The Advantages of Targeted Protein Degradation over Inhibition: a RTK Case Study

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Supporting Information

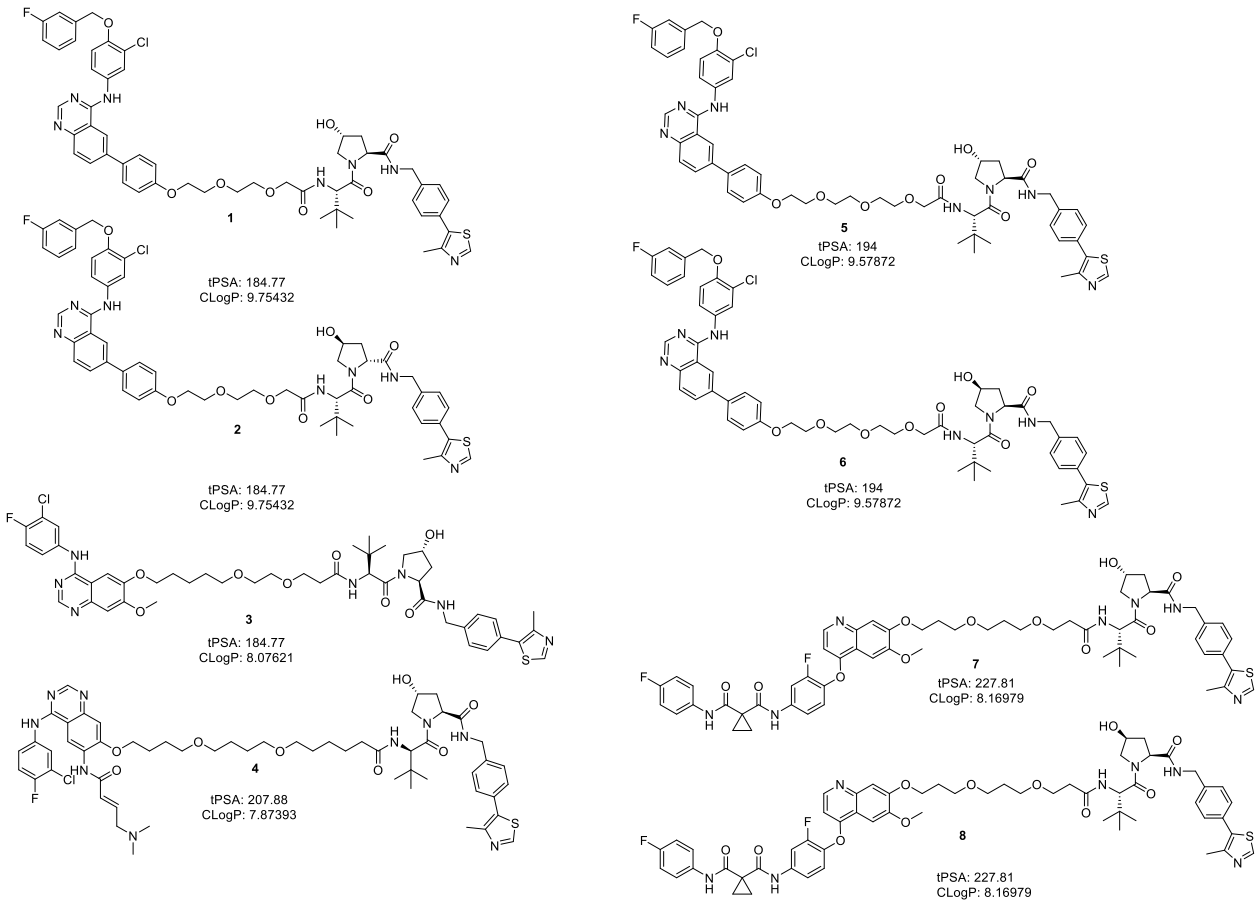


Figure S1 (Related to Figures 1-5) – Structures of Compounds used in this study with calculated total polar surface area and CLogP values. 1 – Lapatinib-based PROTAC (2PEG). 2 – Laptinib-based PROTAC diastereomer (2PEG). 3 – Gefitinib-based PROTAC. 4 – Afatinib-based PROTAC. 5 – Lapatinib-based PROTAC (3PEG). 6 – Lapatinib-based PROTAC diastereomer (3PEG). 7 – Foretinib-based PROTAC. 8 – Foretinib-based PROTAC diastereomer.

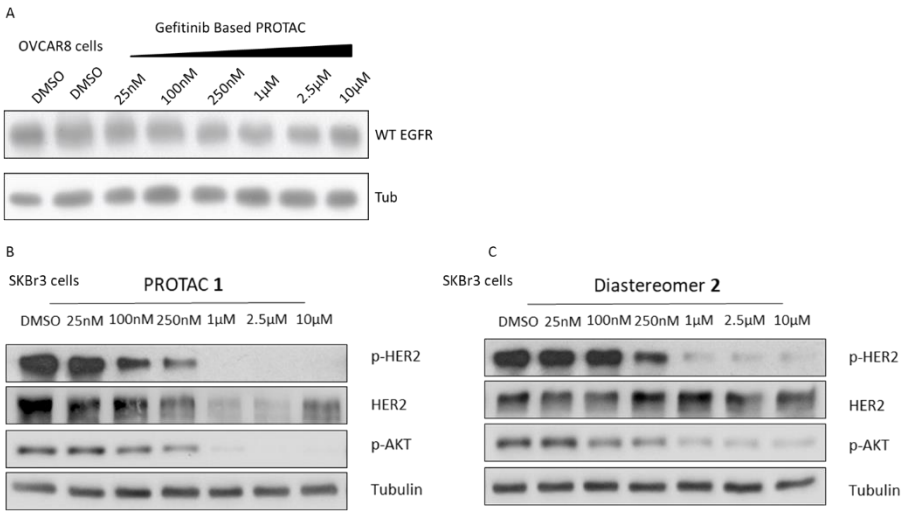


Figure S2 (Related to Figure 1) A – Gefitinib-based PROTAC 3 spares WT EGFR. OVCAR8 Cells were treated for 24 hours with increasing doses of PROTAC 3 or with DMSO control before immunoblotting. B/C – Characterization of PROTAC 1 (B) and diastereomer 2 (C) in SKBr3 cells. Cells were treated for 24 hours in full serum with increasing doses of PROTAC 1 or with diastereomer 2 before immunoblotting.

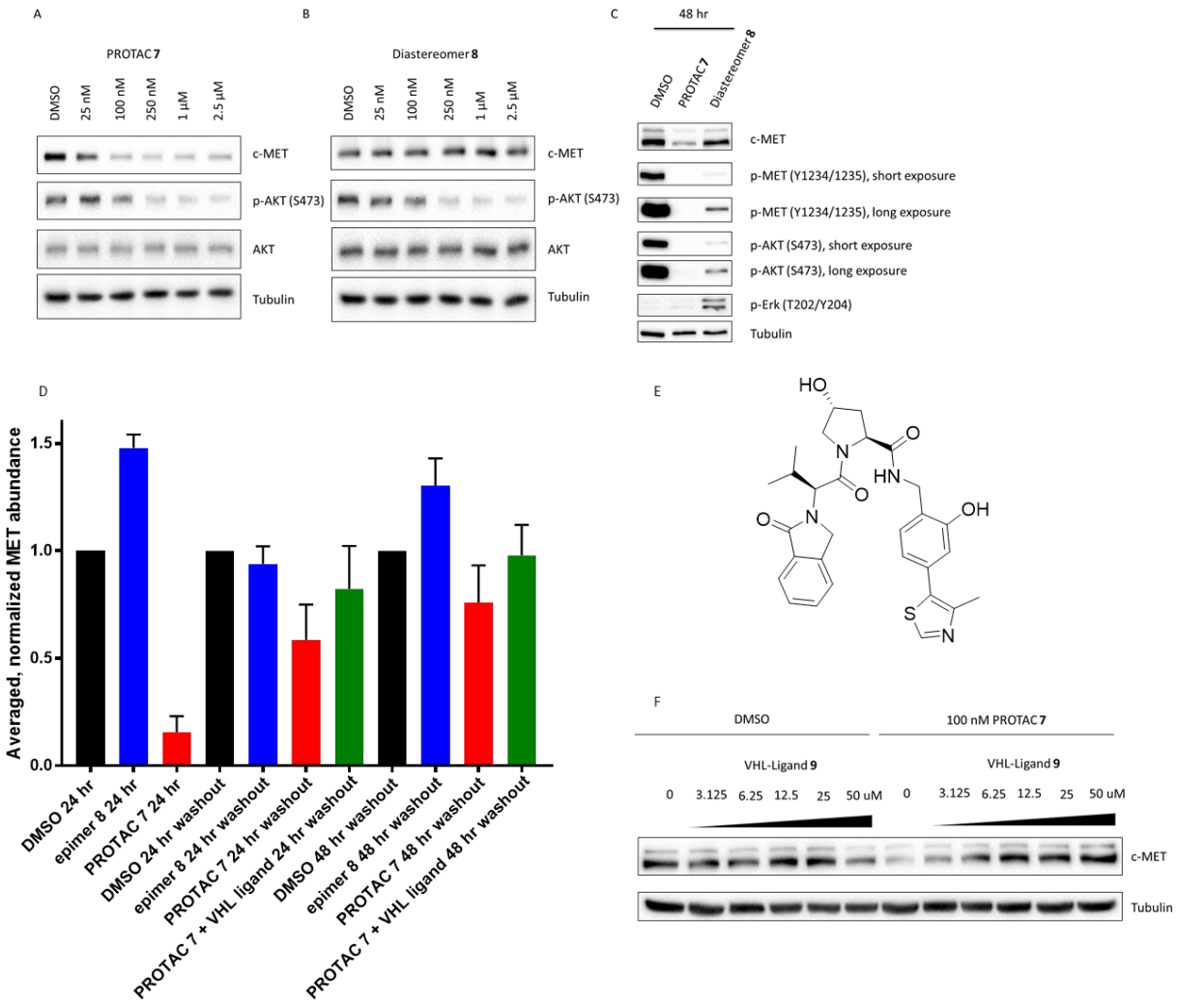


Figure S3 (Related to Figure 3) Characterization of Foretinib-based PROTACs in GTL16 cells. A/B – GTL16 cells were treated with increasing concentrations of PROTAC 7 (A) or diastereomer 8 (B) in media containing full serum for 24 hours before immunoblotting analysis. C - Representative blot of cells treated with 500 nM PROTAC 7 or 500 nM diastereomer 8 for 48 hr before immunoblotting analysis D - Quantification of washout experiments from Fig 3E. c-MET levels normalized to tubulin after treatment with the indicated compounds at the indicated time points. Average of 3 independent repeats and error bars represent S.E.M. E – Structure of VHL-Ligand 9 used in competition experiments. F – Co-treatment competition of PROTAC 7 with VHL-Ligand 9 in MDA-MB-231 cells for 24 hours.

