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Synthesis and conformational studies of peptidomimetics containing a carbocyclic 1,3-diacid

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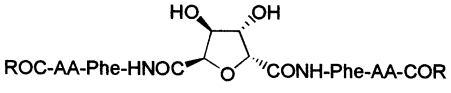
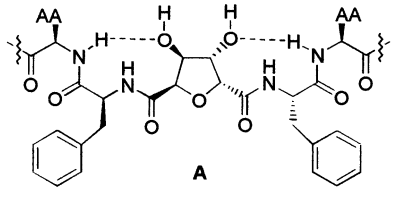
Abstract—A rigid carbocyclic scaffold comprising of an all-*cis* 4,5-dihydroxy-1,3-cyclopentanedicarboxylic acid is developed. Attachment of peptide strands to the carboxylic groups of this novel template led to the peptidomimetics **2** and **3**. Conformational analysis by circular dichroism and NMR studies revealed that these molecules adopt a unique folded structure in nonpolar solvent involving intramolecular hydrogen bonding between PheNH of one strand and LeuC═O (in **2**) or GlyC═O (in **3**) of the other strand. This structure is very different from the structures observed earlier in their sugar counterparts (**1**). The paper describes in detail the synthesis and structural studies of compounds **2** and **3**.

1. Introduction

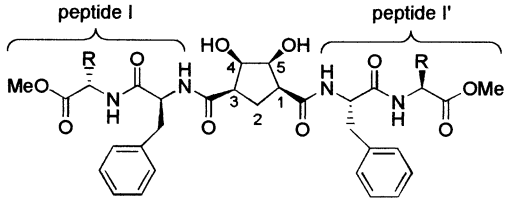
Reverse-turn peptidomimetics constitute one of the most important areas of research in structure–activity relationship studies of peptides.[1] Several conformationally rigid, non-peptidic scaffolds have been designed with different functional groups anchored on a single molecular framework and used for crafting many interesting reverse-turn mimetics.[2] Many of these templates, especially the ones with identical functional groups, have also been used extensively to nucleate parallel β-sheet structures in peptides.[3] Recently, we have developed a structurally rigid, novel molecular framework of a hexose-derived 2,5-anhydrosugar diacid.[4] Bi-directional elongation of its diacid moieties with identical peptide strands led to the formation of architecturally beautiful novel C_2-symmetric peptidomimetics **1**, which displayed very ordered structures consisting of identical intramolecular H-bonds at two ends between AANH→sugar–OH, as shown schematically in structure **A**.[4] The presence of two '*cis*-β-hydroxycarboxyl' moieties, the core structural motif believed to be responsible for such intramolecular H-bonds,[5] on two sides of the tetrahydrofuran ring nucleated identical β-turn-like structures in **1** at both ends. To examine whether similar secondary structure could also be present in other five-membered ring systems, we decided to construct a structurally similar scaffold with a cyclopentane ring, in place of the tetrahydrofuran ring, as the core foundation on which various peptide chains could be anchored. A new molecular entity of an all-*cis* 4,5-dihydroxy-1,3-cyclopentanedicarboxylic acid moiety,[6] carrying the essential '*cis*-β-hydroxycarboxyl' motif on both sides, was conceived as an ideal template that could nucleate topologically simple intramolecular folding. In this paper, we describe the synthesis and detail structural studies of the peptidomimetics **2** and **3** prepared from this novel carbocycle-based template.



1: AA = amino acids; R = ester or amide links



A



2: R = isobutyl; **3**: R = H

Keywords: peptide mimetics; hydrogen bonding; conformation; NMR; Diels–Alder reactions.

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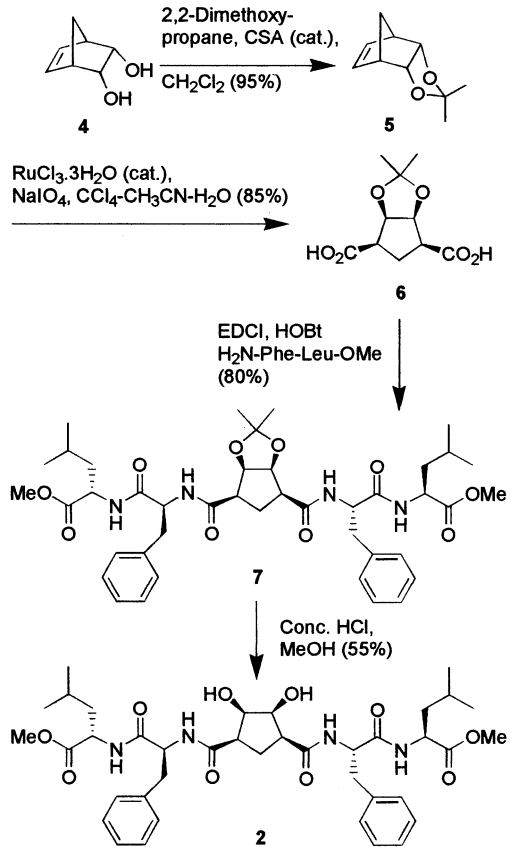

Scheme 1. Synthesis of the peptidomimetic **2**.

