Discovery of a Selective TC-PTP Degrader for Cancer Immunotherapy

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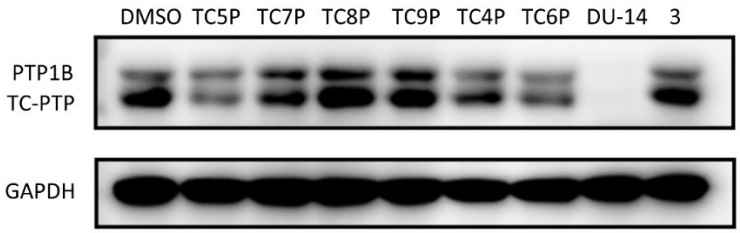


Fig. S1 Degradation assay results of first-generation TC-PTP PROTACs. HEK293 cells were treated with DMSO or 1 μM indicated compounds for 16 hours.

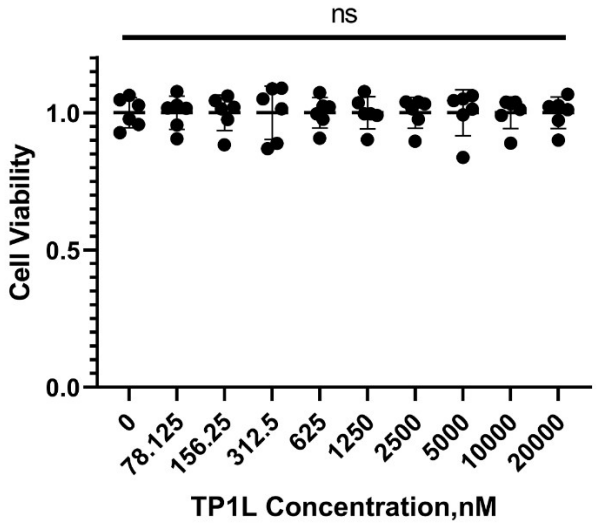


Fig. S2 Cell viability assay of TP1L in HEK 293 cells. No toxicity was observed with TP1L in at up to 20 μM concentration. HEK293 cells were treated with DMSO or TP1L at the indicated concentration for 48 hours, and the cell viability (●) was measured with Cell Counting Kit-8 assay and normalized with DMSO control.

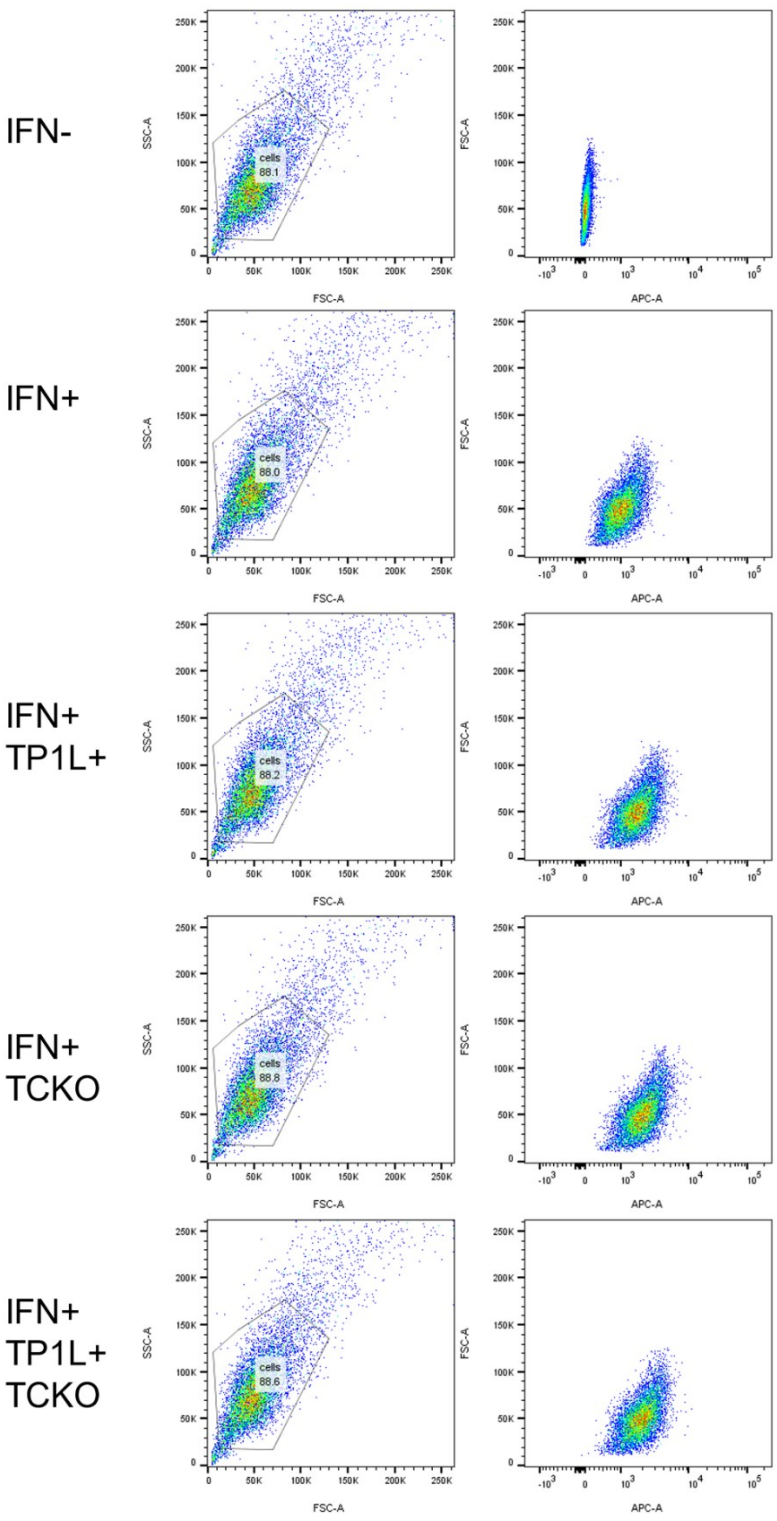


Fig. S3 Examples of FACS plots and gating strategy for flow cytometry analysis of MHC-I. Wildtype and TC-PTP knockout HEK293 cells were treated with DMSO or 500 nM TP1L for 16 hours for TC-PTP degradation then stimulated with 20 ng/ml mouse IFN-γ for 48 hours to induce MHC-I expression. TCKO = TC-PTP knockout.

