Peptide Design Using ω-Amino Acids: Unusual Turn Structures Nucleated by an N-Terminal Single γ-Aminobutyric Acid Residue in Short Model Peptides

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Incorporation of ω-amino acids into peptide sequences plays an important role in designing peptides with modified backbone conformation and enhanced stability against proteolysis. The present study establishes the presence of unusual turns involving 12-membered hydrogen bonded rings in terminally blocked tri- and tetrapeptides. X-ray diffraction analysis of single crystals and NMR studies have been used to probe the three-dimensional structures of two terminally protected short peptides, Boc-γ-Abu(1)-Aib(2)-Ala(3)-OMe **1** and Boc-γ-Abu(1)-Aib(2)-Ala(3)-Aib(4)-OMe **2** (γ-Abu = γ-aminobutyric acid), in which conformationally flexible ω-amino acids (γ-Abu) and conformationally restricted α-aminoisobutyric acid (Aib) residues are positioned contiguously. The crystal structures of both peptides **1** and **2** exhibit unusual turns composed of 12-membered hydrogen bonded rings involving C═O from the Boc-group and Ala(3) NH. A type I′ β-turn was observed in the structure of peptide **2** adjacent to the unusual turn with a hydrogen bond between γ-Abu(1) C═O and Aib(4) NH. The crystals of peptide **1** are in the space group $P2_1$, a = 9.3020(10) Å, b = 23.785(2) Å, c = 10.022(3) Å, β = 101.35°(4), Z = 4, R = 5.7%, and R_w = 14.5%. Similarly, the crystals of peptide **2** are in the space group $C2$, a = 19.0772(6) Å, b = 8.7883(2) Å, c = 16.7758(3) Å, β = 110.7910°(10), Z = 4, R = 6.71%, and R_w = 15.11%. The unusual turn in both peptides **1** and **2** are retained in solution as is evident from NMR studies in $CDCl_3$. The role of the adjacently located Aib residue to nucleate the 12-membered hydrogen bonded ring is also addressed.

Introduction

The use of ω-amino acids in peptide design is a newly emerging area of current research.[1] This is mainly due to the ability of these amino acids to modify the geometry of the peptide backbone[2] and provide proteolytic resistance to bioactive peptide sequences.[3] Incorporation of ω-amino acids into bioactive peptides originally composed of α-amino acids has also been reported.[4] γ-Aminobutyric acid (γ-Abu), a neurotransmitter enzymatically produced[5] in the mammalian brain,[6] has been used to design unusual foldamers.[1b]

Reverse turns play an important role in protein structure[7] and folding.[8] Generally, three types of reverse turns (namely, α-, β-, and γ-turns) have been observed in protein structures. In a polypeptide chain composed of α-amino acids, the ith C═O is hydrogen bonded to i + 2NH for a γ-turn (7 membered), i + 3NH for a β-turn (10 membered), and i + 4NH for 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. Such folds have been reported by Gellman et al., Seebach et al., and Hanessian et al. using substituted, chiral β- or γ-amino acids. Helical structures with 12-membered hydrogen bonded rings have been observed in oligomers of acyclic and cyclic chiral β-amino acids,[9−12]

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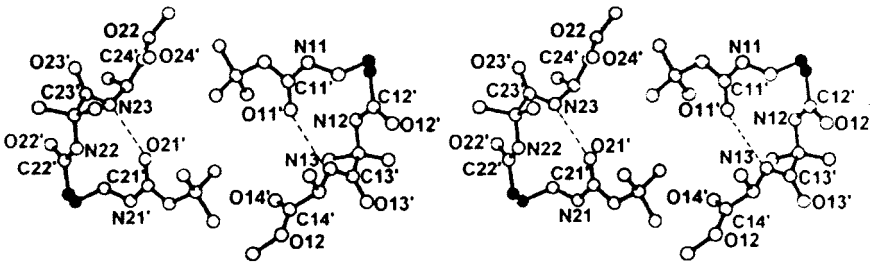

Figure 1. Stereodiagram of peptide **1.** There is a 12-membered, modified 4 → 1 type hydrogen bond between O11′ and N13 in MOL 1 and between O21′ and N23 in MOL 2. For peptide **1**, the number next to the atom type denotes the molecule number in the asymmetric unit of peptide **1** (e.g., N13 → MOL 1, the corresponding atom in MOL 2 → N23). The darkened atoms in the backbone represent the C atoms of the extra CH_2 groups in γ-Abu(1) (C11A and C11B for molecule 1 and C21A and C21B for molecule 2 in the asymmetric unit).

whereas 14-helical structures have been found in cyclic and linear chiral β-amino acids.[13−17] Hetero-oligomers of α- and/or γ-substituted γ-amino acids show 14-helical conformations in solution as evident from their NMR studies.[18,19] A very recent report from Seebach's group describes the formation of 2.6_{14}-helical structures in crystals and solution for γ-peptides composed of 2-, 3-, and 4-substituted chiral γ-amino acids.[20] In most cases, unusual turns are exhibited by substituted chiral residues (namely, β- or γ-amino acids), which are conformationally restricted. Peptides incorporating a centrally positioned β-Ala-γ-Abu segment[1b] also exhibit unusual turns (12-, 14-, 16-, and 19-membered hydrogen bonded rings), thereby forming an exception.

To study of the effects of incorporating conformationally flexible ω-amino acid residues into peptide sequences, we have examined the following three- and four-residue peptides containing N-terminal γ-Abu: Boc-γ-Abu(1)-Aib(2)-Ala(3)-OMe **1** and Boc-γ-Abu(1)-Aib(2)-Ala(3)-Aib(4)-OMe **2**. Here, we also address the additional question of whether an N-terminal γ-Abu can nucleate an unusual turn adjacent to a conformationally restricted Aib residue. A controlled terminally blocked peptide, Boc-γ-Abu(1)-Ala(2)-Aib(3)-OMe **3**, was also synthesized and studied in order to examine whether the N-terminal γ-Abu alone can nucleate a 12-atom hydrogen bonded ring structure.

