Supporting Information

Expanding the Reaction Toolbox for Nanoscale Direct-to-Biology PROTAC Synthesis and Biological Evaluation

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Contents

General Experimental

Solvents and reagents were purchased from commercial suppliers and used as received. If not commercially available, compounds were prepared according to the literature unless stated otherwise. Reactions were carried out under nitrogen and stirred using a magnetic stirrer hotplate unless stated otherwise. Reactions using the glovebox were carried out in an MBraun MB-200B glovebox with an inert N_2 atmosphere. No unexpected or unusually high safety hazards were encountered.

Materials, Reagents and Analytical

NMR spectra were recorded at ambient temperature using standard pulse methods on a Bruker AV-400 instrument at 400 MHz, a Bruker AV4 at 700 MHz or a Bruker AVIIIHD at 600 MHz. Chemical shifts (δ) are reported in parts per million (ppm) and are reported as observed; several PROTACs have peak duplication due to rotamers. Peak assignments were chosen based on chemical shifts, integrations, and coupling constants, considering 2D analyses where necessary, or the following solvent peaks: $CDCl_3$ (^1H = 7.27 ppm), DMSO-d_6 (^1H = 2.50 ppm), CD_3OD (^1H = 4.87 and 3.31 ppm). Coupling constants are quoted to the nearest 0.1 Hz and multiplicities are given by the following abbreviations and combinations thereof: s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), sxt (sextet), m (multiplet), br. (broad).

LCMS analysis was carried out on a Waters Acquity UPLC instrument equipped with a BEH column (50 mm x 2.2 mm, 1.7 µm packing diameter) and Waters micromass ZQ MS using alternate-scan positive and negative electrospray. Analytes were detected as a summed UV wavelength of 210 – 350 nm. Two liquid phase methods were used:

Formic – 40 °C, 1 mL/min flow rate. Gradient elution with the mobile phases as (A) water containing 0.1% (v/v) formic acid and (B) acetonitrile containing 0.1% formic acid. Gradient conditions were initially 1% B, increasing linearly to 97% B over 1.5 min, remaining at 97% for 0.1 min then increasing to 100% B over 0.1 min.

HpH (HpH) – 40 °C, 1 mL/min flow rate. Gradient elution with the mobile phases as (A) 10 Mm aqueous ammonium bicarbonate solution, adjusted to pH 10 with 0.88 M aqueous ammonia and (B) acetonitrile. Gradient conditions were initially 1% B, increasing linearly to 97% B over 1.5 min, remaining at 97% B for 0.4 min then increasing to 100% B over 0.1 min.

HPLC purification was conducted on a ACCQPrep H125 instrument with XSelect CSH C18 column (150 mm x 30 mm internal diameter, 5 µm packing diameter) at ambient temperature, eluting with ammonium bicarbonate or formic acid aqueous solutions with acetonitrile using an appropriate elution gradient determined by LCMS analysis: HpH Method A: Eluting with 0 - 25% acetonitrile in 10 mM aqueous

ammonium bicarbonate; Formic Method A: Eluting 0 - 25% acetonitrile to water with 0.1% formic acid; Formic Method B: Eluting 15 - 55% acetonitrile to water with 0.1% formic acid.

Mass-directed automated purification (MDAP) was performed on a Waters FractionLynx system comprising of a Waters 600 pump with extended pump heads, Waters 2700 autosampler, Waters 996 diode array and Gilson 202 fraction collector. Mass spectra were recorded on a Waters ZQ mass spectrometer using alternate-scan positive and negative electrospray ionisation with a 150-1000 amu scan range, 0.5 s scan time with an 0.2 s inter-scan delay. Automated purification was conducted on a Waters XSelect™ CSH C18 column (75 mm x 30 mm, 5 µm particle diameter) at ambient temperature. The standard injection vehicle was DMSO at a volume of 1 mL. The UV detection was a summed signal from wavelength of 210 nm to 350 nm. Mass spectra were recorded on a Waters QDa mass spectrometer using alternate-scan positive and negative electrospray ionisation method with a scan range of 100 to 1000 amu, sample frequency of 2 Hz, and cone voltage of 5 V.

For low pH methods the solvents employed were: A: 0.1% v/v solution of formic acid in water; B: 0.1% v/v solution of formic acid in acetonitrile. For high pH methods the solvents employed were: A: 10 mM ammonium bicarbonate in water adjusted to pH 10 with ammonia solution; B: acetonitrile. Appropriate elution gradients were determined by LCMS analysis: Method A: Eluting with 0 – 30% gradient; Method B: Eluting with 10 – 40% gradient; Method C: Eluting with 20 – 50% gradient; Method D: Eluting with 30 – 60% gradient; Method E: Eluting with 40 – 70% gradient; Method F: Eluting with 50 – 80% gradient.

Biology Protocols

HiBiT and CellTitre-Glo® assays

Degradation of POIs in cells treated with PROTACs was quantified using the Nano-Glo® HiBiT Lytic Detection System (Promega) in 384 well assay plate format. Clonal cell lines were derived in house, in which the gene was modified using CRISPR/Cas9 editing, so the encoded protein included a HiBiT peptide tag. The HiBiT BRD4 and RIPK2 cell lines were HEK293 (ATCC-CRL-1593) with *N*-terminal and *C*-terminal tags respectively. The AR HiBiT cells were LNCAP with *C*-terminal tag (Promega CS3023266). 10 mM DMSO stock solutions of PROTACs were prepared and diluted across an 11 concentration, 3 fold increment range. Typically 25 nL or 50 nL was dispensed into a white opaque bottomed 384 well assay plate using an acoustic ECHO dispenser (Labcyte). For assay, cells were detached from culture flasks using TrypLE Express enzyme and centrifuged at 400g for 5 min. The cell pellet was resuspended in assay medium (FluoroBrite™ DMEM supplemented with 10% heat inactivated FBS, GlutaMAX™ , penicillin 50 U/mL and streptomycin 50 µg/mL). Alternatively cryopreserved assay ready aliquots of cells were used which were prepared in advance and stored at -150 °C in 90% FBS/10% DMSO. 25 µL of cell suspension containing 10,000 cells was dispensed into each well of the assay plate, which was then incubated at 37 °C/5% CO_2, for 18 h except where stated otherwise. Control wells corresponding to 0% and 100% effect were included. These were cells treated with DMSO vehicle only and assay medium without cells respectively.

25 µL of Nano-Glo® HiBiT lysis buffer supplemented with LgBiT protein and Nano-Glo® substrate was added to each well and the plate shaken at 500 rpm for 10 min at room temperature. Luminescence intensity was measured using a PHERAstar microplate reader (BMG Labtech) and the % POI remaining in each well

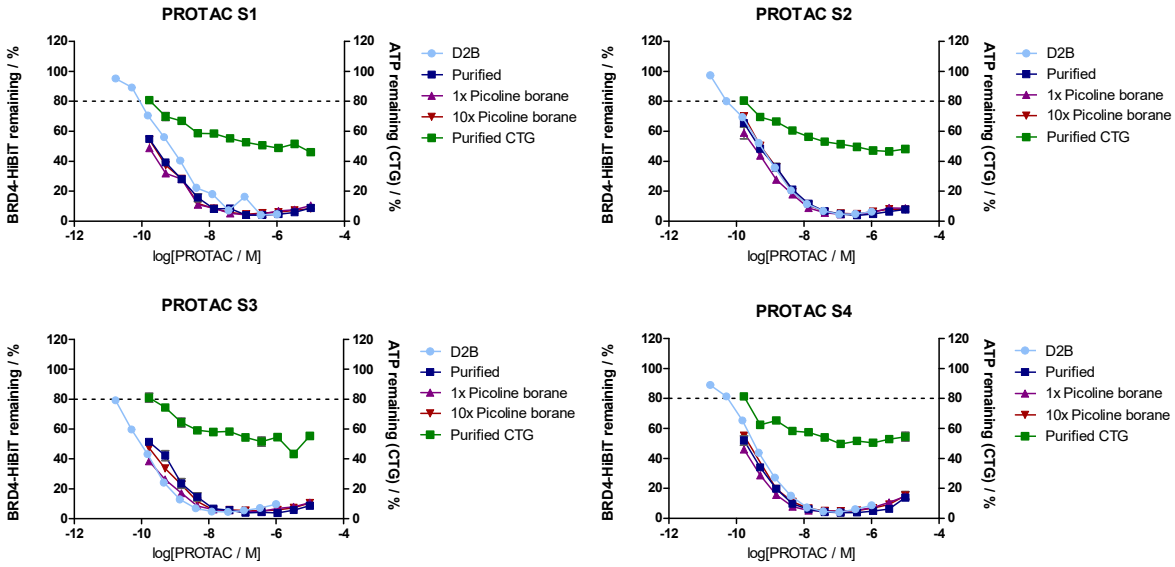
calculated by normalizing the raw luminescence value to the above control wells. Parameters corresponding to the potency and efficacy estimates of the PROTACs were obtained from these values using software (IDBS ActivityBase and GraphPad Prism). Two versions of the DC_{50}, called relative DC_{50} and absolute DC_{50} were typically generated corresponding to the inflection point of a fitted 4-parameter sigmoidal response curve and the interpolated concentration of PROTAC corresponding to 50% of the protein degraded, respectively. The D_{max} parameter was the maximum experimentally observed degradation. All cell culture items and the assay plates were from Thermofisher.

The effects of PROTACs on cell viability were estimated by measuring cellular ATP levels using the CellTiter-Glo® (CTG) Luminescent Cell Viability Assay (Promega). ATP assays were typically conducted with HiBiT assays on a separate assay plate on the same day, following the same general procedure detailed above.

Assessment of tolerance to and potential interference from solvents and chemicals relevant to D2B PROTAC synthesis was achieved by spiking either HiBiT degradation or CTG viability assays. Purified PROTACs were spiked with 1- and 10-times the concentration of reagent used in the D2B reaction, to mimic a 'D2B-relevant' concentration (x1) and to test the robustness of the assay to an excess of reagent (x10). This equated to top assay concentrations of 13 and 130 µM for picoline borane (assuming 1.3 equivalents used in the D2B reductive amination reaction) and 20 and 200 µM for MTBD (assuming 2 equivalents used in the D2B S_NAr or alkylation reactions).

Biological Assays

BRD4 PROTACs



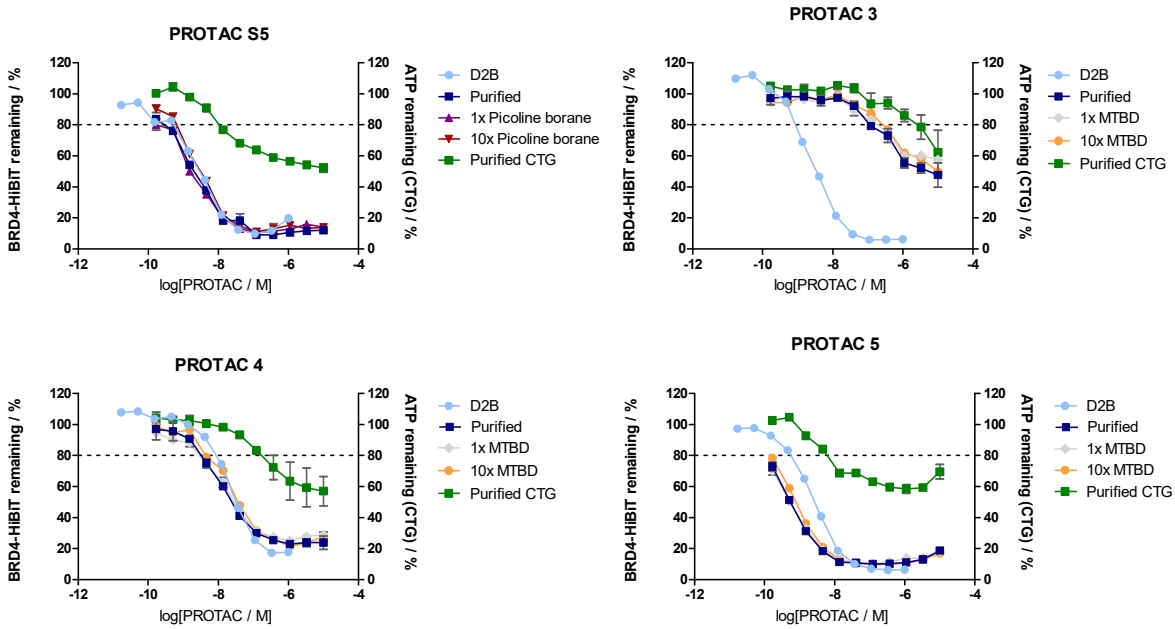


Figure S1. BRD4 PROTACs tested in HiBiT and CTG assays; light blue line corresponds to crude D2B samples; dark blue line corresponds to purified samples; purple, red, grey and yellow lines correspond to spiking experiments with picoline borane or MTBD; green line corresponds to CTG data for purified PROTAC, note that BRD4 PROTACs are known to cause cytotoxicity at high concentrations; all N=2 data (technical replicates) except for D2B data which is N=1; error bars represent SEM.

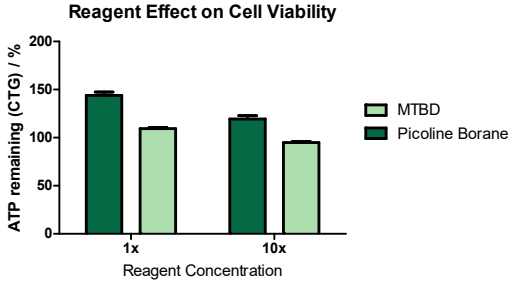


Figure S2. Effect of reagents MTBD and picoline borane on cell viability at 1 and 10x 'D2B relevant' concentration, assessed by CTG assay in a BRD4-HiBiT cell line.

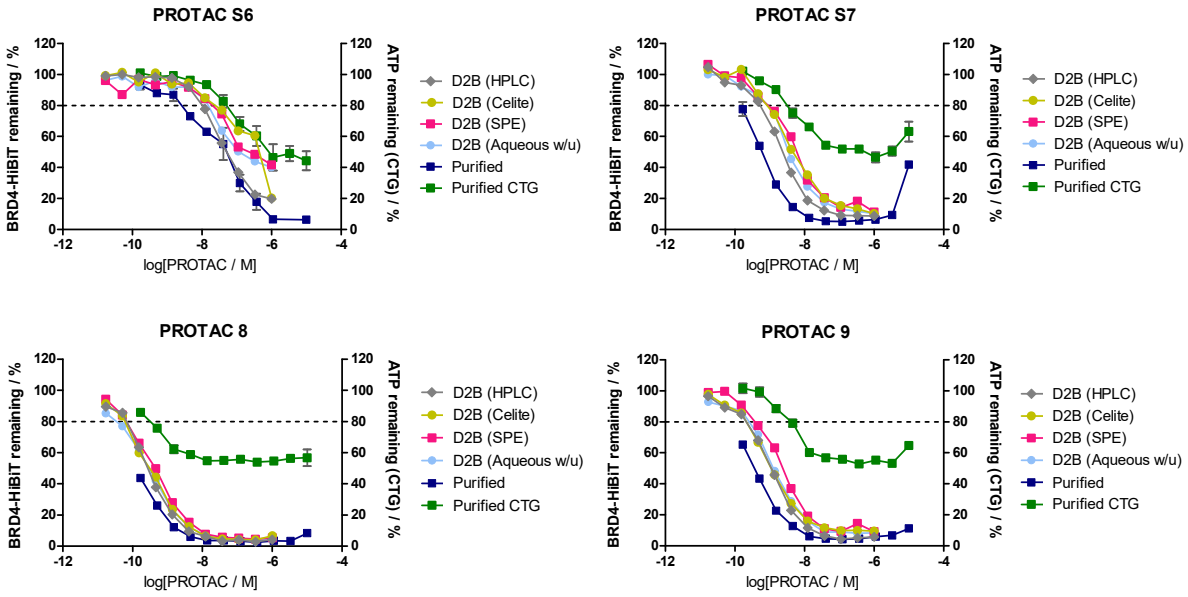
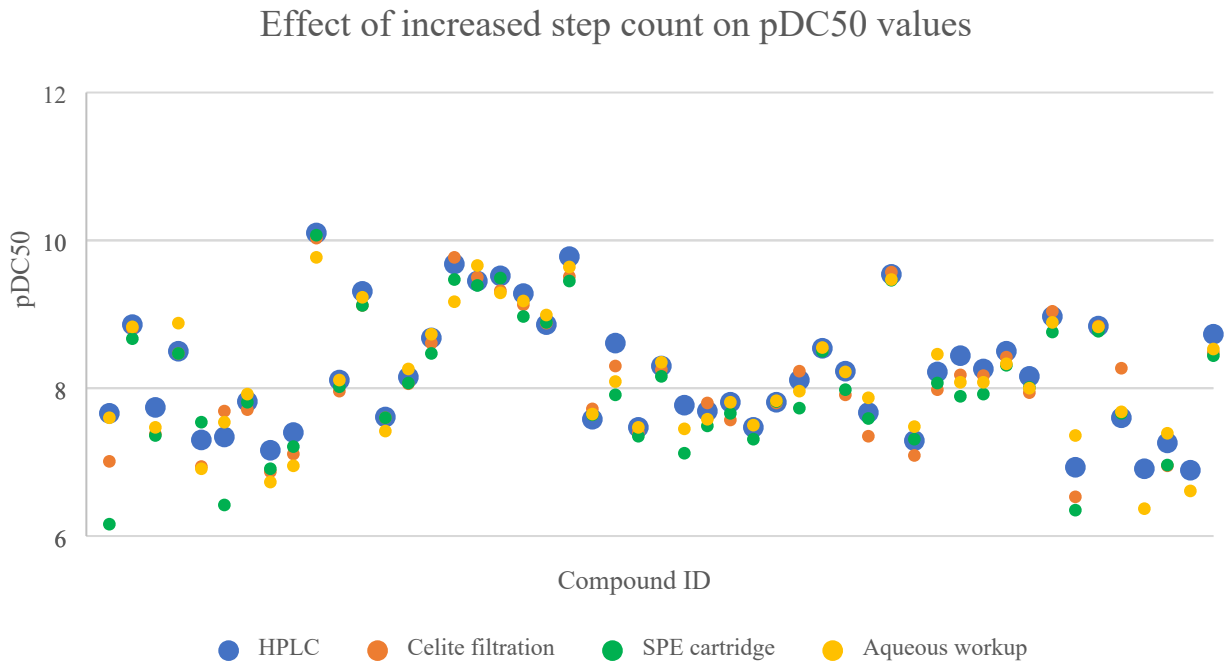


Figure S3. BRD4 PROTACs tested in HiBiT and CTG assays; D2B lines corresponds to samples tested after three steps of chemistry (Pd-mediated cross-coupling, deprotection and amide coupling) with different purification methods after the Pd-mediated sp^2-sp^3 cross-coupling indicated in brackets; dark blue line corresponds to purified samples; green line corresponds to CTG data for purified PROTAC, note that BRD4 PROTACs are known to cause cytotoxicity at high concentrations; all N=2 data (technical replicates) except for D2B data which is N=1; error bars represent SEM.



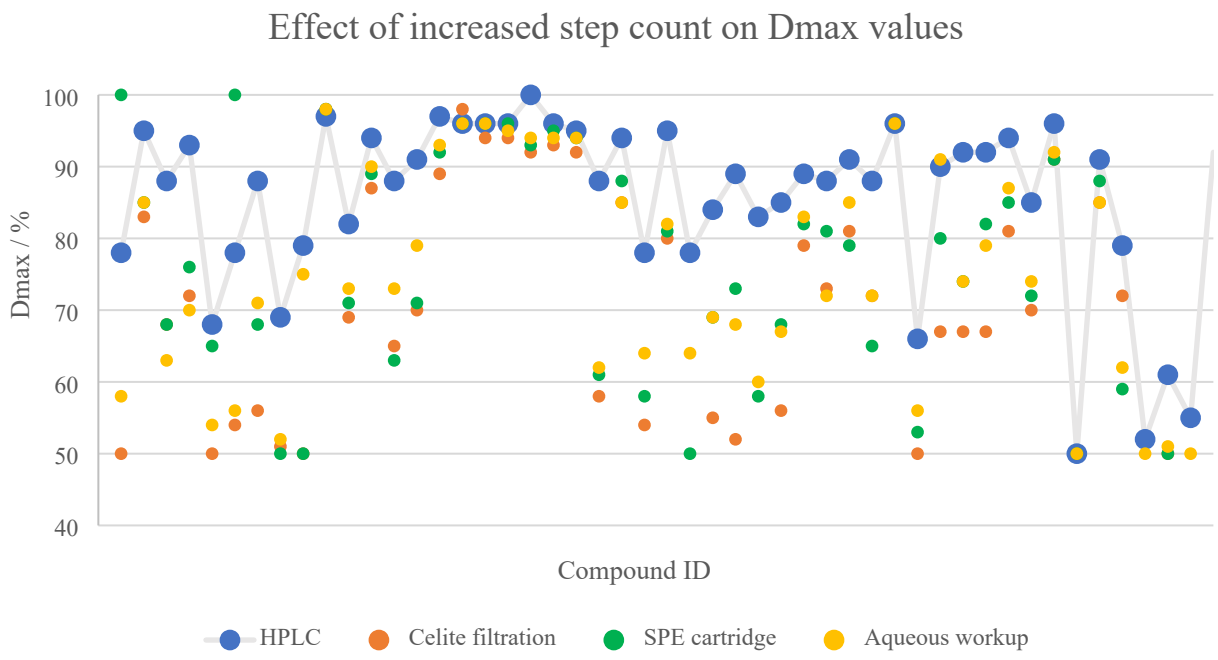


Figure S4. Degradation data after three steps of chemistry in D2B format; transformations consist of Pd-mediated cross coupling (followed by a purification, indicated by different coloured series), deprotection then amide coupling; compound ID corresponds to 49 different PROTACs that were successful in all four libraries; blue points are larger to highlight the HPLC control batch for comparison.

Library analysis across 49 samples that were successful in all four libraries (using different purification methods after Pd-mediated cross-coupling) indicate that celite filtration, SPE cartridge or aqueous workup methods give comparable pDC_{50} values after three steps to the HPLC batches, whereas D_{max} is significantly reduced in the majority of cases.

