Supporting Information

Discovery of Cathepsin S Inhibitor LY3000328 for the Treatment of Abdominal Aortic Aneurysm

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EXPERIMENTAL SECTION

Chemistry. General Methods. All reagents and anhydrous solvents were obtained from commercial sources and used without further purification unless noted otherwise. ^{1}H NMR spectra were recorded on a Bruker 400 MHz spectrometer. ^{1}H NMR chemical shifts are reported in ppm with the solvent as the internal standard (DMSO-d_5 2.49 ppm, $CHCl_3$ 7.26 ppm). Optical rotations were measured with a 341 polarimeter (Perkin-Elmer) at 20 °C and at 589 nm (sodium lamp), except for **5**, for which optical rotation was obtained on a Rudolph Autopol V Automatic Polarimeter at 25 °C and at 589 nm (sodium lamp).

Compounds were analyzed for purity by HPLC and HPLC-MS, and purities of synthesized compounds were all found to be >95% by HPLC methods as specified below. Unless otherwise specified, flow rates were 1.0 mL/min and column temperature was 30 °C.

HPLC Method 1. Agilent 1200 HPLCs with VWD detectors, Waters Exterra MS 4.6 mm × 150 mm × 3.5 µm C18 column; Solvent A: 5 mM aqueous sodium 1-heptanesulfonate containing 0.1% H_3PO_4; Solvent B: acetonitrile; gradient 5–30% B in 10 min, 30–95% B in 10 min, 95–5% B in 1 min, then hold at 5% B for 5 min; Column temp. 40 °C; λ 220 nm.

HPLC Method 2. Shimadzu LC–20 A HPLC, Agilent Zorbax Bonus RP (4.6 mm × 75 mm × 3.5 µm) column; Solvent A: Water with 0.05% (v/v) TFA; Solvent B: acetonitrile with 0.05% (v/v) TFA; gradient 5–20% B in 20 minutes, 20–40% B in 5 minutes, 40–95% B in 5 minutes, followed by a hold at 95% B for 2 min; Flow rate 1.5 mL/min; λ 210 nm.

HPLC Method 3. Shimadzu LC-20 A HPLC, Waters Sunfire C_{18} (4.6 mm × 150 mm × 3.5 µm) column; Solvent A: 0.01% (v/v) TFA in Water; Solvent B: 0.01% (v/v) TFA in acetonitrile; gradient 10–50% B in 15 min, 50–95% B in 5 min, then hold at 95% B for 2 min; λ 235 nm.

Chiral HPLC Method 1. Agilent1200 HPLC with VWD detector, Chiralpak AD-H 4.6 mm × 150 mm × 5 µm eluting with 0.2% (v/v) $(CH_3)_2NEt$ in CH_3OH; Flow rate 0.6 mL/min; λ 220 nm.

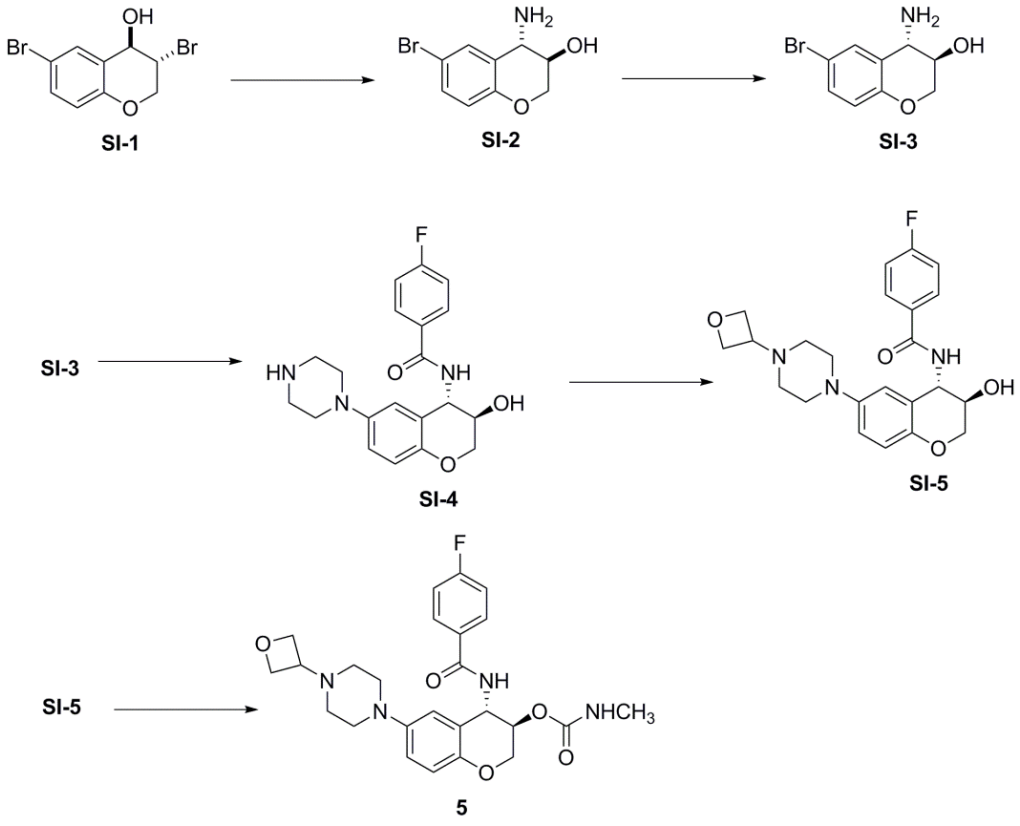
Chiral HPLC Method 2. Shimadzu LC-20 A HPLC, Chiralcel OJ-H (4.6 mm × 150 mm × 5 µm), eluent 0.1% Et_2NH in hexane and EtOH; Flow rate 1.0 mL/min; λ 235 nm.

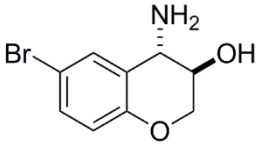
Chiral HPLC Method 3. Shimadzu LC-20 A HPLC, Chiralpak IA (4.6 mm × 250 mm × 5 µm), eluting with 2:8 EtOH: hexanes; λ 235 nm.

Chiral HPLC Method 4. Shimadzu LC-20 A HPLC, Chiralpak IC (4.6 mm × 250 mm × 5 µm) eluting with 3:7 EtOH: hexanes); λ 235 nm.

HRMS Method 1. Thermo Scientific LTQ Orbitrap Discovery Mass Spectrometer. Samples were diluted in a mixture of 90:10 acetonitrile:(0.1% (v/v) TFA in water) and infused under positive mode with a mass range of 70–2000.

Synthesis of 5.

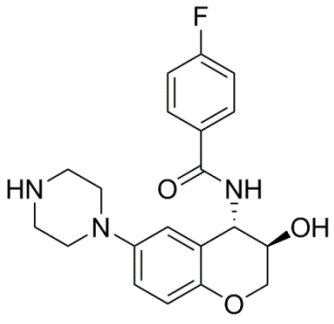




(±)-*trans*-4-amino-6-bromochroman-3-ol (SI-2). To a degassed solution of *trans*-3,6-dibromochroman-4-ol[1] (**SI-1**, 220 kg, 714 mol) and isopropanol (1730 kg) was added ammonia gas (990 kg). The mixture was stirred at 15 °C to 25 °C for 24 h. Water (1467 kg) was added. The mixture was then sparged with nitrogen, heated to 35 °C to 45 °C and concentrated to a total volume of 2000 L to 2500 L. Water (733 kg) was added. The mixture was cooled to 0 °C to 5 °C, stirred for 2 h, and then filtered to furnish the title compound **SI-2** (160 kg, 92%) as a white solid. HPLC = 96.5% purity (t_R = 10.2 min) by HPLC Method 1, mass spectrum (*m*/*z*): 243 (M + 1).

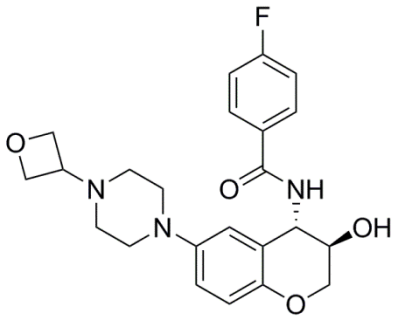
(3*R*,4*S*)-4-amino-6-bromochroman-3-ol (**SI-3**). A mixture of (±)-4-amino-6-bromochroman-3-ol (**SI-2,** 90.0 kg, 369 mol), D-(+)-camphoric acid (73.8 kg, 369 mol), acetonitrile (2202 kg), and water (199 kg) was heated to 70 °C to 75 °C for 1 h. The mixture was cooled to 3 °C to 15 °C at a rate of 4 C° to 6 C°/h and stirred for 4 h. The mixture was filtered, and the filter cake was washed with acetonitrile (193 kg). The undesired stereoisomer was removed by filtration. The combined filtrates, containing the desired stereoisomer, were heated to 40 °C to 50 °C, and a solution of 1.6 N NaOH (900 kg) was added at a rate of 250 to 400 kg/h. After stirring for 1 h, the mixture was concentrated at <50 °C under reduced pressure until the total volume was 1300 L to 1400 L. Water (900 kg) was added, and the mixture was heated to 45 °C to 50 °C and stirred for 1 h. The mixture was then cooled to 0 °C to 10 °C, stirred for 1 h, filtered, and washed with water (50 kg) to afford a white solid that was dried at 40 °C to 50 °C to furnish approximately 34 kg of **SI-3**. The above procedure, starting with 90.0 kg of (±)-*trans*-4-amino-6-bromochroman-3-ol, was repeated four times to furnish a total of 170 kg of **SI-3**. Recrystallization of **SI-3** was then

required. The combined batches of **SI-3** (170 kg) were added to acetonitrile (1294 kg) and water (408 kg). The resulting mixture was heated to 70 °C to 75 °C until a cloudy solution resulted. This solution was maintained at 70 °C to 75 °C and was filtered on a heated filter (60 °C to 70 °C) to furnish a clear solution. The resulting solution was cooled to 3 °C to 15 °C and stirred for 1 h. The mixture was filtered, and the filter cake was washed with water (170 kg) and dried under vacuum while keeping the temperature below 40 °C to yield the title compound **SI-3** (133 kg, 32%, 99.9% ee) as a white solid. HPLC purity = 99.4% (t_R = 10.21 min) by HPLC Method 1. ee = 99.97% at 2.68 min by Chiral HPLC method 1. Specific rotation: $[\alpha]_D^{20}$ = –20.76 (*c* = 10, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.50 (d, *J* = 2.5 Hz, 1H), 7.22 (dd, *J* = 8.7, 2.5 Hz, 1H), 6.68 (dd, *J* = 8.7, 0.7 Hz, 1H), 5.17 (d, *J* = 3.5 Hz, 1H), 4.18–4.09 (m, 1H), 3.93–3.84 (m, 1H), 3.59–3.54 (m, 2H), 1.97 (s, 2H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 153.3, 132.7, 130.7, 129.1, 118.4, 111.8, 68.4, 67.1, 52.3. HRMS (ESI$^+$): calcd. for $C_9H_{10}BrNO_2$ (M+1): 243.9968, found 243.9966.



