Design and Synthesis of Piperazine Sulfonamide Cores Leading to Highly Potent HIV-1 Protease Inhibitors

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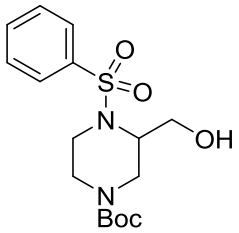
General Methods

Reagents and solvents, including anhydrous THF and dichloromethane, were purchased from Aldrich, Acros or other commercial sources and were used without further purification. Reactions that were moisture sensitive or that required the use of anhydrous solvents were performed under a nitrogen atmosphere. Analytical thin layer chromatography (TLC) was performed on RediSep® pre-coated silica gel plates. Visualization was accomplished with UV light or by staining with basic $KMnO_4$ solution. Compounds were purified by normal phase flash chromatography using an automated purification system (ISCO) using Redisep® disposable flash cartridges with peak detection at 254 nm. Alternatively, compounds were purified by preparative reverse-phase HPLC using a Gilson 215 liquid handler and a Phenomenex Luna C18 column (150 x 20 mm I.D.) with a linear gradient over 15 minutes (95:5 to 0:100 H_2O containing 0.1% trifluoroacetic acid:acetonitrile). The reported yields are for isolated compounds of ≥95% purity, which was determined by HPLC and ^1H NMR. HPLC was carried out with a Waters 2690 Separations Module equipped with a YMC Pro 50 × 3 mm i.d. C18 column interfaced with a Waters Micromass ZMD spectrometer using a gradient of 0.05% TFA in H_2O/CH_3CN with UV detection at 215 and 254 nm. NMR spectra were recorded at 600 MHz for ^1H and 125 MHz for ^{13}C on a Varian spectrometer in the stated solvent. The chemical shifts are given in ppm, referenced to the deuterated solvent signal or tetramethylsilane.

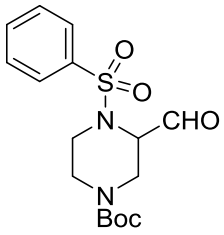
Synthetic Procedures and Compound Characterization

Synthesis of 8



***tert*-Butyl 3-(hydroxymethyl)-4-(phenylsulfonyl)piperazine-1-carboxylate (2).**

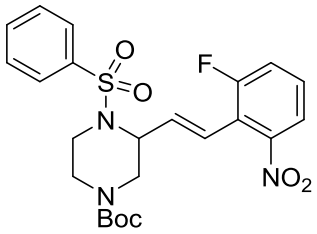
Benzenesulfonyl chloride (1.18 ml, 9.25 mmol) was slowly added to a solution of *tert*-butyl 3-(hydroxymethyl)piperazine-1-carboxylate (2.00 g, 9.25 mmol) and Hunig's base (1.62 ml, 9.25 mmol) in CH_2Cl_2 (46.2 ml) at -78 °C. The reaction was stirred at -78 °C for 1 hour, then warmed directly to RT. The reaction was quenched with aqueous sodium hydrogen carbonate (saturated) and the mixture was extracted with CH_2Cl_2 (x 3). The combined organic fractions were dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. Purification on silica gel, eluting with a gradient of 0-100% EtOAc/hexanes over 15 column volumes, afforded the title compound (2.33 g, 71 % yield) as a white solid. ^{1}H NMR (600 MHz, chloroform-d) δ 7.84 (d, J = 7.6 Hz, 2H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.52 (t, *J* = 7.7 Hz, 2H), 4.20 – 2.60 (br m, 10H), 1.42 (s, 9H); MS (ESI): calcd for $C_{16}H_{24}N_2O_5S$ (M–C_4H_7) 301.1, found 301.1.



***tert*-Butyl 3-formyl-4-(phenylsulfonyl)piperazine-1-carboxylate (3).**

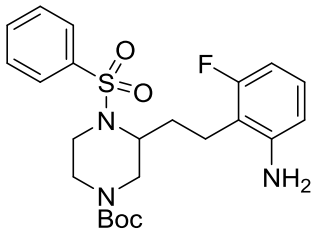
Dess-Martin periodinane (2.95 g, 6.94 mmol) was added portion wise to a solution of **2** (2.25 g, 6.31 mmol) in CH_2Cl_2 (30 ml) at 0 °C and the reaction stirred at this temperature for 1 hour. The reaction was quenched with aqueous sodium hydrogen carbonate (saturated) and the mixture was extracted with dichloromethane (x 3). The combined organic fractions were dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. Purification on silica gel, eluting with a gradient of 0-100% EtOAc/hexanes over 15 column volumes, afforded the

title compound (1.96 g, 5.53 mmol, 88 % yield) as a viscous gum. ^{1}H NMR (600 MHz, chloroform-d) δ 9.48 (s, 1H), 7.79 (d, J = 7.6 Hz, 2H), 7.61 (t, J = 7.4 Hz, 1H), 7.53 (t, J = 7.7 Hz, 2H), 4.41 (s, 2H), 3.86 (br s, 1H), 3.59 (d, J = 12.0 Hz, 1H), 3.27 – 3.19 (m, 1H), 3.12 (dd, J = 4.5, 13.7 Hz, 1H), 2.95 (br s, 1H), 1.39 (s, 9H); not stable to LCMS analysis.



tert-Butyl (*E*)-3-(2-fluoro-6-nitrostyryl)-4-(phenylsulfonyl)piperazine-1-carboxylate (4).

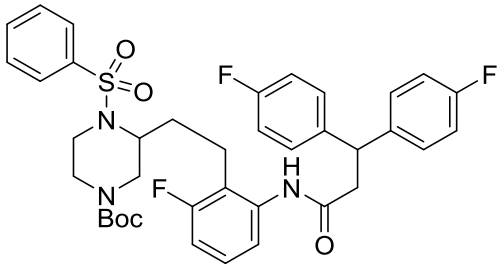
Bromo(2-fluoro-6-nitrobenzyl)triphenylphosphorane (3.50 g, 7.05 mmol), 18-crown-6 (0.169 g, 0.641 mmol), and K_2CO_3 (1.77 g, 12.8 mmol) were stirred at RT in DME (6 ml) for 10 min. A solution of **3** (1.89 g, 5.33 mmol) in DME (6 ml) was added and the reaction stirred for 48h at rt. The reaction was quenched with water and the mixture was extracted with ethyl acetate (x 3). The combined organic fractions were dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. Purification on silica gel, eluting with a gradient of 0-100% EtOAc/hexanes over 15 column volumes, afforded the title compound (650 mg, 1.32 mmol, 21 % yield) as a yellow solid. ^{1}H NMR (600 MHz, chloroform-d) δ 7.81 (d, J = 7.5 Hz, 2H), 7.67 (d, J = 8.1 Hz, 1H), 7.52 (t, J = 7.4 Hz, 1H), 7.46 (t, J = 7.5 Hz, 2H), 7.34 (q, J = 8.0 Hz, 1H), 7.29 (t, J = 9.0 Hz, 1H), 6.51 (d, J = 15.3 Hz, 1H), 6.10 (d, J = 14.2 Hz, 1H), 4.58 (s, 1H), 4.19 – 3.85 (m, 2H), 3.62 (s, 1H), 3.25 (s, 2H), 2.97 (s, 1H), 1.38 (s, 9H). MS (ESI): calcd for $C_{23}H_{26}FN_3O_6S$ (M-CH_2) 477.1, found 477.3.



tert-Butyl 3-(2-amino-6-fluorophenethyl)-4-(phenylsulfonyl)piperazine-1-carboxylate (5).

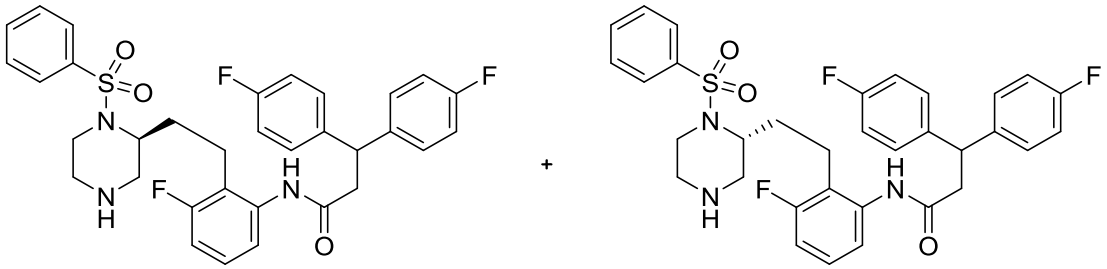
Pearlman's catalyst (416 mg, 0.592 mmol) was added to a nitrogen degassed solution of **4** (582 mg, 1.184 mmol) in trifluoroethanol (6 ml) and the reaction evacuated and backfilled with hydrogen, then stirred under a balloon of hydrogen overnight. The reaction was purged with nitrogen then filtered through a pad of celite and concentrated *in vacuo*. Purification on silica gel, eluting with a gradient of 0-100% EtOAc/hexanes over 15 column

volumes, afforded the title compound (377 mg, 0.813 mmol, 69 % yield) as a white solid. ^{1}H NMR (600 MHz, chloroform-d) δ 7.79 (d, J = 7.4 Hz, 2H), 7.54 (d, J = 7.1 Hz, 1H), 7.49 (t, J = 7.5 Hz, 2H), 6.90 (s, 1H), 6.40 (d, J = 43.2 Hz, 2H), 4.35 – 3.79 (m, 4H), 3.69 (d, J = 9.9 Hz, 1H), 3.00 – 2.81 (m, 2H), 2.71 – 2.32 (m, 2H), 1.75 – 1.50 (m, 2H), 1.42 (s, 9H), (exchangeable NH_2 protons not observed). MS (ESI): calcd for $C_{23}H_{30}FN_3O_4S$ (M+H) 464.2, found 464.3.



tert-Butyl 3-(2-(3,3-bis(4-fluorophenyl)propanamido)-6-fluorophenethyl)-4-(phenylsulfonyl)piperazine-1-carboxylate (6).

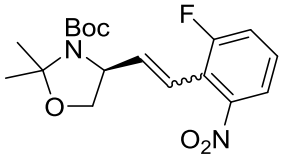
T3P (515 µl, 0.863 mmol) (50% in EtOAc) was added to **5** (200 mg, 0.431 mmol) and 3,3-bis(4-fluorophenyl)propanoic acid (113 mg, 0.431 mmol) in ethyl acetate (863 µl). Once the reactants were dissolved, the reaction was cooled to 0 °C and Hunig's base (151 µl, 0.863 mmol) was added. The reaction was warmed to RT and stirred for 1 hour. The reaction was quenched with water and the mixture was extracted with ethyl acetate (x 3). The combined organic fractions were dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. Purification on silica gel, eluting with a gradient of 0-100% EtOAc/hexanes over 15 column volumes, afforded the title compound (253 mg, 0.357 mmol, 83 % yield) as a white solid. ^{1}H NMR (600 MHz, chloroform-d) δ 8.41 (s, 1H), 7.81 (d, J = 7.6 Hz, 2H), 7.58 (d, J = 6.9 Hz, 1H), 7.53 (t, J = 7.6 Hz, 2H), 7.25 (s, 4H), 7.08 (d, J = 7.0 Hz, 1H), 6.98 – 6.90 (m, 5H), 6.77 (d, J = 8.1 Hz, 1H), 4.69 – 4.64 (m, 1H), 4.21 – 4.11 (m, 1H), 3.97 (d, J = 12.7 Hz, 2H), 3.68 (d, J = 11.5 Hz, 1H), 3.45 – 3.33 (m, 1H), 3.11 – 3.05 (m, 1H), 3.04 – 2.87 (m, 2H), 2.87 – 2.74 (m, 1H), 2.47 (m, 2H), 2.04 (m, 2H), 1.40 (s, 9H). LCMS: 91% pure; MS (ESI): calcd for $C_{38}H_{40}F_3N_3O_5S$ (M+H) 708.3, found 708.5.