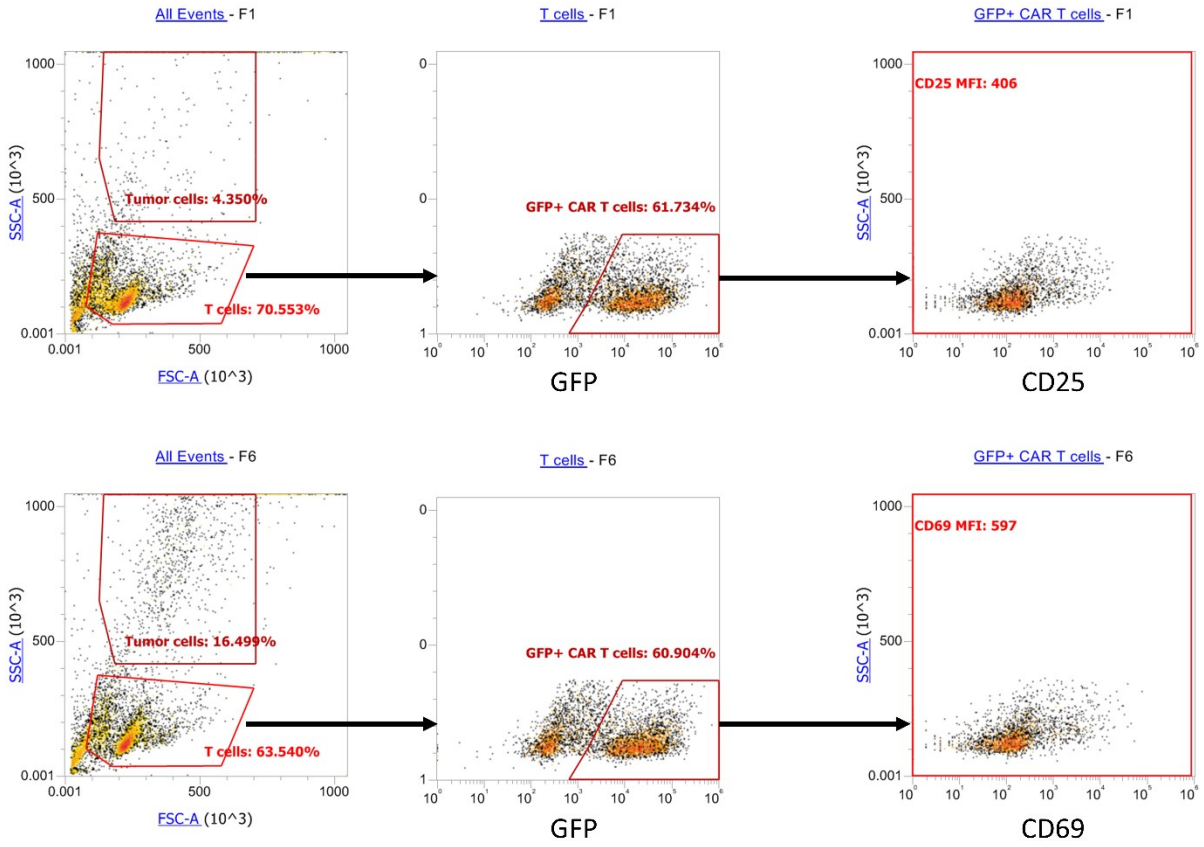


Fig S4 Examples of FACS plots and gating strategy for flow cytometry analysis of KB and CAR-T co-culture. Flow cytometry was used to measure CD25 and CD69 levels in cell lysates from KB and CAR-T cells with and without treatment of TP1L with the addition of bivalent molecule EC17.

Table S1. Enzymatic IC_{50}s of PROTACs against TC-PTP and PTP1B[a]

Compound	TC-PTP IC_{50} (nM)	PTP1B IC_{50} (nM)
TC4P	24.1 ± 0.9	82.3 ± 2.5
TC5P	19.6 ± 0.6	70.2 ± 1.7
TC6P	18.2 ± 0.5	67.9 ± 2.1
TC7P	25.4 ± 0.8	75.8 ± 1.8
TC9P	36.3 ± 0.8	112.5 ± 2.9
TC11P	45.1 ± 1.2	132.6 ± 3.4
TC4L	29.1 ± 1.1	88.3 ± 2.4
TC5L	24.6 ± 0.8	75.6 ± 1.9
TC6L	25.4 ± 0.7	76.9 ± 2.2
TC7L	22.4 ± 0.9	75.8 ± 1.8
TC8L	32.3 ± 1.8	105.8 ± 3.6
TP1L	19.6 ± 1.6	118.2 ± 4.5
TPiL	29.4 ± 0.9	86.4 ± 2.8

[a] TC-PTP activity was assayed using p-nitrophenyl phosphate (pNPP) as substrate with TC-PTP catalytic domain in pH 7 and 3,3-dimethylglutarate (DMG) buffer.

Table S2. IC_{50}s of TP1L for a panel of 14 PTPs[a]

PTP	Ti-01 IC_{50}, μM	Fold selectivity
TC-PTP	0.0196 ± 0.0016	1
PTP1B	0.1182 ± 0.0045	6.0
PTP-MEG2	>10	>500
SHP2	>10	>500
LYP	>10	>500
HePTP	>10	>500
Laforin	>10	>500
STEP	>10	>500
LMW-PTP	>10	>500
CDC-14A	>10	>500
CD45	>10	>500
FAP-1	>10	>500
VHR	>10	>500
PTP-α	>10	>500

[a] PTP activity was assayed using p-nitrophenyl phosphate (pNPP) as substrate in pH 7 and 3,3-dimethylglutarate (DMG) buffer.

Material and Methods

Molecular docking

Molecular modeling studies were conducted using the previously reported crystal structure of the apo TC-PTP catalytic domain (PDB code: 7F5O).[1] Peptide docking was performed using the Schrodinger Molecular Modeling Suite 2021-3 (Schrodinger, LLC, New York, NY, 2021).[2] TC-PTP inhibitor compound **3**[3] was prepared using LigPrep at pH 7.4 ± 1.0, and protein preparation was performed with the protein preparation workflow at pH 7.4 ± 1.0 using default settings unless otherwise stated. Three TC-PTP monomers were present in the crystal structure, and Chains B and C were deleted. Three iodide ions present in Chain A were deleted, and the protein was then preprocessed and optimized for H-bond assignment. The catalytic cysteine residue (C216) exists a thiolate anion due to its low pKa (~5), thus the side chain was manually deprotonated using the 3D builder. Lastly, a restrained minimization was performed, and waters beyond 4Å from heteroatoms were removed. The ligand binding site was established by aligning TC-PTP to the closely related inhibitor bound PTP1B phosphatase cocrystal structure (PDB code: 1PXH).[4] The PTP1B active site inhibitor was extracted, and the coordinates were used to define the grid during TC-PTP receptor grid generation. Ligand docking was performed using standard precision Glide peptide (SP-PEP) docking[2] with an H-bond constraint on the active-site arginine (R222). Data analyses were conducted using both the Schrodinger Maestro interface and the BIOVIA Drug Discovery Studio Visualizer. Figures were generated with PyMOL.

