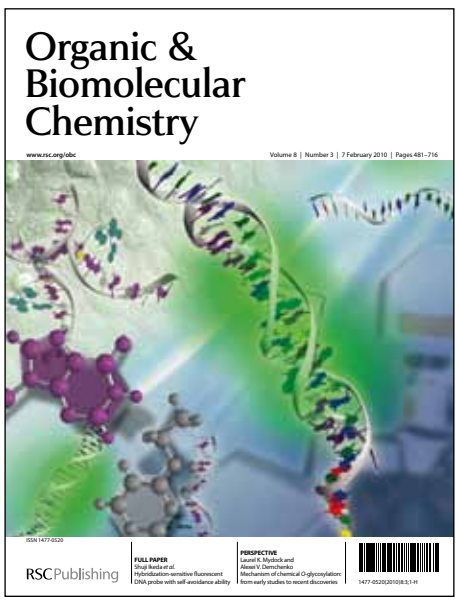
Organic & Biomolecular Chemistry

Accepted Manuscript



Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Conception of Pseudo-β-hairpin Motif Utilizing Ant-Pro Reverse Turn: Consequences of Stereochemical Reordering

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

Herein, we report a special case of *pseudo*-β-hairpin formation by tetrapetide sequences featuring two-membered Ant-Pro dipeptide motif (Ant = anthranilic acid and Pro = proline) at loop region. These folded structures uniquely feature the presence of C9- and C17- H-bonding patterns at reverse turn and interstrand regions, respectively. Their hairpin nucleation and folding propensities have been expounded using solution- and solid state studies of distinct stereochemically altered sequences.

Introduction

Incorporation of diverse synthetic secondary structural elements into functional bioactive peptide core has increasingly meliorated the understanding about the interactions of small molecules with biological targets such as enzymes / receptors, *etc*.[1] This aspect of peptidomimetics has led to rapid development in peptide-based catalysis and therapeutics.[2] In this connection, our group has been keenly involved in developing hybrid aliphatic-aromatic amino acid conjugates utilising natural and unnatural building blocks.[3] *En route,* we achieved interesting conformationally ordered structural architectures like turns,[4] helices,[5] sheets,[6] folds,[7] zipper motifs,[8] *etc*.

Herein, we unveil another striking case of *pseudo*-β-hairpin formation by tetrapeptide sequences comprising Ant-Pro dipeptide (at loop region) adjoining sheet promoting amino acids valine and leucine at N- and C- termini, respectively. These tetrapeptides are shown to exhibit two inter-residual hydrogen-bonding *viz*. (i→i+1)-type C9- and (i-2←i+3)-type C17-networks, between the central and terminal residues, respectively, and are anticipated to promote anti-parallel β-sheet formation.

β-Hairpin is one of the smallest secondary structural folding motifs that causes augmentation of the β-sheet secondary structure in proteins.[9] Further, β- hairpins are amongst the most preferred motifs / candidates for 'protein epitope mimetic' design

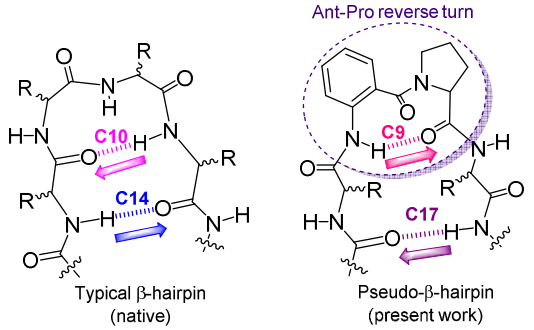

Figure 1: Schematic representation with *intramolecular* H-bonding patterns with their directions (highlighted with shaded arrows) of a typical β-hairpin motif found in proteins (left) and novel *pseudo*-β-hairpin featuring Ant-Pro reverse turn (right). *Note that there are distinctive directional changes in the H-bonding pattern in the native hairpin and pseudo-β-hairpin reported herein.*

owing to their participation in various molecular recognition events.[10] Development of such mimics is expected to further widen the scope of peptides in practical utility.

Ant-Pro unit is evidently known to exhibit a unique nine-membered H-bonding interaction between amide of Ant and C=O of Pro units, driven by the sterically enforced orthogonal arrangement of the rings.[4a] This characteristic feature of this dipeptide unit is in contrast to the native β-turns that display a backward hydrogen-bonding pattern (1←4)-type involving four residues. A juxtaposition of the native β-hairpin and *pseudo*-β-hairpin demonstrating the disparate H-bonding networks have been shown in the figure 1 (*vide supra*). An exhaustive analysis *via* structural and chirality perturbation in-and-around the turn segment confirmed strong reverse turn inducing nature of Ant-Pro motif.[11] Incidentally, the observed Pro ϕ and φ dihedral angles in the Ant-Pro reverse-turn motif described herein are typical of the *polyproline II* semi-extended conformation.[12] Conjoining this dipeptide segment with the sheet preferring amino acids led to formation of β-hairpin mimic with two unusual H-bonding patterns.

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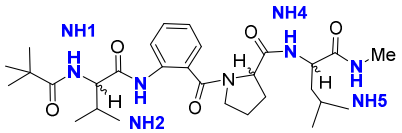
Electronic supplementary information (ESI †) available: Full experimental procedures, ^{1}H NMR, ^{13}C NMR, and ESI mass spectra of all new compounds. See DOI: 10.1039/c0xx00000x

Stereochemical alteration is known to cause conformational modulation in different secondary structures, thus serving as good means to assess the stability and conformational propensity of different peptide chains.[13] Therefore, we designed, synthesized and evaluated the hairpin nucleation tendencies of different stereochemically reordered[14] tetrapeptide sequences: R_1CO-$^{L/D}$Val-Ant-$^{L/D}$Pro-$^{L/D}$Leu-NHR_2, comprising Ant-Pro turn motif at the loop region (Fig. 2). Derivatives with varying stereochemical patterns of α-amino acids *viz* LLL (homochiral) and DLL (heterochiral), LDL and DLD (heterochiral with alternating stereochemisty) were synthesized and their structural pre-disposition was explored. In order to carry out the solution phase investigations, pivolyl- protection was preferred that confers structural rigidity at the N-terminus and methylamide protection at C-terminus for a distinct non-overlapping signal.



1 - 4

1 Piv-^{L}Val-Ant-^{L}Pro-^{L}Leu-NHMe **2** Piv-^{D}Val-Ant-^{L}Pro-^{L}Leu-NHMe
3 Piv-^{L}Val-Ant-^{D}Pro-^{L}Leu-NHMe **4** Piv-^{D}Val-Ant-^{L}Pro-^{D}Leu-NHMe

Figure 2. Designed Ant–Pro oligomers with chirality modulation.

