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Conformational studies of 3,4-dideoxy furanoid sugar amino acid containing analogs of the receptor binding inhibitor of vasoactive intestinal peptide

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Abstract—Conformational analysis of vasoactive intestinal peptide (VIP) receptor binding inhibitor Leu[1]-Met[2]-Tyr[3]-Pro[4]-Thr[5]-Tyr[6]-Leu[7]-Lys[8] **1** by various NMR techniques and constrained molecular dynamics (MD) simulation studies revealed that the molecule had a turn structure involving its Tyr[3]-Pro[4]-Thr[5]-Tyr[6] moiety with intramolecular hydrogen bond between Tyr[6]NH→Tyr[3]CO. In order to mimic the structure of **1**, peptidomimetic analogs **2–4** were synthesized using conformationally constrained scaffolds of 3,4-dideoxy furanoid sugar amino acids (2*S*,5*R*)-ddSaa1 **5** and its enantiomer (2*R*,5*S*)-ddSaa2 **6**. All these analogs displayed well defined three-dimensional structures akin to that found in **1**. Peptides **2** and **3**, which differed only in the sugar amino acid stereochemistry, show propensity of structures with identical intramolecular hydrogen bonds between ThrNH→MetCO. A similar structure with a hydrogen bond between TyrNH→MetCO was observed in **4**.

1. Introduction

Vasoactive intestinal peptide (VIP) is a widely distributed naturally occurring neuropeptide containing 28 amino acids with wide ranging biological activities.[1] It has been found that VIP receptors are over-expressed on a variety of malignant tumor cells that are also associated with the synthesis and secretion of detectable levels of the VIP by the malignant cells themselves.[2] The in vitro studies suggest that VIP acts as a growth factor and plays a dominant role in the sustained or indefinite proliferation of cancer cells. Focus is on the development of VIP receptor binding inhibitors that will have the potential to arrest the growth of malignant cells.[3] The peptide sequence Leu[1]-Met[2]-Tyr[3]-Pro[4]-Thr[5]-Tyr[6]-Leu[7]-Lys[8] **1** is known to be one such VIP receptor binding inhibitor.[4] The role of this octapeptide as a VIP receptor binding inhibitor[5] and its anti cancer activities in combination with other neuropeptide analogs[6] ha1ve been well established. Several novel analogs of this peptide containing α,α-dialkylated amino acids and its lipoconjugates have been synthesized and tested for their anti cancer activities.[7] This prompted us to undertake the development of new peptidomimetic analogs of **1** based on conformationally constrained nonproteinogenic scaffolds in order to increase their physiological stabilities.

It was envisaged that the design of the peptidomimetic analogs of **1** could be greatly facilitated by the knowledge of its three-dimensional structure. This prompted us to undertake first the structural studies of the octapeptide **1**. In this paper, we describe the detailed conformational analysis of **1** using various NMR techniques that established a well-defined turn structure involving Tyr[3]-Pro[4]-Thr[5]-Tyr[6] residues. Based on these structural studies, novel peptidomimetic analogs **2–4** were subsequently developed using 3,4-dideoxy furanoid sugar amino acids **5** (ddSaa1) and **6** (ddSaa2) as building blocks. In recent years, sugar amino acids have emerged as a class of versatile templates that have been used extensively as conformationally constrained scaffolds in many peptidomimetic studies.[8] Insertion of sugar amino acids (2*S*,5*R*)-ddSaa1 **5** and its enantiomer (2*R*,5*S*)-ddSaa2 **6** as dipeptide isosteres in place of the Tyr[3]-Pro[4] segment of **1** led to the formation of analogs **2** and **3**, respectively.

Keywords: 3,4-Dideoxy furanoid sugar amino acids; VIP receptor; Conformation; NMR.
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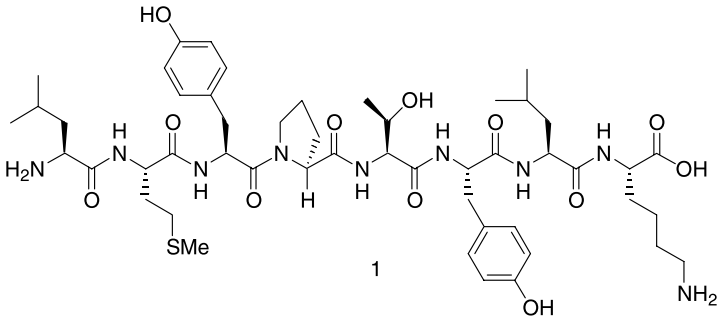

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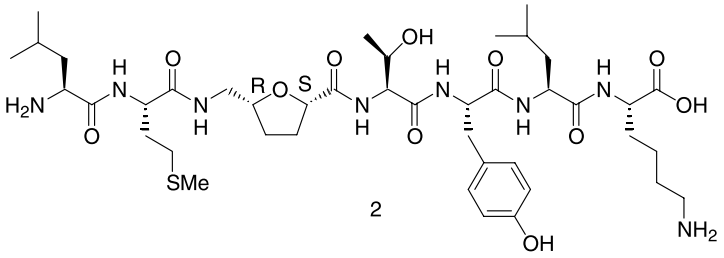

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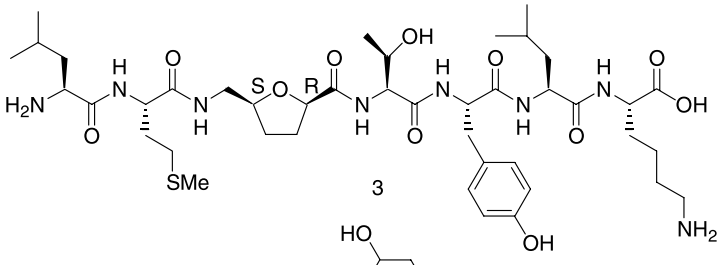

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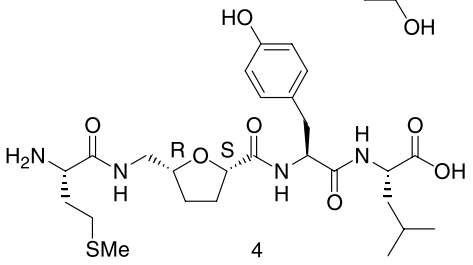

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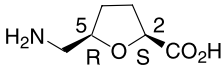

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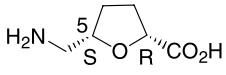


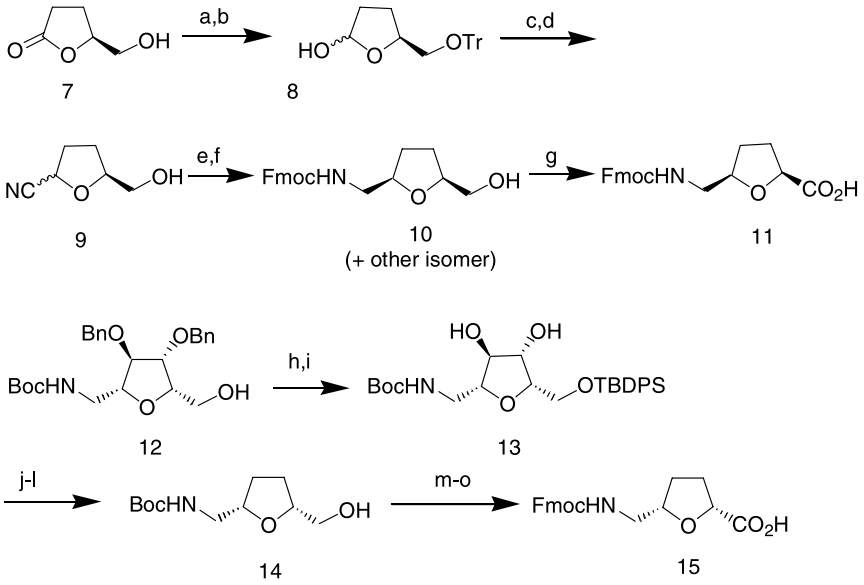
6

Compounds **2** and **3** displayed nucleation of well-defined turn structures, similar to the one found in **1**, with an intramolecular hydrogen bond between ThrNH→MetCO. Further truncation of **1**, replacing three of its amino acids Tyr[3]-Pro[4]-Thr[5] with ddSaa1 and deleting one residue each from both the termini, still retained the essential 10-membered β-turn like structure in the resulting analog **4** with a TyrNH→MetCO hydrogen bond, underlying the turn-inducing effect of 2,5-*syn* furanoid sugar amino acids. The details of the synthesis and structural studies of peptide **1** and its analogs **2**–**4** are described here.