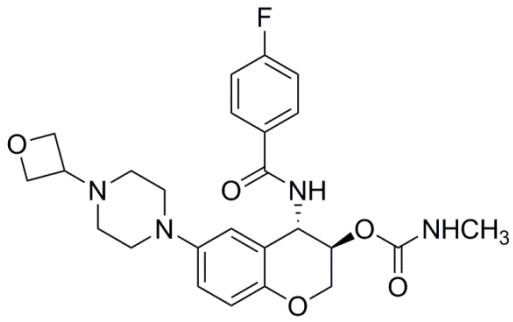
4-fluoro-*N*-((3*R*,4*S*)-3-hydroxy-6-(piperazin-1-yl)chroman-4-yl)benzamide (**SI-4**). To a solution of (3*R*,4*S*)-4-amino-6-bromochroman-3-ol (**SI-3**, 33.0 kg, 135.2 mol) in THF/H_2O (1:1, 330 L) was added $NaHCO_3$ (17.0 kg, 202.8 mol). The resulting mixture was cooled to 0–5 °C and 4-fluorobenzoyl chloride (21.75 kg, 135.2 mol) was added over 1 h. After stirring at 0–5 °C for 30 min, the internal temperature was raised to 20–25 °C, and stirring was continued for 2 h. The mixture was diluted with brine (200 kg) and THF (165 L), and the layers were separated. The organic phase was concentrated under reduced pressure at 50 °C to 66 L, diluted with DMSO (330 L) and concentrated further to

330 kg total weight. Toluene (330 L) was added, and the mixture was degassed using a sub-surface sparge of nitrogen for 1 h. Piperazine (55.2 kg, 635.4 mol) was added, and the nitrogen sparge was continued for 30 min. $Pd_2(allyl)_2Cl_2$ (2.4 kg, 6.56 mol), *t*-Bu_3PHBF_4 (3.98 kg, 13.5 mol), and *t*-BuONa (43.2 kg, 446.2 mol) were added under a nitrogen atmosphere, and the internal temperature was raised to 70 °C. After 4 h, the mixture was cooled to 25 °C and quenched with water (244 L) over 1 h. The pH of the resulting mixture was adjusted to 6.5–7.5 with 6N HCl, and ethyl acetate (330 L) was added. After stirring for 0.5 h, the layers were separated, and the aqueous layer was back extracted with ethyl acetate (5 × 330 L). The combined organics were washed with dilute NaOH (132 L) followed by a 25 % mixture of brine and Na_2CO_3 (990 L). Activated carbon (5 kg) was added to the organic layer, and the temperature of the mixture was raised to 50 °C for 2 h. After cooling to 25 °C, the mixture was filtered, and the filtrate was concentrated to approximately 70 kg. After cooling to 0–5 °C and stirring for 4 h, the resulting solids were isolated by filtration, washed with MTBE (99 L) and dried under vacuum at 50 °C to afford the title compound **SI-4** (29.65 kg, 46% from (3*R*,4*S*)-4-amino-6-bromochroman-3-ol). HPLC purity = 97.3% (t_R = 5.7 min) by HPLC method 2. ee = 100% at 4.68 min by Chiral HPLC method 2. Specific rotation: $[\alpha]_D^{20}$ = 23.02 (*c* = 10, 95% EtOH-5% H_2O). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.75 (d, *J* = 8.2 Hz, 1H), 8.02–7.92 (m, 2H), 7.33–7.22 (m, 2H), 6.80 (dd, *J* = 8.9, 2.9 Hz, 1H), 6.71–6.60 (m, 2H), 5.36 (d, *J* = 3.9 Hz, 1H), 4.95 (dd, *J* = 8.2, 5.2 Hz, 1H), 4.12 (dd, *J* = 10.4, 1.8 Hz, 1H), 3.95–3.83 (m, 2H), 2.84–2.71 (m, 8H), 2.17 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 165.6, 163.1, 148.3, 146.7, 131.3, 130.7, 122.2, 118.2, 117.3, 116.8, 115.4, 67.5, 66.2, 51.5, 46.1, 40.8. HRMS (ESI^+): calcd. for $C_{20}H_{22}FN_3O_3$ (M+1): 372.1718, found 372.1712.



4-fluoro-*N*-((3*R*,4*S*)-3-hydroxy-6-(4-oxetan-3-yl)piperazin-1-yl)chroman-4-yl)benzamide (**SI-5**). A solution of 4-fluoro-*N*-((3*R*,4*S*)-3-hydroxy-6-(piperazin-1-yl)chroman-4-yl)benzamide (**SI-4**, 23.0 kg, 61.9 mol) was prepared in 1:1 MeOH/EtOAc (460 L). 3-Oxetanone (7.6 kg, 106 mol) was added, followed by acetic acid (3.7 kg, 62 mol), and the resulting mixture was purged with nitrogen followed by evacuation under reduced pressure. After repeating the purge process thrice, 5% Pd/C (8.3 kg, 59.8% water wet) was added and the vessel was re-purged with nitrogen, pressurized with hydrogen gas (0.25 MPa), heated to 35 °C, and stirred for 24 h. The reaction mixture was cooled to 15 °C and filtered under nitrogen pressure, and the filtrate was adjusted to pH 7 with 5N NaOH. The filtrate was treated with activated carbon (3.4 kg) and heated to 50 °C for 2 h. After cooling to 20 °C, the mixture was filtered, the cake was washed with 1:1 MeOH/EtOAc (230 L), and the filtrate was concentrated under reduced pressure to 550 L. The filtrate was diluted with ethyl acetate (460 L), and the solution was re-concentrated to 550 L and then washed with water (115 L) and brine (115 L). The organic layer was concentrated to 140 L and heated to 75 °C for 3 h. After cooling to 10 °C, heptane (115 L) was added over 1 h, and the resulting heterogeneous mixture was stirred for 3 h. The mixture was filtered, and the crude solids were dissolved in acetonitrile (920 L) at reflux. The solution was cooled to 40 °C and concentrated under reduced pressure to 120 L. After heating back to reflux and stirring for 2 h, the mixture was cooled slowly (over 15 h) to 20 °C, stirred for 10 h, and filtered to afford the title compound **SI-5** (22.9 kg, 84% yield) as a white solid. HPLC purity = 98.8% (t_R = 6.2 min) by HPLC method 2. ee = 100% (t_R = 24.5 min) by Chiral HPLC method 3. Specific rotation: $[\alpha]_D^{20}$ = 12.23 (*c* = 10, 95% EtOH-5% H_2O). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.77 (d, *J* = 8.1

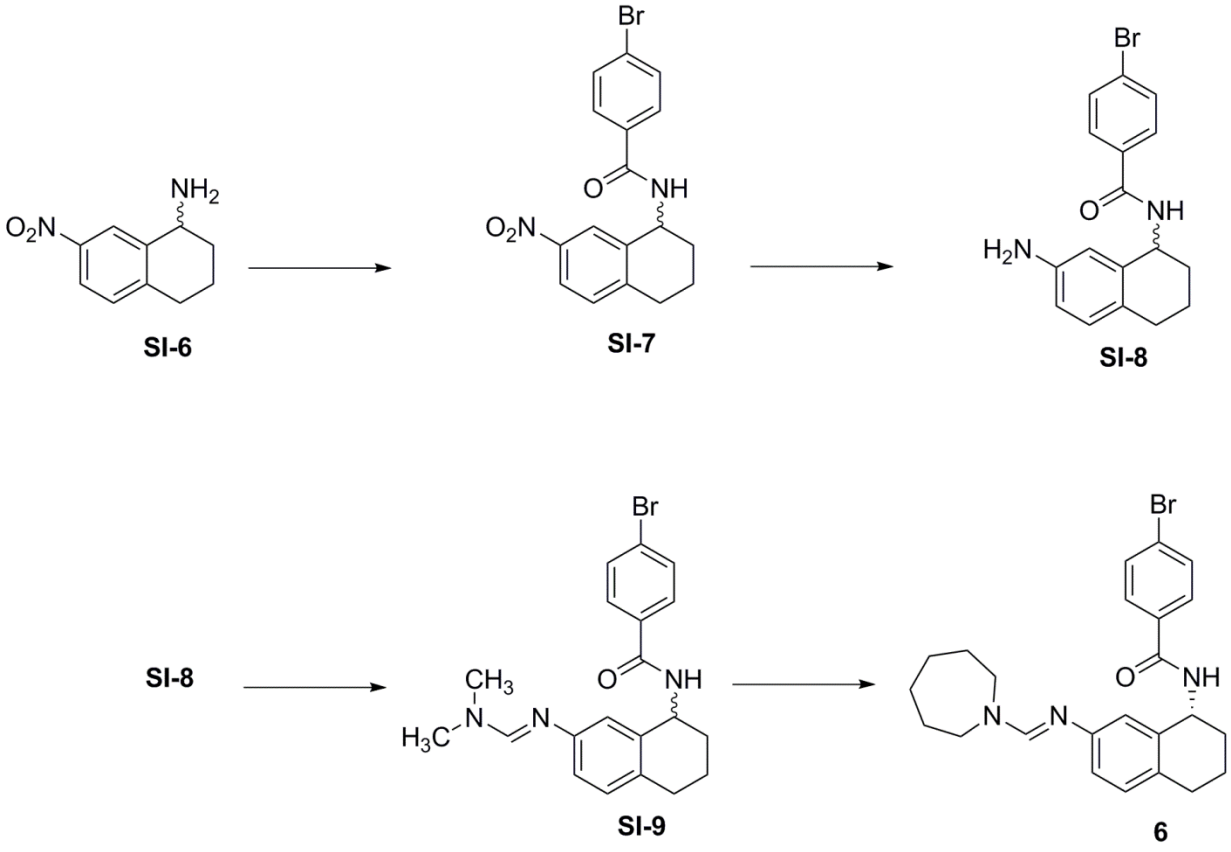
Hz, 1H), 8.02 – 7.92 (m, 2H), 7.33 – 7.22 (m, 2H), 6.83 (dd, *J* = 8.9, 2.9 Hz, 1H), 6.73 – 6.63 (m, 2H), 5.38 (s, 1H), 4.95 (dd, *J* = 8.2, 5.2 Hz, 1H), 4.51 (t, *J* = 6.5 Hz, 2H), 4.40 (t, *J* = 6.0 Hz, 2H), 4.13 (dd, *J* = 10.6, 2.0 Hz, 1H), 3.95 – 3.84 (m, 2H), 3.38 (p, *J* = 6.1 Hz, 1H), 2.94 (t, *J* = 5.0 Hz, 4H), 2.33 (t, *J* = 4.9 Hz, 4H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 165.6, 163.1, 148.4, 145.9, 131.3, 130.7, 122.2, 118.1, 117.4, 116.8, 115.6, 74.8, 67.5, 66.2, 58.9, 50.9, 49.9, 49.5. HRMS (ESI$^+$): calcd. for $C_{23}H_{26}FN_3O_4$ (M+): 428.1980, found 428.1973.