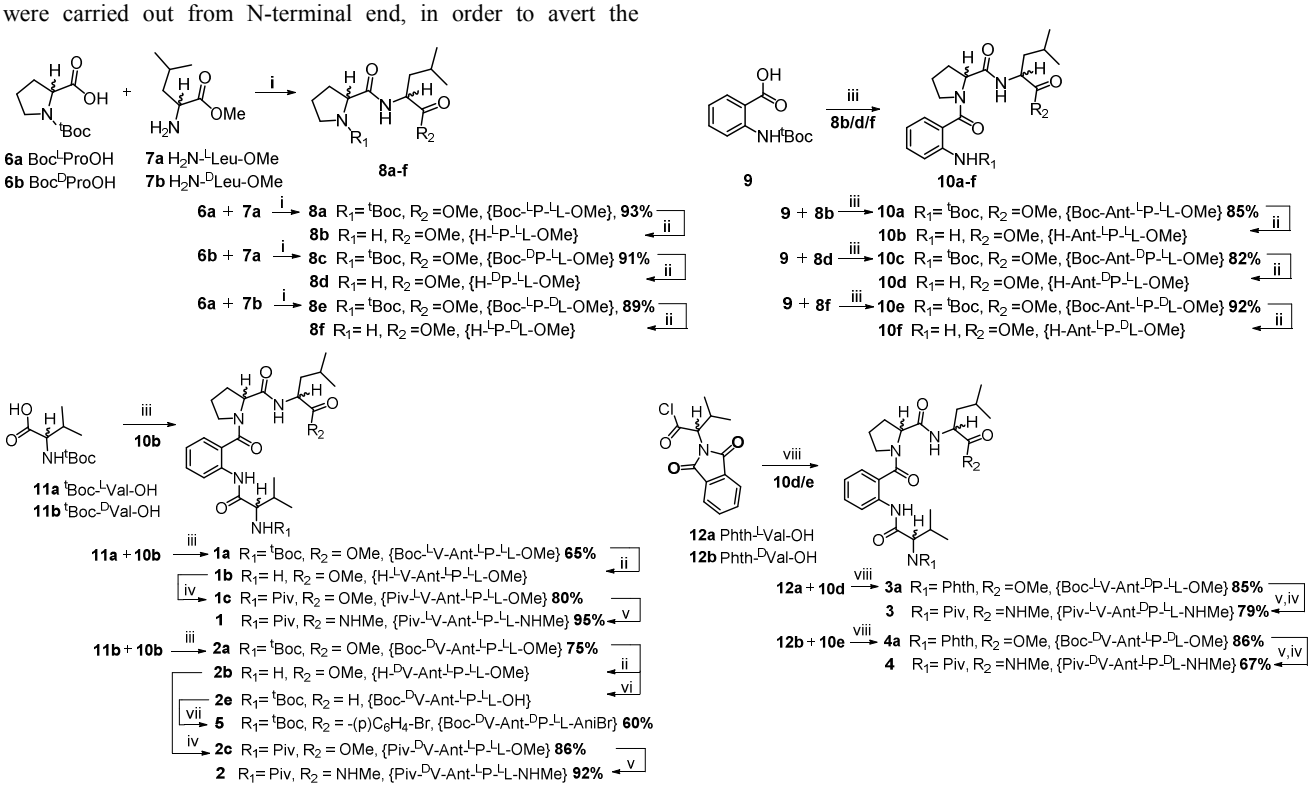
Results and discussion

Synthesis

Syntheses of all the tetrapeptide analogues **1-4** were carried out using conventional solution phase peptide synthesis under standard coupling conditions (Scheme 1). Coupling reactions were carried out from N-terminal end, in order to avert the formation of the benzoxazinone intermediate obtained on activation of anthranilic carboxylic group.[7a,11a,15] After synthesizing dipeptides **8a**, **8c** and **8e**, their corresponding amines were coupled with Boc-protected anthranilic acid **9** using HBTU as the coupling agent in presence of DIEA as base to afford tripeptides **10a**, **10c** and **10e**, respectively. Free amine liberated by deprotection of **10a** was coupled with Boc-^{L}Val-OH and Boc-^{D}Val-OH to afford tetrapeptides **1a** and **2a**, respectively. The N-terminus of tetrapeptide analogues was converted into their corresponding pivaloyl analogs **1c** and **2c**, which was then subjected to amidation by treating it with methanolic methylamine solution to afford **1** and **2**, respectively. For analogues **3** and **4**, the free amines **10d** and **10f** were subjected to coupling with phthalimide protected valine. The tetramers thus obtained were treated with methanolic methylamine for simultaneous phthalimide deprotection and methylamide conversion – followed eventually by N-terminus pivaloyl protection.

Solution-State Structural studies

The interactions arising from the folding pattern of peptides were evaluated by solution-state NMR studies, which were undertaken for all the tetramer analogues **1-4**. First glimpse at the ^{1}H-NMR spectra ($CDCl_3$, 298 K) of all the analogues suggested the presence of nine-membered H-bonding network from the appearance of anthranilamide NH protons at about 9-9.5 ppm (Fig. 3).[16] Similarly, the chemical shift values observed for NH5 provisionally provided a basis for the presence of 17-membered H-bonding interaction.[17]



Scheme 1 *Reagents and conditions:* (i) DCC, DMAP, DCM, 12h; (ii) TFA:DCM, 1:1, 2h; (iii) HBTU, DIEA, DCM, 12h; (iv) Piv-Cl, Et_3N, DCM, 0 °C-rt, 2h; (v) $MeNH_2$, MeOH, 4h; (vi) $LiOH.2H_2O$, $THF:H_2O$, 6h; (vii) H_2N-(p)C_6H_4-Br, HBTU, DIEA, DCM, 12h; (viii) TEA, DCM, 0 °C-rt, 1h.

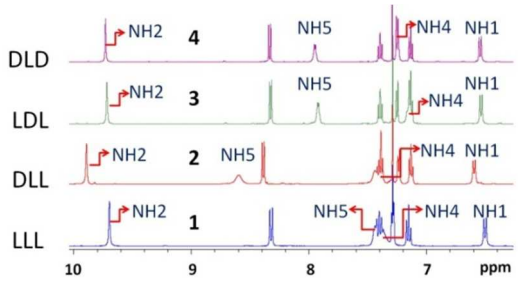


Figure 3. Stacked plot of amide region of tetramer analogues **1**-**4** for the comparison of chemical shift values of NH2 and NH5 amide protons. *Note*: Molecular structures of **1**- **4** are given in figure 2.

In the homochiral tetrapeptide **1**, appearance of relatively upfield and broad NH5 at 7.4 δ, also revealing concentration dependence (see ESI†, page S69, figure S17), clearly suggested the absence of terminal H-bonding interaction. In the case of all the heterochiral analogues **2 - 4**, the downfield chemical shifts for NH5 appearing >7.9 ppm **(**with consistent chemical shift values at different concentrations, see ESI†, page S69-S70, figure S18-S20) were indicative of the presence of terminal H-bonding interaction.

To accrue further evidences to validate these observations, we undertook extensive structural analysis using solution-state NMR studies. 2D NOESY experiments of all tetrapeptide sequences unambiguously confirmed the presence of C9- turn, evident from the prominent nOe cross-peaks observed between the amide NH2 *vs* $Pro_{\delta}H$ protons (C17/17'H) due to the folding induced by the 9-membered H-bonding formation (see ESI†, page S71-S74 for nOe details). Confirmation of 17- membered H-bonding network was obtained from the diagnostic long-range dipolar coupling interactions arising from the spatial proximity between Piv- (C27H) *vs* methylamide NH5 and methyl (C25H) protons (Fig. 4). The heterochiral analogues **3** and **4** displayed intense cross-peaks owing to both these terminal interactions (Fig. 4e-h). But, in case of homochiral analogue **1** and heterochiral tetrapeptide **2**, corresponding long range nOes were notably *weak* (Fig. 4a-c). Contradictory to the expectations from the downfield NH5 proton of compound **2** in its ^{1}H NMR spectrum, the 2D spectral interpretation revealed the partially bound state of NH5 with carbonyl of Piv- group. But, another *medium* dipolar coupling observed between NH5 and $Val_{\alpha}H$ (C2H) confirmed its folded orientation (Fig. 4d).

