

Frustration in the Protein-Protein interface Plays a Central Role in the Cooperativity of PROTAC Ternary Complexes

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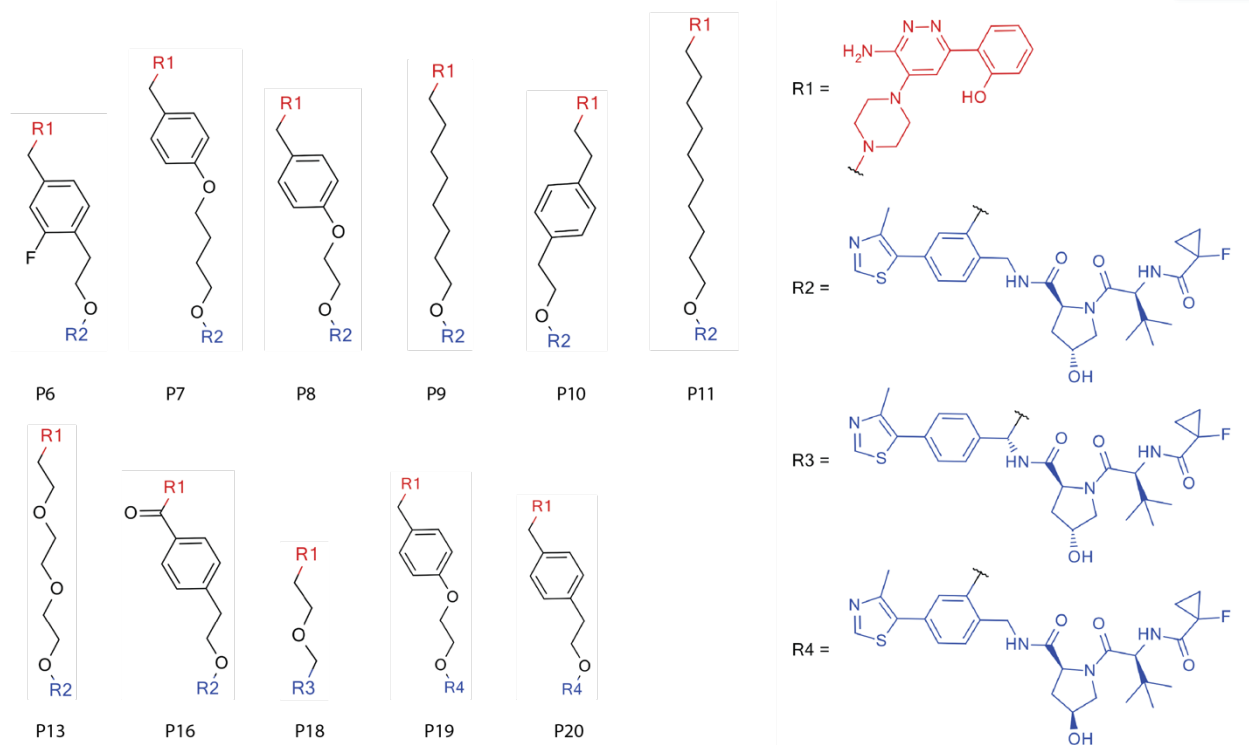
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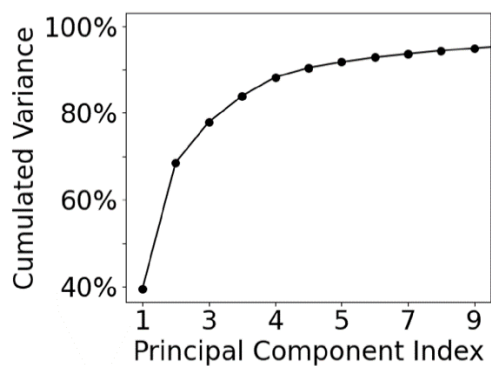
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Supplementary Information

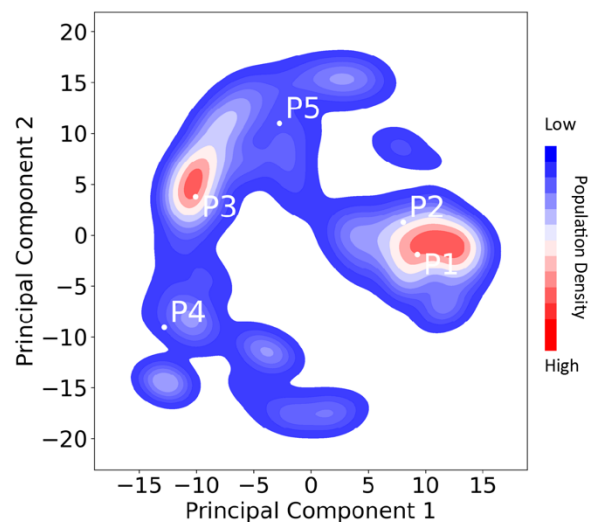
1. Supplementary Figure



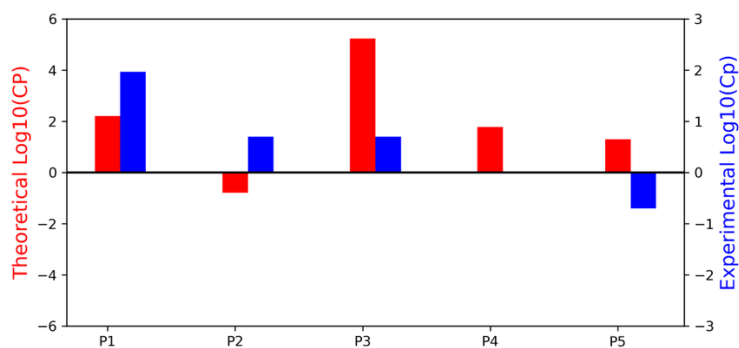
Supplementary Figure 1. Eleven GEN-1 based PROTACs investigated in this work. P9, P10, P11 and P18 have not been described previously, their synthesis is detailed below.



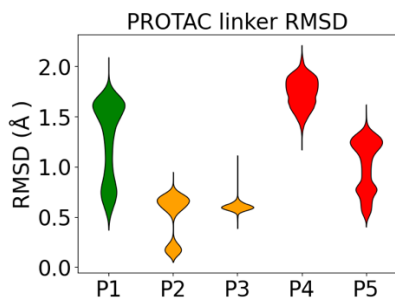
Supplementary Figure 2. The cumulated variance in the Principal Components for the top 10 components. The Principal Component Analysis was performed on the aggregated trajectories of the MD simulations starting from the five crystal structures. This shows that the top 3 PCs recapitulate about 80% of the domain motion of the ternary complexes.



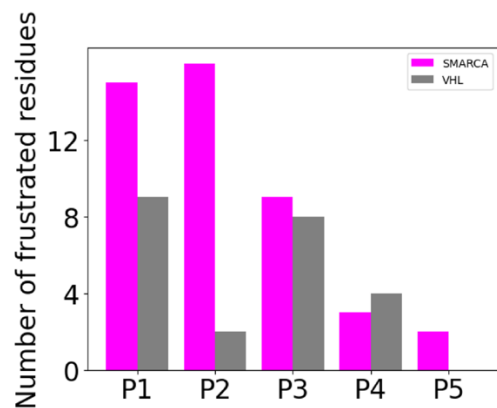
Supplementary Figure 3. Projections of conformations from the MD simulations onto the PC1-PC2 space shown in a contour plot; the respective crystal structures are marked by white dots and labeled P1-P5.



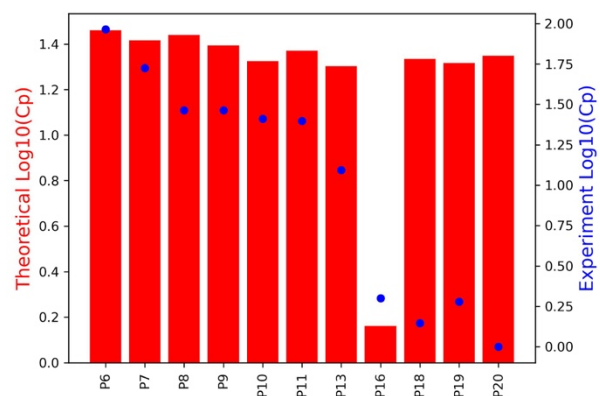
Supplementary Figure 4. Comparison between MMGBSA predicted theoretical LogC_p and experimental LogC_p for P1 to P5.



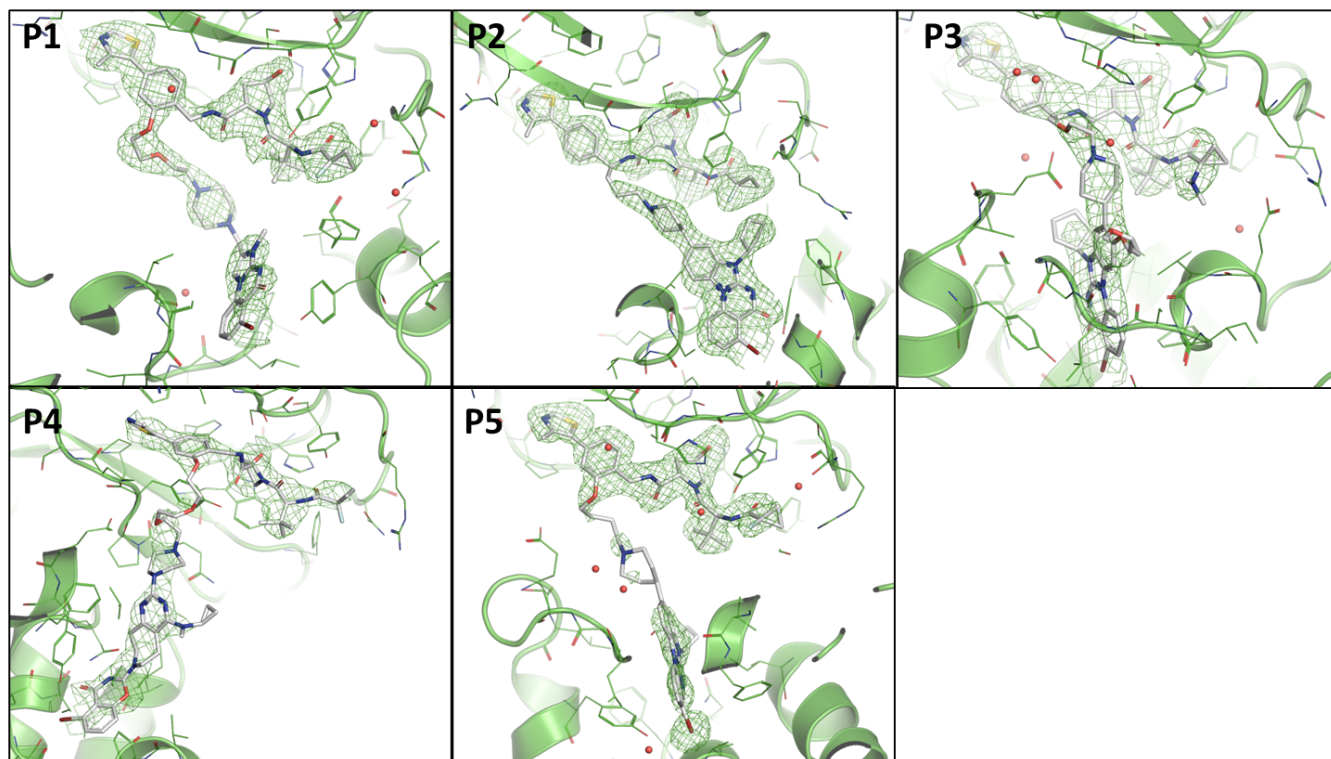
Supplementary Figure 5. the RMSD of linkers in five PROTACs. PROTACs with $\alpha > 10$ are colored in green, those with $2 < \alpha < 10$ in orange, and $\alpha < 2$ cases are colored in red.



Supplementary Figure 6. Number of SMARCA2 or VHL residues involved in the frustrated pairs located in the SMARCA2-VHL interface. SMARCA2 has greater number of frustrated pairs than VHL in all PROTACs except P4.



Supplementary Figure 7. Comparison between MMGBSA predicted theoretical LogC_p and experimental LogC_p for P6 to P20.



Supplementary Figure 8. Omit maps ($f_o - f_c$ maps contoured at 3.0σ) for ligands P1 to P5.

2. Supplementary Methods

Analytical experiments

NMR

NMR experiments were recorded on Bruker Avance 500 MHz or 600 MHz spectrometer at 298 K. Samples were dissolved in 600 μ L DMSO- d_6 and TMS was added as an internal standard. 1D ^1H spectra were acquired with 30° excitation pulses and an interpulse delay of 4.2 s with 64 k datapoints and 20 ppm sweep width. 1D ^{13}C spectra were acquired in modulated mode with broadband composite pulse decoupling (WALTZ16) and an interpulse delay of 3.3 s with 64 k datapoints and a sweep width of 240 ppm. Processing and analysis of 1D spectra was performed with Bruker Topspin 3.6 software. No zero filling was performed, and spectra were manually integrated after automatic baseline correction. ^{13}C DEPT modulated spectra were phased so that C, CH_2 are positive, and CH, CH_3 are negative. 2D HSQC spectra were recorded on all samples to aid the interpretation of the data and to identify signals hidden underneath solvent peaks. Spectra were acquired with sweep widths obtained by automatic sweep width detection from 1D reference spectra in the direct dimension with 1k datapoints and with 210 ppm and 256 datapoints in the indirect dimension. Spectra were processed with Topspin 3.6 software from Bruker and analyzed with ACDlabs NMR workbook 2023.

In certain cases, it was not possible to differentiate between peaks arising from rotamers and those arising from weak intensities due to either slow relaxing quaternary carbons and/or other causes, which is why the number of peaks in the carbon peak list is not always consistent with the number of carbons in the molecule. The same holds true for the proton intensities where due to overlapping rotamers the integrals are sometimes slightly smaller or larger than the expected number of protons.

HPLC-MS

All samples were analyzed on an Agilent 1200 series LC system coupled with an Agilent 6140 mass spectrometer. Purity was determined via UV detection with a bandwidth of 170 nm in the range from 230-400nm. LC parameters were as follows: Waters Xbridge C18 column, 2.5 μ m particle size, 2.1 x 20 mm. Run time 2.1 minutes, flow 1 ml/min, column temperature 60°C and 5 μ L injections. Solvent A (20mM NH_4HCO_3 / NH_3 pH 9), solvent B (MS grade acetonitrile). Start 10% B, gradient 10% - 95% B from 0.0 - 1.5 min, 95% B from 1.5 - 2.0 min, gradient 95% - 10% B from 2.0 – 2.1 min.

HRMS

The mass calibration was performed using the Pierce LTQ Velos ESI positive ion calibration solution from Thermo Scientific (Product Nr. 88323).

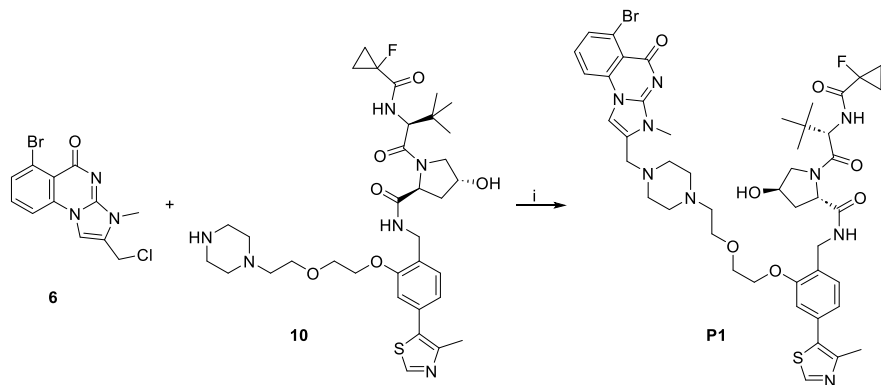
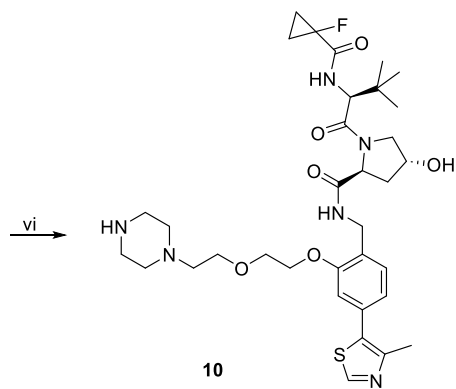
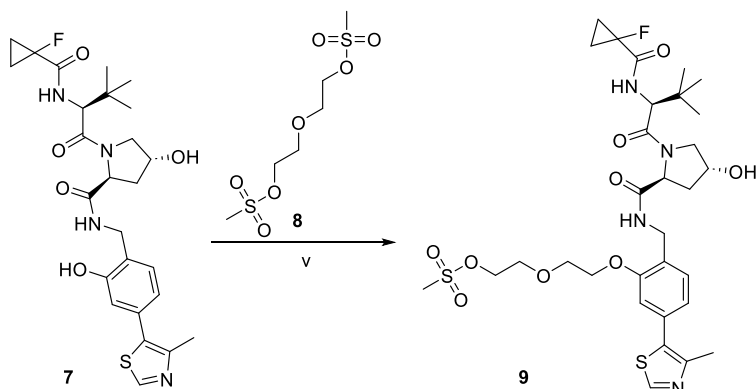
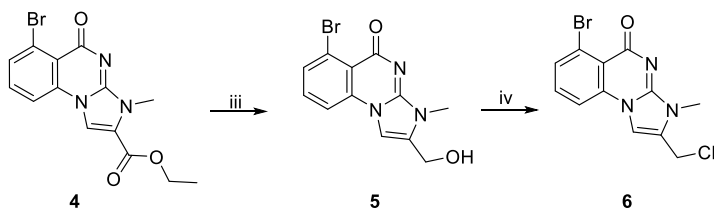
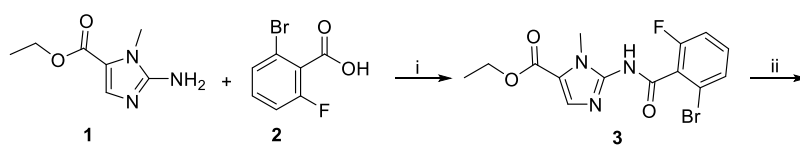
MS parameters: The scan window was set to 200-1200 amu with a maximum injection time of 500 ms and 1 microscan. The resolution of the Orbitrap was 120000 with a mass accuracy ≤ 5 ppm. The ion mode set to positive with a capillary temperature 275 °C and voltage of 60 eV. The tube lens potential was set to 110 eV. 12 NanoESI voltage was 1.45 kV and the N_2 -gas pressure set to 0.45 psi. Total sample volume was 1 μ L and the acquisition time was 0.4 sec, with 10 scans of averaging per spectrum Sample dilution: 10 mM DMSO stock solution was diluted 1:1000 in 25 % acetonitrile +0.01 % formic acid.

Chemical synthesis

General information

All chemicals unless otherwise stated, were commercially available, at least 90% pure, and used without further purification. Commercially available dry solvents were used. All reactions were carried out under nitrogen atmosphere. Normal phase TLC was carried out on pre-coated silica plates (Kieselgel 60 F254, BDH) with visualization via UV light (UV 254 and/or 365 nm) and/or basic potassium permanganate solution. Isolute® phase separator columns from Biotage were used. Flash column chromatography (FCC) was performed using either a Teledyne Isco Combiflash Rf or Rf200i, or a Biotage Isolera One with prepacked Redisep RF Normal phase disposable Columns. Reverse phase chromatography was carried out on a Biotage Isolera One using SNAP-C18 Columns. NMR Spectra were recorded on a Bruker Ascend 400 MHz or 500 MHz as specified. Chemical shifts are quoted in ppm and referenced to the residual solvent signals: ^1H NMR δ (ppm) = 7.26 (CDCl_3), ^{13}C NMR δ (ppm) = 77.16 (CDCl_3); ^1H NMR δ (ppm) = 5.32 (CD_2Cl_2), ^{13}C NMR δ (ppm) = 53.84 (CD_2Cl_2), ^1H NMR δ (ppm) = 2.50 (DMSO- d_6). Signal splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br) or a combination thereof. Coupling constants (J) are measured in Hertz (Hz). High Resolution Mass Spectra (HRMS) were recorded on a Bruker microTOF. Other resolution MS and analytical HPLC traces were recorded on an Agilent Technologies 1200 series HPLC connected to an Agilent Technologies 6130 quadrupole LC/MS with an Agilent diode array detector. The column used was a Waters XBridge column (50 mm $\times$ 2.1 mm, 3.5 μm particle size) and the compounds were eluted with a gradient 5–95% acetonitrile/water + 0.1 formic acid (“acidic method”). Preparative HPLC was performed on a Gilson Preparative HPLC System with a Waters XBridge C18 column (100 mm $\times$ 19 mm; 5 μm particle size) and a gradient of 5 % to 95 % acetonitrile in water over 10 minutes, flow 25 mL/min, with 0.1 % ammonia in the aqueous phase.

