Rational design of potent small molecule SMARCA2/A4 (BRM/BRG1) degraders acting via recruitment of FBXO22

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General methods, procedure and characterization of SMARCA2/A4 ligands and monovalent degraders

General Methods.

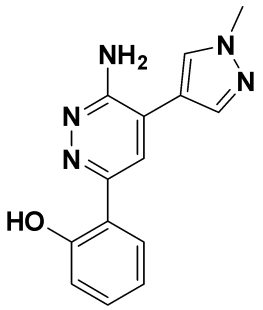
All commercial solvents and reagents were used without additional purification unless indicated otherwise. ^{1}H NMR spectra were recorded on Bruker Avance 400 MHz spectrometer with a Prodigy BBO CryoProbe. ^{13}C NMR spectra were recorded at 101 MHz. Chemical shifts are expressed in δ ppm (NMR description, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad peak). NMR spectra were acquired at 298 K in DMSO-d6, $CDCl_3$ or

CD_3OD and chemical shifts referenced to 2.50 ppm in ^1H and 39.51 ppm in ^{13}C and to 7.26 ppm and 3.31 ppm for ^1H in $CDCl_3$ and CD_3OD respectively. All coupling constants (J) are reported in Hertz. Reaction progress was monitored by either a Shimadzu LCMS/UV system with an LC-30 AD solvent pump, Sil-30 AC autosampler, 2020 MS, SPDM30A UV detector, and CTO-20A column oven, using 2−98% acetonitrile/0.1% formic acid (or 0.01% ammonia) over 2.5 min or a Waters Acquity LCMS system using 2−98% acetonitrile/0.1% formic acid (or 0.1% ammonia) over 2 min. Flash column chromatography purifications were performed using a Teledyne Isco Combiflash Rf and Silicycle HP columns. Reverse-phase purification was done on a Phenomenex Gemini-NX C18 (30 × 100 mm, 5 μm) with a gradient of 5−95% acetonitrile/water (with 0.1% NH_4OH or 0.1% formic acid) at 60 mL/min over 10 min. High-resolution mass spectrometry (HRMS) of final compounds were analyzed on a Dionex Ultimate 3000 coupled with Thermo Scientific Q Exactive HRMS using ESI as ionization source. The LC separation was done on a Phenomenex XB-C18, 1.7μm, 50 × 2.1 mm column at a flow rate of 0.4 ml / minute. MPA (mobile phase A) was water with 0.1% FA and MPB (mobile phase B) was acetonitrile with 0.1% FA. The gradient started at 2 % MPB and ended at 98% MPB over 7 min and held at 98% MPB for 1.5 min following an equilibration for 1.5 min. The LC column temperature was 40 ºC. UV absorbance was collected by a DAD detector and mass spec full scan was applied to all experiments. Unless stated otherwise, analytical purity was >95% as determined by LCMS using UV 254 nm detection.

Procedure and characterization.

Compound 1

2-(6-amino-5-(1-methyl-1H-pyrazol-4-yl)pyridazin-3-yl)phenol



Compound 1 was made according to the reported procedure from WO2016138114A1.

^{1}H NMR (400 MHz, DMSO-d_6): δ 13.81 (s, 1H), 8.39 (s, 1H), 8.18 (s, 1H), 8.17 (s, 1H), 7.99 (d, J = 7.2 Hz, 1H), 7.26 (t, J = 3.6 Hz, 1H), 6.94 - 6.91 (m, 2H), 6.49 (s, 2H), 3.93 (s, 3H). LCMS (ESI): *m/z* 267.9 $(M+H)^+$.

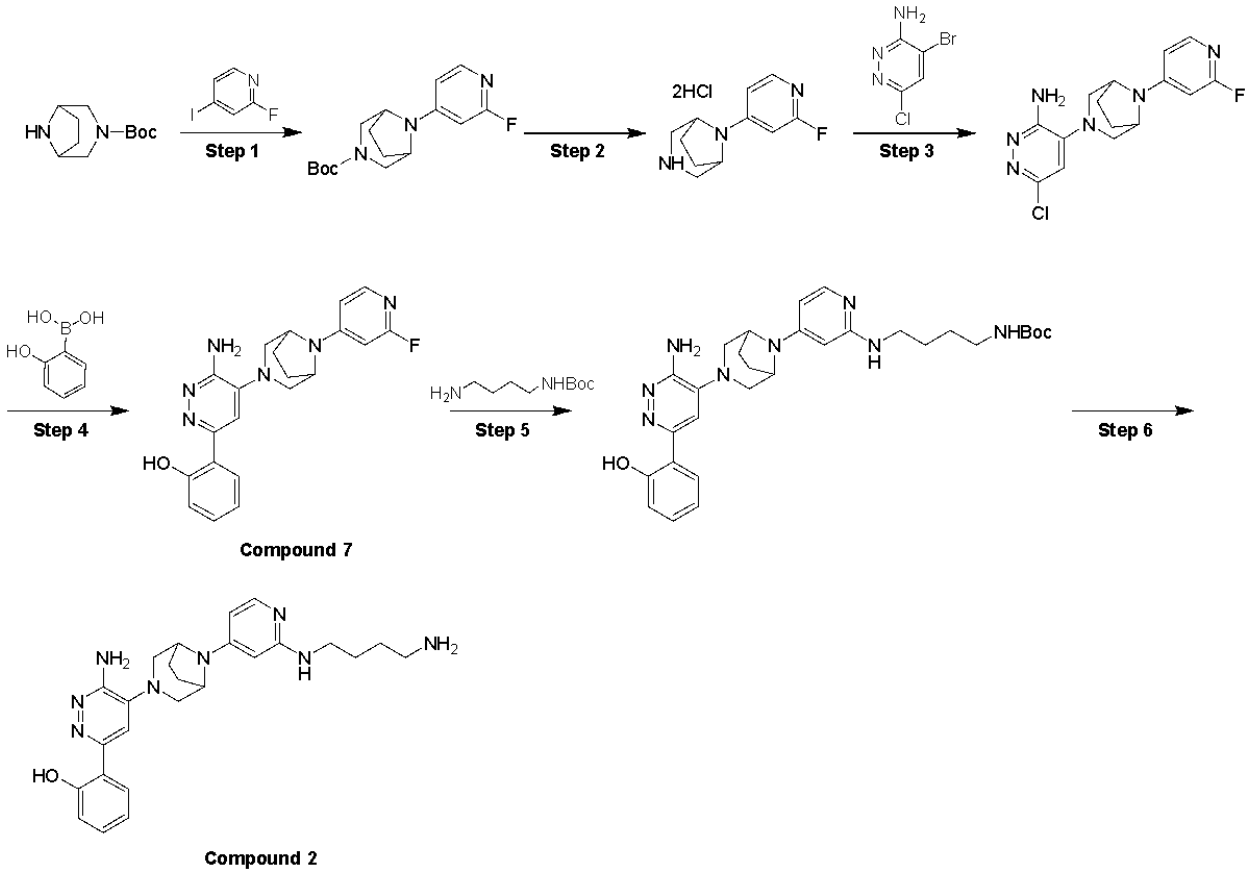
Compound 2 & Compound 7

Compound 2:

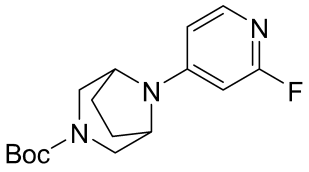
2-(6-amino-5-(8-(2-fluoropyridin-4-yl)-3,8-diazabicyclo[3.2.1]octan-3-yl)pyridazin-3-yl)phenol

Compound 7:

2-(6-amino-5-(8-(2-fluoropyridin-4-yl)-3,8-diazabicyclo[3.2.1]octan-3-yl)pyridazin-3-yl)phenol



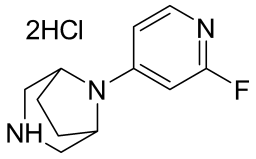
Step 1: tert-butyl 8-(2-fluoropyridin-4-yl)-3,8-diazabicyclo[3.2.1]octane-3-carboxylate



To a mixture of tert-butyl 3,8-diazabicyclo[3.2.1]octane-3-carboxylate (58.0 g, 273 mmol), t-BuONa (39.4 g, 410 mmol), KF (25.4 g, 437 mmol) and xantphos (7.27 g, 12.6 mmol) in 1,4-dioxane (1.20 L) was added 2-fluoro-4-iodopyridine (79.2 g, 355 mmol) in one portion at 25 °C under N_2, then the mixture was bubbled with nitrogen gas. $Pd_2(dba)_3$ was added (5.75 g, 6.28 mmol) and the reaction was stirred under a nitrogen atmosphere at 110 °C for 4 hrs. Two

reactions were combined for workup. The mixture reaction was filtered, the filter cake was washed with EtOAc (1.00 L), and the filtrate concentrated under reduced pressure to give a residue (300 g). The residue was purified by silica gel chromatography (1-30% EtOAc/petroleum ether) to provide the title compound (101 g, 60.1% yield) as a yellow foamy solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 (d, J = 6.0 Hz, 1H), 6.45 (d, J = 6.0 Hz, 1H), 6.10 (s, 1H), 4.20 (d, J = 20.4 Hz, 2H), 3.76 (dd, J = 57.6, 12.4 Hz, 2H), 3.15 (dd, J = 43.6, 12.4 Hz, 2H), 2.12 - 2.01 (m, 2H), 1.92 - 1.87 (m, 2H), 1.45 (s, 9H). LCMS (ESI): *m/z* 308.1 $(M+H)^+$.

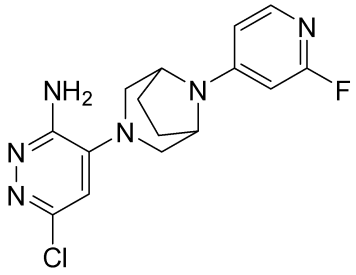
Step 2: 8-(2-fluoropyridin-4-yl)-3,8-diazabicyclo[3.2.1]octane, 2HCl



To a mixture of tert-butyl 8-(2-fluoropyridin-4-yl)-3,8-diazabicyclo[3.2.1]octane-3-carboxylate (100 g, 325 mmol) was added HCl/EtOAc (4 M, 800 mL) at -5-0 °C. Then reaction was heated to 15 °C, and stirred at 15 °C for 2 hrs. The crude reaction mixture was combined with another batch (60.0 g batch) for workup. The reaction mixture was filtered, the filter cake was washed with EtOAc (500 mL), and dried under reduced pressure to afford the title compound (142 g, 97.3% yield, 2HCl) as a light yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.94 (br, s, 1H), 9.51 (br, s, 1H), 7.90 (d, J = 6.0 Hz, 1H), 6.83 (d, J = 6.0 Hz, 1H), 6.61 (s, 1H), 4.57 (s, 2H), 3.04 - 2.95 (m, 4H), 2.18-2.06 (m, 4H). LCMS (ESI): *m/z* 208.2 $(M+H)^+$.

Step 3:

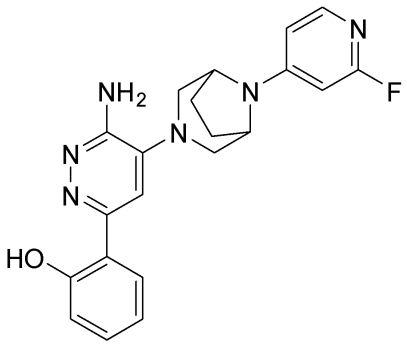
6-chloro-4-(8-(2-fluoropyridin-4-yl)-3,8-diazabicyclo[3.2.1]octan-3-yl)pyridazin-3-amine



To a mixture of 8-(2-fluoropyridin-4-yl)-3,8-diazabicyclo[3.2.1]octane, 2HCl(102 g, 364 mmol, 2HCl), DIPEA (235 g, 1.82 mol) and 4-bromo-6-chloropyridazin-3-amine (91.1 g, 437 mmol) in DMF (700 mL) at 0-5 °C, was added DBU (7.76 g, 51.0 mmol) in one portion under N_2. The mixture was stirred under a nitrogen atmosphere at 100 °C for 16 hrs. The reaction mixture was cooled to 0°C, and then was filtered. The cake was dried under reduced pressure to give a residue. The residue was triturated with H_2O (400 mL) at 15 °C for 30 mins, then filtered. The cake was dried under reduced pressure to give crude product (140 g). The crude product was triturated with CH_3CN (200 mL) at 15 °C for 30 mins, then filtered. The cake was dried under reduced pressure to afford the title compound (86.0 g, 70.5% yield) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.81 (d, J = 5.6 Hz, 1H), 6.91 (s, 1H), 6.76 (d, J = 5.6 Hz, 1H), 6.48 (s, 1H), 5.87 (s, 2H), 4.53 (s, 2H), 3.20 (d, J = 10.8 Hz, 2H), 2.87 (d, J = 11.6 Hz, 2H), 2.16 (d, J = 7.2 Hz, 2H), 1.95-1.93 (m, 2H). LCMS (ESI): *m/z* 335.1 $(M+H)^+$.

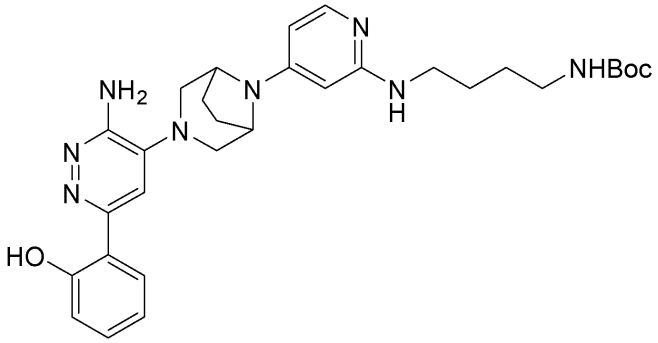
Step 4:

2-(6-amino-5-(8-(2-fluoropyridin-4-yl)-3,8-diazabicyclo[3.2.1]octan-3-yl)pyridazin-3-yl)phenol



To a mixture of 6-chloro-4-(8-(2-fluoropyridin-4-yl)-3,8-diazabicyclo[3.2.1]octan-3-yl)pyridazin-3-amine (40.0 g, 120 mmol), K_2CO_3 (49.5 g, 358 mmol) in 1,4-dioxane (800 mL) and H_2O (160 mL) was added (2-hydroxyphenyl)boronic acid (24.7 g, 179 mmol) in one portion at 15 °C under N_2. The mixture was bubbled with nitrogen gas for 15 mins, then RuPhos-Pd-G3 (5.70 g, 6.81 mmol) was added to the mixture. The reaction flask was placed in a pre-heated oil bath set to 100 °C and stirred under a nitrogen atmosphere at 100 °C for 4 hrs. The reaction was cooled to 20 °C, then the mixture was filtered and filterate was dried under reduced pressure to give a residue. The residue was purified by silica gel chromatography (1-10% MeOH/DCM) to give a crude product. The crude product was triturated with CH_3CN (60.0 mL) at 15 °C for 30 mins, then was filtered. The cake was dried under reduced pressure to afford compound 7 (25.0 g, 52.9% yield) as a yellow foamy solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.11 (s, 1H), 7.90 (d, J = 7.8 Hz, 1H), 7.82 (d, J = 6.0 Hz, 1H), 7.52 (s, 1H), 7.22 (t, J = 8.4 Hz, 1H), 6.88-6.83 (m, 2H), 6.78 (d, J = 5.6 Hz, 1H), 6.50 (s, 1H), 5.99 (s, 2H), 4.58 (s, 2H), 3.28 (d, J = 13.2 Hz, 2H), 3.02 (d, J = 11.6 Hz, 2H), 2.20-2.22 (m, 2H), 2.00 - 1.97 (m, 2H). LCMS (ESI): *m/z* 393.2 $(M+H)^+$.

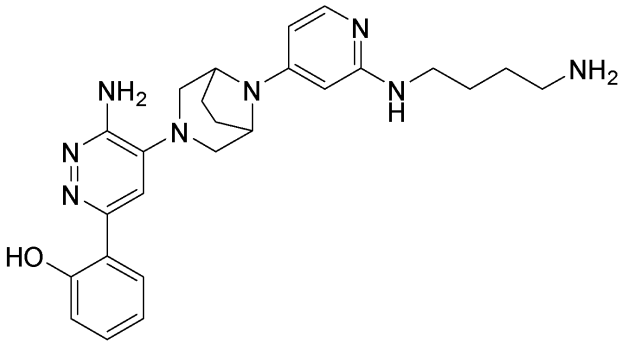
Step 5: tert-butyl (4-((4-(3-(3-amino-6-(2-hydroxyphenyl)pyridazin-4-yl)-3,8-diazabicyclo[3.2.1]octan-8-yl)pyridin-2-yl)amino)butyl)carbamate



A mixture of 2-(6-amino-5-(8-(2-fluoropyridin-4-yl)-3,8-diazabicyclo[3.2.1]octan-3-yl)pyridazin-3-yl)phenol (200 mg, 0.509 mmol), tert-butyl (4-aminobutyl)carbamate (288 mg, 1.53 mmol) and DIPEA (98.8 mg, 0.764 mmol) were taken up into a microwave tube in DMSO (3.00 mL). The sealed tube was heated at 150 °C for 14 hrs in the microwave. The reaction mixture was filtered and the crude product was purified by prep-HPLC (Waters Xbridge Prep OBD C18 150*40mm*10um, water (10mM ammonium bicarbonate and 0.05% ammonia hydroxide v/v)-ACN, 30%-50%) to afford the title compound (70.0 mg, 12% yield) as a yellow solid. LCMS (ESI): *m/z* 561.5 $(M+H)^+$.

Step 6:

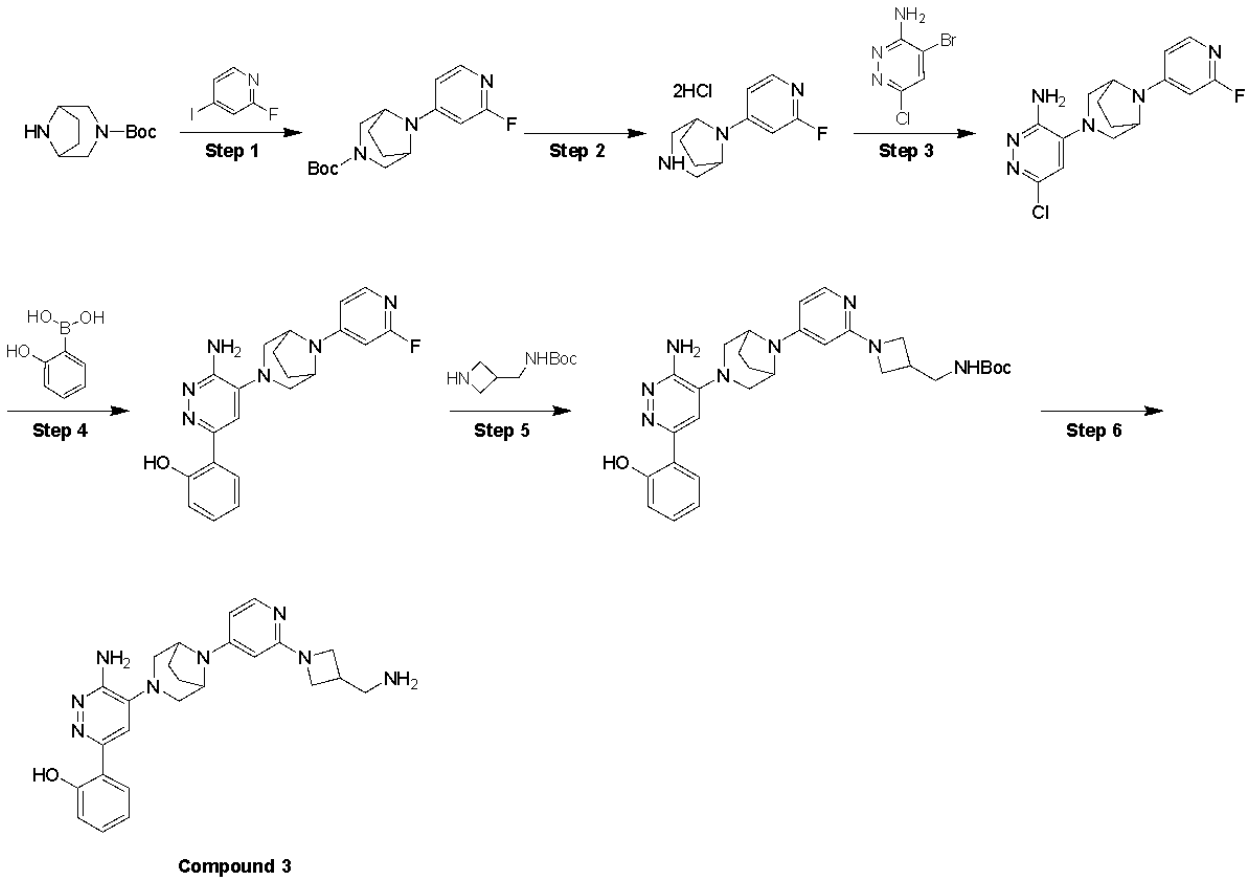
2-(6-amino-5-(8-(2-((4-aminobutyl)amino)pyridin-4-yl)-3,8-diazabicyclo[3.2.1]octan-3-yl)pyridazin-3-yl)phenol



To a solution of tert-butyl (4-((4-(3-(3-amino-6-(2-hydroxyphenyl)pyridazin-4-yl)-3,8-diazabicyclo[3.2.1]octan-8-yl)pyridin-2-yl)amino)butyl)carbamate (70.0 mg, 0.125 mmol) in dioxane (2.00 mL) and MeOH (2.00 mL) was added dropwise 4M HCl/dioxane (0.62 mL) at 0 °C. The mixture was stirred at 15 °C for 12 hrs. The reaction mixture was filtered and the filtrate was concentrated. The crude product was purified by prep-HPLC (Phenomenex luna C18 80*40 mm*3 um, water (0.04% HCl)-ACN, 1%-20%) to afford compound 2 (15.5 mg, 21% yield) as a yellow solid. 1H NMR (400 MHz, CD3OD) δ 7.49-7.45 (m, 3 H), 7.34 (t, J =7.6 Hz, 1 H), 6.96-6.93 (m, 2 H), 6.49 (d, J = 6.8 Hz, 1 H), 6.04 (s, 1 H), 4.67 (s, 1 H), 3.75 – 3.73 (m, 2 H), 3.29-3.21 (m, 3 H), 2.99 (d, J = 6.8 Hz, 2 H), 2.15 – 2.07 (m, 4 H), 1.74 – 1.58 (m, 4H). LCMS (ESI): *m/z* 461.3 $(M+H)^+$.