2. Results and discussions

2.1. Synthesis of sugar amino acids 5 and 6 with Fmoc-protection

Scheme 1 describes the synthesis of Fmoc-protected 3,4-dideoxy furanoid sugar amino acids ddSaa1 and ddSaa2.[9] The starting material for the synthesis of Fmoc-ddSaa1 was (5*S*)-5-(hydroxymethyl)dihydrofuran-2(3*H*)-one **7**, which was prepared from L-glutamic acid in two steps using known methods.[10] Protection of the primary hydroxyl of **7** as the trityl ether, and subsequent reduction of the lactone using DIBAL-H furnished the lactol **8** as a mixture of isomers. Acylation of the lactol hydroxyl group led to the formation of a glycosyl acetate intermediate that was treated with trimethylsilyl cyanide in the presence of BF_3–Et_2O to get a diastereomeric mixture of the glycosyl cyanides **9**.[11] Reduction of the cyanide group of **9** with $LiAlH_4$ gave a primary amine intermediate that was protected in situ using FmocOSu to furnish the intermediate **10**. The faster moving spot on the TLC was the desired (2*S*,5*R*)-isomer that could be separated easily at this stage using standard silica gel column chromatography. The relative stereochemistries of the separated isomers were further confirmed by ^{1}H NOE difference spectroscopic studies. Irradiation of the C2–*H*



Scheme 1. Reagents and conditions: (a) pyridine, TrCl, DMAP (cat.), CH_2Cl_2, 0 °C to rt, 12 h; (b) DIBAL-H, CH_2Cl_2, −78 °C, 10 min; (c) Et_3N, Ac_2O, DMAP (cat.), CH_2Cl_2, 0 °C to rt, 30 min; (d) TMSCN, BF_3–Et_2O, CH_3CN, rt, 4 h; (e) $LiAlH_4$, Et_2O, 0 °C, 5 min, reflux, 3 h; (f) FmocOsu, CH_2Cl_2, 0 °C to rt, 1 h; (g) Jones reagent, acetone, 0 °C to rt, 3 h; (h) H_2, 10% $Pd(OH)_2$–C, MeOH, rt, 8 h; (i) TBDPSCl, Et_3N, DMAP (cat.), DMF, 0 °C to rt, 3 h; (j) Ph_3P, imidazole, I_2, toluene, reflux, 3 h; (k) H_2, 10% $Pd(OH)_2$–C, MeOH, rt, 1 h; (l) TBAF, THF, 0 °C to rt, 3 h; (m) $NaIO_4$, $RuCl_3 \cdot 3H_2O$ (cat.), CH_3CN:CCl_4:H_2O (1:1:1.5), 0 °C, 2 h; (n) TFA, CH_2Cl_2, 0 °C, 2 h; (o) FmocOSu, 10% aqueous Na_2CO_3, dioxane, 0 °C to rt, 12 h.

signal of the (2*S*,5*R*)-isomer **10** enhanced the peak of C5–*H* confirming their *syn*-relationship. Oxidation of the primary hydroxyl of **10** to carboxylic acid using Jones' reagent furnished the target molecule Fmoc-ddSaa1 **11**.

Synthesis of the other enantiomer of **11**, Fmoc-ddSaa2, was started with the known compound **12**, which was prepared by us earlier from D-glucose.[12] Debenzylation of **12** was followed by selective protection of the primary hydroxyl group as the TBDPS ether to get the intermediate diol **13**. Next, the diol **13** was transformed into the 3,4-dideoxy intermediate **14** in three steps—reductive elimination of the 3,4-diol moiety using Ph_3P/I_2/imidazole,[13] hydrogenation of the resulting 3,4-olefin function and finally, desilylation of the C1-hydroxyl group using TBAF. Compound **14** was transformed into the required Fmoc-protected product **15** following a three-step process—oxidation of the primary hydroxyl to get the carboxylic acid, Boc-deprotection using trifluoroacetic acid in dichloromethane, followed by Fmoc protection using FmocOSu to furnish the required product **15**.

2.2. Synthesis of peptides 2–4

Peptides **2**–**4** were synthesized by solid phase method on Wang resin using the Fmoc strategy.[14] Substitution levels for automated synthesis were preferably between 0.9 and 1.2 mmol amino acid per gram resin. Preferably, DIPC/HOBt or HBTU/DIPEA and PyBOP/DIPEA were used as activating reagents in the coupling reactions.[15] The coupling reactions were carried out in DMF, CH_2Cl_2 or NMP[15] or a mixture of these solvents. The Fmoc group was cleaved by using 20% piperidine in DMF for 30 min. Usually, 3–4 equiv of activating agents and 3–4 equiv of Fmoc protected amino acid per resin nitrogen equivalent were used per coupling step. The side chain protecting groups were deprotected and the peptide was simultaneously cleaved from the resin by treatment with trifluoroacetic acid, crystalline phenol, ethanedithiol, thioanisole and deionized water for 2–3 h at room temperature. The crude peptides obtained by precipitation with cold dry ether were purified by preparative HPLC and used for conformational analysis by NMR.

2.3. Conformational analysis. NMR studies

NMR studies of **1**–**4** were carried out in DMSO-d_6. The spectra were well resolved and most of the spectral parameters could be obtained easily and are reported in the Tables 1–4. While the assignments were carried out with the help of total correlation spectroscopy (TOCSY),[16] rotating frame nuclear Overhauser effect spectroscopy (ROESY)[17] experiments provided the information on the proximity of protons, the details of which are provided in Section 4. Variable temperature studies were carried out to measure the temperature coefficients of the amide proton chemical shifts ($\Delta\delta(\Delta($, which provided information about their involvements in intramolecular hydrogen bonds.[18] The cross-peak intensities in the ROESY spectra, shown schematically in Figures 1–4, were used for obtaining the restraints in the simulated molecular dynamics (MD) calculations,[19] the detailed protocol of which is included in Section 4.

2.4. Conformational analysis of 1

Proline, the unique cyclic natural amino acid, can undergo *cis–trans* rotamerisation with preceding amino acid, because of the presence of imide bond. Invariably the *trans* rotamer predominates the *cis* rotamer in solution. Peptide **1** shows two sets of resonances with 6:1 ratio, because of the existence of *cis*/*trans* isomers about Tyr^3-Pro^4 amide linkage. The observation of strong rOe cross-peaks, as shown in **A** in Figure 1, between

Table 1. ^{1}H chemical shifts (δ in ppm), coupling constants (J in Hz) and temperature coefficients ($\Delta\delta/\Delta T$ in ppb/deg K) of **1** in DMSO-d_6 at 500 MHz

