Supporting information

Design and Synthesis of P1-P3 Macrocyclic Tertiary Alcohol Comprising HIV-1 Protease Inhibitors

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1 - General Information

Analytical thin layer chromatography was performed using Merck aluminum sheets precoated with silica gel 60 F₂₅₄. Column chromatography was performed on Merck silica gel 60 (40-63 μm). The microwave reactions were performed in a Biotage Initiator producing controlled irradiation at 2450 MHz with a power of 0–300 W. Reaction temperatures were determined using the built-in on-line IR-sensor. ^1H and ^{13}C NMR spectra were recorded on Varian Mercury Plus instruments; ^1H at 399.9 MHz and ^{13}C at 100.6 MHz at 25 °C. Analytical RPHPLC-MS was performed on a Gilson HPLC system with a Finnigan AQA quadrupole low-resolution mass spectrometer in positive or negative ESI mode using a Onyx Monolithic C₁₈ 4.6 x 50 mm 5 μm (Phenomenex) or ACT C4 4.6 x 50 mm, 5 μm column with MeCN in 0.05% aqueous HCOOH as mobile phase at a flow rate of 4 mL/min. Preparative RPLC-MS was performed on a Gilson HPLC system using a Zorbax SB-C8, 5 μm 21.2 x 150 mm (Agilent technologies) column, with MeCN in 0.05% aqueous HCOOH as mobile phase at a flow rate of 10-15 mL/min or with MeCN in 0.1% aqueous TFA as mobile phase at a flow rate of 5 mL/min. Exact molecular masses were determined on Micromass Q-Tof2 mass spectrometer equipped with an electrospray ion source. Optical rotations were obtained on a Perkin-Elmer 241 polarimeter, specific rotations ($[\alpha]_D$) are reported in deg/dm and the concentration (c) is given in g/100 mL in the specified solvent. All protease inhibitors were >95% pure according to ^1H NMR, LC-UV (254 nm and 214 or 220 nm), and LC-MS (TIC).

2 - Experimental details and spectroscopic data for compounds 2 – (R)-19b

4-(4-Bromobenzylidene)-2-methyloxazol-5(4H)-one¹ (2):

A mixture of 4-bromobenzaldehyde (10.0 g, 54.0 mmol), *N*-acetylglycine (7.6 g, 64.9 mmol), sodium acetate (5.7 g, 70.0 mmol) in acetic anhydride (25 mL) was refluxed at 120 °C for 4 h. After cooling the reaction mixture to room temperature, a yellow precipitate formed that was filtered off and washed with water several times. Drying under vacuum yield **2** as a pale yellow solid (12.7 g, 89%). The obtained compound was used in the next step without further purification. Mp: 108-109 °C.

^1H NMR (400 MHz, CDCl_3) δ 7.85-7.89 (m, 2H), 7.48-7.51 (m, 2H), 6.98 (s, 1H), 2.33 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 167.7, 166.7, 133.6, 133.3, 132.4, 132.2, 130.1, 126.1, 15.9. HRMS $\text{C}_{11}\text{H}_8\text{BrNO}_2$, Calc. m/z $[\text{M}+\text{H}^+]$ 265.9817, found 265.9807.

3-(4-Bromophenyl)-2-hydroxypropanoic acid² (**3**):

A mixture of **2** (10.2 g, 38.22 mmol) in HCl solution (3 M, 230 mL) was refluxed for 24 h. After cooling to room temperature, amalgamated zinc (9.4 g) and toluene (40 mL) were added and the reaction mixture was refluxed at 120 °C for 5 h. After filtration, the reaction mixture was gradually cooled to 0 °C and kept in the refrigerator for 15 h. The precipitate formed was filtered, washed with water (2 $\times$ 25 mL), triturated with *iso*-hexane (2 $\times$ 25 mL) and dried under vacuum to yield **3** as an off-white solid (7.8 g, 83%). The obtained compound was used in the next step without further purification. Mp: 96-97 °C. ^1H NMR (400 MHz, DMSO) δ 12.48 (br s, 1H), 7.41-7.45 (m, 2H), 7.15-7.19 (m, 2H), 5.30 (br s, 1H), 4.11 (t, 1H), 2.93 (d, J = 4.4 Hz, 1H), 2.77 (d, J = 8.4 Hz, 1H). ^{13}C NMR (100 MHz, DMSO) δ 175.5, 138.2, 132.3, 131.4, 120.0, 71.3, 39.9.

5-(4-Bromobenzyl)-2,2-dimethyl-1,3-dioxolan-4-one (**4**):

A mixture of **3** (8.0 g, 32.7 mmol), 2,2-dimethoxypropane (27.2 g, 261.2 mmol) and pyridinium *p*-toluenesulphonate (4.1 g, 16.3 mmol) in CHCl_3 (109 mL) was refluxed at 70 °C for 2 h. The reaction mixture was concentrated *in vacuo* and the residue was purified by flash chromatography (EtOAc/*iso*-hexane 0:100 to 10:90) to yield **4** as a dark yellow oil (7.5 g, 84%). ^1H NMR (400 MHz, CDCl_3) δ 7.43 (d, J = 8.4 Hz, 2H), 7.13 (d, J = 8 Hz, 2H), 4.63 (dd, J = 6.4 Hz, 4.4 Hz, 1H), 3.15 (dd, J = 14.4 Hz, 4.4 Hz, 1H), 3.02 (dd, J = 14.4 Hz, 6.4 Hz, 1H), 1.49 (s, 3H), 1.38 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 172.4, 134.9, 131.9, 131.7, 121.4, 111.1, 74.9, 37.1, 27.2, 26.3. HRMS $\text{C}_{12}\text{H}_{13}\text{BrO}_3$, Calc. m/z $[\text{M}+\text{H}^+]$ 284.0048, found 284.0044.

Methyl-3-[4-(4-bromobenzyl)-2,2-dimethyl-5-oxo-1,3-dioxolan-4-yl] propanoate (5):

Diisopropylamine (3.6 mL, 25.3 mmol) was diluted in dry THF (5 mL) and cooled at -78 °C under nitrogen atmosphere. A solution of *n*BuLi at 2.5 M in hexane (8.4 mL) was slowly added, temperature is allowed to rise to 0 °C for 10 min then cooled again to -78 °C. A solution of **4** (4 g, 14 mmol) in anhydrous THF (12 mL) was added dropwise. 10 min, methyl acrylate (1.3 g, 15.4 mmol) was slowly added and stirred for 10 min at -78 °C. Saturated NH₄Cl was added to the reaction and extracted with EtOAc, dried over MgSO₄ and concentrated. Flash chromatography (EtOAc/*iso*-hexane 0:100 to 15:85) yielded **5** as a light yellow solid (2.8 g, 54%). Mp: 101-102 °C. ¹H NMR (400 MHz, CDCl₃) δ 7.43 (d, *J* = 8.4 Hz, 2H), 7.09 (d, *J* = 8.8 Hz, 2H), 3.68 (s, 3H), 3.06 (d, *J* = 14 Hz, 1H), 2.91 (d, *J* = 14 Hz, 1H), 2.4-2.57 (m, 2H), 2.13 (t, *J* = 8 Hz, 8.4 Hz, 2H), 1.53 (s, 3H), 1.04 (s, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 173.8, 172.9, 134.1, 132.7, 131.6, 121.6, 110.4, 82.9, 52.1, 41.8, 32.9, 28.8, 28.7, 27.9. HRMS C₁₆H₁₉BrO₅, Calc. *m/z* [M+H⁺] 371.0494, found 371.0497.

(S)-tert-Butyl-1-(allylamino)-3-methyl-1-oxobutan-2-yl carbamate (7a):

To a cooled (0 °C) suspension of Boc-L-Valine **6a** (1.20 g, 5.5 mmol), allylamine (0.44 g, 7.7 mmol) and HOBt (0.85 g, 5.5 mmol) in DCM (20 mL) 4-methylmorpholine (1.40 g, 13.8 mmol) was added and stirred for 30 min under nitrogen atmosphere. Then EDC hydrochloride (1.38 g, 7.18 mmol) was added and the reaction mixture was gradually warmed to room temperature and stirred for 15 h. Water was added to the reaction mixture and the separated organic phase was washed with brine (2 × 15 mL), dried over Na₂SO₄ and concentrated *in vacuo*. Flash chromatography (MeOH/DCM 0:100 to 5:95) yielded **7a** as a white solid (1.30 g, 92%). Mp: 106-107 °C. [α]_D²¹ -23.8 (*c* 0.13 MeOH). ¹H NMR (400 MHz, CDCl₃) δ 6.40 (br s, 1H), 5.76-5.84 (m, 1H), 5.11-5.19 (m, 2H), 3.85-3.91 (m, 3H), 2.11 (br s, 1H), 1.41 (d, *J* = 8 Hz, 9H), 0.95 (d, *J* = 6.8 Hz, 3H), 0.92 (d, *J* = 6.8 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃): δ 171.8, 156.1, 134.1, 116.6, 80.1, 60.3, 41.9, 30.9, 28.5, 19.5, 18.1. HRMS C₁₃H₂₄N₂O₃, Calc. *m/z* [M+H⁺] 257.1865, found 257.1872.

