CORRECTION NOTICE

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Catalytic *in vivo* protein knockdown by small-molecule PROTACs

Daniel P Bondeson, Alina Mares, Ian E D Smith, Eunhwa Ko, Sebastien Campos, Afjal H Miah, Katie E Mulholland, Natasha Routly, Dennis L Buckley, Jeffrey L Gustafson, Nico Zinn, Paola Grandi, Satoko Shimamura, Giovanna Bergamini, Maria Faelth-Savitski, Marcus Bantscheff, Carly Cox, Deborah A Gordon, Ryan R Willard, John J Flanagan, Linda N Casillas, Bartholomew J Votta, Willem den Besten, Kristoffer Famm, Laurens Kruidenier, Paul S Carter, John D Harling, Ian Churcher & Craig M Crews

In the version of this supplementary file originally posted online, a Supplementary Note detailing the synthesis and characterization of compounds 1–15 was omitted. This error has been corrected in this file as of 24 August 2015.

Supplemental Information

PROTACs: A System for Catalytic, Small Molecule-induced *in vivo* Protein Knockdown

Daniel P. Bondeson[1†], Alina Mares[5†], Ian E.D. Smith[5†], Eunhwa Ko[1], Sebastien Campos[5], Afjal H. Miah[5], Katie E. Mulholland[5], Natasha Routly[5], Dennis L. Buckley[1], Jeffrey L. Gustafson[1], Nico Zinn[6], Paola Grandi[6], Satoko Shimamura[6], Giovanna Bergamini[6], Maria Faelth-Savitski[6], Marcus Bantscheff[6], Carly Cox[1], Deborah A. Gordon[7], Ryan R. Willard[7], John J. Flanagan[7], Linda N Casillas[8], Bartholomew J Votta[8], Willem den Besten[4], Kristoffer Famm[5], Laurens Kruidenier[5], Paul S. Carter[5], John D. Harling[5], Ian Churcher[5*], Craig M. Crews[1,2,3*]

[†*]These Authors contributed equally to this work

[1]Department of Molecular, Cellular and Developmental Biology**,** Yale University, New Haven, CT.

[2]Department of Chemistry, Yale University, New Haven, CT.

[3]Department of Pharmacology, Yale University, New Haven, CT.

[4]Division of Biology & Biological Engineering, California Institute of Technology, Pasadena, CA.

[5]GSK Medicines Research Centre, Gunnels Wood Road, Stevenage, UK SG1 2NY.

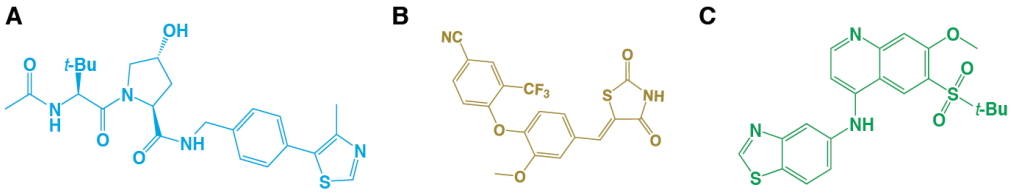
[6]Cellzome, a GSK company, Meyerhofstr. 1, 69117 Heidelberg, Germany.

[7]Arvinas, Inc., New Haven, CT.

[8] Pattern Recognition Receptor Discovery Performance Unit, GlaxoSmithKline, Collegeville, PA

Supplementary Results
Supplementary Figures

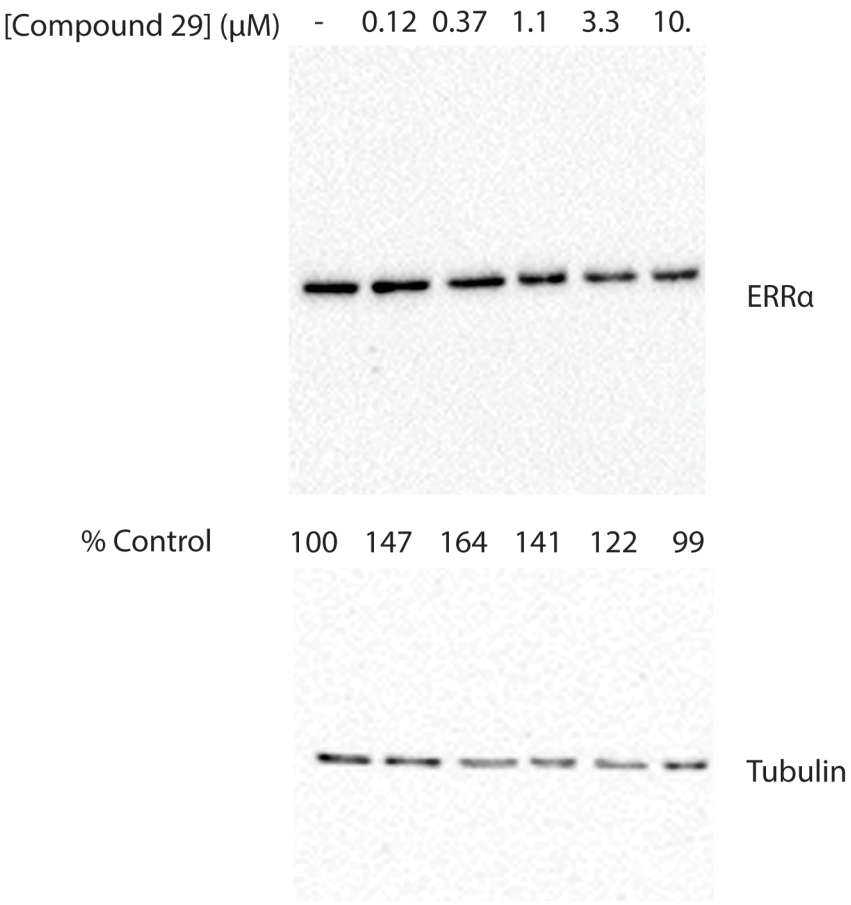
Supplementary Figure 1. PROTAC parent ligand structures



A) VHL Ligand
B) ERRα Ligand (Compound 29 in Reference 21)
C) RIPK2 ligand

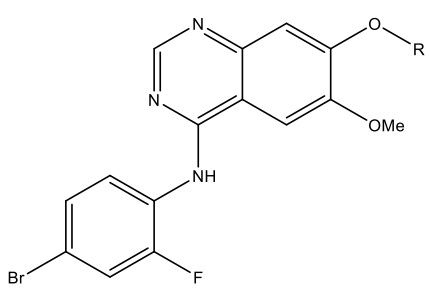
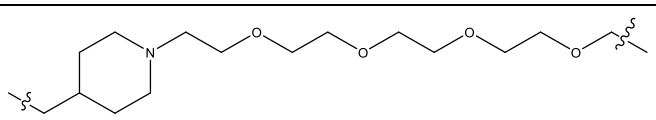
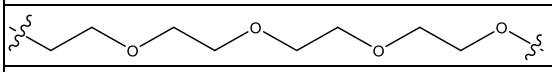
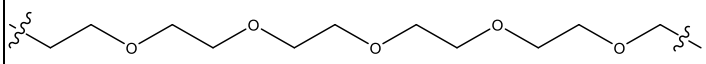
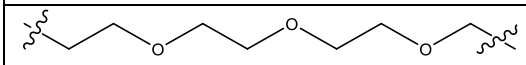
Supplementary Figure 2: The parent Compound 29 does not degrade ERRα

3T3-L1 cells were treated for 16 hours with the indicated concentrations of Compound 29, the parent ligand for PROTAC_ERRα, followed by harvesting and western blot as described in Materials and Methods. Quantification was performed by densitometry, and values are normalized to tubulin and the vehicle control. Values are representative of at least two experiments.

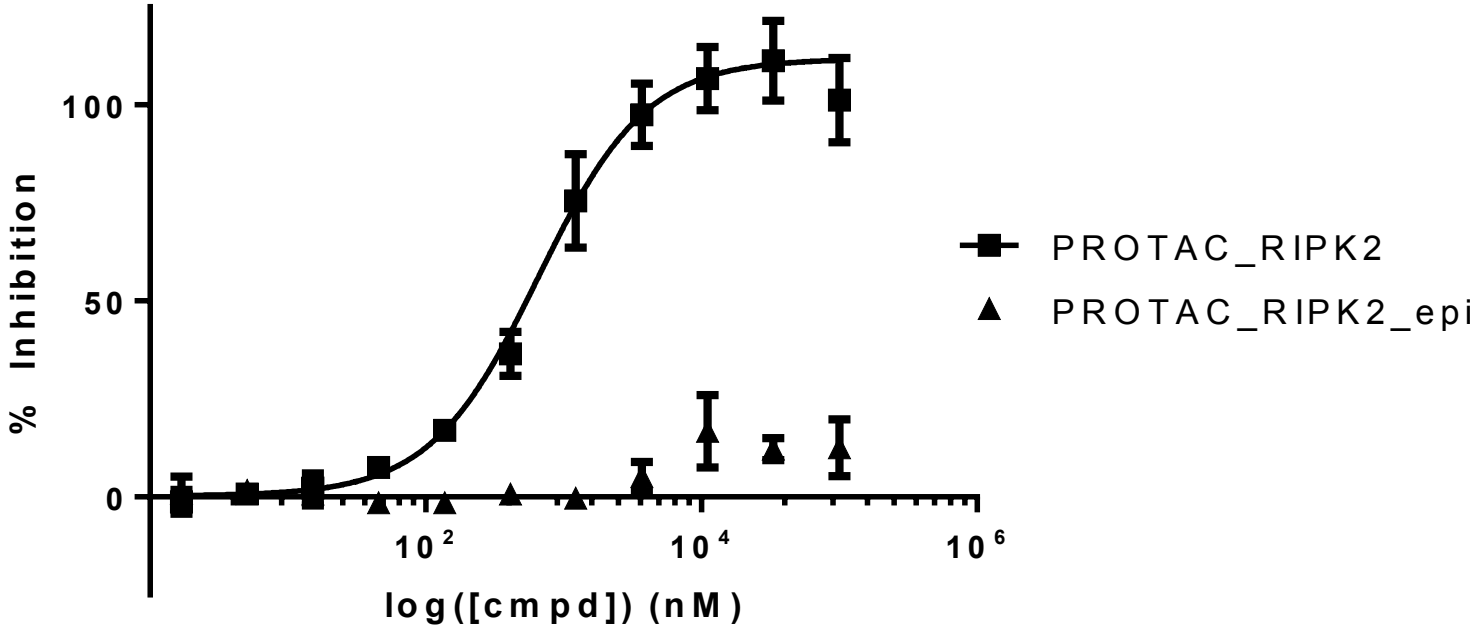


Supplementary Figure 3. Effect of linker on RIPK2 degradation efficiency.

The linker for PROTAC_RIPK2, presented in the main text, was based on a previous generation of RIPK2 PROTACs utilizing the tyrosine kinase inhibitor vandetanib. The Structure-Activity Relationship for different linkers showed that the three PEG linker afforded maximal degradation. Based on these observations, the optimal linker (entry 2) was used in PROTAC_RIPK2.