Amino acid	NH	CαH	CβH	CγH	CδH	Others	$\Delta\delta/\Delta T$
Leu[1]	—	3.71 (m)	1.45–1.49 (m)	1.59 (m)	0.86 (d, J=6.7 Hz), 0.84 (d, J=6.8 Hz)		
Met[2]	8.49 (d) (J=8.5 Hz)	4.39 (m)	1.86, 1.74 (m)	2.41 (m)		2.02 (SCH_3)	−5.5
Tyr[3]	8.20 (d) (J=7.8)	4.55 (ddd) (J=4.5, 8.2 Hz)	2.86 (dd, J=4.5, 14.3), 2.67 (dd, J=8.9, 14.3 Hz)			7.04, 6.61 (Ph), 9.15 (OH)	−7.8
Pro[4]	—	4.43 (m)	1.96, 1.88 (m)	1.82 (m), 1.81 (m)	3.59 (m), 3.43 (m)		
Thr[5]	7.78 (d) (J=8.1 Hz)	4.18 (dd) (J=4.2 Hz)	3.96 (m)	1.01 (d, J=6.4 Hz)		4.91 (OH)	−6.0
Tyr[6]	7.68 (d) (J=8.1 Hz)	4.44 (m)	2.93 (dd, J=4.4, 14.3 Hz), 2.71 (dd, J=9.1, 14.3 Hz)			6.99, 6.60 (Ph)	−3.6
Leu[7]	7.94 (d) (J=8.2 Hz)	4.30 (q) (J=8.2 Hz)	1.45–1.47 (m)	1.58 (m)	0.87, 0.83		−6.9
Lys[8]	7.99 (d) (J=7.9 Hz)	4.13 (dt) (J=5.0 Hz)	1.72 (m)	1.60 (m), 1.54 (m)	1.36 (m)	2.76	−6.9

Table 2. ^{1}H chemical shifts (δ in ppm), coupling constants (J in Hz) and temperature coefficients ($\Delta\delta/\Delta T$ in ppb/deg K) of **2** in DMSO-d_6 at 500 MHz

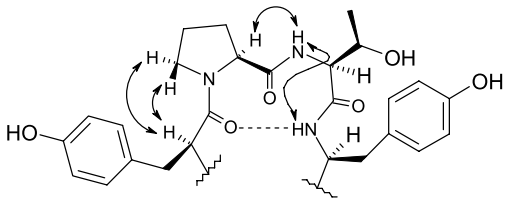
Amino acid	NH	CαH	CβH	CγH	CδH	Others	$\Delta\delta/\Delta T$
Leu	8.08 (bs)	3.80 (m)	1.52–1.61 (m)			0.87 (d), 0.88 (d)	
Met	8.61 (d) (J=8.1 Hz)	4.41 (dt) (J=5.2 Hz)	1.91 (m), 1.80 (m)	2.48 (m), 2.43 (m)		2.03 (SCH_3)	−5.5
ddSaa1	8.33 (d) (J=5.8 Hz)	4.30 (m)	1.87 (m), 2.11 (m)	1.46 (m)	3.96	3.34, 3.16	−7.3
Thr	7.60 (d) (J=8.4 Hz)	4.19 (dd) (J=4.6 Hz)	3.98 (m)	0.99 (m)		4.94 (OH)	−2.8
Tyr	7.87 (d) (J=8.2 Hz)	4.49 (dt) (J=4.4 Hz)	2.90 (dd, J=4.5, 14.2 Hz), 2.67 (dd, J=8.7, 14.2 Hz)			6.98 (d, J=8.4 Hz), 6.59 (d, J=8.4 Hz)	−4.7
Leu	7.96 (d) (J=8.1 Hz)	4.31 (m)	1.45–1.47	1.60 (m)	0.87 (d), 0.84 (d)		−6.5
Lys	8.08 (d) (J=7.7 Hz)	4.15 (dt) (J=5.0 Hz)	1.72	1.60 (m), 1.54 (m)	1.36 (m)	2.76	−7.8

Table 3. ^{1}H chemical shifts (δ in ppm), coupling constants (J in Hz) and temperature coefficients ($\Delta\delta/\Delta T$ in ppb/deg K) of **3** in DMSO-d_6 at 500 MHz

Amino acid	NH	CαH	CβH	CγH	CδH	Others	$\Delta\delta/\Delta T$
Leu	8.07 (bs)	3.80 (m)	1.50 (m)	1.59 (m)	0.87 (d, J=6.5 Hz), 0.87 (d, J=6.5 Hz)		—
Met	8.61 (d) (J=8.1 Hz)	4.39 (ddd) (J=5.0, 8.4 Hz)	1.90 (m), 1.80 (m)	2.47 (m), 2.42 (m)		2.03 (SCH_3)	−5.0
ddSaa2	8.25 (d) (J=5.8 Hz)	4.28 (dd) (J=5.2, 8.3 Hz)	1.88 (m)	1.51 (m)	3.97 (m)	3.24 (m), 3.12 (m)	−6.7
Thr	7.53 (d) (J=8.2 Hz)	4.17 (d) (J=4.6 Hz)	3.95 (m)	0.95 (d, J=6.3 Hz)		5.00 (d, J=5.5 Hz, OH)	−2.2
Tyr	7.93 (d) (J=8.3 Hz)	4.46 (dt) (J=4.3 Hz)	2.92 (dd, J=4.4, 14.1 Hz), 2.67 (dd, J=8.9, 14.1 Hz)			6.98 (d, J=8.5 Hz), 6.60 (d, J=8.5 Hz)	−4.9
Leu	7.94 (d) (J=8.1 Hz)	4.31 (dt) (J=6.1, 8. 1 Hz)	1.45–1.47 (m)	1.60 (m)	0.87 (d, J=6.5 Hz), 0.83 (d, J=6.5 Hz)		−6.1
Lys	8.07 (d) (J=7.5 Hz)	4.14 (m)	1.72 (m)	1.60 (m), 1.54 (m)	1.35	2.75 (m), 7.65 (bs)	−7.4

Table 4. ^{1}H chemical shifts (δ in ppm), coupling constants (J in Hz) and temperature coefficients ($\Delta\delta/\Delta T$ in ppb/deg K) of **4** in DMSO-d_6 at 500 MHz

Amino acid	NH	CαH	CβH	CγH	CδH	Others	$\Delta\delta/\Delta T$
Met	8.16 (bs)	3.84 (m)	2.48 (m), 1.98 (m)	3.30 (m), 3.30 (m)		2.05 (s, SCH_3)	—
ddSaa1	8.69 (t) (J=5.8 Hz)	4.18 (dd) (J=5.4, 8.0 Hz)	1.29 (m), 1.84 (m)	1.66 (m), 2.05 (m)	3.94 (m)	3.12 (m), 3.22 (m)	−4.7
Tyr	7.56 (d) (J=8.8 Hz)	4.58 (ddd) (J=4.1, 9.3 Hz)	2.96 (dd, J=4.4, 14.0 Hz), 2.67 (dd, J=9.8, 14.0 Hz)			6.63 (d, J=8.5 Hz), 7.07 (d, J=8.5 Hz), 9.15 (OH)	−2.8
Leu	8.38 (d) (J=8.0 Hz)	4.23 (ddd) (J=6.0, 8.5 Hz)	1.63–1.52 (m)		0.90 (d, J=6.7 Hz), 0.84 (d, J=6.7 Hz)		−6.9



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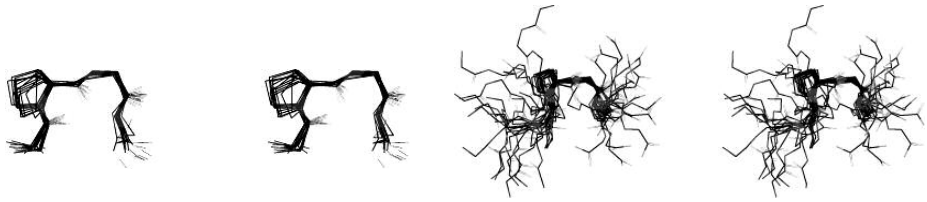
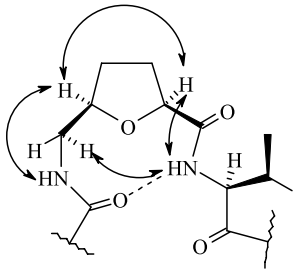


B

Figure 1. (A) Schematic representation of the proposed β-turn involving Tyr^3-Pro^4-Thr^5-Tyr^6 residues in **1** with some of the prominent long-range rOes seen in its ROESY spectrum. (B) Stereo view of the 25 superimposed energy-minimized structures of **1** sampled during 50 cycles of the 300 ps constrained MD simulations following the simulated annealing protocol: H-bonded region (left), full structure (right).