Cloning, expression, and purification of PTP proteins

The proteins (TC-PTP, Aa 1-387; PTP1B, Aa 1-321; SHP-2, Aa 224-528; LYP, Aa 1-294; STEP, Aa 258-539; HePTP, Aa 22-360; FAP-1, Aa 2124-2485; PTPα, Aa 173-793; CD45, Aa 620-1236; CDC14A, Aa 1-413; PTP-MEG2, Aa 286-582; VHR, Aa 1-185; Larforin, Aa 1-331; LMW-PTP, Aa 1-158.) were cloned into pET-21a(+) vector. Bacterial BL21(DE3) (Novagen) was used as an expression host, and the induction of protein expression was carried out in LB media with 1 mM IPTG at 18 °C overnight. Cell pellets were stored at −80 °C for subsequent protein purification. Protein purification was conducted at 4 °C. Frozen cell pellets were lysed by sonication in 40 ml cold lysis buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 5 mM imidazole, and 1 mM PMSF) per liter cell pellet. Cell lysates were clarified by centrifugation using a Bechman JA-18 rotor for 15 min at 6,000 rpm. The supernatant was incubated with HisPur Ni-NTA resin (Thermo Scientific) for 2 h, and then packed onto a column and washed with 50 resin volume of buffer A (50 mM Tris-HCl, pH

8.0, 500 mM NaCl, 5 mM imidazole). The HIS-tagged proteins were eluted with Buffer B (50 mM Tris-HCl, pH=8.0, 500 mM NaCl, 300 mM imidazole,). Pooled HIS-protein-containing fractions were concentrated, loaded onto a HiLoad 26/600 Superdex 75 column (GE Healthcare Biosciences), and eluted with storage buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 mM DTT, 10% glycerol). Proteins used for inhibition assays were purified using Ni-NTA resin (Qiagen) followed by size exclusion column chromatography (ÄKTA pure, cytiva) and the purity was determined to be >95% by SDS-PAGE and Coomassie staining. The protein was aliquoted and stored at −80 °C.

PTP inhibition assay and determination of IC_{50} values

PTP activity was assayed using p-nitrophenyl phosphate (pNPP) as a substrate in DMG buffer (50 mM DMG, pH 7.0, 1 mM EDTA, 150 mM NaCl, 2 mM DTT, 0.1 mg/mL BSA) at 25 °C. The assays were performed in 96-well plates. To determine the IC_{50} values, the reaction was initiated by the addition of enzyme (for PTP1B and TC-PTP, the final concentration was 0.4 nM, for other PTPs, the final concentration was 10 nM) to a reaction mixture (0.2 mL) containing pNPP (at final concentration close to the K_ms of tested enzymes, in specific: 0.05 mM for CDC-14A; 0.5 mM for FAP-1; 2 mM for TC-PTP, PTP1B, STEP, LWM-PTP, and PTPα; 3 mM for SHP2; 4 mM for Laforin; 5 mM for LYP, CD45, and VHR; 6 mM for PTP-MEG2 and HePTP) with various concentrations of inhibitors. The reaction rate was measured using a SpectraMax Plus 384 Microplate Spectrophotometer (Molecular Devices). Data were fitted using SigmaPlot Enzyme Kinetics Module (Systat Software, Inc.).

Cell Culture

HEK293 (ATCC #CRL-1573), U2OS (ATCC #HTB-96), HeLa (ATCC #CRM-CCL-2), and KB cells (ATCC #CRL-3596) were grown in DMEM, while Jurkat and H358 cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum, penicillin (50 units/mL), and streptomycin (50 µg/mL) in a 37°C incubator containing 5% CO2. 20 ng/ml human (Biolegend #713906) or mouse (Biolegend #714006) Interferon-γ were used for JAK/STAT pathway stimulation. Human CAR-T cells were generated as previously described.[5,6]

Cell viability assay

HEK293 cells were seeded in 96-well plates at 5,000 cells per well in 6 replicates and treated with 0, 78.125, 156.25, 312.5, 625, 1250, 2500, 5000, 10000, or 20000 nM of TP1L. After 48h, cell viability was assayed by CCK8 Cell Viability Assay (APExBIO, K1018). The background value (media without cells) was subtracted from the raw data and viability fold change was calculated relative to zero concentration.

Generation of TC-PTP deleted cells

TC-PTP deleted HEK293 cells were generated by transfecting HEK293 cells with TC-PTP CRISPR Plasmids (Santa Cruz Biotechnology #sc—403071-NIC). Transfection was performed using Lipofectamine 2000 (Invitrogen, 11,668,019) according to the manufacturer's recommendations. After transfection, cells were selected in growth media containing 1 µg/mL puromycin for 7 days. Single clones were picked and TC-PTP deletion was verified by western blot.

Immunoblotting

Cells were lysed with ice cold lysis buffer (50 mM Tris (pH 8.0, 150 mM NaCl, 10% Glycerol, 1% Triton-X-100) supplied with phosphatase inhibitor (Bimake) and protease inhibitor mixture (Roche Applied Science). Equal amounts of protein were resolved by SDS-PAGE, transferred to nitrocellulose membrane and subjected to immunoblotting. Antibodies used in this study include anti-following proteins: pSTAT1^{Tyr701} (Cell Signaling Technology #9167, 1:3000), GAPDH (Cell Signaling Technology #97166, 1:5000), pJAK1$^{Tyr1034/1035}$ (Cell Signaling Technology #74129, 1:1000), pJAK2$^{Tyr1007/1008}$ (Cell Signaling Technology #3776, 1:1000), TC-PTP (Abcam #ab180764, 1:3000), PTP1B (Abcam #ab244207, 1:1500), PTP-MEG2 (Invitrogen #MA5-23997, 1:2000), PTEN (Cell Signaling Technology #9188, 1:1000), STAT1 (Cell Signaling Technology #9172, 1:1000), STAT3 (Cell Signaling Technology #4904, 1:1000), STAT5 (Cell Signaling Technology #94205, 1:1000), LCK (Cell Signaling Technology #2984, 1:1000), AKT (Cell Signaling Technology #4691, 1:2000), ZAP70 (Cell Signaling Technology #2705, 1:1000), GSK-3α/β (Cell Signaling Technology #5676, 1:1000), ACTIN (Cell Signaling Technology #4970, 1:3000), SHP2 (Cell Signaling Technology #3397, 1:1000)

Imaging and Immunofluorescence

Cells were cultured directly on glass coverslips in 12- or 24-well plates. After experiments, cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 15 min at room temperature, permeabilized with 0.2% Triton X-100 in PBS for 10 min and blocked with BSA. For immunofluorescence, appropriate antibodies were applied overnight at 4°C, followed by wash and 1h incubation with appropriate secondary antibody. DNA staining (0.5 µg of Hoechst no. 33258/ml; Sigma) was used to identify cell nuclei. After washing with PBS, the coverslips were mounted with anti-fade mounting solution. Images were obtained with a Nikon Inverted Microscope Eclipse Ti-S. Antibodies used in this study include anti-following proteins: PTP1B (Abcam #ab244207, 1:300), TC-PTP (Santa Cruz # sc-373835,

1:100), pSTAT1^{Tyr701} (Cell Signaling Technology #9167, 1:500), Alexa Fluor™ 488 conjugated Goat anti-Rabbit Secondary antibody (ThermoFisher #A-11008, 1:500), Alexa Fluor™ 555 conjugated Goat anti-Mouse Secondary antibody (ThermoFisher # A-21424, 1:500)

Preparation of lentiviral vector encoding anti-FITC CAR

Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll density gradient centrifugation (GE Healthcare Lifesciences, USA) of fresh human blood from healthy volunteers (IRB#: 1702018875). Pure CD3+ T cells were isolated from PBMCs using EasySep™ Human T Cell Isolation Kit (STEM CELL technologies, Canada) and then cultured in TexMACSTM medium (Miltenyi Biotech Inc, CA) containing 1% penicillin and streptomycin sulfate in the presence of human IL-2 (100 IU/ml, Miltenyi Biotech Inc, CA). T cells were divided, and the above media was changed every 2–3 days.

