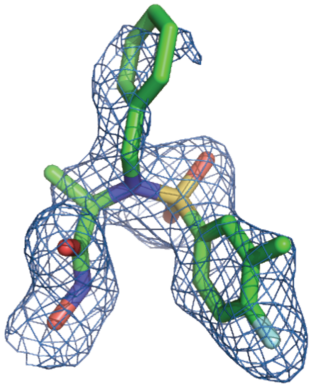
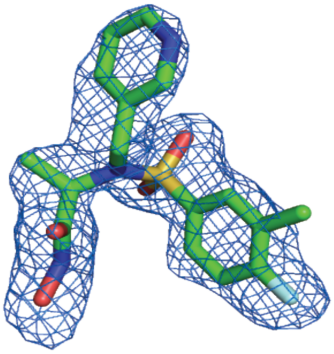
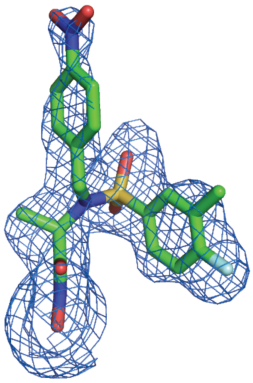
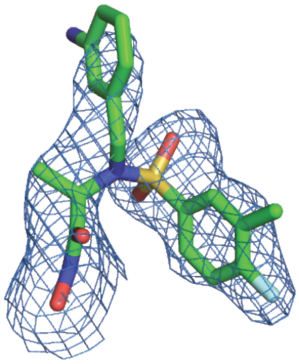
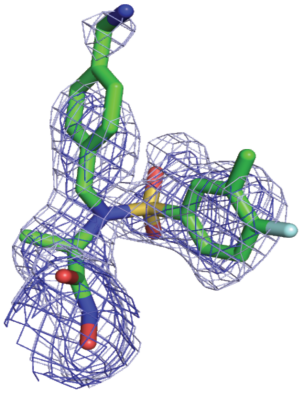
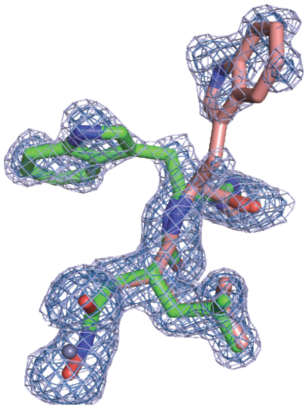
Supporting Information

Anthrax Toxin Lethal Factor Domain 3 is Highly Mobile and Responsive to Ligand Binding

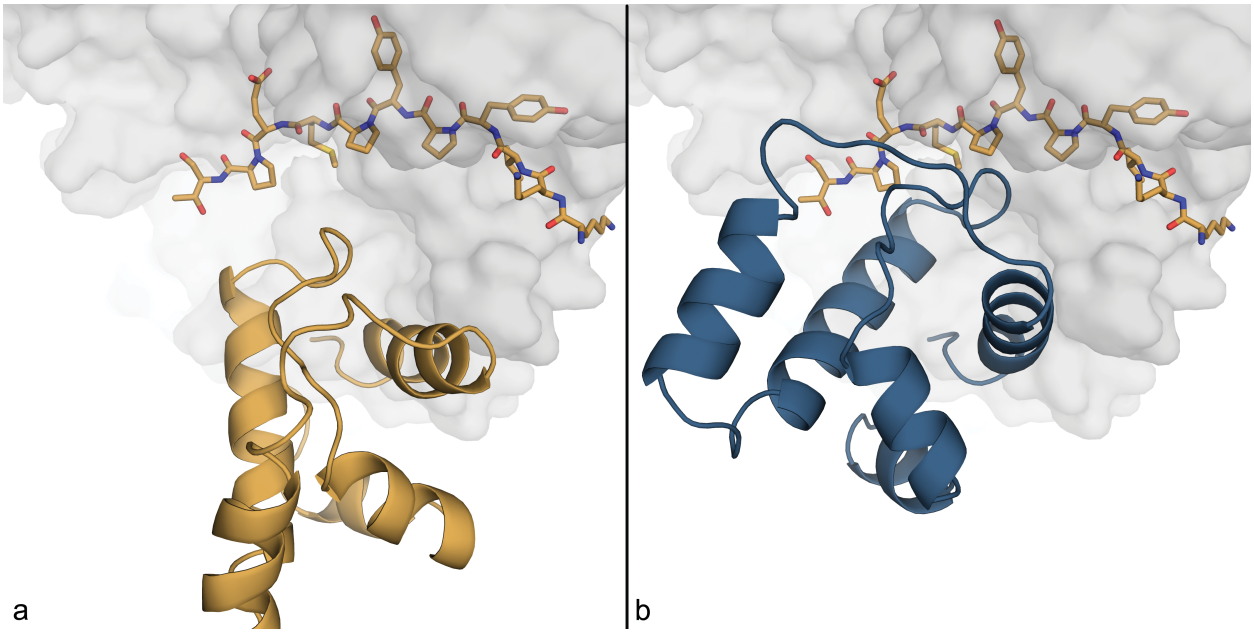
Kimberly M. Maize, Elbek K. Kurbanov, Teresa De La Mora-Rey, Todd W. Geders, Dong-Jin Hwang, Michael A. Walters, Rodney Johnson, Elizabeth A. Amin, Barry C. Finzel

