Conformational studies of C_2 symmetric peptidomimetics based on 2,5-anhydro sugar diacid and 2,5-anhydro sugar diamine scaffolds

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The paper is dedicated to Prof. S. Swaminathan on the occasion of his 80th birthday

(received 23 Aug 04; accepted 08 Nov 04; published on the web 15 Nov 04)

Abstract

Conformational studies of C_2 symmetric peptidomimetics with identical peptide strands attached on both sides of 2,5-anhydro sugar diacid and 2,5-anhydro sugar diamine scaffolds revealed the presence of nine-membered pseudo β-turns in the sugar diacid based molecules consisting of identical intramolecular hydrogen bonds at the two ends between the AA^2NH and sugarOH.

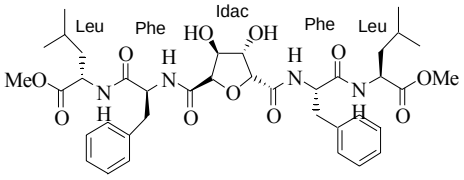
Keywords: C_2 Symmetry, peptidomimetics, NMR, ROESY, molecular dynamics

Introduction

We developed and studied a new class of compounds **1**-**8** (Figure 1), as potential HIV-protease inhibitors that were based on carbohydrate peptide hybrid structures.[1] In this strategy, identical peptide chains were attached on both sides of C_2 symmetric carbohydrate based scaffolds, leading to the formation of C_2-symmetric peptiodomimetics.[2] Carbohydrate based molecular designs are increasingly drawing attention of chemists.[3] Detailed studies on the development of new HIV-1 protease inhibitors based on C_2 symmetric scaffolds and acyclic carbohydrates have recently been reported.[4] It is also being increasingly felt that small molecule protease inhibitors need to have restricted degrees of freedom which is evident from the success of cyclic urea based inhibitors.[5] This prompted us to study cyclic carbohydrate based core foundations as conformationally rigid scaffolds to build a new class of molecular framework as potential protease inhibitors.

Two different types of cyclic carbohydrate frameworks of sugar diacid and sugar diamine were employed in our design. These molecules are 2,5-anhydro-D-idaric acid **9** (Idac), 1,6-diamino-2,5-anhydro-1,6-dideoxy-D-iditol **10** (Idam) and 1,6-diamino-2,5-anhydro-1,6-dideoxy-D-mannitol **11** (Mdam). Attachment of identical peptide strands on both sides of these scaffolds led to the formation of peptidomimetic compounds **1**-**8**, whose syntheses were reported earlier.[1]

The conformational study of **1** was carried out in detail and revealed a C_2 symmetric structure with a nine-membered pseudo β-turn consisting of identical intramolecular hydrogen bonds at the two ends between the LeuNH and sugarOH.[6] A very small temperature coefficient for the LeuNH chemical shift ($\Delta\delta/\Delta T = -1.2$ ppb.K^{-1}) was observed in DMSO-d_6. The presence of two '*cis*-β-hydroxycarboxyl' moieties, the core structural motif believed to be responsible for such intramolecular H-bonds, on two sides of the tetrahydrofuran ring, nucleated identical β-turn-like structures in **1** at both ends. In this paper, we describe detailed conformational analysis of compounds **2-8** achieved by various NMR techniques, which revealed that while all these molecules had C_2 symmetric structures, only the sugar diacid based compounds **2-4**, especially those with free hydroxyl groups on the sugar ring, had the propensity to form nine-membered pseudo β-turn like structures containing intramolecular hydrogen bonds between AA^{2}NH → sugarOH. This supports further the earlier observations that free hydroxyl groups on sugar rings prevent furanoid sugar amino acid based short linear peptides to adopt regular β-turn structures as these hydroxyl groups themselves act as hydrogen bond acceptors.[3a,6]



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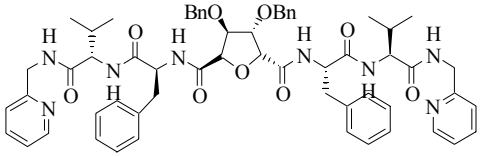

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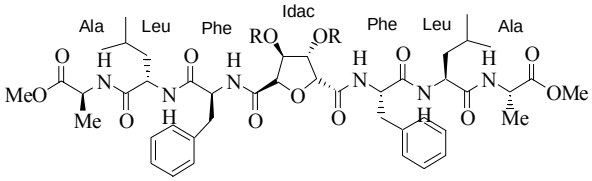
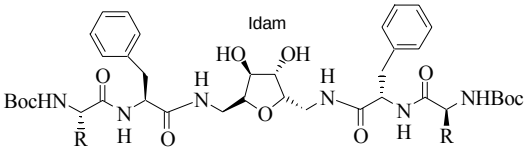
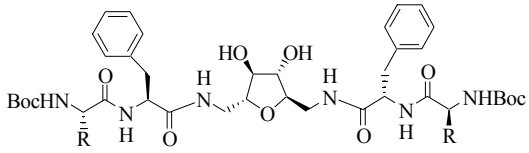


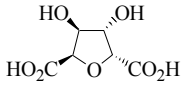
3: R = H **4**: R = CH_2Ph



5: R = isobutyl (Leu) **6**: R = isopropyl (Val)



7: R = isobutyl (Leu) **8**: R = isopropyl (Val)



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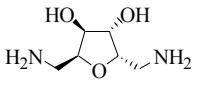

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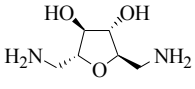

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Figure 1

Results and Discussion

Conformational analysis. NMR studies

NMR studies of compounds **2, 3, 5-8** were carried out in DMSO-d_6. The solvent used for **4** was $CDCl_3$, although its variable temperature studies (VT) were done in DMSO-d_6. The NMR spectra of the peptides described here were quite well resolved, and most of the spectral parameters could be obtained easily for compounds **2-8**. Assignments were carried out with the help of total correlation spectroscopy (TOCSY), rotating frame nuclear Overhauser effect spectroscopy (ROESY) experiments. The cross peak signals in ROESY spectra were used for obtaining the restraints in MD calculations.

