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# Peptidomimetic design of unusual turns by incorporating flexible and rigid ω-amino acids simultaneously

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ABSTRACT

The tripeptides Boc-Gly-Aib-*m*-ABA-OMe (**I**), Boc-βAla-Aib-*m*-ABA-OMe (**II**) and Boc-γAbu-Aib-*m*-ABA-OMe (**III**) (Aib: α-aminoisobutyric acid, βAla: β-alanine, γAbu: γ-aminobutyric acid, *m*-ABA: *meta*-aminobenzoic acid) with homologated amino acids at the N-terminus, the rigid γ-amino acid *m*-ABA at the C-terminus and the helicogenic Aib at the central position have been chosen to create unusual turns. Single crystal X-ray diffraction studies, solvent dependent NMR titrations and 2D NMR analysis reveal that peptides **II** and **III** adopt unusual turns of 11- and 12-membered rings stabilized by modified 4 → 1 type intramolecular hydrogen bonds. Solution phase studies indicate that peptide **I** exists in the β-turn conformation stabilized by 10-membered intramolecular hydrogen bonding.



## 1. Introduction

The interest in the conformational properties of oligopeptides formed by ω-amino acids has been stimulated by the recognition that new classes of folded structures can be formed by homooligomers of backbone homologated amino acids [1,2]. Since these amino acids have extra —$CH_2$— unit(s) compared to α-amino acids, they can modify the geometry of the peptide backbones [3] and provide proteolytic resistance to bioactive peptide sequences [4]. Several reports of incorporation of ω-amino acids into bioactive peptides originally composed of α-amino acids are reported in the literature [5]. The ω-amino acids such as γ-aminobutyric acid (γAbu), a neurotransmitter enzymatically produced [6] in the mammalian brain [7] and β-alanine (βAla), which occurs widely in the animal and plant kingdoms [8–12], have been used in peptide design to create unusual foldamers [13–20].

Reverse turns play important roles in stabilizing secondary and tertiary structures, initiating folding and facilitating intermolecular recognition [21]. Generally in a polypeptide chain constituted of α-amino acids, the *i*th C=O is hydrogen bonded to the *i* + 2 NH to form a γ-turn (7-membered), *i* + 3 NH to form a β-turn (10-membered), and *i* + 4 NH to form an α-turn (13-membered). Insertion of one or more extra carbon atom(s) into the intramolecular hydrogen bonded, folded structures leads to the creation of unusual turns and novel helical folds. As for examples helical structures with 12-membered hydrogen bonded rings have been observed in oligomers of acyclic and cyclic chiral β-amino acids [22–25], and 14-helical structures in cyclic and linear chiral β-amino acids [26–30]. Hetero-oligomers of α- and/or γ-substituted γ-amino acids show 14-helical conformations in solution as evident from their NMR studies [31,32]. Seebach's group has shown the formation of $2.6_{14}$-helical structures in crystals and in solution for γ-peptides comprising of 2-, 3-, and 4-substituted chiral γ-amino acids [33]. In most cases unusual turns are exhibited by substituted chiral residues, which are conformationally restricted. Designs of turns with unsubstituted βAla and γAbu, which are more flexible, have also been reported [17,18]. Peptides incorporating a centrally positioned βAla–γAbu segment have been found to exhibit unusual turns involving *e.g.* 12-, 14-, 16-, and 19-membered hydrogen bonded rings [14].

*meta*-Aminobenzoic acid (*m*-ABA) is considered as a rigid γ-aminobutyric acid with an all-*trans* extended conformation suitable for promoting a β-sheet-like structures [34–37]. In this paper, we are interested to explore the possibility of generating unusual turns in small peptides by inserting both flexible ω-amino acids (βAla, γAbu) as well as rigid ω-amino acid (*m*-ABA) simultaneously. Therefore we have chosen peptides Boc-Gly-Aib-*m*-ABA-OMe (**I**) (Aib, α-aminoisobutyric acid), Boc-βAla-Aib-*m*-ABA-OMe (**II**) and Boc-γAbu-Aib-*m*-ABA-OMe (**III**) (Fig. 1). Insertion of one or more extra carbon atom(s) in the sequence of peptides **II** and **III** has been carried out by changing the N-terminal Gly of peptide **I** with βAla and γAbu. The centrally placed helicogenic Aib is expected to provide the turn structure in the peptide backbone [38–47]. Peptide **I** which contains two α-amino acids and only one ω-amino acid, *m*-ABA at the C-terminus has the highest potentiality among the three peptides to form a turn-like structure. We wanted to establish whether the insertion

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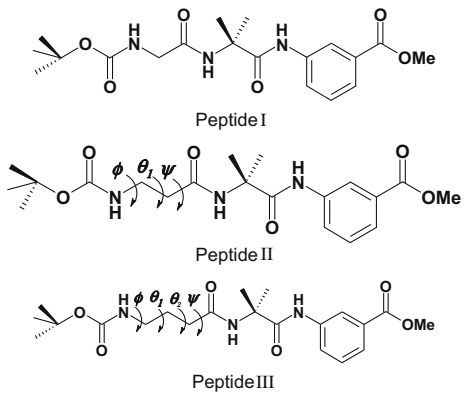


**Fig. 1.** Schematic diagram of peptides **I–III**.

of flexible and rigid ω-amino acids simultaneously as in peptides **II** and **III** can lead to the creation of unusual turns and novel helical folds through intramolecular hydrogen bonding. There are examples of constrained cyclic peptides in which substituted benzenes have been inserted to mimic the turn region of the neurotrophin, a nerve growth factor [48]. Peptides **I–III** will be examples of acyclic analogues if they adopt turn structures. All the peptides were prepared by conventional solution phase synthesis and their conformational studies have been carried out by single crystal X-ray diffraction and NMR studies.