(*S*)-*N*-(3-fluoro-2-(2-(1-(phenylsulfonyl)piperazin-2-yl)ethyl)phenyl)-3,3-bis(4-fluorophenyl)propanamide (7) and (*R*)-*N*-(3-fluoro-2-(2-(1-(phenylsulfonyl)piperazin-2-yl)ethyl)phenyl)-3,3-bis(4-fluorophenyl)propanamide (8).

TFA (288 μl) was added to a solution of **6** (204 mg, 0.288 mmol) in CH_2Cl_2 (1.2 ml) at 0 °C. The reaction was warmed to RT and stirred for 4 hours. The reaction was diluted with CH_2Cl_2 and washed with sodium hydrogen carbonate (saturated), dried over $MgSO_4$ and concentrated *in vacuo*. Purification on silica gel, eluting with a gradient of 0-100% hexanes to 20% EtOH/EtOAc over 15 column volumes, afforded the title compounds as a racemic mixture. ^{1}H NMR (600 MHz, chloroform-d) δ 7.90 (s, 1H), 7.82 (d, J = 7.5 Hz, 2H), 7.61 (t, J = 7.4 Hz, 1H), 7.53 (t, J = 7.7 Hz, 2H), 7.34 (d, J = 7.8 Hz, 1H), 7.28 (d, J = 8.2 Hz, 3H), 7.13 (q, J = 8.1 Hz, 1H), 6.96 (q, J = 8.5 Hz, 4H), 6.83 (t, J = 8.9 Hz, 1H), 4.66 (t, J = 7.9 Hz, 1H), 3.63 (s, 1H), 3.48 (d, J = 13.6 Hz, 1H), 3.26 (t, J = 10.9 Hz, 1H), 3.15 (d, J = 7.7 Hz, 2H), 2.75 (d, J = 12.3 Hz, 1H), 2.69 (d, J = 12.8 Hz, 1H), 2.59 (dd, J = 3.7, 12.5 Hz, 1H), 2.56 – 2.37 (m, 3H), 2.09 – 2.02 (m, 1H), 1.97 – 1.84 (m, 1H), 1.33 – 1.23 (m, 1H), 0.90 – 0.84 (m, 1H). MS (ESI): calcd for $C_{33}H_{32}F_3N_3O_3S$ (M+H) 608.2, found 608.4. The enantiomers were separated under SFC conditions on an AD-H column (3 x 25 cm), eluting with 25 % MeOH (0.1% Et_2NH)/CO_2, 80 ml/min (100 bar) to afford (*R*)-*N*-(3-fluoro-2-(2-(1-(phenylsulfonyl)piperazin-2-yl)ethyl)phenyl)-3,3-bis(4-fluorophenyl)propanamide (79 mg, 45 % yield) (optical rotation: $[\alpha]_D^{25}$ + 40.7 (c 0.94, MeOH)) as a white solid and (*S*)-*N*-(3-fluoro-2-(2-(1-(phenylsulfonyl)piperazin-2-yl)ethyl)phenyl)-3,3-bis(4-fluorophenyl)propanamide (79 mg, 0.130 mmol, 45.1 % yield), optical rotation: $[\alpha]_D^{25}$ – 41.1 (c 0.77, MeOH), as a white solid.

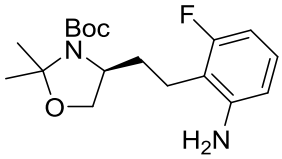
Synthesis of 18-21



***tert*-Butyl (*S*,*E*/*Z*)-4-(2-fluoro-6-nitrostyryl)-2,2-dimethyloxazolidine-3-carboxylate (11).**

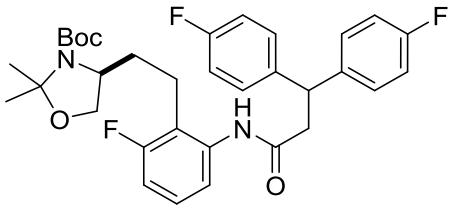
(2-Nitrobenzyl)triphenylphosphonium bromide (12.5 g, 25.3 mmol), potassium carbonate (3.46 g, 25.0 mmol), and 18-crown-6 (1.79 g, 6.76 mmol) were stirred in DME (45 ml) at RT for 10 min. A solution of (*R*)-*tert*-butyl 4-formyl-2,2-dimethyloxazolidine-3-carboxylate (5.17 g, 22.6 mmol) in DME (45 ml) was added and the reaction stirred at RT for 48h. The reaction was quenched with water and the mixture was extracted with ethyl acetate (x

3). The combined organic fractions were dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. Purification on silica gel eluting with a gradient of 0-50% EtOAc/hexanes over 15 column volumes, afforded the title compound (4.08 g, 11.1 mmol, 49 % yield) as a pale yellow oil. ^{1}H NMR was complex due to E/Z isomers; MS (ESI): calcd for $C_{18}H_{23}FN_2O_5$ (M-CH_2) 352.1, found 352.2.



***tert*-Butyl (*S*)-4-(2-amino-6-fluorophenethyl)-2,2-dimethyloxazolidine-3-carboxylate (12).**

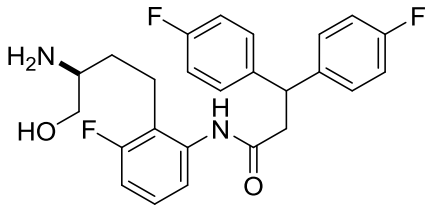
11 (3.92 g, 10.70 mmol) and Pearlman's catalyst (0.376 g, 0.535 mmol) in ethyl acetate (80 ml)/MeOH (40 ml) were shaken on a Parr at 50 psi hydrogen overnight. The reaction was purged with nitrogen then filtered through a pad of celite and concentrated *in vacuo*. Purification on silica gel eluting with a gradient of 0-100% EtOAc/hexanes over 15 column volumes, afforded the title compound (2.52 g, 7.45 mmol, 70 % yield) as a white solid. ^{1}H NMR (600 MHz, chloroform-d) δ 6.93 (d, J = 5.6 Hz, 1H), 6.43 (s, 2H), 4.06 – 3.55 (m, 5H), 2.54 (s, 2H), 1.95 – 1.75 (m, 2H), 1.65 – 1.30, (m, 15H). MS (ESI): calcd for $C_{18}H_{27}FN_2O_3$ (M+H) 339.2, found 339.3. The compound had an optical purity of >95% ee by HPLC (AD-column 20% IPA/hexanes), desired enantiomer rt ~5.55 min, undesired enantiomer rt ~4.55 min.



***tert*-Butyl (*S*)-4-(2-(3,3-bis(4-fluorophenyl)propanamido)-6-fluorophenethyl)-2,2-dimethyloxazolidine-3-carboxylate (13).**

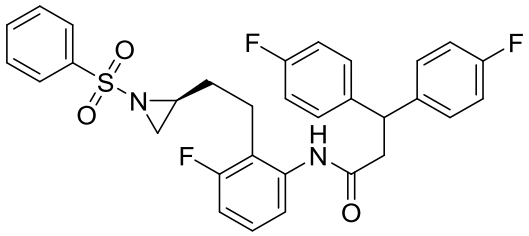
T3P (6.83 ml, 11.5 mmol) was added to **12** and 3,3-bis(4-fluorophenyl)propanoic acid (1.50 g, 5.73 mmol) in ethyl acetate (12 ml). The mixture was stirred at RT until a homogeneous solution was obtained. The solution was cooled to 0 °C and Hunig's base (2.00 ml, 11.5 mmol) was added, the reaction was warmed to RT and stirred for 1 hour. The reaction was quenched with water and the mixture was extracted with ethyl acetate (x 3). The

combined organic fractions were dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. Purification on silica gel eluting with a gradient of 0-100% EtOAc/hexanes afforded the title compound (3.12 g, 93 % yield) as a white solid. ^{1}H NMR (600 MHz, chloroform-d) δ 8.40 (s, 1H), 7.49 (d, J = 8.1 Hz, 1H), 7.30 – 6.73 (m, 10H), 4.70 (t, J = 7.5 Hz, 1H), 3.94 (d, J = 6.3 Hz, 1H), 3.72 (d, J = 8.1 Hz, 2H), 3.38 – 3.09 (m, 2H), 2.65 – 2.35 (m, 2H), 1.90 – 1.75 (m, 2H), 1.57 - 1.45 (m, 15H). MS (ESI): calcd for $C_{33}H_{37}F_3N_2O_4$ (M+H) 583.3, found 583.5; optical rotation: $[\alpha]_D^{25}$ + 25.8 (c 1.94, MeOH).



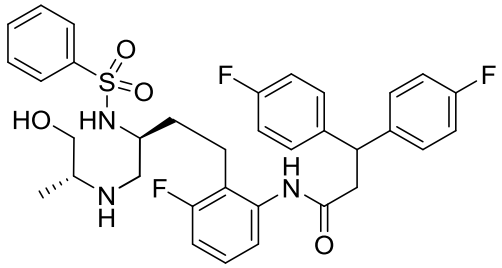
(*S*)-*N*-(2-(3-amino-4-hydroxybutyl)-3-fluorophenyl)-3,3-bis(4-fluorophenyl)propanamide (14).

To a solution of **13** (3.03 g, 5.20 mmol) in CH_2Cl_2 (15 ml) was added water (3.75 ml, 208 mmol) followed by TFA (16.0 ml, 208 mmol) and the reaction was stirred overnight at RT. A 20 g SCX cartridge was conditioned with 2 CV of MeOH. The crude reaction was diluted with MeOH and loaded on the column. The column was flushed with 2 CV of MeOH, then the desired compound eluted with 2 CV of 2M NH3 in MeOH. The solvent was removed *in vacuo* to afford the title compound (2.00 g, 87 % yield) as a white solid. ^{1}H NMR (600 MHz, chloroform-d) δ 10.89 (s, 1H), 7.57 (d, J = 8.2 Hz, 1H), 7.19 (dd, J = 5.6, 8.2 Hz, 4H), 7.12 (q, J = 8.1 Hz, 1H), 6.97 (t, J = 8.6 Hz, 4H), 6.76 (t, J = 8.9 Hz, 1H), 4.68 (t, J = 7.8 Hz, 1H), 3.54 (dd, J = 3.5, 10.6 Hz, 1H), 3.21 (dd, J = 7.9, 10.6 Hz, 1H), 3.00 (d, J = 7.8 Hz, 2H), 2.75 (d, J = 14.5 Hz, 1H), 2.36 – 2.29 (m, 2H), 1.76 – 1.68 (m, 1H), 1.43 (td, J = 4.3, 11.7 Hz, 1H), (exchangeable OH and NH_2 protons not observed). MS (ESI): calcd for $C_{25}H_{25}F_3N_2O_2$ (M+H) 443.2, found 443.3; optical rotation: $[\alpha]_D^{25}$ + 19.6 (c 1.25, MeOH).