2. Results and discussion

2.1. Synthesis of peptidomimetics 2 and 3

Scheme 1 outlines the synthesis of **2**. The starting material, *endo-cis*-bicyclo[2.2.1]hept-5-ene-2,3-diol (**4**), was prepared according to the reported procedure[7] in two steps involving a Diels–Alder reaction in the first step between cyclopentadiene and vinylene carbonate, followed by alkaline hydrolysis of the carbonate ring. The diol **4**, thus obtained, was protected as an acetonide **5** in 95% yield, which was subjected to an oxidative cleavage reaction using a catalytic amount of $RuCl_3{\cdot}3H_2O$ in the presence of $NaIO_4$ to give the diacid **6** in 85% yield. Compound **6** was then treated with an excess of amino-terminal free peptide molecule H_2N–Phe–Leu–OMe under standard solution phase methods using 1-ethyl-3-(3-(dimethylamino)propyl)-carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBt) as coupling agents and amine-free dry DMF and/or dry CH_2Cl_2 as solvent. The purified product **7** (yield 80%) was deprotected under acidic conditions to furnish the final product **2** in 55% yield. The same protocol was followed for the synthesis of the other molecule **3**, by attaching H_2N–Phe–Gly–OMe to the diacid **7** under the same conditions described in Scheme 1 for the synthesis of **2**.

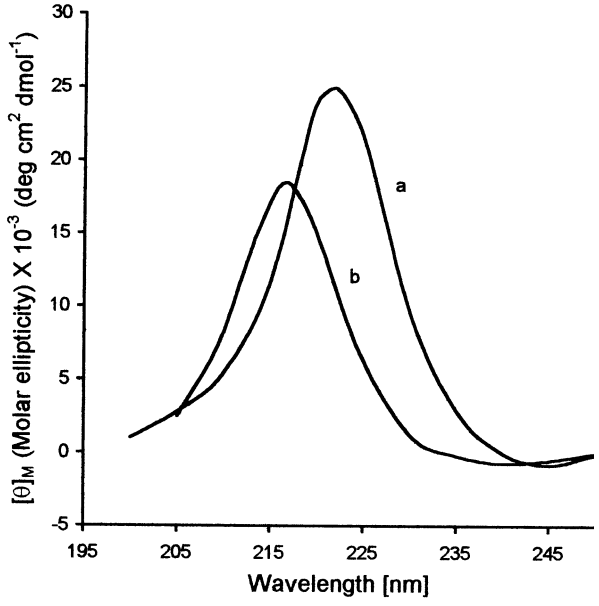

Figure 1. CD spectra (in TFE) of **2** (a) and **3** (b).

2.2. Conformational analysis. Circular dichroism studies

The conformational analysis of the peptidomimetics **2** and **3** were carried out by studying their circular dichroism (CD) spectra in trifluoroethanol (TFE). A strong positive band with very high ellipticity at 222 nm exhibited by **2** in its CD spectrum (Fig. 1) suggested the possibility of the existence of a turn structure in the molecule.[8] Compound **3** also showed a positive band at 216 nm indicating the likely occurrence of a similar structure in solution.

2.3. Conformational analysis. NMR studies

The dimethyl ester of **6** showed a two-fold molecular symmetry in its proton NMR spectrum. In the energy-minimized structure of the molecule, that was supported by the ^1H NMR coupling constants, the C1–C5–C4–C3 dihedral angle was zero making it a perfect *meso* isomer. However, addition of the identical dipeptide residues on both sides resulted in the loss of two-fold molecular symmetry in the peptidomimetics **2** and **3**. The difference of the behavior of the left and right halves of the molecules resulted from their diastereomeric relationship to each other. The protons of the left side peptide chain (peptide I), attached to the C3 end of the cyclopentane ring, with (L)L*RR* configuration (LL*RR* for **3**, L*RR* for **2**, amino acids were all L) showed different chemical shifts than those in the right side peptide (peptide I′) linked to the C1 end of the ring with (L)L*SS* configuration.[9] This was also largely due to the different conformational behavior of the two peptide chains discussed below in detail.

