Supporting Information

HIV-1 Protease Inhibitors with a Tertiary-Alcohol-Containing Transition-State Mimic and Various P2/P1' Substituents

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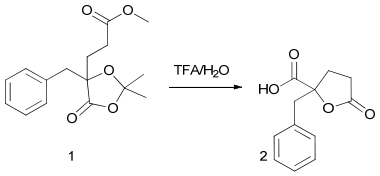
1 General Information

The microwave reactions were performed in a SmithSynthesizer producing controlled irradiation at 2450 MHz with a power of 0–300 W. The reaction temperature was determined using the built-in on-line IR-sensor. Column chromatography was performed on Merck silica gel 60 (40–63 μm). Analytical thin layer chromatography was performed using aluminium sheets precoated with silica gel 60 F_{254}. Analytical RPHPLC-MS was performed on a Gilson HPLC system with a Finnigan AQA ESI quadropole mass spectrometer using a Onyx Monolithic C18 4.6 × 50mm (Phenomenex) with CH_3CN or MeOH in 0.05% aqueous HCOOH as mobile phase at a flow rate of 4 cm^3/min. Preparative RPHPLC-MS was performed on a Gilson HPLC system with a Finnigan AQA ESI quadropole mass spectrometer using a Zorbax SB-C8, 5 μm 21.2 × 150 mm (Agilent technologies) column, with CH_3CN in 0.05% aqueous HCOOH as mobile phase at a flow rate of 10–15 cm^3/min. Preparative RP-HPLC was performed on a either a (A) Gilson HPLC system using a Zorbax SB-C8, 5 μm 21.2 × 150 mm (Agilent technologies) column, with CH_3OH in 0.05% aqueous HCOOH as mobile phase at a flow rate of 10 cm^3/min or a (B) Gilson HPLC system with a Spectra Physics Analytical UV1000 detector using a Zorbax SB-C8, 5 μm 21.2 × 150 mm (Agilent technologies) column, with CH_3CN in 0.05% aqueous HCOOH as mobile phase at a flow rate of 5 cm^3/min. ^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. Exact molecular masses were determined on Micromass Q-Tof2 mass spectrometer equipped with an electrospray ion source. Analytische Laboratorien, Lindlar, Germany performed elemental analyses. Crystallization and collection of X-ray data for compound (*S*)-**4a** were performed at the Latvian Institute of Organic Synthesis, Riga, Latvia. In general, reagents and solvents were used as purchased without further purifications.

1, **2** and **12a–b** were synthesized and characterized as previously preported.[1]

Medivir AB kindly supported us with, the (*S*)-2-amino-*N*-methyl-3-phenylpropanamide (**3e**) and the (*2S*)-2-amino-*N*,4-dimethylhexanamide (**3b**). (*S*)-2-amino-3,3,*N*-trimethyl-butyramide (**3a**) and (*S*)-2-amino-*N*-methyl-propionamide (**3d**) are commercial available.

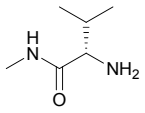
2 Chemistry



1 2

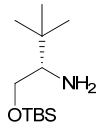
2-Benzyl-5-oxo-tetrahydro-furan-2-carboxylic acid (2)

The solution of **1** (1.758 g, 6.014 mmol) in 6 cm^3 TFA/H_2O (6:1) was stirred at 80 °C overnight. Next, the reaction mixture was concentrated,, dissolved in ethyl acetate and concentrated again to remove all TFA (three times). Dried with vacuum until the crude product solidified. Purified with ethyl acetate-petroleum ether to give 1.258 g (**2**) in 95% yield. δ_H(400 MHz; $CDCl_3$; Me_4Si) 2.00–2.16 (m, 1H), 2.20–2.34 (m, 1H), 2.40–2.54 (m, 2H), 3.13 (d, J = 14.4 Hz, 1H), 3.36 (d, J = 14.4 Hz, 1H), 7.20–7.40 (m, 5H); δ_C(100 MHz; $CDCl_3$; Me_4Si) 28.3, 30.2, 42.3, 86.5, 127.8, 128.8, 130.8, 133.9, 175.3, 176.9; MS (*m/z* 262, M+H^++MeCN, 441, 2 × M+H^+, 661, 3 × M+H^+).



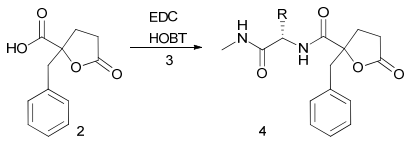
(*S*)-2-amino-*N*,3-dimethylbutanamide (3c)

To a solution of Z-Val-Osu (4.006 g, 1.150 mmol) in dry THF at 0 °C was added 2 M methylamine in THF (10.0 cm^3, 20.0 mmol). The reaction mixture was allowed to come to room temperature and stirred for 5 h. Next the formed white precipitate was filtered off and the residue was evaporated under reduced pressure. The residue was dissolved in DCM and extracted 3 times with saturated aqueous $NaHCO_3$. The aqueous layers were combined and extracted twice with DCM. The organic layers were dried over K_2CO_3 and concentrated under reduced pressure. Pd/C (1.226 g, 1.152 mmol) was added together with 50 cm^3 MeOH and stirred at room temperature under hydrogen gas over night. The Pd/C was filtered off and the residue was concentrated under reduced pressure to give 0.920 g **3c** as yellowish oil in 61% yield. δ_H(400 MHz; $CDCl_3$; Me_4Si) 0.91 (d, J = 7.0 Hz, 3H), 0.94 (d, J = 7.0 Hz, 3H), 1.88–1.97 (m, 1H), 2.75 (s, 3H), 3.05 (d, J = 5.8 Hz, 1H); δ_C(100 MHz; $CDCl_3$; Me_4Si) 17.9. 19.7. 26.1. 33.4. 61.8. 177.3; MS (*m/z* 131.2, M+H^+, 261.2, 2xM+H^+) in accordance with previously reported.[2, 3]



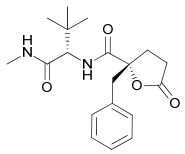
(*S*)-1-(*tert*-Butyl-dimethyl-silanyloxymethyl)-2,2-dimethyl-propylamine (3f)

To the solution of (*S*)-*tert*-Leucinol, (0.20 cm^3, 1.54 mmol) in dry DCM (10 cm^3), triethylamine (1.17 cm^3, 8.39 mmol) was added at 0 °C. The mixture was stirred for five minutes before *tert*-butyl-chlorodimethylsilane (TBSCl) (0.634 g, 4.20 mmol) dissolved in dry DCM (5 cm^3) was added. Thereafter the mixture was stirred at room temperature over night. A white precipitation was formed. Evaporation of solvent resulted in a white solid which was dissolved in water and extracted 3 × DCM. The organic layers were pooled, dried over $MgSO_4$ and evaporated yielding compound **3f** as colourless oil, which was used without further purification.



2.1 General Procedure A: Synthesis of lacton 4

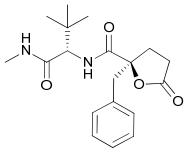
Dry DCM (15-40 mL) was added to the mixture of acid **2** (1.0 equiv), aminoacid methylamide **3** (1.0 equiv), EDC (1.1 equiv) and HOBt (1.1 equiv). The mixture was stirred for 1 h at room temperature. The reaction was quenched with 30 cm^3 water, filtered and extracted with 2 × 30 cm^3 DCM (or ethyl acetate). Combined the organic layers, dried over $MgSO_4$, concentrated and purified on silica gel to give **4** in 16–47% yield.



(*R*)-2-Benzyl-5-oxo-tetrahydro-furan-2-carboxylic acid ((*S*)-2,2-dimethyl-1-methylcarbamoyl-propyl)-amide (4a)

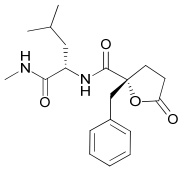
According to the general method **A**, **4a** was prepared using **2** (1.258 g, 5.713 mmol), L- *tert*-leucine methyl amide (0.824 mg, 5.71 mmol), EDC (1.205 g, 6.284 mmol), HOBt (0.849 mg, 6.28 mmol). The crude product was purified by silica flash chromatography using ethyl acetate as the eluent to give 0.792 g **4a** in 40%, and with 5–6% methanol in ethyl acetate to give 0.930 g (*S*)-**4a** in 47% yield. δ_H(400 MHz; $CDCl_3$; Me_4Si) 0.92 (s, 9H), 2.04–2.16 (m, 1H), 2.24–2.42 (m, 2H), 2.50–2.60 (m, 1H),

2.72 (d, J = 5.2 Hz, 3H), 3.06 (d, J = 14.0 Hz, 1H), 3.23 (d, J = 14.0 Hz, 1H), 4.11 (m, 1H), 5.84 (br s, 1H), 6.87 (d, J = 8.8 Hz, 1H), 7.15–7.30 (m, 5H); δ_C(100 MHz; $CDCl_3$; Me_4Si) 26.2, 26.6, 28.2, 30.3, 34.8, 43.4, 60.7, 88.0, 127.5, 128.6, 130.4, 134.0, 169.7, 171.5, 175.1; MS (*m/z* 347, $M+H^+$); Anal. Calcd for $C_{19}H_{26}N_2O_4$: C, 65.87; H, 7.56; N, 8.09; Found: C, 65.72; H, 7.69; N, 8.03.



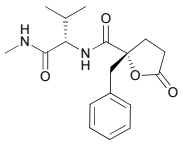
(*S*)-2-Benzyl-5-oxo-tetrahydro-furan-2-carboxylic acid ((*S*)-2,2-dimethyl-1-methylcarbamoyl-propyl)-amide (*S*-4a)

δ_H(400 MHz; $CDCl_3$; Me_4Si) 0.81 (s, 9H), 1.96–2.10 (m, 1H), 2.24–2.50 (m, 3H), 2.77 (m, 3H), 3.10 (d, J = 14.0 Hz, 1H), 3.23 (d, J = 14.0 Hz, 1H), 4.00–4.10 (m, 1H), 6.18 (br s, 1H), 6.92 (d, J = 9.2 Hz, 1H), 7.18–7.30 (m, 5H); δ_C(100 MHz; $CDCl_3$; Me_4Si) 26.2, 26.6, 28.1, 30.4, 34.4, 43.9, 61.0, 87.8, 127.6, 128.7, 130.6, 134.1, 170.3, 171.4, 175.2; MS (*m/z* 347, $M+H^+$); Anal. Calcd for $C_{19}H_{26}N_2O_4 \cdot + H_2O$: C, 62.62; H, 7.74; N, 7.69; Found: C, 62.41; H, 7.62; N, 7.59.



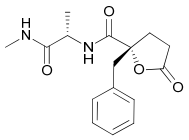
(*R*)-2-Benzyl-5-oxo-tetrahydro-furan-2-carboxylic acid ((*S*)-3-methyl-1-methylcarbamoyl-butyl)-amide (4b)

According to general method **A**, **4b** was prepared using **2** (0.220 g, 1.00 mmol), L-leucine methylamide (0.144 g, 1.00 mmol), EDC (0.211 g, 1.10 mmol), HOBt (0.149 g, 1.10 mmol). The crude product was purified by silica flash chromatography using ethyl acetate as the eluent to give 0.131 g **4b** in 38% yield. δ_H(400 MHz; $CDCl_3$; Me_4Si) 0.80–0.92 (m, 6H), 1.38–1.50 (m, 2H), 1.66–1.76 (m, 1H), 2.16–2.46 (m, 3H), 2.50–2.58 (m, 1H), 2.61 (d, J = 4.8 Hz, 3H), 3.08 (d, J = 14.0 Hz, 1H), 3.27 (d, J = 14.0 Hz, 1H), 4.30–4.40 (m, 1H), 5.40–5.60 (m, 1H), 6.58 (d, J = 8.4 Hz, 1H), 7.20–7.35 (m, 5H); δ_C(100 MHz; $CDCl_3$; Me_4Si) 21.7, 23.0, 26.5, 28.1, 30.8, 40.6, 43.7, 51.6, 88.0, 127.6, 128.6, 130.5, 134.2, 171.3, 171.5, 175.2; MS (*m/z* 347, $M+H^+$, 693, 2 × $M+H^+$); Anal. Calcd for $C_{19}H_{26}N_2O_4 + 1.25H_2O$ + 1/5 MeCN: C, 61.05; H, 7.82; N, 8.08; Found: C, 61.43, H, 7.74, N, 8.13.