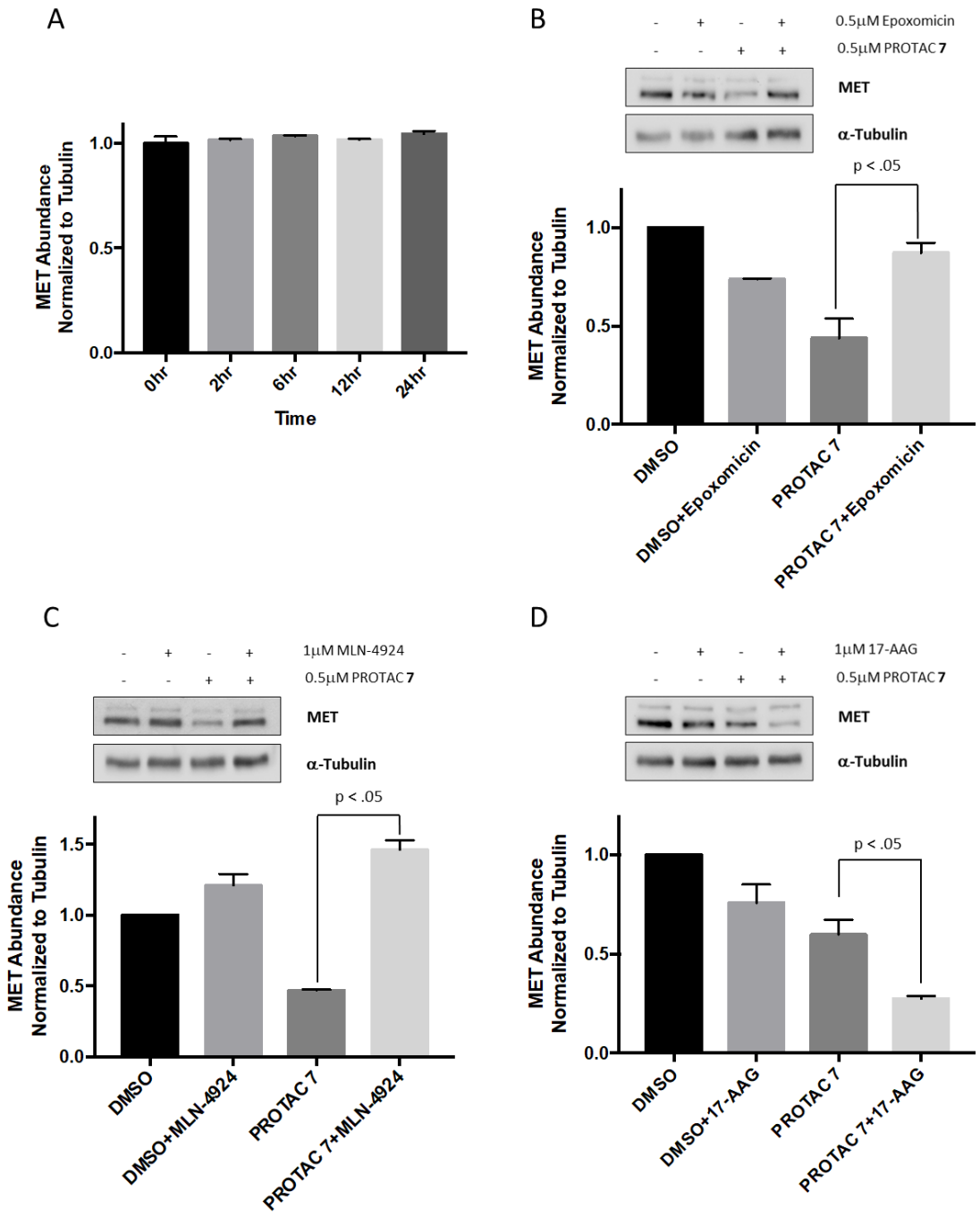


Figure S4 (Related to Figure 4) – A - Quantitative real time PCR was performed at the indicated timepoints after PROTAC treatment (500 nM). Data is normalized to beta-Tubulin. B-D Representative Western blots and quantitation for co-treatment experiments. B - Co-treatment of PROTAC 7 (500 nM) with proteasome inhibitor epoxomicin (500 nM) for 6 hours in MDA-MB-231 cells. Quantified data represent average of 2 repeats. C - Co-treatment of PROTAC 7 (500 nM) with neddylation inhibitor MLN-4924 (1 μM) for 6 hours in MDA-MB-231 cells. Quantified data represent average of 2 repeats. D - Co-treatment of PROTAC 7 (500 nM) with HSP90 inhibitor 17-AAG (1 μM) for 6 hours in MDA-MB-231 cells. Quantified data represent average of 2 repeats.

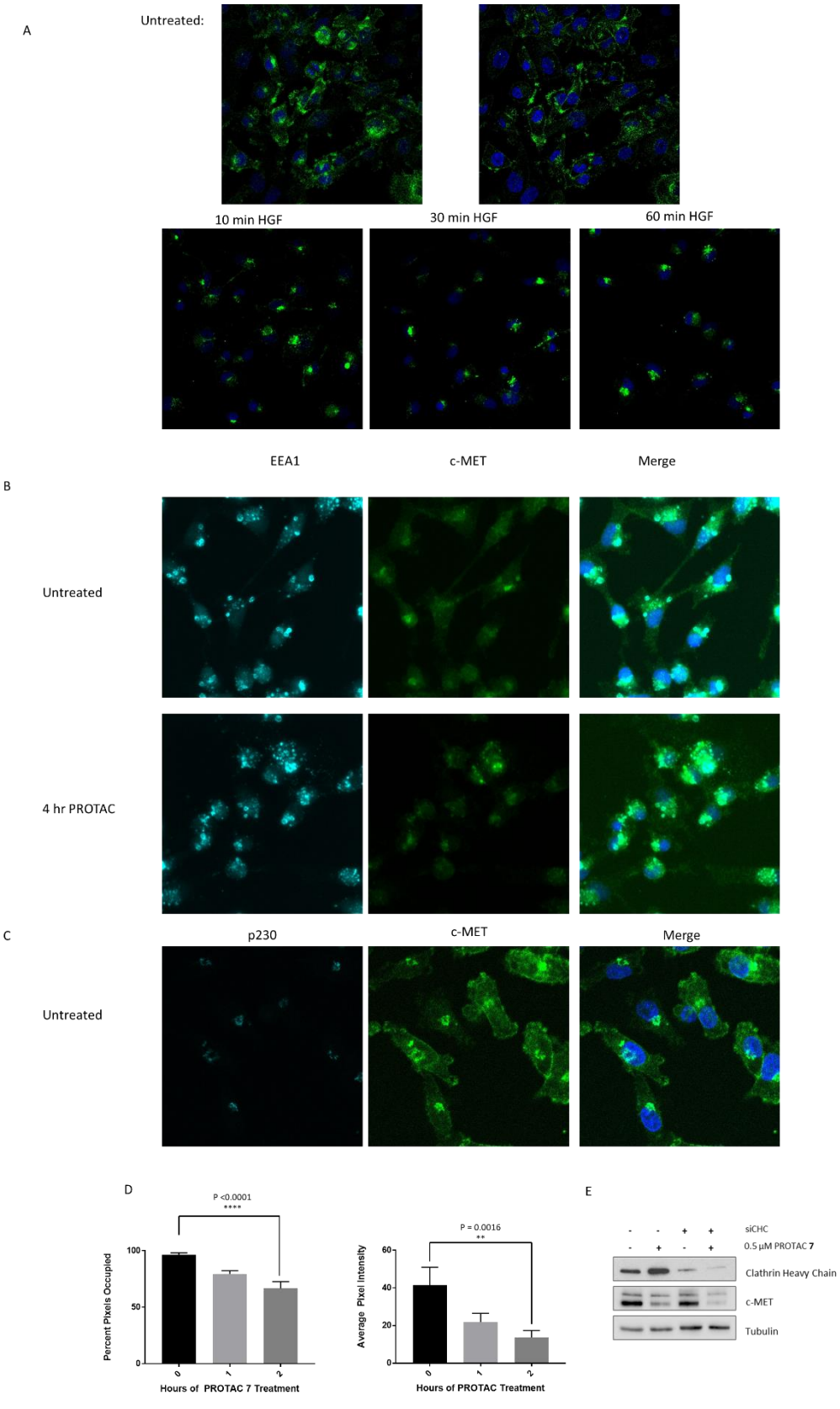
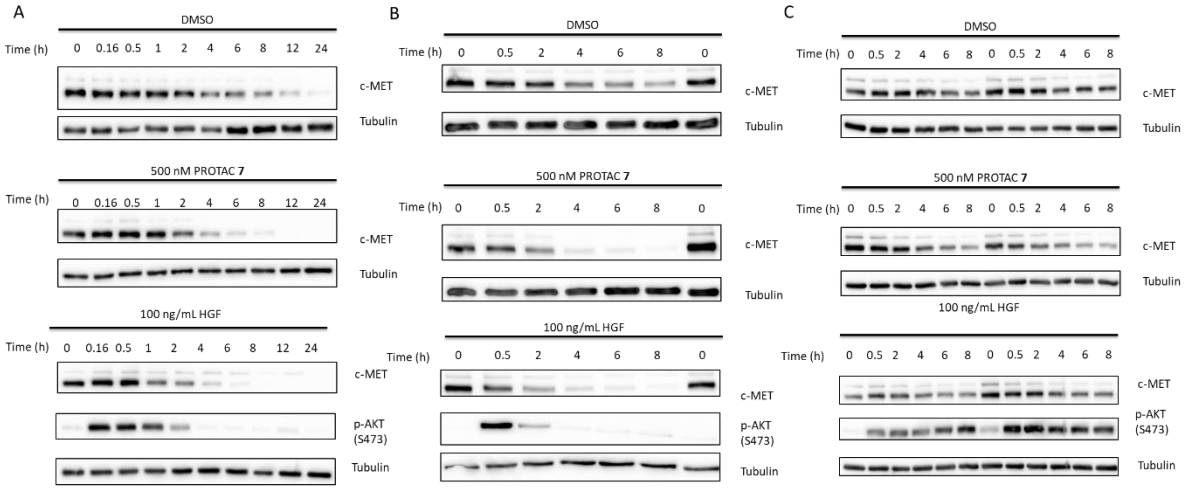

A
Untreated:
10 min HGF
30 min HGF
60 min HGF
EEA1
c-MET
Merge
B
Untreated
4 hr PROTAC
C
p230
c-MET
Merge
Untreated
D
P <0.0001
****
Percent Pixels Occupied
100
50
0
0
1
2
Hours of PROTAC 7 Treatment
P = 0.0016
**
Average Pixel Intensity
60
40
20
0
0
1
2
Hours of PROTAC Treatment
E
siCHC
0.5 μM PROTAC 7
Clathrin Heavy Chain
c-MET
Tubulin

Figure S5 (Related to Figure 4) – A - Representative confocal microscopy images of HGF-mediated internalization of c-Met. MDA-MB-231 cells treated with 100 ng/ml HGF for the indicated times before fixing, permeabilizing, and immunostaining for c-Met. B - Representative confocal microscopy images demonstrating PROTAC-mediated co-localization with early endosome antigen 1 (EEA1). MDA-MB-231 cells treated with 500 nM PROTAC 7 for the indicated times before fixing, permeabilizing, and immunostaining for c-Met and EEA1. C - Representative confocal microscopy images demonstrating c-Met co-localization with p230 (a trans-Golgi marker). D – Quantification of images in Figure 4C. Percentage of cellular pixels occupied by c-Met immunofluorescence and average cellular pixel intensity were used as a proxy for puncta formation and reduction in cell surface c-Met. E – Clathrin heavy chain (CHC) siRNA experiment. MDA-MB-231 cells were transfected with CHC siRNA before treatment with PROTAC 7 for 24 hours prior to lysis and immunoblotting.



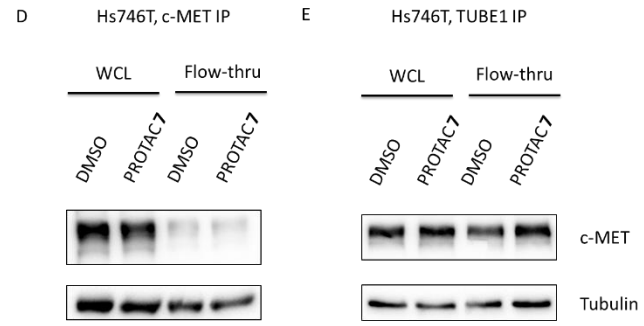
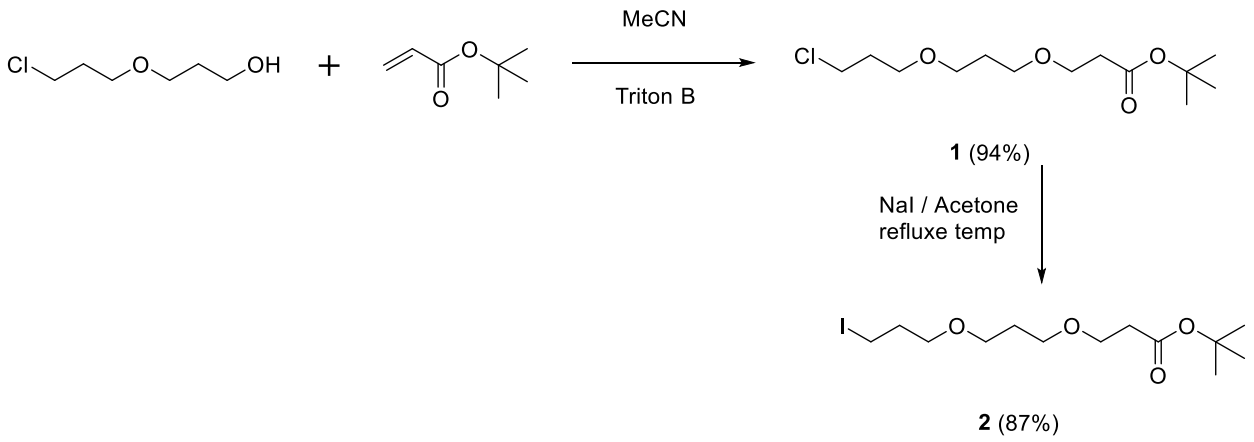


Figure S6 (Related to Figure 5) – Cycloheximide pulse-chase western blots. A – MDA-MB-231 cells were treated with cycloheximide followed by DMSO, PROTAC 7 or HGF and lysed at the indicated incubation times – Set 1. B - MDA-MB-231 cells were treated with cycloheximide followed by DMSO, PROTAC 7 or HGF and lysed at the indicated incubation times – Set 2. C - Hs746T cells were treated with cycloheximide followed by DMSO, PROTAC 7 or HGF and lysed at the indicated incubation times. D – c-Met immunoprecipitation experiments.Hs746T cells were treated with 2 uM epoxomicin for 30 minutes before the addition of PROTAC 7 for 4 hours prior to c-Met immunoprecipitation. (WCL = Whole-cell lysate). E – Hs746T cells were treated as in D prior to TUBE1 immunoprecipitation experiments..

