Discovery of MK-8718, an HIV Protease Inhibitor Containing a Novel Morpholine Aspartate Binding Group

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Assay Protocols

Assay for Inhibition of Microbial Expressed HIV Protease

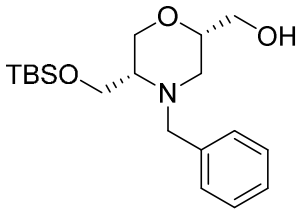
Studies of the inhibition of the wildtype HIV-1 protease (which was expressed in Escherichia coli) were carried out with a peptide substrate [Val-Ser-Gln-Asn-(βnaphtyl)Ala-Pro-Ile-Val (SEQ ID NO 1)]. The inhibitor was first preincubated with the HIV-1 protease (wild type) enzyme in assay buffer (50 mM 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. Representative compounds of the present invention exhibit inhibition of HIV-1 protease in this assay.

Antiviral Assays in Cell Culture

Antiviral potency in a multiple round HIV-1 infection assay was carried out as follows. The assay was performed using MT-4 human T-lymphoid cells. MT-4 cells (2.5 x 105 cells per ml) were infected at a multiplicity of infection of ≤0.01 and incubated overnight in RPMI 1640 culture medium containing 10% fetal bovine serum. The infected cells were then washed twice in fresh culture medium and resuspended in RPMI 1640 containing 50% normal human serum at 4 x 105 cells/ml and seeded into 384-well cell culture plates at 12,000 cells per well. Serial 1:2 dilutions of the test compounds were added to the wells. The test cultures were incubated for an additional 72 h at 37°C at which time virus levels were assayed. IC_{95} values were determined by non-linear 4-parameter curve fitting.

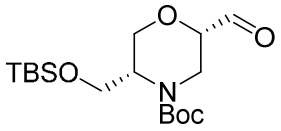
Synthesis of 9b – 9j



((2S,5S)-4-Benzyl-5-(((tert-butyldimethylsilyl)oxy)methyl)morpholin-2-yl)methanol (11).

Lithium perchlorate (18.3 g, 172 mmol) was added portionwise (caution exotherm!) to a solution of (S)-2-(benzylamino)-3-((tert-butyldimethylsilyl)oxy)propan-1-ol (39.0 g, 132 mmol) and (R)-(-)-epichlorohydrin (15.9 g, 172 mmol) in toluene (400 ml) at ambient temperature. The resulting reaction was stirred at ambient temperature for 48 h. MeOH (100 ml) was added followed by slow addition of NaOMe (4M, 83mL) in MeOH. The resulting reaction was stirred at ambient temperature for 48 h. The reaction was quenched with sat. aq. NH_4Cl, and the crude product was extracted with EtOAc (x3). The combined organic fractions washed with brine, dried

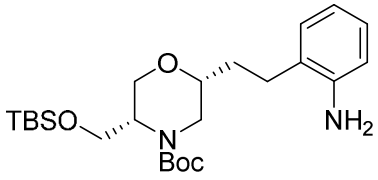
over $MgSO_4$, and concentrated *in vacuo*. Purification on silica gel, eluting with a gradient of 10 to 50 % EtOAc in hexanes, afforded the title compound (25 g, 54 %) as a colorless gum. ^{1}H NMR (400 MHz, Acetone-d6) δ 7.39 (d, J = 7.1 Hz, 2H), 7.36 – 7.29 (m, 2H), 7.28 – 7.20 (m, 1H), 4.03 – 3.95 (m, 3H), 3.92 (d, J = 13.7 Hz, 1H), 3.78 (d, J = 13.7 Hz, 1H), 3.65 (dd, J = 2.8, 11.0 Hz, 1H), 3.61 – 3.51 (m, 3H), 3.50 – 3.40 (m, 1H), 2.73 – 2.64 (m, 1H), 2.55 – 2.48 (m, 2H), 0.91 (s, 9H), 0.09 (s, 3H), 0.07 (s, 3H); MS (ESI): *m*/z = 352.1 (MH^+).



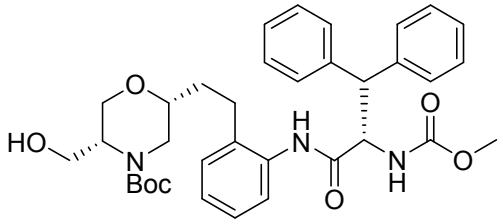
tert-Butyl (2S,5S)-5-(((tert-butyldimethylsilyl)oxy)methyl)-2-formylmorpholine-4-carboxylate (12).