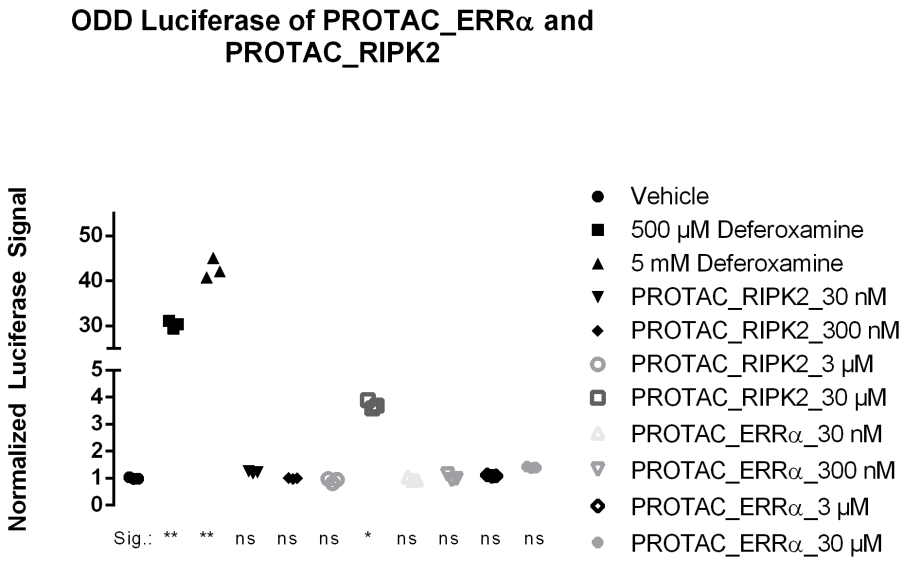
Targeting Ligand	Linker	DC_{50}
Vandetanib 		Weak effect at 10 μM
		0.8 μM
		No effect up to 3µM
		2 μM

Supplementary Figure 4. Inhibition of HIF1a peptide binding to VHL complex by PROTACs



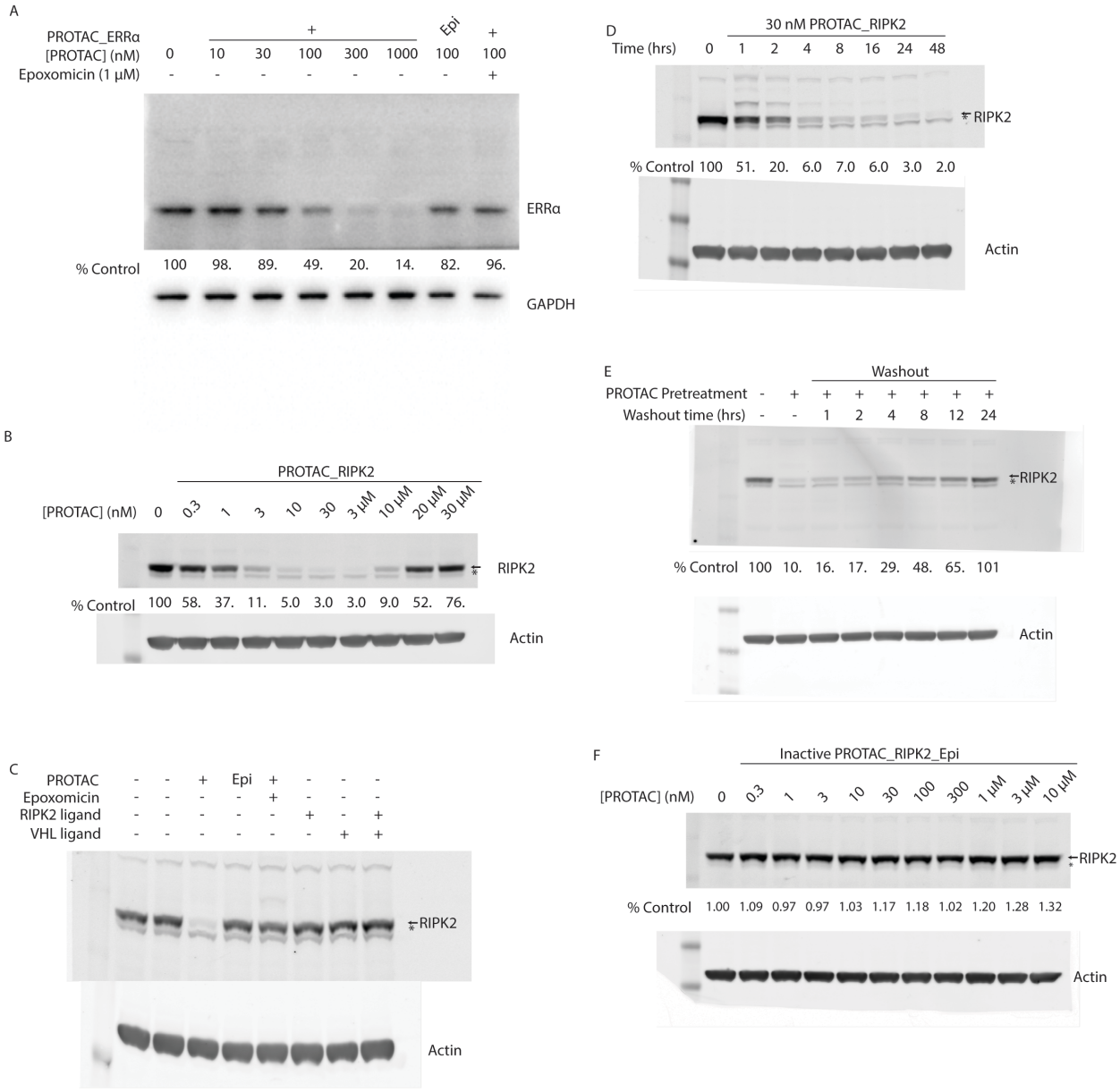
In a competitive fluorescence polarization experiment, a labeled HIF1α probe was in competition with either PROTAC_RIPK2 or PROTAC_RIPK2_epi. The fit sigmoid for PROTAC_RIPK2 demonstrates an IC_{50} of 656 nM. Data shown are mean ± SD of at least 4 experiments.

Supplementary Figure 5. Effect of PROTAC_RIPK2 and PROTAC_ERRα on ODD-luciferase



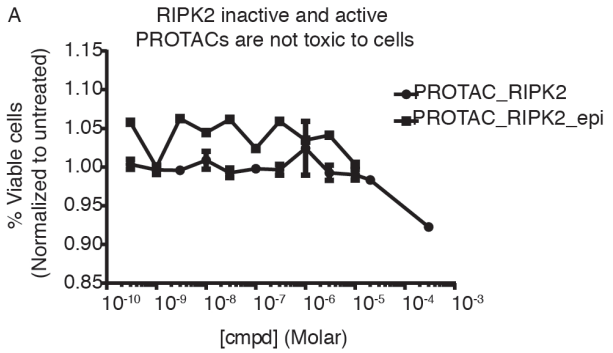
SH-SY5Y cells expressing luciferase fused to the first Oxygen Dependent Degradation (ODD) of HIF1α were treated in triplicate for 16 hours with the indicated compounds. The iron chelator Deferoxamine (Sigma) was used as a positive control for hypoxic response. Luciferase levels were analyzed in whole cell extracts. ** $p<0.0001$, *$p<0.001$, ns: not significant by an ANOVA test compare to vehicle.

Supplementary Figure 6. Full blots of *in cellula* protein degradation.



Nature Chemical Biology: doi:10.1038/nchembio.1858

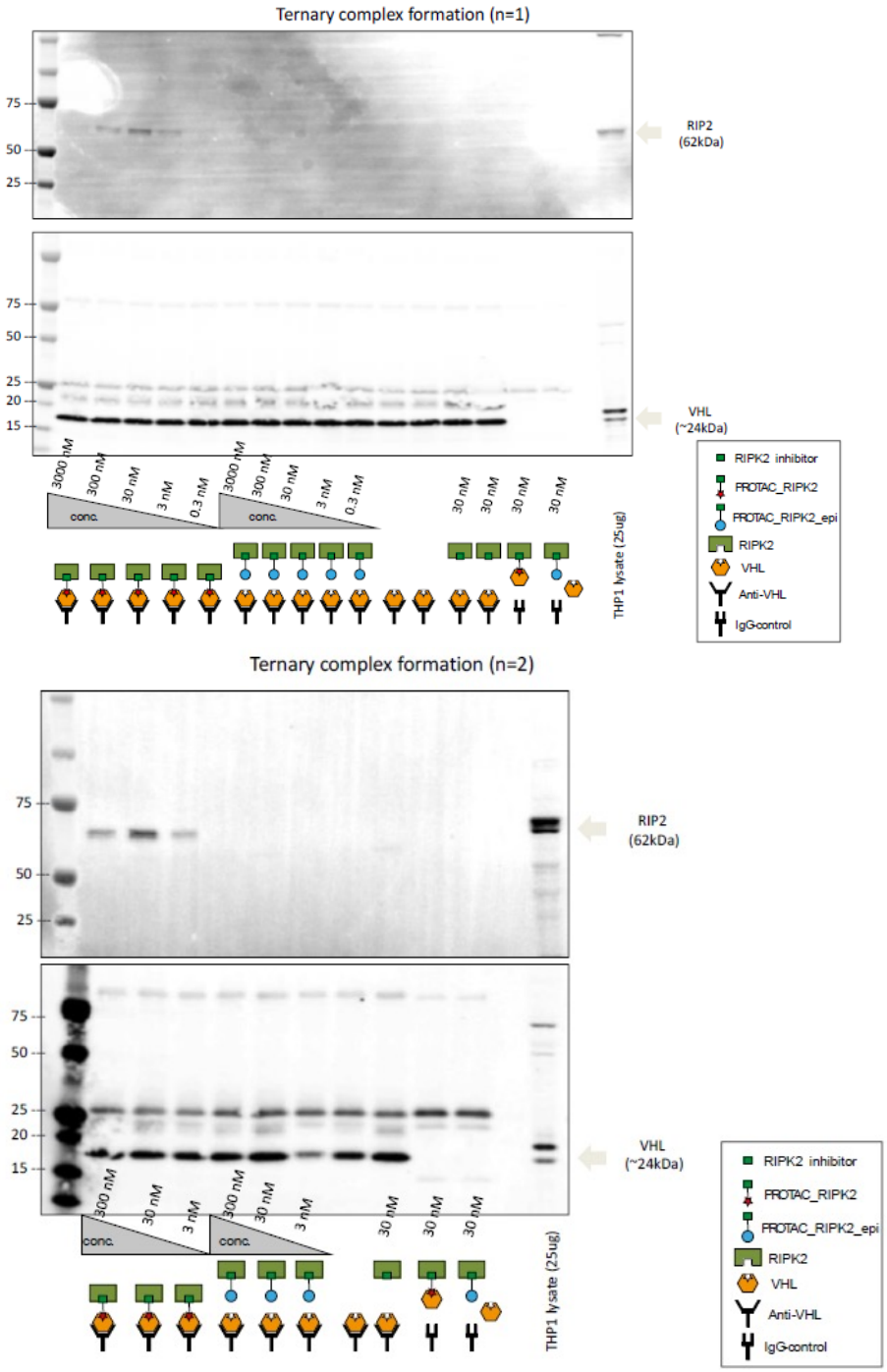
Supplementary Figure 7. PROTAC_RIPK2 is not toxic to cells



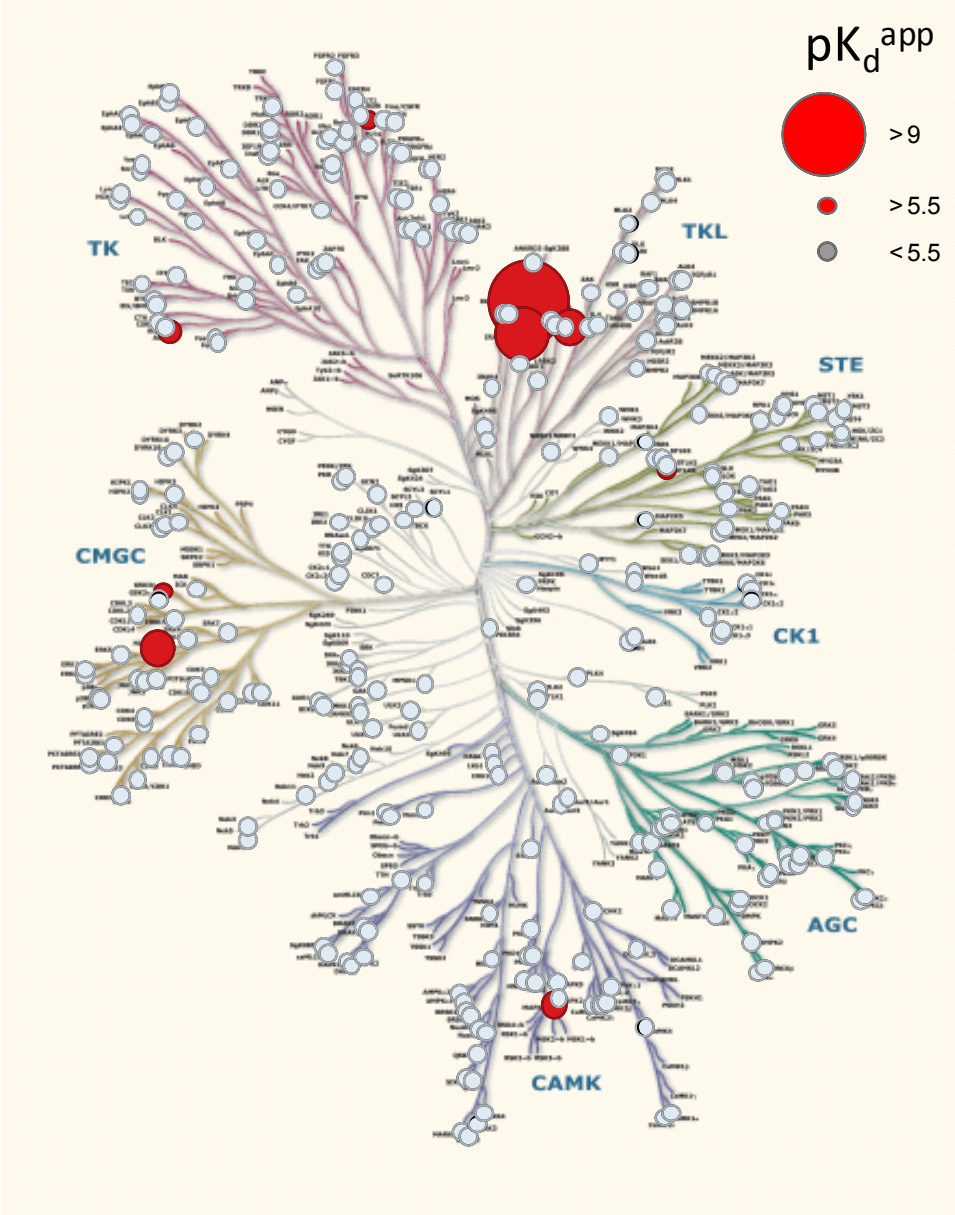
(A) Cell viability with RIPK2 PROTACs. THP-1 cells were treated with the indicated concentrations of PROTAC_RIPK2 or PROTAC_RIPK2_epi for 16 hours, followed by a Trypan Blue assay to determine cell viability. Error bars represent mean and S.D.