(*S*)-*N*-(3-fluoro-2-(2-(1-(phenylsulfonyl)aziridin-2-yl)ethyl)phenyl)-3,3-bis(4-fluorophenyl)propanamide (15).

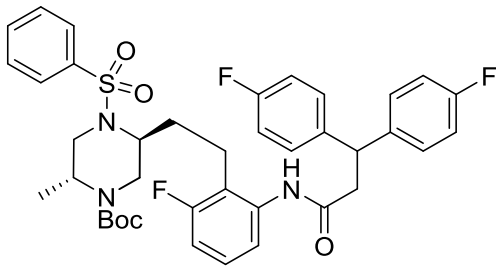
Benzenesulfonylchloride (0.312 ml, 2.420 mmol) was added to a solution of **14** (1.02 g, 2.305 mmol) and TEA (0.643 ml, 4.61 mmol) in DMF (12 ml) at 0 °C. The reaction was stirred at 0 °C for 20 min. The reaction was quenched with aqueous sodium hydrogen carbonate (saturated) and the mixture was extracted with ethyl acetate (x 3). The combined organic fractions were dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. Purification on silica gel, eluting with a gradient of 0-100% EtOAc/hexanes over 15 column volumes, afforded (*S*)-*N*-(3-fluoro-2-(4-hydroxy-3-(phenylsulfonamido)butyl)phenyl)-3,3-bis(4-fluorophenyl)propanamide (1.23 g) as a white solid. MS (ESI): calcd for $C_{31}H_{29}F_3N_2O_4S$ (M+H) 583.2, found 583.4; optical rotation: $[\alpha]_D^{25}$ + 28.3 (c 1.49, MeOH). This material was used in the next reaction as follows. Tri-*n*-butylphosphine (1.42 ml, 5.97 mmol) was added to a solution of (*S*)-*N*-(3-fluoro-2-(4-hydroxy-3-(phenylsulfonamido)butyl)phenyl)-3,3-bis(4-fluorophenyl)propanamide (1.16 g, 1.99 mmol) and diazene-1,2-diylbis(morpholinomethanone) (1.53 g, 5.97 mmol) in THF (20 ml) at rt. The reaction was stirred for 2 hours. The reaction was quenched with water and the mixture was extracted with ethyl acetate (x 3). The combined organic fractions were dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. Purification on silica gel, eluting with a gradient of 0-100% EtOAc/hexanes over 15 column volumes, afforded the title compound (1.02 g, 83 %, 2 steps) as a white solid. ^{1}H NMR (600 MHz, chloroform-d) δ 7.98 (s, 1H), 7.87 (d, J = 7.4 Hz, 2H), 7.67 (t, J = 7.6 Hz, 1H), 7.54 (t, J = 7.9 Hz, 2H), 7.30 – 7.24 (m, 4H), 7.19 (d, J = 7.9 Hz, 1H), 7.17 – 7.11 (m, 1H), 6.97 (q, J = 8.5 Hz, 4H), 6.86 (t, J = 8.9 Hz, 1H), 4.67 (t, J = 7.9 Hz, 1H), 3.16 (d, J = 8.0 Hz, 2H), 2.64 (d, J = 4.3 Hz, 1H), 2.48 (dtt, J = 7.6, 14.6, 20.4 Hz, 3H), 2.25 – 2.20 (m, 1H), 2.04 (d, J = 3.4 Hz, 1H), 1.31 (tt, J = 5.0, 10.1 Hz, 1H). MS (ESI): calcd for $C_{31}H_{27}F_3N_2O_3S$ (M+H) 565.2, found 565.3; optical rotation: $[\alpha]_D^{25}$ + 26.2 (c 2.05, MeOH).



_N_-(3-Fluoro-2-((*S*)-4-(((*R*)-1-hydroxypropan-2-yl)amino)-3-(phenylsulfonamido)butyl)phenyl)-3,3-bis(4-fluorophenyl)propanamide (16).

A solution of **15** (150 mg, 0.266 mmol) and D-alaninol (206 µl, 2.66 mmol) in 1,2-DCE (1.3 ml) was heated at 40 °C for 1 hour. The reaction was loaded directly on silica gel, eluting with a gradient of 0-100% Hexanes to 20%

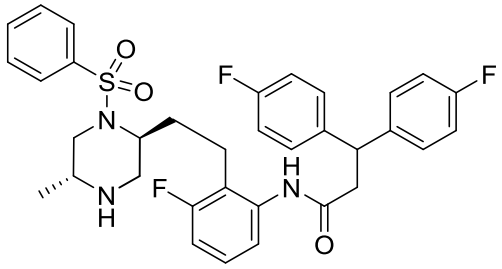
EtOH/EtOAc over 15 column volumes, to afford the title compound (158 mg, 0.247 mmol, 93 % yield) as a white solid. ^{1}H NMR (600 MHz, chloroform-d) δ 8.11 (s, 1H), 7.86 (d, *J* = 7.6 Hz, 2H), 7.63 (t, *J* = 7.5 Hz, 1H), 7.55 (t, *J* = 7.7 Hz, 2H), 7.48 (d, *J* = 8.0 Hz, 1H), 7.33 (ddd, *J* = 5.4, 8.2, 17.8 Hz, 4H), 7.13 (q, *J* = 8.1 Hz, 1H), 7.00 – 6.93 (m, 4H), 6.79 (t, *J* = 8.8 Hz, 1H), 4.67 (t, *J* = 7.9 Hz, 1H), 3.53 (dd, *J* = 3.9, 10.8 Hz, 1H), 3.29 (dd, *J* = 7.1, 15.9 Hz, 2H), 3.19 (dd, *J* = 6.9, 14.1 Hz, 1H), 2.88 (dd, *J* = 4.1, 8.8 Hz, 1H), 2.63 (dd, *J* = 3.9, 12.5 Hz, 1H), 2.58 – 2.52 (m, 1H), 2.52 – 2.44 (m, 3H), 1.96 – 1.88 (m, 1H), 1.60 – 1.54 (m, 2H), 0.86 (d, *J* = 6.6 Hz, 3H), (exchangeable NH and OH protons not observed). MS (ESI): calcd for $C_{34}H_{36}F_3N_3O_4S$ (M+H) 640.3, found 640.4; optical rotation: $[\alpha]_D^{25}$ + 13.2 (c 1.04, MeOH).



***tert*-Butyl (2*R*,5*S*)-5-(2-(3,3-bis(4-fluorophenyl)propanamido)-6-fluorophenethyl)-2-methyl-4-(phenylsulfonyl)piperazine-1-carboxylate (17).**

Boc-anhydride (86.0 µl, 0.370 mmol) was added to a solution of **16** (158 mg, 0.247 mmol) and TEA (68.8 µl, 0.494 mmol) in CH_2Cl_2 (1.2 ml) at RT and the reaction stirred overnight at RT. The solvent was removed *in vacuo* and the residue purified on silica gel, eluting with a gradient of 0-100% EtOAc/hexanes over 15 column volumes, to afforded *tert*-butyl ((*S*)-4-(2-(3,3-bis(4-fluorophenyl)propanamido)-6-fluorophenyl)-2-(phenylsulfonamido)butyl)((*R*)-1-hydroxypropan-2-yl)carbamate (153 mg), as a white solid. MS (ESI): calcd for $C_{39}H_{44}F_3N_3O_6S$ (M+H) 740.3, found 740.5; optical rotation: $[\alpha]_D^{25}$ + 23.7 (c 1.20, MeOH). This material was used in the next reaction as follows. Tri-*n*-butylphosphine (160 µl, 0.641 mmol) was added to a solution of *tert*-butyl ((*S*)-4-(2-(3,3-bis(4-fluorophenyl)propanamido)-6-fluorophenyl)-2-(phenylsulfonamido)butyl)((*R*)-1-hydroxypropan-2-yl)carbamate (158 mg, 0.214 mmol) and diazene-1,2-diylbis(morpholinomethanone) (164 mg, 0.641 mmol) in THF (2 ml) at RT, and the reaction stirred for 2 hours. The reaction was quenched with water and the mixture was extracted with ethyl acetate (x 3). The combined organic fractions were dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. Purification on silica gel, eluting with a gradient of 0-100% EtOAc/hexanes over 15 column volumes, afforded The title compound (119 mg, 0.165 mmol, 56 % yield, 2 steps) as

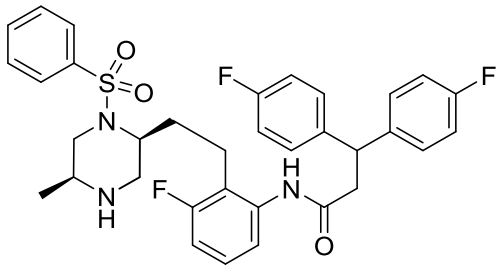
a white solid. ^{1}H NMR (600 MHz, chloroform-d) δ 8.34 (s, 1H), 7.79 (d, J = 7.9 Hz, 2H), 7.58 (t, J = 7.5 Hz, 1H), 7.52 (t, J = 7.7 Hz, 2H), 7.23 (d, J = 7.6 Hz, 4H), 7.08 (s, 1H), 6.98 – 6.90 (m, 5H), 6.75 (s, 1H), 4.69 – 4.63 (m, 1H), 4.29 (br s, 1H), 4.05 (d, J = 14.5 Hz, 1H), 3.96 – 3.86 (m, 1H), 3.46 – 3.36 (m, 2H), 3.13 – 3.03(m, 1H), 3.13 – 3.03 (m, 1H), 2.96 – 2.86 (m, 1H), 2.48 - 2.38 (m, 1H), 2.01 – 1.91 (m, 1H), 1.41 (m, 11H), 1.31 (m, 3H). MS (ESI): calcd for $C_{39}H_{42}F_3N_3O_5S$ (M+H) 722.3, found 722.5; optical rotation: $[\alpha]_D^{25}$ + 8.6 (c 1.42, MeOH).



