Studies on Novel Peptidomimetics Having Bi-directional Dispositions of Hydroxylated D-Pro-Gly Motifs Anchored on a C_2-Symmetric Iminosugar-Based Foundation

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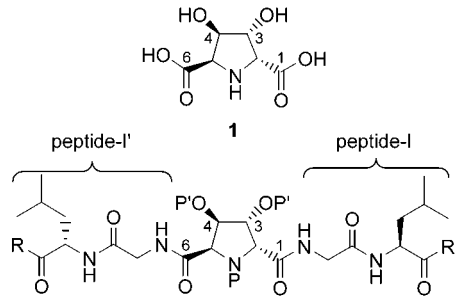
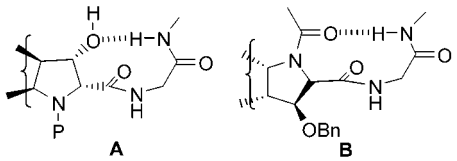
A rigid pyrrolidine based scaffold comprising of 2,5-dideoxy-2,5-imino-D-idaric acid (**1**) is developed. Attachment of peptide strands to the carboxylic groups at both ends of this novel template led to the peptidomimetics **2** and **3**. Conformational analysis by NMR studies revealed that compounds **2b**, **3b** and **2c**, **3c** take interesting turn structures (C_2 symmetric for **2c** and **3c**) in DMSO-d_6 consisting of identical intramolecular hydrogen bonds at two ends between LeuNH → sugar-OH as depicted in structure **A**, whereas **2a** and **3a** display structures with regular β-turns with hydrogen bonds between LeuNH → Boc-C═O in one-half of their molecular frameworks (structure **B**), characteristic of the turn structures commonly observed in "D-Pro-Gly"-containing peptides. These results suggest that a cis hydroxyl group at the 3-position of the proline residue favors a pseudo β-turn-like nine-membered ring structure in hydroxyproline-containing peptides involving an intramolecular hydrogen bond between the hydroxyl and the $i + 2$ backbone amide.

Introduction

For discovering new peptide-based drugs, many structurally rigid nonpeptidic molecular scaffolds have been designed. Insertion of these moieties in appropriate sites, a common approach to restrict the conformational degrees of freedom in small peptides, produces the specific three-dimensional structures required for binding to their receptors.[1] Many of these templates contain diamine or diacid moieties and have been used extensively to nucleate parallel β-sheet structures in peptides.[2] In this paper, we describe the development of a novel structurally constrained molecular scaffold consisting of 2,5-dideoxy-2,5-imino-D-idaric acid (**1**). Bi-directional elongation of the diacid moieties of **1** with identical peptide strands led to the formation of peptides **2** and **3**. Compound **2** was specifically chosen to provide an additional amide proton in the peptide strands that could participate in an intramolecular H-bonding to stabilize further any possible H-bonded structure commonly seen in various peptidomimetic designs.[1,2]





A closer look at compounds **2** and **3** could identify easily the occurrence of the "D-Pro-Gly" type motifs on both sides of the central pyrrolidine dicarboxylic acid framework. It is well established that the type II′ β-turn conformation adopted by a D-Pro-Xxx segment, with a φ value of ca. +60° for the pyrrolidine ring, promotes nucleation of β-hairpin structures in proteins.[3] While induction of proline causes a reversal in the backbone trajectory in peptides, polyproline-based peptides exhibit helical structures as often seen, for example, in collagenous peptides[4] that have repeating units of Gly-X-Y composed predominantly of proline (X) and hydroxypro-

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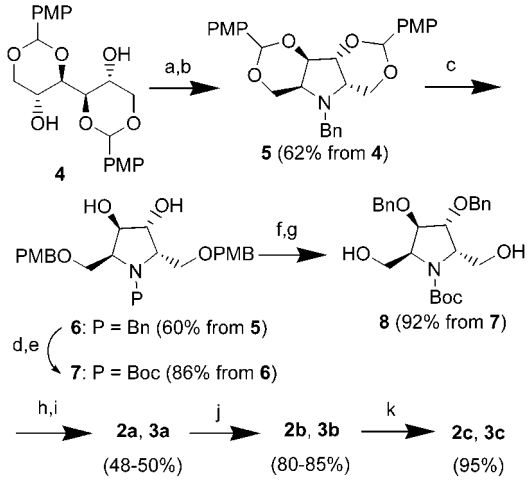
Scheme 1. Synthesis of the Core Building Block 1 and the Peptidomimetics 2 and 3[a]



[a] Reagents and conditions: (a) MsCl, Et_3N, CH_2Cl_2−DMF, 0 °C, 0.5 h; (b) $BnNH_2$, 130 °C, 6 h; (c) LAH−$AlCl_3$ (3:2), CH_2Cl_2−Et_2O, 0 °C to reflux, 2 h; (d) $Pd(OH)_2$−C, MeOH, rt, 4 h; (e) Boc_2O, MeOH; (f) NaH, BnBr, TBAI (cat.), THF, 0 °C to rt, 12 h; (g) DDQ, $CHCl_3$−H_2O, rt, 45 min; (h) PDC, DMF, 0 °C to rt, 24 h; (i) EDCI, HOBt, 0 °C, 0.5 h; then TFA−H_2N−Gly-Leu-COR (R = OMe, or NHMe), DIPEA, DMF−CH_2Cl_2, 0 °C to rt, 12 h; (j) $Pd(OH)_2$−C, MeOH, rt, 6 h; (k) TFA−CH_2Cl_2, 0 °C, 0.5 h.

line (Hyp, Y) residues. The design of the novel molecular framework of **1** with bi-directional dispositions of "hydroxy-D-proline" moieties will enable us to study the conformational bias conferred by it when inserted in peptides. It is also expected to provide an insight into the role of the hydroxyl groups on the structures of hydroxyproline containing peptides.

Results and Discussion

Synthesis of the Core Building Block 1 and the Peptidomimetics 2 and 3. Scheme 1 outlines the synthesis of the core building block **1**, which was finally transformed into the peptides **2** and **3**. Dimesylation of 1,3:4,6-di-*O*-(*p*-methoxy)benzylidene-D-mannitol **4**[5] was followed by the reaction with an excess of benzylamine at 130 °C to get the pyrrolidine compound **5**[6] in 62% yield from **4**. Reductive opening of the *p*-methoxybenzylidene rings using lithium aluminum hydride (LAH) in the presence of anhydrous $AlCl_3$ led to the formation of the diol **6** in 60% yield. Selective removal of the *N*-benzyl group under catalytic hydrogenation conditions was followed by *N*-Boc protection to furnish the intermediate **7** in 86% yield. The secondary hydroxyl groups of **7** were next protected as benzyl ethers, and primary hydroxyl groups were deprotected using DDQ to give **8** in 92% yield from **7**. Oxidation of the primary hydroxyls of **8** using an excess of pyridinium dichromate (PDC) in DMF gave the required diacid. A small amount of the crude diacid was dissolved in Et_2O and esterified by adding an excess of ethereal solution of CH_2N_2 to give the corresponding diester that was purified for characterization purposes.[7] The remaining diacid, after aqueous workup, was directly subjected to the peptide-coupling reaction following the standard solution-phase conditions using 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBt) as coupling agents and dry DMF and CH_2Cl_2 as solvents. The resulting compound **2a** (and **3a**) was subjected to catalytic hydrogenation to give **2b** (and **3b**). Finally, Boc-deprotection furnished the fully deprotected compound **2c** (and **3c**).