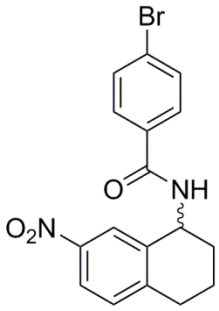


(3*R*,4*S*)-4-(4-fluorobenzamido)-6-(4-(oxetan-3-yl)piperazin-1-yl methylcarbamate (**5**). To a solution of 4-fluoro-*N*-((3*R*,4*S*)-3-hydroxy-6-(4-oxetan-3-yl)piperazin-1-yl)chroman-4-yl)benzamide (**SI-5**, 22.8 kg, 53.3 mol) in THF (456 L) was added 1,1'-carbonyldiimidazole (11.2 kg, 69.3 mol). The resulting mixture was stirred at 15–25 °C for 2 h. After cooling to 0 °C, a solution of methylamine (2.0 M in THF, 46.3 kg, 95.9 mol) was added over 0.5 h, and the mixture was stirred for 4 h. 2 N aqueous NaOH (228 L) was added, and stirring was continued for 0.5 h. The layers were separated, and the organics were washed with 2 N NaOH (3 × 228 L). Water (456 L) was added, and the mixture was concentrated under reduced pressure to 25 L/kg of **5**. THF (45 L) was added, and the mixture was cooled to 10 °C and stirred for 3 h. After filtration, the crude solids were re-dissolved in THF (456 L). Water (456 L) was added and the mixture was re-concentrated to 25 L/kg of **5**. THF (45 L) was added and the mixture stirred at 10 °C for 3 h. After filtration, **5** was isolated as a white crystalline solid (22.8 kg, 90 % yield). HPLC purity = 98.6% (t_R = 24.2 min) by HPLC method 3. ee = 99.9% (t_R = 23.6 min) by Chiral HPLC method 4. Specific rotation: $[\alpha]_D^{25}$ = 55.19 (*c* = 10,

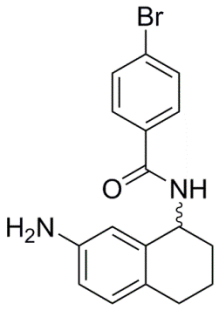
DMSO). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.92 (d, J = 7.8 Hz, 1H), 8.01 – 7.90 (m, 2H), 7.34 – 7.23 (m, 2H), 7.19 (q, J = 4.5 Hz, 1H), 6.87 (dd, J = 9.0, 2.9 Hz, 1H), 6.78 – 6.69 (m, 2H), 5.03 (dd, J = 8.1, 3.7 Hz, 1H), 4.86 (td, J = 4.1, 1.8 Hz, 1H), 4.52 (t, J = 6.5 Hz, 2H), 4.41 (t, J = 6.0 Hz, 2H), 4.23 (dd, J = 11.8, 1.9 Hz, 1H), 4.13 (ddd, J = 11.8, 4.4, 1.6 Hz, 1H), 3.39 (p, J = 6.3 Hz, 1H), 2.96 (t, J = 4.9 Hz, 4H), 2.52 (d, J = 4.5 Hz, 3H), 2.34 (t, J = 4.9 Hz, 4H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 165.4, 164.5 (d, J = 248.7 Hz), 156.1, 148.2, 146.2, 131.0, 130.8 (d, J = 9.5 Hz), 120.9, 118.6, 117.6, 117.2, 115.6 (d, J = 21.3 Hz), 74.8, 68.7, 64.3, 58.9, 49.8, 49.5, 47.7, 27.4. HRMS (ESI^{+}): calcd. for $C_{25}H_{30}FN_4O_5$ (M+1): 485.2195, found 485.2188.

Synthesis of 6.



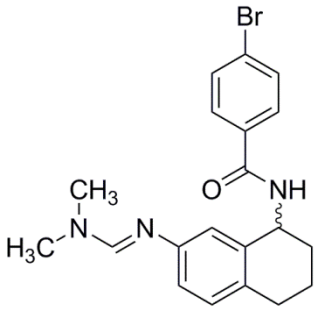


(±)-4-bromo-*N*-(7-nitro-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (SI-7). A solution of 1-amino-7-nitro-1,2,3,4-tetrahydronaphthalene[2] (**SI-6**, 3.5 g, 18.2 mmol) and triethylamine (7.61 mL, 54.6 mmol) in dichloromethane (50 mL) was treated with 4-bromobenzoyl chloride (4.8 g, 21.85 mmol) and stirred at room temperature for three days. The reaction mixture was diluted with dichloromethane and washed with dilute aqueous hydrochloric acid. The organic phase was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to furnish a brown foam. This was recrystallized from EtOH (225 mL). The resulting light tan crystals were dried under reduced pressure at room temperature overnight to furnish the title compound **SI-7** (4.2 g, 61% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (d, J = 2.2 Hz, 1H), 8.01 (dd, J = 8.4, 1.6 Hz, 1H), 7.67, 7.59 (ABq, J_{AB} = 8.8 Hz, 4H), 7.28 (d, J = 8.4 Hz, 1H), 6.37 (d, J = 8.8 Hz, 1H), 5.43 (app q, J = 7.0 Hz, 1H), 2.99–2.85 (m, 2H), 2.25–2.15 (m, 1H), 1.99–1.86 (m, 3H). LC/MS: *m/z* (^{79}Br/^{81}Br) 375/377 ($[M+H]^+$).



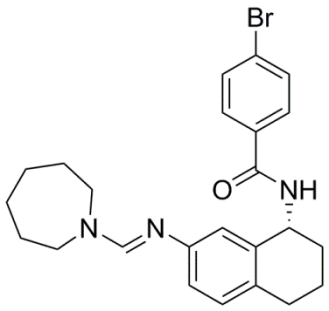
(±)-4-bromo-*N*-(7-amino-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (SI-8). A solution of (±)-4-bromo-*N*-(7-nitro-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (**SI-7**, 4.1 g, 10.9 mmol) in THF (50 mL) and methanol (16 mL) was cooled to 0 °C and treated with $NaBH_4$ (1.65 g, 43.7 mmol), followed by $NiCl_2$ (113.3 mg, 0.874 mmol). After 15 minutes, the mixture was allowed to warm to room temperature and then stirred for 1 h. Then, the mixture was treated with 1 N aqueous NaOH (50 mL) and EtOAc (100 mL). The organic phase was separated and washed with water, then saturated aqueous NaCl. The organic phase was separated and dried over $MgSO_4$, filtered, and concentrated under reduced pressure to furnish a tan foam. This was

subjected to flash chromatography on silica gel, eluting with a gradient of 10–40% EtOAc/hexanes, to furnish two separate products as white foams. The first to elute was the title compound **SI-8** (1.11 g, 29% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, *J* = 8.8 Hz, 2H), 7.55 (d, *J* = 8.8 Hz, 2H), 6.92 (d, *J* = 7.9 Hz, 1H), 6.63 (d, *J* = 2.7 Hz, 1H), 6.57 (dd, *J* = 7.9, 2.7 Hz, 1H), 6.32 (d, *J* = 8.4 Hz, 1H), 5.27 (app dt, *J* = 7.9, 5.8 Hz, 1H), 3.56 (br s, 2H), 2.77–2.63 (m, 2H), 2.13–2.04 (m, 1H), 1.93–1.79 (m, 3H). LC/MS: *m/z* (^{79}Br/^{81}Br) 345/347 ($[M+H]^+$). The second compound to elute was **(±)-*N*-(7-amino-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide** (1.51 g, 52% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80–7.77 (m, 2H), 7.52–7.74 (m, 1H), 7.45–7.40 (m, 2H), 6.92 (d, *J* = 8.4 Hz, 1H), 6.66 (d, 2.6 Hz, 1H), 6.57 (dd, *J* = 8.3, 2.4 Hz, 1H), 6.35 (d, *J* = 8.0 Hz, 1H), 5.30 (app dt, *J* = 8.0, 6.7 Hz, 1H), 3.56 (br s, 2H), 2.78–2.64 (m, 2H), 2.15–2.07 (m, 1H), 1.93–1.81 (m, 3H). LC/MS: *m/z* 267 ($[M+H]^+$).



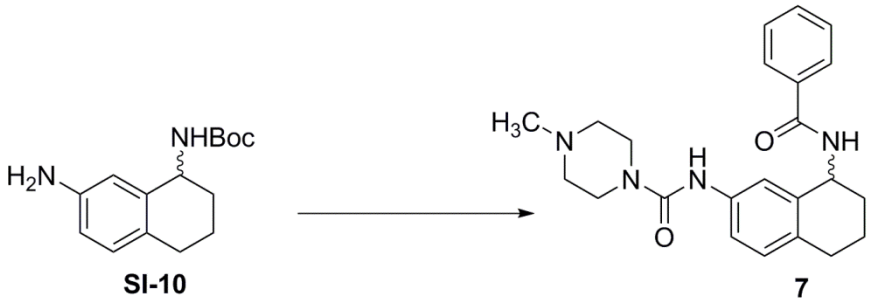
(±)-4-bromo-*N*-(7-(((dimethylamino)methylene)amino)-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (SI-9). A mixture of (±)-4-bromo-*N*-(7-amino-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (**SI-8**, 600 mg, 1.74 mmol), dimethyl formamide dimethyl acetal (1.62 mL, 12.2 mmol), and EtOH (10 mL) was heated to 90 °C with stirring. After 2 h, the mixture was concentrated under reduced pressure, treated with dichloromethane, and concentrated under reduced pressure. The crude material was dried under vacuum, then subjected to flash chromatography on silica gel, eluting with a gradient of 0–15% methanol in DCM to furnish the title compound **SI-9** as a colorless oil. Yield = 80 mg (11%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.82 (d, *J* = 8.8 Hz, 1H), 7.87 (d, *J* = 8.8 Hz, 2H), 7.67 (d, *J* = 8.8 Hz, 2H), 7.60 (s, 1H), 6.95 (d, *J* = 7.8 Hz, 1H), 6.74 (dd, *J* = 7.8, 2.2 Hz, 1H), 6.67 (d, *J* = 2.2

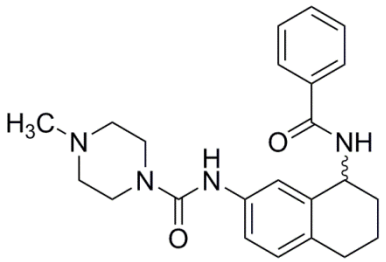
Hz, 1H), 5.20–5.14 (m, 1H), 2.92 (br s, 3H), 2.85 (br s, 3H), 2.76–2.63 (m, 2H), 1.99–1.90 (m, 2H), 1.82–1.66 (m, 2H). LC/MS: *m/z* (^{79}Br/^{81}Br) 400/402 ([M+H]$^+$).



(*R*)-*N*-(7-((azepan-1-ylmethylene)amino)-1,2,3,4-tetrahydronaphthalen-1-yl)-4-bromobenzamide (**6**). A mixture of (±)-4-bromo-*N*-(7-(((dimethylamino)methylene)amino)-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (**SI-9**, 350 mg, 0.87 mmol) and azacycloheptane (5.0 mL, 44.2 mmol) was treated with ammonium sulfate (5.0 mg, 0.04 mmol) and heated to 80 °C with stirring overnight. The mixture was partitioned between water and EtOAc. The organic phase was separated and dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give an orange solid. This was subjected to flash chromatography on silica gel, eluting with a gradient of 0–8% methanol in DCM to furnish a colorless oil that rapidly solidified. This was subjected to preparatory chiral HPLC (Chiralpak AS-H 3 × 25 cm, eluting with 30 mL/min 0.2% *N*,*N*-dimethylethylamine in MeOH). t_R = 2.42 min ((*R*)), 4.12 min ((*S*)). Yield of the (*R*) isomer **6** = 105 mg (26%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.77 (d, *J* = 8.4 Hz, 1H), 7.82 (d, *J* = 8.8 Hz, 2H), 7.62 (d, *J* = 8.8 Hz, 2H), 7.60 (s, 1H), 6.90 (d, *J* = 7.9 Hz, 1H), 6.69 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.62 (d, *J* = 2.2 Hz, 1H), 5.15–5.09 (m, 1H), 3.37 (t, *J* = 6.0 Hz, 2H), 3.32 (t, *J* = 5.8 Hz, 2H), 2.71–2.58 (m, 2H), 1.93–1.85 (m, 2H), 1.76–1.53 (m, 6H), 1.48–1.40 (m, 4H). HRMS (ESI$^+$): calcd. for $C_{24}H_{28}BrN_3O$ (M): 453.1416, found 453.1429.

Synthesis of 7.