Evaluation of strength of Hydrogen-Bonds through NMR studies

Examination of the strength of these H-bonded contacts from MeOD exchange studies faced complications due to plausible differential solvation of amide NHs, which caused the protons to shift downfield and resist NH/D exchange (see ESI†, page S40).[18] Thus, variable temperature NMR experiments at 2 mM concentration for all tetrapeptide sequences were carried out.[19] The comparison of chemical shift values of NH2 (anthranilamide involved in C9- turn formation) and NH5 (methylamide proton that participates in C17- H-bonding network) particularly were thoroughly inspected (see ESI†, page S41-S49). Strikingly, a considerably lower temperature coefficient (ΔδHN/ΔT) of ≈ -2.5 ppb/K was observed for NH2 of tetrapeptides **1** and **2**, but a poor value > -7.5 ppb/K for NH5 indicated solvent exposition of terminal amide proton (see ESI†, page S41-S44). It is noteworthy that ΔδHN/ΔT lower than -4 ppb/K is suggestive of strong intramolecular hydrogen bonding.[19]

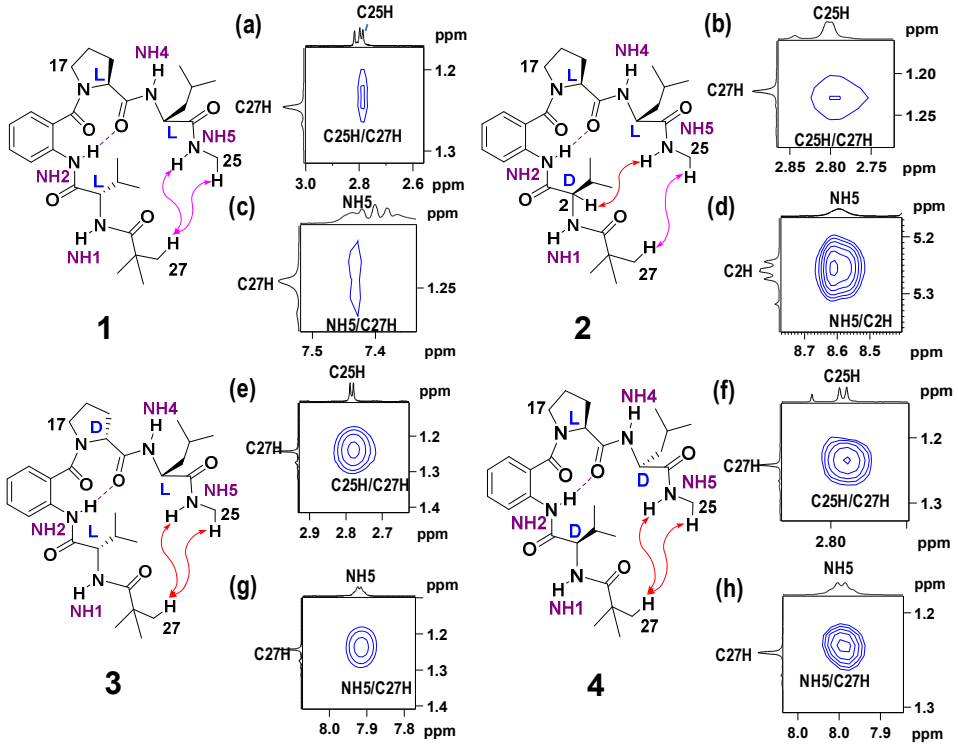


Figure 4. Selected nOe extracts from the 2D NOESY data of **1 - 4** ($CDCl_3$, 400/500 MHz, 298 K): (a), (b), (e) & (f) Piv *vs* methylamide -CH_3; (c), (g) & (h) Piv *vs* NH_5; (d) NH_5 *vs* $Val_{\alpha}H$.

Strangely for **3** and **4**, comparable values of about -5 ppb/K for both NH2 and NH5 were obtained (see ESI†, page S45-S48), revealing both amide protons in equilibrium between hydrogen-bonded and non-hydrogen-bonded states. These observations indicated the fact that alternate heterochiral analogues **3** and **4** exhibit a better stereochemistry, with respect to compounds **1** and **2**, for possible hairpin nucleation.

Variable temperature study was undertaken for tetrapeptide **5**, which strangely revealed temperature coefficient of -5.9 ppb/K (see ESI†, page S49), contradicting the observations of the strong terminal *intra*-molecular H-bonding contact apparent from its crystal structure (*vide infra*). These observations presumably suggest the predominance of packing effects in crystals. However, the collective interpretation of solution-state NMR studies suggest that though the heterochiral tetrapeptides **2** and **5** reveal the sign of hairpin nucleation, the orientation of NH5 poorly drives the chains in parallel fashion.

Solid-state analysis of tetramer 5

Extensive efforts culminated in obtaining crystals of heterochiral DLL derivative **5** (Fig. 5).[20] Analogue **5** was synthesized by coupling acid counterpart of **2a** with 4-bromoaniline. Interestingly, the crystal structure revealed the presence of strong C9-turn between anthranilamide NH2 and C=O of ^{L}Pro residue in *i*→*i+1* forward direction supporting the earlier observations. It also revealed presence of a 17-membered H-bonded ring between the C=O of ^{t}Boc functionality of Val and the methylamide functionality of the Leu residue in *i-2*←*i+3* reverse direction. Strikingly, the observed H-bond distances [d(N-H···O=C)] = 2.1 Å and H- bonding geometry of the C9- and C17- membered H-bonds are characterized by the angles (N-H···O) = 165° and 168°, respectively confirming equal strength for both the interactions.

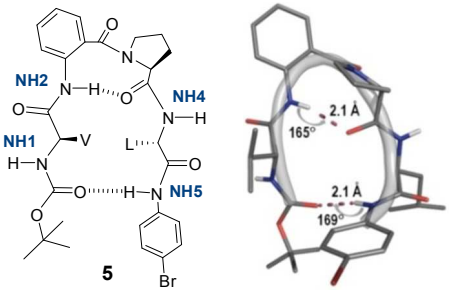


Figure 5: Molecular structure (left) and crystal structure (right) of **5**.

IR and CD studies

IR spectra were recorded in $CHCl_3$ at different concentrations, which displayed single strong absorption band due to N-H stretch about ≈ 3310 cm^{-1}, supporting the presence of intramolecularly H- bonded amide protons of analogues **3** and **4** (see ESI†, page S77-S78). In contrast, tetrapeptide **1** exhibited no sharp bands NH stretching representing the availability of amide protons for intermolecular contacts.[8a] Analogue **2** on the other hand featured strong absorption band at about 3311 cm^{-1} and 3439 cm^{-1} confirming a partially bound state of the amide NHs (see ESI†, page S75-S76). These observations were corroborated well with CD studies which revealed almost similar behaviour.

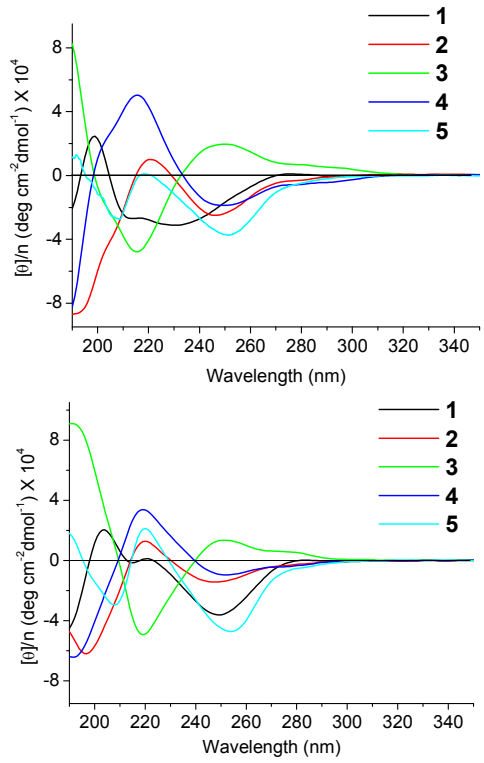


Figure 6. Comparison of CD spectra of tetramer analogues **1**- **5** in TFE (top) and **1**- **5** in acetonitrile (bottom), respectively (0.1 mM).