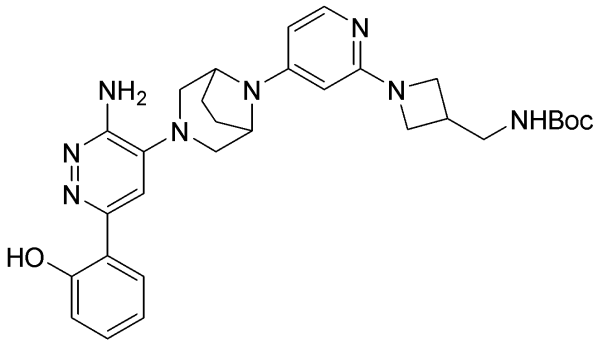
Compound 3

2-(6-amino-5-(8-(2-(3-(aminomethyl)azetidin-1-yl)pyridin-4-yl)-3,8-diazabicyclo[3.2.1]octan-3-yl)pyridazin-3-yl)phenol



Step 1 to 4: procedure similar as described for compound 2 above

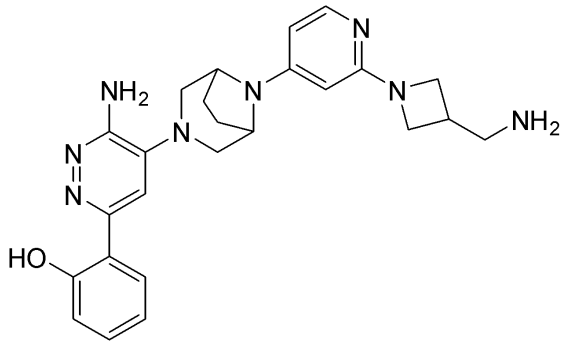
Step 5: tert-butyl ((1-(4-(3-(3-amino-6-(2-hydroxyphenyl)pyridazin-4-yl)-3,8-diazabicyclo[3.2.1]octan-8-yl)pyridin-2-yl)azetidin-3-yl)methyl)carbamate



A mixture of 2-(6-amino-5-(8-(2-fluoropyridin-4-yl)-3,8-diazabicyclo[3.2.1]octan-3-yl)pyridazin-3-yl)phenol (200 mg, 0.509 mmol), tert-butyl (azetidin-3-ylmethyl)carbamate (285 mg, 1.53 mmol) and DIPEA (98.8 mg, 0.764 mmol) were taken up into a microwave tube in DMSO (3.00 mL). The sealed tube was heated at 150 °C for 14 hrs in the microwave. The reaction mixture was filtered and the crude product was purified by prep- HPLC (Waters Xbridge Prep OBD C18 150*40mm*10um, water (10mM ammonium bicarbonate and 0.05% ammonia hydroxide v/v)-ACN, 30%-50%) to afford the title compound (70.0 mg, 12% yield) as a yellow solid. LCMS (ESI): m/z 559.3 (M+H)+.

Step 6:

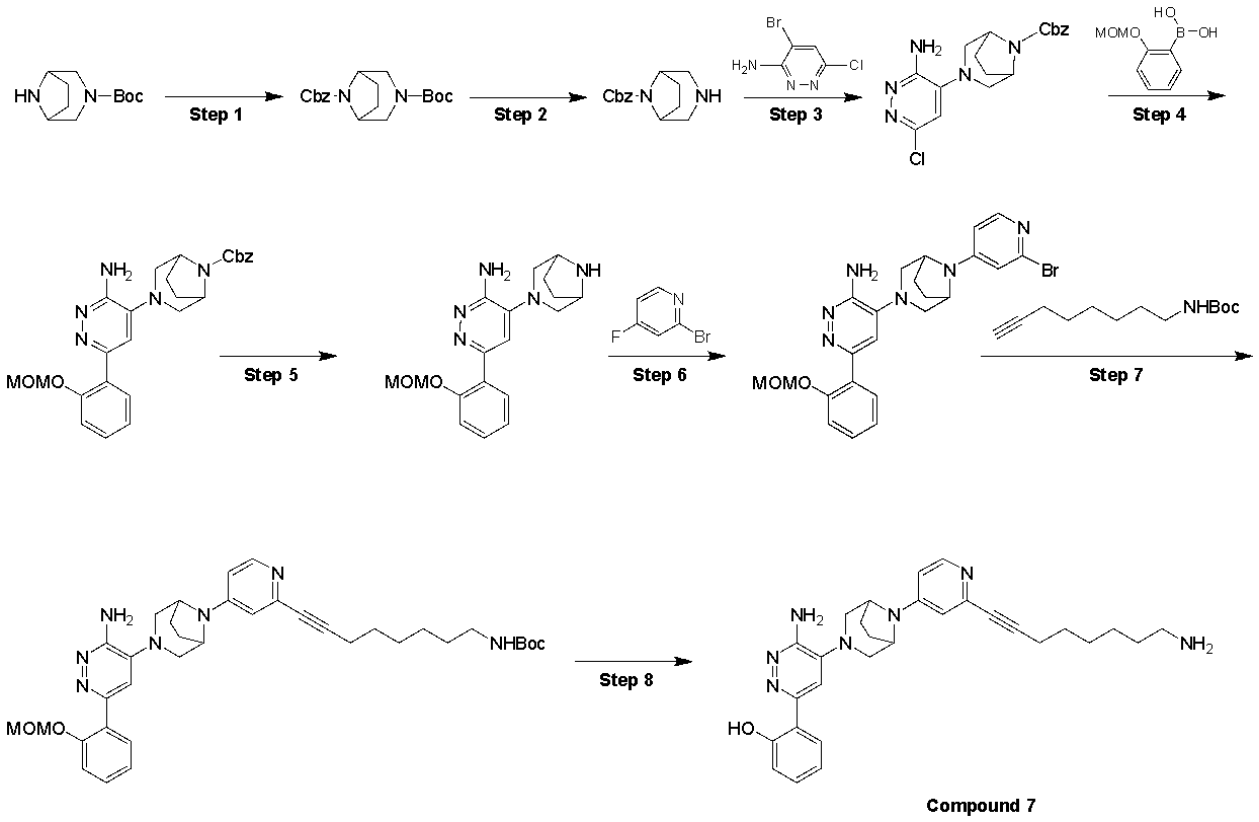
2-(6-amino-5-(8-(2-(3-(aminomethyl)azetidin-1-yl)pyridin-4-yl)-3,8-diazabicyclo[3.2.1]octan-3-yl)pyridazin-3-yl)phenol



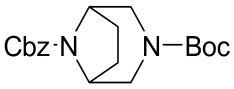
To a solution of tert-butyl ((1-(4-(3-(3-amino-6-(2-hydroxyphenyl)pyridazin-4-yl)-3,8-diazabicyclo[3.2.1]octan-8-yl)pyridin-2-yl)azetidin-3-yl)methyl)carbamate (70.0 mg, 0.125 mmol) in dioxane (1.50 mL) and MeOH (1.50 mL) was added dropwise 4M HCl/dioxane (0.62 mL) at 0 °C. The mixture was stirred at 15 °C for 12 hrs. The reaction mixture was filtered and the filtrate was concentrated. The crude product was purified by prep-HPLC (Phenomenex Gemini-NX C18 75*30mm*3um, water (10mM ammonium bicarbonate and 0.05% ammonia hydroxide v/v)-ACN, 10%-60%) to afford the title compound 3 (112.9 mg, 21% yield) as a yellow solid. 1H NMR (400 MHz, DMSO-d6) δ 14.15 (s, 1H), 7.92 (dd, J = 7.8, 1.6 Hz, 1H), 7.72 (d, J = 5.8 Hz, 1H), 7.48 (s, 1H), 7.27 – 7.19 (m, 1H), 6.90 – 6.81 (m, 2H), 6.20 (dd, J = 5.9, 2.1 Hz, 1H), 5.96 (s, 2H), 5.64 (d, J = 2.1 Hz, 1H), 4.43 (s, 2H), 3.87 (t, J = 7.9 Hz, 2H), 3.54 (dd, J = 8.0, 5.2 Hz, 2H), 3.25 (d, J = 11.9 Hz, 2H), 3.03 (d, J = 11.5 Hz, 2H), 2.73 (d, J = 7.1 Hz, 2H), 2.65 – 2.52 (m, 1H), 2.21 – 2.12 (m, 2H), 1.95 (dd, J = 8.2, 4.1 Hz, 2H). LCMS (ESI): m/z 459.3 (M+H)+.

Compound 4

2-(6-amino-5-(8-(2-(8-aminooct-1-yn-1-yl)pyridin-4-yl)-3,8-diazabicyclo[3.2.1]octan-3-yl)pyridazin-3-yl)phenol



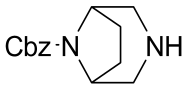
Step 1: 8-benzyl 3-(tert-butyl) 3,8-diazabicyclo[3.2.1]octane-3,8-dicarboxylate



A mixture of tert-butyl 3,8-diazabicyclo[3.2.1]octane-3-carboxylate (125 g, 589 mmol, 1.00 eq) in DCM (620 mL), TEA (179 g, 1.77 mol, 246 mL, 3.00 eq) and CbzCl (151 g, 883 mmol, 126 mL, 1.50 eq) was added, and then the mixture was stirred at 25°C for 3 hrs under N_2 atmosphere. TLC (Petroleum ether: Ethyl acetate = 3: 1) showed new spots (R_f = 0.37) were formed. The solution was washed with water 50.0 mL, extracted with EtOAc 150x3 mL, washed with brine 50.0 mL and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (Petroleum ether/Ethyl acetate = 3: 1) to afford the title

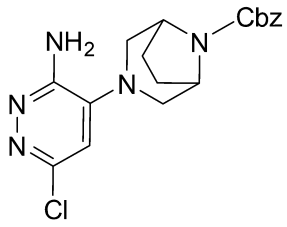
compound (89.0 g, 257 mmol, 43.6% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38-7.34 (m, 5H), 5.15 (s, 2H), 4.30 (s, 2H), 3.87-3.70 (m, 2H), 3.03 (d, J = 28.8 Hz, 2H), 1.95 (s, 2H), 1.88 (d, J = 16.8 Hz, 2H), 1.45 (s, 9H).

Step 2: benzyl 3,8-diazabicyclo[3.2.1]octane-8-carboxylate



To a solution of 8-benzyl 3-(tert-butyl) 3,8-diazabicyclo[3.2.1]octane-3,8-dicarboxylate (89.0 g, 257 mmol, 1.00 eq) in EtOAc (150 mL) was added HCl/EtOAc (4 M, 321 mL, 5.00 eq). The mixture was stirred at 25°C for 3 hrs. The solution was concentrated under reduced pressure to remove most of the solvent and filtered to afford the title compound (59.0 g, 209 mmol, 81.2% yield) as a crude white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39-7.32 (m, 5H), 5.15 (s, 2H), 4.44 (s, 2H), 3.20 (s, 4H), 2.32-2.27 (m, 2H), 2.16-2.00 (m, 2H).

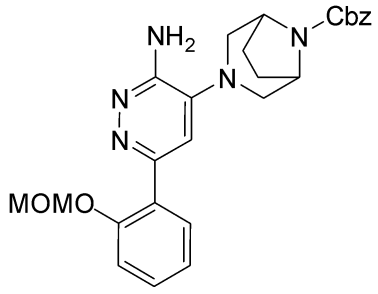
Step 3: benzyl
3-(3-amino-6-chloropyridazin-4-yl)-3,8-diazabicyclo[3.2.1]octane-8-carboxylate



A mixture of benzyl 3,8-diazabicyclo[3.2.1]octane-8-carboxylate (59.0 g, 209 mmol, 1.00 eq) in DMSO (410 mL), 4-bromo-6-chloro-pyridazin-3-amine (45.7 g, 219 mmol, 1.05 eq), DIPEA

(108 g, 835 mmol, 145 mL, 4.00 eq) was added, and then the mixture was stirred at 130°C for 16 hrs under N_2 atmosphere. The solution was added water 100 mL, and combined with another crude reaction mixture of the same product, extracted with EtOAc 150 x 3 mL, and then washed with brine 20.0 mL, concentrated under reduced pressure to afford the title compound (200 g, crude) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39-7.27 (m, 5H), 6.70 (s, 1H), 5.17 (s, 2H), 5.10 (s, 2H), 4.46 (s, 2H), 3.25 (d, J = 9.6 Hz, 2H), 2.89 (d, J = 33.6 Hz, 2H), 2.08-2.05 (m, 2H), 1.95-1.91 (m, 2H).

Step 4: benzyl 3-(3-amino-6-(2-(methoxymethoxy)phenyl)pyridazin-4-yl)-3,8-diazabicyclo[3.2.1]octane-8-carboxylate

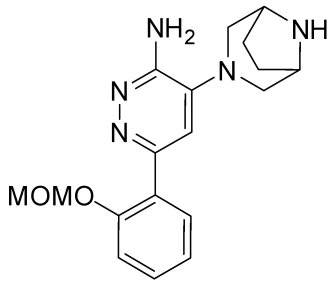


To a mixture of benzyl 3-(3-amino-6-chloropyridazin-4-yl)-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (100 g, 268 mmol, 1.00 eq) in dioxane (800 mL) and H_2O (150 mL) was added (2-(methoxymethoxy)phenyl)boronic acid (73.0 g, 401 mmol, 1.50 eq), $Pd(PPh_3)_4$ (30.9 g, 26.8 mmol, 0.100 eq) and K_2CO_3 (73.9 g, 534 mmol, 2.00 eq), then the mixture was stirred at 100°C for 2 hrs under N_2 atmosphere. The solution was added 100 mL water and combined with another crude reaction mixture of the same reaction. The mixture was extracted with EtOAc 200 mL x 3, the organic was washed with brine 100 mL, dried over Na_2SO_4, concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (Petroleum ether/Ethyl acetate = 0: 1) to afford the title compound (182 g, combined yield:

71.7%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68 (d, J =1.2 Hz, 1H), 7.66-7.25 (m, 6H), 7.17 (d, J = 6.0 Hz, 1H), 7.11-7.10 (m, 1H), 7.04 (s, 1H), 5.08 (s, 2H), 5.06 (s, 2H), 4.92 (s, 2H), 4.39 (s, 2H), 3.32 (s, 3H), 3.17 (d, J = 10.0 Hz, 2H), 2.83 (d, J = 46.8 Hz, 2H), 1.92-2.15 (m, 4H).

Step 5:

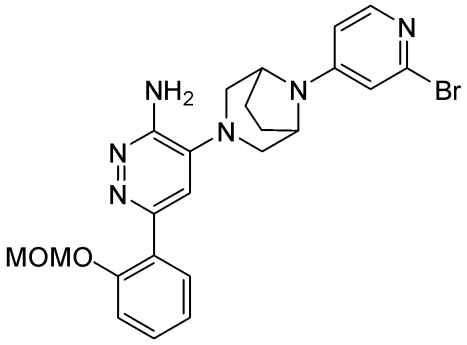
4-(3,8-diazabicyclo[3.2.1]octan-3-yl)-6-(2-(methoxymethoxy)phenyl)pyridazin-3-amine



To a solution of benzyl 3-(3-amino-6-(2-(methoxymethoxy)phenyl)pyridazin-4-yl)-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (182 g, 383 mmol, 1.00 eq) in MeOH (1.27 L) was added $Pd(OH)_2$/C (53.8 g, 38.3 mmol, 10.0% purity, 0.100 eq) under N_2 atmosphere. The suspension was degassed and purged with H_2 3 times. The mixture was stirred under H_2 at 35°C for 16 hrs. The solution was filtered and the filtrate was concentrated under reduced pressure to afford the title compound (100 g, crude) as a brown solid.

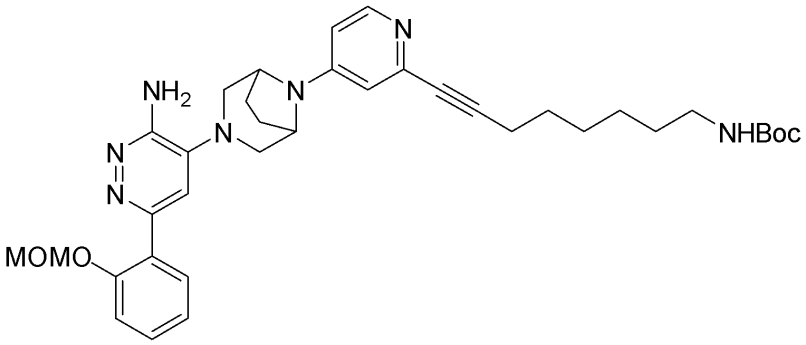
Step 6:

4-(8-(2-bromopyridin-4-yl)-3,8-diazabicyclo[3.2.1]octan-3-yl)-6-(2-(methoxymethoxy)phenyl)pyridazin-3-amine



To a solution of 4-(3,8-diazabicyclo[3.2.1]octan-3-yl)-6-(2-(methoxymethoxy)phenyl)pyridazin-3-amine (16 g, 46.9 mmol) in DMSO (96 mL) was added 2-bromo-4-fluoro-pyridine (8.25 g, 46.9 mmol) and DIEA (60.6 g, 469 mmol, 81.6 mL) then the mixture was stirred for 5 hrs at 130°C. The reaction mixture was quenched by addition H_2O 100 mL, diluted with EtOAc 100 mL and extracted with EtOAc (100 mL x 3). The combined organic layers were washed with brine 100 mL, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. The crude product was triturated with MeOH: DCM = 1: 20 at 15°C for 30 min, and then filtered and concentrated under reduced pressure to afford the title compound (17 g, 72.49% yield) as a light yellow solid. NMR (400 MHz, $CDCl_3$): δ 8.00 (d, *J*=5.96 Hz, 1 H), 7.72 (dd, *J*=7.63, 1.67 Hz, 1 H), 7.22 (s, 1 H) 7.31-7.38 (m, 1 H), 7.15 (d, *J*=7.89 Hz, 1 H), 7.10 (t, *J*=7.37 Hz, 1 H), 6.77 (d, *J*=2.15 Hz, 1 H), 6.54 (dd, *J*=5.96, 2.27 Hz, 1 H), 5.13 (s, 2 H), 5.10 (s, 2 H), 4.37 (s, 2 H), 3.36 (s, 3 H), 3.23 (d, *J*=10.13 Hz, 2 H), 3.02 (d, *J*=11.21 Hz, 2 H), 2.10-2.24 (m, 4 H).