Contents of Supplemental Information

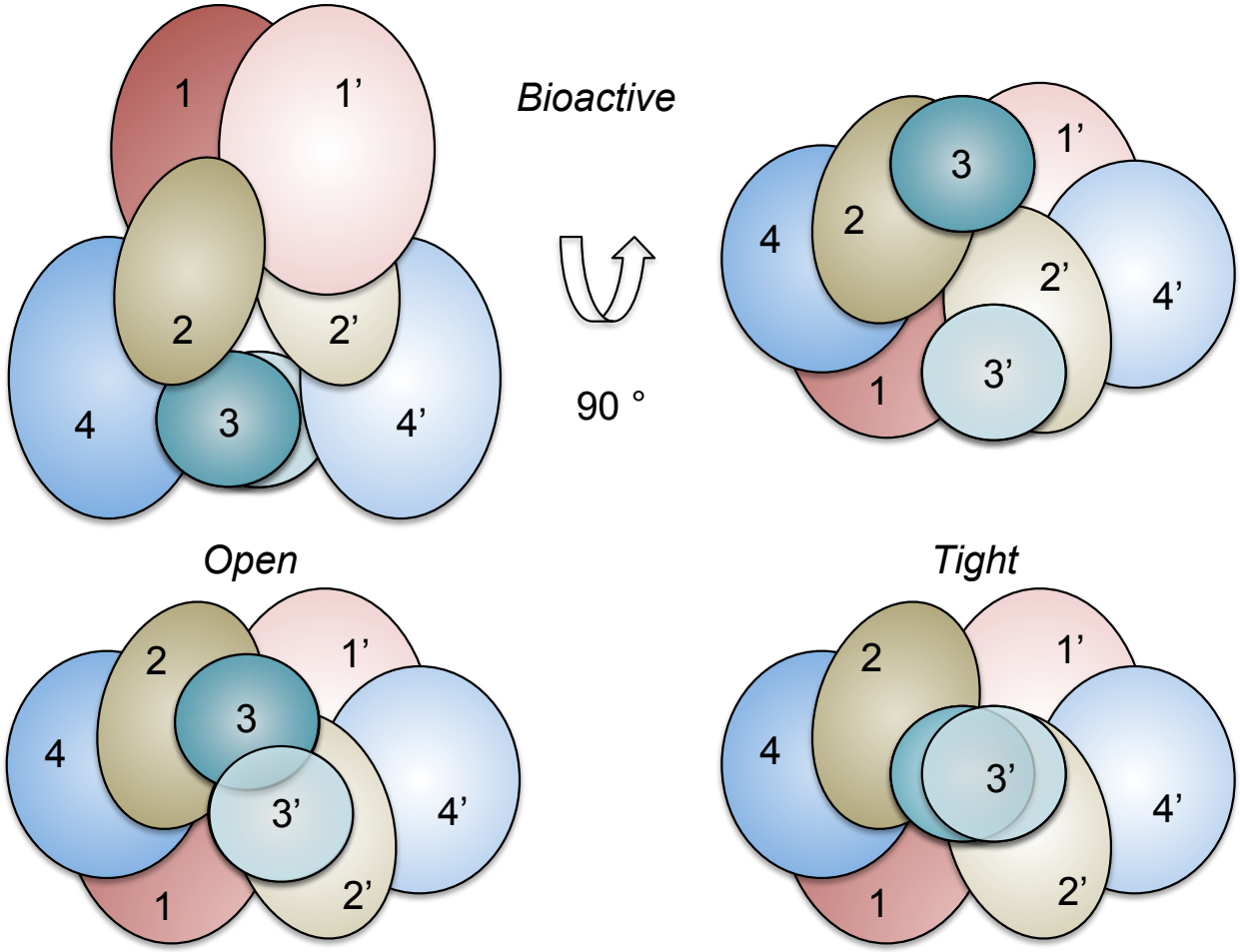
Table S1. Omit Maps of Crystallized Ligands. For all ligands, mFo-DFc density is shown at 3.0σ in dark blue. For compounds **16** and **17**, the 2.0σ density is shown in lighter blue to capture more detailed aspects of the ligand.