((2S,5S)-4-Benzyl-5-(((tert-butyldimethylsilyl)oxy)methyl)morpholin-2-yl)methanol (**11**) (50 g, 142 mmol), Boc_2O (40 ml, 171 mmol), NEt_3 (20 ml, 142 mmol) and $PdOH_2$ (20 g, 28.4 mmol) were suspended in Ethanol (500 ml) under nitrogen in a Parr shaker appartus. The reaction was evacuated and backfilled with hydrogen gas (x 3) and shaken overnight at 45 psi hydrogen. The reaction was evacuated and backfilled with nitrogen (x 3) and the resulting suspension filtered through Solka-Floc®. The filtrate was diluted with EtOAc and the solution washed with water (x 3), then dried over Na_2SO_4 and concentrated *in vacuo*. Purification on silica gel, eluting with a gradient of 5 to 40 % EtOAc in hexanes, afforded the tert-butyl (2S,5S)-5-(((tert-butyldimethylsilyl)oxy)methyl)-2-(hydroxymethyl)morpholine-4-carboxylate (51 g) as a colorless gum. DMSO (22 ml, 312 mmol) was dissolved in CH_2Cl_2 (360 ml) and the resulting solution cooled to -78°C. Oxalyl chloride (21 ml, 241 mmol) was added dropwise at a rate so as the internal temperature did not exceed -55°C. The resulting reaction was stirred at -78°C for 30 min. tert-Butyl (2S,5S)-5-(((tert-butyldimethylsilyl)oxy)methyl)-2-(hydroxymethyl)morpholine-4-carboxylate (51 g, 142 mmol) was dissolved in CH_2Cl_2 (154 ml) and added dropwise at a rate so as the internal temperature did not exceed -60°C. The resulting reaction was stirred at -78°C for 30 min, then a further 1 h between -60 and -45°C. The reaction was cooled to -78°C and NEt_3 (99 ml, 709 mmol) was added dropwise at a rate so as the internal temperature did not exceed -60°C. The resulting reaction was warmed directly to 0°C and stirred for 1 h. The reaction was quenched with water and the crude product was extracted with CH_2Cl_2 (x3). The combined organic fractions washed with water, brine, dried over Na_2SO_4, and concentrated *in vacuo* to afford crude title compound,

tert-butyl (2S,5S)-5-(((tert-butyldimethylsilyl)oxy)methyl)-2-formylmorpholine-4-carboxylate (51 g, 100 %) which was used directly without further purification.

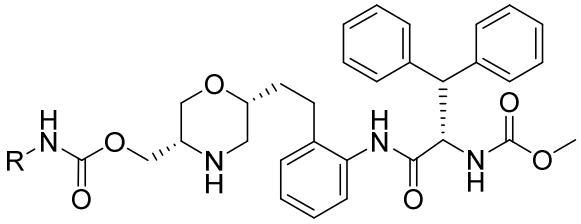


tert-Butyl (2R,5S)-2-(2-aminophenethyl)-5-(((tert-butyldimethylsilyl)oxy)methyl)morpholine-4-carboxylate (13). (2-Nitrobenzyl)triphenylphosphonium bromide monohydrate (77 g, 156 mmol), K_2CO_3 (39.2 g, 284 mmol) and 18-crown-6 (3.75 g, 14.19 mmol) were combined in DME (500 ml) and the reaction stirred at ambient temperature for 5 min. A solution of tert-butyl (2S,5S)-5-(((tert-butyldimethylsilyl)oxy)methyl)-2-formylmorpholine-4-carboxylate (**12**) (51 g, 142 mmol) in DME (500 ml) was added and the resulting reaction stirred overnight at ambient temperature. The resulting suspension was filtered through solka-floc® and the filtrate concentrated *in vacuo*. Purification on silica gel, eluting with a gradient of 10 to 30 % EtOAc in hexanes, afforded tert-butyl (2R,5S)-5-(((tert-butyldimethylsilyl)oxy)methyl)-2-((E and Z)-2-nitrostyryl)morpholine-4-carboxylate (63 g, 92 %) (mixture of *E* and *Z* isomers) as a colorless gum. MS (ESI): m/z = 501.4 (M+Na). The mixture of olefin isomers (63g) was dissolved in nitrogen degassed EtOH and added to a suspension of 10 % Pd/C (11 g) in EtOH, total EtOH (1000 ml). The reaction was evacuated and backfilled with hydrogen and shaken at 50 psi on a Parr reactor overnight.. The reaction was evacuated and backfilled with nitrogen, filtered through a solka-floc®, and concentrated *in vacuo* to afford the title compound (57 g, 97 %) as a colorless gum. ^{1}H NMR (500 MHz, Acetone-d6) δ 6.99 (d, J = 7.3 Hz, 1H), 6.94 (t, J = 7.7 Hz, 1H), 6.70 (d, J = 7.9 Hz, 1H), 6.58 (t, J = 7.4 Hz, 1H), 4.46 (s, 1H), 4.11 (t, J = 11.7 Hz, 1H), 3.98 - 3.70 (m, 4H), 3.68 - 3.60 (m, 1H), 3.53 (dd, J = 11.6, 2.9 Hz, 1H), 3.37 - 3.27 (m, 1H), 2.85 - 2.57 (m, 3H), 1.79 - 1.69 (m, 2H), 1.45 (s, 9H), 0.94 (s, 9H), 0.17 - 0.10 (m, 6H); MS (ESI): m/z = 473.2 (M+Na).



tert-Butyl (2R,5R)-5-(hydroxymethyl)-2-(2-((S)-2-((methoxycarbonyl)amino)-3,3-diphenylpropanamido)phenethyl)morpholine-4-carboxylate (15).