Abbreviations used: EtOAc for ethyl acetate, DMSO for dimethylsulfoxide, MeOH for methanol, EtOH for ethanol, DMF for *N,N*-dimethylformamide, THF for tetrahydrofuran, DCE for 1,2-dichloroethane, STAB for sodium triacetoxyborohydride, sat. for saturated, aq. for aqueous.



Supplementary Figure 9. Synthesis of P1. i) a) **2**, CDI, dioxane, 95°C; b) **1**, HOBt, dioxane, RT; *then* add a) to b), 105°C; ii) K₂CO₃, DMF, 90°C, μ w; iii) LiBH₄, THF/MeOH, RT; iv) MsCl, TEA, DCE/DMSO, 50°C; v) **8**, K₂CO₃, DMF, 60°C; vi) piperazine, TEA, KI, MeCN, 80°C. vii) K₂CO₃, KI, DMF, 100°C.

Synthesis of ethyl 2-(2-bromo-6-fluorobenzamido)-1-methyl-1H-imidazole-5-carboxylate **3**

A solution of 2-Bromo-6-fluorobenzoic acid **2** (11.9 g, 54.4 mmol, 1.15 eq.) and CDI (11.5 g, 70.9 mmol, 1.50 eq.) in 1,4-dioxane (64 mL) was stirred at 95°C for 1 h. In a second vessel, a solution of ethyl 2-amino-1-methyl-1H-imidazole-5-carboxylate **1** (8.0 g, 47.3 mmol) and HOBt (10.2 g, 75.7 mmol, 1.60 eq.) was stirred at RT for 1 h. The activated acid was added to this solution and the resulting mixture was stirred at 105°C for 12 h. The solvent was evaporated, and the crude residue was purified by silica gel column chromatography (0-100% EtOAc in petroleum ether) and again by acidic HPLC-chromatography to obtain **3** (9.0 g, 51% yield).

Synthesis of ethyl 6-bromo-3-methyl-5-oxo-3H,5H-imidazo[1,2-a]quinazoline-2-carboxylate **4**

To a solution of **3** (8.5 g, 23.0 mmol) in DMF (85 mL), potassium carbonate (9.5 g, 68.9 mmol, 3.0 eq.) was added and the mixture was stirred at 90°C for 12 h. It was quenched with sat. NH₄Cl-solution and the resulting precipitate was filtered off, rinsed with EtOAc and dried to give **4** (5.0 g, 58% yield), which was used in the next step without further purification.

Synthesis of 6-bromo-2-(hydroxymethyl)-3-methylimidazo[1,2-a]quinazolin-5(3H)-one **5**

To a solution of **4** (5.0 g, 14.3 mmol) in THF (50 mL) and MeOH (5 mL), lithium borohydride (2M in THF) (14.3 mL, 28.6 mmol, 2.0 eq.) was added dropwise at 0°C over 30 min. After complete addition, the mixture was stirred at RT for 30 min. It was cooled to 0°C and quenched by dropwise addition of 30 mL 1M aq. HCl-solution. The mixture was concentrated under reduced pressure and the residue was purified by acidic prep. HPLC-chromatography to obtain **5** (2.4 g, 53% yield).

Synthesis of 6-bromo-2-(chloromethyl)-3-methylimidazo[1,2-a]quinazolin-5(3H)-one **6**

To a solution of **5** (2.0 g, 6.5 mmol) in chloroform (20 mL), thionyl chloride (2.4 mL, 33 mmol, 5.0 eq.) was added dropwise at RT over 15 min followed by dropwise addition of DMF (505 μ L, 6.5 mmol, 1.0 eq.). The resulting mixture was stirred at RT for 2 h and concentrated under reduced pressure. The crude residue was purified by column chromatography to give **6** (0.8 g, 24% yield), which was immediately used in the following step.

Synthesis of 2-(2-(2-(((2*S*,4*R*)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamido)methyl)-5-(4-methylthiazol-5-yl)phenoxy)ethoxy)ethyl methanesulfonate **9**

To a solution of **7** (500 mg, 939 μ mol) and **8** (370 mg, 1.41 mmol, 1.5 eq.) in DMF (10 mL), potassium carbonate (390 mg, 2.82 mmol, 3.0 eq.) was added. The resulting mixture was stirred at 60°C for 12 h. It was poured into ice-water, stirred for 30 min and extracted with EtOAc. The combined organic layers were washed with brine, dried and concentrated to obtain crude **9** (600 mg, 60% yield), which was taken to the next step without further purification.

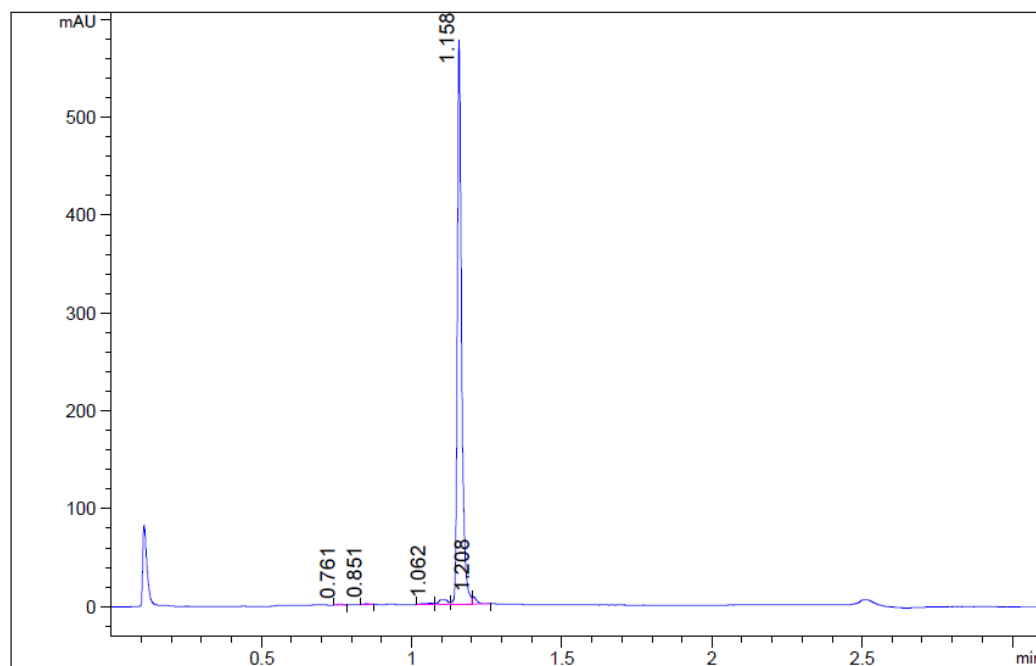
Synthesis of (2*S*,4*R*)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)-2-(2-(2-(piperazin-1-yl)ethoxy)ethoxy)benzyl)pyrrolidine-2-carboxamide **10**

To a solution of **9** (600 mg, 562 μ mol) and piperazine (145 mg, 1.68 mmol, 3.0 eq.) in MeCN (12 mL), TEA (235 μ L, 1.69 mmol, 3.0 eq.) and potassium iodide (47 mg, 283 μ mol, 0.5 eq.) were added. The resulting mixture was stirred at 80°C for 12 h. It was diluted with 1 mL water and purified by acidic HPLC-chromatography to give **10** (230 mg, 58% yield).

Synthesis of (2*S*,4*R*)-*N*-(2-(2-(2-(4-((6-bromo-3-methyl-5-oxo-3,5-dihydroimidazo[1,2-*a*]quinazolin-2-yl)methyl)piperazin-1-yl)ethoxy)ethoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide, **P1**

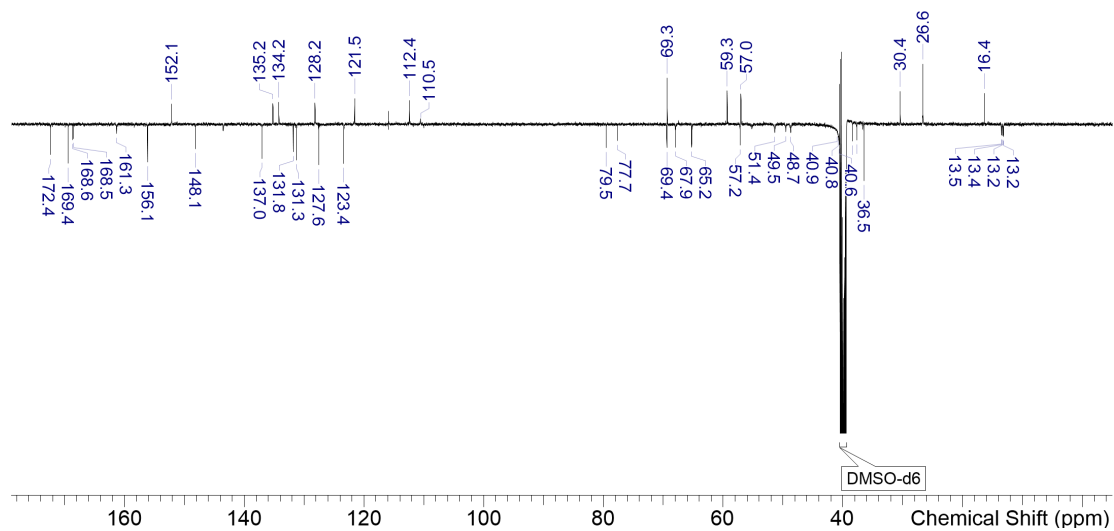
To a solution of **6** (346 mg, 668 μ mol, 2.0 eq.) and **10** (230 mg, 334 μ mol) in DMF (20 mL), potassium carbonate (230 mg, 1.66 mmol, 5.0 eq.) and potassium iodide (160 mg, 964 μ mol, 2.89 eq.) were added. The resulting mixture was stirred at 100°C for 2 h. It was filtered and the filtrate was purified by acidic prep. HPLC to obtain **P1** (38 mg, 11% yield).

HRMS (ESI+) *m/z*: [M+H]⁺ calcd for C₄₆H₅₇BrFN₉O₇S 978.32815; found 978.33543



RT: 0.76 min	Area %: 0.2 %
RT: 0.85 min	Area %: 0.3 %
RT: 1.06 min	Area %: 0.6 %
RT: 1.10 min	Area %: 1.7 %
RT: 1.16 min	Area %: 96.0 %
RT: 1.21 min	Area %: 1.3 %

Supplementary Figure 10. HPLC spectrum of **P1**.



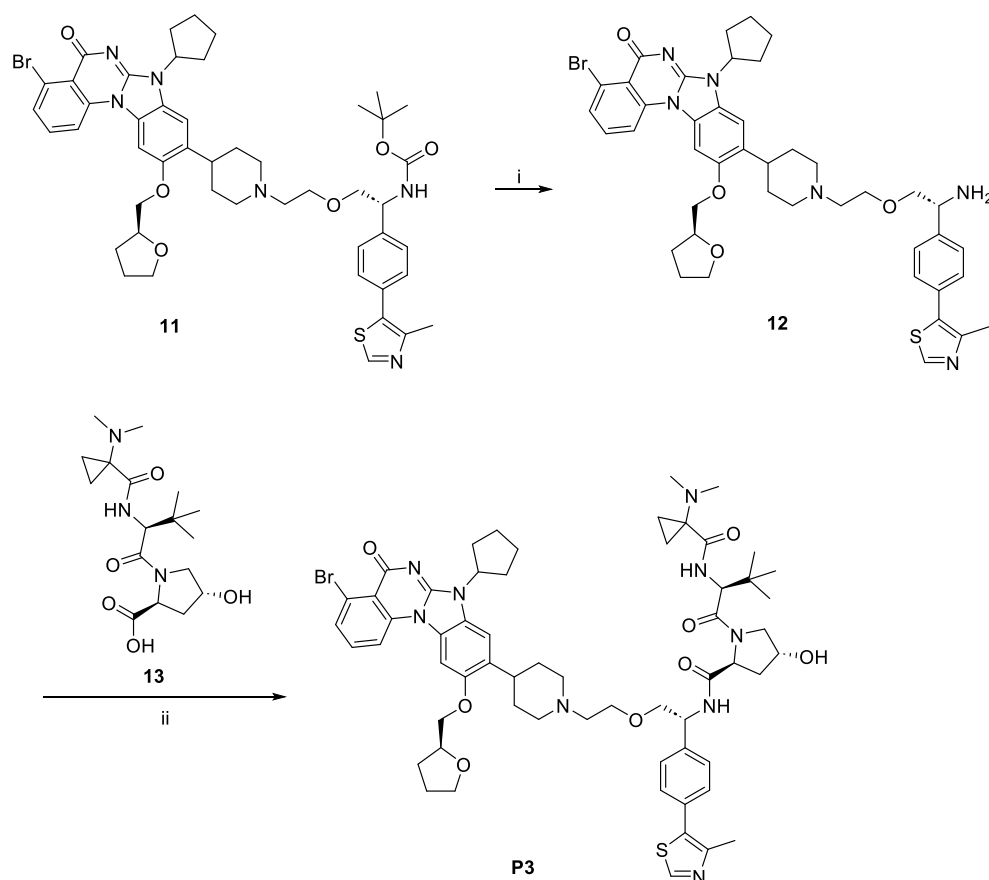
¹H NMR (DMSO-d₆) δ: 10.71-10.96 (m, 1H), 9.02 (s, 2H), 8.71 (t, J=5.5 Hz, 0.1H rotamer), 8.58 (t, J=6.0 Hz, 1H), 8.40 (s, 2H), 8.14 (d, J=8.2 Hz, 1H), 7.90 (d, J=7.9 Hz, 1H), 7.79 (t, J=8.0 Hz, 1H), 7.41 (d, J=7.9 Hz, 1H), 7.28 (dd, J=9.1, 2.5 Hz, 1H), 7.20 (s, 0.1H rotamer), 7.03 (s, 1H),

6.98 (dd, J=7.9, 0.9 Hz, 1H), 4.71 (br t, J=7.4 Hz, 0.1H rotamer), 4.59 (d, J=9.1 Hz, 1H), 4.53 (t, J=8.2 Hz, 1H), 4.44 (br d, J=8.8 Hz, 0.1H rotamer), 4.36 (br s, 1H), 4.30 (dd, J=16.4, 6.0 Hz, 1H), 4.16-4.26 (range, 3H), 3.94 (br t, J=4.4 Hz, 2H), 3.81-3.88 (range, 4H), 3.69 (s, 3H), 3.32-3.39 (range, 3H), 3.04-3.26 (range, 5H), 2.69-2.84 (range, 2H), 2.46 (s, 3H), 2.11 (br dd, J=12.3, 7.9 Hz, 1H), 1.91 (ddd, J=12.9, 8.8, 4.4 Hz, 1H), 1.29-1.43 (m, 2H), 1.22 (dd, J=8.5, 3.2 Hz, 2H), 0.95 (s, 9H)

¹³C NMR (DMSO-d₆) δ: 172.4, 169.4, 168.6 (d, CF=21 Hz), 161.3, 156.1, 152.1, 148.1, 137.0, 135.2, 134.2, 131.8, 131.3, 128.2, 127.6, 123.4, 121.5, 112.4, 78.6 (d, CF=233 Hz), 69.3, 69.4, 67.9, 65.2, 59.3, 57.2, 57.0, 51.4, 49.5, 48.7, 40.9, 40.8, 40.6, 40.6, 38.4, 37.7, 36.5, 30.4, 26.6, 16.4, 13.5 (d, CF=10 Hz), 13.2 (d, CF=11 Hz)

Analytical ¹H NMR note:

The analytical sample contains a protonated form of **P1** which affects the interchangeable peaks NH (broadened) and OH (not apparent)



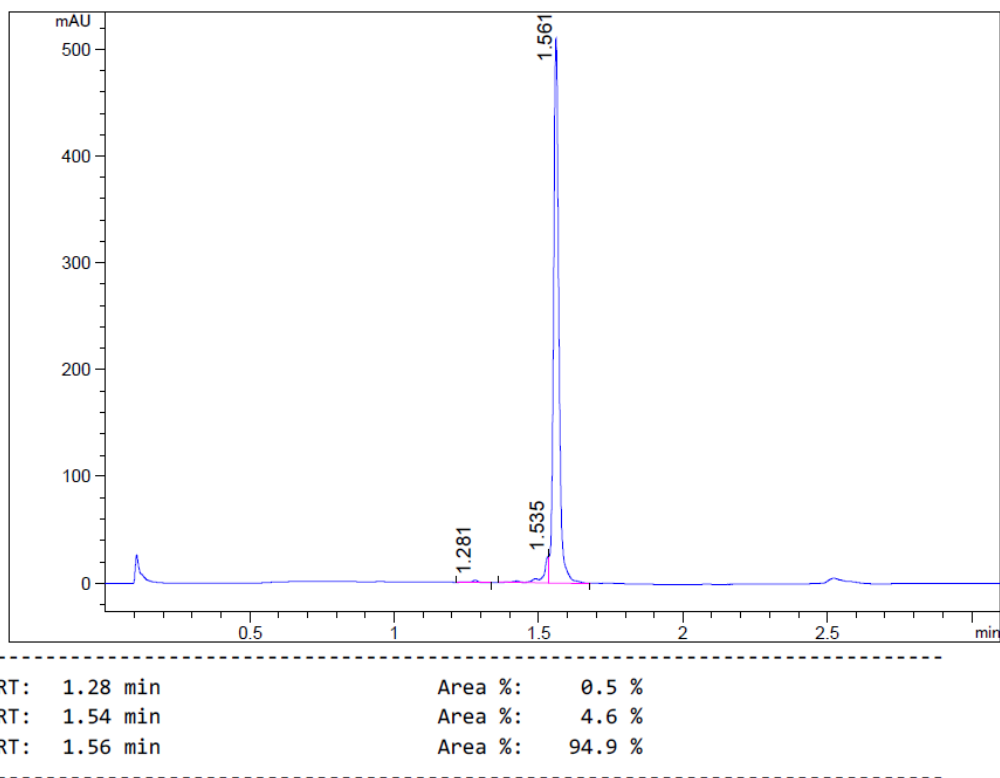
Supplementary Figure 12. Synthesis of P3: i) conc. HCl, MeOH, RT; ii) intermediate **13**, HATU, TEA, DMF, RT.

Synthesis of (2*S*,4*R*)-N-((*R*)-2-(2-(4-(4-bromo-7-cyclopentyl-5-oxo-10-(((*S*)-tetrahydrofuran-2-yl)methoxy)-5,7-dihydrobenzo[4,5]imidazo[1,2-a]quinazolin-9-yl)piperidin-1-yl)ethoxy)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)-1-((*S*)-2-(1-(dimethylamino)cyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide **P3**

11 (42 mg, 45 μ mol) was taken up in methanol (1000 μ L) and conc. aq. HCl (1000 μ L) was added. The mixture was stirred at RT for 1 h. The solvents were removed under reduced pressure to obtain crude **12**.

13 (24 mg, 68 μ mol, 1.5 eq.) was dissolved in DMF (1000 μ L) and TEA (32 μ L, 0.23 mmol, 5.0 eq.) and HATU (52 mg, 0.14 mmol, 3.0 eq.) were added. The resulting mixture was stirred at RT for 30 minutes, then **12** (39 mg, 45 μ mol) was added and stirring was continued at RT for 1 h. The reaction was diluted with water, filtered through a syringe filter and purified by basic HPLC-chromatography to obtain **P3** (32 mg, 61% yield).