Variable temperature studies. Information on the participation of amide protons of compounds **2-8** in intramolecular hydrogen bonding was obtained by variable temperature studies carried out between 30– 70 °C in DMSO-d_6.[7] The temperature coefficients $\Delta\delta/\Delta T$ obtained for various amide protons in these molecules are listed in Table 1. Hydrogenation of compound **2** gave a complex mixture of products and so its structure was studied in the Bn-protected form, which showed high temperature coefficients for the amide proton chemical shifts (Table 1), indicating absence of any H-bonding. The temperature coefficient $\Delta\delta/\Delta T$ of LeuNH peak in **3** was –2.3 ppb.K^{-1} (Figure 2) indicating its significant propensity to form an intramolecularly hydrogen bonded structure.[7] Further support for such a H-bonded structure came from the appearance of the OH signal at an appreciable downfield at 5.96 ppm. In peptide **4**, the propensity of the formation of an intramolecular H-bond was less compared to **3**, as indicated by the relatively higher value of $\Delta\delta/\Delta T$ of –2.8 ppb.K^{-1} for its LeuNH. The decrease in the H-bond forming capability may be due to the replacement of the ring OH groups in **3** by OBn in **4**. Further, the diamine peptidomimetics **5-8** showed large magnitudes of $\Delta\delta/\Delta T$ indicating the absence of any intramolecular H-bonding in these molecules. Further, the OH protons in these compounds resonated upfield as compared to **3** at δ 5.06, 5.05, 5.18, and 5.17 for **5**, **6**, **7**, and **8**, respectively, indicating that they were not involved in H-bonding.[3j]

Table 1. Temperature coefficients (–ppb.K^{-1}) data for Peptides **2-8** in DMSO-d_6

Peptides	Phe	Leu/Val	Ala
2	3.2	4.5	-
3	5.9	2.3	5.0
4	5.2	2.8	3.7
5	4.2	7.7	-
6	4.3	8.3	-
7	3.5	8.0	-
8	4.0	8.3	-

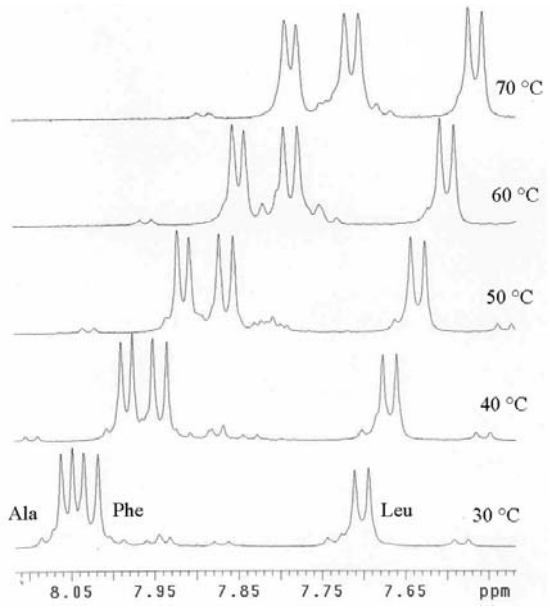

Figure 2. Amide regions of the ^{1}H NMR spectra recorded between 30 – 70 °C for peptide **3**.

Conformational analysis of 2. Perfect C_2 symmetry was observed in **2** showing signals distinctly only from half of the molecule in its NMR spectrum in DMSO-d_6. In the ROESY spectrum of **2** in DMSO-d_6, the characteristic NOE like PheNH ↔ IdacC3H and the sequential NOEs like ArNH ↔ Val CαH, ValNH ↔ Phe CαH, PheNH ↔ IdacC2H were observed (Figure 3). The large magnitudes of $\Delta\delta/\Delta T$ of the amide protons indicate that, due to the bulky Bn-protecting groups as well the sterically crowded Val side-chains, the ValNH did not participate in H-bonding with the OBn group.

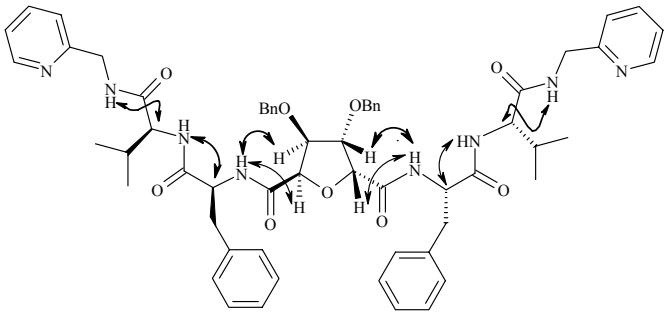

Figure 3. Schematic representation of the NOEs seen for **2**.

Conformational analysis of 3. Peptidomimetic **3** also displayed C_2 symmetry in DMSO-d_6. Various NOEs LeuNH ↔ PheNH, PheNH ↔ IdacC3H (C4H), PheNH ↔ IdacC2H (C5H) and LeuNH ↔ IdacOH were observed along with sequential NOEs AlaNH ↔ Leu CαH, LeuNH ↔ Phe CαH (Figure 4). Expanded ROESY spectrum of **3** with characteristic NOE connectivities is

shown in Figure 5. The temperature coefficient ($\Delta\delta/\Delta T$) of –2.3 ppb.K^{-1} for leucine amide proton indicated its participation in intramolecular hydrogen bonding. The side chains of Leu and Phe appeared to take a predominant conformation about χ_1, which is evident from $J_{\alpha H\text{-}\beta H}$ = 9.8, $J_{\alpha H\text{-}\beta' H}$ = 5.1 for Leu and $J_{\alpha H\text{-}\beta H}$ = 10.4, $J_{\alpha H\text{-}\beta' H}$ = 3.4 Hz for Phe.