Conformational Analysis. NMR Studies. NMR studies of **2** and **3** were carried out in DMSO-d_6, except in case of **3a**, which was studied in $CDCl_3$. For others (**2a**−**c** and **3b**,**c**), NMR studies in $CDCl_3$ could not be carried out either due to poor solubility or exchange broadening of the spectral lines. Some of the experiments in apolar solvents were performed in a mixture of $CDCl_3$ and DMSO-d_6 (95:5, v/v). The NMR spectra of the peptides described here are quite well resolved, and most of the spectral parameters could be obtained easily and are reported in Tables 1−3 for compounds **2a**, **2b**, and **2c**, respectively. For others (**3a**−**c**), the spectral details are provided in the Experimental Section. While the assignments were carried out with the help of two-dimensional double-quantum-filtered correlation spectroscopy (DQF COSY) and total correlation spectroscopy (TOCSY), rotating frame nuclear Overhauser effect spectroscopy (ROESY) experiments provided the information on the proximity of protons, the details of which are provided in the Experimental Section.

The presence of the Boc group attached to the imine of the central pyrrolidine ring caused differences in the behavior of the left and right side peptide chains in **2a**,**b** and **3a**,**b**. For convenience, the right-side peptide chain is designated as peptide-I, while the left one is termed as peptide-I′.

Usually, small peptides exist in solution in several conformations and the NMR parameters show the weighted average of all such structures. However, insertion of the conformationally constrained scaffolds in the designs can give rise to organized structures. Variable-temperature studies allow us to examine such structures

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Table 1. ^{1}H Chemical Shifts (δ in ppm), Coupling Constants (J in Hz), and Temperature Coefficients of the Amide Proton Chemical Shifts ($\Delta\delta/\Delta T$ in ppb/K) of 2a (500 MHz, DMSO-d_6)

protons	Gly(I)	Leu(I)	Gly(I′)	Leu(I′)
NH	8.65 (t, $J_{NH-\alpha H} = 5.5$)	7.42 (d, $J_{NH-\alpha H} = 8.3$)	8.48 (dd, $J_{NH-\alpha'H} = 5.3$, $J_{NH-\alpha H} = 6.0$)	7.86 (d, $J_{NH-\alpha H} = 8.5$)
CαH	3.72 (dd, $J_{\alpha-\alpha'} = 17.8$)	4.22 (dt, $J_{\alpha-\beta} = 7.4$, $J_{\alpha-\beta''} = 7.4$)	3.82 (dd, $J_{\alpha-\alpha'} = 16.6$, $J_{NH-\alpha H} = 6.0$)	4.27 (ddd, $J_{\alpha-\beta} = 5.4$, $J_{\alpha-\beta'} = 10.0$)
Cα′H	3.70 (dd)		3.69 (dd, $J_{NH-\alpha'H} = 5.3$)	
Cβ}H Cγ}H		1.45−1.57 (m)	1.39−1.54 (m)	
CδH		0.85 (d, $J_{\gamma-\delta} = 6.7$)		0.84 (d, $J_{\gamma-\delta} = 6.3$)
Cδ′H		0.81 (d, $J_{\gamma-\delta} = 6.3$)		0.81 (d,$J_{\gamma-\delta} = 6.3$)
-$\Delta\delta/\Delta T$	5.0	3.8	6.4	6.4

others: 7.61 (q, $J = 4.7$ Hz, N*H*-Me(I)), 2.51 (d, NH-*Me*(I)), 7.85 (q, $J = 4.7$ Hz, N*H*-Me(I′)), 2.54 (d, NH-*Me*(I′)); $-\Delta\delta/\Delta T$ (in ppb/K) = 7.9 for NHMe(I), 6.7 for NHMe(I′)

pyrrolidine ring:[a] 7.25−7.20 (m, 10 H, aromatic), 4.83 and 4.53 (m, 4 H, O−C*H*$_2$Ph), 4.64 (d, $J_{C2H-C3H} = 7.8$ Hz, C2-*H*), 4.67 (d, $J_{C5H-C4H} = 7.5$ Hz, C5-*H*), 4.31 (m, $J_{C3H-C4H} = 7.8$ Hz, C3-*H*), 4.32 (m, C4-*H*), 1.30 (s, Boc)

[a] Couplings obtained by simulating the spectrum with the VNMR software.

Table 2. ^{1}H Chemical Shifts (δ in ppm), Coupling Constants (J in Hz), and Temperature Coefficients of the Amide Proton Chemical Shifts ($\Delta\delta/\Delta T$ in Ppb/K) of 2b (500 MHz, DMSO-d_6)

protons	Gly(I)	Leu(I)	Gly(I′)	Leu(I′)
NH	8.42 (t, $J_{NH-\alpha H} = 5.9$)	7.62 (d, $J_{NH-\alpha H} = 8.8$)	8.32 (dd, $J_{NH-\alpha H} = 5.7$, $J_{NH-\alpha'H} = 6.3$)	7.78 (d, $J_{NH-\alpha H} = 8.8$)
CαH	3.80 (dd, $J_{\alpha-\alpha'} = 16.9$)	4.22 (ddd, $J_{\alpha-\beta} = 4.4$, $J_{\alpha-\beta'} = 10.3$)	3.58 (dd, $J_{\alpha-\alpha'} = 16.9$, $J_{NH-\alpha H} = 5.7$)	4.25 (ddd, $J_{\alpha-\beta} = 4.9$, $J_{\alpha-\beta'} = 10.4$)
Cα′H	3.56 (dd)		3.82 (dd, $J_{NH-\alpha'H} = 6.3$)	
CβH		1.44−1.54 (m)		1.43−1.53 (m)
CγH				
CδH		0.85 (d, $J_{\gamma-\delta} = 6.4$)		0.85 (d, $J_{\gamma-\delta} = 6.4$)
Cδ′H		0.80 (d, $J_{\gamma-\delta'} = 6.4$)	0.80 (d, $J_{\gamma-\delta'} = 6.4$)	
$-\Delta\delta/\Delta T$	5.5	3.4	6.4	3.5

others: 7.72 (q, $J = 4.5$ Hz, N*H*-Me(I)), 2.55 (d, NH-*Me*(I)), 7.58 (q, $J = 4.5$ Hz, N*H*-Me(I′)), 2.54 (d, NH-*Me*(I′)), $-\Delta\delta/\Delta T$ (in ppb/K) = 6.6 for both NHMe(I) and NHMe(I′)

pyrrolidine ring:[a] 5.75 (d, $J_{OH-C3H} = 4.8$ Hz, C3-O*H*), 5.83 (d, $J_{OH-C4H} = 4.8$ Hz, C4−O*H*), 4.33 (d, $J_{C2H-C3H} = 8.1$ Hz, C2-*H*), 4.19 (m, $J_{C3H-C4H} = 8.1$ Hz, C3-*H*), 4.31(d, $J_{C4H-C5H} = 7.8$ Hz, C5-*H*), 4.20 (m, C4-*H*), 1.27 (s, Boc)

[a] Couplings obtained by simulating the spectrum with the VNMR software.