AR PROTACs

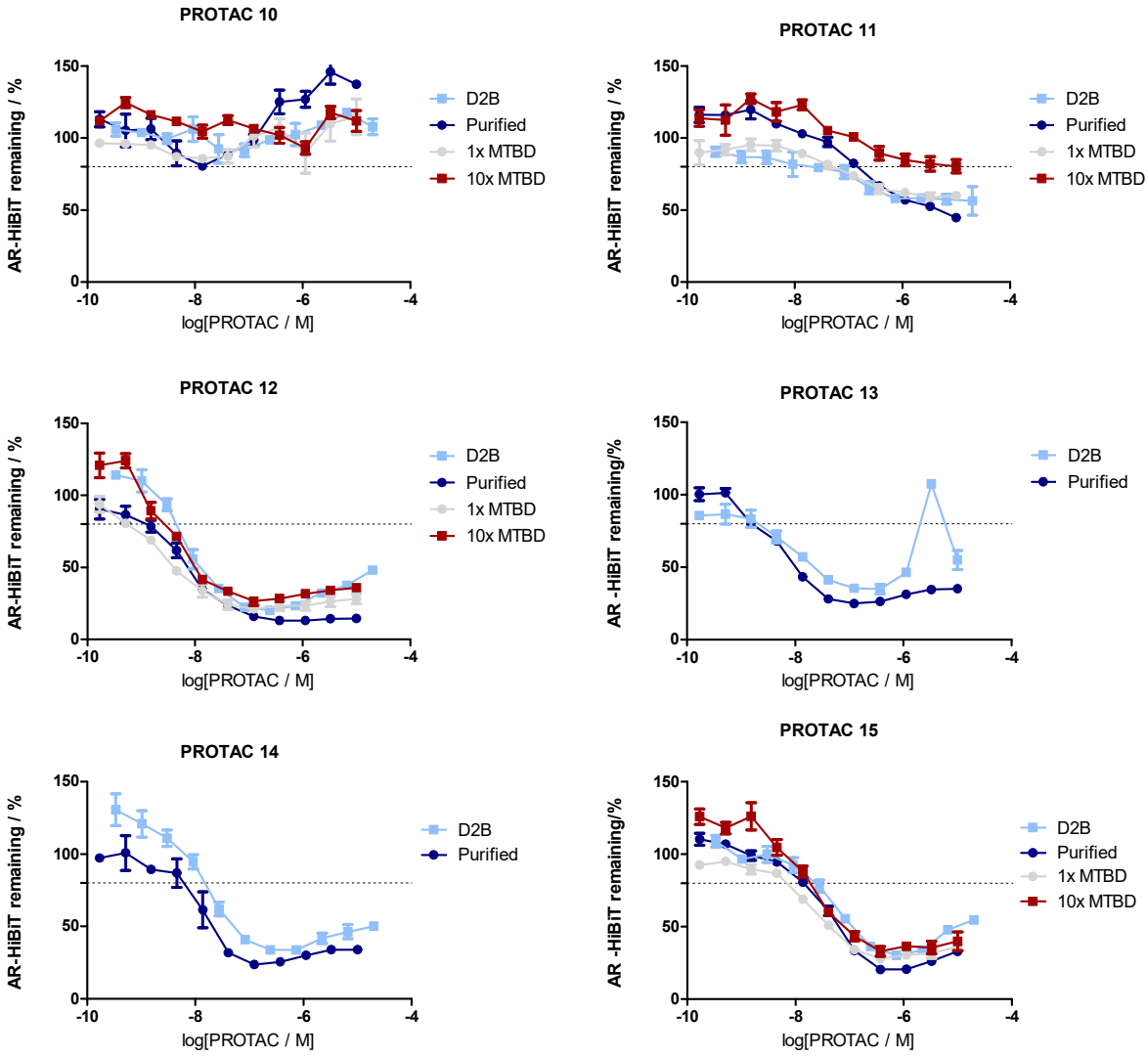


Figure S5. AR PROTACs tested in HiBiT and CTG assays; light blue line corresponds to crude D2B samples; dark blue line corresponds to purified samples; grey and red lines correspond to spiking experiments with MTBD; green line corresponds to CTG data for purified PROTAC; all N=2 data (technical replicates); error bars represent SEM; purified PROTACs were tested at a top concentration 10 µM and D2B samples at 20 µM.

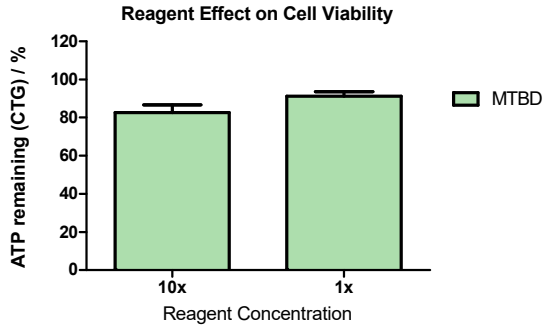


Figure S6. Effect of reagents MTBD on cell viability at 1 and 10x 'D2B relevant' concentration, assessed by CTG assay in an AR-HiBiT cell line.

RIPK2 Cell Viability

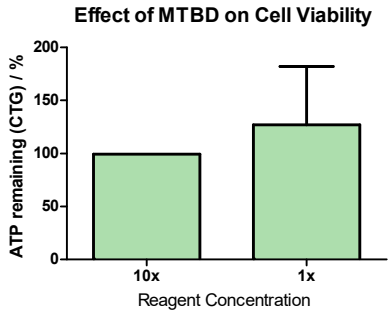


Figure S7. Effect of reagent MTBD on cell viability at 1 and 10x 'D2B relevant' concentration, assessed by CTG assay in a RIPK2-HiBiT cell line.

Chemistry

Direct-to-Biology Standard Protocol

D2B reaction mixtures are dispensed into 1536-well microtitre plates using a mosquito® liquid handler in the glovebox under inert N_2 atmosphere. Reactions are carried out in 4-6 µL DMSO at a concentration of 30 mM. Room temperature reactions are left overnight in a sealed plate without stirring or agitation. Heated reactions are sealed and placed in a thermomixer overnight at the desired temperature with shaking at 300 rpm.

After 18 to 24 h, an aliquot of 0.5 µL of reaction mixture is taken and diluted with 39.5 µL of acetic acid in acetonitrile for LCMS analysis on 2 min Formic method. PROTAC purity is determined by % area in the LCMS UV trace thus is not the same as conversion or product concentration. PyParse is used to automate the analysis process, with the raw data file input and spreadsheet of LCMS purity output.

Compounds are diluted with DMSO and dispensed into columns 1 and 13 of a 384-well assay plate using a mosquito® liquid handler, then a 3-fold incremental 11-point dilution series of each reaction mixture is

prepared using a mosquito® or Bravo liquid handler. Compounds are then tested in the relevant biological assay as crude reaction mixtures. A series of compounds from each D2B experiment are resynthesized with purification and full characterization to validate biological assay results.

BRD4 PROTACs

Reductive Amination D2B

Reactions were carried out according to the Direct-to-Biology Standard Protocol in 1536-well plates.

The following reagents were used for the reductive amination (picoline borane conditions): 0.15 µmol of amine made up to 1.5 µL with DMSO per well (0.1 M, 1 eq.), 0.15 µmol of aldehyde made up to 1.5 µL with DMSO per well (0.1 M, 1 eq.), 0.20 µmol of picoline borane made up to 2 µL with DMSO per well (0.1 M, 1.33 eq.).

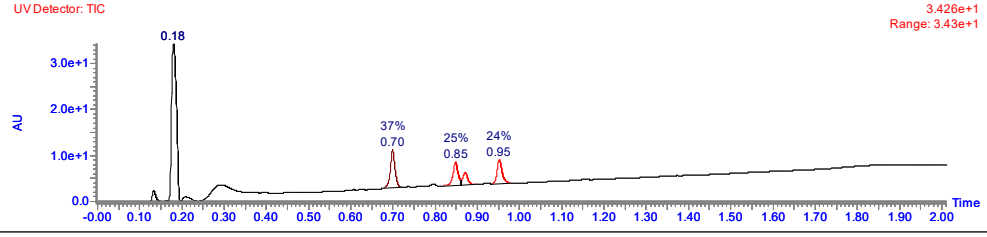
Reactions were carried out at room temperature in the glovebox. 13/24 reactions (54%) were considered to have sufficient conversion to product to be submitted for testing in the BRD4-HiBiT assay.

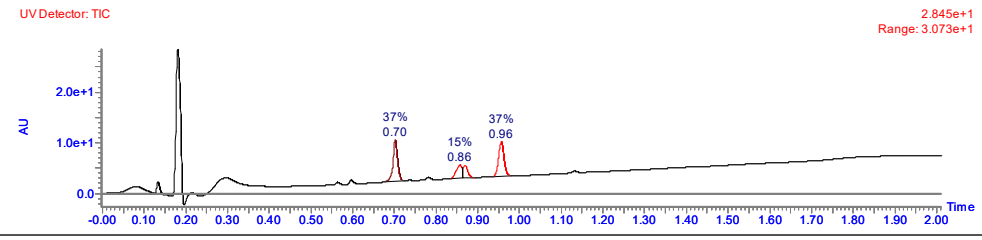
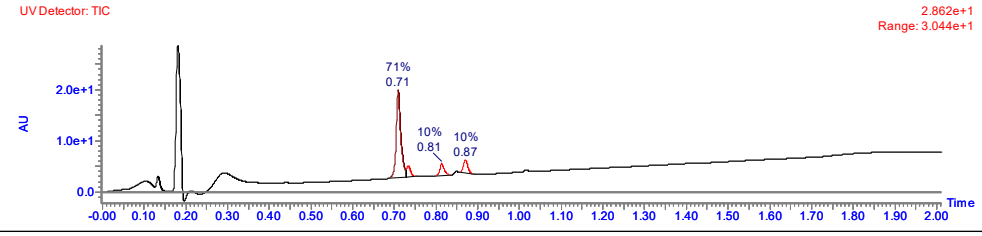
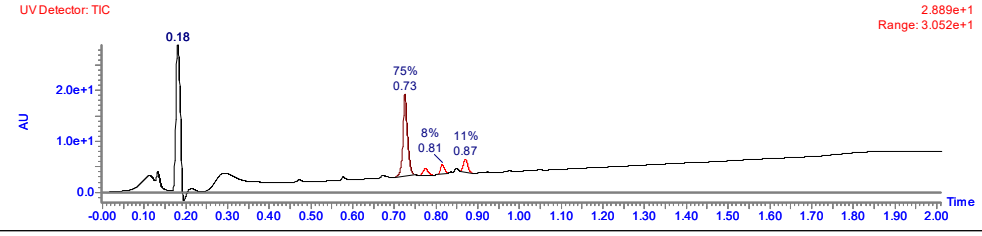
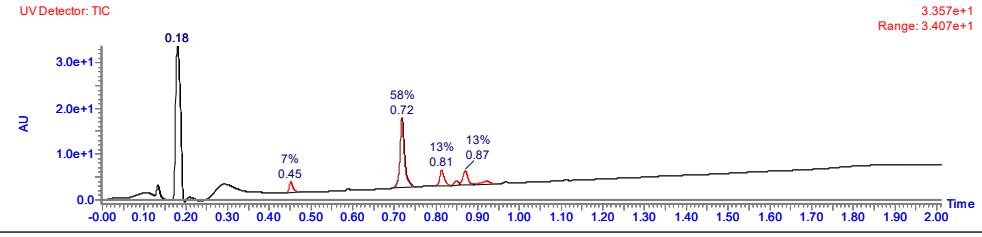
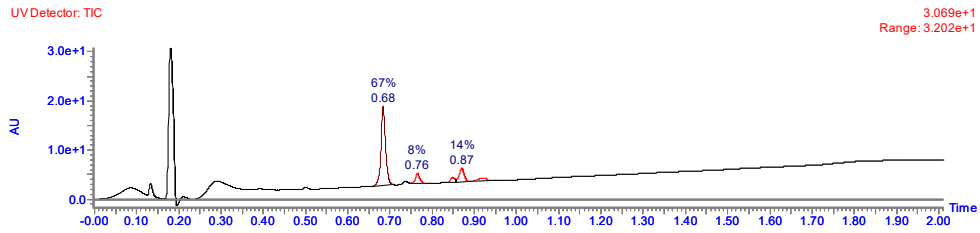
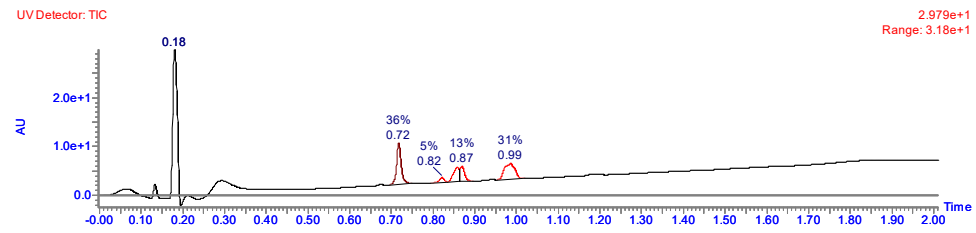
Reaction Plate Layout

1536-well plate	1	2	3	4
A	Reaction 1	Reaction 2	Reaction 3	Reaction 4
B	Reaction 9	Reaction 10	Reaction 11	Reaction 12
C	Reaction 17	Reaction 18	Reaction 19	Reaction 20
D	Empty	Empty	Empty	Empty
E	Reaction 5	Reaction 6	Reaction 7	Reaction 8
F	Reaction 13	Reaction 14	Reaction 15	Reaction 16
G	Reaction 21	Reaction 22	Reaction 23	Reaction 24

Table S1. Layout of 1536-well reaction plate; reactions deemed successful are coloured in green, unsuccessful coloured in red; UV traces shown below.

UV Traces

Reaction number	UV trace from LCMS	Purity / %
1		37

2		37
3		71
4		75
5		58
6		67
7		36

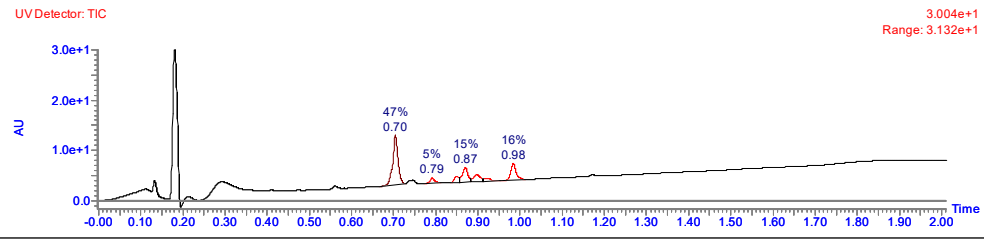
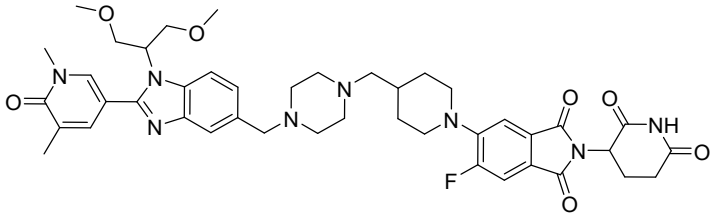
8		47

Table S2. UV traces and product purities from LCMS analysis of crude reaction mixtures are provided for the first eight reactions as examples; reactions deemed successful are coloured in green, unsuccessful coloured in red.

Reductive Amination Purified Compounds

5-(4-((4-((1-(1,3-Dimethoxypropan-2-yl)-2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1*H*-benzo[*d*]imidazol-5-yl)methyl)piperazin-1-yl)methyl)piperidin-1-yl)-2-(2,6-dioxopiperidin-3-yl)-6-fluoroisoindoline-1,3-dione, formic acid salt

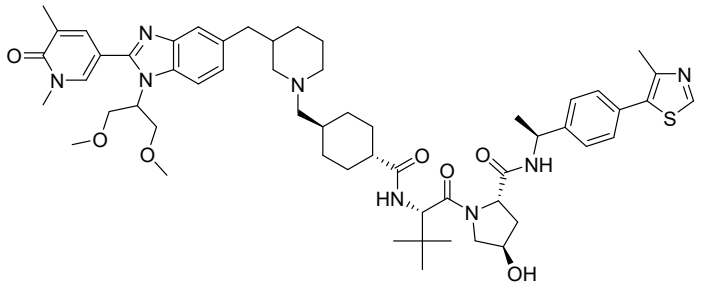


1

To a solution of 1-(2-(2,6-dioxopiperidin-3-yl)-6-fluoro-1,3-dioxoisoindolin-5-yl)piperidine-4-carbaldehyde (59.0 mg, 1 Eq, 152.31 µmol) and 5-(1-(1,3-dimethoxypropan-2-yl)-5-(piperazin-1-ylmethyl)-1*H*-benzo[*d*]imidazol-2-yl)-1,3-dimethylpyridin-2(1*H*)-one (40.169 mg, 0.6 Eq, 91.385 µmol) in DMSO (0.50 mL) was added borane-2-methylpyridine complex (21.2 mg, 1.3 Eq, 198.00 µmol) and the reaction mixture stirred at room temperature for 3 h. The reaction mixture was purified directly by HPLC (Formic Method A) and the relevant fractions combined and concentrated *in vacuo* to give the title product as a bright yellow solid (24 mg, 0.027 mmol, 17.5% yield).

^{1}H NMR: (700 MHz, CD_3OD) δ 8.29 (br s, 2H), 8.04 (d, *J* = 2.3 Hz, 1H), 7.85 (d, *J* = 8.5 Hz, 1H), 7.78 – 7.73 (m, 2H), 7.53 (d, *J* = 11.2 Hz, 1H), 7.47 (d, *J* = 7.4 Hz, 1H), 7.38 (dd, *J* = 8.5, 1.7 Hz, 1H), 5.08 (dd, *J* = 12.8, 5.7 Hz, 1H), 4.98 – 4.91 (m, 1H), 4.12 – 4.06 (m, 4H), 3.81 (dd, *J* = 10.4, 4.2 Hz, 2H), 3.70 – 3.67 (m, 2H), 3.67 (s, 3H), 3.26 (s, 6H), 3.13 – 2.58 (m, 14H), 2.20 (s, 3H), 2.14 – 2.09 (m, 1H), 1.94 – 1.84 (m, 3H), 1.49 – 1.40 (m, 2H); ^{13}C NMR: (176 MHz, CD_3OD) δ 174.7, 171.6, 168.6, 167.2, 164.8, 154.6, 147.8, 144.2, 140.8, 139.7, 138.8, 138.3, 130.2, 126.3, 125.2, 120.8, 115.0, 114.2, 113.8, 113.1, 111.6, 110.7, 105.6, 71.4, 59.5, 53.0 (br s, 1C), 52.3 (br s, 1C), 51.5 (d, *J* = 4.5 Hz, 1C), 50.8, 49.6, 38.9, 33.6, 32.3, 31.6, 23.9, 17.4; ^{19}F{^{1}H} NMR: (376 MHz, CD_3OD) δ -113.69 (s, 1F); LCMS: (2 min Formic): t_R = 0.62 min, $[M+H]^+$ 811.3 (99% purity); HRMS: $C_{44}H_{52}FN_7O_7$ $[M+H]^+$ requires 811.3938, found $[M+H]^+$ 811.3925 (Δ = -1.60 ppm).

(2*S*,4*R*)-1-((2*S*)-2-((1*r*,4*S*)-4-((3-((1-(1,3-Dimethoxypropan-2-yl)-2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1*H*-benzo[*d*]imidazol-5-yl)methyl)piperidin-1-yl)methyl)cyclohexane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide

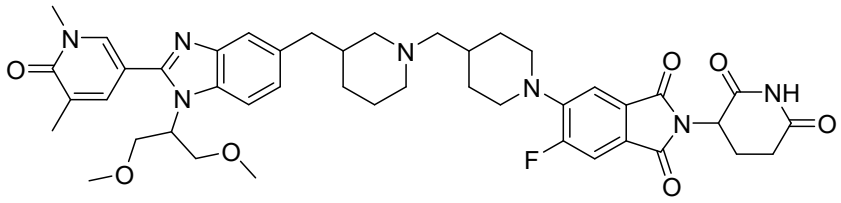


2

To a solution of 5-(1-(1,3-dimethoxypropan-2-yl)-5-(piperidin-3-ylmethyl)-1*H*-benzo[*d*]imidazol-2-yl)-1,3-dimethylpyridin-2(1*H*)-one (48.0 mg, 1 Eq, 109.5 µmol) and (2*S*,4*R*)-1-((*S*)-2-((1*r*,4*S*)-4-formylcyclohexane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl) pyrrolidine-2-carboxamide (41.0 mg, 0.64 Eq, 70.4 µmol) in DMSO (0.50 mL) was added borane-2-methylpyridine complex (15.2 mg, 1.3 Eq, 142.3 µmol) and the reaction mixture stirred at room temperature for 3 h. The reaction mixture was purified directly by HPLC (HpH Method A) and the relevant fractions combined and concentrated *in vacuo* to give the title product as a white solid (20 mg, 0.019 mmol, 17.3% yield).

^{1}H NMR: (700 MHz, CD_3OD) δ 8.87 (s, 1H), 8.03 – 8.01 (m, 1H), 7.77 (dd, *J* = 2.1, 1.1 Hz, 1H), 7.68 (d, *J* = 8.5 Hz, 1H), 7.47 – 7.39 (m, 5H), 7.14 (dd, *J* = 8.5, 1.3 Hz, 1H), 5.00 (q, *J* = 7.1 Hz, 1H), 4.89 – 4.85 (m, 1H), 4.61 – 4.59 (m, 1H), 4.56 (t, *J* = 8.3 Hz, 1H), 4.44 – 4.40 (m, 1H), 4.10 – 4.05 (m, 2H), 3.85 – 3.79 (m, 3H), 3.77 – 3.72 (m, 1H), 3.68 – 3.65 (m, 3H), 3.25 (d, *J* = 3.8 Hz, 6H), 2.88 – 2.77 (m, 2H), 2.68 – 2.61 (m, 2H), 2.49 – 2.46 (m, 3H), 2.28 (tt, *J* = 12.1, 3.4 Hz, 1H), 2.20 (s, 3H), 2.19 – 2.16 (m, 1H), 2.15 – 2.08 (m, 2H), 1.98 – 1.65 (m, 11H), 1.61 – 1.52 (m, 2H), 1.52 – 1.45 (m, 4H), 1.45 – 1.36 (m, 2H), 1.02 (s, 9H), 1.02 – 0.88 (m, 4H); ^{13}C NMR: (176 MHz, CD_3OD) δ 178.8, 173.4, 172.4, 164.8, 164.8, 153.4, 153.0, 149.2, 145.8, 144.1, 140.6, 140.0, 136.7, 133.5, 133.2, 131.7, 130.6, 130.6, 130.0, 127.8, 125.6, 120.2, 113.4, 111.0, 71.5, 71.1, 67.4, 60.7, 59.5, 59.5, 59.4, 58.8, 58.1, 56.0, 50.3, 46.1, 42.2, 39.5, 38.9, 38.9, 36.8, 36.8, 36.8, 35.8, 32.5 (d, *J* = 8.3 Hz, 1C), 32.3 (d, *J* = 12.1 Hz, 1C), 32.1, 31.1 (d, *J* = 3.2 Hz, 1C), 30.1 (d, *J* = 4.5 Hz, 1C), 27.2, 26.1, 22.5, 17.4, 15.9; LCMS: (2 min HpH): t_R = 1.24 min, $[M+H]^+$ 1005.7 (100% purity); HRMS: $C_{56}H_{76}N_8O_7S$ $[M+H]^+$ requires 1005.5631, found $[M+H]^+$ 1005.5612 (Δ = -1.89 ppm).