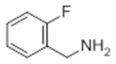
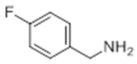
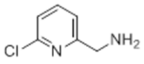
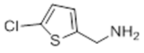
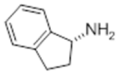
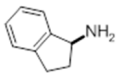
2,6-Lutidine was added to a solution of tert-Butyl (2R,5S)-2-(2-aminophenethyl)-5-(((tert-butyldimethylsilyl)oxy)methyl)morpholine-4-carboxylate (**13**) (4.59 g, 10.2 mmol), HATU® (5.42 g, 14.3 mmol), and (S)-2-((methoxycarbonyl)amino)-3,3-diphenylpropanoic acid (3.66 g, 12.2 mmol) in DMF (70 ml) at ambient temperature and the resulting reaction stirred at this temperature overnight. The reaction was quenched with sat. aq. $NaHCO_3$, and the crude product was extracted with EtOAc (x3). The combined organic fractions washed with 1M HCl, brine, dried over $MgSO_4$, and concentrated *in vacuo*. Purification on silica gel, eluting with a gradient of 0 to 50 % EtOAc in hexanes, afforded tert-butyl (2R,5S)-5-(((tert-butyldimethylsilyl)oxy)methyl)-2-(2-((S)-2-((methoxycarbonyl)amino)-3,3-diphenylpropanamido)phenethyl)morpholine-4-carboxylate (3.95 g, 53%). MS (ESI): m/z = 632.2 (M+2H-Boc). The TBS ether (1.10 g, 1.50 mmol) was dissolved in THF (8 ml) and a 1M solution of TBAF in THF (3 ml) was added dropwise at ambient temperature and the reaction stirred for 1 hour. The reaction was quenched with water, and the crude product was extracted with EtOAc (x3). The combined organic fractions washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. Purification on silica gel, eluting with a gradient of 10 to 100 % EtOAc in hexanes, afforded the title compound (0.780 g, 84 %). ^{1}H NMR (500 MHz, Chloroform-d) δ 8.19 - 8.00 (m, 1H), 7.70 - 7.60 (m, 1H), 7.40 - 7.04 (m, 12H), 5.37 - 5.21 (m, 1H), 5.10 - 4.95 (m, 1H), 4.71 - 4.54 (m, 1H), 4.25 - 4.05 (m, 1H), 4.00 – 3.50 (m, 6H), 3.12 - 2.99 (m, 1H), 2.67 – 2.90 (m, 1H), 2.46 – 2.82 (m, 1H), 2.26 - 2.10 (m, 1H), 1.69 - 1.51 (m, 3H), 1.47 (s, 9H).



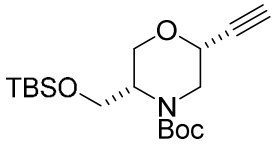
General procedure for the preparation of amines 9b-9j in Table 1.

tert-Butyl (2R,5R)-5-(hydroxymethyl)-2-(2-((S)-2-((methoxycarbonyl)amino)-3,3-diphenylpropanamido) phenethyl)morpholine-4-carboxylate (**15**) (1 eq.) and CDI (2.05 eq) were dissolved in dry Pyridine (0.04 M) and the resulting solution heated at 50 °C for 30 min. The appropriate amine (6 eq.) was added and the resulting reaction heated at 50°C overnight. The mixture was diluted with EtOAc, washed with 5% aq. $KHSO_4$, sat. $NaHCO_3$, dried over Na2SO4, filtered and concentrated. The residues were purified on silica gel eluting with a gradient of 0 to 5% MeOH in DCM. The resulting Boc-protected Morpholines were deprotected by stirring with a 1:1 mixture of DCM:TFA at ambient temperature for 1 hour. The reaction mixtures were concentrated *in vacuo* then co-evaporated with heptane (x2) and triturated with ether to afford the products shown in table 1 as TFA salts. Alternatively the TFA salt could be neutralized with sat. aq. $NaHCO_3$, extracted with EtOAc, dried over MgSO4, concentrated *in vacuo* then purified on silica gel eluting with a gradient of 0 to 5% MeOH in DCM to afford the products shown in table 1.

Table 1. Amine coupling partners and MS data for 9b – 9j.

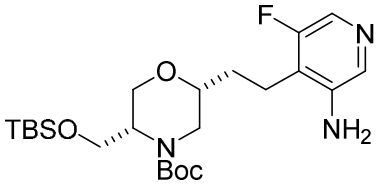
Amine	Product	MS (ESI) (MH+)
	9b	669.3
	9c	669.3
	9d	686.2/688.2
	9e	691.2/693.2
	9f	677.2
	9g	677.2
	9h	603.4
	9i	643.4
	9j	561.0

Synthesis of MK-8718



tert-Butyl (2R,5S)-5-(((tert-butyldimethylsilyl)oxy)methyl)-2-ethynylmorpholine-4-carboxylate (19).