## 2. Experimental

### 2.1. Synthesis of the peptides

The peptides were synthesized by conventional solution phase methods. The *tert*-butyloxycarbonyl (Boc) group was used for N-terminal protection and the C-terminus was protected as methyl ester. Couplings were mediated by *N*,*N*′-dicyclohexylcarbodiimide (DCC)/1-hydroxybenzotriazol (HOBT) [49]. All intermediates were characterized by thin layer chromatography on silica gel and used without further purification. Final peptides were purified by column chromatography using silica gel (100–200 mesh) as the stationary phase. Ethyl acetate and petroleum ether mixture was used as the eluent. The peptides **II** and **III** were fully characterized by X-ray crystallography and NMR studies and peptide **I** with NMR spectroscopy.

#### 2.1.1. Boc-Gly-Aib-OMe (**1**)

Boc-Gly-OH (2.0 g, 11.42 mmol) was dissolved in a mixture of dichloromethane (DCM, 6 ml) and dimethylformamide (DMF, 5 ml). H-Aib-OMe obtained from its hydrochloride (3.5 g, 22.84 mmol) was added to the former solution followed by addition of DCC (3.52 g, 17.13 mmol) and HOBT (1.54 g, 11.42 mmol) in ice-cold condition. The reaction mixture was stirred at room temperature for 1 day. The precipitated *N*,*N*′-dicyclohexylurea (DCU) was filtered off. The organic layer was diluted with 30 ml ethyl acetate and washed with 1 N HCl (3 × 30 ml), brine, 1 M $Na_2CO_3$ solution (3 × 30 ml) and then again with brine. The solvent was dried over anhydrous $Na_2SO_4$ and evaporated *in vacuo* to give a waxy colorless solid. Yield: 2.7 g (86.26%).

#### 2.1.2. Boc-Gly-Aib-OH (**2**)

Peptide **1** (2.7 g, 9.85 mmol) was dissolved in methanol (20 ml) and 2 N NaOH (10 ml) was added to it. The reaction mixture was stirred for 1 day at room temperature. The progress of reaction was monitored by TLC. After completion of reaction the methanol was evaporated. The residue was diluted with water and washed with diethyl ether. The aqueous layer was cooled in ice-bath, neutralized by using 2 N HCl and extracted with ethyl acetate. The solvent was evaporated *in vacuo* to give a waxy colorless solid. Yield: 2.2 g (85.93%).

#### 2.1.3. Boc-Gly-Aib-m-ABA-OMe (peptide **I**)

Peptide **2** (2.2 g, 8.46 mmol) was dissolved in dichloromethane (DCM, 5 ml). H-*m*ABA-OMe obtained from its hydrochloride (3.17 g, 16.92 mmol) was added to former solution, followed by addition of DCC (2.61 g, 12.69 mmol) and HOBT (1.14 g, 8.46 mmol) in ice-cold condition. The reaction mixture was stirred at room temperature for 2 days. The precipitated DCU was filtered off. The organic layer was diluted with 30 ml ethyl acetate and washed with 1 N HCl (3 × 30 ml), brine, 1 M $Na_2CO_3$ solution (3 × 30 ml) and then again with brine. The solvent was then dried over anhydrous $Na_2SO_4$ and evaporated *in vacuo*, to give a colorless solid compound. Purification was done using silica gel as stationary phase and ethyl acetate–petroleum ether mixture (1:4) as eluent.

Yield: 2.8 g (84.33%). Mp = 168 °C; IR (KBr): 3352, 3284.6, 2973.1, 2930.7, 1719.8, 1676.3, 1541.5 $cm^{-1}$; $^1H$ NMR 300 MHz ($CDCl_3$, $\delta$ ppm): 9.16 (*m*-ABA(3)-NH, 1H, s), 8.23 ($H_a$ *m*-ABA(3), 1H, s), 7.97($H_d$ *m*-ABA(3), 1H, d, *J* = 7.8 Hz), 7.75 ($H_b$ *m*-ABA(3), 1H, d, *J* = 7.9 Hz), 7.37 ($H_c$ *m*-ABA(3), 1H, m), 6.60 (Aib(2)-NH, 1H, s), 5.34 (Gly(1)-NH, 1H, s), 4.30 ($C^{\alpha}H$ of Gly(1), 2H, t, *J* = 6.6 Hz), 3.89 (—$OCH_3$, 3H, s), 1.62 and 1.25 ($C^{\beta}H$ of Aib(2), 6H, s), 1.46 (Boc-$CH_3$s, 9H, s); $^{13}C$ NMR 75 MHz ($CDCl_3$, $\delta$ ppm): 172.48, 169.61, 166.87, 138.69, 130.89, 130.63, 128.85, 126.34, 125.07, 124.53, 128.85, 126.34, 125.07, 124.53, 121.03, 81.14, 58.08, 52.06, 28.18, 25.50; Anal. Calcd for $C_{19}H_{27}N_3O_6$ (393.43): C, 57.99; H, 6.91; N, 10.68%; Found: C, 57.91; H, 6.84; N, 10.60%.

#### 2.1.4. Boc-βAla-Aib-OMe (**3**)

Peptide **3** was synthesized following the same procedure as that of peptide **1**, starting with Boc-βAla-OH (2.0 g, 10.57 mmol). Yield: 2.6 g (85.52%).

#### 2.1.5. Boc-βAla-Aib-OH (**4**)

Peptide **4** was synthesized following the same procedure as that of peptide **2**. Yield: 2.1 g (85.02%).

#### 2.1.6. Boc-βAla-Aib-m-ABA-OMe (peptide **II**)

Synthesis and purification of peptide **II** were done following the same procedure as in the case of peptide **I** starting with peptide **4**. Single crystals of peptide **II** were grown from $CHCl_3$–petroleum ether by slow evaporation and were stable at room temperature.