5-(4-((3-((1-(1,3-Dimethoxypropan-2-yl)-2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1*H*-benzo[*d*]imidazol-5-yl)methyl)piperidin-1-yl)methyl)piperidin-1-yl)-2-(2,6-dioxopiperidin-3-yl)-6-fluoroisoindoline-1,3-dione

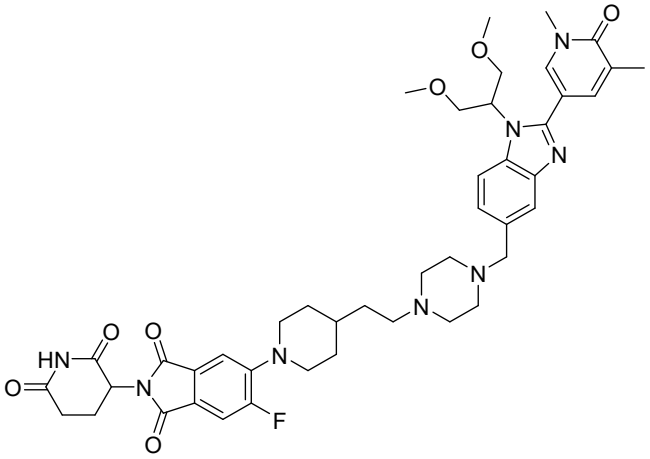


S1

To a solution of 5-(1-(1,3-dimethoxypropan-2-yl)-5-(piperidin-3-ylmethyl)-1*H*-benzo[*d*]imidazol-2-yl)-1,3-dimethylpyridin-2(1*H*)-one (48.0 mg, 1 Eq, 109.5 µmol) and 1-(2-(2,6-dioxopiperidin-3-yl)-6-fluoro-1,3-dioxoisoindolin-5-yl)piperidine-4-carbaldehyde (58.0 mg, 1.37 Eq, 149.73 µmol) in DMSO (0.50 mL) was added borane-2-methylpyridine complex (15.2 mg, 1.3 Eq, 142.28 µmol) and the reaction mixture stirred at room temperature for 3 h. The reaction mixture was purified directly by HPLC (Formic Method A) and the relevant fractions combined and concentrated *in vacuo* to give the title product as a bright yellow solid (34 mg, 0.040 mmol, 36.4% yield).

^{1}H NMR: (700 MHz, CD_3OD) δ 8.36 (s, 2H), 8.02 (d, *J* = 2.3 Hz, 1H), 7.77 – 7.72 (m, 2H), 7.54 – 7.50 (m, 2H), 7.43 (d, *J* = 7.2 Hz, 1H), 7.19 (dd, *J* = 8.5, 1.5 Hz, 1H), 5.07 (dd, *J* = 12.8, 5.4 Hz, 1H), 4.92 (tt, *J* = 9.0, 4.3 Hz, 1H), 4.09 (t, *J* = 9.7 Hz, 2H), 3.80 (ddd, *J* = 10.3, 4.1, 2.8 Hz, 2H), 3.67 – 3.61 (m, 5H), 3.60 – 3.53 (m, 1H), 3.42 (br d, *J* = 10.8 Hz, 1H), 3.26 – 3.23 (m, 6H), 3.10 – 3.01 (m, 2H), 2.95 – 2.65 (m, 9H), 2.29 – 2.21 (m, 1H), 2.18 (s, 3H), 2.14 – 2.08 (m, 1H), 2.06 – 1.94 (m, 2H), 1.93 – 1.80 (m, 4H), 1.52 – 1.42 (m, 2H), 1.38 – 1.27 (m, 1H); ^{13}C NMR: (176 MHz, CD_3OD) δ 174.7, 171.6, 168.5, 168.1, 168.0 (d, *J* = 1.9 Hz, 1C), 164.7, 159.7 (d, *J* = 254.3 Hz, 1C), 153.8, 147.5, 147.4, 144.2, 140.7, 139.8, 134.6, 133.7, 130.6 (d, *J* = 2.5 Hz, 1C), 130.1, 125.7 (d, *J* = 9.5 Hz, 1C), 125.5, 120.3, 115.0 (d, *J* = 5.1 Hz, 1C), 113.9, 112.8 (d, *J* = 25.4 Hz, 1C), 110.8, 71.4, 71.4, 59.5, 59.4, 54.6, 51.1 (d, *J* = 4.5 Hz, 1C), 51.0 (d, *J* = 4.5 Hz, 1C), 50.9, 50.0, 40.8, 38.9, 32.4, 32.3, 31.2, 31.1, 29.5, 23.8, 17.4; ^{19}F{^{1}H} NMR: (376 MHz, CD_3OD) δ -113.55 (s, 1F); LCMS: (2 min Formic): t_R = 0.65 min, $[M+H]^+$ 810.4 (99% purity); HRMS: $C_{44}H_{52}FN_7O_7$ $[M+H]^+$ requires 810.3985, found $[M+H]^+$ 810.3972 (Δ = -1.60 ppm).

5-(4-(2-(4-((1-(1,3-Dimethoxypropan-2-yl)-2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1*H*-benzo[*d*]imidazol-5-yl)methyl)piperazin-1-yl)ethyl)piperidin-1-yl)-2-(2,6-dioxopiperidin-3-yl)-6-fluoroisoindoline-1,3-dione, formic acid salt



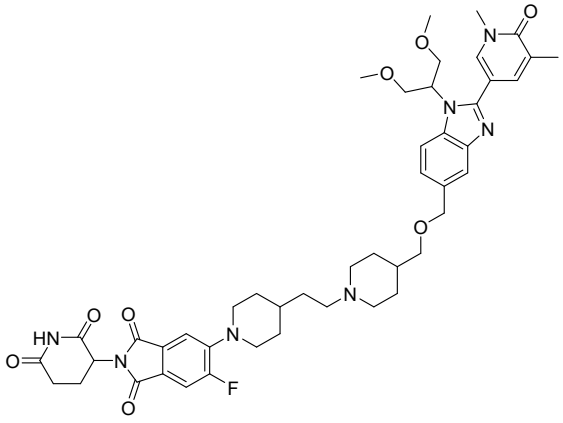
S2

To a solution of 1-(2-(2,6-dioxopiperidin-3-yl)-6-fluoro-1,3-dioxoisoindolin-5-yl)piperidine-4-carbaldehyde (40.0 mg, 1 Eq, 103.26 µmol) and 5-(1-(1,3-dimethoxypropan-2-yl)-5-(piperazin-1-ylmethyl)-1*H*-benzo[*d*]imidazol-2-yl)-1,3-dimethylpyridin-2(1*H*)-one (40 mg, 0.88 Eq, 91.00 µmol) in DMSO (0.50 mL) was added borane-2-methylpyridine complex (14.4 mg, 1.3 Eq, 134.24 µmol) and the reaction mixture stirred at room temperature for 3 h. The reaction mixture was purified directly by HPLC (Formic Method

A) and the relevant fractions combined and concentrated *in vacuo* to give the title product as a bright yellow solid (35 mg, 0.038 mmol, 37.0% yield).

^{1}H NMR: (400 MHz, CD_3OD) δ 8.35 (s, 2H), 8.02 (d, *J* = 2.4 Hz, 1H), 7.79 (d, *J* = 8.3 Hz, 1H), 7.76 (dd, *J* = 2.4, 1.2 Hz, 1H), 7.69 (d, *J* = 0.9 Hz, 1H), 7.51 (d, *J* = 11.2 Hz, 1H), 7.45 (d, *J* = 7.3 Hz, 1H), 7.34 (dd, *J* = 8.6, 1.5 Hz, 1H), 5.07 (dd, *J* = 12.6, 5.5 Hz, 1H), 4.91 (tt, *J* = 9.0, 4.4 Hz, 1H), 4.09 (dd, *J* = 10.3, 9.3 Hz, 2H), 3.87 (s, 2H), 3.81 (dd, *J* = 10.3, 4.2 Hz, 2H), 3.70 – 3.62 (m, 5H), 3.25 (s, 6H), 3.18 (br s, 4H), 3.09 – 3.01 (m, 2H), 2.94 – 2.63 (m, 9H), 2.20 (s, 3H), 2.16 – 2.06 (m, 1H), 1.86 (br d, *J* = 11.0 Hz, 2H), 1.74 – 1.66 (m, 2H), 1.65 – 1.52 (m, 1H), 1.52 – 1.39 (m, 2H); ^{13}C NMR: (176 MHz, CD_3OD) δ 174.7, 171.6, 168.6, 168.1 (d, *J* = 1.9 Hz, 1C), 167.9, 164.8, 159.7 (d, *J* = 253.7 Hz, 1C), 154.1, 147.8, 147.7, 144.1, 140.7, 139.8, 134.6, 131.9, 131.9, 130.6 (d, *J* = 2.5 Hz, 1C), 130.1, 125.8, 125.3 (d, *J* = 10.2 Hz, 1C), 121.2, 114.9 (d, *J* = 5.1 Hz, 1C), 113.9, 112.8 (d, *J* = 25.4 Hz, 1C), 110.8, 71.4, 62.9, 59.5, 56.1, 53.1, 51.7 (br d, *J* = 3.2 Hz, 1C), 51.5, 50.8, 38.9, 34.9, 33.2, 32.3, 32.2, 23.9, 17.4; ^{19}F{^{1}H} NMR: (376 MHz, CD_3OD) δ -113.65 (s, 1F); LCMS: (2 min Formic): t_R = 0.62 min, $[M+H]^+$ 825.4 (99% purity); HRMS: $C_{44}H_{53}FN_8O_7$ $[M+H]^+$ requires 825.4094, found $[M+H]^+$ 825.4076 (Δ = -2.18 ppm).

5-(4-(2-(4-(((1-(1,3-Dimethoxypropan-2-yl)-2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1*H*-benzo[*d*]imidazol-5-yl)methoxy)methyl)piperidin-1-yl)ethyl)piperidin-1-yl)-2-(2,6-dioxopiperidin-3-yl)-6-fluoroisoindoline-1,3-dione



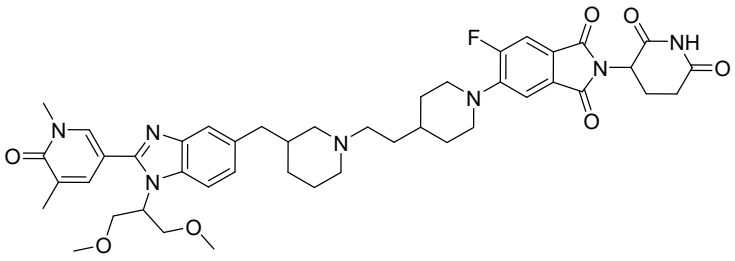
S3

To a solution of 2-(1-(2-(2,6-dioxopiperidin-3-yl)-6-fluoro-1,3-dioxoisoindolin-5-yl)piperidin-4-yl)acetaldehyde (40.0 mg, 1 Eq, 99.65 µmol) and 5-(1-(1,3-dimethoxypropan-2-yl)-5-((piperidin-4-ylmethoxy)methyl)-1*H*-benzo[*d*]imidazol-2-yl)-1,3-dimethylpyridin-2(1*H*)-one (31.0 mg, 0.66 Eq, 66.16 µmol) in DMSO (0.50 mL) was added borane-2-methylpyridine complex (13.9 mg, 1.3 Eq, 129.55 µmol) and the reaction mixture stirred at room temperature for 3 h. The reaction mixture was purified directly by HPLC (Formic Method A) and the relevant fractions combined and concentrated *in vacuo* to give the title product as a bright yellow solid (15 mg, 0.016 mmol, 15.9% yield).

^{1}H NMR: (700 MHz, CD_3OD) δ 8.53 (s, 1H), 8.03 (d, *J* = 2.3 Hz, 1H), 7.78 – 7.75 (m, 2H), 7.66 (d, *J* = 0.8 Hz, 1H), 7.52 (d, *J* = 11.0 Hz, 1H), 7.46 (d, *J* = 7.4 Hz, 1H), 7.29 (dd, *J* = 8.3, 1.5 Hz, 1H), 5.08 (dd, *J* = 12.8, 5.4 Hz, 1H), 4.91 (tt, *J* = 8.9, 4.4 Hz, 1H), 4.65 (s, 2H), 4.09 (dd, *J* = 10.4, 9.1 Hz, 2H), 3.82 (dd, *J* = 10.4, 4.2 Hz,

2H), 3.69 – 3.63 (m, 5H), 3.51 – 3.43 (m, 4H), 3.26 (s, 6H), 3.07 – 3.01 (m, 2H), 2.93 – 2.88 (m, 2H), 2.87 – 2.78 (m, 3H), 2.77 – 2.65 (m, 2H), 2.20 (s, 3H), 2.14 – 2.08 (m, 1H), 2.00 (br d, *J* = 13.8 Hz, 2H), 1.96 – 1.90 (m, 1H), 1.86 (br d, *J* = 12.7 Hz, 2H), 1.73 – 1.68 (m, 2H), 1.63 – 1.53 (m, 3H), 1.51 – 1.42 (m, 2H); ^{13}C NMR (700 MHz, CD_3OD): δ 174.7, 171.6, 170.2, 168.6, 168.1 (d, *J* = 2.5 Hz, 1C), 164.8, 160.4, 159.0, 154.0, 148.6 (d, *J* = 293.7 Hz, 1C), 147.7, 144.0, 140.7, 139.8, 138.8, 134.8, 134.4, 130.7 (d, *J* = 2.5 Hz, 1C), 130.1, 125.4 (d, *J* = 10.2 Hz, 1C), 125.2, 124.4, 122.6, 119.4, 114.9 (d, *J* = 5.1 Hz, 1C), 113.7, 112.8 (d, *J* = 25.4 Hz, 1C), 110.9, 74.9, 74.5, 71.4, 59.5, 53.9 – 53.9 (m, 1C), 51.7 (d, *J* = 3.8 Hz, 1C), 50.8, 38.9, 35.1, 33.2, 32.3, 32.2, 23.9, 17.4; ^{19}F{^{1}H} NMR: (376 MHz, CD_3OD) δ -113.70 (s, 1F); LCMS: (2 min Formic): t_R = 0.68 min, $[M+H]^+$ 854.5 (99% purity); HRMS: $C_{46}H_{56}FN_7O_8$ $[M+H]^+$ requires 854.4247, found $[M+H]^+$ 854.4233 (Δ = -1.64 ppm).

5-(4-(2-(3-((1-(1,3-Dimethoxypropan-2-yl)-2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1*H*-benzo[*d*]imidazol-5-yl)methyl)piperidin-1-yl)ethyl)piperidin-1-yl)-2-(2,6-dioxopiperidin-3-yl)-6-fluoroisoindoline-1,3-dione, formic acid salt

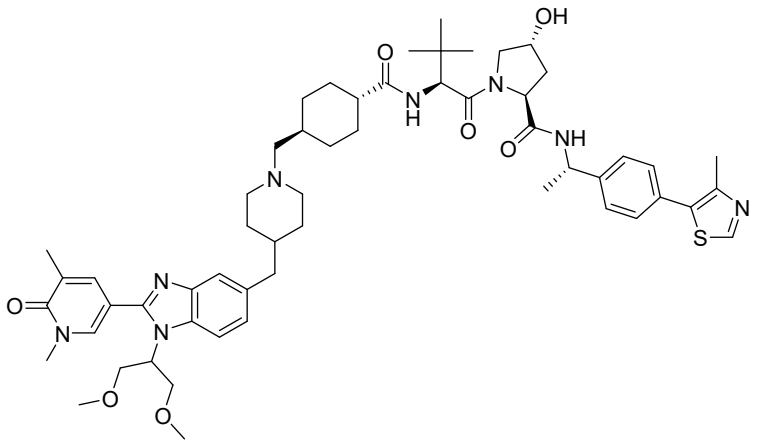


S4

To a solution of 5-(1-(1,3-dimethoxypropan-2-yl)-5-(piperidin-3-ylmethyl)-1*H*-benzo[*d*]imidazol-2-yl)-1,3-dimethylpyridin-2(1*H*)-one (49.0 mg, 1 Eq, 111.73 µmol) and 2-(1-(2-(2,6-dioxopiperidin-3-yl)-6-fluoro-1,3-dioxoisoindolin-5-yl)piperidin-4-yl)acetaldehyde (48.0 mg, 1.1 Eq, 119.58 µmol) in DMSO (0.50 mL) was added borane-2-methylpyridine complex (15.5 mg, 1.3 Eq, 145.24 µmol) and the reaction mixture stirred at room temperature for 3 h. The reaction mixture was purified directly by HPLC (Formic Method A) and the relevant fractions combined and concentrated *in vacuo* to give the title product as a bright yellow solid (19 mg, 0.021 mmol, 18.6% yield).

^{1}H NMR: (700 MHz, CD_3OD) δ 8.38 (br s, 2H), 8.02 (d, *J* = 1.5 Hz, 1H), 7.78 – 7.70 (m, 2H), 7.55 – 7.48 (m, 2H), 7.45 (d, *J* = 7.4 Hz, 1H), 7.18 (dd, *J* = 8.4, 1.2 Hz, 1H), 5.08 (dd, *J* = 12.8, 5.4 Hz, 1H), 4.90 (tt, *J* = 9.0, 4.4 Hz, 1H), 4.08 (t, *J* = 9.6 Hz, 2H), 3.80 (dd, *J* = 10.4, 4.2 Hz, 2H), 3.67 – 3.62 (m, 5H), 3.58 – 3.51 (m, 1H), 3.46 – 3.39 (m, 1H), 3.25 (s, 6H), 3.16 (br t, *J* = 8.3 Hz, 2H), 2.93 – 2.65 (m, 9H), 2.21 – 2.14 (m, 4H), 2.14 – 2.09 (m, 1H), 2.02 – 1.96 (m, 1H), 1.91 (br d, *J* = 12.1 Hz, 1H), 1.84 – 1.64 (m, 5H), 1.58 – 1.51 (m, 1H), 1.47 – 1.39 (m, 2H), 1.37 – 1.27 (m, 1H); ^{13}C NMR: (176 MHz, CD_3OD) δ 174.7, 171.6, 168.6, 168.1, 164.7, 159.7 (d, *J* = 255.0 Hz, 1C), 147.7 (d, *J* = 9.5 Hz, 1C), 144.3, 140.7, 139.8, 134.6, 133.7, 130.7 (d, *J* = 1.3 Hz, 1C), 130.1, 125.5, 125.4 (d, *J* = 10.2 Hz, 1C), 120.3, 114.9 (d, *J* = 5.1 Hz, 1C), 113.9, 112.8 (d, J = 25.4 Hz, 1C), 110.9, 71.4, 59.5, 54.1 – 54.1 (m, 1C), 51.7 – 51.6 (m, *J* = 4.1, 4.1 Hz, 2C), 50.9, 50.0, 40.9, 40.9, 40.8, 38.9, 34.9, 33.1, 33.0, 32.3, 31.7, 29.5, 29.5, 23.9, 17.4; ^{19}F{^{1}H} NMR: (376 MHz, CD_3OD) δ -113.71 (s, 1F); LCMS: (2 min Formic): t_R = 0.68 min, $[M+H]^+$ 824.4 (97% purity); HRMS: $C_{45}H_{54}FN_7O_7$ $[M+H]^+$ requires 824.4142, found $[M+H]^+$ 824.4139 (Δ = -0.36 ppm).

(2*S*,4*R*)-1-((*S*)-2-((1*r*,4*S*)-4-((4-((1-(1,3-Dimethoxypropan-2-yl)-2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1*H*-benzo[*d*]imidazol-5-yl)methyl)piperidin-1-yl)methyl)cyclohexane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide



S5

To a solution of 5-(1-(1,3-dimethoxypropan-2-yl)-5-(piperidin-4-ylmethyl)-1*H*-benzo[*d*]imidazol-2-yl)-1,3-dimethylpyridin-2(1*H*)-one (38.0 mg, 1 Eq, 86.65 µmol) and (2*S*,4*R*)-1-((*S*)-2-((1*r*,4*S*)-4-formylcyclohexane-1-carboxamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl) pyrrolidine-2-carboxamide (41.0 mg, 67% Wt, 0.54 Eq, 47.14 µmol) in DMSO (0.50 mL) was added borane-2-methylpyridine complex (12.0 mg, 1.3 Eq, 112.64 µmol) and the reaction mixture stirred at room temperature for 3 h. The reaction mixture was purified directly by HPLC (HpH Method A) and the relevant fractions combined and concentrated *in vacuo* to give the title product as a white solid (17 mg, 0.016 mmol, 18.5% yield).

^{1}H NMR: (700 MHz, CD_3OD) δ 8.86 (s, 1H), 8.02 (d, *J* = 2.3 Hz, 1H), 7.76 (dd, *J* = 2.4, 1.2 Hz, 1H), 7.66 (d, *J* = 8.3 Hz, 1H), 7.46 – 7.40 (m, 5H), 7.12 (dd, *J* = 8.4, 1.6 Hz, 1H), 5.00 (q, *J* = 7.0 Hz, 1H), 4.89 – 4.85 (m, 1H), 4.61 (s, 1H), 4.57 (t, *J* = 8.3 Hz, 1H), 4.45 – 4.41 (m, 1H), 4.07 (dd, *J* = 10.2, 9.1 Hz, 2H), 3.87 – 3.83 (m, 1H), 3.81 (dd, *J* = 10.3, 4.3 Hz, 2H), 3.75 (dd, *J* = 11.1, 3.9 Hz, 1H), 3.66 (s, 3H), 3.25 (s, 6H), 2.88 (br d, *J* = 11.4 Hz, 2H), 2.67 (d, *J* = 6.8 Hz, 2H), 2.48 (s, 3H), 2.34 – 2.28 (m, 1H), 2.21 – 2.16 (m, 4H), 2.14 (d, *J* = 6.8 Hz, 2H), 1.96 (ddd, *J* = 13.2, 8.9, 4.6 Hz, 1H), 1.91 – 1.76 (m, 6H), 1.64 (br d, *J* = 13.4 Hz, 2H), 1.62 – 1.52 (m, 3H), 1.51 (d, *J* = 7.0 Hz, 3H), 1.49 – 1.41 (m, 2H), 1.39 – 1.32 (m, 2H), 1.03 (s, 9H), 1.01 – 0.92 (m, 3H); ^{13}C NMR: (176 MHz, CD_3OD) δ 178.9, 173.4, 172.4, 164.8, 153.3, 153.0, 149.2, 145.8, 144.1, 140.6, 140.0, 136.8, 133.5, 133.2, 131.7, 130.6, 130.0, 127.8, 125.8, 120.2, 113.3, 111.1, 71.5, 71.1, 67.2, 60.7, 59.5, 59.4, 58.9, 58.1, 55.7 (br d, *J* = 5.1 Hz, 1C), 50.3, 46.1, 44.2, 39.7, 38.9, 38.9, 36.8, 35.9, 32.9, 32.5, 32.3, 31.1, 30.1, 27.2, 22.5, 17.4, 16.0; LCMS: (2 min HpH): t_R = 1.19 min, $[M+H]^+$ 1005.7 (99% purity); HRMS: $C_{56}H_{76}N_8O_7S$ $[M+H]^+$ requires 1005.5631, found $[M+H]^+$ 1005.5611 (Δ = -1.99 ppm).