Results and Discussion

Crystal Structures of Peptides 1 and 2. The two molecules in the asymmetric unit of peptide **1** (MOL 1 and 2, Figure 1) are related by a psuedoinversion center with significant deviation in noncrystallographic symmetry for Ala(3) (Table 2). In peptides **1** and **2**, the insertion of two CH_2 groups into the backbone (γ-Abu) causes an unusual, modified 4→1 type hydrogen bond between CO of the Boc group (O11′, O21′ for peptide **1** and O1′ for peptide **2**) and Ala(3) NH (N13, N23 for peptide **1** and N3 for peptide **2**) forming a 12-membered hydrogen bonded ring (Figures 1 and 2). An additional, 10-membered type I′ β-turn in peptide **2** adjacent to the 12-membered ring involving a hydrogen bond between γ-Abu(1) CO (O2′) and Aib(4) NH (N4) is also formed (Figure-2). The ϕ, ψ, ω, θ_1, and θ_2 of one of the molecules

Table 1. Crystal and Diffraction Parameters of Peptides 1 and 2

	peptide **1** (tripeptide)	peptide **2** (tetrapeptide)
empirical formula	$C_{17}H_{31}N_3O_6$	$C_{21}H_{38}N_4O_7$
crystallizing solvent	ethyl acetate/toluene	ethyl acetate
cocrystallizing solvent	toluene	none
color/habit	colorless needle	colorless, rectangle
crystal size (mm^3)	0.3 × 0.1 × 0.1	0.48 × 0.28 × 0.1
space group	$P2_1$	$C2$
a (Å)	9.3020(10)	19.0772(6)
b (Å)	23.785(2)	8.7883(2)
c (Å)	10.022(3)	16.7758(3)
α (deg)	90	90
β (deg)	101.35(4)	110.7910(10)
γ (deg)	90	90
V ($Å^3$)	2174.0	2629.42
Z	4	4
mw	373.45	458.5
density (calcd, Mg/m^3)	1.141	1.158
$F(000)$	808	992
T (K)	293	293
Independent reflns	4043	5659
no. of obs. reflns	2903, $I > 4\sigma(I)$	2848, $I > 2\sigma(I)$
R	5.7	6.71
R_w	14.5	15.11
S	1.077	0.882
λ (Å)	1.5418	0.71073
no. of param.	580	290

Table 2. Torsion Angles (deg) in Peptides 1 and 2

molecule[a]	residues	ϕ	ψ	ω	θ_1[b]	θ_2[b]
peptide **1**	γ-Abu(1)					
MOL 1		−109.0	−139	175.6	64.9	62.4
MOL 2		107.6	139	−172.8	−62.1	−65.2
peptide **2**		92.7	155.7	−178.7	−69.7	−65.8
peptide **1**	Aib(2)					
MOL 1		−55.0	−36.0	−177.5		
MOL 2		56	37	174.8		
peptide **2**		58.4	26.1	175.8		
peptide **1**	Ala(3)					
MOL 1		63.0	−160.3	−169.9		
MOL 2		−93.0	169.0	168.7		
peptide **2**		77.7	14.8	179.8		
peptide **2**	Aib(4)	−49.9	−40.2	177.5		

[a] MOL 1 and 2 represent the two molecules in the asymmetric unit of peptide **1**. [b] Torsion angle nomenclature follows refs 1b,c.

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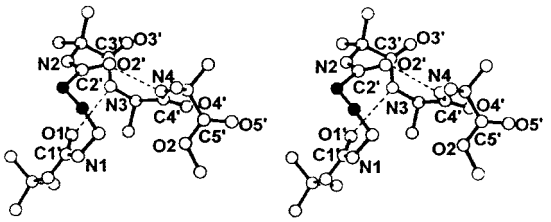

Figure 2. Stereodiagram of peptide **2.** There is a 12-membered, modified 4 → 1 type hydrogen bond between O1′ and N3 (Table 3). The darkened atoms in the backbone represent the C atoms of the extra CH_2 groups in γ-Abu(1) (C1A, C1B).

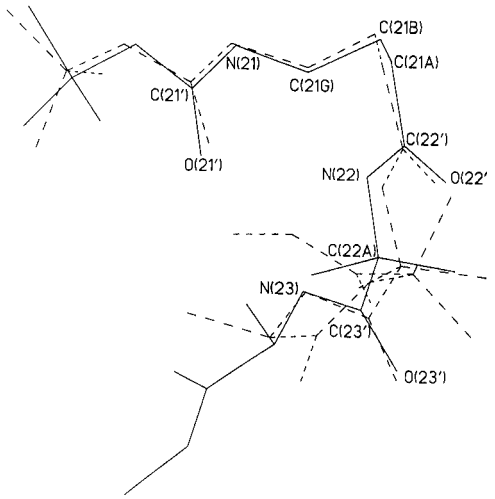

Figure 3. Differences in the conformation of MOL 2 in peptide **1** (solid lines) and peptide **2** (dashed lines). A least-squares fit was made between the coordinates of the labeled backbone atoms O21′−N23.

in the asymmetric unit of peptide **1 (**Mol 2**)** and peptide **2** are similar, with an exception in the case of Ala(3), which is the only chiral residue in both peptides (Table 2 and Figure 3). On the other hand, Mol 1 of peptide **1** and peptide **2** are nonsuperimposable as they differ in chirality. For peptide **2**, ϕ and ψ of Ala(3) lies close to the helical region, whereas the corresponding residue of peptide **1 (**Mol 1 and 2**)** shows a significant departure from helicity. The ϕ and ψ of γ-Abu for both peptides **1** and **2** are in the extended region. The torsions about the methylene groups of the γ-Abu (namely, θ_1 and θ_2) in peptides **1** and **2** are in *gauche* conformations. This facilitates the easy accommodation of CH_2 groups into the folded structure leading to an unusual 12-atom hydrogen bonded ring motif for both peptides. A 12-membered reverse turn has been observed previously in synthetic peptides containing centrally positioned, stereochemically constrained dinipecotic acid segments.[21] But in this study, the ring composed of the same number of atoms has been found in synthetic peptides containing an N-terminal, conformationally flexible γ-Abu residue. For peptide **2**, a β-turn forming Aib(4) falls into the right-handed helical region.