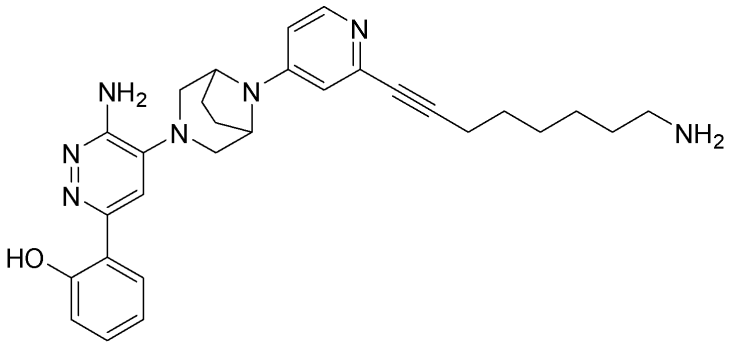
Step 7: tert-butyl (8-(4-(3-(3-amino-6-(2-(methoxymethoxy)phenyl)pyridazin-4-yl)-3,8-diazabicyclo[3.2.1]octan-8-yl)pyridin-2-yl)oct-7-yn-1-yl)carbamate



Under nitrogen, a solution of 4-(8-(2-bromopyridin-4-yl)-3,8-diazabicyclo[3.2.1]octan-3-yl)-6-(2-(methoxymethoxy)phenyl)pyridazin-3-amine (100 mg, 0.200 mmol, 1.0 eq) and tert-butyl oct-7-yn-1-ylcarbamate (74 mg, 0.300 mmol, 1.5 eq) in DMF (2 mL) was added CuI (1.9 mg, 0.010 mmol, 0.030 eq), $Pd(PPh_3)_2Cl_2$ (7 mg, 0.010 mmol, 0.030 eq) and K_2CO_3 (83 mg, 0.600 mmol, 3.0 eq). The resulting solution was shaken at 100 ^oC for 16 hrs. The reaction mixture was concentrated by Speedvac and the residue was purified by prep-TLC and carried on assuming quantitative yield of the title compound.

Step 8:

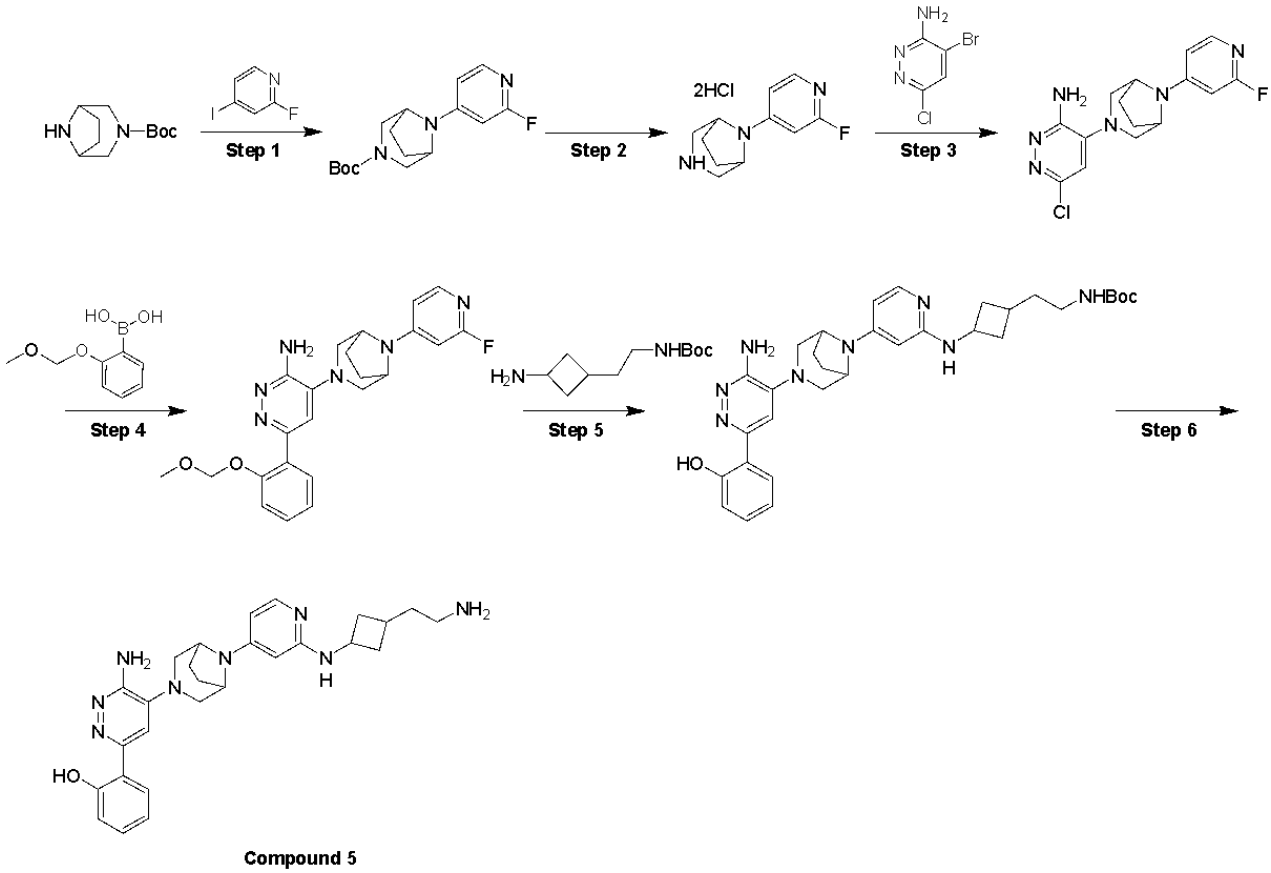
2-(6-amino-5-(8-(2-(8-aminooct-1-yn-1-yl)pyridin-4-yl)-3,8-diazabicyclo[3.2.1]octan-3-yl)pyridazin-3-yl)phenol



A solution of tert-butyl (8-(4-(3-(3-amino-6-(2-(methoxymethoxy)phenyl)pyridazin-4-yl)-3,8-diazabicyclo[3.2.1]octan-8-yl)pyridin-2-yl)oct-7-yn-1-yl)carbamate (128 mg, 0.200 mmol, 1.0 eq.) in HCl (4 M in MeOH, 2 mL) was shaken at 30 °C for 2 hrs. The mixture was evaporated and diluted with 1.5 mL of MeOH. Then the pH was adjusted to 7~8 using aqueous $NH_3.H_2O$. The crude product was purified by prep-HPLC (Xtimate C18 150*25mm*5um, Water (10mM ammonium bicarbonate and 0.05% ammonia hydroxide v/v)-ACN, 51-76%) to afford compound 4 (13.3 mg, 13% yield). 1H NMR (400 MHz, DMSO-d6) δ 8.07 (d, J = 5.8 Hz, 1H), 7.91 (dd, J = 8.0, 1.6 Hz, 1H), 7.50 (s, 1H), 7.22 (ddd, J = 8.4, 7.1, 1.5 Hz, 1H), 6.91 – 6.80 (m, 3H), 6.77 (dd, J = 6.0, 2.5 Hz, 1H), 5.98 (s, 2H), 4.56 (s, 2H), 3.30 – 3.22 (m, 2H), 3.00 (d, J = 11.6 Hz, 2H), 2.62 (t, J = 7.0 Hz, 2H), 2.41 (t, J = 7.0 Hz, 2H), 2.24 – 2.15 (m, 2H), 1.96 (dd, J = 8.1, 4.1 Hz, 2H), 1.60 – 1.48 (m, 2H), 1.48 – 1.37 (m, 4H), 1.36 – 1.26 (m, 2H). LCMS (ESI): m/z 498.3 (M+H)+.

Compound 5

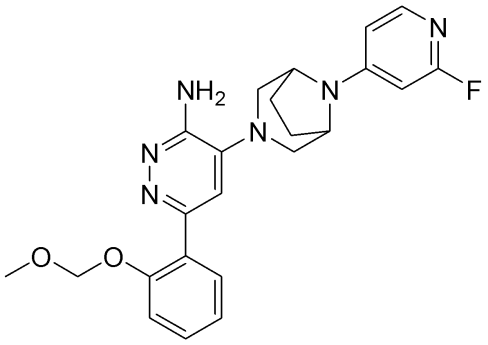
2-(6-amino-5-(8-(2-((3-(2-aminoethyl)cyclobutyl)amino)pyridin-4-yl)-3,8-diazabicyclo[3.2.1]octan-3-yl)pyridazin-3-yl)phenol



Step 1 to 3: procedure similar as described above for compound 2

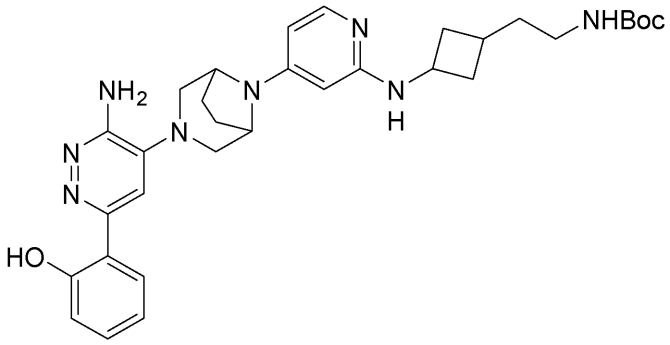
Step 4:

4-(8-(2-fluoropyridin-4-yl)-3,8-diazabicyclo[3.2.1]octan-3-yl)-6-(2-(methoxymethoxy)phenyl)pyridazin-3-amine



To a solution of 6-chloro-4-(8-(2-fluoropyridin-4-yl)-3,8-diazabicyclo[3.2.1]octan-3-yl)pyridazin-3-amine (0.65 g, 1.94 mmol, 1.00 eq) and (2-(methoxymethoxy)phenyl)boronic acid (459 mg, 2.52 mmol, 1.30 eq) in dioxane (10.0 mL) and H_2O (2.00 mL), was added $Pd(dppf)Cl_2.CH_2Cl_2$ (47.5 mg, 58.2 umol, 0.03 eq) and K_2CO_3 (536 mg, 3.88 mmol, 2.00 eq). The mixture was stirred at 100 °C for 5 hrs under N_2. The reaction mixture was poured into water (50.0 mL) and extracted with ethyl acetate (20.0 mL x 2). The combined organic phases were concentrated in vacuo. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate = 3/1 to 0/1) to afford 4-(8-(2-fluoropyridin-4-yl)-3,8-diazabicyclo[3.2.1]octan-3-yl)-6-(2-(methoxymethoxy)phenyl)pyridazin-3-amine (300 mg, 35% yield) as a yellow solid. 1H NMR (400 MHz, DMSO-d6) δ 7.79 (d, J = 6.0 Hz, 1H), 7.57 (dd, J = 1.6, 7.6 Hz, 1H), 7.37 - 7.30 (m, 1H), 7.17 - 7.12 (m, 2H), 7.09 - 7.04 (m, 1H), 6.76 (d, J = 6.0 Hz, 1H), 6.48 (d, J = 1.2 Hz, 1H), 5.73 (s, 2H), 5.14 (s, 2H), 4.55 (s, 2H), 3.22 (s, 3H), 3.19 - 3.16 (m, 2H), 2.86 - 2.83 (m, 2H), 2.25 - 2.16 (m, 2H), 2.00 - 1.90 (m, 2H)

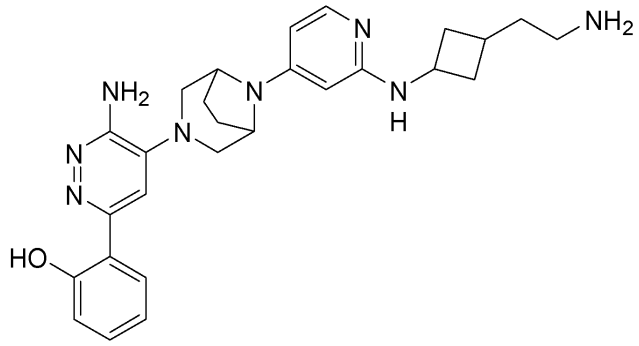
Step 5: tert-butyl (2-(3-((4-(3-(3-amino-6-(2-hydroxyphenyl)pyridazin-4-yl)-3,8-diazabicyclo[3.2.1]octan-8-yl) pyridin-2-yl)amino)cyclobutyl)ethyl)carbamate



Five reactions were carried in parallel in the microwave. To a solution of compound 1 (30.0 mg, 0.0687 mmol, 1.00 eq) in DME (2.00 mL) was added DIEA (44.4 mg, 0.343 mmol, 5.00 eq) and tert-butyl (2-(3-aminocyclobutyl)ethyl)carbamate hydrochloride (137 mg, 0.549 mmol, 8.00 eq.). The mixture was stirred at 150 °C for 10 hrs in the microwave. The reactions were combined and the mixture was concentrated under reduced pressure to afford the title compound (200 mg, crude) was obtained as a yellow oil. LCMS (ESI): m/z 587.5 (M+H)+.

Step 6:

2-(6-amino-5-(8-(2-((3-(2-aminoethyl)cyclobutyl)amino)pyridin-4-yl)-3,8-diazabicyclo[3.2.1] octan-3-yl)pyridazin-3-yl)phenol

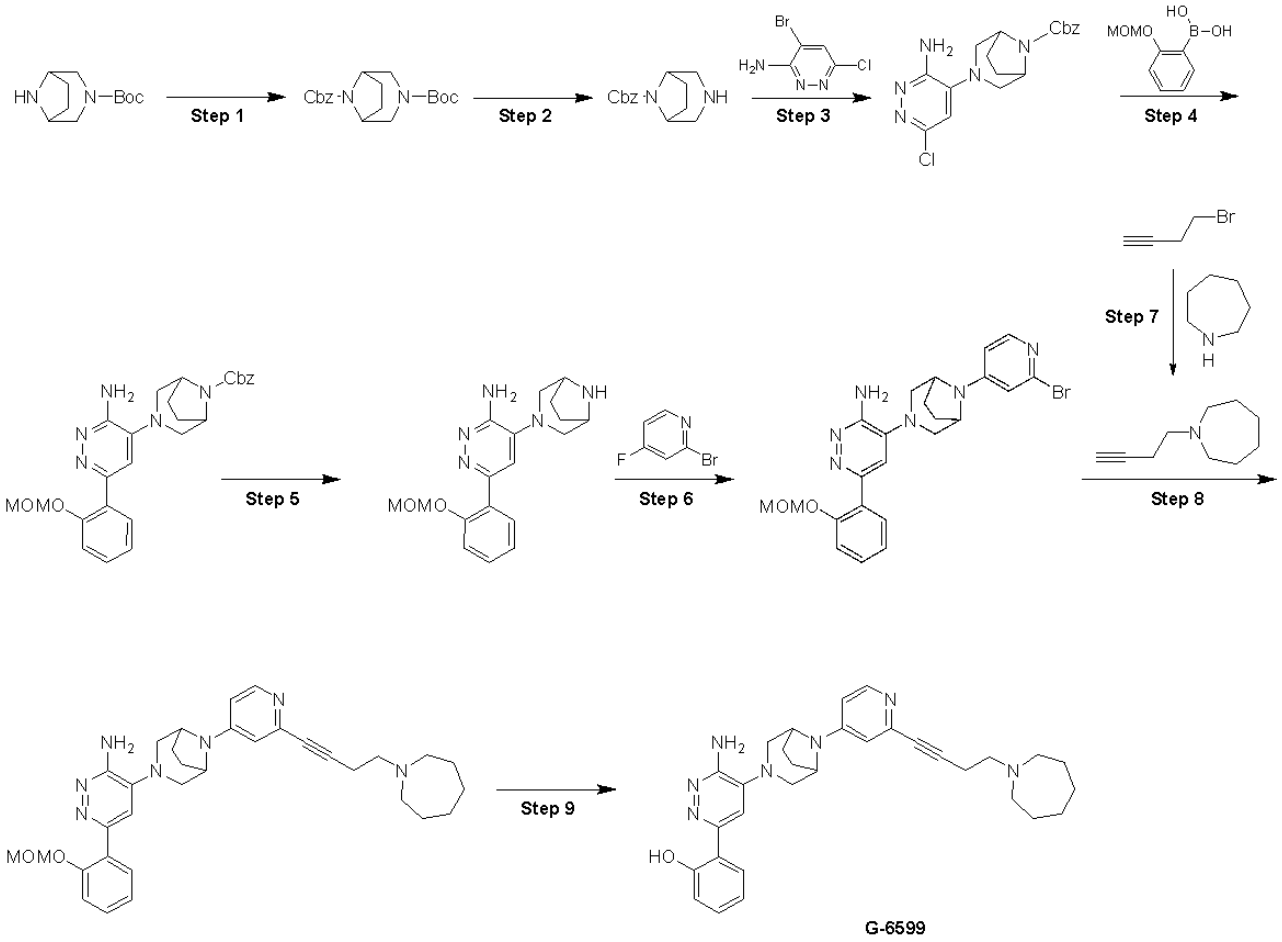


To a solution of tert-butyl (2-(3-((4-(3-(3-amino-6-(2-hydroxyphenyl)pyridazin-4-yl)-3,8-diazabicyclo[3.2.1]octan-8-yl)pyr

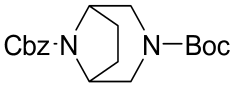
idin-2-yl)amino)cyclobutyl)ethyl)carbamate (100 mg, 0.170 mmol, 1.00 eq) in DCM (2.00 mL) was added TFA (194 mg, 1.70 mmol, 10.0 eq). The mixture was stirred at 25 °C for 5 hrs. The reaction mixture was concentrated under reduced pressure and the residue was purified by prep-HPLC (column: Phenomenex Luna C18 200*40mm*10um; mobile phase: [water(FA)-ACN]; B%: 5%-35%,8min) to afford G'9780 (33.0 mg, 39% yield) as a brown solid. 1H NMR (400 MHz, MeOD) δ 7.67 (d, J = 8.4 Hz, 1H), 7.59 - 7.57 (m, 2H), 7.35 (t, J = 7.6 Hz, 1H), 7.01 - 6.97 (m, 2H), 6.59 (dd, J = 7.6, 2.4, 1H), 5.90 (s, 1H), 4.71 (s, 2H), 4.20 - 4.11 (m, 1H), 3.67 (d, J = 11.6 Hz, 2H), 3.20 (d, J = 11.6 Hz, 2H), 2.92 - 2.81 (m, 2H), 2.49 – 2.41 (m, 1H), 2.31 - 2.14 (m, 8H), 1.94 - 1.91 (m, 2H). LCMS (ESI): m/z 487.3 (M+H)+.

G-6599

2-(6-amino-5-(8-(2-(4-(azepan-1-yl)but-1-yn-1-yl)pyridin-4-yl)-3,8-diazabicyclo[3.2.1]octan-3-yl)pyridazin-3-yl)phenol



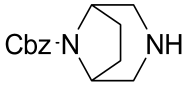
Step 1: 8-benzyl 3-(tert-butyl) 3,8-diazabicyclo[3.2.1]octane-3,8-dicarboxylate



A mixture of tert-butyl 3,8-diazabicyclo[3.2.1]octane-3-carboxylate (125 g, 589 mmol, 1.00 eq) in DCM (620 mL), TEA (179 g, 1.77 mol, 246 mL, 3.00 eq) and CbzCl (151 g, 883 mmol, 126 mL, 1.50 eq) was added, and then the mixture was stirred at 25°C for 3 hrs under N_2 atmosphere. TLC (Petroleum ether: Ethyl acetate = 3: 1) showed new spots (R_f = 0.37) were formed. The solution was washed with water 50.0 mL, extracted with EtOAc 150x3 mL, washed

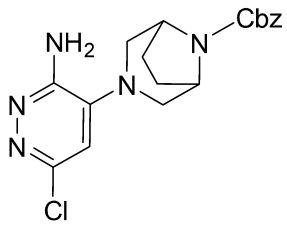
with brine 50.0 mL and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (Petroleum ether/Ethyl acetate = 3: 1) to afford the title compound (89.0 g, 257 mmol, 43.6% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38-7.34 (m, 5H), 5.15 (s, 2H), 4.30 (s, 2H), 3.87-3.70 (m, 2H), 3.03 (d, J = 28.8 Hz, 2H), 1.95 (s, 2H), 1.88 (d, J = 16.8 Hz, 2H), 1.45 (s, 9H).