	n/a			
Compound	**apo**	**10**	**11**	**13**
PDB ID code	4PKQ	4PKR	4PKS	4PKT
				
Compound		**15**	**16**	**17**
PDB ID code		4PKU	4PKV	4PKW

Supplemental Animation S2. (Separate file) Morph of the *bioactive* Domain 3 position (residues 300-385) observed in 1JKY (a; orange) to the *tight* Domain 3 position in the apo structure 4PKQ (b; blue). Possible intermediate *open* positions in yellow. To define the active site, the peptide substrate of 1PWW is shown as orange sticks. Animated movie is in a separate file.

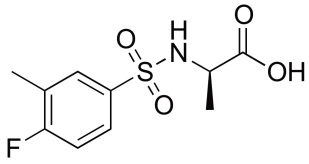


Supplemental Figure S3. Noncrystallographic packing in the Monoclinic crystal form is incompatible with the *open* and *tight* ligand-bounds conformational states. Cartoon representation of the dimer in the asymmetric unit illustrates packing of domains 1-4 in two molecules of the monoclinic a.u., A transition from the *bioactive* (top) to *open* (lower left) or *tight* states (lower right) would require that domain 3 pass through itself.



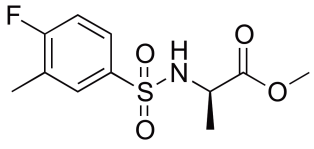
Supplemental Methods

General Synthesis Information. Chemical reagents were purchased from commercial sources and used without additional purification. Bulk solvents were from Fisher Scientific and anhydrous *N,N'*-dimethylformamide (DMF) was purchased from EMD Chemicals. Reactions were performed under an atmosphere of dry N_2 unless otherwise noted. Silica gel chromatography was performed on self-packed columns with SiliaFlash 60Å silica gel (SiliCycle). Preparatory thin layer chromatography (TLC) was performed on plates with glass backed SiliaPlate 60Å silica gel (SiliCycle). Compounds used in biological testing were no less than 90% pure as determined by two-wavelength HPLC analysis (254 and 215 nm). Nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$, CD_3OD, or DMSO-d_6 on a Varian instrument operating at 400 MHz (for ^1H) and 100 MHz (for ^{13}C) at ambient temperature. Chemical shifts are reported in parts per million and normalized to internal solvent peaks or tetramethylsilane (0 ppm).



(*R*)-2-(3-Fluoro-4-methylphenylsulfonamido)propanoic acid (3)

D-Alanine (**1**, 2.5 g, 28.1 mmol) was added to a solution of K_2CO_3 (8.3 g, 59.9 mmol) in dioxane/water (60 mL, 1:1, v/v). A solution of 4-fluoro-3-methylphenyl-sulfonylchloride (5.0 g, 24.0 mmol) in dioxane (4 mL) was added immediately after with vigorous stirring. The mixture was stirred at rt overnight. Upon consumption of the starting material as determined by TLC, the solvent was reduced to one third the reaction volume under reduced pressure, and DCM was added to extract the organic layer. The aq. layer was acidified with conc. HCl to pH 1 and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (1 x 20 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield **3** as a white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 9.57 (bs, 1H), 7.55-7.48 (m, 2H), 7.33 (m, 1H), 5.42 (d, *J* = 8.0 Hz, 1H, NH), 4.03 (m, 1H), 2.33 (s, 3H), 1.33 (d, *J* = 6.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.0, 160.5 (d, *J* = 49.0 Hz), 138.8 (d, *J* = 6.8 Hz), 132.8 (d, *J* = 4.5 Hz), 131.2 (d, *J* = 17.5 Hz), 122.6 (d, *J* = 3.8 Hz), 114.1 (d, *J* = 2.5 Hz), 51.2, 19.6, 14.8.