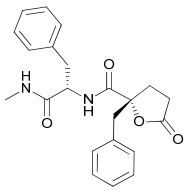
(*R*)-2-Benzyl-5-oxo-tetrahydro-furan-2-carboxylic acid ((*S*)-2-methyl-1-methylcarbamoyl-propyl)-amide (4c)

According to general method **A**, **4c** was prepared using **2** (0.838 g, 3.81 mmol), L-valin methylamide (0.599 g, 4.60 mmol) EDC (0.808 g, 4.22 mmol), HOBt (0.567 g, 4.19 mmol). The crude product was purified by silica flash chromatography using 90–100% ethyl acetate in petroleum ether as the eluent to give 0.413 g **4c** in 33% yield. δ_H(400 MHz; $CDCl_3$; Me_4Si) 0.73 (d, J = 6.9 Hz, 3H), 0.81 (d, J = 6.9 Hz, 3H), 2.17–2.47 (m, 4H), 2.59 (d, J = 4.8 Hz, 3H), 3.05 (d, J = 14.3 Hz, 1H), 3.25 (d, J = 14.3 Hz, 1H), 4.09 (dd, J = 6.0 Hz, 9.3 Hz, 1H), 5.12–5.20 (m, 1H), 6.49 (d, J = 9.3 Hz, 1H), 7.21–7.29 (m, 5H); δ_C(100 MHz; $CDCl_3$; Me_4Si) 17.5, 19.6, 26.5, 28.2, 30.0, 31.1, 43.8, 58.5, 88.4, 127.7, 128.7, 130.6, 134.4, 170.4, 171.7, 175.1; MS (*m/z* 333, $M+H^+$); HRMS: Calcd for *m/z* $C_{24}H_{43}N_2O_4Si^+$: 451.2992. Found: 451.2998.



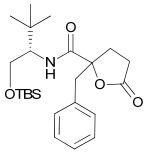
(*R*)-2-Benzyl-5-oxo-tetrahydro-furan-2-carboxylic acid ((*S*)-1-methylcarbamoyl-ethyl)-amide (4d)

According to general method **A**, **4d** was prepared using **2** (0.220 g, 1.00 mmol), H-Ala-NMe·HCl (0.139 g, 1.00 mmol), EDC (0.211 g, 1.10 mmol), HOBt (0.149 mg, 1.10 mmol) and NMM (0.111 g, 1.10 mmol). The crude product was purified by silica flash chromatography using ethyl acetate as the eluent to give 0.0760 g **4d** in 25% yield. δ_H(400 MHz; $CDCl_3$; Me_4Si) 1.30 (d, J = 6.8 Hz, 3H), 2.18–2.29 (m, 1H), 2.30–2.49 (m, 2H), 2.50–2.60 (m, 1H), 2.63 (d, J = 5.5 Hz, 3H), 3.08 (d, J = 14.0 Hz, 1H), 3.27 (d, J = 14.0 Hz, 1H), 4.37 (m, 1H), 5.47 (br s, 1H), 6.69 (d, J = 8.0 Hz, 1H), 7.20–7.35 (m, 5H); δ_C(100 MHz; $CDCl_3$; Me_4Si) 18.1, 26.5, 28.1, 30.6, 43.9, 48.8, 87.9, 127.7, 128.7, 130.6, 134.3, 171.3, 171.5, 175.2; MS (*m/z* 305, $M+H^+$, 609 2 × $M+H^+$); Anal. Calcd for $C_{16}H_{20}N_2O_4 \cdot 1/3H_2O$: C, 61.92; H, 6.71; N, 9.03; Found: C, 62.07; H, 6.75; N, 8.82.



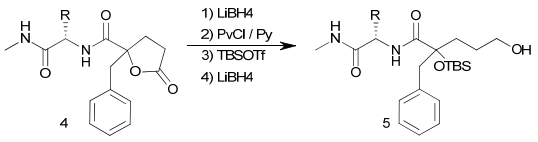
(*R*)-2-Benzyl-5-oxo-tetrahydro-furan-2-carboxylic acid ((*S*)-1-methylcarbamoyl-2-phenyl-ethyl)-amide (4e)

According to general method **A**, **4e** was prepared using **2** (0.220 g, 1.00 mmol), H-Phe-NMe (0.164 g, 1.00 mmol), EDC (0.211 g, 1.10 mmol), HOBt (0.149 g, 1.10 mmol). The crude product was purified by silica flash chromatography using ethyl acetate as the eluent to give 0.139 g **4e** in 37% yield. δ_H(400 MHz; $CDCl_3$; Me_4Si) 1.66–1.82 (m, 1H), 2.00–2.20 (m, 3H), 2.56 (d, J = 4.8 Hz, 3H), 2.73 (dd, J = 14.0 Hz, 10.0 Hz, 1H), 2.98 (d, J = 14.0 Hz, 1H), 3.19 (d, J = 14.0 Hz, 1H), 3.30 (dd, J = 14.0 Hz, 5.2 Hz, 1H), 4.58–4.68 (m, 1H), 5.12–5.22 (m, 1H), 6.49 (d, J = 9.2 Hz, 1H), 7.04–7.14 (m, 2H), 7.16–7.30 (m, 8H); δ_C(100 MHz; $CDCl_3$; Me_4Si) 26.5, 27.5, 31.1, 37.8, 43.9, 54.1, 88.1, 127.0, 127.5, 128.6, 128.8, 129.2, 130.5, 134.6, 137.0, 170.3, 171.4, 175.1; MS (*m/z* 381, $M+H^+$, 761, 2 × $M+H^+$); Anal. Calcd for $C_{22}H_{24}N_2O_4 \cdot 2/3H_2O$: C, 67.33; H, 6.51; N, 7.14; Found: C, 67.19; H, 6.65; N, 6.84.



2-Benzyl-5-oxo-tetrahydro-furan-2-carboxylic acid [(*S*)-1-(*tert*-butyl-dimethyl-silanyloxymethyl)-2,2-dimethyl-propyl]-amide (4f)

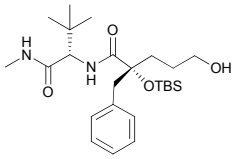
According to general method **A**, **4f** was prepared using compound **2** (0.209 g, 0.949 mmol), **3f** (0.304 g, 1.32 mmol), EDC (0.241 g, 1.26 mmol) and HOBt (0.166 g, 1.23 mmol) The reaction mixture was stirred for 4.5 h at room temperature. The crude product was purified by silica flash chromatography using 25–40% ethyl acetate in petroleum ether as the eluent to give 0.271 g **4f** as a racemate in 66% yield. δ_H(400 MHz; $CDCl_3$; Me_4Si) -0.03 – -0.01 (m, 6H), [0.76 (s, 9H) & 0.88 (s, 9H)], [0.84 (s, 9H) & 0.86 (s, 9H)], 1.97–2.09 (m, 1H), 2.24–2.40 (m, 2H), 2.46–2.55 (m, 1H), [3.08 (d, J = 14.4 Hz, 1H) & 3.12 (d, J = 14.4 Hz, 1H)], 3.24–3.28 (m, 2H), 3.32–3.36 (m, 1H), 3.61–3.70 (m, 2H), 7.22–7.29 (m, 5H); δ_C(100 MHz; $CDCl_3$; Me_4Si) [-5.47 & -5.45], [18.2 & 18.3], [25.93 & 25.95], [27.2 & 27.4], [28.2 & 28.3], [30.3 & 30.5], [34.0 & 34.7], [43.3 & 43.8], [57.4 & 58.0], [62.2 & 62.5], [88.2 & 88.4], 127.5, [128.7 & 128.7], [130.7 & 130.8], 134.4, [171.2 & 171.4], [175.4 & 175.5]; MS (*m/z* 434, M^+). HRMS Calcd for *m/z* $C_{24}H_{39}NO_4Si^+$: 434.2727. Found: 434.2539.



4 5

2.2 General Procedure B: Synthesis of 5

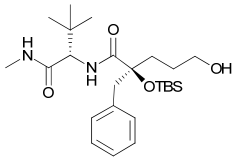
To the solution of **4** (1.0 equiv) in 15 cm^3 diethyl ether was added $LiBH_4$ (3.0 equiv) at room temperature. The reaction mixture was stirred until full conversion. The reaction was quenched with NH_4Cl and extracted with 3 × 30 cm^3 ethyl acetate. Dried with $MgSO_4$, concentrated to give crude diol. The resulting diol was dissolved in 4 cm^3 dry pyridine, then 5.0 equivalent trimethylacetyl chloride was added to the pyridine solution and stirred at room temperature for 1.5 h (sometimes overnight). Then 15 cm^3 water was added to the mixture, extracted with 3 × 15 cm^3 ether, dried over $MgSO_4$, concentrated to give a crude monoester. Thereafter 15 cm^3 dry DCM and triethylamine (6.0 equiv) were added to the intermediate. After 5 min *tert*-butyl dimethyl silyltriflate (TBSOTf) (3.0 equiv) was added at 0 °C and stirred at room temperature for 3 h or overnight. The solution was concentrated, extracted with diethyl ether and water. The ether layer was dried with $MgSO_4$, filtered and concentrated to give a crude oily intermediate. Then intermediate was then dissolved in 15 cm^3 diethyl ether. $LiBH_4$ (3.0 equiv) was added to the ether solution at room temperature. Portions of $LiBH_4$ were added every 2 h until full conversion was achieved. Saturated aqueous NH_4Cl solution was added to quench the reaction, extracted with 3 × 15 cm^3 ether, dried over $MgSO_4$, concentrated and purified on silica gel with 30–100% ethyl acetate in petroleum ether to give the product **5** in 25–85% yield.



(*R*)-2-benzyl-2-(*tert*-butyldimethylsilyloxy)-*N*-((*S*)-3,3-dimethyl-1-(methylamino)-1-oxobutan-2-yl)-5-hydroxypentanamide (5a)

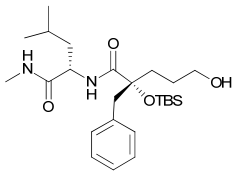
According to the general method **B**, **5a** was prepared using **4a** (1.032 g, 2.98 mmol), $LiBH_4$ (0.213g, 8.95 mmol), trimethylacetyl chloride (1.798 g, 14.9 mmol), pyridine (15 cm^3), triethylamine (3.078 g, 41.8 mmol), TBSOTf (3.231g, 12.3 mmol), $LiBH_4$ (0.313g, 13.2 mmol). The crude product was purified by silica flash chromatography using 50–100% ethyl acetate in petroleum ether as the eluent to give 1.184 g **5a** in 85% yield. δ_H(400 MHz; $CDCl_3$; Me_4Si) 0.16 (s, 3H), 0.24 (s, 3H), 0.93 (s, 9H), 0.97 (s, 9H), 1.45–1.60 (m, 1H), 1.70–1.85 (m, 3H), 2.10–2.20 (m, 1H), 2.69 (d, J = 4.8 Hz, 3H), 2.93

(d, J = 14.4 Hz, 1H), 3.25 (d, J = 14.4 Hz, 1H), 3.55–3.68 (m, 2H), 4.02 (d, J = 10.0 Hz, 1H), 5.81 (m, 1H), 7.05–7.20 (m, 5H), 7.50 (d, J = 10.0 Hz, 1H); δ_C(100 MHz; $CDCl_3$; Me_4Si) -1.7, -1.6, 18.7, 26.1, 26.3, 26.9, 27.5, 34.4, 36.5, 46.8, 60.8, 62.7, 83.0, 126.5, 128.0, 130.0, 136.4, 170.5, 174.1; MS (*m/z* 465, $M+H^+$, 929, 2 × $M+H^+$); Anal. Calcd for $C_{25}H_{44}N_2O_4Si$: C, 64.61; H, 9.54; N, 6.03; Found: C, 64.81; H, 9.62; N, 6.12.



(*S*)-2-benzyl-2-(*tert*-butyldimethylsilyloxy)-*N*-((*S*)-3,3-dimethyl-1-(methylamino)-1-oxobutan-2-yl)-5-hydroxypentanamide ((*S*)-5a)

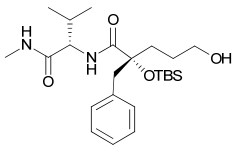
According to the general method **B**, (*S*)-**5a** was prepared using (*S*)-**4a** (0.200 g, 0.57 mmol), $LiBH_4$ (0.041g, 1.23 mmol), trimethylacetyl chloride (0.348 g, 2.89 mmol), pyridine (3 cm^3), triethylamine (0.115 g, 1.73 mmol), TBSOTf (0.229 g, 0.87 mmol), $LiBH_4$ (0.041g, 1.77 mmol). The crude product was purified by silica flash chromatography using 1–2% methanol in ethyl acetate as the eluent to give 0.167 g (*S*)-**5a** in 62% yield. δ_H(400 MHz; $CDCl_3$; Me_4Si) 0.21 (s, 3H), 0.23 (s, 3H), 0.86 (s, 9H), 0.96 (s, 9H), 1.26–1.40 (m, 1H), 1.58–1.75 (m, 2H), 1.93–2.20 (m, 1H), 2.54–2.61 (m, 3H), 2.61–2.78 (m, 1H), 2.95 (d, J = 14.0 Hz, 1H), 3.21 (d, J = 14.0 Hz, 1H), 3.32–3.48 (m, 1H), 4.20–4.27 (m, 1H), 6.94–7.95 (m, 1H), 7.12–7.22 (m, 5H), 7.65 (d, J = 10.0 Hz, 1H); δ_C(100 MHz; $CDCl_3$; Me_4Si) -1.7, -1.5, 18.7, 25.9, 26.3, 26.7, 27.1, 34.4, 36.6, 46.6, 60.5, 62.1, 82.6, 126.6, 128.0, 130.5, 136.6, 171.1, 174.3; MS (*m/z* 465, $M+H^+$, 929, 2 × $M+H^+$); Anal. Calcd for $C_{25}H_{44}N_2O_4Si$: C, 64.61; H, 9.54; N, 6.03; Found: C, 64.95; H, 9.71; N, 6.24.