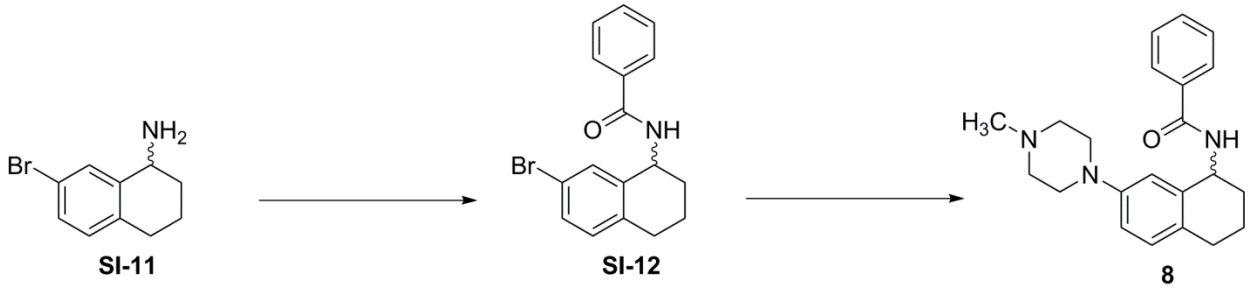


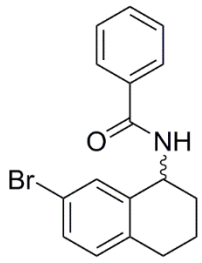


(±)-*N*-(8-benzamido-5,6,7,8-tetrahydronaphthalen-2-yl)-4-methylpiperazine-1-carboxamide (7). A mixture of(7-amino-1,2,3,4-tetrahydronaphthalen-1-yl)carbamic acid *tert*-butyl ester[3] (**SI-10**, 300 mg, 1.14 mmol), THF (5.7 mL), 4-methyl-1-piperazinecarbonyl chloride hydrochloride (455 mg, 2.29 mmol), and Et_3N (797 µL, 5.7 mmol) was stirred at 45 °C overnight. The mixture was concentrated under reduced pressure, diluted with water (20 mL), and extracted with EtOAc (3 × 40 mL). The combined organic phases were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. This material was treated with 1,4-dioxane (5.97 mL) and then HCl (4.0 N solution in 1,4-dioxane, 11.9 mL, 47.7 mmol) and stirred at room temperature overnight. The resulting mixture was concentrated under reduced pressure, dissolved in MeOH, and concentrated under reduced pressure. Then, this material was twice slurried in DCM and concentrated under reduced pressure to furnish 451 mg of a tan solid (quantitative yield, 2 steps). Of this, 100 mg (0.277 mmol) was treated with dichloromethane (2.77 mL), benzoic acid (33.8 mg, 1 equiv), triethylamine (154 µL, 1.1 mmol), and BOP (135 mg, 0.304 mmol). The mixture was stirred at room temperature overnight, then concentrated under reduced pressure, treated with water (25 mL), and extracted with EtOAc (3 × 35 mL). The combined organic extracts were washed with saturated aqueous NaCl (10 mL),

then dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude material was subjected to flash chromatography on silica gel, eluting with a 5–15% MeOH/DCM gradient, to furnish the title compound **7** as a white solid (58 mg, 53% yield over 3 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.0 Hz, 2H), 7.52–7.47 (m, 1H), 7.45–7.40 (m, 3H), 7.06 (dd, *J* = 4.8, 2.6 Hz, 2H), 6.40–6.36 (m, 2H), 5.36–5.30 (m, 1H), 3.53–3.43 (m, 4H), 2.82–2.59 (m, 2H), 2.42 (t, *J* = 5.1 Hz, 4H), 2.31 (s, 3H), 2.15–2.07 (m, 1H), 1.94–1.82 (m, 3H). HRMS (ESI^+): calcd. for $C_{23}H_{28}N_4O_2$ (M): 392.2212, found 392.2222.

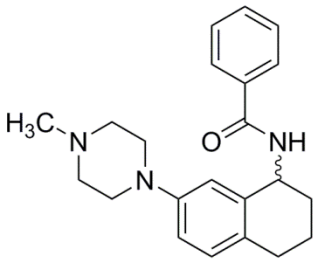
Synthesis of 8.





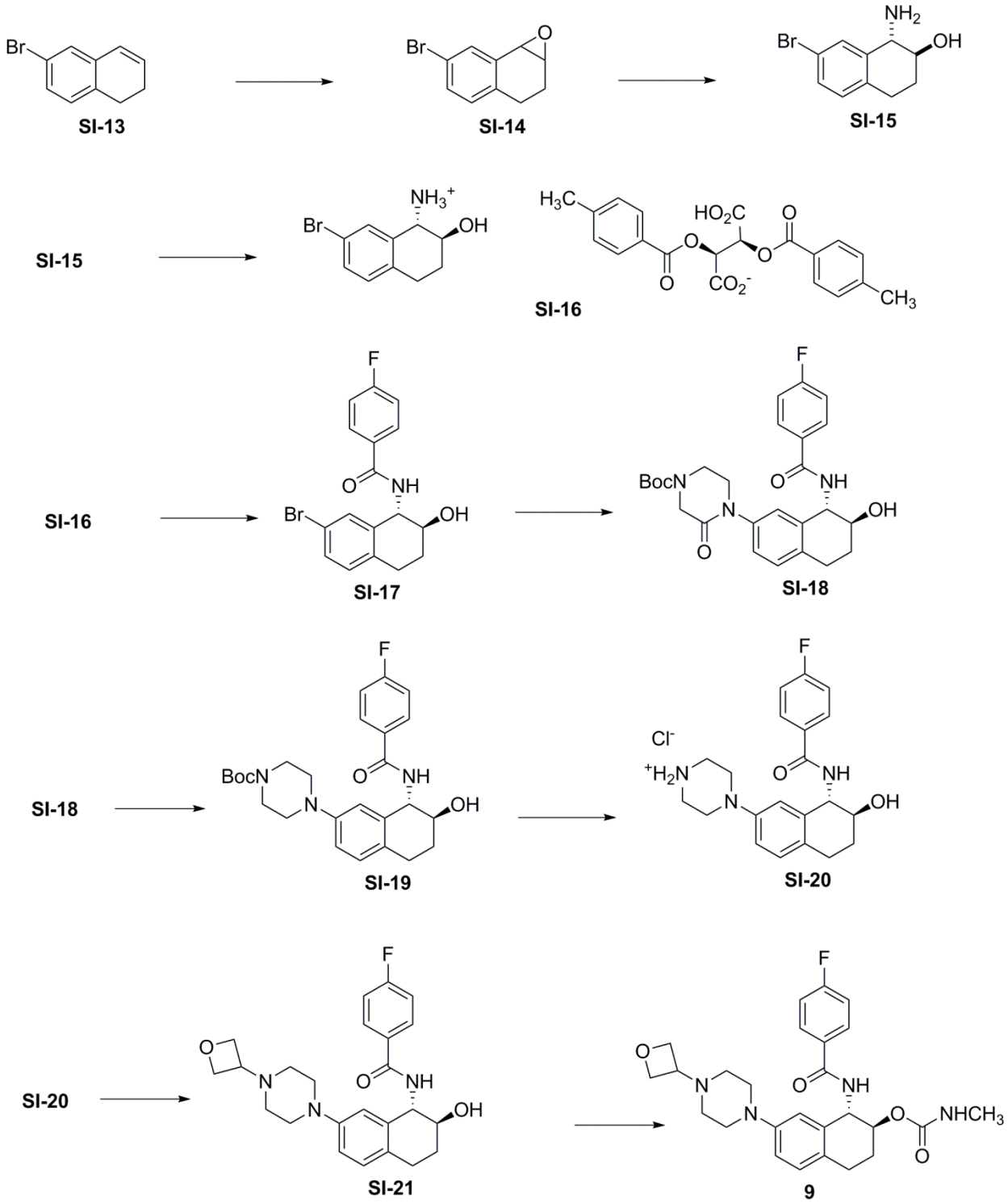
(±)-*N*-(7-bromo-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (SI-12). To a solution of (±)-1-amino-7-bromo-1,2,3,4-tetrahydronapthalene [4](**SI-11**, 1.40 g, 6.19 mmol) in DCM (50 mL) was added triethylamine (2.0 mL, 14.35 mmol), then benzoyl chloride (0.82 mL, 7.07 mmol). The mixture was stirred at room temperature for 1 h, during which time a white precipitate formed. Then, DCM and water were added until all of the solids had dissolved. The organic layer was separated and dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting solids were recrystallized from EtOH (150 mL) to furnish the title compound **SI-12** as a crystalline solid (1.17 g, 57% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.83 (d, *J* = 8.8 Hz, 1H), 7.91 (dd, *J* =

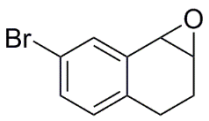
7.4, 2.0 Hz, 2H), 7.56–7.52 (m, 1H), 7.50–7.45 (m, 2H), 7.35 (dd, *J* = 7.9, 2.2 Hz, 1H), 7.29 (d, *J* = 2.2 Hz, 1H), 7.10 (d, *J* = 8.4 Hz, 1H), 5.25–5.19 (m, 1H), 2.72 (app t, *J* = 4.8 Hz, 2H), 2.01–1.91 (m, 2H), 1.85–1.71 (m, 2H). LC/MS *m/z* (^{79}Br/^{81}Br) 330/332 ([M+H]$^+$).



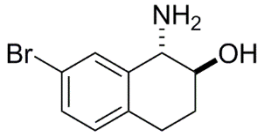
(±)-*N* -(7-(4-methylpiperazin-1-yl)-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (8). Under a nitrogen atmosphere, a flask was charged with tris(dibenzylideneacetone)palladium (20.8 mg, 0.02 mmol),THF (3 mL), and potassium *tert*-butoxide (1.0 M solution in *tert*-butanol, 0.636 mL, 0.64 mmol). (±)-*N*-(7-bromo-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (**SI-12**, 150 mg, 0.45 mmol) was added, followed by *N*-methylpiperazine (61 µL, 0.55 mmol). The mixture was stirred overnight at 50 °C, then partitioned between EtOAc and water. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The material was thrice subjected to flash chromatography on silica gel, first eluting with a 2–15% MeOH/DCM gradient, then eluting with a 0–5% MeOH/THF gradient, then eluting with a 50–100% THF/hexanes gradient to furnish the title compound **8** (12.9 mg, 8% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.73 (d, *J* = 8.8 Hz, 1H), 7.90 (dd, *J* = 7.6, 1.8 Hz, 1H), 7.54–7.49 (m, 1H), 7.47–7.43 (m, 2H), 6.96 (d, *J* = 8.3 Hz, 1H), 6.81 (dd, *J* = 8.4, 2.6 Hz, 1H), 6.72 (d, *J* = 2.6 Hz, 1H), 5.20–5.14 (m, 1H), 2.99 (dd, *J* = 5.4, 4.4 Hz, 4H), 2.69–2.64 (m, 2H) 2.39 (dd, *J* = 5.4, 4.4 Hz, 4H), 2.17 (s, 3H), 1.97–1.90 (m, 2H), 1.81–1.68 (m, 2H). HRMS (ESI$^+$): calcd. for $C_{22}H_{28}N_3O$ (M): 349.2154, found 349.2170.

Synthesis of 9.



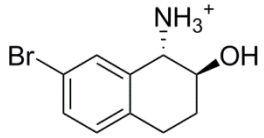


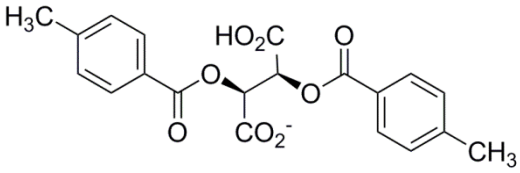
(±)-*cis*-6-bromo-1a,2,3,7b-tetrahydronaphtho[1,2-b]oxirene (SI-14). To a solution of 6-bromo-1,2-dihydronaphthalene[1a,6] (**SI-13**, 250 g, 1.20 mol) in dichloromethane (3.75 L), saturated aqueous sodium bicarbonate (1250 mL)was added, and the mixture was cooled to 0–5 °C. Then, *m*CPBA (77%, 275 g, 1.23 mol, 1.03 eq) was added in portions. The mixture was mechanically stirred at 0–5 °C for 1.5 hr, and then allowed to warm to room temperature. The mixture was stirred at room temperature for approximately 1.5 hr, and add additional mCPBA (77%, 15.0 g, 67 mmol, 0.05 eq) was added at room temperature, then the mixture was stirred for one hour. The mixture was diluted with dichloromethane (3750 mL) and the layers were separated. The organic layer was washed with saturated aqueous sodium bicarbonate (3 L), then with a mixture of a 10% aqueous sodium bisulfite solution (3750 mL) and an aqueous saturated sodium bicarbonate (1250 mL) at room temperature for about 10 min. The layers were separated, and the organic layer was washed with saturated aqueous sodium bicarbonate (3 L) and brine (3 L). The mixture was concentrated under reduced pressure to furnish the title compound **SI-14** as an oil (263 g, 98% yield). GC/MS m/z (^{79}Br/^{81}Br) 224/226 [M^+].