(S)-tert-Butyl-1-(allylamino)-3,3-dimethyl-1-oxobutan-2-yl carbamate (7b):

To a cooled (0 °C) suspension of Boc-Tle-OH **6b** (1.90 g, 8.4 mmol), allylamine (0.72 g, 12.6 mmol) and HOBt (1.79 g, 11.7 mmol) in DCM (25 mL) 4-methylmorpholine (3.38 g, 33.5 mmol) was added and the mixture was stirred for 30 min under nitrogen atmosphere. Then EDC hydrochloride (2.10 g, 10.9 mmol) was added and the reaction mixture was gradually warmed to room temperature and stirred for 15 h. Water was added to the reaction mixture and the separated organic phase was washed with brine (2 × 20 mL), dried over Na₂SO₄ and concentrated *in vacuo*. Flash chromatography (MeOH/DCM 0:100 to 3:97) yielded **7b** as a white solid (2.10 g, 92%). Mp: 111-112 °C. $[\alpha]_D^{21}$ -12.0 (c 0.1 MeOH). ¹H NMR (400 MHz, CDCl₃) δ 6.5 (br s, 1H), 5.73-5.8 (m, 1H), 5.37 (d, *J* = 9.6 Hz, 1H), 5.17 (dd, *J* = 1.6 Hz, 17.2 Hz, 1H), 5.08 (dd, *J* = 1.2 Hz, 10.4 Hz, 1H), 3.8-3.93 (m, 3H), 1.39 (s, 9H), 0.97 (s, 9H). ¹³C NMR (100 MHz, CDCl₃) δ 171.1, 156.2, 134.2, 116.7, 79.7, 62.4, 41.9, 34.6, 28.6, 26.8. HRMS C₁₄H₂₆N₂O₃, Calc. *m/z* [M+H⁺] 271.2021, found 271.2015.

(S)-N-Allyl-2-amino-3-methylbutanamide (8a):

A solution of **7a** (1.30 g, 4.9 mmol) in DCM (4 mL) was added dropwise to cold (0 °C) TFA (2.79 g, 24.5 mmol) and the reaction mixture was warmed to room temperature and stirred for 30 min. The mixture was concentrated *in vacuo* to yield the TFA salt of **8a** and was used in the next step without further purification. MS (ESI) *m/z* 157.1 [M+1]⁺.

(S)-N-Allyl-2-amino-3,3-dimethylbutanamide (8b):

A solution of **7b** (1.26 g, 4.7 mmol) in DCM (4 mL) was added dropwise to cold (0 °C) TFA (2.65 g, 23.2 mmol) and the reaction mixture was warmed to room temperature and stirred for 30 min. The mixture was concentrated *in vacuo* to yield the TFA salt of **8b** and was used in the next step without further purification. MS (ESI) *m/z* 171.2 [M+1]⁺.

(*R* or *S*)-*N*-((*S*)-1-(Allylamino)-3-methyl-1-oxobutan-2-yl)-2-(4-bromobenzyl)-5-oxotetrahydrofuran-2-carboxamide ((*R*)-9a**, (*S*)-**9a**):**

(*R*)-**9a**

(*S*)-**9a**

A solution of **5** (1.00 g, 2.69 mmol) in TFA/H₂O (6:1.6 mL) was stirred at 80 °C overnight and then concentrated. The residue was dissolved in EtOAc followed by concentration of the

solution in order to get rid of excess of TFA yielding a lactone intermediate as TFA salt. The intermediate, **8a** (3.23 mmol) and HOBt (0.45 g, 2.96 mmol) were dissolved in DCM (20 mL), then 4-methylmorpholine (1.09 g, 10.77 mmol) was added and the mixture was stirred for 30 min under nitrogen atmosphere. Then at 0 °C, EDC hydrochloride (0.67 g, 3.5 mmol) was added and the reaction mixture was gradually warmed to room temperature and stirred for 15 h. Water (20 mL) was added to the reaction mixture and the separated organic phase was washed with brine (2 × 20 mL), dried over MgSO₄ and concentrated. Flash chromatography (DCM/EtOAc 95:5) yielded (*R*)-**9a** (0.52 g, 45%) as a white solid and subsequently (EtOAc) (*S*)-**9a** (0.47 g, 40%) as white solid.

For (*R*)-**9a**: Mp: 174-175 °C. $[\alpha]_D^{21} +177.7$ (*c* 0.13 MeOH). ¹H NMR (400 MHz, CDCl₃) δ 7.32-7.36 (m, 2H), 7.03-7.06 (m, 2H), 6.66 (d, *J* = 9.2 Hz, 1H), 5.68-5.77 (m, 1H), 5.64 (t, *J* = 5.6 Hz, 6 Hz, 1H), 5.06-5.13 (m, 2H), 4.02-4.08 (m, 1H), 3.66-3.81 (m, 2H), 3.19 (d, *J* = 14 Hz, 1H), 2.98 (d, *J* = 14.4 Hz, 1H), 2.18-2.58 (m, 4H), 2.06-2.15 (m, 1H), 0.83 (d, *J* = 6.4 Hz, 3H), 0.77 (d, *J* = 6.8 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 174.9, 171.6, 169.7, 133.9, 133.3, 132.2, 131.9, 121.9, 116.7, 87.9, 58.8, 43.1, 42.0, 30.8, 30.6, 28.2, 19.6, 18.0. HRMS C₂₀H₂₅BrN₂O₄, Calc. *m/z* [M+H⁺] 437.1076, found 437.1078.

For (*S*)-**9a**: Mp: 47-48 °C. $[\alpha]_D^{21} +5.2$ (*c* 0.14 MeOH). ¹H NMR (400 MHz, CDCl₃) δ 7.32-7.36 (m, 2H), 7.04-7.07 (m, 2H), 6.69 (d, *J* = 8 Hz, 1H), 5.94 (t, *J* = 5.6 Hz, 1H), 5.68-5.78 (m, 1H), 5.04-5.13 (m, 2H), 3.91 (dd, *J* = 6.8 Hz, 8.8 Hz, 1H), 3.72-3.87 (m, 2H), 3.19 (d, *J* = 14 Hz, 1H), 2.98 (d, *J* = 14 Hz, 1H), 2.17-2.48 (m, 4H), 1.79-1.9 (m, 1H), 0.69 (d, *J* = 6.4 Hz, 3H), 0.61 (d, *J* = 7.2 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 175.1, 171.3, 170.2, 133.9, 133.3, 132.3, 131.9, 121.9, 116.9, 87.8, 59.2, 43.4, 42.1, 31.0, 30.6, 28.1, 19.2, 18.2. HRMS C₂₀H₂₅BrN₂O₄, Calc. *m/z* [M+H⁺] 437.1076, found 437.1072.

(*R* or *S*)-*N*-((*S*)-1-(Allylamino)-3,3-dimethyl-1-oxobutan-2-yl)-2-(4-bromobenzyl)-5-oxotetrahydrofuran-2-carboxamide ((*R*)-9b**, (*S*)-**9b**):**

A solution of **5** (2.00 g, 5.39 mmol) in TFA/H₂O (4:1 mL) was stirred at 80 °C overnight and then concentrated. The residue is dissolved in EtOAc followed by concentration of the solution in order

to get rid of excess of TFA. **8b** (8.14 mmol) and HOBt (0.91 g, 5.93 mmol) in DCM (40 mL) and 4-methylmorpholine (2.18 g, 21.6 mmol) were added and stirred for 30 min under nitrogen atmosphere. Then at 0 °C, EDC hydrochloride (1.34 g, 7.00 mmol) was added and the reaction mixture was gradually warmed to room temperature and stirred for 15 h. Water (20 mL) was added to the reaction mixture and the separated organic phase was washed with brine (2 × 20 mL), dried over Na₂SO₄ and concentrated *in vacuo*. Flash chromatography (EtOAc/isohexane 1:4) yielded (*R*)-**9b** (1.04 g, 43%) as a white solid and subsequently (EtOAc/isohexane 1:1) (*S*)-**9b** (1.06 g, 44%) as white solid.

For (*R*)-**9b**: Mp: 147-148 °C. $[\alpha]_D^{21} +6.0$ (*c* 0.10 MeOH). ¹H NMR (400 MHz, CDCl₃) δ 7.31-7.34 (m, 2H), 7.02 (d, *J* = 8.4 Hz, 2H), 6.81 (d, *J* = 9.2 Hz, 1H), 5.71-5.80 (m, 2H), 5.09-5.17 (m, 2H), 4.05 (d, *J* = 9.6 Hz, 1H), 3.82-3.90 (m, 1H), 3.67-3.75 (m, 1H), 3.15 (d, *J* = 14.4 Hz), 2.96 (d, *J* = 14.4 Hz, 1H), 2.15-2.56 (m, 4H), 0.87 (s, 9H). ¹³C NMR (100 MHz, CDCl₃) δ 174.9, 171.3, 168.9, 134.0, 133.2, 132.1, 131.9, 121.9, 117.2, 87.9, 60.8, 43.0, 42.1, 34.9, 30.6, 28.3, 26.7. HRMS (ESI) calcd for C₂₁H₂₇BrN₂O₄ [M + H]⁺ 451.1232, found 451.1230. For (*S*)-**9b**: Mp: 60-62 °C. $[\alpha]_D^{21} +13.3$ (*c* 0.12 MeOH). ¹H NMR (400 MHz, CDCl₃) δ 7.33-7.35 (m, 2H), 7.09 (d, *J* = 8 Hz, 2H), 6.8 (d, *J* = 9.2 Hz, 1H), 5.99 (t, *J* = 5.6 Hz, 1H), 5.69-5.79 (m, 1H), 5.15 (dd, *J* = 1.2 Hz, 17.2 Hz, 1H), 5.08 (dd, *J* = 1.2 Hz, 10.4 Hz, 1H), 3.96 (d, *J* = 8.8 Hz, 1H), 3.83-3.89 (m, 1H), 3.70-3.77 (m, 1H), 3.18 (d, *J* = 14.4 Hz, 1H), 2.99 (d, *J* = 14 Hz, 1H), 2.12-2.46 (m, 4H), 0.74 (s, 9H). ¹³C NMR (100 MHz, CDCl₃) δ 175.1, 171.1, 169.6, 134.0, 133.4, 132.4, 131.9, 121.9, 117.1, 87.8, 61.3, 43.4, 42.1, 34.3, 30.9, 28.2, 26.6. HRMS C₂₁H₂₇BrN₂O₄, Calc. m/z [M+H]⁺ 451.1232, found 451.1236.