(*R*)-2-benzyl-2-(*tert*-butyldimethylsilyloxy)-5-hydroxy-*N*-((*S*)-4-methyl-1-(methylamino)-1-oxopentan-2-yl)-pentanamide (5b)

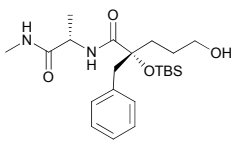
According to the general method **B**, **5b** was prepared using **4b** (0.121 g, 0.349 mmol), $LiBH_4$ (0.025 g, 1.05 mmol), trimethylacetyl chloride (0.211 g, 1.75 mmol), pyridine (4 cm^3), triethylamine (0.106 g, 1.05 mmol), TBSOTf (0.139 g, 0.524 mmol), $LiBH_4$ (0.146 g, 6.14 mmol). The crude product was purified by silica flash chromatography using 50–100% ethyl acetate in petroleum ether as the eluent to

give 0.097 g **5b** 60% yield. δ_H(400 MHz; $CDCl_3$; Me_4Si) 0.23 (s, 3H), 0.24 (s, 3H), 0.86–0.93 (m, 15H), 1.38–1.60 (m, 3H), 1.70–1.84 (m, 3H), 1.93 (br s, 1H), 2.10–2.20 (m, 1H), 2.58 (d, J = 4.8 Hz, 3H), 2.94 (d, J = 14.4 Hz, 1H), 3.23 (d, J = 14.4 Hz, 1H), 3.60 (t, J = 6.4 Hz, 2H), 4.24 (m, 1H), 5.72 (m, 1H), 6.98 (d, J = 8.8 Hz, 1H), 7.15–7.30 (m, 5H); δ_C(100 MHz; $CDCl_3$; Me_4Si) -1.6, 18.7, 21.6, 23.2, 24.8, 26.3, 26.4, 27.2, 36.6, 40.5, 46.5, 51.3, 62.5, 83.2, 126.8, 128.1, 130.3, 136.5, 171.9, 174.4; MS (*m/z* 465, $M+H^+$, 929, 2 × $M+H^+$); Anal. Calcd for $C_{25}H_{44}N_2O_4Si$: C, 64.61; H, 9.54; N, 6.03; Found: C, 64.69; H, 9.72; N, 5.92.



(*R*)-2-benzyl-2-(*tert*-butyldimethylsilyloxy)-5-hydroxy-*N*-((*S*)-3-methyl-1-(methylamino)-1-oxobutan-2-yl)-pentanamide (5c)

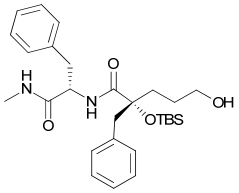
According to general procedure **B**, **5c** was prepared using **4c** (0.393 g, 1.18 mmol), $LiBH_4$ (0.257 g, 11.8 mmol), trimethylacetyl chloride (0.715 g, 5.93 mmol), pyridine (6 cm^3), trimethylamine (0.668 g, 6.60 mmol), TBSOTf (0.944 g, 3.57 mmol), litiumborohydride (0,105 g, 4.80 mmol). The crude product was purified by silica flash chromatography using 90% ethyl acetate in petroleum ether as the eluent to give 0.345 g **5c** in 65% yield. δ_H(400 MHz; $CDCl_3$; Me_4Si) 0.25 (s, 3H), 0.26 (s, 3H), 0.85 (d, J = 6.8 Hz, 3H), 0.91 (d, J = 6.8 Hz, 3H), 0.93 (s, 9H), 1.46–1.58 (m, 1H), 1.70–1.85 (m, 2H), 2.13–2.20 (m, 1H), 2.23–2.31 (m, 1H), 2.60 (d, J = 4.8 Hz, 3H), 2.96 (d, J = 14.2Hz, 1H), 3.25 (d, J = 14.2 Hz, 1H), 3.58–3.65 (m, 2H), 4.09 (dd, J = 5.9 Hz, 9.2 Hz, 1H), 5.41–5.50 (m, N-H, 1H), 7.15–7.25 (m, 5H); δ_C(100 MHz; $CDCl_3$; Me_4Si) -1.5, -1.3, 17.8, 18.7, 19.7, 26.39, 26.4, 27.5, 29.8, 36.7, 46.6, 58.4, 62.6, 83.5, 126.8, 128.1, 130.3, 136.6, 171.1, 174.3; MS (*m/z* 450 M^++H^+); HRMS Calcd for *m/z* $C_{24}H_{43}N_2O_4Si^+$: 451.2992. Found: 451.2998.



(*R*)-2-benzyl-2-(*tert*-butyldimethylsilyloxy)-5-hydroxy-*N*-((*S*)-1-(methylamino)-1-oxopropan-2-yl)-pentanamide (5d)

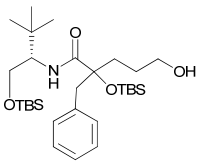
According to the general method **B**, **5d** was prepared using **4d** (0.078 g, 0.234 mmol), $LiBH_4$ (0.017 g, 0.71 mmol), trimethylacetyl chloride (0.143 g, 1.18 mmol), pyridine (3 cm^3), trimethylamine (0.287 g, 2.84 mmol), TBSOTf (0.343 g, 1.30 mmol), $LiBH_4$ (0.056 g, 2.37 mmol), The crude product was

purified by silica flash chromatography using 0–5% methanol in ethyl acetate as the eluent to give 25 mg **5d** in 25% yield. δ_H(400 MHz; $CDCl_3$; Me_4Si) 0.01 (s, 6H), 0.68 (s, 9H), 1.08 (d, J = 7.2 Hz, 3H), 1.43–1.72 (m, 2H), 1.87–1.95 (m, 2H), 2.37 (s, 3H), 2.71 (d, J = 14.0 Hz, 1H), 3.00 (d, J = 14.0 Hz, 1H), 3.36–3.44 (m, 2H), 4.03–4.10 (m, 1H), 5.50–5.51 (br s, 1H), 6.86 (d, J = 8.4 Hz, 1H), 6.94–7.03 (m, 4H); δ_C(100 MHz; $CDCl_3$; Me_4Si) -1.7, -1.6, 17.9, 18.7, 26.3, 26.4, 27.2, 36.5, 46.4, 48.5, 62.5, 83.0, 126.8, 128.1, 130.3, 136.5, 172.1, 174.3; MS (*m/z* 423, $M+H^+$, 845, 2 × $M+H^+$); HRMS Calcd for *m/z* $C_{22}H_{39}N_2O_4Si^+$: 423.2679. Found: 423.2673.



(*R*)-2-benzyl-2-(*tert*-butyldimethylsilyloxy)-5-hydroxy-*N*-((*S*)-1-(methylamino)-1-oxo-3-phenylpropan-2-yl)-pentanamide (5e)

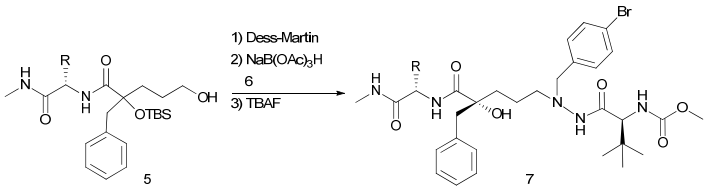
According to the general method **B**, **5e** was prepared using **4e** (0.129 g, 0.339 mmol), $LiBH_4$ (0.059 g, 2.40 mmol), trimethylacetyl chloride (0.204 g, 1.70 mmol), pyridine (3 cm^3), trimethylamine (0.137 g, 1.36 mmol), TBSOTf (0.179 g, 0.68 mmol), $LiBH_4$ (0.123 g, 5.17 mmol), The crude product was purified by silica flash chromatography using 50–100% ethyl acetate in petroleum ether as the eluent to give 0.103 g **5e** in 61% yield. δ_H(400 MHz; $CDCl_3$; Me_4Si) 0.13 (s, 3H), 0.19 (s, 3H), 0.84 (s, 9H), 1.20–1.30 (m, 1H), 1.44–1.54 (m, 1H), 1.56–1.66 (m, 1H), 1.86 (br s, 1H), 1.90–2.02 (m, 1H), 2.57 (d, J = 4.8 Hz, 3H), 2.80–2.90 (m, 2H), 3.20–3.32 (m, 2H), 3.40 (t, J = 6.0 Hz, 2H), 4.62 (m, 1H), 5.53 (m, 1H), 7.03 (d, J = 8.8 Hz, 1H), 7.10–7.30 (m, 10H); δ_C(100 MHz; $CDCl_3$; Me_4Si) -1.7,-1.5, 18.7, 26.4, 26.5, 26.7, 36.9, 37.7, 46.0, 53.9, 62.5, 83.2, 126.7, 126.9, 128.1, 128.8, 129.4, 130.3, 136.6, 137.2, 171.1, 174.4; MS (*m/z* 499, $M+H^+$, 997, 2 × $M+H^+$); Anal. Calcd for $C_{28}H_{42}N_2O_4Si$: C, 67.43; H, 8.49; N, 5.62; Found: C, 67.58; H, 8.61; N, 5.77.



2-Benzyl-2-(*tert*-butyl-dimethyl-silanyloxy)-5-hydroxy-pentanoic acid [(*S*)-1-(*tert*-butyl-dimethyl-silanyloxymethyl)-2,2-dimethyl-propyl]-amide (5f)

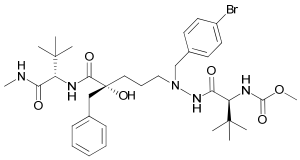
According to the general method **B**, **5f** was prepared using **4f** (0.237 g, 0.547 mmol), $LiBH_4$ (4 cm^3, 2 M solution in THF) trimethylacetyl chloride (0.3 cm^3, 2.44 mmol), pyridine (3 cm^3), triethylamine

(0.4 cm^3, 2.870 mmol), TBSOTf (0.4 cm^3, 1.74 mmol) $LiBH_4$ (5 cm^3 of a 2 M solution in THF) was added by syringe. The reaction mixture was stirred at room temperature for 48 h and then additional $LiBH_4$ was added every 2 h until full conversion of the starting material was achieved. The crude product was purified by silica flash chromatography using 1–4% MeOH in DCM as the eluent followed by purification by RPHPLC-MS (85–100% MeOH in 0.05% aqueous formic acid) to give 0.0817 g **5f** in 27% crude yield. Used in next step without further purification.



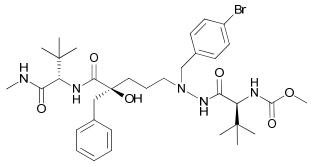
2.3 General Procedure C: Reductive amination (7)

To the mixture of **5** (1.0 equiv) and Dess-Martin reagent (1.1–1.2 equiv) was added 5 cm^3 dry DCM. The mixture was stirred at room temperature for about 40 min. Then 4 cm^3 saturated $NaHCO_3$ solution and 4 cm^3 saturated $Na_2S_2O_3$ solution were added, extracted with 3 × 15 cm^3 DCM, dried with $MgSO_4$, concentrated, to give the crude aldehyde. Then {(*S*)-1-[*N*'-(4-bromo-benzyl)-hydrazinocarbonyl]-2,2-dimethyl-propyl}-carbamic acid methyl ester (**6**) (0.5 equiv), synthesised as previously reprted,[4] and 10 cm^3 THF were added to the mixture. To the solution was added acetic acid (1.0–1.5 equiv) and stirred for 15 min at room temperature. Then $Na(OAc)_3BH$ (1.5–3.0 equiv) was added at room temperature. The mixture was stirred at room temperature overnight. The reaction was quenched with saturated NH_4Cl aqueous solution, extracted with 3 × 15 cm^3 DCM. Dried with $MgSO_4$, concentrated and purified on silica gel with 50–100% ethyl acetate-petroleum ether, combined the fractions with right MS, deprotected with 10.0 equiv 1.0 M TBAF in THF overnight at room temperature. Purified with ethyl acetate, 5% methanol in DCM on silica gel or on RP-HPLC and thereafter freeze dried to give product **7** in 3–81% yield.