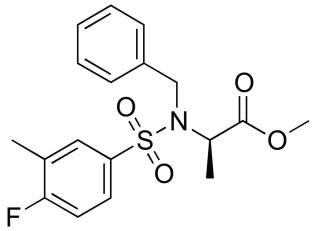


(*R*)-Methyl 2-(4-fluoro-3-methylphenylsulfonamido)propanoate (4)

To a solution of **3** (4.9 g, 17.8 mmol) in MeOH (10 mL) was added a catalytic amount of conc. H_2SO_4. The reaction was heated to reflux. After 6 h, the solution was concentrated under reduced pressure and the resulting residue was dissolved in EtOAc (10 mL). This solution was washed with water (20 mL), sat. aq. $NaHCO_3$ (20 mL), and brine (20 mL), then dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to yield **4** as a white solid (5.15 g, 78%, two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (m, 1H), 7.67 (m, 1H), 5.54 (d, *J* = 8.4 Hz, 1H), 4.01 (m, 1H), 3.50 (s, 3H), 2.33 (s, 3H), 1.43 (d, *J* = 7.4 Hz, 0.5 H), 1.38 (d, *J* = 7.4 Hz, 2.5 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 163.8 (d, *J* = 251.9 Hz), 135.4 (d, *J* = 3.0 Hz), 130.9 (d, *J* = 6.8 Hz), 127.1 (d, *J* = 9.8 Hz), 126.4 (d, *J* = 18.3 Hz), 115.8 (d, *J* = 24.2 Hz), 52.7, 51.5, 19.9, 14.6.

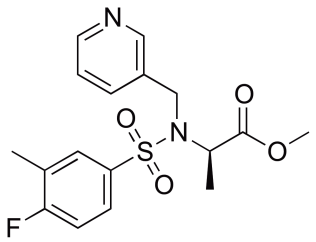
General Procedure for N-Alkylation (5-9)

To a solution of **4** (110 mg, 0.4 mmol) and benzyl bromide (80 µL, 0.5 mmol) in anhydrous DMF (2 mL) was added K_2CO_3 (275.0 mg, 2.0 mmol). The reaction mixture was stirred at rt. After 48 h, the solvent was removed under reduced pressure and the resulting residue was mixed with H_2O. This mixture was extracted with EtOAc (3 x 15 mL) and the combined extracts were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified over SiO_2 using an eluent of EtOAc/hexane (1/4, v/v).

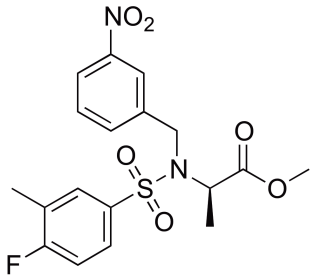


(R)-methyl 2-(N-benzyl-4-fluoro-3-methylphenylsulfonamido)propanoate (5)

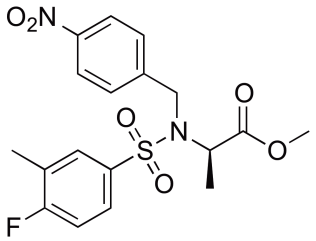
Colorless oil (120 mg, 82.0%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (m, 2H), 7.32-7.23 (m, 5H), 7.08 (t, *J* = 9.2 Hz, 1H), 4.65 (q, *J* = 7.2 Hz, 1H), 4.57 (d, *J* = 16.0 Hz, 1H), 4.40 (d, *J* = 16.0 Hz, 1H), 3.47 (s, 3H), 2.30 (s, 3H), 1.30 (d, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 163.6 (d, *J* = 252 Hz), 137.1, 135.7 (d, *J* = 3.0 Hz), 131.1 (d, *J* = 6.0 Hz), 128.4, 128.1, 127.6, 127.3 (d, *J* = 9.1 Hz), 126.0 (d, *J* = 18.2 Hz), 115.6 (d, *J* = 23.6 Hz), 55.3, 52.1, 49.2, 16.6, 14.6 (d, *J* = 3.8 Hz).