Supplementary Figure 8. Demonstration of a PROTAC-mediated ternary complex between RIPK2 and VHL

Immunoaffinity enrichment of endogenous VHL from THP-1 cell extract in the presence of RIPK2 inhibitor, PROTAC_RIPK2 and PROTAC_RIPK2_epi. Immunoblotting demonstrates enrichment of VHL (bottom) using an anti-VHL antibody as compared to IgG control. In the presence of various concentrations of PROTAC_RIPK2 but not with PROTAC_RIPK2_epi and RIPK2-binding ligand, RIPK2 is selectively co-precipitated (top). Shown are replicate n=1 and replicate n=2.



Supplementary Figure 9A. Kinome specificity of RIPK2 ligand



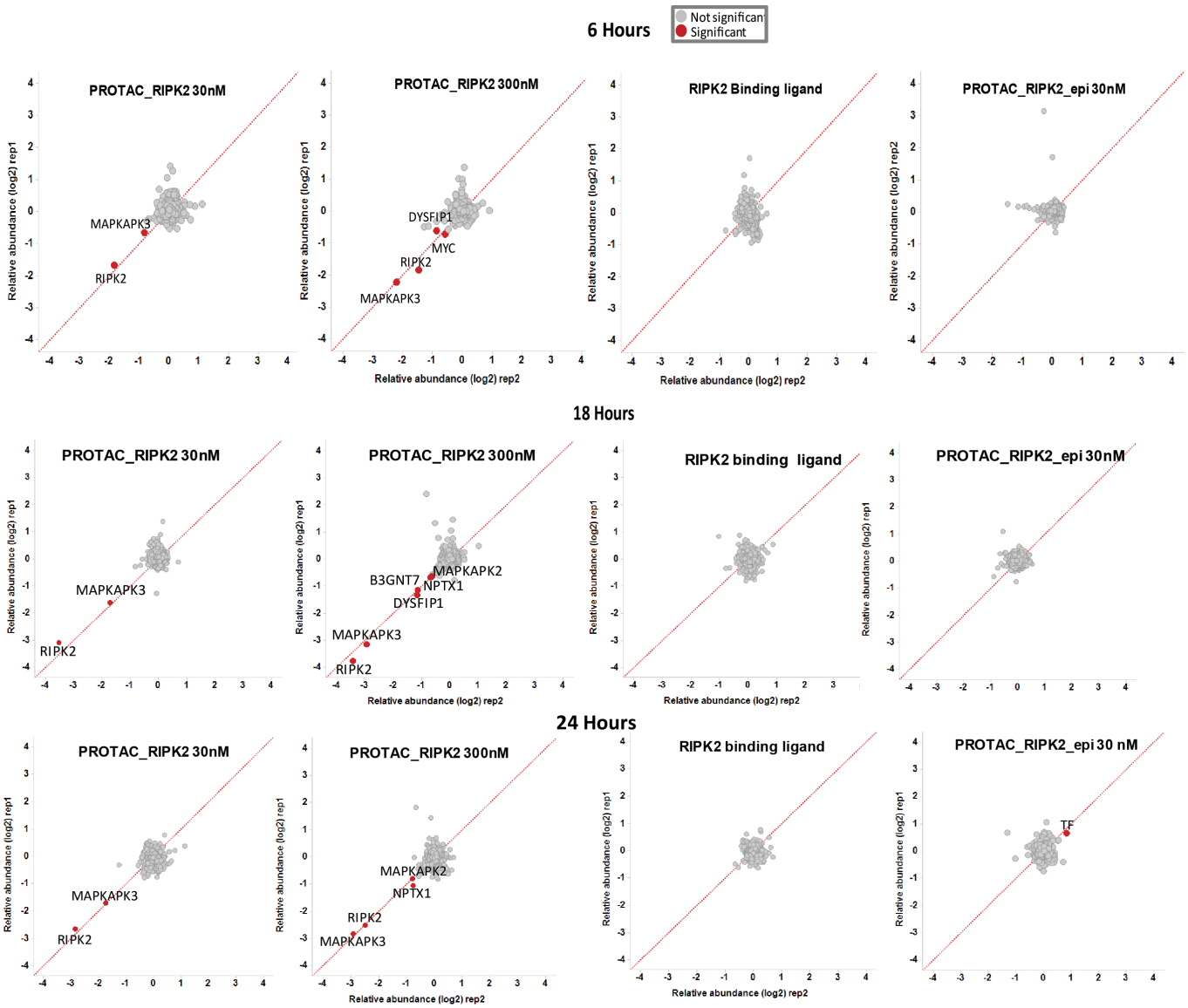
The specificity of the RIPK2 ligand was profiled against 371 kinases by KinoBead profiling (average of 2 determinations).

Supplementary Figure 9B. Summary of significant activities from kinobead profiling of RIPK2-Ligand, PROTAC_RIPK2 and PROTAC_RIPK2_epi

Protein	pK_D^{app}		
	RIPK2-Ligand	PROTAC RIPK2	PROTAC RIPK2_epi
RIPK2	9.3	8.6	8.5
RIPK3	7.7	6.6	6.7
NLK	6.7	<5.5	<5.5
ABL1/BCR-ABL	6.5	6.2	6.1
TESK2	6.7	6.1	6.3
TGFBR2	6.2	<5.5	<5.5
MAPKAPK3	6.1	<5.5	<5.5

The PROTACS interact with similar kinases to the parent ligand though absolute pK_D^{app} values (mean of n=2) are slightly lower. Both PROTACs show almost identical profiles to each other.

Supplementary Figure 10A. RIPK2 Proteomics time and concentration



dependence

Proteomic analysis of protein abundances in response to treatment of THP1 cells with the indicated agent at the indicated time. Scatter plots depicting relative abundances of detected proteins (circles) from THP-1 cells incubated for 6 h with 30 and 300 nM PROTAC_RIPK2, 30 nM Protac_RIPK2_epi and RIPK2-binding ligand compared to vehicle treated samples. . Proteins significantly ($p<0.05$) regulated in the two biological replicate experiments are indicated in red. Relative abundance is plotted replicate 1 vs replicate 2 on a Log2 scale.

Supplementary Figure 10B. ERRα Proteomics time and concentration

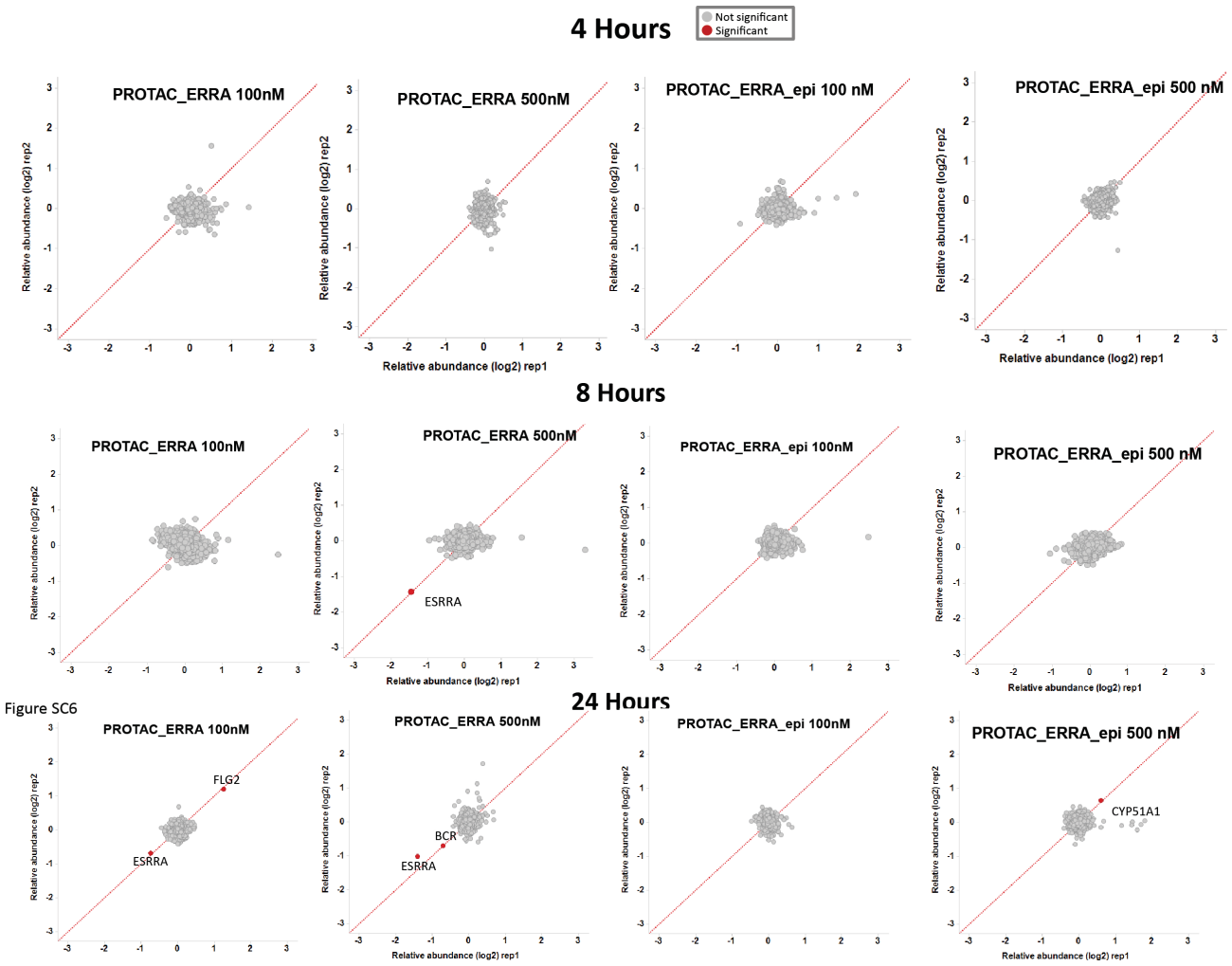


Figure SC6

dependence

Proteomic analysis of protein abundances in response to treatment of MCF-7 cells with the indicated agent at the indicated time. Scatter plots depicting relative abundances of detected proteins (circles) from MCF-7 cells incubated for 4 h with 100 and 500 nM PROTAC_ ERRα and ERRα_epi compared to vehicle treated samples. Proteins significantly ($p<0.05$) regulated in the two biological replicate experiments are indicated in red. Relative abundance is plotted replicate 1 vs replicate 2 on a Log2 scale.

Tables

Supplementary Table 1**:** Relative abundance of proteins immunoprecipitated with the active or inactive VHL ligands. This data table is graphically represented in Figure 3A. See file “Table S1 Bondeson et al”.

Supplementary Table 2**:** Results summary for ternary complex formation. This data table is graphically represented in Figure 3C. See file “Table S4 Bondeson et al”

Supplementary Table 3**:** Proteomic analysis of RIPK2 phosphorylation sites. LC/MS/MS analysis of RIPK2 recombinant protein after incubation in kinase buffer with or without ATP. See file “Table S3 Bondeson et al”.

Supplementary Table 4**:** Results summary for Kinobead competition binding experiments. This data table is graphically represented in Supplementary Figure 8. See file “Table S2 Bondeson et al”.

Supplementary Table 5: Results summary for expression proteomics. This data table is graphically represented in Figure 4 and Supplementary Figure 9. See file “Table S5 Bondeson et al”.