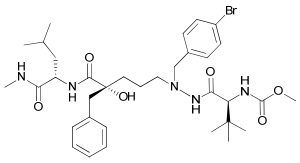
((*S*)-1-{*N*'-(4-Bromo-benzyl)-*N*'-[(*R*)-4-((S)-2,2-dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-hydroxy-5-phenyl-pentyl]-hydrazinocarbonyl}-2,2-dimethyl-propyl)-carbamic acid methyl ester (7a)

Compound **7a** was prepared according to general procedure **C**, using alcohol **3a** (0.140 g, 0.301 mmol), Dess-Martin reagent (0.141 g, 0.331 mmol), bromohydrazide **6** (0.056 g, 0.151 mmol), AcOH (0.014 mg, 0.226 mmol), $Na(OAc)_3BH$ (0.126 g, 0.595 mmol) and 1.0 M TBAF (0.75 cm^3) in THF. The crude product was purified by silica flash chromatography using ethylacetate as eluent to give 0.071 mg **7a** in 66% yield. δ_H(400 MHz; CD_3OD; Me_4Si) 0.82 (s, 9H), 0.89 (s, 9H), 1.40–1.54 (m, 1H), 1.64–1.86 (m, 2H), 1.94–2.06 (m, 1H), 2.59 (s, 3H), 2.70–2.86 (m, 3H), 2.99 (d, J = 13.2 Hz, 1H), 3.63 (s, 3H), 3.71 (s, 1H), 3.83 (br s, 2H), 3.99 (s, 1H), 7.11–7.20 (m, 5H), 7.25–7.30 (m, 2H), 7.36–7.42 (m, 2H); δ_C(100 MHz; CD_3OD; Me_4Si) 22.6, 26.0, 26.9, 27.1, 34.9, 35.6, 37.9, 47.1, 52.8, 58.5, 61.5, 62.0, 62.9, 79.5, 122.1, 127.3, 128.8, 131.3, 132.2, 132.4, 137.8, 158.9, 171.9, 172.2, 176.4; MS (*m/z* 704, $M+H^+$, 706 $M+H^+$); Anal. Calcd for $C_{34}H_{50}BrN_5O_6 \cdot 2H_2O$: C, 55.13; H, 7.35; N, 9.45; Found: C, 55.03; H, 6.96; N, 9.29.



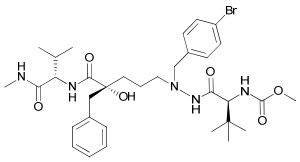
((*S*)-1-{*N'*-(4-Bromo-benzyl)-*N'*-[(*S*)-4-((*S*)-2,2-dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-hydroxy-5-phenyl-pentyl]-hydrazinocarbonyl}-2,2-dimethyl-propyl)-carbamic acid methyl ester ((*S*)-7a)

Compound (*S*)-**7a** was prepared according to general procedure **C**, using alcohol (*S*)-**5a** (0.150 g, 0.323 mmol), Dess-Martin reagent (0.151 g, 0.355 mmol), bromohydrazide **6** (0.061 mg, 0.161 mmol), AcOH (0.029 mg, 0.484 mmol), $Na(OAc)_3BH$ (0.205 g, 0.968 mmol) and 1.0 M TBAF (0.85 cm^3) in THF. The crude product was purified by silica flash chromatography using 0–5% methanol in ethyl acetate as eluent to give 0.032 g (*S*)-**7a** in 28% yield. δ_H(400 MHz; CD_3OD; Me_4Si) 0.66 (s, 9H), 0.79 (s, 9H), 1.34–1.50 (m, 1H), 1.60–1.84 (m, 2H), 1.88–2.00 (m, 1H), 2.67 (s, 3H), 2.72–2.86 (m, 3H), 3.02 (d, J = 13.6 Hz, 1H), 3.63 (s, 3H), 3.71 (s, 1H), 3.76–3.86 (m, 2H), 3.89 (s, 1H), 7.11–7.32 (m, 7H), 7.36–7.42 (m, 2H); δ_C(100 MHz; CD_3OD; Me_4Si) 21.9, 26.1, 26.88, 26.92, 34.8, 34.9, 38.5, 46.7, 52.8, 58.3, 61.3, 61.8, 63.0, 79.7, 122.1, 127.4, 128.8, 131.8, 132.2, 132.5, 137.8, 138.1, 158.9, 171.7, 173.1, 176.2; MS (*m/z* 704, $M+H^+$, 706 $M+H^+$); Anal. Calcd for $C_{34}H_{50}BrN_5O_6 \cdot 1/2H_2O$: C, 57.22; H, 7.20; N, 9.81; Found: C, 57.28; H, 7.07; N, 9.72.



((*S*)-1-{*N*'-(4-Bromo-benzyl)-*N*'-[(*R*)-4-hydroxy-4-((*S*)-3-methyl-1-methylcarbamoyl-butylcarbamoyl)-5-phenyl-pentyl]-hydrazinocarbonyl}-2,2-dimethyl-propyl)-carbamic acid methyl ester (7b)

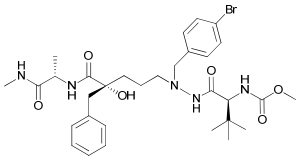
Compound **7b** was prepared according to general procedure **C**, using alcohol **5b** (0.091 g, 0.196 mmol), Dess-Martin reagent (0.091 g, 0.215 mmol), bromohydrazide **6** (0.046 g, 0.124 mmol), AcOH (0.015 g, 0.247 mmol), $Na(OAc)_3BH$ (0.105 g, 0.495 mmol) and 1.0 M TBAF (1.24 cm^3) in THF. The crude product was purified by silica flash chromatography using 2–5% methanol in DCM as eluent to give 0.071 g **7b** in 81% yield. δ_H(400 MHz; CD_3OD; Me_4Si) 0.80 (s, 9H), 0.84–0.92 (m, 6H), 1.38–1.58 (m, 4H), 1.60–1.84 (m, 2H), 1.92–2.02 (m, 1H), 2.56 (s, 3H), 2.70–2.86 (m, 3H), 3.02 (d, J = 13.6 Hz, 1H), 3.62 (s, 3H), 3.70 (s, 1H), 3.82 (br s, 2H), 4.28 (dd, J = 8.0 Hz, 6.4 Hz, 1H), 7.12–7.25 (m, 5H), 7.25–7.30 (m, 2H), 7.38–7.42 (m, 2H); δ_C(100 MHz; CD_3OD; Me_4Si) 22.1, 22.3, 23.4, 25.9, 26.5, 26.9, 34.9, 38.2, 42.5, 46.8, 52.6, 52.8, 58.6, 61.7, 63.0, 79.4, 122.1, 127.5, 128.9, 131.6, 132.2, 132.4, 137.8, 138.0, 158.9, 171.9, 174.5, 176.7; MS (*m/z* 704, $M+H^+$, 706 $M+H^+$); Anal. Calcd for $C_{34}H_{50}BrN_5O_6 \cdot H_2O$: C, 56.50; H, 7.25; N, 9.69; Found: C, 56.80; H, 7.09; N, 9.58.



((*S*)-1-{*N*'-(4-Bromo-benzyl)-*N*'-[(*R*)-4-hydroxy-4-((*S*)-2-methyl-1-methylcarbamoyl-propyl-carbamoyl)-5-phenyl-pentyl]-hydrazinocarbonyl}-2,2-dimethyl-propyl)-carbamic acid methyl ester (7c)

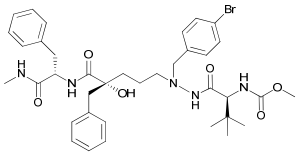
Compound **7c** was prepared according to general procedure **C**, using alcohol **5c** (0.050 g, 0.111 mmol), Dess-Martin reagent (0.057 g, 0.014 mmol), bromohydrazide **6** (0.040 g, 0.011 mmol), $Na(OAc)_3BH$ (0.095 g, 0.448 mmol) and 1.0 M TBAF (1.4 cm^3) in THF. Purified with RP-HPLC water-acetonitrile 20–80% to give 0.0136 g **7c** in 18% yield. δ_H(400 MHz; CD_3OD; Me_4Si) 0.81 (s, 9H), 0.84 (d, J = 5.1 Hz, 3H), 0.86 (d, J = 5.1 Hz, 3H), 1.44–1.54 (m, 1H), 1.66–1.82 (m, 2H), 1.93–2.06 (m, 2H), 2.60 (s, 3H), 2.82 (d, J = 13.6 Hz, 1H), 2.84–2.91 (m, 2H), 3.02 (d, J = 13.6 Hz, 1H), 3.64 (s, 3H), 3.72 (s, 1H), 3.91 (s, 2H), 3.97 (d, J = 7.2 Hz, 1H), 7.15–7.23 (m, 5H), 7.28–7.33 (m, 2H), 7.40–7.44 (m, 2H); δ_C(100 MHz; CD_3OD; Me_4Si) 18.8, 19.7, 22.2, 26.2, 26.9, 32.3, 34.9, 38.0, 47.0, 52.8, 58.8, 60.0, 62.1,

63.0, 79.5, 122.6, 127.5, 128.8, 131.5, 132.4, 132.6, 136.8, 137.8, 158.9, 171.8, 173.3, 176.6; MS (*m/z* 690, M^++H^+, 692, M+H^+); HRMS Calcd for *m/z* $C_{33}H_{49}BrN_5O_6^+$: 690.2866. Found: 690.2864.



((*S*)-1-{*N*'-(4-Bromo-benzyl)-*N*'-[(*R*)-4-hydroxy-4-((*S*)-1-methylcarbamoyl-ethylcarbamoyl)-5-phenyl-pentyl]-hydrazinocarbonyl}-2,2-dimethyl-propyl)-carbamic acid methyl ester (7d)

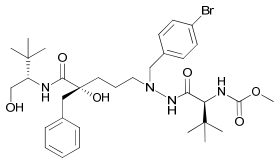
Compound **7d** was prepared according to general procedure **C**, using alcohol **5d** (0.025 g, 0.059 mmol), Dess-Martin reagent (0.026 g, 0.062 mmol), bromohydrazide **6** (0.022 g, 0.059 mmol), AcOH (0.0071 g, 0.12 mmol), $Na(OAc)_3BH$ (0.050 g, 0.43 mmol) and 1.0 M TBAF (0.19 cm^3) in THF. The crude product was purified by silica flash chromatography using 60–100% ethyl acetate in petroleumeter and 2–5% methanol in ethyl acetate as eluents to give 0.0115 g **7d** in 29% yield. δ_H(400 MHz; CD_3OD; Me_4Si) 0.80 (s, 9H), 1.23 (d, *J* = 7.2 Hz, 3H), 1.36–1.48 (m, 1H), 1.62–1.72 (m, 2H), 1.90–2.00 (m, 1H), 2.58 (s, 3H), 2.70–2.86 (m, 3H), 3.02 (d, *J* = 13.6 Hz, 1H), 3.63 (s, 3H), 3.70 (s, 1H), 3.78–3.88 (m, 2H), 4.22 (q, *J* = 6.8 Hz, 1H), 7.16–7.24 (m, 5H), 7.25–7.30 (m, 2H), 7.36–7.41 (m, 2H); δ_C(100 MHz; CD_3OD; Me_4Si) 19.0, 22.3, 26.4, 26.9, 34.9, 38.2, 46.8, 52.8, 58.7, 62.1, 63.0, 79.3, 122.2, 127.5, 128.9, 131.6, 132.2, 132.5, 137.7, 138.0, 158.9, 171.9, 174.8, 176.5; MS (*m/z* 662, M+H^+, 664, M+H^+); Anal. Calcd for $C_{31}H_{44}BrN_5O_6$: C, 56.19; H, 6.69; N, 10.57; Found: C, 56.22; H, 6.88; N, 10.39.