$Tyr^3C\alpha H \leftrightarrow Pro^4C\delta H$ and $Tyr^3C\alpha H \leftrightarrow Pro^4C\delta' H$ in the ROESY spectrum show that the major isomer has a *trans* imide bond preceding Pro^4. Moderate magnitude of $\Delta\delta/\Delta T = -3.6$ ppb/deg K for Tyr^6NH indicates the propensity of a structure, with its participation in intramolecular hydrogen bonding. Observation of rOe cross-peaks between $Thr^5NH \leftrightarrow Tyr^6NH$ and $Tyr^6NH \leftrightarrow Leu^7NH$ as well as the participation of Tyr^6NH in hydrogen bonding shows the existence of a 10-membered β-turn around Pro^4-Thr^5 residues. The presence of similar intensities of rOe cross-peaks of $Thr^5NH \leftrightarrow Pro^4C\alpha H$ and $Thr^5NH \leftrightarrow Thr^5C\alpha H$ imply that the observed turn is a type-II β-turn.

The molecular dynamics calculations on **1** clearly show structures with a type-II β-turn about Pro^4-Thr^5 residues. Figure 1 depicts the assembly **B** of the backbone superimposed turn structures of the 25 samples collected during 300 ps simulated annealing protocol (detailed protocol has been given in the Supporting Information). It clearly shows a type-II β-turn about Pro^4-Thr^5 residues, where as the other



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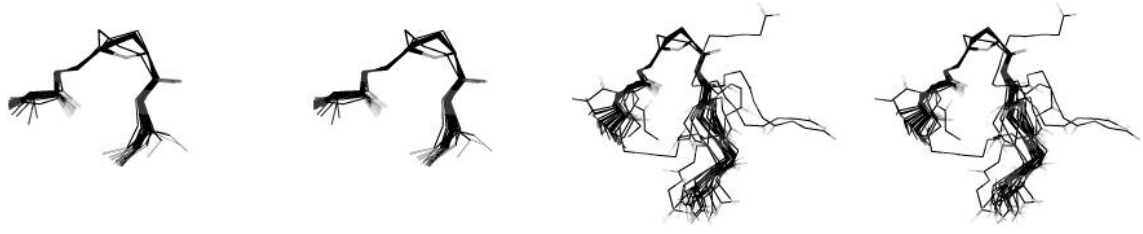

B

Figure 2. (A) Schematic representation of the proposed β-turn like structures involving Met-ddSaa1-Thr residues in **2** with some of the prominent long-range rOes seen in its ROESY spectrum. (B) Stereo view of the 25 superimposed energy-minimized structures of **2** sampled during 50 cycles of the 300 ps constrained MD simulations following the simulated annealing protocol: H-bonded region (left), full structure (right).

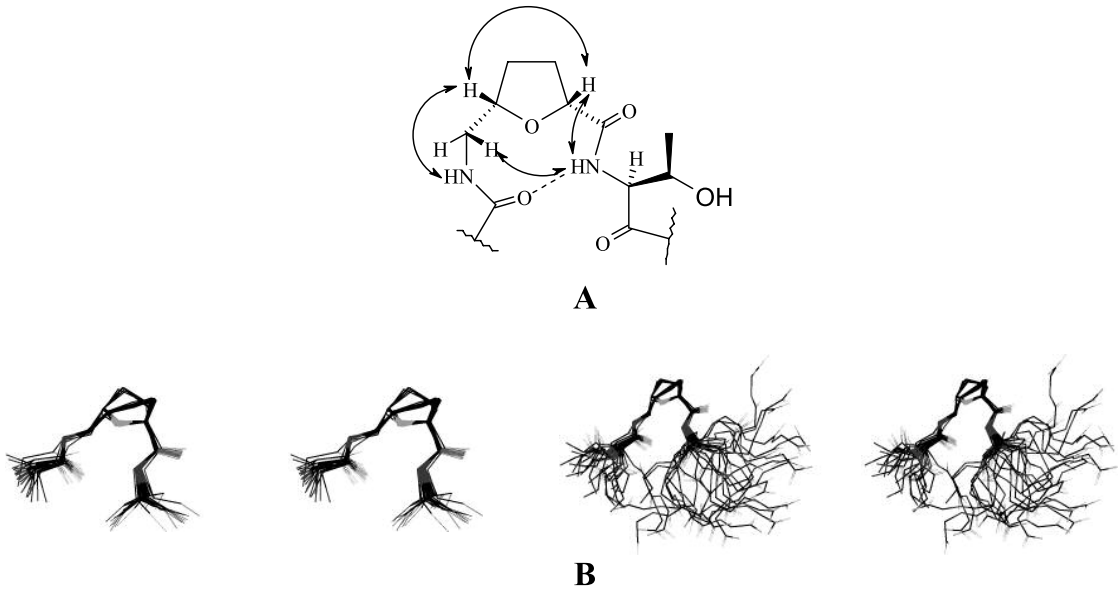

Figure 3. (A) Schematic representation of the proposed β-turn like structures involving Met-ddSaa2-Thr residues in **3** with some of the prominent long-range rOes seen in its ROESY spectrum. (B) Stereo view of the 25 superimposed energy-minimized structures of **3** sampled during 50 cycles of the 300 ps constrained MD simulations following the simulated annealing protocol: H-bonded region (left), full structure (right).

part of the peptide backbone shows an irregular (extended) conformation. The average pair wise backbone RMSD for the structures is 0.47 ± 0.16 Å.

2.5. Conformational analysis of 2

As compared to **1**, in peptide **2**, the dipeptide isostere (2*S*,5*R*)-ddSaa1 **5**, has been inserted in place of the Tyr^3-Pro^4 residues. The relative configuration of the ddSaa1 at the C2 and C5 carbons was confirmed by the observation of the rOe cross-peak between ddSaa1C2–H↔C5–H, which imply that these protons are on the same side of the five-membered sugar ring. Temperature coefficient, $\Delta\delta/\Delta T = -2.8$ ppb/deg K, for Thr^5NH showed that it participates in intramolecular hydrogen bonding. Appearances of the sequential $NH_i \leftrightarrow NH_{i+1}$ rOe cross-peaks (MetNH↔ddSaa1NH, ddSaa1NH↔ThrNH, ThrNH↔TyrNH, TyrNH↔LeuNH and LeuNH↔LysNH) in the ROESY spectrum show that there is a propensity towards a helical structure. The presence of rOes between ddSaa1NH↔ThrNH, ThrNH↔TyrNH, ThrNH↔ddSaa1C6–$H_{2(\alpha,\alpha')}$ (**A** in Fig. 2) coupled with the intramolecular hydrogen bonding of ThrNH imply that the molecule has a β-turn like structure around Met-ddSaa1-Thr residues,