Supplemental Note for Syntheses

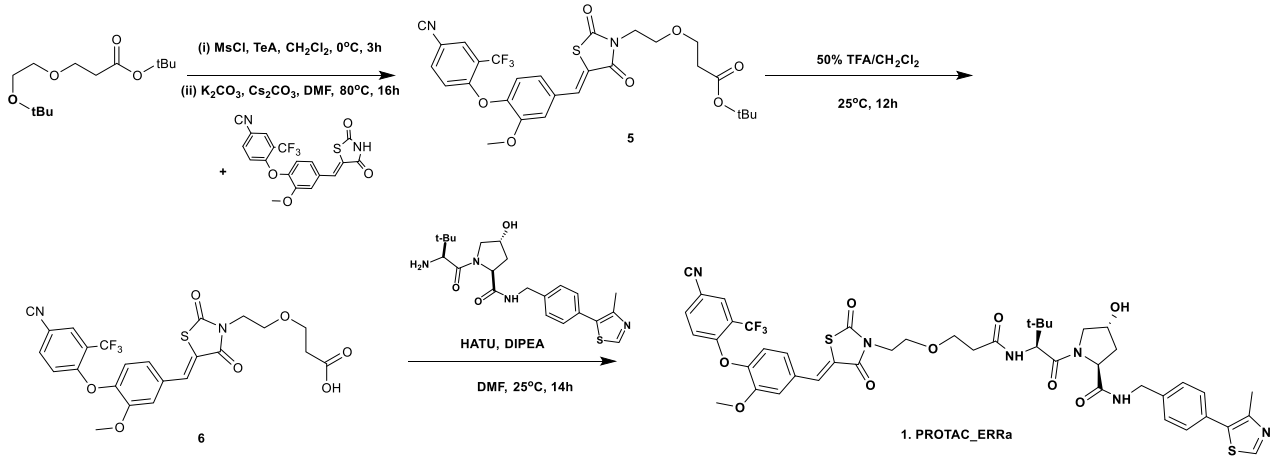
All reactions were carried out under an atmosphere of dry nitrogen or argon. Glassware was oven-dried prior to use. Unless otherwise indicated, common reagents or materials were obtained from commercial source and used without further purification. Dichloromethane (CH_2Cl_2) and dimethylformamide (DMF) were dried by a PureSolv^{TM} solvent drying system.

Flash column chromatography was performed using silica gel 60 (230-400 mesh). Analytical thin layer chromatography (TLC) was carried out on Merck silica gel plates with QF-254 indicator and visualized by UV. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DPX 500 (500 MHz ^{1}H; 125 MHz ^{13}C) or Bruker Avance DPX 400 (400 MHz ^{1}H; 100 MHz ^{13}C) spectrometer at room temperature. Chemical shifts were reported in ppm relative to the residual $CDCl_3$ (δ 7.26 ppm ^{1}H; δ 77.0 ppm ^{13}C). NMR chemical shifts were expressed in ppm relative to internal solvent peaks, and coupling constants were measured in Hz. Mass spectra were obtained using Perkin-Elmer API 150 EX LC/MSD spectrometers.

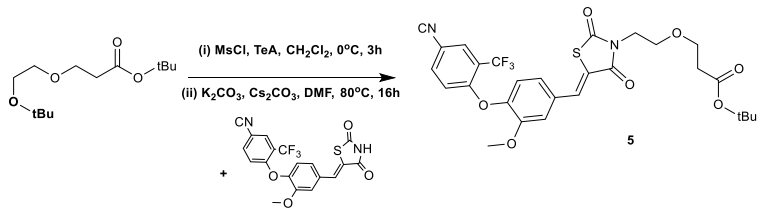
General Procedure and Preparation of PROTAC_ERRα

The parent ligand for PROTAC_ERRα was prepared via the previously published methodology in Reference 19.

Scheme S1. Syntheses of PROTAC_ERRα

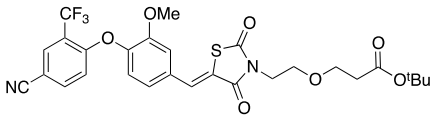


General Procedure for Compound 5. tert-butyl (Z)-3-(2-(5-(4-(4-cyano-2-(trifluoromethyl)phenoxy)-3-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)ethoxy) propanoate



To a solution of tert-butyl 3-(2-hydroxyethoxy)propanoate (1.0 eq.) and TEA (1.3 eq.) in CH_2Cl_2 (0.2 M) was added slowly MsCl (1.3 eq.) at 0 ^{o}C. The mixture was stirred for 3 h at 0 ^{o}C. After

the reaction, the solvent was removed under vacuum, and then the resulting was dissolved with EtOAc. The solution was filtered through celite, and washed with EtOAc. The combined organic phase was dried over $MgSO_4$. After completely removing the solvent, the crude product was used directly to next step. The crude compound was dissolved in DMF (0.2 M), and then compound 29 (1.0 eq), K_2CO_3 (2.5 eq.) and Cs_2CO_3 (0.1 eq.) were added to the solution at 25 ^oC. The mixture was heated to 80 ^oC, and then stirred for 16 h. After the reaction, the mixture was cooled down to 25 ^oC, diluted with H_2O, and then extracted with EtOAc (x3). The combined organic phase was dried over $MgSO_4$. After completely removing the solvent, the crude product was purified by prep-TLC (2:98 $MeOH/CH_2Cl_2$) to give compound 5 as a yellowish oil.

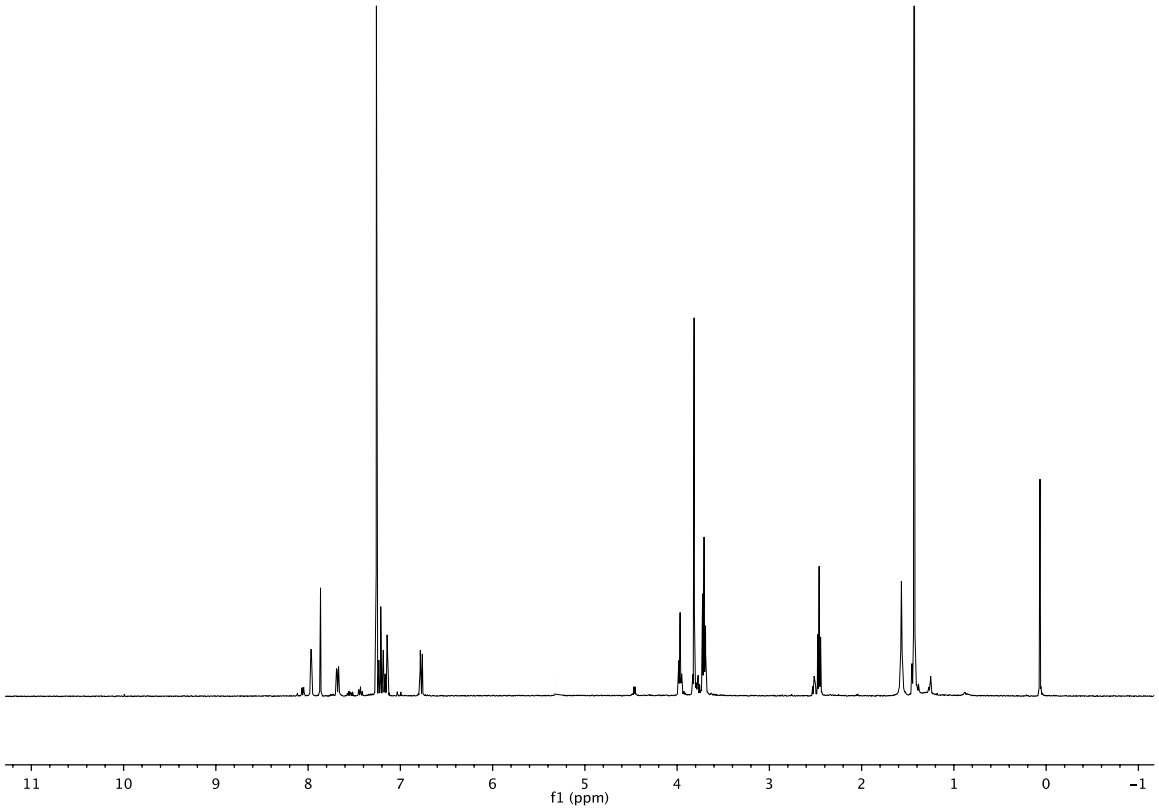


Yield (33 %)

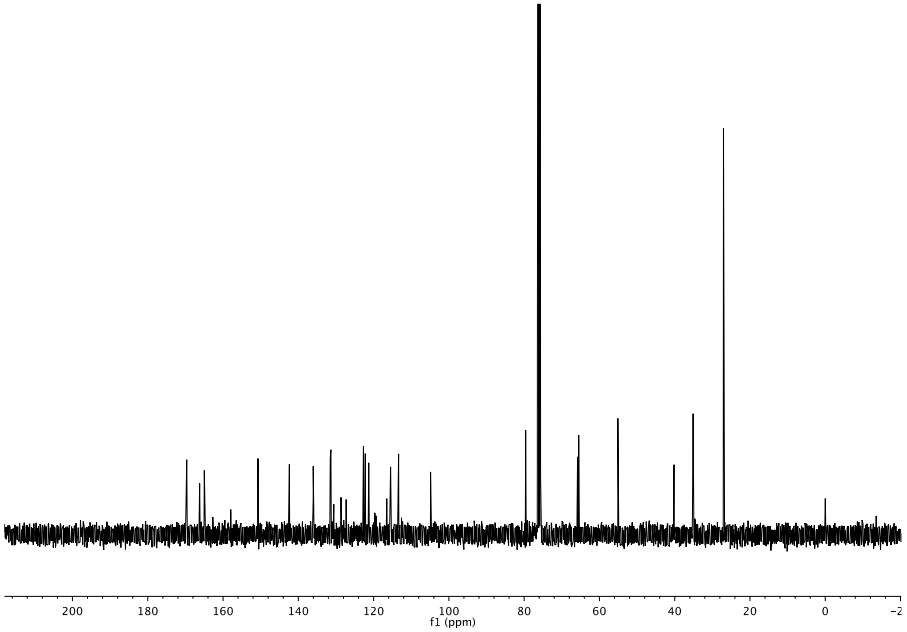
^1H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, 1H, J = 2.0 Hz), 7.87 (s, 1H), 7.68 (dd, 1H, J = 8.8, 2.0 Hz), 7.18 (m, 3H), 6.77 (d, 1H, J = 84 Hz), 3.97 (t, 2H, J = 5.6 Hz), 3.82 (s, 3H), 3.71 (m, 4H), 2.46 (t, 2H, J = 6.4 Hz), 1.43 (s, 9H)

^{13}C NMR (100 MHz, $CDCl_3$) δ 169.6, 166.2, 162.7, 150.7, 142.4, 136.0, 131.5, 131.3, 128.7, 127.3, 122.7, 122.2, 121.3, 119.7, 119.3, 116.4, 115.5, 113.3, 104.9, 79.6, 65.8, 65.5, 55.1, 40.2, 35.1, 27.0

MS (ESA, m/z) calcd for $C_{28}H_{27}F_3N_2NaO_7S$ $(M+Na)^+$ 615.1, found 615.0

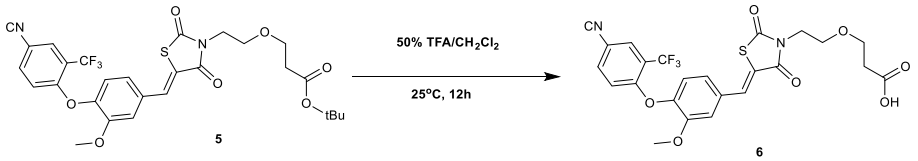


^{1}H NMR of **5**

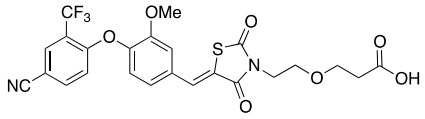


^{13}C NMR of **5**

General Procedure for Compound 6. (Z)-3-(2-(5-(4-(4-cyano-2-(trifluoromethyl)phenoxy)-3-methoxybenzylidene)-2,4-dioxothiazolidin-3-yl)ethoxy)propanoic acid