Nucleophilic Aromatic Substitution D2B

Reactions were carried out according to the Direct-to-Biology Standard Protocol in 1536-well plates.

The following reagents were used for the S_NAr library: 0.15 µmol of amine made up to 1.5 µL with DMSO per well (0.1 M, 1 eq.), 0.15 µmol of aryl chloride made up to 1.5 µL with DMSO per well (0.1 M, 1 eq.), 0.45 µmol of MTBD made up to 2 µL with DMSO per well (0.225 M, 3 eq.).

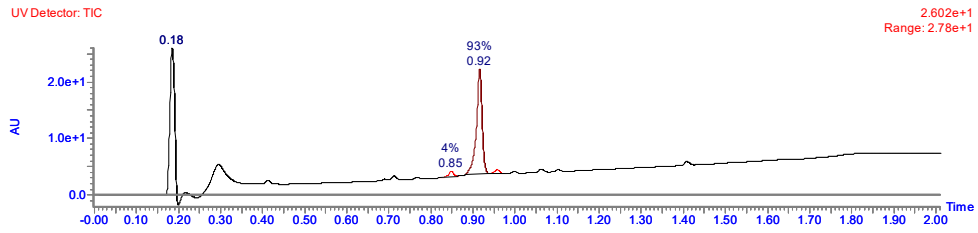
Reactions were carried out at 60 °C at 300 rpm using the thermomixer in the glovebox. 48/48 reactions (100%) were considered to have sufficient conversion to product to be submitted for testing in the BRD4-HiBiT assay.

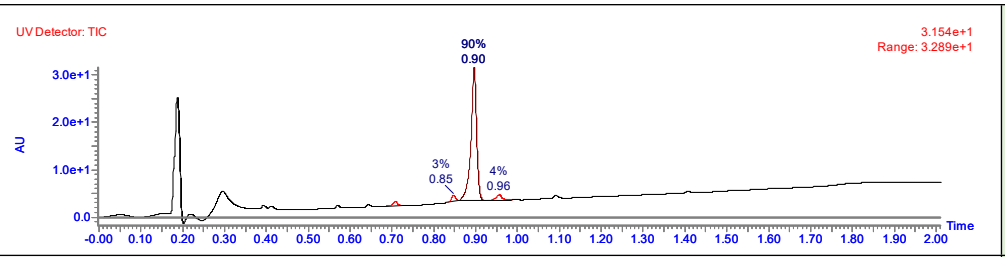
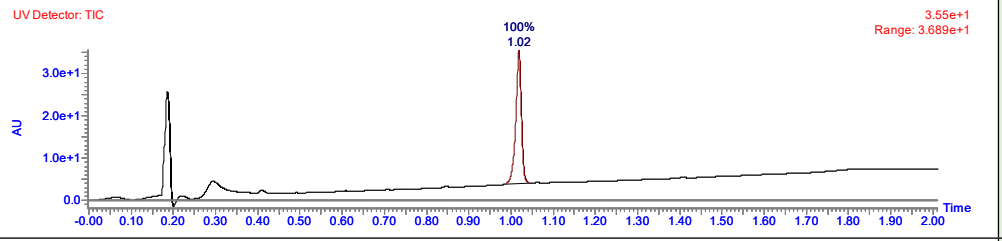
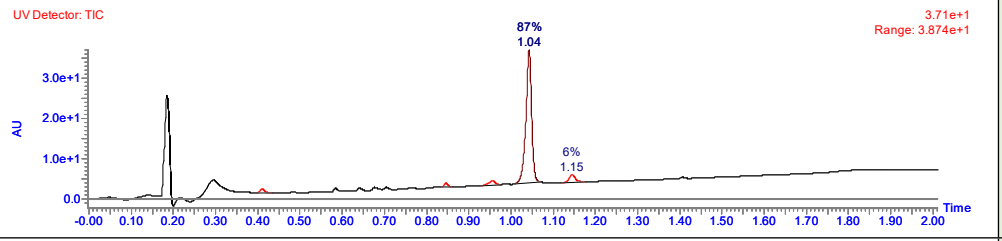
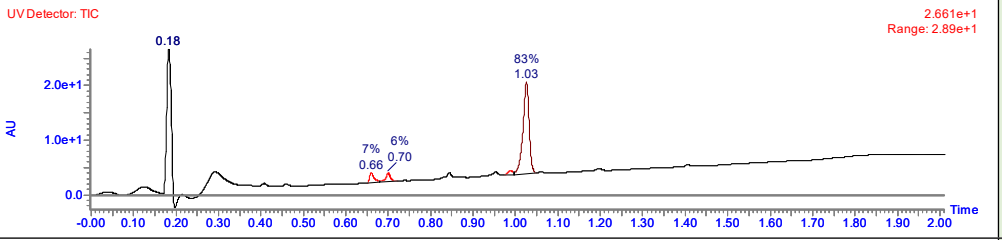
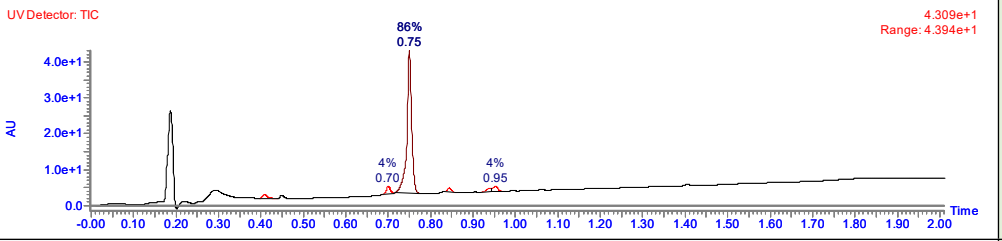
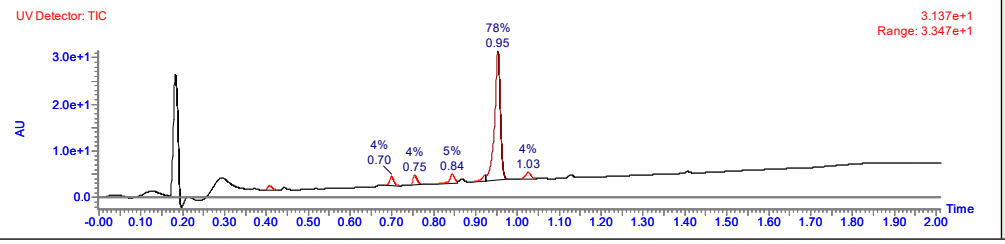
Reaction Plate Layout

1536-well plate	1	2	3	4
A	Reaction 1	Reaction 2	Reaction 3	Reaction 4
B	Reaction 9	Reaction 10	Reaction 11	Reaction 12
C	Reaction 17	Reaction 18	Reaction 19	Reaction 20
D	Reaction 25	Reaction 26	Reaction 27	Reaction 28
E	Reaction 33	Reaction 34	Reaction 35	Reaction 36
F	Reaction 37	Reaction 38	Reaction 39	Reaction 40
G	Reaction 5	Reaction 6	Reaction 7	Reaction 8
H	Reaction 13	Reaction 14	Reaction 15	Reaction 16
I	Reaction 21	Reaction 22	Reaction 23	Reaction 24
J	Reaction 29	Reaction 30	Reaction 31	Reaction 32
K	Reaction 37	Reaction 38	Reaction 39	Reaction 40
L	Reaction 45	Reaction 46	Reaction 47	Reaction 48

Table S3. Layout of 1536-well reaction plate; reactions deemed successful are coloured in green; UV traces shown below.

UV Traces

Reaction number	UV trace from LCMS	Purity / %
1		93

2		90
3		100
4		87
5		83
6		86
7		78

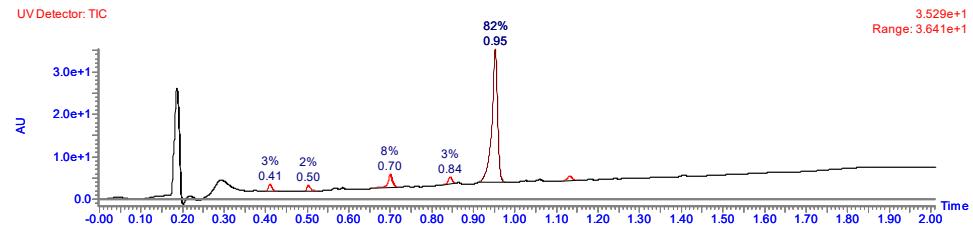
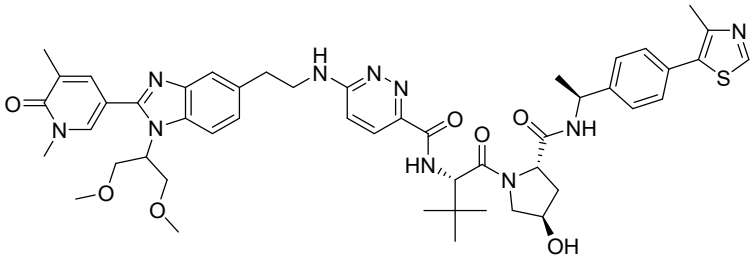
8		82

Table S4. UV traces and product purities from LCMS analysis of crude reaction mixtures are provided for the first eight reactions as examples; reactions deemed successful are coloured in green.

Nucleophilic Aromatic Substitution Purified Compounds

6-((2-(1-(1,3-Dimethoxypropan-2-yl)-2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1*H*-benzo[*d*]imidazol-5-yl)ethyl)amino)-*N*-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)pyridazine-3-carboxamide

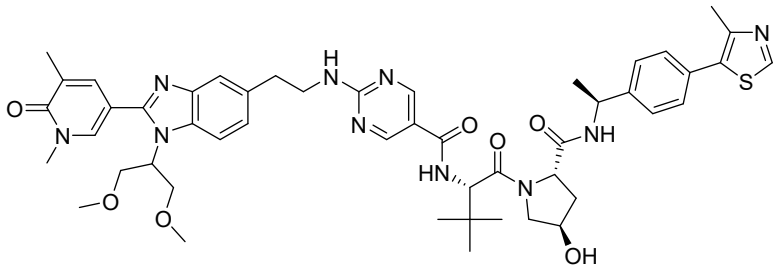


3

To a solution of 5-(5-(2-aminoethyl)-1-(1,3-dimethoxypropan-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)-1,3-dimethylpyridin-2(1*H*)-one (31.0 mg, 1 Eq, 80.63 µmol) in DMSO (0.75 mL) was added 6-chloro-*N*-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)pyridazine-3-carboxamide (33.2 mg, 0.7 Eq, 56.70 µmol) followed by 1-methyl-2,3,4,6,7,8-hexahydro-1*H*-pyrimido[1,2-*a*]pyrimidine (37.63 µL, 3.25 Eq, 262.04 µmol) and the reaction mixture was heated to 60 °C for 24 h. The reaction mixture was cooled to room temperature then purified by HPLC (Formic method B). The relevant fractions were combined and concentrated *in vacuo* to give the title compound.

^{1}H NMR: (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.50 (d, *J* = 8.3 Hz, 1H), 7.90 (d, *J* = 2.2 Hz, 1H), 7.82 (d, *J* = 9.3 Hz, 1H), 7.75 (br d, *J* = 7.8 Hz, 1H), 7.69 (dd, *J* = 2.3, 1.2 Hz, 1H), 7.63 (br s, 1H), 7.48 (d, *J* = 8.3 Hz, 1H), 7.45 – 7.34 (m, 4H), 7.11 (dd, *J* = 8.4, 1.2 Hz, 1H), 6.62 (d, *J* = 9.3 Hz, 1H), 5.30 (br s, 1H), 5.13 – 5.02 (m, 1H), 4.89 – 4.77 (m, 2H), 4.67 (d, *J* = 8.3 Hz, 1H), 4.53 (br s, 1H), 4.22 (br d, *J* = 11.2 Hz, 1H), 3.99 – 3.92 (m, 2H), 3.89 – 3.78 (m, 4H), 3.66 (br dd, *J* = 11.5, 3.7 Hz, 1H), 3.62 (s, 3H), 3.28 (s, 6H), 3.11 (br t, *J* = 6.5 Hz, 2H), 2.60 – 2.52 (m, 5H), 2.22 (s, 3H), 2.09 – 2.00 (m, 1H), 1.49 (d, *J* = 7.1 Hz, 3H), 1.14 (s, 9H); ^{13}C NMR: (176 MHz, $CDCl_3$) δ 171.9, 169.6, 164.0, 162.8, 159.8, 152.3, 150.2, 148.4, 144.4, 143.6, 143.3, 138.6, 137.4, 133.0, 132.5, 131.7, 130.8, 129.6, 129.5, 126.9, 126.4, 123.7, 119.7, 112.1, 108.7, 70.8, 70.2, 59.2, 58.2, 58.1, 57.7, 56.6, 49.0, 43.3, 38.3, 35.3, 35.1, 35.0, 29.7, 26.6, 22.4, 17.4, 16.1; LCMS: (2 min Formic): t_R = 0.87 min, $[M+H]^+$ 933.5 (99% purity); HRMS: $C_{49}H_{60}N_{10}O_7S$ $[M+H]^+$ requires 933.4440, found $[M+H]^+$ 933.4410 (Δ = -3.21 ppm).

2-((2-(1-(1,3-Dimethoxypropan-2-yl)-2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1*H*-benzo[*d*]imidazol-5-yl)ethyl)amino)-*N*-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)pyrimidine-5-carboxamide

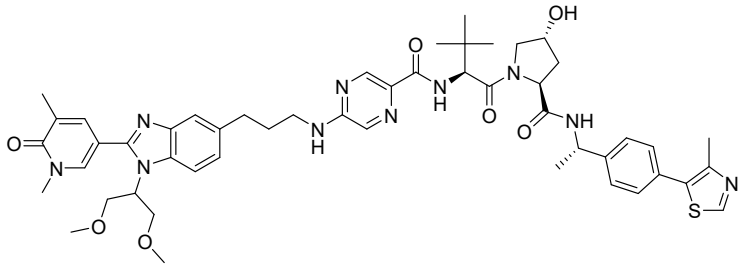


4

To a solution of 5-(5-(2-aminoethyl)-1-(1,3-dimethoxypropan-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)-1,3-dimethylpyridin-2(1*H*)-one (31.00 mg, 1 Eq, 80.63 µmol) in DMSO (0.75 mL) was added 2-chloro-*N*-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)pyrimidine-5-carboxamide (44.2 mg, 0.94 Eq, 75.50 µmol) followed by 1-methyl-2,3,4,6,7,8-hexahydro-1*H*-pyrimido[1,2-*a*]pyrimidine (37.6 µL, 3.25 Eq, 262.04 µmol) and the reaction mixture was heated to 60 °C for 24 h. The reaction mixture was cooled to room temperature then purified by MDAP (Formic method B). The relevant fractions were combined and concentrated *in vacuo* to give the title compound.

^{1}H NMR: (400 MHz, $CDCl_3$) δ 8.68 (s, 1H), 7.91 (d, *J* = 2.4 Hz, 1H), 7.70 (dd, *J* = 2.3, 1.1 Hz, 1H), 7.62 (d, *J* = 1.2 Hz, 1H), 7.50 – 7.44 (m, 2H), 7.42 – 7.34 (m, 4H), 7.11 (dd, *J* = 8.4, 1.6 Hz, 1H), 6.88 (br d, *J* = 8.8 Hz, 1H), 5.92 – 5.83 (m, 1H), 5.16 – 5.06 (m, 1H), 4.88 – 4.70 (m, 3H), 4.52 (br s, 1H), 4.15 (br d, *J* = 11.2 Hz, 1H), 3.99 – 3.90 (m, 2H), 3.84 (dd, *J* = 9.9, 4.8 Hz, 2H), 3.82 – 3.72 (m, 2H), 3.68 (dd, *J* = 11.2, 3.7 Hz, 1H), 3.63 (s, 3H), 3.28 (s, 6H), 3.08 – 2.84 (m, 5H), 2.52 (s, 3H), 2.51 – 2.46 (m, 1H), 2.22 (s, 3H), 2.13 – 2.04 (m, 1H), 1.49 (d, *J* = 6.8 Hz, 3H), 1.14 – 1.06 (m, 9H); ^{13}C NMR: (176 MHz, $CDCl_3$) δ 171.9, 169.7, 164.8, 162.9, 162.8, 158.0 (br d, *J* = 138.6 Hz, 2C), 152.0, 150.3, 148.4, 143.4, 143.1, 138.6, 137.5, 133.3, 132.3, 131.6, 130.9, 129.6, 129.5, 126.4, 123.8, 119.7, 116.1, 111.9, 108.7, 70.8, 70.8, 70.1, 59.2, 58.6, 57.7, 57.0, 48.9, 43.1, 38.3, 36.0, 35.6, 35.3, 26.7, 22.2, 17.4, 16.0; LCMS: (2 min Formic): t_R = 0.84 min, $[M+H]^+$ 933.5 (97% purity); HRMS: $C_{49}H_{60}N_{10}O_7S$ $[M+H]^+$ requires 933.4440, found $[M+H]^+$ 933.4417 (Δ = -2.46 ppm).

5-((3-(1-(1,3-Dimethoxypropan-2-yl)-2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1*H*-benzo[*d*]imidazol-5-yl)propyl)amino)-*N*-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)pyrazine-2-carboxamide



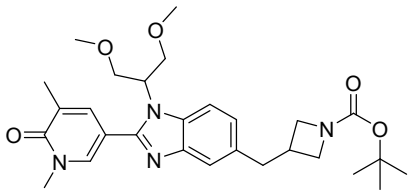
5

To a solution of 5-chloro-*N*-((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-(((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl) carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)pyrazine-2-carboxamide (29.3 mg, 1 Eq, 50.0 µmol) in DMSO (0.75 mL) was added 5-(5-(3-aminopropyl)-1-(1,3-dimethoxypropan-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)-1,3-dimethylpyridin-2(1*H*)-one (19.9 mg, 1 Eq, 50.00 µmol) followed by 1-methyl-2,3,4,6,7,8-hexahydro-1*H*-pyrimido[1,2-*a*]pyrimidine (23.3 µL, 3.25 Eq, 162.50 µmol) and the reaction mixture was heated to 60 °C for 24 h. The reaction mixture was cooled to room temperature then purified by MDAP (Formic method B). The relevant fractions were combined and concentrated *in vacuo* to give the title compound.

^{1}H NMR: (400 MHz, $CDCl_3$) δ 8.62 – 8.52 (m, 2H), 8.03 (d, *J* = 8.3 Hz, 1H), 7.80 (d, *J* = 2.2 Hz, 1H), 7.63 – 7.52 (m, 3H), 7.48 (s, 1H), 7.36 (d, *J* = 8.3 Hz, 1H), 7.31 – 7.23 (m, 4H), 7.03 – 6.94 (m, 1H), 5.43 – 5.17 (m, 1H), 5.05 – 4.93 (m, 1H), 4.79 – 4.56 (m, 3H), 4.42 (br s, 1H), 4.09 (br d, *J* = 11.2 Hz, 1H), 3.86 (t, *J* = 9.5 Hz, 2H), 3.74 (dd, *J* = 9.8, 4.9 Hz, 2H), 3.60 – 3.49 (m, 4H), 3.40 – 3.29 (m, 2H), 3.23 – 3.13 (m, 6H), 2.74 (br t, *J* = 7.5 Hz, 2H), 2.42 (s, 3H), 2.40 – 2.33 (m, 1H), 2.11 (s, 3H), 1.99 – 1.86 (m, 3H), 1.39 (d, *J* = 6.8 Hz, 3H), 1.20 – 1.13 (m, 1H), 1.02 (s, 9H); ^{13}C NMR: (176 MHz, $CDCl_3$) δ 172.0, 169.8, 164.6, 162.8, 155.8, 152.0, 150.3, 148.4, 143.3, 143.1, 138.6, 137.5, 135.7, 132.5, 132.1, 131.6, 130.7, 129.5, 129.4, 126.4, 123.5, 119.0, 111.8, 108.7, 70.8, 70.0, 59.2, 58.5, 58.4, 57.7, 57.5, 56.7, 48.9, 40.9, 38.2, 35.5, 35.4, 35.3, 33.0, 31.0, 26.6, 22.3, 17.4, 16.1; LCMS: (2 min Formic): t_R = 0.90 min, $[M+H]^+$ 947.6 (86% purity); HRMS: $C_{50}H_{62}N_{10}O_7S$ $[M+H]^+$ requires 947.4597, found $[M+H]^+$ 947.4586 (Δ = -1.16 ppm).

Pd-Mediated C(sp^2)-C(sp^3) Cross-Coupling D2B

Tert-butyl 3-((1-(1,3-dimethoxypropan-2-yl)-2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1*H*-benzo[*d*]imidazol-5-yl)methyl)azetidine-1-carboxylate



7

To a solution of 5-(5-bromo-1-(1,3-dimethoxypropan-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)-1,3-dimethylpyridin-2(1*H*)-one (100 mg, 1 Eq, 237.92 µmol), ((1-(tert-butoxycarbonyl)azetidin-3-yl)methyl)trifluoroborate, potassium salt (92 mg, 1.4 Eq, 333.09 µmol) and RuPhos Pd G3 (119.40 mg, 0.3 Eq, 142.76 µmol) in toluene (2.14 mL) was added tripotassium phosphate (101 mg, 4 Eq, 0.95 mmol) and water (238 µL) and the reaction mixture was stirred at 100 °C for 24 h.

Compound **S1** was split into four batches for varied purification processes: HPLC, celite cartridge, solid-phase extraction (SPE) cartridge, and aqueous work up.

For the HPLC batch: The solvent was removed under a stream of nitrogen and the crude product was dissolved in methanol and filtered through a celite cartridge then purified by MDAP (HpH Method C). The

relevant fractions were combined and concentrated *in vacuo* to give the title product (57 mg, 0.097 mmol, 40.8% yield).