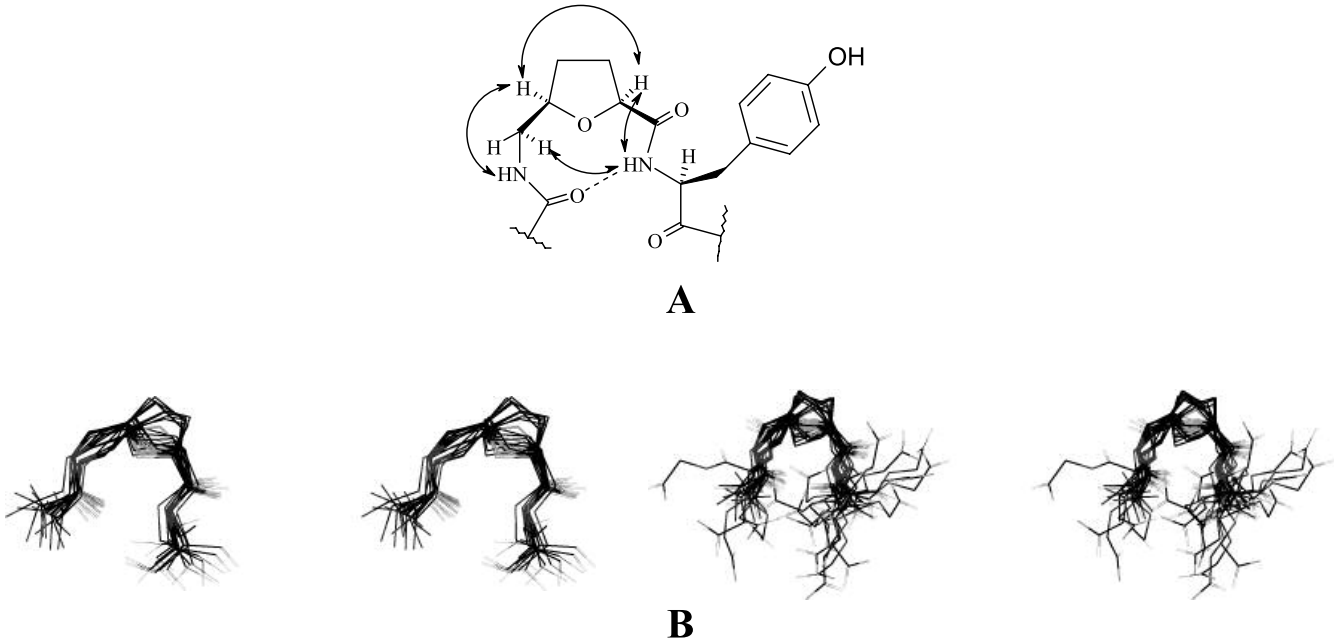

Figure 4. (A) Schematic representation of the proposed β-turn like structures involving Met-ddSaa1-Tyr residues in **4** with some of the prominent long-range rOes seen in its ROESY spectrum. (B) Stereo view of the 25 superimposed energy-minimized structures of **4** sampled during 50 cycles of the 300 ps constrained MD simulations following the simulated annealing protocol: H-bonded region (left), full structure (right).

which is stabilized by the ThrNH→MetCO H-bond. The observed β-turn is similar to that observed earlier by us[20] and others[21] for oligomers containing furanoid sugar amino acids. The MD calculations on **2** show the existence of a 10-membered hydrogen bonded turn structure between ThrNH→MetCO, which mimics a regular β-turn structure, where as the rest of the peptide backbone seem to take an extended conformation. Figure 2 shows an ensemble **B** of 25 conformations superimposed at the turn structure during 50 cycles of 300 ps simulated annealing MD run. The average pair wise backbone RMSD is 0.87±0.39 Å.

2.6. Conformational analysis of 3

In compound **3**, containing the dipeptide isostere (2*R*,5*S*)-ddSaa2 **6**, the observed conformation of the peptide is similar to that of **2**, which had the isomeric sugar amino acid. The small magnitude of $\Delta\delta/\Delta T = -2.2$ ppb/deg K for Thr^5NH confirms its participation in hydrogen bonding probably with $Met^2C{=}O$. Similar to **2**, all the sequential $NH_i \leftrightarrow NH_{i+1}$ rOe cross-peaks (MetNH↔ddSaa2NH, ddSaa2NH↔ThrNH, ThrNH↔TyrNH, TyrNH↔LeuNH and LeuNH↔LysNH) in the ROESY spectrum were observed. It supports the presence of an incipient helix. Participation of the ThrNH in hydrogen bonding, as well as the observation of rOe cross-peaks between ddSaa2NH↔ThrNH, ThrNH↔TyrNH and ThrNH↔ddSaa2C6–$H_{2(\alpha,\alpha')}$ (**A** in Fig. 3) indicate that the molecule has a β-turn like structure involving Met-ddSaa2-Thr residues. The observed β-turn is similar to that found in **2** and in various oligomers containing furanoid sugar amino acids.[20,21]

Molecular mechanics calculations clearly show the existence of a 10-membered H-bond between ThrNH→MetCO. Figure 3 shows an ensemble **B** of 25 conformations superimposed at the turn structure during 50 cycles of 300 ps simulated annealing MD run. The average pair wise backbone RMSD is 0.41±0.23 Å.

2.7. Conformational analysis of 4

In compound **4**, TyrNH shows small magnitude of $\Delta\delta/\Delta T$ (−2.8 ppb/deg K), indicating its participation in intramolecular hydrogen bonding. The presence of ROESY cross-peaks between TyrNH↔ddSaa1C2–H and TyrNH↔ddSaa1C6–H coupled with the participation of TyrNH in hydrogen bonding suggests that a 10-membered β-turn like structure, similar to the one observed earlier by us and others,[20,21] is stabilized by a hydrogen bond between TyrNH→MetCO. Appearance of a rOe cross-peak between the ddSaa1C2–H↔ddSaa1C5–H indicates that the two protons are on the same side of the five-membered ring. The $^3J_{NH-C\alpha H}$ of 8.8 and 8.0 Hz for Tyr and Leu residues, respectively, indicate that there is propensity of structures with backbone ϕ angles in the β-region of the Ramachandran plot. The cross-peak intensities in the ROESY spectrum of **4** (**A** in Fig. 4) were used for obtaining the restraints in the MD calculations. The molecular dynamics calculations showed the existence of a β-turn like structure as shown in **B** in Figure 4 having a 10-membered intramolecular hydrogen bond. The average pair wise backbone RMSD is 1.20±0.33 Å.

3. Conclusion

3,4-Dideoxy furanoid sugar amino acids **5** and **6**, having a *syn* relationship between C2–H and C5–H, belong to the growing family of very useful molecular building blocks of sugar amino acids and display remarkable propensities to induce well defined turn structures in small peptides. The β-turn structure found in VIP anatagonist **1** was successfully reproduced by introducing the nonproteinogenic dipeptide isostere **5** and **6** in the molecule, resulting in the development of novel peptidomimetic analogs **2**–**4**. Although both ddSaa1 **5** and ddSaa2 **6** gave rise to the similar 10-membered hydrogen bonded structures, the turn induced by the latter with (2*R*,5*S*) stereochemistry was more pronounced than those derived from the former having the (2*S*,5*R*) stereochemistry. These studies may help in designing mimics of the bioactive peptide conformations of small peptides, where proline residue participates in well-defined β-turn structures.

4. Experimental

4.1. General experimental procedures

All reactions were carried out in oven or flame-dried glassware with magnetic stirring under a 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 or 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 on FT-IR Nicolet-740. Mass spectra were obtained on Micromass Autospec and Quattro spectrometers under liquid secondary ion mass spectrometric (LSIMS) and electron spray ionisation (ESI) techniques, respectively. Optical rotations were measured with a Jasco Dip-370 and Horiba Sepa-300 digital polarimeters.

4.2. NMR spectroscopy

NMR spectra of the peptides **1**–**4** were recorded on Varian Unity-Inova 500 MHz spectrometer at 30 °C with 2–10 mM solutions in appropriate solvents using TMS 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 on Bruker Avance-300 spectrometer at 75 MHz with complete proton decoupling. The chemical shift assignments were carried out with the help of two-dimensional total correlation spectroscopy (TOCSY)[16] and rotating frame nuclear Overhauser effect spectroscopy (ROESY) experiments,[17] the later also provided the information on the proximity of protons. All the experiments were carried out in the phase sensitive mode.[22] The spectra were acquired

with 2×256 or 2×192 free induction decays (FID) containing 8–16 transients with relaxation delays of 1.0–1.5 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. To obtain the temperature coefficients of NH-chemical shifts, the spectra were recorded between 30 and 70 °C (at 30, 40, 50, 60, and 70 °C) in DMSO-d_6. The temperature coefficients $\Delta\delta/\Delta T$ were determined from the slopes of the linear regression lines obtained from the chemical shift versus temperature plots (see Supplementary Information).[18]

4.3. Molecular dynamics

Molecular mechanics/dynamics calculations were carried out using the Sybyl 6.8 program on a Silicon Graphics O2 workstation. The Tripos force field, with default parameters, was used throughout the simulations.