((*S*)-1-{*N*'-(4-Bromo-benzyl)-*N*'-[(*R*)-4-hydroxy-4-((*S*)-1-methylcarbamoyl-2-phenyl-ethylcarbamoyl)-5-phenyl-pentyl]-hydrazinocarbonyl}-2,2-dimethyl-propyl)-carbamic acid methyl ester (7e)

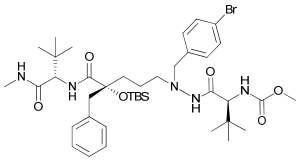
Compound **7e** was prepared according to general procedure **C**, using alcohol **5e** (0.099 g, 0.199 mmol), Dess-Martin reagent (0.092 g, 0.22 mmol), bromohydrazide **6** (0.044 g, 0.12 mmol), AcOH (0.014 g, 0.24 mmol), $Na(OAc)_3BH$ (0.105 g, 0.43 mmol) and 1.0 M TBAF (1.19 cm^3) in THF. The crude product was purified by silica flash chromatography using 2–5% methanol in DCM as eluent to give 0.0061 g **7e** in 76% yield. δ_H(400 MHz; CD_3OD; Me_4Si) 0.81 (s, 9H), 0.98–1.08 (m, 1H), 1.34–1.46

(m, 1H), 1.62–1.72 (m, 1H), 1.78–1.90 (m, 1H), 2.51 (s, 3H), 2.56–2.70 (m, 1H), 2.72–2.86 (m, 2H), 2.94–3.04 (m, 2H), 3.61 (s, 3H), 3.71 (s, 1H), 3.77 (br s, 2H), 4.47 (dd, J = 8.8 Hz, 6.0 Hz, 1H), 7.10–7.24 (m, 10H), 7.24–7.30 (m, 2H), 7.36–7.42 (m, 2H); δ_C(100 MHz; CD_3OD; Me_4Si) 22.1, 26.4, 26.9, 34.9, 38.2, 46.8, 52.8, 55.5, 58.5, 61.8, 63.0, 79.4, 122.1, 127.5, 127.8, 128.8, 129.5, 130.4, 131.6, 132.2, 132.4, 137.9, 138.0, 138.2, 158.9, 171.8, 173.3, 176.6; MS (*m/z* 738, $M+H^+$, 740, $M+H^+$); Anal. Calcd for $C_{37}H_{48}BrN_5O_6 \cdot H_2O$: C, 58.73; H, 6.66; N, 9.25; Found: C, 58.92; H, 6.47; N, 9.10;



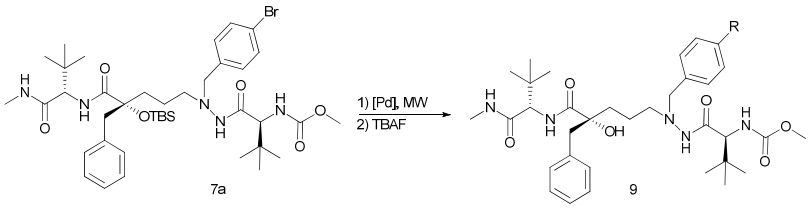
((*S*)-1-{*N*'-(4-Bromo-benzyl)-*N*'-[(*R*)-4-hydroxy-4-((*S*)-1-hydroxymethyl-2,2-dimethyl-propyl-carbamoyl)-5-phenyl-pentyl]-hydrazinocarbonyl}-2,2-dimethyl-propyl)-carbamic acid methyl ester (7f)

Compound **7f** was prepared according to general procedure **C**, using alcohol **5f** (0.0617 g, 0.112 mmol), Dess-Martin reagent (0.060 mg, 0.14 mmol) bromohydrazide **6** (0.0399 mg, 0.107 mmol) $Na(OAc)_3BH$ (0.1736 g, 0.819 mmol). The TBS-protected intermediate was purified by RP-HPLC-MS (50–100% MeOH in 0.05% aqueous formic acid) and the treated with TBAF (1 M solution in THF) until full conversion. Purification and separation of the two epimers were performed by repeated RP-HPLC with 50–100% CH_3CN in 0.05% aqueous formic acid to give 0.0026 g **7f** and 0.0019 g (*S*)-**7f** in 3% and 2% respectively. δ_H(400 MHz; $(CD_3)_2CO$; Me_4Si) 0.84 (s, 9H), 0.88 (s, 9H), 1.44–1.72 (m, 2H), 1.88–2.10 (m, 2H), 2.70–3.18 (m, 4H), 3.38–3.42 (m 1H), 3.56 (s, 3H), 3.63 (m, 2H), 3.74 (d, J = 9.7 Hz, 1H), 3.94 (s, 2H), 7.16–7.35 (m, 7H), 7.41 (m, 2H); δ_C(100 MHz; $(CD_3)_2CO$; Me_4Si) 22.3, 26.9, 27.6, 34.4, 34.7, 37.4, 46.7, 52.3, 57.7, 59.6, 61.2, 62.4, 62.8, 79.0, 121.2, 127.0, 128.4, 131.5, 131.8, 131.8, 138.3, 138.4, 157.8, 171.1, 175.4; MS (*m/z* 678, $[M+H]^+$, 680 $[M+H]^+$); HRMS Calcd for *m/z* $C_{33}H_{50}BrN_4O_6^+$: 677.2914. Found: 677.2908.



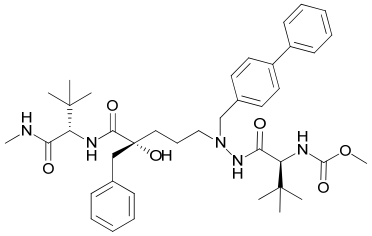
((*S*)-1-{*N*'-(4-Bromo-benzyl)-*N*'-[(*R*)-4-(*tert*-butyl-dimethyl-silanyloxy)-4-((*S*)-2,2-dimethyl-1-methylcarbamoyl-propylcarbamoyl)-5-phenyl-pentyl]-hydrazinocarbonyl}-2,2-dimethyl-propyl)-carbamic acid methyl ester (**8**).

Compound **8** was prepared according to general procedure **C**, using alcohol **5a** (0.761 g, 1.64 mmol), Dess-Martin reagent (0.834 g, 1.97 mmol), bromohydrazide **6** (0.370 g, 0.995 mmol), AcOH (0.120 mg, 1.99 mmol), $Na(OAc)_3BH$ (0.422 g, 1.99 mmol). The crude product was purified by silica flash chromatography using 4–5% methanol in DCM as eluemt to give 0.580 g **8** in 71% yield. δ_H(400 MHz; $CDCl_3$; Me_4Si) 0.07 (s, 3H), 0.08 (s, 3H), 0.86 (s, 9H), 0.87 (s, 9H), 0.94 (s, 9H), 1.50–1.70 (m, 2H), 2.04–2.20 (m, 2H), 2.66–2.78 (m, 4H), 2.85 (d, J = 14.4 Hz, 1H), 2.94–3.04 (m, 1H), 3.28 (d, J = 14.4 Hz, 1H), 3.62 (s, 3H), 3.76–3.90 (m, 2H), 3.99 (d, J = 13.2 Hz, 1H), 4.10 (d, J = 9.6 Hz, 1H), 5.40 (d, J = 9.6 Hz, 1H), 6.34 (br s, 1H), 7.00–7.20 (m, 8H), 7.30–7.40 (m, 2H), 7.48 (d, J = 9.6 Hz, 1H), 7.74 (br s, 1H); δ_C(100 MHz; $CDCl_3$; Me_4Si) -1.9, -1.7, 18.6, 22.3, 26.1, 26.2, 26.6, 26.8, 26.9, 34.4, 34.7, 38.3, 46.4, 52.4, 55.3, 60.1, 60.7, 61.1, 82.7, 121.3, 126.5, 128.0, 130.0, 130.9, 131.4, 136.2, 136.6, 157.0, 170.1, 170.4, 174.2; MS (*m/z* 819, M+H$^+$, 821, M+H$^+$); Anal. Calcd for $C_{40}H_{64}BrN_5O_6Si$: C, 58.66; H, 7.88; N, 8.55; Found: C, 58.77; H, 8.07; N, 8.45.



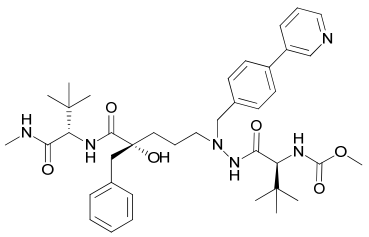
2.4 General Procedure D: Palladium-Catalysed Suzuki Reactions (9a–e)

Aryl bromide **8** (1.0 equiv), boronic acid (3.0 equiv), Herrmann's palladacycle[5] (0.05 equiv), HP(*t*-Bu)$_3$BF$_4$[6] (0.10 equiv), K_2CO_3 (3.0 equiv), 1.0 mL DME and 0.3 cm^3 H_2O were added to the 2–5 cm^3 vial. The vial was sealed and the mixture was irradiated with microwaves to 120 °C for 20 min. The cold mixture was then extracted with ethyl acetate. The organic layer was dried with $MgSO_4$ and concentrated. Then 10.0 equiv TBAF in THF was added and stirred at room temperature overnight. To the solution was added 10 cm^3 water, extracted with DCM, dried with $MgSO_4$, concentrated and purified on silica and thereafter freeze dried.



((*S*)-1-{*N*'-Biphenyl-4-ylmethyl-*N*'-[(*R*)-4-((*S*)-2,2-dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-hydroxy-5-phenyl-pentyl]-hydrazinocarbonyl}-2,2-dimethyl-propyl)-carbamic acid methyl ester (9a)

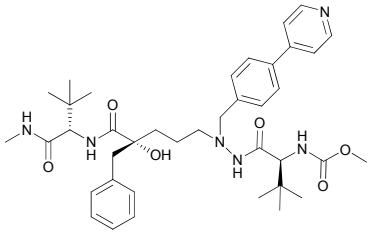
Compound **9a** was prepared according to general procedure **D** using **8** (0.0495 g, 0.0604 mmol), phenylboronic acid (0.0261 g, 0.214 mmol), Herrmann's palladacycle (0.0030 g, 0.0032 mmol), HP(*t*-Bu)$_3$BF$_4$ (0.0020 g, 0.0068 mmol), K_2CO_3(0.0292 g, 0.211 mmol), DME, (1.0 cm^3), H_2O (0.3 cm^3), TBAF in THF (0.61 cm^3). Extended purification on RP-HPLC with 35–85% acetonitrile in water to give **9a** 0.0188 g in 44% yield. δ_H(400 MHz; CD_3OD; Me_4Si) 0.82 (s, 9H), 0.89 (s, 9H), 1.44–1.57 (m, 1H), 1.67–1.88 (m, 2H), 1.96–2.06 (m, 1H), 2.16 (s, 1H), 2.59 (s, 3H), 2.80 (d, *J* = 13.3 Hz, 1H), 2.83–2.87 (m, 2H), 3.00 (d, *J* = 13.3 Hz, 1H), 3.52 (s, 3H), 3.74 (s, 1H), 3.91 (s, 2H), 3.99 (s, 1H), 4.59 (s, 1H), 7.12–7.20 (m, 5H), 7.28–7.35 (m, 1H), 7.39–7.46 (m, 4H), 7.48–7.54 (m, 2H), 7.56–7.61 (m, 2H); δ_C(100 MHz; CD_3OD; Me_4Si) 22.2, 26.0, 26.9, 27.2, 35.0, 35.6, 37.9, 47.1, 52.7, 58.7, 61.2, 61.6, 62.7, 63.0, 79.6, 127.3, 127.88, 127.94, 128.4, 128.8, 129.9, 131.3, 136.1, 137.7, 142.1, 142.1, 159.0, 171.8, 172.2, 176.3; MS (*m/z* 702, M+H$^+$); HRMS Calcd for *m/z* $C_{40}H_{56}N_5O_6^+$: 702.4231. Found: 702.4243.



{(*S*)-1-[*N*'-[(*R*)-4-((*S*)-2,2-Dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-hydroxy-5-phenyl-pentyl]-*N*'-(4-pyridin-3-yl-benzyl)-hydrazinocarbonyl]-2,2-dimethyl-propyl}-carbamic acid methyl ester (9b)

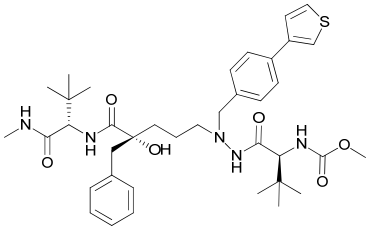
Compound **9b** was prepared according to general procedure **D** using **8** (0.070 g, 0.0855 mmol), 3-pyridine boronic acid (0.0315 g, 0.256 mmol), Herrmann's palladacycle (0.0040 g, 0.0043 mmol), HP(*t*-Bu)$_3$BF$_4$ (0.0025 g, 0.0085 mmol), K_2CO_3(0.0354 g, 0.256 mmol), DME, (0.9 cm^3), H_2O (0.2 cm^3), TBAF in THF (0.85 cm^3). Purification on silica to give 0.040 g **9b** in 82% yield. δ_H(400 MHz; CD_3OD; Me_4Si) 0.80 (s, 9H), 0.88 (s, 9H), 1.44–1.56 (m, 1H), 1.66–1.88 (m, 2H), 1.96–2.06 (m, 1H),

2.58 (s, 3H), 2.74–2.88 (m, 3H), 2.98 (d, J = 14.0 Hz, 1H), 3.51 (s, 3H), 3.73 (s, 1H), 3.92 (br s, 2H), 4.00 (s, 1H), 7.10–7.18 (m, 5H), 7.46–7.58 (m, 5H), 8.02–8.08 (m, 1H), 8.46–8.52 (m, 1H), 8.75 (s, 1H); δ_C(100 MHz; CD_3OD; Me_4Si) 22.5, 26.0, 26.9, 27.1, 35.0, 35.6, 38.0, 47.1, 52.7, 58.5, 61.4, 62.4, 62.9, 79.5, 125.5, 127.3, 127.8, 128.7, 131.3, 131.4, 136.4, 137.6, 137.7, 138.4, 138.8, 148.2, 148.6, 158.9, 171.8, 172.2, 176.3; MS (*m/z* 703, M^+); Anal. Calcd for $C_{39}H_{54}N_6O_6 \cdot H_2O$: C, 64.98; H, 7.83; N, 11.66; Found: C, 65.18; H, 8.11; N, 11.45.