^{1}H NMR: (400 MHz, $CDCl_3$) δ 7.85 (d, *J* = 2.4 Hz, 1H), 7.71 – 7.66 (m, 1H), 7.50 (s, 1H), 7.42 (d, *J* = 8.3 Hz, 1H), 7.02 (dd, *J* = 8.3, 1.7 Hz, 1H), 4.86 – 4.75 (m, 1H), 3.98 (t, *J* = 8.3 Hz, 2H), 3.92 (dd, *J* = 9.8, 8.1 Hz, 2H), 3.82 (dd, *J* = 9.8, 4.9 Hz, 2H), 3.67 (dd, J = 8.8, 5.4 Hz, 2H), 3.58 (s, 3H), 3.25 (s, 6H), 3.00 (d, J = 7.8 Hz, 2H), 2.92 – 2.79 (m, 1H), 2.19 (s, 3H), 1.41 (s, 9H); ^{13}C NMR: (101 MHz, $CDCl_3$) δ 162.7, 156.4, 152.0, 143.7, 138.3, 137.5, 133.6, 132.3, 129.3, 123.3, 119.1, 111.6, 108.9, 79.1, 70.8, 59.1, 57.6, 54.1, 40.1, 38.1, 30.1, 28.3, 17.2; LCMS: (2 min HpH): t_R = 1.08 min, $[M+H]^+$ 511.2 (87% purity); HRMS: $C_{28}H_{38}N_4O_5$ $[M+H]^+$ requires 511.2915, found $[M+H]^+$ 511.2929 (Δ = 2.74 ppm).

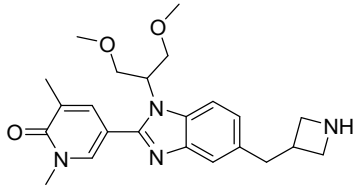
For the celite cartridge batch: Material was dissolved in DCM (5 mL) and filtered through a celite filtration cartridge, then flushed with methanol (5 mL) and concentrated under a stream of nitrogen to give the title product (62 mg, 0.075 mmol, 62% purity).

For the SPE cartridge batch: Material was dissolved in DCM (5 mL), loaded onto bond elute SPE filtration cartridge, and flushed with methanol (5 mL) and acetonitrile (5 mL), then concentrated under a stream of nitrogen to give the title product (68 mg, 0.083 mmol, 62% purity).

For the aqueous workup batch: Material was dissolved in DCM (5 mL) and washed with water (3 x 5 mL) and the DCM layer was dried using a hydrophobic frit then concentrated under a stream of nitrogen to give the title product (59 mg, 0.070 mmol, 61% purity).

Each batch was subsequently deprotected with TFA and passed through a strong cation exchange (SCX) cartridge according to the following procedure (example given for HPLC batch).

5-(5-(Azetidin-3-ylmethyl)-1-(1,3-dimethoxypropan-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)-1,3-dimethylpyridin-2(1*H*)-one



S8

To a vial containing *tert*-butyl 3-((1-(1,3-dimethoxypropan-2-yl)-2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1*H*-benzo[*d*]imidazol-5-yl)methyl)azetidine-1-carboxylate (87 mg, 1 Eq, 170.37 µmol) in DCM (425 µL) was added 2,2,2-trifluoroacetic acid (130 µL, 10 Eq, 1.70 mmol) and the reaction mixture stirred at room temperature for 23 h. TFA and DCM were removed under a stream of nitrogen then the crude product was dissolved in methanol and passed through an SCX cartridge. The cartridge was flushed with methanol (3 mL), then 4 M ammonia in methanol (3 mL) to elute the title product as the free base (71 mg, 0.15 mmol, 88% yield).

The subsequent amide coupling step was carried out in 1536-well plates using the following reagents: 0.15 µmol of amine made up to 1.5 µL with DMSO per well (0.1 M, 1 Eq), 0.225 µmol of acid made up to

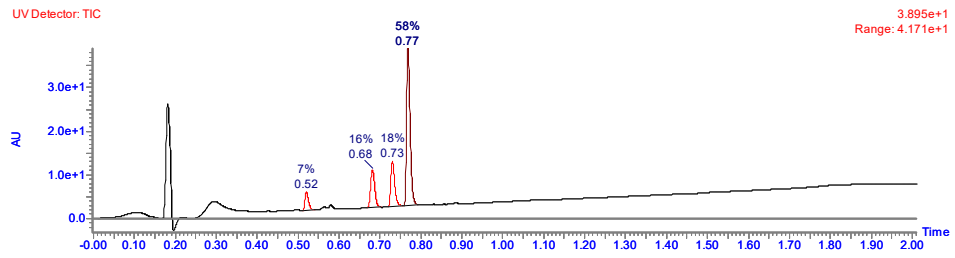
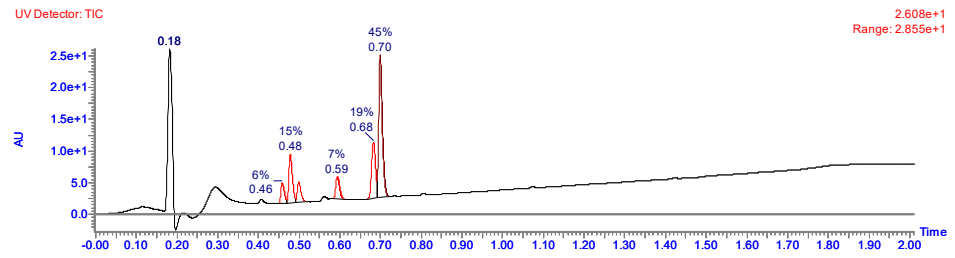
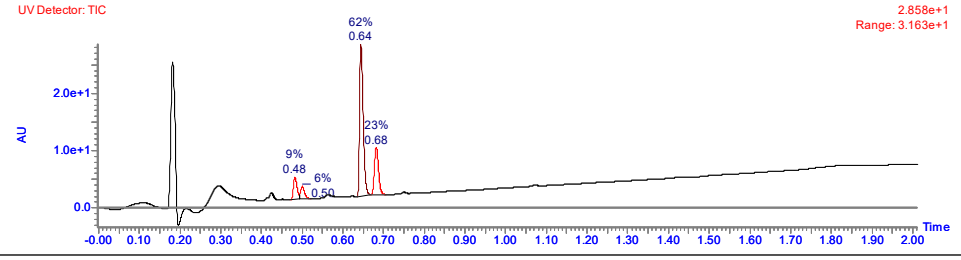
1.5 µL with DMSO per well (0.15 M, 1 Eq), 0.225 µmol of EDC.HCl made up to 1.28 µL with DMSO per well (0.176 M, 1.5 Eq), 0.3 µmol of OxymaPure made up to 0.589 µL with DMSO per well (0.509 M, 2 Eq), and 131 nL neat NMM per well (8 Eq, 1.2 µmol).

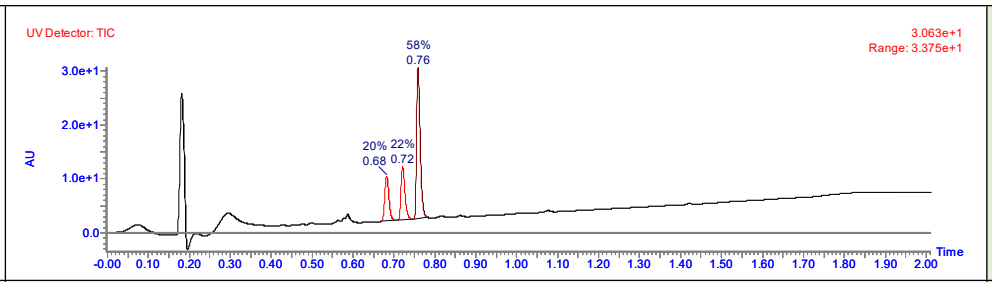
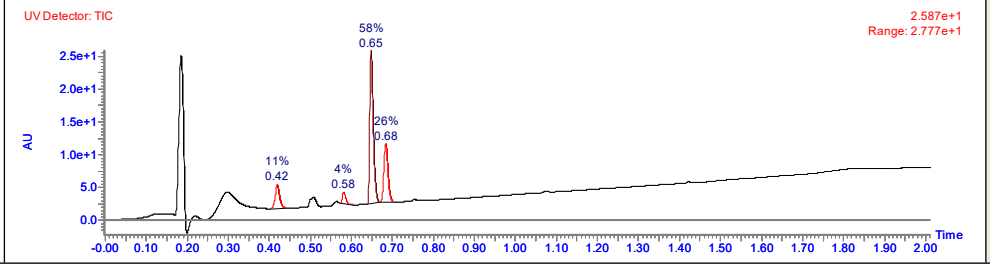
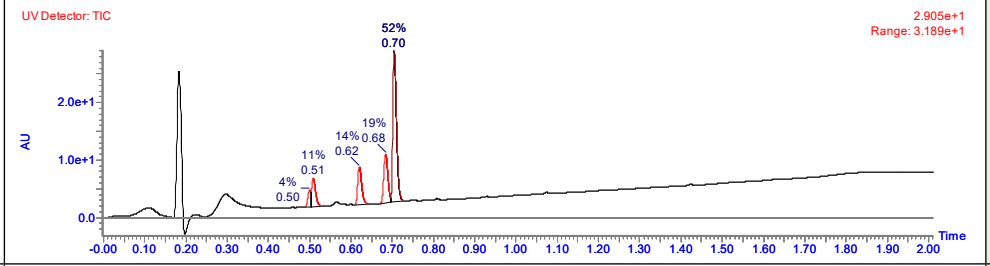
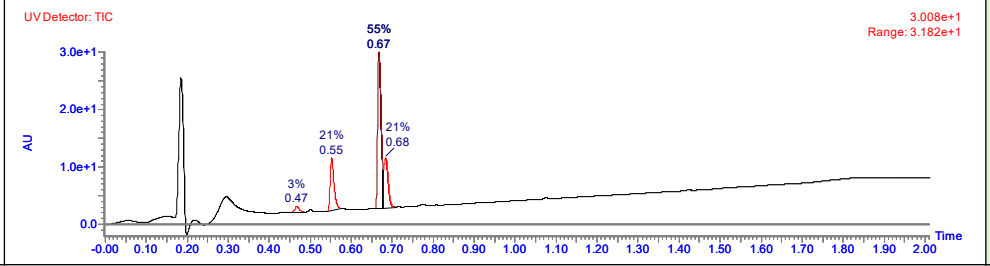
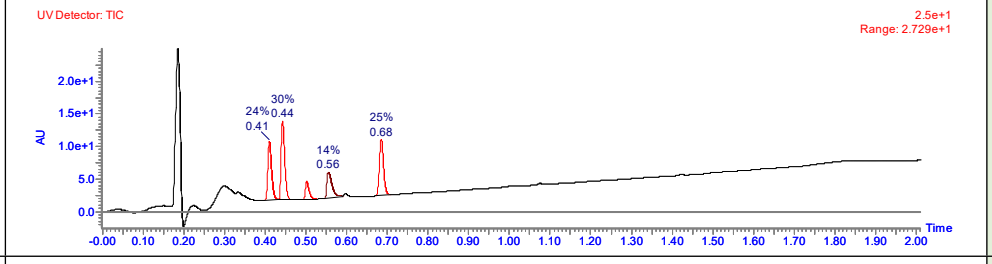
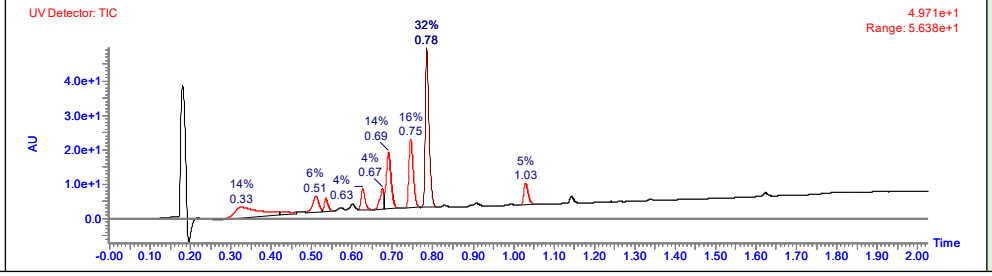
Reaction Plate Layout

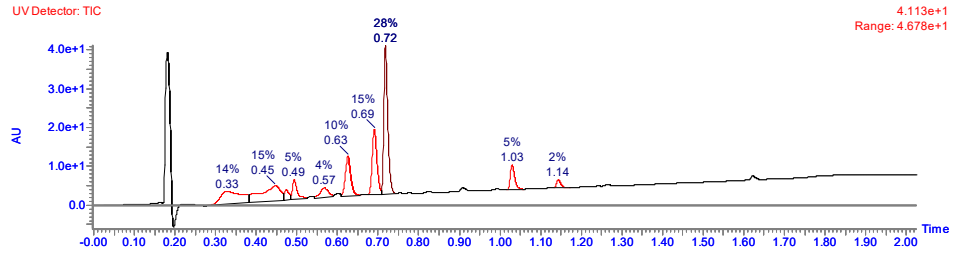
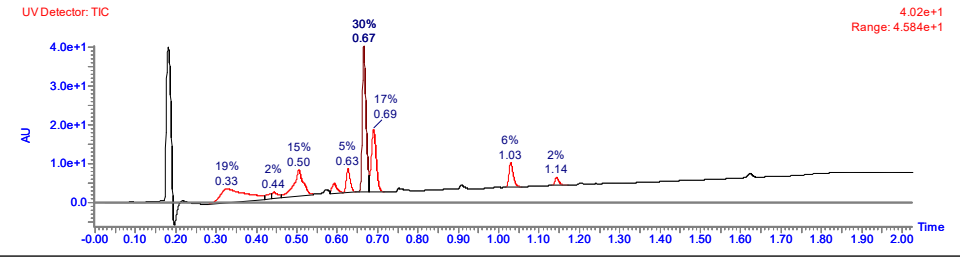
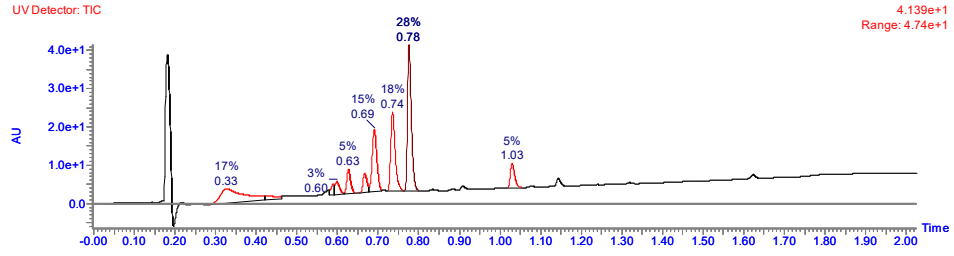
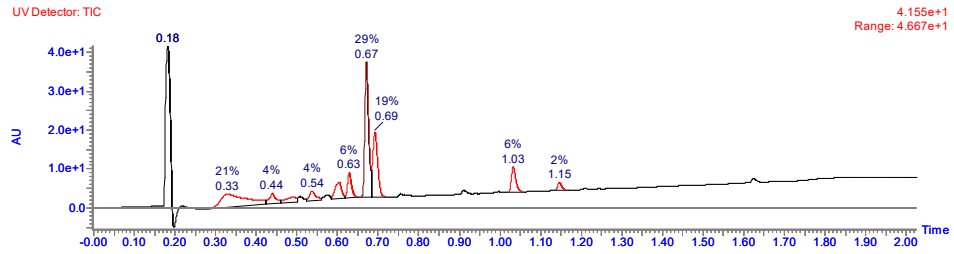
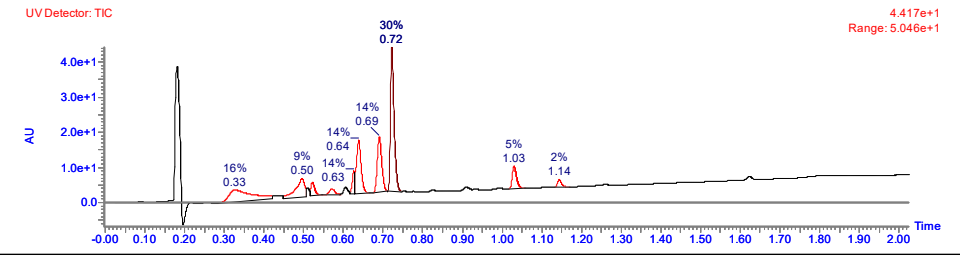
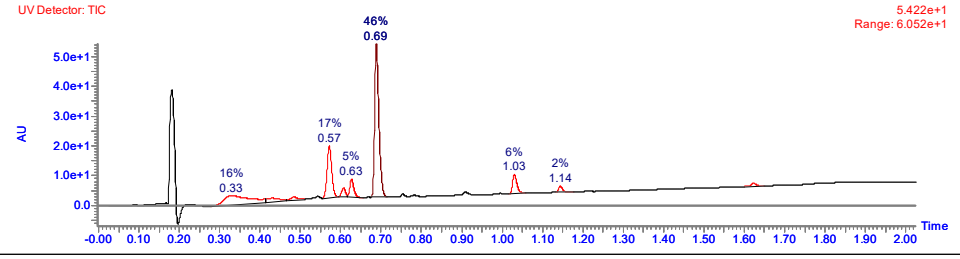
1536-well plate	22	23	24	25
A	Reaction 1	Reaction 33	Reaction 65	Pattern repeated in batches of 3 rows for each purification type across rows 25-33
B	Reaction 2	Reaction 34	Reaction 66	
C	Reaction 3	Reaction 35	Reaction 67	
D	Reaction 4	Reaction 36	Reaction 68	
E	Reaction 5	Reaction 37	Reaction 69	
…through to…			…to Reaction 87	
AF	Reaction 32	Reaction 64		

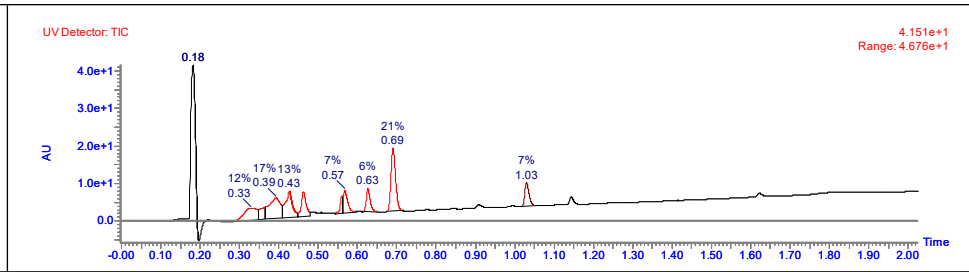
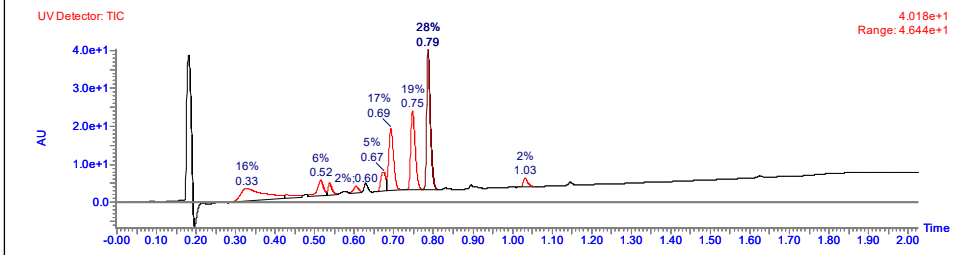
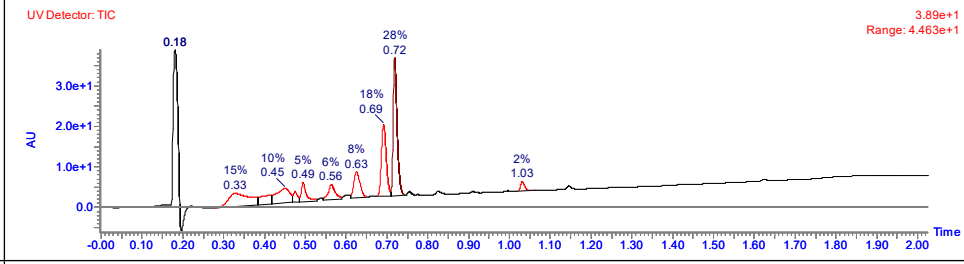
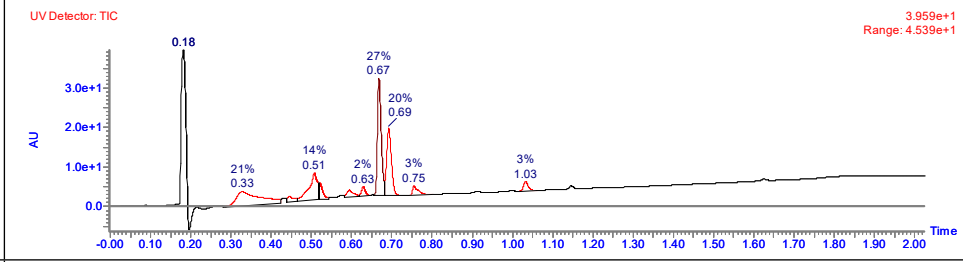
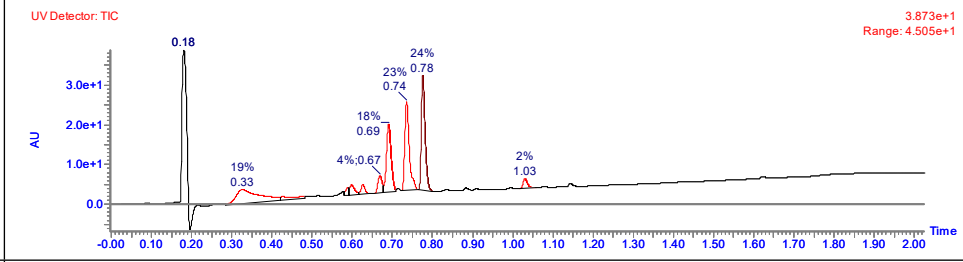
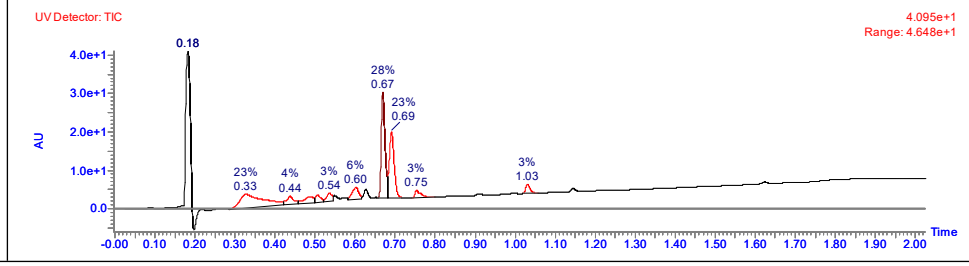
Table S5. Layout of 1536-well reaction plate; UV traces shown below.