Packing of the Peptides 1 and 2. Apart from van der Waals interactions, the packing of both peptides are stabilized by N−H···O type intermolecular hydrogen bonds (Table 3). The packing of peptide **1** in the cell is in the form of layers that are perpendicular to the *a*-axis (Figure 4). Two such adjacent layers are stabilized by two

Table 3. Hydrogen Bonds in Peptides 1 and 2

molecule[a]	type	donor	aceptor	N···O (Å)	H···O (Å)	N−H···O (deg)
peptide **1**						
MOL 1	intra	N13	O11′	2.837	2.003	162.02
MOL 2	intra	N23	O21′	2.934	2.099	163.24
peptide **2**	intra	N3	O1′	3.077	2.256	159.70
	intra	N4	O2′	2.875	2.047	161.39
		N22	O12′	3.006	2.238	148.67
peptide **1**	inter	N12	O22′	3.009	2.195	176.39
		N11	O13′	2.839	1.946	163.51
		N21	O23′	2.847	2.205	164.30
peptide **2**	inter	N1	O4′	2.930	2.127	155.35
		N2	O5′	2.874	2.275	126.79

[a] For peptide **1**, the number next to the atom type denotes the molecule number in the asymmetric unit of peptide **1 (**e.g., N13 → MOL 1, the corresponding atom in MOL 2 → N23).

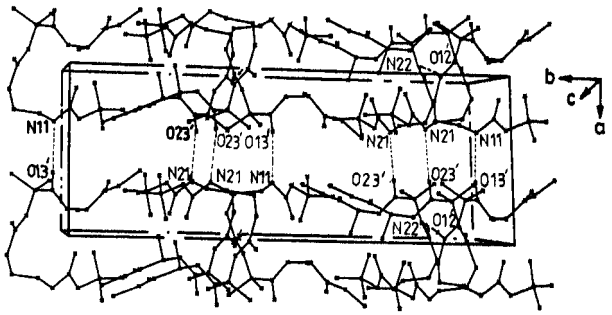

Figure 4. Crystal packing diagram of peptide **1** illustrating intermolecular hydrogen bonding in the solid state.

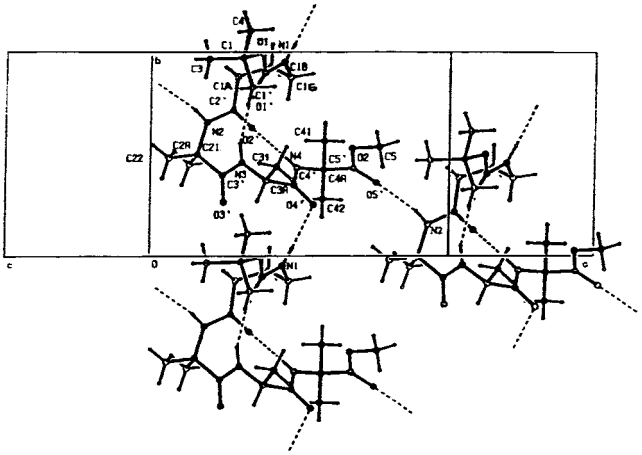

Figure 5. Crystal packing diagram of peptide **2** showing intramolecular and intermolecular hydrogen bonding involved in crystal packing.

intermolecular hydrogen bonds (Table 3), which are N11−H···O13′ and N21−H···O23′.

Within a layer, two extra hydrogen bonds N22−H···O23′ and N22−H···O22′ are present. Additional stabilization is provided by van der Waals contacts between O16, O11 and methyl atoms C14, C15. For peptide **2**, two intermolecular hydrogen bonds N1−H···O4′ and N2−H···O5′ primarily mediate packing of the molecules in crystals (Figure 5).

Solution Structures for Peptides 1 and 2. NMR studies of peptides **1** and **2** were performed in $CDCl_3$ solution. Complete assignments of the ^{1}H NMR spectra for both peptides were carried out using a combination of DQF-COSY and ROESY. Table 4 summarizes chemical shifts of the various resonances for peptides **1** and **2**.

Table 4. Characteristic ^{1}H NMR Parameters for Peptides 1 and 2 (Chemical Shifts δ)[a]

residues	NH	C$^\alpha$H	C$^\beta$H	C$^\gamma$H	$\Delta\delta$[b]
γ-Abu(1)	4.74	2.21	1.75−1.85	3.18	0.76
	(4.84)	(2.18, 2.26)	(1.55, 2.0)	(3.08, 3.3)	(0.71)
Aib(2)	6.46	−	1.54, 1.56		0.58
	(6.21)	(−)	(2.2, 1.49)		(0.68)
Ala(3)	7.35	3.3	1.39−1.40		0.19
	(7.73)	(4.41)	(1.24)		(0.14)
Aib(4)	−	−	−		−
	(7.45)	(−)	(1.56)		(0.04)

[a] Chemical shift values of proton resonances for peptides **1** and **2** in $CDCl_3$. Values in parentheses correspond to peptide **2.** Chemical shift values of proton resonances for peptide **3** are mentioned in the Experimental Section (synthesis of peptide **3**). [b] $\Delta\delta$ is the chemical shift difference for NH protons in $CDCl_3$ and 8.25% $(CD_3)_2SO/CDCl_3$ for peptide **1** and 9.09% $(CD_3)_2SO/CDCl_3$ for peptide **2**. For peptide **3**, $\Delta\delta$ values of γAbu(1) NH, Ala(2) NH, and Aib(3) NH are 0.53, 0.50, and 0.38, respectively, at 7.40% of $(CD_3)_2SO$ in $CDCl_3$.

