β-Hairpin peptides containing 3-amino benzoic acid, a constrained γ-amino acid

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Abstract

β-Hairpin consists of two β-strands joined by an appropriate di-peptide loop. In the designed hairpin the strands consist of hydrophobic residues, while the turn is a β- turn, invariably consisting of Pro-Gly residues. Incorporation of 3-aminobenzoic acid (Aba) in the β-hairpin peptides does not compromise the stability of the secondary structure. Unlike some of the designs, these hairpins permit accommodation of both enantiomers of Pro-Gly turn motifs. Formation of β-hairpin with ^{L}Pro in the turn region is unique, which is generally not favoured due the incompatible twist constraints arising in natural amino acids. We have also found that these strands can accommodate ^{D}Pro-^{L}Pro, ^{L}Pro-^{L}Pro and achiral Aib-Aib turn motifs in the hairpin. These results point to the dampening of the twist in the strands due to the presence of Aba.

Keywords: β-Hairpin, NMR spectroscopy, molecular dynamics, γ amino acid, 3-amino benzoic acid, hydrogen bonding

Introduction

Oligomers of higher homologues of natural α-amino acids have been subject matter of intense research activity in recent years. The reason behind is their ability to fold into variety of secondary structures, referred to as foldamers.[1] Though, β-amino acids have received lot of attention, their higher homologues, γ-amino acids, have been less studied. Theoretical studies on oligomers of γ-amino acids, the γ-peptides, have been predicted to have a wide variety of structures with H-bonds formed both in the forward as well as backward directions.[2] Experimental studies have shown that they have interesting helical structure.[3,4] Recent capability of designing β-hairpins,[5] enabled studies of hybrid peptides containing α-and β amino acids.[6] It has been observed that the inclusion of β-amino amino acids is tolerated without disrupting the

designed fold. There are, however, very few studies on the incorporation of γ-amino acids in peptides generating regular secondary structures.[7] Schrieber *et al* showed that the vinylogus γ-amino acids (v-AA) participate in nucleation of parallel β-sheet conformation in solid state and generate a "minimal" β-hairpin when placed adjacent to the turn forming Pro-Gly di-peptide.[8] Vinylogous proline has also been used as turn inducer, which participates in 12-membered hydrogen bonding involving a di-peptide loop with a *cis*-amide bond preceding to vinylogous proline and thereby stabilizes a hairpin conformation in solution.[9] We have incorporated 3-Aminobenzoic acid (Aba), **(1)**, in designs containing natural amino acids and shown that it is accommodated in the hairpin exceedingly well and very stable secondary structures result.[10] In the present communication we have reviewed some of these results and incorporated a variety of turn inducing motifs to investigate the stability of the hairpin containing an Aba in each strand.

Results and Discussion

Aba, a γ amino acid, mimics a vinylogue, having a rigid extended frame work with the two intervening dihedral angles θ_1 (C2-C3) and θ_2 (C1-C2), between φ (N-C3) and ψ (C1-CO), having a fixed value of about 180°, which may promote a β-sheet like structure.[11] Aba was first introduced in cyclic peptides that behave as artificial receptors for anions and recently it has been incorporated in other peptides.[12] Alternating L-Pro-Aba containing cyclic tetra- and hexa-peptides have been investigated for their ion binding capabilities. Cation-π interaction with the aromatic ring leads to cation inclusion into the shallow disk shaped receptor cavity. Substituted Aba has also been used as a constrained residue in hetero duplexes and nanocavities of tunable sizes.[13] Aba has capability of having hydrogen bond donor as well as acceptor sites facing the same side of an extended sheet, which may enable the residue to participate in H-bonding simultaneously.

Aba
1
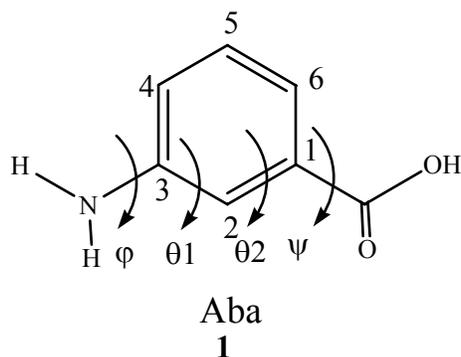
The β-hairpin consists of two antiparallel strands connected by a suitable turn. While the major contributions towards the stabilization of these structures come from hydrogen bonds (H-bonds) across the strands, the other non-covalent interactions like hydrophobic, hydrophilic, aromatic and salt bridges also contribute significantly. The initial developments in the design of

the hairpins were based on the observations, that most of the hairpin turns in the proteins are type I' / type II'. Among the various nucleating chain reversal dipeptide motifs, Pro-Gly is very often used to generate β-turns for stabilization of hairpins.[5,14] Among the proteogenic amino acids, Pro is conformationally restricted, with a φ value in the vicinity of -60°. A consequence of this, the values of ψ also get restricted to about -30° or 120°. This however leads to induction of type I / type II turns. The need to have β-turns of the type I' / type II' in the design would be naturally met by the dipeptide enantiomer, [D]Pro-Gly, and thus the designed β-hairpins invariably have this turn motif. It has been observed that the [D]Pro-Gly containing turns lead to stable β-hairpins, while those with [L]Pro-Gly do not provide adequate control of the stereochemistry for the stability of the hairpin. The relationship between β-turn and the β-hairpin promotion in peptides arises from the right handed twist preference of the β sheets, which must be matched in the loop. [L]Pro-Gly lead to left-handed type I / type II β-turns, which are not compatible with the twist of the strand for L-residues. Indeed replacing [D]Pro with the [L]Pro enantiomer causes the destruction of the β-hairpin folds in several-designed sequences.[15]

Based on the residues involved in the nucleation of β-sheet secondary structures in the proteins, the β-strands in the present study, consist of hydrophobic residues, because they have the highest propensities for nucleating the β-sheets. This permits them to associate and come close to each other. The strands in the present study are tri- peptides containing an Aba and two hydrophobic residues, like Val, Leu, Ile and Gly.

In our design a variety of turn inducing motifs like [L]Pro-Gly, [D]Pro-Gly, [L]Pro-[L]Pro, [D]Pro-[L]Pro and Aib-Aib were used. Interestingly the results showed that all these turn inducers stabilize the hairpin conformation in solution. The peptides **2-10**, were synthesized by standard method of peptide coupling using DCC/HOBt and EDCI/HOBt and by anhydride method using etylchloroformate.[16] The results of structural studies carried out by NMR in about 2-5 mM solutions in $CDCl_3$ at 30 °C and Molecular dynamics (MD) are reported below.

2 : Boc-Gly-*Aba*-[L]Pro-Gly-*Aba*-Val-OMe
3 : Boc-Gly-*Aba*-[D]Pro-Gly-*Aba*-Val-OMe
4 : Boc-Ala-Gly-*Aba*-[L]Pro-Gly-*Aba*-Val-Val-OMe
5 : Boc-Ala-Gly-*Aba*-[D]Pro-Gly-*Aba*-Val-Val-OMe
6 : Boc-Ile-Val-*Aba*-[L]Pro-Gly-*Aba*-Ile-Val-OMe
7 : Boc-Ile-Val-*Aba*-[D]Pro-Gly-*Aba*-Ile-Val-OMe
8 : Boc-Ile-Val-*Aba*-[L]Pro-[L]Pro-*Aba*-Ile-Val-OMe
9 : Boc-Ile-Val-*Aba*-[D]Pro-[L]Pro-*Aba*-Ile-Val-OMe
10 : Boc-Ile-Val-*Aba*-Aib-Aib-*Aba*-Ile-Val-OMe

The NMR spectra demonstrated wide dispersion of backbone amide and αH chemical shifts, supporting a well-defined organized structure for these peptides in solution. The spectra were well resolved and most of the spectral parameters could be obtained from one-dimensional spectra. The spectral assignments were carried out with the help of total correlation spectroscopy

(TOCSY) experiments and the rotating frame nuclear Overhauser effect spectroscopy (ROESY) experiments, which in addition provided the information on the proximity of the protons. The information on hydrogen bonding was derived from solvent titration studies. Upto 33% of polar solvent, DMSO-d_6 (v/v), was added sequentially in steps of 50 μl CDCl$_3$ solution (600 μl) during the solvent titration. Small magnitude of amide chemical shift temperature coefficients were used as indication of their participation in hydrogen bonding. Low field shifts of the amide proton chemical shifts was further used as the confirmation of their participation in H-bonding.

Peptides 2 and 3

Hexamers **2** and **3** were first studied for understanding the implications of insertion of Aba. The presence of characteristic NOEs Gly4NH / Aba5NH, Pro3αH / Aba5NH as well as the fact that sequential Pro3αH / Gly4NH peaks are much stronger than the Gly4αH / Aba5NH support a type II β turn structure around [L]Pro-Gly for hexapeptide **2** and a type II' β turn involving [D]Pro-Gly in **3.** The evidence of extended structure for Aba2 and Aba5 is derived from Aba2H6 / Pro3δH and Aba5H6 / Val6NH cross peaks in the ROESY spectrum. The signatures of incipient β-hairpin are indicated by the NOE cross peaks Aba2H2 / Aba5H2, Gly1αH / Val6αH and Boc / OMe (Figure 1). Only Aba5NH participates in hydrogen bonding as it showed small change in its chemical shift during solvent titration. No other evidence of hairpin was observed because of fraying at the termini. These observations encouraged us to investigate the larger octa peptides.
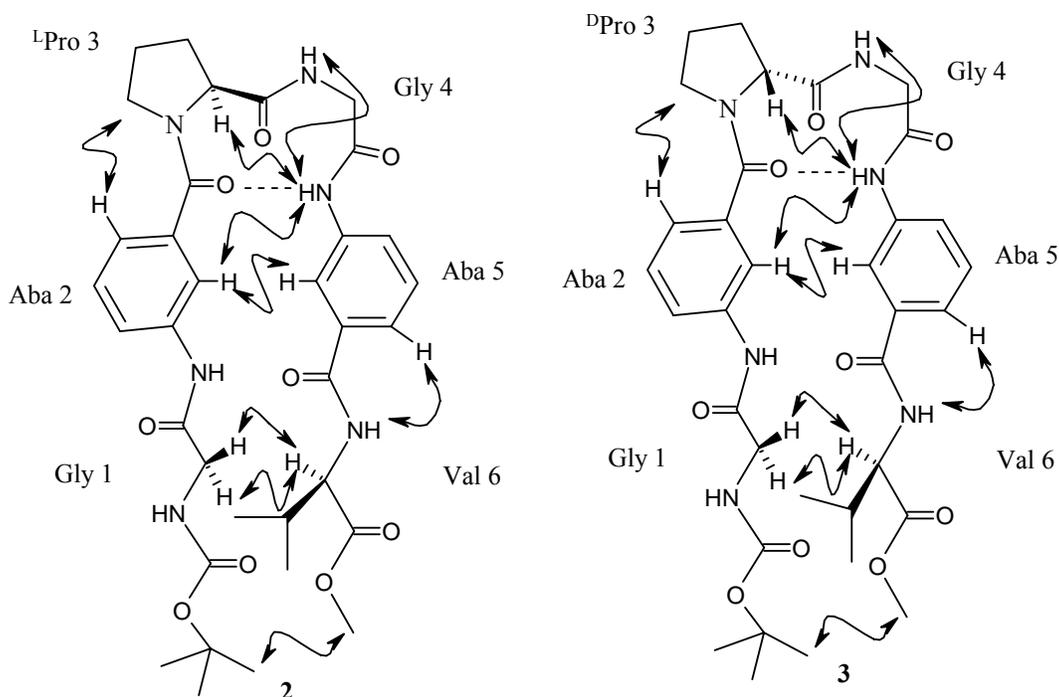
Figure 1. Schematic representation of NOEs and H-bonds for **2** and **3**.