Step 2: benzyl 3,8-diazabicyclo[3.2.1]octane-8-carboxylate



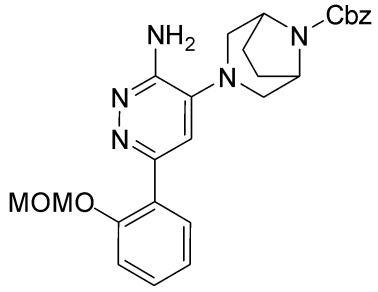
To a solution of 8-benzyl 3-(tert-butyl) 3,8-diazabicyclo[3.2.1]octane-3,8-dicarboxylate (89.0 g, 257 mmol, 1.00 eq) in EtOAc (150 mL) was added HCl/EtOAc (4 M, 321 mL, 5.00 eq). The mixture was stirred at 25°C for 3 hrs. The solution was concentrated under reduced pressure to remove most of the solvent and filtered to afford the title compound (59.0 g, 209 mmol, 81.2% yield) as a crude white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39-7.32 (m, 5H), 5.15 (s, 2H), 4.44 (s, 2H), 3.20 (s, 4H), 2.32-2.27 (m, 2H), 2.16-2.00 (m, 2H).

Step 3: benzyl
3-(3-amino-6-chloropyridazin-4-yl)-3,8-diazabicyclo[3.2.1]octane-8-carboxylate



A mixture of benzyl 3,8-diazabicyclo[3.2.1]octane-8-carboxylate (59.0 g, 209 mmol, 1.00 eq) in DMSO (410 mL), 4-bromo-6-chloro-pyridazin-3-amine (45.7 g, 219 mmol, 1.05 eq), DIPEA (108 g, 835 mmol, 145 mL, 4.00 eq) was added, and then the mixture was stirred at 130°C for 16 hrs under N_2 atmosphere. The solution was added water 100 mL, and combined with another crude reaction mixture of the same product, extracted with EtOAc 150 x 3 mL, and then washed with brine 20.0 mL, concentrated under reduced pressure to afford the title compound (200 g, crude) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39-7.27 (m, 5H), 6.70 (s, 1H), 5.17 (s, 2H), 5.10 (s, 2H), 4.46 (s, 2H), 3.25 (d, J = 9.6 Hz, 2H), 2.89 (d, J = 33.6 Hz, 2H), 2.08-2.05 (m, 2H), 1.95-1.91 (m, 2H).

Step 4: benzyl 3-(3-amino-6-(2-(methoxymethoxy)phenyl)pyridazin-4-yl)-3,8-diazabicyclo[3.2.1]octane-8-carboxylate

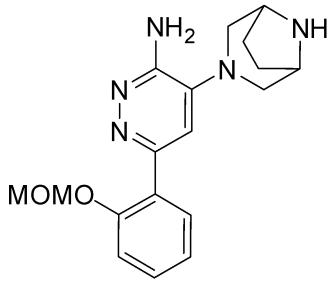


To a mixture of benzyl 3-(3-amino-6-chloropyridazin-4-yl)-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (100 g, 268 mmol, 1.00 eq) in dioxane (800 mL) and H_2O (150 mL) was added (2-(methoxymethoxy)phenyl)boronic acid (73.0 g, 401 mmol, 1.50 eq), $Pd(PPh_3)_4$ (30.9 g, 26.8 mmol, 0.100 eq) and K_2CO_3 (73.9 g, 534 mmol, 2.00 eq), then the mixture was stirred at 100°C for 2 hrs under N_2 atmosphere. The solution was added 100 mL water and combined with another crude reaction mixture of the same reaction. The mixture was extracted with EtOAc 200 mL x 3, the organic was washed with brine 100 mL, dried over Na_2SO_4, concentrated under

reduced pressure to give a residue. The residue was purified by column chromatography (Petroleum ether/Ethyl acetate = 0: 1) to afford the title compound (182 g, combined yield: 71.7%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68 (d, J =1.2 Hz, 1H), 7.66-7.25 (m, 6H), 7.17 (d, J = 6.0 Hz, 1H), 7.11-7.10 (m, 1H), 7.04 (s, 1H), 5.08 (s, 2H), 5.06 (s, 2H), 4.92 (s, 2H), 4.39 (s, 2H), 3.32 (s, 3H), 3.17 (d, J = 10.0 Hz, 2H), 2.83 (d, J = 46.8 Hz, 2H), 1.92-2.15 (m, 4H).

Step 5:

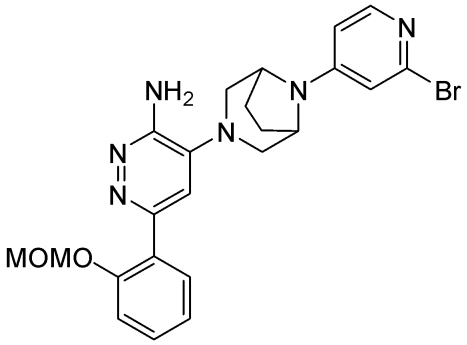
4-(3,8-diazabicyclo[3.2.1]octan-3-yl)-6-(2-(methoxymethoxy)phenyl)pyridazin-3-amine



To a solution of benzyl 3-(3-amino-6-(2-(methoxymethoxy)phenyl)pyridazin-4-yl)-3,8-diazabicyclo[3.2.1]octane-8-carboxylate (182 g, 383 mmol, 1.00 eq) in MeOH (1.27 L) was added $Pd(OH)_2$/C (53.8 g, 38.3 mmol, 10.0% purity, 0.100 eq) under N_2 atmosphere. The suspension was degassed and purged with H_2 3 times. The mixture was stirred under H_2 at 35°C for 16 hrs. The solution was filtered and the filtrate was concentrated under reduced pressure to afford the title compound (100 g, crude) as a brown solid.

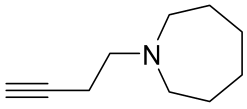
Step 6:

4-(8-(2-bromopyridin-4-yl)-3,8-diazabicyclo[3.2.1]octan-3-yl)-6-(2-(methoxymethoxy)phenyl)pyridazin-3-amine



To a solution of 4-(3,8-diazabicyclo[3.2.1]octan-3-yl)-6-(2-(methoxymethoxy)phenyl)pyridazin-3-amine (16 g, 46.9 mmol) in DMSO (96 mL) was added 2-bromo-4-fluoro-pyridine (8.25 g, 46.9 mmol) and DIEA (60.6 g, 469 mmol, 81.6 mL) then the mixture was stirred for 5 hrs at 130°C. The reaction mixture was quenched by addition H_2O 100 mL, diluted with EtOAc 100 mL and extracted with EtOAc (100 mL x 3). The combined organic layers were washed with brine 100 mL, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a residue. Thev crude product was triturated with MeOH: DCM = 1: 20 at 15°C for 30 min, and then filtered and concentrated under reduced pressure to afford the title compound (17 g, 72.49% yield) as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00 (d, *J*=5.96 Hz, 1 H), 7.72 (dd, *J*=7.63, 1.67 Hz, 1 H), 7.22 (s, 1 H) 7.31-7.38 (m, 1 H), 7.15 (d, *J*=7.89 Hz, 1 H), 7.10 (t, *J*=7.37 Hz, 1 H), 6.77 (d, *J*=2.15 Hz, 1 H), 6.54 (dd, *J*=5.96, 2.27 Hz, 1 H), 5.13 (s, 2 H), 5.10 (s, 2 H), 4.37 (s, 2 H), 3.36 (s, 3 H), 3.23 (d, *J*=10.13 Hz, 2 H), 3.02 (d, *J*=11.21 Hz, 2 H), 2.10-2.24 (m, 4 H).

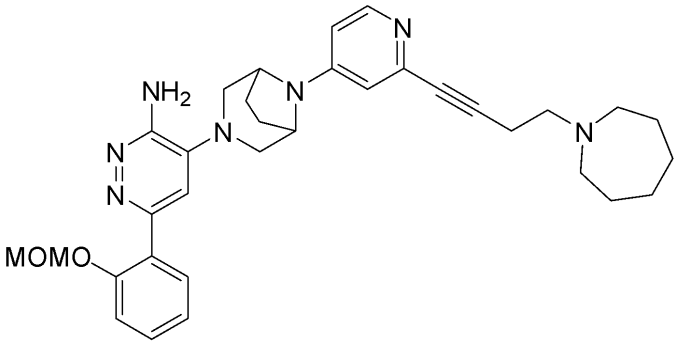
Step 7: 1-(but-3-yn-1-yl)azepane



To a solution of 4-bromobut-1-yne (4.00 g, 30.0 mmol, 1.00 eq) in MeCN (30.0 mL) was added K_2CO_3 (4.16 g, 30.0 mmol, 1.00 eq) and azepane (3.64 g, 36.7 mmol, 4.14 mL, 1.22 eq). The mixture was stirred at 80 °C for 5 hrs. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO_2, Petroleum ether/Ethyl acetate=20/1 to 10/1) to afford the title compound (2.00 g, 44% yield) as a yellow oil.

Step 8:

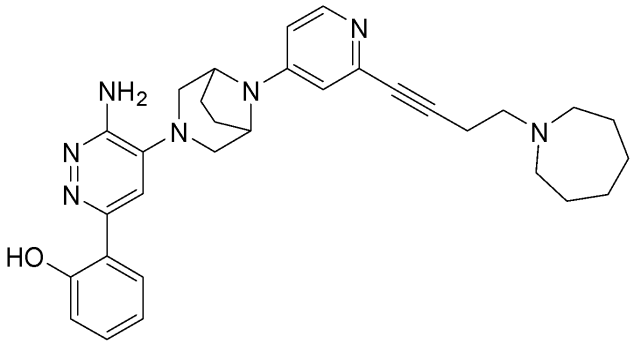
4-(8-(2-(4-(azepan-1-yl)but-1-yn-1-yl)pyridin-4-yl)-3,8-diazabicyclo[3.2.1]octan-3-yl)-6-(2-(methoxymethoxy)phenyl)pyridazin-3-amine



To a solution of 4-(8-(2-bromopyridin-4-yl)-3,8-diazabicyclo[3.2.1]octan-3-yl)-6-(2-(methoxymethoxy)phenyl)pyridazin-3-amine (300 mg, 0.60 mmol, 1.00 eq) and 1-(but-3-yn-1-yl)azepane (456 mg, 3.02 mmol, 5.00 eq) in DMF (5.00 mL) was added PPh_3 (31.6 mg, 0.12 mmol, 0.20 eq), TEA (183 mg, 1.81 mmol, 3.00 eq), $Pd(PPh_3)_4$ (69.7 mg, 0.060 mmol, 0.10 eq) and CuI (11.5 mg, 0.060 mmol, 0.10 eq) under N_2. The mixture was stirred at 80°C for 5 hrs under N_2. The reaction mixture was quenched by addition H_2O (20.0 mL) at 25°C, and then filtered. The filtrate was extracted with EtOAc (20 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford the desired product (330 mg, crude) as a crude brown gum. LCMS (ESI): m/z 568.3 (M+H)+.

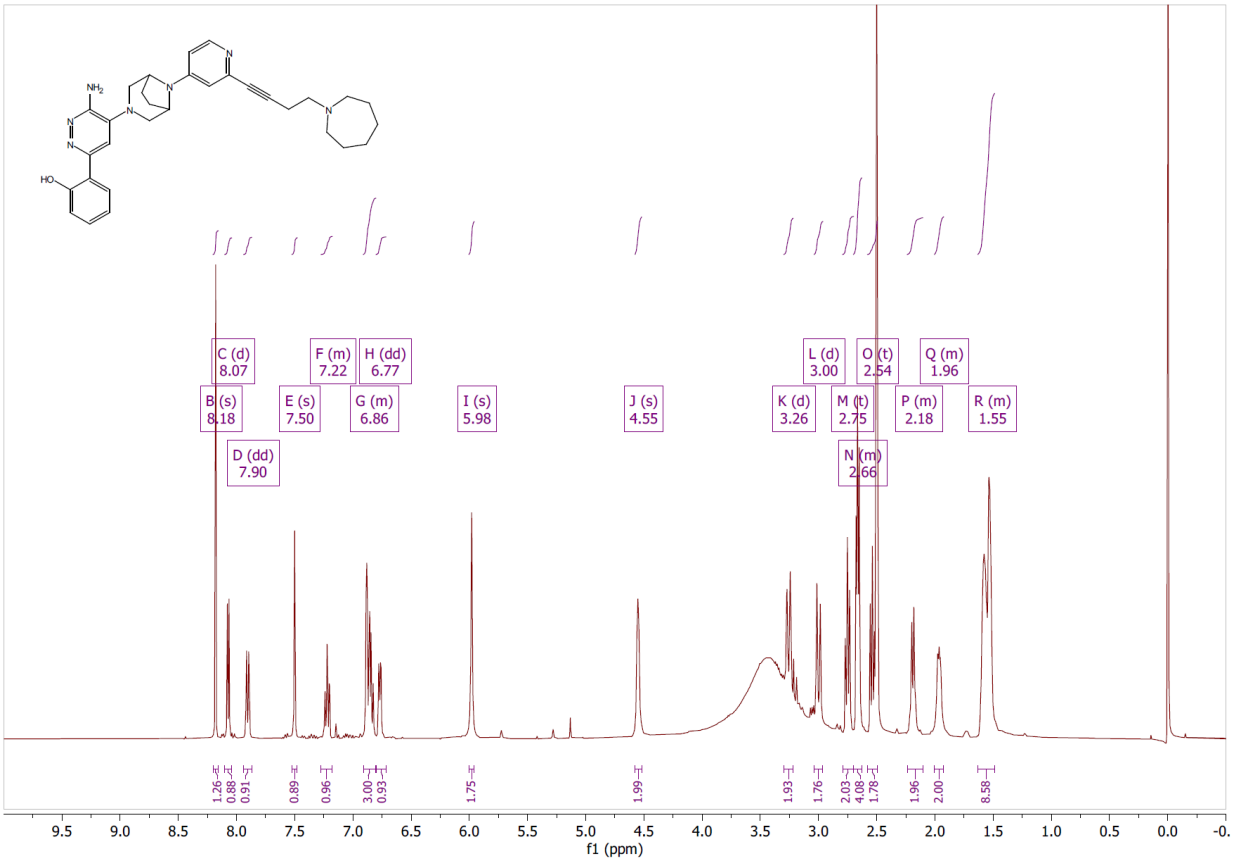
Step 9:

2-(6-amino-5-(8-(2-(4-(azepan-1-yl)but-1-yn-1-yl)pyridin-4-yl)-3,8-diazabicyclo[3.2.1]octan-3-yl)pyridazin-3-yl)phenol

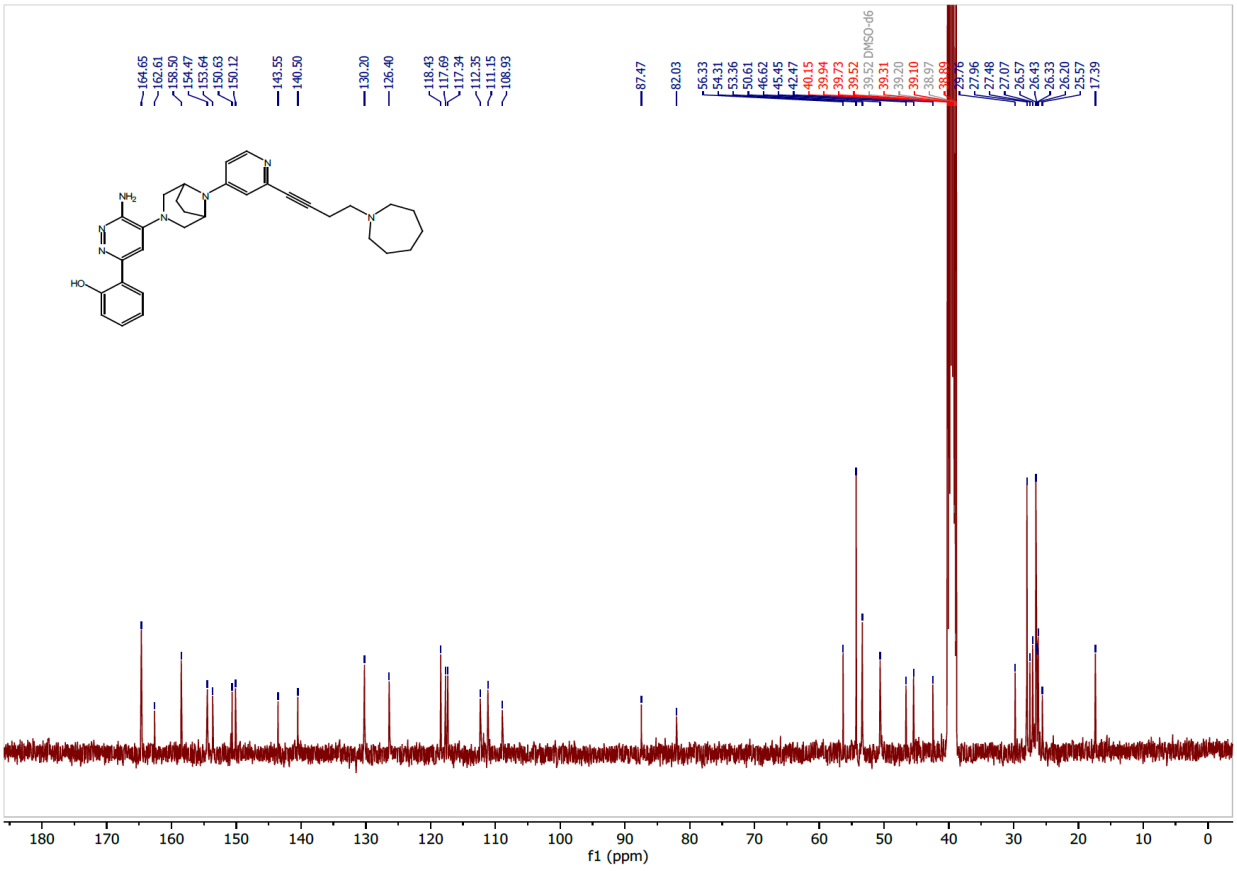


To a solution of 4-(8-(2-(4-(azepan-1-yl)but-1-yn-1-yl)pyridin-4-yl)-3,8-diazabicyclo[3.2.1]octan-3-yl)-6-(2-(methoxymethoxy)phenyl)pyridazin-3-amine (250 mg, 0.44 mmol, 1.00 eq) in DCM (5.00 mL) was added HCl/EtOAc (4 M, 20.0 mL, 181 eq). The mixture was stirred at 25°C for 5 hrs. The reaction mixture was concentrated under reduced pressure to and purified by prep-HPLC (column: Phenomenex Luna C18 200*40mm*10um; mobile phase: [water(FA)-ACN];B%: 1%-25%, 8min) to afford G-6599 (47.3 mg, 20% yield) as yellow solid. ^{1}H NMR (400 MHz, DMSO) δ 8.18 (s, 1H), 8.07 (d, *J* = 5.8 Hz, 1H), 7.90 (d, *J* = 7.9 Hz, 1H), 7.50 (s, 1H), 7.27 – 7.18 (m, 1H), 6.91 – 6.80 (m, 3H), 6.77 (dd, *J* = 6.0, 2.4 Hz, 1H), 5.98 (s, 2H), 4.55 (s, 2H), 3.26 (d, *J* = 12.0, 2.3 Hz, 2H), 3.00 (d, *J* = 11.6 Hz, 2H), 2.75 (t, *J* = 7.4 Hz, 2H), 2.70 – 2.63 (m, 4H), 2.54 (t, *J* = 7.4 Hz, 2H), 2.24 - 2.10 (m, 2H), 2.00 – 1.93 (m, 2H), 1.63 - 1.49 (m, 8H). ^{13}C NMR (101 MHz, DMSO) δ 164.65, 162.61, 158.50, 154.47, 153.64, 150.63, 150.12, 143.55, 140.50, 130.20, 126.40, 118.43, 117.69, 117.34, 112.35, 111.15, 108.93, 87.47, 82.03, 56.33, 54.31, 53.36, 50.61, 46.62, 45.45, 42.47, 40.15, 39.94, 39.73, 39.52, 39.31, 39.10, 38.89, 29.76, 27.96, 27.48, 27.07, 26.57, 26.43, 26.33, 26.20, 25.57, 17.39. HRMS (ESI+): m/z calcd for C31H37N7O (M+H)+ 524.3132., found. 524.3131.