Yield: 2.7 g (86.53%). Mp = 108 °C; IR (KBr): 3357.9, 3322.6, 2981.9, 1710.3, 1668.5, 1532.2 $cm^{-1}$; $^1H$ NMR 300 MHz ($CDCl_3$, $\delta$ ppm): 9.32 (*m*-ABA(3)-NH, 1H, s), 8.14 ($H_a$ *m*-ABA(3), 1H, s), 7.77 ($H_d$ *m*-ABA(3), 1H, d, *J* = 8.1 Hz), 7.75 ($H_b$ *m*-ABA(3), 1H, d, *J* = 7.9 Hz), 7.37 ($H_c$ *m*-ABA(3), 1H, m), 6.4 (Aib(2)-NH, 1H, s), 5.10 (βAla(1)-NH, 1H, s), 3.90 (—$OCH_3$, 3H, s), 3.39–3.45 ($C^{\beta}H$ of βAla(1), 2H, m), 2.35–2.49 ($C^{\alpha}H$ of βAla(1), 2H, m), 1.62 ($C^{\beta}H$ of Aib(2), 6H, s), 1.42 (Boc-$CH_3$s, 9H, s); $^{13}C$ NMR 75 MHz ($CDCl_3$, $\delta$ ppm): 173.02, 172.45, 166.80, 156.42, 138.53, 130.78, 128.94, 125.16, 124.61, 121.04, 79.83, 58.57, 52.11, 37.48, 36.96, 28.34, 25.36; Anal. Calcd for $C_{20}H_{29}N_3O_6$ (407.47): C, 58.90; H, 7.17; N, 10.31%; Found: C, 58.82; H, 7.08; N, 10.25%.

#### 2.1.7. Boc-γAbu-Aib-OMe (**5**)

Peptide **5** was synthesized following the same procedure as that of peptide **1**, starting with Boc-γAbu-OH (2.0 g, 9.84 mmol). Yield: 2.6 g (87.54%).

#### 2.1.8. Boc-γAbu-Aib-OH (**6**)

Peptide **6** was synthesized following the same procedure as that of peptide **2**. Yield: 2.2 g (89.06%).

*2.1.9. Boc-γAbu-Aib-m-ABA-OMe (peptide **III**)*

Synthesis and purification of peptide **III** were done following the same procedure as in the case of peptide **I** starting with peptide **6**. Single crystals of peptide **III** were grown from $CHCl_3$–petroleum ether by slow evaporation and were stable at room temperature.

Yield: 2.8 g (87.22%). Mp = 134 °C; IR (KBr): 3345.1, 3288.5, 2979.5, 1675, 1536.1 $cm^{-1}$; $^1H$ NMR 300 MHz ($CDCl_3$, $\delta$ ppm): 9.78 (*m*-ABA(3)-NH, 1H, s), 8.19 ($H_a$ *m*-ABA(3), 1H, s), 7.92 ($H_d$ *m*-ABA(3), 1H, d, *J* = 8.1 Hz), 7.73 ($H_b$ *m*-ABA(3), 1H, d, *J* = 7.8 Hz), 7.35 ($H_c$ *m*-ABA(3), 1H, t, *J* = 7.95), 6.49 (Aib(2)-NH, 1H, s), 4.84 (γAbu(1)-NH, 1H, t, *J* = 6 Hz), 3.88 (—$OCH_3$, 3H, s), 3.13 ($C^{\gamma}H$ of γAbu(1), 2H, m), 2.25($C^{\alpha}H$ of γAbu(1), 2H, m), 1.79 ($C^{\beta}H$ of γAbu(1), 2H, m), 1.61 ($C^{\beta}H$ of Aib(2), 6H, s), 1.48 (Boc-$CH_3$s, 9H, s); $^{13}C$ NMR 75 MHz ($CDCl_3$, $\delta$ ppm): 173.01, 172.90, 166.90, 157.26, 139.09, 130.65, 128.80, 124.76, 124.46, 120.88, 79.90, 58.13, 52.00, 33.22, 28.39, 26.95, 25.41; Anal. Calcd for $C_{21}H_{31}N_3O_6$ (421.48): C, 59.83; H, 7.41; N, 9.97%; Found: C, 59.76; H, 7.32; N, 9.88%.

*2.2. FT-IR spectroscopy*

IR spectra were examined using a Perkin-Elmer-782 model spectrophotometer. The solid-state FT-IR measurements were performed using the KBr disk technique.

*2.3. NMR experiments*

All $^1H$ NMR and $^{13}C$ NMR studies were recorded on a Bruker Avance 300 model spectrometer operating at 300, 75 MHz, respectively. The peptide concentrations were 10 mM in $CDCl_3$ for $^1H$ NMR and 40 mM in $CDCl_3$ for $^{13}C$ NMR. Solvent titration experiments were carried out at a concentration of 10 mM in $CDCl_3$ with gradual addition of $d_6$-DMSO from 0% to 10% v/v approximately. Resonance assignments were done using two-dimensional ROSEY spectra [50].

*2.4. Crystal data for peptides **II** and **III***

Peptide **II**: $C_{20}H_{29}N_3O_6$, 0.5$CHCl_3$, *M* = 467.15, monoclinic, space group $P2_1/n$, *Z* = 8, *a* = 12.3329(8) Å, *b* = 27.6445(12) Å, *c* = 14.6166(5) Å, $\beta$ = 97.987(4)°, *V* = 4935.0(4) $Å^3$. Peptide **III**: $C_{21}H_{31}N_3O_6$, *M* = 421.49, monoclinic, space group $P2_1/c$, *Z* = 4, *a* = 9.677(1) Å, *b* = 24.207(4) Å, *c* = 9.953(1) Å, $\beta$ = 104.03(1)°, *V* = 2261.8(5) $Å^3$. Diffraction data for the two peptides **II** and **III**, were obtained with MoKα radiation at 150 K using the Oxford Diffraction X-Calibur CCD System. The crystals were positioned at 50 mm from the CCD. 321 frames were measured with a counting time of 10 s. Data analyses were carried out with the Crysalis program [51]. The structures were solved and refined using direct methods with the SHELXS-97 programs [52]. The non-hydrogen atoms were refined with anisotropic thermal parameters. The hydrogen atoms bonded to carbon were included in geometric positions and given thermal parameters equivalent to 1.2 times (or 1.5 times for methyl hydrogen atoms) those of the atom to which they were attached. Crystallographic details have been deposited at the Cambridge Crystallographic Data Centre, reference CCDC Nos. are 742973 and 742974.