The absence of molecular symmetry leads to the spectral complexity due to the appearances of the resonances close to each other, having very similar chemical shifts. It was, therefore, not possible to derive all the spectral parameters, especially the couplings involved in the Leucine side-chains. The sequential αH–NH, H3–Phe(I)NH and H1–Phe(I′)NH cross-peaks in the ROESY spectra were used to make assignments of the resonances in the peptide chains I and I′. The spectral parameters for **2** and **3** are given in Tables 1 and 2, respectively.

Table 1. ^{1}H chemical shifts (δ) in ppm, Coupling constants (J) in Hz, and temperature coefficients ($\Delta\delta/\Delta T$, in ppb/K) of the amide protons of **2** (500 MHz, $CDCl_3$)

Protons	Phe (I)	Leu (I)	Phe (I′)	Leu (I′)
NH	7.60 (d, $J_{NH-\alpha H}$=7.6 Hz)	6.67 (d, $J_{NH-\alpha H}$=8.6 Hz)	6.37 (d, $J_{NH-\alpha H}$=7.7 Hz)	6.69 (d, $J_{NH-\alpha H}$=8.6 Hz)
CαH	4.63 (ddd, J=7.9, 7.6, 6.0 Hz)	4.58 (ddd, $J_{\alpha-\beta}$=9, 5 Hz)[a]	4.73 (ddd, J=7.7, 6.5, 6.2 Hz)	4.59 (ddd, $J_{\alpha-\beta}$=9, 5 Hz)[a]
CβH	3.17 (dd, $J_{\alpha-\beta}$=6.0 Hz)	{1.55–1.43 (m)	3.26 (dd, $J_{\alpha-\beta}$=6.2 Hz)	{1.55–1.43 (m)
CβH′	3.09 (dd, $J_{\alpha-\beta'}$=7.9 Hz, $J_{\beta-\beta'}$= 13.9 Hz)		3.08 (dd, $J_{\alpha-\beta'}$=6.5 Hz, $J_{\beta-\beta'}$= 14.0 Hz)	
CγH				
CδH		0.86 (d, CH_3, $J_{\gamma-\delta}$=6.3 Hz) 0.87 (d, CH_3, $J_{\gamma-\delta}$=6.3 Hz)		0.89 (d, CH_3, $J_{\gamma-\delta}$=6.3 Hz) 0.90 (d, CH_3, $J_{\gamma-\delta}$=6.3 Hz)
Others	7.20–7.36 (m, Ar*H*)	3.69 (s, CO_2CH_3)[b]	7.20–7.36 (m, Ar*H*)	3.71 (s, CO_2CH_3)[b]
$-\Delta\delta/\Delta T$ (ppb/K) for NH	9.1	6.5	+0.7	5.2
Cyclopentane ring: 4.47 (d, 4-O*H*, J_{H4-OH}=7.4 Hz), 4.28 (d, 5-O*H*, J_{H5-OH}=6.1 Hz), 4.21 (m, *H*4), 4.19 (m, *H*5), 2.81 (m, *H*1), 2.79 (m, *H*3), 2.26 (dt, *H*2, $J_{2-2'}$=14.3 Hz, J_{1-2}=J_{3-2}=7.2 Hz), 2.06 (dt, *H*2′, $J_{2-2'}$=14.3 Hz, $J_{1-2'}$=$J_{3-2'}$=9.3 Hz)				

[a] Errors in these couplings are ±1 Hz.
[b] These assignments are interchangeable.

Table 2. ^{1}H Chemical shifts (δ) in ppm, coupling constants (J) in Hz, and temperature coefficients ($\Delta\delta/\Delta T$, in ppb/K) of the amide protons of **3** (500 MHz, $CDCl_3$)