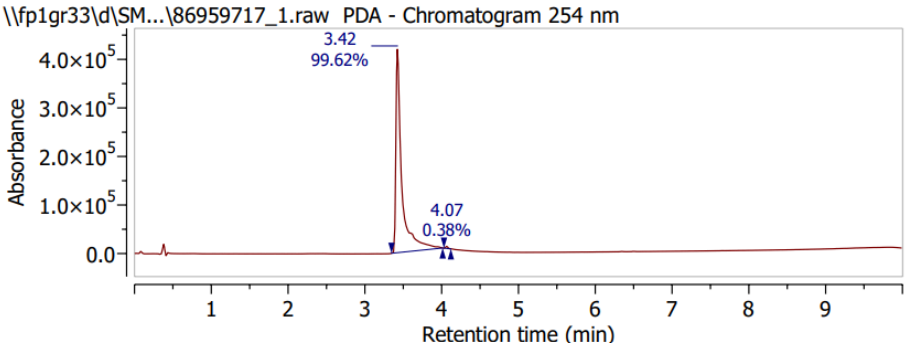
G-6599 ^{1}H NMR



G-6599 ^{13}C NMR



G-6599 HRMS & LCMS



DAD, Sig=254 (>=1.5%)

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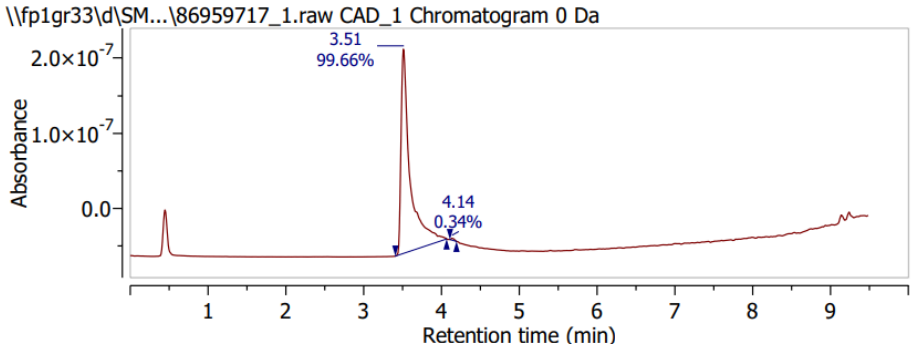
4.07	42639.00	0.38	413.21



CAD_1 (>=5%)

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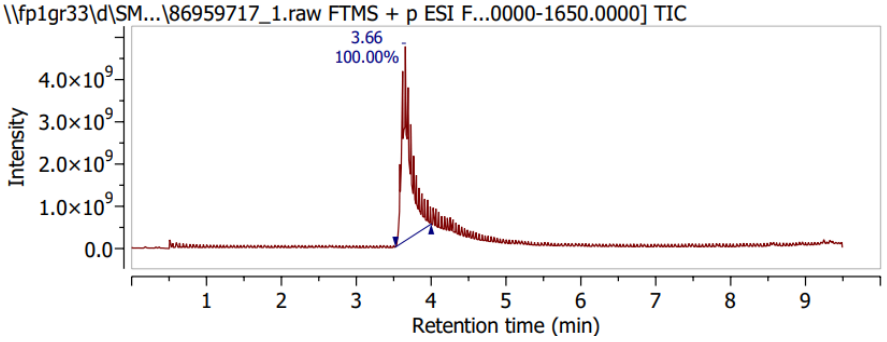

TIC (>=2%)

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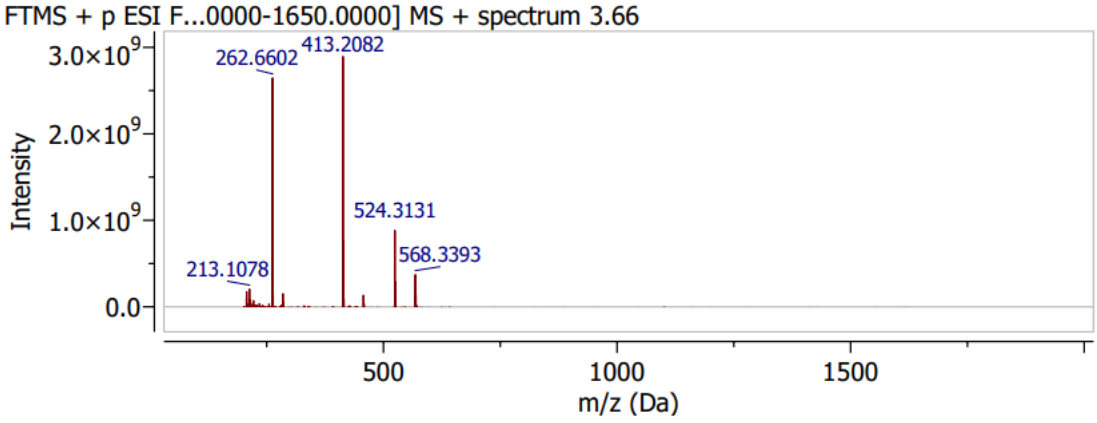