Peptides 4 and 5

Peptides **4** and **5** were studied in $CDCl_3$ + 4% DMSO-d_6 solvent mixture to improve resolution of the spectra. Solvent titration studies[17] show Aba3NH, Aba6NH and Val8NH participate in hydrogen bonding, having very small chemical shift change of –0.32, 0.12 and –0.31 ppm for **4** and –0.34, 0.14 and 0.38 ppm for **5** respectively. Further support for this conclusion comes from the down field appearance of their chemical shifts. Strong NOE cross peaks Aba3H6 / Pro4δH and Aba6H6 / Val7NH show that Aba3 and Aba6 take an extended structure. The presence of Aba6NH / Gly5NH, Pro4αH / Aba6NH and the fact that Pro4αH / Gly5NH are stronger than Gly5NH / Aba NH6 clearly show the existence of a type-IIβ turn about [L]Pro5-Gly6 in **4** and type-II'β turn about [D]Pro5- Gly6 in **5**. The evidence for a stable β-hairpins in both peptides comes from inter strand NOE cross peaks Aba3H2 / Aba6H2, Gly2αH / Val7αH, Boc / OMe (Figure 2) as well as participation of Aba3NH, Aba6NH and Val8NH in intra molecular cross-strand hydrogen bondings. Ala1NH does not participate in hydrogen bonding due to fraying at the ends. For **4** the large values of $^3J_{NH-\alpha H}$ (8.6 / Val7 and 7.8 / Val8 Hz) in addition to the down field appearance of αH protons (4.42 / Gly2, 4.80 / Val7 and 4.55 ppm / Val8) are noteworthy. Similarly for **5** large $^3J_{NH-\alpha H}$ (8.5 / Val7 and 7.8 / Val8 Hz) as well as down field appearance of Hα protons (4.83 / Val7 and 4.43 ppm / Val8) are observed. Additionally the wide dispersion of strong inter-residue αH-NH and weak intra-residue NH-αH cross peaks, strongly support backbone dihedral angles for the two strands to be in the β region of the Ramachandran plot. To further establish our design, less polar octa peptides **6** and **7**, were studied.
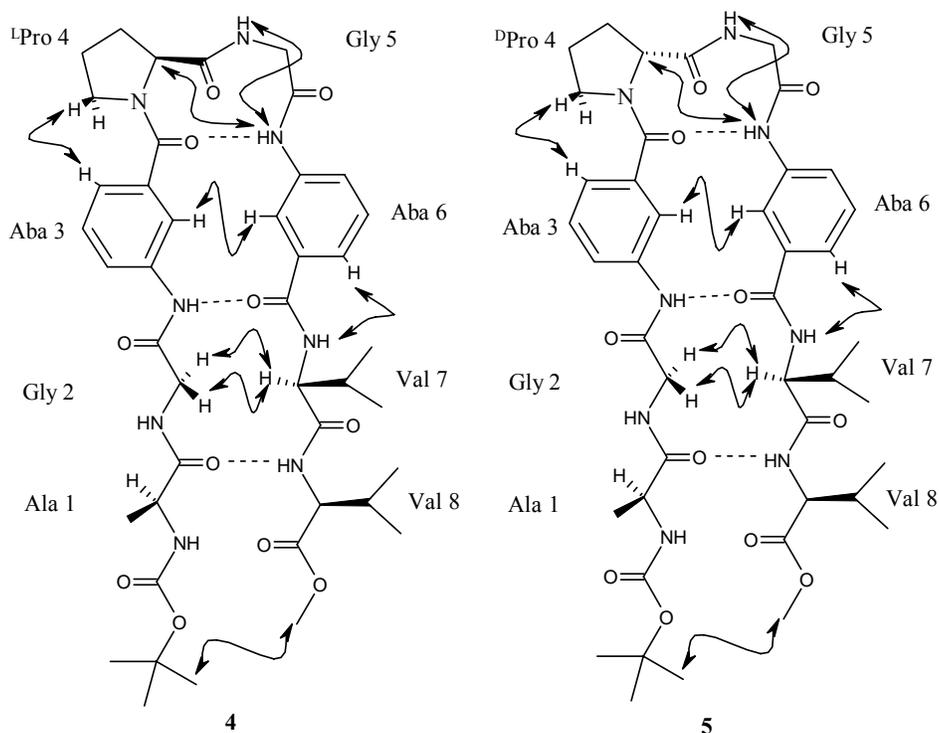
Figure 2. Schematic representation of NOEs and H-bonds for **4** and **5**.

Table 1. [1]H chemical shifts of NH and αH for peptides 2-10 (in ppm)

		Residue 1	Residue 2	Residue 3	Residue 4	Residue 5	Residue 6	Residue 7	Residue 8
2	NH	5.35	9.02	-	7.32	9.30	6.80	-	-
	αH	4.01,3.92	-	4.44	4.30, 3.82	-	4.64	-	-
3	NH	5.51	9.06	-	7.30	9.30	6.75	-	-
	αH	4.06, 3.95	-	4.41	4.39, 3.81	-	4.62	-	-
4	NH	5.78	7.65	9.88	-	8.37	9.53	7.30	8.01
	αH	4.25	4.42, 4.12		4.49	4.38, 3.80	-	4.80	4.45
5	NH	5.82	7.71	9.88	-	8.43	9.53	7.22	8.06
	αH	4.27	4.28, 4.16	-	4.47	4.42, 3.76	-	4.83	4.43
6	NH	5.41	6.97	10.30	-	6.42	9.35	7.18	8.47
	αH	4.16	4.91	-	4.30	4.68, 3.73	-	5.02	4.55
7	NH	5.68	6.42	10.16	-	6.74	9.35	7.33	8.46
	αH	4.03	4.40	-	4.36	4.72, 3.69	-	4.80	4.55
8	NH	5.86	6.52	10.54	-	-	9.71	6.71	8.39
	αH	3.93	4.43	-	4.44	4.49	-	4.97	4.56
9	NH	5.72	6.49	10.16	-	-	9.15	6.78	8.43
	αH	4.03	4.42	-	4.72	4.84	-	4.94	4.50
10	NH	5.68	6.61	10.01	6.77	6.16	9.35	6.98	8.30
	αH	4.97	4.59	-	-	-	-	4.95	4.55

Peptides 6 and 7

For peptides **6** and **7** the solvent titration studies[17] show that Ile1NH, Aba3NH, Aba6NH and Val8NH are hydrogen bonded as their chemical shifts change by small amount. Further support for this deduction comes from the appreciable down field appearance of their resonances in CDCl$_3$. Like the peptides studied earlier, Aba3 and Aba6 were found to take an extended structure. For **6** the presence of the cross peaks Gly5NH /Aba6NH as well as much stronger Pro4αH / Gly5NH compared to Gly5αH / Aba6NH clearly show the existence of a type-II β-turn involving [L]Pro5-Gly6 residues. The unequivocal evidence for a stable β-hairpin comes from inter-strand NOE cross peaks Aba3H2 / Aba6H2, Val2αH / Ile7αH, Ile1NH / Val8NH, Val2αH / Ile7γCH$_3$ and Boc/OCH$_3$ (Figure 3) as well as participation of inter-strand hydrogen bonds of Ile1NH, Aba3NH, Aba6NH and Val8NH. The large value of $^3J_{NH-\alpha H}$ (8.4 / Ile1, 9.0 / Val2, 8.9 / Ile7 and 8.2 / Val8, Hz) (Table 2) as well as the down field appearance of αH protons (4.91 / Val2, 5.02 / Ile7 and 4.55 / Val8, ppm) and the wide dispersion of strong inter-residue αH-NH and weak intra-residue αH-NH cross peaks, strongly support the presence of well defined beta sheets in **6**. Very similar observations have been made for peptide **7,** with [D]Pro-Gly as the turn inducing motif, which takes a "mirror image" type-II' β-turn in the β-hairpin. Interestingly the β-