Protons	Phe (I)	Gly (I)	Phe (I′)	Gly (I)
NH	8.06 (d, $J_{NH-\alpha H}$=8.5 Hz)	7.30 (bs)	6.74 (d, $J_{NH-\alpha H}$=8.0 Hz)	7.2 (bs)
CαH	4.61 (dt, J=8.5, 5.5 Hz)	α=4.08 (dd, $J_{NH-\alpha H}$=6.0 Hz), α′=3.86 (dd, $J_{NH-\alpha' H}$=5.0 Hz) ($J_{\alpha-\alpha'}$=18.0 Hz)	4.86 (dt, J=8.0, 5.8 Hz)	α=4.04 (dd, $J_{NH-\alpha H}$= 5.6 Hz), α′=3.88 (dd, $J_{NH-\alpha' H}$=5.0 Hz) ($J_{\alpha-\alpha'}$=18.3 Hz)
CβH	3.24 (dd, $J_{\alpha-\beta}$=5.5 Hz)		3.23 (dd, $J_{\alpha-\beta}$=5.8 Hz)	
CβH′	3.04 (dd, $J_{\alpha-\beta'}$=8.5 Hz, $J_{\beta-\beta'}$=13.9 Hz)		3.08 (dd, $J_{\alpha-\beta'}$=8.0 Hz, $J_{\beta-\beta'}$=14.2 Hz)	
Others	7.18–7.32 (m, Ar*H*)	3.68 (s, CO_2CH_3)[a]	7.18–7.32 (m, Ar*H*)	3.69 (s, CO_2CH_3)[a]
$-\Delta\delta/\Delta T$ (ppb/K) for NH	14.1	9.1	2.45	9.3
Cyclopentane ring: 4.56 (bs, O*H*), 4.42 (bs, O*H*), 4.21 (m, *H*4), 4.22 (m, *H*5), 2.82 (m, *H*1), 2.83 (m, *H*3), 2.32 (dt, *H*2, $J_{2-2'}$=14.5 Hz, J_{1-2}=J_{3-2}=5.2 Hz), 1.9 (dt, *H*2′, $J_{1-2'}$=$J_{3-2'}$=9.3 Hz)				

[a] These assignments are interchangeable.

In noncompetitive solvents like CD_2Cl_2, $CDCl_3$, intramolecularly hydrogen bonded amide protons resonate downfixeld and typically exhibit a relatively large temperature dependence of the chemical shifts compared to the free amide protons.[3c] The temperature coefficients of the amide proton chemical shifts ($\Delta\delta/\Delta T$) are listed in Tables 1 and 2. The Phe(I)NH signals in both **2** and **3** show larger temperature coefficients than the other amide protons indicating their possible involvements in intramolecular H-bonding.

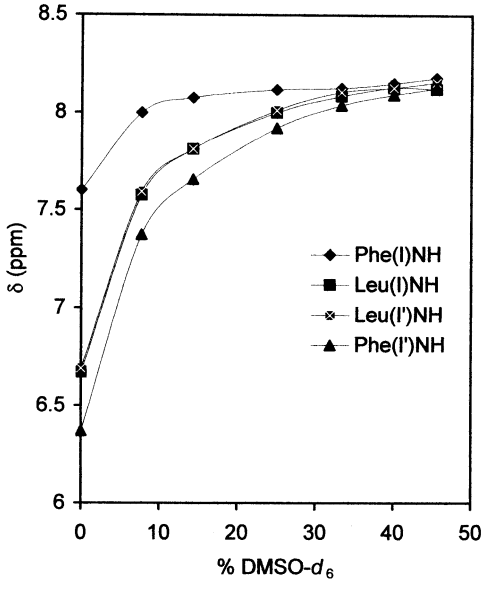

Figure 2. Solvent titration plots for the amide protons of **2**.

The variations in the chemical shifts of the amide protons in nonpolar ($CDCl_3$)-polar solvent (DMSO-d_6) titration also provide information on their participation in the intramolecular hydrogen bondings.[10] Addition of strongly hydrogen bonding solvents like DMSO-d_6 to $CDCl_3$ solution of the peptides perturbs all the exposed amide protons, which start forming H-bonds with the added polar solvent molecules causing substantial downfield shifts in their NMR signals. The intramolecularly hydrogen bonded amide proton chemical shifts, on the other hand, show relatively smaller solvent dependence. The solvent induced shifts of the amide proton resonances of **2** and **3** are shown in Figs. 2 and 3, respectively. Only Phe(I)NH showed small shifts in both the compounds indicating its participation in intramolecular H-bonds.

2.4. Conformational analysis of 2

The $^3J_{NH-\alpha H}$ were about 8.6 Hz for the Leucines in **2**, implying the predominance of φ values of approximately −120°, whereas for its Phe residues, the $^3J_{NH-\alpha H}$ were about 7.5 Hz, which may arise due to either the propensity of a structure