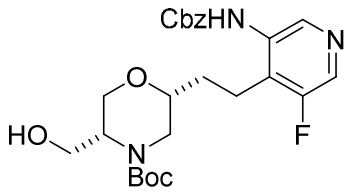
Dimethyl (1-diazo-2-oxopropyl)phosphonate (3.90 ml, 26.0 mmol) was added to tert-butyl (2S,5S)-5-(((tert-butyldimethylsilyl)oxy)methyl)-2-formylmorpholine-4-carboxylate (**12**) (7.78 g, 21.6 mmol) and K_2CO_3 (5.98 g, 43.3 mmol) in anhydrous MeOH (216 ml) at ambient temperature. The resulting reaction was stirred for 2 h at ambient temperature, then filtered and the MeOH removed *in vacuo*. Sat. aq. KH_2PO_4 was added and the mixture was extracted with EtOAc (x 3). The combined organic fractions were dried over $MgSO_4$ and concentrated *in vacuo*. Purification on silica gel, eluting with a gradient of 0 to 30 % EtOAc in hexanes, afforded the title compound (4.6 g, 60 %) as a colorless gum. ^{1}H NMR (500 MHz, DMSO-d6) δ 4.16 (d, J = 10.9 Hz, 1H), 3.85 (d, J = 11.8 Hz, 1H), 3.90 – 3.67 (m, 3H), 3.60 – 3.45 (m, 3H), 3.02 – 2.78 (br m, 1H), 1.40 (s, 9H), 0.87 (s, 9H), 0.05 (s, 6H); MS (ESI): *m/z* = 256.01 (M+H-Boc).



tert-Butyl (2R,5S)-2-(2-(3-amino-5-fluoropyridin-4-yl)ethyl)-5-(((tert-butyldimethylsilyl)oxy)methyl)morpholine-4-carboxylate (21).

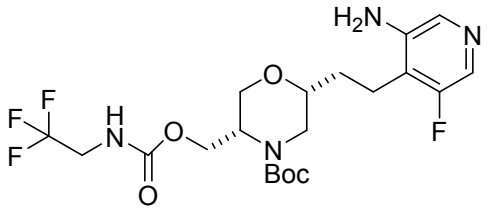
tert-Butyl (2R,5S)-5-(((tert-butyldimethylsilyl)oxy)methyl)-2-ethynylmorpholine-4-carboxylate (**19**) (6.80 g, 19.1 mmol), 5-fluoro-4-iodopyridin-3-amine (4.55 g, 19.1 mmol), $(PPh_3)_2PdCl_2$ (0.940 g, 1.34 mmol), NEt_3 (80 ml, 574 mmol), and CuI (0.364 g, 1.91 mmol) were heated at 70 °C overnight. The reaction was quenched with sat. aq. KH_2PO_4, and the crude product was extracted with EtOAc (x3). The combined organic fractions were dried over $MgSO_4$, and concentrated *in vacuo*. Purification on silica gel, eluting with a gradient of 0 to 100 % EtOAc in hexanes, afforded tert-butyl (2R,5S)-2-((3-amino-5-fluoropyridin-4-yl)ethynyl)-5-(((tert-butyldimethylsilyl)oxy)methyl)morpholine-4-carboxylate (6.5 g) as a viscous oil contaminated with 5-fluoro-4-iodopyridin-3-amine. The impure material was taken into the next reaction as follows. PtO_2 (1.58 g, 6.96 mmol)

was suspended in nitrogen degassed trifluorethanol and tert-butyl (2R,5S)-2-((3-amino-5-fluoropyridin-4-yl)ethynyl)-5-(((tert-butyldimethylsilyl)oxy)methyl)morpholine-4-carboxylate (6.5 g, 14 mmol) (~50% pure) was dissolved in nitrogen degassed trifluorethanol and added to the suspension of PtO_2, total trifluorethanol 280 ml. The reaction was evacuated and backfilled with hydrogen and shaken on the a Parr at 50 psi hydrogen for 48 h. The reaction was degassed with nitrogen, filtered through celite and the solvent removed *in vacuo*. Purification on silica gel, eluting with a gradient of 0 to 100 % EtOAc in hexanes, afforded the title compound (2.1 g, 24 %) as a white solid. ^{1}H NMR (500 MHz, DMSO-d6) δ 7.79 (s, 1H), 7.63 (s, 1H), 5.46 (s, 2H), 3.92 (d, J = 11.7 Hz, 1H), 3.85 – 3.67 (m, 3H), 3.56 – 3.50 (m, 1H), 3.44 (dd, J = 2.8, 11.7 Hz, 1H), 3.32 (s, 1H), 3.30 – 3.19 (br m, 1H), 2.73 – 2.54 (m, 2H), 1.63 – 1.47 (m, 2H), 1.40 (s, 9H), 0.87 (s, 9H), 0.06 (s, 6H); MS (ESI): *m/z* = 470.5 (MH^+), 97% pure by LCMS.