A dielectric constant of 47 Debye was used in all minimizations as well as in MD runs. Minimizations were done first with steepest decent, followed by conjugate gradient methods for a maximum of 2000 iterations each or RMS deviation of 0.005 kcal/mol, whichever was earlier. The energy-minimized structures were then subjected to MD studies. A number of inter atomic distance constraints (more than three bond away) were used in the MD studies that were derived from the rOe cross-peaks (see Supplementary Information) on the basis of two-spin approximation by taking the TyrCβH protons distance (1.8 Å) as an internal standard. For all the amide bonds, the torsional angle 180° was used as a constraint. No H-bonding constraint was used. For distance constraints, a force constant of 15 kcal/Å was applied in the form of flat bottom potential well and a force constant of 5 kcal/Å was employed for the dihedral angle constraints.[19] The energy-minimized structures were subjected to constrained MD simulations for duration of 300 ps using 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 deg K.[23]

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. Structures were sampled after every two cycle, leading to an ensemble of total 25 structures. The sampled structures were energy-minimized using the above-mentioned protocol and the superimposed structures obtained by backbone alignment are shown in Figures 1–4. To determine the backbone and the average pair-wise heavy atom RMSD, the structures were analyzed using the MOLMOL program.[24]

4.3.1. (5*S*)-Tetrahydro-5-[(triphenylmethoxy)methyl]-2-furanol (8). To a solution of **7** (46 g, 396 mmol) in dry CH_2Cl_2 (800 mL), Et_3N (82.8 mL, 594 mmol), trityl chloride (121.4 g, 435.6 mmol) and DMAP (9.68 g, 79.2 mmol) were sequentially added at 0 °C and stirred at room temperature for 12 h. The reaction was then quenched with saturated NH_4Cl solution, the organic layer was separated and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with water, brine, dried (Na_2SO_4) and concentrated in vacuo. Purification by column chromatography afforded (5*S*)-5-[(triphenylmethoxy)methyl]dihydrofuran-2(3*H*)-one (110.6 g) in 77% yield.

The product (110 g, 307 mmol) was dissolved in CH_2Cl_2 (600 mL) and the solution was cooled to −78 °C. DIBAL-H (1.2 M in toluene, 281 mL, 337.7 mmol) was added dropwise and stirred for 15 min at this temperature. The reaction mixture was then quenched with MeOH followed by saturated sodium potassium tartrate solution and stirred for 1 h. The organic layer was separated and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with water, brine, dried (Na_2SO_4) and concentrated in vacuo. Purification by column chromatography afforded the title compound **8** (95 g, 86%) as a mixture of diastereomers at the anomeric position. Data for **8**: R_f=0.35 (silica gel, 40% EtOAc in petroleum ether); IR (neat) ν_{max} 3448, 3350, 1479, 1433, 1219, 1075 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz, mixture of isomers 1:1) δ 7.48–7.22 (m, 15H, Ar*H*), 5.62 and 5.48 (two m, 1H, anomeric *H*), 4.45 (dq, J=7.5, 5.3 Hz) and 4.27 (m) (total 1H), 3.30 and 3.22 (two dd, J=9.8, 3.7, 9.8, 4.5 Hz) and 3.10 (d, J=5.3 Hz) (total 2H), 2.83 (d, J=6.0 Hz) and 2.50 (m) (total 1H), 2.08–1.82 (m, 3H), 1.70 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz, mixture of isomers) δ 144.00, 143.78, 128.74, 128.68, 127.77, 127.71, 127.02, 126.89, 98.91, 98.68, 79.31, 77.57, 66.84, 66.10, 34.03, 32.74, 25.93, 25.25; MS (ESI): m/z (%): 383 (100) $[M+Na]^+$, 399 (55) $[M+K]^+$; HRMS (ESI): Calcd for $C_{24}H_{24}O_3Na$ $[M+Na]^+$ 383.1623, found 383.1606.

4.3.2. (5*S*)-Tetrahydro-5-(hydroxymethyl)-2-furancarbonitrile (9). The lactol **8** (95 g, 264 mmol) was dissolved in CH_2Cl_2 (500 mL) and the solution was cooled to 0 °C. Et_3N (55.2 mL, 396 mmol) was added drop wise. After 10 min, Ac_2O (30 mL, 317 mmol) was added followed by DMAP (6.48 g, 53 mmol) and stirred for 30 min. The reaction was then quenched with saturated NH_4Cl solution, the organic layer was separated and the aqueous layer was extracted with EtOAc. The combined organic extracts were washed with water, brine, dried (Na_2SO_4) and concentrated in vacuo. Purification by column chromatography afforded the expected acetate intermediate (102 g) in 96% yield as a mixture of diastereomers at the anomeric position.

The acetate (100 g, 249 mmol) was dissolved in acetonitrile (500 mL) and to it trimethylsilyl cyanide (50 mL, 375 mmol) was added at room temperature. This was followed by the addition of $BF_3.Et_2O$ (25.2 mL, 199 mmol) and the reaction mixture was stirred for 4 h. The solution was then concentrated and purified by column chromatography to afford the title compound **9** (20.2 g) as a mixture of diastereomers in 64% yield. Data for **9**: R_f=0.39 (silica gel, 70% EtOAc in petroleum ether); IR (neat) ν_{max} 3410 (br), 2896, 1752, 1704, 1432, 1204, 1166, 1120, 1040, 784 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz, mixture of isomers 1:1) δ 4.79 and 4.71 (two dd, J=4.2, 7.8, 3.62, 7.8 Hz, total 1H), 4.33–4.28 and 4.21–4.15 (two m, total 1H), 3.78 (m,

1H), 3.66 and 3.56 (two dd, J=6.03, 12.0, 4.83, 12.0 Hz, 2H), 2.38–2.25 and 2.13–2.00 (two m, 2H), 2.21–2.14 and 1.94–1.86 (two m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz, mixture of isomers) δ 119.40, 119.00, 82.19, 80.82, 66.72, 66.40, 64.27, 63.90, 31.99, 31.61, 26.52, 26.03; MS (ESI): m/z (%): 150 (12) $[M+Na]^+$; HRMS (ESI): Calcd for $C_6H_9NO_2Na$ $[M+Na]^+$ 150.0531, found 150.0531.