## 3. Results and discussion

*3.1. Peptides conformations in the crystal state*

Although we were able to grow single crystals of peptides **II** and **III** suitable for X-ray diffraction studies, we were not able to generate single crystals for peptide **I**. Peptide Boc-βAla-Aib-*m*-ABA-OMe (**II**) crystallizes in the centrosymmetric space group $P2_1/n$ with two molecules in the crystallographic asymmetric unit designated as **A** and **B** together with one molecule of solvent chloroform. Both peptide molecules adopt turn structures (Fig. 2). The insertion of one extra $CH_2$ group into the peptide backbone due to the presence of β-Ala causes an unusual, modified 4 → 1 type hydrogen bond between CO of the Boc group and *m*-ABA(3)-NH forming 11-membered hydrogen bonded rings. Since peptide **II** is achiral the choice of the signs of the backbone torsion angles is arbitrary (Table 1). Inspection of the torsion angles shows that molecules **A** and **B** are structurally similar where the centrally placed Aib adopts helical conformations ($\phi$, $\psi$: 53.3(3)°, 38.3(3)° in **A**, 59.0(3)°; 36.1(3)° in **B**) (Table 1). The $\phi$ and $\psi$ angles of βAla of both molecules are in the semi-extended region. The values of the torsion angles ($\theta_1$) about the —$CH_2$—$CH_2$— unit of βAla are slightly higher than the ideal values of *gauche* conformations, *e.g.* +60° and −60° (Table 1). In all cases the strong intramolecular hydrogen bonds (N(10)—H(10)...O(2)=C(2)) characterized by short distances (2.917(3) Å in **A**, 2.975(3) Å in **B**), are well complemented

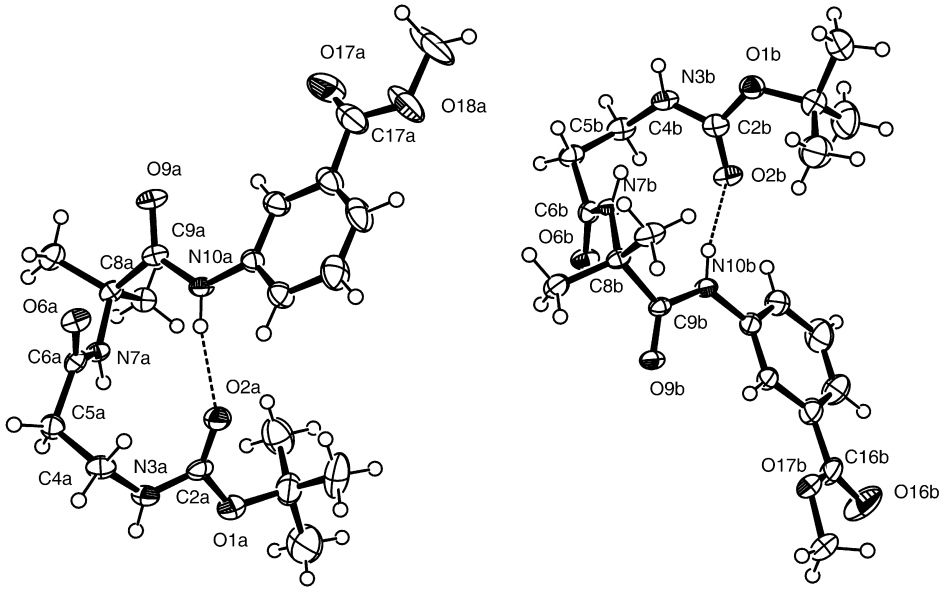


**Fig. 2.** ORTEP diagrams of peptide **II** (**A** and **B**) with atom numbering scheme. Thermal ellipsoids are shown at the 30% probability. Hydrogen bonds are shown as dotted lines.

**Table 1**
Selected backbone torsion angles (°) for peptides **II** and **III**.[a]

| Peptide | Residue | $\phi$ | $\theta_1$ | $\theta_2$ | $\psi$ | $\omega$ |
|---|---|---|---|---|---|---|
| **IIA** | βAla(1) | 92.5(3) | −84.5(3) | – | 98.9(3) | 169.8(2) |
| | Aib(2) | 53.3(3) | | | 38.3(3) | 171.9(2) |
| **IIB** | βAla(1) | 99.7(3) | −75.0(3) | – | 89.6(3) | 166.6(2) |
| | Aib(2) | 59.0(3) | – | – | 36.1(3) | 175.2(3) |
| **III** | γAbu(1) | 114.2(2) | −60.7(2) | −62.4(2) | 142.2(1) | 169.1(1) |
| | Aib(2) | 50.8(2) | – | – | 41.8(2) | −174.6(1) |

[a] Peptides **II** and **III** are achiral and therefore the choice of the signs of the backbone torsion angles is arbitrary.

**Table 2**
Hydrogen bonds parameters for peptides **II** and **III**.

| Type | N…O/(Å) | H…O/(Å) | O…H—N/(°) |
|---|---|---|---|
| Peptide **IIA** | | | |
| *Intramolecular* | | | |
| N(10A)—H(10A)…O(2A) | 2.917(3) | 2.11 | 156 |
| *Intermolecular* | | | |
| N(3A)—H(3A)…O(9B)[a] | 2.852(3) | 2.06 | 154 |
| N(7A)—H(7A)…O(6B)[b] | 2.874(3) | 2.21 | 134 |
| Peptide **IIB** | | | |
| *Intramolecular* | | | |
| N(10B)—H(10B)…O(2B) | 2.975(3) | 2.19 | 153 |
| *Intermolecular* | | | |
| N(7B)—H(7B)…O(9A)[c] | 2.837(3) | 2.19 | 132 |
| N(3B)—H(3B)…O(6A)[c] | 2.890(3) | 2.04 | 169 |
| Peptide **III** | | | |
| *Intramolecular* | | | |
| N11—H11…O2 | 3.033(2) | 2.25 | 152 |
| *Intermolecular* | | | |
| N3—H3…O10[d] | 3.003 (2) | 2.18 | 160 |
| N8—H8…O7[e] | 2.977(2) | 2.31 | 135 |