(R)-methyl 2-(4-fluoro-3-methyl-N-(pyridin-3-ylmethyl)phenylsulfonamido)propanoate (6)
Colorless oil (126 mg, 79 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (m, 2H), 7.90 (m, 1H), 7.62 (m, 2H), 7.27-7.23 (m, 1H), 7.10 (t, *J* = 8.8 Hz, 1H), 4.71 (q, *J* = 7.2 Hz, 1H), 4.60 (d, *J* = 16.8 Hz, 1H), 4.44 (d, *J* = 16.4 Hz, 1H), 3.50 (s, 3H), 2.31 (s, 3H), 1.32 (d, *J* = 6.8 Hz, 3.0 H).^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 163.7 (d, *J* = 252.8 Hz), 149.2, 149.0, 135.9, 135.3 (d, *J* = 3.0 Hz), 133.2, 131.0 (d, *J* = 6.1 Hz), 127.3 (d, *J* = 9.1 Hz), 126.3 (d, *J* = 18.2 Hz), 123.4, 115.8 (d, *J* = 24.3 Hz), 55.3, 52.2, 46.6, 16.9, 14.6 (d, *J* = 3.1 Hz).

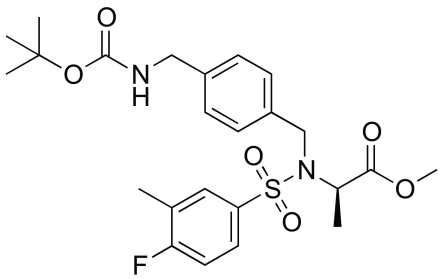


(*R*)-Methyl 2-(4-fluoro-3-methyl-*N*-(3-nitrobenzyl)phenylsulfonamido)propanoate (7)
Colorless oil (147 mg, 83.3%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14 (m, 2H), 7.77 (m, 1H), 7.63 (m, 2H), 7.51 (m, 1H), 7.08 (t, *J* = 7.6 Hz, 1H), 4.76 (m, 1H), 4.69 (d, *J* = 17.2Hz, 1H), 4.52 (d, *J* = 17.2Hz, 1H), 3.52 (s, 3H), 2.31 (s, 3H), 1.32 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 163.8 (d, *J* = 252.7 Hz), 148.2, 140.1, 135.5 (d, *J* = 3.8 Hz), 133.8, 131.0 (d, *J* = 6.1 Hz), 129.4, 127.3 (d, *J* = 9.2 Hz), 126.4 (d, *J* = 18.2 Hz), 122.5, 122.4, 115.7 (d, *J* = 23.5 Hz), 55.5, 52.2, 48.3, 17.0, 14.6.



(R)-methyl 2-(4-fluoro-3-methyl-N-(4-nitrobenzyl)phenylsulfonamido)propanoate (8)
Colorless oil (155 mg, 52 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, *J* = 8.0 Hz 2H), 7.66-7.57 (m, 4H), 7.13 (t, *J* = 8.0 Hz, 1H), 4.77-4.71 (m, 2H), 4.52 (d, *J* = 16.0 Hz, 1H), 3.50 (s, 3H), 2.33

(s, 3H), 1.30 (d, *J* = 4.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 163.9 (d, *J* = 253.0 Hz), 147.32, 145.82, 134.9 (d, *J* = 3.0 Hz), 131.1 (d, *J* = 7.0 Hz), 128.35, 127.3 (d, *J* = 9.0 Hz), 126.4 (d, *J* = 19.0 Hz), 123.6, 115.8 (d, *J* = 24.0 Hz), 55.5, 52.2, 48.5, 17.1, 14.6 (d, *J* = 3 Hz).