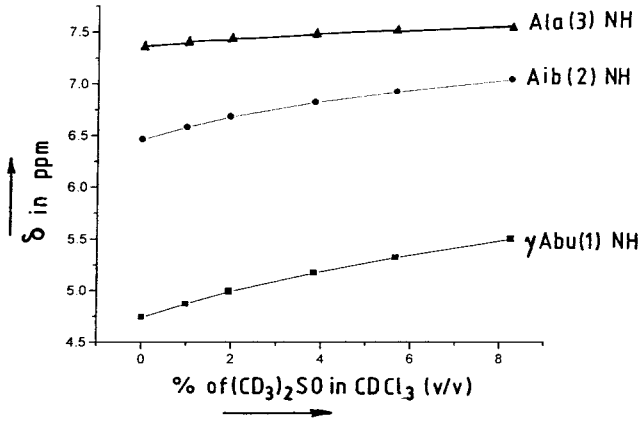

Figure 6. Plot of the solvent dependence of NH chemical shifts of peptide **1** at varying concentrations of $(CD_3)_2SO$ in $CDCl_3$.

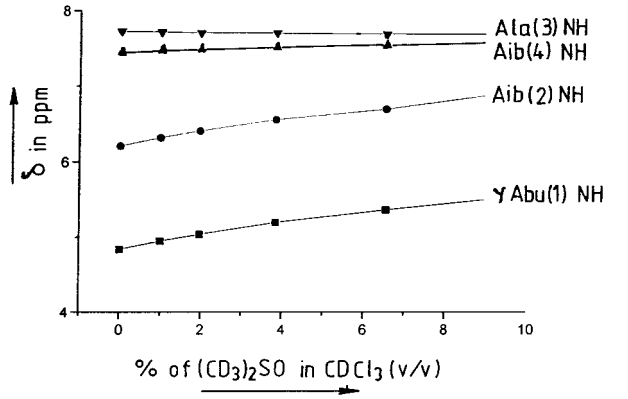

Figure 7. Plot of the solvent dependence of NH chemical shifts of peptide **2** at varying concentrations of $(CD_3)_2SO$ in $CDCl_3$.

The effects of adding a hydrogen bond accepting solvent like $(CD_3)_2SO$ to $CDCl_3$ solutions of peptides **1** and **2** are represented in Figures 6 and 7, respectively. Generally, addition of small amounts of $(CD_3)_2SO$ in $CDCl_3$ brings about monotonic downfield shifts of exposed NH groups in peptides, leaving solvent-shielded NH groups largely unaffected. This allows identification of intramolecular hydrogen bonded NH groups in peptides using solvent titration experiments.[22] Figure 6 shows the solvent-shielded nature of only Ala(3) NH for peptide **1**, and

Table 5. Important Inter-Residue NOEs for Peptides 1 and 2

peptide **1**	peptide **2**
Ala(3) NH ↔ Aib(2) C$^\beta$H$_3$	Ala(3) NH ↔ Aib(4) NH
Aib(2) NH ↔ Aib(2) C$^\beta$H$_3$	Ala(3) NH ↔ Aib(2) NH
Aib(2) NH ↔ γAbu(1) C$^\alpha$H	Ala(3) NH ↔ Ala(3) C$^\alpha$H
Ala(3) NH ↔ Ala(3) C$^\alpha$H	Ala(3) C$^\alpha$H ↔ Ala(3) C$^\beta$H
γAbu(1) NH ↔ γAbu(1) C$^\gamma$H	Aib(4) NH ↔ Ala(3) C$^\alpha$H
γAbu(1) C$^\gamma$H ↔ γAbu(1) C$^\alpha$H	Aib(4) NH ↔ Aib(4) C$^\beta$H
γAbu(1) C$^\gamma$H ↔ γAbu(1) C$^\beta$H	Aib(2) NH ↔ γAbu(1) C$^\alpha$H
γAbu(1) C$^\alpha$H ↔ γAbu(1) C$^\beta$H	Aib(4) NH ↔ Ala(3) C$^\alpha$H
Ala(3) C$^\alpha$H ↔ γAbu(1) C$^\beta$H	

Figure 7 exhibits the solvent-shielded behavior of Ala(3) and Aib(4) NH groups for peptide **2**, evident from their nonsignificant change in chemical shifts upon the addition of $(CD_3)_2SO$ in $CDCl_3$ solutions. Therefore, Ala(3) NH in peptide **1** and Ala(3) and Aib(4) NHs in peptide **2** are involved in intramolecular hydrogen bonding. Table 4 illustrates $\Delta\delta$ values of all NHs for peptides **1** and **2**. Several inter-residue NOEs for peptide **1** and **2** are listed in Table 5. Furthermore, the ROESY spectrum (supplied in Supporting Information) of peptide **1** in $CDCl_3$ reveals two important inter-residue NOEs, namely, γ-Abu(1) C$^\alpha$H ↔ Aib(2) NH and Aib(2) C$^\beta$H$_3$ ↔ Ala(3) NH. This NOE data and the solvent-shielded nature of Ala(3) NH are supportive of a 12-membered hydrogen bonded ring involving C═O from the Boc group and Ala(3) NH for peptide **1** in solution. Several inter-residue key NOEs have been found for peptide **2** in $CDCl_3$. The NOEs of Aib(2) NH ↔ γ-Abu(1) C$^\alpha$H, Aib(2) NH ↔ Ala(3) NH, and Aib(2) C$^\beta$H$_3$ ↔ Ala(3) NH and the solvent-shielded nature of Ala(3) NH are indicative of an unusual turn involving a 12-membered hydrogen bonded ring, with similar atoms as in peptide **1**. The observation of d_{NN} $(i, i + 1)$ NOEs for residues 2 and 3 and for residues 3 and 4 and $d_{\alpha N}$ $(i, i + 1)$ NOEs for residues 2 and 3 in peptide **2** clearly suggests a β-turn involving γ-Abu(1) C═O and Aib(4) NH. Thus, the crystallographic and NMR data for both peptides are in mutual agreement.