Table 3. ^{1}H Chemical Shifts (δ in ppm), Coupling Constants (J in Hz), and Temperature Coefficients of the Amide Proton Chemical Shifts ($\Delta\delta/\Delta T$ in ppb/K) of 2c (500 MHz, DMSO-d_6)

protons	Gly	Leu
NH	8.35 (dd,$J_{NH-\alpha H} = 6.2$, $J_{NH-\alpha'H} = 5.6$)	7.79 (d, $J_{NH-\alpha H} = 8.5$)
CαH	3.79 (dd, $J_{\alpha-\alpha'} = 16.7$, $J_{NH-\alpha H} = 6.2$)	4.19 (ddd, $J_{\alpha-\beta(pro-S)} = 4.9$, $J_{\alpha-\beta(pro-R)} = 9.9$)
Cα′H	3.67 (dd, $J_{NH-\alpha'H} = 5.6$)	
Cβ*H*(*pro-R*)		1.39 (ddd, $J_{\gamma-\beta(pro-R)} = 5.2$, $J_{\beta(pro-R)-\beta(pro-S)} = 12.9$)[a]
Cβ*H*(*pro-S*)		1.45 (ddd, $J_{\gamma-\beta(pro-S)} = 8.2$, $J_{\beta(pro-S)-\beta(pro-R)} = 12.9$)[a]
Cγ*H*		1.52 (m)
Cδ*H*(*pro-S*)		0.80 (d, $J_{\gamma-\delta(pro-S)} = 6.3$)
Cδ*H*(*pro-R*)		0.85 (d, $J_{\gamma-\delta(pro-R)} = 6.5$)
$-\Delta\delta/\Delta T$	3.9	3.9

others: 7.71 (q, $J = 4.6$ Hz, N*H*-Me), 2.55 (d, NH-*Me*); $-\Delta\delta/\Delta T = 5.0$ (NHMe)

pyrrolidine ring:[a] 3.18 (bs, N*H*), 3.96 (bs, 2 H, C2(C5)-*H*), 4.18 (t, $J = 3.4$ Hz, 2 H, C3(C4)-*H*), 5.35 (d, $J = 3.5$ Hz, 2 H, C3(C4)-O*H*).

[a] Couplings obtained by simulating the spectrum with the VNMR software.

in detail where the temperature coefficients of amide proton chemical shifts ($\Delta\delta/\Delta$T) are used to measure their involvement in intramolecular hydrogen bonds. While the LeuNH protons showed medium values of the temperature coefficients in both **2** and **3**, all other amide protons had larger values of $\Delta\delta/\Delta$T as shown in Tables 1−3 and in the Experimental Section. 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.[8] The addition of strongly hydrogen-bonding solvents such as DMSO-d_6 to a$CDCl_3$ 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 cross-peak intensities in the ROESY spectra of **2a** and **2b**, as shown schematically in Figure 1, were used for obtaining the restraints in the MD calculations. The

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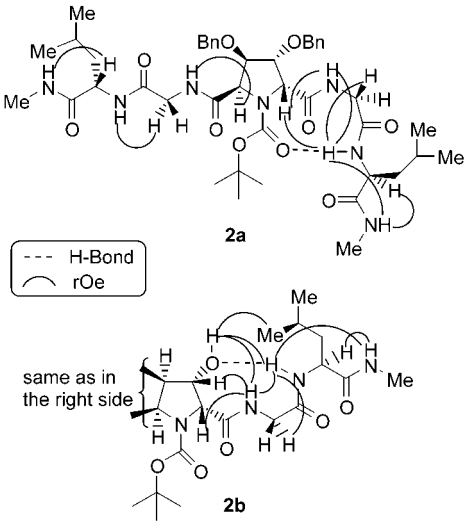

Figure 1. Schematic representation of the hydrogen-bonded ring structures of **2a** and **2b** with the long-range ROEs seen in their ROESY spectra.

two-spin approximation was used to obtain the intermolecular distances.[9] The upper and lower bounds of these distance restraints were fixed at ±15% of the derived distances. Several long-range (more than four bonds) distance constraints and torsional restraints (all ω dihedrals and the H−C3−C4−H torsion) were used in the energy calculations and MD studies. The results derived from the 100 ps MD runs for compound **2a** and **2b** are shown in Figures 2 and 3, respectively. The MD structures of **2c** were very similar to those of **2b**.

Conformational Analysis of 2a and 3a. Detail NMR studies of compounds **2a** (in DMSO-d_6) and **3a** (in $CDCl_3$), having their hydroxyl groups protected as benzyl ethers, revealed that they behave like true "D-Pro-Gly"-containing peptides,[3] which nucleate type II′ β-turns. The medium value of $\Delta\delta/\Delta T$ for Leu(I)NH in **2a** (−3.8 ppb/K) and its downfield shift in **3a** that changed very little in solvent titration studies ($\Delta\delta$ = 0.3 ppm, 0−30% v/v of DMSO-d_6 in $CDCl_3$) indicate the propensity of Leu(I)NH → BocC═O hydrogen bond in these molecules. In addition, the Leu(I)NH-Gly(I)NH and Leu(I)NH-pyrrolidineC2−H ROE cross-peaks, as shown in Figure 1, confirm the presence of such turn structures. The other peptide chain, peptide-I′, consisting of Leu(I′) and Gly(I′) residues, does not show any well-defined structure in both these molecules. The couplings between the protons in the pyrrolidine ring are consistent with a twist conformation (^3_4T, where 3 and 4 refer to C3 and C4, respectively) for both **2a** and **3a**.[10] The Leu(I′) side chain takes a single predominant conformation about the Cα−Cβ bond.[11] As against this, Leu(I) shows the presence of substantial populations of more than one isomer about Cα-Cβ.

Several long-range restraints derived from the ROESY cross-peaks of **2a** in DMSO-d_6 were used in the MD calculations. The superimposed display of the 10 structures out of 20 samples collected at regular intervals of 5 ps during a 100 ps MD run, subsequently energy-minimized and superimposed aligning the hydrogen bonded parts clearly reveal type II′ β-turns in one-half of the molecule while the other side peptide strand displays random structures (Figure 2). The twist conformation of the pyrrolidine ring is clearly brought out by these studies.

The solution structure of **2a** was also investigated in $CDCl_3$ containing 5% DMSO-d_6. Solvent titration again showed participation of Leu(I)NH in intramolecular hydrogen bonding as indicated by small change in its chemical shift by adding up to 30% v/v of DMSO-d_6.

Conformational Analysis of 2b and 3b. The structures of **2b** and **3b** in DMSO-d_6 were found to be very similar to that of a sugar diacid, 2,5-dideoxy-D-idaric acid, containing peptidomimetic, which had bi-directionally elongated Phe-Leu residues.[12] They take an unusual structure with a pseudo-β-turn, consisting of a LeuNH → pyrrolidineC3−OH hydrogen bond, reminiscent of the main-chain NH → side-chain OH type H-bond seen sometimes in serine- and threonine-containing peptides.[13] However, the magnitudes of the temperature coefficients of the amide proton chemical shifts ($\Delta\delta/\Delta T$) for LeuNH (−3.4 and −3.5 ppb/K for **2b**; −3.4 and −3.3 ppb/K for **3b**) imply that these hydrogen bonds are only of medium strength. The turn structure is further supported by the proximity of GlyNH, LeuNH, and LeuδH to pyrrolidine-OH protons on either side of the ring as shown in the ROESY spectra. For the Leu side chains in **2b** on both sides, the couplings between α and β protons correspond to about 75% predominance of a single conformation about Leu χ_1.[14] Very similar populations for the rotamers were obtained about the Leu χ_1 of **3b** also. The sugar ring conformation for both **2b** and **3b** are similar to those observed in **2a** and **3a**. A twist conformation for the pyrrolidine ring is deduced from the couplings.