Symmetry elements: [a]1 − *x*, 1 − *y*, 1 − *z*; [b]½ − *x*, ½ + *y*, ½ − *z*; [c]1 − *x*, 1 − *y*, −*z*; [d]*x* − 1, *y*, *z*; [e]*x*, ½ − *y*, ½ + *z*.

by the N—H…O=C angles of 156° in **A**, 153° in **B** to form stable 11-membered unusual turns (Fig. 2 and Table 2). Interestingly a previous report shows that peptide Boc-βAla-Aib-βAla-OMe where *m*-ABA of peptide **II** has been replaced by flexible ω-amino acid βAla adopts a fully extended conformation and does not possess any intramolecular hydrogen bond [38]. The result indicates that when the third residue is a flexible ω-amino acid like βAla in the above sequence, the centrally placed Aib can not dictate its stereochemical preference fully to produce a turn structure through cooperative steric interactions amongst the amino acid residues. But replacement of βAla with rigid *m*-ABA as in peptide **II** helps sterically to form turn structure. Peptide **II** provides the first example of the formation of an 11-membered unusual turn stabilized by an intramolecular hydrogen bond in a tripeptide containing βAla at the N-terminus. The conformational heterogeneity as it is observed in case of peptide **II (A and B)** in the solid state has biological implications since interconversion between β-turn types have been observed in HIV protease [53].

The peptide Boc-γAbu-Aib-*m*-ABA-OMe (**III**) crystallizes with one molecule in the crystallographic asymmetric unit, again in a centrosymmetric space group ($P2_1/c$). The crystal structure reveals that it adopts an unusual turn structure stabilized by 12-membered 4 → 1 type hydrogen bond between the C(2)=O(2) of the Boc group and the N(11)—H(11) of *m*-ABA(3) (Fig. 3). Like peptide

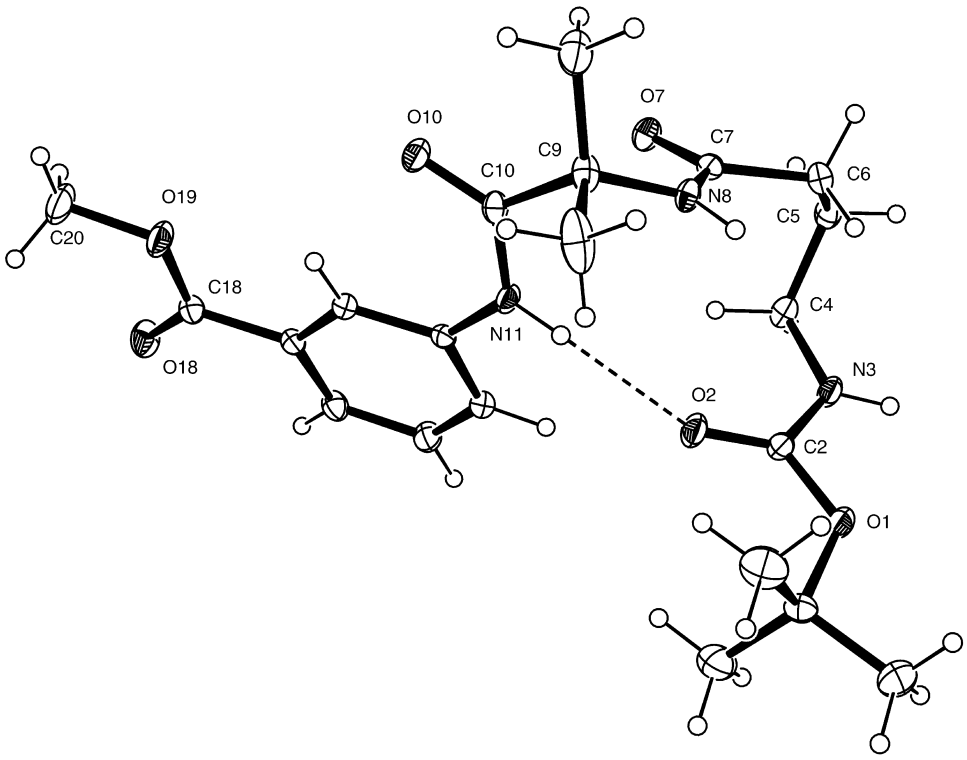


**Fig. 3.** ORTEP diagram of peptide **III** with atom numbering scheme. Thermal ellipsoids are shown at 30% probability. Hydrogen bond is shown as dotted line.

**II**, peptide **III** is also achiral and therefore the choice of the signs of the backbone torsion angles is arbitrary (Table 1). The centrally placed Aib is found to adopt a helical conformation with $\phi$ values as 50.8(2)°, 41.8(2)°, similar to the values observed in **II**. The $\phi$ and $\psi$ angles of γAbu in peptide **III** are found to be in the extended region, 114.2(2)°, 142.2(1)°. The torsion angles $\theta_1$, $\theta_2$ about the —$CH_2$—$CH_2$—$CH_2$— unit of γAbu are in *gauche* conformation, −60.7(2)°, −62.4(2)°. This disposition facilitates the easy accommodation of the $CH_2$ groups into the folded peptide backbone leading to an unusual 12-membered hydrogen bonded ring in peptide **III**. This strong intramolecular hydrogen bond from N(11)—H(11) to O(2)═C(2) is characterized by the short distance 3.033(2) Å (N...O), well complemented by the N—H...O angle of 152° to form a stable 12-membered ring (Fig. 3 and Table 2). Generation of 12-membered reverse turns has also been observed previously in synthetic peptides containing γAbu at the N-terminus [18] and also in peptides with centrally positioned, sterically constrained dinipecotic acid segments [54,55]. Peptide **III** provides a unique example in which a 12-membered turn has been stabilized in a tripeptide in spite of having two ω-amino acids in the sequence.