hairpins in these peptides does not fray much in the termini. Compared to **4** and **5** the beta sheet structure in these peptides is better defined, as the $^3J_{\text{NH-}\alpha\text{H}}$ in the strands are generally larger and also the chemical shifts of the αH appear at much lower fields. We have therefore used these β-strands in our subsequent studies.
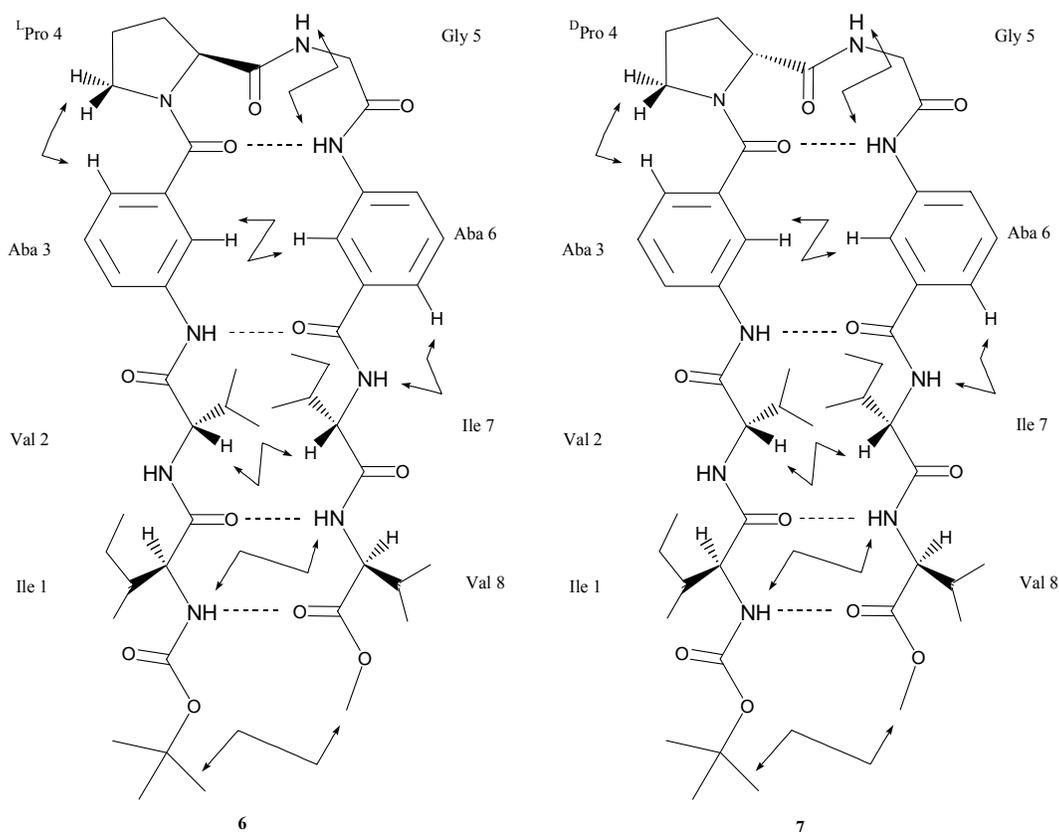
Figure 3. Schematic representation of NOEs and H-bonds for **6** and **7**.

Table 2. $^3J_{\text{NH-}\alpha\text{H}}$ couplings* (Hz) for peptides **2-10**

Peptide	Residue 1	Residue 2	Residue 4	Residue 5	Residue 6	Residue 7	Residue 8
2	5.9, 5.9	-	5.2, 5.2	-	8.6	-	-
3	5.9, 5.9	-	7.8, 5.0	-	8.3	-	-
4	7.1	3.4, 3.4	-	7.8, 4.8	-	8.6	7.8
5	6.9	5.0, 5.0	-	7.4, 4.7	-	8.5	7.8
6	8.4	9.0	-	9.1, 4.0	-	8.9	8.2
7	8.4	7.8	-	9.3, 3.5	-	8.5	9.1
8	8.2	7.9	-	-	-	9.5	9.0
9	8.3	7.9	-	-	-	9.6	9.2
10	8.4	8.0	-	-	-	8.9	8.8

*Residue 3 does not have $^3J_{\text{NH-}\alpha\text{H}}$ and therefore has not been included in the table.

Peptides 8 and 9

Kopple *et.al.* have used two successive proline residues, to limit conformational freedom in peptides.[18] They observed that [D]Pro-[L]Pro is a stronger and less ambiguous turn determinant compared to turns with a single proline residue. Unlike the earlier peptides, **8** and **9** contain [L]Pro-[L]Pro and [D]Pro-[L]Pro as the turn inducers. Solvent titration studies[17] suggest that Ile1, Aba3, Aba6 and Val8 amide protons participate in hydrogen bonding.

   Due to the absence of amide protons in the two Pro residues in the turn, there are not many characteristic signatures of the turn available in these peptides. However, for **8,** the down field appearance of αH protons 4.43 / Val2, 4.97 / Ile7, 4.56 / Val8 as well as large $^3J_{\text{NH-}\alpha\text{H}}$ coupling for Ile1, Val2, Ile7, Val8 being 8.2, 7.9, 9.5, 9.0 Hz respectively, support the presence of β sheet structure in the strands. Similar pattern is observed in **9**. Like peptides **4-7**, the β-hairpins in **8** and **9** are amply supported by characteristic NOE connectivities Aba3H2 / Aba6H2, Aba3H2 / Aba6NH, Pro4αH / Aba6NH, Val2αH / Ile7αH, Ile1NH / Val8NH, Boc / OMe, across the two strands (Figure 4). Figure 5 shows expanded ROESY spectrum with characteristic NOEs. Due to the conformational restraints on the Pro residues, the β-turns are expected to be type I for **8** and type II' for **9**.
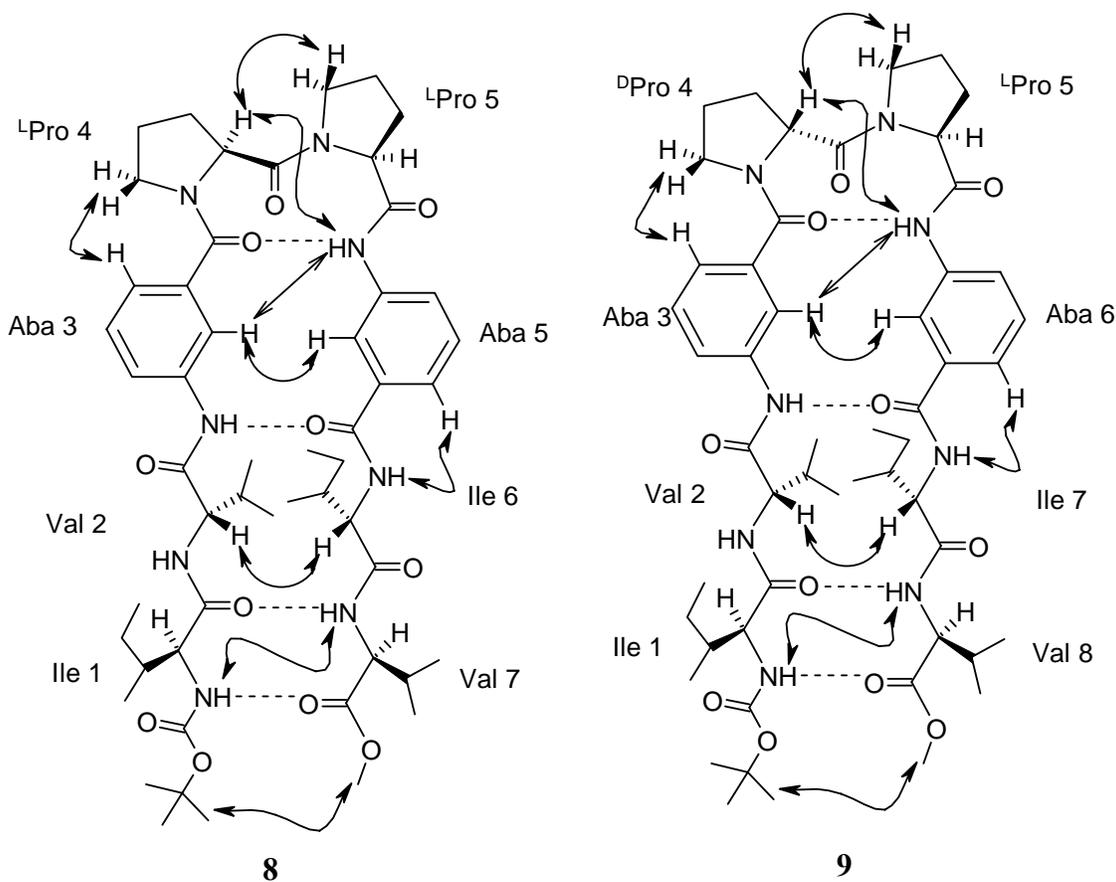
Figure 4. Schematic representation of NOEs and H-bonds for **8** and **9**.
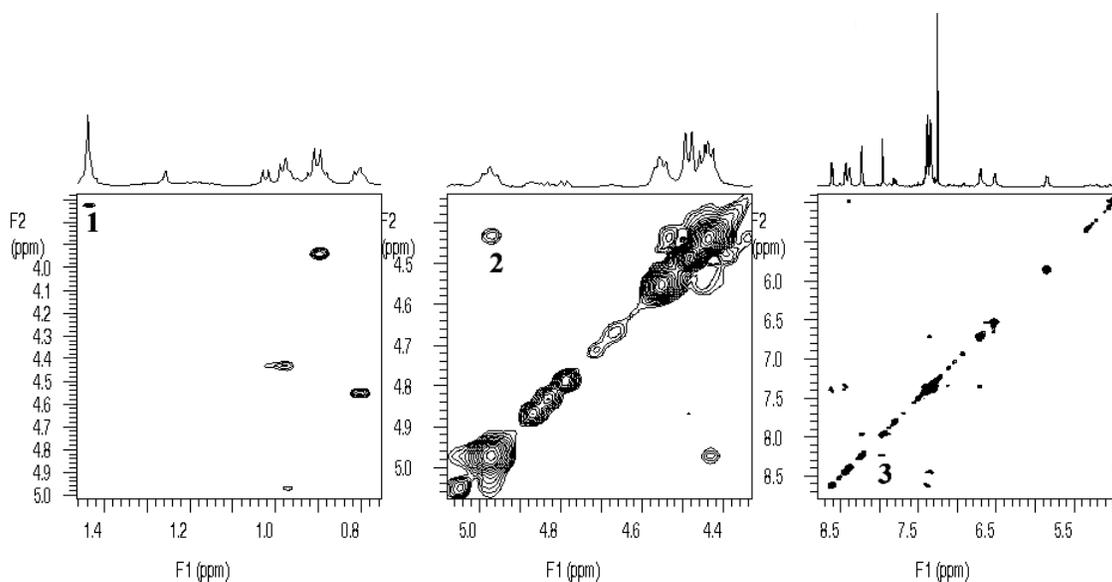
Figure 5. Expanded ROESY spectrum of peptide **8**. The NOE cross peaks are: (1) Boc / OCH$_3$, (2) Val2αH / Ile7αH and (3) Aba3H2 / Aba6H2.