HRMS (ESI+) *m/z*: [M+H]⁺ calcd for C₆₀H₇₆BrN₉O₈S 1162.47441; found 1162.48169

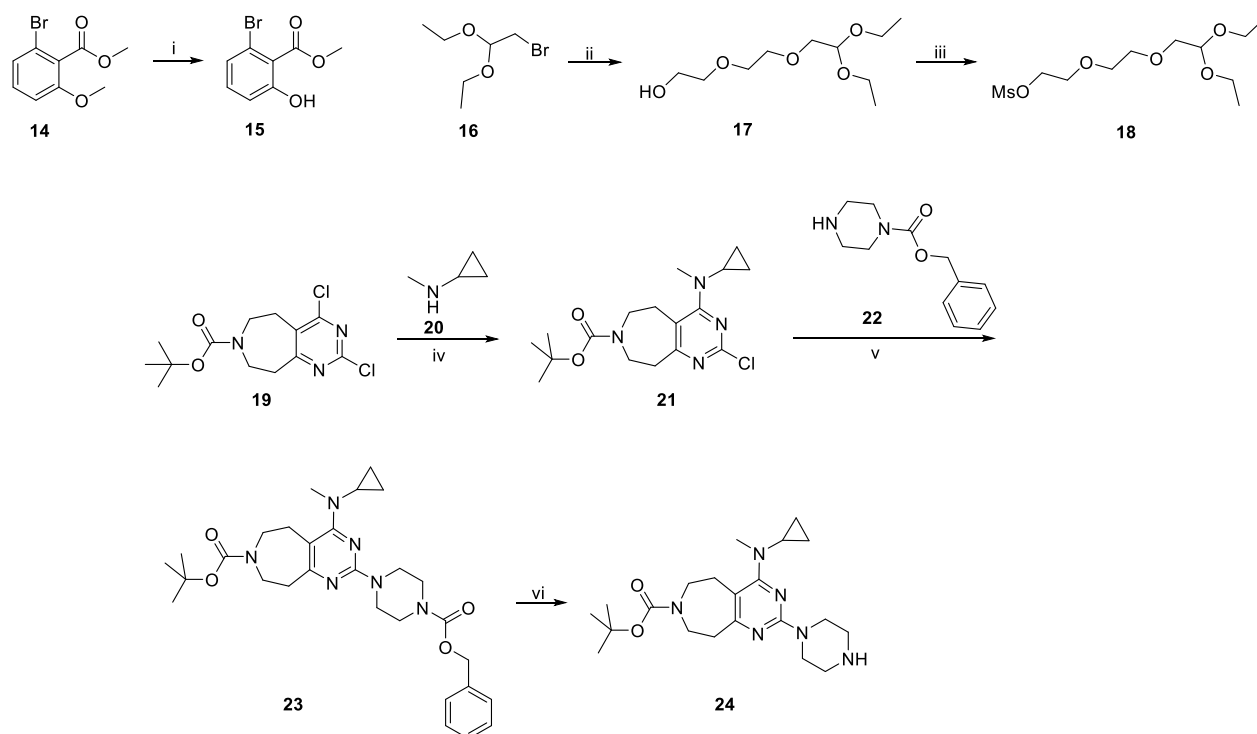


Supplementary Figure 13. HPLC spectrum of P3.

¹H NMR (DMSO-*d*₆) δ: 8.96 (s, 1H), 8.95 (br s, 0.1H rotamer), 8.69 (d, J=7.9 Hz, 0.1H rotamer), 8.51 (d, J=7.9 Hz, 1H), 8.48 (d, J=8.4 Hz, 1H), 8.12 (d, J=9.7 Hz, 1H), 7.95 (d, J=9.2 Hz, 0.1H rotamer), 7.83 (s, 1H), 7.80 (d, J=7.9 Hz, 1H), 7.69 (t, J=8.2 Hz, 1H), 7.44 (s, 4H), 7.41 (s, 1H), 7.38 (s, 0.1H rotamer), 5.25 (quin, J=8.7 Hz, 1H), 5.14 (d, J=3.5 Hz, 1H), 5.00-5.06 (m, 1H), 4.98

(d, J=2.8 Hz, 0.1H rotamer), 4.91-4.96 (m, 0.1H rotamer), 4.75 (t, J=7.4 Hz, 0.1H rotamer), 4.52 (t, J=8.3 Hz, 1H), 4.48 (d, J=9.7 Hz, 1H), 4.34 (d, J=9.4 Hz, 0.1H rotamer), 4.27-4.31 (m, 1H), 4.17-4.27 (range, 3H), 3.81-3.88 (m, 1H), 3.72-3.77 (m, 1H), 3.64-3.70 (m, 1H), 3.52-3.64 (range, 5H), 3.43-3.48 (m, 0.1H rotamer), 3.37-3.42 (m, 0.1H rotamer), 2.94-3.05 (range, 3H), 2.44 (s, 3H), 2.19-2.26 (m, 2H), 2.16 (s, 6H), 1.93-2.12 (range, 10H), 1.76-1.92 (range, 3H), 1.68-1.76 (range, 6H), 1.00-1.04 (m, 1H), 0.96 (s, 9H), 0.93-0.95 (m, 3H)

¹³C NMR (DMSO-d₆) δ: 172.6, 171.5, 169.7, 164.4, 152.8, 152.0, 149.0, 148.2, 141.2, 139.1, 133.8, 132.7, 131.5, 130.5, 129.1, 127.9, 125.3, 124.6, 123.2, 117.1, 116.0, 109.0, 99.9, 77.2, 73.4, 72.5, 69.3, 69.0, 68.2, 59.0, 57.8, 57.0, 56.8, 54.9, 54.3, 52.5, 47.9, 42.1, 40.5, 38.2, 36.6, 35.7, 32.2, 28.6, 28.1, 26.8, 26.7, 26.1, 25.2, 16.4, 12.8, 10.8



Supplementary Figure 15. Synthesis of key intermediates for P4: i) BBr₃, DCM, RT; ii) diethylene glycol, KOH, 115 °C; iii) methanesulfonyl chloride, TEA, DCM, 0 °C-RT; iv) **20**, TEA, DMSO, RT; v) **22**, DIPEA, NMP, 150 °C, μ w; vi) Pd(OH)₂/C, H₂, MeOH, RT.

Synthesis of methyl 2-bromo-6-hydroxybenzoate **15**

A solution of boron tribromide (1M in DCM, 31.6 mL, 31.6 mmol, 2.5 eq.) in DCM (15 mL) was cooled to 0°C and a solution of methyl 2-bromo-6-methoxybenzoate **14** (3.1 g, 12.6 mmol) in DCM (60 mL) was added dropwise. The mixture was stirred at 0°C for 1 h and quenched with water. It was diluted with DCM and the layers were separated. The aqueous layer was extracted with EtOAc. The combined organics were dried and concentrated. The crude residue was purified by silica gel column chromatography (0-15% EtOAc in cyclohexane) to obtain **15** (2.1 g, 72% yield).

Synthesis of 2-[2-(2,2-Diethoxy-ethoxy)-ethoxy]-ethanol **17**

Potassium hydroxide (1.0 g, 18 mmol, 2.7 eq) was added to diethylene glycol (4.7 g, 44 mmol, 6.6 eq.) at 80°C and the resulting mixture was stirred until all solids were dissolved. Then 2-Bromo-1,1-diethoxyethane **16** (1.3 g, 6.7 mmol) was added, and the mixture was stirred at 115°C for 72 h. It was cooled to RT, quenched with water, extracted with DCM, dried over MgSO₄ and concentrated. The crude residue was purified by silica gel column chromatography (0-10% MeOH in DCM) to obtain **17** (0.8 g, 54% yield).

Synthesis of 2-[2-(2,2-diethoxyethoxy)ethoxy]ethyl methanesulfonate **18**

17 (917 mg, 4.1 mmol) was dissolved in DCM (9 mL) and TEA (1.2 mL, 8.3 mmol, 2.0 eq.) was added. It was cooled to 0°C and methanesulfonyl chloride (740 mg, 4.1 mmol, 1.0 eq.) was added. The resulting mixture was stirred at RT for 2 h and quenched with water. It was extracted

with DCM, washed with water and brine, dried and concentrated to give crude **18** (513 mg, 41% yield), which was used in the next step without further purification.

Synthesis of 2-Chloro-4-(cyclopropyl-methyl-amino)-5,6,8,9-tetrahydro-pyrimido[4,5-d]azepine-7-carboxylic acid tert-butyl ester **21**

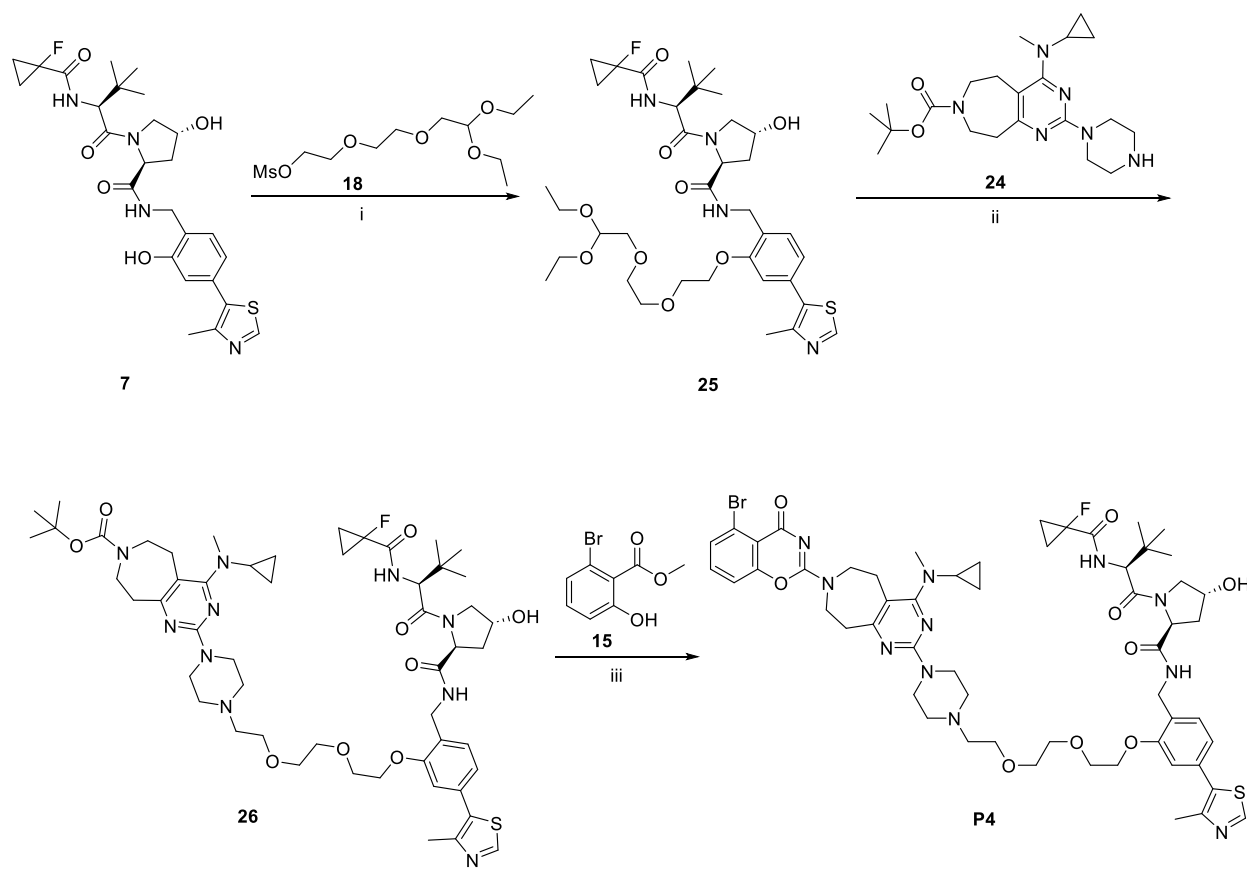
To a solution of 2,4-Dichloro-5,6,8,9-tetrahydro-pyrimido[4,5-d]azepine-7-carboxylic acid tert-butyl ester **19** (100 mg, 314 μ mol) in DMSO (1000 μ L), TEA (131 μ L, 943 μ mol, 3.0 eq.) and Cyclopropyl-methyl-amine **20** (45 mg, 629 μ mol, 2.0 eq.) were added. The resulting mixture was stirred at RT for 16 h. The reaction was diluted with MeCN and water, filtered through a syringe filter and purified by acidic HPLC-chromatography to obtain **21** (82 mg, 74% yield).

Synthesis of benzyl 4-{7-[(tert-butoxy)carbonyl]-4-[cyclopropyl(methyl)amino]-5H,6H,7H,8H,9H-pyrimido[4,5-d]azepin-2-yl}piperazine-1-carboxylate **23**

To a solution of **21** (233 mg, 584 μ mol) in NMP (1000 μ L), DIPEA (124 μ L, 730 μ mol, 1.3 eq.) and 1-(Benzyloxycarbonyl)piperazine **22** (287 μ L, 1.46 mmol, 2.5 eq.) were added. The resulting mixture was irradiated in the microwave at 150°C for 1.5 h. The mixture was diluted with sat. aq. potassium carbonate solution and extracted with DCM. The combined organic layers were dried and concentrated. The crude was dissolved in MeCN and water, filtered through a syringe filter and purified by acidic HPLC-chromatography to give **23** (164 mg, 52% yield).

Synthesis of tert-butyl 4-[cyclopropyl(methyl)amino]-2-(piperazin-1-yl)-5H,6H,7H,8H,9H-pyrimido[4,5-d]azepine-7-carboxylate **24**

To a solution of **23** (164 mg, 306 μ L) in MeOH (10 mL) in a steel reactor, palladium hydroxide (20% on charcoal) (69 mg, 98 μ mol, 0.32 eq.) was added. The reactor was flushed with nitrogen and filled with hydrogen. The reaction mixture was stirred under 6 bar of hydrogen atmosphere for 2.5 h. The catalyst was filtered off over a Celite pad and rinsed with MeOH. The filtrate was concentrated under reduced pressure and the resulting crude **24** was taken to the next step without further purification.



Supplementary Figure 16. Synthesis of P4: i) **18**, K₂CO₃, DMF, 75°C; ii) (a) 1M HCl, dioxane, 50°C; (b) TEA, **24**, DMF *then* STAB, RT; iii) (a) 4M HCl, dioxane, RT; (b) **15**, TEA, CNBr, acetone, 0°C-RT, *then* (a).

Synthesis of (2S,4R)-N-[(2-{2-[2-(2,2-diethoxyethoxy)ethoxy]ethoxy}-4-(4-methyl-1,3-thiazol-5-yl)phenyl)methyl]-1-[(2S)-2-[(1-fluorocyclopropyl)formamido]-3,3-dimethylbutanoyl]-4-hydroxypyrrolidine-2-carboxamide **25**

To a solution of **7** (100 mg, 188 μmol) in DMF (1000 μL), potassium carbonate (65 mg, 469 μmol, 2.5 eq.) and **18** (85 mg, 282 μmol, 1.5 eq.) were added and the resulting mixture was stirred at 75°C for 11 h. It was diluted with MeCN and water, filtered through a syringe filter and purified by basic HPLC-chromatography to give **25** (68 mg, 49% yield).

Synthesis of tert-butyl 4-[cyclopropyl(methyl)amino]-2-{4-[2-(2-{2-[2-((2S,4R)-1-[(2S)-2-[(1-fluorocyclopropyl)formamido]-3,3-dimethylbutanoyl]-4-hydroxypyrrolidin-2-yl]formamido)methyl)-5-(4-methyl-1,3-thiazol-5-yl)phenoxy]ethoxy]ethoxy}ethyl]piperazin-1-yl}-5H,6H,7H,8H,9H-pyrimido[4,5-d]azepine-7-carboxylate **26**

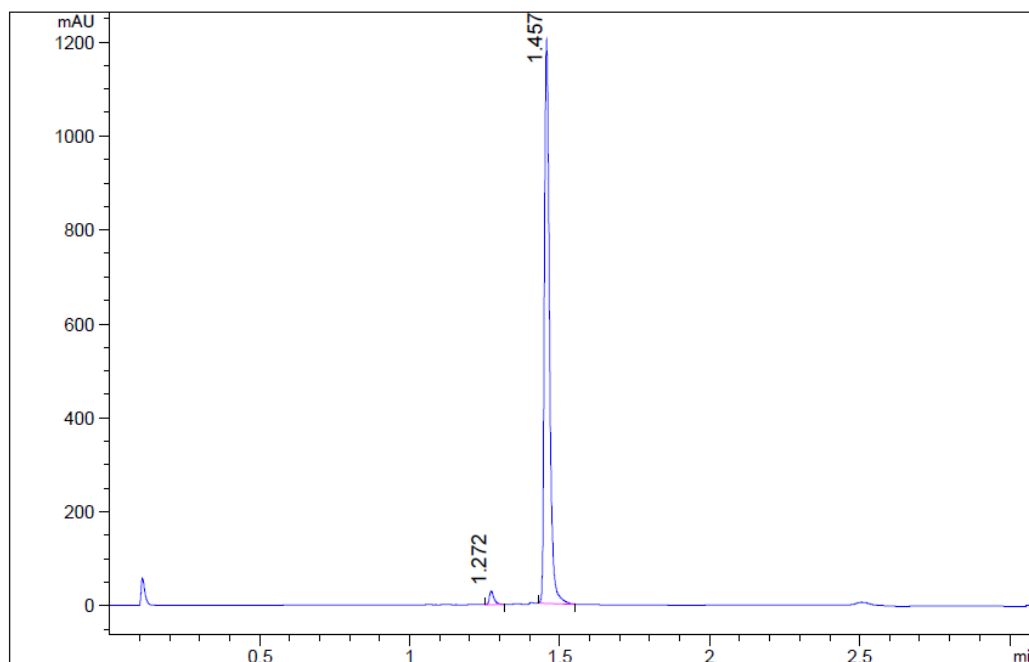
To a solution of **25** (100 mg, 122 μmol) in 1,4-dioxane (2 mL), 1M aq. HCl (122 μL, 122 μmol, 1.0 eq.) was added and the mixture was stirred at 50°C for 16 h. Then TEA (188 μL, 1.4 mmol, 11 eq.) was added, followed by a solution of **24** (55 mg, 137 μmol, 1.1 eq.) in DMF (550 μL) and a tip of spatula of Mg₂SO₄. The resulting mixture was stirred at RT for 5 min before STAB (153 mg, 700 μmol, 5.7 eq.) was added and stirring was continued at RT for 1 h. The reaction was

quenched with water and the solvents were removed under reduced pressure. The residue was dissolved in MeCN and water, filtered through a syringe filter and purified by basic HPLC-chromatography to give **26** (43 mg, 34% yield).

Synthesis of (2S,4R)-N-[(2-{2-[2-(2-{4-[7-(5-bromo-4-oxo-4H-1,3-benzoxazin-2-yl)-4-[cyclopropyl(methyl)amino]-5H,6H,7H,8H,9H-pyrimido[4,5-d]azepin-2-yl]piperazin-1-yl}ethoxy)ethoxy]ethoxy}-4-(4-methyl-1,3-thiazol-5-yl)phenyl)methyl]-1-[(2S)-2-[(1-fluorocyclopropyl)formamido]-3,3-dimethylbutanoyl]-4-hydroxypyrrolidine-2-carboxamide P4

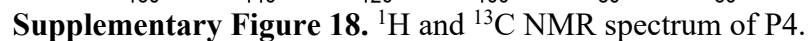
A solution of **15** (14 mg, 61 μ mol, 1.5 eq.) and TEA (29 μ L, 205 μ mol, 5.0 eq.) in acetone (0.5 mL) was cooled to 0°C and cyanogen bromide (5M in MeCN) (12 μ L, 61 μ mol, 1.5 eq.) was added slowly. The mixture was stirred at RT for 1 h. In a second vessel, **26** (43 mg, 41 μ mol) was taken up in 4M HCl in dioxane (2 mL, 8 mmol, 195 eq.) and stirred at RT for 10 min. It was concentrated under reduced pressure, the residue was dissolved in acetone (1 mL) and added dropwise to the above reaction mixture. The resulting reaction mixture was stirred at RT for 1 h. It was concentrated to dryness, the residue was dissolved in MeCN and water, filtered through a syringe filter and purified by basic HPLC-chromatography to give **P4** (43 mg, 34% yield).