The molecular packing arrangements of peptides **II** and **III** are shown in Figs. 4 and 5. Each molecule **A** of peptide **II** is linked with three neighbouring **B** molecules through intermolecular hydrogen bonding. These interactions involve βAla(B)-NH...O═C-βAla(A), Aib(B)-NH...O═C-Aib(A), Aib(B)-C═O...HN-βAla(A) and Aib(A)-NH...O═C-βAla(B) (Fig. 4 and Table 2). The same is true for each **B** molecule which interacts with three **A** molecules in a similar fashion. The molecules of peptide **II** are interlinked in *ac* plane to form two-dimensional sheet-like structure (Fig. 4). Each molecule of peptide **III** is linked with four neighbouring molecules through one hydrogen bond to each such as γAbu-NH...O═C-Aib and Aib-NH...O═C-γAbu resulting in a two-dimensional sheet-like structure in *ac* plane (Fig. 5 and Table 2).

### 3.2. Solution phase conformations

In absence of a crystal structure the solution phase conformation of the peptide Boc-Gly-Aib-*m*-ABA-OMe (**I**) was probed by

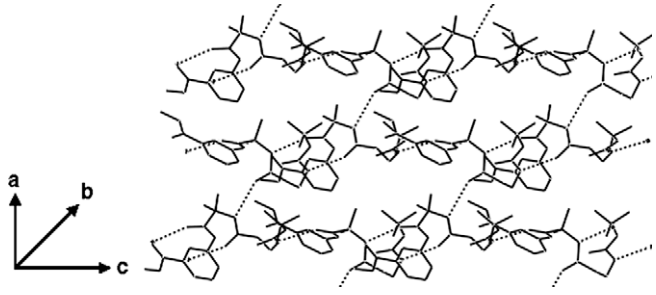


**Fig. 4.** The packing arrangement of peptide **II** in *ac* plane. Intermolecular hydrogen bonds are shown as dotted lines. Hydrogen atoms are omitted for clarity.

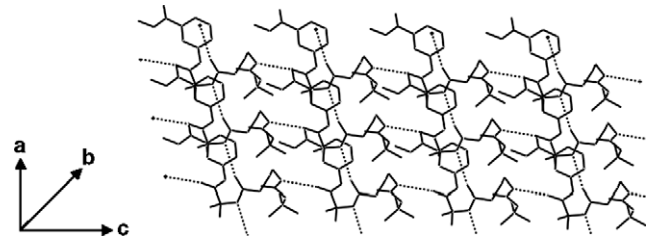


**Fig. 5.** The packing arrangement of peptide **III** in the *ac* plane forming β-sheet-like structure. Hydrogen bonds are shown as dotted lines. Hydrogen atoms are omitted for clarity.

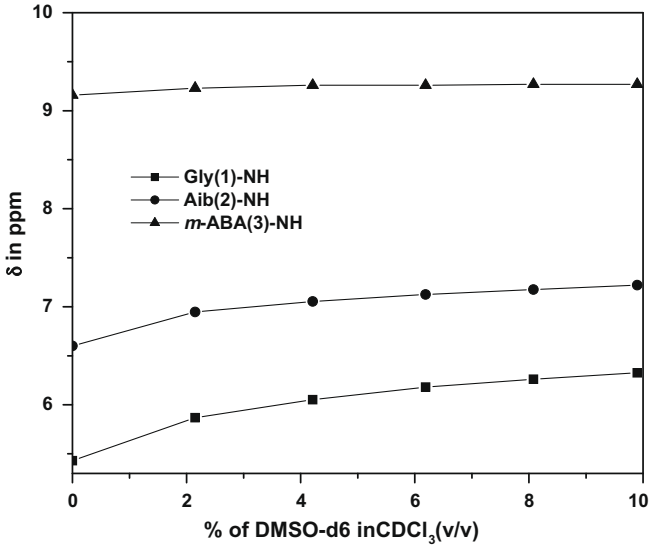


**Fig. 6.** NMR solvent titration curves for NH protons in peptide **I**.

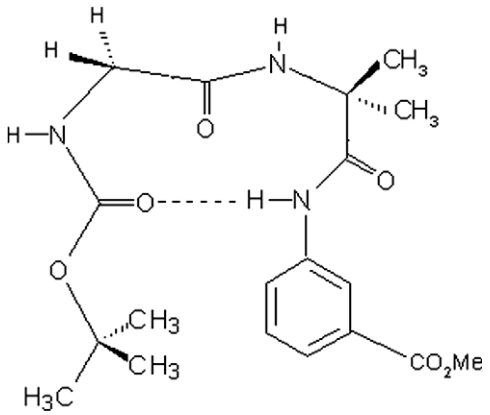


**Fig. 7.** Schematic representation of β-turn structure of peptide **I** stabilized by 10-membered intramolecular hydrogen bond.