Peptide 10

We tested our strategy further by incorporating non-proline moieties in the turn, such as α-amino isobutyric acid (Aib). Balaram *et. al.,* have used novel dipeptide chain reversal nucleating segment, Aib-D-amino acid (Aib-^{D}Xaa), in their β-hairpin design.[19] The choice of achiral Aib was used due to their strong conformational preferences with φ=-60° and ψ=-30° or φ=60° and ψ=30°. In addition the incorporation of ^{D}Xaa favors a type-I' β-turn. However Aib-Aib achiral turn motif has not been used in designed β-hairpins. Due to the overwhelming constraints on Aib conformation, the turns are expected to be type-I/type-III or type-I'/type-III'. Similar to earlier octa peptides **10**, Ile1, Aba3, Aba6 and Val8 amide protons participate in the hydrogen bonding. Wide dispersion and appreciable down field shift of αH proton chemical shifts (Ile1 / 4.97, Val2 / 4.59, Ile7 / 4.95, Val8 / 4.55) and large $^3J_{NH-αH}$ (8.4 / Ile1, 8.0 / Val2, 8.9 / Ile7, 8.8 / Val8) is observed indicating that the β-strands are well defined. Further support for the β-strands comes from the wide dispersion of strong inter-residue αH-NH and weak intra residue αH-NH cross peaks. The hairpin is confirmed very well by characteristic NOEs Ile1NH / Val8NH, Aba3H2 / Aba6H2, Val2αH / Ile7αH, Boc / OMe (Figure 6).

　　We also investigated these peptides in DMSO-d_6. The structures were found to be not very well defined. The VT studies between 30-70 °C showed that the temperature coefficients of amide chemical shifts, which is used for obtaining information on H-bonding, have magnitudes > 3.5 ppb/°C. This implies that the intra molecular H-bonds do not exist in DMSO-d_6 solvent. The evidence of β-turns and β-strands are also not very compelling. The lack of structure is due to the solvent molecules getting involved, in intermolecular H-bonding with the peptides and thus disrupting the structure.
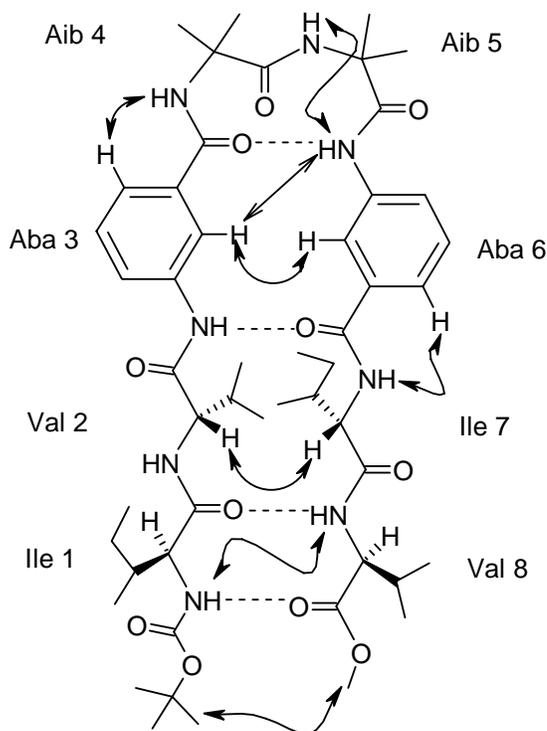
Figure 6. Schematic representation of NOEs and H-bonding for **10**.

Molecular dynamics studies

The MD calculations on **5** show that Aba takes an extended structure, which, facilitates accommodation of two additional carbons in the backbone without disrupting the hairpin. Figure 7 shows twenty superimposed minimum energy structures obtained from a 600 ps MD run for **4**, **6**, **8**, **9** and **10**. Since Aba is achiral, φ and ψ take both signs with same propensity. We discuss specifically peptide **6** in detail and the general discussions are valid for other peptides as well. The structures for **6** show that the magnitudes of φ and ψ for Aba(3) and Aba(6) are 132±4°, 132±9° and 140±5° and 150±6° respectively, which are very similar to that for β-anti parallel sheets. Four cross-strand hydrogen bonds stabilize the hairpin. Significant fraying at the termini is reflected in the large variations for φ and ψ of the terminal residues. The angle between the Aba(3) and Aba(6) aromatic rings is around 13-80°. It has been shown that the hydrophobic effects involving side chain-side chain interactions are critical determinants of anti-parallel β-sheet stability.[20] Such interactions among the aromatic rings of peptides and proteins lead to the stability of the secondary structures.[21] The interactions between the two Aba rings across the strands might also contribute to additional stabilization of the hairpins. For peptide **10**, we were unable to get information on the nature of the turn from NMR data. MD calculations, however, very clearly demonstrate that most of the structures shown Figure 7 take a type III' β-turn.
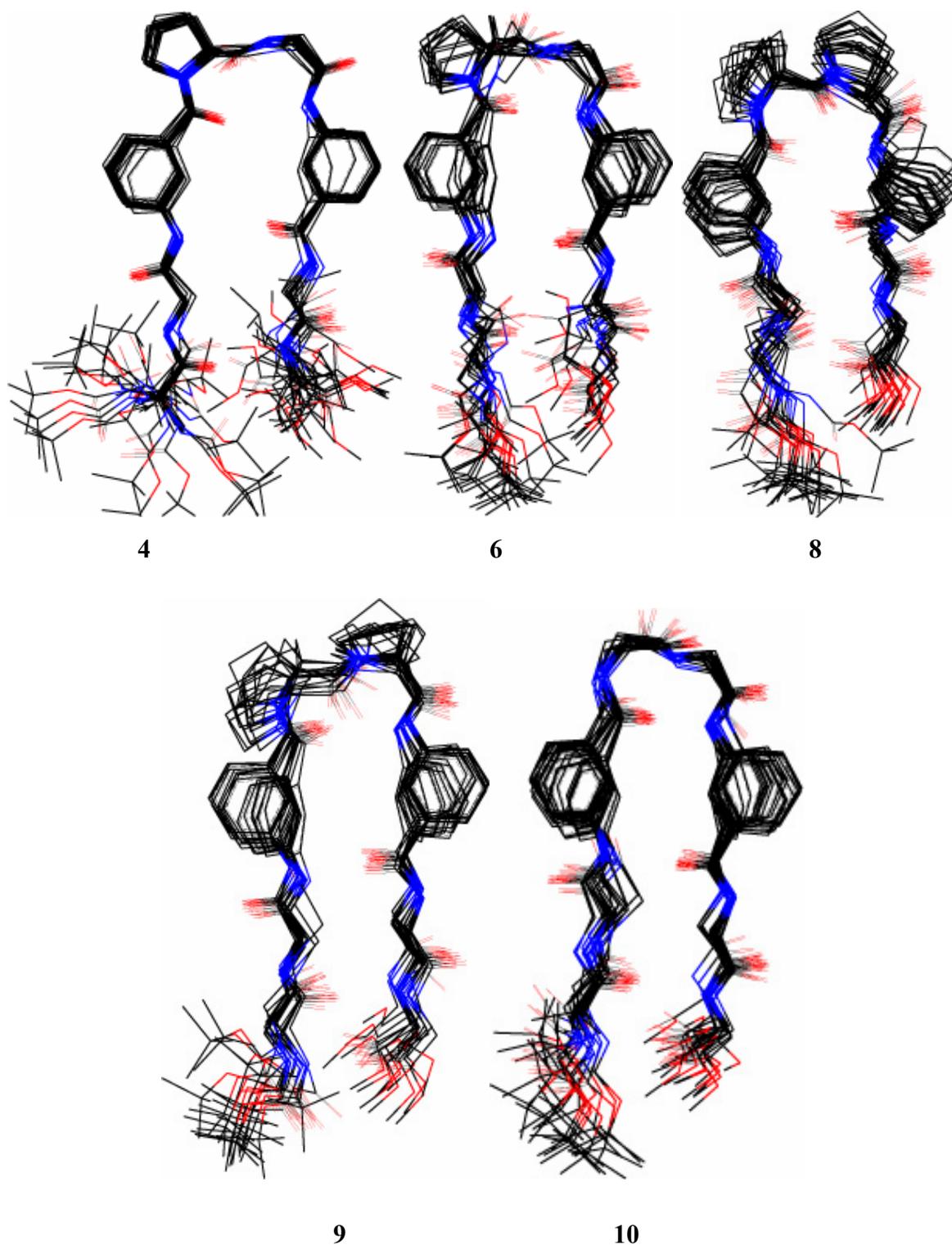
Figure 7. 20 Overlapped structures obtained from molecular dynamics simulation studies for **4**, **6**, **8**, **9** and **10**. For clarity in visualization the side chain groups are not shown in the figure except for proline.

The β-hairpin with [D]Pro-Gly turns containing only α residues take a type II' turn,[15] where as the β-hairpins containing β-amino acids can take both type I' and type II' turns.[6] In our studies, however all the turns with [D]Pro-Gly as well as [D]Pro-[L]Pro, are type II' where as the ones with [L]Pro-Gly are type II and [L]Pro-[L]Pro are type I. The turns exert deferring influences on the relative twist of the anti parallel strands. While the extent of twist in type I' turn matches that of the anti parallel strands, in contrast type II' turns result in substantially "planar twist", leading to flattening of the hairpin considerably. It has been shown that incorporation of vinylogous amino acids in peptides does accommodate [L]Pro-Gly turn and result in minimal hairpin due to the dampening of the right handed twist constraint of the strands.[8] It is thus very likely that the β-hairpin stability in the **2-9** is due to the absence of any perceptible twist of the strands, arising due to the presence of Aba, a mimic of the vinylogus amino acid, which permits both enantiomeric turns in the β-hairpin. The presence of NOE across the residues diagonally placed has been attributed to the curvature / twist of the sheets.[22] For peptides **2-10** nOe's are observed only between residues placed directly across each other and not between the diagonal ones, further supporting absence of the curvature. Additional support for the absence of the twist of the β-strands comes from the report where peptide containing [L]Pro-Ala turn motif has been shown to exist as a three strand mono-layer film of β-hairpins extended parallel to the plane of air water interface.[23]

Conclusions

It has been shown that inclusion of Aba, a γ amino acid, is very well tolerated in designed hairpins, containing a variety of turn motifs. Observation of β-hairpin in peptides containing the turn inducing motifs, [L]Pro-Gly, [L]Pro-[D]Pro and Aib-Aib, is quite unusual and novel and indicates flattening of the sheets due to incorporation of Aba.