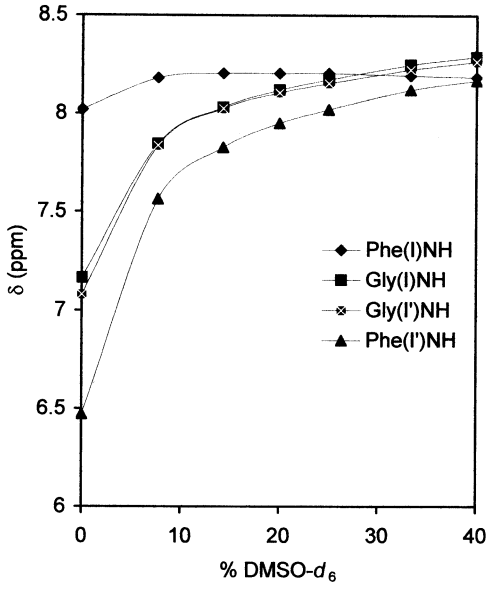

Figure 3. Solvent titration plots for the amide protons of **3**.

with $\varphi \approx -120°$ or the average of several conformations. The presence of a strong sequential αH–NH connectivity is consistent with the $\varphi-\psi$ values in the β-region of the Ramachandran plots. Yet, the presence of NH–NH sequential connectivity indicates that some fraction of the molecules have $\varphi-\psi$ values in the α-region. The $^3J_{\alpha H-\beta H}$ for the Phe residues were about 7 Hz. This is a result of more than one significant rotamers about Cα–Cβ. For Leu, the two $J_{\alpha H-\beta H}$ are 5 and 9 Hz (due to spectral overlap these values are accurate to about 1 Hz). Inability to make stereospecific assignments for the β-protons in these residues precluded obtaining information on the rotamer populations about Cα–Cβ.

Phe(I)NH of **2** appeared at very low field (7.6 ppm) compared to Phe(I′)NH indicating its participation in an intramolecular hydrogen bond.[11,12] This was confirmed by the temperature coefficient of Phe(I)NH (−9.1 ppb/K) that was larger than those of other amide protons. The solvent-titration also provided further support for intramolecular H-bonding. Addition of DMSO-d_6 in the $CDCl_3$ solution of **2** (Fig. 2) resulted in a very small shift of its Phe(I)NH signal compared to the other amide proton chemical shifts, which exhibited large solvent dependence.

The rOe cross-peaks suggested the existence of a 14-membered intramolecularly hydrogen bonded ring involving Phe(I)NH and Leu(I′)CO, as depicted schematically in Fig. 4.

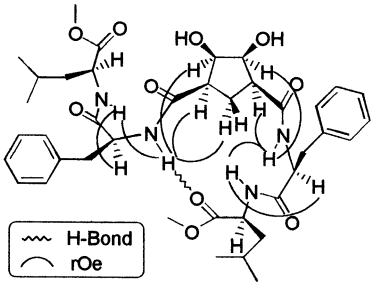

Figure 4. Schematic representation of the 14-membered hydrogen bonded ring structure of **2** with the long range ROEs seen in its NMR spectrum.

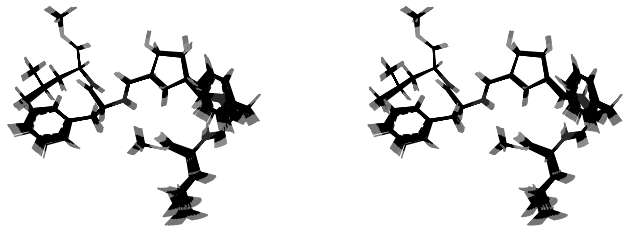

Figure 5. Stereoview of the 20 superimposed energy-minimized structures sampled during 20 ps MD simulations of **2**.

The cross-peak intensities in the ROESY spectra were used for obtaining the restraints in the MD calculations. The two-spin approximation was used to obtain the intermolecular distances. The lower and upper bounds of these distance restraints were fixed as 15% less and 15% more, respectively, than the derived distances. Several long-range distance constraints, torsional restraints and an H-bonding restraint were used in the energy calculations. The results derived from the 20 ps dynamics run for compound **2** are shown in Fig. 5. The structures are consistent with NMR restraints and have an H-bond between Phe(I)NH and the Leu(I′)-carbonyl. The cyclopentane ring showed a C2-*endo* structure, which is in agreement with the NMR data. Interestingly, the dimethyl ester of **6** also showed such a cyclopentane ring geometry.

2.5. Conformational analysis of 3

The solution of structure of **3** as determined by NMR studies was found to be very similar to that of **2**. The ^{1}H NMR data of **3** is shown in Table 2. Its Phe(I)NH also showed a downfield chemical shift (8.06 ppm) that had a very large temperature coefficient (−14.1 ppb/K) and was less solvent-dependent compared to the other amide proton chemical shifts as can be seen from its solvent titration plots (Fig. 3). The structure of **3** as determined from the various coupling constants, ROESY cross-peaks and subsequent MD calculations was found to be very similar to that of **2**, involving an intramolecular H-bond between Phe(I)NH and Gly(I′)-carbonyl. In fact, the Phe(I)NH in **3** had a more downfield chemical shift, a much larger temperature coefficient and also lesser solvent dependence compared to those exhibited by the Phe(I)NH in **2**, suggesting a stronger H-bonded ring structure in the former.