(±)-*trans*-1-amino-7-bromo-1,2,3,4-tetrahydronaphthalen-2-ol (SI-15). In a 2 L pressure reactor equipped with a mechanical stirrer, (±)-*cis*-6-bromo-1a,2,3,7b-tetrahydronaphtho[1,2-b]oxirene (**SI-14**, 67.0 g, 298 mmol) was dissolved in tetrahydrofuran (200 mL). Ammonia (7 M solution in methanol, 350 mL, 2.45 mol) was added, and the reactor was sealed and stirred at 70 °C for 2 days. Additional ammonia (7 M solution in methanol, 140 mL, 0.98 mol) was added, and the reactor was sealed and stirred at 70 °C for 1 day. The reaction mixture was concentrated under reduced pressure to furnish a residue (68.1 g).

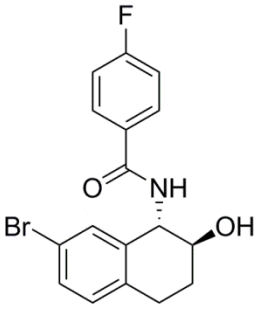
Diethyl ether (760 mL) was added, and the slurry was stirred at room temperature for approximately 5 hr. The solid was collected by filtration, rinsed with diethyl ether (2 × 215 mL), and dried overnight to furnish the title compound **SI-15** (58.1 g, 81% yield). GC/MS m/z (^{79}Br/^{81}Br) 224/226 $[M\text{-}NH_2]^+$.





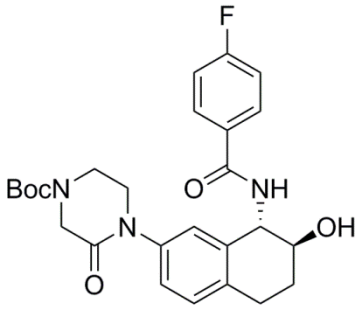
(1*S*,2*S*)-1-Amino-7-bromo-1,2,3,4-tetrahydronaphthalen-2-ol (2*S*,3*S*)- 2,3-bis(4-methylbenzoyloxy)succinate (SI-16). In one portion, (+)-*O,O'*-di-4-toluoyl-D-tartaric acid (2.30 kg, 5.95 mol) was added to a solution of (±)-*trans*-1-amino-7-bromo-1,2,3,4-tetrahydronaphthalen-2-ol (**SI-15**, 1.40 kg, 5.78 mol) in acetonitrile (16.0 L) and water (3.50 L). The thick suspension was heated to reflux (77 °C) under nitrogen for 30 min. (The stirrer did not start to stir until the internal temperature reached 46 °C.) Then, then mixture was allowed to cool to room temperature with stirring. After 5 hr., the thick slurry was filtered through a Büchner funnel and rinsed with acetonitrile (2 L). The mixture was filtered for one hour until no more solvent was collected. The wet cake was dried in air at room temperature overnight to obtain the desired salt as a yellow solid (1.70 kg). The solid was added into acetonitrile (15 L) and water (3.3 L) and heated as a thick slurry to reflux (77 °C) under nitrogen with stirring for 30 min. Then, then mixture was allowed to cool to room temperature with stirring. After 5 hr., the thick slurry was filtered through a Büchner funnel and rinsed with acetonitrile (2 L). The mixture was filtered for 30 min until no more solvent was collected. The wet cake was dried at room temperature for 48 h to obtain the title compound **SI-16** as a pale yellow solid (800 g, 22% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.84 (d, J = 8.2 Hz, 4H),

7.72 (d, *J* = 1.5 Hz, 1H), 7.41 (dd, *J* = 8.2, 1.9 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 4H), 7.09 (d, *J* = 8.2 Hz, 1H), 5.64 (s, 2H), 4.08 (d, *J* = 6.8 Hz, 1H), 3.83 (ddd, *J* = 9.5, 6.6, 3.0 Hz, 1H), 2.75 (A of ABqdd, *J* = 17.3, 5.6, 5.6 Hz, 1H), 2.65 (B of ABqdd, *J* = 17.2, 8.6, 6.0 Hz, 1H), 1.95 (dddd, *J* = 13.3, 5.5, 4.8, 3.3 Hz, 1H), 1.67 (dddd, J = 13.4, 9.0, 9.0, 5.7 Hz, 1H). LC-ES/MS m/z (^{79}Br/^{81}Br) 242/244 $[M+H]^+$ for the free base. 99.0% ee. Analytical conditions for enantiomeric excess determination by supercritical fluid chromatography (SFC): Column: CHIRALPAK® AD-H (0.46 × 250 mm× 5 μm), carbon dioxide flow rate: 2.55 mL/min., co-solvent: methanol with 0.1% diethyl amine, co-solvent flow rate: 0.45 mL/min., back pressure: 150 bar, column temperature: 40.9 °C. Isomer 1 T_R = 7.36 min, 0.5% area and isomer 2 (title compound) T_R = 8.46 min., 99.5% area.



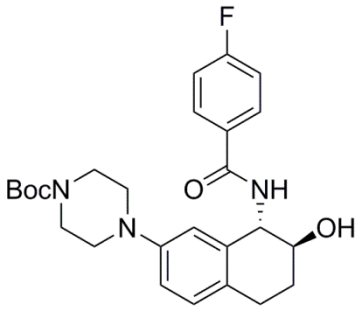
***N*-((1*S*,2*S*)-7-bromo-2-hydroxy-1,2,3,4-tetrahydronaphthalen-1-yl)-4-fluorobenzamide (SI-17).** In a 3-necked 5000 mL flask equipped with a mechanical stirrer was placed THF (1.8 L) and (1*S*,2*S*)-1-Amino-7-bromo-1,2,3,4-tetrahydronaphthalen-2-ol (2*S*,3*S*)- 2,3-bis(4-methylbenzoyloxy)succinate (**SI-16**, 85.6 g, 136.2 mmol). The mixture was cooled to 0–5 °C and treated with saturated aqueous $NaHCO_3$ (1.8 L), which resulted in a temperature increase to 21 °C. The mixture was allowed to cool back to 0–5 °C, then was treated with 4-fluorobenzoyl chloride (20.0 mL, 169.15 mmol) and stirred vigourously. After 1 hour, the mixture was allowed to warm to 16 °C and was treated with water (800 mL) and DCM (800 mL), then stirred at 17 °C for 10 min. The resulting emulsion was divided into two equal parts, each of which was treated with water (200 mL) and DCM (200 mL), shaken, and separated. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated

under reduced pressure to furnish the title compound **SI-17** as an orange solid (50.2 g, 101% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.73 (d, *J* = 8.6 Hz, 1H), 8.00 (dd, *J* = 8.9, 5.6 Hz, 2H), 7.35–7.27 (m, 3H), 7.23 (d, *J* = 1.4 Hz, 1H), 7.09 (d, 8.2 Hz, 1H), 5.09 (d, *J* = 4.4 Hz, 1H), 4.99 (t, *J* = 7.8 Hz, 1H), 3.89 (dddd, *J* = 9.2, 7.0, 4.0, 3.2 Hz, 1H), 2.82 (A of ABqdd, *J* = 17.1, 5.6, 5.6 Hz, 1H), 2.75 (B of ABqdd, *J* = 17.1, 9.0, 5.5 Hz, 1H), 2.05 (dddd, *J* = 12.9, 4.0, 4.0, 3.0 Hz, 1H), 1.77 (dddd, *J* = 12.7, 8.8, 8.8, 5.6 Hz, 1H).



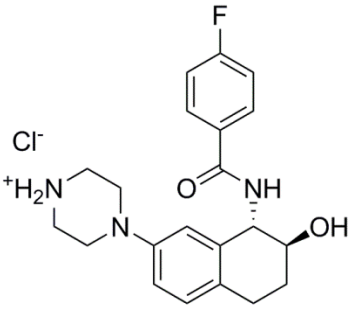
***tert*-butyl 4-((7*S*,8*S*)-8-(4-fluorobenzamido)-7-hydroxy-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxopiperazine-1-carboxylate (SI-18).** A mixture of *N*-((1*S*,2*S*)-7-bromo-2-hydroxy-1,2,3,4-tetrahydronaphthalen-1-yl)-4-fluorobenzamide (**SI-17**, 29.8 g, 81.8 mmol), *tert*-butyl 3-oxopiperazine-1-carboxylate (17.8 g, 88.9 mmol), and K_2CO_3 (22.9 g, 164.0 mmol) in toluene (400 mL) was sparged with nitrogen for 30 minutes, then treated with copper(I) iodide (7.80 g, 40.6 mmol) and *sym*-dimethylethylenediamine (9.00 mL, 84.1 mmol). The resulting brown slurry was stirred mechanically at 110 °C. After 1 h, the mixture had turned blue. Stirring was continued at 110 °C for an additional 16 h, at which time it had turned brown and contained thick solids. The mixture was cooled to room temperature and extracted between EtOAc (1050 mL) and water (1050 mL). The organic phase was separated and washed with water (2 × 650 mL) and saturated aqueous NaCl (650 mL), then dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting crude material was subjected to flash chromatography on silica gel, eluting with a 4–7% *i*-PrOH/CH_2Cl_2 gradient, to furnish the title compound **SI-18** as a yellow foam (31.5 g, 80% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.71 (d, *J* = 8.7 Hz, 1H), 7.99

(dddd, *J* = 8.9, 5.5, 3.0, 3.0 Hz, 2H), 7.28 (app tt, *J* = 8.9, 2.0 Hz, 2H), 7.16–7.07 (m, 3H), 5.04–4.99 (m, 2H), 3.99 (s, 2H), 3.88 (dddd, *J* = 9.0, 7.3, 4.4, 3.0 Hz, 1H), 3.63–3.56 (m, 4H), 2.85 (A of ABqdd, *J* = 17.2, 5.7, 5.7 Hz, 1H), 2.74 (B of ABqdd, *J* = 17.2, 7.6, 5.4 Hz, 1H), 2.05 (dddd, *J* = 13.1, 5.7, 5.7, 2.8 Hz, 1H), 1.76 (dddd, *J* = 13.5, 8.0, 8.0, 5.6 Hz, 1H), 1.38 (s, 9H). LC-ES/MS *m/z* 428 $[M+2H–t\text{-Bu}]^+$, 484 $[M+H]^+$, 506 $[M+Na]^+$.