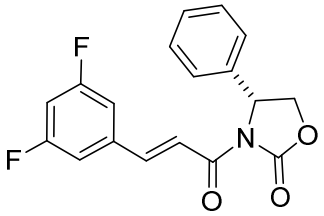
tert-Butyl (2R,5R)-2-(2-(3-(((benzyloxy)carbonyl)amino)-5-fluoropyridin-4-yl)ethyl)-5-(hydroxymethyl)morpholine-4-carboxylate (23)

Cbz-Cl (806 µl, 5.64 mmol) was added dropwise to a solution of tert-butyl (2R,5S)-2-(2-(3-amino-5-fluoropyridin-4-yl)ethyl)-5-(((tert-butyldimethylsilyl)oxy)methyl)morpholine-4-carboxylate (**21**) (1.0 g, 2.13 mmol) in pyridine (5 ml) at 0 ^{o}C. The reaction was allowed to warm slowly to ambient temperature overnight. The reaction was quenched with water and the crude product was extracted with CH_2Cl_2 (x3). The combined organic fractions were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue was dissolved in THF (7 ml) and 1M TBAF (4.26 ml, 4.26 mmol) in THF was added dropwise at ambient temperature. The resulting reaction was stirred at this temperature for 2 h. The solution was diltuted with EtOAc then washed with water and brine dried over $MgSO_4$, and concentrated *in vacuo*. Purification on silica gel, eluting with a gradient of 0 to 100 % A to B (A = $CHCl_3$, B = 7:2:1 $CHCl_3$:EtOAc:MeOH) afforded the title compound (615 mg, 59 %) as a glassy solid. ^{1}H NMR (399 MHz, Chloroform-d) δ 9.05 (s, 1H), 8.18 (s, 1H), 8.03 (s, 1H), 7.47 – 7.31 (m, 5H), 5.28 – 5.16 (m, 2H), 4.06 – 3.60 (m, 5H), 3.52 (dd, J = 3.5, 11.9 Hz, 1H), 3.22 – 3.10 (m, 1H), 2.88 – 2.63 (m, 3H), 1.91 – 1.64 (m, 2H), 1.44 (s, 9H); MS (ESI): *m/z* = 490.6 (MH^+), 82% pure by LCMS.



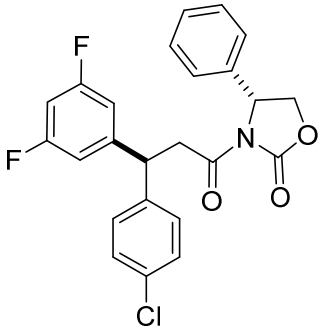
tert-Butyl (2R,5S)-2-(2-(3-amino-5-fluoropyridin-4-yl)ethyl)-5-((((2,2,2-trifluoroethyl)carbamoyl)oxy)methyl)morpholine-4-carboxylate (25)

A solution of tert-butyl (2R,5R)-2-(2-(3-(((benzyloxy)carbonyl)amino)-5-fluoropyridin-4-yl)ethyl)-5-(hydroxymethyl)morpholine-4-carboxylate (**23**) (652 mg, 1.33 mmol) and CDI (270 mg, 1.67 mmol) in pyridine (7 ml) was heated at 60 ^{o}C for 90 min. 2,2,2-Trifluoroethylamine (2.09 ml, 26.6 mmol) was added and the reaction heated at 60 ^{o}C overnight. The solution was diltuted with EtOAc then washed with water and brine, then dried over $MgSO_4$ and concentrated *in vacuo*. Purification on silica gel, eluting with a gradient of 0 to 60 % EtOAc in hexanes, afforded 402 mg of tert-butyl (2R,5S)-2-(2-(3-(((benzyloxy)carbonyl)amino)-5-fluoropyridin-4-yl)ethyl)-5-((((2,2,2-trifluoroethyl)carbamoyl)oxy)methyl)morpholine-4-carboxylate. This material was dissolved in nitrogen degassed EtOH and added to a suspension of 10 % Pd/C (60mg) in EtOH, total EtOH (10 ml). The reaction was evacuated and backfilled with hydrogen and stirred overnight under a balloon of hydrogen. The reaction was evacuated and backfilled with nitrogen and filtered through a pad of celite. The solvent was removed in vacuo to afford the title compound (286 mg, 45%) as a foam. ^{1}H NMR (399 MHz, Chloroform-d) 7.86 (d, J = 2.4 Hz, 1H), 7.84 (s, 1H), 5.18 – 5.00 (m, 1H), 4.45 – 3.58 (m, 11H), 3.33 – 3.20 (m, 1H), 2.89 – 2.62 (m, 2H), 1.79 – 1.67 (m, 2H), 1.44 (s, 9H); MS (ESI): *m*/*z* = 481.6 (MH^+), 76% pure by LCMS.



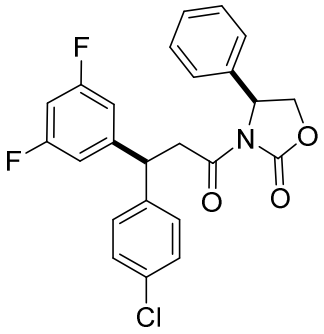
(R,E)-3-(3-(3,5-Difluorophenyl)acryloyl)-4-phenyloxazolidin-2-one (32)