CD spectra were recorded in non-polar solvent like 2,2,2-triflouroethanol (TFE) and in a polar competing solvent like acetonitrile for a comparison (Fig. 6). From the patterns obtained, the maxima/minima ca. 218 nm were indicative of the hairpin-like folding pattern that was consistently observed in alternate heterochiral analogues **3** and **4** featuring an exact mirror image of the absorption peaks, irrespective of the solvent variation.

The homochiral tetrapeptide **1** displayed totally distinct CD signatures on solvent variation with no defined cotton effect, though a slight conformational ordering was witnessed in acetonitrile. Analogues **2** and **5** displayed slight changes in the absorption values *i.e.* with a weak maxima ≈ 221 nm in TFE which appears at 218 nm in acetonitrile with enhanced intensity, revealing solvent dependence. Intense absorbance peaks at around 250 nm were consistently observed for all the tetrapeptide derivatives, owing to the backbone aromatic groups/aromatic electronic transitions.[21]

Conclusion

In conclusion, we have successfully demonstrated the essential requisites for the design of novel *pseudo*-β-hairpin mimics based on the Ant-Pro reverse turn motif. Detailed structural investigations suggest that configurational control has an immense role to play in orientating of the arms of the hairpin motif in parallel fashion to support β-sheet formation. Amongst the tetrapeptide sequences evaluated, heterochiral sequences with alternating stereochemistry exhibited the best hairpin nucleation propensity. We anticipate that this understanding will deeply benefit in developing the locale of *β*-hairpin mimetics.

Experimental procedures

Single crystal X-ray crystallographic studies of 5

Single crystals of **5** were grown by slow evaporation of the solution mixture of ethyl acetate and pet-ether. Colorless needle-type crystal of approximate size 0.26 x 0.21 x 0.03 mm^3, was used for data collection. Total runs = 3, total frames = 1265, θ range = 2.13 to 25.00°, completeness to θ of 25.00 º is 99.9 %, $C_{34}H_{46}BrN_5O_5$, M = 702.69. Crystals system: Orthorhombic, space group $P2_12_12_1$, a = 9.6704(5), b = 9.8238(6), c = 38.263(2) Å, V = 3634.9(3) $Å^3$, Z = 4, D_c = 1.280 g/cc, μ (Mo–Kα) = 1.179 mm^{-1}, 46085 reflections measured, 6400 unique reflections, 5166 observed [I>2σ(I)], R value 0.0447, wR_2 = 0.1143, largest diff. peak and hole 0.45 and -0.21 e. $Å^{-3}$.

General method for the synthesis of compounds 10a, 10c and 10e.

(*S*)-methyl 2-((*S*)-1-(2-((tert-butoxycarbonyl)amino)benzoyl) pyrrolidine-2-carboxamido)-4-methylpentanoate 10a.

To a solution of compound **8b** (1.8 g, 7.258 mmoles) and Boc-Ant-OH (1.37 g, 5.806 mmoles, 0.8 equiv) in DCM (30 mL), DIPEA (1.89 mL, 10.887 mmol, 1.5 equiv) and HBTU (3.3 g, 8.709 mmol, 1.2 equiv) were added at 0 °C. After 12 h, the reaction mixture was diluted with DCM (30 mL) and washed sequentially with saturated solutions of $NaHCO_3$ (20 mL), water (20 mL) and $KHSO_4$ (20 mL). The washings were extracted with DCM (10 mL x 3) and the combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to obtain the crude residue, which was purified by column chromatography (eluent: 30% AcOEt/pet. ether, Rf: 0.3) to afford **10a** as a waxy solid (2.3 g, 85%). $[\alpha]_D^{25.3}$: -121.10° (c = 1, $CHCl_3$); IR ($CHCl_3$, ν (cm^{-1}): 3364, 3020, 2977, 2401, 1725, 1683, 1624, 1521, 1417, 1159, 1046, 929; ^{1}H NMR ($CDCl_3$/200MHz): δ ppm 8.35 (s, 1H, amide), 8.17-8.13 (d, J = 8.34Hz, 1H), 7.41-7.31 (m, 2H), 7.04-6.92 (m, 2H), 4.81-4.74 (m, 1H), 4.66-4.54 (m, 1H), 3.73 (s, 3H), 3.57-3.47 (m, 2H), 2.33-1.78 (m, 4H), 1.71-1.60 (m, 2H), 1.50 (s, 9H), 0.93-0.88 (m, 6H); ^{13}C NMR ($CDCl_3$, 50MHz): δ ppm 173.2, 170.9, 168.9, 153.0, 137.2, 131.0, 127.4, 123.6, 121.8, 120.4, 80.4, 59.6, 52.2, 50.9, 50.6, 41.2, 28.3, 27.7, 25.3, 24.8, 22.7, 21.9; MALDI-TOF/TOF: 484.8722 $(M+Na)^+$; 500.8892 $(M+K)^+$; Elemental Analysis calculated for $C_{24}H_{35}N_3O_6$: C, 62.45; H, 7.64; N, 9.10; Found: C, 62.36; H, 7.56; N, 9.18.

(*S*)-methyl 2-((*R*)-1-(2-((tert-butoxycarbonyl)amino)benzoyl) pyrrolidine-2-carboxamido)-4-methylpentanoate 10c.

Compound **10c** was synthesized following the procedure for **10a**. The crude product was purified by column chromatography (eluent: 30% AcOEt/pet. ether, Rf: 0.3) to furnish **10c** (82%) as a waxy solid. $[\alpha]_D^{25.9}$: 111.728° (c = 1, $CHCl_3$); IR ($CHCl_3$, ν (cm^{-1}): 3355, 3017, 2975, 2897, 2401, 1724, 1683, 1625, 1590,1521, 1413, 1158, 1050, 928; ^{1}H NMR ($CDCl_3$/200MHz): δ ppm 8.23 (s, 1H, amide), 8.11-8.07 (d, J=8.34Hz, 1H), 7.42-7.27 (m, 3H), 7.08-7.01 (m, 1H), 4.90-4.84 (m, 1H), 4.67-4.58 (m, 1H), 3.70 (s, 3H), 3.60-3.48 (m, 2H), 2.46-2.38 (m, 1H), 2.15-1.82 (m, 4H), 1.70-1.60 (m, 2H), 1.50 (s, 9H), 0.95-0.93 (m, 6H); ^{13}C NMR ($CDCl_3$, 50MHz): δ ppm 173.3, 170.9, 170.2, 153.1, 136.9, 131.0, 127.5, 124.3, 122.2, 121.1, 80.4, 59.2, 52.3, 50.9, 50.3, 49.0, 41.2, 28.3, 27.0, 25.2, 24.9, 22.8, 21.7; MALDI-TOF/TOF: 485.0148 $(M+Na)^+$; 501.0496 $(M+K)^+$; Elemental Analysis calculated for $C_{24}H_{35}N_3O_6$: C, 62.45; H, 7.64; N, 9.10; Found: C, 62.36; H, 7.56; N, 9.18.

(*S*)-methyl 2-((*R*)-1-(2-((*tert*-butoxycarbonyl)amino)benzoyl) pyrrolidine-2-carboxamido)-4-methylpentanoate 10e.