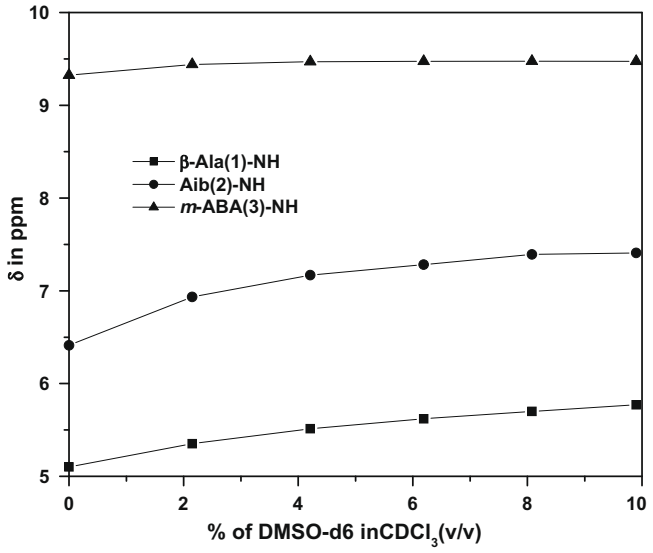


**Fig. 8.** NMR solvent titration curves for NH protons in peptide **II**.

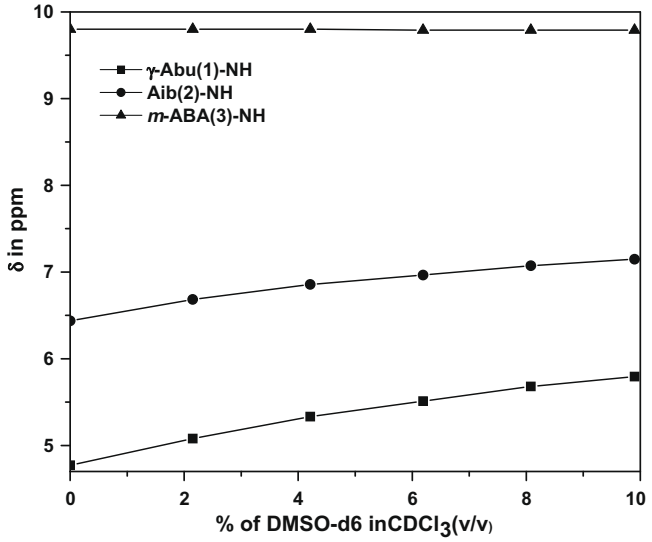


**Fig. 9.** NMR solvent titration curves for NH protons in peptide **III**.

the NMR solvent titration method. In this experiment a solution of peptide **I** in nonpolar $CDCl_3$ (10 mM in 0.5 ml) was gradually titrated against polar $(CD_3)_2SO$ and the changes in the chemical shifts of NHs were recorded by $^1H$ NMR (Fig. 6) [56–58]. The solvent titration shows that by increasing the percentage of $(CD_3)_2SO$ in $CDCl_3$ from 0 to 10% the net changes in the chemical shift ($\Delta\delta$) values for Gly(1)-NH, Aib(2)-NH and *m*-ABA(3)-NH are 0.89, 0.56 and 0.11 ppm, respectively. The $\Delta\delta$ values demonstrate that *m*-ABA(3)-NH is solvent shielded, and the other two NH groups are solvent exposed, which is a characteristic feature of a β-turn structure where the *m*-ABA(3)-NH is involved in an intramolecular hydrogen bond to Boc-CO (Fig. 7). In $^1H$ NMR solvent titration of peptide **II** the net changes in the chemical shift ($\Delta\delta$) values for βAla(1)-NH, Aib(2)-NH and *m*-ABA(3)-NH are 0.67, 0.99 and 0.14 ppm, respectively (Fig. 8), indicating a turn structure where *m*-ABA(3)-NH is hydrogen bonded to Boc-CO as it is observed in the crystal structure (Fig. 2). In a similar experiment the solvent dependence of the NH chemical shifts, that is demonstrated in this $CDCl_3$-$(CD_3)_2SO$ titration experiment of peptide **III** (Fig. 9), indicates that γAbu(1)-NH and Aib(2)-NH are free with $\Delta\delta$ values as 1.02 and 0.71 ppm, respectively, and *m*-ABA(3)-NH is intramolecularly hydrogen bonded with $\Delta\delta$ value as −0.01 ppm, indicating a turn structure as depicted in the crystal structure (Fig. 3).

**Table 3**
Important inter-residue NOEs for peptides **II** and **III**.

| Peptide **II** | Peptide **III** |
|---|---|
| *m*ABA(3)NH ↔ Aib(2)$C^{\beta}H$ | *m*ABA(3)NH ↔ Aib(2)$C^{\beta}H$ |
| Aib(2)$C^{\beta}H$ ↔ Aib(2)NH | Aib(2)$C^{\beta}H$ ↔ Aib(2)NH |
| Aib(2)NH ↔ βAla(1)$C^{\alpha}H$ | Aib(2)NH ↔ γAbu(1)$C^{\alpha}H$ |
| βAla(1)$C^{\alpha}H$ ↔ βAla(1)NH | Aib(2)NH ↔ γAbu(1)$C^{\beta}H$ |
| βAla(1)NH ↔ βAla(1)$C^{\beta}H$ | γAbu(1)$C^{\alpha}H$ ↔ γAbu(1)$C^{\beta}H$ |
| | γAbu(1)$C^{\alpha}H$ ↔ γAbu(1)$C^{\gamma}H$ |
| | γAbu(1)$C^{\beta}H$ ↔ γAbu(1)$C^{\gamma}H$ |

The formation of unusual turns by peptides **II** and **III** in the solution phase was further confirmed by 2D NMR analysis. Several inter-residue NOEs for peptides **II** and **III** are listed in Table 3. Furthermore, the ROESY spectrum [50] of peptide **II** in $CDCl_3$ (Fig. 10) reveals two important inter-residue NOEs, namely, *m*ABA(3)NH ↔ Aib(2)$C^{\beta}H$ and Aib(2)NH ↔ βAla(1)$C^{\alpha}H$. This NOE data and the solvent-shielded nature of *m*ABA(3)-NH are supportive of a 11-membered hydrogen bonded ring involving C═O from the Boc group and *m*ABA(3)-NH for peptide **II** in solution. Several inter-residue key NOEs have been found for peptide **III** in $CDCl_3$ (Fig. 11). The NOEs of *m*ABA(3)NH ↔ Aib(2)$C^{\beta}H$ and Aib(2)NH ↔ γAbu(1)-$C^{\alpha}H$ and the solvent-shielded nature of *m*ABA(3)-NH are indicative of an unusual turn involving a 12-membered hydrogen bonded ring, with C═O from the Boc group and *m*ABA(3)-NH. Thus, the crystallographic and NMR data for both peptides **II** and **III** are in mutual agreement.