Experimental Section

Standard amide coupling using DCC and HOBt. Solid 1-hydroxybenzotriazole (HOBt, 1 eq.) and dicyclohexylcarbodiimide (DCC, 1 eq.) were added sequentially at 0 °C to a stirred solution of *N*-protected amino acid / peptide in dry CH_2Cl_2 or in a mixture of dry DMF and CH_2Cl_2 in cases where the solubility was poor in CH_2Cl_2, under N_2. After a period of ~0.25 h, the reaction mixture was mixed with amino acid methyl ester / amine-free peptide methyl ester in dry CH_2Cl_2. The combined mixture was stirred at room temperature for 6 h, the precipitate dicylohexyl urea filtered and the residue washed with CH_2Cl_2 and the combined filtrates were washed sequentially with cold 0.5 N HCl, saturated $NaHCO_3$ and NaCl solutions. The organic extract was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by column chromatography using EtOAc / hexane as eluent over 60-120 silica gel.

General procedure for peptide coupling using ethyl chloroformate

N-Methyl morpholine (NMM, 1 eq.) and ethyl chloroformate (1 eq.) were added sequentially at -20 °C to a stirred solution of *N*-protected amino acid / peptide in dry CH_2Cl_2 or in a mixture of dry DMF and CH_2Cl_2 in cases where the solubility was poor in CH_2Cl_2, under N_2. After a period of ~0.25 h, the reaction mixture was mixed with amino acid methyl ester / amine free peptide methyl ester (freshly generated from its hydrochloride salt/TFA salt, using triethylamine) in dry CH_2Cl_2. The combined mixture was stirred at room temperature for 6 h, the residue washed with CH_2Cl_2 and the combined filtrates were worked up as above. The organic extract was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was purified by column chromatography using EtOAc/hexane as eluent over 60-120 silica gel.

Peptide 2. White solid; mp 136°C. ^1H NMR (CDCl₃, 500 MHz): δ 9.30 (s, 1H, Aba5-N*H*), 9.02 (s, 1H, Aba2-N*H*), 8.14 (d, *J*=8.0 Hz, 1H, Aba5-C4*H*), 8.02 (s, 1H, Aba5-C2*H*), 7.89 (d, *J*=8.0 Hz, 1H, Aba2-C4*H*), 7.61 (s, 1H, Aba5-C2*H*), 7.41 (d, *J*=8.0 Hz, 1H, Aba5-C6*H*), 7.32 (dd, *J*=5.2, 5.5 Hz, 1H, Gly4-N*H*), 7.31 (t, *J*=8.0 Hz, 1H, Aba5-C5*H*), 7.28 (d, *J*=8.0 Hz, 1H, Aba2-C6*H*), 7.28 (t, *J*=8.0 Hz, 1H, Aba2-C5*H*), 6.80 (d, *J*=8.6 Hz, 1H, Val6-N*H*), 5.55 (t, *J*=5.9 Hz, 1H, Gly1-N*H*), 4.64 (dd, *J*=5.6, 8.6 Hz, 1H, Val6-Cα*H*), 4.44 (dd, *J*=5.6, 7.5 Hz, 1H, Pro3-Cα*H*), 4.30 (dd, *J*=5.5, 18.6 Hz, 1H, Gly4-Cα*H*), 4.01 (dd, *J*=5.9, 16.9 Hz, 1H, Gly1-Cα*H*), 3.92 (dd, *J*=5.9, 16.9 Hz, 1H, Gly1-Cα*H'*), 3.82 (dd, *J*=5.5, 18.6 Hz, 1H, Gly4-Cα*H'*), 3.76 (s, 3H, OC*H₃*), 3.68 (m, 2H, Pro3-Cδ*H* & Cδ*H'*), 2.21-1.90 (m, 4H, Pro3-Cβ*H*, Cβ*H'*, Cγ*H*, & Cγ*H'*), 2.18 (m, 1H, Val6-Cβ*H*), 1.44 (s, 9H, *Boc*), 0.93 (d, *J*=6.8 Hz, 3H, Val6-CγC*H₃*), 0.93 (d, *J*=6.8 Hz, 3H, Val6-CγC*H3'*);13C NMR(CDCl3,75MHz) δ 172.8, 172.6, 169.9, 168.4, 168.11, 156.2, 138.5, 138.1, 135.6, 134.4, 128.9, 128.8, 123.2, 122.6, 122.3, 119.1, 118.7, 80.0, 77.2, 61.5, 58.0, 52.2, 50.6, 44.5, 43.5, 31.1, 29.4, 28.2, 25.5, 19.0, 18.1. IR(KBr, cm^{-1}: 3450, 2970, 2925, 1659, 1592, 1547, 1440, 1252, 1163, 1025. FABMS (m/z, %) M$^+$+H (681, 24). ESI HRMS: (M$^+$) calcd. for $C_{34}H_{44}O_9N_6$+Na (703.3067), found 703.3099.

Peptide 3. White solid; mp 115°C. ^1H NMR (CDCl₃, 500 MHz): δ 9.30 (s, 1H, Aba5-N*H*), 9.06 (s, 1H, Aba2-N*H*), 8.16 (d, *J*=8.0 Hz, 1H, Aba5-C4*H*), 8.02 (s, 1H, Aba5-C2*H*), 7.93 (d, *J*=8.0 Hz, 1H, Aba2-C4*H*), 7.63 (s, 1H, Aba5-C2*H*), 7.41 (d, *J*=8.0 Hz, 1H, Aba5-C6*H*), 7.33 (t, *J*=8.0 Hz, 1H, Aba5-C5*H*), 7.30 (dd, *J*=5.0, 7.8 Hz, 1H, Gly4-N*H*), 7.29 (t, *J*=8.0 Hz, 1H, Aba2-C5*H*), 7.29 (d, *J*=8.0 Hz, 1H, Aba2-C6*H*), 6.75 (d, *J*=8.3 Hz, 1H, Val6-N*H*), 5.51 (t, *J*=5.9 Hz, 1H, Gly1-N*H*), 4.62 (dd, *J*=6.0, 8.3 Hz, 1H, Val6-Cα*H*), 4.41 (t, *J*=5.9 Hz, 1H, Pro3-Cα*H*), 4.39 (dd, *J*=7.8, 17.2 Hz, 1H, Gly4-Cα*H*), 4.06 (dd, *J*=5.9, 16.7 Hz, 1H, Gly1-Cα*H*), 3.95 (dd, *J*=5.9, 16.7 Hz, 1H, Gly1-Cα*H'*), 3.81 (dd, *J*=5.0, 17.2 Hz, 1H, Gly4-Cα*H'*), 3.71 (s, 3H, OC*H₃*), 3.70 (m, 2H, Pro3-Cδ*H* & Cδ*H'*), 2.22 (m, 1H, Val6-Cβ*H*), 2.20-1.91 (m, 4H, Pro3-Cβ*H*, Cβ*H'*, Cγ*H*, & Cγ*H'*), 1.45 (s, 9H, *Boc*), 0.99 (d, *J*=6.9 Hz, 3H, Val6-CγC*H₃*), 0.97 (d, *J*=6.9 Hz, 3H, Val6-CγC*H3'*); ^{13}CNMR(CDCl3,75MHz) δ 172.9, 172.6, 169.9, 168.55, 168.52, 168.1, 156.2, 138.4, 138.1, 134.4, 128.9, 128.7, 123.3, 122.7, 122.3, 119.3, 118.8, 80.0, 61.5, 58.2, 52.1, 50.6, 46.1, 44.6, 43.6, 31.0, 29.6, 29.3, 28.2, 25.5, 19.0, 18.2. IR(KBr, cm^{-1})⁻: 3308, 2973, 1684, 1617, 1589,

1544, 1487, 1438, 1367, 1251, 1203, 1165, 1024. FABMS(m/z, %) M^++Na(703, 4). ESI HRMS: (M^+) calcd. for $C_{34}H_{44}O_9N_6$ +Na (703.3067), found: 703.3093.

Peptide 4. White solid; mp 130°C. ^{1}H NMR (CDCl$_3$ + 50μl DMSO-$d6$, 500 MHz): δ 9.88 (s, 1H, Aba3-NH), 9.53 (s, 1H, Aba6-NH), 8.37 (dd, J=4.8, 7.8 Hz, 1H, Gly5-NH), 8.34 (d, J=8.0 Hz, 1H, Aba6-C4H), 8.21 (d, J=7.5 Hz, 1H, Aba3-C4H), 8.08 (s, 1H, Aba6-C2H), 8.01 (d, J=7.8 Hz, 1H, Val8-NH), 7.90 (s, 1H, Aba3-C2H), 7.65 (t, J=3.4 Hz, 1H, Gly2-NH), 7.44 (m, 1H, Aba6-C6H), 7.40 (m, 1H, Aba3-C6H), 7.38 (m, 1H, Aba6-C5H), 7.37 (m, 1H, Aba3-C5H), 7.30 (d, J=8.6 Hz, 1H, Val7-NH), 5.78 (d, J=7.1 Hz, 1H, Ala1-NH), 4.80 (t, J=8.6 Hz, 1H, Val7-CαH), 4.49 (dd, J=5.6, 7.3 Hz, 1H, Pro4-CαH), 4.45 (dd, J=5.8, 7.8 Hz, 1H, Val8-CαH), 4.42 (dd, J=3.4, 16.6 Hz, 1H, Gly2-CαH), 4.38 (dd, J=7.8, 17.3 Hz, 1H, Gly5-CαH), 4.25 (m, 1H, Ala1-CαH), 4.12 (dd, J=3.4, 16.6 Hz, 1H, Gly2-CαH'), 3.83 (m, 2H, Pro4-CδH & CδH'), 3.80 (dd, J=4.8, 17.3 Hz, 1H, Gly5-CαH'), 3.74 (s, 3H, OCH_3), 2.24-1.95 (m, 4H, Pro4-CβH, CβH', CγH & CγH'), 2.19 (m, 1H, Val8-CβH), 2.16 (m, 1H, Val7-CβH), 1.44 (s, 9H, Boc), 1.38 (d, J=7.2 Hz, 3H, Ala1-CβCH_3)**,** 0.97 (d, J=6.8 Hz, 3H, Val8-CγCH_3), 0.97 (d, J=6.8 Hz, 3H, Val8-CγCH_3'), 0.94 (d, J=6.8 Hz, 3H, Val7-CγCH_3), 0.92 (d, J=6.8 Hz, 3H, Val8-CγCH_3'); IR(KBr, cm^{-1}): 3448.7, 2959.4, 2926.1, 2852.9, 1737.4, 1700.8, 1654.2, 1587.6, 1561.0, 1527.7, 1254.7, 1164.8, 1021.9. FABMS(m/z, %) M^++Na(873.8, 72). ESI HRMS: (M^+) calcd. for $C_{42}H_{58}O_{11}N_8$+Na (873.4122) found: 873.4156.