An scFv with high affinity for fluorescein was synthesized (GeneScript), and plasmids encoding human CD8a, 4-1BB, and CD3z chain were purchased from GeneScript. Overlapping PCR method was then used to generate the final 1551 bp anti-FITC CAR construct and inserted into a pCDH-EF1-MCS-(PGK-GFP) lentiviral expression vector (System Biosciences). The sequence of the anti-FITC CAR construct was confirmed by DNA sequencing (Purdue Genomic Core Facility). Purified human T cells were first activated using Dynabeads coupled to anti-CD3/CD28 antibodies (Life Technologies) for 12 to 24 hours in the presence of human IL2 (100 IU/mL) and then infected with the aforementioned lentivirus. After 3 to 5 days of transduction, T cells were harvested and analyzed for GFP fluorescence by flow cytometry to determine transduction efficiency.

Analysis of cytotoxic activity and activation of anti-FITC CAR-T cells in vitro

Folate receptor-expressing KB cells were stained with viability dye (Sartorius, #4839) and seeded on 96 wells plates (~8000 cells / well), and 4M5.3 (anti-FITC) CAR-T cells at day 18 post transduction were incubated with or without PROTAC (TP1L) at 1uM overnight. 4M5.3 CAR-T cells were then added to the target cells at 1:3 effector to target cell (E:T) ratio in the absence or presence of 1 nM FITC-folate small molecule bispecific adaptor (EC17) and with the absence or presence 1 uM TP1L.

After coincubation for 48 hours, supernatants were collected from each culture well. Culture wells were then gently washed with PBS to collect unattached CAR-T cells and combined with the supernatants for staining of CAR-T cell markers. Suspension of CAR-T cells were centrifuged at 350 x g for 10 mins, and resuspended in PBS + 2% FBS for staining of CD25 (BioLegend, #302610) and CD69 (BioLegend, #310910).

Cells were then washed 3 times prior proceeding to flow cytometry for measurement of mean fluorescent intensity.

To analyze the in vitro lysis of KB cells, KB cells were collected by incubating with 0.25% trypsin (Fisher Scientific, MT25053CI) in each culture well and followed by washing the culture wells twice with complete RPMI 1640 media (Fisher Scientific, #27-016-021). Cell suspension from each culture wells were then proceeded to flow cytometry for analysis of live cell counts by the viability dye. Percent KB cell lysis was determined by comparing treatment wells to control wells with KB cells alone.

Antigen presentation complex evaluation

After incubation as described in results, HEK293 cells were trypsinized and washed in PBS + 2% FBS, stained with Anti-HLA Class 1 ABC antibody (Abcam #225636, 1:400) against human MHC-I complex and subsequently with Alexa Fluor™ 488 conjugated Goat anti-Rabbit Secondary antibody (ThermoFisher #A-11008, 1:500 as per the manufacturer's instructions and then analysed on an BD Fortessa LSR flow cytometry cell analyzer. To measure CAR-T cell activation, CAR-T cells were collected by centrifuge and stained with anti-human CD3 APC antibody (Biolegend #300312, 1:200), anti-human CD25 FITC Antibody (Biolegend #302604, 1:200), anti-human CD69 Pacific Blue Antibody (Biolegend #310920, 1:200) and then analysed on an BD Fortessa LSR flow cytometry cell analyzer.

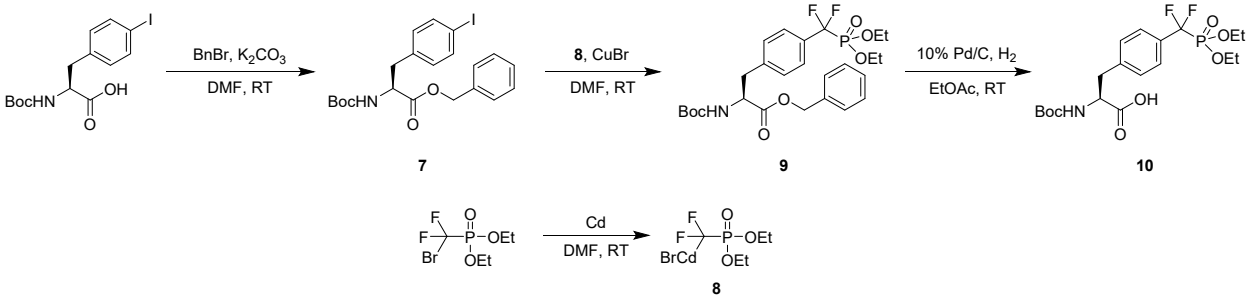
Chemistry

General Information

Unless otherwise noted, all reagents were purchased from commercial suppliers and used without further purification. Thin-layer chromatography was performed using glass precoated Merck silica gel 60 F254 plates. Flash column chromatography was performed on Biotage prepacked columns using the automated flash chromatography system Biotage Isolera One. Normal phase column chromatography was performed using KP-SIL silica gel (Biotage, USA), and reverse phase column chromatography was performed using Teledyne Isco RediSepRf Gold columns. HPLC purification was performed using column: Phenomenex Kinetex C18 5 µm 150 x 21.2 mm; Eluent A: water + 0.1% trifluoroacetic acid (99%), Eluent B: methanol; DAD scan: 210-400 nm). The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 500 MHz spectrometer using chloroform-D ($CDCl_3$) or dimethyl sulfoxide (DMSO-d6) as the solvents. Chemical shifts are expressed in ppm (δ scale) and referenced to the residual protonated solvent. Peak multiplicities are reported using the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad singlet). Mass spectra and purity data were obtained using an Agilent Technologies 6470 series, triple quadrupole LC–MS. The purity of all final tested compounds was determined to be >95% (UV, λ = 254 nm). High-resolution mass analysis was performed on an Agilent 6550 iFunnel Q-TOF mass LC–MS. PNPP was purchased from Thermo Scientific (catalog# PI34045).

Preparation of TC-PTP ligand 16.

Scheme S1. Synthesis of **Intermediate 10**.



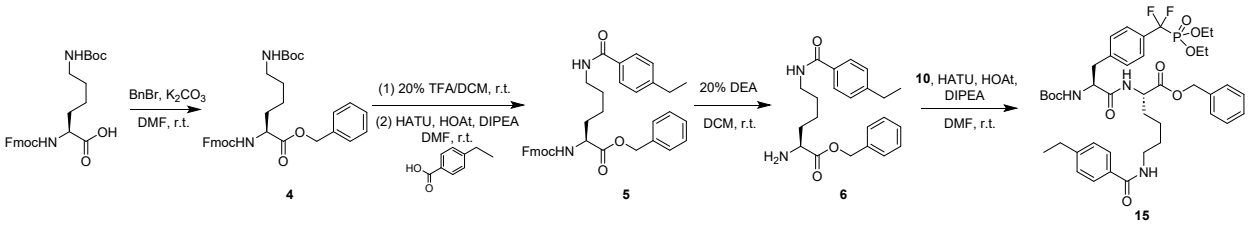
Benzyl (*S*)-2-((tert-butoxycarbonyl)amino)-3-(4-iodophenyl)propanoate (**7**). To a stirred mixture of (*S*)-2-((tert-butoxycarbonyl)amino)-3-(4-iodophenyl)propanoic acid (10.00 g, 25.6 mmol, 1.0 eq.) and K_2CO_3