***N*-(3-Fluoro-2-(2-((2*S*,5*R*)-5-methyl-1-(phenylsulfonyl)piperazin-2-yl)ethyl)phenyl)-3,3-bis(4-fluorophenyl)propanamide (18).**

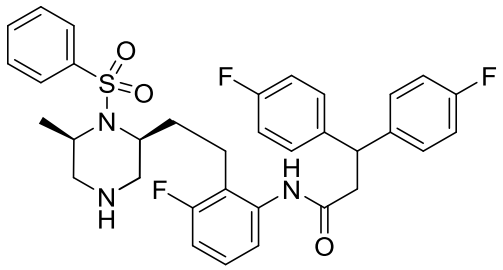
TFA (165 µl) was added to a solution of **17** (119 mg, 0.165 mmol) in CH_2Cl_2 (659 µl) at 0 °C. The reaction was warmed to RT and stirred for 4 hours. The reaction was diluted with CH_2Cl_2 and washed with sodium hydrogen carbonate (saturated), dried over $MgSO_4$ and concentrated *in vacuo*. Purification on silica gel, eluting with a gradient of 0-100% Hexanes to 20% EtOH/EtOAc over 15 column volumes, afforded the title compound (93 mg, 0.150 mmol, 91 % yield) as a white solid. ^{1}H NMR (600 MHz, chloroform-d) δ 8.43 (s, 1H), 7.75 (d, J = 7.5 Hz, 2H), 7.65 (t, J = 7.6 Hz, 2H), 7.57 (t, J = 7.7 Hz, 2H), 7.34 – 7.27 (m, 2H), 7.25 (d, J = 10.7 Hz, 2H), 7.13 (q, J = 8.1 Hz, 1H), 6.93 (dt, J = 8.7, 26.7 Hz, 4H), 6.77 (t, J = 9.0 Hz, 1H), 4.64 (t, J = 7.8 Hz, 1H), 3.78 (d, J = 9.5 Hz, 1H), 3.17 (dd, J = 9.3, 13.8 Hz, 1H), 3.10 (dd, J = 6.8, 13.8 Hz, 1H), 3.07 – 3.00 (m, 2H), 3.00 – 2.94 (m, 1H), 2.78 (br s, 1H), 2.70 – 2.60 (m, 1H), 2.47 (t, J = 12.6 Hz, 1H), 2.33 (t, J = 13.8 Hz, 1H), 2.21 (t, J = 10.0 Hz, 1H), 1.78 - 1.68(m, 1H), 1.06 (d, J = 6.3 Hz, 3H), 0.99 – 0.81 (m, 1H). LCMS: 96% pure; MS (ESI): calcd for $C_{34}H_{34}F_3N_3O_3S$ (M+H) 622.2, found 622.4; optical rotation: $[\alpha]_D^{25}$ + 42.8 (c 0.53, MeOH).

Compounds **19**, **20**, and **21** were prepared in a similar fashion to **18**, starting from aziridine **15** and the appropriate chiral amine.



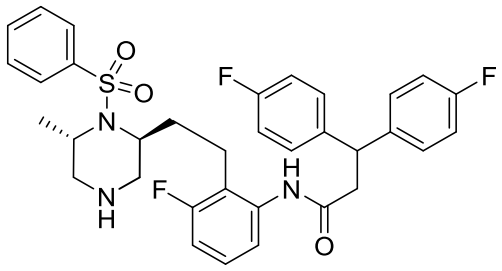
***N*-(3-Fluoro-2-(2-((2*S*,5*S*)-5-methyl-1-(phenylsulfonyl)piperazin-2-yl)ethyl)phenyl)-3,3-bis(4-fluorophenyl)propanamide (19).**

^{1}H NMR (600 MHz, chloroform-d) δ 7.87 (s, 1H), 7.81 (d, *J* = 7.4 Hz, 2H), 7.59 (t, *J* = 7.3 Hz, 1H), 7.52 (t, *J* = 7.7 Hz, 2H), 7.31 (d, *J* = 8.0 Hz, 1H), 7.30 – 7.25 (m, 4H), 7.13 (q, *J* = 8.1 Hz, 1H), 6.96 (q, *J* = 8.5 Hz, 4H), 6.83 (t, *J* = 9.0 Hz, 1H), 4.66 (t, *J* = 7.9 Hz, 1H), 3.68 (br s, 1H), 3.56 (dd, *J* = 2.8, 13.7 Hz, 1H), 3.15 (d, *J* = 7.8 Hz, 2H), 2.78 – 2.71 (m, 1H), 2.67 (d, *J* = 12.6 Hz, 1H), 2.60 (dd, *J* = 3.7, 12.5 Hz, 1H), 2.55 – 2.35 (m, 3H), 2.02 (dd, *J* = 6.3, 14.3 Hz, 1H), 1.85 (dd, *J* = 5.9, 13.9 Hz, 1H), 0.94 (d, *J* = 6.3 Hz, 3H), 0.98 – 0.80 (m, 1H). LCMS: 94% pure; MS (ESI): calcd for $C_{34}H_{34}F_3N_3O_3S$ (M+H) 622.2, found 622.5; optical rotation: $[\alpha]_D^{25}$ + 38.0 (c 0.98, MeOH).



***N*-(3-fluoro-2-(2-((2*S*,6*R*)-6-methyl-1-(phenylsulfonyl)piperazin-2-yl)ethyl)phenyl)-3,3-bis(4-fluorophenyl)propanamide (20).**

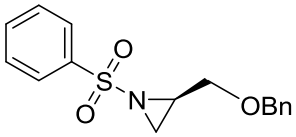
^{1}H NMR (600 MHz, chloroform-d) δ 7.79 – 7.74 (m, 2H), 7.65 (s, 1H), 7.59 (t, *J* = 7.4 Hz, 1H), 7.50 (t, *J* = 7.8 Hz, 2H), 7.40 (d, *J* = 8.0 Hz, 1H), 7.31 – 7.22 (m, 4H), 7.13 (q, *J* = 8.1 Hz, 1H), 7.00 – 6.90 (m, 4H), 6.82 (t, *J* = 8.8 Hz, 1H), 4.67 (t, *J* = 7.9 Hz, 1H), 3.95 – 3.89 (m, 1H), 3.55 – 3.50 (m, 1H), 3.19 (qd, *J* = 8.0, 14.3 Hz, 2H), 2.79 (d, *J* = 12.2 Hz, 1H), 2.69 (d, *J* = 12.1 Hz, 1H), 2.66 – 2.58 (m, 1H), 2.52 (ddd, *J* = 4.3, 12.5, 17.5 Hz, 2H), 2.40 – 2.32 (m, 1H), 2.12 – 2.05 (m, 1H), 1.96 – 1.86 (m, 1H), 1.42 (d, *J* = 6.9 Hz, 3H), 0.91 – 0.81 (m, 1H). LCMS: 100% pure; MS (ESI): calcd for $C_{34}H_{34}F_3N_3O_3S$ (M+H) 622.2, found 622.; optical rotation: $[\alpha]_D^{25}$ + 48.6 (c 0.48, MeOH).



***N*-(3-Fluoro-2-(2-((2*S*,6*S*)-6-methyl-1-(phenylsulfonyl)piperazin-2-yl)ethyl)phenyl)-3,3-bis(4-fluorophenyl)propanamide (21).**

^{1}H NMR (600 MHz, chloroform-d) δ 7.98 (s, 1H), 7.85 (d, *J* = 7.6 Hz, 2H), 7.60 (t, *J* = 7.3 Hz, 1H), 7.54 (q, *J* = 7.6, 8.2 Hz, 3H), 7.21 (q, *J* = 7.5 Hz, 4H), 7.12 (q, *J* = 8.2 Hz, 1H), 6.90 (td, *J* = 6.1, 8.5 Hz, 4H), 6.80 (t, *J* = 8.8 Hz, 1H), 4.63 (t, *J* = 8.0 Hz, 1H), 3.87 (br s, 1H), 3.66 (br s, 1H), 3.13 (d, *J* = 8.0 Hz, 2H), 2.93 – 2.83 (m, 3H), 2.75 – 2.65 (m, 1H), 2.50 (t, *J* = 11.9 Hz, 1H), 2.43 (dd, *J* = 8.5, 12.6 Hz, 1H), 2.18 – 2.08 (m, 1H), 2.00 – 1.90 (m, 1H), 1.10 (d, *J* = 6.5 Hz, 3H), 0.91 – 0.81 (m, 1H). LCMS: 90% pure; MS (ESI): calcd for $C_{34}H_{34}F_3N_3O_3S$ (M+H) 622.2, found 622.4; optical rotation: $[\alpha]_D^{25}$ + 32.3 (c 0.76, MeOH).

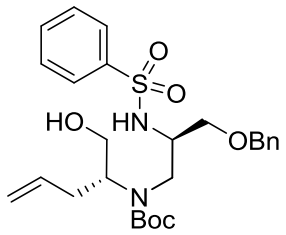
Synthesis of 35



(*R*)-2-((Benzyloxy)methyl)-1-(phenylsulfonyl)aziridine (23).

Benzenesulfonyl chloride (0.329 ml, 2.55 mmol) was added to a solution of (*R*)-2-amino-3-(benzyloxy)propan-1-ol (440 mg, 2.43 mmol) and TEA (0.677 ml, 4.86 mmol) in DMF (12 ml) at 0 °C. The reaction was stirred at 0 °C for 20 min. The reaction was quenched with aqueous sodium hydrogen carbonate (saturated) and the mixture was extracted with ethyl acetate (x 3). The combined organic fractions were dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. Purification on silica gel, eluting with a gradient of 0-100% EtOAc/hexanes over 15 column volumes, afforded (*R*)-*N*-(1-(benzyloxy)-3-hydroxypropan-2-yl)benzenesulfonamide (677 mg) as a white solid. MS (ESI): calcd for $C_{16}H_{19}NO_4S$ (M+H) 322.1, found 322.1; optical rotation: $[\alpha]_D^{25}$ + 13.2 (c 0.65, MeOH). This material was used in the next reaction as follows. Tri-*n*-butylphosphine (0.753 ml, 3.02 mmol) was added to a

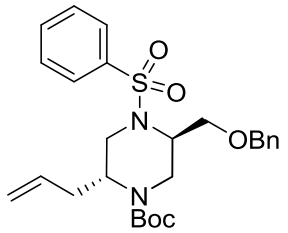
solution of DIAD (0.586 ml, 3.02 mmol) and (*R*)-*N*-(1-(benzyloxy)-3-hydroxypropan-2-yl)benzenesulfonamide (646 mg, 2.01 mmol) in THF (10 ml) at 0 °C. The reaction was stirred for 2 hours. The reaction was quenched with water and the mixture was extracted with ethyl acetate (x 3). The combined organic fractions were dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. Purification on silica gel, eluting with a gradient of 0-100% EtOAc/hexanes over 15 column volumes, afforded the title compound (569 mg, 81 % yield, 2 steps) as a colorless oil. ^{1}H NMR (600 MHz, chloroform-d) δ 7.97 (d, J = 8.4 Hz, 2H), 7.66 – 7.60 (m, 1H), 7.53 (t, J = 7.7 Hz, 2H), 7.29 (dt, J = 6.7, 14.5 Hz, 3H), 7.19 (d, J = 7.4 Hz, 2H), 4.44 (s, 2H), 3.61 (dd, J = 4.0, 11.2 Hz, 1H), 3.42 (dd, J = 6.2, 11.2 Hz, 1H), 3.06 (ddd, J = 4.3, 6.3, 10.6 Hz, 1H), 2.70 (d, J = 7.1 Hz, 1H), 2.22 (d, J = 4.5 Hz, 1H). MS (ESI): calcd for $C_{16}H_{17}NO_3S$ (M+H) 304.1, found 304.1; optical rotation: $[\alpha]_D^{25}$ + 12.6 (c 0.63, MeOH).