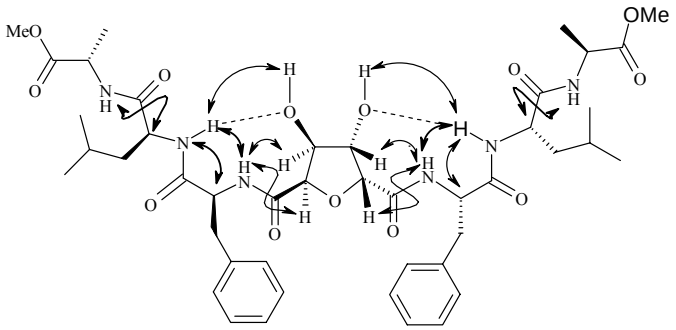

Figure 4. Schematic representation of the proposed H-bonded structure of **3** with some of the prominent long-range NOEs seen in its ROESY spectrum.

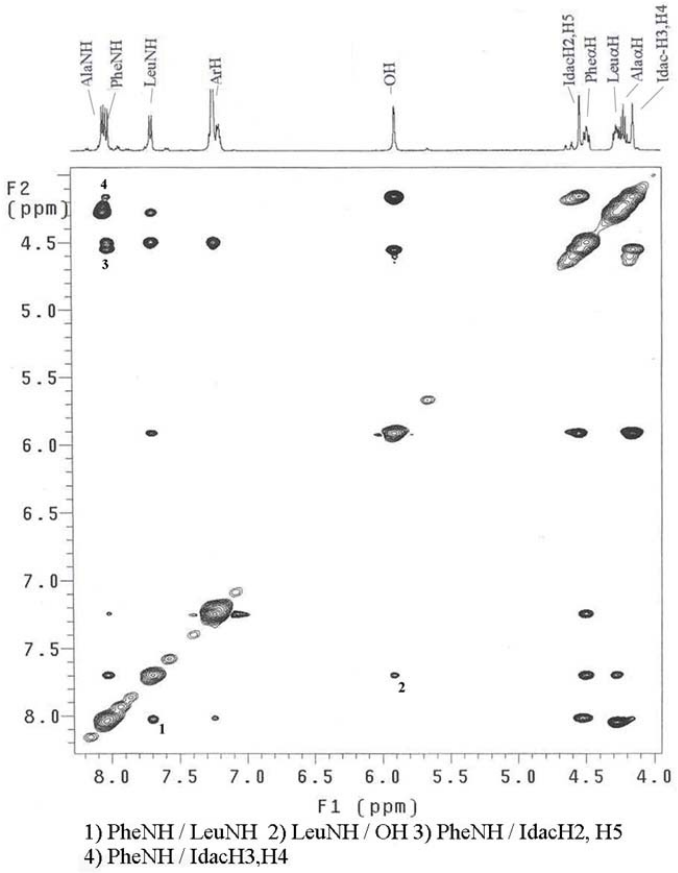

Figure 5. Expanded ROESY spectrum of **3** in DMSO-d_6.

The structures sampled during the restrained MD calculations,[8] using distance constraints based on the ROESY cross-peak intensities[9] for **3** in DMSO-d_6, are in agreement with the structure obtained from NMR studies. The two peptide-chains form a pseudo β-turn involving H-bond between LeuNH and IdacOH. The central portions of these structures are fairly conserved with the variations localized mainly at the Leu side-chain (Figure 6). The five-membered sugar rings take twist conformations that are in agreement with the NMR data.

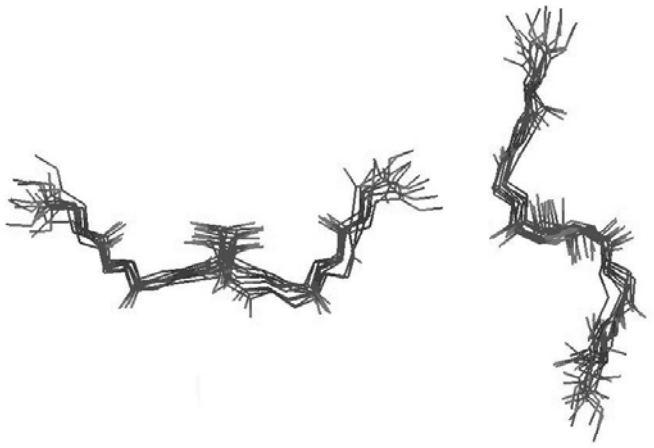

Figure 6. Ten superimposed structures sampled during MD simulation studies for **3**. Structures on left and right represent views from two different angles.

Conformational analysis of 4. Similar to **3,** organized structure was also observed in compound **4**. The ROESY spectrum of **4** in $CDCl_3$ displayed C_2 symmetry for half of the molecule. Characteristic NOEs like PheNH ↔ LeuNH, AlaNH ↔ LeuNH, LeuNH ↔ IdacC2H were observed along with the sequential connectivities PheNH ↔ IdacC2H, AlaNH ↔ Leu$C\alpha$H and LeuNH ↔ Phe$C\alpha$H (Figure 7). Compound **4** showed restricted rotation for Leu χ_1 with $J_{\alpha H\text{-}\beta H}$ = 9.0, and $J_{\alpha H\text{-}\beta' H}$ = 5.7 Hz. For the Phe side-chain, there was no restricted rotation about χ_1.

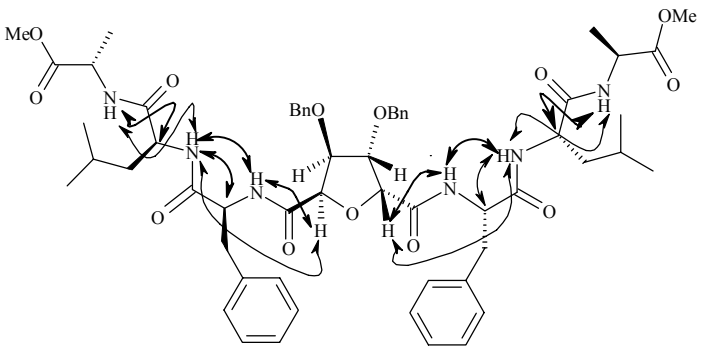

Figure 7. Schematic representation of some of the prominent long-range NOEs seen for **4** in $CDCl_3$.