HRMS (ESI+) m/z : $[M+H]^+$ calcd for $C_{56}H_{71}BrFN_{11}O_9S$ 1172.43571; found 1172.44299



RT: 1.27 min	Area %: 2.1 %
RT: 1.46 min	Area %: 97.9 %

Supplementary Figure 17. HPLC spectrum of P4.



¹H NMR (DMSO-d₆) δ: 8.98 (s, 1H), 8.55 (t, J=5.5 Hz, 0.1H rotamer), 8.49 (t, J=5.8 Hz, 1H), 8.11 (d, J=7.9 Hz, 0.1H rotamer), 7.64 (d, J=7.6 Hz, 1H), 7.56 (t, J=8.0 Hz, 1H), 7.49 (t, J=8.0 Hz, 1H), 7.42 (d, J=7.6 Hz, 1H), 7.35-7.40 (m, 0.1H rotamer), 7.29 (dd, J=9.1, 2.2 Hz, 1H), 7.20 (d, J=7.9 Hz, 0.1H rotamer), 7.16 (t, J=7.4 Hz, 0.1H rotamer), 7.03-7.07 (m, 1H), 6.97 (d, J=7.9 Hz, 1H), 5.17 (d, J=3.8 Hz, 1H), 5.04 (d, J=2.8 Hz, 0.1H rotamer), 4.69 (t, J=7.6 Hz, 0.1H rotamer), 4.60 (d, J=9.1 Hz, 1H), 4.52 (t, J=8.2 Hz, 1H), 4.45 (d, J=9.1 Hz, 0.1H rotamer), 4.32-4.38 (m,

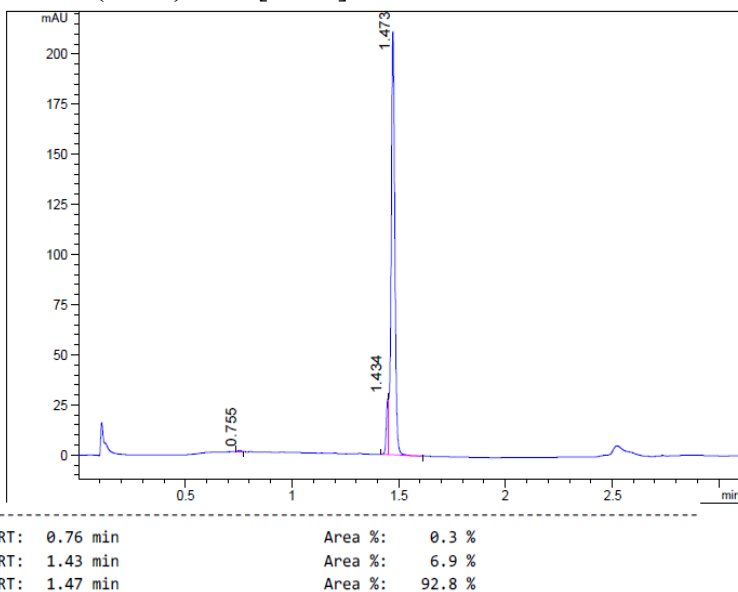
¹H), 4.31 (br d, J=6.3 Hz, 1H), 4.17-4.25 (m, 3H), 4.14 (br d, J=5.0 Hz, 0.1H rotamer), 4.11 (br d, J=5.0 Hz, 0.1H rotamer), 3.88 (br d, J=4.4 Hz, 2H), 3.76-3.85 (m, 4H), 3.66 (br d, J=3.8 Hz, 0.3H rotamer), 3.62-3.66 (range, 2H), 3.59-3.62 (range, 4H), 3.52-3.57 (range, 4H), 3.47-3.49 (m, 0.19H rotamer), 2.89-2.98 (range, 4H), 2.86 (br d, J=8.2 Hz, 4H), 2.46 (s, 5H), 2.38-2.43 (m, 4H), 2.21-2.29 (m, 0.1H rotamer), 2.09 (br dd, J=12.5, 7.7 Hz, 1H), 1.99-2.05 (m, 0.1H rotamer), 1.92 (ddd, J=12.9, 8.8, 4.4 Hz, 1H), 1.31-1.46 (m, 2H), 1.16-1.27 (m, 2H), 0.96 (s, 9H), 0.68 (br d, J=6.0 Hz, 2H), 0.41 (br s, 2H)

¹³C NMR (DMSO-d₆) δ: 172.3, 169.4, 168.5 (d, CF=20 Hz), 168.1, 166.5, 166.2, 163.7, 158.9, 158.9, 156.3, 155.7, 155.5, 148.3, 134.6, 132.1, 131.7, 131.3, 128.2, 127.6, 125.9, 121.5, 121.0, 120.6, 118.9, 116.8, 115.9, 112.6, 111.4, 108.7, 108.7, 78.6 (d, CF=232 Hz), 70.5, 70.2, 69.4, 69.4, 68.8, 68.4, 59.3, 57.8, 57.2, 57.0, 53.5, 47.7, 46.9, 45.1, 44.4, 43.7, 39.4, 38.8, 38.4, 37.7, 36.5, 35.1, 34.9, 28.0, 27.5, 26.6, 16.5, 13.5(d, CF=10 Hz), 13.2(d, CF=11 Hz), 8.8, 8.7

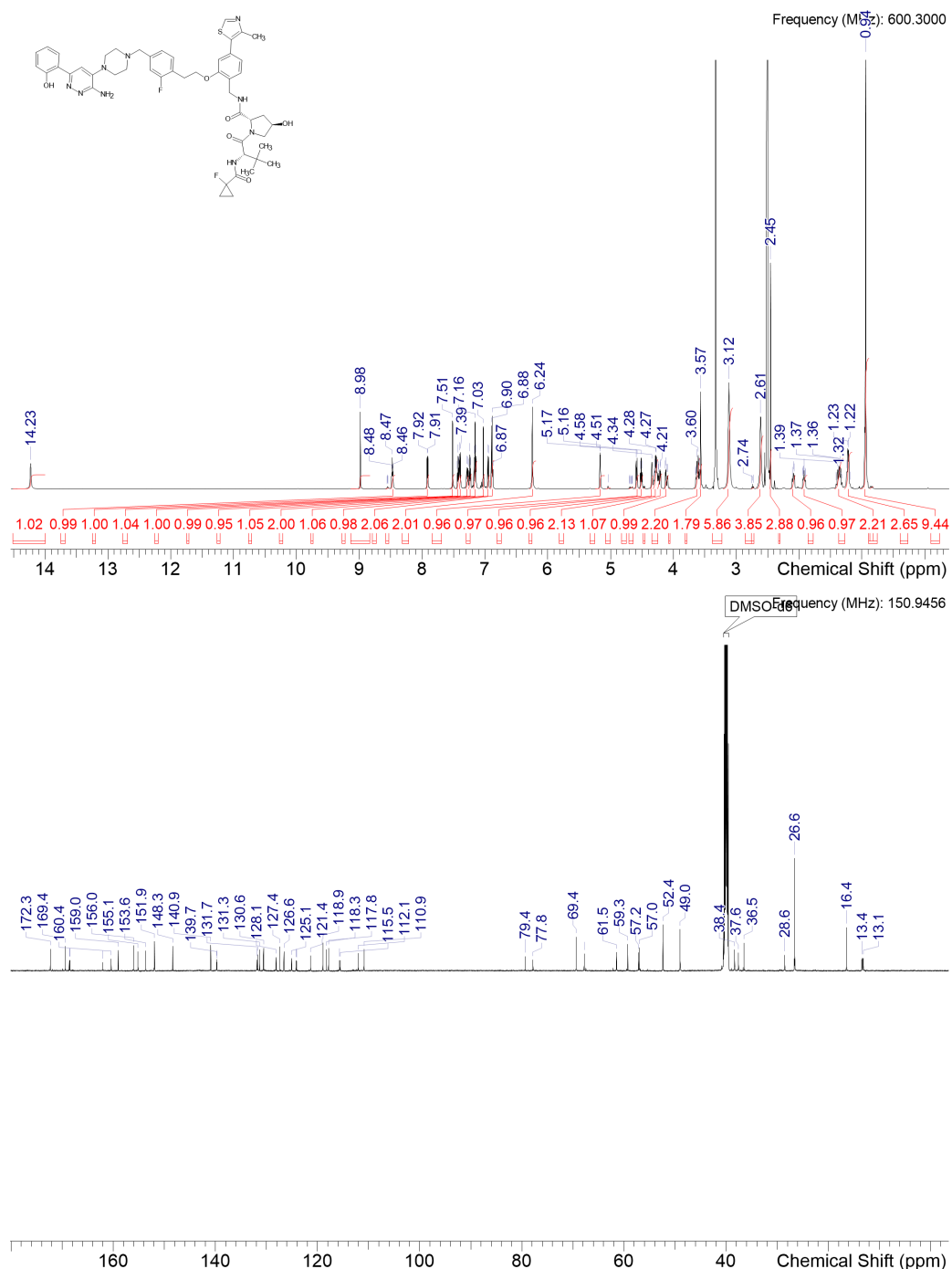
Synthesis of (2*S*,4*R*)-N-(2-(4-((4-(3-amino-6-(2-hydroxyphenyl)pyridazin-4-yl)piperazin-1-yl)methyl)-2-fluorophenoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide P6

Synthesis described in [2].

HRMS (ESI+) m/z : $[M+H]^+$ calcd for C₄₉H₅₇F₂N₉O₆S 938.41392; found 938.4212



Supplementary Figure 19. HPLC spectrum of P6.



Supplementary Figure 20. ¹H and ¹³C NMR spectrum of P6.

¹H NMR (DMSO-d₆) δ : 14.23 (br s, 1H), 8.98 (s, 1H), 8.55 (br t, J=5.5 Hz, 0.1H rotamer), 8.47 (br t, J=5.9 Hz, 1H), 7.91 (br d, J=7.9 Hz, 1H), 7.51 (s, 1H), 7.43 (br t, J=7.9 Hz, 1H), 7.40 (br d, J=7.7 Hz, 1H), 7.28 (br d, J=9.2 Hz, 1H), 7.24 (br t, J=7.7 Hz, 1H), 7.13-7.18 (range, 2H), 7.03 (s, 1H), 6.95 (br d, J=7.7 Hz, 1H), 6.85-6.92 (range, 2H), 6.24 (s, 2H), 5.17 (br d, J=2.8 Hz, 1H), 5.04 (br s, 0.1H rotamer), 4.64-4.71 (m, 0.1H rotamer), 4.59 (br d, J=9.0 Hz, 1H), 4.51 (br t, J=8.3 Hz, 1H), 4.46-4.48 (m, 0.1H rotamer), 4.32-4.37 (m, 1H), 4.25-4.32 (m, 2H), 4.22 (br dd, J=16.7, 6.4 Hz, 1H), 4.11 (br dd, J=16.6, 5.2 Hz, 1H), 4.05-4.07 (m, 0.1H rotamer), 3.58-3.67 (m, 2H), 3.57

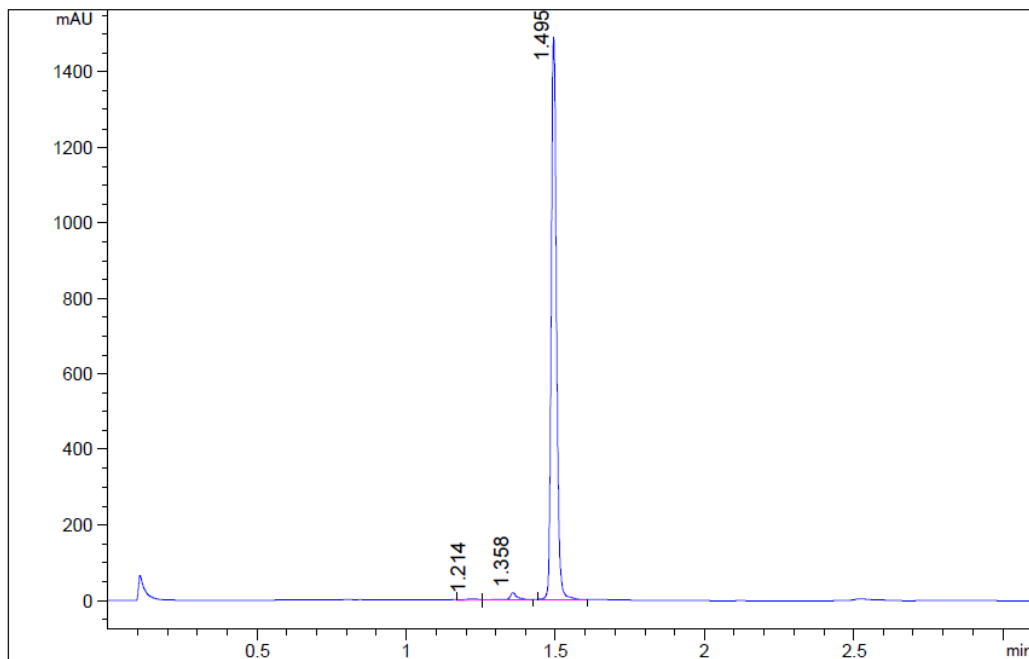
(s, 2H), 3.05-3.21 (range, 6H), 2.74 (t, J=6.9 Hz, 0.1H rotamer), 2.57-2.67 (range, 4H), 2.45 (s, 3H), 2.06-2.14 (m, 1H), 1.87-1.96 (m, 1H), 1.30-1.43 (m, 2H), 1.16-1.28 (m, 2H), 0.94 (s, 9H), 0.82-0.88 (m, 0.1H rotamer)

¹³C NMR (DMSO-d₆) δ: 172.3, 169.4, 168.5 (d, CF=20 Hz), 161.2 (d, CF=243 Hz), 159.0, 156.0, 155.1, 153.6, 151.9, 148.3, 140.9, 139.7 (d, CF=8 Hz), 131.9 (d, CF=5 Hz), 131.7, 131.3, 130.6, 128.1, 127.4, 126.6, 125.1, 124.2 (d, CF=17 Hz), 121.4, 118.9, 118.3, 117.8, 115.6 (d, CF=23 Hz), 112.1, 110.9, 78.6 (d, CF=233 Hz), 69.4, 67.8, 61.5, 59.3, 57.2, 57.0, 52.4, 49.0, 38.4, 37.6, 36.5, 28.6, 26.6, 16.4, 13.4 (d, CF=10 Hz), 13.2 (d, CF=10 Hz)

Synthesis of (2*S*,4*R*)-N-[(2-{4-[4-({4-[3-amino-6-(2-hydroxyphenyl)pyridazin-4-yl]piperazin-1-yl}methyl)phenoxy]butoxy}-4-(4-methyl-1,3-thiazol-5-yl)phenyl)methyl]-1-[(2*S*)-2-[(1-fluorocyclopropyl)formamido]-3,3-dimethylbutanoyl]-4-hydroxypyrrolidine-2-carboxamide P7

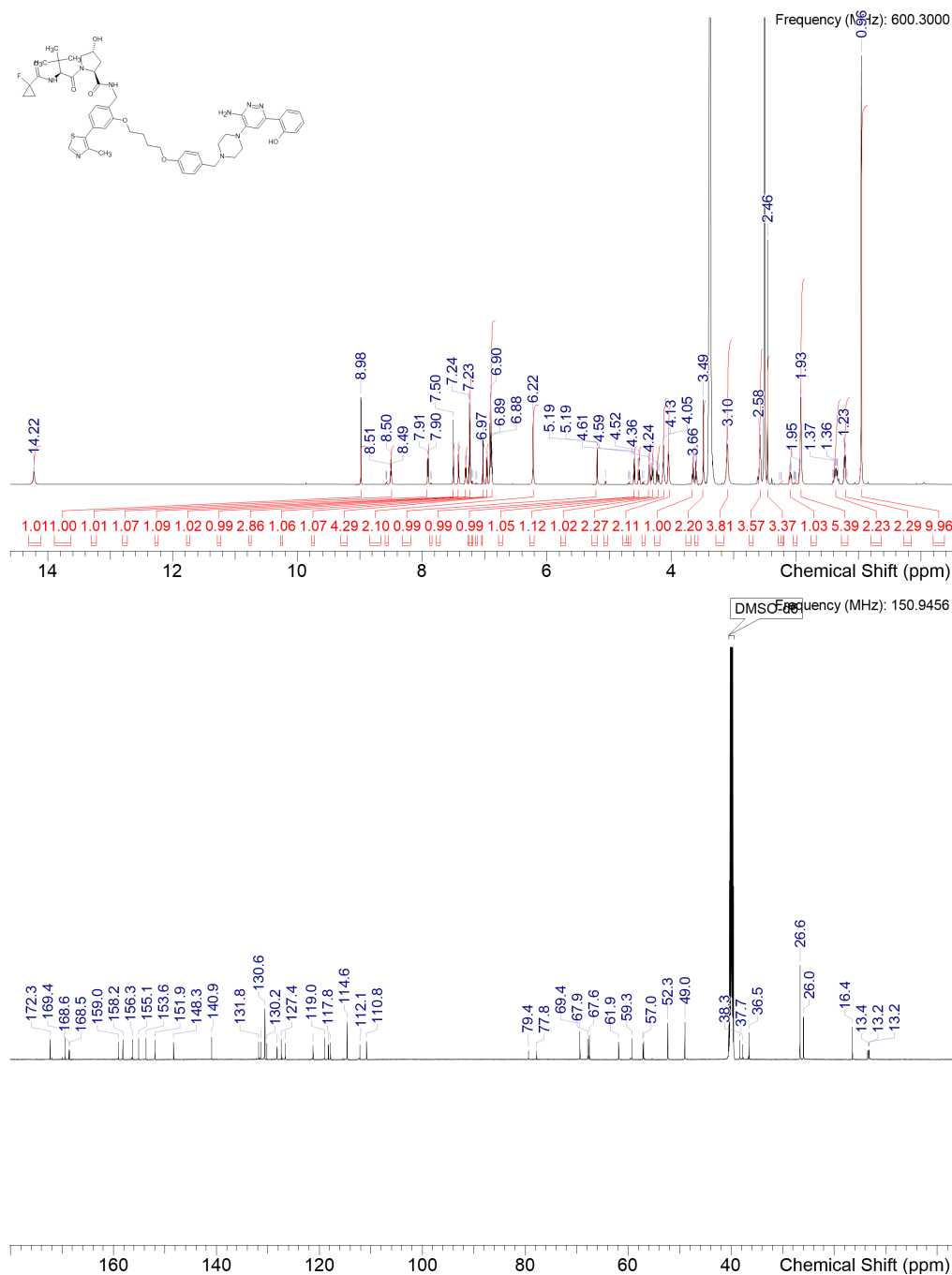
Synthesis described in [2].

HRMS (ESI+) m/z : $[M+H]^+$ calcd for C₅₁H₆₂FN₉O₇S 964.44951; found 964.45679



RT: 1.21 min	Area %: 0.6 %
RT: 1.36 min	Area %: 1.9 %
RT: 1.49 min	Area %: 97.5 %

Supplementary Figure 21. HPLC spectrum of P7.