***tert*-Butyl ((*R*)-3-(benzyloxy)-2-(phenylsulfonamido)propyl)((*R*)-1-hydroxypent-4-en-2-yl)carbamate (24).** (*R*)-2-Aminopent-4-en-1-ol hydrochloride (958 mg, 6.96 mmol) was dissolved in MeOH and loaded onto a 20 g Isolute Flash SCX-2 cartridge (preconditioned with MeOH). The cartridge was washed with 2 CV of MeOH, then the free amine was eluted with 2 CV of 2M NH_3 in MeOH. The eluent was concentrated *in vacuo* and used directly in the reaction below.

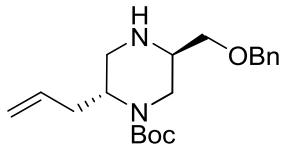
23 (528 mg, 1.74 mmol) and the amine from above were heated at 45 °C in THF (9 ml). The reaction was diluted with EtOAc and washed with water, then brine (saturated). The organic fraction was dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure to afford *N*-((*R*)-1-(benzyloxy)-3-(((*R*)-1-hydroxypent-4-en-2-yl)amino)propan-2-yl)benzenesulfonamide (691 mg), as a colorless oil, taken on crude. MS (ESI): m/z = 405.3 (MH^+); optical rotation: $[\alpha]_D^{25}$ - 5.5 (c 0.60, MeOH). This material was used in the next reaction as follows. Boc-anhydride (735 µl, 3.16 mmol) was added to a solution of *N*-((*R*)-1-(benzyloxy)-3-(((*R*)-1-hydroxypent-4-en-2-yl)amino)propan-2-yl)benzenesulfonamide (640 mg, 1.58 mmol) and TEA (441 µl, 3.16 mmol) in acetonitrile (8 ml) at 45 °C and the reaction was heated at 45 °C for 6 hours. The reaction was quenched with water and the mixture was extracted with ethyl acetate (x 3). The combined organic fractions were dried ($MgSO_4$), filtered and

the solvent was evaporated under reduced pressure. Purification on silica gel, eluting with a gradient of 0-100% EtOAc/hexanes over 15 column volumes, afforded the title compound (520 mg, 1.030 mmol, 64 % yield, 2 steps) as a colorless oil. The ^{1}H NMR of this compound was difficult to interpret due to broad poorly resolved peaks. MS (ESI): calcd for $C_{26}H_{36}N_2O_6S$ (M+H) 505.2, found 505.3; optical rotation: $[\alpha]_D^{25}$ + 6.1 (c 0.66, MeOH).



***tert*-Butyl (2*R*,5*R*)-2-allyl-5-((benzyloxy)methyl)-4-(phenylsulfonyl)piperazine-1-carboxylate (25).**

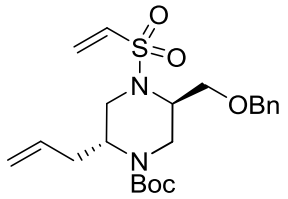
Tri-*n*-butylphosphine (359 µl, 1.454 mmol) was added to a solution of DIAD (283 µl, 1.45 mmol) and **24** (489 mg, 0.969 mmol) in THF (5 ml) at 0 °C and the reaction warmed immediately to RT and stirred for 20 min. The reaction was quenched with water and the mixture was extracted with ethyl acetate (x 3). The combined organic fractions were dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. Purification on silica gel, eluting with a gradient of 0-100% EtOAc/hexanes over 15 column volumes, afforded the title compound (398 mg, 84 % yield) as a white solid. ^{1}H NMR (600 MHz, chloroform-d) δ 7.78 (s, 2H), 7.51 (s, 1H), 7.41 (s, 2H), 7.34 – 7.22 (m, 3H), 7.22 – 7.15 (m, 2H), 5.72 – 5.58 (m, 1H), 5.08 – 4.88 (m, 2H), 4.47 – 4.19 (m, 3H), 4.21 – 3.93 (m, 3H), 3.62 – 3.47 (m, 1H), 3.45 – 3.32 (m, 1H), 3.23 (d, J = 40.0 Hz, 1H), 3.15 – 2.95 (m, 1H), 2.28 (s, 2H), 1.46 – 1.31 (m, 9H). MS (ESI): calcd for $C_{26}H_{34}N_2O_5S$ (M+H) 487.2, found 487.2; optical rotation: $[\alpha]_D^{25}$ + 3.4 (c 0.44, MeOH).



***tert*-Butyl (2*R*,5*R*)-2-allyl-5-((benzyloxy)methyl)piperazine-1-carboxylate (26).**

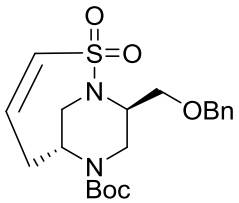
25 (368 mg, 0.756 mmol) and magnesium (368 mg, 15.1 mmol) in MeOH (7.5 ml) were sonicated for 1 hour at RT, after which time the magnesium was consumed. The solvent was removed *in vacuo* and the reaction was quenched with aqueous ammonium chloride (saturated) and the mixture was extracted with ethyl acetate (x 3). The combined organic fractions were dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. Purification on silica gel, eluting with a gradient of 0-100% Hexanes to 20% EtOH/EtOAc over 15

column volumes, afforded the title compound (248 mg, 95 %) as a colorless oil. ^{1}H NMR (600 MHz, chloroform-d) δ 7.40 – 7.22 (m, 5H), 5.80 – 5.70 (m, 1H), 5.10 (d, J = 16.1 Hz, 1H), 5.05 -5.00 (m, 1H), 4.54 (ddt, J = 5.0, 11.9, 16.7 Hz, 2H), 4.06 (br s, 1H), 3.76 (d, J = 13.9 Hz, 1H), 3.70 – 3.64 (m, 1H), 3.41 – 3.36 (m, 1H), 3.23 – 3.17 (m, 1H), 3.12 (br s, 1H), 3.02 – 2.96 (m, 1H), 2.61 – 2.51 (m, 2H), 2.46 – 2.41 (m, 1H), 1.49 – 1.39 (m, 9H). MS (ESI): calcd for $C_{20}H_{30}N_2O_3$ (M+H) 347.2, found 347.3; optical rotation: $[\alpha]_D^{25}$ – 57.1 (c 0.59, MeOH).



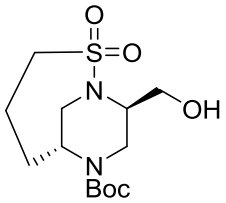
***tert*-Butyl (2*R*,5*R*)-2-allyl-5-((benzyloxy)methyl)-4-(vinylsulfonyl)piperazine-1-carboxylate (27).**

26 (239 mg, 0.690 mmol) and TEA (481 µl, 3.45 mmol) in CH_2Cl_2 were added drop wise to a solution of 2-chloroethanesulfonyl chloride (144 µl, 1.38 mmol) in CH_2Cl_2, total CH_2Cl_2 (7 ml) at 0 °C. The reaction was warmed directly to RT and stirred for 30 min. The reaction was quenched with aqueous potassium phosphate monobasic (saturated) and the mixture was extracted with ethyl acetate (x 3). The combined organic fractions were dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. Purification on silica gel, eluting with a gradient of 0-100% EtOAc/hexanes over 15 column volumes, afforded the title compound (239 mg, 0.547 mmol, 79 % yield) as a colorless oil. ^{1}H NMR (600 MHz, chloroform-d) δ 7.45 – 7.15 (m, 5H), 6.55 – 6.45 (m, 1H), 6.17 – 6.10 (m, 1H), 5.80 – 5.65 (m, 2H), 5.16 (d, J = 16.8 Hz, 1H), 5.08 (s, 1H), 4.60 – 4.55 (m, 1H), 4.50 – 4.45 (m, 1H), 4.24 (d, J = 49.6 Hz, 1H), 4.14 – 3.91 (m, 2H), 3.70 – 3.60 (m, 1H), 3.46 (s, 1H), 3.43 – 3.32 (m, 1H), 3.14 (dd, J = 13.5, 40.3 Hz, 1H), 3.08 – 3.02 (m, 1H), 2.50 – 2.32 (m, 2H), 1.43 (dd, J = 4.9, 14.2 Hz, 9H). MS (ESI): calcd for $C_{22}H_{32}N_2O_5S$ (M+H) 437.2, found 437.2; optical rotation: $[\alpha]_D^{25}$ – 43.1 (c 0.57, MeOH).



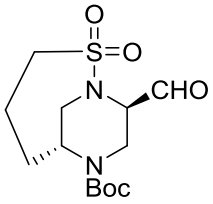
***tert*-Butyl (6*R*,9*R*)-9-((benzyloxy)methyl)-2-thia-1,7-diazabicyclo[4.3.1]dec-3-ene-7-carboxylate 2,2-dioxide (28).**

A solution of **27** (233 mg, 0.534 mmol) in DCE (53 ml) was degassed with nitrogen. Zhan Catalyst-1B (78 mg, 0.107 mmol) was added and the reaction heated at 50 °C for 4 hours. The reaction was cooled to RT and DMSO (0.379 ml, 5.34 mmol) was added and the reaction stirred at RT overnight. The solvent was removed *in vacuo*. Purification on silica gel, eluting with a gradient of 0-100% EtOAc/hexanes over 15 column volumes, afforded the title compound (152 mg, 0.372 mmol, 70 % yield) as a white solid. ^{1}H NMR (600 MHz, chloroform-d) δ 7.39 – 7.26 (m, 5H), 6.65 (d, J = 11.9 Hz, 1H), 6.30 – 6.22 (m, 1H), 4.60 – 4.50 (m, 2H), 4.15 – 3.73 (m, 4H), 3.69 – 3.57 (m, 3H), 3.53 – 3.40 (m, 1H), 3.30 – 3.10 (m, 1H), 2.75 – 2.63 (m, 1H), 1.51 – 1.41 (m, 9H). MS (ESI): calcd for $C_{20}H_{28}N_2O_5S$ (M+H) 409.2, found 409.2; optical rotation: $[\alpha]_D^{25}$ – 48.8 (c 0.67, MeOH).