(*R*)-*N*-((*S*)-1-(Allylamino)-3-methyl-1-oxobutan-2-yl)-2-(4-bromobenzyl)-2-(*tert*-butyldimethylsilyloxy)-5-hydroxypentanamide ((*R*)-10a):

To a 2 M solution of lithium borohydride in THF (5.5 mL) was added (**R**)-**9a** (1.2 g, 2.8 mmol) and stirred at room temperature under nitrogen atmosphere for 15 h. The reaction mixture was cooled in ice bath, quenched with saturated NH_4Cl solution and extracted with EtOAc (2 $\times$ 20 mL). The organic layer was washed with brine (1 $\times$ 10 mL), dried over Na_2SO_4 and concentrated under reduced pressure to yield the diol compound as a white solid, which was used in the next step without purification. MS (ESI) m/z 443.2 ($\text{M}+2$)⁺. To a cooled (0 °C) solution of the diol compound (1.0 g, 2.3 mmol) in anhydrous DCM (10 mL) was added anhydrous pyridine (10 mL) and stirred for 5 min under nitrogen atmosphere. Trimethylacetyl chloride (0.55 g, 4.5 mmol) was added to this solution in a drop-wise manner and the reaction mixture was gradually warmed to room temperature and stirred for 15 h. The reaction mixture was concentrated under reduced pressure and a fast flash column (EtOAc/*iso*-hexane 1:1) was performed to yield the primary alcohol protected compound as a pale colored oil which solidified upon cooling. MS (ESI) m/z 527.2 ($\text{M}+2$)⁺. A solution of the previous compound (0.65 g, 1.2 mmol) in anhydrous DCM (10 mL) was cooled in an ice bath and triethylamine (0.75 g, 7.5 mmol) was added dropwise under nitrogen atmosphere and stirred for 5 min after which TBSOTf (0.99 g, 3.7 mmol) was added in a drop-wise manner. The reaction mixture was gradually warmed to room temperature and stirred for 15 h. Saturated NaHCO_3 solution (15 mL) was added to the reaction mixture and the aqueous layer was extracted with DCM (2 $\times$ 15 mL), washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to yield a colorless oil which was purified by a fast flash column (EtOAc/*iso*-hexane 4:6) providing the primary and tertiary alcohol protected compound as a yellow colored oil. MS (ESI) m/z 639.5 ($\text{M}+2$)⁺. To a 2 M solution of lithium borohydride in THF (6 mL) was added the previous compound (0.73 g, 1.1 mmol) and stirred at room temperature under nitrogen atmosphere for 15 h. The reaction mixture was cooled in ice bath, quenched with saturated NH_4Cl solution and extracted with EtOAc (2 $\times$ 15 mL). The organic layer was washed with brine (1 $\times$ 15 mL), dried over Na_2SO_4 and concentrated under reduced pressure. Flash chromatography (MeOH/DCM 1:9) yielded (**R**)-**10a** (0.63 g, 42% yield over 4 steps) as a pale yellow colored oil. ^1H NMR (400 MHz, CDCl_3) δ 7.26 (d, J = 8.4 Hz, 2H), 6.99 (d, J = 8.4 Hz, 2H), 5.64-5.74 (m, 2H), 5.03-5.09 (m, 2H), 4.02 (dd, J = 3.2, 15.2 Hz, 1H), 3.70-3.73 (m,

2H), 3.51-3.57 (m, 2H), 3.15 (d, $J = 14.4$ Hz, 1H), 2.84 (d, $J = 14.4$ Hz, 1H), 2.03-2.15 (m, 2H), 1.65-1.75 (m, 2H), 1.41-1.48 (m, 1H), 0.76-0.91 (m, 15H), 0.18 (s, 3H), 0.14 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 175.7, 171.8, 137.2, 135.6, 133.9, 133.5, 132.9, 132.8, 122.3, 118.1, 84.7, 64.2, 60.1, 47.5, 43.4, 38.3, 32.1, 29.0, 28.0, 27.9, 27.8, 27.4, 21.3, 21.2, 20.3, 19.7, 0.02, 0.00. HRMS $\text{C}_{26}\text{H}_{43}\text{BrN}_2\text{O}_4\text{Si}$, Calc. m/z $[\text{M}+\text{H}^+]$ 555.2254, found 555.2251.

(*R*)-*N*-((*S*)-1-(Allylamino)-3,3-dimethyl-1-oxobutan-2-yl)-2-(4-bromobenzyl)-2-((*tert*-butyldimethylsilyl)oxy)-5-hydroxypentanamide ((*R*)-10b):

To a 2 M solution of lithium borohydride in THF (5.4 mL) was added (**R**)-**9b** (1.2 g, 2.7 mmol) and the mixture was stirred at room temperature under nitrogen atmosphere for 15 h. The reaction mixture was cooled in ice bath, quenched with saturated NH_4Cl solution and extracted with EtOAc (2×20 mL). The organic layer was washed with brine (1×10 mL),

dried over Na_2SO_4 and concentrated under reduced pressure to yield the diol as a white solid, which was used in the next step without purification. MS (ESI) m/z 457.1 $[\text{M}+1]^+$. To a cooled (0°C) solution of the diol (1.0 g, 2.2 mmol) in anhydrous DCM (10 mL) was added anhydrous pyridine (10 mL) and stirred for 5 min under nitrogen atmosphere. Trimethylacetyl chloride (0.53 g, 4.4 mmol) was added dropwise to this solution and the reaction mixture was gradually warmed to room temperature and stirred for 15 h. The reaction mixture was concentrated under reduced pressure and a fast flash column (EtOAc/*iso*-hexane 4:6) was performed to yield the primary alcohol protected compound as a light yellow foamy solid. MS (ESI) m/z 541.2 $(\text{M}+2)^+$. This compound (0.67 g, 1.2 mmol) was dissolved in anhydrous DCM (10 mL), was cooled in an ice bath and triethylamine (0.75 g, 7.4 mmol) was added under nitrogen atmosphere and stirred for 5 min after which TBSOTf (0.98 g, 3.7 mmol) was added in a drop-wise manner. The reaction mixture was gradually warmed to room temperature and stirred for 15 h. Saturated NaHCO_3 solution (15 mL) was added to the reaction mixture and the aqueous layer was extracted with DCM (2×15 mL), washed with brine (1×10 mL), dried over Na_2SO_4 and concentrated under reduced pressure. A fast flash column (EtOAc/*iso*-hexane 4:6) provided the primary and tertiary alcohol protected compound as a yellow colored oil. MS (ESI) m/z 653.8 $(\text{M}+2)^+$. To a 2 M solution of lithium borohydride in THF (6 mL) was added the previous compound (0.82 g, 1.3 mmol) and the

mixture was stirred at room temperature under nitrogen atmosphere for 15 h. The reaction mixture was cooled in ice bath, quenched with saturated NH_4Cl solution (15 mL) and extracted with EtOAc (2×15 mL). The organic layer was washed with brine (1×15 mL), dried over Na_2SO_4 and concentrated under reduced pressure. Flash chromatography (MeOH/DCM 5:95) yielded **(R)-10b** (0.68 g, 45% yield over 4 steps) as a pale colored oil. ^1H NMR (400 MHz, CDCl_3) δ 7.41 (d, $J = 9.2$ Hz, 1H), 7.10-7.12 (m, 2H), 6.84 (d, $J = 8$ Hz, 2H), 5.82 (t, $J = 5.6$ Hz, 1H), 5.59-5.67 (m, 1H), 4.95-5.05 (m, 2H), 3.89 (d, $J = 9.6$ Hz, 1H), 3.75-3.82 (m, 1H), 3.54-3.61 (m, 1H), 3.40-3.49 (m, 2H), 3.05 (d, $J = 14.4$ Hz, 1H), 2.75 (d, $J = 14.8$ Hz, 1H), 1.94-2.01 (m, 1H), 1.81 (br s, 1H), 1.55-1.64 (m, 2H), 1.32-1.40 (m, 1H), 0.77-0.82 (m, 18H), 0.09 (s, 3H), 0.00 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 175.5, 171.3, 137.3, 135.9, 133.3, 132.8, 122.1, 118.6, 84.5, 64.3, 62.5, 47.7, 43.6, 38.4, 36.2, 29.1, 28.6, 27.9, 20.4, 0.1, 0.0. HRMS $\text{C}_{27}\text{H}_{45}\text{BrN}_2\text{O}_4\text{Si}$, Calc. m/z $[\text{M}+\text{H}^+]$ 569.2410, found 569.2415.

(R)-N-((S)-1-(Allylamino)-3-methyl-1-oxobutan-2-yl)-2-((tert-butyldimethylsilyl)oxy)-5-hydroxy-2-(4-vinylbenzyl)pentanamide ((R)-11a):

A 10-20 mL microwave vial was charged with **(R)-10a** (0.63 g, 1.15 mmol), 2,4,6-trivinylcyclotriboroxane – pyridine complex (0.55 g, 2.3 mmol), $\text{Pd}(\text{OAc})_2$ (26 mg, 0.12 mmol), $\text{HP}(\text{tBu})_3\text{BF}_4$ (67 mg, 0.24 mmol), potassium carbonate (0.95 g, 6.9 mmol), 1,2-dimethoxyethane (10 mL) and distilled water (3 mL) and irradiated in the microwave reactor cavity

at 100 °C for 30 min. The reaction mixture filtered off a short Celite plug and the plug was rinsed with EtOAc (20 mL). The organic layer was washed with brine (2×15 mL), dried over Na_2SO_4 and concentrated under reduced pressure. Flash chromatography (MeOH/DCM 10:90) yielded **(R)-11a** (0.46 g, 81% yield) as a yellow colored oil. ^1H NMR (400 MHz, CDCl_3) δ 7.27 (d, $J = 8.4$ Hz, 2H), 7.19 (d, $J = 9.6$ Hz, 1H), 7.09 (d, $J = 8.4$ Hz, 2H), 6.57 (dd, $J = 11.2, 17.6$ Hz, 1H), 5.51-5.65 (m, 3H), 5.12-5.15 (m, 1H), 4.97-5.02 (m, 2H), 4.02-4.08 (m, 1H), 3.62-3.67 (m, 1H), 3.55 (t, $J = 6.4$ Hz, 2H), 3.19 (d, $J = 14.4$ Hz, 1H), 2.89 (d, $J = 14.4$ Hz, 1H), 2.05-2.23 (m, 2H), 1.64-1.77 (m, 3H), 1.41-1.49 (m, 2H), 0.73-0.88 (m, 15H), 0.19 (s, 3H), 0.01 (s, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 175.8, 171.9, 138.1, 137.8, 137.6, 135.6, 132.0, 131.9, 127.5, 127.4, 117.7, 115.2, 85.0, 64.2, 60.0, 47.8, 43.2, 38.3, 31.6, 29.1,

28.0, 27.9, 27.6, 21.3, 20.3, 19.4, 0.01, 0.0. HRMS $C_{28}H_{46}N_2O_4Si$, Calc. m/z $[M+H^+]$ 503.3305, found 503.3301.