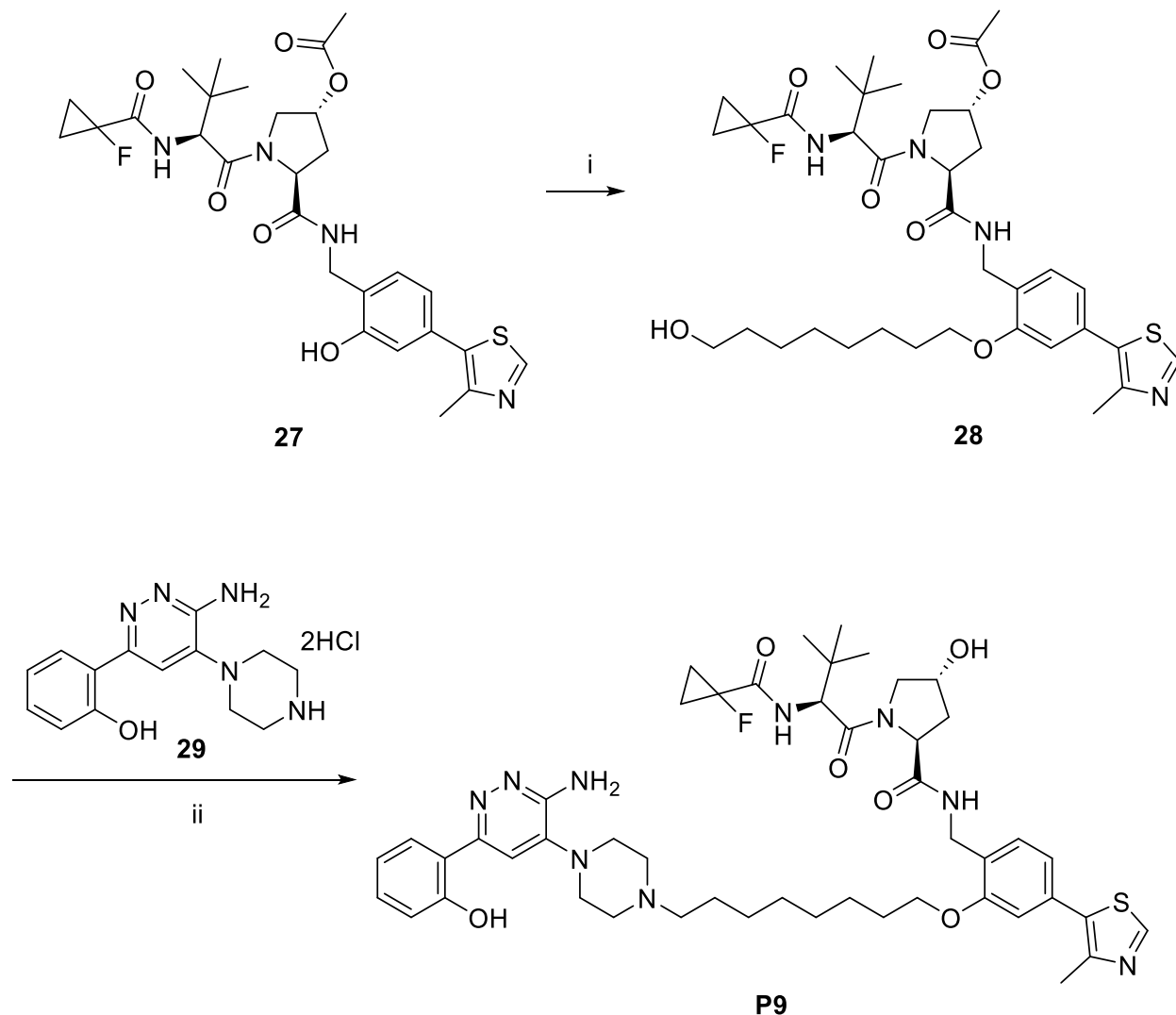


Supplementary Figure 22. ¹H and ¹³C NMR spectrum of P7.

¹H NMR (DMSO-*d*₆) δ : 14.22 (br s, 1H), 8.98 (s, 1H), 8.57 (br t, *J*=5.6 Hz, 0.1H rotamer), 8.50 (t, *J*=5.9 Hz, 1H), 7.91 (dd, *J*=8.3, 1.3 Hz, 1H), 7.86 (d, *J*=8.8 Hz, 0.05H rotamer), 7.50 (s, 1H), 7.42 (d, *J*=7.7 Hz, 1H), 7.30 (dd, *J*=9.2, 2.2 Hz, 1H), 7.25 (d, *J*=1.5 Hz, 0.33H rotamer), 7.23 (d, *J*=8.4 Hz, 3H), 7.20 (d, *J*=7.7 Hz, 0.1H rotamer), 7.13 (d, *J*=8.6 Hz, 0.1H rotamer), 7.03 (br s, 0.1H rotamer), 7.02 (d, *J*=1.1 Hz, 1H), 6.96 (dd, *J*=7.8, 1.2 Hz, 1H), 6.83-6.93 (range, 4H), 6.22 (s, 2H), 5.19 (d, *J*=3.5 Hz, 1H), 5.06 (d, *J*=2.8 Hz, 0.1H rotamer), 4.68 (t, *J*=7.4 Hz, 0.1H rotamer), 4.60 (d, *J*=9.4 Hz, 1H), 4.52 (t, *J*=8.3 Hz, 1H), 4.46 (br t, *J*=9.4 Hz, 0.1H rotamer), 4.36 (br s, 1H), 4.31 (dd, *J*=16.3, 6.1 Hz, 1H), 4.22 (dd, *J*=16.3, 5.5 Hz, 1H), 4.13 (br t, *J*=4.1 Hz, 2H),

4.05 (br t, $J=4.1$ Hz, 2H), 3.65 (dd, $J=10.8, 3.8$ Hz, 1H), 3.61 (br d, $J=3.6$ Hz, 1H), 3.49 (s, 2H), 3.04-3.16 (m, 4H), 2.55-2.61 (m, 4H), 2.46 (s, 3H), 2.25 (br t, $J=9.9$ Hz, 0.1H rotamer), 2.10 (br dd, $J=12.5, 7.9$ Hz, 1H), 2.02 (ddd, $J=12.4, 7.0, 5.0$ Hz, 0.1H rotamer), 1.87-1.97 (range, 4H), 1.28-1.45 (m, 2H), 1.14-1.27 (m, 2H), 0.96 (s, 9H)

^{13}C NMR (DMSO- d_6) δ : 172.3, 169.4, 168.6 (d, CF=20 Hz), 159.0, 158.2, 156.3, 155.1, 153.6, 151.9, 148.3, 140.9, 131.8, 131.4, 130.6, 130.2, 128.2, 127.4, 126.6, 121.2, 119.0, 118.3, 117.8, 114.6, 112.1, 110.8, 78.6 (d, CF=233 Hz), 69.4, 67.6, 61.9, 59.3, 57.1, 57.0, 52.3, 49.0, 38.3, 37.7, 36.5, 26.6, 26.0, 16.4, 13.4 (d, CF=10 Hz), 13.2 (d, CF=10 Hz)



Supplementary Figure 23. Synthesis of P9: i) 8-bromo octanol-1-ol, K_2CO_3 , DMF, 90°C ; ii) COCl_2 , DMSO, TEA, DCM, -78°C ; then **29**, TEA, DCE, STAB, RT; then NaOH, EtOH, 50°C .

Starting material syntheses are reported in the literature:

(2*S*,4*R*)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide was synthesized as discussed in [3],

(3*R*,5*S*)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-5-((2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-3-yl acetate was synthesised from (2*S*,4*R*)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide as in [2].

Synthesis of (3*R*,5*S*)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-5-((2-((8-hydroxyoctyl)oxy)-4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-3-yl acetate **28**

A mixture of (3*R*,5*S*)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-5-((2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-3-yl acetate **27** (80 mg, 0.139 mmol) and K₂CO₃ (22 mg, 0.159 mmol) in DMF (2 mL) was pre-stirred at 90 °C for 45 minutes before addition of 8-bromo-octan-1-ol (2 µL, 0.146 mmol). The resulting reaction mixture was stirred at 90 °C for 5 hours. The reaction mixture was cooled to room temperature, diluted with H₂O and DCM, and passed through a phase separator. The filtrate was concentrated under reduced pressure. The remaining residue was passed through a silica column (0-10% MeOH in CH₂Cl₂) and the desired fractions concentrated under reduced pressure to afford **28** (79.7 mg) containing octan-1-ol related impurities which was taken directly into the next reaction without further purification.

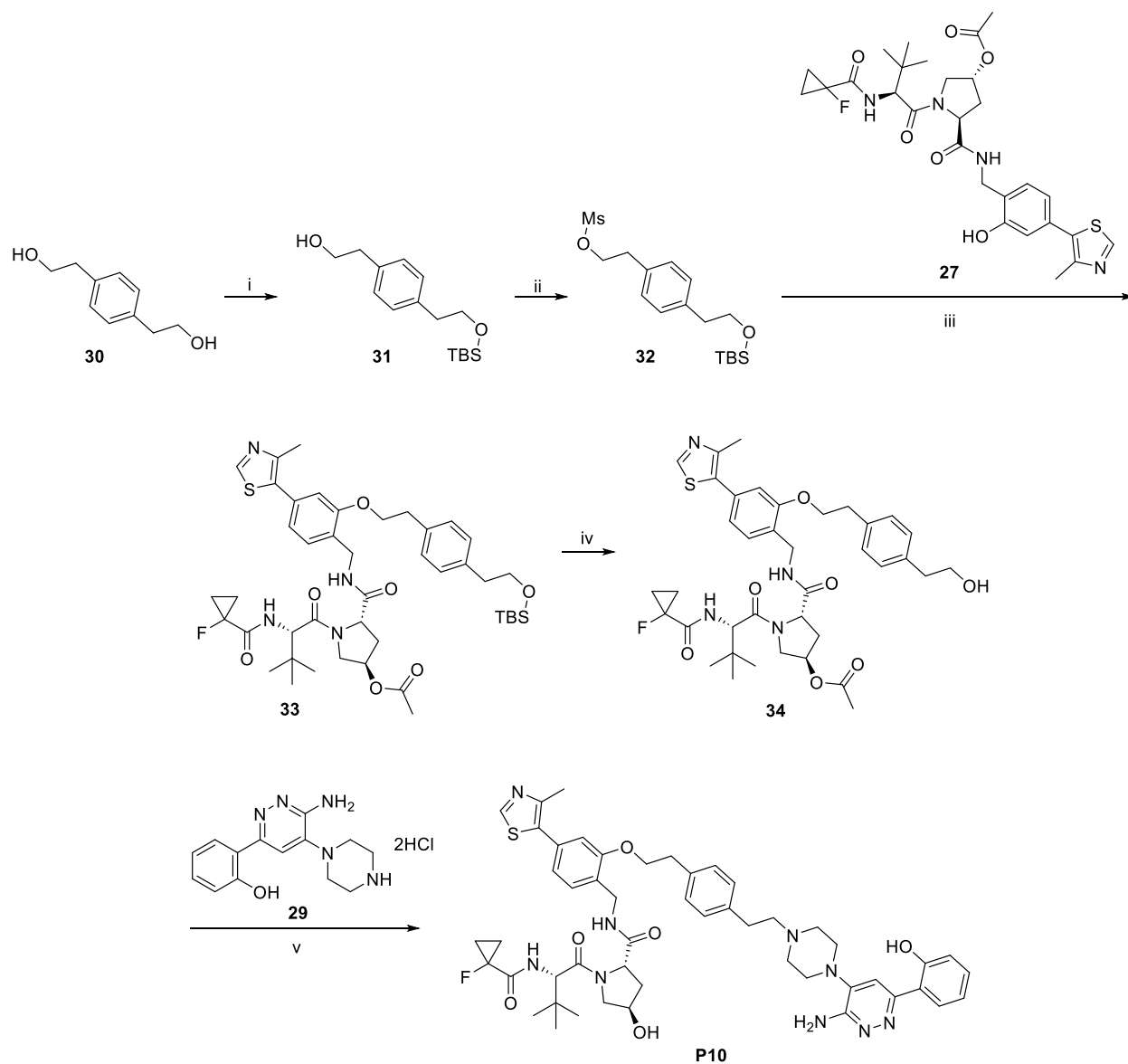
Synthesis of (2*S*,4*R*)-*N*-(2-((8-(4-(3-amino-6-(2-hydroxyphenyl)pyridazin-4-yl)piperazin-1-yl)octyl)oxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide **P9**

DMSO (15 µL, 0.211 mmol) was added to a solution of oxalyl chloride (15 µL, 0.175 mmol) in CH₂Cl₂ (1 mL) at -78 °C. The resulting reaction mixture was stirred at -78 °C for 15 min, after which (3*R*,5*S*)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-5-((2-((8-hydroxyoctyl)oxy)-4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-3-yl acetate **28** (79.7 mg) in CH₂Cl₂ (1 mL) was added dropwise. The reaction mixture was stirred at -78 °C for 1 hour, after which triethylamine (76 µL, 0.525 mmol) was added. The reaction mixture was allowed to warm up to room temperature and stirred at ambient temperature for 1 hour. The reaction mixture was then concentrated under reduced pressure. The crude residue was used directly without purification.

The crude residue was added to a solution of 2-(6-amino-5-(piperazin-1-yl)pyridazin-3-yl)phenol dihydrochloride **29** (37 mg, 0.107 mmol) and triethylamine (500 µL, 0.526 mmol) in DCE (3 mL) and DMSO (0.6 mL). STAB (116 mg, 0.547 mmol) was added shortly after, followed by magnesium sulfate. The reaction mixture was stirred at room temperature overnight. The reaction mixture was filtered and concentrated under reduced pressure. The residue was purified by reverse phase chromatography under acidic conditions (0-60% CH₃CN in 0.1% aq. HCO₂H). Desired fractions were concentrated under reduced pressure. The resulting residue was dissolved in EtOH (3.000 mL) and aq. 2M NaOH (3 mL, 6 mmol) and stirred at 50 °C for 1 hour. The reaction mixture was neutralized to pH 6 with aq. 1M HCl and concentrated under reduced pressure. The residue was purified by reverse phase chromatography on acidic conditions (0-60% CH₃CN in 0.1% aq. HCO₂H) and the desired fractions concentrated under reduced pressure to afford **P9** (46.5 mg,

0.051 mmol, 37 % over 3 steps from (3*R*,5*S*)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-5-((2-hydroxy-4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-3-yl acetate).

^1H NMR (400 MHz, CD_2Cl_2) δ 8.69 (s, 1H), 7.65 (d, $J = 7.9$ Hz, 1H), 7.39 (s, 1H), 7.33 (d, $J = 7.7$ Hz, 1H), 7.28 (t, $J = 7.7$ Hz, 1H), 7.20 (t, $J = 5.7$ Hz, 1H), 7.09 – 7.01 (m, 1H), 6.97 (t, $J = 7.9$ Hz, 2H), 6.94 – 6.87 (m, 2H), 5.30 (s, 2H), 4.67 (t, $J = 7.7$ Hz, 1H), 4.59 (d, $J = 8.9$ Hz, 1H), 4.55 – 4.35 (m, 3H), 4.04 (t, $J = 6.2$ Hz, 2H), 3.89 (d, $J = 11.0$ Hz, 1H), 3.64 (dd, $J = 11.1, 3.9$ Hz, 1H), 3.36 – 3.26 (m, 4H), 3.02 (s, 3H), 2.73 (dd, $J = 9.4, 5.2$ Hz, 2H), 2.51 (s, 3H), 2.48 – 2.39 (m, 1H), 2.13 – 2.04 (m, 1H), 1.91 – 1.79 (m, 2H), 1.66 (s, 2H), 1.53 (s, 2H), 1.46 – 1.21 (m, 11H), 0.94 (s, 9H); LC-MS: $t_{\text{Ret.}} = 1.33$ min; MS ($\text{M}+\text{H}$) $^+ = 914$



Supplementary Figure 24. Synthesis of P10: i) TBSCl, imidazole, DMF, 50°C; ii) MsCl, TEA, DCM, 0°C; iii) **27**, K_2CO_3 , DMF, 70°C; iv) TBAF, THF, RT; v) COCl_2 , DMSO, TEA, DCM, -78°C; then **29**, TEA, DCE, STAB, RT; then NaOH, EtOH, 50°C.

Synthesis of 2-(4-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)phenyl)ethan-1-ol **31**

tert-Butyl-chloro-dimethyl-silane (1.50 g, 0.010 mol) was added to a mixture of 2,2'-(1,4-phenylene)bis(ethan-1-ol) **30** (1.60 g, 0.010 mol) and imidazole (1.30 g, 0.019 mol) in DMF (20 mL). The resulting reaction mixture was stirred at 50°C overnight. The reaction mixture was cooled to room temperature, diluted with H₂O, and extracted with EtOAc. The combined organics were washed with brine, dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (0-50% EtOAc in heptane) and the desired fractions concentrated under reduced pressure to afford **31** (1.00 g, 3.56 mmol, 37%).

Synthesis of 4-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)phenethyl methanesulfonate **32**

Methanesulfonyl chloride (400 µL, 5.24 mmol) was added to a solution of **31** (775 mg, 2.76 mmol) and triethylamine (2.0 mL, 13.8 mmol) in CH₂Cl₂ (8 mL) and THF (8 mL) at 0°C. The reaction mixture was stirred at 0°C for 120 minutes. Sat. aq. NaHCO₃ was added, and the reaction mixture filtered through a phase separator. The filtrate was concentrated under reduced pressure and the residue purified by column chromatography on silica gel (0-50% EtOAc in Heptane). Desired fractions were concentrated under reduced pressure to afford **32** (161 mg, 0.449 mmol, 16%).

Synthesis of (3*R*,5*S*)-5-((2-(4-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)phenethoxy)-4-(4-methylthiazol-5-yl)benzyl)carbamoyl)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)pyrrolidin-3-yl acetate **33**

32 (161 mg, 0.449 mmol) was added to a solution of **27** (258 mg, 0.449 mmol) and K₂CO₃ (150 mg, 1.08 mmol) in DMF (5 mL). The resulting reaction mixture was stirred at 70°C overnight. The reaction mixture was diluted with CH₂Cl₂, quenched with water and passed through a phase separator. The organic phase was concentrated under reduced pressure and the residue purified by column chromatography on silica gel (0-10% MeOH in CH₂Cl₂) to afford **33** (237 mg, 0.282 mmol, 63%).

Synthesis of (3*R*,5*S*)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-5-((2-(4-(2-hydroxyethyl)phenethoxy)-4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-3-yl acetate **34**

33 (236 mg, 0.283 mmol) was dissolved in THF (5 mL) and TBAF (300 µL, 1M in THF, 0.3 mmol) was added. The reaction mixture was stirred at room temperature for 2 hours before being diluted with CH₂Cl₂, dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (0-5% MeOH in CH₂Cl₂) and the desired fractions concentrated under reduced pressure to afford **34** (172 mg, 0.237 mmol, 84%).