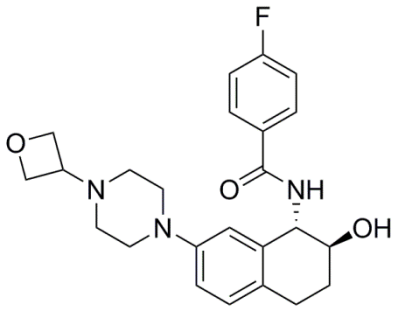


***tert*-butyl 4-((7*S*,8*S*)-8-(4-fluorobenzamido)-7-hydroxy-5,6,7,8-tetrahydronaphthalen-2-yl)piperazine-1-carboxylate (SI-19).** A solution of *tert*-butyl 4-((7*S*,8*S*)-8-(4-fluorobenzamido)-7-hydroxy-5,6,7,8-tetrahydronaphthalen-2-yl)-3-oxopiperazine-1-carboxylate (**SI-18**, 28.3 g, 58.5 mmol) in THF (200 mL) was cooled to 0–5 °C. Over 5 minutes, this solution was charged with borane-dimethylsulfide complex (2.0 M in THF, 49.0 mL, 98.0 mmol) at such a rate that the internal temperature did not exceed 4 °C (some foaming was observed). The resulting light yellow solution was mechanically stirred at room temperature for 1 h. Then, the mixture was cooled to –20 °C and was transferred *via* cannula over 15 minutes to a flask containing –20 °C absolute ethanol (195 mL) (*Caution! Foaming and exotherm!*) at such a rate that the internal temperature did not exceed –10 °C. At the end of this cannulation, the resulting mixture, still at –20 °C, was transferred *via* cannula over 15 minutes to a flask containing 0–5 °C water (1260 mL) at such a rate that the internal temperature did not exceed 7 °C. After the addition, the mixture was allowed to warm to room temperature with stirring, and then was extracted with EtOAc (2 × 680 mL), and the combined organic layers were washed with saturated aqueous NaCl (490 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting material

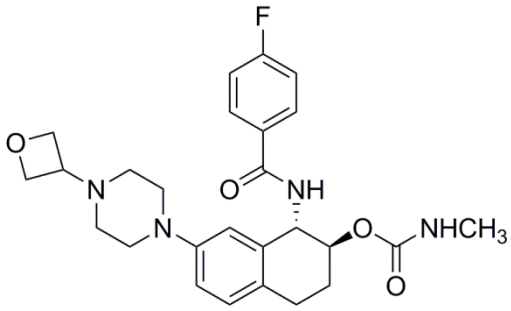
was subjected to flash chromatography on silica gel, eluting with 75% EtOAc/heptane, to furnish the title compound **SI-19** (17.6 g, 64% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.62 (d, J = 8.7 Hz, 1H), 7.99 (dddd, J = 8.9, 5.4, 2.0, 2.0 Hz, 2H), 7.28 (app tt, J = 8.9, 2.0 Hz, 2H), 6.98 (d, J = 8.4 Hz, 1H), 6.82 (dd, J = 8.5, 2.5 Hz, 1H), 6.70 (d, J = 2.4 Hz, 1H), 4.99–4.94 (m, 2H), 3.88–3.82 (m, 1H), 3.41–3.35 (m, 4H), 2.95–2.88 (m, 4H), 2.77 (A of ABqdd, J = 16.7, 5.8, 5.8 Hz, 1H), 2.68 (B of ABqdd, J = 16.7, 7.9, 5.6 Hz, 1H), 2.07–2.00 (m, 1H), 1.78–1.68 (m, 1H), 1.38 (s, 9H). LC-ES/MS *m/z* 414 $[M+2H-t\text{-Bu}]^+$, 470 $[M+H]^+$, 492 $[M+Na]^+$.



4-fluoro-*N*-((1*S*,2*S*)-2-hydroxy-7-(piperazin-1-yl)-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide hydrochloride (SI-20). A solution of *tert*-butyl 4-((7*S*,8*S*)-8-(4-fluorobenzamido)-7-hydroxy-5,6,7,8-tetrahydronaphthalen-2-yl)piperazine-1-carboxylate (**SI-19**, 19.4 g, 41.3 mmol) in 1,4-dioxane (430 mL) was treated with hydrogen chloride (4N solution in 1,4-dioxane, 135 mL, 540 mmol) at room temperature. Then, the mixture was heated to 50 °C with stirring for 13 h, then concentrated under reduced pressure to furnish the title compound **SI-20** as a white solid in ca. 60% purity (24.8 g, 89% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.43 (br s, 2H), 8.65 (d, J = 8.6 Hz, 1H), 7.99 (dddd, J = 8.9, 5.6, 2.1, 2.1, 2H), 7.28 (app tt, J = 8.9, 2.1, 2H), 7.04 (d, J = 8.5 Hz, 1H), 6.92 (dd, J = 8.3, 2.4 Hz, 1H), 6.79 (d, J = 2.2 Hz, 1H), 4.96 (app t, J = 7.6 Hz, 1H), 3.86 (ddd, J = 9.7, 6.9, 2.7 Hz, 1H), 3.29–3.23 (m, 4H), 3.18 (br s, 4H), 2.79 (A of ABqdd, J = 16.8, 5.7, 5.7 Hz, 1H), 2.69 (B of ABqdd, J = 16.8, 7.9, 5.6 Hz, 1H), 2.03 (dddd, J = 13.2, 6.8, 6.8, 2.9 Hz, 1H), 1.73 (dddd, J = 13.3, 8.7, 8.7, 5.7 Hz, 1H). LC-ES/MS *m/z* 370 $[M+H]^+$.



4-fluoro-*N*-((1*S*,2*S*)-2-hydroxy-7-(4-(oxetan-3-yl)piperazin-1-yl)-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (SI-21). A mixture of 4-fluoro-*N*-((1*S*,2*S*)-2-hydroxy-7-(piperazin-1-yl)-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide hydrochloride (**SI-20**, 24.8 g of 60% purity, 36.7 mmol), acetonitrile (520 mL), and 3-oxetanone (4.4 g, 61.1 mmol) was treated with sodium triacetoxyborohydride (29.6 g, 140 mmol) and stirred mechanically at room temperature for 3 h. Then, 3-oxetanone (0.44 g, 0.61 mmol) and sodium triacetoxyborohydride (3.0 g, 1.4 mmol) were added, and the mixture was stirred at room temperature for another 4 h. A saturated aqueous solution of $NaHCO_3$ (1300 mL) was added, and the mixture was stirred for 10 min. Then, water (100 mL) was added, and the mixture was extracted with DCM (3 × 650 mL). The combined organic layers were washed with saturated aqueous NaCl (650 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting off-white solid was subjected to flash chromatography on silica gel, eluting with a gradient of 5–10% *i*-PrOH in DCM, to furnish the title compound **SI-21** as a white solid (12.0 g, 77% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.62 (d, *J* = 8.7 Hz, 1H), 7.98 (dddd, *J* = 9.0, 5.6, 3.0, 3.0 Hz, 2H), 7.28 (app tt, *J* = 8.9, 2.0 Hz, 2H), 6.96 (d, *J* = 8.4 Hz, 1H), 6.80 (dd, *J* = 8.3, 2.5 Hz, 1H), 6.67 (d, *J* = 2.4 Hz, 1H), 4.98–4.93 (m, 2H), 4.52 (app t, *J* = 6.5 Hz, 2H), 4.41 (app t, *J* = 6.5 Hz, 2H), 3.84 (dddd, *J* = 9.7, 7.2, 4.3, 3.2 Hz, 1H), 3.38 (app p, *J* = 6.3 Hz, 1H), 2.99 (dd, *J* = 5.0, 4.7 Hz, 4H), 2.76 (A of ABqdd, *J* = 16.7, 5.8, 5.8 Hz, 1H), 2.67 (B of ABqdd, *J* = 16.7, 8.5, 5.6 Hz, 1H), 2.33 (dd, *J* = 5.0, 4.8 Hz, 4H), 2.03 (dddd, *J* = 13.0, 7.3, 4.4, 3.0 Hz, 1H), 1.72 (dddd, *J* = 13.0, 8.9, 8.9, 5.7 Hz, 1H). LC-ES/MS *m/z* 426 $[M+H]^+$.



(1*S*,2*S*)-1-(4-fluorobenzamido)-7-(4-(oxetan-3-yl)piperazin-1-yl)-1,2,3,4-tetrahydronaphthalen-2-yl methylcarbamate (9). A solution of 4-fluoro-*N*-((1*S*,2*S*)-2-hydroxy-7-(4-(oxetan-3-yl)piperazin-1-yl)-1,2,3,4-tetrahydronaphthalen-1-yl)benzamide (**SI-21**, 9.00 g, 21.2 mmol) in THF (270 mL) was treated with 1,1'-carbonyldiimidazole (4.20 g, 25.9 mmol) and stirred at room temperature for 8 h. Then, methylamine (2.0 M in THF, 108 mL, 316 mmol) was added, and the mixture was stirred at room temperature for 15 min. The reaction mixture was filtered, and the filter cake was rinsed with THF (2 × 100 mL). The combined filtrates were concentrated under reduced pressure to furnish a white solid. This was dissolved in DCM (500 mL) and washed with water (500 mL) (a small amount of brine was used to break up the emulsion). The organic phase was separated, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to furnish 9.1 g of white solid. The resulting material was combined with 3.6 g of material made by the same method and slurried with EtOAc (255 mL) at 70 °C with stirring for 4 h, then was allowed to cool to room temperature, filtered, and rinsed with chilled EtOAc (85 mL). The resulting solids were dried in a 45 °C vacuum oven overnight to furnish the title compound **9** as a white solid. (10.7 g, 75% yield on a batch-proportional basis). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.79 (d, *J* = 8.6 Hz, 1H), 7.93 (dddd, *J* = 8.9, 5.3, 3.0, 3.0 Hz, 2H), 7.29 (app tt, *J* = 8.9, 2.0 Hz, 2H), 7.01–6.97 (m, 2H), 6.84 (dd, *J* = 8.8, 2.6 Hz, 1H), 6.67 (d, *J* = 2.4 Hz, 1H), 5.14 (app t, *J* = 7.4 Hz, 1H), 4.93 (ddd, *J* = 9.2, 7.2, 2.0 Hz, 1H), 4.52 (app t, *J* = 6.2 Hz, 2H), 4.41 (app t, *J* = 6.2 Hz, 2H), 3.41–3.35 (m, 1H), 3.02–2.96 (m, 4H), 2.76–2.71 (m, 1H), 2.36–2.30 (m, 4H), 2.16–2.07 (m, 1H), 1.90–1.80 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.6, 164.5 (d, *J* = 278.4 Hz), 156.7, 150.0, 136.4, 131.4 (d, *J* = 3.1 Hz), 130.6 (d, *J* = 9.2

Hz), 129.3, 127.5, 116.0, 115.6 (d, J = 21.9 Hz), 115.3, 74.7, 72.4, 58.9, 52.3, 49.4, 48.8, 27.3, 26.6, 25.5. HRMS (ESI^+): calcd. for $C_{26}H_{31}FN_4O_4$ (M): 482.2329, found 482.2372.

Crystallization and Structure Determination. Cat S was expressed in the baculovirus system and purified by Ni-NTA and size exclusion chromatography. The protein construct contained a W113R mutation and had a His tag at the C-terminus. A complex with inhibitor was produced by adding 2 mM **5** to 11.4 mg/mL protein 1 hour prior to crystallization. Crystallization was setup at 295K in 24-well VDX hanging-drop format containing 1 µL protein (11.4 mg/mL Cat S, 150 mM NaCl, 20 mM Tris pH 8.0, 5 mM DTT, 2 mM **5**, and 2% DMSO) + 1.5 µl crystallization solution (21% PEG-8000, 150 mM Ammonium Sulfate, 100 mM Sodium Acetate pH 4.6 and 1 mM EDTA) suspended over 500 µl of crystallization solution. Crystals (300 × 200 µm plates) grew to full size within 1 week and were frozen in 25% glycerol and 75% crystallization solution for data collection. X-ray diffraction data was collected at beamline LRL-CAT at APS (Advanced Photon Source). The structures were determined by molecular replacement using a prior internal structure as a template. The structure of Cat S with **5** was determined to a resolution of 1.6 Å. The crystallographic refinement was done by Refmac 5.6 with bulk solvent correction while the model building was carried out by Coot. The final refinement R-factors were R_{work} = 0.198, R_{free} = 0.222.

Cysteine Protease Enzyme Assays. Human Cat S enzyme (Cat# 219343), human Cat L enzyme (Cat# 219382), human Cathepsin B enzyme (Cat # 219364), human Cathepsin K enzyme (Cat# 342001) and human Cathepsin V enzyme (Cat# 219467) were acquired from Calbiochem. Mouse Cat S enzyme was cloned in Baculovirus using a mCat S-pAN51(T760)

construct and affinity purified. Assays were performed for Cat S, mCat S, Cat B and Cat L by adding enzyme to a concentration of FRET substrate (Benzyloxycarbonyl-L-Leucyl-L-Arginine 4-Methyl-Coumaryl-7-Amide, Cat # 3210-v; Peptide Institute) equal to the Km in a 50 mM sodium phosphate buffer (pH 6.5) containing 2.5 mM DTT, 2.5 mM EDTA plus 0.01% Triton X-100. The Cat V and Cat K assays were performed as above, but in a 0.1 M sodium acetate (pH 5.5) buffer containing 2.5 mM DTT, 2.5 mM EDTA plus 0.01% Triton X-100. K_m values for the FRET substrate were 25 µM, 25 µM, 20 µM, 37.5 µM, 35 µM, 35 µM and 25 µM for human Cat S, mouse Cat S, human Cat L, human Cat B, human Cat K, human Cat V and human Cat F respectively. For all enzyme assays, the 10-point inhibition curve was plotted and fitted with the four-parameter logistic equation to obtain the IC_{50} values.