(*R*)-*N*-((*S*)-1-(Allylamino)-3,3-dimethyl-1-oxobutan-2-yl)-2-((*tert*-butyldimethylsilyl)oxy)-5-hydroxy-2-(4-vinylbenzyl)pentanamide ((*R*)-11b):

A 10-20 mL microwave vial was charged with (**R**)-**10b** (0.68 g, 1.2 mmol), 2,4,6-trivinylcyclotriboroxane – pyridine complex (0.57 g, 2.4 mmol), $Pd(OAc)_2$ (27 mg, 0.12 mmol), $HP(tBu)_3BF_4$ (69 mg, 0.24 mmol), potassium carbonate (0.99 g, 7.1 mmol), 1,2-dimethoxyethane (10 mL) and distilled water (3 mL) and irradiated in the microwave reactor cavity at 100 °C

for 30 min. The reaction mixture was passed through a short Celite plug and the plug was rinsed with EtOAc (20 mL). The organic layer was washed with brine (2×15 mL), dried over Na_2SO_4 and concentrated under reduced pressure. Flash chromatography (MeOH/DCM 5:95) yielded (**R**)-**11b** (0.60 g, 97% yield) as a dark yellow colored oil. 1H NMR (400 MHz, $CDCl_3$) δ 7.39 (d, J = 9.6 Hz, 1H), 7.06 (d, J = 8.4 Hz, 2H), 6.9 (d, J = 8 Hz, 2H), 6.46 (dd, J = 10.8, 17.6 Hz, 1H), 5.47-5.67 (m, 3H), 4.91-5.02 (m, 3H), 3.89 (d, J = 9.6 Hz, 1H), 3.67-3.72 (m, 1H), 3.42-3.53 (m, 3H), 3.09 (d, J = 14.8 Hz, 1H), 2.79 (d, J = 14.4 Hz, 1H), 1.95-2.02 (m, 1H), 1.56-1.66 (m, 3H), 1.32-1.39 (m, 1H), 0.76-0.82 (m, 18H), 0.08 (s, 3H), 0.00 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.7, 171.4, 138.5, 137.9, 137.5, 135.9, 131.8, 127.6, 118.5, 114.9, 84.7, 64.4, 62.6, 48.3, 43.6, 38.3, 36.2, 29.2, 28.7, 28.0, 20.4, 0.1, 0.0. HRMS $C_{29}H_{48}N_2O_4Si$, Calc. m/z $[M+H^+]$ 517.3462, found 517.3456.

(*R*)-*N*-((*S*)-1-(Allylamino)-3-methyl-1-oxobutan-2-yl)-2-(4-allylbenzyl)-2-((*tert*-butyldimethylsilyl)oxy)-5-hydroxypentanamide ((*R*)-12a):

A 2-5 mL microwave vial was charged (**R**)-**10a** (70 mg, 0.13 mmol), allyltributylstannane (98 μ L, 0.31 mmol), $Pd(PPh_3)_2Cl_2$ (9 mg, 10% mol), CuO (15 mg, 0.19 mmol), DMF (1 mL) and irradiated in the microwave reactor cavity at 150 °C for 30 min. The reaction mixture was passed through a short Celite plug and

the plug was rinsed with EtOAc. The organic layer was concentrated, dissolved in acetonitrile. Washing with isohexan (3 times) was conducted and acetonitrile phase was concentrated. Flash chromatography (MeOH/DCM 2:98 to 5:95) yielded **(R)-12a** (40 mg, 62% yield) as a yellow colored oil. ^1H NMR (400 MHz, CDCl_3) δ 7.25–6.90 (m, 6H), 5.95–5.76 (m, 1H), 5.65 (m, 1H), 5.10–4.91 (m, 4H), 4.06 (m, 1H), 3.71–3.42 (m, 4H), 3.29–3.12 (m, 2H), 3.03 (d, J = 13.4 Hz, 1H), 2.75 (d, J = 13.4 Hz, 1H), 2.30–2.01 (m, 3H), 1.78–1.51 (m, 3H), 0.96–0.72 (m, 15H), 0.23–0.04 (m, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 179.08, 174.25, 142.12, 140.81, 137.42, 133.99, 133.60, 132.04, 131.84, 119.44, 86.67, 67.53, 62.02, 48.87, 45.17, 43.42, 40.22, 33.20, 30.07, 29.78, 29.48, 23.17, 22.09, 0.00.

(R)-N-((S)-1-(Allylamino)-3,3-dimethyl-1-oxobutan-2-yl)-2-(4-allylbenzyl)-2-((tert-butyldimethylsilyl)oxy)-5-hydroxypentanamide ((R)-12b):

A 2-5 mL microwave vial was charged **(R)-10b** (74 mg, 0.13 mmol), allyltributylstannane (103 μL , 0.33 mmol), $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ (9 mg, 10 %mol), CuO (16 mg, 0.20 mmol), DMF (2 mL) and irradiated in the microwave reactor cavity at 150 $^\circ\text{C}$ for 30 min. The reaction mixture was passed through a short Celite plug and the plug was rinsed with EtOAc. The organic layer was

concentrated, dissolved in acetonitrile. Washing with isohexan (3 times) was conducted and acetonitrile phase was concentrated. Flash chromatography (MeOH/DCM 2:98 to 5:95) yielded **(R)-12b** (37 mg, 55% yield) as a yellow colored oil. ^1H NMR (400 MHz, CDCl_3) δ 7.56 (d, J = 9.57 Hz, 1H), 6.91–7.08 (m, 4H), 5.85–5.99 (m, 1H), 5.70 – 5.84 (m, 1H), 5.06–5.22 (m, 2H), 4.96–5.06 (m, 2H), 4.07–4.20 (m, 1H), 3.78–4.00 (m, 2H), 3.52–3.77 (m, 2H), 3.19–3.35 (m, 3H), 2.89 (d, J = 14.64 Hz, 1H), 2.05–2.21 (m, 1H), 1.70–1.99 (m, 2H), 1.46–1.61 (m, 1H), 1.00 (s, 9H), 0.89 (s, 9H), 0.23 (s, 3H), 0.15 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 176.06, 171.58, 139.79, 139.42, 135.92, 132.28, 131.75, 130.02, 118.51, 117.53, 84.64, 64.46, 62.62, 48.25, 43.65, 41.71, 38.32, 36.22, 29.24, 28.73, 28.04, 20.41, 0.11, 0.0.

Methyl ((*S*)-1-(2-((*R*)-5-(((*S*)-1-(allylamino)-3-methyl-1-oxobutan-2-yl)amino)-4-(4-bromobenzyl)-4-hydroxy-5-oxopentyl)-2-(4-bromobenzyl)hydrazinyl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate ((*R*)-14a):

To a solution of (**R**)-**10a** (0.10 g, 0.18 mmol) in anhydrous DCM (5 mL) Dess-Martin Periodinane (0.15 g, 0.36 mmol) was added and the mixture was stirred at room temperature under nitrogen atmosphere for 2 h. The reaction mixture was washed with saturated Na₂S₂O₃ solution (2 × 10 mL), brine (2 × 10 mL) and dried over Na₂SO₄. The organic layer was concentrated under reduced pressure to yield the corresponding aldehyde. To the solution of this crude aldehyde in 1,2-dichloroethane (5 mL) **13** was added (0.08 g, 0.22 mmol), molecular sieves (4 Å) and stirred for 1 h at room temperature after which sodium triacetoxyborohydride (0.12 g, 0.54 mmol) was added and the reaction mixture was stirred for 15 h at room temperature under nitrogen atmosphere. Saturated NaHCO₃ solution (10 mL) was added to the reaction mixture and the aqueous layer was extracted with DCM (2 × 15 mL), washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure. Flash chromatography (EtOAc/*iso*-hexane 40:60) yielded the OTBS-protected intermediate (0.11 g, 67% yield) as a light yellow solid.

A solution (2 M) of TBAF in anhydrous THF (2 mL) was added to the OTBS-protected intermediate (0.11 g, 0.12 mmol) under nitrogen atmosphere at 0 °C and the reaction mixture was warmed to room temperature and stirred for 30 min. Distilled water (5 mL) was added to the reaction mixture and the aqueous layer was extracted with EtOAc (2 × 10 mL). The organic layer was washed with brine (1 × 10 mL), dried over Na₂SO₄ and concentrated under reduced pressure. Flash chromatography (MeOH/DCM 5:95) followed by preparative LC-MS yielded (**R**)-**14a** (0.08 g, 90% yield) as a pale yellow colored oil. ¹H NMR (400 MHz, CDCl₃) δ 7.35 (d, *J* = 8.4 Hz, 2H), 7.31 (d, *J* = 8.4 Hz, 2H), 7.10-7.15 (m, 4H), 5.66-5.76 (m, 2H), 5.29 (d, *J* = 9.2 Hz, 1H), 5.03-5.12 (m, 2H), 4.28-4.42 (m, 1H), 3.99-4.10 (m, 1H), 3.61-3.87 (m, 4H), 3.58 (s, 3H), 3.02 (d, *J* = 13.6 Hz, 1H), 2.80 (d, *J* = 13.2 Hz, 1H), 2.70 (t, *J* = 10 Hz, 2H), 2.19-2.28 (m, 1H), 1.94-2.05 (m, 2H), 1.18-1.53 (m, 3H), 0.62-0.97 (m, 15H). ¹³C NMR (100 MHz, CDCl₃) δ 175.3, 173.3, 171.7, 157.1, 135.4, 132.5, 131.5, 131.2, 131.1, 131.0, 120.9, 116.4, 78.3, 61.2, 60.8, 59.3, 52.7, 45.2, 41.6, 34.6, 29.9, 26.2, 22.4, 19.4, 17.1. HRMS C₃₅H₄₉Br₂N₅O₆, Calc. *m/z* [M+H⁺] 794.2128, found 794.2134.