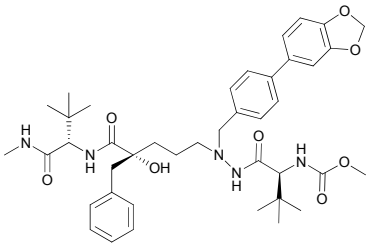
{(*S*)-1-[*N*'-[(*R*)-4-((*S*)-2,2-Dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-hydroxy-5-phenyl-pentyl]-*N*'-(4-pyridin-4-yl-benzyl)-hydrazinocarbonyl]-2,2-dimethyl-propyl}-carbamic acid methyl ester (9c)

Compound **9c** was prepared according to general procedure **D** using **8** (0.070 g, 0.0855 mmol), 4-pyridine boronic acid (0.0315 g, 0.256 mmol), Herrmann's palladacycle (0.0040 g, 0.0043 mmol), $HP(t\text{-}Bu)_3BF_4$ (0.0025 g, 0.0085 mmol), K_2CO_3(0.0354 g, 0.256 mmol), DME, (0.9 cm^3), H_2O (0.2 cm^3), TBAF in THF (0.86 cm^3). Purification on silica to give 0.044 g **9c** in 73% yield. δ_H(400 MHz; CD_3OD; Me_4Si) 0.80 (s, 9H), 0.88 (s, 9H), 1.42–1.56 (m, 1H), 1.66–1.88 (m, 2H), 1.96–2.08 (m, 1H), 2.58 (s, 3H), 2.72–2.88 (m, 3H), 2.98 (d, J = 13.2 Hz, 1H), 3.51 (s, 3H), 3.72 (s, 1H), 3.86–3.96 (m, 2H), 3.99 (s, 1H), 7.10–7.18 (m, 5H), 7.48–7.54 (m, 2H), 7.62–7.70 (m, 4H), 8.52–8.58 (m, 2H); δ_C(100 MHz; CD_3OD; Me_4Si) 22.6, 26.0, 26.9, 27.2, 35.0, 35.6, 38.0, 47.1, 52.7, 58.6, 61.5, 62.4, 63.0, 79.6, 123.0, 127.3, 127.8, 128.8, 131.3, 131.4, 137.76, 137.78, 140.1, 150.48, 150.54, 158.9, 171.9, 172.2, 176.3; MS (*m/z* 703, M^+). Anal. Calcd for $C_{39}H_{54}N_6O_6 \cdot H_2O$: C, 64.98; H, 7.83; N, 11.66; Found: C, 65.20; H, 8.15; N, 11.54.



{(*S*)-1-[*N*'-[(*R*)-4-((*S*)-2,2-Dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-hydroxy-5-phenyl-pentyl]-*N*'-(4-thiophen-3-yl-benzyl)-hydrazinocarbonyl]-2,2-dimethyl-propyl}-carbamic acid methyl ester (9d)

Compound **9d** was prepared according to general procedure **D** using **8** (0.050 g, 0.0607 mmol), 3-thienylboronic acid (0.0233 g, 0.182 mmol), Herrmann's palladacycle (0.0032 g, 0.0034 mmol), HP(*t*-Bu)$_3$BF$_4$ (0.0020 g, 0.0068 mmol), K_2CO_3(0.0253 g, 0.183 mmol), DME, (1.0 cm^3), H_2O (0.3 cm^3), TBAF in THF (0.60 cm^3). Extended purification on RP-HPLC with 35–85% acetonitrile in water to give 0.0092 g **9d** in 21% yield. δ_H(400 MHz; CD_3OD; Me_4Si) 0.82 (s, 9H), 0.89 (s, 9H), 1.46–1.59 (m, 1H), 1.69–1.86 (m, 2H), 1.96–2.07 (m, 1H), 2.56 (s, 3H), 2.79 (d, J = 13.4 Hz, 1H), 2.81–2.93 (m, 2H), 3.00 (d, J = 13.4 Hz, 1H), 3.54 (s, 3H), 3.74 (s, 3H), 3.97 (s, 2H), 3.98–4.00 (m, 1H), 7.10–7.18 (m, 4H), 7.39–7.49 (m, 5H), 7.55–7.60 (m, 3H); δ_C(100 MHz; CD_3OD; Me_4Si) 22.3, 26.0, 26.9, 27.2, 35.0, 35.6, 37.9, 47.1, 52.7, 58.6, 61.6, 62.7, 63.0, 79.6, 121.1, 127.1, 127.2, 127.30, 127.34, 128.8, 131.28, 131.33, 136.2, 136.7, 137.7, 143.3, 159.0, 171.8, 172.3, 176.3; MS (*m/z* 708, M+H$^+$); HRMS Calcd for *m/z* $C_{38}H_{54}N_5O_6S^+$: 708.3795. Found: 708.3784.



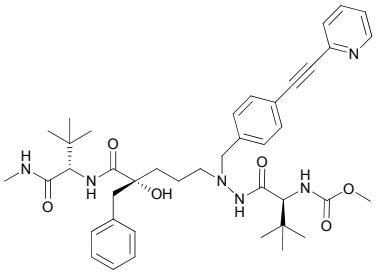
((*S*)-1-{*N*'-(4-Benzo[1,3]dioxol-5-yl-benzyl)-*N*'-[(*R*)-4-((*S*)-2,2-dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-hydroxy-5-phenyl-pentyl]-hydrazinocarbonyl}-2,2-dimethyl-propyl)-carbamic acid methyl ester (9e)

Compound **9e** was prepared according to general procedure **D** using **8** (0.050 g, 0.0614 mmol), 3,4-(methylenedioxy)phenylboronic acid (0.0302 g, 0.182 mmol), Herrmann's palladacycle (0.0030 g, 0.0032 mmol), HP(*t*-Bu)$_3$BF$_4$ (0.0024 g, 0.0083 mmol), K_2CO_3 (0.0259 g, 0.187 mmol), DME (1.0 cm^3), H_2O (0.3 cm^3), TBAF in THF (1.0 cm^3). Extended purification on RP-HPLC with 35–85% acetonitrile in water to give 0.0159 g **9e** in 35% yield. δ_H(400 MHz; CD_3OD; Me_4Si) 0.81 (s, 9H), 0.89

(s, 9H), 1.47–1.61 (m, 1H), 1.72–1.85 (m, 2H), 1.97–2.08 (m, 1H), 2.59 (s, 3H), 2.80 (d, J = 13.4 Hz, 1H), 2.87–2.97 (m, 2H), 3.00 (d, J = 13.4 Hz, 1H), 3.56 (s, 1H), 3.75 (s, 1H), 3.98–4.06 (m, 3H), 5.98 (s, 2H), 6.86–6.91 (m, 1H), 7.04–7.09 (m, 2H), 7.12–7.19 (m, 5H), 7.40–7.49 (m, 4H); δ_C(100 MHz; CD_3OD; Me_4Si) 22.0, 26.0, 27.0, 27.2, 35.0, 35.6, 37.8, 47.1, 52.8, 58.8, 61.6, 62.8, 63.0, 79.5, 102.5, 108.3, 109.5, 121.5, 127.4, 127.7, 128.8, 131.3, 131.4, 135.1, 136.4, 137.7, 142.0, 148.7, 149.7, 159.0, 171.7, 172.2, 176.3; MS (*m/z* 746, $M+H^+$); HRMS Calcd for *m/z* $C_{41}H_{56}N_5O_8^+$: 746.4129. Found: 746.4113.

2.5 General Procedure E: Palladium-Catalysed Sonogashira Reactions (9f-h)

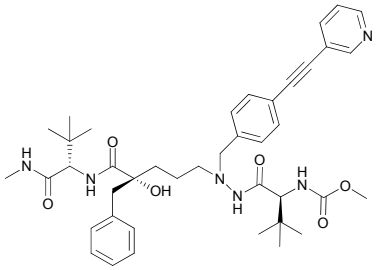
Aryl bromide **8** (1.0 equiv), ethynylpyridine (2.5 equiv), Bis(triphenylphosphine)palladium(II) chloride (0.10 equiv), piperidine (4.0 equiv), 1.3 cm^3 H_2O and 1.6 cm^3 acetone were added to the 2–5 cm^3 vial. The vials was sealed and the mixture was irradiated with microwavs to 140 °C for 30 min. The cold mixture was then extracted with DCM, washed with brine and the organic layer was dried with $MgSO_4$ and concentrated. Then 10.0 equiv TBAF in THF was added and stirred at room temperature overnight. To the solution was added 10 cm^3 water, extracted with DCM, dried organic layer with $MgSO_4$, concentrated and passed a plug of silica to get rid of TBAF before purified on RP-HPLC with acetonitrile in water to yield 14–31%.



{(*S*)-1-[*N*'-[(*R*)-4-((*S*)-2,2-Dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-hydroxy-5-phenyl-pentyl]-*N*'-(4-pyridin-2-ylethynyl-benzyl)-hydrazinocarbonyl]-2,2-dimethyl-propyl}-carbamic acid methyl ester (9f)

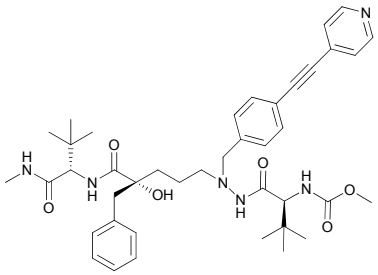
Compound **9f** was prepared according to general procedure **E** using **8** (0.050 g, 0.0615 mmol), 2-ethynylpyridine (0.0157 g, 0.152 mmol), Bis(triphenylphosphine)palladium(II) chloride (0.0044 g, 0.0063 mmol), piperidine (0.0208 g, 0.244 mmol), H_2O, acetone, TBAF in THF (2.41 cm^3). Purification on RP-HPLC with 25–90% acetonitrile in water to give 0.00133 g **9f** in 30% yield. δ_H(400 MHz; CD_3OD; Me_4Si) 0.82 (s, 9H), 0.89 (s, 9H), 1.44–1.58 (m, 1H), 1.66–1.84 (m, 2H), 1.97–2.07 (m, 1H), 2.59 (s, 3H), 2.80 (d, J = 13.3 Hz, 1H), 2.76–2.89 (m, 2H), 3.00 (d, J = 13.3 Hz, 1H), 3.62 (s, 3H),

3.70–3.75 (m, 1H), 3.94 (s, 2H), 4.00 (d, J = 9.6 Hz, 1H), 7.12–7.20 (m, 5H), 7.46 (d, J = 8.4 Hz, 2H), 7.55 (d, J = 8.4 Hz, 2H), 7.59 (ddd, J = 1.3 Hz, 2.2 Hz, 7.9 Hz, 1H), 7.81 (dt, J = 1.3 Hz, 7.9 Hz, 1H), 8.09 (dt, J = 1.7 Hz, 7.9 Hz, 1H), 8.61–8.63 (m, 1H); δ_C(100 MHz; CD_3OD; Me_4Si) 22.6, 26.0, 26.9, 27.2, 34.9, 35.6, 38.0, 47.1, 52.8, 58.7, 61.5, 62.4, 63.0, 79.6, 87.0, 93.5, 121.5, 125.3, 127.3, 128.8, 129.4, 130.8, 131.3, 132.9, 137.8, 140.8, 141.5, 142.5, 149.1, 158.9, 171.9, 172.3, 176.4; MS (*m/z* 727, $M+H^+$); HRMS Calcd for *m/z* $C_{41}H_{55}N_6O_6^+$: 727.4183. Found: 727.4185.