Methods S1

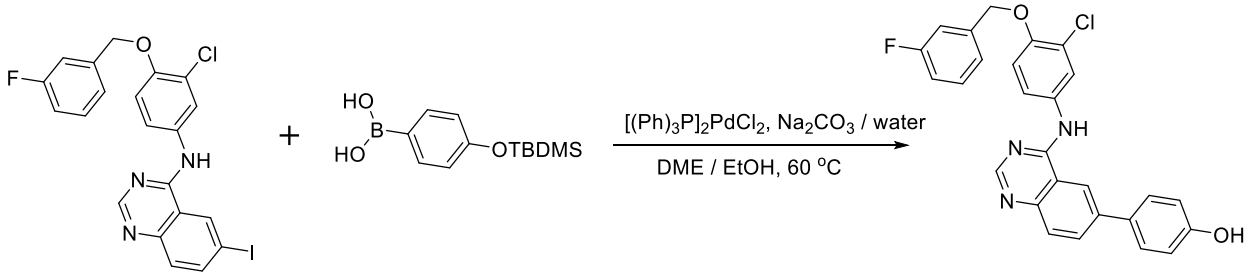
Synthesis of PROTAC 1 and 2



***tert*-Butyl 3-(3-(3-chloropropoxy)propoxy)propanoate (1).** 3-(3-chloropropoxy)propan-1-ol (66 mg, 0.43 mmol) in acetonitrile (3 mL) was added tert-butyl prop-2-enoate (0.31 ml, 2.16 mmol) followed by Triton B (54 mg, 0.1 mmol, 40% by weight in water). The mixture was stirred at room temperature for 72 hour. The mixture was concentrated under vacuum and crude product was purified by column chromatography (SiO_2, gradient Hex:EtOAc, 95:5 to 9:1) to give 115 mg of product (**1**) as an oil (94% yield). ^{1}H NMR (500 MHz, Chloroform-d) δ 3.70 – 3.59 (m, 4H), 3.59 – 3.42 (m, 6H), 2.47 (t, J = 6.5 Hz, 2H), 2.04 – 1.96 (m, 2H), 1.82 (p, J = 6.3 Hz, 2H), 1.45 (s, 9H). ^{13}C NMR (151 MHz, Chloroform-d) δ 171.13, 80.63, 68.02, 67.97, 67.27, 66.64, 42.17, 36.50, 32.88, 30.09, 28.25. LC-MS (ESI); m/z $[M+Na]^+$: Calcd. for $C_{13}H_{25}ClO_4Na$, 303.1339. Found 303.1381.

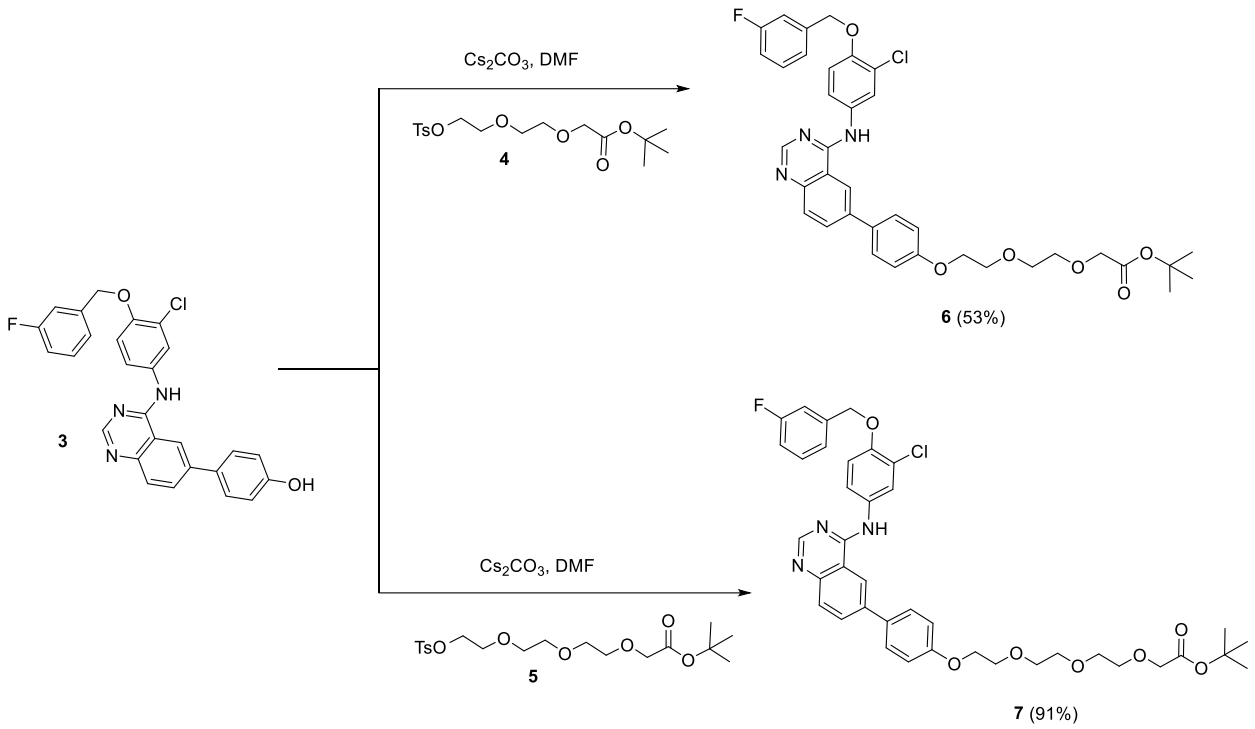
***tert*-Butyl 3-(3-(3-iodopropoxy)propoxy)propanoate (2)**. To a solution of tert-butyl 3-[3-(3-chloropropoxy)propoxy]propanoate (161 mg, 0.57 mmol) in Acetone (5 ml) was added NaI (429 mg, 2.87 mmol). The reaction mixture was stirred at reflux temperature for 24 h, then the solvent was removed under vacuum and crude product was dissolved in EtOAc (15 mL), washed with water (10 mL), and with an aqueous solution of Na_2SO_3 (10%, 10 mL). Organic layer was separated, washed with water (10 mL), dried

(Na_2SO_4) and evaporated under vacuum. Crude product was pure by NMR (>98% purity, 186 mg, 87% yield), product (**2**) was used in the next step without any further purification. ^{1}H NMR (400 MHz, Chloroform-d) δ 3.66 (t, J = 6.5 Hz, 2H), 3.57 – 3.40 (m, 6H), 3.27 (t, J = 6.8 Hz, 2H), 2.48 (t, J = 6.5 Hz, 2H), 2.08 – 1.99 (m, 2H), 1.82 (p, J = 6.4 Hz, 2H), 1.45 (s, 9H). ^{13}C NMR (151 MHz, Chloroform-d) δ 171.13, 80.64, 70.18, 68.01, 67.98, 66.65, 36.50, 33.57, 30.10, 28.26, 3.72. LC-MS (ESI): m/z $[M+Na]^+$ Calcd. for $C_{13}H_{25}IO_4Na$: 395.0695, Found: 395.0719.



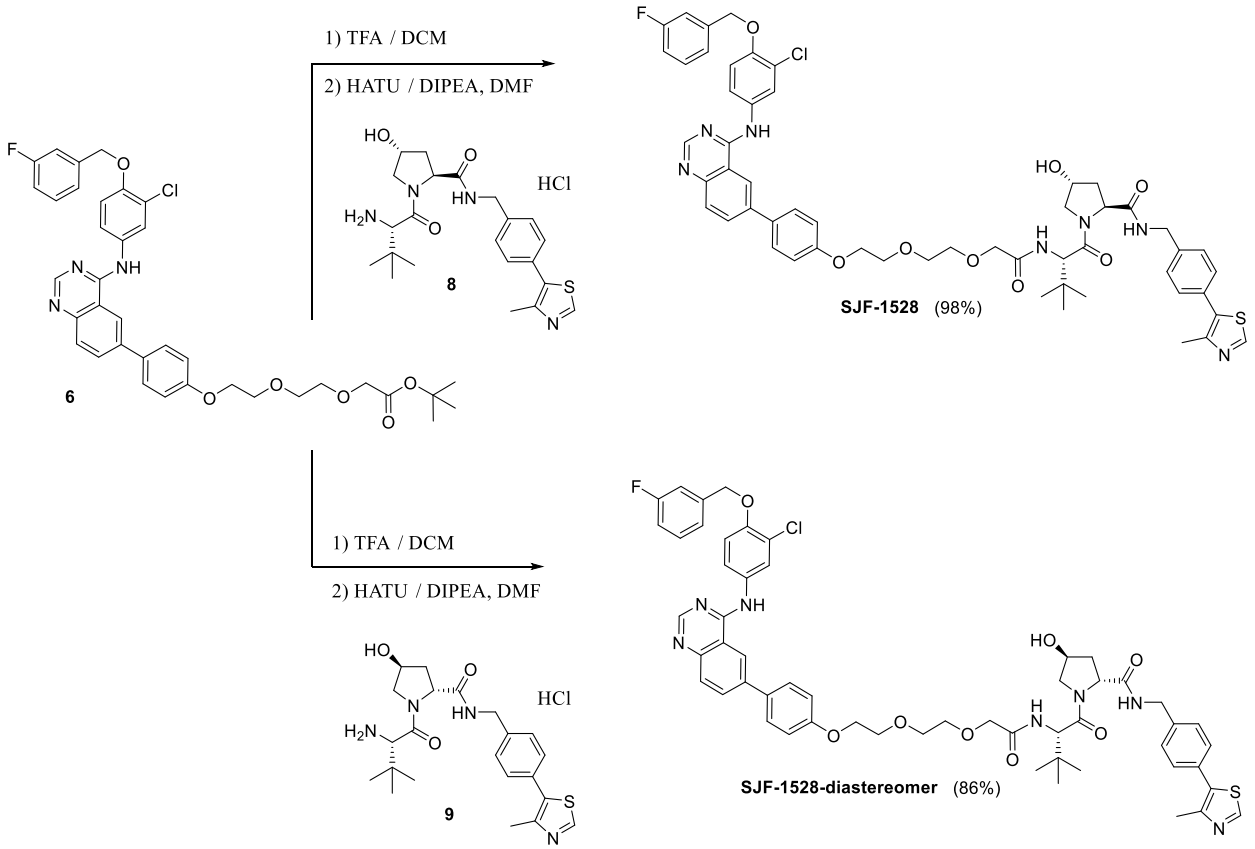
3 (75%)