Methyl ((*S*)-1-(2-((*R*)-5-(((*S*)-1-(allylamino)-3,3-dimethyl-1-oxobutan-2-yl)amino)-4-(4-bromobenzyl)-4-hydroxy-5-oxopentyl)-2-(4-bromobenzyl)hydrazinyl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate ((*R*)-14b):

To a solution of (**R**)-**10b** (0.05 g, 0.09 mmol) in anhydrous DCM (5 mL) Dess-Martin Periodinane was added (0.07 g, 0.18 mmol) and the mixture was stirred at room temperature under nitrogen atmosphere for 2 h. The reaction mixture was washed with saturated Na₂S₂O₃ solution (1 × 5 mL), brine (1 × 5 mL) and dried over Na₂SO₄. The organic layer was concentrated under reduced pressure to yield the corresponding aldehyde. To the solution of this crude aldehyde in 1,2-dichloroethane (5 mL) was added **13** (0.04 g, 0.11 mmol) and molecular sieves (4 Å) and the mixture was stirred for 1 h at room temperature after which sodium triacetoxyborohydride (0.06 g, 0.27 mmol) was added and the reaction mixture was stirred for 15 h at room temperature under nitrogen atmosphere. Saturated NaHCO₃ solution (5 mL) was added to the reaction mixture and the aqueous layer was extracted with DCM (2 × 10 mL), washed with brine (1 × 10 mL), dried over Na₂SO₄ and concentrated under reduced pressure. Flash chromatography (EtOAc/*iso*-hexane 40:60) yielded the OTBS-protected intermediate (0.06 g, 70% yield over 2 steps) as a pale yellow colored oil.

A solution (2 M) of TBAF in anhydrous THF (3 mL) was added to the OTBS-protected intermediate (0.04 g, 0.04 mmol) under nitrogen atmosphere at 0 °C and the reaction mixture was warmed to room temperature and stirred for 30 min. Distilled water (5 mL) was added to the reaction mixture and the aqueous layer was extracted with EtOAc (2 × 10 mL). The organic layer was washed with brine (1 × 10 mL), dried over Na₂SO₄ and concentrated under reduced pressure. Flash chromatography (MeOH/DCM 5:95) followed by preparative LC-MS yielded (**R**)-**14b** (0.03 g, 80% yield) as a pale colored oil. ¹H NMR (400 MHz, CDCl₃) δ 7.35 (d, *J* = 8.4 Hz, 4H), 7.07-7.12 (m, 4H), 5.69-5.82 (m, 1H), 5.06-5.18 (m, 2H), 3.62-4.02 (m, 5H), 3.57 (s, 3H), 3.00 (d, *J* = 13.6 Hz, 1H), 2.79 (d, *J* = 13.6 Hz, 1H), 2.66 (t, *J* = 10.8, 10.4 Hz, 2H), 1.89-1.99 (m, 2H), 1.20-1.52 (m, 3H), 0.87-0.89 (m, 9H), 0.78 (s, 9H). ¹³C NMR (100 MHz, CDCl₃) δ 175.1, 170.9, 170.3, 157.3, 135.8, 134.1, 132.5, 131.7, 131.2, 131.1, 130.8, 120.8, 116.9, 77.9, 61.3, 56.2, 52.8, 45.4, 42.0, 41.5, 37.0, 34.4, 34.0, 27.0, 26.5, 23.0, 21.2. HRMS C₃₆H₅₁Br₂N₅O₆, Calc. *m/z* [M+H⁺] 808.2284, found 808.2279.

Methyl ((S)-1-(2-(((S)-5-(((S)-1-(allylamino)-3-methyl-1-oxobutan-2-yl)amino)-4-(4-bromobenzyl)-4-hydroxy-5-oxopentyl)-2-(4-bromobenzyl)hydrazinyl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate ((S)-14a):

Compound **(S)-14a** was prepared from diastereoisomer **(S)-9a** using the same pathway and similar protocols as **(R)-14a** from diastereoisomer **(S)-9a**. **(S)-14a** is obtained as a pale yellow colored oil. ^1H NMR (400 MHz, CDCl_3) δ 7.35 (d, J = 8.4 Hz, 2H), 7.31 (d, J = 8.0 Hz, 2H), 7.14-7.19 (m, 2H), 7.08 (d, J = 8.4 Hz, 2H), 6.13 (br s, 1H), 5.68-5.73 (m, 1H), 5.03-5.14 (m, 2H), 4.04-4.10 (m, 1H), 3.61-3.88 (m, 3H), 3.57 (s, 3H), 3.17-3.26 (m, 1H), 2.92-3.07 (m, 2H), 2.67-2.81 (m, 2H), 2.14-2.17 (m, 1H), 1.80-1.98 (m, 2H), 1.65-1.68 (m, 2H), 1.27-1.45 (m, 4H), 0.49-0.84 (m, 15H). ^{13}C NMR (100 MHz, CDCl_3) δ 175.4, 173.6, 171.6, 157.3, 135.9, 132.5, 131.7, 131.4, 131.1, 131.0, 120.8, 116.8, 78.8, 61.2, 60.7, 59.3, 52.6, 45.7, 41.6, 34.2, 29.9, 26.4, 22.9, 19.3, 17.9. HRMS $\text{C}_{35}\text{H}_{49}\text{Br}_2\text{N}_5\text{O}_6$, Calc. m/z $[\text{M}+\text{H}^+]$ 794.2128, found 794.2134.

Methyl ((S)-1-(2-(((S)-5-(((S)-1-(allylamino)-3,3-dimethyl-1-oxobutan-2-yl)amino)-4-(4-bromobenzyl)-4-hydroxy-5-oxopentyl)-2-(4-bromobenzyl)hydrazinyl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate ((S)-14b):

Compound **(S)-14b** was prepared from diastereoisomer **(S)-9b** using the same pathway and similar protocols as **(R)-14b** from diastereoisomer **(S)-9b**. **(S)-14b** is obtained as a pale colored oil. ^1H NMR (400 MHz, CDCl_3) δ 7.31 (t, J = 8.0 Hz, 4H), 7.10 (dd, J = 8.0, 8.4 Hz, 4H), 6.18 (br s, 1H), 5.66-5.74 (m, 1H), 5.41 (d, J = 9.6 Hz, 1H), 5.13 (dd, J = 1.2, 17.2 Hz, 1H), 5.03 (dd, J = 1.2, 10.4 Hz, 1H), 4.32 (d, J = 12 Hz, 1H), 3.72-3.85 (m, 3H), 3.59 (s, 3H), 3.4 (br s, 1H), 3.1 (br s, 1H), 2.92 (d, J = 13.2 Hz, 1H), 2.76 (d, J = 13.6 Hz, 1H), 2.62 (br s, 1H), 2.19-2.22 (m, 1H), 1.61-1.66 (m, 2H), 1.20-1.44 (m, 3H), 0.74-0.79 (m, 9H), 0.60 (s, 9H). ^{13}C NMR (100 MHz, CDCl_3) δ 175.2, 171.4, 170.9, 157.1, 136.0, 133.9, 132.5, 131.7, 131.5, 131.1, 120.8, 117.1, 78.9, 61.9, 60.9, 56.6, 52.6, 45.7, 42.3, 38.2, 34.3, 33.1, 26.5, 26.4, 21.9. HRMS $\text{C}_{36}\text{H}_{51}\text{Br}_2\text{N}_5\text{O}_6$, Calc. m/z $[\text{M}+\text{H}^+]$ 808.2284, found 808.2276.

Methyl ((*S*)-1-(2-((*R*)-5-(((*S*)-1-(allylamino)-3-methyl-1-oxobutan-2-yl)amino)-4-hydroxy-5-oxo-4-(4-vinylbenzyl)pentyl)-2-(4-bromobenzyl)hydrazinyl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate ((*R*)-15a):

To a solution of (**R**)-**11a** (0.46 g, 0.91 mmol) in anhydrous DCM (20 mL) Dess-Martin Periodinane was added (0.77 g, 1.8 mmol) and the mixture was stirred at room temperature under nitrogen atmosphere for 2 h. The reaction mixture was washed with saturated Na₂S₂O₃ solution (2 × 10 mL), brine (2 × 10 mL) and dried over Na₂SO₄. The organic layer was concentrated under reduced pressure to yield the corresponding aldehyde. To the solution of this crude aldehyde in 1,2-dichloroethane (20 mL) **13** was added (0.41 g, 1.1 mmol), and molecular sieves (4 Å) and the mixture was stirred for 1 h at room temperature after which sodium triacetoxyborohydride (0.58 g, 2.7 mmol) was added and the reaction mixture was stirred for 15 h at room temperature under nitrogen atmosphere. Saturated NaHCO₃ solution (20 mL) was added to the reaction mixture and the aqueous layer was extracted with DCM (2 × 15 mL), washed with brine, dried over Na₂SO₄ and concentrated under reduced pressure. Flash chromatography (EtOAc/*iso*-hexane 40:60) yielded the OTBS-protected intermediate (0.47 g, 60% yield) as a light yellow solid.