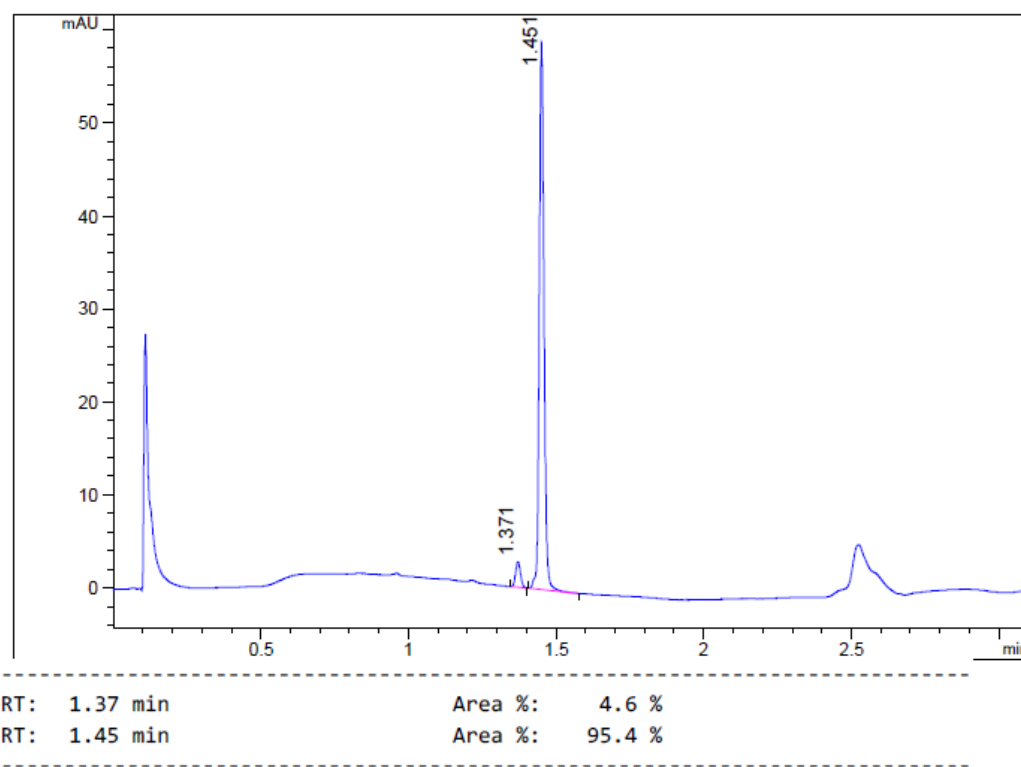
Synthesis of (2*S*,4*R*)-*N*-(2-(4-(2-(4-(3-amino-6-(2-hydroxyphenyl)pyridazin-4-yl)piperazin-1-yl)ethyl)phenethoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide **P10**

DMSO (34 µL, 0.478 mmol) was added to a solution of oxalyl chloride (30 µL, 0.350 mmol) in CH₂Cl₂ (2 mL) at -78°C. The resulting reaction mixture was stirred at -78°C for 15 min before dropwise addition of a solution of **34** (171 mg, 0.237 mmol) in CH₂Cl₂ (2 mL). The reaction mixture is stirred at -78°C for 1 hour, after which triethylamine (137 µL, 0.946 mmol) was added. The reaction mixture is allowed to warm up to room temperature and is stirred at the same

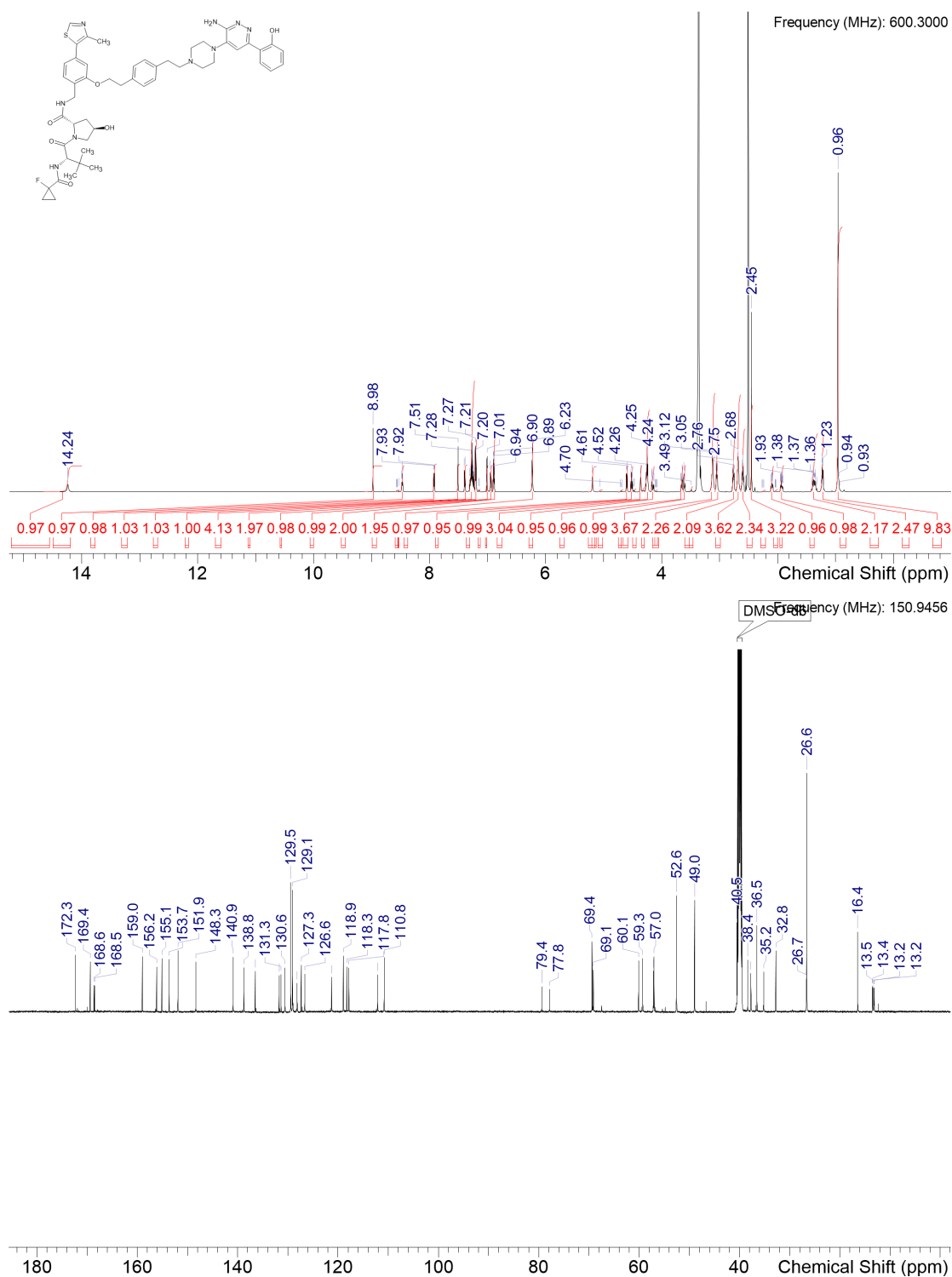
temperature for 1 hour. The reaction mixture was warmed to room temperature and concentrated under reduced pressure. The crude residue was used directly without purification.

The crude residue was added to a solution of **29** (81 mg, 0.237 mmol) and triethylamine (1.03 mL, 7.11 mmol) in DCE (4 mL) and DMSO (1 mL). STAB (251 mg, 1,186 mmol) was added, followed by magnesium sulfate. The reaction mixture was stirred at room temperature overnight before being filtered and concentrated under reduced pressure. The residue was purified by reverse phase chromatography under acidic conditions (0-60% CH₃CN in 0.1% aq. HCO₂H). Desired fractions were concentrated under reduced pressure and the residue dissolved in ethanol (3 mL) and 2M aq. NaOH (3 mL, 6 mmol). The resulting reaction mixture was stirred at 50°C for 1 hour. The reaction mixture was neutralized to pH 6 with 1M aq. HCl and concentrated under reduced pressure. The residue was purified by preparative HPLC under basic conditions (5-95% CH₃CN in 0.1% aq. NH₃) to afford **P10** (41.6 mg, 0.044 mmol, 19%).

HRMS (ESI+) *m/z*: [M+H]⁺ calcd for C₅₀H₆₀FN₉O₆S 934.43925; found 934.44653



Supplementary Figure 25. HPLC spectrum of P10.

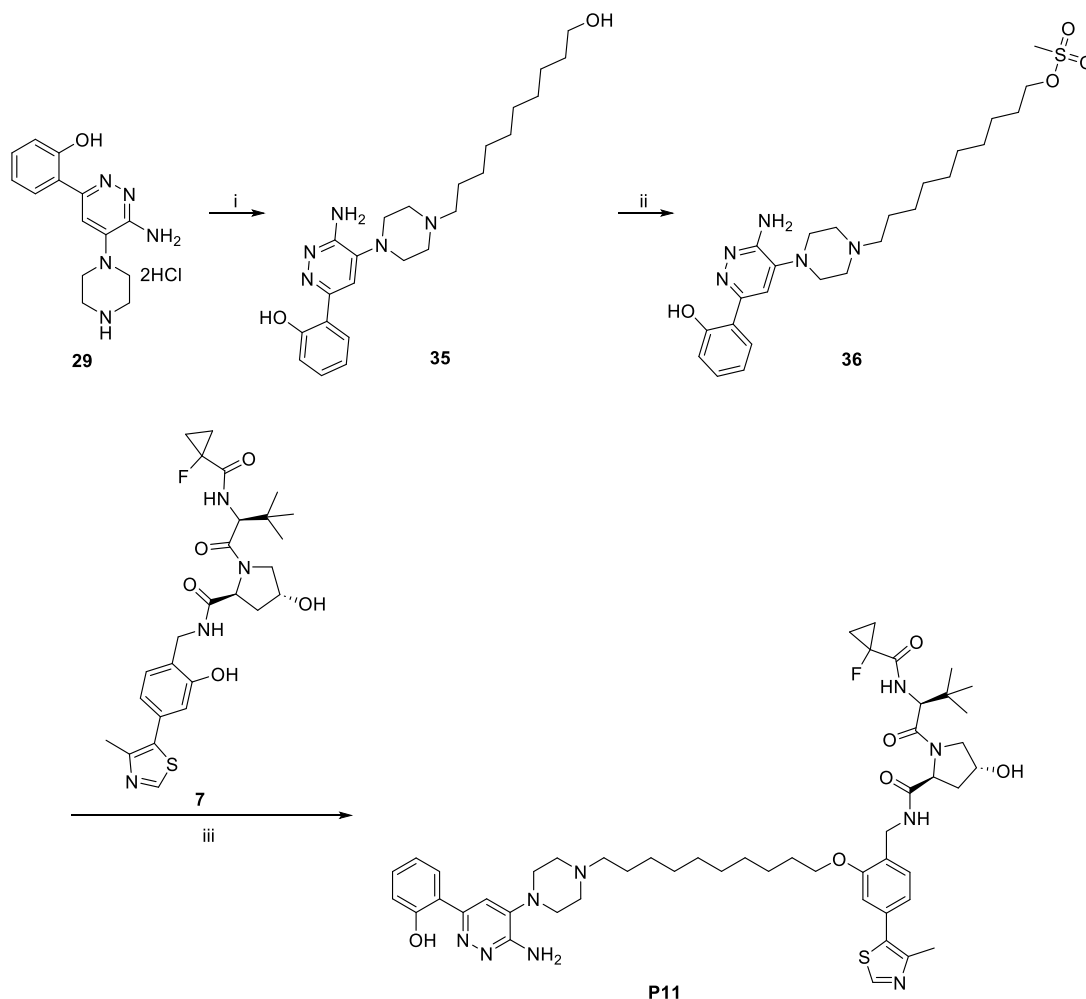


Supplementary Figure 26. ¹H and ¹³C NMR spectrum of P10.

¹H NMR (DMSO-d₆) δ: 14.24 (br s, 1H), 8.98 (s, 1H), 8.57 (br t, J=5.6 Hz, 0.1H rotamer), 8.53 (s, 0.1H rotamer), 8.47 (t, J=5.9 Hz, 1H), 7.92 (dd, J=8.3, 1.3 Hz, 1H), 7.51 (s, 1H), 7.39 (d, J=7.9 Hz, 1H), 7.23-7.32 (range, 4H), 7.21 (d, J=7.9 Hz, 2H), 7.15 (d, J=8.1 Hz, 0.3H rotamer),

7.02 (br s, 0.1H rotamer), 7.01 (d, J=1.1 Hz, 1H), 6.95 (dd, J=7.8, 1.2 Hz, 1H), 6.85-6.92 (range, 2H), 6.23 (s, 2H), 5.19 (br s, 1H), 5.06 (br s, 0.1H rotamer), 4.70 (t, J=7.4 Hz, 0.1H rotamer), 4.60 (d, J=9.2 Hz, 1H), 4.52 (t, J=8.3 Hz, 1H), 4.48 (br d, J=8.8 Hz, 0.1H rotamer), 4.36 (br s, 1H), 4.30-4.34 (m, 0.1H rotamer), 4.20-4.29 (range, 3H), 4.15 (br dt, J=16.5, 5.5 Hz, 1H), 4.09 (br dd, J=16.2, 4.9 Hz, 0.1H rotamer), 3.65 (dd, J=10.8, 3.8 Hz, 1H), 3.60 (d, J=6.6 Hz, 1H), 3.46-3.52 (m, 0.1H rotamer), 3.08-3.19 (range, 4H), 3.05 (br t, J=6.2 Hz, 2H), 2.76 (br t, J=7.6 Hz, 2H), 2.64-2.72 (range, 4H), 2.56-2.64 (m, 2H), 2.45 (s, 3H), 2.20-2.29 (m, 0.1H rotamer), 2.10 (br dd, J=12.6, 7.8 Hz, 1H), 2.00-2.06 (m, 0.1H rotamer), 1.93 (ddd, J=12.9, 8.8, 4.4 Hz, 1H), 1.31-1.45 (m, 2H), 1.16-1.28 (m, 2H), 0.96 (s, 9H)

¹³C NMR (DMSO-*d*₆) δ: 172.3, 169.4, 168.6, 159.0, 156.2, 155.1, 153.7, 151.9, 148.3, 140.9, 138.8, 136.5, 131.7, 131.3, 130.6, 129.5, 129.1, 128.1, 127.3, 126.6, 121.3, 118.9, 118.3, 117.8, 112.1, 110.8, 78.6, 69.4, 69.1, 60.1, 59.3, 57.2, 57.0, 52.6, 49.0, 40.5, 38.4, 37.7, 36.5, 35.2, 32.8, 26.7, 26.6, 16.4, 13.5, 13.2



Supplementary Figure 27. Synthesis of P11: i) 10-chlorodecan-1-ol, K₂CO₃, DMF, 70°C; ii) MsCl, TEA, THF, RT; iii) 7, K₂CO₃, DMF, 75°C.

Synthesis of 2-{6-amino-5-[4-(10-hydroxydecyl)piperazin-1-yl]pyridazin-3-yl}phenol 35

29 (100 mg, 0.37 mmol) and 10-chlorodecan-1-ol (75 μ L, 0.37 mmol, 1.0 eq.) were dissolved in DMF (500 μ L) and potassium carbonate (61 mg, 0.44 mmol, 1.2 eq.) was added. The resulting mixture was stirred at 70°C for 36 h. It was diluted with MeCN and water, filtered through a syringe filter and purified by basic HPLC-chromatography to obtain **35** (32 mg, 20% yield).

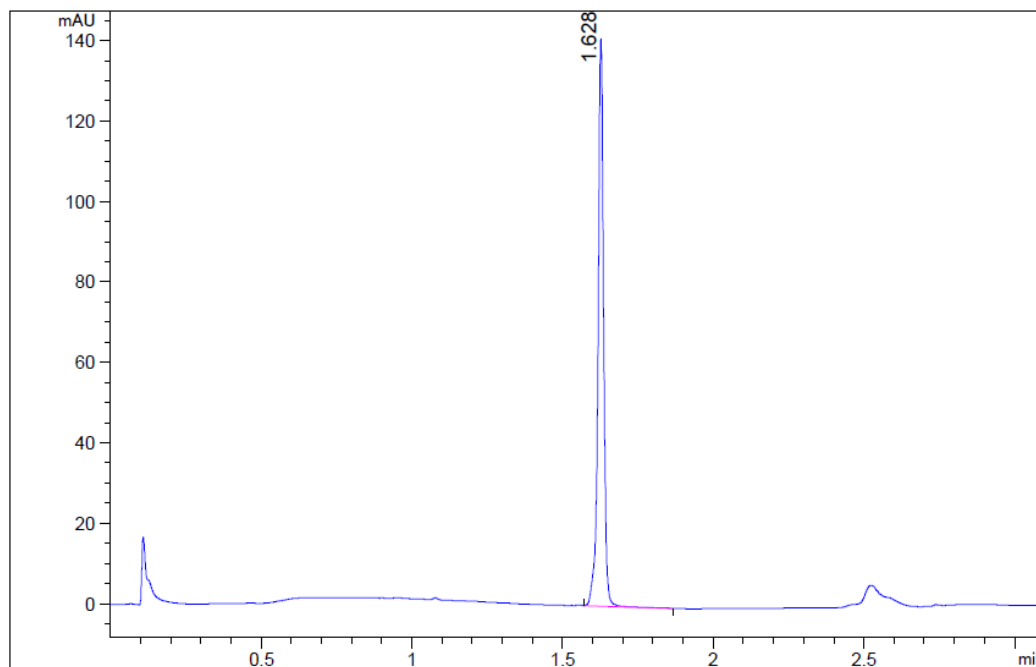
Synthesis of 10-{4-[3-amino-6-(2-hydroxyphenyl)pyridazin-4-yl]piperazin-1-yl}decyl methanesulfonate **36**

35 (32 mg, 75 μ mol) was dissolved in THF (500 μ L) and TEA (13 μ L, 90 μ mol, 1.2 eq.) was added, followed by addition of methanesulfonyl chloride (12 μ L, 0.16 mmol, 2.1 eq.). The resulting mixture was stirred at RT for 1 h. It quenched with sat. sodium bicarbonate solution, diluted with DCM and stirred for 1 h. The reaction mixture was extracted with DCM, dried over MgSO_4 and concentrated to obtain crude **36**.

Synthesis of (2*S*,4*R*)-N-(2-((10-(4-(3-amino-6-(2-hydroxyphenyl)pyridazin-4-yl)piperazin-1-yl)decyl)oxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide **P11**

To a mixture of **7** (20 mg, 38 μ mol) and potassium carbonate (16 mg, 0.11 mmol, 3.0 eq.) in DMF (200 μ L), **36** (19 mg, 38 μ mol, 1.0 eq), dissolved in DMF (200 μ L), was added. The resulting mixture was stirred at 75°C for 1 h. It was diluted with MeCN and water, filtered through a syringe filter and purified by basic HPLC-chromatography to obtain **P11** (19 mg, 54% yield).

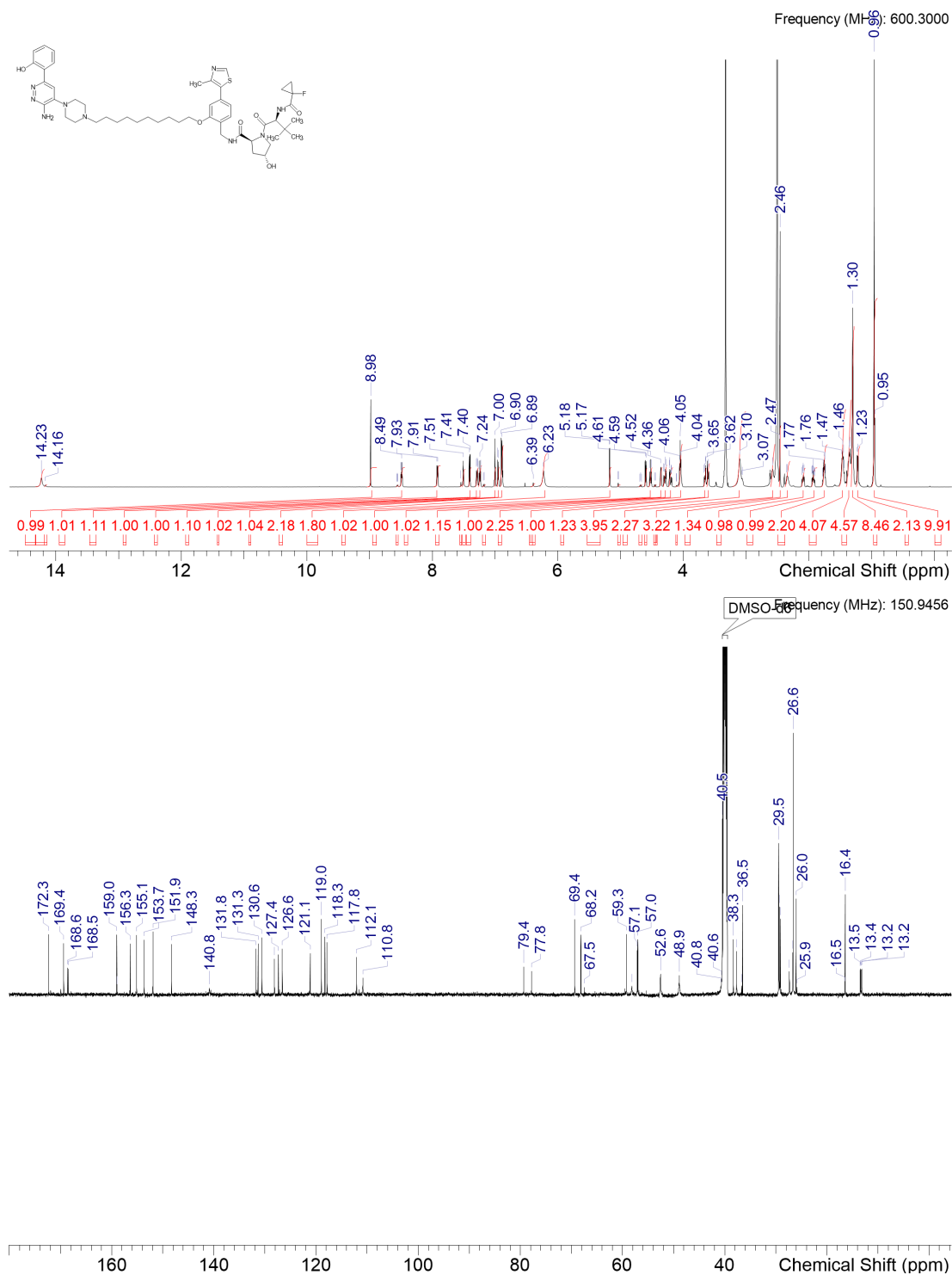
HRMS (ESI⁺) *m/z*: $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{50}\text{H}_{68}\text{FN}_9\text{O}_6\text{S}$ 942.50181; found 942.50909



RT: 1.63 min

Area %: 100.0 %

Supplementary Figure 28. HPLC spectrum of **P11**.