Conformational analysis of 5-8. Peptidomimetics **5-8** displayed perfect C_2 symmetric structures in their NMR spectra in DMSO-d_6. However, as the temperature coefficients of all the amide protons in these molecules were very high (Table 1), they were not studied for further structural details. The ROESY spectra of **6** and **8** did not show many long range NOEs (Figure 8) and definite structural information on their structures could not be obtained. This may partly be due to the additional methylene groups attached to the sugar ring.

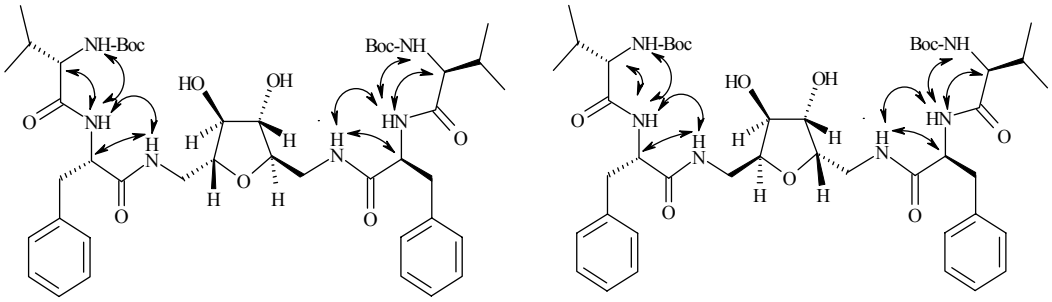

Figure 8. Diagrammatic representations of some of the NOEs for **6** (left) and **8** (right) in DMSO-d_6.

Discussion

The large values (7.6-8.5 Hz) of $^3J_{\text{NH-}\alpha\text{H}}$ for Phe and Leu in **3** and **4**, and for Val in **2** were observed. Similarly, for the diamine peptidomimetics **5-8**, the $^3J_{\text{NH-}\alpha\text{H}}$s were large (> 8 Hz) for Phe and Leu in **5** and **7**, and for Val in **6** and **8**, which correspond to a value of φ in the vicinity of –120°. The existence of a complete set of sequential CαH ↔ NH NOE connectivities, wherever possible, implied the propensity of structures with large population of conformers in the β-region of φ-ψ space (φ =–180° to –60° and ψ = 60° to 180°). The side-chains of Phe, Leu and Val point to the existence of large population of a single conformation in several of these peptides. For peptides **3**, **4**, **5**, and **7**, the LeuNH ↔ LeuCβH NOEs, stronger than the LeuNH ↔ LeuCβ'H NOEs, were observed. Further LeuCαH ↔ LeuCδH and LeuNH ↔ LeuCγH NOEs were also seen. Large $J_{\alpha\text{H-}\beta\text{H}}$ of about 9 Hz and small $J_{\alpha\text{H-}\beta'\text{H}}$ of about 5 Hz in Phe, Leu and Val further indicated restricted rotation about χ_1.

The observation of $J_{\text{H2-H3}} = J_{\text{H4-H5}} = 3.5$ Hz in **2**, **3**, and **4** and $J_{\text{H3-H4}} \approx 0$ are consistent with a twist conformation of $^4{}_3T$ (where 4 and 3 refer to C3 and C4, respectively) for the five-membered ring with C3 and C4 both being *exo* to the respective peptide chains.

Conclusion

The anticipated nucleation of the nine-membered β-turn like ring structures at both ends of these C_2 symmetric assemblies involving identical intramolecular H-bonding, as observed in **1**

between LeuNH → sugarOH, was realised in some compounds. The observation of $\Delta\delta/\Delta T$ for LeuNH being 2.3 and 2.8 in **3** and **4**, respectively, support the formation of incipient intramolecular LeuNH → sugarOH hydrogen bonds in these molecules, relatively stronger in the former. In the diamine peptidomimetics, the absence of connectivities between sugar ring and the peptide sequences, along with the disappearance of the H-bonding character, which was consistently observed in the diacid-based molecules, indicate the deviation from the designed structure. Higher degree of freedom created in Idam and Mdam by the methylene group in the sugar ring might be responsible for the absence of any ordered structure. These observations reveal the importance of the constraints imparted in these structures for obtaining the designed reverse turn mimetics.

In summary, introduction of C_2 symmetric scaffolds **9-11** helped molecules **1-8** adopt C_2 symmetric structures and thus fulfilled the primary objective of our study. Although, these compounds did not show any activity towards HIV protease inhibition up to 2 mM inhibitor concentration,[1] positioning of more appropriate residues at the P1/P1′ positions in these molecules will probably lead to substrates with improved biological activities.[10] Further work is currently in progress.

Experimental Section

General Procedures. All reactions were carried out in oven or flame-dried glassware with magnetic stirring under nitrogen atmosphere using dry, freshly distilled solvents, unless otherwise noted. Reactions were monitored by thin layer chromatography (TLC) carried out on 0.25 mm silica gel plates with UV light, I_2, 7% ethanolic phosphomolybdic acid-heat and 2.5% ethanolic anisaldehyde (with 1% AcOH and 3.3% conc. H_2SO_4)-heat as developing agents. Silica gel finer than 200 mesh was used for flash column chromatography. Yields refer to chromatographically and spectroscopically homogeneous materials unless otherwise stated. IR spectra were recorded as neat liquids or KBr pellets. NMR spectra were recorded on 200, 300 and 400 MHz spectrometers at room temperature of ~ 21 °C in $CDCl_3$ using tetramethylsilane as internal standard or the solvent signal as secondary standard and the chemical shifts are shown in δ scales. Multiplicities of NMR signals are designated as s (singlet), d (doublet), t (triplet), q (quartet), br (broad), m (multiplet, for unresolved lines), etc. ^{13}C NMR spectra were recorded with complete proton decoupling. Mass spectra were obtained under electron impact (EI), electron spray ionisation (ESI) and liquid secondary ion mass spectrometric (LSIMS) techniques.