The structures sampled during the restrained MD calculations on the basis of the ROESY cross-peaks found for **2b** in DMSO-d_6 reveal an ensemble of "scorpion"-like structures as shown in Figure 3, where the two peptide chains form cyclic conformations at both ends involving hydrogen bonds between LeuNH and pyrrolidine-OH. The central portions of these structures are fairly conserved with the variations localized mainly at the Leu side-chains. Like in **2a**, here also the pyrrolidine ring structures take twist conformations that are in agreement with the NMR data.

The structures of **2b** and **3b** could not be obtained in pure $CDCl_3$ due to their poor solubility. However, addition of 5% DMSO-d_6 rendered clear solutions. Solvent titrations performed on these solutions by adding sequentially increased amounts of DMSO-d_6 (upto 30% v/v) showed that the chemical shifts of LeuNH change by small amounts during the titrations. This indicates that the hydrogen bonding patterns are similar to those noticed in DMSO-d_6 solutions and the structures of **2b** and **3b** are probably similar in both solvents.

Conformational Analysis of 2c and 3c. Compounds **2c** and **3c**, with unprotected hydroxyl and imine groups

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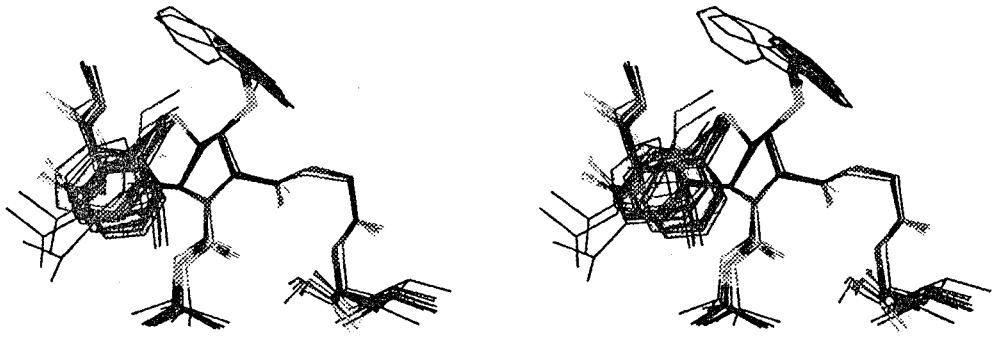

Figure 2. Stereoview of the 10 superimposed structures taken from the 20 samples collected at 5 ps intervals during 100 ps MD simulations of **2a**, subsequently energy-minimized and superimposed aligning the hydrogen-bonded parts, revealing clearly type II′ β-turns in one-half of the molecule with the other side peptide strand displaying random structures.

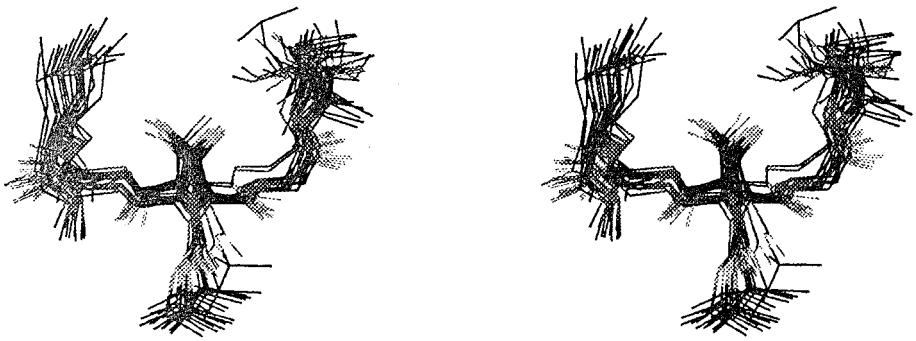

Figure 3. Stereoview of the 20 superimposed structures, sampled at 5 ps intervals during 100 ps MD simulations of **2b**, subsequently energy-minimized and superimposed aligning the hydrogen bonded parts, revealing an ensemble of "scorpion"-like structures where the two peptide chains form cyclic conformations at both ends involving hydrogen bonds between LeuNH and pyrrolidine-OH with fairly conserved structures observed in the central portion of the molecule and the variations localized mainly at the Leu side chains.

show the presence of C_2 symmetries in them. The medium strengths of the LeuNH hydrogen bonds ($\Delta\delta/\Delta T = -3.9$ ppb/K, for both **2c** and **3c**) along with the rOe cross-peaks between pyrrolidine-OH and LeuNH, LeuβH(*pro-R*), LeuγH, LeuδH(*pro-R*) and NHMe (in **2c**) support the presence of nine-membered pseudo β-turn structures similar to those seen in **2b** and **3b**. The temperature coefficients of the GlyNH protons in these molecules were comparable to those of the LeuNH protons ($\Delta\delta/\Delta T = -3.9$ ppb/K in **2c** and -4.2 ppb/K in **3c**) indicating that they may be involved in some GlyNH → pyrrolidine-OH type H-bonds similar to those seen earlier by others in serine, threonine containing peptides.[13] The rOe cross-peaks between GlyNH and pyrrolidine-OH further support these possibilities. The Leu side-chains of these molecules are quite rigid and show the rotamer populations of g^- about χ_1 nearly 71% and 69% for **2c** and **3c**, respectively. The predominance of the g^- rotamers about Cα-Cβ bond is further supported by much stronger rOe cross-peaks between LeuNH-LeuβH(*pro-R*) than LeuNH-LeuβH(*pro-S*) and the LeuβH(*pro-S*)-NHMe cross-peak. The rigidity of Leu side-chain is also observed between Cβ-Cγ (χ2) bond with about 55% and 62% rotamer populations having anti relationships between LeuβH(*pro-S*) and LeuγH for **2c** and **3c**, respectively. The cross-peaks between LeuαH-LeuδH(*pro-S*) and LeuNH-LeuγH provide additional evidences of the rigidity of the Leu side chains.

The small values of the coupling constants of the pyrrolidine ring protons in **2c** and **3c** imply change in the ring puckering compared to **2a**, **3a** and **2b**, **3b**. The $J_{H2-H3} = J_{H4-H5} = 3.4$ Hz and $J_{H3-H4} \approx 0$ Hz 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 C4 and C3 both being exo to the peptide chains I and I′, respectively.[10]

Discussion

The ROESY spectra of **2b** and **3b** showed exchange peaks between the resonances for two peptide chains as a result of the cis−trans isomerism involving amide bond containing the Boc group. For **2a** and **3a**, the H-bonded structures resulted in these molecules locking into one predominant conformer. However, the presence of other minor conformers contributed to some exchange peaks between the peptide chains in these molecules as well. The possibility of two isomers did not exist for Boc-deprotected compounds **2c** and **3c**, both of which showed C_2 symmetric structures. The bulky benzyl protective groups on the hydroxyls of the pyrrolidine rings prevented the nucleation of the LeuNH → pyrrolidineC3−OBn hydrogen bonds in compounds **2a** and **3a**, which were seen in free hydroxyl containing peptides **2b** and **3b**. Such an observation was also made earlier on benzyl-protected sugar diacid containing peptide,[12] and this steric crowding forced the peptide chains in **2a** and **3a** to fold in opposite direction and adopt regular type II′ β-turns involving 10-membered hydrogen bonded structures between Leu(I)NH and the carbonyl of *N*-Boc group. It is remarkable that protection−deprotection of the hydroxyl groups can make these peptidomimetic molecules switch from one conformation to another that has the potential to lead to many useful applications.