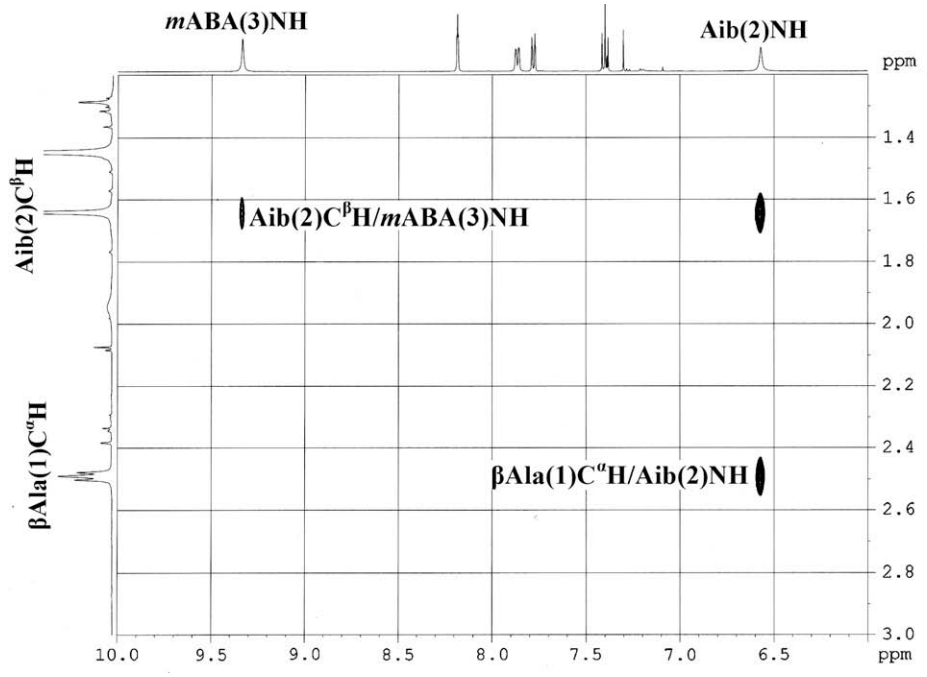


**Fig. 10.** Partial view of $^1H$–$^1H$ ROESY spectrum of peptide **II** in $CDCl_3$ (peptide concn: $1 \times 10^{-2}$ M), showing only $d_{\alpha N}(i, i+1)$, where $i = 1$ and $d_{\beta N}(i, i+1)$, where $i = 2$ crosspeaks, diagnostic of turn structure.

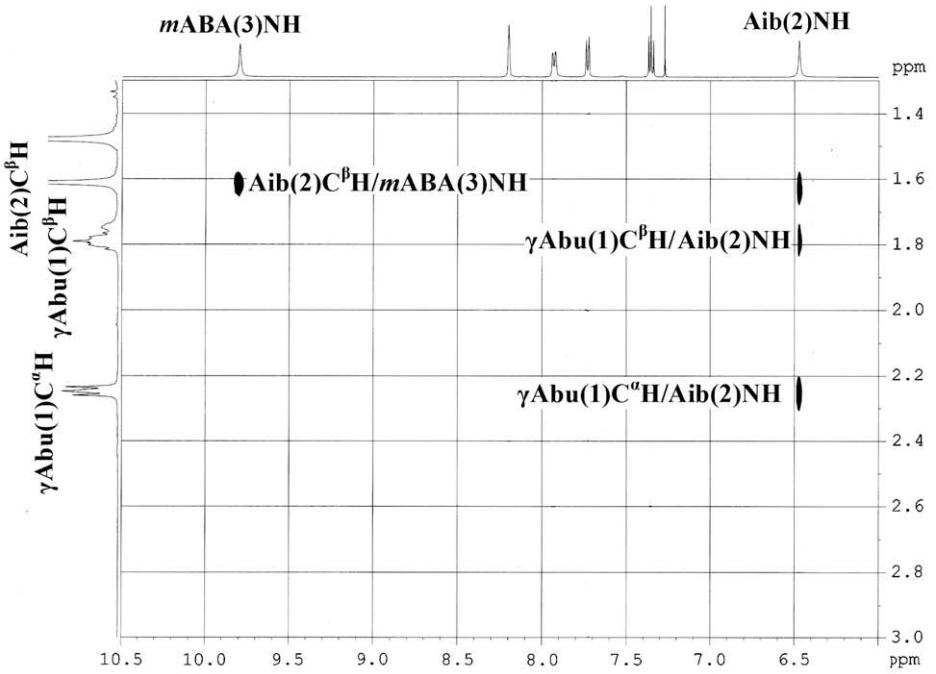


**Fig. 11.** Partial view of $^1H$–$^1H$ ROESY spectrum of peptide **III** in $CDCl_3$ (peptide concn: $1 \times 10^{-2}$ M), showing only $d_{\alpha N}(i, i+1)$, where $i = 1$ and $d_{\beta N}(i, i+1)$, where $i = 1$ and 2, crosspeaks, diagnostic of turn structure.

## 4. Conclusions

It has been shown that the incorporation of flexible ω-amino acids such as βAla, γAbu at the N-terminus and rigid γ-amino acid such as *m*-amino benzoic acid at the C-terminus in acyclic tripeptides with centrally placed helicogenic Aib can lead to the formation of unusual turns of 11- and 12-membered rings stabilized by modified 4 → 1 type intramolecular hydrogen bonds. *m*-amino benzoic acid, a rigid γ-aminobutyric acid, in spite of having high propensity for β-sheet formation, has been nicely accommodated into the turn structures generated by peptides **I–III**. The conformational heterogeneity as it is observed in case of peptide **II** with two isomeric structures provides insights for designing bioactive peptides of conformationally flexible turns. Peptides **II** and **III** represent a potentially useful way for peptidomimetic design of unusual turns which will help to develop biologically active peptides resistant to proteolytic hydrolysis.

## Supporting informations

Supplementary data contains $^1H$ NMR of peptides **I–III**, $^1H$–$^1H$-ROESY spectra of peptides **II** and **III** and crystallographic data in CIF formats of peptides **II** and **III**.

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