For peptide **3**, all three peptide NHs are solvent exposed (Figure 8) as evident from relatively high $\Delta\delta$ values of its NHs compared to corresponding $\Delta\delta$ values of the NHs of peptides **1** and **2** (Table 4). The $\Delta\delta$ values of Ala(3) NH for peptides **1** and **2** are lower (peptide **1**, 0.19; peptide **2**, 0.14), even at higher percentages of $(CD_3)_2SO$ (peptide **1**, 8.25%; peptide **2**, 9.09%), than the $\Delta\delta$ value of Ala(3) NH of peptide **3** at a lower percentage of the same solvent (7.40%). This demonstrates that peptide **3** cannot form any intramolecular hydrogen bonded, folded structure in solution.

Conclusion

Structures of peptides **1** and **2** obtained by both crystallographic and NMR studies are mutually consistent. This indicates that both peptides have a 12-atom hydrogen bonded ring as a common conformational feature. Comparison with peptide **3** suggests that contiguously located γ-Abu and Aib residues are essential

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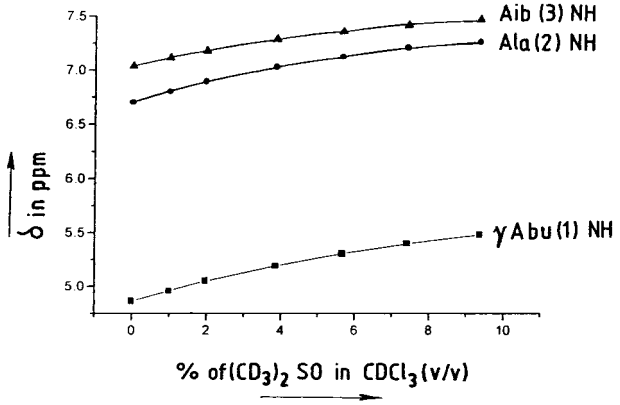

Figure 8. Plot of the solvent dependence of NH chemical shifts of peptide **3** at varying concentrations of $(CD_3)_2SO$ in $CDCl_3$.

in the 12-membered ring formation. The ability of an Aib residue to promote the formation of different types of helices[23] (α and 3_{10}) and β- and γ-turn structures[24,25] in synthetic peptides is well-known. Creation of unusual turns incorporating noncoded amino acids is endowed with significant potential in peptide design. The design of such turns bearing requisite functionalities at appropriate side chains holds considerable promise in biological sciences.

Experimental Section

Peptide Synthesis. Peptides **1**−**3** were synthesized by conventional solution-phase methods by using a racemization-free fragment condensation strategy. The Boc group was used for N-terminal protection, and the C-terminus was protected as a methyl ester. Deprotections were performed using a saponification method. Couplings were mediated by dicyclohexylcarbodiimide-1-hydroxybenzotriazole (DCC/HOBt). All intermediates were characterized by ^{1}H NMR (300 and 500 MHz) and thin-layer chromatography (TLC) on silica gel and used without further purification. The final products were purified by column chromatography using silica (100−200 mesh size) gel as the stationary phase and ethyl acetate−toluene mixture as the eluent. The purified final compounds were fully characterized by 500 and 300 MHz ^{1}H NMR spectroscopy.