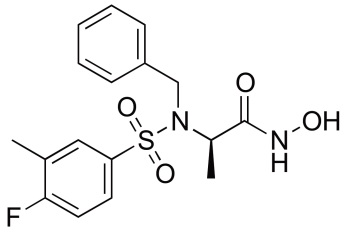


(R)-methyl 2-(N-(4-(((tert-butoxycarbonyl)amino)methyl)benzyl)-4-fluoro-3-methylphenylsulfonamido)propanoate (9)

Colorless oil (380 mg, 71 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65-7.62 (m, 2H), 7.28 (d, *J* = 8.4 Hz, 2H), 7.20 (d, *J* = 8.0 Hz, 2H), 7.09 (t, *J* = 8.4 Hz, 1H), 4.91 (s, 1H), 4.63 (q, *J* = 7.6 Hz, 1H), 4.55 (d, *J* = 16.8 Hz, 1H), 4.38 (d, *J* = 16.4 Hz, 1H), 4.28 (s, 2H), 3.48 (s, 3H), 2.32 (s, 3H), 1.46 (s, 9H), 1.30 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.57, 163.65 (d, *J* = 252.0 Hz), 155.89, 138.47, 136.29, 136.10 (d, *J* = 3.7 Hz), 131.12 (d, *J* = 6.1 Hz), 128.21, 127.46, 127.33 (d, *J* = 9.9 Hz), 126.08 (d, *J* = 18.2 Hz), 115.63 (d, *J* = 23.6 Hz), 79.55, 55.27, 52.06, 48.94, 44.27, 28.40, 16.75, 14.59 (d, *J* = 3.0 Hz).

General Procedure for conversion of esters to hydroxamic acids (10-14)

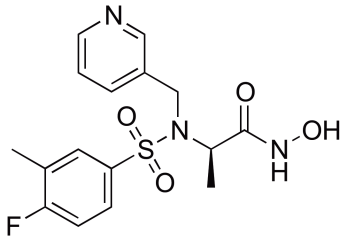
To a solution of **5** in MeOH (1.0 mL) was added hydroxylamine hydrochloride (46 mg, 0.7 mmol) and NaOMe (225 µL, 1.0 mmol, 25 wt %) in MeOH at 273 K. The reaction was allowed to gradually warm to rt and was stirred overnight. After 16 h, the solvent was removed under reduced pressure and the resulting residue suspended in brine. The aq. layer was extracted with EtOAc (3 x 10 mL), and the combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by preparatory TLC using DCM/MeOH (19/1, v/v).



(R)-2-(N-benzyl-4-fluoro-3-methylphenylsulfonamido)-N-hydroxypropanamide (10)

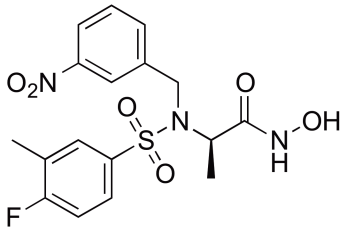
White foam (65 mg, 54%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.63-7.58 (m, 2H), 7.35-7.20 (m, 5H), 7.13 (t, *J* = 8.8 Hz, 1H), 4.69 (m, 2H), 4.53 (q, *J* = 6.8 Hz 1H), 2.26 (s, 3H), 1.21 (d, *J* = 7.2 Hz,

3H). ^{13}C NMR (100 MHz, CD_3OD) δ 168.68, 163.56 (d, *J* = 250.5 Hz), 138.40, 135.75 (d, *J* = 3.1 Hz), 130.80 (d, *J* = 6.1 Hz), 127.82, 127.60, 127.09 (d, *J* = 9.9 Hz), 126.81, 125.97 (d, *J* = 19.0 Hz), 115.23 (d, *J* = 23.5 Hz), 53.1, 16.4, 13.07 (d, *J* = 3.8 Hz). MS (ESI) 367.22 $[M + H]^+$.



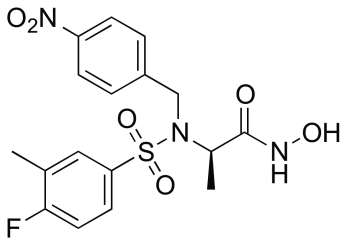
(R)-2-(4-fluoro-3-methyl-N-(pyridin-3-ylmethyl)phenylsulfonamido)-N-hydroxypropanamide (11)

White foam (35 mg, 82%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.53-8.40 (m, 2H), 7.88-7.86 (m, 1H), 7.66-7.64 (m, 2H), 7.37-7.35 (m, 1H), 7.17 (t, *J* = 9.2 Hz, 1H), 4.80-4.69 (m, 2H), 4.60-4.55 (m, 1H), 2.30 (s, 3H), 1.22 (d, *J* = 7.2 Hz, 3.0 H). ^{13}C NMR (100 MHz, CD_3OD) δ 168.4, 163.7 (d, *J* = 250.5 Hz), 148.2, 147.2, 136.6, 135.3 (d, *J* = 3.8 Hz), 130.8 (d, *J* = 6.0 Hz), 127.2 (d, *J* = 9.9 Hz), 126.3 (d, *J* = 19.0 Hz), 123.5, 115.4 (d, *J* = 24.3 Hz), 52.9, 45.5, 16.1, 13.0 (d, *J* = 3.0 Hz). MS (ESI) 368.26 $[M + H]^+$.