A solution (2 M) of TBAF in anhydrous THF (3 mL) was added to the OTBS-protected intermediate (0.10 g, 0.12 mmol) under nitrogen atmosphere at 0 °C and the reaction mixture was warmed to room temperature and stirred for 30 min. Distilled water (5 mL) was added to the reaction mixture and the aqueous layer was extracted with EtOAc (2 × 10 mL). The organic layer was washed with brine (1 × 10 mL), dried over Na₂SO₄ and concentrated under reduced pressure. Flash chromatography (MeOH/DCM 10:90) followed by preparative HPLC yielded (**R**)-**15a** (0.08 g, 92% yield) as a pale yellow colored oil. ¹H NMR (400 MHz, CDCl₃) δ 7.12-7.40 (m, 8H), 6.54-6.69 (m, 1H), 6.22 (br s, 1H), 5.63-5.79 (m, 1H), 5.57 (d, *J* = 10 Hz, 1H), 5.00-5.12 (m, 3H), 3.93-4.09 (m, 1H), 3.72-3.81 (m, 4H), 3.51 (s, 3H), 3.03 (d, *J* = 13.6 Hz, 1H), 2.91 (d, *J* = 13.2 Hz, 1H), 2.69 (br s, 1H), 2.11-2.18 (m, 1H), 1.82-1.91 (m, 2H), 1.31-1.63 (m, 4H), 0.71 (s, 9H), 0.61 (d, *J* = 6 Hz, 3H), 0.56 (d, *J* = 6 Hz, 3H). ¹³C NMR (100 MHz, CDCl₃) δ 175.1, 171.3, 170.1, 157.7, 136.5, 136.4, 136.2, 134.8, 131.5, 131.4, 130.5, 126.3, 120.7, 116.1, 113.8, 79.7, 75.2, 61.2, 60.4, 59.4, 52.1, 46.3, 42.8, 34.1, 30.3, 26.3, 21.5, 19.4, 18.6, 15.2. HRMS C₃₇H₅₂BrN₅O₆, Calc. *m/z* [M+H⁺] 742.3179, found 742.3173.

Methyl ((*S*)-1-(2-((*R*)-5-(((*S*)-1-(allylamino)-3,3-dimethyl-1-oxobutan-2-yl)amino)-4-hydroxy-5-oxo-4-(4-vinylbenzyl)pentyl)-2-(4-bromobenzyl)hydrazinyl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate ((*R*)-15b):

To a solution of (**R**)-**11b** (0.66 g, 1.3 mmol) in anhydrous DCM (20 mL) was added Dess-Martin Periodinane (1.1 g, 2.6 mmol) and stirred at room temperature under nitrogen atmosphere for 2 h. The reaction mixture was washed with saturated Na₂S₂O₃ solution (2 × 10 mL), brine (2 × 10 mL) and dried over Na₂SO₄. The organic layer was concentrated under reduced pressure to yield the corresponding aldehyde. To the solution of this crude aldehyde in 1,2-

dichloroethane (20 mL) was added **13** (0.57 g, 1.5 mmol) and molecular sieves (4 Å) and stirred for 1 h at room temperature after which sodium triacetoxyborohydride (0.81 g, 3.8 mmol) was added and the reaction mixture was stirred for 15 h at room temperature under nitrogen atmosphere. Saturated NaHCO₃ solution (20 mL) was added to the reaction mixture and the aqueous layer was extracted with DCM (2 × 15 mL), washed with brine (1 × 10 mL), dried over Na₂SO₄ and concentrated under reduced pressure. Flash chromatography (EtOAc/*iso*-hexane 40:60) yielded the OTBS-protected intermediate (0.54 g, 50% yield) as a white solid.

A solution (2 M) of TBAF in anhydrous THF (2 mL) was added to the OTBS-protected intermediate (0.04 g, 0.05 mmol) under nitrogen atmosphere at 0 °C and the reaction mixture was warmed to room temperature and stirred for 30 min. Distilled water (10 mL) was added to the reaction mixture and the aqueous layer was extracted with EtOAc (2 × 15 mL). The organic layer was washed with brine (1 × 15 mL), dried over Na₂SO₄ and concentrated under reduced pressure. Flash chromatography (MeOH/DCM 5:95) followed by preparative LC-MS yielded (**R**)-**15b** (0.03 g, 95% yield) as a pale colored oil. ¹H NMR (400 MHz, CDCl₃) δ 7.32 (d, *J* = 8 Hz, 2H), 7.24 (d, *J* = 8 Hz, 2H), 7.17 (d, *J* = 8.4 Hz, 2H), 7.09 (d, *J* = 8.4 Hz, 2H), 6.61 (dd, *J* = 10.8, 17.6 Hz, 1H), 5.61-5.73 (m, 2H), 5.03-5.19 (m, 3H), 4.05 (d, *J* = 9.6 Hz, 1H), 3.84 (s, 2H), 3.63-3.70 (m, 3H), 3.58 (s, 3H), 3.05 (d, *J* = 13.6 Hz, 1H), 2.84 (d, *J* = 13.6 Hz, 1H), 2.66 (t, *J* = 9.6 Hz, 2H), 1.85-2.01 (m, 2H), 1.47-1.53 (m, 2H), 1.30 (br s, 1H), 0.91 (s, 9H), 0.78 (s, 9H). ¹³C NMR (100 MHz, CDCl₃) δ 21.3, 26.4, 27.1, 34.1, 34.3, 36.9, 41.9, 45.6, 52.7, 56.2, 61.2, 61.5, 66.7, 68.5, 78.1, 113.5, 116.6, 121.6, 126.0, 130.8, 130.9, 131.6, 134.1, 136.0, 136.4, 136.9, 157.3, 170.3, 170.8, 175.4. HRMS C₃₈H₅₄BrN₅O₆, Calc. *m/z* [M+H⁺] 756.3336, found 756.3332.

Methyl ((S)-1-(2-(((S)-5-(((S)-1-(allylamino)-3-methyl-1-oxobutan-2-yl)amino)-4-hydroxy-5-oxo-4-(4-vinylbenzyl)pentyl)-2-(4-bromobenzyl)hydrazinyl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate ((S)-15a):

Compound **(S)-15a** was prepared from diastereoisomer **(S)-9a** using the same pathway and similar protocols as **(R)-15a** from diastereoisomer **(S)-9a**. **(S)-15a** is obtained as a pale yellow colored oil. ^1H NMR (400 MHz, CDCl_3) δ 7.76 (br s, 1H), 7.63 (br s, 1H), 7.09-7.35 (m, 8H), 6.58-6.66 (m, 1H), 6.31 (br s, 1H), 5.60-5.76 (m, 1H), 5.49 (d, $J = 10$ Hz, 1H), 5.02-5.16 (m, 3H), 3.98-4.05 (m, 1H), 3.73-3.88 (m, 4H), 3.56 (s, 3H), 3.01 (d, $J = 13.6$ Hz, 1H), 2.86 (br s, 1H), 2.79 (d, $J = 13.2$ Hz, 1H), 2.65 (br s, 1H), 2.14-2.17 (m, 1H), 1.85-1.90 (m, 2H), 1.36-1.67 (m, 4H), 0.74 (s, 9H), 0.63 (d, $J = 6$ Hz, 3H), 0.54 (d, $J = 6$ Hz, 3H). ^{13}C NMR (100 MHz, CDCl_3) δ 175.7, 171.6, 170.9, 157.3, 136.9, 136.5, 136.2, 134.1, 131.6, 131.3, 130.9, 126.0, 121.9, 116.8, 113.4, 79.0, 75.6, 61.3, 60.7, 59.3, 52.6, 46.1, 42.1, 34.3, 30.0, 26.4, 21.6, 19.4, 18.1, 15.1. HRMS $\text{C}_{37}\text{H}_{52}\text{BrN}_5\text{O}_6$, Calc. m/z $[\text{M}+\text{H}^+]$ 742.3179, found 742.3169.

Methyl ((S)-1-(2-(((S)-5-(((S)-1-(allylamino)-3,3-dimethyl-1-oxobutan-2-yl)amino)-4-hydroxy-5-oxo-4-(4-vinylbenzyl)pentyl)-2-(4-bromobenzyl)hydrazinyl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate ((S)-15b):

Compound **(S)-15b** was prepared from diastereoisomer **(S)-9b** using the same pathway and similar protocols as **(R)-15b** from diastereoisomer **(S)-9b**. **(S)-15b** is obtained as a pale yellow colored oil. ^1H NMR (400 MHz, CDCl_3) δ 7.06-7.32 (m, 8H), 6.53-6.66 (m, 2H), 5.61-5.65 (m, 2H), 5.39 (t, $J = 8$ Hz, 1H), 5.01-5.16 (m, 2H), 3.41-3.89 (m, 6H), 3.17-3.19 (m, 1H), 2.92-2.98 (m, 2H), 2.81 (d, $J = 13.6$ Hz, 1H), 2.6 (br s, 1H), 2.21-2.23 (m, 1H), 1.38-1.62 (m, 6H), 0.53-0.87 (m, 18H). ^{13}C NMR (100 MHz, CDCl_3) δ 175.8, 171.3, 170.9, 156.8, 136.9, 136.7, 136.3, 136.2, 135.3, 131.7, 130.9, 130.7, 125.9, 122.0, 113.7, 113.3, 79.1, 68.8, 67.6, 63.7, 56.9, 52.6, 46.3, 46.1, 42.8, 34.7, 34.4, 26.5, 26.4, 26.3, 25.8, 22.9. HRMS $\text{C}_{38}\text{H}_{54}\text{BrN}_5\text{O}_6$, Calc. m/z $[\text{M}+\text{H}^+]$ 756.3336, found 756.3344.

Methyl ((*S*)-1-(2-((*R*)-5-(((*S*)-1-(allylamino)-3-methyl-1-oxobutan-2-yl)amino)-4-(4-allylbenzyl)-4-hydroxy-5-oxopentyl)-2-(4-bromobenzyl)hydrazinyl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate ((*R*)-16a):

To a solution of (*R*)-**12a** (0.16 g, 0.31 mmol) in anhydrous DCM (5 mL) was added Dess-Martin Periodinane (0.27 g, 0.63 mmol) and stirred at room temperature under nitrogen atmosphere for 2 h. The reaction mixture was washed with saturated Na₂S₂O₃ solution (2 × 10 mL), brine (2 × 10 mL) and dried over MgSO₄. The organic layer was concentrated under reduced pressure to yield the corresponding aldehyde. To the solution of this crude aldehyde in 1,2-

dichloroethane (5 mL) was added **13** (0.14 g, 0.38 mmol), 2 drops of acetic acid and molecular sieves (4 Å) and stirred for 1 h at room temperature after which sodium triacetoxymethylborohydride (0.20 g, 0.95 mmol) was added and the reaction mixture was stirred for 15 h at room temperature under nitrogen atmosphere. Saturated NaHCO₃ solution (20 mL) was added to the reaction mixture and the aqueous layer was extracted with DCM (2 × 15 mL), washed with brine (1 × 10 mL), dried over MgSO₄ and concentrated under reduced pressure. Purification by preparative HPLC yielded the OTBS-protected intermediate (0.10 g, 37% yield) as a colorless oil.