Synthesis of the peptides. The peptides were synthesized[1,6b] by conventional solution phase methods using 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBt) as coupling agents and dry CH_2Cl_2 and/or amine-free dry DMF as solvents.[11] While the *tert*-butoxycarbonyl (Boc) group was used for *N*-protection, the C-terminal was protected as a methyl ester (OMe). Deprotection of the former was done in TFA-CH_2Cl_2

(1:1) and saponification of the later was performed using LiOH in THF-MeOH-H_2O. Deprotection of the Bn-protected compounds was done by hydrogenation using $Pd(OH)_2$-C in MeOH. The final products were purified by silica gel column chromatography and fully characterized by spectroscopic methods before using them in the conformational studies.

Selected physical data of 2. $[\alpha]_D^{20}$ 2.9° (*c* 0.24, $CHCl_3$); IR (KBr) ν_{max} 3282, 1639, 1546, 1359, 1219, 1104, 771 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.51 (t, 2 H, J = 5.8 Hz, ArN*H*), 8.46 (d, 2 H, J = 4.2 Hz, Ar*H*), 8.08 (d, 2 H, $J_{NH\text{-}\alpha H}$ = 8.7 Hz, ValN*H*), 7.79 (d, 2 H, $J_{NH\text{-}\alpha H}$ = 8.3 Hz, PheN*H*), 7.71 (dt, 2 H, J = 1.7, 7.7, 7.7 Hz, Ar*H*), 7.30-7.06 (m, 24 H, Ar*H*), 4.76-4.69 (m, 4 H, PhCH_2), 4.71 (ddd, 2 H, $J_{NH\text{-}\alpha H}$ = 8.3, $J_{\beta H\text{-}\alpha H}$ = 8.4, $J_{\beta H\text{-}\alpha H}$ = 4.4 Hz, PheCα*H*), 4.53 (d, 2 H, J = 3.5 Hz, IdacC2*H*, C5*H*), 4.38-4.28 (m, 2 H, ArCH_2), 4.19 (dd, 2 H, $J_{\alpha H\text{-}\beta H}$ = 7.4, $J_{NH\text{-}\alpha H}$ = 8.7 Hz, ValCα*H*), 4.13 (t, 2 H, J = 3.5 Hz, IdacC3*H*, C4*H*), 3.05 (dd, 2 H, $J_{\beta H\text{-}\alpha H}$ = 4.4, $J_{\beta H\text{-}\beta' H}$ = 13.7 Hz, PheCβ'*H*), 2.88 (dd, 2 H, $J_{\beta H\text{-}\alpha H}$ = 8.4, $J_{\beta H\text{-}\beta' H}$ = 13.7 Hz, PheCβ*H*), 1.95 (m, 2 H, ValCβ*H*), 0.83 (d, 12 H, J = 6.7 Hz, Val CγCH_3, ValCγ'CH_3); MS (LSIMS) *m/z* (%) 1045 (54) $[M + H]^+$, 1067 (24) $[M + Na]^+$.

Selected physical data of 3. $[\alpha]_D^{20}$ –12.8° (*c* 0.52, $CHCl_3$); IR (KBr) ν_{max} 3410, 2920, 1790, 1740, 1540, 1200, 960, 720 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.09 (d, 2 H, $J_{NH\text{-}\alpha H}$ = 6.8 Hz, AlaN*H*), 8.07 (d, 2 H, $J_{NH\text{-}\alpha H}$ = 8.5 Hz, PheN*H*), 7.72 (d, 2 H, $J_{NH\text{-}\alpha H}$ = 8.2 Hz, LeuN*H*), 7.28-7.19 (m, 10 H, Ar*H*), 5.96 (d, 2 H, J = 2.8 Hz, O*H*), 4.56 (d, 2 H, J = 3.5 Hz, IdacC2*H*, C5*H*), 4.50 (ddd, 2 H, $J_{\alpha H\text{-}\beta H}$ = 10.4, $J_{NH\text{-}\alpha H}$ = 8.5, $J_{\alpha H\text{-}\beta' H}$ 3.4 Hz, PheCα*H*), 4.28 (ddd, 2 H, $J_{NH\text{-}\alpha H}$ = 8.2, $J_{\alpha H\text{-}\beta H}$ = 9.8, $J_{\alpha H\text{-}\beta' H}$ = 5.1, LeuCα*H*), 4.23 (m, 2 H, AlaCα*H*), 4.16 (dd, 2 H, J = 2.8, 3.5 Hz, IdacC3*H*, C4*H*), 3.60 (s, 6 H, $J_{NH\text{-}\alpha H}$ = 8.2, OCH_3), 3.19 (dd, 2 H, $J_{\alpha H\text{-}\beta' H}$ = 3.4, $J_{\beta H\text{-}\beta' H}$ = 14.1 Hz, PheCβ'*H*), 2.96 (dd, 2 H, $J_{\alpha H\text{-}\beta H}$ = 10.4, $J_{\beta H\text{-}\beta' H}$ = 14.1 Hz, PheCβ*H*), 1.63 (m, 2 H, LeuCγ*H*), 1.44 (ddd, 2 H, $J_{\alpha H\text{-}\beta' H}$ = 5.1, $J_{\beta' H\text{-}\gamma H}$ = 8.6, $J_{\beta H\text{-}\beta' H}$ = 13.5 Hz, LeuCβ'*H*), 1.38 (ddd, 2 H, $J_{\alpha H\text{-}\beta H}$ = 9.8, $J_{\beta H\text{-}\gamma H}$ = 5.6, $J_{\beta H\text{-}\beta' H}$ = 13.5 Hz, LeuCβ*H*), 1.30 (d, 6 H, $J_{\alpha H\text{-}\beta CH3}$ = 6.4 Hz, AlaCβCH_3) 0.89 (d, 6 H, $J_{\gamma H\text{-}\delta H}$ = 7.2 Hz, LeuCδCH_3), 0.80 (d, 6 H, $J_{\gamma H\text{-}\delta' H}$ = 7.2 Hz, LeuCδ'CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.92, 172.25, 170.51, 169.47, 135.79, 128.94, 128.91, 127.51, 83.26, 77.92, 54.19, 52.43, 51.35, 48.46, 40.39, 36.55, 24.48, 22.89, 21.72, 17.49; MS (LSIMS) *m/z* (%) 883 (100) $[M + H]^+$, 905 (80) $[M + Na]^+$.