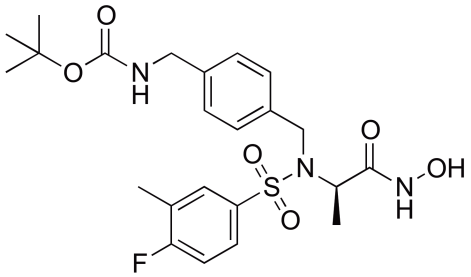
(*R*)-2-(4-Fluoro-3-methyl-*N*-(3-nitrobenzyl)phenylsulfonamido)-*N*-hydroxypropanamide (12)

White foam (92 mg, 73%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.95 (s, 1H), 8.87 (s, 1H), 8.17 (s, 1H), 8.07 (d, *J* = 8.4 Hz, 1H), 7.78 (d, *J* = 8 Hz, 1H), 7.70 (m, 1H), 7.65 (m, 1H), 7.58 (t, *J* = 8 Hz, 1H), 7.30 (t, *J* = 9.0 Hz, 1H), 4.80 (d, *J* = 17.4 Hz, 1H), 4.72 (d, *J* = 17.4 Hz, 1H), 4.46 (m, 1H), 2.25 (s, 3H), 1.10 (d, *J* = 7.2 Hz, 3.0 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.8, 164.1 (d, *J* = 252.8 Hz), 148.2, 139.1, 134.6 (d, *J* = 3.2 Hz), 134.2, 130.8 (d, *J* = 6.1 Hz), 129.6, 127.1 (d, *J* = 9.9 Hz), 127.0, 122.9, 122.8, 116.3 (d, *J* = 23.5 Hz), 53.0, 47.5, 14.9, 14.6. MS (ESI) 412.22 $[M + H]^+$.



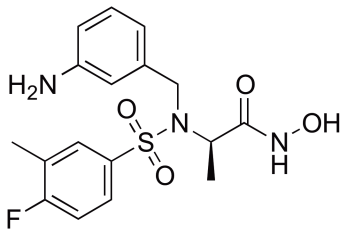
(R)-2-(4-fluoro-3-methyl-N-(4-nitrobenzyl)phenylsulfonamido)-N-hydroxypropanamide (13)

White foam (70 mg, 45 %). ^{1}H NMR (400 MHz, CD_3OD) δ 8.12 (d, *J* = 8.4 Hz 2H), 7.68-7.58 (m, 4H), 7.17 (t, *J* = 9.2 Hz, 1H), 4.88-4.72 (m, 2H), 4.57 (q, *J* = 6.4 Hz, 1H), 2.29 (s, 3H), 1.19 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 168.39, 163.80 (d, *J* = 250.4 Hz), 147.01, 146.84, 135.13 (d, *J* = 3.8 Hz), 130.86 (d, *J* = 6.0 Hz), 128.24, 127.25 (d, *J* = 9.1 Hz), 126.30 (d, *J* = 18.2 Hz), 122.87, 115.50 (d, *J* = 23.6 Hz), 52.89, 16.02, 13.04 (d, *J* = 3.8 Hz). MS (ESI) 412.1 $[M + H]^+$.