Synthesis of Peptide 1. (a) Synthesis of Boc-γ-Abu-OH **4**. A solution of γ-aminobutyric acid (4.12 g, 40 mmol) in a mixture of dioxan (80 mL), water (40 mL), and 1N NaOH (40 mL) was stirred and cooled in an ice−water bath. Di-tertiarybutylpyrocarbonate (9.6 g, 44 mmol) was added, and stirring was continued at room temperature for 6 h. Then, the solution was concentrated under vacuum to about 40−60 mL, cooled in an ice−water bath, covered with a layer of ethyl acetate (about 50 mL), and acidified with a dilute solution of $KHSO_4$ to pH 2−3 (congo red). The aqueous phase was extracted with ethyl acetate, and this operation was done repeatedly. The ethyl acetate extracts were pooled, washed with water, dried over anhydrous Na_2SO_4, and evaporated under vacuum. The pure material was obtained. Yield: 7.308 g (36 mM, 90%). Anal. Calcd for $C_9H_{17}NO_4$ (203): C, 53.2; H, 8.37; N, 6.8. Found: C, 53.3; H, 8.4; N, 6.7. (b) Boc-γ-Abu(1)-Aib(2)-OMe **5**. A 7.03 g (36 mM) sample of Boc-γ-Abu-OH was dissolved in a mixture of 30 mL of dichloromethane (DCM) in an ice−water bath. H-Aib-OMe was isolated from 11.65 g (72 mM) of the corresponding methyl ester hydrochloride by neutralization, subsequent extraction with ethyl acetate, and concentration (10 mL), and this was added to the reaction mixture, followed immediately by 7.42 g (36 mM) of dicyclohexylcarbodiimide (DCC). The reaction mixture was allowed to come to room temperature and stirred for 24 h. DCM was evaporated, and the residue was taken in ethyl acetate (60 mL); dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 N HCl (3 × 50 mL), brine, 1 M sodium carbonate (3 × 50 mL), and brine (2 × 50 mL), dried over anhydrous sodium sulfate, and evaporated under vacuum to yield 10.26 g (34 mmol, 94%). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 6.73 [Aib-NH, 1H, s], 4.79 [γ-Abu NH, 1H, t], 3.75 [-OCH_3, 3H, s], 3.2 [C^{γ}Hs of γ-Abu, 2H, m], 2.18−2.21 [C^{α}Hs γ-Abu, 2H, m], 1.77−1.82 [C^{β}-Hs of γ-Abu, 2H, m], 1.65 [$C^{\beta}H_3$ of Aib, 3H, s], 1.62 [$C^{\beta}H_3$ of Aib, 3H, s], 1.44 [Boc-CH_3s, 9H, s]. Anal. Calcd for $C_{14}H_{26}N_2O_5$ (302): C, 55.62; H, 8.6; N, 9.27. Found: C, 55.7; H, 8.5; N, 9.2. (c) Boc-γ-Abu(1)-Aib(2)-OH **6**. To 9.06 g (30 mmol) of **5** were added 75 mL MeOH and 30 mL of 2 N NaOH, and the progress of saponification was monitored by thin-layer chromatography (TLC). The reaction mixture was stirred. After 10 h, methanol was removed under vacuum, and the residue was taken in 50 mL of water and washed with diethyl ether (2 × 50 mL). Then, the pH of the aqueous layer was adjusted to 2 using 1 N HCl, and the aqueous layer was extracted with ethyl acetate (3 × 50 mL). The extracts were pooled, dried over anhydrous sodium sulfate, and evaporated in vacuum to yield 8 g of **6**. Yield: 8 g, 27.77 mmol, 92.59%. ^{1}H NMR ($(CD_3)_2SO$, δ): 11.8 [−COOH, 1H, b], 7.76 [Aib-NH, 1H, s], 6.58 [γ-Abu NH, 1H, t], 2.69 [C^{γ}Hs of γ-Abu, 2H, m], 2.30 [C^{α}Hs γ-Abu, 2H, m], 1.80, 1.85 [C^{β}Hs of γ-Abu, 2H, m], 1.17 [Boc-CH_3s, 9H, s], 1.11 [$C^{\beta}H_3$s of Aib, 6H, s]. Anal. Calcd for $C_{13}H_{24}N_2O_5$ (288): C, 54.16; H, 8.3; N, 9.7. Found: C, 54.2; H, 8.4; N, 9.55. (d) Boc-γ-Abu(1)-Aib(2)-Ala(3)-OMe **1**. A 5.8 g (20 mmol) sample of Boc-γ-Abu(1)-Aib(2)-OH in 20 mL of DMF was cooled in an ice−water bath; H-Ala-OMe was isolated from 5.58 g (40 mmol) of the corresponding methyl ester hydrochloride by neutralization, subsequent extraction with ethyl acetate, and concentration (10 mL), and it was added to the reaction mixture, followed immediately by 4.12 g (20 mmol) of DCC and 2.7 g (20 mmol) of HOBt. The reaction mixture was stirred for 3 days. The residue was taken in ethyl acetate (60 mL), and the DCU was filtered off. The organic layer was washed with 2 N HCl (3 × 50 mL), brine, 1 M sodium carbonate (3 × 50 mL), and brine (2 × 50 mL), dried over anhydrous sodium sulfate, and evaporated under vacuum to yield 6.34 g (17 mmol) of a white solid. Purification was done on a silica gel column (100−200 mesh) using ethyl acetate as the eluent. Yield: 85%. Single crystals were grown from ethyl acetate−toluene (3:1) mixture by slow evaporation. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 7.35 [Ala(3) NH, 1H, d], 6.46 [Aib NH(2), 1H, s], 4.74 [γ-Abu(1)NH, 1H, t], 4.55 [Ala(3) C^{α}H, 1H, m], 3.73 [OCH_3, 3H, s], 3.18 [C^{γ}Hs of γ-Abu(1), 2H, m], 2.21 [C^{α}Hs of γ-Abu(1), 2H, m], 1.80 [C^{β}Hs of γ-Abu(1), 2H, m], 1.54, 1.56 [C^{β}Hs of Aib(2), 6H, s], 1.51 [Boc-CH_3s, 9H, s], 1.39 [Ala(3) C^{β}Hs, 3H, d]. Mass spectral data: $M + H^+ = 374$, $M_{calcd} = 373$. Anal. Calcd for $C_{17}H_{31}N_3O_6$ (373): C, 54.69; H, 8.31; N, 11.26. Found: C, 54.5; H, 8.4; N, 11.2.

Synthesis of Peptide 2. (e) Boc-γ-Abu(1)-Aib(2)-Ala(3)-OH **7**. To 3.73 g (10 mmol) of Boc-γ-Abu(1)-Aib(2)-Ala(3)-OMe **1** were added 50 mL of methanol and 20 mL of 2 N NaOH, and the progress of saponification was monitored by thin-layer chromatography (TLC). The reaction mixture was stirred. After 10 h, methanol was removed under vacuum, and the residue was taken in 50 mL of water and washed with diethyl ether (2 × 50 mL). Then, the pH of the aqueous layer was adjusted to 2 by adding 1 N HCl, and the aqueous layer was