***In Vitro* Enzyme Inhibition Data (all values in nM)**

Cpd	hCat S IC_{50}	mCat S IC_{50}	Cat L IC_{50}	Cat K IC_{50}	Cat B IC_{50}	Cat V IC_{50}
6	1170 ± 227 (n=7)	1570 ± 108 (n=3)	>167000 (n=3)	>100000 (n=3)	>100000 (n=3)	>100000 (n=3)
7	1290 ± 453 (n=6)	2780 ± 230 (n=3)	>167000 (n=3)	>100000 (n=4)	>100000 (n=4)	>100000 (n=4)
8	4000 ± 1630 (n=4)	3930 ± 553 (n=3)	>167000 (n=3)	>100000 (n=3)	>100000 (n=3)	>100000 (n=3)
9	12.4 ± 6.48 (n=10)	3.91 ± 0.71 (n=8)	>167000 (n=3)	>100000 (n=5)	52200 ± 20700 (n=4)	15900 ± 2020 (n=3)
5	7.70 ± 5.85 (n=11)	1.67 ± 1.17 (n=9)	>100000 (n=3)	>100000 (n=6)	4390 ± 660 (n=4)	21500 ± 6550 (n=4)

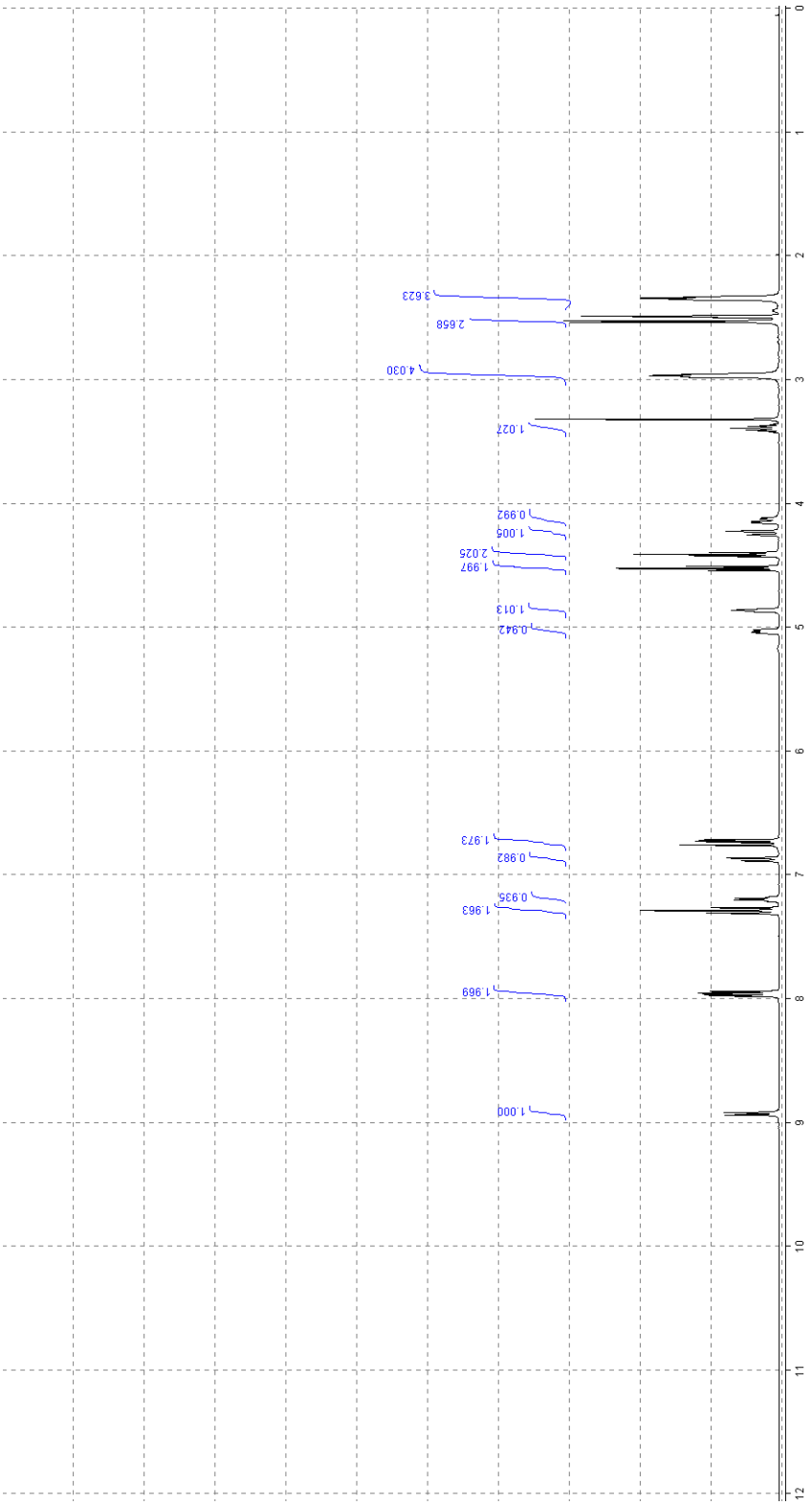
$CaCl_2$-induced AAA animal efficacy model. The abdominal aortic aneurysm (AAA) animal efficacy model using $CaCl_2$ induction to study the effect of MMP-2 and MMP-9 inhibitors on AAA[5] was modified as described below.

Wild-type male 129SvEv mice (10 weeks old) from Taconic (Cambridge City, Indiana) were divided into AAA surgical or sham operated groups (n=10-13 mice/group). Sham operated and AAA untreated groups were administered a vehicle solution of 1% NATROSOL® (hydroxyethylcellulose)/0.25% TWEEN® 80 (polysorbate 80)/0.05% Antifoam-1510® (Dow Corning). The remaining AAA surgical groups were administered 1, 3, 10, or 30 mg/kg of test compound in vehicle solution. All groups were dosed by oral gavage BID for 4 weeks with the first dose given one day prior to surgery (p.m.) and the second dose given the morning of surgery. Animals did not receive a p.m. dose on the day of surgery. The day after surgery, dosing continued BID for 28 days. On the day of surgery, animals received analgesia (BUPRENEX®, 0.1 mg/kg) subcutaneously 10 minutes pre-operatively and 3 hours post-operatively. Mice were anesthetized with isoflurane and a laparotomy was performed for the $CaCl_2$ -stimulated induction of the AAA. The abdominal aorta from the level of the renal arteries to the iliac bifurcation was isolated from the inferior vena cava and surrounding connective tissues. Once isolated, the region of interest (ROI) of the abdominal aorta was wrapped with a sterile cotton gauze soaked in a 0.25 M aqueous $CaCl_2$ solution. In sham control animals, 0.9% saline was substituted for $CaCl_2$. After 7 minutes, the gauze was removed, and a second $CaCl_2$ (or saline if a sham) soaked gauze was reapplied. Following the second 7-minute period, the gauze was removed, the aorta was rinsed with 0.9% saline, and the abdomen was closed. Animals were returned to general housing at the end of their surgical day, remaining individually housed with *ad lib* access to a standard rodent diet (Purina 2014) and water. After 4 weeks of dosing, the aortic lumenal

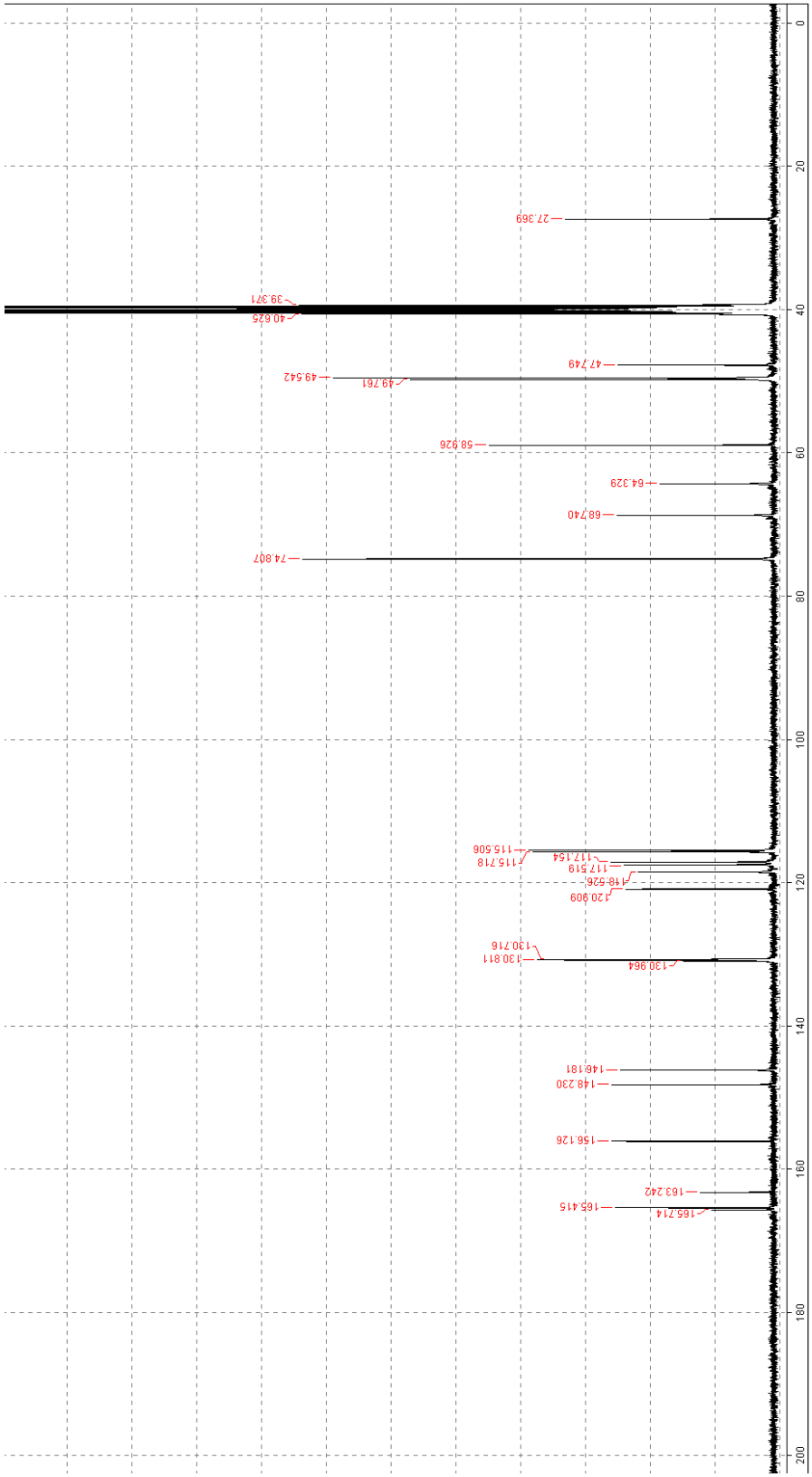
perimeter and the area and diameter of the aortic segment that was wrapped with gauze were determined by ultrasound (Biosound Ultrasound – 7.5 MHz) and statistically analyzed with JMP® 7 software (Cary, North Carolina). Percentage reductions of AAA determined by measurement of the aortic lumenal perimeter (which represents in this instance a more accurate measurement of the abdominal aorta due to the irregular geometry associated with the aortic segment being measured) were represented as means ± standard deviation.