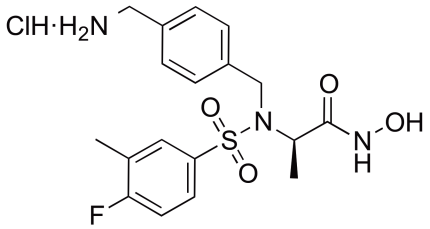
(R)-tert-butyl 4-((4-fluoro-N-(1-(hydroxyamino)-1-oxopropan-2-yl)-3-methylphenylsulfonamido)methyl)benzylcarbamate (14)

White foam (190 mg, 50 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.1 (s, 1H), 7.60-7.58 (m, 2H), 7.27 (d, *J* = 8.4 Hz, 2H), 7.21 (d, *J* = 7.6 Hz, 2H), 7.11 (t, *J* = 8.0 Hz, 1H), 4.99 (s, 1H), 4.62 (d, *J* = 15.6 Hz, 1H), 4.43 (brs, 1H), 4.28 (brs, 3H), 2.30 (s, 3H), 1.46 (s, 9H), 1.19 (d, *J* = 5.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.98, 163.86 (d, *J* = 253.5 Hz), 156.10, 138.80, 135.31, 135.04 (d, *J* = 3.7 Hz), 130.86 (d, *J* = 6.1 Hz), 128.80, 127.58, 127.06 (d, *J* = 9.1 Hz), 126.70 (d, *J* = 18.2 Hz), 116.06 (d, *J* = 23.5 Hz), 79.73, 53.17, 48.12, 44.30, 28.42, 14.61 (d, *J* = 3.0 Hz), 14.42.



(R)-2-(N-(3-aminobenzyl)-4-fluoro-3-methylphenylsulfonamido)-N-hydroxypropanamide (15)

To a solution of **12** (130 mg, 0.3 mmol) in DCM (10 mL) under an H_2 atmosphere was added palladium on activated carbon (Pd/C) (130.0 mg, 10 wt. %). The reaction was shaken in a Parr apparatus at 4 atm at rt. After 4 h, the reaction mixture was filtered through a Celite pad and the filtrate was concentrated under reduced pressure. The crude residue was purified by flash column chromatography on SiO_2 using acetone/hexane (1/3, v/v) to yield the **15** as white foam (98 mg, 82%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.61 (s, 1H), 8.78 (s, 1H), 7.62 (m, 2H), 7.26 (t, *J* = 9.2 Hz, 1H), 6.90 (t, *J* = 7.6 Hz, 1H), 6.53 (s, 1H), 6.40 (m, 2H), 5.30-4.95 (bs, 2H, NH_2), 4.55 (d, *J* = 16.8 Hz, 1H), 4.46 (d, *J* = 16.8 Hz, 1H), 4.37 (m, 1H), 1.17 (d, *J* = 6.8 Hz, 3.0 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.2, 163.8 (d, *J* = 263.5 Hz), 146.2, 137.4, 135.1, 130.9 (d, *J* = 6.0 Hz), 129.5, 127.1 (d, *J* = 9.1 Hz), 126.6 (d, *J* = 17.4 Hz), 119.1, (d, *J* = 24.3 Hz), 53.5, 48.5, 14.6, 14.1. MS (ESI) 382.33 $[M + H]^+$.



(R)-2-(N-(4-(aminomethyl)benzyl)-4-fluoro-3-methylphenylsulfonamido)-N-hydroxypropanamide hydrochloride (16)

Compound **14** (190 mg, 0.4 mmol) was treated with 4N HCl in dioxane (2 mL) at rt. After 30 min, the solvent was removed under reduced pressure, and the resulting residue was triturated with diethyl ether (3 x 3 mL) to yield **16** as white solid (160.0 mg, 97 %). ^{1}H NMR (400 MHz, CD_3OD) δ 7.72-7.65 (m, 2H), 7.48 (d, *J* = 8.0 Hz, 2H), 7.40 (d, *J* = 8.0 Hz, 2H), 7.20 (t, *J* = 8.8 Hz, 1H), 4.79 (d, *J* = 16.8 Hz, 1H), 4.65 (d, *J* = 16.8 Hz, 1H), 4.51 (q, *J* = 7.2 Hz, 1H), 4.10 (s, 2H), 2.32 (s, 3H), 1.16 (d, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 168.56, 163.75 (d, *J* = 250.5 Hz), 140.27, 135.33 (d, *J* = 3.0 Hz), 131.79, 130.81 (d, *J* = 6.0 Hz), 128.50, 128.11, 127.20 (d, *J* = 9.9 Hz), 126.26 (d, *J* = 18.2 Hz), 115.42 (d, *J* = 23.5 Hz), 52.88, 42.64, 16.09, 13.05 (d, *J* = 3.8 Hz). MS (ESI) 396.36 $[M + H]^+$.