Peptide 5. White solid; mp 137°C. ^{1}H NMR (CDCl$_3$ + 50μl DMSO-$d6$, 500 MHz): δ 9.88 (s, 1H, Aba3-NH), 9.53 (s, 1H, Aba6-NH), 8.43 (dd, J=4.7, 7.4 Hz, 1H, Gly5-NH), 8.20 (d, J=8.0 Hz, 1H, Aba3-C4H), 8.18 (d, J=8.0 Hz, 1H, Aba6-C4H), 8.06 (d, J=7.8 Hz, 1H, Val8-NH), 7.97 (s, 1H, Aba6-C2H), 7.83 (s, 1H, Aba3-C2H), 7.71 (t, J=5.0 Hz, 1H, Gly2-NH), 7.45 (m, 1H, Aba6-C6H), 7.37 (m, 1H, Aba6-C5H), 7.35 (m, 1H, Aba3-C6H), 7.35 (m, 1H, Aba6-C5H), 7.22 (d, J=8.5 Hz, 1H, Val7-NH), 5.82 (d, J=4.8 Hz, 1H, Ala1-NH), 4.83 (dd, J=6.3, 8.5 Hz, 1H, Val7-CαH), 4.47 (dd, J=4.7, 7.1 Hz, 1H, Pro4-CαH), 4.43 (dd, J=5.3, 7.8 Hz, 1H, Val8-CαH), 4.42 (m, 1H, Gly5-CαH), 4.28 (dd, J=5.0, 16.8 Hz, 1H, Gly2-CαH), 4.27 (m, 1H, Ala1-CαH), 4.16 (dd, J=5.0, 16.8 Hz, 1H, Gly2-CαH'), 3.79 (m, 2H, Pro4-CδH & CδH'), 3.76 (m, 1H, Gly5-CαH'), 3.73 (s, 3H, OCH_3), 2.23-1.94 (m, 4H, Pro4-CβH, CβH', CγH & CγH'), 2.17 (m, 1H, Val7-CβH), 2.16 (m, 1H, Val8-CβH), 1.45 (s, 9H, Boc), 1.39 (d, J=7.0 Hz, 3H, Ala1-CβCH_3)**,** 0.98 (d, J=6.8 Hz, 3H, Val7-CγCH_3)**,** 0.94 (d, J=6.8 Hz, 3H, Val7-CγCH_3')**,** 0.93 (d, J=7.1 Hz, 3H, Val8-CγCH_3), 0.93 (d, J=7.1 Hz, 3H, Val8-CγCH_3'); ^{13}CNMR(CDCl3,75MHz) δ 173.7, 172.8, 172.3, 171.6, 169.7, 168.5, 167.7, 167.3, 155.6, 138.6, 138.4, 135.3, 134.8, 128.9, 128.6, 124.4, 122.6, 122.0, 120.1, 119.8, 118.7, 80.0, 61.6, 58.4, 57.7, 52.0, 50.7, 43.7, 32.0, 31.8, 30.0, 29.6, 29.3, 28.2, 25.6, 24.8, 22.6, 19.0, 18.8, 18.3, 18.0, 14.0. IR(KBr, cm^{-1}: 3306, 2964, 2930, 1661, 1645, 1590, 1557, 1540, 1486, 1444, 1306, 1251, 1218, 1163, 1021. FABMS(m/z, %) M^++Na(873.7, 14). ESI HRMS: (M^+) calcd. for $C_{42}H_{58}O_{11}N_8$ + Na (873.4122) found: 873.4153.

Peptide 6. White solid; mp 152°C. ^{1}H NMR (CDCl$_3$, 500 MHz): δ 10.30 (s, 1H, Aba3-NH), 9.35 (s, 1H, Aba6-NH), 8.48(d, J=7.4 Hz, 1H, Aba6-C4H), 8.47 (d, J=8.2 Hz, 1H, Val8-NH), 8.35 (d, J=7.5 Hz, 1H, Aba3-C4H), 8.00 (s, 1H, Aba6-C2H), 7.84 (s, 1H, Aba3-C2H), 7.41 (m, 1H, Aba6-C5H), 7.39 (m, 1H, Aba3-C6H), 7.38 (m, 1H, Aba6-C6H), 7.37 (m, 1H, Aba3-C5H), 7.18

(d, *J*=8.9 Hz, 1H, Ile7-N*H*), 6.97 (d, *J*=9.0 Hz, 1H, Val2-N*H*), 6.42 (dd, *J*=4.0, 9.1 Hz, 1H, Gly5-N*H*), 5.41 (d, *J*=8.4 Hz, 1H, Ile1-N*H*), 5.02 (dd, *J*=5.5, 8.9 Hz, 1H, Ile7-CαH), 4.91 (dd, *J*=6.2, 9.0 Hz, 1H, Val2-CαH), 4.68 (d, *J*=9.1, 17.4 Hz, 1H, Gly5-CαH), 4.55 (dd, *J*=5.6, 8.2 Hz, 1H, Val8-CαH), 4.30 (dd, *J*=3.8, 8.1 Hz, 1H, Pro4-CαH), 4.16 (m, 1H, Ile1-CαH), 3.84 (m, 2H, Pro4-Cδ*H* & Cδ*H'*), 3.75 (s, 3H, OC*H₃*), 3.73 (dd, *J*=4.0, 17.3 Hz, 1H, Gly5-CαH'), 2.35 (m, 1H, Pro4-Cγ*H*), 2.22 (m, 1H, Pro4-Cβ*H*), 2.20 (m, 1H, Val8-Cβ*H*), 2.17 (m, 1H, Pro4-Cβ*H'*), 2.07 (m, 1H, Val2-Cβ*H*), 2.00 (m, 1H, Pro4-Cγ*H'*),1.97 (m, 1H, Ile1-Cβ*H*), 1.73 (m, 1H, Ile7-Cβ*H*),1.50 (m, 1H, Ile1-Cγ*H*), 1.44 (s, 9H, *Boc*), 1.42 (m, 1H, Ile1-Cγ*H*), 1.16 (m, 1H, Ile1-Cγ*H'*), 0.97 (d, 3H, Val8-CγC*H₃*), 0.97 (d, 3H, Val8-CγC*H₃'*), 0.96 (m, 1H, Ile8-Cγ*H'*), 0.95 (d, 3H, Val2-CγC*H₃*), 0.95 (d, 3H, Val2-CγC*H₃'*), 0.82 (d, *J*=6.9 Hz, 3H, Ile7-CγC*H₃*), 0.72 (t, *J*=7.4 Hz, 3H, Ile7-CδC*H₃*); ^{13}CNMR(CDCl₃,75MHz) δ 172.6, 172.1, 171.7,171.4, 169.7, 169.5 168.2, 167.6 ,155.7, 139.1, 138.8, 135.3, 134.8, 129.1, 128.4, 124.0, 123.4, 122.6 121.6, 121.5, 119.8, 77.2, 62.2, 59.6, 58.7, 57.5, 51.8, 51.0, 44.0, 39.8, 37.44, 33.02, 30.8, 29.7, 29.2, 28.31, 25.8, 24.6, 24.4, 22.6, 19.4, 19.0, 18.2, 18.0, 15.7, 15.2, 14.0, 11.6, 11.5. IR (KBr, *cm⁻¹*): 3286.9, 2966.2, 2926.6, 1745.8, 1718.2, 1637.2, 1588.1, 1544.8, 1489.6, 1442.3, 1387.1, 1312.2, 1249.1, 1205.7, 1165.7, 1020.4. FABMS(m/z, %) M⁺+H(949, 12).