Compound **10e** was synthesized following the procedure for **10a**. The crude product was purified by column chromatography (eluent: 30% AcOEt/pet. ether, Rf: 0.3) to furnish **10e** (92%) as a waxy solid. $[\alpha]_D^{26.1}$: -112.868° (c = 1, $CHCl_3$); IR ($CHCl_3$, ν (cm^{-1}): 3348, 3018, 2897, 2401, 1724, 1682, 1625, 1590,1520, 1412, 1369, 1158, 1052, 928; ^{1}H NMR ($CDCl_3$/200MHz): δ ppm 8.23 (s, 1H, amide), 8.10-8.06 (d, J=8.34Hz, 1H), 7.41-7.27 (m, 3H), 7.07-7.00 (m, 1H), 4.89-4.80 (m, 1H), 4.66-4.55 (m, 1H), 3.69 (s, 3H), 3.53-3.44 (m, 2H), 2.45-2.32 (m, 1H), 2.15-1.82 (m, 4H), 1.69-1.55 (m, 2H), 1.49 (s, 9H), 0.94-0.91 (m, 6H); ^{13}C NMR ($CDCl_3$, 50MHz): δ ppm 173.3, 170.9, 170.1, 153.1, 136.9, 131.0, 127.5, 124.2, 122.2, 121.0, 80.4, 59.2, 52.3, 50.8, 50.3, 41.2, 28.3, 27.0, 25.2, 24.8, 22.8, 21.7; MALDI-TOF/TOF: 484.8722 $(M+Na)^+$; 500.8892 $(M+K)^+$; Elemental Analysis calculated for $C_{24}H_{35}N_3O_6$: C, 62.45; H, 7.64; N, 9.10; Found: C, 62.43; H, 7.69; N, 8.98.

(*S*)-methyl 2-((*S*)-1-(2-((S)-2-((tert-butoxycarbonyl)amino)-3-methylbutanamido) benzoyl) pyrrolidine-2-carboxamido)-4-methylpentanoate 1a.

To a solution of compound **10b** (0.4 g, 1.108 mmoles, 1 equiv) and Boc-^{L}Val-OH (0.29 g, 1.329 mmoles, 1.2 equiv) in DCM (5mL), HBTU (0.546 g, 1.44 mmol, 1.3 equiv) and DIEA (0.287 mL, 1.662 mmol, 1.5 equiv) were added. After 12 h, the reaction mixture was diluted with DCM (30 mL) and washed sequentially with saturated solutions of $NaHCO_3$ (20 mL), water (20 mL) and $KHSO_4$ (20 mL). The washings were extracted with DCM (10 mL x 3) and the combined organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to obtain the crude residue, which was purified by column chromatography (eluent: 40% AcOEt/pet. ether, Rf: 0.4) to afford **1a** (0.40 g, 65%) as a sticky liquid. $[\alpha]_D^{26.9}$: -44.216° (c = 1, $CHCl_3$); IR ($CHCl_3$, ν (cm^{-1}): 3280, 3069, 2963, 2931, 2875, 1745, 1715, 1668, 1589, 1541, 1457, 1421, 1391, 1370, 1275, 1247, 1162, 1092, 1016, 987, 873; ^{1}H NMR ($CDCl_3$/200MHz): δ ppm 9.39 (s, 1H), 8.26-8.28 (d, J = 7.96 Hz, 1H), 7.37-7.21 (m, 2H), 7.09-7.02 (m, 1H), 6.78-6.74 (d, J = 7.96 Hz, 1H), 5.34-5.30 (d, J = 8.97 Hz, 1H), 4.68-4.53 (m, 2H), 4.28-4.21 (m, 1H), 3.68 (s, 3H), 3.48-3.17 (m, 2H), 2.21-2.11 (m, 3H), 1.98-1.5 (m, 5H), 1.36 (s, 9H), 0.97-0.94 (d, J = 6.69 Hz, 3H), 0.88-0.84 (m, 9H); ^{13}C NMR ($CDCl_3$, 50MHz): δ ppm 173.6, 171.6, 168.5, 165.6, 155.9, 134.8, 130.4, 126.6, 124.0, 121.9, 79.4, 68.6, 60.2, 59.9, 52.3, 51.0, 49.1, 43.9, 41.2, 31.3, 29.2, 28.2, 25.1, 24.7, 22.7, 21.8, 19.3, 17.5; MALDI-TOF/TOF: 584.1642 $(M+Na)^+$; 600.1794 $(M+K)^+$; Elemental Analysis calculated for $C_{29}H_{44}N_4O_7$: C, 62.12; H, 7.91; N, 9.99; Found: C, 62.15; H, 7.69; N, 9.88.

(*S*)-methyl 2-((*S*)-1-(2-((R)-2-((*tert*-butoxycarbonyl)amino)-3-methylbutanamido)benzoyl) pyrrolidine-2-carboxamido)-4-methylpentanoate 2a.

Compound **2a** was synthesized following the procedure for **1a.** The crude product was purified by column chromatography (eluent: 40% AcOEt/pet. ether, Rf: 0.4) to furnish **2a** (75%) as a sticky liquid. $[\alpha]_D^{26.9}$: -66.352° (c = 1, $CHCl_3$); IR ($CHCl_3$, ν (cm^{-1}): 3300, 2960, 2925, 1747, 1667, 1626, 1514, 1456, 1414, 1368, 1246, 1204, 1160, 1017, 875; IR ($CHCl_3$, ν (cm^{-1}): 3280, 3069, 2963, 2931, 2875, 1745, 1715, 1668, 1589, 1541, 1457, 1421, 1391, 1370, 1275, 1247, 1162, 1092, 1016, 987, 873; ^{1}H NMR ($CDCl_3$/200MHz): δ ppm 9.22 (s, 1H), 8.39-8.35 (d, J = 8.21 Hz, 1H), 7.41-7.26 (m, 2H), 7.11-7.03 (m, 1H), 6.97-6.93 (bs, 1H),

5.82-5.77 (d, J = 8.84 Hz, 1H), 4.71-4.54 (m, 2H), 4.19-4.12 (m, 1H), 3.72 (s, 3H), 3.42-3.37 (m, 2H), 2.26-2.19 (m, 4H), 2.02-1.51 (m, 4H), 1.40 (s, 9H), 0.99-0.87 (m, 12H); ^{13}C NMR ($CDCl_3$, 50MHz): δ ppm 173.1, 171.5, 171.0, 168.6, 165.6, 155.6, 135.3, 130.6, 126.8, 125.3, 123.4, 121.3, 79.3, 61.2, 59.7, 52.1, 51.1, 49.6, 41.0, 31.1, 28.7, 28.2, 25.2, 24.6, 22.6, 21.7, 19.2, 17.8; MALDI-TOF/TOF: 584.0563 $(M+Na)^+$; 600.0703 $(M+K)^+$; Elemental Analysis calculated for $C_{29}H_{44}N_4O_7$: C, 62.12; H, 7.91; N, 9.99; Found: C, 62.20; H, 7.72; N, 9.91.

(*S*)-2-((*S*)-1-(2-((*R*)-2-((*tert*-butoxycarbonyl)amino)-3-methyl butanamido)benzoyl) pyrrolidine-2-carboxamido)-4-methyl pentanoic acid 2e:

To a solution of **2a** (0.5 g, 0.091 mmol, 1 equiv.) in THF:H_2O (2:1 v/v), LiOH.$2H_2O$ (0.01 g, 0.182 mmol, 2 equiv.) was added and the reaction mixture was stirred for 12 h. After completion of the reaction, THF was removed *in vacuo* and the solution was neutralized using $KHSO_4$ solution. The crude product was extracted into ethyl acetate and was washed with brine solution. The organic layer was dried over Na_2SO_4 and evaporated *in vacuo* to afford **2e**. The crude product obtained was used for the next step without further purification.