(5.31 g, 38.4 mmol, 1.5 eq.) in DMF (100 ml) was added Benzyl bromide (6.57 g, 38.4 mmol, 1.5 eq.). Then the mixture was stirred at r.t. for 4 hours. After completion, the reaction mixture was diluted with EtOAc (500 ml) and washed with brine (3 x 500 ml). The organic layer was dried over anhydrous Na_2SO_4 and evaporated in vacuo. The product was then purified by flash chromatography (EtOAc/n-Hexane, 0%→10%). Yield: 8.80 g (72%). ^{1}H NMR (500 MHz, DMSO) δ 7.63 – 7.57 (m, 2H), 7.37 – 7.29 (m, 4H), 7.29 – 7.22 (m, 2H), 7.07 – 7.02 (m, 2H), 5.11 – 5.00 (m, 2H), 4.18 (ddd, *J* = 9.8, 8.0, 5.6 Hz, 1H), 2.94 (dd, *J* = 13.7, 5.6 Hz, 1H), 2.83 (dd, *J* = 13.7, 9.8 Hz, 1H), 1.30 (s, 9H). LC/MS m/z calculated $[M+H]^+$ 482.08, found 482.18.

Benzyl (*S*)-2-((tert-butoxycarbonyl)amino)-3-(4-((diethoxyphosphoryl)difluoromethyl)phenyl)propanoate (**9**). Intermediate **7** (10.00 g, 20.8 mmol, 1.0 eq.) was dissolved in anhydrous DMF (100 mL), then CuBr (5.97 g, 41.6 mmol, 2 equiv) and half of the supernatant solution of the Cd reagent **8** (ca. 1.7 equiv, prepared according to reported method[7]) was added in a dropwise manner. After 3 hours, further CuBr (2.98 g, 20.8 mmol, 1 equiv) and the other half of the Cd reagent solution were added. The reaction was allowed to stir at r.t. for 19 hours in total. The progress of the reaction was monitored by LC-MS. Upon completion, the reaction mixture was diluted with EtOAc (500 mL), filtered through Celite, and extracted with aq. NH4Cl (2 x 500 mL) and brine (500 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated in vacuo. The product was then purified by flash chromatography (EtOAc/n-Hexane, 0%→10%). Yield 11.86 g (57%). ^{1}H NMR (500 MHz, DMSO) δ 7.45 (d, *J* = 7.9 Hz, 2H), 7.41 – 7.26 (m, 8H), 5.09 (s, 2H), 4.26 (ddd, *J* = 9.8, 8.1, 5.3 Hz, 1H), 4.16 – 3.99 (m, 4H), 3.09 (dd, *J* = 13.8, 5.4 Hz, 1H), 2.95 (dd, *J* = 13.8, 10.0 Hz, 1H), 1.29 (s, 9H), 1.19 (td, *J* = 7.1, 1.5 Hz, 6H). LC/MS m/z calculated $[M+H]^+$ 542.21, found 542.28.

(*S*)-2-((tert-butoxycarbonyl)amino)-3-(4-((diethoxyphosphoryl)difluoromethyl)phenyl)propanoic acid (**10**). To a solution of intermediate **9** (8.98 g, 16.6 mmol, 1.0 eq.) in EtOAc (50 mL) was added Pd/C (10 wt. % loading, 750.0 mg). The reaction mixture was evacuated and filled with H_2 gas three times. The mixture was then stirred at r.t. for 12 hours. Upon completion, the reaction mixture was concentrated in vacuo. The product was then purified by flash chromatography (MeOH/DCM, 0%→5%). Yield 6.66 g (89%). ^{1}H NMR (500 MHz, DMSO) δ 7.45 (d, *J* = 8.0 Hz, 2H), 7.39 (d, *J* = 8.1 Hz, 2H), 7.12 (d, *J* = 8.5 Hz, 1H), 4.17 – 3.98 (m, 4H), 3.07 (dd, *J* = 13.8, 4.7 Hz, 2H), 2.91 – 2.83 (m, 1H), 1.29 (s, 9H), 1.19 (td, *J* = 7.0, 1.9 Hz, 6H). LC/MS m/z calculated $[M+H]^+$ 452.16, found 452.23.

Scheme S2. Synthesis of **Intermediate 15**.



Benzyl N^2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(tert-butoxycarbonyl)-L-lysinate (**4**). To a mixture of N^2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N^6-(tert-butoxycarbonyl)-L-lysine (10.00 g, 21.34 mmol, 1.0 eq.) and K_2CO_3 (4.42 g, 32.01 mmol, 1.5 eq.) in DMF (100 ml) was added Benzyl bromide (5.47 g, 32.01 mmol, 1.5 eq.). Then the mixture was stirred at r.t. for 4 hours. After completion, the reaction mixture was diluted with EtOAc (500 ml) and washed with brine (3 x 500 ml). The organic layer was dried over anhydrous Na_2SO_4 and evaporated in vacuo. The product was then purified by flash chromatography (EtOAc/n-Hexane, 0%→30%). Yield 9.06 g (76%). ^{1}H NMR (500 MHz, DMSO) δ 7.90 – 7.82 (m, 2H), 7.78 (d, *J* = 7.7 Hz, 1H), 7.69 (d, *J* = 7.5 Hz, 2H), 7.43 – 7.36 (m, 2H), 7.35 – 7.26 (m, 7H), 6.74 (t, *J* = 5.8 Hz, 1H), 5.10 (d, *J* = 1.3 Hz, 2H), 4.33 – 4.23 (m, 2H), 4.23 – 4.17 (m, 1H), 4.06 – 3.99 (m, 1H), 2.88 – 2.81 (m, 2H), 1.74 – 1.57 (m, 2H), 1.36 – 1.27 (m, 13H). LC/MS m/z calculated $[M+H]^+$ 559.28, found 559.39.

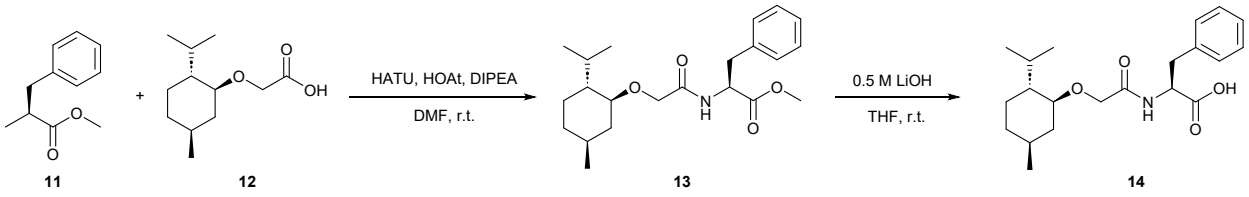
Benzyl N^6-(4-ethylbenzoyl)-*L*-lysinate (**5**). To a solution of Intermediate **4** (7.50 g, 13.42 mmol, 1.0 eq.) in DCM (80 mL), trifluoroacetic acid (20 mL) was added and stirred for 4 hours at rt. Then the excess reagent and solvent were evaporated under reduced pressure to give the deprotected amine, which was used in the next step without further purification. The deprotected amine, 4-ethyl benzoic acid (2.22 g, 14.76 mmol, 1.1 eq.), HOAt (2.19 g, 16.10 mmol, 1.2 eq.) and DIPEA (12.14g, 93.94 mmol, 7.0 eq.) were then dissolved in DMF (100 ml) and stirred at 0 °C. To the stirred solution HATU (7.65 g 20.13 mmol, 1.5 eq.) was added slowly. After 15 minutes, the reaction mixture was diluted with EtOAc (800 ml) and washed with brine (3 x 500 ml). The organic layer was dried over anhydrous Na_2SO_4 and evaporated in vacuo. The product was then purified by flash chromatography (EtOAc/n-Hexane, 0%→40%) to afford **5**. 2 steps yield 6.39 g (81%). LC/MS m/z calculated $[M+H]^+$ 591.28, found 591.27.