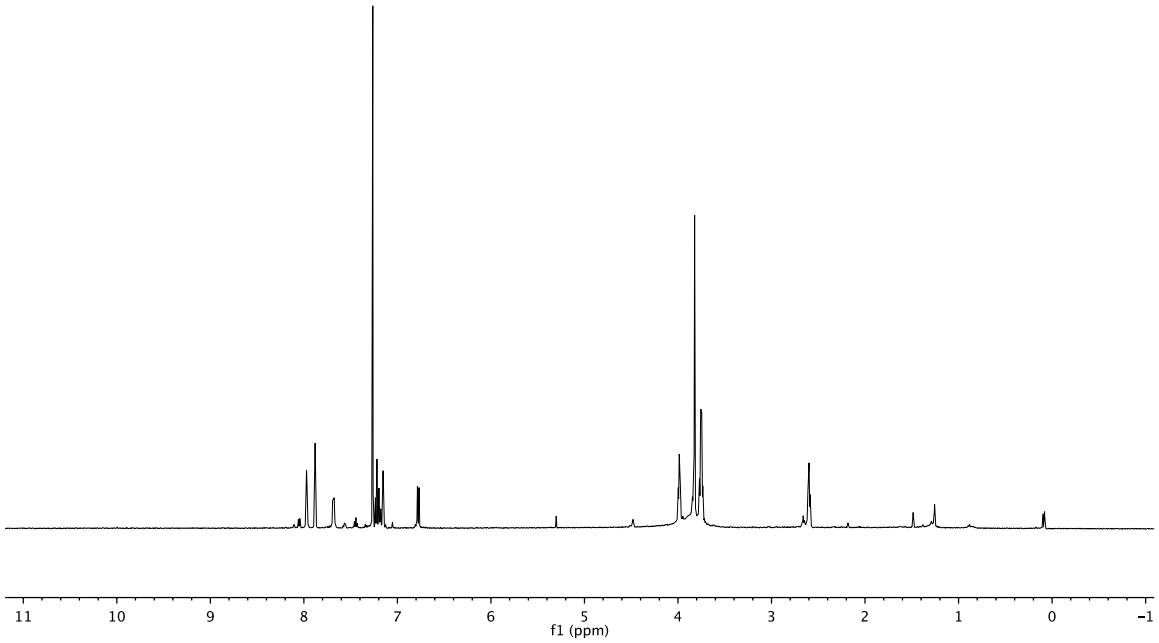
The compound 5 (1.0 eq.) was dissolved in 50 % TFA in CH_2Cl_2 at 25 ^{o}C, and then stirred for 12 h. After the reaction, the solvent was removed under vacuum and then the crude product was used to next step without further purification.



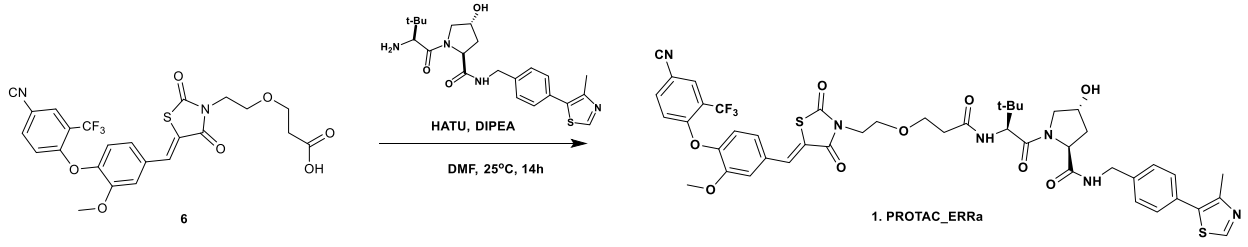
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (d, 1H, J = 2.5 Hz), 7.88 (s, 1H), 7.68 (dd, 1H, J = 9.0, 2.5 Hz), 7.19 (m, 3H), 6.78 (d, 1H, J = 8.0 Hz), 3.99 (t, 2H, J = 5.5 Hz), 3.83 (s, 3H), 3.75 (m, 4H), 2.60 (t, 2H, J = 6.0 Hz)

^{13}C NMR (125 MHz, $CDCl_3$) δ N/A

MS (ESA, m/z) calcd for $C_{24}H_{20}F_3N_2O_7S$ $(M+H)^+$ 537.5, found 537.3

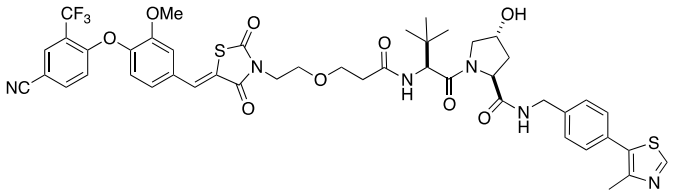


^{1}H NMR of **6**



General Procedure for PROTAC_ERRα

To a solution of compound 2 (1.0 eq.) in DMF (0.1 M) were HATU (1.0 eq.), DIPEA (3.0 eq.) and (2S,4R)-1-((S)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (1.0 eq.) at 25 °C. The mixture was stirred for 14 h. After the reaction, the mixture was diluted with sat. $NaHCO_3$ (aq), and then extracted with EtOAc (x3). The combined organic phase was washed with sat. NH_4Cl (aq), and brine, and then dried over $MgSO_4$. After completely removing the solvent, the crude product was purified by flash chromatography (3:97 to 7:93 MeOH/CH_2Cl_2) to give PROTAC_ERRα as a yellowish oil.

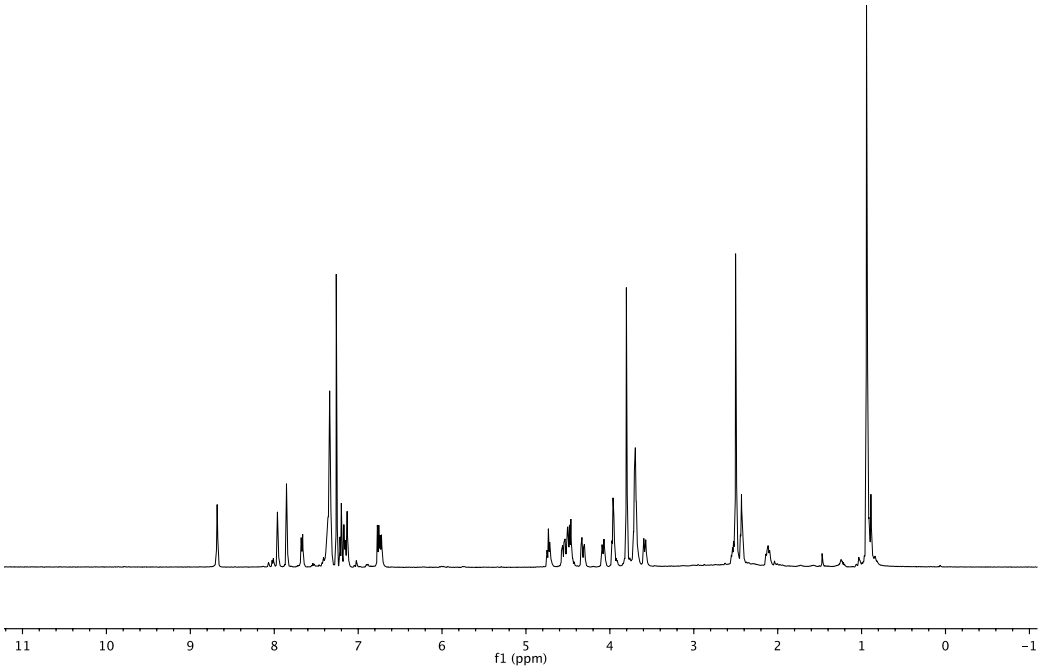


Yield (62 %)

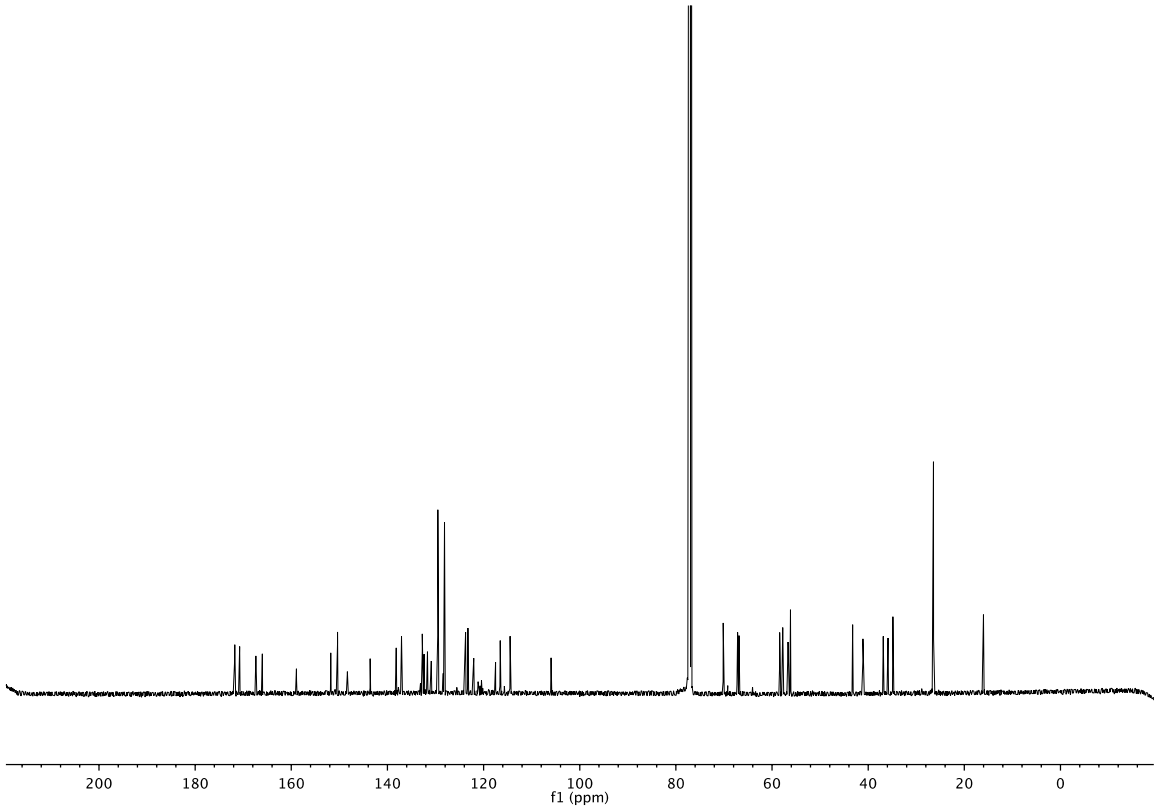
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.67 (s, 1H), 7.96 (s, 1H), 7.85 (s, 1H), 7.67 (d, 1H, J = 8.5 Hz), 7.33 (m, 4H), 7.17 (m, 2H), 6.76 (d, 1H, J = 7.5 Hz), 6.73 (d, 1H, J = 7.5 Hz), 4.73 (m, 1H), 4.55 (dd, 1H, J = 15.0, 6.5 Hz), 4.47 (m, 2H), 4.32 (dd, 1H, J = 15.0, 5.0 Hz), 4.08 (d, 1H, J = 11.0 Hz), 3.96 (m, 2H), 3.80 (s, 3H), 3.70 (m, 4H), 3.58 (d, 1H, J = 11.5 Hz), 2.54 (m, 1H), 2.50 (s, 3H), 2.43 (t, 2H, J = 5.5 Hz), 2.12 (m, 1H), 0.94 (s, 9H)

^{13}C NMR (125 MHz, $CDCl_3$) δ 171.8, 171.7, 170.7, 167.4, 166.0, 158.9, 151.8, 150.4, 148.3, 143.6, 138.2, 137.1, 132.8, 132.4, 131.7, 130.9, 129.7, 129.5, 128.4, 128.1, 123.7, 123.3, 122.0, 121.1, 120.1, 120.4, 117.5, 116.5, 114.5, 106.0, 70.2, 67.2, 66.9, 58.4, 57.8, 56.7, 56.2, 43.2, 41.1, 36.9, 35.9, 34.9, 26.5, 16.0