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extracted with ethyl acetate (3 × 40 mL). The extracts were pooled, dried over anhydrous sodium sulfate, and evaporated under vacuum to yield a pure solid. Yield: 2.5 g (7 mmol, 70%). ^{1}H NMR (300 MHz, $(CD_3)_2SO$, δ): 12.73 [−COOH, 1H, b], 7.8 [Ala(3) NH, 1H, d], 7.45 [Aib NH(2), 1H, s], 6.79 [γ-Abu(1)-NH, 1H, t], 3.3 [Ala C$^\alpha$H, 1H, q], 2.93−3.3 [C$^\gamma$Hs of γ-Abu(1), 2H, m], 2.14−2.19 [C$^\alpha$Hs of γ-Abu(1), 2H, m], 1.54−1.61 [C$^\beta$-Hs of γ-Abu(1), 2H, m], 2.48 [C$^\beta$Hs Aib(2), 6H, s], 1.35 [Boc CH_3, 9H, s], 1.27−1.29 [Ala(3) C$^\beta$Hs, 3H, d]. Anal. Calcd for $C_{16}H_{29}N_3O_6$ (359): C, 53.48; H, 8.07; N, 11.69. Found: C, 53.5; H, 8; N, 11.6. (f) Boc-γ-Abu(1)-Aib(2)-Ala(3)-Aib(4)-OMe **2**. A 1.8 g (5 mmol) sample of **7** in 10 mL of DMF was cooled in an ice−water bath. H-Aib-OMe was isolated from 1.53 g (10 mmol) of the corresponding methyl ester hydrochloride by neutralization, subsequent extraction with ethyl acetate, and concentration (7 mL), and this was added to the reaction mixture, followed immediately by 1 g (5 mM) of DCC and 0.7 g (5 mM) of HOBt. The reaction mixture was stirred for 3 days. The residue was taken in ethyl acetate (50 mL), and DCU was filtered off. The organic layer was washed with 2 N HCl (3 × 50 mL), brine, 1 M sodium carbonate (3 × 50 mL), and brine (2 × 50 mL), dried over anhydrous sodium sulfate, and evaporated under vacuum to yield 1.84 g (4 mmol, 80%) of a white solid. Purification was done by crystallization from ethyl acetate. Single crystals were obtained from ethyl acetate by slow evaporation. ^{1}HNMR (500 MHz, $CDCl_3$, δ): 7.73 [Ala(3) NH, 1H, d], 7.45 [Aib(4) NH, 1H, s], 6.21 [Aib(2) NH, 1H, s], 4.84 [γ-Abu(1) NH, 1H, t], 4.41 [Ala(3) C$^\alpha$H, 1H, m], 3.68 [OCH_3, 3H, s], 3.08, 3.3 [C$^\gamma$Hs of γ-Abu(1), 2H, m], 2.18, 2.27 [C$^\alpha$Hs of γ-Abu(1), 2H, m], 1.55, 2.00 [C$^\beta$Hs of γ-Abu(1), 2H, m], 1.56 [C$^\beta$Hs of Aib(4), 6H, s], 2.2, 1.49 [C$^\beta$Hs of Aib(2)], 1.45 [Boc-CH_3s, 9H, s], 1.24 [Ala(3) C$^\beta$Hs, 3H, d]. Mass spectral data: $M + H^+ = 459$, $M_{calcd} = 458$. Anal. Calcd for $C_{21}H_{38}N_4O_7$ (458): C, 55.02; H, 8.29; N, 12.22. Found: C, 55.3; H, 8.4; N, 12.2.