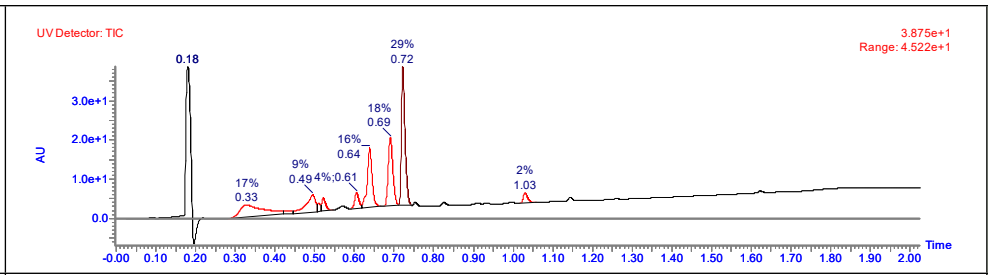
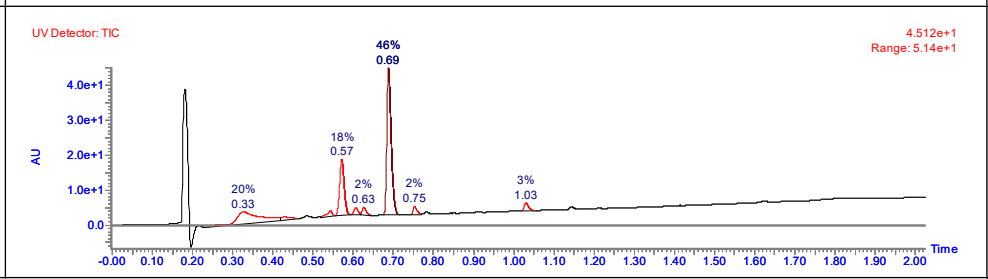
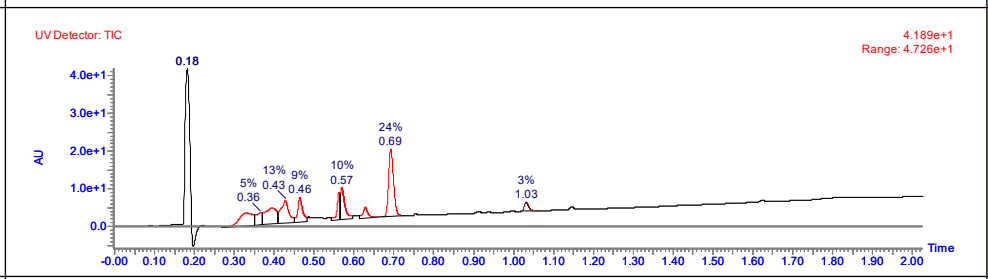
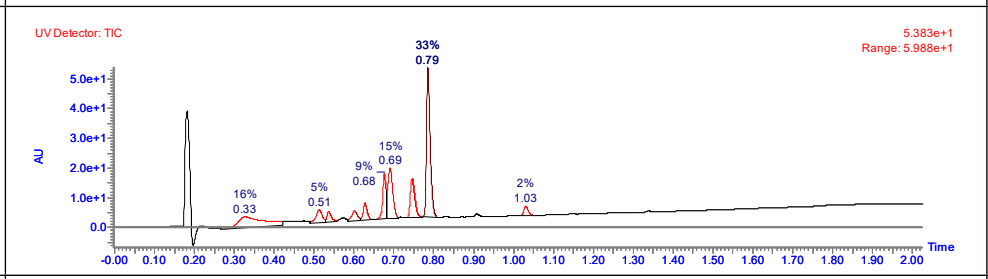
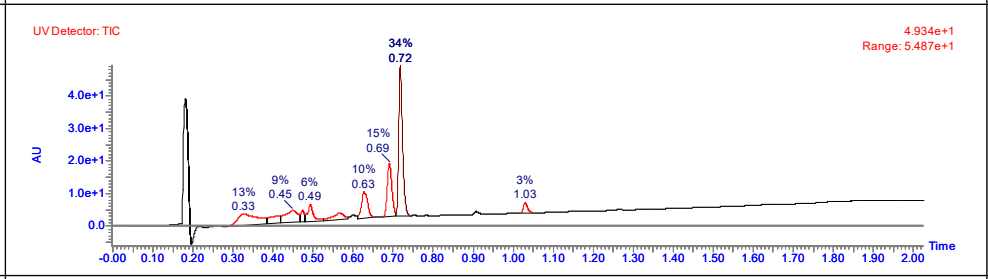
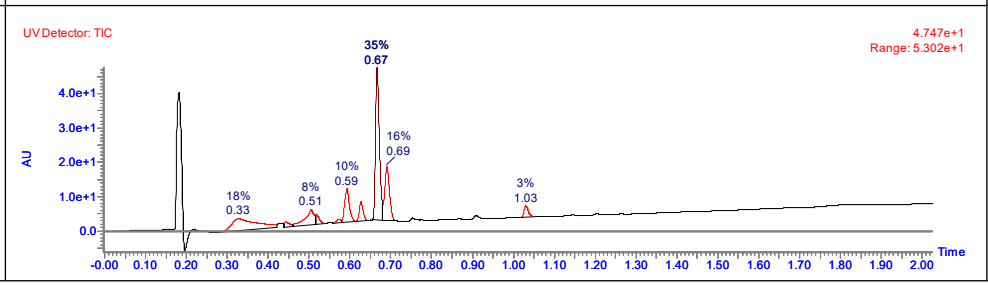
UV Traces

Reaction	UV trace	Purity / %
HPLC batch – Reaction 1		58
HPLC batch – Reaction 2		45
HPLC batch – Reaction 3		62

HPLC batch – Reaction 4		58
HPLC batch – Reaction 5		58
HPLC batch – Reaction 6		52
HPLC batch – Reaction 7		55
HPLC batch – Reaction 8		14
Celite batch – Reaction 1		32

Celite batch – Reaction 2		28
Celite batch – Reaction 3		30
Celite batch – Reaction 4		28
Celite batch – Reaction 5		29
Celite batch – Reaction 6		30
Celite batch – Reaction 7		46

Celite batch – Reaction 8		7
SPE batch – Reaction 1		28
SPE batch – Reaction 2		28
SPE batch – Reaction 3		27
SPE batch – Reaction 4		24
SPE batch – Reaction 5		28

SPE batch – Reaction 6		29
SPE batch – Reaction 7		46 (peak overlap)
SPE batch – Reaction 8		10
Aq. w/u batch – Reaction 1		33
Aq. w/u batch – Reaction 2		34
Aq. w/u batch – Reaction 3		35

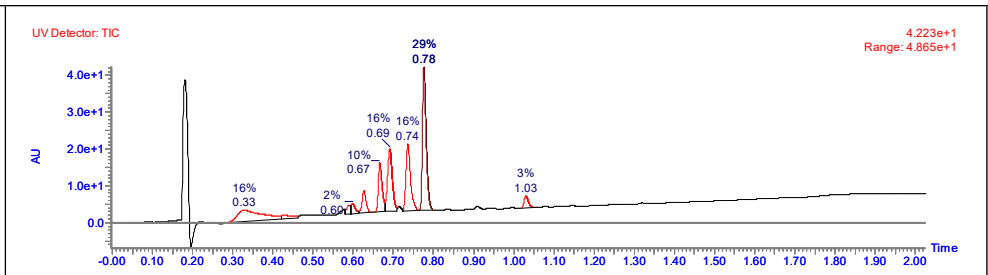
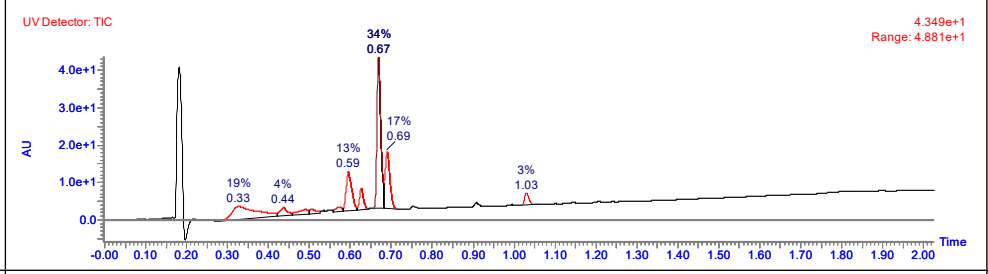
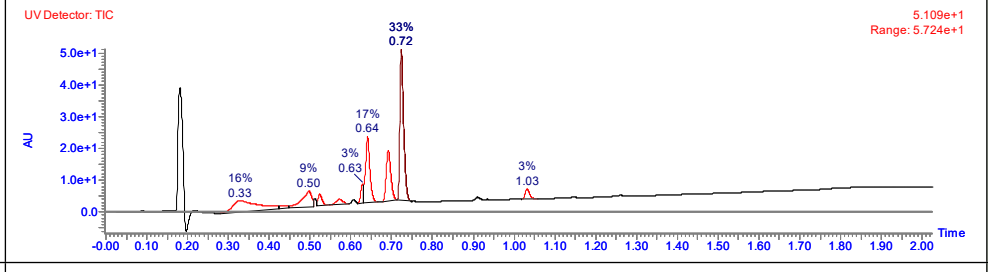
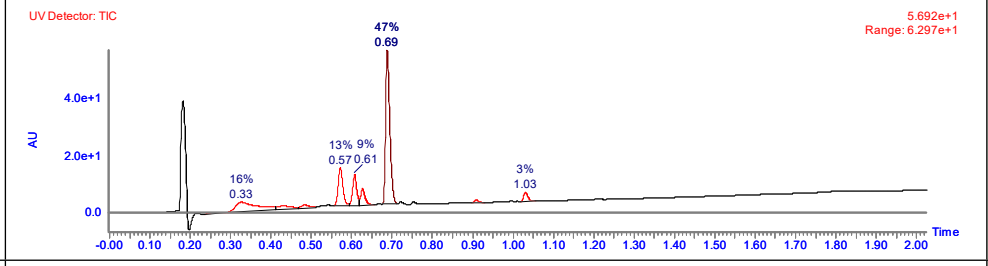
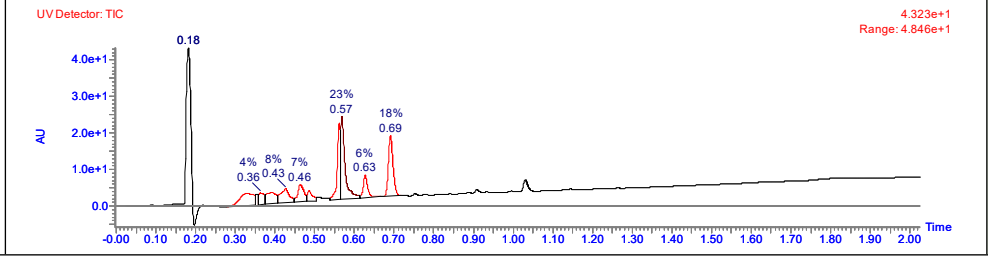
Aq. w/u batch – Reaction 4		29
Aq. w/u batch – Reaction 5		34
Aq. w/u batch – Reaction 6		33
Aq. w/u batch – Reaction 7		47 (peak overlap)
Aq. w/u batch – Reaction 8		23 (peak overlap)

Table S6. UV traces and product purities from LCMS analysis of crude reaction mixtures are provided for the first 8 reactions with each purification method as examples; reactions deemed successful are coloured in green, unsuccessful coloured in red.

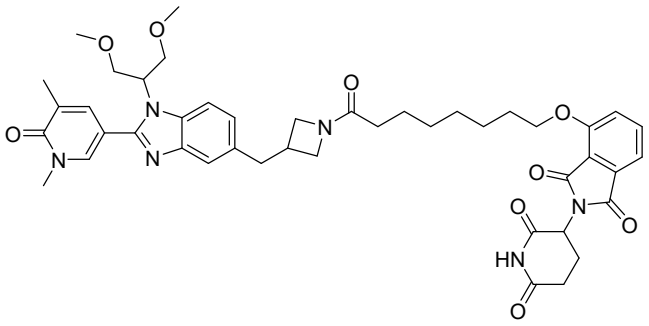
Pd-Mediated C(sp^2)-C(sp^3) Cross-Coupling Purified Compounds

The following BRD4 PROTACs were prepared according to standard procedure B:

To a solution of 5-(5-(azetidin-3-ylmethyl)-1-(1,3-dimethoxypropan-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)-1,3-dimethylpyridin-2(1*H*)-one (44.5 mg, 1 Eq, 108.40 µmol) and E3 ligase ligand acid (5.4 mg, 1.1 Eq, 119.24 µmol) in DMF (0.75 mL) was added HATU (45.3 mg, 1.1 Eq, 119.24 µmol) and DIPEA (56.6 µL, 3 Eq, 325.20

μmol) and the reaction mixture was stirred at room temperature for 72 h. The reaction mixtures were purified directly by HPLC and concentrated *in vacuo* to give the title products.

4-((8-(3-((1-(1,3-Dimethoxypropan-2-yl)-2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1*H*-benzo[*d*]imidazol-5-yl)methyl)azetidin-1-yl)-8-oxooctyl)oxy)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione

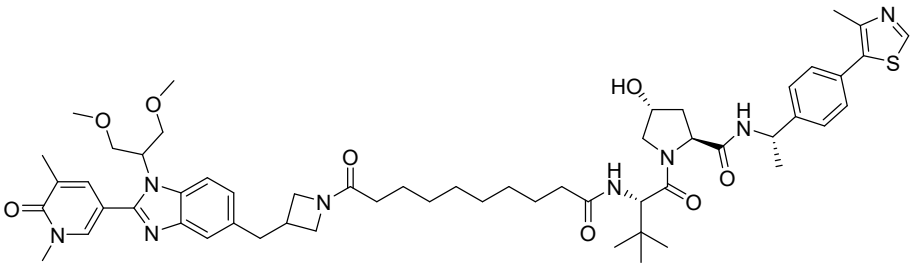


8

According to the standard method B, with purification by HPLC (HpH Method A), the title compound was synthesised (12 mg, 0.014 mmol, 13.0% yield).

^{1}H NMR: (600 MHz, CD_3OD) δ 8.10 – 8.07 (m, 1H), 8.07 (d, *J* = 2.3 Hz, 1H), 7.77 – 7.74 (m, 2H), 7.72 (dd, *J* = 8.5, 7.3 Hz, 1H), 7.53 (br s, 1H), 7.40 (t, *J* = 7.3 Hz, 2H), 7.21 (dd, *J* = 8.5, 1.3 Hz, 1H), 5.06 (dd, *J* = 12.4, 5.6 Hz, 1H), 4.92 (tt, *J* = 8.9, 4.2 Hz, 1H), 4.25 (t, *J* = 8.5 Hz, 1H), 4.21 (t, *J* = 6.4 Hz, 2H), 4.09 (dd, *J* = 10.0, 9.2 Hz, 2H), 4.03 (t, *J* = 9.0 Hz, 1H), 3.94 (dd, *J* = 8.7, 5.3 Hz, 1H), 3.81 (dd, *J* = 10.2, 4.1 Hz, 2H), 3.75 – 3.71 (m, 1H), 3.66 (s, 3H), 3.25 (s, 6H), 3.07 – 3.04 (m, 2H), 3.03 – 2.97 (m, 1H), 2.86 – 2.77 (m, 1H), 2.73 – 2.64 (m, 2H), 2.20 (s, 3H), 2.12 (t, *J* = 7.7 Hz, 2H), 2.10 – 2.04 (m, 1H), 1.88 – 1.82 (m, 2H), 1.63 – 1.51 (m, 4H), 1.45 – 1.34 (m, 4H); ^{13}C NMR: (151 MHz, CD_3OD) δ 176.2, 174.7, 171.6, 168.8, 167.4, 164.7, 158.2, 153.2, 142.7, 141.1, 139.6, 138.1, 136.2, 135.3, 133.2, 130.2, 125.7, 120.7, 119.1, 118.3, 116.4, 114.2, 111.6, 109.8, 71.3, 70.6, 59.7, 59.5, 56.4, 53.9, 50.5, 40.8, 39.0, 32.3, 32.1, 31.2, 30.3, 30.1, 30.0, 27.0, 26.0, 23.8, 17.4; LCMS: (2 min Formic): t_R = 0.86 min, $[M+H]^+$ 809.4 (96% purity); HRMS: $C_{44}H_{52}N_6O_9$ $[M+H]^+$ requires 809.3869, found $[M+H]^+$ 809.3894 (Δ = 3.09 ppm).

(2*S*,4*R*)-1-((*S*)-2-(10-(3-((1-(1,3-Dimethoxypropan-2-yl)-2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1*H*-benzo[*d*]imidazol-5-yl)methyl)azetidin-1-yl)-10-oxodecanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide

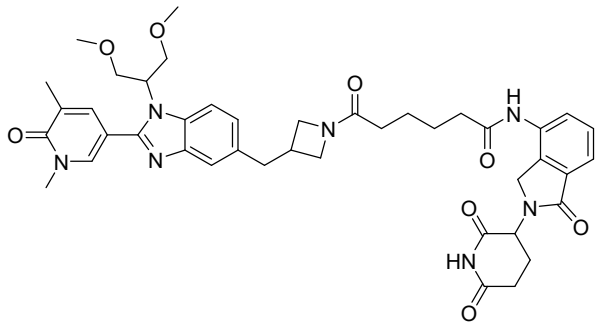


9

According to the standard method B, with purification by HPLC (Formic Method B), the title compound was synthesised (19 mg, 0.018 mmol, 16.3% yield).

^{1}H NMR: (600 MHz, CD_3OD) δ 8.87 (s, 1H), 8.02 (d, *J* = 1.9 Hz, 1H), 7.78 – 7.75 (m, 1H), 7.71 (d, *J* = 8.3 Hz, 1H), 7.52 (s, 1H), 7.46 – 7.35 (m, 4H), 7.18 (dd, *J* = 8.7, 1.1 Hz, 1H), 5.00 (q, *J* = 7.2 Hz, 1H), 4.91 – 4.87 (m, 1H), 4.63 (s, 1H), 4.57 (t, *J* = 8.3 Hz, 1H), 4.42 (br s, 1H), 4.29 – 4.20 (m, 1H), 4.10 – 4.01 (m, 3H), 3.96 (dd, *J* = 8.7, 5.3 Hz, 1H), 3.87 (br d, *J* = 11.3 Hz, 1H), 3.80 (dd, *J* = 10.4, 4.3 Hz, 2H), 3.76 – 3.71 (m, 2H), 3.66 (s, 3H), 3.25 (s, 6H), 3.08 – 2.98 (m, 3H), 2.47 (s, 3H), 2.34 – 2.22 (m, 2H), 2.20 – 2.16 (m, 4H), 2.14 – 2.08 (m, 2H), 1.95 (ddd, *J* = 13.2, 8.8, 4.7 Hz, 1H), 1.64 – 1.53 (m, 4H), 1.50 (d, *J* = 6.8 Hz, 3H), 1.33 (br s, 8H), 1.06 – 1.00 (m, 9H); ^{13}C NMR: (151 MHz, CD_3OD) δ 176.2, 176.1, 173.4, 172.4, 164.8, 153.6, 153.0, 149.2, 145.8, 144.2, 140.7, 139.9, 135.4, 133.6, 133.5, 131.7, 130.6, 130.6, 130.0, 127.8, 125.2, 119.7, 113.9, 110.9, 71.4, 71.1, 60.7, 59.5, 59.4, 59.1, 58.1, 56.4, 53.9, 50.3, 49.7, 40.9, 40.3, 38.9, 38.9, 37.7, 36.8, 36.6, 32.2, 31.3, 30.4, 30.4, 30.4, 27.2, 27.1, 26.2, 25.1, 22.6, 17.4, 16.0, 14.6; LCMS: (2 min Formic): t_R = 0.93 min, $[M+H]^+$ 1021.6 (97% purity); HRMS: $C_{56}H_{76}N_8O_8S$ $[M+H]^+$ requires 1021.5580, found $[M+H]^+$ 1021.5587 (Δ = 0.69 ppm).

6-(3-((1-(1,3-Dimethoxypropan-2-yl)-2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1*H*-benzo[*d*]imidazol-5-yl)methyl)azetidin-1-yl)-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)-6-oxohexanamide

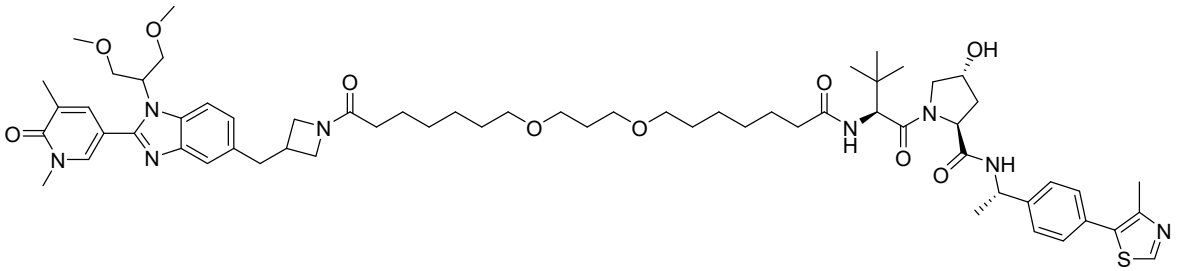


S6

According to the standard method B, with purification by HPLC (HpH Method A followed by Formic Method A), the title compound was synthesised (12 mg, 0.015 mmol, 13.5% yield).

^{1}H NMR: (600 MHz, CD_3OD) δ 8.10 (s, 2H), 8.03 (d, *J* = 1.8 Hz, 1H), 7.77 – 7.73 (m, 1H), 7.71 (d, *J* = 8.4 Hz, 2H), 7.64 (br d, *J* = 7.7 Hz, 1H), 7.53 – 7.46 (m, 2H), 7.20 – 7.15 (m, 1H), 5.14 (br dd, *J* = 13.2, 5.1 Hz, 1H), 4.92 – 4.87 (m, 1H), 4.53 – 4.45 (m, 2H), 4.25 (t, *J* = 8.1 Hz, 1H), 4.10 – 3.98 (m, 3H), 3.95 (br dd, *J* = 8.4, 5.1 Hz, 1H), 3.80 (dd, *J* = 10.3, 4.4 Hz, 2H), 3.75 – 3.70 (m, 1H), 3.65 (s, 3H), 3.25 (s, 6H), 3.07 – 2.97 (m, 3H), 2.91 – 2.83 (m, 1H), 2.74 (ddd, *J* = 17.7, 4.3, 2.2 Hz, 1H), 2.50 – 2.39 (m, 3H), 2.20 – 2.10 (m, 6H), 1.75 – 1.63 (m, 4H); ^{13}C NMR: (151 MHz, CD_3OD) δ 175.6, 174.8, 174.4, 172.2, 171.2, 164.9, 164.7, 153.6, 143.9, 140.8, 139.8, 136.6, 135.6, 134.7, 134.1, 133.5, 130.2, 130.1, 128.0, 125.3, 121.6, 119.6, 114.0, 110.7, 71.4, 59.5, 56.4, 53.9, 53.8, 40.8, 38.9, 37.1, 32.5, 31.8, 31.3, 26.5, 25.6, 24.3, 17.4; LCMS: (2 min Formic): t_R = 0.66 min, $[M+H]^+$ 780.6 (100% purity); HRMS: $C_{42}H_{49}N_7O_8$ $[M+H]^+$ requires 780.3716, found $[M+H]^+$ 780.3712 (Δ = -0.51 ppm).