4-(4-((3-Chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)quinazolin-6-yl)phenol (3). A suspension of N-[3-chloro-4-[(3-fluorophenyl)methoxy]phenyl]-6-iodo-quinazolin-4-amine (300 mg, 0.59 mmol) in a mixture of 1,2-Dimethoxyethane (12 ml) and Ethanol (8 ml) was evacuated in vacuum and purged with argon (5x), then 2M Na_2CO_3 in water (6.5 ml) was added and the reaction mixture was again evacuated in vacuum and purged with argon (5x), then [4-[tert-butyl(dimethyl)silyl]oxyphenyl]boronic acid (209 mg, 0.831 mmol) was added into, and $[(Ph)_3P]_2PdCl_2$ (70 mg, 0.08 mmol). The reaction mixture was heated to 60 °C for 3h. The reaction mixture was cooled to room temperature and the reaction mixture was poured into an aqueous saturated solution of $NaHCO_3$ (30 mL) and product was extracted with AcOEt (2x30 mL). Organic extracts were combined, dried (Na_2SO_4), filtered over a celite pad, and evaporated under vacuum. Crude product was purified by flash chromatography (SiO_2-25g. dry silica-dispersion loading, gradient Hex:AcOEt, 9:1 to 100% AcOEt in 15 min) to give 210 mg of product (75% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ 9.85 (s, 1H), 9.68 (s, 1H), 8.69 (s, 1H), 8.56 (s, 1H), 8.12 (d, J = 8.7 Hz, 1H), 8.03 (s, 1H), 7.87 – 7.67 (m, 4H), 7.48 (td, J = 8.0, 6.0 Hz, 1H), 7.39 – 7.25 (m, 3H), 7.19 (tt, J = 7.8, 1.4 Hz, 1H), 6.93 (d, J = 8.6 Hz, 2H), 5.27 (s, 2H). ^{13}C NMR (151 MHz, dmso) δ 163.06, 161.44, 157.66, 157.59, 154.07, 149.69, 148.44, 139.73, 139.69, 138.23, 133.18, 131.45, 130.69, 130.64, 129.86, 128.36, 128.32, 124.20, 123.45, 123.43, 122.38, 121.01, 118.95, 115.88, 115.33, 114.87, 114.73, 114.28, 114.21, 114.07, 69.37. LC-MS (ESI): m/z $[M+H]^+$ Calcd. for $C_{27}H_{20}ClFN_3O_2$, 472.1228. Found 472.1283.



tert-Butyl 2-(2-(2-(4-(4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)quinazolin-6-yl)phenoxy)-ethoxy)ethoxy)acetate (6). To a mixture of 4-[4-[3-chloro-4-[(3-fluorophenyl)methoxy]anilino]-quinazolin-6-yl]phenol (10.4 mg, 0.022 mmol) and tert-butyl 2-[2-[2-(p-tolylsulfonyloxy)ethoxy]ethoxy]-acetate (**4**) (10.8 mg, 0.03 mmol) in N,N-Dimethylformamide (2 mL) was added Cs_2CO_3 (21.7 mg, 0.067 mmol). Reaction mixture was heated at 50 ^oC for 6 h. Reaction mixture was diluted with AcOEt (20 mL), washed with water (4x15 mL), dried Na_2SO_4 and evaporated under vacuum. Crude product was purified by PTLC (DCM:MeOH:NH_4OH, 92:7:1) to give 8 mg of pure product (**6**) (53% yield). ^1H NMR (500 MHz, DMSO-d6) δ 9.88 (s, 1H), 8.74 (s, 1H), 8.57 (s, 1H), 8.16 (d, J = 8.7 Hz, 1H), 8.03 (s, 1H), 7.83 (dd, J = 8.7, 6.6 Hz, 3H), 7.76 (dd, J = 8.9, 2.6 Hz, 1H), 7.52 – 7.40 (m, 1H), 7.38 – 7.25 (m, 3H), 7.21 – 7.15 (m, 1H), 7.13 (d, J = 8.8 Hz, 2H), 5.26 (s, 2H), 4.26 – 4.12 (m, 2H), 4.01 (s, 2H), 3.83 – 3.71 (m, 2H), 3.63 (s, 4H), 1.42 (s, 9H).^{13}C NMR (151 MHz, dmso) δ 169.37, 163.01, 161.39, 158.54, 157.60, 154.15, 149.70, 148.58, 139.69, 139.64, 137.71, 133.15, 131.46, 131.44, 130.60, 130.55, 128.33, 128.25, 124.19, 123.36, 123.34, 122.36, 121.03, 119.29, 115.29, 115.00, 114.78, 114.64, 114.30, 114.12, 113.98, 80.66, 69.88, 69.40, 69.38, 68.91, 68.12, 67.28, 27.77. LC-MS (ESI): m/z $[M+H]^+$ Calcd. for $C_{37}H_{38}ClFN_3O_6$, 674.2433. Found 674.2411.

tert-Butyl 2-(2-(2-(2-(4-(4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)quinazolin-6-yl)phenoxy)-ethoxy)ethoxy)ethoxy)acetate (7). To a mixture of 4-[4-[3-chloro-4-[(3-fluorophenyl)methoxy]anilino]-quinazolin-6-yl]phenol (7.2 mg, 0.015 mmol) and tert-butyl 2-[2-[2-[2-(p-tolylsulfonyloxy)ethoxy]ethoxy] -ethoxy]-acetate (8.3 mg, 0.02 mmol) in N,N-Dimethylformamide (2 mL) was added Cs_2CO_3 (14.91 mg, 0.05 mmol). Reaction mixture was heated at 50 ^oC for 2 h. Reaction mixture was diluted with AcOEt (20 mL), washed with water (4x15 mL), dried Na_2SO_4 and evaporated under vacuum. Crude product was purified by PTLC (DCM:MeOH:NH_4OH, 92:7:1) to give 10 mg of product (**7**) (91% yield). ^1H NMR (500 MHz, DMSO-d6) δ 9.87 (s, 1H), 8.74 (d, J = 2.1 Hz, 1H), 8.57 (s, 1H), 8.16 (dd, J = 8.8, 1.9 Hz, 1H), 8.03 (s, 1H), 7.83 (dd, J = 8.6, 5.7 Hz, 3H), 7.76 (dd, J = 9.0, 2.6 Hz, 1H), 7.47 (td, J = 8.0, 6.1 Hz, 1H), 7.38 – 7.25 (m, 3H), 7.22 – 7.14 (m, 1H), 7.13 (d, J = 8.8 Hz, 2H), 5.27 (s, 2H), 4.23 – 4.13 (m, 2H), 3.98 (s, 2H), 3.84 – 3.74 (m, 2H), 3.68 – 3.46 (m, 8H), 1.41 (s, 9H). ^{13}C NMR (151 MHz, DMSO-d6) δ 169.36, 163.01, 161.39, 158.54, 157.60, 154.15, 149.70, 148.57, 139.69, 139.64, 137.72, 133.14, 131.44, 130.61, 130.55, 128.32, 128.25, 124.19, 123.36, 123.34, 122.37, 121.03, 119.29, 115.28, 115.02, 114.78, 114.64, 114.30, 114.13, 113.98, 80.64, 69.95, 69.86, 69.78, 69.72, 69.38, 68.95, 68.09, 67.29, 27.76. LC-MS (ESI): m/z $[M+H]^+$ Calcd. For $C_{39}H_{42}ClFN_3O_7$, 718.2695. Found 718.3026.

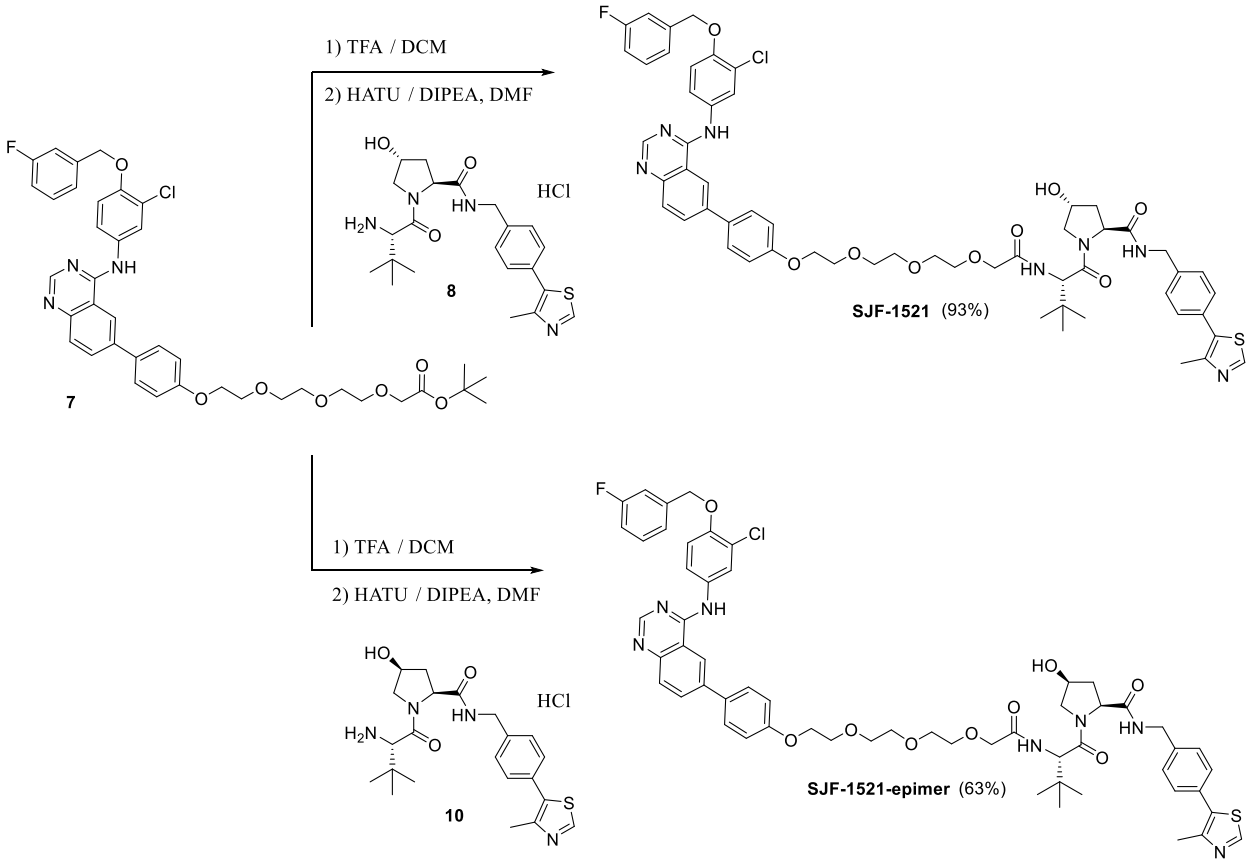


(2S,4R)-1-((S)-2-(2-(2-(2-(4-(4-((3-Chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)quinazolin-6-yl)phenoxy)ethoxy)ethoxy)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (SJF-1528, PROTAC 1). A solution of tert-butyl 2-[2-[2-[4-[4-[3-chloro-4-[(3-fluorophenyl)methoxy]anilino]quinazolin-6-yl] -phenoxy]ethoxy]ethoxy]acetate (**6**) (8 mg, 0.01 mmol) in a mixture of TFA (1 ml, 13.46 mmol) and Dichloromethane (3 ml) was stirred for 2 h. Then the solvent was removed under vacuum and crude product was dried under high vacuum for 2 h. The crude product was used in the next step without any further purification (7.3 mg, quantitative yield). LC-MS (ESI): m/z $[M+H]^+$ Calcd. for $C_{33}H_{30}ClFN_3O_6$, 618.1807. Found 618.1917. To a solution of 2-[2-[2-[4-[4-[3-chloro-4-[(3-fluorophenyl)methoxy]anilino]quinazolin-6-yl]phenoxy]- ethoxy]ethoxy]acetic acid (7.3 mg, 0.01 mmol), and (2S,4R)-1-[(2S)-2-amino-3,3-dimethyl-butanoyl]-4-hydroxy-N-[[4-(4-methylthiazol-5-yl)phenyl] methyl]pyrrolidine-2-carboxamide;hydrochloride (**8**) (8.27 mg, 0.02 mmol) in N,N-Dimethylformamide (2 ml) was added DIPEA (0.2 ml, 1.14 mmol) and HATU (8.98 mg, 0.02 mmol) at room temperature. The reaction mixture was stirred for 12 h (overnight) at the same temperature. Reaction mixture was diluted with AcOEt (20 mL), washed with water (4x15 mL), dried (Na_2SO_4) and evaporated under vacuum. Crude product was purified by PTLC (DCM:MeOH:NH_4OH, 92:7:1), to give 12 mg of the expected product(**SJF-1528**) (98 % yield).^{1}H NMR (500 MHz, DMSO-d6) δ 9.87 (s, 1H), 8.95 (s, 1H), 8.72 (s, 1H), 8.57 (d, J = 2.6 Hz, 2H), 8.13 (dd, J = 8.7, 1.9 Hz, 1H), 8.02 (d, J = 2.4 Hz, 1H), 7.81 (dd, J = 8.6, 4.9 Hz, 3H), 7.76 (dd, J = 9.0, 2.6 Hz, 1H), 7.55 – 7.24 (m, 9H), 7.18 (t, J = 8.6 Hz, 1H), 7.10 (d, J = 8.7 Hz, 2H), 5.26 (s, 2H), 5.15 (d, J = 3.4 Hz, 1H), 4.58 (d, J = 9.6 Hz, 1H), 4.50 – 4.22 (m, 5H), 4.19 (t, J = 4.6 Hz, 2H), 4.00 (s, 2H), 3.89 – 3.77 (m, 2H), 3.75 – 3.54 (m, 6H), 2.41 (s, 3H), 2.11 – 2.01 (m, 1H), 1.95 – 1.87 (m, 1H), 0.95 (s, 9H).^{13}C NMR (151 MHz, DMSO-d6) δ 171.75, 169.16, 168.62, 163.01, 161.40, 158.51, 157.60, 154.15, 151.43, 149.70, 148.58, 147.72, 139.69, 139.64, 139.40, 137.73, 133.15, 131.43, 131.12, 130.61, 130.56, 129.69, 128.67, 128.32, 128.24, 128.15, 127.43, 124.18, 123.37, 123.35, 122.35, 121.04, 119.28, 115.29, 114.99, 114.79, 114.65, 114.30, 114.13, 113.99, 70.48, 69.79, 69.63, 69.40, 69.02, 68.89, 67.21, 58.76, 56.62, 55.73, 41.69, 37.96, 35.74, 26.21, 15.92. LC-MS (ESI): m/z $[M+H]^+$ Calcd. for $C_{55}H_{58}ClFN_7O_8S$, 1030.3740. Found 1030.4004.