Synthesis of Peptide 3. (a) Boc-γ-Abu(1)-Ala(2)-OMe **8**. A 1.62 g (8 mM) sample of Boc-γ-Abu-OH was dissolved in a mixture of 10 mL of dichloromethane (DCM)/DMF in an ice−water bath. H-Ala-OMe was isolated from 2.2 g (16 mM) of methyl ester hydrochloride by neutralization, subsequent extraction with ethyl acetate, and concentration (5 mL), and it was added to the reaction mixture, followed immediately by 1.64 g (8 mM) of dicyclohexylcarbodiimide (DCC) and 1.08 g (8 mM) of HOBt. The reaction mixture was allowed to come to room temperature and stirred for 24 h. DCM was evaporated; the residue was taken in ethyl acetate (30 mL), and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 2 N HCl (3 × 30 mL), brine, 1 M sodium carbonate (3 × 30 mL), and brine (2 × 30 mL), dried over anhydrous sodium sulfate, and evaporated under vacuum to yield 1.4 g (4.9 mmol, 61.2%). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 6.62 [Ala(2) NH, 1H, d], 4.79 [γ-Abu(1) NH, 1H, t], 4.53−4.63 [C$^\alpha$H of Ala(2), 1H, q], 3.75 [−OCH_3, 3H, s], 3.15−3.26 [C$^\gamma$Hs of γ-Abu(1), 2H, m], 2.24−2.29 [C$^\alpha$Hs of γ-Abu(1), 2H, m], 1.77−1.82 [C$^\beta$Hs of γ-Abu(1), 2H, m], 1.44 [Boc-CH_3s, 9H, s], 1.41 [Ala(2) C$^\beta$Hs, 3H, d]. Anal. Calcd for $C_{13}H_{24}N_2O_5$ (288): C, 54.16; H, 8.33; N, 9.72. Found: C, 54.2; H, 8.4; N, 9.7. (b) Boc-γ-Abu(1)-Ala(2)-OH **9**. To 1.3 g (4.5 mmol) of **8** were added 10 mL of MeOH and 3 mL of 2 N NaOH, and the progress of saponification was monitored by thin-layer chromatography (TLC). The reaction mixture was stirred. After 10 h, methanol was removed under vacuum, and the residue was taken in 20 mL of water and washed with diethyl ether (2 × 20 mL). Then, the pH of the aqueous layer was adjusted to 2 using 1 N HCl, and the aqueous layer was extracted with ethyl acetate (3 × 15 mL). The extracts were pooled, dried over anhydrous sodium sulfate, and evaporated under vacuum to yield 0.82 g of **9**. Yield: 0.82 g (3 mM, 66.7%). ^{1}H NMR (300 MHz, $(CD_3)_2$-SO, δ): 11.9 [−COOH, 1H, b], 7.71 [Ala(2) NH, 1H, d], 6.1 [γ-Abu(1) NH, 1H, t], 3.6 [C$^\alpha$H of Ala(2), 1H, q], 2.65 [C$^\gamma$Hs of γ-Abu(1), 2H, m], 2.4 [C$^\alpha$Hs of γ-Abu(1), 2H, m], 1.8 [C$^\beta$Hs of γ-Abu(1), 2H, m], 1.2 [Boc-CH_3s, 9H, s], 1.1 [Ala(2) C$^\beta$Hs, 3H, d]. Anal. Calcd for $C_{12}H_{22}N_2O_5$ (274): C, 52.55; H, 8.03; N, 10.2. Found: C, 52.6; H, 8.1; N, 10.1. (c) Boc-γ-Abu(1)-Ala(2)-Aib-(3)-OMe **3**. A 0.74 g (2.71 mmol) sample of Boc-γ-Abu(1)-Ala-(2)-OH in 20 mL of DMF was cooled in an ice−water bath. H-Aib-OMe was isolated from 0.83 g (5.4 mmol) of the corresponding methyl ester hydrochloride by neutralization, subsequent extraction with ethyl acetate, and concentration (5 mL), and this was added to the reaction mixture, followed immediately by 0.55 g (2.7 mmol) of DCC and 0.37 g (2.7 mmol) of HOBt. The reaction mixture was stirred for 3 days. The residue was taken in ethyl acetate (20 mL), and the DCU was filtered off. The organic layer was washed with 2 N HCl (3 × 20 mL), brine, 1 M sodium carbonate (3 × 20 mL), and brine (2 × 20 mL), dried over anhydrous sodium sulfate, and evaporated under vacuum to yield 0.55 g (1.5 mmol) of a white solid. Purification was done on a silica gel column (100−200 mesh) using ethyl acetate as the eluent. Yield: 55.55%. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 6.86 [Aib(3) NH, 1H, s], 6.5 [Ala-(2) NH, 1H, d], 4.76 [γ-Abu(1) NH, 1H, t], 4.42−4.45 [Ala(2) C$^\alpha$H, 1H, q], 3.72 [OCH_3, 3H, s], 3.16−3.17 [C$^\gamma$Hs of γ-Abu(1), 2H, m], 2.23−2.26 [C$^\alpha$Hs of γ-Abu(1), 2H, m], 1.81−1.82 [C$^\beta$-Hs of γ-Abu(1), 2H, m], 1.55, 1.54 [C$^\beta H_3$s of Aib(2), 6H, s], 1.43 [Boc-CH_3s, 9H, s], 1.31−1.37 [Ala(3) C$^\beta$Hs, 3H, d]. Anal. Calcd for $C_{17}H_{31}N_3O_6$ (373): C, 54.69; H, 8.31; N, 11.26. Found: C, 54.71; H, 8.42; N, 11.22.

X-ray Diffraction: Peptide 1. Single crystals were grown from an ethyl acetate−toluene mixture by slow evaporation and were stable at room temperature. Crystal and data collection parameters are listed in Table 1. A CAD4 automatic diffractometer was used to collect X-ray diffraction data. The structure was solved by a direct method using SHELXS-97[26] and refined by full matrix anisotropic least squares by SHELXL-97.[27] Hydrogen atoms were both selected from electron density and fixed at ideal geometrical positions. The final structure was refined to an R factor of 5.7 ($I > 4\sigma$). Fractional coordinates and related data are available as Supporting Information. Bond length and bond angles do not show significant differences from expected values. Torsion angles and hydrogen bond parameters are listed in Tables 2 and 3, respectively. **Peptide 2.** Single crystals were grown by slow evaporation from ethyl acetate solution. The X-ray diffraction data were collected from a single crystal at room temperature (293 K) on a Siemens SMART CCD area detector diffractometer equipped with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) using an ω scan mode. Pertinent parameters concerning data collection and the crystal are listed in Table 1. The structure was solved by direct methods using SHELXS-97.[26] Refinement was carried out with a full matrix least-squares method using SHELXL-97.[27] The anisotropic refinement was performed on all the non-hydrogen atoms before fixing hydrogen. The hydrogen atoms were fixed by riding atom model and allowing to riding over on the adjutant atoms. The final R factor was converged to 6.7% (R_w = 15.11) for 2848 observed reflections with $I \geq 2\sigma(I)$. Fractional coordinates and related data are available as Supporting Information. Neither bond lengths nor bond angles show significant differences from expected values. Torsion angles and hydrogen bond parameters are listed in Tables 2 and 3, respectively.

Spectroscopic Studies: NMR. All NMR studies were carried out on Brüker DRX 500 MHz and DPX 300 MHz spectrometers. Peptide concentrations were in the range of 10−15 mM in $CDCl_3$. Resonance assignments were done using double-quantum-filtered COSY and ROESY at 300 K. All the two-dimensional experiments were performed using 1K data points, 512 increments, and 24 scans. The mixing time of ROESY experiments was set to 300 ms. The two-dimensional data were processed using Brüker UX NMR software in a 1K × 512 data matrix. The FIDs were multiplied by a phase-shifted square Q sine bell function prior to Fourier transformations. **Mass.** Mass spectra were recorded on a Micromass

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Zabspec Hybrid Sector-TOF instrument by positive mode electronspray ionization using a 1% solution of acetic acid in methanol−water as a liquid carrier.

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Supporting Information Available: 500 MHz $^1H-^1H$ DQF COSY, ROESY spectra of peptides **1** and **2**, mass spectra of peptides **1** and **2**, and tables of atomic coordinates, bond lengths, bond angles, anisotropic thermal parameters, and coordinates for hydrogen atoms for peptides **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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