***tert*-butyl ((*R*)-1-((2-((*S*)-2-(((*S*)-1-((4-bromophenyl)amino)-4-methyl-1-oxopentan-2-yl) carbamoyl)pyrrolidine-1-carbonyl) phenyl)amino)-3-methyl-1-oxobutan-2-yl)carbamate 5:**

To a solution of acid **2e** (0.1g, 0.183 mmol, 1 equiv) and 4-bromoaniline (0.031 g, 0.183 mmol, 1 equiv) in dry DCM (10 mL), HBTU (0.083g, 0.219 mmol, 1.2 equiv) and DIEA (0.041 mL, 0.238 mmol, 1.3 equiv) were added and the reaction was maintained at 0 °C. The mixture was stirred at room temperature for an additional 12 h. DCM (10 mL) was added to the reaction mixture and the combined organic layers were washed sequentially with solutions of $KHSO_4$, $NaHCO_3$ and brine. Organic layer was then dried over Na_2SO_4 and was evaporated *in vacuo*. The crude product was purified by column chromatography (eluent: 40% AcOEt/pet. ether, Rf: 0.4) to furnish **5** (0.75 g, 60%). as a white crystalline solid. mp: 229-231 °C; $[\alpha]_D^{27.1}$: -73.588° (*c* 1, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}): 3272, 2962, 2930, 2403, 1682.4, 1621, 1538, 1490, 1457, 1394, 1302, 1246, 1163, 1075, 1010, 828; ^{1}H NMR ($CDCl_3$,500MHz): δ ppm 9.64 (s, 1H, amide), 9.58 (s, 1H, amide), 8.34-8.32 (d, J = 8.24 Hz, 1H), 7.59-7.57 (m, 2H), 7.39-7.37 (m, 3H), 7.24-7.22 (m, 3H), 7.14-7.09 (m, 2H), 5.56-5.54 (d, J = 9.46 Hz, 1H), 4.96-4.88 (dd, J = 6.71Hz, J = 9.16 Hz, 1H), 4.72-4.69 (m, 1H), 4.60-4.56 (m, 1H), 3.39-3.31 (m, 2H), 2.30-2.24 (m, 1H), 2.07-1.84 (m, 4H), 1.74-1.64 (m, 3H), 1.46 (s, 9H), 0.98-0.96 (d, J = 6.10 Hz, 3H), 0.91-0.86 (m, 9H); ^{13}C NMR ($CDCl_3$, 125MHz): δ ppm 173.1, 171.6, 171.0, 168.6, 156.1, 137.6, 134.5, 131.6, 130.0, 127.0, 125.3, 123.9, 121.3, 121.25, 116.5, 79.9, 59.8, 58.9, 54.0, 49.2, 40.9, 33.3, 29.6, 28.5, 24.8, 22.6, 22.3, 18.9, 18.4; MALDI-TOF/TOF: 725.4464 $(M+Na)^+$; 741.5165 $(M+K)^+$; Anal. calcd for $C_{34}H_{46}BrN_5O_6$: C, 58.28; H, 6.62; Br, 11.40; N, 10.00; Found: C, 58.36; H, 6.69; Br, 11.41; N, 9.94.

General method for the synthesis of compounds 3a and 4a.

(*S*)-methyl 2-((*R*)-1-(2-((*S*)-2-(1,3-dioxoisoindolin-2-yl)-3-methylbutanamido) benzoyl) pyrrolidine-2-carboxamido)-4-methylpentanoate 3a.

To a solution of Phth-^{L}Val-OH (0.576g, 2.333 mmol, 1.2 equiv) in dry DCM (10 mL) and cat. amount of DMF, oxalyl chloride (0.20 mL, 2.527 mmol, 1.3equiv) was added dropwise at 0 °C. Later, the reaction was allowed to continue at rt for 1h. DCM was then stripped off *in vacuo*. The solution containing **10d** (0.7g, 1.944 mmol, 1 equiv) and Et_3N (0.394 mL, 2.916 mmol, 1.5 equiv) in dry DCM (15 mL) was cooled in an ice bath with stirring. A solution of Phth-^{L}Val-COCl in DCM (10 mL) was added dropwise for 15 min to the above reaction mixture. The mixture was stirred at room temperature for an additional 1 h. DCM (10 mL) was added to the mixture which was then washed with $KHSO_4$ solution (10 mL), saturated aqueous $NaHCO_3$ (10 mL), and brine (10 mL). The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude product was purified by column chromatography (eluent: 40% AcOEt/pet. ether, Rf: 0.4) to furnish **3a** (0.96 g, 85%) as a white crystalline solid. mp: 157-160 °C; $[\alpha]_D^{26.9}$: -117.856° (*c* 1, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}): IR ($CHCl_3$) ν (cm^{-1}): 3063, 2959, 2927, 2874, 1722, 1622, 1532, 1453, 1417, 1384, 1247, 1153, 1071, 988; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 9.54 (1H, amide), 8.08-8.04 (d, J = 8.08 Hz, 1H), 7.91-7.85 (m, 2H), 7.78-7.74 (m, 2H), 7.43-7.33 (m, 2H), 7.17-7.10 (m, 2H), 4.63-4.52 (m, 3H), 3.69 (s, 3H), 3.50-3.42 (m, 2H), 3.04-2.92 (m, 1H), 2.46-2.35 (m, 1H), 2.1-1.92 (m, 2H), 1.85-1.46 (m, 4H), 1.19-1.16 (d, J = 6.57 Hz, 3H), 0.93-0.90 (m, 9H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 173.1, 170.6, 170.2, 168.0, 166.8, 135.2, 134.3, 131.6, 130.9, 127.5, 126.2, 124.0, 123.5, 123.2, 61.6, 59.0, 52.2, 50.8, 50.4, 41.0, 38.6, 29.6, 27.5, 26.8, 25.2, 24.8, 22.8, 21.8, 20.5, 19.4; MALDI-TOF: 613.3940 $(M+Na)^+$, 629.4184 $(M+K)^+$; Anal. calcd for $C_{32}H_{38}N_4O_7$: C, 64.85; H, 6.80; N, 9.45; Found: C, 64.98; H, 6.69; N, 9.49.

(*R*)-methyl 2-((*S*)-1-(2-((*R*)-2-(1,3-dioxoisoindolin-2-yl)-3-methylbutanamido) benzoyl) pyrrolidine-2-carboxamido)-4-methylpentanoate 4a.

Compound **4a** was synthesized following the procedure for **3a.** The crude product was purified by column chromatography (eluent: 40% AcOEt/pet. ether, Rf: 0.4) to furnish **4a** (86%) as a white solid. mp: 151-153 °C; $[\alpha]_D^{26.2}$: -107.592° (*c* 1, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}): 3062, 2959, 2923, 2873, 2951, 1721, 1623, 1532, 1456, 1428, 1383, 1249, 1153, 1071; ^{1}H NMR (200 MHz, $CDCl_3$) δ: 9.55 (1H, amide), 8.08-8.04 (d, J = 7.96 Hz, 1H), 7.91-7.87 (m, 2H), 7.79-7.75 (m, 2H), 7.44-7.34 (m, 2H), 7.17-7.10 (m, 2H), 4.63-4.53 (m, 3H), 3.69 (s, 3H), 3.51-3.42 (m, 2H), 3.04-2.93 (m, 1H), 2.49-2.32 (m, 1H), 2.07-1.93 (m, 2H), 1.86-1.47 (m, 4H), 1.19-1.16 (d, J = 6.69 Hz, 3H), 0.94-0.90 (m, 9H); ^{13}C NMR (50 MHz, $CDCl_3$) δ: 173.1, 170.6, 170.2, 168.0, 166.9, 135.2, 134.32, 131.6, 130.9, 127.5, 126.3, 124.0, 123.5, 123.3, 61.6, 59.1, 52.2, 50.9, 50.4, 41.0, 38.6, 27.6, 26.8, 25.2, 24.8, 22.8, 21.8, 20.5, 19.5; MALDI-TOF: 613.7018 $(M+Na)^+$, 629.7104 $(M+K)^+$; Anal. calcd for $C_{32}H_{38}N_4O_7$: C, 64.85; H, 6.80; N, 9.45; Found: C, 64.97; H, 6.71; N, 9.51.

