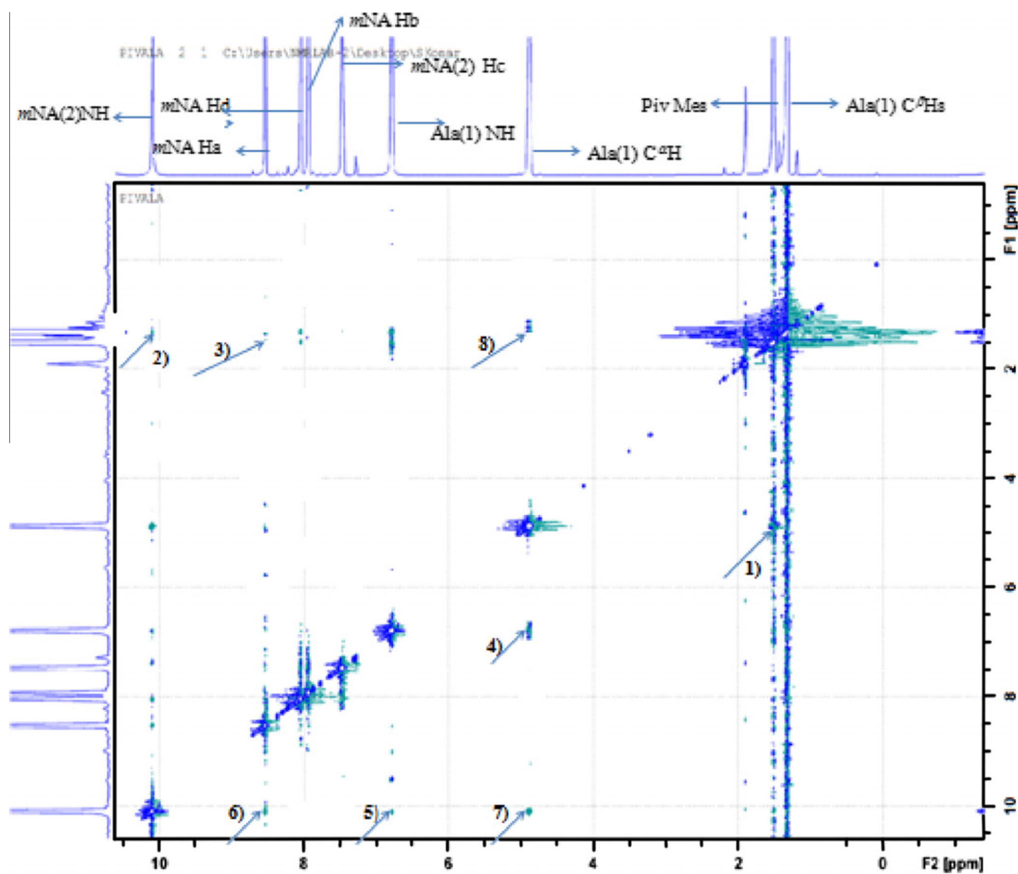


Fig. 7. ROESY spectrum of the proposed γ-turn in peptide **III** in $CDCl_3$.