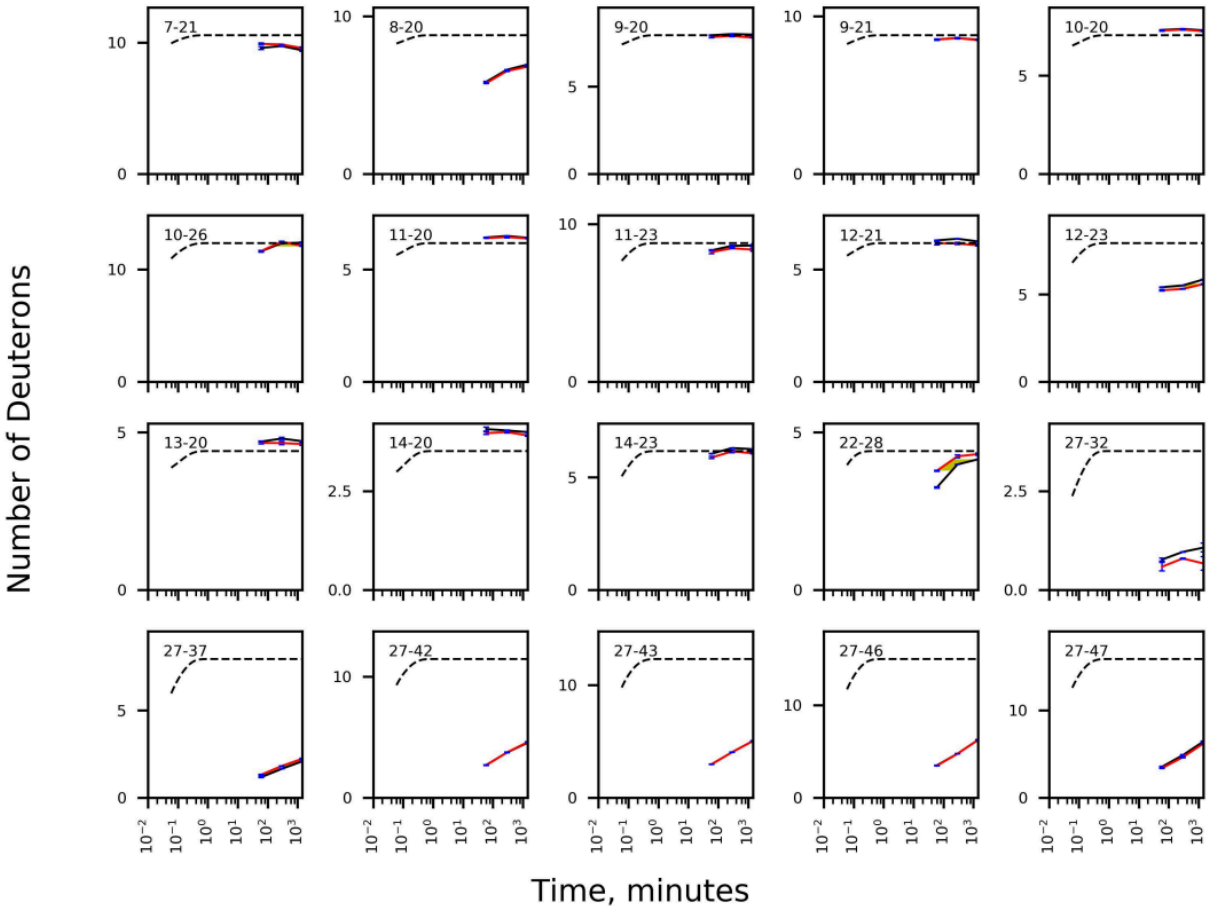
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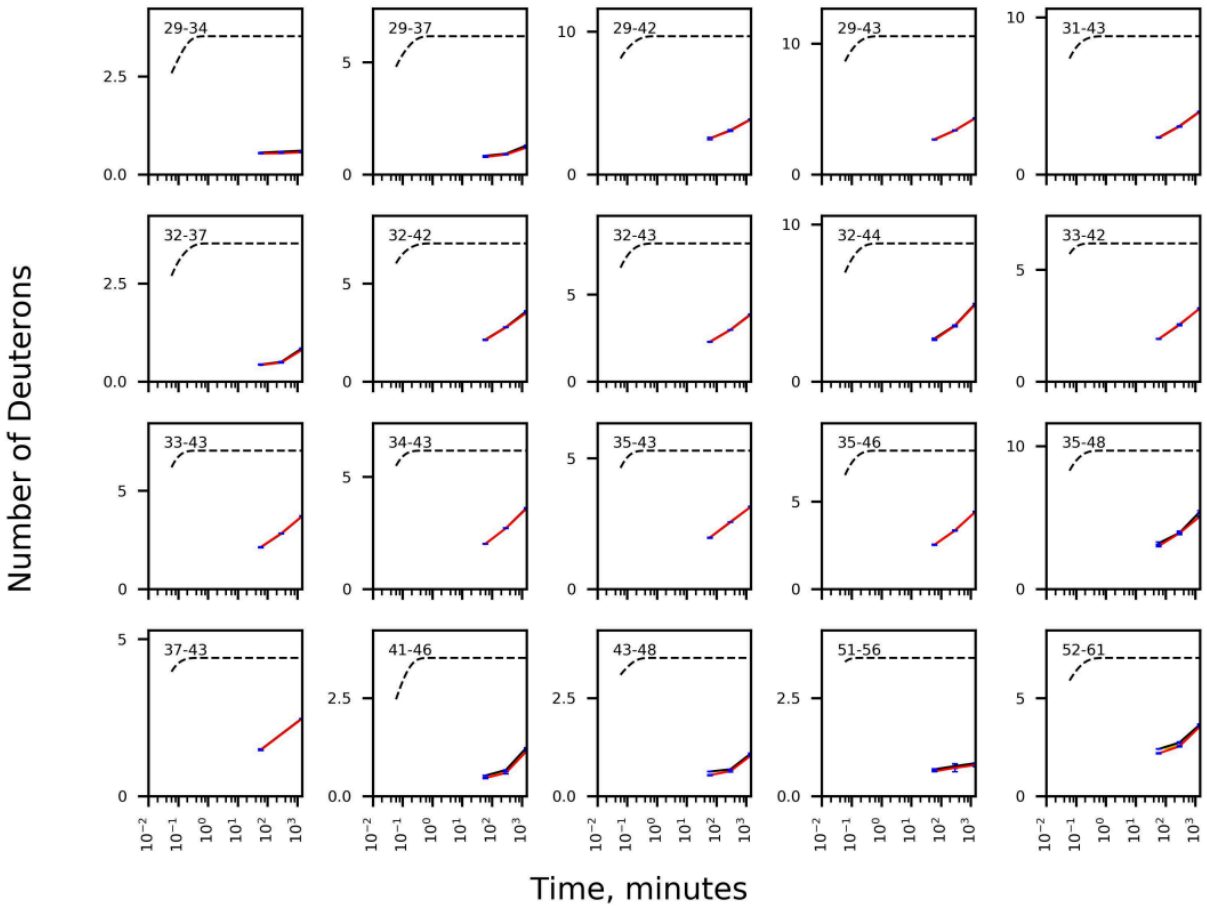
27-47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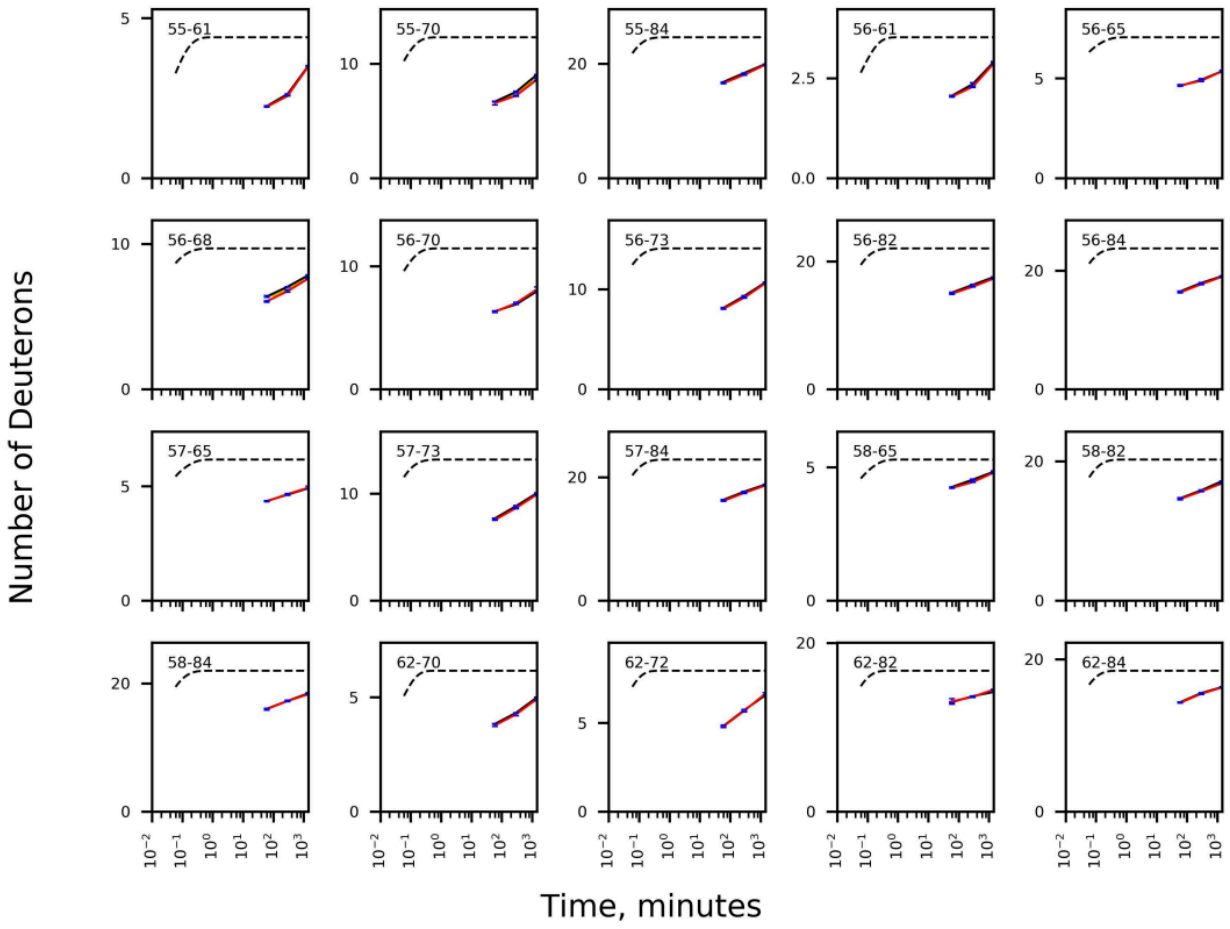
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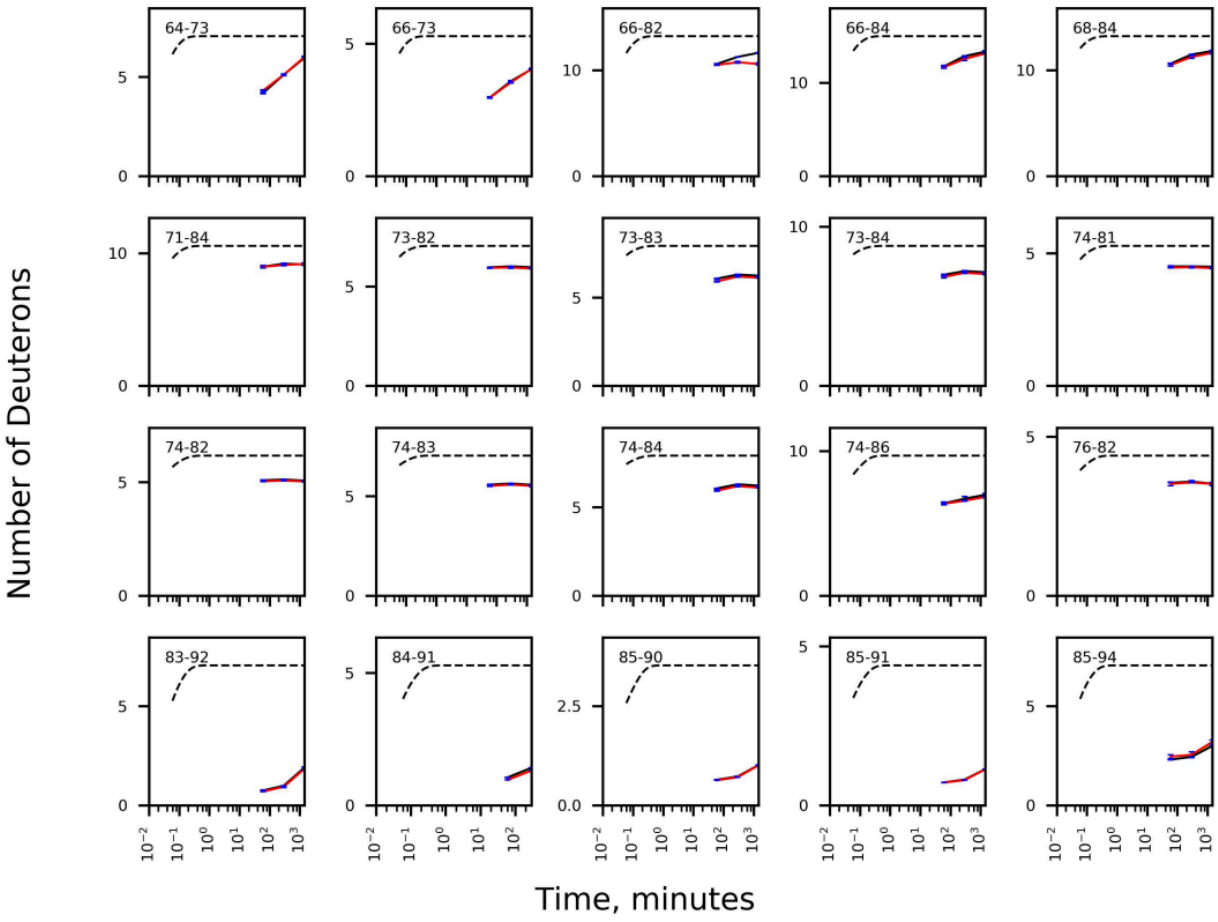
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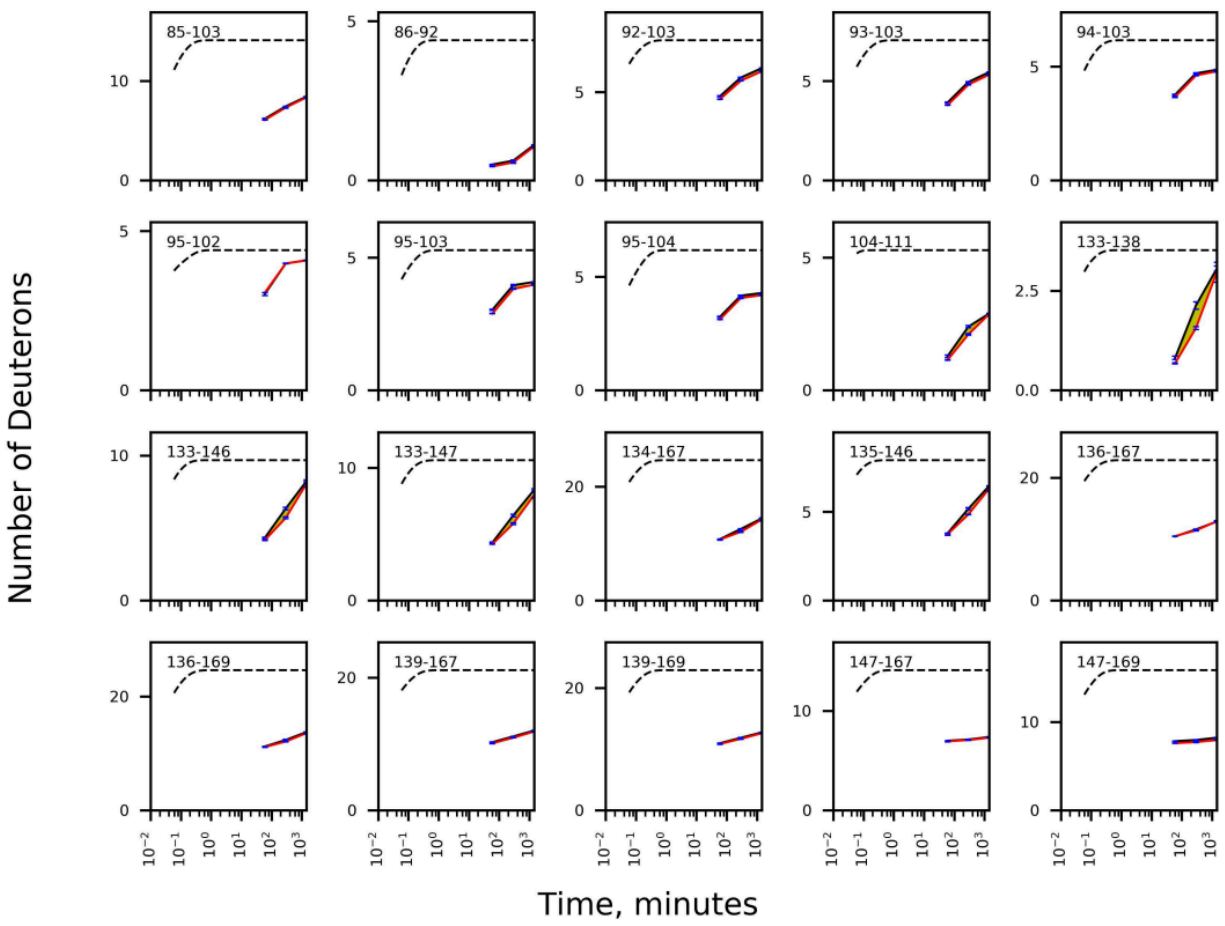
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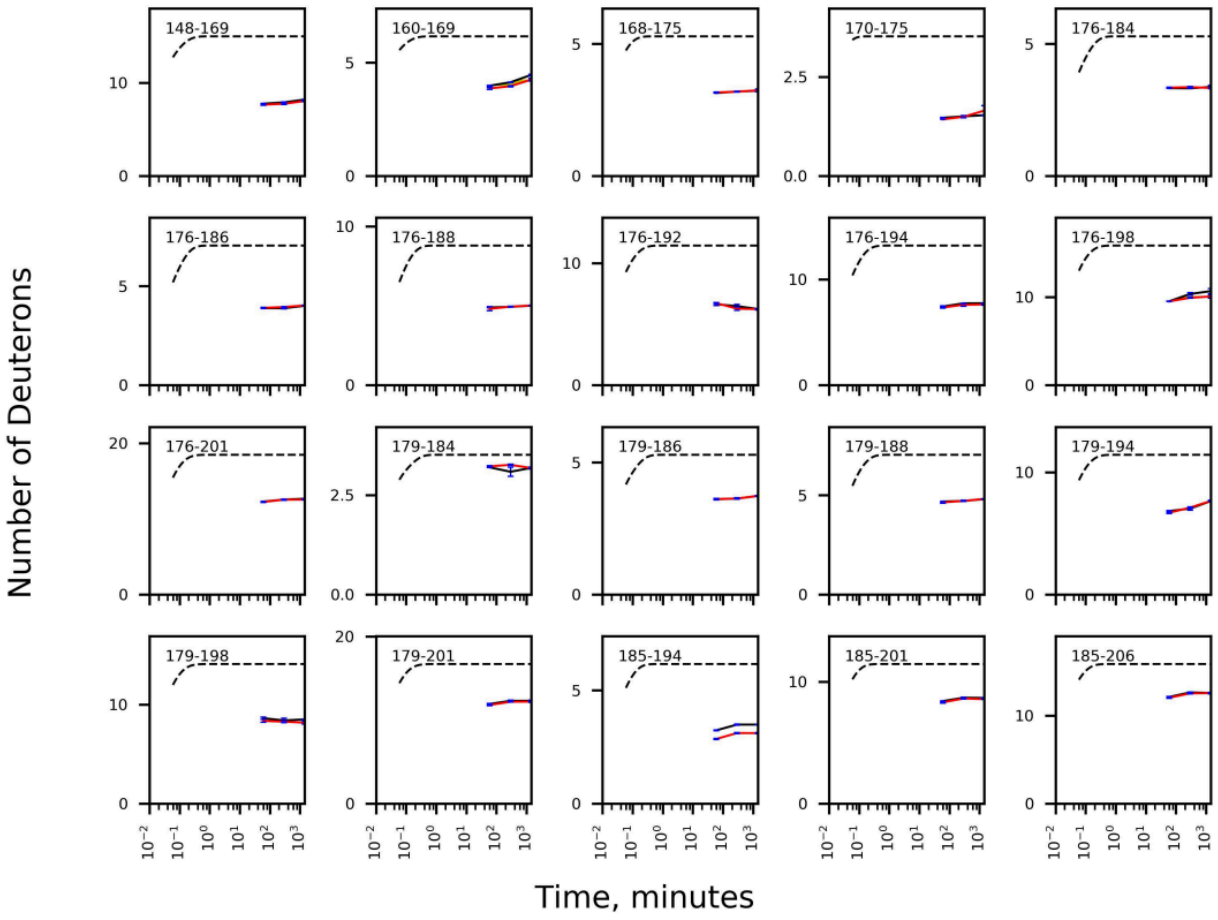
147-169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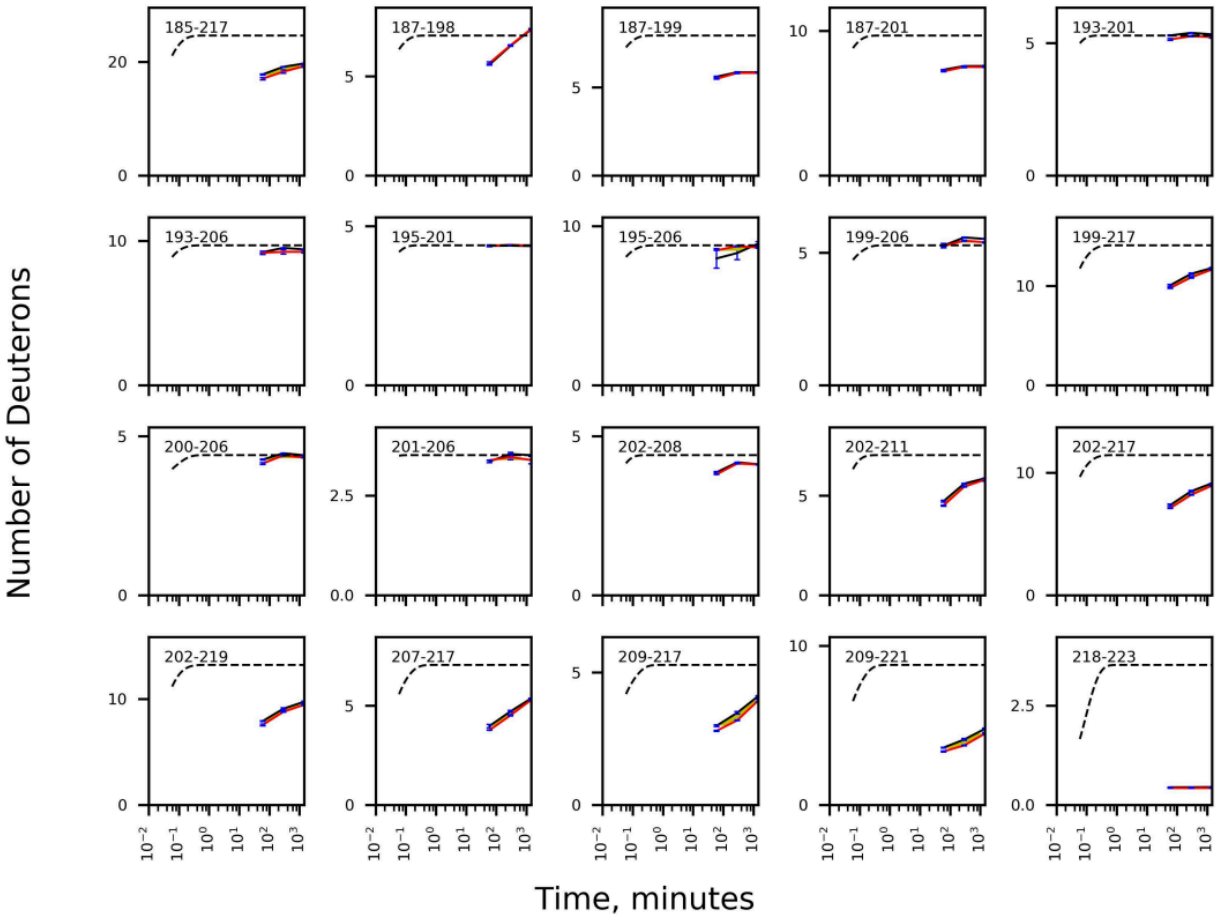
Number of Deuterons