A 2.5M solution of ^{n}BuLi in hexanes (28.6 ml, 71.5 mmol) was added dropwise to a solution of (R)-4-phenyloxazolidin-2-one (11.7 g, 71.5 mmol) in THF (400 ml) at -10 ^{o}C and the resulting solution stirred at this temperature for 10 min. A solution of (E)-3-(3,5-difluorophenyl)acryloyl chloride (14.5 g, 71.5 mmol) in THF (100 ml) was added dropwise and the resulting reaction stirred at 0^{o}C for 30 min. The reaction was quenched with 20% aq. NH_4Cl, and the crude product was extracted with EtOAc (x3). The combined organic fractions washed with water, sat. aq. $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated *in vacuo*. Purification on silica gel, eluting with a gradient of 0 to 50 % A to B (A = 1:1 hexanes:$CHCl_3$, B = EtOAc) afforded the title compound (20 g, 86 %) as a solid. ^{1}H NMR (399 MHz, Chloroform-d) δ 7.91 (d, J = 15.7 Hz, 1H), 7.64 (d, J = 15.7 Hz, 1H), 7.46-7.32 (m, 5H), 7.15 – 7.03 (m, 2H), 6.84 (tt, J = 2.3, 8.7 Hz, 1H), 5.55 (dd, J = 3.9, 8.7 Hz, 1H), 4.76 (t, J = 8.8 Hz, 1H), 4.35 (dd, J = 3.9, 8.9 Hz, 1H); MS (ESI): *m/z* = 330.3 (MH^+), 96% pure by LCMS.



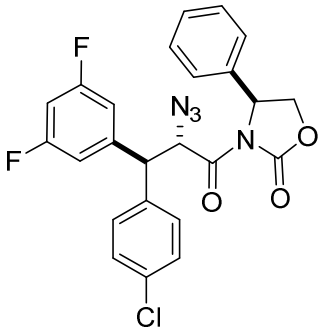
(R)-3-((S)-3-(4-chlorophenyl)-3-(3,5-difluorophenyl)propanoyl)-4-phenyloxazolidin-2-one (34)

A 1M solution of (4-Chlorophenyl)magnesium bromide in THF (76 ml, 76 mmol) was added to $CuBr.SMe_2$ (15.6 g, 76 mmol) in THF (100 ml) at -40 ^{o}C. A solution of (R,E)-3-(3-(3,5-difluorophenyl)acryloyl)-4-phenyloxazolidin-2-one (**32**) (10.0 g, 30.4 mmol) in THF (100 ml) was added dropwise and the resulting reaction stirred at -40 ^{o}C for 1.5 h, then allowed to warm slowly to ambient temperature. The reaction was quenched with 20% aq. NH_4Cl, and the crude product was extracted with EtOAc (x3). The combined organic fractions washed with water and brine, dried over $MgSO_4$, and concentrated *in vacuo*. Purification on silica gel, eluting with a gradient of 0 to 50 % A to B (A = 1:1 hexanes:$CHCl_3$, B = EtOAc) afforded the title compound (13.6 g, 100 %) as a solid. ^{1}H NMR (399 MHz, Chloroform-d) δ 7.35 – 7.16 (m, 5H), 7.18 – 7.04 (m, 4H), 6.79 – 6.69 (m, 2H), 6.63 (tt, J = 2.3, 8.9 Hz, 1H), 5.36 (dd, J = 3.9, 8.7 Hz, 1H), 4.67 (t, J = 8.8 Hz, 1H), 4.53 (t, J = 7.7 Hz, 1H), 4.25 (dd, J = 3.9, 8.9 Hz, 1H), 3.80 (dd, J = 7.4, 16.9 Hz, 1H), 3.58 (dd, J = 8.1, 16.9 Hz, 1H); MS (ESI): *m/z* = 442.4 (MH^+), 88% pure by LCMS.



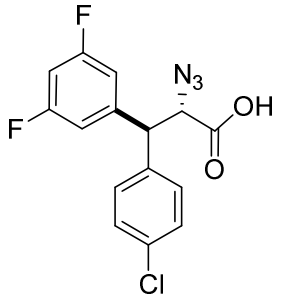
(S)-3-((S)-3-(4-chlorophenyl)-3-(3,5-difluorophenyl)propanoyl)-4-phenyloxazolidin-2-one (36)