MS (ESA, m/z) calcd for $C_{46}H_{48}F_3N_6O_9S_2$ $(M+H)^+$ 949.3, found 948.9

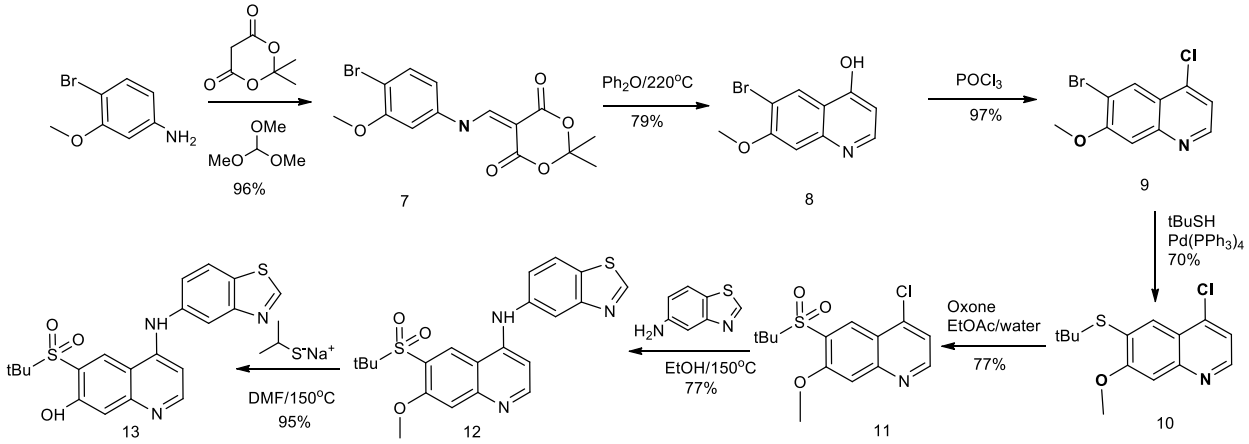


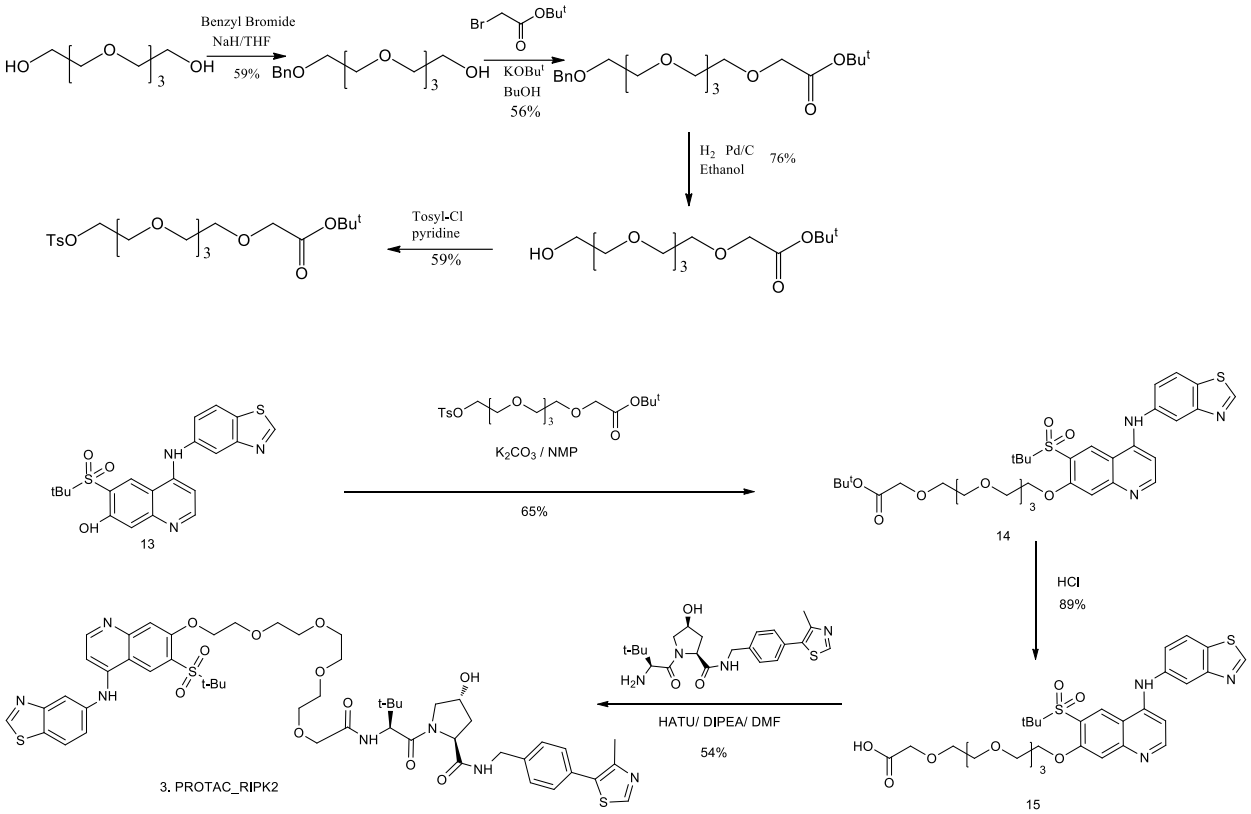
^{1}H NMR of PROTAC_ ERRα



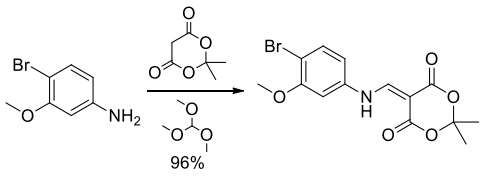
^{13}C NMR of PROTAC_ ERRα

General Procedure and Preparation of PROTAC_RIPK2 and PROTAC_RIPK2_epi





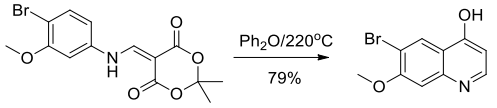
General Procedure for Compound 7. 5-({[4-bromo-3-(methyloxy)phenyl]amino} methylidene)-2,2- dimethyl-l,3-dioxane-4,6-dione



7

2,2-Dimethyl-1,3-dioxane-4,6-dione (8.5 g, 58 mmol) in trimethyl orthoformate (50 mL, 450 mmol) was refluxed at 105 °C for 1 hr. 4-Bromo-3- methoxyaniline (10.5 g, 50.4 mmol) was then added and refluxing was continued for an additional hour. The suspension was filtered, and the solid was washed with MeOH and vacuum dried to yield 5-({[4-bromo-3-(methyloxy)phenyl]amino}methylidene)-2,2- dimethyl-l,3-dioxane-4,6-dione (17 g, 49 mmol, 96 % yield). ^{1}H NMR (400 MHz, DMSO- d) δ ppm 1.68 (s, 6H), 3.90 (s, 3H), 7.11 (dd, J= 8.6 Hz, 2 Hz, 1H), 7.43 (d, J= 2 Hz, 1H), 7.59 (d, J= 8.6 Hz, 1H), 8.64 (s, IH), 11.23 (br. s., 1H).

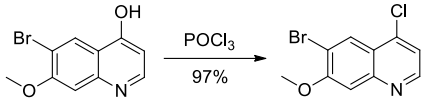
General Procedure for Compound 8. 6-bromo-7-(methyloxy)-4-quinolinol



7 8

To diphenyl ether (68 mL, 420 mmol) at 230°C was added 5-({[4-bromo-3-(methyloxy)phenyl]amino}methylidene)-2,2-dimethyl- l,3-dioxane-4,6-dione (15 g, 42 mmol), and the mixture was stirred for 1 hr. The reaction mixture was poured into hexane after being cooled to room temperature. The precipitate was filtered and washed with hexane. The brown solid was dried under vacuum overnight to afford 6-bromo-7-(methyloxy)-4-quinolinol (10 g, 33 mmol, 79 % yield). ^{1}H NMR (400 MHz, DMSO-d) δ ppm 3.94 (s, 3H), 5.99 (dd, J= 7.4 Hz, 1.2 Hz, 1H), 7.05 (s, 1H), 7.86 (dd, J= 7.4 Hz, 5.8 Hz, 1H), 8.16 (s, 1H), 11.68 (br. s., 1H). MS (m/z) 254, 256 ($M+H^+$).

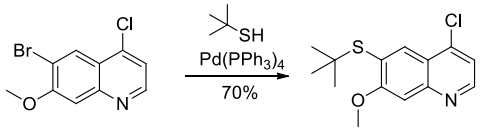
General Procedure for Compound 9. 6-bromo-4-chloro-7-(methyloxy)quinoline



8 9

6-Bromo-7-(methyloxy)-4- quinolinol (4.17 g, 16.41 mmol) in phosphorus oxychloride (8 mL, 82 mmol) was stirred at 110 °C for 1 hr. The reaction mixture was cooled and cautiously poured into saturated aqueous sodium carbonate with ice while stirring. The resulting suspension was filtered, the solid was washed with water and vacuum-dried overnight to yield 6-bromo-4-chloro-7-(methyloxy)quinoline (4.6 g, 16 mmol, 97 % yield). ^{1}H NMR (400 MHz, DMSO-d) δ ppm 4.05 (s, 3H), 7.61 (s, 1H), 7.65 (d, J= 4.8 Hz, 1H), 8.38 (s, 1H), 8.81 (d, J= 4.8 Hz, 1H). MS (m/z) 272, 274 ($M+H^+$).

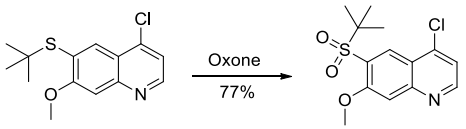
General Procedure for Compound 10. 6-(tert-butylthio)- 4-chloro-7-methoxyquinoline



9 10

A mixture of 6-bromo-4- chloro-7-methoxyquinoline (50 g, 183 mmol), $Pd(PPh_3)_4$ (5.30 g, 4.59 mmol), Na_2C0_3 (48.6 g, 459 mmol) and 1,4-dioxane (895 mL) was purged with nitrogen for 10 minutes. 2- methyl-2-propanethiol (tBuSH; 22.75 mL, 202 mmol) was added and the reaction was heated at 70°C for 4 d. The reaction was cooled to rt and flushed through a silica gel plug that had been pre-wetted with EtOAc using 100% EtOAc as the eluent. The product- containing fractions were combined and triturated with MeOH to afford 6-(tert-butylthio)- 4-chloro-7-methoxyquinoline (37.5 g, 128 mmol, 70 % yield). ^{1}H NMR (400 MHz, DMSO-d) δ ppm 8.79 (d, J=4.8 Hz, 1 H), 8.25 (s, 1 H), 7.63 (d, J=4.8 Hz, 1 H), 7.54 (s, 1 H), 3.99 (s, 3 H), 1.31 (s, 9 H). MS (m/z) 282

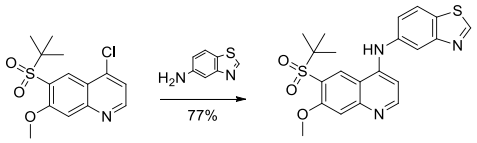
General Procedure for Compound 11. 6-(tert-butylsulfonyl)-4-chloro-7-methoxyquinoline



10 11

(Tert-butylthio)-4-chloro-7-methoxyquinoline (18.5 g, 63.0 mmol) in EtOAc (315 mL) and water (315 mL) was treated with Oxone (44.6 g, 72.5 mmol) and stirred at rt for 18h. The layers were separated and the aqueous phase was extracted twice with EtOAc. The combined organic extracts were concentrated to dryness. The residue was dissolved in a minimal amount of 10% MeOH/DCM, loaded onto a 340g pre-packed silica cartridge and purified via column chromatography (100% EtOAc, then 0-20% MeOH/EtOAc). Product-containing fractions were concentrated to dryness and triturated with EtOAc to yield 6-(tert-butylsulfonyl)-4-chloro-7-methoxyquinoline (15.2g, 48.4 mmol, 77% yield). ^{1}H NMR (400 MHz, DMSO- d) δ ppm 8.95 (d, J=4.8 Hz, 1 H), 8.65 (s, 1 H), 7.71 - 7.79 (m, 2 H), 4.04 (s, 3 H), 1.31 (s, 9 H).MS (m/z) 314.