Selected physical data of 4. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.32-7.18 (m, 18 H, Ar*H*), 7.06-7.01 (m, 2 H, Ar*H*), 6.94 (d, 2 H, $J_{NH\text{-}\alpha H}$ = 7.8 Hz, PheN*H*), 6.66 (d, 2 H, $J_{NH\text{-}\alpha H}$ = 7.3 Hz, AlaN*H*), 6.28 (d, 2 H, $J_{NH\text{-}\alpha H}$ = 8.3 Hz, LeuN*H*), 4.61 (d, 2 H, J = 3.5 Hz, Idac-C2*H*, C5*H*), 4.50 (ddd, 2 H, PheCα*H*), 4.42 (m, 2 H, AlaCα*H*), 4.34 (ddd, 2 H, $J_{\alpha H\text{-}\beta H}$ = 9.0, $J_{\alpha H\text{-}\beta' H}$ = 5.7 Hz, LeuCα*H*), 4.05 (d, 2 H, J = 3.5 Hz, IdacC3*H*, C4*H*), 4.34 (ABq, 4 H, J = 12.9 Hz, PhCH_2), 3.72 (s, 6 H, OCH_3), 3.28 (dd, 2 H, $J_{\alpha H\text{-}\beta' H}$ = 6.2, $J_{\beta H\text{-}\beta' H}$ = 13.8 Hz, PheCβ'*H*), 3.14 (dd, 2 H, $J_{\alpha H\text{-}\beta H}$ = 10.4, $J_{\beta H\text{-}\beta' H}$ = 14.1 Hz, PheCβ*H*), 1.57 (ddd, 2 H, $J_{\alpha H\text{-}\beta' H}$ = 5.9, $J_{\beta' H\text{-}\gamma H}$ = 8.2, $J_{\beta H\text{-}\beta' H}$ = 13.9 Hz, LeuCβ'*H*), 1.35 (d, 6 H, $J_{\alpha H\text{-}\beta H}$ = 7.3 Hz, AlaCβCH_3), 1.29 (m, 2 H, LeuCγ*H*), 1.12 (ddd, 2 H, $J_{\alpha H\text{-}\beta H}$ = 8.9, $J_{\beta H\text{-}\gamma H}$ = 5.7, $J_{\beta H\text{-}\beta' H}$ = 13.9 Hz, LeuCβ*H*), 0.79 (d, 6 H, $J_{\gamma H\text{-}\delta H}$ = 6.5 Hz, LeuCδCH_3), 0.75 (d, 6 H, $J_{\gamma H\text{-}\delta' H}$ = 6.5 Hz, LeuCδ'CH_3).

Selected physical data of 5. $[\alpha]_D^{20}$ –43.1° (*c* 0.5, $CHCl_3$), IR (KBr) ν_{max} 3410, 3350, 2910, 2900, 1660, 1640, 1540, 1160, 1100, 1075, 763, 712 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.06 (t, 2

H, J = 6.2 Hz, IdamN*H*), 7.71 (d, 2 H, $J_{NH\text{-}\alpha H}$ 8.1 Hz, PheN*H*), 7.28-7.14 (m, 10 H, Ar*H*), 6.88 (d, 2 H, $J_{NH\text{-}\alpha H}$ = 8.3 Hz, LeuN*H*), 5.06 (d, 2 H, J = 4.5 Hz, O*H*), 4.52 (ddd, 2 H, $J_{NH\text{-}\alpha H}$ = 8.1, $J_{\alpha H\text{-}\beta' H}$ = 5.0, $J_{\alpha H\text{-}\beta H}$ = 8.9 Hz, PheCα*H*), 3.93 (dt, 2 H, J = 3.0, 6.2, 6.2 Hz, IdamC2*H*, C5*H*), 3.86 (ddd, 2 H, $J_{NH\text{-}\alpha H}$ = 8.3, $J_{\alpha H\text{-}\beta H}$ = 9.9, $J_{\alpha H\text{-}\beta' H}$ 5.2 Hz, LeuCα*H*), 3.83 (dd, 2 H, J = 3.0, 4.5 Hz, IdamC3*H*, C4*H*), 3.25 (dt, 2 H, J = 6.2, 6.2, 13.6 Hz, IdamC1H, C6H), 3.12 (dt, 2 H, J = 6.2, 6.2, 13.6 Hz, IdamC1*H′*, C6*H′*), 2.94 (dd, 2 H, $J_{\alpha H\text{-}\beta' H}$ = 5.0, $J_{\beta H\text{-}\beta' H}$ = 13.7 Hz, PheCβ'*H*), 2.80 (dd, 2 H, $J_{\alpha H\text{-}\beta H}$ = 8.9, $J_{\beta H\text{-}\beta' H}$ = 13.7 Hz, PheCβ*H*), 1.46 (m, 2 H, LeuCγ*H*), 1.37 (s, 18 H, Boc), 1.31 (m, 2 H, $J_{\alpha H\text{-}\beta H}$ = 9.9, $J_{\beta H\text{-}\gamma H}$ = 5.4, $J_{\beta H\text{-}\beta' H}$ = 13.5 Hz, LeuCβ*H*), 1.22 (m, 2 H, $J_{\alpha H\text{-}\beta' H}$ = 5.2, $J_{\beta' H\text{-}\gamma H}$ = 8.5, $J_{\beta H\text{-}\beta' H}$ = 13.5 Hz, LeuCβ'*H*), 0.82 (d, 6 H, $J_{\delta CH3\text{-}\gamma H}$ = 6.6 Hz, LeuCδCH_3), 0.78 (d, 6 H, $J_{\delta' CH3\text{-}\gamma H}$ = 6.6 Hz, LeuCδCH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.86, 172.35, 155.89, 136.23, 129.24, 128.57, 126.97, 80.32, 79.36, 75.86, 53.94, 53.56, 41.04, 38.08, 37.91, 28.23, 24.66, 22.85, 21.74; MS (LSIMS) *m/z* 884 (100) $[M + H]^+$, 906 (90) $[M + Na]^+$.