A solution of aq. 35 % H_2O_2 (5.15 ml, 58.8 mmol) then solid LiOH (705 mg, 29.4 mmol) were added to a solution of (R)-3-((S)-3-(4-chlorophenyl)-3-(3,5-difluorophenyl)propanoyl)-4-phenyloxazolidin-2-one (**34**) (6.50 g, 14.7 mmol) in THF:H_2O 3:1 (120 ml) at 0 ^oC. The reaction was stirred at this temperature for 30 min. A solution of Na_2SO_3 (7.42 g, 58.8 mmol) in H_2O was added followed by 0.5M $NaHCO_3$ (147 ml, 73.6 mmol) and the resulting reaction stirred for 5min. The THF was removed *in vacuo* and the residue diluted with water and washed with CH_2Cl_2 (x3). The aqueous layer was acidified with 6M HCl and the crude product was extracted with EtOAc (x3). The combined organic fractions were dried over $MgSO_4$, and concentrated *in vacuo* to afford (S)-3-(4-chlorophenyl)-3-(3,5-difluorophenyl)propanoic acid as a solid. The crude acid was dissolved in CH_2Cl_2 (50 ml) and SO_2Cl_2 (2.88 ml, 39.4 mmol) was added. The resulting reaction was heated at reflux for 4 h. The solvent was removed *in vacuo* to afford (S)-3-(4-chlorophenyl)-3-(3,5-difluorophenyl)propanoyl chloride as an oil, used crude in the following reaction. A 2.5M solution of ^nBuLi in hexanes (5.25 ml, 13.1 mmol) was added dropwise to a solution of (S)-4-phenyloxazolidin-2-one (2.14 g, 13.1 mmol) in THF (90 ml) at -10 ^oC and the resulting solution stirred at this temperature for 10 min. The crude acid chloride isolated above was dissolved in THF (60 ml) and added dropwise and the resulting reaction stirred at 0^oC for 30 min. The reaction was quenched with 20% aq. NH_4Cl, and the crude product was extracted with EtOAc (x3). The combined organic fractions washed with water, sat. aq. $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated *in vacuo*. Purification on silica gel, eluting with a gradient of 0 to 50 % A to B (A = 1:1 hexanes:$CHCl_3$, B = EtOAc) afforded the title compound (4.7 g, 81 %) as a solid. ^1H NMR (399 MHz, Chloroform-d) δ 7.34 – 7.28 (m, 3H), 7.26 – 7.18 (m, 2H), 7.18 – 7.05 (m, 4H), 6.77 – 6.64 (m, 2H), 6.60 (tt, J = 2.3, 8.8 Hz, 1H), 5.34 (dd, J = 3.9, 8.7 Hz, 1H), 4.65 (t, J = 8.9 Hz, 1H), 4.55 (t, J = 7.6 Hz, 1H), 4.26 (dd, J = 4.0, 8.9 Hz, 1H), 3.87 – 3.49 (m, 2H).



(S)-3-((2S,3S)-2-azido-3-(4-chlorophenyl)-3-(3,5-difluorophenyl)propanoyl)-4-phenyloxazolidin-2-one (38)

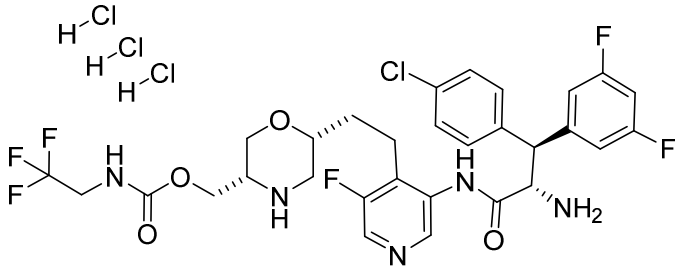
A solution of (S)-3-((S)-3-(4-chlorophenyl)-3-(3,5-difluorophenyl)propanoyl)-4-phenyloxazolidin-2-one (**36**) (4.70 g, 10.6 mmol) in THF(30 ml) was added dropwise to a 1M solution of NaHMDS in THF (11.7 ml, 11.7 mmol) at -78 ^{o}C. The resulting reaction was stirred at this temperature for 30 min. Solid trisylazide (4.28 g, 13.8 mmol) was added in one portion and the reaction stirred for 2 min, then acetic acid (3.65 ml, 63.8 mmol) and tetramethylamonium acetate (5.67 g, 42.5 mmol) were added. The cooling bath was removed and the reaction stirred at ambient temperature for 4 h. The reaction was quenched with brine and the crude product was extracted with EtOAc (x3). The combined organic fractions washed with water, sat. aq. $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated *in vacuo*. Purification on silica gel, eluting with a gradient of 0 to 40 % EtOAc in hexanes, afforded the title compound (3.23 g, 63 %) as a white solid. ^{1}H NMR (399 MHz, Chloroform-d) δ 7.44 – 7.33 (m, 3H), 7.32 – 7.27 (m, 2H), 7.27 – 7.25 (m, 1H), 7.25 – 7.23 (m, 1H), 7.23 – 7.17 (m, 2H), 6.95 – 6.85 (m, 2H), 6.73 (tt, J = 2.2, 8.8 Hz, 1H), 5.98 (d, J = 11.1 Hz, 1H), 5.13 (dd, J = 3.8, 8.6 Hz, 1H), 4.50 – 4.36 (m, 2H), 4.23 (dd, J = 3.8, 9.0 Hz, 1H); MS (ESI): *poor ionization,* 95% pure by LCMS.



(2S,3S)-2-azido-3-(4-chlorophenyl)-3-(3,5-difluorophenyl)propanoic acid (40)

A solution of aq. 35 % H_2O_2 (2.34 ml, 26.8 mmol) then solid LiOH (320 mg, 13.4 mmol) were added to a solution of (S)-3-((2S,3S)-2-azido-3-(4-chlorophenyl)-3-(3,5-difluorophenyl)propanoyl)-4-phenyloxazolidin-2-one (**38**)

(3.23 g, 6.69 mmol) in THF:H_2O 3:1 (60 ml) at 0 °C. The reaction was stirred at this temperature for 30 min. A solution of Na_2SO_3 (3.37 g, 26.8 mmol) in H_2O was added followed by 0.5 M $NaHCO_3$ (66.5 ml, 33.2 mmol) and the resulting reaction stirred for 5min. The THF was removed *in vacuo* and the residue diluted with water and washed with CH_2Cl_2 (x3). The aqueous layer was acidified with 6M HCl and the crude product was extracted with EtOAc (x3). The combined organic fractions were dried over $MgSO_4$, and concentrated *in vacuo* to afford the title compound (2.26 g, 100 %) as a white solid. ^{1}H NMR (399 MHz, Chloroform-d) δ 7.33 – 7.29 (m, 2H), 7.22 – 7.17 (m, 2H), 6.90 – 6.82 (m, 2H), 6.73 (tt, J = 2.3, 8.8 Hz, 1H), 4.53 – 4.45 (m, 2H).