To a solution of Intermediate **5** (6.39 g, 10.83 mmol, 1.0 eq.) in DCM (80 mL), diethyl amine (20 mL) was added and stirred for 4 hours at rt. Then the excess reagent and solvent were evaporated under reduced

pressure to give the crude deprotected amine **6**, which was then purified by flash chromatography (MeOH/DCM, 0%→10%). yield 3.60 g (90%).

Benzyl N^2-((S)-2-((tert-butoxycarbonyl)amino)-3-(4-((diethoxyphosphoryl)difluoromethyl)phenyl) propanoyl)-N^6-(4-ethylbenzoyl)-L-lysinate (**15**). The amine intermediate **6** (3.60 g, 9.77 mmol, 1.0 eq.), intermediate **10** (4.85 g, 10.74 mmol, 1.1 eq.), HOAt (1.60 g, 12.98 mmol, 11.72 mmol, 1.2 eq.) and DIPEA (5.04 g, 39.08 mmol, 4.0 eq.) were then dissolved in DMF (100 ml) and stirred at 0 °C. To the stirred solution HATU (6.18 g, 16.24 mmol, 1.5 eq.) was added slowly. After 15 minutes, the reaction mixture was diluted with EtOAc (800 ml) and washed with brine (3 x 500 ml). The organic layer was dried over anhydrous Na_2SO_4 and evaporated in vacuo. The product was then purified by flash chromatography (EtOAc/n-Hexane, 10%→50%). 2 steps yield 6.18 g (79%). ^{1}H NMR (500 MHz, DMSO) δ 8.39 – 8.32 (m, 2H), 7.77 – 7.71 (m, 2H), 7.48 – 7.37 (m, 4H), 7.37 – 7.27 (m, 5H), 7.27 – 7.21 (m, 2H), 6.92 (d, *J* = 8.9 Hz, 1H), 5.10 (s, 2H), 4.35 – 4.21 (m, 2H), 4.16 – 3.98 (m, 4H), 3.22 (q, *J* = 7.1 Hz, 2H), 3.16 (d, *J* = 5.3 Hz, 1H), 3.00 – 2.92 (m, 1H), 2.77 – 2.68 (m, 1H), 2.61 (q, *J* = 7.6 Hz, 2H), 1.83 – 1.74 (m, 1H), 1.73 – 1.64 (m, 1H), 1.57 – 1.45 (m, 2H), 1.42 – 1.31 (m, 2H), 1.25 (s, 9H), 1.23 – 1.10 (m, 11H). LC/MS m/z calculated $[M+H]^+$ 802.36, found 802.42.

Scheme S9. Synthesis of **Intermediate 14**.

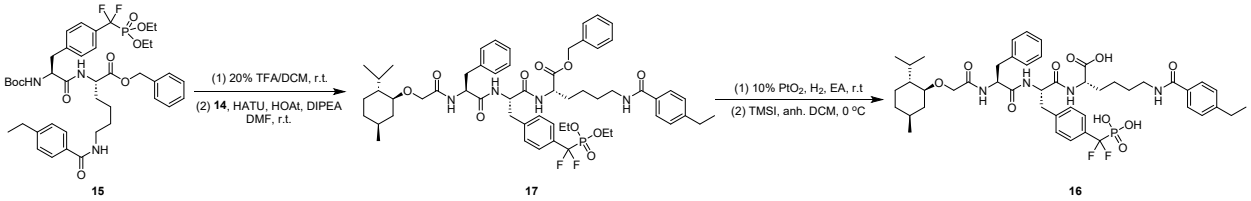


Methyl (2-(((1*S*,2*R*,5*S*)-2-isopropyl-5-methylcyclohexyl)oxy)acetyl)-L-phenylalaninate (**13**). Methyl L-phenylalaninate (**11**, 5.00 g, 27.90 mmol, 1.0 eq.), (+)-Menthoxyacetic acid (**12**, 6.58 g, 30.69 mmol, 1.1 eq.), HOAt (4.56 g, 33.48 mmol, 1.2 eq.) and DIPEA (14.42, 111.60 mmol, 4.0 eq.) were then dissolved in DMF (100 ml) and stirred at 0 °C. To the stirred solution HATU (15.91 g, 41.85 mmol, 1.5 eq.) was added slowly. After 15 minutes, the reaction mixture was diluted with EtOAc (600 ml) and washed with brine (3 x 500 ml). The organic layer was dried over anhydrous Na_2SO_4 and evaporated in vacuo. The product was then purified by flash chromatography (EtOAc/n-Hexane, 10%→50%) to afford **13**. Yield 7.91 g (76%). ^{1}H NMR (500 MHz, DMSO) δ 7.39 (d, *J* = 8.0 Hz, 1H), 7.28 – 7.13 (m, 5H), 4.51 (td, *J* = 8.0, 4.9 Hz, 1H), 3.93 (d, *J* = 15.0 Hz, 1H), 3.72 (d, *J* = 15.0 Hz, 1H), 3.62 (s, 3H), 3.11 – 2.96 (m, 3H), 2.07 (ddq, *J* = 9.6, 6.9, 3.5 Hz,

1H), 2.01 (d, *J* = 11.0 Hz, 1H), 1.61 – 1.50 (m, 2H), 1.34 – 1.24 (m, 1H), 1.21 – 1.12 (m, 1H), 0.94 – 0.67 (m, 9H), 0.67 – 0.60 (m, 3H). LC/MS m/z calculated $[M+H]^+$ 376.24, found 376.22.

(2-(((1*S*,2*R*,5*S*)-2-isopropyl-5-methylcyclohexyl)oxy)acetyl)-L-phenylalanine (**14**). To a solution of intermediate **13** (5.71 g, 15.20 mmol) in THF (50 ml), 0.5 M LiOH (aq.) was added. The mixture was stirred at r.t. for 4 hours. Then 50 ml 1.0 M HCl (aq.) was added to the reaction mixture. The mixture was extracted with EtOAc (3 x 250 ml) and washed with brine (2 x 250 ml). The organic layer was combined, dried over anhydrous Na_2SO_4, and evaporated in vacuo to give crude intermediate **14**. Yield 5.18 g (94%). This crude product was used in the following synthesis without further purification.

Scheme S10. Synthesis of **compound 16 (TC-PTP ligand)**.