Peptide 7. White solid; mp 149°C. ^{1}H NMR (CDCl₃, 500 MHz): δ 10.16 (s, 1H, Aba3-N*H*), 9.35 (s, 1H, Aba6-N*H*), 8.46 (d, *J* = 9.1, 1H, Val8-N*H*), 8.43 (d, *J* =7.4, 1H, Aba6-C4*H*), 8.22 (d, *J* =7.2, 1H, Aba3-C4*H*), 7.35 (t, *J*=7.2, 1H, Aba3-C5*H*), 7.34 (d, *J*=7.2, 1H, Aba3-C6*H*), 7.34 (d, *J*=7.2, 1H, Aba6-C6*H*), 7.33 (d, *J*=8.5,1H, Ile7-N*H*) 7.23 (t, *J*= 7.4, 1H, Aba6-C5*H*), 6.74 (dd, *J*=9.3, *J*=3.5, 1H, Gly5-N*H*), 6.42 (d, *J*= 7.8, 1H, Val2-N*H*), 5.68 (d, *J*= 8.4, 1H, Ile1-N*H*), 4.80 (t, *J*= 8.5, 1H, Ile7- CαH), 4.72 (dd, *J*=9.3 *J*=17.4, 1H, Gly5-CαH), 4.55 (dd, *J*= 9.1, *J*= 5.6, 1H, Val8- CαH), 4.36 (dd, *J*= 3.9, *J*= 8.1, 1H, ^{D}Pro4-CαH), 4.03 (t, *J*= 8.4, 1H, Ile1- CαH), 3.85 (m, 2H, ^{D}Pro4- δH, δ'H), 3.72 (s, 3H, OC*H₃*), 3.69 (dd, *J*= 3.5, *J*=17.4, 1H, Gly5- α'*H*), 2.32 (m, 1H, ^{D}Pro4-γH), 2.21 (m, 1H, ^{D}Pro4-β*H*), 2.13 (m, 1H, ^{D}Pro4-β'*H*), 2.07 (m, 1H, Val 2- β*H*), 2.05 (m, 1H, Val 8-β*H*), 1.98 (m, 1H, ^{D}Pro4-γ'*H*), 1.89 (m, 1H, Ile1-β*H*), 1.88 (m, 1H, Ile7-β*H*), 1.65 (m, 1H, Ile7-γ*H*), 1.59 (m, 1H, Ile1-γ*H*), 1.45 (s, 9H, *Boc*), 1.01 (d, 3H, *J*= 6.9, Val 2-γC*H₃*), 1.01 (d, 3H, *J*=6.9, Val 2-γ'C*H₃*), 0.96 (d, *J*=7.5, 3H, Ile7-γC*H₃*), 0.95 (d, *J*=6.9, Ile1-γC*H₃*), 0.72 (t, *J*=7.4, 3H, Ile1-δC*H₃*), 0.72 (d, *J*=7.1, Val 8-γ'C*H₃*), 0.71 (d, *J*=7.1, 3H, Val 8-γC*H₃*). IR (Neat, *cm⁻¹*): 3415.2, 2964,5 2360.2, 1639.5, 1553.1, 1031.9, 618.4. FABMS(m/z, %) M⁺+H(949, 12). ESI HRMS: (M⁺) calcd. for C₄₉H₇₂O₁₁N₈ + Na (971.5218) found: 971.5227.

Peptide 8. White solid; mp 140°C. ^{1}H NMR (CDCl₃, 500 MHz): δ 10.54 (s, 1H, Aba-3 N*H*), 9.71 (s, 1H, Aba-6 N*H*), 8.62 (d, *J*=7.9, 1H, Aba3-C4*H*), 8.44 (d, *J*= 8.2, 1H, Aba6-C4*H*), 8.39 (d, *J*= 9.0, 1H, Val 8-N*H*), 8.23 (s, 1H, Aba3-C2*H*), 7.96 (s, 1H, Aba6-C2*H*), 7.40 (t, *J*= 7.9, 1H, Aba3-C5*H*), 7.39 (d, *J*= 8.2, 1H, Aba6-C6*H*), 7.35 (d, *J*= 7.9, 1H, Aba3-C6*H*), 7.35 (t, *J*= 8.2, Aba6-C5*H*), 6.71 (d, 1H, *J*= 9.5, 1H, Ile7-N*H*), 6.52 (d, *J*= 7.9, 1H, Val 2-N*H*), 5.86 (d, *J*=8.2, 1H, Val 1-N*H*), 4.97 (t, *J*=9.5, 1H, Ile7-CαH), 4.56 (dd, *J*=9.0, *J*= 5.2, 1H, Val8-CαH), 4.49 (dd, *J*=3.9, *J*= 8.1, 1H, ^{L}Pro5-CαH), 4.44 (dd, 1H, *J*= 3.9, *J*=8.1, ^{L}Pro4-CαH), 4.43 (t, *J*= 7.9, *J*= 7.9, 1H, Val 2- CαH), 3.94 (m, 2H, ^{L}Pro4-δH, δ'*H*), 3.93 (t, *J*=8.2, 1H, Ile1-CαH), 3.73 (s, 3H, OC*H₃*), 3.70 (m, 2H, ^{L}Pro5-δH, δ'*H*), 2.25 (m, 1H, ^{L}Pro5-γ*H*), 2.24 (m, 1H, ^{L}Pro4-β*H*), 2.16 (m,

1H, ^{L}Pro4-βH), 2.08 (m, 1H, Val 2-βH), 1.96 (m, 1H, ^{L}Pro4-β'H), 1.85 (m, 1H, ^{L}Pro4-γ'H), 1.85 (m, 1H, Val 1-βH), 1.82 (m, 1H, Val 7-βH), 1.70 (m, 1H, Ile7-γH), 1.58 (m, 1H, Ile1-γH), 1.44 (s, 9H, *Boc*), 1.19 (m, 1H, Ile7-γ'H), 1.17 (m, 1H, Ile1-γ'H)**,** 1.00 (d, J= 6.7, 6H, Val2-γCH_3, γ'CH_3), 0.97 (d, J=7.3, 3H, Ile7-γCH$_3$), 0.95 (d, J=6.9, Ile1-γCH$_3$), 0.92 (t, J=7.4, Ile7-δ CH_3), 0.90 (t, J=7.4, 3H, Ile1-δCH_3), 0.80 (d, J =6.8, Val8-γCH_3, γ'CH_3). IR(Neat cm^{-1}); 3286.1, 2966.9, 1646.0, 1553.6, 1169.6, 1019.1, 755.1. FABMS(m/z, %) M$^+$+H (990, 8). ESI HRMS: (M$^+$) calcd. for $C_{52}H_{76}O_{11}N_8$ + Na (1011.5531) found: 1011.5573.

Peptide 9. White solid; mp 148°C. ^{1}H NMR (CDCl$_3$, 500 MHz): δ 10.16 (s, 1H, Aba3-NH), 9.15 (s,1H, Aba6-NH), 8.43 (d, J=9.2, 1H, Val8-NH), 8.38 (d, J=8.2, 1H, Aba6-C4H), 8.25 (d, J=7.6, 1H , Aba3-C4H), 7.82 (s, 1H, Aba6-C2H), 7.69 (s, 1H, Aba3-C2H), 7.37 (t, J=8.2, 1H, Aba6-C5H), 7.33 (d, J=8.2, 1H, Aba6-C6H), 7.31 (t, J=7.9, 1H, Aba3-C5H), 7.26 (d, J=7.5, 1H, Aba3-C6H), 6.78 (d, J=9.6, 1H, Ile7-NH), 6.49 (d, J=7.9, 1H, Val2-NH), 5.72 (d, J= 8.3, 1H, Ile1-NH), 4.94 (t, J=9.6, 1H, Ile7-CαH), 4.84 (dd, J=1.5, J=7.9, 1H, ^{L}Pro5-**α**H), 4.72 (dd, J=3.2, J=8.3, 1H, ^{D}Pro4-αH), 4.50 (dd, J=9.2, J=5.6, 1H, Val8-αH), 4.42 (t, J=7.9, 1H, Val2-αH), 4.08 (m, 2H, ^{L}Pro5-δH, δ'H),

4.03 (t, J=8.3, 1H, Ile1-αH), 3.75 (m, 1H, ^{D}Pro4-δH, δ'H), 3.72 (s, 3H, OCH_3), 3.59 (m, 1H,^{L}Pro5-γH), 2.59 (m, 1H,^{L}Pro5-βH), 2.23 (m, γH), 2.13 (m, 1H, ^{D}Pro4-β'H), 2.10 (m, 1H, ^{D}Pro4-βH), 2.09 (m, 1H, ^{L}Pro5-β'H), 2.08 (m, 1H, Val 2-βH), 2.03 (m, 1H, Val8-βH), 1.99 (m, 1H, ^{D}Pro4-γ'H), 1.98 (m, 1H, ^{L}Pro5-γ'H), 1.88 (m, 1H, Ile1-βH), 1.84 (m, 1H, Ile7-βH), 1.67 (m, 1H, Ile7-βH), 1.59 (m, 1H, Ile1-βH), 1.45 (s, 9H, *Boc*), 1.21 (m, 1H, Ile1-γ'H), 1.04 (d, J= 6.8, 6H, Val2-γCH$_3$, γ'CH_3), 0.98 (d, J= 6.9, 3H, Ile1-γCH_3), 0.96 (d, J=6.9, 1H, Ile7-γCH_3), 0.95 (t, J=7.4, 3H, Ile1-δCH$_3$), 0.88 (t, J=7.4, 1H, Ile7-δCH_3), 0.71 (d, J= 6.8, 6H, Val8-γCH_3, γ'CH_3); ^{13}CNMR(CDCl3,75MHz) δ 172.6, 172.5, 171.1, 170.2, 169.5, 168.4, 155.8, 139.1, 138.8, 135.7, 135.2, 128.6, 127.9, 124.4, 123.5, 122.2, 121.6, 121.0, 120.2, 79.4, 60.9, 60.7, 59.1, 58.6, 57.0, 51.8, 50.8, 47.3, 38.3, 37.2, 31.8, 30.9, 30.7, 29.6, 29.5, 29.3, 28.5, 28.3, 25.6, 25.1, 24.8, 24.1, 22.6, 19.2, 19.0, 18.6, 17.7, 15.4, 15.2, 14.0, 11.2, 11.0. IR(Neat cm^{-1}); 3729.6, 3668.0, 3522.6, 2966.6, 2357.7, 1651.4, 1305.1, 754.0, 553.8. FABMS(m/z, %) M$^+$+H (990, 12). ESI HRMS: (M$^+$) calcd. for $C_{52}H_{76}O_{11}N_8$ + Na (1011.5531) found: 1011.5536.