4.3.3. (2*S*,5*R*)-5-[*N*-(9-Fluorenylmethoxycarbonyl)-aminomethyl]tetrahydro-2-furanmethanol (10). To the solution of **9** (20 g, 157.48 mmol) in dry ether (350 mL) at 0 °C, $LiAlH_4$ (14.94 g, 393.7 mmol) was added portion wise. After the addition was over the solution was refluxed for 3 h. It was then cooled to 0 °C and quenched by the sequential addition of water (15 mL), 3 M NaOH (15 mL) and water (45 mL). Stirring was continued till free flowing solids were formed. Then the mixture was filtered through a sintered funnel, and washed thoroughly with EtOAc. The combined organic filtrate and washings were concentrated in vacuo to dryness. The resulting crude amine was dissolved in CH_2Cl_2 (250 mL), FmocOSu (58.4 g, 173.23 mmol) was added at 0 °C and stirred for 1 h at rt. Then the solution was concentrated in vacuo and directly subjected to purification by column chromatography to separate the isomers furnishing the title compound **10** (15.1 g, 57% yield) as colorless oil. Data for **10**: R_f=0.33 (silica gel, 70% EtOAc in petroleum ether); $[\alpha]^{26}_D$=−4.47 (c 1.9, $CHCl_3$); IR (neat) ν_{max} 3426 (br), 2924, 2850, 2337, 1704, 1538, 1436, 1220, 1085, 772, 656 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.76 (d, J=7.84 Hz, 2H, Ar*H*), 7.60 (d, J=7.24 Hz, 2H, Ar*H*), 7.39 (t, J=7.24 Hz, 2H, Ar*H*), 7.31 (t, J=7.24, Hz, 2H, Ar*H*), 5.22 (bs, 1H, N*H*), 4.45–4.39 (m, 2H, OCH_2 of Fmoc), 4.22 (t, J=6.64 Hz, 1H, OCH_2CH of Fmoc), 4.08–4.01 (m, 2H), 3.75 (dd, J=11.5, 1.8 Hz, 1H), 3.48 (dd, J=11.5, 4.83 Hz, 1H), 3.36–3.22 (m, 2H), 2.45 (bs, 1H, O*H*), 2.02–1.95 (m, 1H), 1.93–1.86 (m, 1H), 1.84–1.78 (m, 1H), 1.68–1.55 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 157.07, 144.53, 143.90, 141.25, 128.48, 127.97, 127.61, 127.00, 125.03, 119.89, 80.30, 78.88, 66.58, 64.37, 47.16, 45.29, 28.44, 26.47, 22.65; MS (ESI): m/z (%): 376 (95) $[M+Na]^+$; HRMS (ESI): Calcd for $C_{21}H_{23}NO_4Na$ $[M+Na]^+$ 376.1525, found 376.1491.

4.3.4. (2*S*,5*R*)-5-[*N*-(9-Fluorenylmethoxycarbonyl)-aminomethyl]tetrahydro-2-furancarboxylic acid (11). To a solution of **10** (14 g, 39.62 mmol) in acetone (100 mL) at 0 °C, was added freshly prepared Jones reagent carefully and drop by drop till the orange color persisted. The reaction mixture was stirred for 0.5 h at 0 °C, then warmed to rt, and stirred for an additional 3 h. Then the reaction mixture was quenched with isopropanol (80 mL) and diluted with EtOAc, washed with water, brine, dried (Na_2SO_4) and concentrated in vacuo. Purification by colomun chromatography afforded the title compound **11** (9.72 g) in 67% yield. Data for **11**: R_f=0.4 (10% MeOH in $CHCl_3$); $[\alpha]^{26}_D$=−6.4 (c 0.74, MeOH); IR (neat) ν_{max} 3418 (br), 2898, 2841, 2363, 1628, 1436, 1372, 1256, 1134, 1070, 762, 651 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.87 (d, J=7.81 Hz, 2H, Ar*H*), 7.68 (d, J=7.21 Hz, 2H, Ar*H*), 7.64 (br s, 1H, N*H*), 7.40 (t, J=7.2 Hz, 2H, Ar*H*), 7.32 (t, J=7.2 Hz, 2H, Ar*H*), 4.34–4.19 (m, 4H), 4.01 (t, J=6.01 Hz, 1H), 3.22–3.10 (m, 2H), 2.16 (td, J=8.4, 20.4 Hz, 1H), 1.95 (m, 1H), 1.88 (m, 1H), 1.58 (td, J=8.4, 19.8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 174.97, 156.53, 144.00, 140.86, 127.79, 127.23, 125.37, 120.25, 79.22, 76.51, 65.66, 46.84, 44.34, 29.98, 27.80; MS (ESI): m/z (%): 390 (100) $[M+Na]^+$, 406 (10) $[M+K]^+$; HRMS (ESI): Calcd for $C_{21}H_{21}NO_5Na$ $[M+Na]^+$ 390.1317, found 390.1310.

4.3.5. *N*-(9-Fluorenylmethoxycarbonyl)-6-amino-2,5-anhydro-1-*O*-(*tert*-butyldiphenyl)silyl-6-deoxy-D-glucitol (13). To a solution of compound **12** (9.56 g, 21.55 mmol) in MeOH was added 10% $Pd(OH)_2$ on C (1.08 g). It was hydrogenated for 8 h under atmospheric pressure using a H_2 balloon. The reaction mixture was then filtered through a short pad of Celite and the filter cake was washed with MeOH. The filtrate and washings were combined and concentrated in vacuo. The residue was azeotroped with dry toluene and used directly in the next step without further purification.

The above-prepared intermediate triol was dissolved in dry DMF (65 mL) and treated at 0 °C under a nitrogen atmosphere with Et_3N (4.5 mL, 32.33 mmol). After 10 min, TBDPSCl (6.08 mL, 23.71 mmol) followed by DMAP (264 mg, 2.16 mmol) were added. After stirring for 3 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, 30–35% EtOAc in petroleum ether eluant) afforded the title compound **13** (9.84 g, 91% in two steps). Data for **13**: R_f=0.6 (silica gel 60% EtOAc in petroleum ether); $[\alpha]^{26}_D$=−22.83 (c 2.37, $CHCl_3$); IR (neat) ν_{max} 3418 (br), 3214, 2970, 2932, 2360, 1695, 1513, 1428, 1391, 1366, 1252, 1221, 1167, 1111, 824, 770, 704, 613, 505 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.71 (d, J=7.7 Hz, 2H, Ar*H*), 7.65 (d, J=7.7 Hz, 2H, Ar*H*), 7.44–7.37 (m, 6H, Ar*H*), 4.89 (br s, 1H, N*H*), 4.24 (m, 1H), 4.01 (dd, J=3.6, 8.3 Hz, 2H), 3.99 (dd, J=4.7, 8.9 Hz, 1H), 3.94 (dd, J=4.7, 8.3 Hz, 1H), 3.73 (q, J=4.7 Hz, 1H), 3.63 (br s, 1H, O*H*), 3.41 (m, 1H), 3.37 (m, 1H), 2.58 (br s, 1H, O*H*), 1.43 (s, 9H, Boc), 1.06 (s, 9H, Si^{t}Bu); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.58, 135.58, 135.49, 132.51, 132.30, 129.94, 127.80, 83.01, 79.63, 79.43, 79.08, 63.35, 42.41, 28.33, 26.73, 18.98; MS (LSIMS): m/z (%): 402 (95) $[M+H-C_5H_8O_2]^+$; HRMS (LSIMS): Calcd for $C_{22}H_{32}NO_4Si$ $[M+H-C_5H_8O_2]^+$ 402.2101, found 402.2108.

4.3.6. (2*R*,5*S*)-5-[*N*-(*tert*-Butoxycarbonyl)-aminomethyl]tetrahydro-2-furanmethanol (14). To a solution of compound **13** (9.84 g, 19.61 mmol), Ph_3P (20.58 g, 78.45 mmol) and imidazole (5.34 g, 78.45 mmol) at reflux in toluene (200 mL), I_2 (14.93 g, 58.84 mmol) was added in small portions with stirring. The white finely dispersed complex initially formed was transformed into a clear yellow solution that darkened as iodine was liberated at the bottom of the reaction vessel, a dark tarry complex was formed from which the product was gradually dissolved. After 3 h, the reaction mixture was cooled and iodine (4.98 g, 19.6 mmol) was added, followed by aqueous sodium hydroxide (6.28 g, 156.88 mmol, 120 mL water). The mixture was stirred until virtually all of the tarry red deposits were dissolved. The mixture was transferred into a separating funnel. The aqueous layer was separated and the organic layer was washed successively with water, saturated aqueous sodium thiosulphate, saturated aqueous $NaHCO_3$

and brine. The organic layer was dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by column chromatography (SiO_2, 15–18% EtOAc in petroleum ether eluant) afforded the olefin intermediate (7.79 g, 85%).