The OTBS-protected intermediate (0.10 g, 0.12 mmol) was dissolved in anhydrous THF (4 mL). At 0 °C, a solution (2 M) of TBAF in anhydrous THF (4.6 mL) was slowly added. The reaction mixture was then kept under nitrogen atmosphere and was warmed to room temperature. Stirring was maintained overnight after which TLC show no starting material left. Distilled water (5 mL) was added to the reaction mixture and the aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried over MgSO₄ and concentrated under reduced pressure. Purification was made by preparative HPLC gave a pale colored oil (0.06 g, 74% yield). ¹H NMR (400 MHz, CD₃OD) δ 7.91 (t, *J* = 5.66 Hz, 1H), 7.53 (d, *J* = 8.98 Hz, 1H), 7.42 (d, *J* = 8.59 Hz, 2H), 7.30 (d, *J* = 8.59 Hz, 2H), 7.12 (d, *J* = 8.20 Hz, 2H), 7.05 (d, *J* = 8.20 Hz, 2H), 5.97 (tdd, *J* = 6.69, 10.20, 17.04 Hz, 1H), 5.75 - 5.88 (m, 1H), 5.01 - 5.25 (m, 4H), 4.09 (dd, *J* = 7.03, 8.98 Hz, 1H), 3.85 (s, 2H), 3.69 - 3.78 (m, 3H), 3.65 (s, 3H), 3.37 (s, 1H), 3.03 (d, *J* = 13.67 Hz, 1H), 2.72 - 2.86 (m, 3H), 1.92 - 2.06 (m, 2H), 1.64 - 1.82 (m, 2H), 1.39 - 1.57 (m, 2H), 0.87 - 0.96 (m, 6H), 0.85 (s, 9H). ¹³C NMR (101 MHz, CD₃OD) δ 175.59, 171.25, 170.42, 157.16, 138.20, 137.24, 134.16, 133.75, 131.30, 130.54, 130.49, 128.19, 121.33, 115.96, 115.84, 77.84, 61.37, 60.72, 59.29, 55.72,

52.53, 45.27, 41.63, 39.85, 33.73, 29.27, 26.19, 20.55, 19.53, 17.99. HRMS $C_{38}H_{54}BrN_5O_6$, Calc. m/z $[M+H^+]$ 756.3336, found 756.3335.

Methyl ((*S*)-1-(2-((*R*)-5-(((*S*)-1-(allylamino)-3,3-dimethyl-1-oxobutan-2-yl)amino)-4-(4-allylbenzyl)-4-hydroxy-5-oxopentyl)-2-(4-bromobenzyl)hydrazinyl)-3,3-dimethyl-1-oxobutan-2-yl)carbamate ((*R*)-16b):

To a solution of (*R*)-**12b** (0.11 g, 0.21 mmol) in anhydrous DCM (5 mL) was added Dess-Martin Periodinane (0.17 g, 0.41 mmol) and stirred at room temperature under nitrogen atmosphere for 2 h. The reaction mixture was washed with saturated $Na_2S_2O_3$ solution (2×10 mL), brine (2×10 mL) and dried over $MgSO_4$. The organic layer was concentrated under reduced pressure to yield the corresponding aldehyde. To the solution of this crude aldehyde in 1,2-dichloroethane (5 mL) was added **13** (0.09 g, 0.25 mmol), 2 drops of acetic acid and molecular sieves (4 Å) and stirred for 1 h at room temperature after which sodium triacetoxyborohydride (0.13 g, 0.62 mmol) was added and the reaction mixture was stirred for 15 h at room temperature under nitrogen atmosphere. Saturated $NaHCO_3$ solution (20 mL) was added to the reaction mixture and the aqueous layer was extracted with DCM (2×15 mL), washed with brine (1×10 mL), dried over $MgSO_4$ and concentrated under reduced pressure. Purification by preparative HPLC yielded the OTBS-protected intermediate (0.07 g, 41% yield) as a colorless oil.

The OTBS-protected intermediate (0.07 g, 0.09 mmol) is dissolved in anhydrous THF (4 mL). At 0 °C, a solution (2 M) of TBAF in anhydrous THF (6.8 mL) was slowly added. The reaction mixture was then kept under nitrogen atmosphere and was warmed to room temperature. Stirring was maintained for 30 min after which TLC show no more starting material left. Distilled water (5 mL) was added to the reaction mixture and the aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. Purification was made by preparative HPLC gave a pale colored oil (0.04 g, 70% yield). 1H NMR (400 MHz, CD_3OD) δ 8.15 (t, J = 5.66 Hz, 1H), 7.54 (d, J = 9.76 Hz, 1H), 7.42 (d, J = 8.40 Hz, 2H), 7.30 (d, J = 8.40 Hz, 2H), 7.10 (d, J = 8.01 Hz, 2H), 7.03 (d, J = 8.20 Hz, 2H), 5.97 (m, 1H), 5.83 (m, 1H), 5.25 (dq, J = 17.18 Hz, J = 1.56 Hz, 1H), 5.14 (dq, J = 10.35 Hz, J = 1.56 Hz, 1H), 5.02 - 5.11 (m, 2H), 4.13 (d, J =

9.57 Hz, 1H), 3.87 (s, 2H), 3.73 (m, 3H), 3.65 (s, 3H), 3.36 (m, 2H), 3.00 (d, $J = 13.47$ Hz, 1H), 2.83 (m, 3H), 2.00 (m, 1H), 1.77 (m, 3H), 1.51 (m, 1H), 1.28 (s, 3H), 0.95 (s, 9H), 0.85 (s, 9H). ^{13}C NMR (101 MHz, CD_3OD) δ 176.79, 176.72, 172.02, 159.05, 139.30, 139.14, 135.62, 135.41, 132.56, 132.37, 131.56, 129.19, 122.34, 116.93, 116.06, 79.64, 63.09, 61.67, 58.69, 56.19, 52.95, 46.66, 42.94, 41.08, 38.05, 36.78, 35.07, 29.69, 27.38, 27.07, 22.65. HRMS $\text{C}_{39}\text{H}_{56}\text{BrN}_5\text{O}_6$, Calc. m/z $[\text{M}+\text{H}^+]$ 770.3492, found 770.3494.

((R)-17b) and ((R)-18b):

A 0.5-2.0 mL microwave vial was charged **(R)-15b** (15 mg, 1.2 mmol) and Hoveyda-Grubbs 2nd generation catalyst (10

mol%) in 1,2-dichloroethane (3 mL) and irradiated in the microwave reactor cavity at 130 °C for 1 h. The reaction mixture was passed through a short Celite plug and the plug was rinsed with EtOAc (15 mL). The organic layer was washed with brine (2×15 mL), dried over Na_2SO_4 and concentrated under reduced pressure. Preparative HPLC purification yielded **(R)-17b** (5 mg, 42% yield) and **(R)-18b** (3 mg, 20% yield) as light brown colored oils that were freeze dried.

For (R)-17b:

^1H NMR (400 MHz, CDCl_3): δ 7.34-7.51 (m, 3H), 6.93-7.17 (m, 8H), 6.12-6.6.16 (m, 1H), 6.31 (dd, $J = 14.4, 15.6$ Hz, 1H), 5.63 (d, $J = 10.8$ Hz, 1H), 5.05-5.13 (m, 1H), 3.67-4.03 (m, 3H), 3.58 (s, 3H), 3.26-3.43 (m, 2H), 2.55-3.01 (m, 4H), 1.98-2.34 (m, 4H), 1.80 (d, $J = 6.4$ Hz, 1H), 0.67-1.01 (m, 18H). ^{13}C NMR (100 MHz, CDCl_3): δ 18.7, 26.5, 27.0, 27.3, 33.9, 35.4, 36.5, 42.1, 45.7, 52.8, 57.0, 60.4, 61.5, 66.7, 68.2, 79.8, 121.8, 125.7, 128.6, 129.6, 130.8, 131.0, 131.4, 131.8, 135.0, 135.9, 137.9, 156.9, 167.8, 171.1, 174.6. HRMS $\text{C}_{36}\text{H}_{50}\text{BrN}_5\text{O}_6$, Calc. m/z $[\text{M}+\text{H}^+]$ 728.3023, found 728.3021.

For (R)-18b:

^1H NMR (400 MHz, CDCl_3): δ 6.98-7.47 (m, 8H), 6.76-7.03 (m, 1H), 6.29 (d, $J = 16.4$ Hz, 1H), 5.09-5.15 (m, 1H), 3.76-3.93 (m, 4H), 3.57 (s, 3H), 3.24-3.47 (m, 2H), 3.01-3.08 (m, 1H), 2.69-2.80 (m, 3H), 2.11-2.42 (m, 4H), 1.57-1.84 (m, 4H), 0.64-1.41 (m, 18H). ^{13}C NMR

(100 MHz, CDCl₃): δ 21.2, 22.5, 26.5, 26.9, 29.5, 33.9, 35.3, 36.5, 42.1, 46.2, 52.8, 57.4, 60.3, 61.9, 66.8, 68.4, 79.7, 103.8, 121.9, 123.1, 125.9, 126.4, 128.2, 130.8, 131.4, 131.7, 136.3, 137.2, 139.3, 157.3, 158.4, 167.1, 177.5. HRMS C₃₆H₅₀BrN₅O₆, Calc. m/z [M+H⁺] 728.3023, found 728.3027.