The interstrand H-bonded turn structure observed here for these cabocycle-based peptidomimetics **2** and **3** is quite different from the structure observed in the corresponding sugar analogue Idac-(Phe–Leu–OMe)$_2$ (**1**) that displayed a very symmetrical structure (**A**) consisting of identical intramolecular H-bonds at two ends between LeuNH⟶sugar–OH and had a different sugar pucker consisting of a twist conformation.[4] A close look at the structures obtained from the MD simulations of Idac-(Phe–Leu–OMe)$_2$ showed that in this molecule the distance between the Phe amide-proton and the sugar ring oxygen is ∼2.2Å and the resulting electrostatic interaction was possibly responsible for the

induction of the observed pseudo β-turn in the molecule.[4a] Replacement of the ring oxygen with carbon resulted in the loss of similar electrostatic interactions in **2** and **3** forcing the peptide chains to adopt different orientations. However, the '1,3-*cis*' relationship between the peptide chains anchored on the carbocyclic ring in these molecules could still bring them into close proximity that probably induced the observed interstrand H-bond. These three factors, namely, the '1,3-*cis*' relationship of the peptide chains, the absence of the oxygen atom in the core five-membered ring and a different puckering of the cyclopentane ring probably worked synergistically to instill this unique structure in these molecules which is distinctly different from their sugar-based counterparts.

The rigid scaffold of the cyclopentane ring described here can serve as a novel template to design interesting peptidomimetics that may lead to the development of de novo molecular entities with well-defined three dimensional structures and useful properties.

3. Experimental

3.1. 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. Melting points are uncorrected.

IR spectra were recorded as neat liquids or KBr pellets. Mass spectra were obtained under electron impact (EI) and liquid secondary ion mass spectrometric (LSIMS) techniques, respectively. For LSIMS *m*-nitrobenzyl alcohol was used as a matrix.

The CD spectra were recorded in quartz cell of 1 mm path length at 25°C using a peptide concentration of 0.7 mM in TFE.

3.2. NMR spectroscopy

NMR spectra were recorded on 200 (21°C) and 500 MHz (30°C) spectrometers with 7–10 mM solutions in appropriate solvents using tetramethylsilane as internal standard or the solvent signals as secondary standards and the chemical shifts are shown in δ scales. ^{13}C NMR spectra were recorded with complete proton decoupling. The assignments were carried out with the help of two-dimensional total correlation spectroscopy (TOCSY).[13] For some cases the rotating frame nuclear Overhauser effect spectroscopy (ROESY) experiments,[13] which provide the information on the proximity of protons, were additionally used to confirm the assignments made. All the experiments were carried out in the phase sensitive mode using the procedure of States et al.[14] The spectra were acquired with 2×256 or 2×192 free induction decays (FID) containing 8–16 transients with relaxation delays of 1.5–2.0 s. The ROESY experiments were performed with mixing time of 0.3 s. For ROESY experiments a spin-locking field of about 2 kHz and pulsed field locking with 30° pulses were used. The TOCSY experiments were performed with the spin locking fields of about 10 kHz and a mixing time of 0.08 s. The two-dimensional data were processed with gaussian apodization in both the dimensions. The chemical shifts, coupling constants and temperature coefficients ($\Delta\delta/\Delta T$) of amide proton chemical shifts of **2** and **3** are given in Tables 1 and 2, respectively. To obtain the temperature coefficients of NH-chemical shifts, the spectra were recorded between 30 and 50°C.

3.3. Molecular dynamics

All molecular mechanics/dynamics calculations were carried out using Sybyl 6.7 program on a Silicon Graphics O2 workstation. The Tripos force field with default parameters was used throughout the simulations. Minimizations were done first with steepest decent, followed by conjugate gradient methods for a maximum of 2000 iterations each or RMS deviation of 0.05 kcal mol^{-1}, whichever was earlier. The energy-minimized structures were then subjected to MD simulations. A number of inter-atomic distance and torsional angle constraints[15] obtained from NMR data were used as restraints in the minimization as well as MD runs. For MD runs a temperature of 300 K was used. The energy-minimized molecules were initially equilibrated for 1 ps and subsequently subjected to a 20 ps dynamics with a step size of 1 fs, sampling the trajectory at equal intervals of 1 ps. The samples were minimized using the above mentioned energy minimization protocol. The twenty energy-minimized structures were compared and superimposed as shown in Fig. 4.