(2R,4S)-1-((S)-2-(2-(2-(2-(4-(4-((3-Chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)quinazolin-6-yl)phenoxy)ethoxy)ethoxy)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**SJF-1528-Diasteromer, PROTAC 2**). It was prepared from (2R,4S)-1-((S)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-

yl)benzyl)pyrrolidine-2-carboxamide hydrochloride (**9**) and following the same procedure than above. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.62 (s, 1H), 8.54 (s, 1H), 7.96 (dd, *J* = 8.7, 1.7 Hz, 1H), 7.91 (d, *J* = 8.6 Hz, 1H), 7.84 (dd, *J* = 8.8, 2.6 Hz, 1H), 7.71 (d, *J* = 2.5 Hz, 1H), 7.56 (t, *J* = 6.8 Hz, 3H), 7.34 (dd, *J* = 8.0, 6.2 Hz, 2H), 7.22 (t, *J* = 9.3 Hz, 2H), 7.14 (t, *J* = 8.3 Hz, 4H), 7.07 – 6.99 (m, 3H), 6.96 (d, *J* = 8.9 Hz, 1H), 5.14 (s, 2H), 4.88 (dd, *J* = 8.7, 5.0 Hz, 1H), 4.62 (p, *J* = 5.4 Hz, 1H), 4.36 (dt, *J* = 15.4, 7.3 Hz, 2H), 4.29 (d, *J* = 6.6 Hz, 1H), 4.17 – 4.06 (m, 5H), 3.91 – 3.81 (m, 3H), 3.73 – 3.58 (m, 4H), 3.47 – 3.36 (m, 2H), 2.41 (s, 3H), 2.38 (t, *J* = 5.3 Hz, 1H), 2.27 (ddd, *J* = 13.8, 8.7, 5.9 Hz, 1H), 1.12 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.85, 171.65, 170.60, 163.79, 162.16, 158.70, 158.07, 154.36, 150.50, 150.20, 148.29, 139.19, 139.14, 139.01, 137.91, 133.24, 132.47, 131.70, 131.65, 130.41, 130.18, 130.12, 129.14, 128.38, 127.54, 124.48, 122.92, 122.45, 122.44, 122.00, 119.24, 115.69, 115.55, 114.94, 114.80, 114.24, 114.04, 113.89, 71.39, 70.69, 70.44, 70.43, 70.17, 69.68, 67.94, 59.67, 58.55, 54.89, 42.89, 38.16, 33.85, 26.66, 16.10. LC-MS (ESI): m/z $[M+H]^+$: Calcd. for $C_{55}H_{58}ClFN_7O_8S$, 1030.3740. Found 1030.3821.

Synthesis of PROTAC 5 and 6

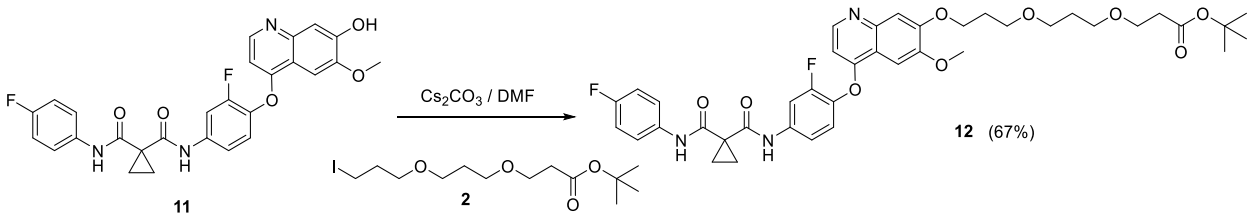


(2S,4R)-1-((S)-2-(tert-Butyl)-14-(4-(4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)quinazolin-6-yl)phenoxy)-4-oxo-6,9,12-trioxa-3-azatetradecanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**SJF-1521, PROTAC 5**). It was prepared from (2R,4S)-1-((S)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide hydrochloride (**8**) and tert-butyl 2-(2-(2-(2-(4-(4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)-quinazolin-6-yl)phenoxy)ethoxy)ethoxy)ethoxy)acetate (**7**), following the same procedure than above (93% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ 9.87 (s, 1H), 8.95 (s, 1H), 8.72 (s, 1H), 8.59 (t, J = 6.0 Hz, 1H), 8.55 (s, 1H), 8.13 (dd, J = 8.7, 1.9 Hz, 1H), 8.00 (d, J = 2.5 Hz, 1H), 7.89 – 7.76 (m, 3H), 7.73 (dd, J = 9.0, 2.6 Hz, 1H), 7.54 – 7.23 (m, 8H), 7.22 – 7.12 (m, 1H), 7.09 (d, J = 8.8 Hz, 2H), 5.24 (s, 2H), 5.15 (d, J = 3.5 Hz, 1H), 4.55 (d, J = 9.6 Hz, 1H), 4.48 – 4.18 (m, 5H), 4.18 – 4.06 (m, 2H), 3.95 (s, 2H), 3.80 – 3.69 (m, 2H), 3.69 – 3.51 (m, 8H), 2.41 (s, 3H), 2.08 – 2.00 (m, 1H), 1.93 – 1.82 (m, 1H), 0.92 (s, 9H).^{13}C NMR (151 MHz, DMSO-d6) δ 171.76, 169.12, 168.59, 163.01, 161.39, 158.53, 157.60, 154.15, 151.45, 149.70, 148.57, 147.73, 139.68, 139.63, 139.42, 137.72, 133.14, 131.44, 131.42, 131.13, 130.60, 130.55, 129.68, 128.68, 128.32, 128.24, 127.44, 124.19, 123.36, 123.34, 122.36, 121.03, 119.27, 115.28, 114.99, 114.78, 114.64, 114.29, 114.13, 113.98, 70.48, 69.97, 69.90, 69.63, 69.59, 69.38, 68.95, 68.88, 67.26, 58.75, 56.60, 55.69, 41.68, 37.94, 35.73, 26.19, 15.93. LC-MS (ESI): m/z $[M+H]^+$: Calcd. for $C_{57}H_{62}ClFN_7O_9S$, 1074.4002. Found 1074.4285.

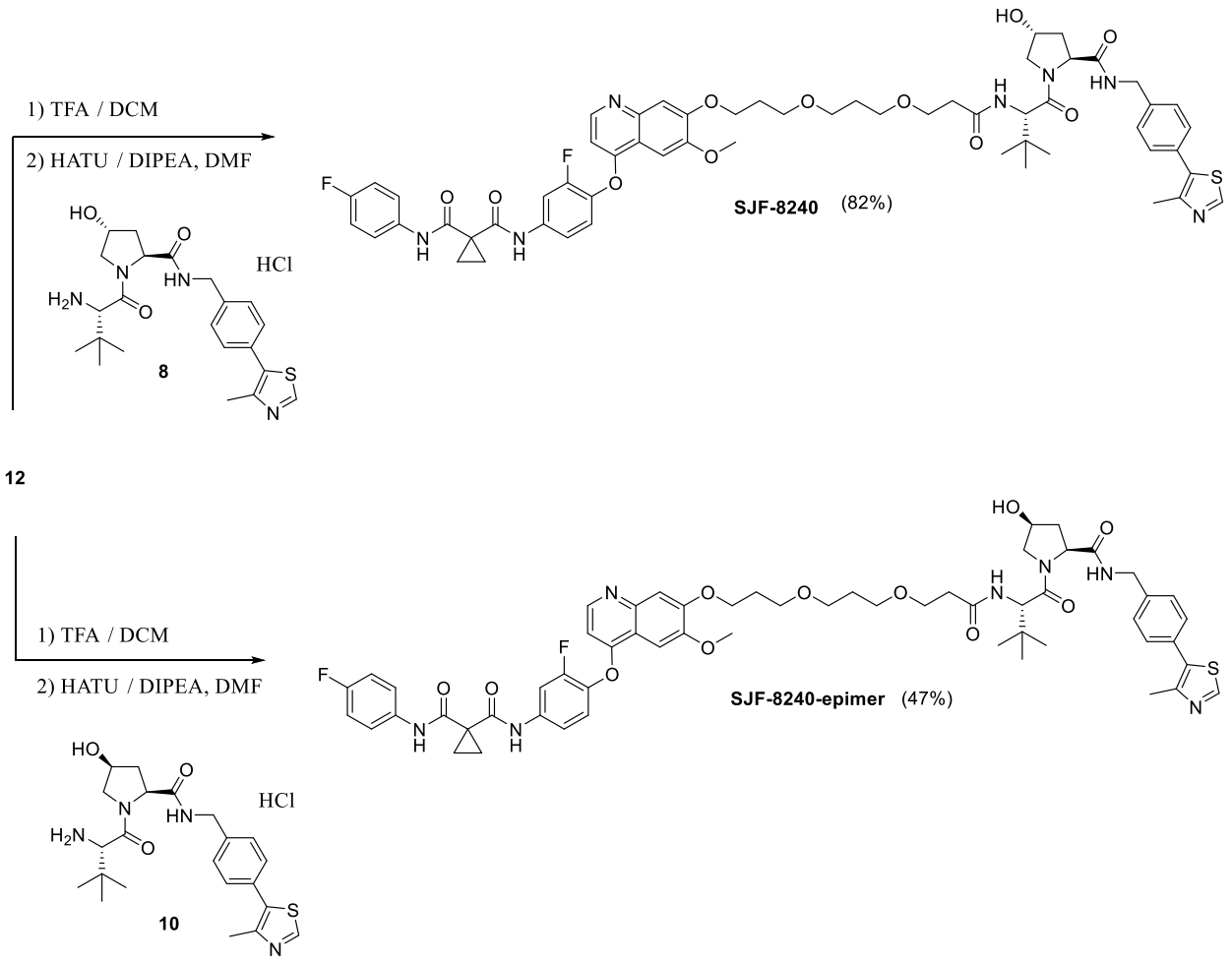
(2S,4S)-1-((S)-2-(tert-Butyl)-14-(4-(4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)quinazolin-6-yl)phenoxy)-4-oxo-6,9,12-trioxa-3-azatetradecanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (**SJF-1521-diastereomer, PROTAC 6**).). It was prepared from (2S,4S)-1-((S)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide hydrochloride (**10**) and tert-butyl 2-(2-(2-(2-(4-(4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)amino)-quinazolin-6-yl)phenoxy)ethoxy)ethoxy)ethoxy)acetate (**7**), following the same procedure than above (63% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ 9.95 (s, 1H), 9.04 (s, 1H), 8.84 – 8.77 (m, 1H), 8.73 (t, J = 6.0 Hz, 1H), 8.64 (s, 1H), 8.22 (dd, J = 8.8, 1.8 Hz, 1H), 8.10 (d, J = 2.6 Hz, 1H), 7.89 (dd, J = 8.8, 1.8 Hz, 3H), 7.83 (dd, J = 9.0, 2.6 Hz, 1H), 7.65 – 7.30 (m, 9H), 7.25 (td, J = 8.8, 8.3, 2.6 Hz, 1H), 7.18 (d, J = 8.8 Hz, 2H), 5.51 (d, J = 7.2 Hz, 1H), 5.33 (s, 2H), 4.59 (d, J = 9.2 Hz, 1H), 4.52 – 4.41 (m, 2H), 4.40 – 4.25 (m, 2H), 4.26 – 4.19 (m, 2H), 4.03 (s, 2H), 3.99 – 3.91 (m, 1H), 3.88 – 3.80 (m, 2H), 3.68 (ddt, J = 6.7, 5.2, 3.3 Hz, 8H), 3.59 – 3.44 (m, 1H), 2.50 (s, 3H), 2.44 – 2.35 (m, 1H), 1.81 (dt, J = 12.4, 6.1 Hz, 1H), 1.03 (s, 9H).^{13}C NMR (151 MHz, dmso) δ 172.26, 169.38, 168.91, 163.02, 161.40, 158.53, 157.61, 154.16, 151.48, 149.71, 148.58, 147.76, 139.69, 139.64, 139.14, 137.72, 133.15, 131.45, 131.11, 130.61, 130.56, 129.77, 128.70, 128.34, 128.25, 127.47, 124.20, 123.37, 123.35, 122.38,

121.04, 119.29, 115.29, 115.00, 114.79, 114.65, 114.31, 114.13, 113.99, 70.46, 69.96, 69.88, 69.64, 69.52, 69.40, 69.03, 68.97, 67.27, 58.59, 55.84, 55.62, 41.82, 36.92, 35.19, 26.18, 15.94. LC-MS (ESI): m/z $[M+H]^+$: Calcd. for $C_{57}H_{62}ClFN_7O_9S$, 1074.4002. Found 1074.3920.