***tert*-Butyl (6*R*,9*R*)-9-(hydroxymethyl)-2-thia-1,7-diazabicyclo[4.3.1]decane-7-carboxylate 2,2-dioxide (29).**

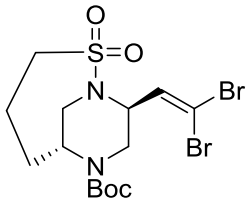
A solution of **28** (126 mg, 0.308 mmol) and Pearlman's catalyst (43.3 mg, 0.062 mmol) were stirred overnight under a balloon of hydrogen. The reaction was purged with nitrogen then filtered through a pad of celite and concentrated *in vacuo*. Purification on silica gel, eluting with a gradient of 0-100% Hexanes to 20% EtOH/EtOAc over 15 column volumes, afforded the title compound (89 mg, 0.278 mmol, 90 % yield) as a white solid. ^{1}H NMR (600 MHz, chloroform-d) δ 4.35 – 4.15 (m, 1H), 4.05 – 3.97 (m, 1H), 3.77 – 3.68 (m, 3H), 3.63 (br s, 2H), 3.53 – 3.42 (m, 2H), 3.41 – 3.32 (m, 1H), 2.33 – 2.20 – 1.90 (m, 5H), 1.45 (s, 9H). MS (ESI): calcd for $C_{13}H_{24}N_2O_5S$ (M+H) 321.2, found 321.2; optical rotation: $[\alpha]_D^{25}$ + 21.7 (c 0.72, MeOH).



***tert*-Butyl (6*R*,9*R*)-9-formyl-2-thia-1,7-diazabicyclo[4.3.1]decane-7-carboxylate 2,2-dioxide (30).**

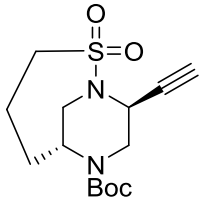
Dess-Martin periodinane (6.26 g, 14.8 mmol) was added to a 0 °C solution of **29** (3.94 g, 12.3 mmol) in CH_2Cl_2 (62 ml). The reaction was stirred overnight at room temp. The reaction was diluted with CH_2Cl_2, then filtered. The filtrate was washed with 10% w/v aq. sodium thiosulphate, sodium hydrogen carbonate (saturated (x 2), then

brine. The organic fraction was dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure to afford the title compound (3.92 g, 100 % yield), as a white foam, taken on crude. ^{1}H NMR (600 MHz, chloroform-d) δ 9.60 (s, 1H), 4.50 – 4.18 (m, 3H), 3.5 – 3.80 (m, 1H), 3.56 (dd, J = 6.7, 11.7 Hz, 1H), 3.39 (dd, J = 4.7, 14.4 Hz, 1H), 3.33 – 3.17 (m, 2H), 2.28 – 2.22 (m, 1H), 2.08 – 1.98 (m, 2H), 1.80 (br s, 1H), 1.45 (s, 9H).



***tert*-Butyl (6*R*,9*S*)-9-(2,2-dibromovinyl)-2-thia-1,7-diazabicyclo[4.3.1]decane-7-carboxylate 2,2-dioxide (31).**

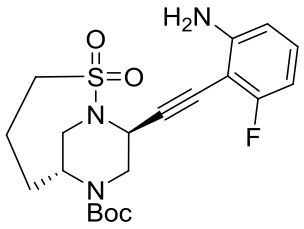
Carbon tetrabromide (8.17 g, 24.6 mmol) was added to a 0 °C solution of **30** (3.92 g, 12.3 mmol) and triphenylphosphine (12.9 g, 49.2 mmol) in CH_2Cl_2 (82 ml) at 0 °C. The reaction was allowed to warm slowly to RT overnight. The reaction was quenched with water and the mixture was extracted with CH_2Cl_2 (x 3). The combined organic fractions were dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. Purification on silica gel, eluting with a gradient of 0-100% EtOAc/hexanes over 15 column volumes, afforded the title compound (4.17 g, 71 % yield) as a white solid. ^{1}H NMR (600 MHz, chloroform-d) δ 6.57 (d, J = 7.5 Hz, 1H), 4.64 (br s, 1H), 4.48 – 4.17 (m, 1H), 3.75 – 3.60 (m, 2H), 3.51 (dd, J = 4.5, 14.2 Hz, 2H), 3.39 (dd, J = 3.2, 15.8 Hz, 1H), 3.30 – 3.23 (m, 1H), 2.23 (br s, 1H), 2.05 – 1.95 (m, 2H), 1.84 (br s, 1H), 1.46 (s, 9H). MS (ESI): calcd for $C_{14}H_{22}Br_2N_2O_4S$ (M+H)(^{79}Br, ^{81}Br) 475.0, found 475.0; optical rotation: $[\alpha]_D^{25}$ + 18.7 (c 0.46, MeOH).



***tert*-Butyl (6*R*,9*S*)-9-ethynyl-2-thia-1,7-diazabicyclo[4.3.1]decane-7-carboxylate 2,2-dioxide (32).**

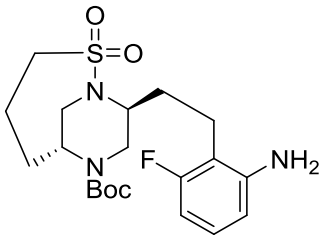
3M Ethylmagnesium bromide (5.86 ml, 17.6 mmol) in ether was added drop wise to a solution of **31** (4.17 g, 8.79 mmol) in THF (88 ml) at 0 °C at a rate to maintain the internal temperature <5°C. The reaction was stirred at 0 °C for 1 hour then quenched with aqueous ammonium chloride (saturated) and the mixture was extracted with ethyl acetate (x 3). The combined organic fractions were dried ($MgSO_4$), filtered and the solvent was evaporated under

reduced pressure. Purification on silica gel, eluting with a gradient of 0-100% EtOAc/hexanes over 15 column volumes, afforded the title compound (2.71 g, 98 % yield) as a white solid. ^{1}H NMR (600 MHz, chloroform-d) δ 4.95 – 4.75 (m, 1H), 4.60 – 4.30 (m, 1H), 4.06 – 3.96 (m, 1H), 3.65 (s, 2H), 3.53 (dd, J = 8.1, 14.5 Hz, 1H), 3.47 – 3.22 (m, 1H), 3.16 – 3.09 (m, 1H), 2.40 – 2.26 (m, 2H), 2.10 – 1.93 (m, 2H), 1.70– 1.62 (m, 1H), 1.45 (s, 9H). MS (ESI): calcd for $C_{14}H_{22}N_2O_4S$ (M-CH_2) 300.1, found 300.1; optical rotation: $[\alpha]_D^{25}$ - 32.0 (c 0.62, MeOH).



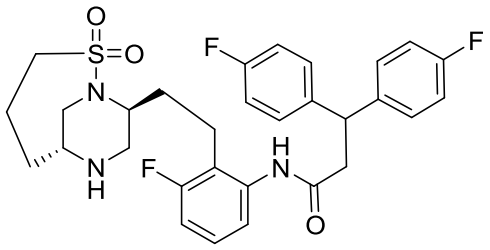
***tert*-Butyl (6*R*,9*S*)-9-((2-amino-6-fluorophenyl)ethynyl)-2-thia-1,7-diazabicyclo[4.3.1]decane-7-carboxylate 2,2-dioxide (33).**

A solution of **32** (500 mg, 1.59 mmol), 3-fluoro-2-iodoaniline (452 mg, 1.91 mmol), and TEA (6.65 ml, 47.7 mmol) in acetonitrile (8 ml) was degassed with nitrogen bubbling. Copper(I) iodide (30.3 mg, 0.159 mmol) and bis(triphenylphosphine)palladium(II) chloride (78 mg, 0.111 mmol) were added and the vessel evacuated and backfilled with nitrogen. The reaction was heated at 70 °C for 4 hours. The reaction was quenched with aqueous potassium phosphate monobasic (saturated) and the mixture was extracted with ethyl acetate (x 3). The combined organic fractions were dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. Purification on silica gel, eluting with a gradient of 0-100% EtOAc/hexanes over 15 column volumes, afforded the title compound (327 mg, 0.772 mmol, 49 % yield) as a white solid. ^{1}H NMR (600 MHz, chloroform-d) δ 7.05 – 7.00 (m, 1H), 6.41 (d, J = 7.6 Hz, 1H), 6.36 (t, J = 8.0 Hz, 1H), 5.12 (s, 1H), 4.65 – 4.11 (m, 4H), 3.71 (q, J = 15.2 Hz, 2H), 3.60 – 3.38 (m, 2H), 3.22 – 3.15 (m, 1H), 2.38 – 2.32 (m, 1H), 2.13 – 1.97 (m, 2H), 1.76 – 1.66 (m, 1H), 1.43 (s, 9H). MS (ESI): calcd for $C_{20}H_{26}FN_3O_4S$ (M+H) 424.2, found 424.2; optical rotation: $[\alpha]_D^{25}$ – 12.8 (c 0.26, MeOH).



***tert*-Butyl (6*R*,9*S*)-9-(2-amino-6-fluorophenethyl)-2-thia-1,7-diazabicyclo[4.3.1]decane-7-carboxylate 2,2-dioxide (34).**

Pearlman's catalyst (90 mg, 0.13 mmol) was added to a nitrogen degassed solution of **33** (543 mg, 1.28 mmol) in EtOH (26 ml) and the reaction evacuated and backfilled with hydrogen, then stirred under a balloon of hydrogen overnight. The reaction was purged with nitrogen then filtered through a pad of celite and concentrated *in vacuo*. Purification on silica gel, eluting with a gradient of 0-100% EtOAc/hexanes over 15 column volumes, afforded the title compound (316 mg, 0.739 mmol, 58 % yield) as a white solid. ^{1}H NMR (600 MHz, chloroform-d) δ 6.95 – 6.90 (m, 1H), 6.46 – 6.38 (m, 2H), 4.50 – 4.25 (m, 1H), 4.05 – 3.76 (m, 2H), 3.69 (d, J = 15.5 Hz, 1H), 3.56 – 3.48 (m, 1H), 3.45 – 3.35 (m, 2H), 3.25 – 3.16 (m, 1H), 2.70 – 2.45 (m, 2H), 2.35 – 2.26 (m, 1H), 2.04 – 1.99 (m, 2H), 1.97 – 1.68 (m, 3H), 1.43 (s, 9H), (exchangeable NH_2 protons not observed). MS (ESI): calcd for $C_{20}H_{30}FN_3O_4S$ (M+H) 428.2, found 428.3; MS (ESI): m/z = 428.3 (MH^+); optical rotation: $[\alpha]_D^{25}$ + 20.8 (c 0.29, MeOH).