3.3.1. The acetonide 5. To a solution of **4** (1.5 g, 11.9 mmol) in dry CH_2Cl_2 (5 mL) was added 2,2-dimethoxypropane (2.92 mL, 23.8 mmol) followed by the addition of camphorsulphonic acid (28 mg, 0.12 mmol) at 0°C. The reaction mixture was stirred at room temperature for 4 h. It was then diluted with $CHCl_3$, washed with saturated $NaHCO_3$ and brine, dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by column chromatography (SiO_2, 5% EtOAc in petroleum ether eluant) afforded **5** (1.877 g, 95%) as a syrupy liquid. R_f=0.5 (silica gel, 25% EtOAc in petroleum ether); IR (neat): ν_{max} 3425, 3000, 2950, 1500, 1050 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 6.1 (s, 2H, olefinic*H*), 4.65 (s, 2H, C*H*–O), 2.93 (s, 2H, tertiary C–*H*), 1.65 and 1.43 (two d, *J*=7.5 Hz, 2H, CH_2), 1.3 and 1.2 (two s, 6H, acetonide methyls); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 134.03, 114.5, 81.27, 46.21, 45.58, 25.68, 25.22; MS (EI): *m/z* (%): 151 (10) [M^+-CH_3]; HRMS (EI): calcd for $C_9H_{11}O_2$ [M^+-CH_3]: 151.0759, found: 151.0758.

3.3.2. The diacid 6. To a solution of **5** (1.5 g, 9.036 mmol) in $CCl_4/CH_3CN/H_2O$ (1:1:1.5, 28 mL) was added $NaIO_4$ (8.697 g, 40.66 mmol) at 0°C. To this biphasic solution, $RuCl_3\cdot 3H_2O$ (52.3 mg, 0.2 mmol) was added at the same

temperature and the reaction mixture was stirred vigorously for 12 h at room temperature. It was then diluted with acetone (100 mL), filtered through a short pad of Celite® and the filter cake was washed with acetone. The filtrate and washings were combined and concentrated in vacuo. The residue was again dissolved in acetone (30 mL), filtered and the filtrate was concentrated in vacuo to get the diacid **6** (1.76 g, 85%) as a white solid. R_f=0.5 (silica gel, EtOAc); mp 238–240°C; IR (neat): ν_{max} 3450, 3275, 2975, 2940, 2890, 1375, 1040 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ 12.06 (br s, 2H, COO*H*), 4.72 (m, 2H, C*H*–O), 2.70 (m, 2H, C*H*CO_2H), 2.08 (dt, J=13.1, 12.6 Hz, 1 H, methyleneC*H*), 1.64 (dt, J=13.1, 6.1 Hz, 1H, methyleneC*H′*), 1.26 and 1.19 (two s, 6H, acetonide methyls); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 171.60, 109.38, 80.17, 46.98, 25.62, 25.57, 24.11; MS (LSIMS): *m/z* (%): 231 (42) [M^++H]; HRMS (LSIMS): calcd for $C_{10}H_{15}O_6$ [M^++H]: 231.0868, found: 231.0867.