Synthesis of PROTAC 7 and 8



tert-Butyl 3-(3-(3-((4-(2-fluoro-4-(1-((4-fluorophenyl)carbamoyl)cyclopropane-1-carboxamido)-phenoxy)-6-methoxyquinolin-7-yl)oxy)propoxy)propoxy)propanoate (**12**). To a mixture of N1'-[3-fluoro-4-[(7-hydroxy-6-methoxy-4-quinolyl)oxy]phenyl]-N1-(4-fluorophenyl)cyclopropane -1,1-dicarboxamide (**11**) (15 mg, 0.03 mmol) and tert-butyl 3-[3-(3-iodopropoxy)propoxy]propanoate (**2**) (16.57 mg, 0.04 mmol) in N,N-Dimethylformamide (1 mL) was added Cs_2CO_3 (29.01 mg, 0.09 mmol). After stirring at room temperature for 12 hrs (overnight), the reaction mixture was diluted with AcOEt (20 mL) and washed with water (5x10 mL), organic phase was evaporated under vacuum. Crude product was purified by PTLC (DCM:MeOH:NH_4OH, 92:7:1) to give 15 mg of product (**12**) (67% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ 10.39 (s, 1H), 10.01 (s, 1H), 8.46 (d, J = 5.2 Hz, 1H), 7.90 (d, J = 13.2 Hz, 1H), 7.71 – 7.58 (m, 2H), 7.51 (d, J = 7.4 Hz, 2H), 7.46 – 7.35 (m, 2H), 7.15 (t, J = 8.9 Hz, 2H), 6.41 (d, J = 5.1 Hz, 1H), 4.21 (t, J = 6.2 Hz, 2H), 3.95 (s, 3H), 3.60 – 3.37 (m, 8H), 2.37 (d, J = 12.2 Hz, 2H), 2.04 (p, J = 6.4 Hz, 2H), 1.71 (p, J = 6.4 Hz, 2H), 1.47 (s, 4H), 1.37 (s, 9H). ^{13}C NMR (151 MHz, DMSO-d6) δ 170.45, 168.27, 167.87, 159.29, 159.07, 157.48, 154.07, 152.44, 151.89, 149.56, 148.82, 146.37, 138.05, 137.98, 135.70, 135.61, 135.20, 135.19, 123.82, 122.46, 122.41, 116.90, 115.11, 115.09, 114.96, 114.47, 109.04, 108.88, 108.50, 101.95, 99.01, 79.64, 67.07, 66.55, 65.92, 65.45, 55.79, 35.87, 31.93, 29.53, 28.90, 27.76, 27.73, 15.31. LC-MS (ESI): m/z $[M+H]^+$ Calcd. for $C_{40}H_{46}F_2N_3O_9$, 750.3202 . Found 750.3509.



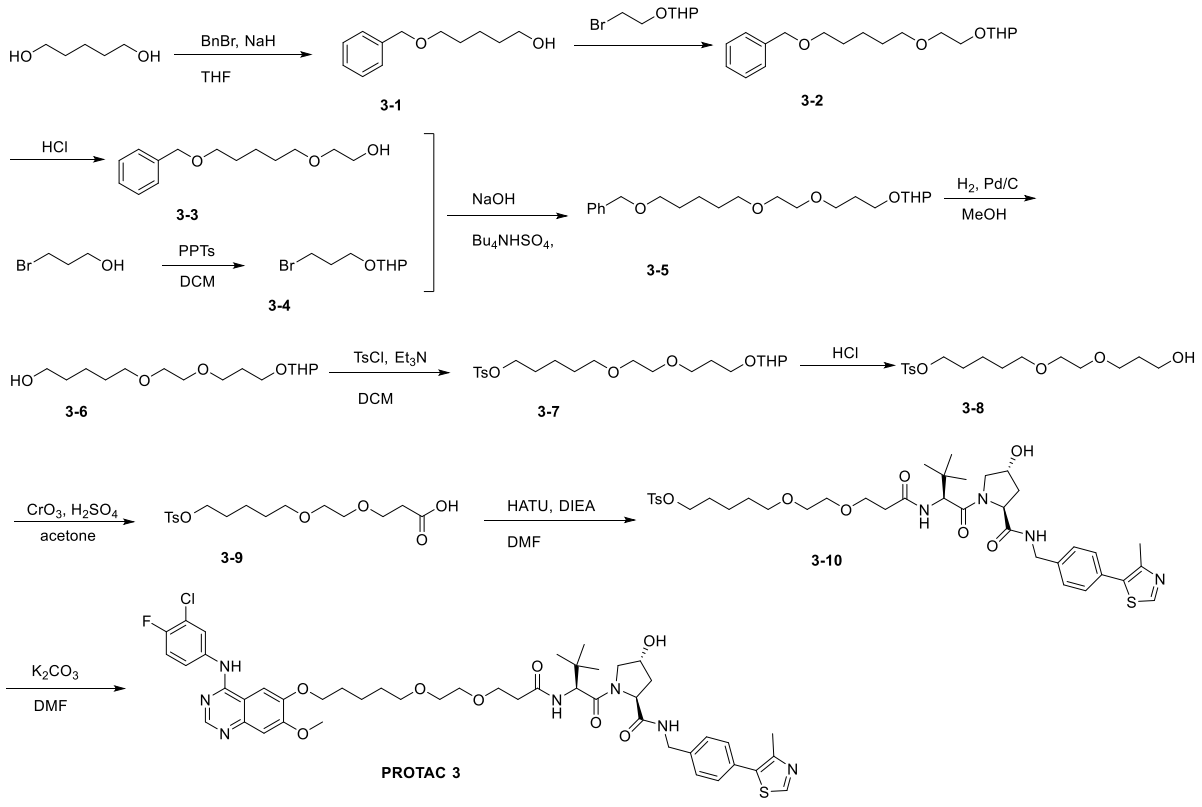
N-(3-Fluoro-4-((7-(3-(3-(3-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)-pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-3-oxopropoxy)propoxy)propoxy)-6-methoxyquinolin-4-yl)oxy)phenyl)-N-(4-fluorophenyl)cyclopropane-1,1-dicarboxamide (**SJF-8240, PROTAC 7**). A solution of tert-butyl 3-[3-[3-[[4-[2-fluoro-4-[[1-[(4-fluorophenyl)carbamoyl]cyclopropanecarbonyl] amino]phenoxy]-6-methoxy-7-quinolyl]oxy]propoxy]propoxy]propanoate (**12**) (15 mg, 0.02 mmol) in a mixture of TFA (1 ml, 13.46 mmol) and Dichloromethane (3 ml) was stirred for 2 h. Then the solvent was removed under vacuum and crude product was dried under high vacuum for 2 h. Crude product was used in the next step without any further purification (13.8 mg, quantitative yield). LC-MS (ESI): m/z [M+H]+ Calcd. for $C_{36}H_{38}F_2N_3O_9$, 694.2576. Found 694.2324. To a solution of crude product from above (13.8 mg, 0.02 mmol) and (2S,4R)-1-[(2S)-2-amino -3,3-dimethyl-butanoyl]-4-hydroxy-N-[[4-(4-methylthiazol-5-yl)phenyl]methyl]-pyrrolidine-2-carboxamide;hydrochloride (**8**) (11.15 mg, 0.02 mmol) in N,N-Dimethylformamide (2 ml)

was added DIPEA (0.17 ml, 0.99 mmol) and HATU (11.35 mg, 0.03 mmol) at room temperature. The reaction mixture was stirred for 12 h (overnight) at the same temperature. Reaction mixture was diluted with ACOEt (20 mL), washed with water (4x15 mL), dried (Na_2SO_4) and evaporated under vacuum. Crude product was purified by PTLC (DCM:MeOH:NH_4OH, 90:9:1), to give 18 mg of product (82 % yield). ^{1}H NMR (500 MHz, DMSO-d6) δ 10.38 (s, 1H), 10.00 (s, 1H), 8.97 (s, 1H), 8.56 (t, J = 6.1 Hz, 1H), 8.46 (d, J = 5.2 Hz, 1H), 7.96 – 7.85 (m, 2H), 7.69 – 7.59 (m, 2H), 7.51 (d, J = 8.8 Hz, 2H), 7.45 – 7.33 (m, 5H), 7.15 (t, J = 8.9 Hz, 2H), 6.41 (d, J = 5.1 Hz, 1H), 5.12 (d, J = 3.3 Hz, 1H), 4.55 (d, J = 9.4 Hz, 1H), 4.43 (ddd, J = 10.9, 6.7, 3.3 Hz, 2H), 4.27 – 4.16 (m, 3H), 3.94 (s, 3H), 3.76 – 3.33 (m, 10H), 2.58 – 2.51 (m, 1H), 2.43 (s, 3H), 2.35 – 2.25 (m, 1H), 2.03 (p, J = 5.7 Hz, 3H), 1.95 – 1.83 (m, 1H), 1.72 (p, J = 6.4 Hz, 2H), 1.48 (d, J = 3.9 Hz, 4H), 0.92 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d6) δ 171.89, 169.97, 169.51, 168.26, 167.88, 159.31, 159.22, 157.31, 154.21, 152.26, 151.90, 151.39, 149.56, 148.75, 147.69, 146.29, 139.47, 138.01, 137.94, 135.70, 135.60, 135.17, 135.15, 131.13, 129.61, 128.81, 128.61, 127.40, 123.77, 122.46, 122.40, 116.90, 115.09, 114.92, 114.47, 109.53, 109.05, 108.87, 108.45, 101.94, 99.03, 68.85, 67.16, 67.09, 66.62, 66.54, 65.47, 58.69, 56.35, 56.24, 55.77, 41.64, 37.92, 35.69, 35.36, 31.87, 29.60, 28.89, 26.28, 15.91, 15.31. LC-MS (ESI): m/z $[M+H]^+$ Calcd. for $C_{58}H_{66}F_2N_7O_{11}S$, 1106.4509. Found 1106.4510.