Time, minutes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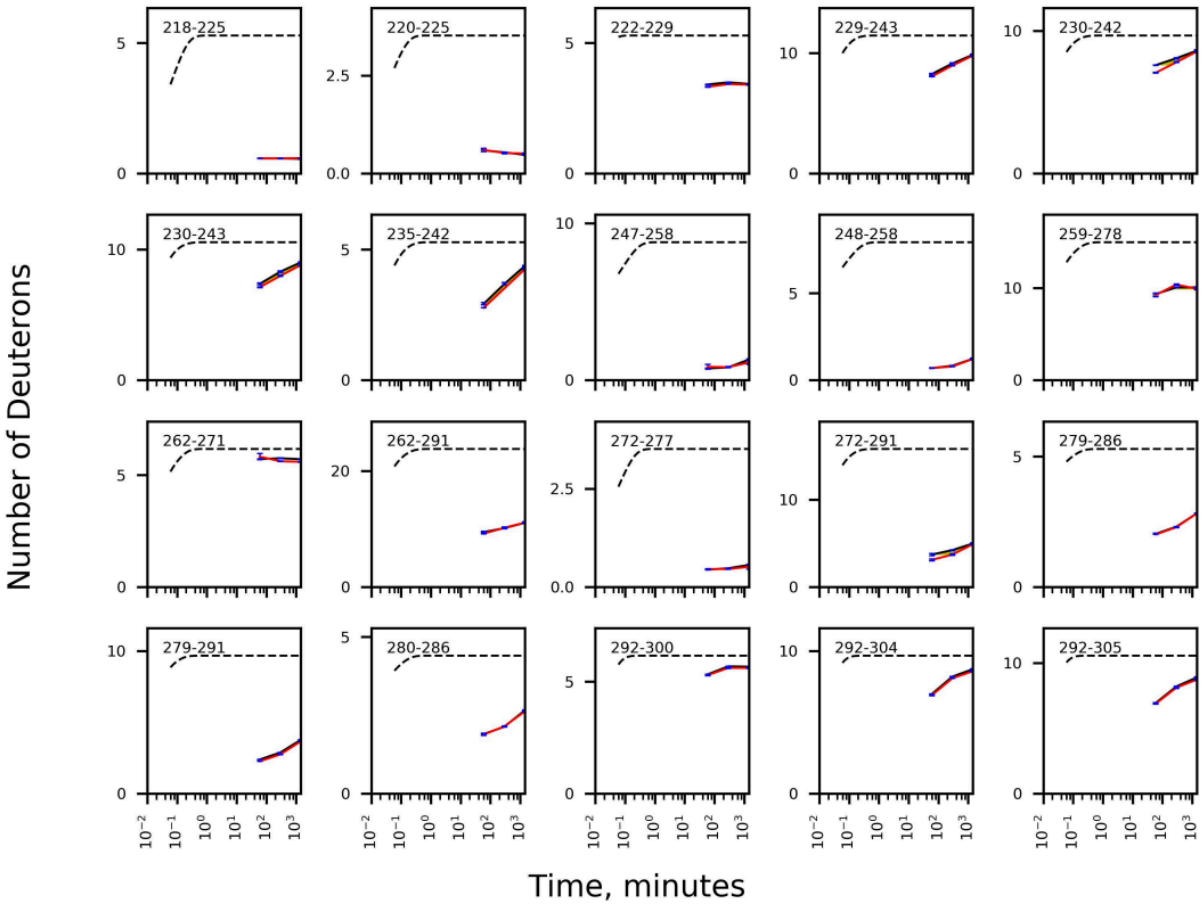
Number of Deuterons
Time, minutes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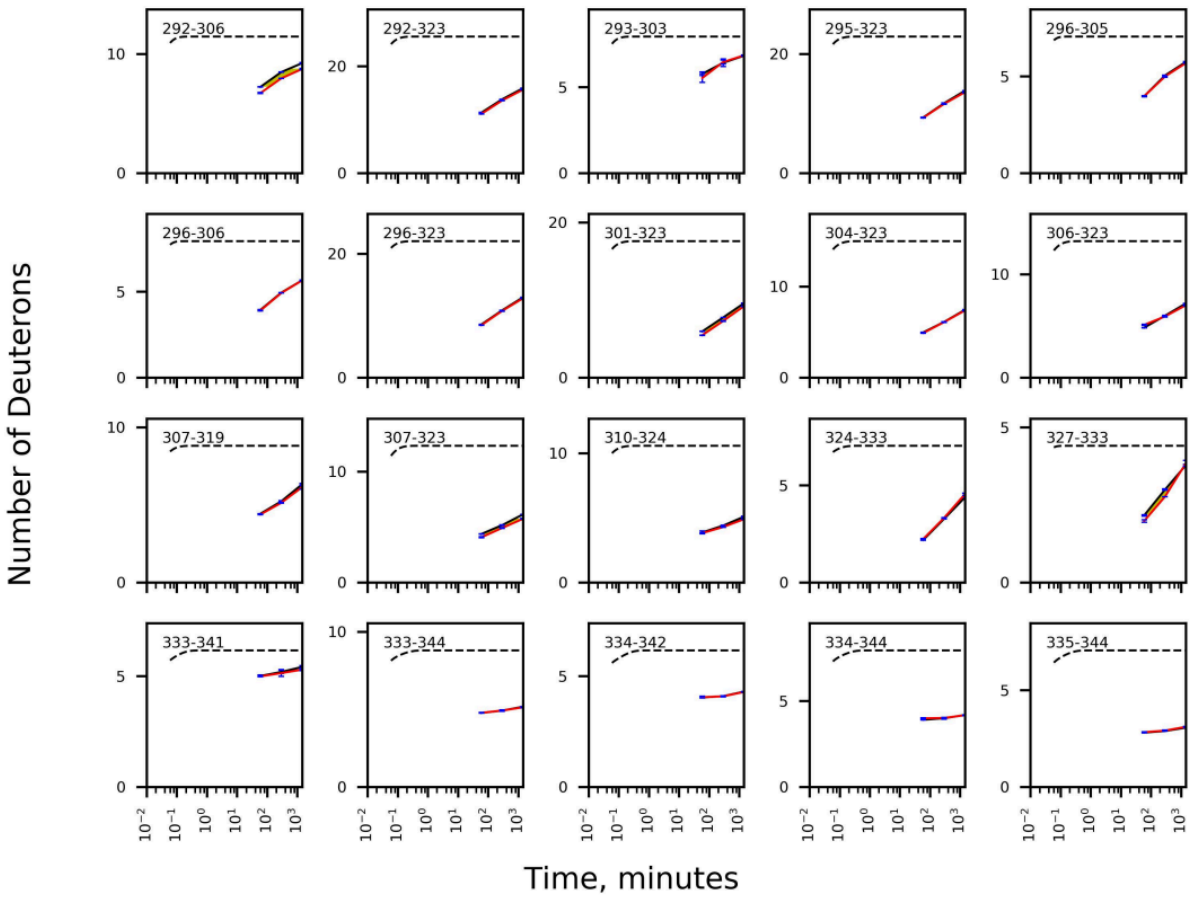
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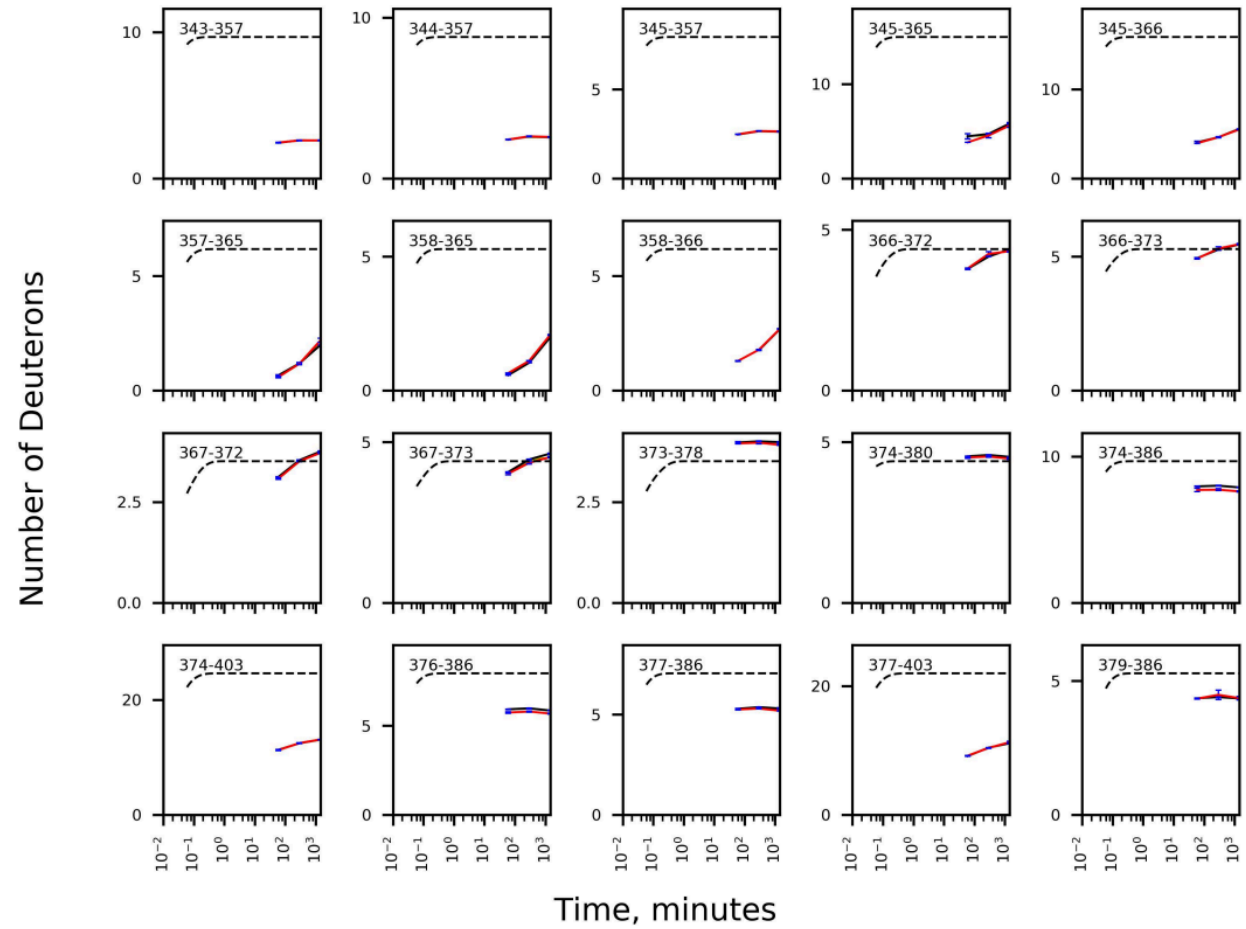
Number of Deuterons
Time, minutes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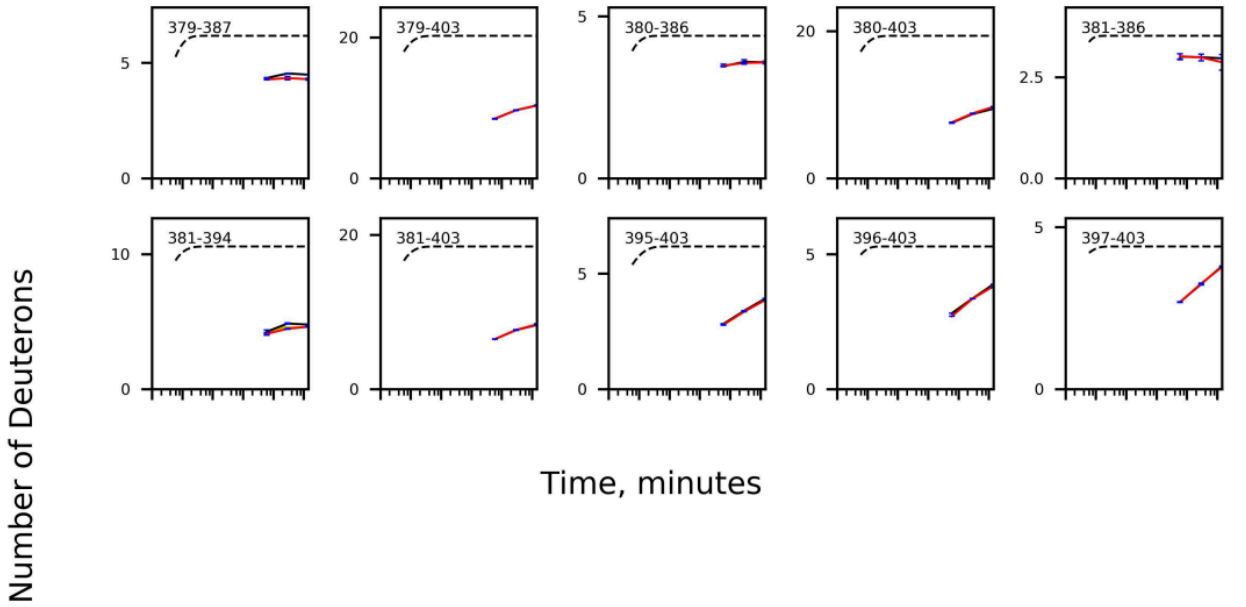
Number of Deuterons
Time, minutes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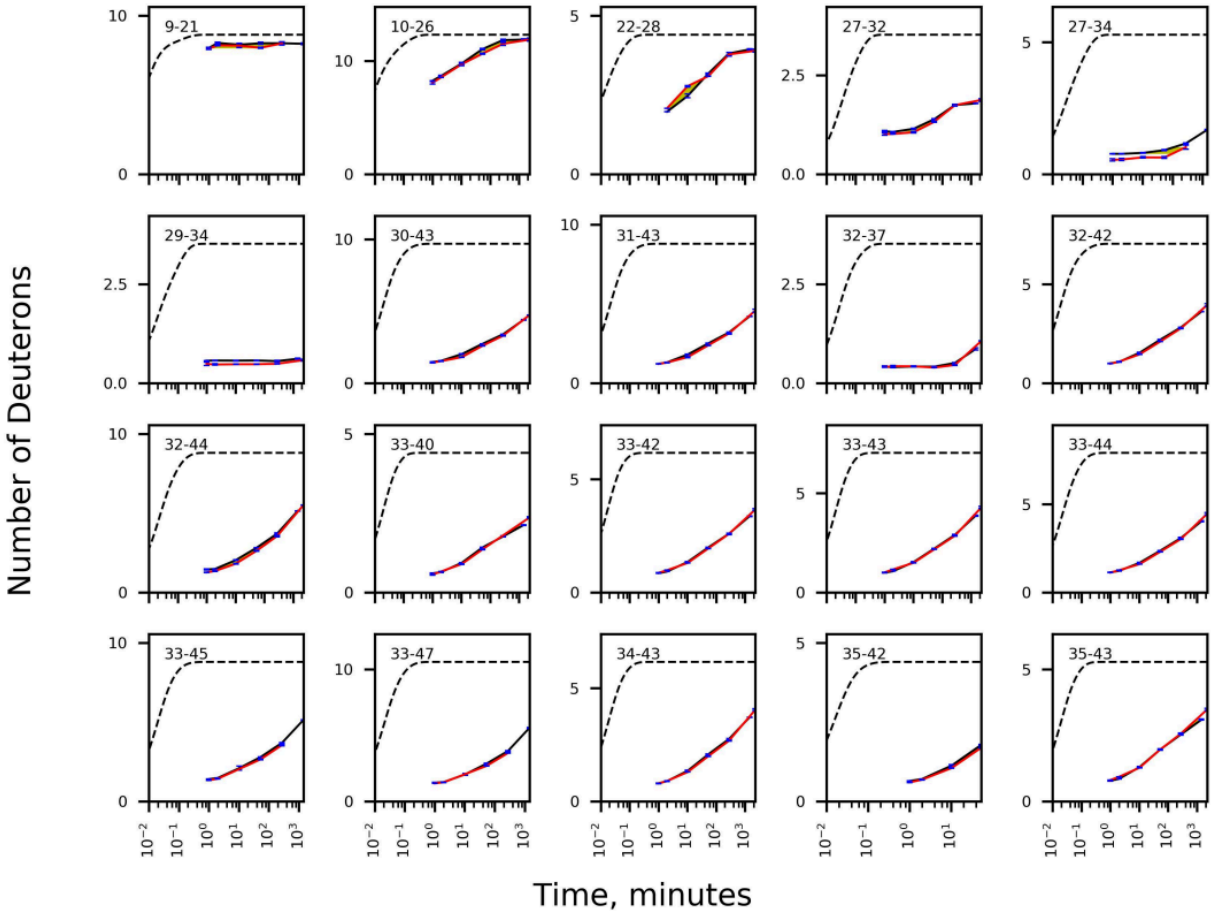
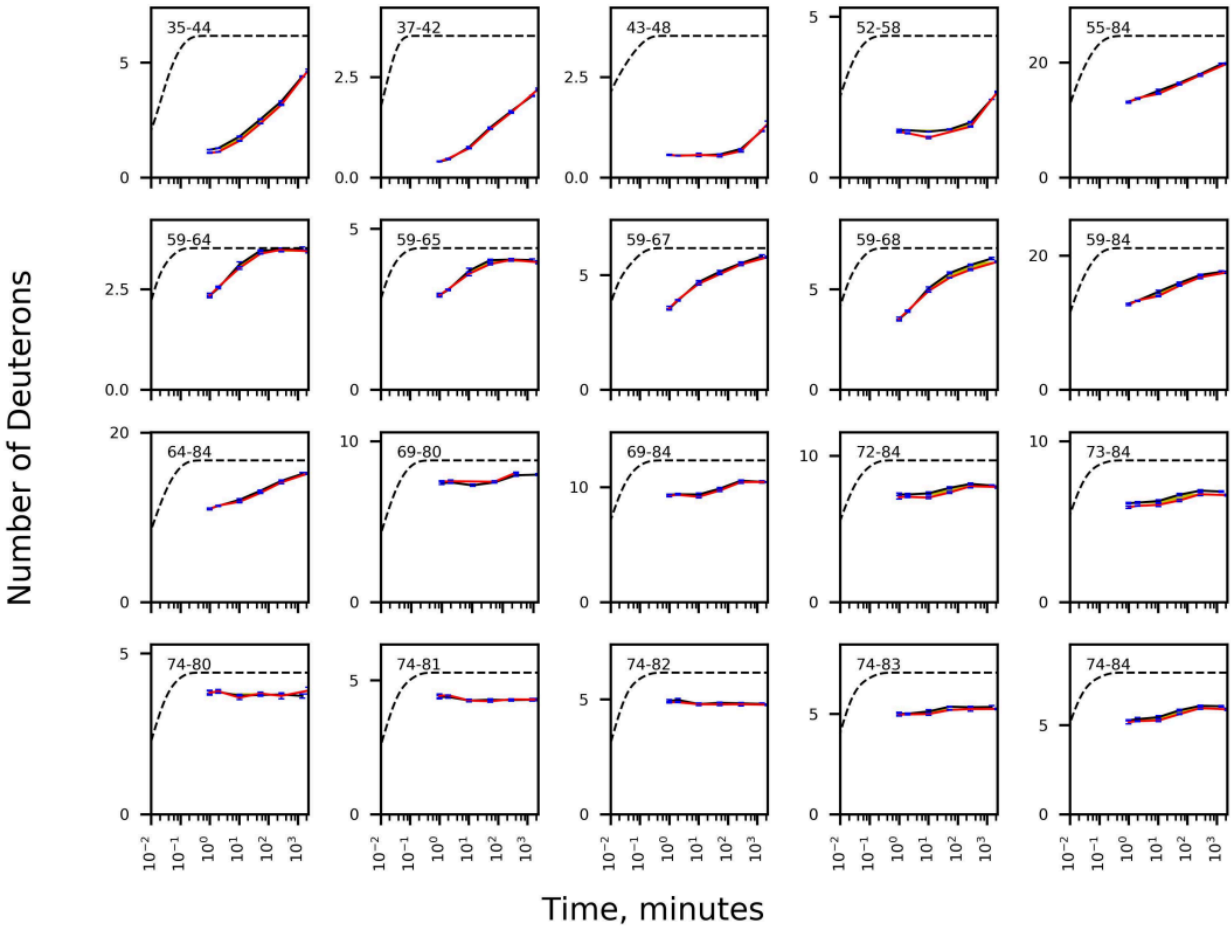
Number of Deuterons
Time, minutes