Conclusion

A novel pyrrolidine-based scaffold comprising 2,5-dideoxy-2,5-imino-D-idaric acid (**1**) is developed for the first time. The various functional groups present on this designed scaffold, especially the hydroxyl groups on the pyrrolidine ring, can play important roles in designing new molecular entities. It is noteworthy that the protected/unprotected hydroxyl groups not only control the hydrophobic/hydrophilic nature of such molecular assemblies, they can also influence the three-dimensional structures such assemblies will adopt. We hope that the studies described here will help to understand the structures of hydroxyproline containing peptides. The nonproteinogenic properties of the design will probably make compounds built on this scaffold physiologically more stable as well. This iminosugar based diacid compound is expected to find wide ranging applications in peptidomimetic studies, especially in designing bioactive conformations of small peptides and in creating de novo molecular entities with well-defined structures and useful properties.

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% concd 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 liquid secondary ion mass spectrometric (LSIMS) technique.

NMR Spectroscopy. NMR spectra were recorded on 500 MHz spectrometers at 30 °C 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. 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. The assignments were carried out with the help of two-dimensional total correlation spectroscopy (TOCSY).[15] For some cases, the rotating frame nuclear Overhauser effect spectroscopy (ROESY) experiments,[15] 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.[16] 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 was 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 **2a**, **2b**, and **2c** are given in Tables 1, 2, and 3, respectively. For compounds **3a**−**c**, these data are provided in the Experimental Section. To obtain the temperature coefficients of NH-chemical shifts, the spectra were recorded between 30 and 70 °C.

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. A dielectric constant of 47 D was used in all minimizations as well as MD runs. A number of interatomic distances, all the ω dihedrals, all of which were fixed as trans, and the H−C3−C4−H torsional angle as 150 ± 20° were used as restraints in the minimization as well as during the MD runs.[17] Force constants of 15 kcal/Å and 5 kcal/rad were employed for ROE cross-peak-derived distance and dihedral angle constraints, respectively. An additional H-bond distance constraint of 2.2 Å with a force constant of 30 kcal/Å was initially used to derive the starting energy-minimized structures of these molecules. Minimizations were done first with steepest decent, followed by conjugate gradient methods for a maximum of 2000 iterations each or root mean square (RMS) deviation of 0.05 kcal/mol, whichever was earlier. The energy-minimized structures were then subjected to MD simulations. All the above-mentioned restraints, excluding the H-bond restraint, were employed during the MD simulations. The energy-minimized structures were initially equilibrated for 10 ps and subsequently subjected to molecular dynamics at 300 K for 100 ps with a step size of 1 fs, sampling the trajectory at equal intervals of 5 ps. The samples collected were next minimized using the above-mentioned energy minimization protocol with the same set of restraints used during simulations. The energy-minimized structures thus obtained were compared and superimposed as shown in Figures 2 and 3.[18] For the sake of clarity in viewing, only 10 best matching structures are included in Figure 2.

Synthesis of 5. To a solution of **4** (10 g, 23.9 mmol) in dry CH_2Cl_2 (50 mL) and dry DMF (25 mL) was added Et_3N (9.9 mL, 71.7 mmol) at 0 °C. After 5 min, methanesulfonyl chloride (4.07 mL, 52.6 mmol) was added and the mixture stirred for 0.5 h. It was then diluted with $CHCl_3$, washed with saturated NH_4Cl solution, water, and brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. The resulting crude dimesylated product was thoroughly dried under high vacuum, dissolved in dry benzylamine (78 mL, 717 mmol), and heated at 130 °C for 6 h. The excess benzylamine was distilled off under reduced pressure. The resulting crude was diluted with EtOAc, washed with saturated NH_4Cl solution, water, and brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. Purification by column chromatography (SiO_2, 20% EtOAc in petroleum ether eluant) afforded **5** (7.25 g, 62%) as a white solid: $R_f = 0.5$ (silica gel, 30% EtOAc in petroleum ether); $[\alpha]^{20}_D$ 72.9 (*c* 1.48, $CHCl_3$); mp 144−145 °C; IR (KBr) ν_{max} 2846, 1615, 1492, 1276, 1123 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.53 (d, $J = 7.5$ Hz, 2 H, NCH_2-Ar2*H* and 6*H*), 7.44 (d, $J = 8.6$ Hz, 4 H, PMP-*H*), 7.32 (t, $J = 7.5$ Hz, 2 H, NCH_2-Ar3*H* and 5*H*), 7.22 (t, $J = 7.5$ Hz, 1 H, NCH_2-Ar4*H*), 6.92 (d, $J = 8.6$ Hz, 4 H, PMP-*H*), 5.47 (s, 2 H, −OC*H*O− of *p*-methoxybenzylidene), 4.46 (d, $J = 16.1$ Hz, 1 H, NC*H*Ph), 4.38 (d, $J = 2.4$ Hz, 2 H, C2-*H*, C5-*H*), 4.24 (d, $J = 16.1$ Hz, 2 H, C1-*H*, C6-*H*), 4.06 (d, $J = 16.1$ Hz, 1 H, NC*H*′Ph), 3.94 (dd, $J = 16.1$, 2.4 Hz, 2 H, C1-*H*′, C6-*H*′), 3.82 (s, 6 H, OCH_3), 3.49 (bs, 2 H, C3-*H*, C4-*H*); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 160.13, 141.79, 130.97, 128.39, 127.53, 127.31, 126.52, 113.70,113.64, 99.49, 79.40, 66.74, 57.63, 55.26, 51.80; MS (LSIMS) *m/z* 490 (40) $[M^+ + H]$, 512 (8) $[M^+ + Na]$; HRMS (LSIMS) calcd for $C_{29}H_{31}NO_6$ $[M^+]$ 489.2151, found 489.2152.

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(18) MD simulations, using all the specified restraints including those for the H-bond, at 1000 K for 100 ps following the same protocol described in the Experimental Section gave structures that after annealing and minimization appeared similar to those shown in Figures 2 and 3.

Synthesis of 6. To a solution of **5** (7 g, 14.2 mmol) in dry CH_2Cl_2 (140 mL) and dry diethyl ether (140 mL) was added $LiAlH_4$ (6.5 g, 171.5 mmol) portionwise at 0 °C. The mixture was slowly heated to reflux temperature, after which time anhydrous $AlCl_3$ (15.2 g, 114.3 mmol) in dry diethyl ether (140 mL) was added slowly to the hot solution over a 45 min period and reflux was continued for another 1.5 h. The mixture was cooled to 0 °C, and the excess $LiAlH_4$ was decomposed carefully with slow addition of EtOAc (70 mL), followed by the addition of water (210 mL). The solution was decanted from the precipitated solid. To the residual slurry, again EtOAc (200 mL) was added, stirred for 15 min, allowed to settle, and then decanted. From the combined decanted solutions, organic layer was separated, washed with water, brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. The crude was purified by column chromatography (SiO_2, 60% EtOAc in petroleum ether eluant) to afford **6** (4.2 g, 60%) as a syrupy liquid: R_f = 0.5 (silica gel, 80% EtOAc in petroleum ether); $[\alpha]^{20}_D$ 42.9 (*c* 1.0, $CHCl_3$); IR (neat) ν_{max} 3415, 2846, 1600, 1508, 1238 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.27−7.19 (m, 5 H, NCH_2−Ar*H*), 7.22 (d, *J* = 8.6 Hz, 4 H, PMP-*H*), 6.87 (d, *J* = 8.6 Hz, 4 H, PMP-*H*), 4.4 (ABq, 4 H, PMPCH_2O-), 4.29 (m, 2 H, C2-*H*, C5-*H*), 3.80 (s, 8 H, OCH_3, NCH_2Ph), 3.58 (dd, *J* = 10.1, 4.3 Hz, 2 H, C1-*H*, C6-*H*), 3.49 (dd, *J* = 10.1, 2.5 Hz, 2 H, C1-*H′*, C6-*H′*), 3.30 (m, 2 H, C3-*H*, C4-*H*), 2.85 (bs, 2 H, C3−O*H*, C4−O*H*); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 159.28, 129.97, 129.31, 128.16, 128.0, 126.72, 113.83, 78.93, 73.08, 67.41, 61.55, 55.21, 52.68; MS (LSIMS) *m*/*z* 492 (8) [M^+ − H], 516 (2) [M^+ + Na]; HRMS (LSIMS) calcd for $C_{29}H_{36}NO_6$ [M^+ + H] 494.2543, found 494.2544.