N-(3-Fluoro-4-((7-(3-(3-(3-(((S)-1-((2S,4S)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)-pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-3-oxopropoxy)propoxy)propoxy)-6-methoxyquinolin-4-yl)oxy)phenyl)-N-(4-fluorophenyl)cyclopropane-1,1-dicarboxamide (**SJF-8240-diastereomer, PROTAC 8**). It was prepared from (2S,4R)-1-[(2S)-2-amino -3,3-dimethyl-butanoyl]-4-hydroxy-N-[[4-(4-methylthiazol-5-yl)phenyl]methyl]pyrrolidine-2-carboxamide;hydrochloride (**10**) (10.42 mg, 0.022 mmol) and following the same procedure than above. Crude product was purified by PTLC (DCM:MEOH:NH_4OH, 90:9:1), to give 9.7 mg of the expected product (47 % yield). ^{1}H NMR (500 MHz, DMSO-d6) δ 10.38 (s, 1H), 10.00 (s, 1H), 8.97 (s, 1H), 8.63 (t, J = 6.0 Hz, 1H), 8.46 (d, J = 5.2 Hz, 1H), 7.90 (d, J = 9.7 Hz, 2H), 7.71 – 7.57 (m, 2H), 7.51 (d, J = 8.7 Hz, 2H), 7.44 – 7.28 (m, 5H), 7.15 (t, J = 8.9 Hz, 2H), 6.41 (d, J = 5.1 Hz, 1H), 5.43 (d, J = 7.2 Hz, 1H), 4.56 – 4.38 (m, 2H), 4.36 (dd, J = 8.6, 6.1 Hz, 1H), 4.32 – 4.13 (m, 4H), 3.94 (s, 3H), 3.97 – 3.82 (m, 1H), 3.64 – 3.46 (m, 4H), 3.48 – 3.35 (m, 4H), 2.57 – 2.45 (m, 2H), 2.43 (s, 3H), 2.36 – 2.26 (m, 2H), 2.03 (p, J = 6.3 Hz, 2H), 1.73 (dp, J = 13.0, 6.2 Hz, 3H), 1.55 – 1.38 (m, 4H), 0.93 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d6) δ 171.89, 169.97, 169.51, 168.26, 167.88, 159.31, 159.22, 157.31, 154.21, 152.26, 151.90, 151.39, 149.56, 148.75, 147.69, 146.29, 139.47, 138.01, 137.94, 135.70, 135.60, 135.17, 135.15, 131.13, 129.61, 128.81, 128.61, 127.40, 123.77, 122.46, 122.40, 116.90, 115.09, 114.92, 114.47,

109.53, 109.05, 108.87, 108.45, 101.94, 99.03, 68.85, 67.16, 67.09, 66.62, 66.54, 65.47, 58.69, 56.35, 56.24, 55.77, 41.64, 37.92, 35.69, 35.36, 31.87, 29.60, 28.89, 26.28, 15.91, 15.31. LC-MS (ESI): m/z $[M+H]^+$ Calcd. for $C_{58}H_{66}F_2N_7O_{11}S$, 1106.4509. Found 1106.5096.

Synthesis of PROTAC 3



5-(Benzyloxy)pentan-1-ol (3-1). Into a 1000 mL round-bottom flask, was placed pentane-1, 5-diol (30 g, 288.05 mmol, 1.00 equiv), tetrahydrofuran (500 mL). This was followed by the addition of sodium hydride (13.8 g, 575.00 mmol, 2.00 equiv) in several batches. The mixture was stirred for 1 h at 25 ºC. To this was added BnBr (58 g, 339.12 mmol, 1.20 equiv) dropwise with stirring. The resulting solution was stirred overnight at 25 ºC. The reaction was then quenched by the addition of 50 mL of water. The resulting solution was extracted with 3x500 mL of ethyl acetate and the organic layers were combined and dried over anhydrous sodium sulfate. The solids were filtered out. The resulting mixture was concentrated under vacuum. The residue was applied onto a silica gel column eluted with ethyl acetate/petroleum ether (1:5). This resulted in 28 g (50%) of 5-(benzyloxy)pentan-1-ol as colorless oil. LC-MS m/z: (ES^+) $[M+H]^+$ = 195; Retention time: 1.01 min; ^1H NMR (300 MHz, $CDCl_3$, 25 ºC): 7.35 (s, 5H), 4.52 (s, 2H), 3.65 (t, 2H), 3.51 (t, 2H), 1.69-1.40 (m, 6H).

2-(2-[[5-(Benzyloxy)pentyl]oxy]ethoxy)oxane (3-2). Into a 100 mL round-bottom flask, was placed 5-(benzyloxy)pentan-1-ol (3 g, 15.44 mmol, 1.00 equiv), 50% sodium hydroxide solution (20 mL), 2-(2-bromoethoxy)oxane (12.8 g, 61.22 mmol, 4.00 equiv), Bu_4NHSO_4 (0.5 g, 0.10 equiv). The resulting solution was stirred for 12 h at 65ºC. The reaction mixture was cooled. The resulting mixture was washed with 20 mL of water and 20 mL of brine. The mixture was dried over anhydrous sodium sulfate. The solids were filtered out. The resulting mixture was concentrated under vacuum. This resulted in 4 g (80%) of 2-(2-[[5-(benzyloxy)pentyl]oxy]ethoxy)oxane as red oil. LC-MS m/z: (ES^+) $[M+H]^+$ = 323; Retention time: 1.25 min.

2-[[5-(Benzyloxy)pentyl]oxy]ethan-1-ol (3-3). Into a 100 mL round-bottom flask, was placed 2-(2-[[5-(benzyloxy)pentyl]oxy]ethoxy)oxane (4 g, 12.41 mmol, 1.00 equiv), methanol (40 mL), hydrogen chloride (2 mL). The resulting solution was stirred overnight at 50ºC. The reaction mixture was cooled. The resulting mixture was washed with water and brine. The mixture was dried over anhydrous sodium sulfate. The solids were filtered out. The resulting mixture was concentrated under vacuum. The residue was applied onto a silica gel column eluted with ethyl acetate/petroleum ether (1:2). This resulted in 3 g (100%) of 2-[[5-(benzyloxy)pentyl]oxy]ethan-1-ol as colorless oil. LC-MS m/z: (ES^+) $[M+H]^+$ = 239; Retention time: 1.12 min.

2-(3-Bromopropoxy)oxane (3-4). Into a 250 mL round-bottom flask, was placed 3-bromopropan-1-ol (4.75 g, 34.17 mmol, 1.00 equiv), dichloromethane (100 mL), PPTs (10 mg, 0.04 mmol, 0.10 equiv), 3,4-dihydro-2H-pyran (3.32 g, 39.47 mmol, 1.16 equiv). The resulting solution was stirred for 5 h at room temperature. The mixture was dried over anhydrous magnesium sulfate. The solids were filtered out. The resulting mixture was concentrated under vacuum. The residue was applied onto a silica gel column eluted

with ethyl acetate/petroleum ether (1:5). This resulted in 5 g (66%) of 2-(3-bromopropoxy)oxane as colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$, 25 ^{o}C): 4.62 (t, 1H), 3.95-3.85 (m, 2H), 3.59-3.48 (m, 4H), 2.18-2.10 (m, 2H), 1.90-1.45 (m, 6H).

2-[3-(2-[[5-(benzyloxy)pentyl]oxy]ethoxy)propoxy]oxane (3-5). Into a 50 mL round-bottom flask, was placed 2-[[5-(benzyloxy)pentyl]oxy]ethan-1-ol (150 mg, 0.63 mmol, 1.00 equiv), 2 mL of 50% NaOH solution, 4 equivalents of 2-(3-bromopropoxy)oxane, and catalytic amount of Bu_4NHSO_4 (0.1 eq). The resulting solution was stirred overnight at 65^{o}C. The reaction mixture was cooled. The resulting solution was extracted with 3x50 mL of ethyl acetate and the organic layers were combined and dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was applied onto a silica gel column eluted with ethyl acetate/petroleum ether (1:2). This resulted in 200 mg (84%) of 2-[3-(2-[[5-(benzyloxy)pentyl]oxy]ethoxy)propoxy]oxane as colorless oil. LC-MS m/z: (ES^+) $[M+Na]^+$ = 403; Retention time: 1.53 min; ^{1}H NMR (300 MHz, $CDCl_3$, 25 ^{o}C): 7.35 (s, 5H), 4.62 (t, 1H), 4.52 (s, 2H), 3.95-3.85 (m, 4H), 3.59-3.48 (m, 10H), 1.90-1.45 (m, 14H).

5-[2-[3-(Oxan-2-yloxy)propoxy]ethoxy]pentan-1-ol (3-6). Into a 50 mL round-bottom flask, was placed 2-[3-(2-[[5-(benzyloxy)pentyl]oxy]ethoxy)propoxy]oxane (80 mg, 0.21 mmol, 1.00 equiv), methanol (5 mL), palladium on carbon (200 mg, 0.20 equiv). To this mixture H_2(g) was introduced in. The resulting solution was stirred overnight at room temperature. The solids were filtered out. The resulting mixture was concentrated under vacuum. This resulted in 64 mg (crude) of 5-[2-[3-(oxan-2-yloxy)propoxy]ethoxy]pentan-1-ol as colorless oil. LC-MS m/z: (ES^+) $[M+H]^+$ = 291; Retention time: 1.32 min; ^{1}H NMR (300 MHz, $CDCl_3$, 25 ^{o}C): 4.62 (t, 1H), 3.98-3.79 (m, 2H), 3.65-3.47 (m, 8H), 1.90-1.45 (m, 14H).

5-[2-[3-(Oxan-2-yloxy)propoxy]ethoxy]pentyl 4-methylbenzene-1-sulfonate (3-7). Into a 50 mL round-bottom flask, was placed 5-[2-[3-(oxan-2-yloxy)propoxy]ethoxy]pentan-1-ol (60 mg, 0.21 mmol, 1.00 equiv), dichloromethane (2 mL), triethylamine (47 mg, 0.46 mmol, 3.00 equiv), TsCl (30 mg, 0.16 mmol, 1.50 equiv). The resulting solution was stirred overnight at room temperature. The resulting mixture was washed with water and brine. The mixture was dried over anhydrous sodium sulfate. The solids were filtered out. The resulting mixture was concentrated under vacuum. This resulted in 90 mg (98%) of 5-[2-[3-(oxan-2-yloxy)propoxy]ethoxy]pentyl 4-methylbenzene-1-sulfonate as colorless oil. LC-MS m/z: (ES^+) $[M+H]^+$ = 445, Retention time: 1.25 min.

3-[2-[(5-[[(4-Methylbenzene)sulfonyl]oxy]pentyl)oxy]ethoxy]propan-1-ol (3-8). Into a 50 mL round-bottom flask, was placed 5-[2-[3-(oxan-2-yloxy)propoxy]ethoxy]pentyl 4-methylbenzene-

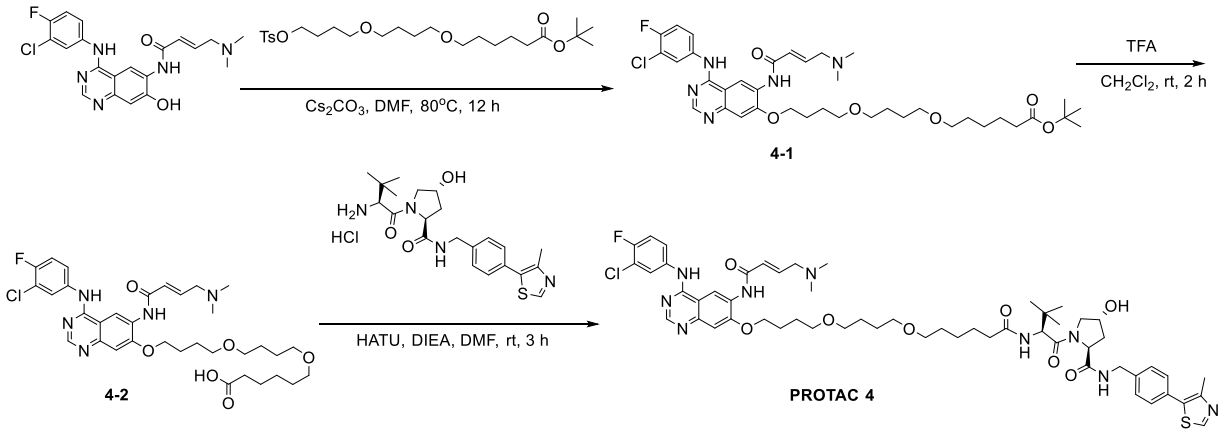
1-sulfonate (90 mg, 0.20 mmol, 1.00 equiv), methanol (2 mL), hydrogen chloride (0.5 mL). The resulting solution was stirred for 2 h at room temperature. The resulting mixture was concentrated under vacuum. The resulting solution was extracted with 3x20 mL of ethyl acetate and the organic layers were combined and dried over anhydrous sodium sulfate. The solids were filtered out. The resulting mixture was concentrated under vacuum. This resulted in 45 mg (62%) of 3-[2-[(5-[[(4-methylbenzene)sulfonyl]oxy]pentyl)oxy]ethoxy]propan-1-ol as colorless oil. LC-MS m/z: (ES^+) $[M+H]^+$ = 291; Retention time: 0.93 min; ^1H NMR (400 MHz, $CDCl_3$, 25 ^oC): 7.83 (d, 2H), 7.35 (d, 2H), 4.05 (t, 2H), 3.80 (t, 2H), 3.70 (t, 2H), 3.64 (d, 2H), 3.58 (d, 2H), 3.45 (t, 2H), 2.92 (brs, 1H), 2.47 (s, 3H), 1.91-1.82 (m, 2H), 1.73-1.65 (m, 2H), 1.58-1.52 (m, 2H), 1.45-1.35 (m, 2H).