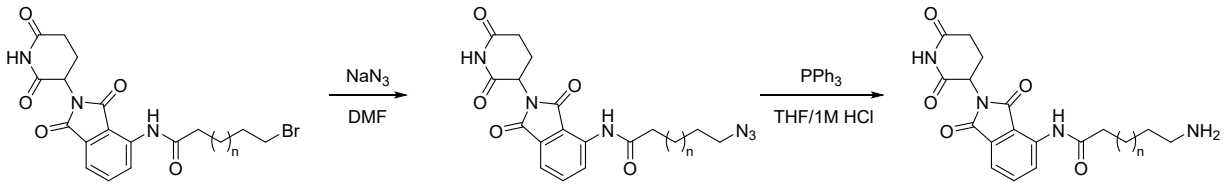


benzyl *N*^{2}-((S)-3-(4-((diethoxyphosphoryl)difluoromethyl)phenyl)-2-((S)-2-(2-(((1S,2R,5S)-2-isopropyl-5-methylcyclohexyl)oxy)acetamido)-3-phenylpropanamido)propanoyl)-N6-(4-ethylbenzoyl)-L-lysinate (**17**). To a solution of Intermediate **15** (5.36 g, 6.68 mmol, 1.0 eq.) in DCM (80 mL), trifluoroacetic acid (20 mL) was added and stirred for 6 hours at rt. Then the excess reagent and solvent were evaporated under reduced pressure to give the crude deprotected amine, which was used in the next step without further purification. The deprotected amine, intermediate **14** (2.66 g, 7.35 mmol, 1.1 eq.), HOAt (1.09 g, 8.02 mmol, 1.2 eq.) and DIPEA (6.04 g, 46.76 mmol, 7.0 eq.) were then dissolved in DMF (50 ml) and stirred at 0 °C. To the stirred solution HATU (3.81 g, 10.02 mmol, 1.5 eq.) was added slowly. After 15 minutes, the reaction mixture was diluted with EtOAc (300 ml) and washed with brine (3 x 300 ml). The organic layer was dried over anhydrous Na_2SO_4 and evaporated in vacuo. The product was then purified by flash chromatography (EtOAc/n-Hexane, 50%→100%) to give **17**. 2 steps yield 4.19 g (60%). 1H NMR (500 MHz, DMSO) δ 8.55 (d, *J* = 7.4 Hz, 1H), 8.40 – 8.32 (m, 2H), 7.74 (d, *J* = 8.3, 2H), 7.43 – 7.27 (m, 3H), 7.26 – 7.20 (m, 3H), 7.18– 7.05 (m, 5H), 5.12 (s, 2H), 4.64 (ddd, *J* = 9.9, 8.5, 4.1 Hz, 1H), 4.52 (td, J = 8.3, 4.4 Hz, 1H), 4.35 – 4.29 (m, 1H), 4.10 – 3.95 (m, 4H), 3.87 (d, *J* = 15.0 Hz, 1H), 3.61 (d, *J* = 15.0 Hz, 1H), 3.27 – 3.13 (m, 2H), 3.07 – 2.98 (m, 2H), 2.92 (dd, J = 13.8, 4.3 Hz, 1H), 2. 2.84 – 2.76 (m, 2H), 2.61 (q, *J* = 7.6 Hz, 2H), 2.03 – 1.91 (m, 2H), 1.84 – 1.74 (m, 1H), 1.73 – 1.63 (m, 1H), 1.59 – 1.47 (m, 4H), 1.42 – 1.32 (m, 2H), 1.30 – 1.08 (m, 11H), 0.92 – 0.58 (m, 12H). LC/MS m/z calculated [M+H]+ 1045.52, found.1045.53.

N^2-((*S*)-3-(4-(difluoro(phosphono)methyl)phenyl)-2-((S)-2-(2-(((1S,2R,5S)-2-isopropyl-5-methylcyclohexyl) oxy)acetamido)-3-phenylpropanamido)propanoyl)-N6-(4-ethylbenzoyl)-L-lysine (**15**). To a solution of intermediate **17** (2.42 g, 2.32 mmol, 1.0 eq.) in EtOAc (50 mL) was added PtO_2 (10 wt. % loading, 242.0 mg). The reaction mixture was evacuated and filled with H_2 gas three times. The mixture was then stirred at r.t. for 12 hours. Upon completion, the reaction mixture was concentrated in vacuo to give crude deprotected carboxylic acid, which was directly used in the next step without further purification. The solution of the deprotected carboxylic acid in anhydrous DCM (25 ml) was cooled to 0 °C and stirred vigorously. Then trimethylsilyl iodide (3.25 g, 16.24 mmol, 7.0 eq.) was added to the solution dropwise. The reaction was kept at 0 °C and monitored with LCMS. Upon completion, the reaction mixture was added to a 50% MeCN/H_2O mixture dropwise and stirred at r.t. for 30 minutes. Then the water and organic solvent was evaporated in vacuo to give crude intermediate 10, which was further purified by prep HPLC (50-90% gradient of MeOH in 0.1% TFA/H_2O) to give **15**. 2 steps yield 0.91 g (44%). ^{1}H NMR (500 MHz, DMSO) δ 8.39 – 8.31 (m, 3H), 7.93 (s, 1H), 7.74 (d, *J* = 8.2 Hz, 2H), 7.43 – 7.33 (m, 4H), 7.28 – 7.22 (m, 3H), 7.20 – 7.09 (m, 5H), 4.66 – 4.53 (m, 2H), 4.24 – 4.17 (m, 1H), 3.88 (d, *J* = 15.1 Hz, 1H), 3.62 (d, *J* = 15.0 Hz, 1H), 3.29 – 3.16 (m, 2H), 3.10 – 2.94 (m, 3H), 2.89 – 2.78 (m, 2H), 2.62 (q, *J* = 7.6 Hz, 2H), 2.04 – 1.92 (m, 2H), 1.81 – 1.72 (m, 1H), 1.68 – 1.59 (m, 1H), 1.58 – 1.47 (m, 4H), 1.42 – 1.33 (m, 2H), 1.31 – 1.20 (m, 1H), 1.19 – 1.09 (m, 4H), 0.92 – 0.65 (m, 9H), 0.65 – 0.57 (m, 3H). LC/MS m/z calculated $[M+H]^+$ 955.48, found.955.52. LC/MS m/z calculated [M-H]- 897.41, found 897.39.

Preparation of PROTAC linkers with E3 ligands

Preparation of PROTAC linkers with pomalidomide



A1: n = 1; **A2**: n = 2; **A3**: n = 3; **A4**: n = 4; **A5**: n = 5; **A6**: n = 6.

N1: n = 1; **N2**: n = 2; **N3**: n = 3; **N4**: n = 4; **N5**: n = 5; **N6**: n = 6.

P1: n = 1; **P2**: n = 2; **P3**: n = 3; **P4**: n = 4; **P5**: n = 5; **P6**: n = 6.

General procedure to synthesize P1-P6 from B1-B6

To a solution of 0.5 mmol **A1-A6** (prepared according to previous reports[8,9]) in DMF (5 ml) was added NaN_3 (1.0 mmol, 97.5 mg, 2 eq.), and the resulting mixture was stirred at 80 °C for 12h. Then the reaction was cooled, and 10 mL water was added. The mixture was extracted with EtOAc (3x 15 ml), and the combined organic phase was washed with water (20 ml) and brine (3 x 20 ml). The organic phase was dried with

Na_2SO_4, filtered, and concentrated. The resulting yellow oil (**N1-N6**) was used in the next step without further purification.