Synthesis of 7. To a solution of **6** (4.0 g, 8.1 mmol) in MeOH (30 mL) was added $Pd(OH)_2$ on C (20%, 450 mg). It was hydrogenated under atmospheric pressure using a H_2 balloon. After 4 h, the reaction mixture was filtered through a short pad of Celite, and the filter cake was washed with MeOH. The filtrate and the washings were combined and concentrated in vacuo. The residue was dissolved in dry MeOH (30 mL), and Boc_2O (2.79 mL, 12.15 mmol) was added and stirred overnight at room temperature. The reaction mixture was concentrated in vacuo. Purification by column chromatography (SiO_2, 50% EtOAc in petroleum ether eluant) afforded **7** (3.5 g, 86%): R_f = 0.4 (silica gel, 50% EtOAc in petroleum ether); $[\alpha]^{20}_D$ 37.82 (*c* 1.7, $CHCl_3$); IR (neat) ν_{max} 3408, 2923, 1677, 1500, 1384, 1238 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.19 (d, *J* = 8.7 Hz, 4 H, PMP-*H*), 6.86 (d, *J* = 7.4 Hz, 4 H, PMP-*H*), 4.43 (d, *J* = 11.7 Hz, 1 H, PMPC*H*O), 4.42 (s, 2 H, PMPCH_2O), 4.40 (d, *J* = 11.7 Hz, 1 H, PMPC*H′*O), 4.30 (m, 2 H, C3-*H*, C4-*H*), 4.01 (m, 2 H, C5-*H*, C6-*H*), 3.89 (m, 1 H, C2-*H*), 3.79 (s, 6 H, OCH_3), 3.77 (m, 1 H, C1-*H*), 3.66 (m, 2 H, C1-*H′*, C6-*H′*), 2.75 (d, *J* = 8.2 Hz, 1 H, C4−O*H*), 2.66 (d, *J* = 8.2 Hz, 1 H, C3−O*H*), 1.39 (s, 9 H, Boc); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.33, 159.26, 153.70, 129.90, 129.65, 129.12, 113.92, 113.85, 79.92, 77.32, 73.12, 67.24, 66.37, 57.21, 57.11, 55.19, 28.37; MS (LSIMS) *m*/*z* 504 (28) [M^+ + H], 526 (38) [M^+ + Na]; HRMS (LSIMS) calcd for $C_{27}H_{38}NO_8$ [M^+ + H] 504.2597, found 504.2596.

Synthesis of 8. To a solution of **7** (3 g, 5.9 mmol) in dry THF (18 mL) under nitrogen atmosphere at 0 °C was added portionwise NaH (60% dispersion in oil, 714 mg, 17.8 mmol). After the mixture was stirred for 10 min at the same temperature, BnBr (1.5 mL, 13.1 mmol) followed by TBAI (220 mg, 0.59 mmol) were added, and stirring was continued from 0 °C to room temperature for 12 h. The mixture was then diluted with EtOAc, washed with saturated NH_4Cl solution, water, and brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. Purification by column chromatography (SiO_2, 16% EtOAc in petroleum ether eluant) afforded the Bn-protected intermediate (3.99 g, 98%) that was used directly in the next step without characterization.

To a solution of the above Bn-protected intermediate (3.9 g, 5.7 mmol) in $CHCl_3$ (132 mL) and water (6.6 mL) was added DDQ (3.23 g, 14.2 mmol) at room temperature and the mixture stirred for 45 min. The mixture was then diluted with $CHCl_3$, washed with saturated $NaHCO_3$, water, and brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. Purification by column chromatography (SiO_2, 50% EtOAc in petroleum ether eluant) afforded **8** (2.42 g, 96%): R_f = 0.3 (silica gel, 30% EtOAc in petroleum ether); $[\alpha]^{20}_D$ −11.9 (*c* 1.7, $CHCl_3$); IR (neat) ν_{max} 3438, 2915, 1684, 1392, 1100 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.37−7.30 (m, 10 H, Ar*H*), 4.76 and 4.66 (ABq, 2 H, PhCH_2O), 4.67 (s, 2 H, PhCH_2O), 4.34 (m, 2 H, C3-*H*, C4-*H*), 4.11 (m, 1 H, C5-*H*), 4.01 (m, 1 H, C6-*H*), 3.95 (m, 1 H, C2-*H*), 3.84 (m, 1 H, C1-*H*), 3.78 (m, 1 H, C1-*H′*), 3.69 (m, 1 H, C6-*H′*), 3.26 (m, 1 H, C6-O*H*), 2.29 (t, *J* = 7.2 Hz, 1 H, C1-O*H*), 1.47 (s, 9 H, Boc); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 154.98, 137.36, 128.56, 128.06, 127.77, 127.68, 81.95, 81.32, 80.85, 73.49, 73.42, 61.92, 61.20, 58.63, 57.47, 28.37; MS (LSIMS) *m*/*z* 344 (30) [M^+ + H − $C_5H_8O_2$]; HRMS (LSIMS) calcd for $C_{25}H_{34}NO_6$ [M^+ + H] 444.2386, found 444.2387.

Synthesis of 2a. To a solution of **8** (1 g, 2.25 mmol) in dry DMF (30 mL), PDC (8.4 g, 22.5 mmol) was added at 0 °C. The reaction mixture was stirred at room temperature for 24 h under nitrogen atmosphere. It was then diluted with EtOAc, washed with saturated $CuSO_4$ solution, water, and brine, dried (Na_2SO_4), filtered, and concentrated in vacuo to get the diacid.

A small amount of the crude diacid (1/9 portion, 0.25 mmol) was dissolved in Et_2O (1 mL) and treated with an ethereal solution of CH_2N_2 until all the acids were converted into the diester. It was then concentrated in vacuo, and purification by column chromatography (SiO_2, 14% EtOAc in petroleum ether eluant) afforded the dimethyl diester (89 mg, 71%) as a white solid: R_f = 0.45 (silica gel, 20% EtOAc in petroleum ether); $[\alpha]^{20}_D$ −114.2 (*c* 1.025, $CHCl_3$); mp 111−113 °C; IR (neat) ν_{max} 2930, 1746, 1700, 1384, 1353, 1192, 1115 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.31−7.22 (m, 10 H, Ar*H*), 4.74 (d, *J* = 8.0 Hz, 1 H, C5-*H*), 4.73 and 4.60 (two d, *J* = 11.5 Hz, 2 H, PhCH_2O), 4.71 and 4.60 (two d, *J* = 11.5 Hz, 2 H, PhCH_2O), 4.68 (d, *J* = 8.0 Hz, 1 H, C2-*H*), 4.44 (t, *J* = 8.0 Hz, 1 H, C3-*H*), 4.40 (t, *J* = 8.0 Hz, 1 H, C4-*H*), 3.70 and 3.69 (two s, 6 H, OCH_3), 1.40 (s, 9 H, Boc); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.81, 170.69, 153.18, 137.50, 128.32, 127.73, 127.44, 81.39, 80.69, 80.13, 73.35, 59.92, 59.03, 52.23, 52.04, 28.11; MS (LSIMS) *m*/*z* 500 (4) [M^+ + H], 400 (46) [M^+ + H − $C_5H_8O_2$].