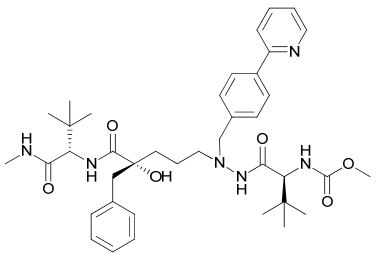
{(*S*)-1-[*N*'-[(*R*)-4-((*S*)-2,2-Dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-hydroxy-5-phenyl-pentyl]-*N*'-(4-pyridin-3-ylethynyl-benzyl)-hydrazinocarbonyl]-2,2-dimethyl-propyl}-carbamic acid methyl ester (9g)

Compound **9g** was prepared according to general procedure **E** using **8** (0.050 g, 0.0613 mmol), 3-ethynylpyridine (0.0129 g, 0.125 mmol), Bis(triphenylphosphine)palladium(II) chloride (0.0020 g, 0.0028 mmol), copper iodide (0.0010 g, 0.0053 mmol), triethylamine (0.085 cm^3 g, 0.610 mmol), DMF (2.1 cm^3), TBAF in THF (0.610 cm^3). Purification on RP-HPLC with 35–85% acetonitrile in water to give 0.00634 g **9g** in 14% yield. δ_H(400 MHz; CD_3OD; Me_4Si) 0.82 (s, 9H), 0.89 (s, 9H), 1.44–1.56 (m, 1H), 1.66–1.88 (m, 2H), 1.96–2.07 (m, 1H), 2.59 (s, 3H), 2.75–2.89 (m, 2H), 2.80 (d, J = 13.2 Hz, 1H), 3.00 (d, J = 13.2 Hz, 1H), 3.62 (s, 3H), 3.72 (s, 1H), 3.93 (s, 2H), 4.00 (d, J = 9.5 Hz,1H), 7.14–7.16 (m, 5H), 7.43–7.47 (m, 2H), 7.49–7.52 (m, 2H), 7.74–7.78 (m, 1H), 8.32 (dt, J = 1.8 Hz, 8.1 Hz, 1H), 8.66 (dd, J = 1.6 Hz, 5.4 Hz, 1H), 8.87–8.88 (m, 1H); δ_C(100 MHz; CD_3OD; Me_4Si) 22.6, 26.0, 26.9, 27.2, 34.9, 35.6, 38.0, 47.1, 52.8, 58.7, 61.5, 62.4, 63.0, 79.6, 84.8, 98.5, 122.0, 123.8, 126.6, 127.3, 128.8, 130.8, 131.3, 132.7, 137.8, 140.4, 144.2, 146.1, 149.4, 158.9, 171.9, 172.3, 176.4; MS (*m/z* 727, $M+H^+$); HRMS Calcd for *m/z* $C_{41}H_{55}N_6O_6^+$: 727.4183. Found: 727.4199.



{(*S*)-1-[*N*'-[(*R*)-4-((*S*)-2,2-Dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-hydroxy-5-phenyl-pentyl]-*N*'-(4-pyridin-4-ylethynyl-benzyl)-hydrazinocarbonyl]-2,2-dimethyl-propyl}-carbamic acid methyl ester (9h)

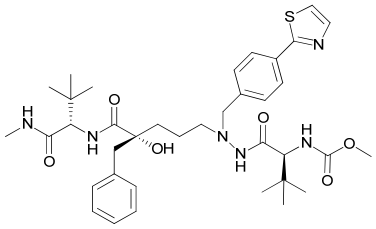
Compound **9h** was prepared according to general procedure **E** using **8** (0.050 g, 0.0608 mmol), 4-ethynylpyridine (0.0217 g, 0.155 mmol), Bis(triphenylphosphine)palladium(II) chloride (0.0045 g, 0.0064 mmol), piperidine (0.0338 g, 0.397 mmol), H_2O, acetone, TBAF in THF (2.00 cm^3). Purification on RP-HPLC with 20–85% acetonitrile in water to give 0.00127 g **9h** in 29% yield. δ_H(400 MHz; $(CD_3)_2CO$; Me_4Si) 0.86 (s, 9H), 0.92 (s, 9H), 1.26–1.34 (m, 1H), 1.42–1.55 (m, 1H), 1.63–1.76 (m, 1H), 2.06–2.07 (m, 1H), 2.65 (s, 3H), 2.83 (d, J = 13.4 Hz, 1H), 2.81–2.89 (m, 2H), 2.92–3.00 (m, 1H), 3.05 (d, J = 13.4 Hz, 1H), 3.57 (s, 3H), 3.77 (s, 1H), 4.04–4.10 (m, 3H), 7.12–7.23 (m, 5H), 7.41–7.45 (m, 2H), 7.49–7.53 (m, 2H), 7.57–7.69 (m, 2H), 8.62–8.74 (m, 2H); δ_C(100 MHz; $(CD_3)_2CO$; Me_4Si) 22.4, 25.7, 26.9, 27.2, 34.7, 35.2, 37.4, 46.6, 52.2, 57.6, 60.8, 61.5, 62.2, 78.9, 87.0, 95.9, 121.2, 126.8, 128.4, 130.1, 131.3, 132.5, 133.5, 138.1, 141.0, 149.5, 157.5, 171.0, 171.1, 174.8; MS (*m/z* 727, $M+H^+$); HRMS Calcd for *m/z* $C_{41}H_{55}N_6O_6^+$: 727.4183. Found: 727.4188.



{(*S*)-1-[*N*'-[(*R*)-4-((*S*)-2,2-Dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-hydroxy-5-phenyl-pentyl]-*N*'-(4-pyridin-2-yl-benzyl)-hydrazinocarbonyl]-2,2-dimethyl-propyl}-carbamic acid methyl ester (13a)

Compound **13a** was prepared according to general procedure **C**, using hydrazide **12a** (synthesized as presented previously[1]) to give 0.051 mg **13a** in 67% yield. δ_H(400 MHz; CD_3OD; Me_4Si) 0.81 (s, 9H), 0.88 (s, 9H), 1.42–1.56 (m, 1H), 1.66–1.88 (m, 2H), 1.94–2.08 (m, 1H), 2.58 (s, 3H), 2.70–2.88 (m,

3H), 2.99 (d, J = 13.6 Hz, 1H), 3.50 (s, 3H), 3.73 (s, 1H), 3.93 (br s, 2H), 3.99 (s, 1H), 7.08–7.20 (m, 5H), 7.30–7.38 (m, 1H), 7.46–7.52 (m, 2H), 7.79–7.92 (m, 4H), 8.56–8.62 (m, 1H); δ_C(100 MHz; CD_3OD; Me_4Si) 22.6, 26.0, 26.9, 27.1, 35.0, 35.6, 38.0, 47.1, 52.6, 58.5, 61.5, 62.4, 63.0, 79.6, 122.5, 123.7, 127.3, 127.9, 128.8, 131.0, 131.3, 137.8, 138.9, 139.5, 139.6, 150.2, 158.7, 158.9, 171.9, 172.2, 176.4; MS (*m/z* 703, $M+H^+$); Anal. Calcd for $C_{39}H_{54}N_6O_6 \cdot H_2O$: C, 64.98; H, 7.83; N, 11.66; Found: C, 65.29; H, 8.08; N, 11.57.



{(*S*)-1-[*N*'-[(*R*)-4-((*S*)-2,2-Dimethyl-1-methylcarbamoyl-propylcarbamoyl)-4-hydroxy-5-phenyl-pentyl]-*N*'-(4-thiazol-2-yl-benzyl)-hydrazinocarbonyl]-2,2-dimethyl-propyl}-carbamic acid methyl ester (13b)

Compound **13b** was prepared according to general procedure **C**, using hydrazide **12b** (synthesized as presented previously[1]) to give 0.058 mg **13b** in 75% yield. δ_H(400 MHz; CD_3OD; Me_4Si) 0.80 (s, 9H), 0.88 (s, 9H), 1.42–1.56 (m, 1H), 1.64–1.88 (m, 2H), 1.94–2.08 (m, 1H), 2.58 (s, 3H), 2.72–2.88 (m, 3H), 2.99 (d, J = 13.6 Hz, 1H), 3.52 (s, 3H), 3.71 (s, 1H), 3.91 (br s, 2H), 4.00 (s, 1H), 7.10–7.20 (m, 5H), 7.42–7.50 (m, 2H), 7.54–7.60 (m, 1H), 7.80–7.88 (m, 3H); δ_C(100 MHz; CD_3OD; Me_4Si) 22.6, 26.0, 26.9, 27.2, 34.9, 35.6, 37.9, 47.1, 52.7, 58.6, 61.5, 62.3, 62.9, 79.5, 120.6, 127.3, 128.8, 131.25, 131.33, 133.6, 137.7, 141.0, 144.4, 158.9, 170.0, 171.9, 172.2, 176.4; MS (*m/z* 709, M^+). Anal. Calcd for $C_{37}H_{52}N_6O_6S \cdot H_2O$: C, 61.13; H, 7.49; N, 11.56; Found: C, 60.83; H, 7.70; N, 11.35;

3 HIV protease inhibition

The HIV-1 protease was cloned and heterologously expressed in *Escherichia coli* and purified as described elsewhere.[7] The K_i values for the synthesized compounds were determined from two individual measurements by a fluorometric assay using the fluorescent substrate DABCYL- • -Abu-Ser-Gln-ASN-Tyr-Pro-Ile-Val-Gln-EDANS (Bachem, Bubendorf, Switzerland).[8] Measurements were performed in 96-well plates with a Fluoroskan plate reader (Labsystems, Helsinki, Finland). Excitation and emission wavelengths were 355 and 500 nm, respectively. All incubations were performed at 30 ºC in 0.1 M sodium acetate-1 M NaCl-1 mM dithiothreitol (DTT)-1 mM EDTA-3% DMSO at pH 5.0 with 5 μM substrate. In order to allow substrate and inhibitor to be dissolved completely, all

components (300 µL) were preincubated for at least 20 min before the reaction was started by adding enzyme. Initial rates were measured over 5 min. Data were analyzed by nonlinear regression by using SIMFIT and an equation for tightly binding inhibitors. The kinetic constants (k_{cat} and K_m) were determined by using the spectrophotometric assay with a chromophoric peptide substrate. The substrate concentration was varied over as wide a range as permitted with respect to sensitivity and soluability. The kinetic constants were estimated by nonlinear regression analysis by using SIMFIT and the equation for simple Michaelis-Menten kinetics. 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 procedure[8] 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 10^4 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) assay[9] and the concentration causing 50% decrease in cell proliferation (CC_{50}) was determined.

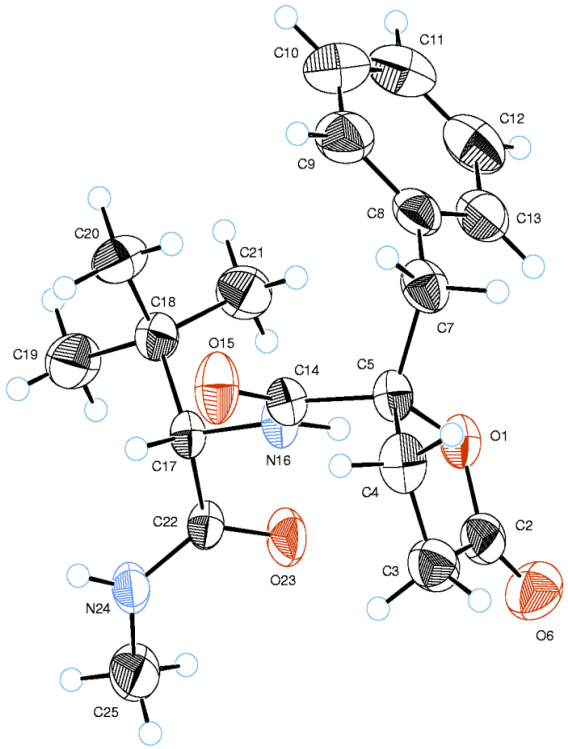
6 Caco-2 cell penetration assay

The transport was measured in one direction (apical to basolateral compartment). 0.1 mL of the apical 1X HBSS buffer (pH 7.4) with a final concentration of 10µM test compound (0.5% DMSO) was added to the apical compartment and after 60 minutes samples were withdrawn from the basolateral compartment. The basolateral buffer (0.6 mL) contained 1% BSA and 1X HBSS.

7 Metabolic stability

In the stability assay the final volume for each sample was 100µL containing Tris-HCl (pH 7.4), NADPH regenerating system, human liver microsomes and test compound, 1µL of a 100µM solution (final DMSO concentration 0.5%). The test compounds were incubated for 30 minutes at 37 °C. The parent compound remaining was analysed by LC-MS/MS.