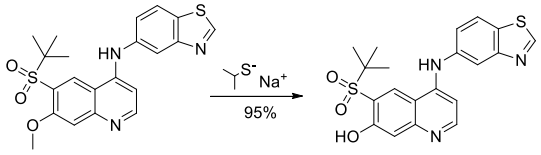
General Procedure for Compound 12. N-(6-(tert-butylsulfonyl)-7-methoxyquinolin-4-yl) benzo[d]thiazol-5-amine



11 12

A mixture of 6-(tert-butylsulfonyl)-4-chloro-7-methoxyquinoline (2 g, 6.37 mmol) and benzo[d]thiazol-5-amine (0.957 g, 6.37 mmol) in ethanol (10 mL) was irradiated by microwave at 150ºC for 15 mins. The reaction mixture was partitioned between ethyl acetate and saturated sodium bicarbonate. The aqueous layer was extracted with EtOAc twice and the combined EtOAc layers were dried over magnesium sulfate, filtered, concentrated. The residue was purified via flash chtomatography (100 g pre-packed silica cartridge, 0-75%EtOAc/cyclohexane) to yield N-(6-(tert-butylsulfonyl)-7-methoxyquinolin-4-yl)benzo[d]thiazol-5-amine (2.11 g, 4.94 mmol, 77 % yield). ^{1}H NMR (400 MHz, DMSO-d) δ ppm 1.32 (s, 9H), 3.98 (s, 3H), 6.90 (d, J= 5.3 Hz, 1H), 7.47 (s, 1H), 7.54 (dd, J= 8.6 Hz, 2 Hz, 1H), 8.03 (d, J= 2 Hz, 1H), 8.20 (d, J= 8.6 Hz, 1H), 8.50 (d, J= 5.6 Hz, 1H), 8.94 (s, 1H), 9.43 (s, 1H), 9.69 (s, 1H). MS (m/z) 428

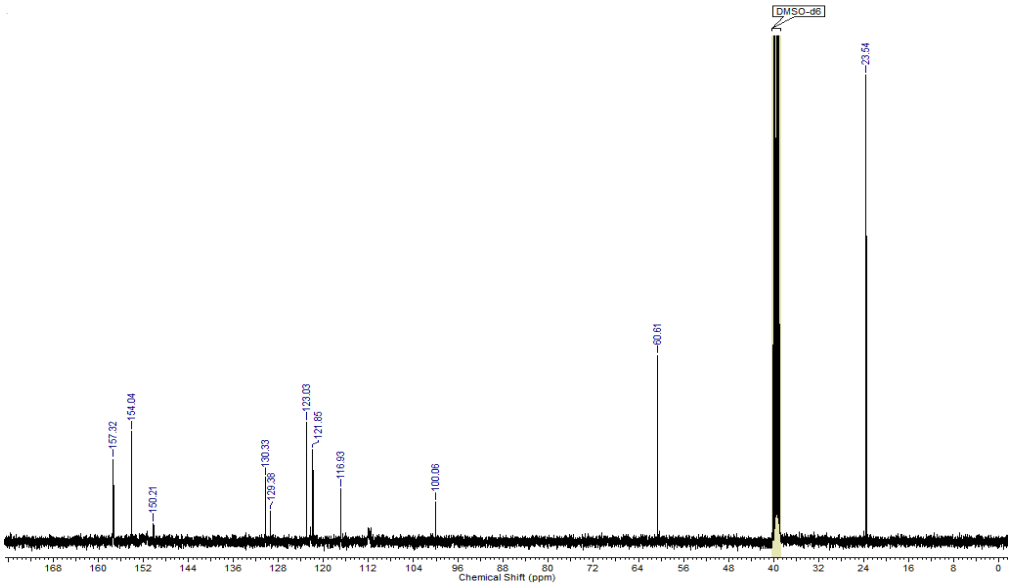
General Procedure for Compound 13. 4-(benzo[d]thiazol-5-ylamino)-6-(tert-butylsulfonyl)quinolin-7-ol



12 13

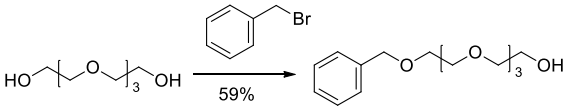
To a solution of N-(6-(tert-butylsulfonyl)-7-methoxyquinolin-4-yl)benzo[d]thiazol-5-amine hydrochloride (5.35g, 11.53 mmol) in DMF (50mL) was added sodium propane-2-thiolate (5.66 g, 57.7 mmol). The reaction was then heated to 150 °C for 1 hour. It was cooled to rt and concentrated under vacuum. The residue was treated with ethanol (70 mL) and stirred at 60ºC for 15 minutes, cooled in ice then the yellow precipitated product was filtered off, washed with minimum ethanol and dried under vacuum to afford 4-(benzo[d]thiazol-5-ylamino)-6-(tert-butylsulfonyl)quinolin-7-ol (4.55 g, 11mmol, 95 % yield). ^{1}H NMR (400 MHz, DMSO-d) δ ppm 1.35 (s, 9H), 6.74 (d, J= 5.3 Hz, 1H), 7.26 (s, 1H), 7.52 (dd, J= 8.6 Hz, 2 Hz, 1H),

8.02 (d, J= 2 Hz, 1H), 8.20 (d, J= 8.6 Hz, 1H), 8.36 (d, J= 5.6 Hz, 1H), 8.87 (s, 1H), 9.43 (s, 1H). MS (m/z) 414

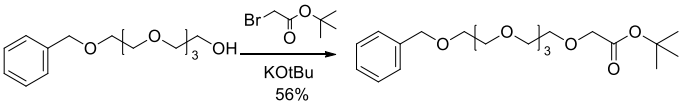


^{13}C of **13**

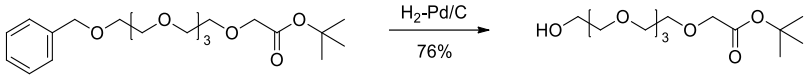
General Procedure for 14. tert-butyl 14-((4-(benzo[d]thiazol-5-ylamino)-6-(tert-butylsulfonyl)quinolin-7-yl)oxy)-3,6,9,12-tetraoxatetradecan-1-oate



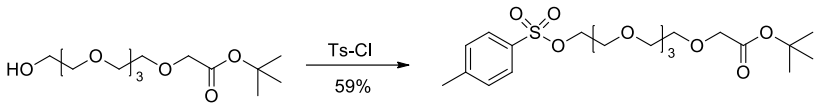
An ice-cooled solution of 2,2'-((oxybis(ethane-2,1-diyl))bis(oxy))diethanol (27.3 g, 140 mmol) in tetrahydrofuran (30 mL) under an atmosphere of nitrogen was treated with sodium hydride, 60 % w/w in mineral oil (1.54 g, 38.6 mmol). After stirring for 25 minutes at 0°C, the reaction mixture was treated dropwise with (bromomethyl)benzene (4.17 mL, 35.1 mmol). The mixture was heated to reflux under nitrogen for 24 hr then concentrated in vacuo. The residue was taken up in EtOAc (50 mL) and washed with brine (3x30 mL), dried ($MgSO_4$)and evaporated under vacuum. The product was purified using reverse phase chromatography (C18) eluting with 5-95% acetonitrile (+ 0.1% formic acid) and water (+ 0.1% formic acid). The appropriate fractions were combined and evaporated in vacuo to yield 1-phenyl-2,5,8,11-tetraoxatridecan-13-ol (5.76 g, 0.020 mmol, 58% yield) as a colourless oil. ^1H NMR (400 MHz, $CDCl_3$) δ ppm 3.69 (m, 16H), 4.59 (s, 2H), 7.29 (m, 1H), 7.35 (m, 4H). MS (m/z) 285



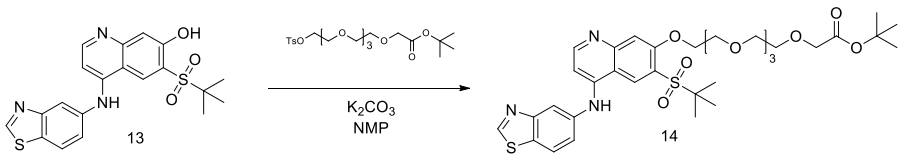
Potassium tert-butoxide (2.23 g, 19.8 mmol) was added to a stirred solution of 1-phenyl-2,5,8,11-tetraoxatridecan-13-ol (5.76 g, 18.0 mmol) in tert-butanol (60 mL) and the mixture was stirred at room temperature for 2 h. Tert-butyl 2-bromoacetate (4.79 mL, 32.5 mmol) was then added, and the mixture was stirred at room temperature overnight. The mixture was diluted with DCM (50 mL) and washed with water (50 mL) and then brine (2 x 50 mL). The organic extract was dried and concentrated under vacuum. The product was purified via flash chromatography using a gradient of 0-100% TBME-cyclohexane to yield tert-butyl 1-phenyl-2,5,8,11,14-pentaoxahexadecan-16-oate (4.22 g, 10.1 mmol, 56 % yield) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ ppm 1.47 (s, 9H), 3.66 (m, 16H), 4.01 (s, 2H), 4.57 (s, 2H), 7.27 (m, 1H), 7.34 (m, 4H). MS (m/z) 399



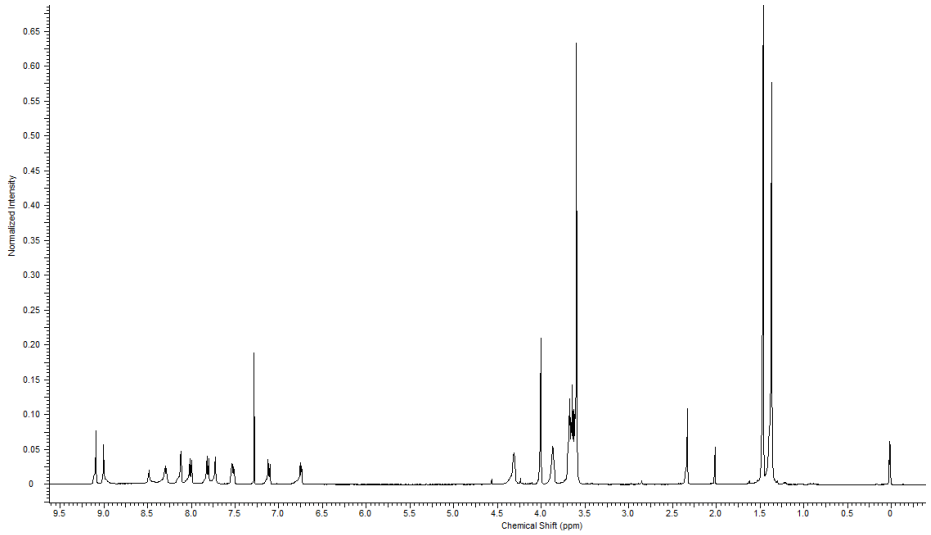
A mixture of tert-butyl 1-phenyl-2,5,8,11,14-pentaoxahexadecan-16-oate (4.22 g, 10.1 mmol) and palladium on carbon (10%) (1.61 g, 1.51 mmol) in ethanol (40 mL) was stirred at room temperature under an atmosphere of hydrogen for 1.5 hours. The catalyst was filtered through celite and the fitrate evaporated under vacuum to yield tert-butyl 14-hydroxy-3,6,9,12-tetraoxatetradecan-1-oate (2.96 g, 7.68 mmol, 76 % yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.44 (s, 9H), 3.66 (m, 16H), 3.99 (s, 2H). MS (m/z) 309

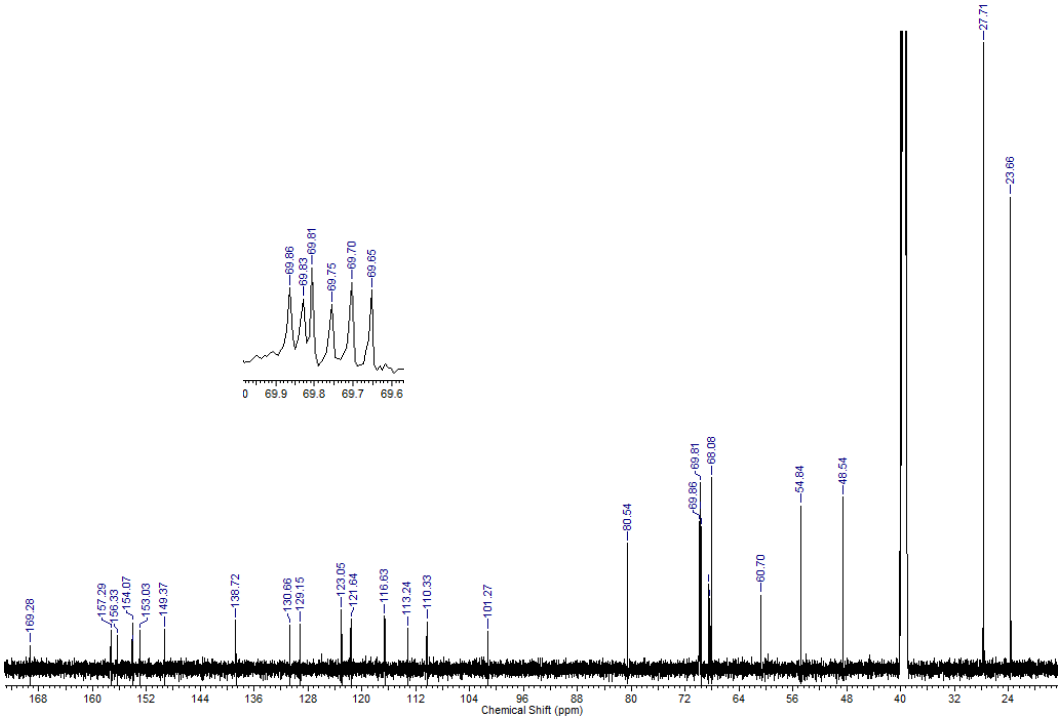


Tosyl-Cl (2.49 g, 13.1 mmol) was added to an ice-cooled solution of tert-butyl 14-hydroxy-3,6,9,12-tetraoxatetradecan-1-oate (2.96 g, 7.68 mmol) in pyridine (30 mL). The reaction was stirred at room temperature for 5 hours. The reaction mixture was partitioned between ethyl acetate (50 mL) and 2 M aq. HCl (40 mL). The organic extract was separated, washed with 2M aq HCl (40 mL), followed by saturated sodium bicarbonate (50 mL) and brine (50 mL). The organic extract was then dried and concentrated under reduced pressure and the product was purified by chromatography on silica 330g, using a gradient elution from 0-100% TBME in cyclohexane to yield tert-butyl 14-(tosyloxy)-3,6,9,12-tetraoxatetradecan-1-oate (2.17 g, 4.50 mmol, 59 % yield), as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.47 (s, 9H), 2.45 (s, 3H), 3.58 (s, 4H), 3.63 (m, 4H), 3.68 (m, 8H), 4.01 (s, 2H), 4.16 (m, 2H), 7.34 (d, 2H, J=8.3Hz), 7.78 (d, 2H, J=8.3Hz) . MS (m/z) 463