To a solution of BocNH-Gly-Leu-CONHMe (1.32 g, 4.4 mmol) in dry CH_2Cl_2 (6 mL) was added trifluoroacetic acid (3 mL) at 0 °C and the mixture stirred for 1 h. The reaction mixture was then concentrated in vacuo to give TFA.H_2N-Gly-Leu-CONHMe.

In another round-bottom flask, the remaining portion of the diacid (2 mmol), prepared above and dissolved in DMF−CH_2Cl_2 (1:2, 9 mL), was sequentially treated with HOBt hydrate (541 mg, 4.0 mmol) and EDCI (767 mg, 4.0 mmol) at 0 °C. After 0.5 h, TFA·H_2N-Gly-Leu-CONHMe, prepared above and dissolved in dry DMF (4 mL), was added to the reaction mixture followed by the addition of DIPEA (1.53 mL, 8.8 mmol). After being stirred for 12 h at room temperature, the reaction mixture was diluted with EtOAc, washed with saturated NH_4Cl solution, water, and brine, dried (Na_2SO_4), filtered, and concentrated in vacuo. Purification by column chromatography (SiO_2, 7% MeOH in $CHCl_3$ eluant) afforded **2a** (804 mg, 48%) as a white solid: R_f = 0.45 (silica gel, 8% MeOH in $CHCl_3$); $[\alpha]^{20}_D$ −80.65 (*c* 0.15, MeOH); mp 251−253 °C; IR (KBr) ν_{max} 3294, 2958, 1641, 1528, 1388, 1122 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz) δ 172.10, 171.92, 169.87, 169.51, 168.28, 168.01, 153.40, 137.96, 128.10, 127.57, 127.44, 127.37, 80.15, 79.88, 79.40, 79.12, 72.14, 72.02, 59.63, 50.86, 50.73, 42.57, 42.15, 41.07, 40.43, 27.75, 25.48, 24.03, 22.97, 21.47, 21.30; MS (LSIMS) *m*/*z* 738 (100) [M^+ + H − $C_5H_8O_2$]; HRMS (LSIMS) calcd for $C_{38}H_{56}N_7O_8$ [M^+ + H − $C_5H_8O_2$] 738.4190, found 738.4202.

Synthesis of 2b. To a solution of **2a** (250 mg, 0.29 mmol) in MeOH (3 mL) was added $Pd(OH)_2$ on C (20%, 50 mg), and the mixture was hydrogenated under atmospheric pressure using an H_2-filled balloon for 6 h. It was then filtered through a short pad of Celite, and the filter cake was washed with MeOH. The filtrate and the washings were combined and concentrated in vacuo. Purification by column chromatography (SiO_2, 10% MeOH in $CHCl_3$ eluant) afforded **2b** (156 mg, 80%): R_f = 0.5 (silica gel, 12% MeOH in $CHCl_3$); $[\alpha]^{20}_D$ 10.6 (*c* 0.5, MeOH); mp 220−222 °C; IR (KBr) ν_{max} 3328, 2960, 1657,

1545, 1407, 1254, 1169, 1113 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) see Table 2; ^{13}C NMR (DMSO-d_6, 125 MHz) δ 172.14, 172.06, 170.42, 170.38, 168.66, 168.46, 153.38, 79.51, 74.11, 73.40, 61.50, 61.05, 50.91, 50.80, 42.67, 42.53, 40.72, 40.36, 27.84, 25.59, 25.58, 24.01, 23.95, 23.08, 21.43, 21.33; MS (LSIMS) *m/z* 680 (39) $[M^+ + Na]$, 558 (22) $[M^+ + H - C_5H_8O_2]$; HRMS (LSIMS) calcd for $C_{29}H_{52}N_7O_{10}$ $[M^+ + H]$ 658.3776, found 658.3746.

Synthesis of 2c. To a solution of **2b** (100 mg, 0.15 mmol) in dry CH_2Cl_2 (1 mL) was added trifluoroacetic acid (1 mL) at 0 °C and the mixture stirred under nitrogen atmosphere for 0.5 h. The reaction mixture was concentrated in vacuo. The resulting crude was dissolved in MeOH, cooled to 0 °C, and then slowly neutralized with aqueous ammonia. It was then concentrated in vacuo. Purification by column chromatography (SiO_2, 14% MeOH in $CHCl_3$ eluant) afforded **2c** (80 mg, 95%): $R_f = 0.4$ (silica gel, 16% MeOH in $CHCl_3$); $[\alpha]^{20}_D$ 35.5 (*c* 0.63, MeOH); mp 145−148 °C; IR (KBr) ν_{max} 3415, 2923, 1646, 1423 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) see Table 3; ^{13}C NMR (DMSO-d_6, 125 MHz) δ 172.17, 171.66, 168.88, 78.31, 64.10, 50.96, 42.31, 40.63, 25.57, 24.13, 22.97, 21.44; MS (LSIMS) *m/z* 558 (10) $[M^+ + H]$; HRMS (LSIMS) calcd for $C_{24}H_{44}N_7O_8$ $[M^+ + H]$ 558.3251, found 558.3233.

Synthesis of 3a. Compound **3a** was synthesized following the same method as described above for the synthesis of **2a**: $R_f = 0.5$ (silica gel, 6% MeOH in $CHCl_3$); $[\alpha]^{20}_D$ −74.4 (*c* 1.8, MeOH); mp 222−224 °C; IR (KBr) ν_{max} 3323, 2946, 1723, 1653, 1515, 1361 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.2−7.25 (m, 10 H, aromatic), 6.95 (d, $J = 8.1$ Hz, 1 H, Leu(I)N*H*), 6.63 (d, $J = 8.4$ Hz, 1 H, Leu(I′)N*H*), 6.15 (dd, $J = 6.9$, 5.9 Hz, 1 H, Gly(I)N*H*), 6.10 (dd, $J = 5.7$, 5.3 Hz, 1 H, Gly(I′)N*H*), 4.81 (m, 2 H, PhCH_2O), 4.72 (m, 2 H, PhCH_2O), 4.55 (ddd, $J = 8.9$, 8.1, 5.4 Hz, 1 H, Leu(I)α*H*), 4.54 (dd, $J = 8.1$, 7.8 Hz, 1 H, C4-*H*), 4.51 (dd, $J = 8.1$, 8.0 Hz, 1H, C3-*H*), 4.44 (d, $J = 8.0$ Hz, 1 H, C2-*H*), 4.29 (d, $J = 7.8$ Hz, 1 H, C5-*H*), 4.27 (ddd, $J = 9.4$, 8.4, 5.1 Hz, 1 H, Leu(I′)α*H*), 4.07 (dd, $J = 17.0$, 5.7 Hz, 1 H, Gly(I′)α*H*′), 3.97 (dd, $J = 17.3$, 5.9 Hz, 1 H, Gly(I)α*H*), 3.84 (dd, $J = 17.3$, 6.9 Hz, 1 H, Gly(I)α*H*′), 3.79 (dd, $J = 17.0$, 5.3 Hz, 1 H, Gly(I′)α*H*), 3.71 (s, 3 H, Leu(I′)-OCH_3), 3.66 (s, 3 H, Leu-(I)-OCH_3), 1.66 (m, 1 H, Leu(I)γ*H*), 1.59 (m, 1 H, Leu(I′)γ*H*), 1.58 (m, 2 H, Leu(I)β*H*), 1.43 (m, 2 H, Leu(I′)β-*H*), 1.33 (s, 9 H, Boc), 0.92 (d, $J = 6.4$ Hz, 3 H, Leu(I′)δ*H*), 0.92 (d, $J = 6.4$ Hz, 3 H, Leu(I′)δ*H*′), 0.91 (d, $J = 6.4$ Hz, 3 H, Leu(I)δ*H*′), 0.89 (d, $J = 6.4$ Hz, 3 H, Leu(I)δ*H*); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 173.14, 172.97, 170.11, 169.60, 169.05, 168.31, 154.19, 137.53, 137.34, 128.54, 128.41, 128.02, 127.88, 81.72, 81.06, 80.93, 73.90, 73.77, 60.87, 60.70, 52.13, 52.01, 50.90, 50.70, 43.14, 42.99, 40.81, 40.23, 28.15, 28.04, 24.60, 22.80, 21.57, 21.47; MS (LSIMS) *m/z* 862 (40) $[M^+ + Na]$, 840 (12) $[M^+ + H]$, 740 (50) $[M^+ + H - C_5H_8O_2]$.