8 X-ray evaluation of stereochemistry for (*S*)-4a



Compound reference	(*S*)-**4a**
Chemical formula	$C_{19}H_{26}N_2O_4$
Formula Mass	346.42
Crystal system	Orthorhombic
a/Å	9.8793(1)
b/Å	11.1873(2)
c/Å	17.4480(4)
α/°	90.00
β/°	90.00
γ/°	90.00
Unit cell volume/Å^3	1928.40(6)
Temperature/K	293
Space group	*P*212121
No. of formula units per unit cell, *Z*	4
No. of reflections measured	16378
No. of independent reflections	5607
R_{int}	0.0307
Final R_1 values ($I > 2\sigma(I)$)	0.0471
Final $wR(F^2)$ values ($I > 2\sigma(I)$)	0.1314
Final R_1 values (all data)	0.0639
Final $wR(F^2)$ values (all data)	0.1451

9 Inhibitors co-crystallised with HIV-1 Protease

The HIV-1 protease mutant L63P, V82T, I84V was co-crystallized with the inhibitors **9a** and **9d**. The protein concentration was 4 mg/cm^3 and the inhibitors were added in a twofold molar excess and incubated for two minutes prior to addition of the crystallization solution consisting of 0.7 M NaCl and 50 mM Mes at pH 5.0. 25% glycerol was used as cryo-protectant and flash freezing had to be done immediately before mounting in the X-ray beam. By crystal seeding the experiments were triggered to generate crystals in the space group $P2_12_12$. X-ray diffraction data were collected at MAX-lab Lund and the beam lines I911.1-3. Data were processed with Mosfilm[10] and scaled with *SCALA*.[11] The protein model coordinates from 1AJV were used for the molecular replacement calculations. Refinements were done using the program package *CNS*.[12] The structures were refined to the $R_{crystal}$ (R_{free})-factors† of 22.9% (25.3%) and 21.6% (24.8%) respectively. Model building was done with the program *O*[13] and refined with the CNS programs.[12, 13] The **9a** structure was refined to 2.0 Å resolution and with $R_{crystal}$ (R_{free})-factors† of 22.1% (25.7%), and the **9d** structure was refined to 1.8 Å resolution and with $R_{crystal}$ (R_{free})-factors† of 22.3% (25.0%).Pictures were prepared with *O* and *MOLRAY*.[14]

†$R_{merge} = [\Sigma_h\Sigma_i | I_{hi} - \langle I_h \rangle | / \Sigma_{hkl}\Sigma_i | I_{hi} |]$

9.1 Table of statistics for the crystallographic structure determination

Values within parenthesis correspond to the highest resolution bin. Molprobity[15] was used to calculate the Ramachandran statistics, residues with bad bonds, residues with bad angles, and poor rotamers.

Data set	9a	9d
Data collection		
Wavelength	1.021375	0.97268
Resolution range (Å)	57.7-2.0(2.08–1.97)	40.7-1.8 (1.86–1.76)
No. of measured reflections	97441(11938)	147904 (8436)
No. of unique reflections	16091 (2050)	21982 (2618)
Average multiplicity	6.06 (5.8)	6.73 (3.2)
Completeness (%)	97.4 (86.7)	94.8 (80.4)
Mean I/σ(I)	4.5 (3.7)	5.4 (4.1)
Rmergea (%)	10.4 (16.1)	8.6 (17.5)
Rp.i.mb (%)	4.7 (7.2)	3.3 (10.6)
Crystal parameters		
Solvent content (%)	53.8	54.0
Vm (Å3•Da-1)	2.7	2.7
No. of molecules in the asymmetric unit	2	2
Spacegroup	P21212	P21212
Unit cell lengths (Å)	58.13 85.86 46.11	58.12 85.88 46.17
Unit-cell angles (°)	90 90 90	90 90 90
Mosaicity (°)	0.33	0.36
Refinement statistics		
Resolution range	25.0–2.0	25.0–1.8
No. of reflections in working set	15121	20142
No. of reflections in test set	799	1066
R-factor (%)	22.1	22.3
Rfree (%)	25.7	25.0
No. of nonhydrogen atoms	1695	1704
No. of solvent molecules	136	142
Mean B factor, protein (Å)	13.0	13.2
Mean B-factor, ligand (Å)	23.2	19.3
Mean B-factor, water (Å)	21.5	23.6
Ramachandran plot outliers	0	0
Ramchandran favoured (%)	100	100
Residues with bad bonds	0	0
Residues with bad angles	0	0
Poor rotamers (%)	1.2	3.1

R_{merge} and $R_{p.i.m}$ are defined a [a] $R_{merge}=\Sigma_h\Sigma_I |I_{hI}-\langle I_h\rangle| / \Sigma_h\Sigma\langle I_h\rangle$, [b] $R_{P.I.M} =\Sigma_{hkl}[1/(N-1)]^{1/2}\Sigma_i |I_i(hkl)-\langle I(hkl)\rangle| / \Sigma_{hkl}\Sigma_i I(hkl)$ respectively.

9.2 X-ray crystallisation

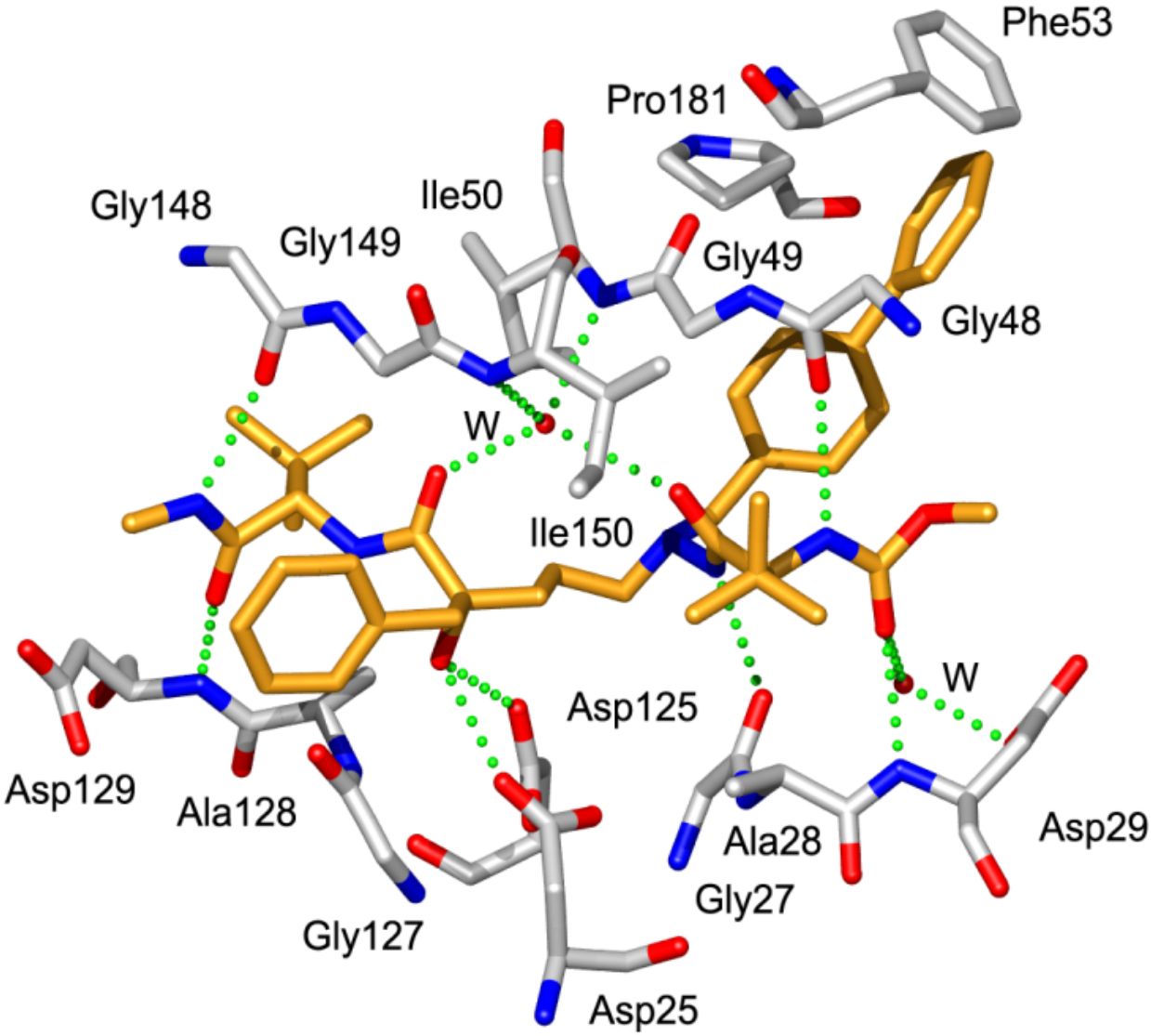


Figure 1 Inhibitor 9a (PDC code 2xye) co-crystallised with HIV-1 protease

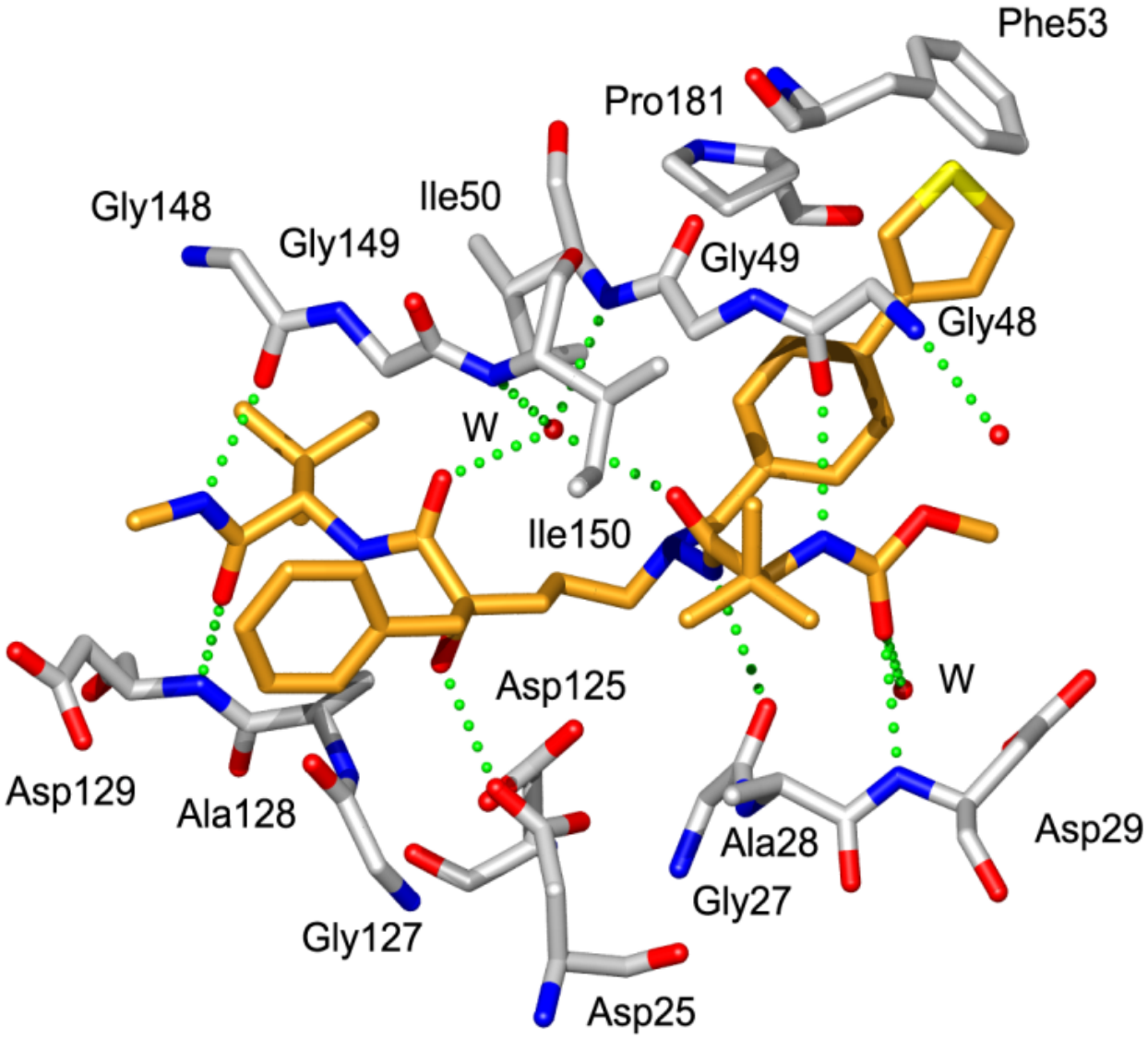


Figure 2 Inhibitor 9d (PDC code 2xyf) co-crystallised with HIV-1 protease

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