Analytical data for LY3000328 (5).

^{1}H NMR (400 MHz, DMSO-d_6)

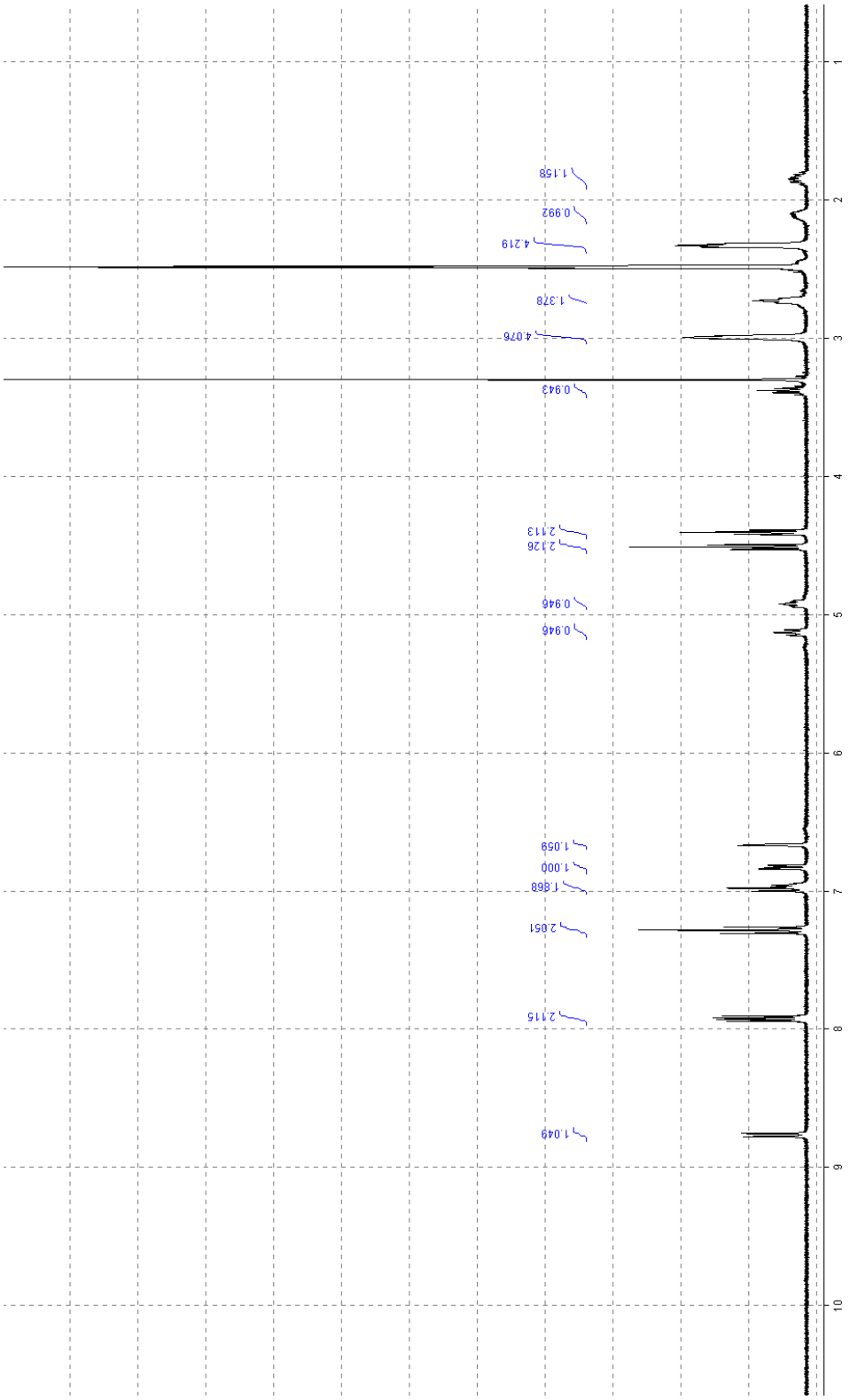


^{13}C NMR (100 MHz, DMSO-d_6)

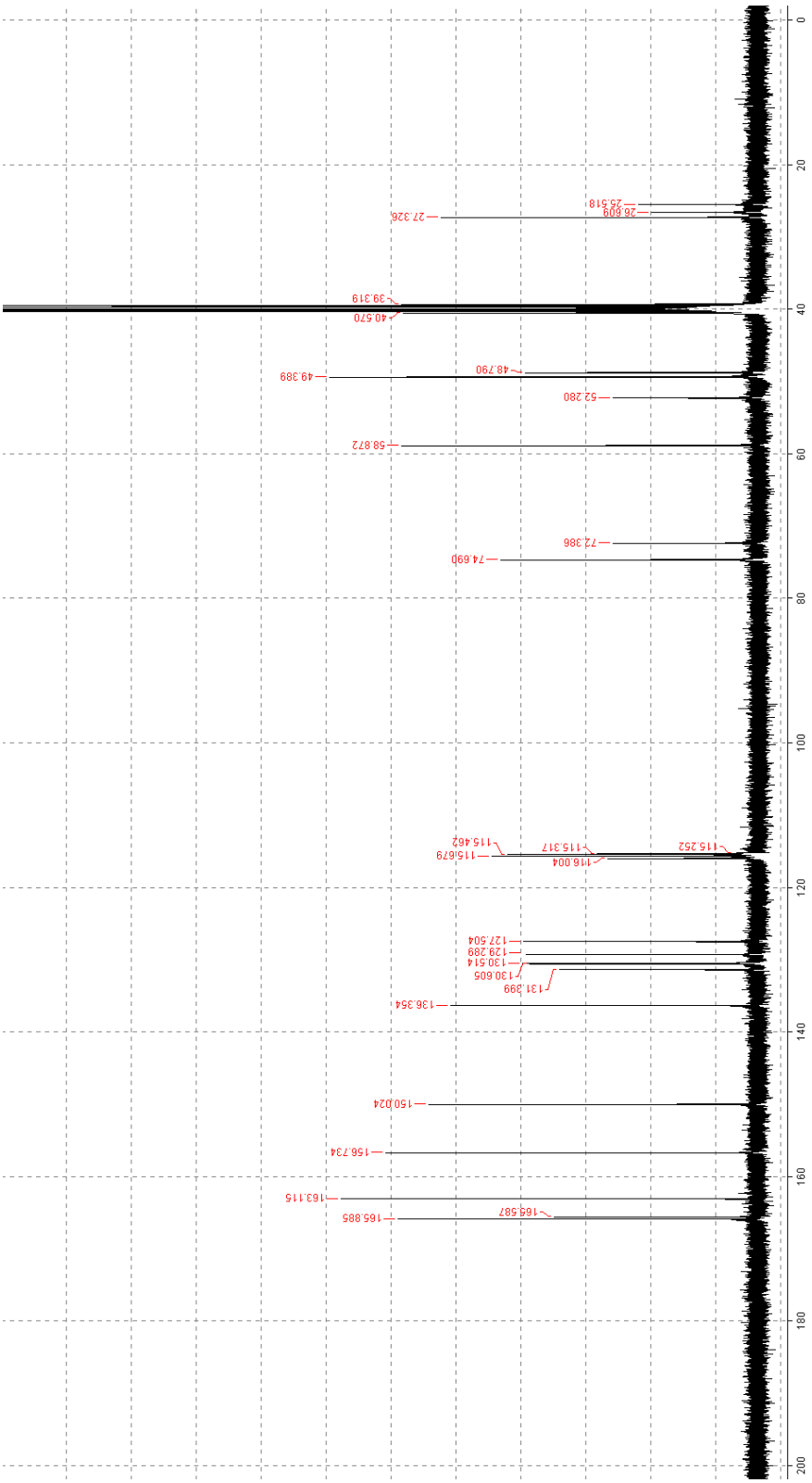


Analytical data for 9.

^{1}H NMR (400 MHz, DMSO-d_6)



^{13}C NMR (100 MHz, DMSO-d_6)



Pharmacokinetic (PK) properties for 5 and 9 in Rat and Dog.

Cpd.	Species	CL (mL/min/kg)	V_d (L/kg)	$T_{1/2,po}$ (hr)	AUC_{po} (ng.hr/ml)	C_{max} (ng/ml)	F (%)
5	Rat[a]	12.7	2	2.9	2400	483	92
	Dog[b]	3.5	0.5	2.1	28000	3271	97
9	Rat[a]	35.7	5	2.6	700	728	78
	Dog[b]	3.0	0.5	2.7	35700	4255	100

[a] Rat (Male Sprague-Dawley, 3 animals per group) 1 mg/kg iv, 1 mg/kg po; [b] Dog (Male Beagle, 3 animals per group) 1 mg/kg iv, 3 mg/kg po. All values were within 25% of the mean value.

***In vitro* ADME Characteristics of 5 and 9.**

Cpd.	[a]CYP3A4	[a]CYP2D6	[a]CYP2C9	[b]Human	[b]Dog	[b]Rat	[b]Mouse	[c]MDCK
5	0.6	0.0	1.7	6.2	6.4	8.5	12.2	4.0
9	0.0	0.0	13.4	17.3	5.5	7.4	10.2	9.3

Each value represents a mean of n=2

[a] % inhibition at 10 µM substrate CYP3A4/midazolam probe, 10 µM substrate CYP2D6/bufuralol probe, or 10 µM substrate CYP2C9/diclofenac probe

[b] % microsomal metabolism after 30 minute incubation at 4 µM

[c] % A to B transport at 2.5 µM substrate; Madin Darby canine kidney cell line expressing Pgp; incubation 1 hour

Abbreviations. AAA, abdominal aortic aneurysm; ANOVA, analysis of variance; ApoE, apolipoprotein E; Asn, asparagine; AUC, area under the curve; BID, twice daily; calcd, calculated; Cat B, human cathepsin B; Cat K, human cathepsin K; Cat L, human cathepsin L; Cat S, human cathepsin S; Cat V, human cathepsin V; CD4, cluster of differentiation 4; CDI, *N*,*N*'-carbonyldiimidazole; cm, centimeter(s); CL, clearance; Cpd., compound; CYP450, cytochrome P450; Cys, cysteine; d, doublet; dd, doublet of doublets; ddd, doublet of doublets of doublets; DMSO, dimethylsulfoxide; DPBS, Dulbecco's phosphate buffered saline; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; ee, enantiomeric excess; ESI, electrospray ionization; F, bioavailability; FRET, fluorescence energy resonance transfer; GI, gastrointestinal; Gly, glycine; h, hour(s); His, histidine; HPLC, high performance liquid chromatography; HRMS, high resonance mass spectrometry; IC_{50}, half-maximal inhibitory concentration; IgG, immunoglobulin G; Inh., inhibition; iv, intravenous; kDa, kilodalton; kg, kilogram(s); L, liter(s); LDL, low-density lipoprotein; m, multiplet; mCat S, mouse cathepsin S; Met. Stab., metabolic stability; mg, milligram(s); MHC, major histocompatibility complex; MHz, megahertz; min, minute(s); mL, milliliter(s); mm, millimiter(s); mM, millimolar; MPa, megapascal(s); MS, mass spectrometry; Ni-NTA, nickel nitrilotriacetic acid; nm, nanometer(s); nM, nanomolar; NMR, nuclear magnetic resonance; NT, not tested; p, pentet; PBS, phosphate-buffered saline; PEG-8000, poly(ethylene glycol), average molecular weight 8000; Phe, phenylalanine; po, *per os* (by mouth); PWBC, peripheral white blood cells; rCat S, rat cathepsin S; RP, reverse phase; s, singlet; SDS, sodium dodecyl sulfate; SEM, standard error of the mean; SMC, smooth muscle cells; t, triplet; *t*-Bu, tertiary butyl; $T_{1/2}$, half-life; TFA, trifluoroacetic acid; THF, tetrahydrofuran; µm, micrometers; µM, micromolar.

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