Selected physical data of 6. $[\alpha]_D^{20}$ –23.3° (*c* 0.5, $CHCl_3$); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.04 (t, 2 H, J = 6.8 Hz, IdamN*H*), 7.85 (d, 2 H, $J_{NH\text{-}\alpha H}$ = 8.4 Hz, PheN*H*), 7.24-7.14 (m, 10 H, Ar*H*), 6.64 (d, 2 H, $J_{NH\text{-}\alpha H}$ = 8.4 Hz, ValN*H*), 5.05(s, 2 H, J = 3.6 Hz, O*H*), 4.56 (ddd, 2 H, $J_{NH\text{-}\alpha H}$ = 8.4, $J_{\alpha H\text{-}\beta' H}$ = 5.1, $J_{\alpha H\text{-}\beta H}$ = 9.2 Hz, PheCα*H*), 3.91 (dt, 2 H, J = 2.8, 6.8, 6.8 Hz, IdamC2*H*, C5*H*), 3.82 (dd, 2 H, J = 2.8, 3.6 Hz, IdamC3*H*, C4*H*), 3.70 (dd, 2 H, $J_{NH\text{-}\alpha H}$ = 8.4 Hz, $J_{\alpha H\text{-}\beta H}$ = 6.8 Hz, ValCα*H*), 3.24 (ddd, 2 H, J = 5.8, 6.8, 13.6 Hz, IdamC1*H*, C6*H*), 3.12 (dt, 2 H, J = 5.6, 6.8, 13.6 Hz, IdamC1*H′*, C6*H′*), 2.94 (dd, 2 H, $J_{\alpha H\text{-}\beta' H}$ = 5.1, $J_{\beta H\text{-}\beta' H}$ = 13.7 Hz, PheCβ'*H*), 2.79 (dd, 2 H, $J_{\alpha H\text{-}\beta H}$ = 9.2, $J_{\beta H\text{-}\beta' H}$ = 13.7 Hz, PheCβ*H*), 1.81(m, 2 H, ValCβ*H*), 1.37 (s, 18 H, Boc), 0.71 (d, 6 H, $J_{\beta H\text{-}\gamma CH3}$ = 6.8 Hz, ValCγCH_3), 0.67 (d, 6 H, $J_{\beta H\text{-}\gamma CH3}$ = 6.8 Hz, ValCγ'CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.38, 170.87, 155.27, 137.47, 129.11, 127.91, 126.15, 78.53, 78.06, 75.84, 59.99, 53.48, 38.29, 37.92, 30.36, 28.11, 19.02, 18.05; MS (LSIMS) *m/z* (%) 856 (100) $[M + H]^+$, 878 (40) $[M + Na]^+$.

Selected physical data of 7. $[\alpha]_D^{20}$ –34.8° (*c* 0.5, $CHCl_3$); IR (Neat) ν_{max} 3400, 2970, 1680, 1635, 1219, 1530, 1160 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.10 (t, 2 H, J = 6.2 Hz, MdamN*H*), 7.66 (d, 2 H, $J_{NH\text{-}\alpha H}$ = 8.1 Hz, PheN*H*), 7.13-7.25 (m, 10 H, Ar*H*), 6.89 (d, $J_{NH\text{-}\alpha H}$ = 8.6 Hz, LeuN*H*), 5.18 (d, 2 H, J = 4.0 Hz, O*H*), 4.56 (ddd, 2 H, $J_{NH\text{-}\alpha H}$ = 8.1 Hz, $J_{\alpha H\text{-}\beta H}$ = 9.5, $J_{\alpha H\text{-}\beta' H}$ = 4.7 Hz, PheCα*H*), 3.86 (ddd, 2 H, J = 6.2, 8.6, 9.7 Hz, MdamC2*H*, C5*H*), 3.76 (ddd, 2 H, $J_{NH\text{-}\alpha H}$ = 8.6 Hz, $J_{\alpha H\text{-}\beta H}$ = 9.7, $J_{\alpha H\text{-}\beta' H}$ = 5.2 Hz, LeuCα*H*), 3.71 (ddd, 2 H, J = 2.4, 4.0, 8.6 Hz, MdamC3*H*, C4*H*), 3.28 (m, 2 H, MdamC1*H*, C6*H*), 3.20 (dt, 2 H, J = 6.2, 6.2, 13.8 Hz, MdamC1*H′*, C6*H′*), 2.97 (dd, 2 H, $J_{\alpha H\text{-}\beta' H}$ = 4.7, $J_{\beta H\text{-}\beta' H}$ = 13.7 Hz, PheCβ'*H*), 2.78 (dd, 2 H, $J_{\alpha H\text{-}\beta H}$ = 9.5, $J_{\beta H\text{-}\beta' H}$ = 13.7 Hz, PheCβ*H*), 1.44 (m, 2 H, LeuCγ*H*), 1.37 (s, 18 H, Boc), 1.31 (m, 2 H, $J_{\alpha H\text{-}\beta H}$ = 9.7, $J_{\beta H\text{-}\gamma H}$ = 5.5, $J_{\beta H\text{-}\beta' H}$ = 13.5 Hz, LeuCβ*H*), 1.20 (m, 2 H, $J_{\alpha H\text{-}\beta' H}$ = 5.4, $J_{\beta' H\text{-}\gamma H}$ = 8.9, $J_{\beta H\text{-}\beta' H}$ = 13.5 Hz, LeuCβ'*H*), 0.82 (d, 6 H, $J_{\delta CH3\text{-}\gamma H}$ = 6.6 Hz, LeuCδCH_3), 0.77 (d, 6 H, $J_{\delta' CH3\text{-}\gamma H}$ = 6.6 Hz, LeuCδ'CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.25, 171.90, 155.88, 136.66, 129.35, 128.45, 126.78, 81.31, 80.19, 78.45, 54.44, 53.49, 41.22, 38.07, 28.28, 24.60, 22.94, 21.74; MS (LSIMS) *m/z* (%) 783 (82) $[M + H - C_5H_8O_2]^+$, 883 (48) $[M + H]^+$, 905 (100) $[M + Na]^+$.