((R)-19a):

A 10-20 mL microwave vial was charged **(R)-16a** (24 mg, 0.32 mmol) and Hoveyda-Grubbs 2nd generation catalyst (10 mol%) in DCM (9 mL) and irradiated in the microwave reactor cavity at 60 °C for 30 min. The reaction mixture was passed through a short Celite plug and the plug was rinsed with EtOAc (15 mL). The organic layer was concentrated under reduced pressure. Preparative HPLC purification yielded **(R)-19a** (8 mg, 35% yield) as a colorless oil. ¹H NMR (400 MHz, CD₃OD) δ 7.44–.36 (m, 2H), 7.30 (m, 3H), 7.07 (m, 2H), 6.87 (m, 1H), 6.32 (dt, J = 10.2, 7.4 Hz, 1H), 5.70 (td, J = 10.2, 7.6 Hz, 1H), 3.93–3.81 (m, 3H), 3.75–3.66 (m, 2H), 3.63 (s, 3H), 3.48–3.33 (m, 2H), 2.80 (m, 4H), 2.03 (m, 1H), 1.87 (m, 2H), 1.72 (m, 1H), 1.48 (m, 1H), 1.30 (m, 3H), 0.83–0.71 (m, 15H). ¹³C NMR (101 MHz, CD₃OD) δ 176.15, 172.23, 172.07, 142.49, 137.87, 136.38, 135.47, 132.60, 132.37, 131.55, 131.22, 129.47, 129.38, 128.30, 122.31, 80.57, 63.15, 62.18, 59.51, 58.86, 56.20, 52.95, 47.14, 38.25, 37.61, 35.04, 32.97, 32.31, 32.24, 29.68, 27.05, 22.72, 19.82, 18.58. HRMS C₃₆H₅₀BrN₅O₆, Calc. m/z [M+H⁺] 728.3023, found 728.3002.

((R)-19b):

A microwave vial was charged **(R)-16b** (15 mg, 0.19 mmol) and Hoveyda-Grubbs 2nd generation catalyst (10 mol%) in DCM (3 mL) and irradiated in the microwave reactor cavity at 60 °C for 30 min. The reaction mixture was passed through a short Celite plug and the plug was rinsed with EtOAc (15 mL). The organic layer was concentrated under reduced pressure. Preparative HPLC purification yielded **(R)-19b** (5 mg, 36% yield) as a colorless oil. ¹H NMR (400 MHz, CD₃OD) δ 7.40 (m, 2H), 7.31 (m, 2H), 7.21 (m, 1H), 7.06 (dd, J = 7.9, 1.9 Hz, 1H), 6.97 (dd, J = 8.0, 1.9 Hz, 1H), 6.83 (dd, J = 7.9, 1.9 Hz, 1H), 6.30 (ddd, J = 10.4, 8.3, 6.6 Hz, 1H), 5.79 (ddt, J = 8.9, 6.2, 4.4 Hz, 1H), 4.04 (m, 1H), 3.85 (s, 2H), 3.71 (s, 1H), 3.62 (m, 4H), 3.49–3.32 (m, 2H), 3.12–3.02 (m, 1H),

2.89–2.74 (m, 4H), 2.09–1.96 (m, 1H), 1.86 (m, 1H), 1.73 (m, 1H), 1.48 (m, 1H), 1.35–1.22 (m, 3H), 0.81 (m, 18H). ¹³C NMR (101 MHz, CD₃OD) δ 175.77, 172.08, 170.85, 159.09, 141.63, 137.88, 135.81, 135.18, 132.62, 132.36, 131.26, 130.89, 129.44, 128.89, 128.36, 122.31, 80.61, 63.15, 62.16, 61.41, 58.89, 52.95, 38.07, 37.43, 35.87, 35.06, 32.78, 30.90, 29.69, 27.19, 27.07, 22.73. HRMS C₃₇H₅₂BrN₅O₆, Calc. m/z [M+H⁺] 742.3179, found 742.3180

3 - HIV protease inhibition

The HIV-1 protease was cloned and heterologously expressed in *Escherichia coli* and purified as described elsewhere.³ The K_i values for the synthesized compounds were determined from two individual measurements by a fluorometric assay.⁴ Assay variability was checked by inclusion of a known inhibitor and the standard deviation for the enzyme assays was ±50% of the mean.

4 - In vitro anti-HIV activity

The in vitro anti-HIV activity was assayed in MT4 cells according to a previously published procedure⁴ using the colorimetric XTT assay to monitor the cytopathogenic effects.

5 - Determination of cytotoxicity

MT4 cells were maintained in RPMI supplemented with 10% heat inactivated fetal calf serum, penicillin (100 U/mL) and streptomycin (100 µg/mL). Briefly, compounds in serial dilutions were added to MT4 cells (2 x 10⁴ cells/well) in microplates. After five days of incubation at 37 °C, the number of viable cells in each well was assessed by using a soluble formazan (XTT) assay⁵ and the concentration causing 50% decrease in cell proliferation (CC₅₀) was determined.

6 - X-ray structure determination details

Production of the protease material and co-crystallization of the inhibitor with the protease was performed at +4 °C by hanging drop procedure according to the procedures described earlier.⁶ The crystals belonged to the space group P21212.

X-ray data were recorded on MarResearch CCD-detectors on the synchrotron beam lines at I911 at MAX-lab Lund, Sweden. Data processing and scaling was performed with MOSFLM⁷ and SCALA⁸ from the CCP4 program suite.⁹ The structure was built in COOT¹⁰ and refined with the CCP4 programs. PDB-ID for structures (*R*)-**17b**, (*R*)-**19a**, (*R*)-**19b** are 3zps, 3zpt and 3zpu respectively. Figures were made in Swiss-PdbViewer¹¹ and rendered in Molray¹²

7 - Table of crystallographic structure determination statistics

Compound in complex	(<i>R</i>)- 17b	(<i>R</i>)- 19a	(<i>R</i>)- 19b
Data collection			
Cell axis length (Å)	57.84, 85.94, 46.28	58.20, 85.92, 46.27	58.33, 86.12, 46.29
Resolution range (Å)	28.9–1.55	29.1–1.54	27.7–1.80
No. of reflections measured	147807	237106	104399
No. of unique reflections	34063	35356	21480
Average multiplicity	4.3	6.7	4.9
Completeness (%)	99.3	99.8	96.7
R _{merge} (%)	7.6	11.4	9.6
R _{p.i.m.} (%)	3.5	4.9	4.8
<I>/s(<I>)	6.3	3.2	6.7
Refinement			
No. of reflections, working set	30637	31712	19307
No. of reflections, R _{free}	1740	1799	1067
R _{conv.} (%)	18.7	22.0	18.1
R _{free} (%)	21.0	24.2	22.4
No. of atoms	1859	1859	1860
No. of solvent water atoms	289	289	290
Mean B factor (Å ²)	15.6	13.3	15.2
Mean B factor (Å ²)			
r.m.s deviation from ideal bond lengths (Å)	0.006	0.009	0.03
r.m.s deviation from ideal bond angle	0.90	1.1	2.1

8 - X-ray pictures

(*R*)-**17b**, PDB code 3zps

(*R*)-**19a**, PDB code 3zpt

(*R*)-**19b**, PDB code 3zpu

9 - References

- 1) B.S. Furniss, A.J. Hannaford, P.W.G. Smith, Vogel's Text Book of Organic Chemistry, fifth ed. Longman Group Limited, London, **1989**, p. 1155.
- 2) Wong, Henry N. C. et al, *Synthesis*, **1992**, (8), 793-797
- 3) Danielson, U. H.; Lindgren, M. T.; Markgren, P.-O.; Nillroth, U. Investigation of an allosteric site of HIV-1 proteinase involved in inhibition by Cu^{2+} . *Adv. Exp. Med. Biol.* **1998**, 436, 99-103
- 4) Nillroth, U.; Vrang, L.; Markgren, P.-O.; Hulten, J.; Hallberg, A.; Danielson, U. H. Human immunodeficiency virus type 1 proteinase resistance to symmetric cyclic urea inhibitor analogs. *Antimicrob. Agents and Chemother.* **1997**, 41, 2383-2388.
- 5) Weislow, O. S.; Kiser, R.; Fine, D. L.; Bader, J.; Shoemaker, R. H.; Boyd, M. R. New soluble-formazan assay for HIV-1 cytopathic effects : application to high-flux screening of synthetic and natural products for AIDS-antiviral activity. *J. Natl. Cancer Inst.* **1989**, 81, 577-586.
- 6) Andersson, H. O.; Fridborg, K.; Löwgren, S.; Alterman, M.; Muhlman, A.; Björnsne, M.; Garg, N.; Kvarnström, I.; Schaal, W.; Classon, B.; Karlen, A.; Danielsson, U. H.; Ahlsen, G.; Nillroth, U.; Vrang, L.; Oberg, B.; Samuelsson, B.; Hallberg, A.; Unge, T. Optimization of P1-P3 groups in symmetric and asymmetric HIV-1 protease inhibitors. *Eur. J. Biochem.* **2003**, 270, 1746-1758.
- 7) Leslie, A. Integration of macromolecular diffraction data. *Acta Crystallographica Section D* **1999**, 55, 1696-1702.
- 8) Evans, P. R. Data reduction Proceedings of CCP4 Study Weekend on Data Collection and Processing, Daresbury Laboratory, Warrington, England. **1993**, 114-122.
- 9) Bailey, S. The CCP4 suite: programs for protein crystallography. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **1994**, D50, 760-763.
- 10) Emsley, P. and Cowtan, K., Coot: model-building tools for molecular graphics" *Acta Crystallographica Section D-Biological Crystallography* **60**: 2126-2132 Part 12 Sp. Iss. 1 DEC 2004
- 11) Guex, N. and Peitsch, M.C. Swiss model and the Swiss-PdbViewer: An environment for comparative protein modeling. *Electrophoresis* **1997**, 18, 2714-2723.
- 12) Harris, M.; Jones, T. A. Molray - a web interface between O and the POV-Ray ray tracer. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2001**, D57, 1201-1203