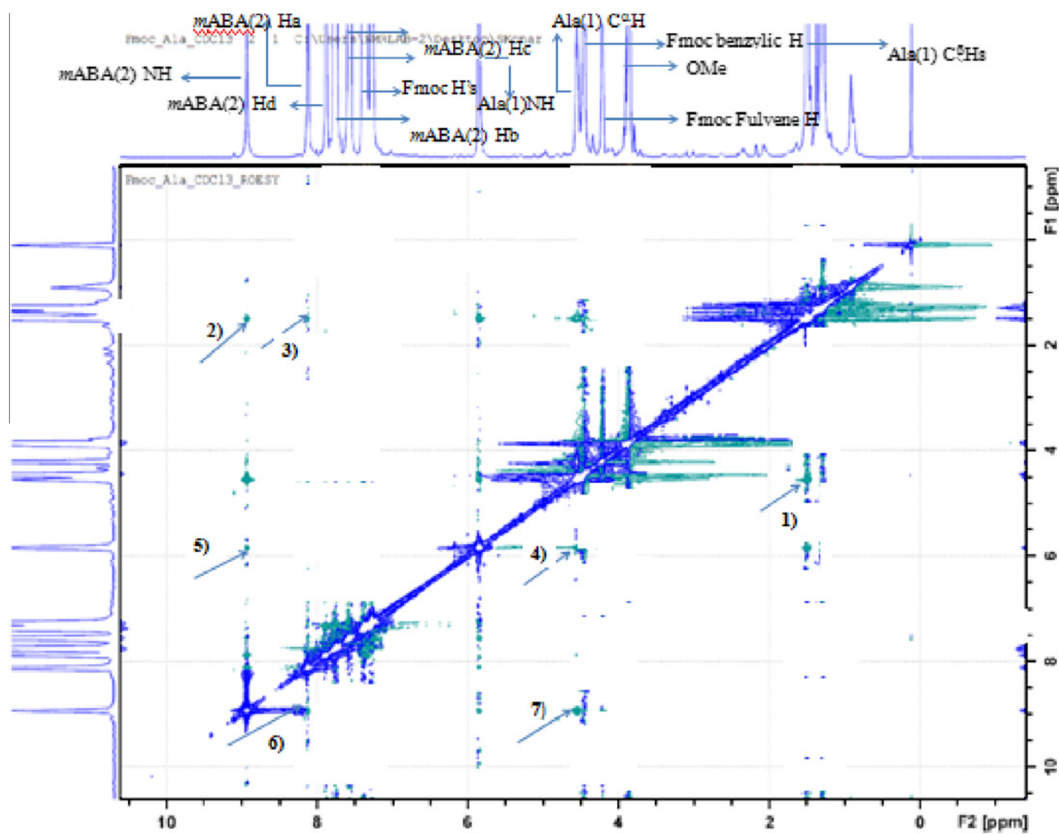


Fig. 8. ROESY spectrum of the proposed γ-turn in peptide **IV** in $CDCl_3$.

Table 4
Important interresidue NOEs for peptide **I–V** in $CDCl_3$.

*Peptide **I***		*Peptide **II***	
(1)	Ala(1) C^{β}Hs ↔ Ala(1) C^{α}Hs	(1)	Ala(1) C^{β}Hs ↔ Ala(1) C^{α}Hs
(2)	Ala(1) C^{β}Hs ↔ *m*NA(2) NH	(2)	Ala(1) C^{β}Hs ↔ Ala(1) NH
(3)	Ala(1) C^{β}Hs ↔ *m*NA(2) H_a	(3)	Ala(1) C^{α}Hs ↔ Ala(1) NH
(4)	Ala(1) C^{α}Hs ↔ Ala(1) NH	(4)	Ala(1) NH ↔ *m*NA(2) NH
(5)	Ala(1) NH ↔ *m*NA(2) NH	(5)	Ala(1) C^{β}Hs ↔ *m*NA(2) NH
(6)	*m*NA(2) H_d ↔ *m*NA(2) NH	(6)	Ala(1) NH ↔ *m*NA(2) NH
(7)	Ala(1) C^{α}Hs ↔ *m*NA(2) NH		
*Peptide **III***		*Peptide **V***	
(1)	Ala(1) C^{β}Hs ↔ Ala(1) C^{α}Hs	(1)	β-CN-Ala(1) C^{β}Hs ↔ β-CN-Ala(1) C^{α}H
(2)	Ala(1) C^{β}Hs ↔ *m*NA(2) NH	(2)	β-CN-Ala(1) C^{β}Hs ↔ β-CN-Ala(1) NH
(3)	Ala(1) C^{β}Hs ↔ *m*NA(2) H_a	(3)	β-CN-Ala(1) C^{α}H ↔ *m*ABA(2) NH
(4)	Ala(1) C^{α}H ↔ Ala(1) NH	(4)	β-CN-Ala(1) C^{α}H ↔ β-CN-Ala(1) NH
(5)	Ala(1) NH ↔ *m*NA(2) NH	(5)	β-CN-Ala(1) NH ↔ *m*ABA(2) NH
(6)	*m*NA(2) H_a ↔ *m*NA(2) NH		
(7)	Ala(1) C^{α}H ↔ *m*NA(2) NH		
(8)	Ala(1) C^{β}Hs ↔ Ala(1) NH		
*Peptide **IV***			
(1)	Ala(1) C^{β}Hs ↔ Ala(1) C^{α}Hs		
(2)	Ala(1) C^{β}Hs ↔ *m*ABA(2) NH		
(3)	*m*ABA(2) H_a ↔ Ala(1) C^{β}Hs		
(4)	Ala(1) C^{α}H ↔ Ala(1) NH		
(5)	Ala(1) C^{β}Hs ↔ Ala(1) NH		
(6)	*m*ABA(2) H_a ↔ *m*ABA(2) NH		
(7)	Ala(1) NH ↔ *m*ABA(2) NH		