General method for the synthesis of compounds 1c and 2c:

(*S*)-methyl 4-methyl-2-((*S*)-1-(2-((*S*)-3-methyl-2-pivalamido butanamido)benzoyl) pyrrolidine-2-carboxamido)pentanoate 1c.

To a solution of **1b** (0.33 g, 0.713 mmol, 1 equiv) in dry DCM (5 mL), Et_3N (0.145 mL, 1.071 mmol, 1.5 equiv) was added. The reaction mixture was kept under N_2 atmosphere and the temperature was maintained at 0 °C. Piv-Cl (0.1 mL, 0.855 mmol, 1.2 equiv) was added drop wise slowly into the reaction mixture. After 15 min, reaction was allowed to continue at rt for 1 hr. DCM (10 mL) was added to the reaction mixture and DCM layer was washed with $NaHCO_3$ solution followed by water and brine. DCM layer was dried over Na_2SO_4 and was concentrated *in vacuo*. The crude product was purified by column chromatography (eluent: 45% AcOEt/pet. ether, Rf: 0.4) to furnish **1c** (0.30 g, 80%) as a sticky liquid. $[\alpha]_D^{25.7}$: -53.748° (*c* 1,

$CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}): 3408, 3020, 2400, 1635, 1420, 1318, 1079; ^1H NMR ($CDCl_3$/200MHz): δ ppm 9.44 (s, 1H), 8.25-8.21 (d, J = 7.96 Hz, 1H), 7.42-7.26 (m, 2H), 7.16-7.11 (m, 1H), 7.0-6.96 (d, J = 8.46 Hz, 1H), 6.43-6.39 (d, J = 8.59 Hz, 1H), 4.76-4.55 (m, 3H), 3.73 (s, 3H), 3.46-3.21 (m, 2H), 2.28-2.12 (m, 2H), 2.03-1.51 (m, 6H), 1.22 (s, 9H), 1.02-0.98 (d, J = 6.69 Hz, 3H), 0.93-0.85 (m, 9H); ^{13}C NMR ($CDCl_3$, 50MHz): δ ppm 178.6, 173.8, 171.7, 171.3, 168.4, 134.6, 130.4, 127.0, 126.7, 124.1, 122.1, 60.5, 58.3, 52.3, 50.8, 49.1, 41.2, 38.8, 31.2, 29.6, 29.2, 27.4, 25.1, 24.9, 22.7, 21.7, 19.3, 17.8; MALDI-TOF/TOF: 570.2427 $(M+K)^+$; Anal. calcd for $C_{29}H_{44}N_4O_6$: C, 63.95; H, 8.14; N, 10.29; Found: C, 63.88; H, 8.20; N, 10.25.

(*S*)-methyl 4-methyl-2-((*S*)-1-(2-((*R*)-3-methyl-2-pivalamido butanamido)benzoyl) pyrrolidine-2-carboxamido)pentanoate 2c.

Compound **2c** was synthesized following the procedure for **1c** from **2b.** The crude product was purified by column chromatography (eluent: 45% AcOEt/pet. ether, Rf: 0.4) to furnish **2c** (86%) as a sticky liquid. mp: 58-60 °C; $[\alpha]_D^{26.3}$: -89.084° (*c* 1, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}): 3283, 3073, 2960, 2873, 1747, 1667, 1626, 1588, 1524, 1455, 1417, 1370, 1301, 1201, 1155, 1025, 986, 914; ^1H NMR ($CDCl_3$/200MHz): δ ppm 9.53 (s, 1H), 8.32-8.28 (d, J = 8.21 Hz, 1H), 7.44-7.32 (m, 2H), 7.15-7.07 (m, 1H), 6.82-6.79 (d, J = 7.83 Hz, 1H), 6.51-6.47 (d, J = 8.21 Hz, 1H), 4.80-4.73 (m, 1H), 4.63-4.49 (m, 2H), 3.74 (s, 3H), 3.58-3.42 (m, 2H), 2.31-2.16 (m, 3H), 2.04-1.6 (m, 5H), 1.23 (s, 9H), 1.02-0.89 (m, 12H); ^{13}C NMR ($CDCl_3$, 50MHz): δ ppm 178.4, 173.3, 171.1, 170.4, 169.2, 135.9, 131.0, 127.3, 124.8, 123.4, 121.9, 59.7, 58.6, 52.3, 51.1, 50.5, 41.1, 38.9, 31.8, 29.6, 28.1, 27.5, 25.3, 24.8, 22.7, 21.9, 19.3, 17.7; MALDI-TOF/TOF: 570.1554 $(M+K)^+$; Anal. calcd for $C_{29}H_{44}N_4O_6$: C, 63.95; H, 8.14; N, 10.29; Found: C, 63.88; H, 8.20; N, 10.25.

(*S*)-*N*-((*S*)-4-methyl-1-(methylamino)-1-oxopentan-2-yl)-1-(2-((*S*)-3-methyl-2-pivalamidobutanamido)benzoyl)pyrrolidine-2-carboxamide 1.

Compound **1c** (0.2g, 0.25 mmol) was stirred in saturated solution of methanolic methylamine for 2h at rt. Solvent was evaporated *in vacuo* and the crude product was purified by column chromatography (eluent: 45% AcOEt/pet. ether, Rf: 0.4) to furnish **1** (95%) as a white fluffy solid. mp: 100-102 °C; $[\alpha]_D^{27.1}$: -50.672° (*c* 0.5, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}): 3440, 3318, 3020, 2967, 2401, 1648, 1589, 1509, 1457, 1420, 1369; ^1H NMR (400 MHz, $CDCl_3$) δ: 9.68 (1H, amide), 8.32-8.29 (d, J = 8.28 Hz, 1H), 7.42-7.35 (m, 3H), 7.28-7.26 (m, 1H), 7.16-7.12 (t, J = 7.53 Hz, 1H), 6.50-6.48 (d, J = 8.28 Hz, 1H), 4.98-4.94 (m, 1H), 4.74-4.71 (dd, J = 5.27 Hz, J = 8.03 Hz, 1H), 4.64-4.58 (m, 1H), 3.37-3.23 (m, 2H), 2.78-2.77 (d, J = 4.52 Hz, 3H), 2.35-2.26 (m, 1H), 2.17-1.07 (m, 3H), 1.85-1.80 (m, 1H), 1.70-1.57 (m, 3H), 1.23 (s, 9H), 1.02-1.0 (d, J = 6.78 Hz, 3H), 0.96-0.91 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 178.4, 173.9, 171.8, 171.3, 168.6, 134.5, 130.1, 127.7, 125.9, 124.1, 121.6, 60.5, 58.0, 52.2, 48.9, 40.4, 38.8, 32.3, 29.8, 29.7, 27.5, 26.4, 24.7, 24.6, 22.7, 22.3, 19.3, 18.2; MALDI-TOF: 567.2104 $(M+Na)^+$, 583.2536 $(M+K)^+$; Anal. calcd for $C_{29}H_{45}N_5O_5$: C, 64.06; H, 8.34; N, 12.88; Found: C, 64.17; H, 8.44; N, 12.87.