Supplementary Figure 29. ¹H and ¹³C NMR spectrum of P11.

¹H NMR (DMSO-d₆) δ: 14.23 (br s, 1H), 14.16 (br s, 0.1H rotamer), 8.98 (s, 1H), 8.56 (br t, J=5.5 Hz, 0.1H rotamer), 8.49 (br t, J=6.0 Hz, 1H), 7.92 (br d, J=7.5 Hz, 1H), 7.55 (s, 0.1H rotamer), 7.51 (br s, 1H), 7.40 (d, J=7.7 Hz, 1H), 7.29 (dd, J=9.2, 2.4 Hz, 1H), 7.24 (t, J=8.3 Hz,

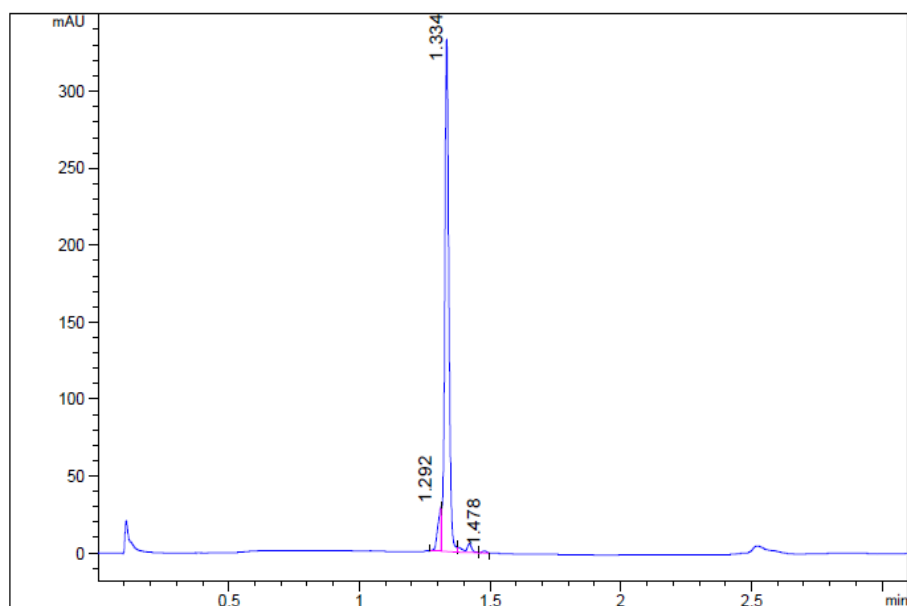
1H), 7.18 (d, J=8.1 Hz, 0.1H rotamer), 7.00 (d, J=1.3 Hz, 1H), 6.95 (dd, J=7.7, 1.3 Hz, 1H), 6.87-6.92 (range, 2H), 6.39 (s, 0.2H rotamer), 6.23 (br s, 2H), 5.17 (d, J=3.7 Hz, 1H), 5.04 (d, J=2.8 Hz, 0.1H rotamer), 4.68 (t, J=7.4 Hz, 0.1H rotamer), 4.60 (d, J=9.2 Hz, 1H), 4.52 (t, J=8.3 Hz, 1H), 4.45 (d, J=9.4 Hz, 0.1H rotamer), 4.36 (br s, 1H), 4.29 (dd, J=16.5, 6.2 Hz, 1H), 4.20 (dd, J=16.5, 5.7 Hz, 1H), 4.11 (br d, J=5.0 Hz, 0.1H rotamer), 4.02-4.07 (range, 2H), 3.65 (dd, J=6.2, 3.7 Hz, 2H), 3.58-3.63 (m, 2H), 3.01-3.21 (range, 4H), 2.54-2.61 (m, 2H), 2.45-2.47 (m, 3H), 2.35 (br d, J=5.9 Hz, 1H), 2.09 (br dd, J=12.6, 7.8 Hz, 1H), 1.93 (ddd, J=12.8, 8.7, 4.5 Hz, 1H), 1.70-1.81 (m, 2H), 1.41-1.52 (range, 4H), 1.33-1.40 (range, 4H), 1.28-1.33 (range, 8H), 1.22 (dd, J=8.3, 2.8 Hz, 2H), 0.91-1.01 (m, 9H)

¹³C NMR (DMSO-d₆) δ: 172.3, 169.4, 168.6 (d, CF=20 Hz), 159.0, 158.9, 156.3, 155.1, 153.7, 151.9, 148.3, 140.8, 131.8, 131.3, 130.6, 128.1, 127.4, 126.6, 121.1, 119.0, 118.3, 117.8, 112.1, 110.8, 78.6 (d, CF=233 Hz), 69.4, 68.2, 67.5, 59.3, 58.2, 57.1, 57.0, 52.6, 48.9, 48.9, 40.8, 40.6, 40.5, 38.3, 37.7, 36.7, 36.5, 29.5, 29.3, 29.2, 29.1, 27.4, 26.7, 26.6, 26.0, 25.9, 16.5, 16.4, 13.4 (d, CF=10 Hz), 13.2 (d, CF=10 Hz)

Synthesis of (2*S*,4*R*)-*N*-(2-(4-(4-(3-amino-6-(2-hydroxyphenyl)pyridazin-4-yl)piperazine-1-carbonyl)phenethoxy)-4-(4-methylthiazol-5-yl)benzyl)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide P16

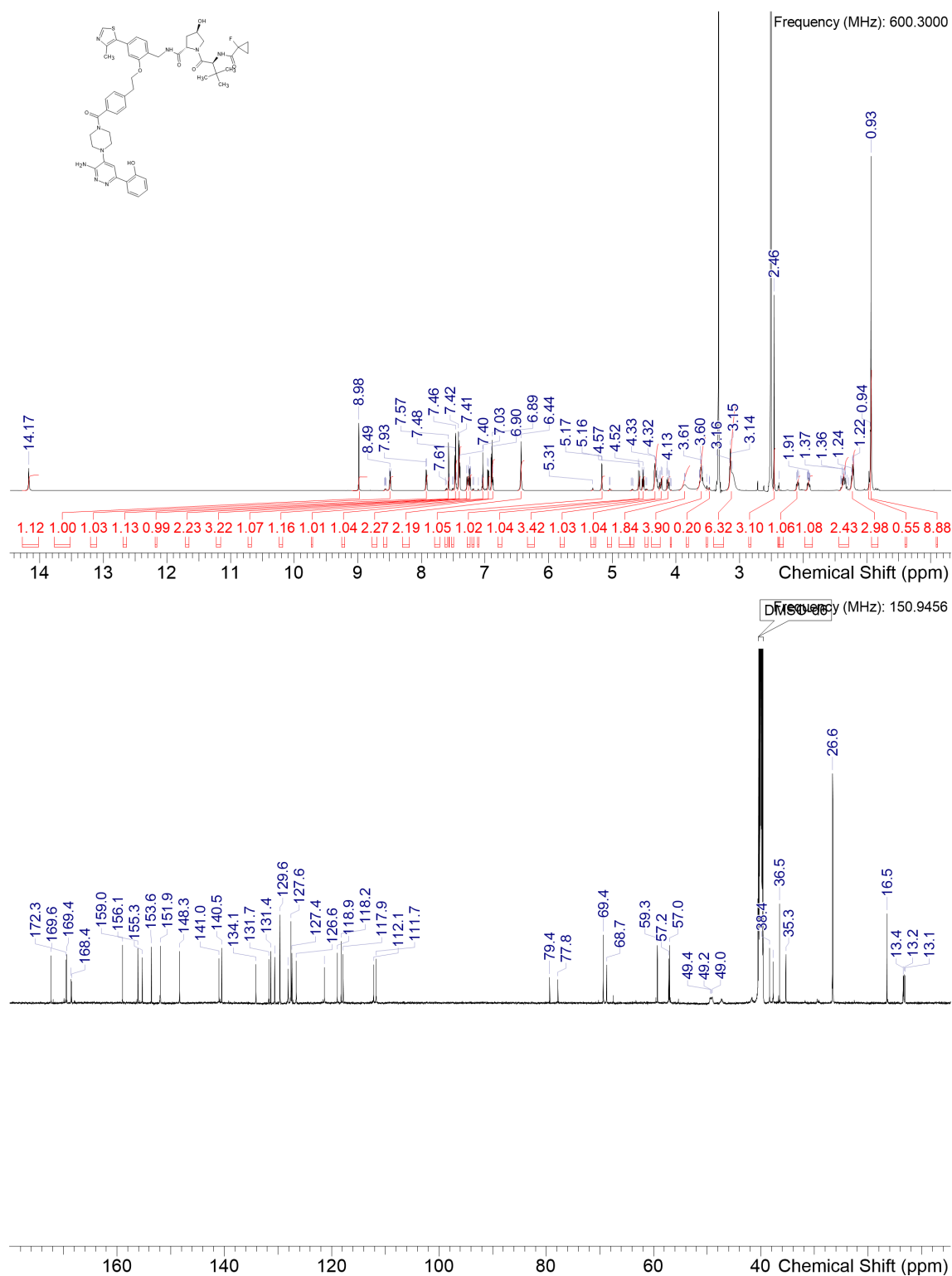
Synthesis in [2].

HRMS (ESI+) *m/z*: [M+H]⁺ calcd for C₄₉H₅₆FN₉O₇S 934.40330; found 934.41058



RT: 1.29 min	Area %: 5.8 %
RT: 1.33 min	Area %: 91.4 %
RT: 1.42 min	Area %: 2.5 %
RT: 1.48 min	Area %: 0.3 %

Supplementary Figure 30. HPLC spectrum of P16.

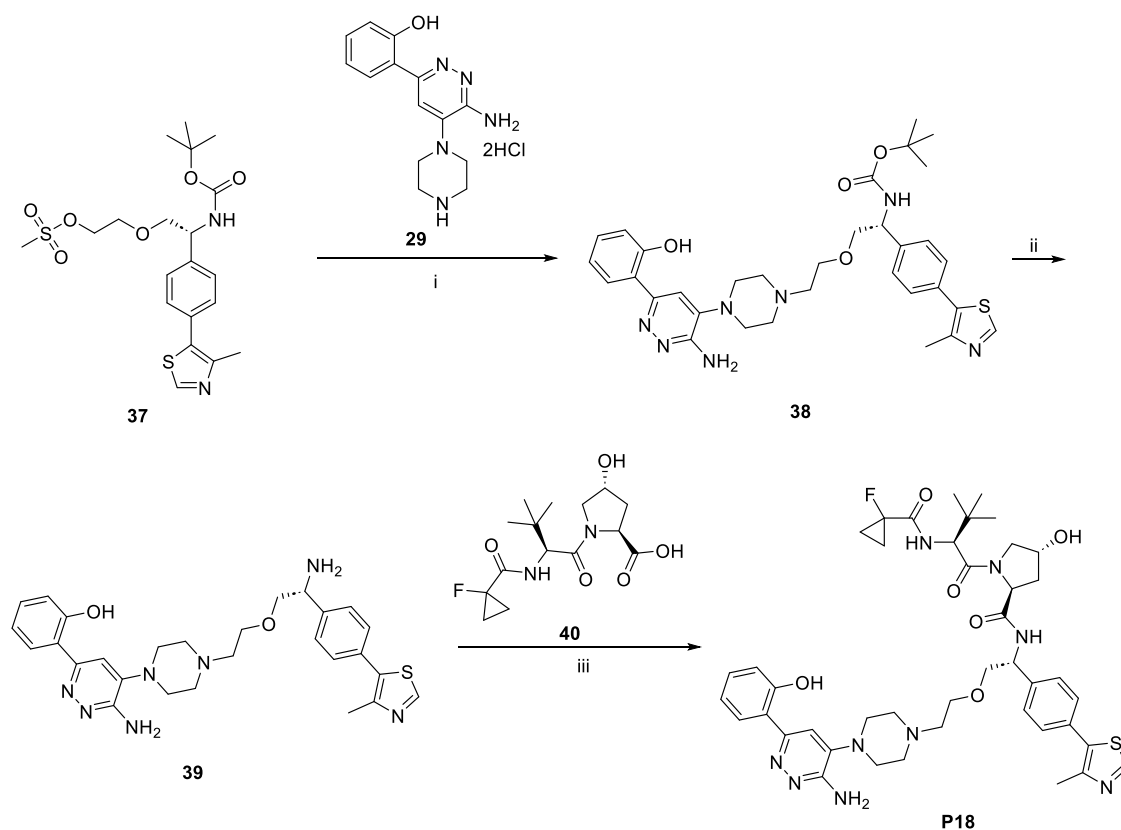


Supplementary Figure 31. ¹H and ¹³C NMR spectrum of P16.

¹H NMR (DMSO-d₆) δ: 14.17 (br s, 1H), 8.98 (s, 1H), 8.57 (q, J=5.4 Hz, 0.1H rotamer), 8.49 (t, J=6.0 Hz, 1H), 7.92 (dd, J=8.2, 1.2 Hz, 1H), 7.60-7.63 (m, 0.1H rotamer), 7.57 (s, 1H), 7.56 (br s, 0.1H rotamer), 7.51 (d, J=7.9 Hz, 0.1H rotamer), 7.44-7.49 (range, 2H), 7.37-7.44 (range, 3H),

7.28 (br dd, $J=9.3, 2.3$ Hz, 1H), 7.24 (dt, $J=8.4, 1.6$ Hz, 1H), 7.19 (d, $J=7.7$ Hz, 0.1H rotamer), 7.11 (s, 0.1H rotamer), 7.04 (s, 1H), 6.95 (dd, $J=7.8, 1.0$ Hz, 1H), 6.85-6.92 (range, 2H), 6.44 (s, 2H), 5.31 (s, 0.1H rotamer), 5.16 (d, $J=3.5$ Hz, 1H), 5.04 (d, $J=2.8$ Hz, 0.1H rotamer), 4.69 (t, $J=7.3$ Hz, 0.1H rotamer), 4.58 (d, $J=9.4$ Hz, 1H), 4.52 (t, $J=8.3$ Hz, 1H), 4.47 (br d, $J=9.2$ Hz, 0.1H rotamer), 4.28-4.38 (range, 3H), 4.24 (dd, $J=16.6, 6.3$ Hz, 1H), 4.12 (dd, $J=16.6, 5.4$ Hz, 1H), 4.08 (br d, $J=4.8$ Hz, 0.1H rotamer), 3.78-3.96 (range, 2H), 3.54-3.68 (range, 4H), 3.51 (s, 0.2H rotamer), 3.46-3.50 (m, 0.2H rotamer), 3.05-3.20 (range, 6H), 2.46 (s, 3H), 2.38 (s, 0.1H rotamer), 2.09 (br dd, $J=12.6, 7.8$ Hz, 1H), 1.85-1.97 (m, 1H), 1.29-1.44 (m, 2H), 1.18-1.28 (m, 2H), 0.97 (s, 0.55H rotamer), 0.92-0.95 (m, 9H)

^{13}C NMR (DMSO- d_6) δ : 172.3, 169.6, 169.4, 168.5 (d, CF=20 Hz), 159.0, 156.1, 155.3, 153.6, 151.9, 148.3, 141.0, 140.5, 134.1, 131.7, 131.4, 130.6, 129.6, 128.1, 127.6, 127.4, 126.6, 121.3, 118.9, 118.2, 117.9, 112.1, 111.7, 78.6 (d, CF=233 Hz), 69.4, 68.7, 59.3, 57.2, 57.0, 49.2, 47.2, 41.7, 38.4, 37.7, 36.5, 35.3, 26.6, 16.5, 13.4 (d, CF=10 Hz), 13.2 (d, CF=10 Hz)



Supplementary Figure 32. Synthesis of P18: i) **29**, DIPEA, DMF, 80°C; ii) conc. HCl, MeOH, RT; iii) **40**, HATU, TEA, DMF, RT.

Synthesis of *tert*-butyl (*R*)-(2-(2-(4-(3-amino-6-(2-hydroxyphenyl)pyridazin-4-yl)piperazin-1-yl)ethoxy)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamate **38**

37 (35 mg, 77 μmol) and **29** (21 mg, 77 μmol , 1.0 eq.) were dissolved in DMF (1000 μL) and DIPEA (66 μL , 0.38 mmol, 5 eq.) was added. The mixture was stirred at 80°C for 14 h. It was

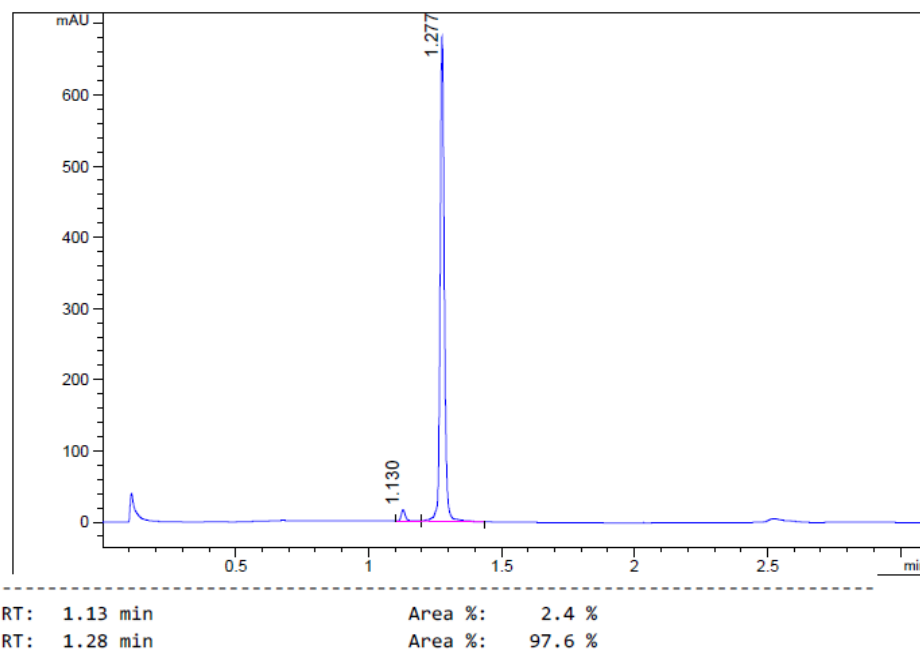
diluted with MeCN and water, filtered through a syringe filter and purified by basic HPLC-chromatography to obtain **38** (23 mg, 48% yield).

Synthesis of (2*S*,4*R*)-N-((*R*)-2-(2-(4-(3-amino-6-(2-hydroxyphenyl)pyridazin-4-yl)piperazin-1-yl)ethoxy)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)-1-((*S*)-2-(1-fluorocyclopropane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxypyrrolidine-2-carboxamide **P18**

38 (23 mg, 36 μ mol) was taken up in methanol (1000 μ L) and conc. aq. HCl (400 μ L) was added. The mixture was stirred at RT for 1 h. The solvents were removed under reduced pressure to obtain crude **39**.