3.3.3. The acetonide protected peptidomimetic 7. To a solution of BocNH–Phe–Leu–OMe (1.078 g, 2.75 mmol) in dry CH_2Cl_2 (4 mL) was added trifluoroacetic acid (2 mL) at 0°C and stirred under nitrogen for 1 h. The reaction mixture was then concentrated in vacuo. In another round-bottom flask, the diacid **6** (0.287 g, 1.25 mmol) dissolved in dry DMF (2 mL) and dry CH_2Cl_2 (4 mL) was sequentially treated with HOBt (0.337 g, 2.5 mmol) and EDCI (0.479 g, 2.5 mmol) at 0°C. After 15 min, TFA·H_2N–Phe–Leu–OMe prepared above and dissolved in dry DMF (2 mL) was added to the reaction mixture followed by the addition of DIPEA (0.97 mL, 5.5 mmol). After stirring for 12 h at room temperature, the reaction mixture was diluted with EtOAc, washed with saturated NH_4Cl and brine, dried (Na_2SO_4) and concentrated in vacuo. Purification by column chromatography (SiO_2, 2.5% MeOH in $CHCl_3$ eluant) afforded **7** (0.78 g, 80%) as a white solid. R_f=0.43 (silica, $CHCl_3$/MeOH/AcOH, 80:8:2); $[\alpha]_D^{22}$=−33.9 (*c* 0.32, $CHCl_3$); mp 266–268°C; IR (neat): ν_{max} 3425, 3275, 1720, 1635 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 7.31–7.20 (10H, Ar*H*), 6.58 and 6.57 (two d, J=7.9 and 7.7 Hz, 2H, PheN*H*), 6.34 and 6.32 (two d, J=8.4 and 8.2 Hz, 2H, LeuN*H*), 4.74 (m, 2H, C4–*H* and C5–*H*), 4.71 (m, 2H, Pheα*H*), 4.55 (m, 2H, Leuα*H*), 3.70 and 3.69 (two s, 6H, CO_2CH_3), 3.21 and 3.17 (two dd, J=14.0, 6.4 Hz, 2H, Pheβ*H*), 3.07 and 3.06 (two dd, J=14, 6.4 Hz, 2H, Pheβ*H′*), 2.49 (m, 2H, carbocyclic C1–*H* and C3–*H*), 2.09 (dt, J=13.1, 6.1 Hz, 1H, C2–*H*), 2.01 (dt, J=13.1, 12.6 Hz, 1H, C2–*H′*), 1.62–1.40 (m, 6H, Leuβ*H* and Leuγ*H*), 1.27 and 1.25 (two s, 6H, acetonide methyls), 0.90 and 0.89 (four d, J=6.6 Hz, 12H, Leu methyls); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 172.75, 170.37, 169.62, 169.48, 136.58, 136.40, 129.46, 129.42, 128.68, 128.60, 127.08, 126.99, 111.38, 79.88, 79.79, 54.10, 54.00, 52.23, 50.88, 50.81, 49.27, 49.10, 41.54, 41.37, 37.51, 28.94, 25.33, 24.75, 24.68, 23.89, 22.72, 21.94; MS (LSIMS): *m/z* (%): 779 (100) [M^++H], 801 (72) [M^++Na]; HRMS (LSIMS): calcd for $C_{42}H_{59}N_4O_{10}$ [M^++H]: 779.4231, found: 779.4225.

3.3.4. Peptidomimetic 2. To a solution of **7** (0.399 g, 0.5 mmol) in MeOH (4 mL) was added conc. HCl (4 mL) at 0°C. The reaction mixture was stirred at room temperature for 3 h. It was then quenched with saturated $NaHCO_3$ solution and extracted with 10% MeOH in EtOAc (2×50 mL). The organic extracts were combined, washed with brine, dried (Na_2SO_4) and concentrated in vacuo. Purification by column chromatography (SiO_2, 5% MeOH in $CHCl_3$ eluant) afforded **2** (0.202 g, 55%) as a white solid. R_f=0.41 (silica, $CHCl_3$/MeOH/AcOH, 80:8:2); $[\alpha]_D^{22}$=−36.3 (*c* 0.31, $CHCl_3$); mp 193–194°C; IR (neat): ν_{max} 3300, 2950, 1735, 1650, 1530 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz): δ 173.40, 173.27, 172.85, 170.77, 170.25, 136.80, 136.28, 129.42, 129.35, 128.81, 128.56, 127.25, 126.89, 74.75, 74.50, 55.03, 54.40, 52.37, 52.26, 50.89, 50.73, 47.59, 46.83, 41.37, 41.28, 37.80, 37.46, 28.34, 24.70, 22.75, 22.71, 21.86, 21.79; MS (LSIMS): *m/z* (%): 739 (24) [M^++H], 761 (16) [M^++Na]; HRMS (LSIMS): calcd for $C_{39}H_{55}N_4O_{11}$ [M^++H]: 739.3918, found: 739.3917.

3.3.5. Peptidomimetic 3. This compound was synthesized following the same method as described above for the synthesis of **2**. R_f=0.31 (silica, $CHCl_3$/MeOH/AcOH, 80:8:2); $[\alpha]_D^{22}$=−21.3 (*c* 0.11, MeOH); mp 206–208°C; IR (neat): ν_{max} 3300, 2925, 1735, 1650, 1525 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): see Table 2; ^{13}C NMR (DMSO-d_6, 125 MHz): δ 172.76, 172.70, 171.48, 169.98, 138.00, 137.59, 129.15, 128.96, 128.00, 127.96, 126.15, 74.34, 74.11, 53.84, 53.79, 51.64, 51.61, 46.72, 45.88, 40.64, 40.57, 37.33, 36.93, 27.05; MS (LSIMS): *m/z* (%): 649 (58) [M^++Na]; HRMS (LSIMS): calcd for $C_{31}H_{39}N_4O_{10}$ [M^++H]: 627.2666, found: 627.2660.

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