(2*S*,4*R*)-1-((*S*)-2-(7-(3-((7-(3-((1-(1,3-Dimethoxypropan-2-yl)-2-(1,5-dimethyl-6-oxo-1,6-dihydropyridin-3-yl)-1*H*-benzo[*d*]imidazol-5-yl)methyl)azetidin-1-yl)-7-oxoheptyl)oxy)propoxy)heptanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide



S7

According to the standard method B, with purification by HPLC (Formic Method B), the title compound was synthesised (10 mg, 0.008 mmol, 7.2% yield).

^{1}H NMR: (600 MHz, CD_3OD) δ 8.87 (s, 1H), 8.02 (d, *J* = 2.3 Hz, 1H), 7.79 – 7.74 (m, 1H), 7.71 (d, *J* = 8.7 Hz, 1H), 7.52 (s, 1H), 7.46 – 7.39 (m, 4H), 7.18 (dd, *J* = 8.3, 1.1 Hz, 1H), 5.00 (q, *J* = 7.0 Hz, 1H), 4.91 – 4.87 (m, 1H), 4.62 (s, 1H), 4.60 – 4.54 (m, 1H), 4.45 – 4.35 (m, 1H), 4.26 (t, *J* = 8.3 Hz, 1H), 4.10 – 4.00 (m, 3H), 3.96 (dd, *J* = 8.8, 5.1 Hz, 1H), 3.87 (br d, *J* = 10.9 Hz, 1H), 3.83 – 3.79 (m, 2H), 3.77 – 3.71 (m, 2H), 3.66 (s, 3H), 3.51 – 3.45 (m, 4H), 3.41 (q, *J* = 6.7 Hz, 4H), 3.25 (s, 6H), 3.08 – 2.99 (m, 3H), 2.94 (s, 1H), 2.48 (s, 3H), 2.35 – 2.21 (m, 2H), 2.21 – 2.16 (m, 4H), 2.12 (t, *J* = 7.5 Hz, 2H), 1.95 (ddd, *J* = 13.1, 8.8, 4.5 Hz, 1H), 1.79 (quin, *J* = 6.4 Hz, 2H), 1.66 – 1.53 (m, 8H), 1.50 (d, *J* = 6.8 Hz, 3H), 1.42 – 1.30 (m, 8H), 1.06 – 0.99 (m, 9H). ^{13}C NMR: (151 MHz, CD_3OD) δ 176.1, 176.1, 173.4, 172.4, 164.8, 153.6, 153.0, 149.2, 145.8, 144.3, 140.7, 139.9, 135.4, 133.5, 133.5, 131.7, 130.6, 130.0, 127.8, 125.2, 119.7, 113.9, 111.6, 110.9, 72.1, 71.4, 71.1, 68.9, 60.7, 59.6, 59.5, 59.5, 59.4, 59.1, 58.1, 56.4, 53.9, 50.3, 49.7, 40.9, 40.1, 38.9, 38.9, 36.7, 36.6, 32.1, 31.3, 31.3, 30.8, 30.8, 30.3, 30.3, 27.2, 27.2, 27.2, 27.2, 26.1, 22.6, 17.4, 16.0; LCMS: (2 min Formic): t_R = 1.02 min, $[M+H]^+$ 1151.6 (90% purity); HRMS: $C_{63}H_{90}N_8O_{10}S$ $[M+H]^+$ requires 1151.6574, found $[M+H]^+$ 1151.6583 (Δ = 0.78 ppm).

AR PROTACs

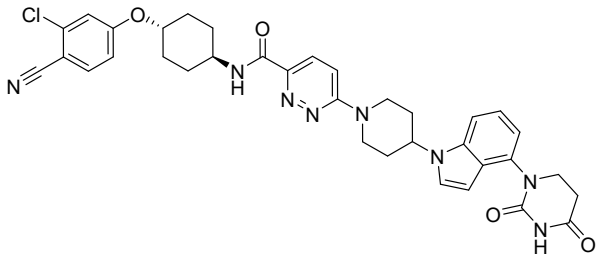
Nucleophilic Aromatic Substitution D2B

The following reagents were used for the S_NAr library: 0.15 µmol of amine made up to 1.5 µL with DMSO per well (0.1 M, 1 eq.), 0.15 µmol of aryl chloride made up to 1.5 µL with DMSO per well (0.1 M, 1 eq.), 0.17 µmol of MTBD made up to 3 µL with DMSO per well (0.057 M, 1.13 eq.).

Reactions were carried out at 90 °C at 300 rpm using the thermomixer in the glovebox. 65/76 reactions (86%) were considered to have sufficient conversion to product to be submitted for testing in the AR-HiBiT assay.

Nucleophilic Aromatic Substitution Purified Compounds

N-((1*r*,4*r*)-4-(3-Chloro-4-cyanophenoxy)cyclohexyl)-6-(4-(4-(2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)-1*H*-indol-1-yl)piperidin-1-yl)pyridazine-3-carboxamide

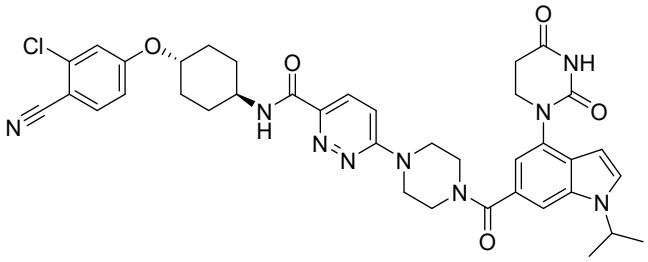


10

6-Chloro-*N*-((1*r*,4*r*)-4-(3-chloro-4-cyanophenoxy)cyclohexyl)pyridazine-3-carboxamide (20 mg, 1 Eq, 0.051 mmol), 1-(1-(piperidin-4-yl)-1*H*-indol-4-yl)dihydropyrimidine-2,4(1*H*,3*H*)-dione (15.97 mg, 1 Eq, 0.051 mmol) and DIPEA (0.018 mL, 2 Eq, 0.10 mmol) were combined in DMSO (0.4 mL) and the reaction mixture was heated to 70 °C for 1 h, then allowed to cool to give a dense suspension. This was diluted with methanol (0.5 mL) and filtered. The filtrate was evaporated *in vacuo* and the residue dissolved in DMSO (1 mL), then purified by MDAP (Formic Extended Method C) to give the title product (9 mg, 0.013 mmol, 26.4 % yield) as a colourless solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.29 (s, 1H), 8.61 (d, *J* = 8.3 Hz, 1H), 7.90 – 7.81 (m, 2H), 7.59 (d, *J* = 8.3 Hz, 1H), 7.52 (d, *J* = 3.4 Hz, 1H), 7.46 (d, *J* = 9.8 Hz, 1H), 7.38 (d, *J* = 2.4 Hz, 1H), 7.22 – 7.11 (m, 2H), 6.98 (dd, *J* = 7.6, 0.5 Hz, 1H), 6.41 (d, *J* = 3.2 Hz, 1H), 4.88 – 4.77 (m, 1H), 4.72 (br d, *J* = 13.2 Hz, 2H), 4.59 – 4.48 (m, 1H), 3.94 – 3.82 (m, 1H), 3.78 (t, *J* = 6.7 Hz, 2H), 3.28 (br t, *J* = 11.6 Hz, 2H), 2.76 (t, *J* = 6.7 Hz, 2H), 2.16 – 1.87 (m, 8H), 1.73 – 1.59 (m, 2H), 1.58 – 1.45 (m, 2H); LCMS: (2 min HpH): t_R = 1.17 min, $[M+H]^+$ 667.1, 669.1 (100% purity).

N-((1*r*,4*r*)-4-(3-Chloro-4-cyanophenoxy)cyclohexyl)-6-(4-(4-(2,4-dioxotetrahydropyrimidin-1(2*H*)-yl)-1-isopropyl-1*H*-indole-6-carbonyl)piperazin-1-yl)pyridazine-3-carboxamide



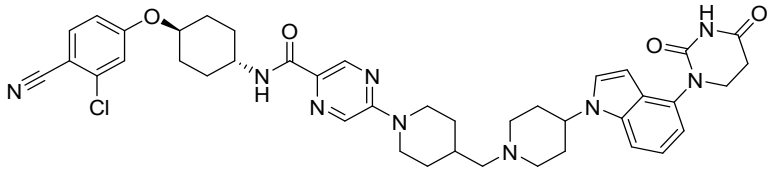
11

A mixture of 6-chloro-*N*-((1*r*,4*r*)-4-(3-chloro-4-cyanophenoxy)cyclohexyl)pyridazine-3-carboxamide (10 mg, 1 Eq, 0.026 mmol), 1-(1-isopropyl-6-(piperazine-1-carbonyl)-1*H*-indol-4-yl)dihydropyrimidine-2,4(1*H*,3*H*)-dione (9.8 mg, 1 Eq, 0.026mmol), and *N*-methylmorpholine (8.43 µL, 3 Eq, 0.077 mmol) in DMSO (85 µL) was stirred at 60 °C for 72 h. The reaction mixture diluted in DMSO (0.5 mL) and purified by

automated purification (HpH Method E). The solvent was removed under a stream of nitrogen to give the title compound (5.4 mg, 7.31 µmol, 28.6 % yield).

^{1}H NMR (700 MHz, DMSO-d_6) δ 10.37 (s, 1H), 8.62 (d, *J* = 8.5 Hz, 1H), 7.86 (dd, *J* = 17.2, 9.1 Hz, 2H), 7.66 (d, *J* = 3.4 Hz, 1H), 7.64 (s, 1H), 7.40 – 7.36 (m, 2H), 7.13 (dd, *J* = 8.7, 2.3 Hz, 1H), 7.10 (d, *J* = 1.3 Hz, 1H), 6.50 (d, *J* = 3.4 Hz, 1H), 4.85 (spt, *J* = 6.6 Hz, 1H), 4.57 – 4.50 (m, 1H), 3.90 – 3.62 (m, 11H), 2.78 (t, *J* = 6.6 Hz, 2H), 2.10 (br d, *J* = 10.6 Hz, 2H), 1.93 – 1.87 (m, 2H), 1.68 – 1.60 (m, 2H), 1.55 – 1.49 (m, 2H), 1.48 (d, *J* = 6.8 Hz, 6H); LCMS: (2 min HpH): t_R = 1.04 min, $[M+H]^+$ 738.3, 740.3 (100% purity).

_N_-((1_r_,4_r_)-4-(3-Chloro-4-cyanophenoxy)cyclohexyl)-5-(4-((4-(4-(2,4-dioxotetrahydropyrimidin-1(2_H_)-yl)-1_H_-indol-1-yl)piperidin-1-yl)methyl)piperidin-1-yl)pyrazine-2-carboxamide

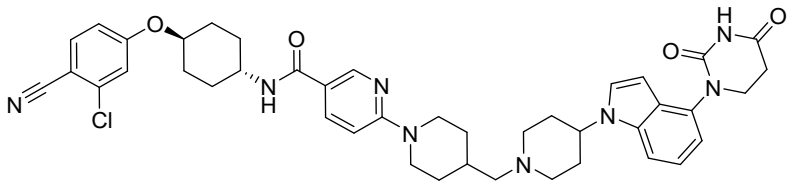


12

To *N*-((1*r*,4*r*)-4-(3-chloro-4-cyanophenoxy)cyclohexyl)-5-(4-formylpiperidin-1-yl)pyrazine-2-carboxamide (1 Eq, 0.085 mmol) was added 1-(1-(piperidin-4-yl)-1*H*-indol-4-yl)dihydropyrimidine-2,4(1*H*,3*H*)-dione (1 Eq, 0.085 mmol) in anhydrous DCM (0.50 mL) and borane-2-methylpyridine complex (0.027 g, 3 Eq, 0.255 mmol) in anhydrous DCM (0.50 mL) and the reaction mixture was stirred at room temperature for 18 h. Acetic acid (100 µL) was added and the mixture stirred for 2 h. The mixture was dissolved in DMSO (0.5 mL) and purified by automated purification (HpH method). The solvent was evaporated *in vacuo* to give the title product (8 mg, 0.010 mmol, 11.2% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (d, *J* = 1.0 Hz, 1H), 7.98 (d, *J* = 1.5 Hz, 1H), 7.63 (s, 1H), 7.56 (d, *J* = 8.9 Hz, 1H), 7.41 – 7.35 (m, 2H), 7.29 (d, *J* = 3.4 Hz, 1H), 7.24 (t, *J* = 7.9 Hz, 1H), 7.04 (d, *J* = 6.9 Hz, 1H), 7.00 (d, *J* = 2.5 Hz, 1H), 6.85 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.40 (d, *J* = 3.0 Hz, 1H), 4.48 (br d, *J* = 13.3 Hz, 2H), 4.36 – 4.18 (m, 2H), 4.10 – 3.99 (m, 1H), 3.94 (t, *J* = 6.6 Hz, 2H), 3.10 – 2.96 (m, 4H), 2.89 (t, *J* = 6.6 Hz, 2H), 2.30 (d, *J* = 6.9 Hz, 2H), 2.25 – 2.12 (m, 6H), 2.12 – 2.05 (m, 3H), 1.95 (br d, *J* = 13.3 Hz, 2H), 1.91 – 1.82 (m, 1H), 1.76 – 1.63 (m, 3H), 1.54 – 1.40 (m, 2H), 1.32 – 1.19 (m, 2H); ^{13}C NMR: (151 MHz, DMSO-d_6) δ 170.8, 162.8, 161.8, 155.0, 152.0, 141.6, 137.0, 136.5, 135.7, 134.1, 132.2, 128.5, 125.3, 124.9, 120.9, 117.1, 116.8, 116.4, 115.4, 109.0, 103.2, 99.1, 75.6, 63.5, 53.0, 53.0, 46.8, 45.0, 44.1, 33.1, 32.2, 31.4, 29.9, 29.8, 29.5; LCMS: (2 min Formic): t_R = 0.84 min, $[M+H]^+$ 764.7, 766.7 (92% purity); HRMS: $C_{41}H_{46}ClN_9O_4$ $[M+H]^+$ requires 764.3434, found $[M+H]^+$ 764.3427 (Δ = -0.92 ppm).

_N_-((1_r_,4_r_)-4-(3-Chloro-4-cyanophenoxy)cyclohexyl)-6-(4-((4-(4-(2,4-dioxotetrahydropyrimidin-1(2_H_)-yl)-1_H_-indol-1-yl)piperidin-1-yl)methyl)piperidin-1-yl)nicotinamide

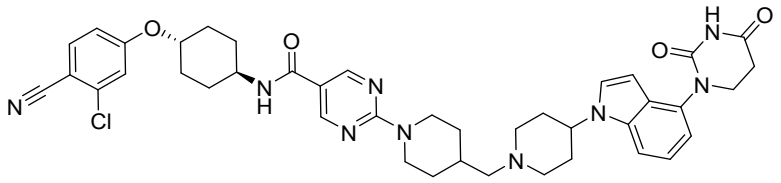


13

To *N*-((1*r*,4*r*)-4-(3-chloro-4-cyanophenoxy)cyclohexyl)-6-(4-formylpiperidin-1-yl)nicotinamide (1 Eq, 0.085 mmol) was added 1-(1-(piperidin-4-yl)-1*H*-indol-4-yl)dihydropyrimidine-2,4(1*H*,3*H*)-dione (1 Eq, 0.085 mmol) in anhydrous DCM (0.50 mL) and borane-2-methylpyridine complex (0.027 g, 3 Eq, 0.255 mmol) in anhydrous DCM (0.50 mL) and the reaction mixture was stirred at room temperature for 18 h. Acetic acid (100 µL) was added and the mixture stirred for 2 h. The mixture was dissolved in DMSO (0.5 mL) and purified by automated purification (HpH method). The solvent was evaporated *in vacuo* to give the title product (8 mg, 0.010 mmol, 11.1% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (d, *J* = 2.5 Hz, 1H), 7.88 (dd, *J* = 8.9, 2.5 Hz, 1H), 7.59 – 7.51 (m, 2H), 7.38 (br d, *J* = 8.4 Hz, 1H), 7.30 (d, *J* = 3.4 Hz, 1H), 7.26 – 7.20 (m, 1H), 7.04 (d, *J* = 6.9 Hz, 1H), 7.00 (d, *J* = 2.5 Hz, 1H), 6.85 (dd, *J* = 8.4, 2.5 Hz, 1H), 6.65 (d, *J* = 8.9 Hz, 1H), 6.40 (d, *J* = 3.0 Hz, 1H), 5.78 (d, *J* = 7.9 Hz, 1H), 4.43 (br d, *J* = 12.8 Hz, 2H), 4.34 – 4.19 (m, 2H), 4.10 – 3.99 (m, 1H), 3.94 (t, *J* = 6.6 Hz, 2H), 3.07 (br d, *J* = 11.3 Hz, 2H), 2.99 – 2.86 (m, 4H), 2.29 (d, *J* = 6.9 Hz, 2H), 2.25 – 2.13 (m, 6H), 2.12 – 2.05 (m, 4H), 1.91 (br d, *J* = 13.3 Hz, 2H), 1.88 – 1.79 (m, 1H), 1.75 – 1.66 (m, 2H), 1.47 – 1.35 (m, 2H), 1.31 – 1.17 (m, 2H); ^{13}C NMR: (151 MHz, DMSO-d_6) δ 171.29, 164.97, 162.30, 160.13, 152.48, 148.61 – 148.43 (m, 1C), 144.18, 137.47, 136.98, 136.20, 134.57, 125.85, 125.36, 121.39, 118.57, 117.61, 117.32, 116.90, 115.95, 115.95, 109.95, 109.99, 105.71, 103.68, 99.58, 76.14, 53.48, 47.72, 45.48, 44.95, 40.55, 32.66, 31.86, 30.48, 30.37, 30.01; LCMS: (Formic): t_R = 0.76 min, $[M+H]^+$ 763.8, 765.7 (100% purity); HRMS: $C_{42}H_{47}ClN_8O_4$ $[M+H]^+$ requires 763.3482, found $[M+H]^+$ 763.3476 (Δ = -0.79 ppm).

_N_-((1_r_,4_r_)-4-(3-Chloro-4-cyanophenoxy)cyclohexyl)-2-(4-((4-(4-(2,4-dioxotetrahydropyrimidin-1(2_H_)-yl)-1_H_-indol-1-yl)piperidin-1-yl)methyl)piperidin-1-yl)pyrimidine-5-carboxamide

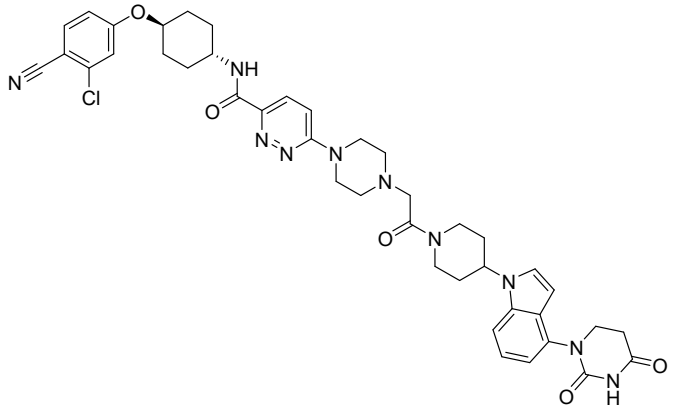


14

To *N*-((1*r*,4*r*)-4-(3-chloro-4-cyanophenoxy)cyclohexyl)-2-(4-formylpiperidin-1-yl)pyrimidine-5-carbox amide (1 Eq, 0.085 mmol) was added 1-(1-(piperidin-4-yl)-1*H*-indol-4-yl)dihydropyrimidine-2,4(1*H*,3*H*)-dione (1 Eq, 0.085 mmol) in anhydrous DCM (0.50 mL) and borane-2-methylpyridine complex (0.027 g, 3 Eq, 0.255 mmol) in anhydrous DCM (0.50 mL) and the reaction mixture was stirred at room temperature for 18 h. Acetic acid (100 µL) was added and the mixture stirred for 2 h. The mixture was dissolved in DMSO (0.5 mL) and purified by automated purification (HpH method). The solvent was evaporated *in vacuo* to give the title product (7 mg, 0.008 mmol, 9.0% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 2H), 7.56 (d, *J* = 8.9 Hz, 1H), 7.38 (d, *J* = 8.4 Hz, 1H), 7.30 (d, *J* = 3.4 Hz, 1H), 7.23 (t, *J* = 7.9 Hz, 1H), 7.04 (d, *J* = 6.9 Hz, 1H), 7.00 (d, *J* = 2.5 Hz, 1H), 6.85 (dd, *J* = 8.9, 2.5 Hz, 1H), 6.40 (d, *J* = 3.0 Hz, 1H), 5.74 (d, *J* = 7.9 Hz, 1H), 4.87 (br d, *J* = 13.3 Hz, 2H), 4.33 – 4.17 (m, 2H), 4.09 – 3.98 (m, 1H), 3.94 (t, *J* = 6.6 Hz, 2H), 3.07 (br d, *J* = 11.3 Hz, 2H), 3.01 – 2.92 (m, 2H), 2.89 (t, *J* = 6.6 Hz, 2H), 2.29 (br d, *J* = 6.9 Hz, 2H), 2.25 – 2.12 (m, 6H), 2.12 – 2.04 (m, 3H), 1.96 – 1.81 (m, 3H), 1.74 – 1.59 (m, 4H), 1.49 – 1.34 (m, 2H), 1.28 – 1.13 (m, 2H); LCMS: (2 min HpH): t_R = 0.82 min, $[M+H]^+$ 764.7, 766.7 (94% purity).