40 (14 mg, 41 μ mol, 1.2 eq.) was dissolved in DMF (500 μ L) and TEA (21 μ L, 0.14 mmol, 4.0 eq.) and HATU (41 mg, 0.11 mmol, 3.0 eq.) were added. The resulting mixture was stirred at RT for 15 minutes, then **39** (19 mg, 36 μ mol) was added and stirring was continued at RT for 3 h. The reaction was diluted with water, filtered through a syringe filter and purified by basic HPLC-chromatography to obtain **P18** (17 mg, 56% yield).

HRMS (ESI+) m/z : $[M+H]^+$ calcd for $C_{43}H_{54}FN_9O_6S$ 844.39134; found 844.39862



Supplementary Figure 33. HPLC spectrum of P18.

1H), 4.16-4.20 (m, 0.1H rotamer), 3.75 (dd, J=10.1, 4.8 Hz, 0.1H rotamer), 3.65 (s, 0.1H rotamer), 3.63 (br d, J=2.8 Hz, 0.1H rotamer), 3.58-3.62 (m, 1H), 3.47-3.57 (range, 5H), 3.44 (t, J=6.1 Hz, 0.1H rotamer), 3.40 (br d, J=12.5 Hz, 0.1H rotamer), 2.88-3.11 (range, 4H), 2.62 (s, 0.1H rotamer), 2.51-2.59 (range, 4H), 2.49 (br t, J=4.7 Hz, 2H), 2.39 (s, 0.3H rotamer), 2.37 (s, 3H), 2.06 (s, 0.28H rotamer), 2.02 (br t, J=10.3 Hz, 1H), 1.79-1.85 (m, 0.1H rotamer), 1.71 (ddd, J=12.9, 8.7, 4.5 Hz, 1H), 1.18-1.35 (m, 2H), 1.08-1.18 (m, 2H), 0.89-0.93 (m, 9H)

¹³C NMR (DMSO-d₆) δ: 171.4, 169.3, 168.5 (d, CF=20 Hz), 159.0, 155.1, 153.6, 152.0, 148.3, 141.2, 140.9, 131.5, 130.6, 130.6, 129.1, 127.9, 126.6, 118.9, 118.3, 117.9, 110.7, 78.6 (d, CF=231 Hz), 73.4, 69.3, 68.8, 59.1, 57.5, 57.1, 57.0, 53.0, 52.6, 49.0, 38.2, 36.6, 26.7, 16.5, 13.4 (d, CF=10 Hz), 13.1 (d, CF=10 Hz)

Protein production and purification

Following wild type versions of human proteins were used for protein expression: VHL (UniProt accession number: P40337), ElonginC (Q15369), ElonginB (Q15370) and the bromodomain (BD) of SMARCA2 (SMARCA2^{BD}; P51531-2, residues 1373-1493 with additional N-terminal SM residues (cloning artefact)).

SMARCA2^{BD} was synthesized by GeneArt, subcloned into pDEST15 vectors (Invitrogen) and expressed in *E. coli* BL21(DE3) as an N-terminal GST fusion protein with a TEV protease cleavage site. Cells were cultured overnight in LB medium with ampicillin (100 µg/mL), then diluted 1:100 into Terrific Broth containing the same antibiotic and grown at 37 °C to OD₆₀₀ ≈ 1. Protein expression was induced by addition of 0.3 mM IPTG followed by overnight growth at 23 °C. Cells were harvested by centrifugation and stored at -80 °C prior to purification.

Cells were resuspended in Lysis buffer and lysed by sonication (20 pulses of 5 s for 4 min) using a Sonopuls HD 3080 (Bandelin, Berlin, Germany) or by homogenization using a Stansted Pressure Cell Homogeniser (Stansted Fluid Power). The Lysis buffers were as follows: for SMARCA2^{BD}: 50 mM HEPES, 500 mM sodium chloride, 5% glycerol, 5 mM dithiothreitol (DTT), supplemented with complete protease inhibitors (Roche). Affinity purification was performed using Glutathione Sepharose 4B (GE Healthcare) in batch mode or on-column. Cleavage of the GST-tag was performed using TEV protease for 16 h at 4 °C, either on column, or in solution following elution of the GST-tagged BDs with Lysis buffer containing 20 mM reduced L glutathione (Sigma Aldrich). For SMARCA2^{BD}, prior to TEV protease cleavage the eluted GST-tagged BD was first dialysed into desalting buffer (20 mM HEPES, 250 mM sodium chloride, pH 7.0 + 0.5% glycerol). Where TEV cleavage was performed in solution, a second affinity (GST-trap) column purification step was carried out to remove the GST-tag and uncleaved GST-tagged protein. Proteins were further purified by size exclusion chromatography (HiLoad Superdex-75, 16/600) (GE Healthcare) and stored in Storage buffer (for SMARCA2^{BD}: 20 mM HEPES, 150 mM sodium chloride, pH 7.5). All chromatography purification steps were performed either at room temperature or 4 °C using an ÄKTA FPLC purification system (GE Healthcare) or a plastic Econo-Pac column (Bio-Rad).

The VCB complex was expressed and purified as described previously [4], with the modification that 0.3 mM IPTG was used for induction of expression. Briefly, N terminally His₆ tagged VHL (54–213), ElonginC (17–112) and ElonginB (1–104) were co-expressed and the complex isolated by Ni affinity chromatography. After removal of the His₆ tag by TEV protease, the complex was further purified by anion exchange and size exclusion chromatography and finally stored in a buffer containing 20 mM HEPES pH 7.5, 150 mM NaCl, and 1 mM TCEP.

TR-FRET cooperativity assay

All assays were performed at room temperature in a Proxiplate-384 PLUS, white (PerkinElmer) plate. Reagents were diluted in buffer, consisting of 50 mM HEPES, 50 mM sodium chloride, 2 mM DTT, 0.008% Brij, 0.01% BSA at pH 7.3, and were allowed to equilibrate to room temperature before addition to plates. The final concentrations in the assay were as follows: 40 nM SMARCA2^{BD}, a concentration series of each PROTAC (10 μ M to 1 nM, 11-point half-log-fold serial dilution), 16.6 nM biotinylated ACBI2 [3] (K_d for SMARCA2^{BD} ~400 nM), 2.5 nM Lance Eu-W1024 labeled streptavidin (PerkinElmer Cat No. AD0062, 50 μ g, 500 μ g mL⁻¹, 8.3 μ M), 50 nM ULIGHT-anti 6xHis AB (PerkinElmer TRF0105-M, 5 μ M, 800 μ g mL⁻¹) and either assay buffer or 5 μ M VCB at a final DMSO concentration of 1%. SMARCA2^{BD}, streptavidin-Eu and ULIGHT AB were mixed in assay buffer (SMARCA2 solution) and kept at room temperature before use. Then, 5 nL of the biotinylated probe solution was added to rows 1–23 (with the Labcyte Echo 55x) and 187.5 nL VCB (or assay buffer) was transferred with the Labcyte Echo 55x to rows 1–23. Then, 15 μ L of the SMARCA2 solution was added to rows 1–24. Plates were incubated at room temperature for 60 min and the signal was measured in a PerkinElmer Envision HTS Multilabel Reader using the TR-FRET LANCE Ultra specs from PerkinElmer (665 nm/615 nm $\times$ 10,000).

Each plate contained 16 wells of a negative control (diluted DMSO instead of test compound with biotinylated probe) and 16 wells of a positive control (diluted DMSO instead of test compound without biotinylated probe). The average of the background control (positive control, without enzyme) was calculated and subtracted from the measured values for each plate. IC₅₀ values were calculated and analyzed using a four-parametric logistic model for each PROTAC in the absence or presence of saturating concentrations of VCB.

3. Supplementary Tables

Supplementary Table 1: Measurement of PROTAC cooperativity (α) and ternary complex affinity (ternary IC₅₀) via TR-FRET competition for SMARCA2^{BD} $\pm$ VCB. Experimental details are given in the Supplementary Methods and in [1]. PROTAC cooperativity (α) is calculated as ratio between TR-FRET IC₅₀ / TR-FRET IC₅₀ + VCB values. IC₅₀ data represent geometric means with errors stated as geometric standard deviation and number of experimental replicates (n) given in parentheses. Cooperativity uncertainties were calculated from IC₅₀ geometric standard deviations using standard error propagation rules.

Compound	TR-FRET			X-ray structure	Reference
	SMARCA2 ^{BD} IC ₅₀ (nM)	Ternary SMARCA2 ^{BD} IC ₅₀ (nM)	α	Ternary SMARCA2 ^{BD} :PROTAC:VCB complex	

		(PROTAC + VCB)		PDB code	
P1	$8,300 \cdot 1.2^{\pm 1}$ (n = 3)	$89 \cdot 1.5^{\pm 1}$ (n = 3)	93 ± 41	9HYN	this study
P2	$57 \cdot 1.2^{\pm 1}$ (n = 9)	$14 \cdot 1.5^{\pm 1}$ (n = 7)	4.1 ± 1.8	7Z77	6 in [1]
P3	$84 \cdot 1.3^{\pm 1}$ (n = 6)	$17 \cdot 1.9^{\pm 1}$ (n = 6)	5.0 ± 3.5	9HYB	this study
P4	$380 \cdot 1.3^{\pm 1}$ (n = 3)	$280 \cdot 1.2^{\pm 1}$ (n = 2)	1.3 ± 0.4	9HYO	this study
P5	$98 \cdot 1.1^{\pm 1}$ (n = 4)	$160 \cdot 1.7^{\pm 1}$ (n = 4)	0.6 ± 0.3	9HYP	23 in [1]
P6	$> 10,000$ (n = 3)	$110 \cdot 1.3^{\pm 1}$ (n = 6)	> 91	-	[2]
P7	$> 10,000$ (n = 5)	$190 \cdot 1.1^{\pm 1}$ (n = 7)	> 53	-	[2]
P8	$1,100 \cdot 1.5^{\pm 1}$ (n = 86)	$45 \cdot 1.3^{\pm 1}$ (n = 80)	25 ± 12	-	ACBI1 in [3]
P9	$> 10,000$ (n = 2)	$370 \cdot 1.5^{\pm 1}$ (n = 5)	> 27	-	this study
P10	$> 10,000$ (n = 4)	$230 \cdot 1.1^{\pm 1}$ (n = 2)	> 43	-	this study
P11	$> 10,000$ (n = 2)	$490 \cdot 1.6^{\pm 1}$ (n = 5)	> 20	-	this study
P13	$4,000 \cdot 1.0^{\pm 1}$ (n = 4)	$320 \cdot 1.1^{\pm 1}$ (n = 3)	12 ± 1	6HAY	PROTAC 1 in [3]
P16	$590 \cdot 1.3^{\pm 1}$ (n = 4)	$330 \cdot 1.9^{\pm 1}$ (n = 4)	1.8 ± 1.2	-	[2]
P18	1,700 (n = 1)	1,600 (n = 1)	1.1	-	this study
P19	$1,400 \cdot 1.1^{\pm 1}$ (n = 2)	$1,300 \cdot 1.1^{\pm 1}$ (n = 2)	1.0 ± 0.1	-	cis-ACBI1 in [3].
P20	5,600 (n = 2)	$> 10,000$ (n = 2)	< 0.56	-	cis-PROTAC 2 in [3]

Supplementary Table 2: The population percentage of each cluster for all the five PROTAC simulations. These population percentages were used to calculate the Shannon entropy for each PROTAC.

	P1	P2	P3	P4	P5
Cluster 1	0%	0%	7%	42%	0%
Cluster 2	1%	0%	7%	15%	39%
Cluster 3	22%	60%	0%	2%	12%

Cluster 4	0%	0%	85%	40%	19%
Cluster 5	0%	40%	0%	0%	0%
Cluster 6	1%	0%	1%	1%	27%
Cluster 7	76%	0%	0%	0%	3%

Supplementary Table 3: Data collection and refinement statistics for VCB : P1 : SMARCA2^{BD}
ternary
complex co-crystal structure

Crystallography data collection and refinement statistics

	SMARCA2 ^{BD} :P1:VCB (PDB: 9HYN)
Data collection	
Space group	P2(1)2(1)2(1)
Cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	79.94, 118.23, 122.70
<i>a</i> , <i>b</i> , <i>c</i> (°)	90.0, 90.0, 90.0
Resolution (Å)	85.14-2.37 (2.491-2.366) *
<i>R</i> _{merge}	12.9 (153.4)
CC (1/2)	0.998 (0.608)
<i>I</i> / <i>sI</i>	16.6 (1.4)
Completeness (spherical, %)	84.9 (22.6)
Completeness (ellipsoidal, %)	94.9 (55.1)
Multiplicity	10.3 (11.5)
Refinement	
Resolution (Å)	45.01-2.37
No. reflections	40856
<i>R</i> _{work} / <i>R</i> _{free}	24.6/26.2
No. atoms	
Protein	7330
Ligand/ion	130
Water	157
<i>B</i> -factors	
Protein	53.86
Ligand/ion	33.83
Water	36.19
R.m.s. deviations	
Bond lengths (Å)	0.008
Bond angles (°)	0.97

Values as output by autoPROC and autoBUSTER

*Values in parentheses are for highest-resolution shell.

Supplementary Table 4: Data collection and refinement statistics for VCB : P3 : SMARCA2^{BD}
ternary
complex co-crystal structure

	SMARCA2 ^{BD} :P3:VCB (PDB: 9HYB)
Data collection	
Space group	P 2(1)

Cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	108.38, 78.54, 171.58
<i>a</i> , <i>b</i> , <i>c</i> (°)	90.0, 91.43, 90.0
Resolution (Å)	108.34-2.86 (3.225-2.856) *
<i>R</i> _{merge}	15.5 (87.2)
CC (1/2)	0.995 (0.568)
<i>I</i> / <i>sI</i>	8.6 (1.7)
Completeness (spherical, %)	63.5 (10.5)
Completeness (ellipsoidal, %)	92.8 (60.2)
Multiplicity	4.4 (4.2)
Refinement	
Resolution (Å)	32.47-2.86
No. reflections	42742
<i>R</i> _{work} / <i>R</i> _{free}	25.1/26.5
No. atoms	
Protein	14697
Ligand/ion	316
Water	299
<i>B</i> -factors	
Protein	73.38
Ligand/ion	66.09
Water	48.95
R. m. s. deviations	
Bond lengths (Å)	0.008
Bond angles (°)	0.92

Values as output by autoPROC and autoBUSTER

*Values in parentheses are for highest-resolution shell.

Supplementary Table 5: Data collection and refinement statistics for VCB : P4 : SMARCA2^{BD}
ternary
complex co-crystal structure

	SMARCA2 ^{BD} :P4:VCB (PDB: 9HYO)
Data collection	
Space group	P 1 2 ₁ 1
Cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	48.525, 64.096, 89.476
<i>a</i> , <i>b</i> , <i>c</i> (°)	90 96.866 90
Resolution (Å)	48.18 - 3.741 (3.874 - 3.741)
<i>R</i> _{meas}	0.188 (0.775)
<i>I</i> / <i>sI</i>	5.9 (1.9)
Completeness (%)	98.56 (96.32)
Multiplicity	3.2
Refinement	
Resolution (Å)	48.18 - 3.7
No. reflections	5685
<i>R</i> _{work} / <i>R</i> _{free}	22.0/34.8

No. atoms (non-hydrogen)	
Protein	3606
Ligand	79
Water	3
<i>B</i> -factors	
Protein	60.87
Ligand	20
Water	30
R. m. s. deviations	
Bond lengths (Å)	0.010
Bond angles (°)	1.29

Supplementary Table 6: Data collection and refinement statistics for VCB : P5 : SMARCA2^{BD} ternary complex co-crystal structure

	SMARCA2 ^{BD} :P5:VCB (PDB: 9HYP)
Data collection	
Space group	P 1 2 ₁ 1
Cell dimensions	
<i>a</i> , <i>b</i> , <i>c</i> (Å)	46.624, 81.585, 58.117
<i>a</i> , <i>b</i> , <i>c</i> (°)	90 97.752 90
Resolution (Å)	46.2 - 2.2 (2.279 - 2.2)
<i>R</i> _{meas}	0.102 (0.603)
<i>I</i> / <i>sI</i>	9.3 (2.1)
Completeness (%)	99.40 (95.35)
Multiplicity	2.9
Refinement	
Resolution (Å)	46.2 - 2.2
No. reflections	21863
<i>R</i> _{work} / <i>R</i> _{free}	21.2/25.4
No. atoms (non-hydrogen)	
Protein	3537
Ligand	71
Water	60
<i>B</i> -factors	
Protein	48.1
Ligand	47.7
Water	37.0
R. m. s. deviations	
Bond lengths (Å)	0.010

Supplementary Table 7: MD simulation check list.

Reliability and reproducibility checklist for molecular dynamics simulations *All boxes must be marked YES by acceptance unless an N/A option is available	Yes	N/A	Response (Please state where this information can be found in the text)
1. Convergence of simulations and analysis			
1a. Is an evaluation presented in the text to show that the property being measured has equilibrated in the simulations <i>(e.g. time-course analysis)?</i>	☒		In the main text above Figure 2: “The root mean square deviation (RMSD) in the coordinates of the backbone atoms of the SMARCA2 domain ranges from 2.8 Å to 15.6 Å when aligned by the VHL domain of the P1 ternary complex crystal structure.”
1b. Then, is it described in the text how simulations are split into equilibration and production runs and how much data were analyzed from production runs?	☒		In the method section, MD simulation protocol: “Six random velocities were employed to generate six independent production runs. Each production run lasted for 500 ns, and the complete production trajectories (30,000 snapshots in total) were used for subsequent analysis.”
1c. Are there at least 3 simulations per simulation condition with statistical analysis?	☒		There are six velocities/simulations per condition
1d. Is evidence provided in the text that the simulation results presented are independent of initial configuration?	☒		To strengthen the error estimation, we increased the MD simulation sample size by generating 10 different conformation ensembles using bootstrapping methods.

2. Connection to experiments		
2a. Are calculations provided that can connect to experiments (e.g. loss or gain in function from mutagenesis, binding assays, NMR chemical shifts, J-couplings, SAXS curves, interaction distances or FRET distances, structure factors, diffusion coefficients, bulk modulus and other mechanical properties, etc.)?	☒	Figure 3D, Figure 6A and Figure 7.
3. Method choice		
3a. Is it described in the text what force field and water model are used and why?	☒	In method section, MD simulation protocol: “. The protein atoms were parameterized using the FF99SB-ILDN force field ⁴³ . The PROTAC TC complex was solvated in a truncated octahedron SPC ⁴⁴ water box with at least 14 Å margin from the protein surface using tleap from the AmberTools 18 package ²⁵ .”
3b. Do simulations contain membranes, membrane proteins, intrinsically disordered proteins, glycans, nucleic acids, polymers, or cryptic ligand binding?	☐	☒ Response not needed if N/A
If 3b is YES, are enhanced sampling methods used?	☐	☐ Response not needed if N/A
If enhanced sampling methods are used, are the convergence criteria clearly stated?	☐	
If 3b is YES, is it explained in the text why or why not enhanced sampling methods are used?	☐	
4. Code and reproducibility		
4a. Is a table provided describing the system setup, such as simulation box dimensions, total number of atoms, total number of water molecules, salt concentration, lipid composition (number of molecules and type)?	☐	Information is offered as written text in the method section, without a table.
4b. Is it described in the text what simulation and analysis software and which versions are used?	☒	In the method section: “AmberTools 18 package”
4c. Are initial coordinate and simulation input files and a coordinate file of the final output provided as supplementary files or in a public repository?	☒	All simulation files and sample trajectories are attached as attachment to this manuscript.

4d. Is there custom code or custom force field parameters?	☐	☒	Response not needed if N/A
If YES, are they provided as supplementary profiles or in a public repository?	☐		

4. Supplementary References

1. Kofink, C. *et al.* A selective and orally bioavailable VHL-recruiting PROTAC achieves SMARCA2 degradation in vivo. *Nat Commun* **13**, 5969 (2022).
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3. Farnaby, W. *et al.* BAF complex vulnerabilities in cancer demonstrated via structure-based PROTAC design. *Nat Chem Biol* **15**, 672–680 (2019).
4. Gadd, M. S. *et al.* Structural basis of PROTAC cooperative recognition for selective protein degradation. *Nat Chem Biol* **13**, 514–521 (2017).