Selected physical data of 8. $[\alpha]_D^{20}$ 29.5° (*c* 0.55, $CHCl_3$); IR (Neat) ν_{max} 3400, 3300, 2995, 1685, 1640, 1580, 1540, 1200 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.07 (t, 2 H, J = 6.3 Hz,

MdamN*H*), 7.81 (d, 2 H, $J_{NH\text{-}\alpha H}$ = 8.7 Hz, PheN*H*), 7.23-7.14 (m, 6 H, Ar*H*), 6.65 (d, 2 H, $J_{NH\text{-}\alpha H}$ = 9.2 Hz, ValN*H*), 5.17 (d, 2 H, *J* = 3.9 Hz, O*H*), 4.60 (ddd, 2 H, $J_{NH\text{-}\alpha H}$ = 8.7, $J_{\alpha H\text{-}\beta' H}$ = 9.7, $J_{\alpha H\text{-}\beta' H}$ = 4.3 Hz, PheC*αH*), 3.77-3.70 (m, 4 H, MdamC2*H*, C3*H*, C4*H*, C5*H*), 3.69 (dd, 2 H, $J_{NH\text{-}\alpha H}$ = 9.2, $J_{\alpha H\text{-}\beta H}$ = 6.8 Hz, ValC*αH*), 3.27 (dt, 2 H, *J* = 6.3, 6.3 13.2 Hz, MdamC2*H*, C6*H*), 3.19 (dt, 2 H, *J* = 6.3, 6.3,13.2 Hz, MdamC1*H′*, C6*H′*), 2.97 (dd, 2 H, $J_{\alpha H\text{-}\beta' H}$ = 4.3, $J_{\beta H\text{-}\beta' H}$ = 13.7 Hz, PheC*β′H*), 2.77 (dd, 2 H, $J_{\alpha H\text{-}\beta' H}$ = 9.7, $J_{\beta H\text{-}\beta' H}$ = 13.7 Hz, PheC*βH*), 1.79(m, 2 H, ValC*βH*), 1.37 (s, 18 H, Boc), 0.69 (d, 6 H, $J_{\beta H\text{-}\gamma CH3}$ = 6.8 Hz, ValC*γ*CH_3), 0.64 (d, 2 H, $J_{\beta H\text{-}\gamma CH3}$ = 6.8 Hz, ValC*γ′*CH_3); ^{13}C NMR (DMSO-d_6, 125 MHz) *δ* 171.10, 170.86, 155.27, 137.64, 129.18, 127.87, 126.11, 82.12, 78.18, 78.08, 60.10, 53.41, 41.10, 38.13, 30.41, 28.13, 19.04, 18.08; MS (LSIMS) *m/z* (%) 755 (22) $[M + H - C_5H_8O_2]^+$, 855 (15) $[M + H]^+$, 877 (18) $[M + Na]^+$.

Acknowledgements

We thank CSIR, New Delhi for research fellowships (MHVRR and SG) and DST, New Delhi for financial support.

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8. Protocol used for MD Simulation Studies: Molecular mechanics/dynamics calculations were carried out using Sybyl 6.8 program on a Silicon Graphics O2 workstation. The Tripos force field, with default parameters, was used throughout the simulations. Energy minimizations were first carried out with steepest decent, followed by conjugate gradient method for a maximum of 1000 iterations each or RMS deviation of 0.005 kcal/mol, whichever was earlier. The energy-minimized structures were then subjected to MD. A number of interatomic distances obtained from NMR data were used as constraints. Distance constraints with a force constant of 15 kcal/Å were applied in the form of flat bottom potential well with a common lower bound of 2.0 Å and the upper bound of 2.8, 3.5, and 4.0 Å, in accordance with the NOE intensities for strong (s), medium (m), and weak (w), respectively. Force constant of 5 kcal/Å was employed for dihedral angle constraints. The energy-minimized structures were subjected to constrained MD simulations for duration of 300 ps (50 cycles each of 6 ps period, of the Simulated Annealing protocol). The atomic velocities were applied following Boltzmann distribution about the center of mass, to obtain a starting temperature of 700 K. After simulating for 1 ps at high temperature, the system temperature

was reduced exponentially over a 5 ps period to reach a final temperature of 300 K. Resulting structures were sampled for each and every cycle, leading to an ensemble of total 50 structures, out of which 10 structures, saved at 30 ps intervals, were minimized using the above mentioned protocol.

9. Distance constraints used in MD simulation studies for Peptide **3**: PheNH ↔ IdacC2H (w), PheNH ↔ IdacC3H (w), PheNH ↔ LeuNH (m), LeuNH ↔ IdacOH (m), LeuNH ↔ PheCαH (m), AlaNH ↔ LeuCαH (m). Similar constraints were used on either side of the C_2 symmetric molecule.
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