To a solution of the olefin intermediate (6.52 g, 13.94 mmol) in MeOH (100 mL) was added 10% $Pd(OH)_2$ on C (700 mg). It was hydrogenated for 1 h under atmospheric pressure using a H_2 balloon. The reaction mixture was then filtered through a short pad of Celite and the filter cake was washed with MeOH. The filtrate and washings were combined and concentrated in vacuo. The residue was azeotroped with dry toluene to afford the 3,4-dideoxy intermediate, which was used directly in the next step without further purification.

A solution of the above-prepared dideoxy compound in THF (42 mL) was treated at 0 °C with TBAF (1 M in THF, 15.33 mL, 15.33 mmol). The reaction mixture was stirred at room temperature for 3H, quenched with saturated NH_4Cl solution, and extracted with EtOAc. The combined organic extracts were washed with brine, dried (Na_2SO_4) and concentrated in vacuo. Purification by column chromatography (SiO_2, 65–70% EtOAc in petroleum ether eluant) afforded the title compound **14** (2.09 g, 65% in two steps). Data for **14**: R_f=0.4 (silica gel, 70% EtOAc in petroleum ether); $[\alpha]_D^{26}$=(55.59 (*c* 1.6, $CHCl_3$); IR (neat) ν_{max} 3322 (br), 3014, 2976, 1692, 1516, 1393, 1366, 1251, 1218, 1169, 1080, 978, 941, 771, 697, 665 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 4.89 (t, *J*=5.6 Hz, 1H, N*H*), 4.05 and 4.01 (two m, 2H), 3.74 (dd, *J*=2.7, 11.7 Hz, 1H), 3.49 (dd, *J*=4.9, 11.7 Hz, 1H), 3.24 (ddd, *J*=3.7, 5.5, 13.5 Hz, 1H), 3.18 (dd, *J*=6.7, 13.5 Hz, 1H), 2.66 (br s, 1H), 1.99 (m, 1H), 1.88 (m, 1H), 1.69 (m, 1H), 1.64 (m, 1H), 1.45 (s, 9H, Boc); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.58, 80.35, 79.36, 78.99, 64.33, 44.94, 28.47, 28.31, 26.41; MS (LSIMS): *m*/*z* (%): 132 (95) $[M+H-C_5H_8O_2]^+$, 232 (35) $[M+H]^+$; HRMS (ESI): Calcd for $C_{11}H_{21}NO_4Na$ $[M+Na]^+$ 254.1368, found 254.1369.

4.3.7. (2*R*,5*S*)-5-[*N*-(9-Fluorenylmethoxycarbonyl)-aminomethyl]tetrahydro-2-furancarboxylic acid (15). A mixture of $NaIO_4$ (5.58 g, 26.07 mmol) and $RuCl_3 \cdot 3H_2O$ (22.5 mg, 0.087 mmol) in $CH_3CN/CCl_4/H_2O$ (1:1:1.5, 53 mL) was stirred at rt for 45 min and then added to a solution of the alcohol (2.01 g, 8.69 mmol) in CH_3CN (25 mL) at 0 °C. After stirring for 5 min, an additional amount of $NaIO_4$ (1.86 g, 8.69 mmol) was added to the reaction mixture. After 2 h, it was diluted with EtOAc, washed with saturated aqueous NH_4Cl, brine, dried (Na_2SO_4) and concentrated in vacuo. Purification by column chromatography afforded the acid (1.71 g, 80%).

To a solution of the acid in dry CH_2Cl_2 (16 mL) at 0 °C, trifluoroacetic acid (5 mL) was added and stirred for 2 h at rt. The excess trifluoroacetic acid was then evaporated off on rotary evaporator and dried thoroughly under high vacuum. The resulting TFA salt was dissolved in dioxane and cooled to 0 °C. Then 10% aqueous Na_2CO_3 (17.4 mL) followed by FmocOSu (2.35 g, 6.97 mmol) were added and stirred for 12 h at rt. The dioxane was then evaporated in vacuo and the residual aqueous layer was washed with EtOAc. The aqueous layer was acidified with 1 M HCl pH≈2, extracted with EtOAc, washed with water, brine, dried (Na_2SO_4) and concentrated in vacuo. Purification by column chromatography (SiO_2, 12–15% MeOH in chloroform eluant) afforded the title compound **15** (2.17 g, 85% yield). Data for **15**: R_f=0.4 (10% MeOH in $CHCl_3$); $[\alpha]_D^{26}$=+8.13 (*c* 1.01, MeOH); IR (neat) ν_{max} 3304 (br), 3019, 1698, 1622, 1449, 1331, 1216, 1099, 977, 943, 753, 696 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) δ 8.25 (br s, 1H, N*H*), 7.87 (d, *J*=7.2 Hz, 2H, Ar*H*), 7.71 (d, *J*=7.2 Hz, 2H, Ar*H*), 7.40 (t, *J*=7.2 Hz, 2H, Ar*H*), 7.31 (t, *J*=7.2 Hz, 2H, Ar*H*), 4.34–4.19 (m, 4H), 4.05 (t, *J*=6.01 Hz, 1H), 3.25–3.15 (m, 2H), 2.08 (m, 1H), 1.94 (m, 1H), 1.80 (m, 1H), 1.61 (td, *J*=8.4, 19.8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 176.24, 156.64, 143.93, 140.69, 127.62, 127.12, 125.38, 120.10, 78.55, 78.08, 65.57, 46.70, 43.97, 30.28, 27.28; MS (LSIMS): *m*/*z* (%): 390 (65) $[M+Na]^+$; HRMS (LSIMS): Calcd for $C_{21}H_{21}NO_5Na$ $[M+Na]^+$ 390.1317, found 390.1304.

4.3.8. Data for 1. ^1H NMR (DMSO-d_6, 500 MHz): see Table 1; quant. HPLC profile: RP-18 column, 50×300 mm, mobile phase: A=water (0.1% TFA), B=acetonitrile (0.1% TFA), gradient: 20–40% B in 20 min, detection at 220 nm, retention time=7.71 min; MS (LSIMS): *m*/*z* (%): 1029 (4.6) $[M+H]^+$.

4.3.9. Data for 2. ^1H NMR (DMSO-d_6, 500 MHz): see Table 2; quant. HPLC profile: RP-18 column, 50×300 mm, mobile phase: A=water (0.1% TFA), B=acetonitrile (0.1% TFA), gradient: 20–40% B in 20 min, detection at 220 nm, retention time=6.96 min; MS (LSIMS): *m*/*z* (%): 895 (70) $[M+H]^+$, 917 (20) $[M+Na]^+$; HRMS (LSIMS): Calcd for $C_{42}H_{71}N_8O_{11}S$ $[M+H]^+$ 895.4963, found 895.4970.

4.3.10. Data for 3. ^1H NMR (DMSO-d_6, 500 MHz): see Table 3; quant. HPLC profile: RP-18 column, 50×300 mm, mobile phase: A=water (0.1% TFA), B=acetonitrile (0.1% TFA), gradient: 20–40% B in 20 min, detection at 220 nm, retention time=6.60 min; MS (LSIMS): *m*/*z* (%): 895 (100) $[M+H]^+$, 917 (11) $[M+Na]^+$; HRMS (LSIMS): Calcd for $C_{42}H_{71}N_8O_{11}S$ $[M+H]^+$ 895.4963, found 895.4982.

4.3.11. Data for 4. ^1H NMR (DMSO-d_6, 500 MHz): see Table 4; quant. HPLC profile: RP-18 column, 50×300 mm, mobile phase: A=water (0.1% TFA), B=acetonitrile (0.1% TFA), gradient: 20–40% B in 20 min, detection at 220 nm, retention time=7.33 min; MS (LSIMS): *m*/*z* (%): 553 (98) $[M+H]^+$, 575 (9) $[M+Na]^+$; HRMS (LSIMS): Calcd for $C_{26}H_{41}N_4O_7S$ $[M+H]^+$ 553.2695, found 553.2686.

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Supplementary data

Supplementary data associated with this article can be found at doi:10.1016/j.tet.2004.07.032.

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