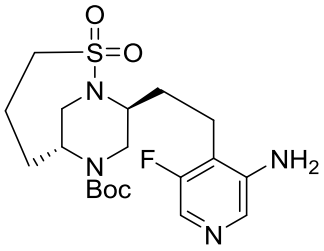


***N*-(2-(2-((6*R*,9*S*)-2,2-Dioxido-2-thia-1,7-diazabicyclo[4.3.1]decan-9-yl)ethyl)-3-fluorophenyl)-3,3-bis(4-fluorophenyl)propanamide (35).**

T3P (278 µl, 0.468 mmol) was added to **34** (100 mg, 0.234 mmol) and 3,3-bis(4-fluorophenyl)propanoic acid (61.3 mg, 0.234 mmol) in ethyl acetate (468 µl). The mixture was stirred at RT until a homogeneous solution was obtained. The solution was cooled to 0 °C and Hunig's base (82.0 µl, 0.468 mmol) was added, the reaction was warmed to RT and stirred for 1 hour. The reaction was quenched with water and the mixture was extracted with ethyl acetate (x 3). The combined organic fractions were dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. Purification by on silica gel, eluting with a gradient of 0-100% EtOAc/hexanes afforded (6R,9S)-*tert*-butyl 9-(2-(3,3-bis(4-fluorophenyl)propanamido)-6-fluorophenethyl)-2-thia-1,7-diazabicyclo[4.3.1]decane-7-carboxylate 2,2-dioxide (111 mg) as a white solid. LCMS: 100% pure; MS (ESI): calcd for $C_{35}H_{40}F_3N_3O_5S$ (M+H) 672.3, found 672.4; optical rotation: $[\alpha]_D^{25}$ + 8.5 (c 0.25, MeOH). This material was used in the next reaction as follows. 4M HCl in dioxane (1.5 ml) was added to (6*R*,9*S*)-tert-butyl 9-(2-(3,3-bis(4-

fluorophenyl)propanamido)-6-fluorophenethyl)-2-thia-1,7-diazabicyclo[4.3.1]decane-7-carboxylate 2,2-dioxide (100 mg, 0.149 mmol) at 0 °C and the reaction warmed to RT and stirred for 1 hour. The reaction was diluted with CH_2Cl_2 and washed with aqueous sodium hydrogen carbonate (saturated). The combined organic fractions were dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. Purification on silica gel, eluting with a gradient of 0-100% Hexanes to 20% EtOH/EtOAc over 15 column volumes, afforded the title compound (64 mg, 53% yield, 2 steps) as a white solid. ^{1}H NMR (600 MHz, chloroform-d) δ 7.97 (s, 1H), 7.35 – 7.27 (m, 4H), 7.20 (d, J = 8.2 Hz, 1H), 7.11 (q, J = 8.1 Hz, 1H), 6.97 (dt, J = 8.7, 11.7 Hz, 4H), 6.83 (t, J = 9.0 Hz, 1H), 4.64 (t, J = 8.0 Hz, 1H), 3.7 – 3.65 (m, 2H), 3.50 (d, J = 15.6 Hz, 1H), 3.39 – 3.33 (m, 2H), 3.27 (dd, J = 5.2, 15.6 Hz, 1H), 3.14 (d, J = 7.9 Hz, 2H), 3.02 (dd, J = 6.3, 12.8 Hz, 1H), 2.48 – 2.32 (m, 3H), 2.27 – 2.16 (m, 1H), 2.14 – 2.00 (m, 2H), 1.98 – 1.88 (m, 1H), 1.72 – 1.862 (m, 2H). MS (ESI): calcd for $C_{30}H_{32}F_3N_3O_3S$ (M+H) 572.2, found 572.3; optical rotation: $[\alpha]_D^{25}$ + 48.5 (c 0.31, MeOH).

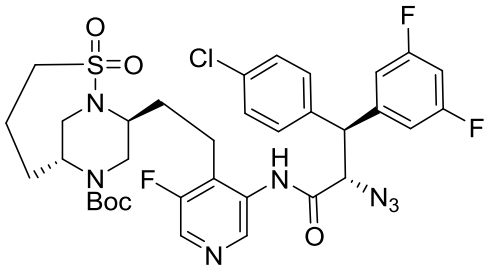
Synthesis of 38



***tert*-Butyl (6*R*,9*S*)-9-(2-(3-amino-5-fluoropyridin-4-yl)ethyl)-2-thia-1,7-diazabicyclo[4.3.1]decane-7-carboxylate 2,2-dioxide (36).**

A solution of **32** (519 mg, 1.65 mmol), 5-fluoro-4-iodopyridin-3-amine (471 mg, 1.98 mmol), and TEA (6.9 ml, 49.5 mmol) in acetonitrile (8.3 ml) was degassed with nitrogen bubbling. Copper(I) iodide (31.4 mg, 0.165 mmol) and bis(triphenylphosphine)palladium(II) chloride (81.0 mg, 0.116 mmol) were added and the vessel evacuated and backfilled with nitrogen. The reaction was heated at 70 °C for 4 hours. The reaction was quenched with aqueous potassium phosphate monobasic (saturated) and the mixture was extracted with ethyl acetate (x 3). The combined organic fractions were dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. Purification on silica gel, eluting with a gradient of 0-100% Hexanes to 20% EtOH/EtOAc over 15 column

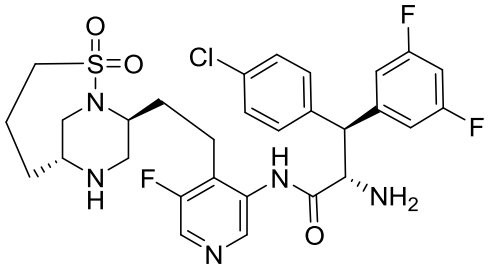
volumes, afforded (6*R*,9*S*)-*tert*-butyl 9-((3-amino-5-fluoropyridin-4-yl)ethynyl)-2-thia-1,7-diazabicyclo[4.3.1]decane-7-carboxylate 2,2-dioxide (403 mg) as a white solid. MS (ESI): calcd for $C_{19}H_{25}FN_4O_4S$ (M+H) 425.2, found 425.2; optical rotation: $[\alpha]_D^{25}$ + 8.2 (c 0.78, MeOH). This material was used in the next reaction as follows. Pearlman's catalyst (61 mg, 0.086 mmol) was added to a nitrogen degassed solution of (6*R*,9*S*)-*tert*-butyl 9-((3-amino-5-fluoropyridin-4-yl)ethynyl)-2-thia-1,7-diazabicyclo[4.3.1]decane-7-carboxylate 2,2-dioxide (366 mg, 0.862 mmol) in EtOH (17 ml) and the reaction evacuated and backfilled with hydrogen, then stirred under a balloon of hydrogen overnight. The reaction was purged with nitrogen then filtered through a pad of celite and concentrated *in vacuo*. Purification on silica gel, eluting with a gradient of 0-100% Hexanes to 20% EtOH/EtOAc over 15 column volumes, afforded the title compound (272 mg, 43 % yield, 2 steps) as a white solid. ^{1}H NMR (600 MHz, DMSO-d6) δ 7.77 (s, 1H), 7.62 (s, 1H), 5.50 (br s, 2H), 4.32 – 4.15 (m, 1H), 3.94 – 3.79 (m, 1H), 3.60 (t, J = 14.1 Hz, 2H), 3.53 – 3.48 (m, 1H), 3.45 – 3.34 (m, 1H), 3.20 (d, J = 14.3 Hz, 1H), 2.55 – 2.45 (m, 1H), 2.40 (br s, 1H), 2.22 (br s, 1H), 1.90 – 1.80 (m, 2H), 1.75 – 1.50 (m, 2H), 1.35 (s, 9H), (exchangeable NH_2 protons not observed). MS (ESI): calcd for $C_{19}H_{29}FN_4O_4S$ (M+H) 429.2, found 429.2; optical rotation: $[\alpha]_D^{25}$ - 61.4 (c 0.85, MeOH).



***tert*-Butyl (6*R*,9*S*)-9-(2-(3-((2*S*,3*S*)-2-azido-3-(4-chlorophenyl)-3-(3,5-difluorophenyl)propanamido)-5-fluoropyridin-4-yl)ethyl)-2-thia-1,7-diazabicyclo[4.3.1]decane-7-carboxylate 2,2-dioxide (37).**

$POCl_3$ (26.1 µl, 0.280 mmol) was added to a solution of **36** (120 mg, 0.280 mmol) and (2*S*,3*S*)-2-azido-3-(4-chlorophenyl)-3-(3,5-difluorophenyl)propanoic acid (95 mg, 0.280 mmol) in pyridine (1.4 ml) at -15 °C and the reaction stirred at this temperature for 30 min, then warmed to 0°C and stirred for 1 hour. The reaction was quenched with brine (saturated) and the mixture was extracted with ethyl acetate (x 3). The combined organic fractions were dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. Purification on silica gel, eluting with a gradient of 0-100% EtOAc/hexanes over 15 column volumes, afforded the title compound (173 mg, 83 % yield) as a white foam. ^{1}H NMR (600 MHz, chloroform-d) δ 8.13 (br s, 1H), 7.28 – 7.33 (m, 3H), 7.12

(q, *J* = 7.8 Hz, 1H), 6.99 (d, *J* = 6.4 Hz, 2H), 6.90 (t, *J* = 9.0 Hz, 1H), 6.87 – 6.80 (m, 1H), 6.72 (t, *J* = 8.9 Hz, 1H), 4.74 – 4.61 (m, 2H), 4.38 (br s, 1H), 3.78 (br s, 1H), 3.68 (d, *J* = 15.5 Hz, 1H), 3.62 (dd, *J* = 6.9, 13.9 Hz, 1H), 3.39 (d, *J* = 14.0 Hz, 1H), 3.29 – 3.20 (m, 2H), 2.60 – 2.40 (m, 2H), 2.40 – 2.32 (m, 1H), 2.16 – 1.98 (m, 3H), 1.83 – 1.71 (m, 2H), 1.47 (s, 9H). MS (ESI): calcd for $C_{34}H_{37}ClF_3N_7O_5S$ (M+H) 748.2, found 748.3; optical rotation: $[\alpha]_D^{25}$ + 78.5 (c 0.57, MeOH).



(2*S*,3*S*)-2-Amino-3-(4-chlorophenyl)-3-(3,5-difluorophenyl)-*N*-(4-(2-((6*R*,9*S*)-2,2-dioxido-2-thia-1,7-diazabicyclo[4.3.1]decan-9-yl)ethyl)-5-fluoropyridin-3-yl)propanamide (38).