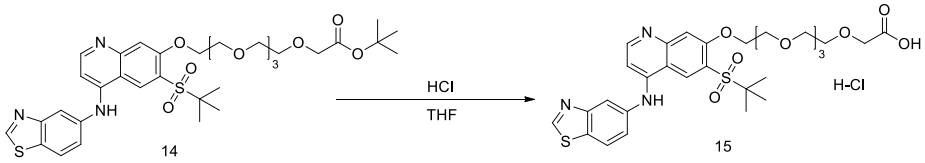
A mixture of 4-(benzo[d]thiazol-5-ylamino)-6-(tert-butylsulfonyl)quinolin-7-ol (50 mg, 0.12 mmol), tert-butyl 14-(tosyloxy)-3,6,9,12-tetraoxatetradecan-1-oate (84 mg, 0.18 mmol) and potassium carbonate (50 mg, 0.36 mmol) in NMP (2.4mL) was stirred and heated at 95^{o}C for 4 hours. The product was subjected directly to purification by mass-directed automated preparative HPLC (formic acid modifier) to afford tert-butyl 14-((4-(benzo[d]thiazol-5-ylamino)-6-(tert-butylsulfonyl)quinolin-7-yl)oxy)-3,6,9,12-tetraoxatetradecan-1-oate (55 mg, 0.078 mmol, 65% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.37 (s, 9H), 1.46 (s, 9H), 3.64 (m, 14H), 3.87 (s, 2H), 4.01 (s, 2H), 4.31 (s, 2H), 6.75 (d, 1H, J=6.4Hz), 7.12 (d, 1H, J=8.1Hz), 7.52 (d, 1H, J=7.3Hz) , 7.73 (s, 1H) , 7.52 (d, 1H, J=7.3Hz) , 7.81 (d, 1H, J=7.3Hz) , 8.01 (d, 1H, J=8.6Hz) , 8.12 (s, 1H), 8.29 (d, 1H, J=6.4Hz), 8.12 (s, 1H), 9.00 (s, 1H), 9.09 (s, 1H). MS (m/z) 704





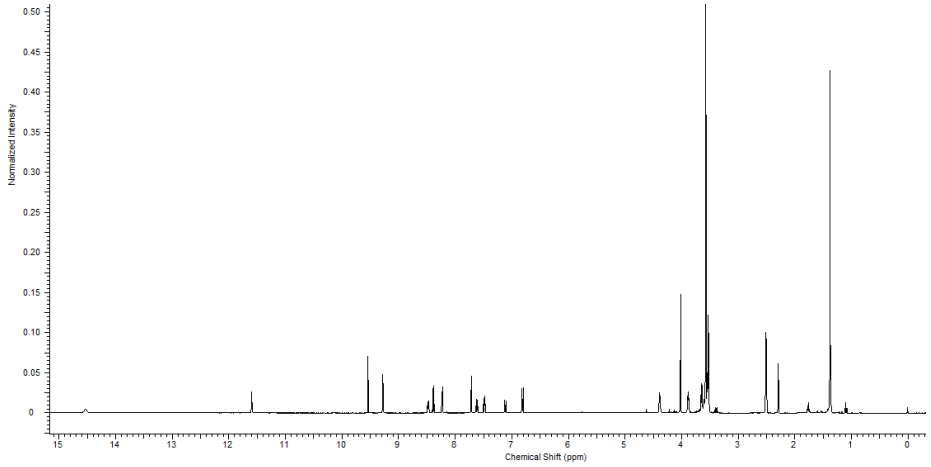
^{13}C NMR of **14**

General Procedure for 15. 14-((4-(benzo[d]thiazol-5-ylamino)-6-(tert-butylsulfonyl)quinolin-7-yl)oxy)-3,6,9,12-tetraoxatetradecan-1-oic acid, Hydrochloride

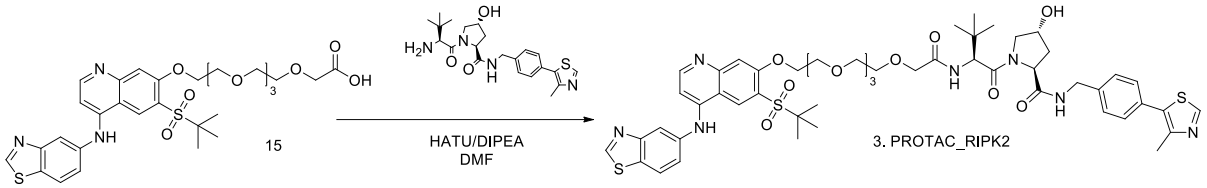


14 15

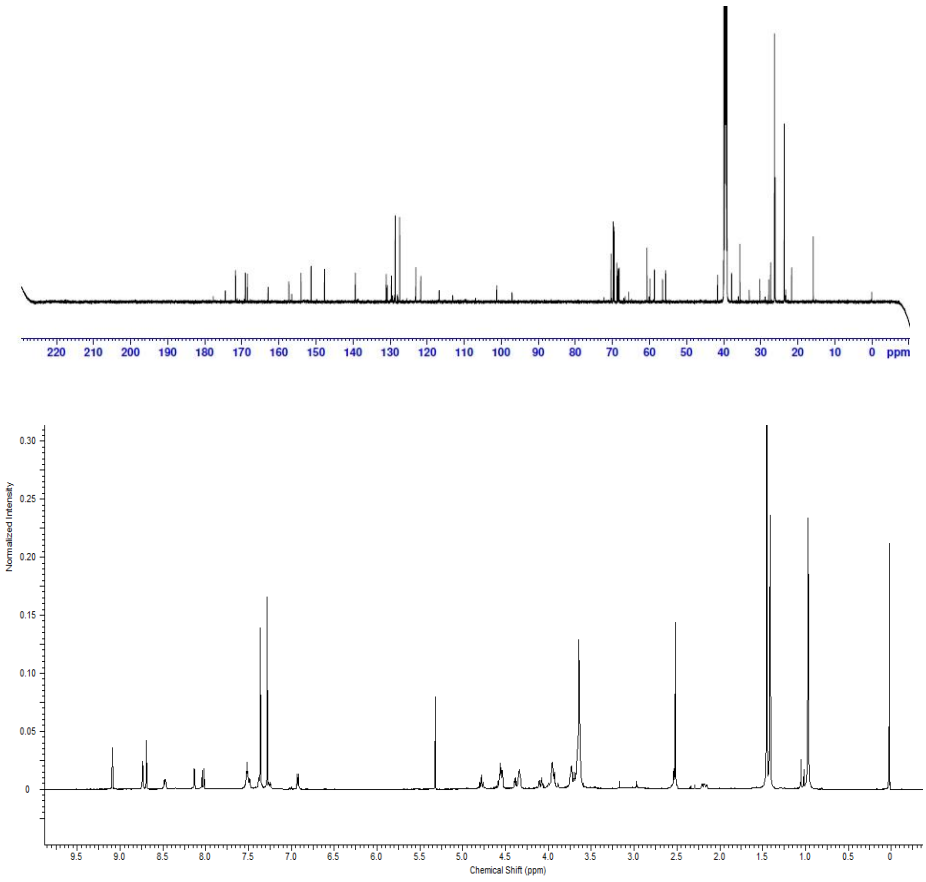
A solution of tert-butyl 14-((4-(benzo[d]thiazol-5-ylamino)-6-(tert-butylsulfonyl)quinolin-7-yl)oxy)-3,6,9,12-tetraoxatetradecan-1-oate (52 mg, 0.074 mmol) in THF (2 mL) was treated with hydrochloric acid (4M in 1,4-dioxane) (0.018 mL, 0.074 mmol), After 3 hours the mixture was evaporated to dryness to afford 14-((4-(benzo[d]thiazol-5-ylamino)-6-(tert-butylsulfonyl)quinolin-7-yl)oxy)-3,6,9,12-tetraoxatetradecan-1-oic acid, Hydrochloride (45 mg, 0.066 mmol, 89 % yield). ^{1}H NMR (400 MHz, DMSO-d) δ ppm 1.38 (s, 9H), 3.53 (m, 6H), 3.57 (m, 12H), 3.65 (m, 2H), 3.88 (m, 2H), 4.02 (s, 2H), 4.39 (t, 3H, J=3.7 Hz), 6.81 (d, 1H, J=7.3Hz), 7.12 (d, 1H, J=7.8Hz), 7.49 (d, 1H, J=8.1Hz) , 7.62 (dd, 1H, J=8.3Hz, 2.0Hz) , 7.72 (s, 1H), 8.23 (d, 1H, J=2.0Hz) , 8.38 (s, 1H) , 8.40(s, 1H), 8.47(s, 1H), 8.49(s, 1H), 9.28 (s, 1H), 9.54 (s, 1H), 11.60 (s, 1H), 14.54 (bs, 1H). MS (m/z) 648



General Procedure for 3. PROTAC_RIPK2



A mixture of 14-((4-(benzo[d]thiazol-5-ylamino)-6-(tert-butylsulfonyl)quinolin-7-yl)oxy)-3,6,9,12-tetraoxatetradecan-1-oic acid (70 mg, 0.11 mmol) and (2S,4R)-1-((S)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide, Hydrochloride (61 mg, 0.130 mmol) in DMF (0.7 mL) was treated with DIPEA (0.075 mL, 0.43 mmol) and then with HATU (45 mg, 0.12 mmol) and stirred at ambient temperature for 30 minutes. The product was subjected directly to purification by mass-directed automated preparative HPLC (formic acid modifier) to afford (2S,4R)-1-((S)-17-((4-(benzo[d]thiazol-5-ylamino)-6-(tert-butylsulfonyl)quinolin-7-yl)oxy)-2-(tert-butyl)-4-oxo-6,9,12,15-tetraoxa-3-azaheptadecan-1-oyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (62 mg, 0.058 mmol, 54.1 % yield). ^{13}C NMR (150 MHz, DMSO-d6) δ 178.2, 175.0, 172.2, 169.9, 169.0, 163.4, 157.9, 157.1, 154.6, 151.9, 148.3, 148.2, 139.9, 131.6, 131.3, 130.2, 129.9, 129.3, 129.1, 128.6, 128.5, 127.9, 123.6, 122.2, 117.3, 113.6, 101.7, 70.9, 70.4, 70.3, 70.3, 70.1, 69.4, 69.0, 68.9, 68.7, 66.2, 61.2, 60.4, 59.2, 57.0, 56.2, 42.2, 38.4, 36.6, 36.2, 33.6, 30.8, 29.4, 28.4, 27.9, 26.8, 26.8, 26.7, 24.2, 24.0, 23.8, 22.2, 16.4, 0.6. ^{1}H NMR (400 MHz, DMSO-d) δ ppm 0.97 (s, 9H), 1.41 (s, 9H), 2.52 (s, 3H), 3.64 (m, 12H), 3.73 (m, 2H), 3.96 (m, 4H), 4.09 (m, 1H), 4.34 (m, 2H), 4.39 (m, 2H), 4.56 (m, 3H), 4.78 (m, 1H), 5.32 (s, 1H), 6.92 (d, 1H, J=6.8Hz), 7.28 (s, 2H), 7.36 (m, 4H), 7.52 (m, 3H), 8.03 (d, 1H, J=8.3Hz), 8.13 (m, 1H), 8.48 (d, 1H, J=5.6Hz) , 8.69 (s, 1H), 8.74(s, 1H), 9.09(s, 1H). MS (m/z) 1061. NB spectral analysis subject to presence of rotameric forms.



PROTAC_RIPK2_epi was prepared using an identical sequence except (2R,4S)-1-((R)-2-amino-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide, Hydrochloride was used in the final step