((3S,6R)-6-(2-(3-((2S,3S)-2-Amino-3-(4-chlorophenyl)-3-(3,5-difluorophenyl)propanamido)-5-fluoropyridin-4-yl)ethyl)morpholin-3-yl)methyl (2,2,2-trifluoroethyl)carbamate trihydrochloride (9t – MK-8718)

$POCl_3$ (35 µl, 0.375 mmol) was added dropwise to a solution of (2S,3S)-2-azido-3-(4-chlorophenyl)-3-(3,5-difluorophenyl)propanoic acid (**40**) (105 mg, 0.312 mmol) and tert-butyl (2R,5S)-2-(2-(3-amino-5-fluoropyridin-4-yl)ethyl)-5-((((2,2,2-trifluoroethyl)carbamoyl)oxy)methyl)morpholine-4-carboxylate (**25**) (150 mg, 0.312 mmol) in pyridine (0.5 ml) at -10 °C and the reaction stirred for 30 min while allowing to warm to 0 °C. The reaction was quenched with sat. aq. $NaHCO_3$ and the crude product was extracted with CH_2Cl_2 (x3). The combined organic fractions washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification on silica gel, eluting with a gradient of 0 to 70 % EtOAc in hexanes, afforded 150 mg of tert-butyl (2R,5S)-2-(2-(3-((2S,3S)-2-azido-3-(4-chlorophenyl)-3-(3,5-difluorophenyl)propanamido)-5-fluoropyridin-4-yl)ethyl)-5-((((2,2,2-trifluoroethyl)carbamoyl)oxy)methyl)morpholine-4-carboxylate. This compond (150 mg, 0.187 mmol) and PPh_3 (73.8 mg, 0.281 mmol) were heated at reflux in 4:1 THF:H_2O (5 ml) overnight. The solvent was removed *in vacuo* and purification on silica gel, eluting with a gradient of 0 to 10 % MeOH in $CHCl_3$, afforded 136 mg of tert-butyl (2R,5S)-2-(2-(3-((2S,3S)-2-amino-3-(4-chlorophenyl)-3-(3,5-difluorophenyl)propanamido)-5-fluoropyridin-4-yl)ethyl)-5-((((2,2,2-trifluoroethyl)carbamoyl)oxy)methyl)morpholine-4-carboxylate. This material was dissolved

4M HCl in dioxane (1 ml) and stirred for 1 hour. The solvent was removed *in vacuo* to afford the title compound (59 mg, 55 %) as a white solid. ^{1}H NMR (600 MHz, DMSO-d6) δ 8.27 (s, 1H), 8.08 (s, 1H), 7.92 – 7.86 (m, 1H), 7.41 (d, J = 8.4 Hz, 2H), 7.33 (d, J = 8.4 Hz, 2H), 7.10 (d, J = 6.9 Hz, 2H), 7.05 (t, J = 9.3 Hz, 1H), 4.31 (d, J = 9.6 Hz, 1H), 4.26 (d, J = 9.6 Hz, 1H), 4.20 – 4.10 (m, 1H), 4.09 – 4.00 (m, 1H), 3.82 – 3.70 (m, 2H), 3.65 (d, J = 11.8 Hz, 1H), 3.52 (d, J = 8.8 Hz, 1H), 3.22 – 3.16 (m, 1H), 2.81 – 2.76 (m, 1H), 2.61 – 2.49 (m, 4H), 2.41 – 2.32 (m, 1H), 1.61 – 1.51 (m, 1H), 1.41 – 1.31 (m, 1H); ^{13}C NMR (126 MHz, DMSO-d6) δ 174.3, 164.2 (d, J_{C-F} = 14 Hz), 162.2 (d, J_{C-F} = 13 Hz), 159.8, 157.6 (d, J_{C-F} = 53 Hz), 147.4 (t, J_{C-F} = 9 Hz), 143.8, 141.3, 135.7 (d, J_{C-F} = 24 Hz), 134.5 (d, J_{C-F} = 3 Hz), 133.1 (d, J_{C-F} = 14 Hz), 132.4, 131.0, 125.7 (q, J_{C-F} = 279 Hz), 112.9 – 112.6 (m), 102.8 (t, J_{C-F} = 26 Hz), 75.9, 77.0, 63.8, 59.2, 54.9, 50.9, 46.2, 42.7 (q, J_{C-F} = 33 Hz), 31.7, 20.4; Optical rotation: $[\alpha]_D^{25}$ + 37.2 (c 1.12, MeOH); HRMS: Calcd for $C_{30}H_{32}ClF_6N_5O_4$ (MH)+ 674.1963, found 674.1950; 97% pure by LCMS.

Enzyme bound conformation of MK-8718 extracted from crystal structure of MK-8718 bound to HIV Protease