Supplementary Figure 1. Black and red traces highlight the deuterium added on each peptide for Apo FBXO22, or SMARCA2+FBXO22, respectively. Each datapoint is plotted with error bars +/- SEM, N=3 replicates. Yellow area is highlighted to draw attention to the region of uptake used to compute empirical protection factors.

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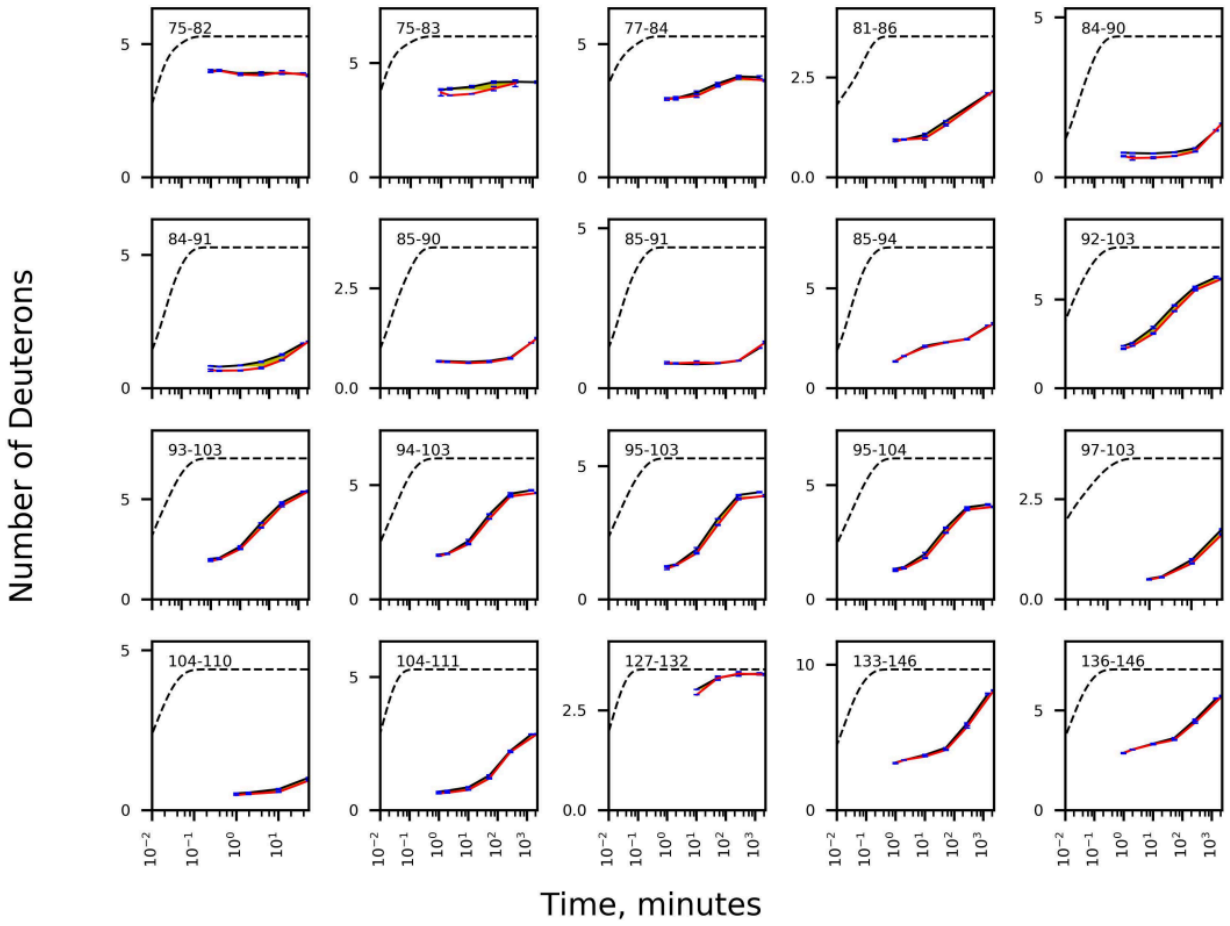
Number of Deuterons
Time, minutes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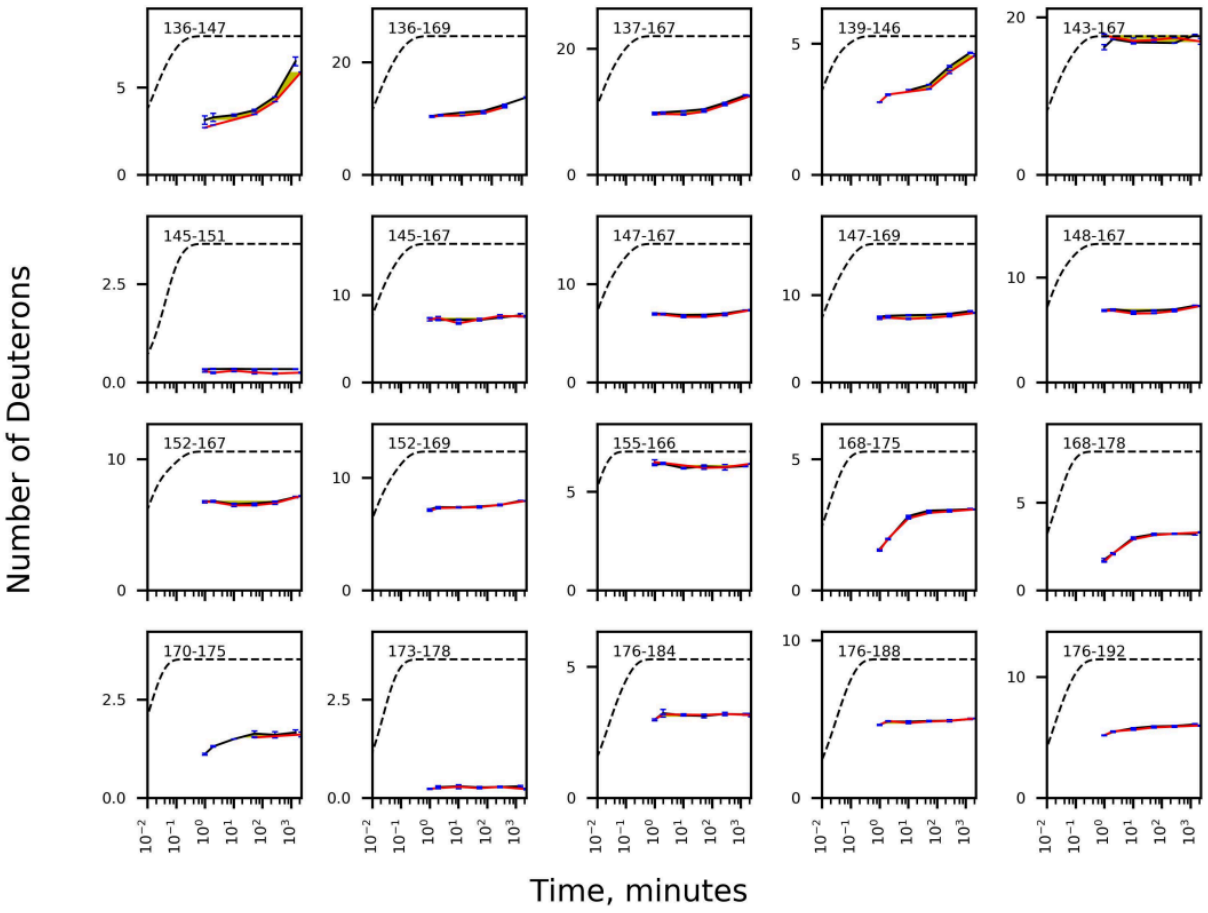
136-146
Time, minutes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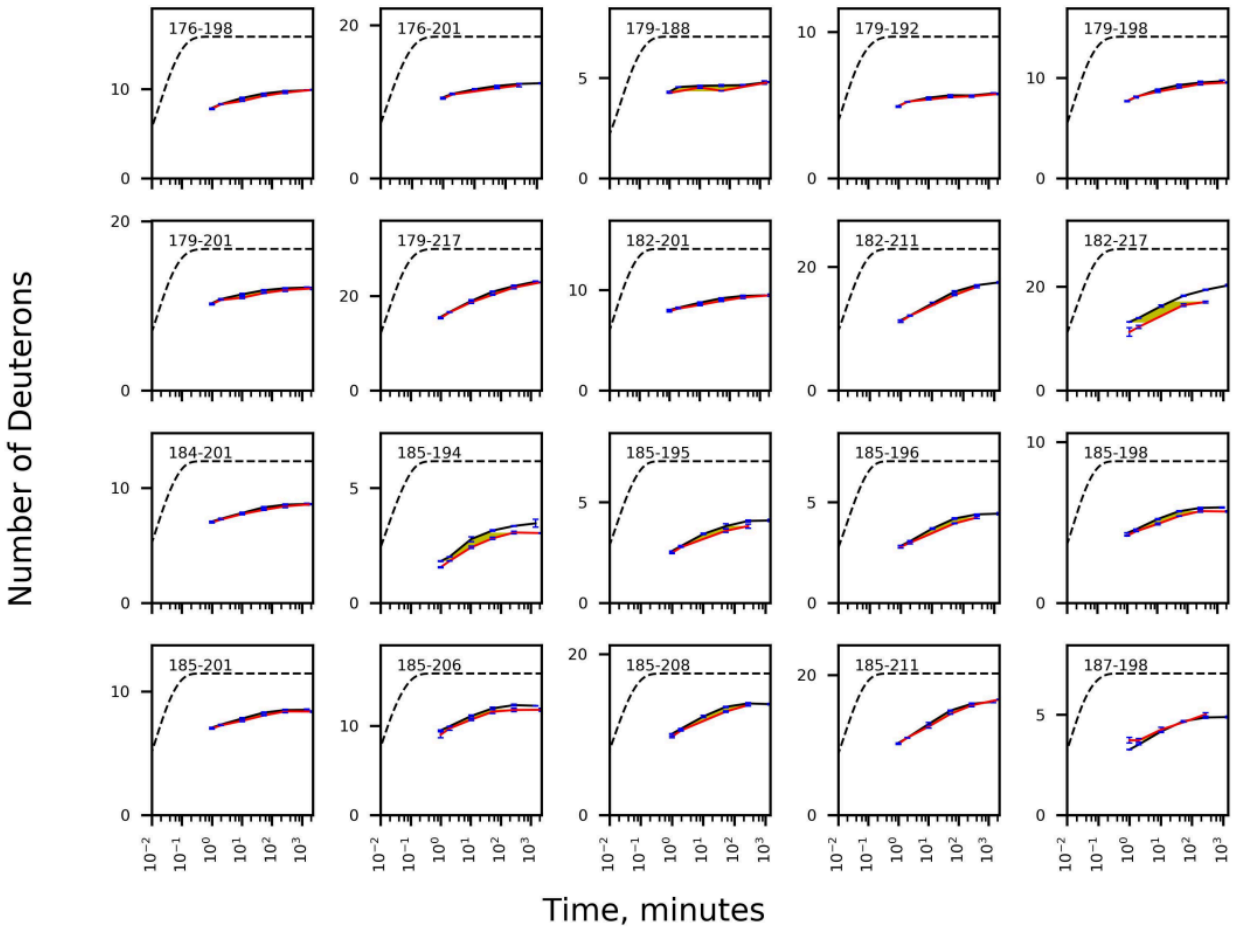
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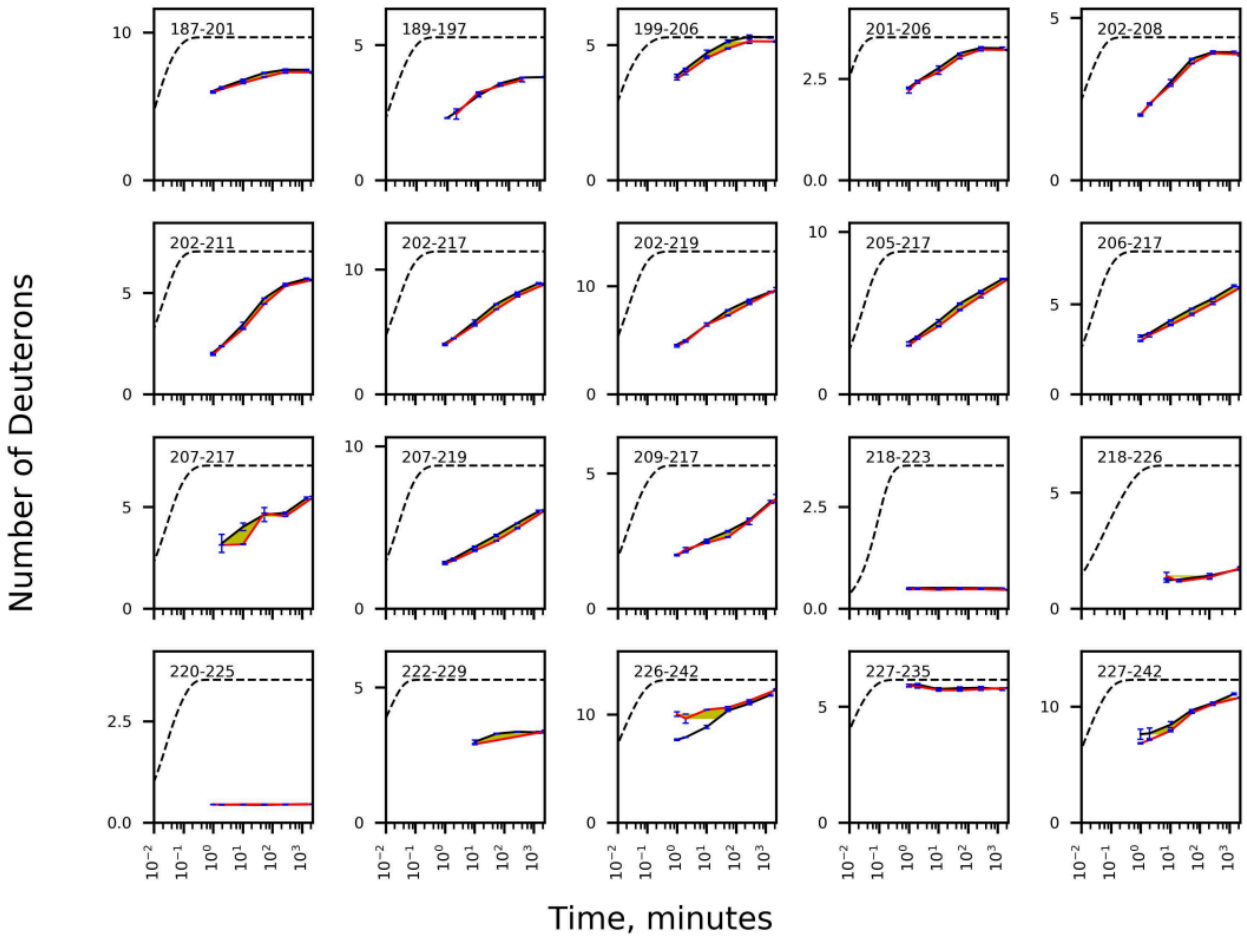
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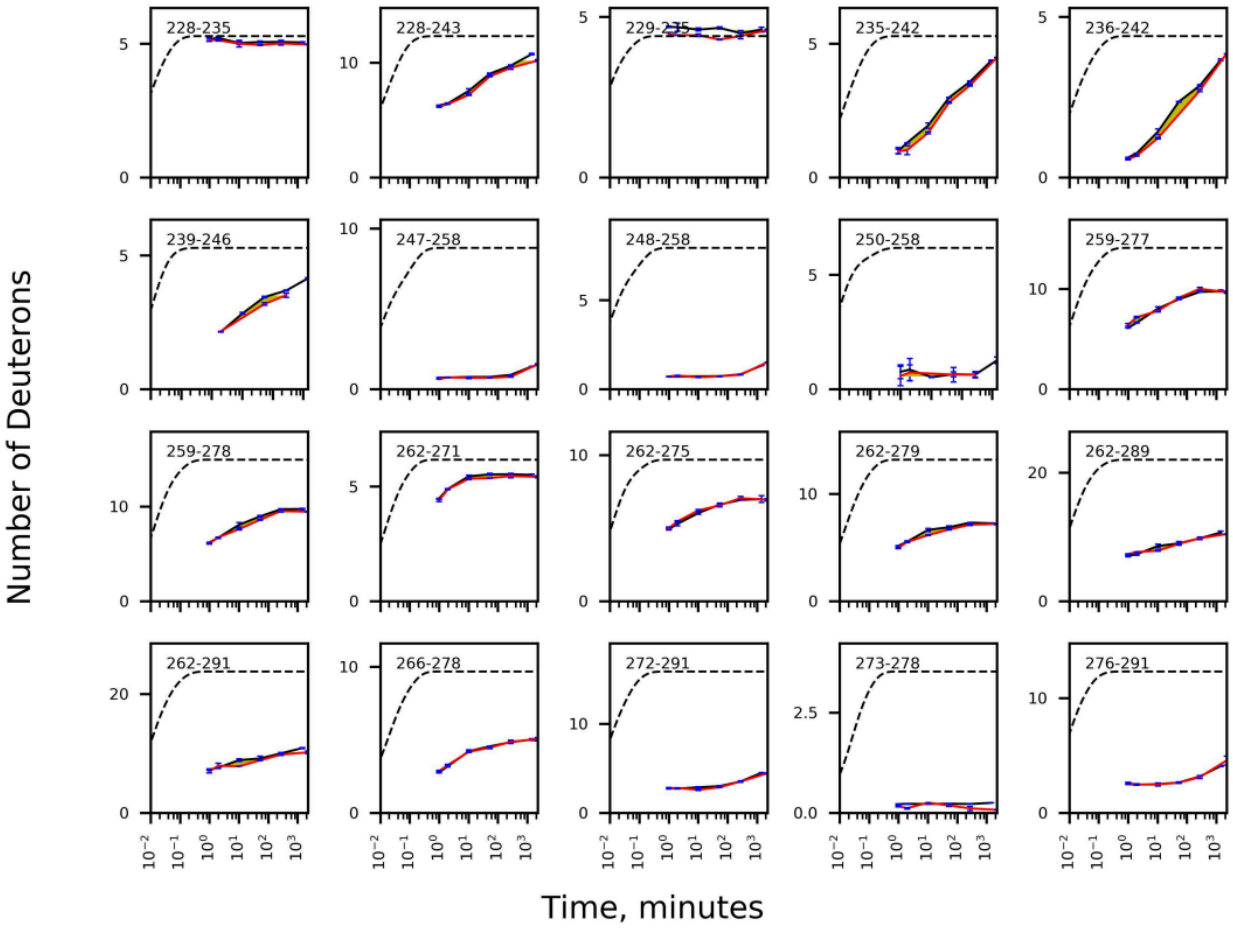
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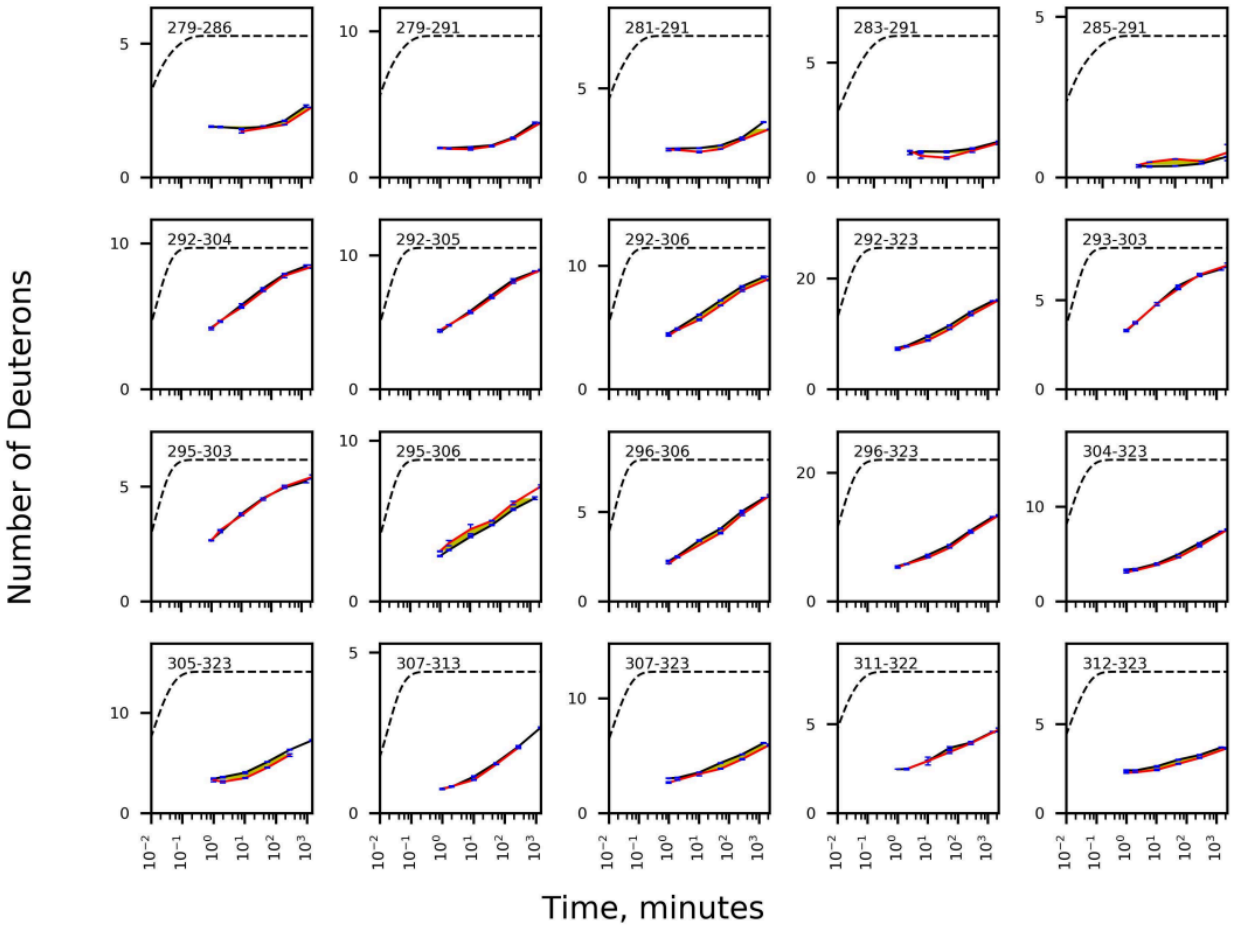
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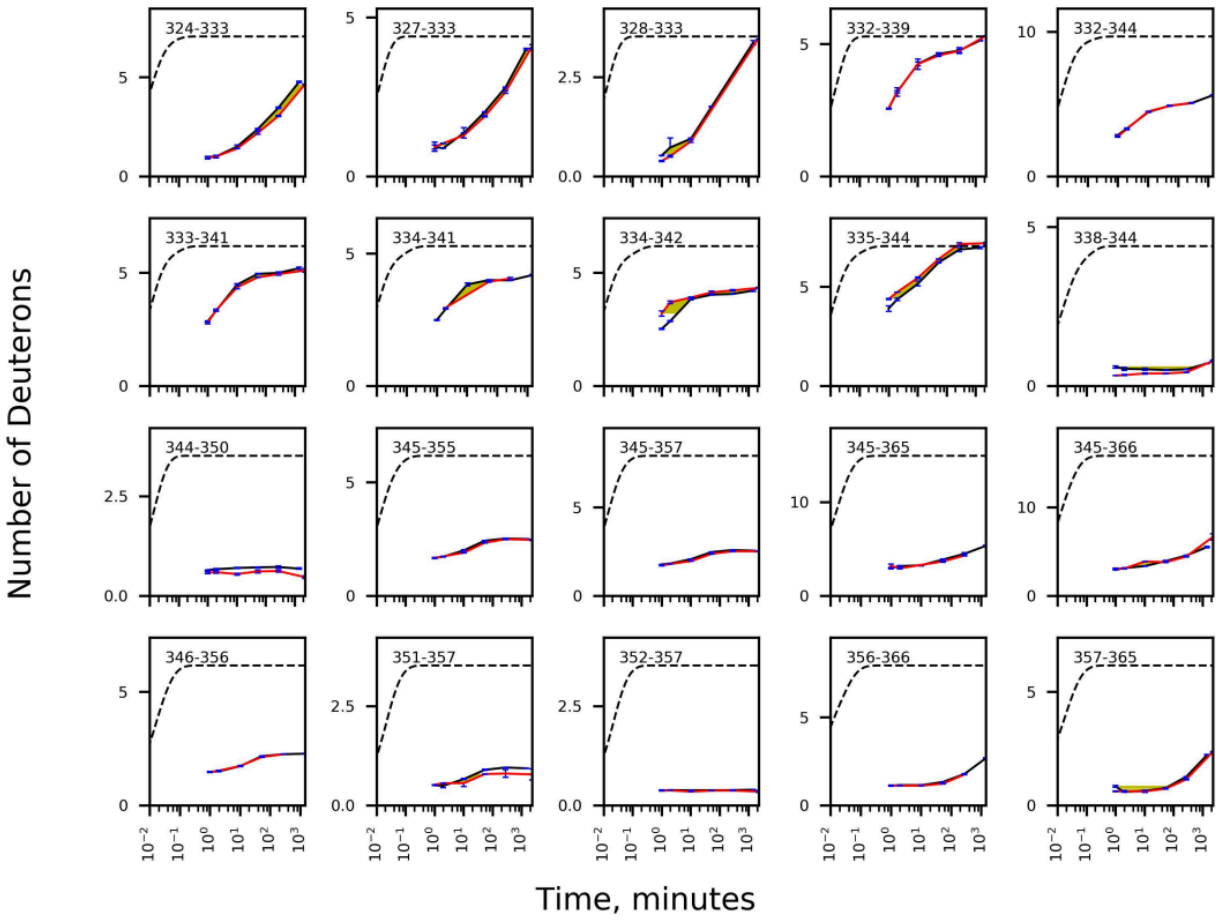
312-323
Number of Deuterons
Time, minutes


Number of Deuterons
Time, minutes
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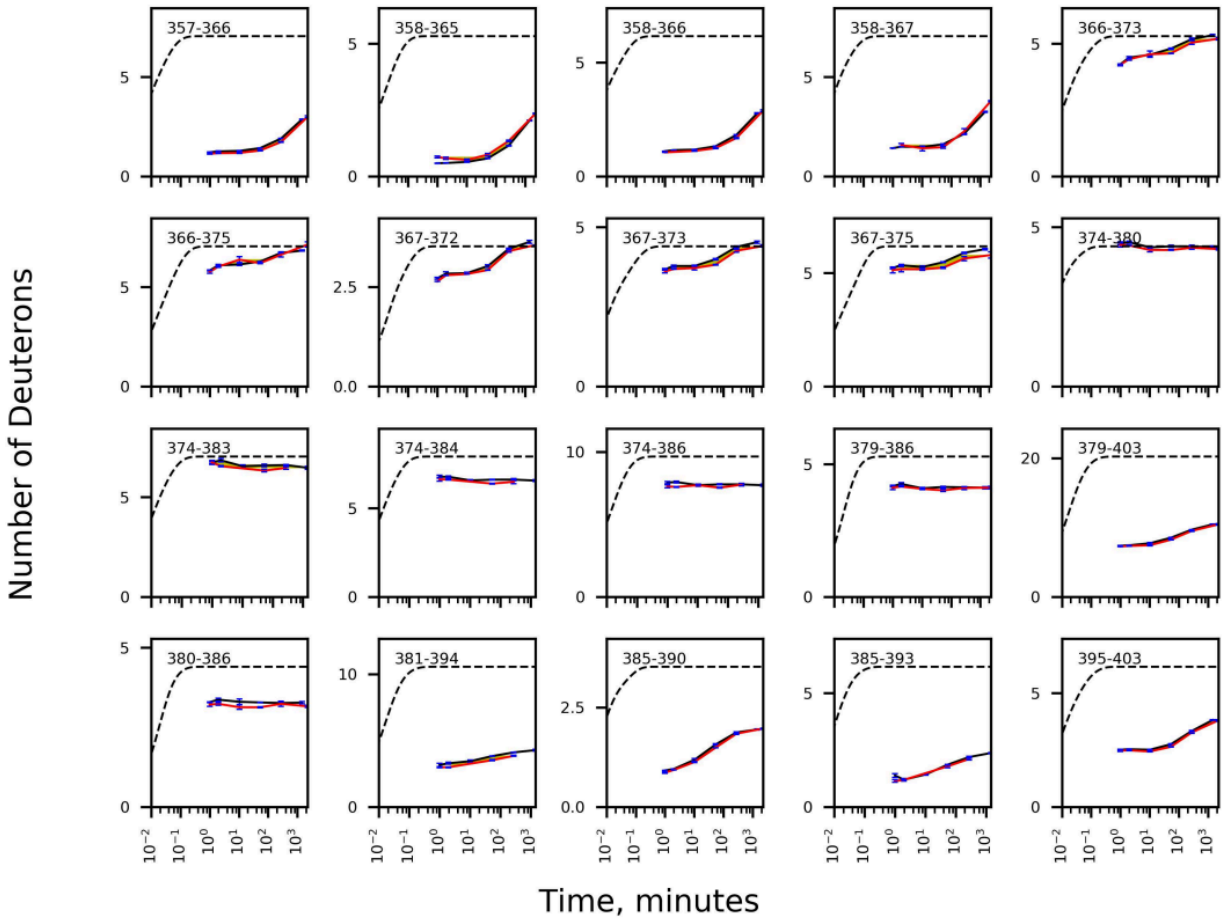
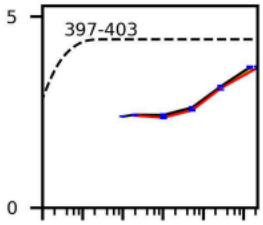
356-366
357-365





Number of Deuterons

Time, minutes

Supplementary Figure 2. Black and red traces highlight the deuterium added on each peptide for Apo FBXO22, or SMARCA2+G-6599+FBXO22, respectively. Each datapoint is plotted with error bars +/- SEM, N=3 replicates. Yellow area is highlighted to draw attention to the region of uptake used to compute empirical protection factors.