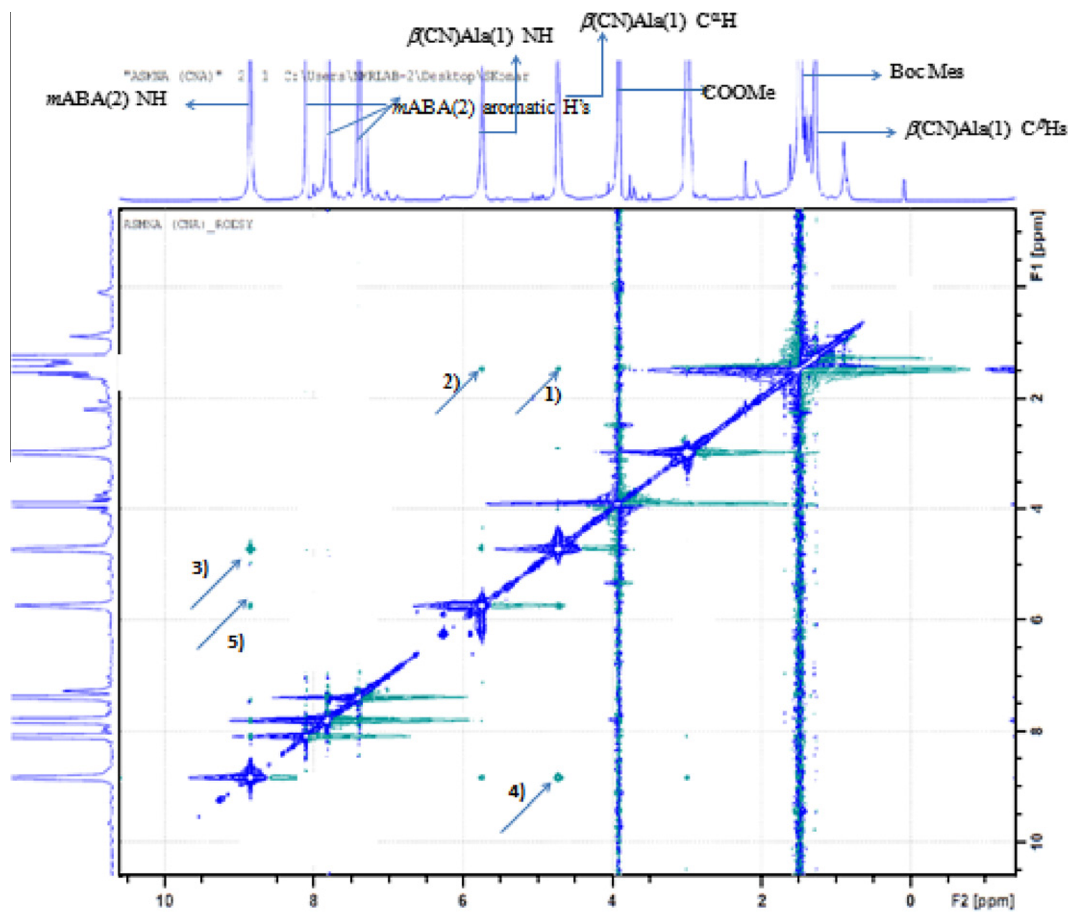


Fig. 9. ROESY spectrum of the proposed γ-turn in peptide **V** in $CDCl_3$.

Table 5
Crystallographic refinement details of Peptide **I–III**, **V** and **VI** (CCDC: **I**, 876272; **II**: 876271; **III**: 876273; **V**: 876274; **VI**: 876275).

	Peptide **I**	Peptide **II**	Peptide **III**	Peptide **V**	Peptide **VI**
Formula	$C_{14}H_{19}N_3O_5$	$C_{14}H_{19}N_3O_5$	$C_{14}H_{19}N_3O_4$	$C_{17}H_{21}N_3O_5$	$C_{16}H_{22}N_2O_5$
Molecular Wt(gm)	309.32	309.32	293.32	347.37	322.36
Crystal system	Triclinic	Triclinic	Monoclinic	Monoclinic	Monoclinic
Space group	$P1$	$P1$	$P2_1/n$	$P2_1$	$P2_1/c$
a (Å)	5.0385(3)	5.0685(3)	6.9562(2)	5.1032(2)	15.6945(7)
b (Å)	5.6116(3)	5.6696(4)	21.3693(6)	11.6839(4)	5.8390(3)
c (Å)	13.3741(9)	13.5082(8)	10.5537(3)	14.8422(6)	18.1326(8)
α (°)	97.152(4)	97.192(4)	90.00	90.00	90.00
β (°)	91.665(4)	91.116(5)	97.752(2)	98.153(3)	100.295(2)
γ (°)	95.767(4)	94.903(5)	90.00	90.00	90.00
Z	1	1	4	2	4
V (Å^3)	372.97(4)	383.53(4)	1554.46(8)	876.03(6)	
Density (gm/cm^3)	1.377	1.339	1.253	1.317	1.310
Measured reflns	2740	2770	4651	3693	3650
$R1 > 2\sigma(I)$,	0.0341	0.0444	0.0551	0.0431	0.0489
w$R2$	0.1027	0.1250	0.1805	0.1406	0.1437

to Gly by decreasing the steric bulk of the sidechain displays a completely extended motif [29] (Table 1, Entry h). Even homologation of the —CH_2— groups in the *N*-terminal residue of the peptide supports the formation of extended conformation [22] (Table 1, Entry *i, j*). Studies reveal that the *C*-terminal protecting group have almost negligible effect in determining the conformation in small synthetic peptides [30] (Table 1, Entry *k, l*). Recent report from the group of Pramanik displays that pseudopeptides comprising of α and ω-amino acids in the $i+1$th position and *p*-nitro aniline/*m*ABA in the $i+2$th position fails to support the reverse turn architecture [31,32] (Table 1, Entry m–q).

3.2. Solution conformational analysis

In order to examine the existence of reverse turn (β-turn & γ-turn) conformation of peptides **I**–**V** in solution phase various NMR experiments were performed.