Trimethylphosphine (307 µl, 0.307 mmol) was added to a solution of **37** in THF (1.7 ml)/Water (0.34 ml) at 0 °C and the reaction stirred at this temperature for 20 min. The reaction was quenched with brine (saturated) and the mixture was extracted with ethyl acetate (x 3). The combined organic fractions were dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. Purification on silica gel eluting with a gradient of 0-100% Hexanes to 20% EtOH/EtOAc over 15 column volumes, afforded (6*R*,9*S*)-*tert*-butyl 9-(2-(3-((2*S*,3*S*)-2-amino-3-(4-chlorophenyl)-3-(3,5-difluorophenyl)propanamido)-5-fluoropyridin-4-yl)ethyl)-2-thia-1,7-diazabicyclo[4.3.1]decane-7-carboxylate 2,2-dioxide (93 mg) as a white solid. MS (ESI): calcd for $C_{34}H_{39}ClF_3N_5O_5S$ (M+2H) 723.3, found 723.3; optical rotation: $[\alpha]_D^{25}$ + 12.6 (c 0.41, MeOH). This material was used in the next reaction as follows. 4M HCl (500 µl, 2.00 mmol) in dioxane was added to a solution of (6*R*,9*S*)-tert-butyl 9-(2-(3-((2*S*,3*S*)-2-amino-3-(4-chlorophenyl)-3-(3,5-difluorophenyl)propanamido)-5-fluoropyridin-4-yl)ethyl)-2-thia-1,7-diazabicyclo[4.3.1]decane-7-carboxylate 2,2-dioxide (72.2 mg, 0.100 mmol) in CH_2Cl_2 (500 µl) at 0 °C and the reaction was warmed directly to RT and stirred for 4hrs. The solvent was removed *in vacuo* and aqueous sodium hydrogen carbonate (saturated) was added and the mixture was extracted with CH_2Cl_2 (x 3). The combined organic fractions were dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. Purification on silica gel, eluting with a gradient of 0-100% Hexanes to 20% EtOH/EtOAc over 15 column volumes, afforded the title compound (59 mg, 60 % yield, 2 steps). ^{1}H NMR (600 MHz, chloroform-d) δ 9.09 (s, 1H), 7.40 (d, *J* = 8.2 Hz, 1H), 7.31 – 7.27 (m, 3H), 7.12 (q, *J* = 7.6 Hz, 1H), 6.88 (d, *J* = 6.9 Hz, 2H), 6.82 (t, *J* = 8.9 Hz,

1H), 6.68 (t, *J* = 9.0 Hz, 1H), 4.69 (d, *J* = 6.3 Hz, 1H), 4.26 (d, *J* = 6.2 Hz, 1H), 3.88 (br s, 1H), 3.74 (t, *J* = 12.9 Hz, 1H), 3.41 (d, *J* = 15.6 Hz, 1H), 3.38 – 3.34 (m, 1H), 3.29 (dd, *J* = 7.8, 14.2 Hz, 1H), 3.22 (dd, *J* = 5.1, 15.6 Hz, 1H), 3.05 (dd, *J* = 6.4, 12.7 Hz, 1H), 2.68 – 2.57 (m, 2H), 2.49 (dd, *J* = 7.4, 12.5 Hz, 1H), 2.20 – 2.12 (m, 1H), 2.10 – 2.02 (m, 1H), 2.02 – 1.94 (m, 1H), 1.80 – 1.70 (m, 2H), 1.31 – 1.24 (m, 1H), 0.92 – 0.82 (m, 1H). ^{13}C NMR (125 MHz, chloroform-d) δ 171.9, 164.0 (d, J_{C-F} = 14 Hz), 162.0 (d, J_{C-F} = 13 Hz), 160.4, 144.1 (t, J_{C-F} = 9 Hz), 139.1, 136.3 (d, J_{C-F} = 7 Hz), 133.0, 129.8, 128.9, 127.5 (d, J_{C-F} = 10 Hz), 121.0 (d, J_{C-F} = 17 Hz), 119.1 (d, J_{C-F} = 3 Hz), 112.2 (d, J_{C-F} = 14 Hz), 112.1 (t, J_{C-F} = 24 Hz), 102.8 (t, J_{C-F} = 25 Hz), 59.0, 56.7, 53.3, 51.0, 43.9, 40.8, 32.5, 31.1, 20.6, 20.5, 19.6. LCMS: 95% pure; MS (ESI): calcd for $C_{29}H_{31}ClF_3N_5O_3S$ (M+2H) 623.2, found 623.3; optical rotation: $[\alpha]_D^{25}$ + 86.3 (c 0.64, MeOH).

Assay Protocols

Assay for Inhibition of Microbial Expressed HIV Protease

Studies of the inhibition of the wild type HIV-1 protease (which was expressed in Escherichia coli) were carried out with a peptide substrate [Val-Ser-Gln-Asn-(βnaphtyl)Ala-Pro-Ile-Val (SEQ ID NO 1)]. The inhibitor was first preincubated with the HIV-1 protease (wild type) enzyme in assay buffer (50 mM sodium acetate, pH 5.5, 100 mM NaCl, and 0.1% BSA) for 30 minutes at room temperature. Substrate was added to 400 micromolar in a total volume of 20 microliters containing 20 picomolar HIV-1 protease (final) and the reaction is incubated for 1 hour at 30°C. The reaction was quenched with the addition of formic acid and indinavir to 0.012% and 150 nM final concentrations, respectively. Product formation was determined after separation of product and substrate on a Zorbax Eclipse XDB-C18 column connected to an API 4000 mass spectrometer (Applied Biosystems) with multiple reaction monitoring (transitions were 644.5/428.9 and 615.4/422.2 (M1/M3) for product and indinavir respectively). The extent of inhibition of the reaction was determined from the peak area of the products. Analysis of the products, independently synthesized, provided quantitation standards and confirmation of the product composition.

Assessing Antiviral Potency in a Multiple Round HIV-1 Infection Assay

HIV-1 replication was monitored using MT4-gag-GFP clone D3 (hereafter designate MT4-GFP), which are MT-4 cells modified to harbor a GFP reporter gene, the expression of which is dependent on the HIV-1 expressed

proteins tat and rev. Productive infection of an MT4-GFP cell with HIV-1 results in GFP expression approximately 24h post-infection.

MT4-GFP cells were maintained at 37ºC/5% CO2/90% relative humidity in RPMI 1640 supplemented with 10% fetal bovine serum, 100 U/mL penicillin/streptomycin, and 400µg/mL G418 to maintain the reporter gene. For infections, MT4-GFP cells were placed in the same medium lacking G418 and infected overnight with H9IIIB virus at an approximate multiplicity of infection of 0.01 in the same incubation conditions. Cells were then washed and re-suspended in RPMI 1640 containing 50% normal human serum at 1.6 x 105 cells/mL. Compound plates were prepared by dispensing compounds dissolved in DMSO into wells of 384 well poly D lysine-coated plates (0.2µl/well) using an ECHO acoustic dispenser. Each compound was tested in a 10 point serial 3-fold dilution (typical final concentrations: 8.4 µM – 0.43 nM). Controls included no inhibitor (DMSO only) and a combination of three antiviral agents (efavirenz, indinavir, and an integrase strand transfer inhibitor at final concentrations of 4µM each). Cells were added (50µL/well) to compound plates and the infected cells were maintained at 37ºC/5% CO2/90% relative humidity.

Infected cells were quantified at two time points, ~48h and~ 72h post-infection, by counting the number of green cells in each well using an Acumen eX3 scanner. The increase in the number of green cells over ~24h period gives the reproductive ratio, Ro, which is typically 5-15 and has been shown experimentally to be in logarithmic phase (data not shown). Inhibition of Ro is calculated for each well, and Potency (EC_{50}) was determined by non-linear 4-parameter curve fitting.

Rat Pharmacokinetic Profiles[a]

Compound	Cl (mL/min/kg)	Vd (L/kg)	$t_{1/2}$ (hours)	Bioavailability (%F)	Rat PPB[b] (% bound)
7	18	2.1	2.6	8.1	99.8
18	23	2.1	1.6	3.1	99.8
20	16	2.9	4.1	2.7	99.9
21	13	1.6	2.3	14	>99.9

[a] 2 mpk IV (1:1 DMSO:PEG400), 10 mpk PO (10% Tween 80), n=2 Wistar Hannover rats. [b] PPB = plasma protein binding

All procedures related to the use of animals in these studies were reviewed and approved by the Institutional Animal Care and Use Committee at Merck Research Laboratories and conform with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996).

Procedures for Co-crystallization Studies

HIV Protease, containing three amino acid substitutions (Q7K, L33I, L63I), was crystallized by the hanging drop vapor diffusion method. The reservoir solution contained 100 mM sodium acetate, pH 5.2, and 350-500 mM sodium chloride. Larger crystals were obtained by streak seeding immediately after crystallization. Crystals were soaked overnight with 200 uM compound from a 10mM DMSO stock in a buffer containing 100 mM sodium acetate and 750 mM sodium chloride. For cryo-protection, crystals were transferred into a solution containing 100 mM sodium acetate, 500 mM sodium chloride, and 25% glycerol. The crystals were then vitrified for data collection by quick submersion in liquid nitrogen.

Table of Crystallographic Statistics

Compound (pdb)	**7 (6B36)**	**20 (6B38)**	**18 (6B3C)**	**19 (6B3F)**	**21 (6B3G)**	**35 (6B3H)**
Wavelength	1	1	1	1	1	1
Resolution range	19.28 - 1.63 (1.69 - 1.63)	21.41 - 1.48 (1.53 - 1.48)	20.50 - 1.60 (1.66 - 1.60)	20.95 - 1.46 (1.52 - 1.46)	21.46 - 1.50 (1.554 - 1.50)	19.53 - 1.62 (1.68 - 1.62)
Space group	P 2_12_12	P 2_12_12	P 2_12_12	P 2_12_12	P 2_12_12	P 2_12_12
Unit cell	57.59 85.71 46.56 90 90 90	58.33 85.78 46.55 90 90 90	58.62 86.06 46.4 90 90 90	58.55 85.94 46.49 90 90 90	58.39 85.83 46.45 90 90 90	58.43 86.2 46.23 90 90 90
Total reflections	153812 (7296)	252390 (25311)	199309 (20092)	263225 (26171)	244118 (23997)	165967 (6882)
Unique reflections	26221 (1776)	39568 (2853)	31234 (2684)	41156 (2729)	38085 (2736)	28148 (1906)
Multiplicity	5.9 (4.1)	6.4 (6.5)	6.4 (6.5)	6.4 (6.5)	6.4 (6.4)	5.9 (3.8)
Completeness (%)	89.04 (60.84)	99.66 (99.54)	99.00 (98.87)	99.89 (99.90)	99.90 (99.89)	92.60 (60.71)
Mean I/sigma(I)	19.94 (1.92)	20.46 (2.80)	16.61 (2.40)	19.24 (2.83)	17.31 (3.25)	21.46 (2.05)
Wilson B-factor	24.25	19.66	23.47	18.34	18.2	23.31
R-merge	0.0478 (0.581)	0.0516 (0.650)	0.0640 (0.702)	0.0539 (0.650)	0.0654 (0.672)	0.0507 (0.585)
R-work	18.2%	18.3%	18.3%	18.3%	18.5%	17.7%
R-free	20.2%	19.6%	19.4%	19.6%	20.2%	19.9%
# non-H atoms						
macromolecules	1516	1516	1516	1516	1516	1516
ligands	47	91	47	47	91	84
solvent	147	165	136	158	148	158
RMS deviation						
bonds	0.01	0.01	0.01	0.01	0.01	0.01
angles	1.12	1.25	1.17	1.21	1.25	1.09
Ramachandran						
favored (%)	99.48	100	100	99.48	100	100
allowed (%)	0.52	0	0	0.52	0	0
outliers (%)	0	0	0	0	0	0
Average B-factor						
Overall	26.43	22.16	25.6	20.96	20.56	25.05
macromolecules	25.47	21.04	24.78	19.87	19.51	24.32
ligands	30.54	22.89	27.45	22.05	22.31	21.89
solvent	35.11	32.07	34.24	31.13	30.17	33.64

Numbers in parentheses represent statistics in the highest resolution bin