(*S*)-*N*-((*S*)-4-methyl-1-(methylamino)-1-oxopentan-2-yl)-1-(2-((*R*)-3-methyl-2-pivalamidobutanamido)benzoyl)pyrrolidine-2-carboxamide 2.

Compound **2** was synthesized following the procedure for **1** from **2c.** The crude product was purified by column chromatography (eluent: 45% AcOEt/pet. ether, Rf: 0.4) to furnish **2** (67%) as a sticky liquid. $[\alpha]_D^{27.1}$: -79.944° (*c* 0.5, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}): 3439, 3314, 3020, 2965, 2874, 2401, 1646, 1589, 1550, 1515, 1456, 1417, 1370, 1296; ^1H NMR (500 MHz, $CDCl_3$) δ: 9.89 (1H, amide), 8.59 (m, 1H, amide), 8.40-8.38 (d, J = 8.54 Hz, 1H), 7.44-7.37 (m 2H), 7.24-7.23 (d, J = 7.32 Hz, 1H), 7.14-7.11 (m, 1H), 6.60-6.58 (d, J = 8.54 Hz, 1H), 5.27-5.24 (dd, J = 6.71 Hz, J = 8.54 Hz, 1H), 4.78-4.77 (m, 1H), 4.48-4.47 (m, 1H), 3.37-3.32 (m, 2H), 2.8-2.8 (d, J = 2.75 Hz, 3H), 2.33 (m, 1H), 2.08-1.96 (m, 3H), 1.98-1.88 (m, 1H), 1.79-1.63 (m, 3H), 1.22 (s, 9H), 0.99-0.96 (m, 9H), 0.93-0.92 (d, J = 6.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 178.3, 174.0, 172.9, 171.0, 168.4, 134.5, 129.9, 127.2, 125.4, 123.9, 121.0, 59.7, 57.3, 53.3, 49.2, 40.6, 38.9, 33.4, 28.8, 27.6, 26.4, 24.84, 24.82, 22.7, 22.2, 18.9, 18.3; MALDI-TOF: 566.9368 $(M+Na)^+$, 583.1334 $(M+K)^+$; Anal. calcd for $C_{29}H_{45}N_5O_5$: C, 64.06; H, 8.34; N, 12.88; Found: C, 64.09; H, 8.39; N, 12.77.

(*R*)-*N*-((*S*)-4-methyl-1-(methylamino)-1-oxopentan-2-yl)-1-(2-((*S*)-3-methyl-2-pivalamidobutanamido)benzoyl)pyrrolidine-2-carboxamide 3.

Compound **3** was synthesized following the procedure for **1** to afford the free amine which was further protected with pivaloyl group following the procedure for **1c.** The crude product was purified by column chromatography (eluent: 45% AcOEt/pet. ether, Rf: 0.4) to furnish **3** (79%) as a white solid. mp: 80-82 °C; $[\alpha]_D^{26.1}$: 22.608° (*c* 0.5, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}): 3312, 3019, 2966, 2874, 2401, 1648, 1588, 1518, 1456, 1420, 1369, 1297; ^1H NMR (500 MHz, $CDCl_3$) δ: 9.7 (1H, amide), 8.32-8.30 (dd, J = 1.53 Hz, J = 8.54 Hz, 1H), 7.91-7.90 (m, 1H, amide), 7.39-7.36 (dd, J = 1.53 Hz, J = 8.54 Hz, 1H), 7.24-7.22 (dd, J = 1.53 Hz, J = 7.32 Hz, 1H), 7.15-7.10 (m, 2H), 6.53-6.51 (d, J = 9.16 Hz, 1H), 5.09-5.06 (dd, J = 7.02 Hz, J = 8.85 Hz, 1H), 4.94-4.90 (m, 1H), 4.72-4.69 (m, 1H), 3.40-3.31 (m, 2H), 2.77-2.76 (d, J = 4.58 Hz, 3H), 2.36-2.29 (m, 1H), 2.12-1.86 (m, 4H), 1.7-1.61 (m, 3H), 1.23 (s, 9H), 1.03-1.02 (d, J = 6.71 Hz, 3H), 0.98-0.95 (m, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 178.2, 172.5, 172.2, 171.1, 168.4, 134.7, 129.8, 127.5, 125.2, 123.8, 121.7, 60.1, 57.4, 51.9, 49.2, 42.0, 38.9, 33.2, 30.1, 29.7, 27.7, 26.3, 25.0, 24.6, 22.8, 22.4, 19.3, 18.0; MALDI-TOF: 566.7384 $(M+Na)^+$, 583.7939 $(M+K)^+$; Anal. calcd for $C_{29}H_{45}N_5O_5$: C, 64.06; H, 8.34; N, 12.88; Found: C, 64.09; H, 8.39; N, 12.77.

(*R*)-*N*-((*S*)-4-methyl-1-(methylamino)-1-oxopentan-2-yl)-1-(2-((*S*)-3-methyl-2-pivalamidobutanamido)benzoyl)pyrrolidine-2-carboxamide 4.

Compound **4** was synthesized following the procedure for **3** from **4a**. The crude product was purified by column chromatography (eluent: 45% AcOEt/pet. ether, Rf: 0.4) to furnish **4** (67%) as a white solid. mp: 75-77 °C; $[\alpha]_D^{26.4}$: (*c* 0.5, $CHCl_3$); IR ($CHCl_3$) ν (cm^{-1}): 3312, 3018, 2966, 2874, 2401, 1648, 1588, 1551, 1513, 1456, 1420, 1369, 1297; ^1H NMR (500 MHz, $CDCl_3$) δ: 9.71 (1H, amide), 8.32-8.31 (d, J = 8.24 Hz, 1H), 7.93-7.92 (m, 1H, amide), 7.39-7.36 (dd, J = 1.53 Hz, J = 8.54 Hz, 1H), 7.24-7.22 (m, 2H), 7.13-7.10 (m, 1H), 6.53-6.51 (d, J = 9.16 Hz, 1H), 5.10-5.06 (dd, J = 6.71 Hz, J = 9.16 Hz, 1H), 4.94-4.89 (m, 1H), 4.72-4.69 (m, 1H), 3.40-3.31 (m, 2H), 2.77-2.76 (d, J = 4.88 Hz, 3H), 2.37-2.30 (m, 1H), 2.11-1.88 (m, 4H), 1.7-1.61 (m, 3H), 1.22 (s, 9H), 1.03-1.02 (d, J = 6.71 Hz, 3H), 0.98-0.95 (m, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 178.2, 172.6, 172.2, 171.1, 168.4, 134.6, 129.8, 127.5, 125.2, 123.8, 121.6, 60.1, 57.4, 51.8, 49.1, 42.0, 38.9, 33.1, 30.1, 27.6, 26.3, 25.0, 24.6, 22.7, 22.3, 19.2, 18.0; MALDI-TOF: 566.8729 $(M+Na)^+$, 583.0687 $(M+K)^+$; Anal. calcd for $C_{29}H_{45}N_5O_5$: C, 64.06; H, 8.34; N, 12.88; Found: C, 64.09; H, 8.39; N, 12.77.

Acknowledgements

Dedicated to Professor Goverdhan Mehta on the occasion of his 70th birthday. This work was funded by NCL-IGIB-JRI. RV, ASK and RLG thank CSIR, New Delhi, for research fellowships.

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