Peptide 10. White solid; mp 108°C. ^{1}H NMR (CDCl$_3$, 500 MHz): δ 10.01 (s, 1H, Aba3-NH), 9.35 (s, 1H, Aba6-NH), 8.51 (d, J=8.2, 1H, Aba6-C4H), 8.46 (d, J=7.6, Aba3-4H), 8.30 (d, J=8.8, 1H, Val8-NH), 8.03 (s, 1H, Aba6-C2H), 7.41 (t, J=8.2, 1H, Aba6-C5H), 7.37 (t, J=7.9, 1H, Aba3-C5H), 7.35 (d, J=7.5, 1H, Aba3-C6H), 7.34 (d, J=8.2, Aba6- C6H), 6.98 (d, J=8.9, 1H, Ile7-NH), 6.77 (s, 1H, Aib4-NH), 6.61 (d, J=8.0, 1H, Val2-NH), 6.16 (s, 1H, Aib5-NH), 5.68 (d, J=8.4, 1H, Ile1-NH), 4.97 (t, J=8.4, 1H, Ile1-αH), 4.95 (t, J=8.9, 1H, Ile7-αH), 4.59 (dd, J=8.0, J=6.9, 1H, Val2-αH), 4.55 (dd, J=8.8, J= 5.7, 1H, Val8-αH), 3.72 (s, 3H, OCH_3), 2.09 (m, 1H, Val2-βH), 2.08 (m, 1H, Val8-βH), 1.87 (m, 1H, Ile7-βH), 1.65(s, 3H, Aib4-CH_3), 1.63 (s, 3H, Aib5-CH_3), 1.60 (s, 3H, Aib4-CH_3), 1.55 (s, 3H, Aib5-CH_3), 1.42 (m, 1H, Ile1-γH), 1.20 (m, 1H, Ile7-γH), 1.16 (m, 1H, Ile1-γ'H), 1.01 (d, J=6.8, 6H, Val2-γCH_3, γ'CH_3), 0.98 (m, 1H, Ile7-γ'H), 0.98 (d, J=6.9, 3H, Ile1-γCH_3), 0.90 (d, J=6.9, 3H, Ile7-γCH3), 0.89 (t, J=7.4, Ile1-δCH_3), 0.82 (d, J=6.8, 6H, Val8-γCH_3, γ'CH_3), 0.72 (t, J=7.4, 3H, Ile7-δCH_3); ^{13}CNMR

(CDCl3,75MHz); δ 173.3, 173.3, 172.5, 172.4, 172.3, 170.4, 168.5, 167.1, 155.8, 139.6, 139.1, 134.8, 132.2, 129.1, 128.6, 123.4, 122.8, 121.3, 121.0, 120.3, 119.6, 79.5, 59.9, 59.2, 58.3, 57.7, 57.4, 51.9, 37.3, 37.1, 31.0. 30.8, 29.6, 29.3, 29.2, 28.2, 27.3, 26.1 25.5, 24.7, 24.4, 23.8, 19.0, 18.8, 17.7, 15.4, 15.1, 11.3, 11.2, 11.1. IR(Neat cm^{-1}); 3305.3, 2924.5, 2854.3, 1648.2, 1590.8, 1551.4, 1487.2, 1303.7, 1216.3, 1167.1, 757.7. FABMS(m/z, %) M^{+}+H(965, 20). ESI HRMS: (M^{+}) calcd. for $C_{50}H_{76}O_{11}N_8$ + Na (987.5631) found: 987.5513.

Acknowledgments

References and Notes

1. (a) Seebach, D.; Matthews, J.L. *J. Chem. Soc., Chem. Commun.* **1997**, 2015. (b) Banerjee, A; Balram, P. *Current Science* **1997**, *73*, 1067. (c) Gellman, S. H. *Acc. Chem. Res*. **1998**, *31*, 173. (d) DeGrado, W.F.; Schneider, J. P.; Hamuro, Y. *J. Pept. Res*. **1999**, *54*, 206. (e) Kirshenbaum, K.; Zukermann, R. N.; Dill, K.A. *Curr. Opin. Struct. Biol*. **1999**, 9, 530. (f) Stigers, K.D.; Soth, M.J.; Nowick, J.S. *Curr. Opin. Chem. Biol*. **1999**, *3*, 714. (g) Gademann, K.; Hintermann, T.; Schreiber, J.V. *Curr. Med. Chem*. **1999**, *6*, 905. (h) Cheng, R. P.; Gellman, S.H.; Degrado, Cr. F. *Chem. Rev*. **2001**, *101*, 3219. (i) Sharma, G. V. M.; Reddy, K. V.; Krishna, P. R.; Sankar, A. R.; Narsimulu, K.; Kumar, S. K.; Jayaprakash, P.; Jagannadh, B.; Kunwar, A. C. *J. Am. Chem. Soc*. **2003**, *125*, 1367.
2. (a) Baldauf, C.; Gunter, R.; Hofmann, H. *Angew. Chem., Int. Ed.* **2004**, *43*, 1594. (b) J. Baldauf, C; Gunther, R.; Hofmann, H. J.; *Helv. Chim. Acta* **2003**, *86*, 2573.
3. Hanessian, S.; Luo, X.; Schaum, R., Michnick, S. *J. Am. Chem. Soc*. **1998**, *120*, 8569.
4. (a) Hinterman, T; Gademann, K.; Jann, B.; Seebach, D. *Helv. Chim. Acta* **1998**, *81*, 983. (b) Brenner, M.; Seebach, D. *Helv. Chim. Acta* **2001**, *84*, 1181. (c) Seebach, D.; Brenner, M.; Rueping, M.; Schweizer, B.; Jaun, B. *J. Chem.Soc., Chem. Commun.* **2001**, 207. (d) Seebach, D.; Brenner, M.; Rueping, M.; Jaun, B. *Chem. Eur. J*. **2002**, *8*, 573.
5. (a) Blanco, F.J,; Jimenez, M. A.; Herranz, J.; Rico, M.; Santoro, J.; Nieto, J. L. *J. Am. Chem. Soc*. **1993**, *115*, 5887. (b) Haque, T.S.; Little, J.C.; Gellman, S.H. *J. Am. Chem. Soc*. **1994**, *116*, 4105. (c) Awasthi, S.K.; Raghothama, S.; Balaram, P. *Biochem. Biophys. Res. Commun*. **1995***, 216,*375. (e) Dortemme, T.; Ramirez-Alvarado, J.; Serrano. L. *Science* **1998**, *281*, 253. (f) Schenck, H. L; Gellman, S. H. *J. Am. Chem. Soc*. **1998**, *120*, 4869.
6. (a) Karle, I.L.; Gopi, H.N.; Balaram, P. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 3716. (b) Karle, I.L.; Gopi, H.N.; Balaram, P. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5160. (c) Roy, R. S.; Raghothama, S. R.; Karle, I. L.; Balaram, P. *Helv. Cheim. Acta* **2002**, *85*, 3313.

7. Karle, I. L.; Pramanik, A.; Banerjee, A.; Bhattacharya, S.; Balaram, P. *J. Am. Chem. Soc*. **1997**, *119, 9087.

8. Hagihara, M.; Anthony, N.J.; Stout, T.J.; Clardy, J.; Schreiber, S. L. *J. Am. Chem. Soc*. **1992**, *114, 6568.

9. Chakraborty, T. K.; Ghosh, A.; Kumar, S. K.; Kunwar, A. C. *J. Org. Chem*. **2003**, *68*, 6459.

10. Ramana Rao, M. H. V.; Kiran Kumar, S.; Kunwar, A. C. *Tetrahedron Lett*. **2003,** *44*, 7369.

11. Calculations using MOPAC showed that the lowest energy conformations on 1N(3-Methyl Carbamoyl Phenyl) acetamide, correspond to φ= ± 136°and ψ= ± 154°, which are very close to the values of antiparallel β-sheets.

12. (a) Ishida, H.; Singh, M.; Donowaki, K.; Ohkubo, K. *J. Org. Chem*. **1995**, *60*, 5374. (b) Ishida, H.; Donowaki, K.; Singh, M.; Ohkubo, K. *Tetrahedron Lett*. **1995**, *36*, 8987. (c) Kubik, S,; Goddard, R. *J. Chem. Soc., Chem Commun*. **2000**, 633. (d) Pohl, S.; Goddard, R.; Kubik, S. *Tetrahedron. Lett*. **2001**, *42*, 7555.

13. (a) Zeng,H; Yang,X.; Flowers,r-II. R. A.; Gong,B. *J.Am.Chem. Soc*. **2**002, *124*, 2903. (b) Nowick, J.S.; Lam, K.S.; Khasanova, T.V.; Kemnitzer, W.E.; Maitra S.; Mee, H.T.; Liu, R. *J. Am. Chem. Soc*. **2002**, *124*, 4972. (c) Gong, B.; Yan, Y.; Zeng, H.; Skrzypczak-Jankunn, E.; Kim, Y. W; Zhu, J.; Ickes, H. *J. Am. Chem. Soc*. **1999**, *121*, 5607. (d) Gong, B; Zeng, H; Zhu, J; Yua, L; Han, Y; Cheng, S; Furukawa, M; Parra, R. D; Kovalevsky, A. Y; Mills, J. L; Skrzypczak-Jankun, E; Martinovic, S; Smith, R. D; Zheng, C; Szyperski, T; Zeng, X. C., *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 11583.

14. (a) Gibbs, A. C; Bjorndahl, T. C; Hodges, R. S; Wishart, D. S., *J.Am. Chem. Soc*. **2002**, *124*, 1203; (b) Stanger, H.E.; Gellman, S.E. *J. Am. Chem. Soc*. **1998**, *120*, 4236.

15. Venkatraman, J.; Shankaramma, S. C.; Balaram, P. *Chem. Rev*. **2001**, *101*, 3131.

16. Bodanszky, M. *Peptide Chemistry – A Practical Textbook*. Springer Verlag: Berlin 1988.

17. Solvent titration was carried out by adding up to 33% v/v of DMSO-d_6 in CDCl$_3$ solution sequentially.

18. Bean, J. W; Kopple, K. P.; Peishoff, C. E. *J. Am. Chem. Soc*. **1992**, *114*, 5328.

19. Aravinda, S.; Shamala, B.; Rajkishore, R.; Gopi, H. N.; Balaram, P. *Angew. Chem., Int. Ed.* **2002**, *41*, 3863.

20. Maynard, A. J.; Sharman, G. J.; Searle, M.S. *J. Am. Chem. Soc*. **1998**, *120*, 2903.

21. Burley, S.K.; Petsko, G. A. *Science* **1985**, *229*, 23.

22. Syud, F.A; Stanger, H. E; Gellman, S.H. *J. Am. Chem. Soc*. **2001**, *123*, 8667.

23. Rapaport, H.; Moller, G.; Knobler, C.M.; Jensen, T.R.; Kjaer, K.; Leiserowitz, L.; Tirrell, D.A. *J. Am. Chem. Soc*. **2002**, *124*, 9342.