Complete ^{1}H NMR assignment of peptides **I**–**V** were obtained using a combination of 2D COSY and ROESY methods in $CDCl_3$. In order to investigate the existence of intramolecular hydrogen bonding and peptide conformation in solution phase, the solvent dependence of the NH chemical shifts were examined by NMR titration [33]. In this experiment a solution of the peptide in non-polar $CDCl_3$ (10 mM in 0.5 ml) was gradually titrated against polar $(CD_3)_2SO$. The changes in the chemical shifts are presented in Fig. 3. The solvent titration experiment shows that by increasing the percentage of $(CD_3)_2SO$ in $CDCl_3$ from 0% to 6% (v/v) the net changes in the chemical shift ($\Delta\delta$) values for Ala(1)-NH, *m*NA (2) NH for peptide **I**, D-Ala(1)-NH, *m*NA-(2)-NH for peptide **II**, Ala(1)-NH, *m*NA-(2) NH for peptide **III**, Ala(1)-NH, *m*ABA(2)-NH for peptide **IV** and β-CN-Ala(1)-NH, *m*ABA(2)-NH for peptide **V** are 0.55, 0.66; 0.49, 0.65; 0.05, 0.18; 0.76, 0.50; and 0.73, 0.64 respectively. From the $\Delta\delta$ values it is evident that both the NH groups of peptides **I**–**II** & **IV**–**V** are solvent exposed, indicating their non-involvement in intramolecular hydrogen bonding. However these observations are consistent with the existence of a very weak intramolecular hydrogen bonding in a small acyclic peptide containing a conformationally constrained system at $i+2$ position which on addition of a strong polar solvent d_6-DMSO in a nonpolar environment $CDCl_3$, gets disrupted. Interestingly the NH groups of peptide **III** are solvent shielded indicating their involvement in intramolecular hydrogen bonding in solution.

Further attempts to characterize the reverse turn conformation in $CDCl_3$ using NOEs reveal that peptides **I**–**V** exhibits the NN(i, $i+1$) pattern, i.e. (Ala(1)NH $\leftrightarrow$ *m*NA(2)NH for peptides **I–III;** Ala(1)NH $\leftrightarrow$ *m*ABA(2)NH for peptide **IV** and β-CN-Ala(1)NH $\leftrightarrow$ *m*ABA(2)NH for peptide **V**) indicative of a reverse turn conformation in solution($CDCl_3$) (Figs. 5–9) irrespective of the configuration of the α-amino acid. Moreover the ROESY spectrum clearly shows a short range connectivity $d_{\alpha N}$ (i, $i+1$) [Ala(1)C$^{\alpha}$-H $\leftrightarrow$ *m*NA(2)NH] and $d_{\beta N}$ (i, $i+1$) [Ala(1)C$^{\beta}$H $\leftrightarrow$ *m*NA(2)NH] (Figs. 4–9, Table 4), diagnostic of a reverse turn conformation [34,35]. Since peptides **I**–**V** are small acyclic peptides with only two amino acids, there is no possibility for the presence of a β-turn conformation. Hence it is reasonable to assume that the structure adopted by the peptides **I**–**V** in solution is a γ-turn. The solution conformational analysis of peptide **VI** is under study.

4. Conclusions

In summary, the results display that pseudopeptides **I** and **II** with methyl as the sidechain substituent; *m*NA, the conformationally constrained nitro gamma amino moiety at the C-terminus and tertiary butyloxy carbonyl at the *N*-terminus, forms gamma turn conformation both in the solid state as well as in solution, irrespective of the configuration of the α-amino acid (L or D). Our solid state systematic conformational analysis further reveals that slight change in modification of any of the stated conditions donot support the formation of this γ-turn architecture. However this solid state conformational hypothesis doesnot hold true in solution for the remaning peptides (**III–V**) as is revealed by NMR measurements.

Thus the role of methyl group and methyl derivatives at the *N*-terminal residue of a dipeptide containing Ala (irrespective of configuration L or D) coupled with conformationally constrained *m*ABA/mNA is crucial for dictating this overall conformational γ-turn architecture. The importance of co-operative steric interactions in stabilizing a particular secondary motif in peptides is clearly evident from our systematic studies which may open a new avenue in introducing γ-turn motifs within the bioactive conformation of selected peptides.The function of other sidechain containing residues at the *N*-terminus of dipeptide in gamma turn formation is yet to be explored.

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