_N_-((1_r_,4_r_)-4-(3-Chloro-4-cyanophenoxy)cyclohexyl)-6-(4-(2-(4-(4-(2,4-dioxotetrahydropyrimidin-1(2_H_)-yl)-1_H_-indol-1-yl)piperidin-1-yl)-2-oxoethyl)piperazin-1-yl)pyridazine-3-carboxamide



15

To a stirring solution of 2-(4-(6-(((1*r*,4*r*)-4-(3-chloro-4-cyanophenoxy)cyclohexyl)carbamoyl)pyridazin-3-yl)piperazin-1-yl)aceticacid (218 mg, 0.44 mmol) and 1-(1-(piperidin-4-yl)-1*H*-indol-4-yl)dihydro pyrimidine-2,4(1*H*,3*H*)-dione (96 mg, 0.306 mmol) and HATU (199 mg, 0.524 mmol) in DMF (3 mL) was added DIPEA (0.229 mL, 1.31 mmol), followed by stirring of the yellow coloured solution for for 2 h. The solvent was partially removed using a flow of nitrogen then 1 mL of remaining solution was purified directly by MDAP (HpH Extended Method C). The desired fractions were combined and concentrated using a flow of nitrogen to give the title compound (42 mg, 0.053 mmol, 12.1% yield) as a white coloured solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 10.30 (s, 1H), 8.58 (d, *J* = 8.1 Hz, 1H), 7.85 (dd, *J* = 9.2, 4.8 Hz, 2H), 7.56 (d, *J* = 8.3 Hz, 1H), 7.51 (d, *J* = 3.4 Hz, 1H), 7.38 (d, *J* = 2.2 Hz, 1H), 7.35 (d, *J* = 9.5 Hz, 1H), 7.19 – 7.10 (m, 2H), 6.97 (dd, *J* = 7.6, 0.5 Hz, 1H), 6.42 (d, *J* = 3.2 Hz, 1H), 4.78 – 4.65 (m, 1H), 4.64 – 4.50 (m, 2H), 4.26 (br d, *J* = 12.7 Hz, 1H), 3.92 – 3.81 (m, 1H), 3.80 – 3.69 (m, 6H), 3.42 – 3.34 (m, 1H), 3.27 – 3.17 (m, 2H), 2.85 – 2.79 (m, 1H), 2.75 (t, *J* = 6.7 Hz, 2H), 2.65 – 2.54 (m, 4H), 2.15 – 2.06 (m, 2H), 2.05 – 1.82 (m, 6H), 1.71 – 1.58 (m, 2H), 1.57 – 1.45 (m, 2H); ^{13}C NMR: (151 MHz, DMSO-d_6) δ 170.8, 167.2, 162.4, 161.8, 160.1, 152.0, 144.8, 137.0, 136.4, 135.7, 134.2, 126.3, 125.4, 124.8, 121.0, 117.2, 116.8, 116.4, 115.4, 112.6, 109.0, 103.2, 99.3, 75.6, 60.4, 52.5, 52.0, 48.6, 47.1, 45.0, 44.4, 40.7, 32.8, 31.9, 31.4, 29.8, 29.4; LCMS: (2 min HpH): t_R = 1.10 min, $[M+H]^+$ 793.2, 795.1 (100% purity); HRMS: $C_{41}H_{45}N_{10}O_5$ $[M+H]^+$ requires 793.3336, found $[M+H]^+$ 793.3325 (Δ = -1.39 ppm).

RIPK2 PROTACs

Alkylation D2B

Reactions were carried out according to the Direct-to-Biology Standard Protocol in 1536-well plates.

The following reagents were used for the alkylation library: 0.12 µmol of phenol made up to 1.2 µL with DMSO per well (0.1 M, 1 eq.), 0.13 µmol of alkyl chloride made up to 1.2 µL with DMSO per well (0.11 M, 1.1 eq.), 0.24 µmol of MTBD made up to 1.6 µL with DMSO per well (0.15 M, 2 eq.).

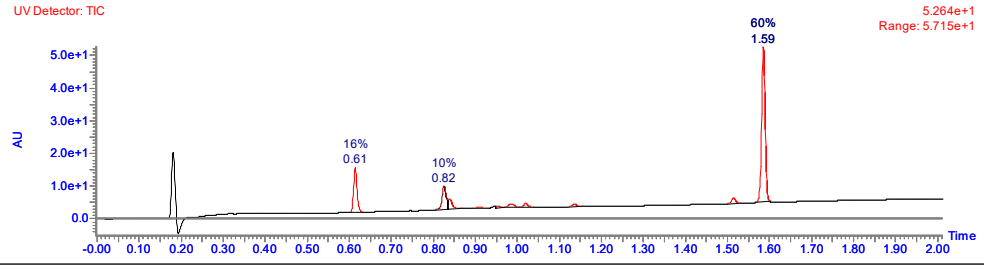
Reactions were carried out at 90 °C at 300 rpm using the thermomixer in the glovebox. 32/32 reactions (100%) were considered to have sufficient conversion to product to be submitted for testing in the RIPK2-HiBiT assay.

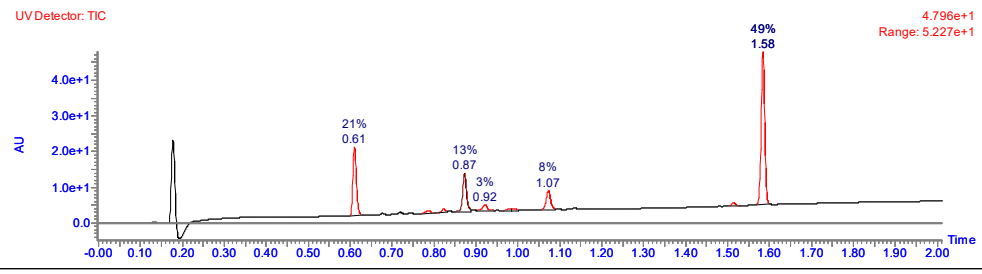
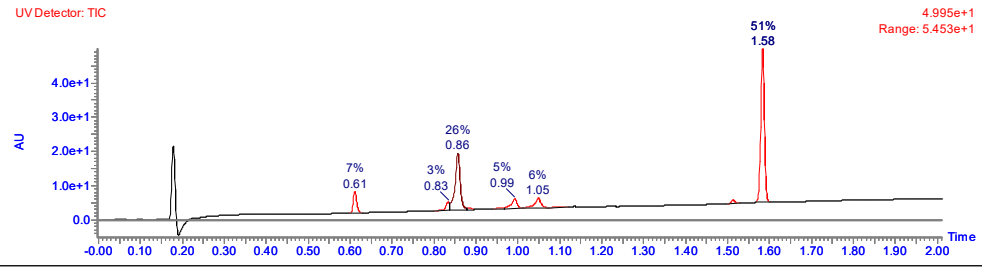
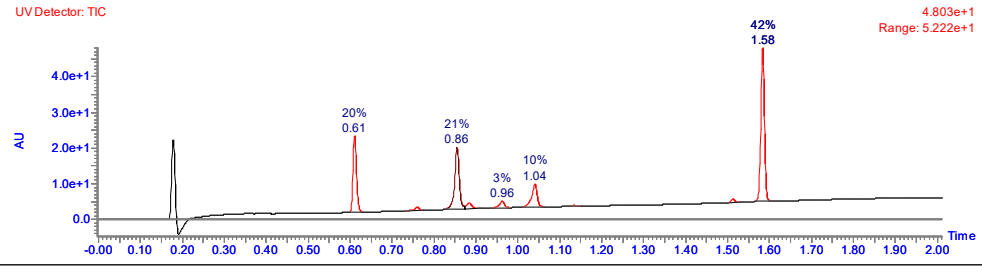
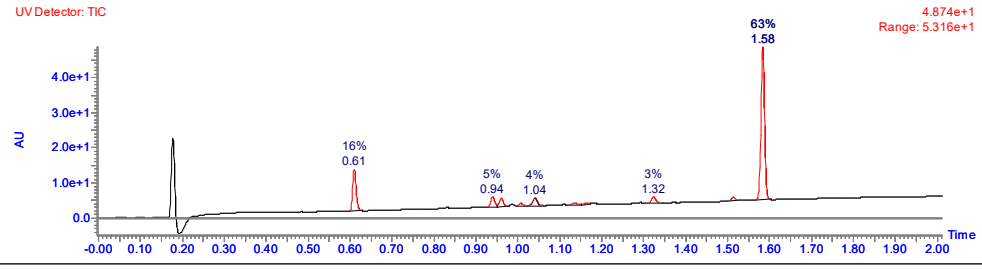
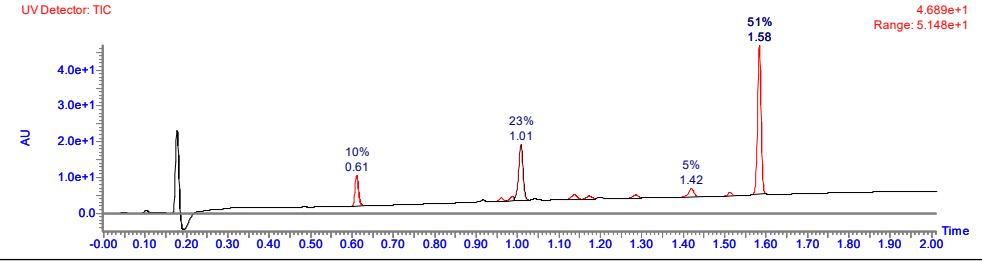
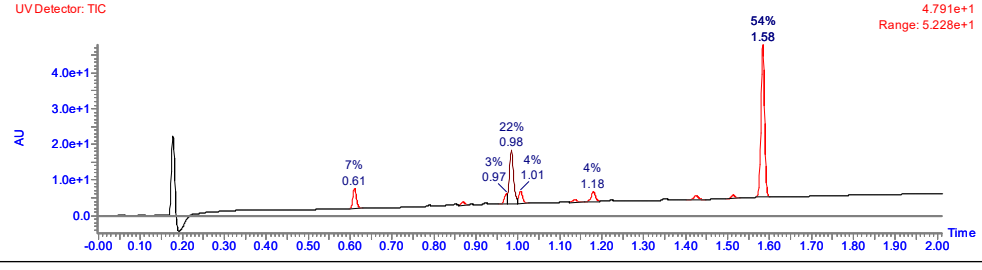
Reaction Plate Layout

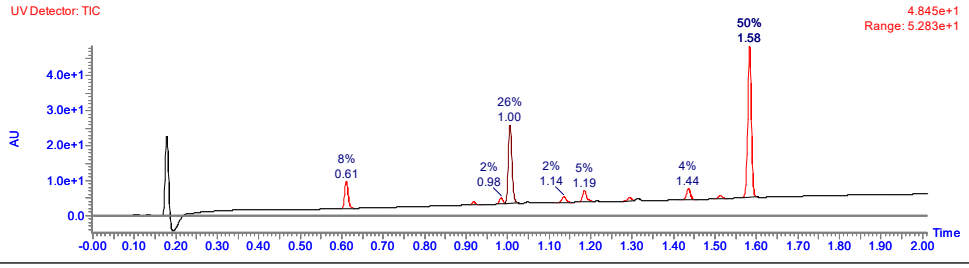
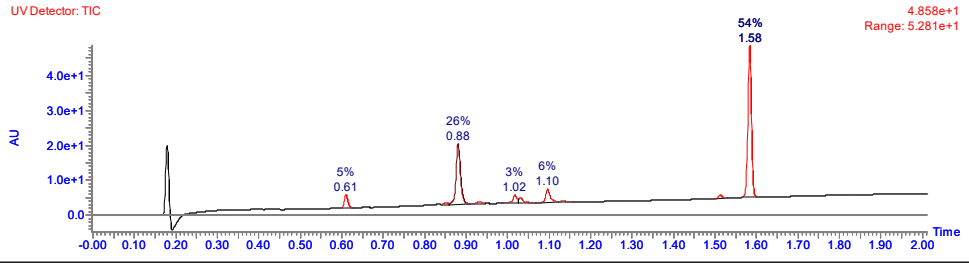
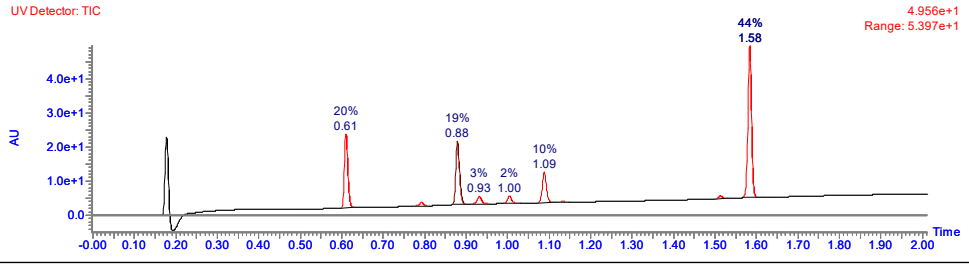
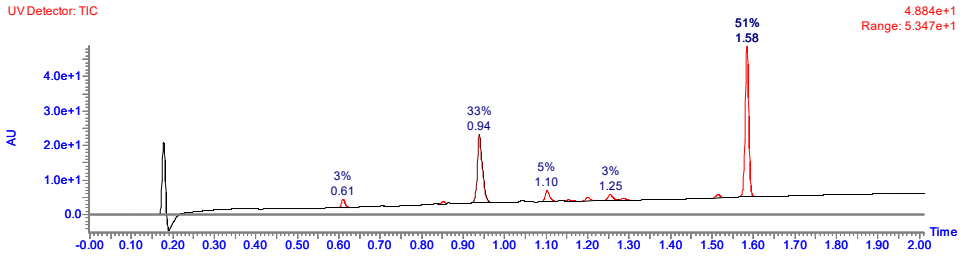
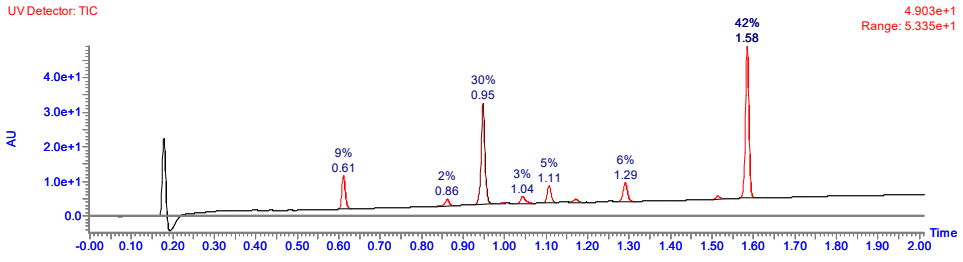
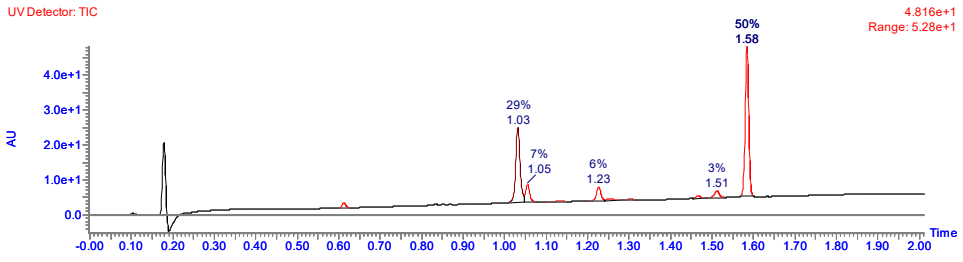
1536-well plate	P2-Et		MTBD	
	1	2	25	26
A	Reaction 1	Reaction 17	Reaction 1	Reaction 17
B	Reaction 2	Reaction 18	Reaction 2	Reaction 18
C	Reaction 3	Reaction 19	Reaction 3	Reaction 19
D	Reaction 4	Reaction 20	Reaction 4	Reaction 20
E	Reaction 5	Reaction 21	Reaction 5	Reaction 21
…through to…				
P	Reaction 16	Reaction 32	Reaction 16	Reaction 32

Table S7. Layout of 1536-well reaction plate; UV traces shown below.

UV Traces

Reaction number	UV trace from LCMS	Purity / %
1 (P2-Et)		10% (0.82 min)

1 (MTBD)		13% (0.87 min)
2 (P2-Et)		26% (0.86 min)
2 (MTBD)		21% (0.86 min)
3 (P2-Et)		4% (1.04 min)
3 (MTBD)		23% (1.01 min)
4 (P2-Et)		22% (0.98 min)

4 (MTBD)		26% (1.00 min)
5 (P2-Et)		26% (0.88 min)
5 (MTBD)		19% (0.88 min)
6 (P2-Et)		33% (0.94 min)
6 (MTBD)		30% (0.95 min)
7 (P2-Et)		29% (1.03 min)

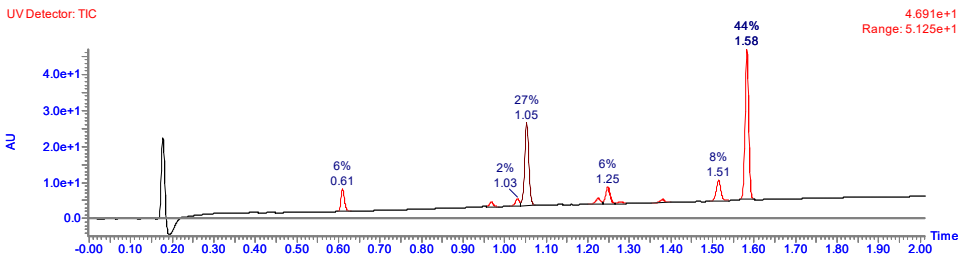
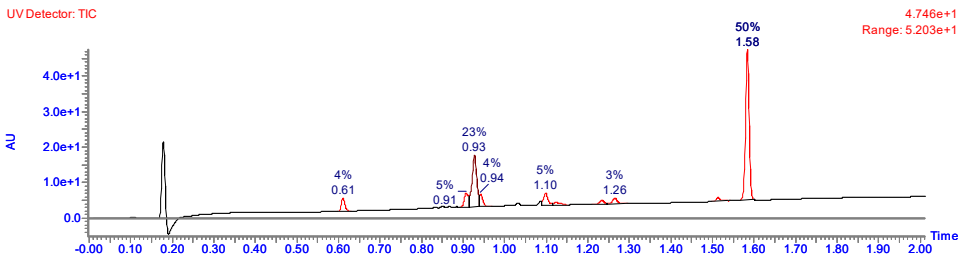
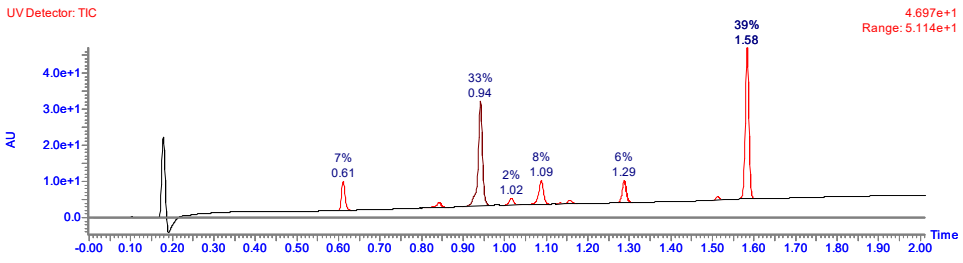
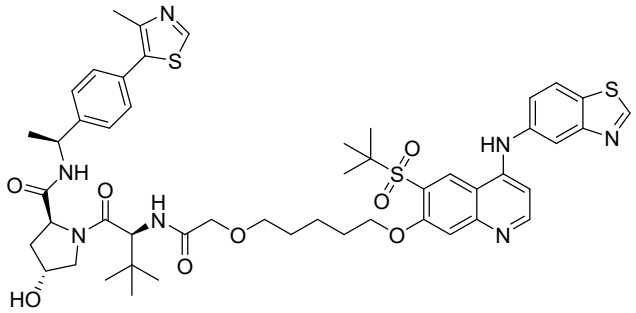
7 (MTBD)		27% (1.05 min)
8 (P2-Et)		23% (0.93 min)
8 (MTBD)		33% (0.94 min)

Table S8. UV traces and product purities from LCMS analysis of crude reaction mixtures are provided for the first eight reactions as examples; reactions deemed successful are coloured in green; note internal standard is added to LCMS at 1.58/1.59 min.

Alkylation Purified Compound

(2*S*,4*R*)-1-((*S*)-2-(2-((5-((4-(Benzo[*d*]thiazol-5-ylamino)-6-(tert-butylsulfonyl)quinolin-7-yl)oxy)pentyl)oxy)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-((*S*)-1-(4-(4-methylthiazol-5-yl)phenyl)ethyl)pyrrolidine-2-carboxamide



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To a solution of VHL ligand with alkyl chloride (1.1 Eq) in DMSO (1.13 mL) was added 4-(benzo[*d*]thiazol-5-ylamino)-6-(tert-butylsulfonyl)quinolin-7-ol (30.0 mg, 1 Eq, 72.55 µmol) and 1-methyl-2,3,4,6,7,8-hexahydro-1*H*-pyrimido[1,2-*a*]pyrimidine (20.8 µL, 2 Eq, 145.10 µmol) and the reaction mixture was

stirred at 90 °C for 20 h. The reaction mixture was purified by automated purification (HpH Method E) and concentrated *in vacuo* to give the title compound (37 mg, 0.036 mmol, 49.1% yield).

^{1}H NMR: (600 MHz, CD_3OD) δ 9.31 (s, 1H), 8.95 (s, 1H), 8.85 (s, 1H), 8.41 (d, *J* = 5.6 Hz, 1H), 8.16 – 8.12 (m, 1H), 8.06 (d, *J* = 1.5 Hz, 1H), 7.56 (dd, *J* = 8.7, 1.9 Hz, 1H), 7.46 (s, 1H), 7.43 – 7.36 (m, 5H), 6.90 (d, *J* = 5.6 Hz, 1H), 5.01 – 4.97 (m, 1H), 4.69 (s, 1H), 4.61 (dd, *J* = 9.0, 7.9 Hz, 1H), 4.45 – 4.43 (m, 1H), 4.31 – 4.25 (m, 2H), 4.06 – 3.96 (m, 2H), 3.84 (br d, *J* = 10.9 Hz, 1H), 3.75 (dd, *J* = 11.3, 3.8 Hz, 1H), 3.66 – 3.62 (m, 2H), 3.33 (dd, *J* = 3.6, 1.7 Hz, 1H), 2.46 (s, 3H), 2.23 – 2.18 (m, 1H), 2.02 – 1.94 (m, 3H), 1.81 – 1.71 (m, 5H), 1.47 (d, *J* = 7.2 Hz, 3H), 1.43 (s, 9H), 1.05 (s, 9H); ^{13}C NMR: (151 MHz, CD_3OD) δ 173.3, 172.1, 171.7, 159.1, 158.6, 155.5, 154.8, 154.2, 153.0, 152.6, 149.2, 145.7, 140.2, 133.5, 132.3, 131.7, 131.6, 130.6, 130.6, 127.8, 125.0, 124.3, 123.8, 118.7, 114.8, 111.6, 110.3, 102.6, 73.0, 71.1, 70.9, 70.5, 69.2, 62.9, 61.0, 60.8, 58.3, 58.2, 50.3, 41.2, 38.9, 37.9, 37.4, 30.4, 30.3, 30.0, 29.9, 27.1, 24.6, 23.9, 22.8, 22.5, 16.0; LCMS: (2 min Formic): t_R = 0.83 min, $[M+H]^+$ 984.4 (100% purity); HRMS: $C_{50}H_{61}N_7O_8S_3$ $[M+H]^+$ requires 984.3817, found $[M+H]^+$ 984.3801 (Δ = -1.63 ppm).