Synthesis of 3b. Compound **3b** was synthesized from **3a** following the same method as described for the synthesis of **2b**: $R_f = 0.4$ (silica gel, 8% MeOH in $CHCl_3$); $[\alpha]^{20}_D$ 5.23 (*c* 0.78, MeOH); mp 120−123 °C; IR (KBr) ν_{max} 3338, 2961, 1746, 1653, 1546, 1415 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.45 (d, $J = 6.1$ Hz, 1 H, Gly(I′)N*H*), 8.42 (t, $J = 5.9$ Hz, 1 H, Gly-(I)N*H*), 7.96 (d, $J = 8.3$ Hz, 1 H, Leu(I)N*H*), 7.81 (d, $J = 7.9$ Hz, 1 H, Leu(I′)N*H*), 5.97 (d, $J = 4.6$ Hz, 1 H, C3-O*H*), 5.85 (d, $J = 4.7$ Hz, 1 H, C4-O*H*), 4.35 (ddd, $J = 10.3$, 8.3, 4.8 Hz, 1 H, Leu(I)α*H*), 4.31 (d, $J = 8.1$ Hz, 1 H, C2-*H*), 4.31 (d, $J = 8.1$ Hz, 1 H, C5-*H*), 4.27 (m, 1 H, Leu(I′)α*H*), 4.20 (ddd, $J = 8.6$, 8.1, 4.6 Hz, 1 H, C3-*H*), 4.19 (ddd, $J = 8.6$, 8.1, 4.7 Hz, 1 H, C4-*H*), 3.82 (dd, $J = 17.1$, 5.9 Hz, 1 H, Gly(I)α*H*), 3.78 (dd, $J = 17.1$, 6.1 Hz, 1 H, Gly(I′)α*H*), 3.61 (s, 3 H, Leu(I′)-OCH_3), 3.59 (s, 3 H, Leu(I)-OCH_3), 3.59 (dd, $J = 17.1$, 6.1 Hz, 1 H, Gly(I′)α*H*′), 3.54 (dd, $J = 17.1$, 5.9 Hz, 1 H, Gly(I)α*H*′), 1.60 (m, 1 H, Leu(I′)γ*H*), 1.55 (m, 1 H, Leu(I)γ*H*), 1.44 (m, 2 H, Leu(I′)βH_2), 1.43 (m, 2 H, Leu(I)βH_2), 1.28 (s, 9 H, Boc), 0.87 (d, $J = 6.4$ Hz, 3 H, Leu(I)δ*H*), 0.87 (d, $J = 6.4$ Hz, 3 H, Leu-(I′)δ*H*), 0.81 (d, $J = 6.4$ Hz, 3 H, Leu(I)δ′*H*), 0.81 (d, $J = 6.4$ Hz, 3 H, Leu(I′)δ′*H*); $-\Delta\delta/\Delta T$ (in ppb/K) = 7.0 for Gly(I)NH, 7.4 for Gly(I′)NH, 3.4 for Leu(I)NH and 3.3 for Leu(I′)NH; ^{13}C NMR (DMSO-d_6, 125 MHz): δ 172.68, 172.61, 170.25, 169.95, 168.84, 168.56, 153.26, 79.36, 73.96, 73.25, 61.21, 60.93, 51.81, 51.77, 49.99, 49.87, 42.22, 42.16, 27.79, 23.83, 22.78, 21.13; MS (LSIMS) *m/z* 682 (25) $[M^+ + Na]$, 660 (11) $[M^+ + H]$, 560 (100) $[M^+ + H - C_5H_8O_2]$; HRMS (LSIMS) calcd for $C_{29}H_{50}N_5O_{12}$ $[M^+ + H]$ 660.3456, found 660.3463.

Synthesis of 3c. It was synthesized from **3b** following the same method as described for the synthesis of **2c**: $R_f = 0.4$ (silica gel, 10% MeOH in $CHCl_3$); $[\alpha]^{20}_D$ 30.8 (*c* 0.44, MeOH); mp 95−98 °C; IR (KBr) ν_{max} 3307, 2938, 1738, 1653, 1530 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.27 (d, $J = 5.9$ Hz, 2 H, GlyN*H*), 8.03 (d, $J = 7.6$ Hz, 2 H, LeuN*H*), 5.29 (d, $J = 3.6$ Hz, 2 H, O*H*), 4.27 (ddd, $J = 9.7$, 7.6, 5.3 Hz, 2 H, Leuα*H*), 4.17 (m, 2 H, C3-*H*, C4-*H*), 3.76 (dd, $J = 17.2$, 5.9 Hz, 2 H, Glyα*H*), 3.73 (dd, $J = 14.0$, 6.0 Hz, 2 H, Glyα*H*′), 3.92 (bs, 2 H, C2-*H*, C5-*H*), 3.61 (s, 6 H, Leu-OCH_3), 1.59 (m, 2 H, Leuγ*H*), 1.51 (ddd, $J = 13.4$, 9.7, 5.2 Hz, 2 H, Leuβ*H*′), 1.47 (ddd, $J = 13.4$, 8.9, 5.3 Hz, 2 H, Leuβ*H*), 0.87 (d, $J = 6.6$ Hz, 6 H, Leuδ*H*), 0.82 (d, $J = 6.5$ Hz, 6 H, Leuδ′*H*); $-\Delta\delta/\Delta T$ (in ppb/K) = 4.2 for GlyNH, and 3.9 for LeuNH; ^{13}C NMR (DMSO-d_6, 125 MHz) δ 172.69, 171.07, 169.14, 78.14, 64.00, 51.76, 50.17, 41.81, 39.66, 24.01, 22.64, 21.26; MS (LSIMS) *m/z* 560 (37) $[M^+ + H]$; HRMS (LSIMS) calcd for $C_{24}H_{42}N_5O_{10}$ $[M^+ + H]$ 560.2931, found 560.2916.

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Supporting Information Available: ^1H, ^{13}C, TOCSY, ROESY, and expansion of ROESY NMR spectra of **2a**−**c** and **3a**−**c**, ^1H VT NMR spectra of **2a**−**c**, and a listing of the distance constraints data used in the MD studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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