3-[2-[(5-[[(4-Methylbenzene)sulfonyl]oxy]pentyl)oxy]ethoxy]propanoic acid (3-9). Into a 50 mL round-bottom flask, was placed 3-[2-[(5-[[(4-methylbenzene)sulfonyl]oxy]pentyl)oxy]ethoxy]propan-1-ol (100 mg, 0.28 mmol, 1.00 equiv), acetone (2 mL). To this was added CrO_3 (55 mg, 2.00 equiv), sulfuric acid (0.1 mL), water (0.6 mL) under ice bath. The resulting solution was stirred for 2 h at 5-10 ^oC. The reaction was then quenched by the addition of iso-propanol. The resulting solution was extracted with 2x10 mL of ethyl acetate and the organic layers were combined and dried over anhydrous sodium sulfate. The solids were filtered out. The resulting mixture was concentrated under vacuum. This resulted in 90 mg (87%) of 3-[2-[(5-[[(4-methylbenzene)sulfonyl]oxy]pentyl)oxy]ethoxy]propanoic acid as colorless oil. LC-MS m/z: (ES^+) $[M+H]^+$ = 375; Retention time: 0.92 min.

(2*S*,4*R*)-1-[(2*S*)-3,3-Dimethyl-2-(3-[2-[(5-[[(4-methylbenzene)-sulfonyl]oxy]pentyl)oxy]ethoxy]propanamido)butanoyl]-4-hydroxy-N-[[4-(4-methyl-1,3-thiazol-5-yl)phenyl]methyl]pyrrolidine-2-carboxamide (3-10). Into a 50 mL round-bottom flask, was placed 3-[2-[(5-[[(4-methylbenzene)sulfonyl]oxy]pentyl)oxy]ethoxy]propanoic acid (112 mg, 0.30 mmol, 1.00 equiv), (2*S*,4*R*)-1-[(2*S*)-2-amino-3,3-dimethylbutanoyl]-4-hydroxy-N-[[4-(4-methyl-1,3-thiazol-5-yl)phenyl]methyl]pyrrolidine-2-carboxamide (90 mg, 0.21 mmol, 1.00 equiv), HATU (137 mg, 0.36 mmol, 1.50 equiv), N,N-dimethylformamide (2 mL), DIEA (124 mg, 0.96 mmol, 4.00 equiv). The resulting solution was stirred for 2 h at room temperature. The resulting solution was extracted with 2x10 mL of ethyl acetate and the organic layers were combined. The resulting mixture was washed with 4x5 mL of water. The mixture was dried over anhydrous sodium sulfate. The solids were filtered out. The resulting mixture was concentrated under vacuum. This resulted in 110 mg (47%) of (2*S*,4*R*)-1-[(2*S*)-3,3-dimethyl-2-(3-[2-[(5-[[(4-methylbenzene)-sulfonyl]oxy]pentyl)oxy]ethoxy]propanamido)butanoyl]-4-hydroxy-N-[[4-(4-methyl-1,3-thiazol-5-yl)phenyl]methyl]pyrrolidine-2-carboxamide as colorless oil. LC-MS m/z: (ES^+) $[M+H]^+$ = 787; Retention time: 1.03 min.

(2*S*,4*R*)-1-[(2*S*)-2-[3-(2-[[5-([4-[(3-Chloro-4-fluorophenyl)amino]-7-methoxyquinazolin-6-yl]oxy)pentyl]oxy]ethoxy)propanamido]-3,3-dimethylbutanoyl]-4-hydroxy-N-[[4-(4-methyl-1,3-thiazol-5-yl)phenyl]methyl]pyrrolidine-2-carboxamide (PROTAC 3). Into a 50 mL round-bottom flask, was placed 4-[(3-chloro-4-fluorophenyl)amino]-7-methoxyquinazolin-6-ol (45 mg, 0.14 mmol, 1.00 equiv), (2*S*,4*R*)-1-[(2*S*)-3,3-dimethyl-2-(3-[2-[(5-[[(4-methylbenzene)sulfonyl]oxy]-pentyl)oxy]ethoxy]propanamido)butanoyl]-4-hydroxy-N-[[4-(4-methyl-1,3-thiazol-5-yl)phenyl]methyl]pyrrolidine-2-carboxamide (110 mg, 0.14 mmol, 1.00 equiv), potassium carbonate (58 mg, 0.42 mmol, 3.00 equiv), N,N-dimethylformamide (2 mL). The resulting solution was stirred for 4 h at 80 ºC. The solids were filtered out. The crude product was purified by Prep-HPLC with the following conditions: XBridge Prep C18 OBD Column, 19 x 100 mm, 5 micron; mobile phase, water with 0.1% TFA and MeCN (25.0% MeCN up to 45.0% in 10 min); Detector, UV 254 nm. HPLC purification resulted in 16.3 mg (12%) of (2*S*,4*R*)-1-[(2*S*)-2-[3-(2-[[5-([4-[(3-chloro-4-fluorophenyl)amino]-7-methoxyquinazolin-6-yl]oxy)pentyl]oxy]ethoxy)propanamido]-3,3-dimethylbutanoyl]-4-hydroxy-N-[[4-(4-methyl-1,3-thiazol-5-yl)phenyl]methyl]pyrrolidine-2-carboxamide as a white solid. LC-MS m/z: (ES$^+$) [M+H] $^+$ = 934; Retention time: 1.97 min; ^{1}H NMR (300 MHz, CD_3OD, 25 ºC): 8.89 (s, 1H), 8.45 (s, 1H), 7.95-8.05 (d, 1H), 7.65-7.74 (m, 2H), 7.36-7.49 (m, 3H), 7.17-7.31 (m, 2H), 4.68-4.34 (m, 5H), 4.15-4.23 (m, 2H), 4.02 (s, 3H), 3.91-3.72 (m, 4H), 3.62 (s, 4H), 3.52-3.54 (m, 2H), 2.58-2.45 (m, 4H), 2.25-1.52 (m, 8H) , 1.05 (s, 9H).

Synthesis of PROTAC 4.



***tert*-Butyl 6-[4-[4-([4-[(3-chloro-4-fluorophenyl)amino]-6-[(2*E*)-4-(dimethylamino)but-2-enamido]quinazolin-7-yl]oxy)butoxy]butoxy]hexanoate (4-1).** Into a 25 mL round-bottom flask, was placed a solution of (2*E*)-N-[4-[(3-chloro-4-fluorophenyl)amino]-7-hydroxyquinazolin-6-yl]-4-

(dimethylamino)but-2-enamide (200.0 mg, 0.48 mmol, 1.00 equiv) in N,N-dimethylformamide (10 mL), cesium carbonate (314.0 mg, 0.96 mmol, 2.00 equiv), *tert*-butyl 6-(4-(4-(tosyloxy)butoxy)butoxy)hexanoate (281.0 mg, 1.20 equiv). The resulting solution was stirred for 12 h at 80 °C in an oil bath. The reaction was then quenched by the addition of water (20 mL). The resulting mixture was extracted with ethyl acetate (10 mL x 3) and the organic layers were combined. The resulting mixture was washed with brine (10 mL). The mixture was dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was applied onto a silica gel column eluted with dichloromethane/methanol (10:1). This resulted in 77.0 mg (22%) of *tert*-butyl 6-[4-[4-([4-[(3-chloro-4-fluorophenyl)amino]-6-[(2*E*)-4-(dimethylamino)but-2-enamido]quinazolin-7-yl]oxy)butoxy]butoxy]hexanoate as a brown solid. LC-MS (ES^+): *m/z* 730.31 $[M+H]^+$

6-[4-[4-([4-[(3-Chloro-4-fluorophenyl)amino]-6-[(2*E*)-4-(dimethylamino)but-2-enamido]quinazolin-7-yl]oxy)butoxy]butoxy]hexanoic acid (4-2). Into a 25 mL round-bottom flask, was placed a solution of tert-butyl 6-[4-[4-([4-[(3-chloro-4-fluorophenyl)amino]-6-[(2*E*)-4-(dimethylamino)but-2-enamido]quinazolin-7-yl]oxy)butoxy]butoxy]hexanoate (77.0 mg, 0.11 mmol, 1.00 equiv) in dichloromethane/trifluoroacetic acid (10/2 mL). The resulting solution was stirred for 2 h at room temperature. The resulting mixture was concentrated under vacuum. This resulted in 71.0 mg (100%) of 6-[4-[4-([4-[(3-chloro-4-fluorophenyl)amino]-6-[(2*E*)-4-(dimethylamino)but-2-enamido]quinazolin-7-yl]oxy)butoxy]butoxy]hexanoic acid as brown oil. LC-MS (ES^+): *m/z* 674.30 $[M+H]^+$

(2*S*,4*R*)-1-[(2*S*)-2-(6-[4-[4-([4-[(3-chloro-4-fluorophenyl)amino]-6-[(2*E*)-4-(dimethylamino)but-2-enamido]quinazolin-7-yl]oxy)butoxy]butoxy]hexanamido)-3,3-dimethylbutanoyl]-4-hydroxy-N-[[4-(4-methyl-1,3-thiazol-5-yl)phenyl]methyl]pyrrolidine-2-carboxamide (PROTAC 4). Into a 25 mL round-bottom flask under ice bath, was placed a solution of 6-[4-[4-([4-[(3-chloro-4-fluorophenyl)amino]-6-[(2*E*)-4-(dimethylamino)but-2-enamido]quinazolin-7-yl]oxy)butoxy]butoxy]hexanoic acid (71.0 mg, 0.11 mmol, 1.00 equiv) in N,N-dimethylformamide (10 mL), *N*-ethyl-*N*-isopropylpropan-2-amine (68.0 mg, 0.53 mmol, 5.00 equiv), o-(7-azabenzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate (52.0 mg, 0.14 mmol, 1.30 equiv). The mixture was stirred for 20 min. To this was added (2*S*,4*R*)-1-[(2*S*)-2-amino-3,3-dimethylbutanoyl]-4-hydroxy-*N*-[4-(4-methyl-1,3-thiazol-5-yl)phenyl]methylpyrrolidine-2-carboxamide hydrochloride (64.0 mg, 1.30 equiv) at 0℃. The resulting solution was stirred for 2 h at room temperature. The resulting solution was extracted with ethyl acetate (10 mL x 3) and the organic layers were combined. After washing with brine (10

mL), the mixture was dried over anhydrous sodium sulfate, filtered, and concentrated under vacuum. The residue was purified by prep-HPLC under the following condition: X Bridge RP18 column, 19 x 150 mm, 5 micron; Mobile Phase A: water/0.05% ammonium bicarbonate; Mobile Phase B: acetonitrile; Flow rate: 20 mL/min; Gradient: 38% B to 52% B in 15 min; Detector: 254 nm. This resulted in 27.0 mg (24%) of (2S,4R)-1-[(2S)-2-(6-[4-[4-([4-[(3-chloro-4-fluorophenyl)amino]-6-[(2*E*)-4-(dimethylamino)but-2-enamido]quinazolin-7-yl]oxy)butoxy]butoxy]hexanamido)-3,3-dimethylbutanoyl]-4-hydroxy-N-[[4-(4-methyl-1,3-thiazol-5-yl)phenyl]methyl]pyrrolidine-2-carboxamide as a white solid. ^{1}H NMR (300 MHz, CD_3OD): δ 8.88 (s, 1H), 8.49 (s, 1H), 8.04-8.02 (d, *J* = 6.9 Hz, 1H), 7.75-7.65 (m, 1H), 7.47-7.41 (m, 4H), 7.38-7.26 (m, 2H), 7.05-7.00 (m, 1H), 6.55-6.50 (d, *J* = 15.3 Hz, 1H), 4.63 (s, 1H), 4.56-4.49 (m, 3H), 4.37-4.30 (m, 3H), 3.92-3.78 (m, 2H), 3.57-3.53 (m, 2H), 3.47-3.46 (m, 2H), 3.46-3.40 (m, 4H), 3.24-3.21 (m, 2H), 2.47 (s, 3H), 2.32 (s, 6H), 2.30-2.23 (m, 3H), 2.08-2.01 (m, 3H), 1.84-1.82 (m, 2H), 1.61-1.52 (m, 8H), 1.38-1.25 (m, 2H), 1.00 (s, 9H); LC-MS (ES^+): m/z 544.25 [$(M+2H^+)/2$].