Triphenyl phosphine (1 mmol, 262.3 mg, 2 eq.) was added to a solution of azide **N1-N6** (0.5 mmol) in 5 mL THF/1 M HCl (4:1). The reaction mixture was allowed to stir for 4 h at room temperature before it was concentrated to remove THF. Then the solution was extracted with EtOAc. The water layer was concentrated and purified with HPLC (20-60% gradient of MeOH in 0.1% TFA/H_2O) to give the products **P1-P6** as a light-yellow solid.

5-amino-*N*-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)pentanamide (**P1**, 49% yield over 2 steps). ^{1}H NMR (500 MHz, DMSO) δ ^{1}H NMR (400 MHz, DMSO-d6) δ 11.15 (s, 1H), 9.74 (s, 1H), 8.42 (d, *J* = 8.4 Hz, 1H), 7.91 – 7.78 (m, 3H), 7.63 (d, *J* = 7.3 Hz, 1H), 5.13 (dd, *J* = 12.7, 5.4 Hz, 1H), 2.94–2.71 (m, 3H), 2.66–2.45 (m, 4H), 2.10–2.01 (m, 1H), 1.68 – 1.50 (m, 4H). LC/MS m/z calculated $[M+H]^+$ 373.15, found 373.21.

6-amino-*N*-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)hexanamide (**P2**, 45% yield over 2 steps). ^{1}H NMR (500 MHz, DMSO) δ 11.14 (s, 1H), 9.71 (s, 1H), 8.43 (d, *J* = 8.4 Hz, 1H), 7.90 – 7.75 (m, 3H), 7.60 (d, *J* = 7.4 Hz, 1H), 5.12 (dd, *J* = 12.9, 5.3 Hz, 1H), 2.94 – 2.71 (m, 3H), 2.64 – 2.44 (m, 4H), 2.10–2.00 (m, 1H), 1.66 – 1.51 (m, 4H), 1.40 – 1.31 (m, 2H). LC/MS m/z calculated $[M+H]^+$ 387.17, found 387.16.

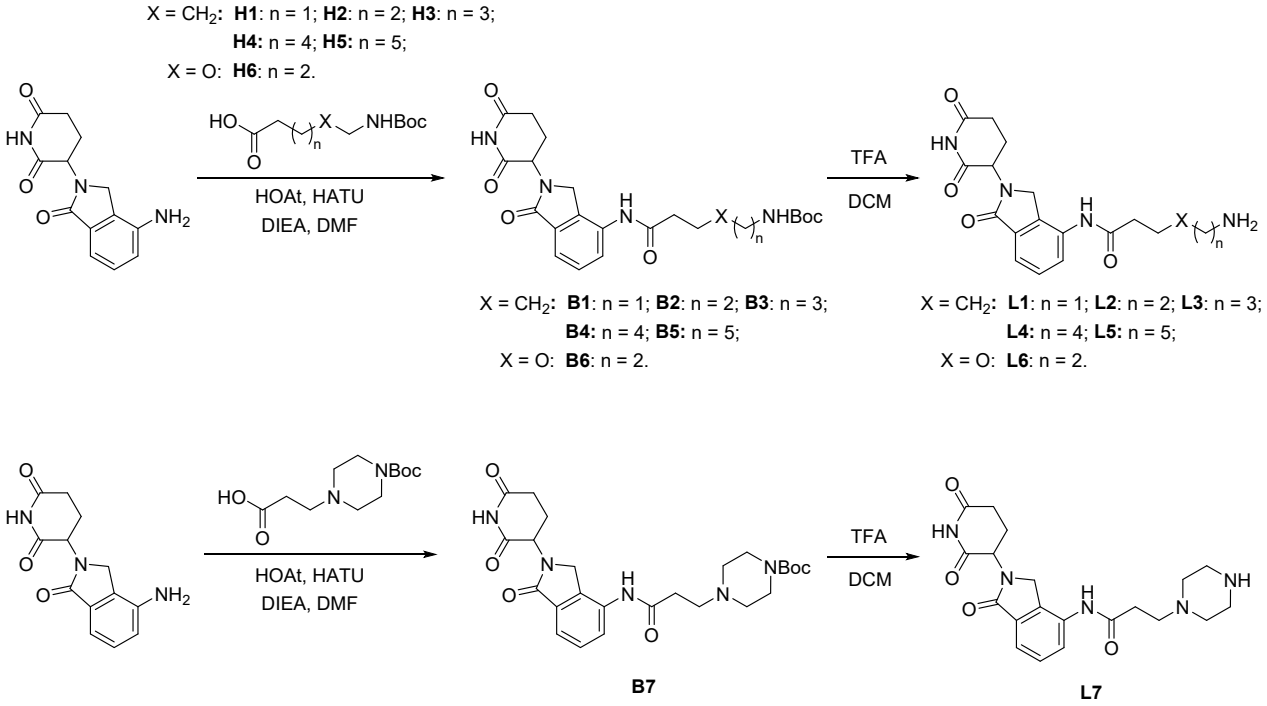
7-amino-*N*-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)heptanamide (**P3**, 51% yield over 2 steps). ^{1}H NMR (500 MHz, DMSO) δ 11.14 (s, 1H), 9.71 (s, 1H), 8.43 (d, *J* = 8.4 Hz, 1H), 7.90 – 7.74 (m, 3H), 7.60 (d, *J* = 7.3 Hz, 1H), 5.12 (dd, *J* = 12.9, 5.2 Hz, 1H), 2.93 – 2.74 (m, 3H), 2.64–2.51 (m, 2H), 2.45 (t, *J* = 7.3 Hz, 2H), 2.09–1.99 (m, 1H), 1.67 – 1.51 (m, 4H), 1.39 – 1.26 (m, 4H). LC/MS m/z calculated $[M+H]^+$ 401.18, found 401.11.

8-amino-*N*-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)octanamide (**P4**, 53% yield over 2 steps). ^{1}H NMR (500 MHz, DMSO) δ 11.14 (s, 1H), 9.71 (s, 1H), 8.43 (d, *J* = 8.4 Hz, 1H), 7.88 – 7.72 (m, 3H), 7.60 (d, *J* = 7.3 Hz, 1H), 5.12 (dd, *J* = 12.8, 5.4 Hz, 1H), 2.93 – 2.70 (m, 3H), 2.63–2.51 (m, 2H), 2.44 (t, *J* = 7.3 Hz, 2H), 2.10–1.99 (m, 1H), 1.65 – 1.49 (m, 4H), 1.35 – 1.23 (m, 6H). LC/MS m/z calculated $[M+H]^+$ 415.20, found 415.24.

9-amino-*N*-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)nonanamide (**P5**, 60% yield over 2 steps). ^{1}H NMR (500 MHz, DMSO) δ 11.14 (s, 1H), 9.71 (s, 1H), 8.43 (d, *J* = 8.4 Hz, 1H), 7.82 (t, *J* = 7.9 Hz, 1H), 7.60 (d, *J* = 7.4 Hz, 1H), 5.12 (dd, *J* = 12.8, 5.3 Hz, 1H), 2.93 – 2.69 (m, 3H), 2.62–2.50 (m, 2H), 2.43 (t, *J* = 7.3 Hz, 2H), 2.09–1.99 (m, 1H), 1.63 – 1.49 (m, 4H), 1.34– 1.21 (m, 8H). LC/MS m/z calculated $[M+H]^+$ 429.21, found 429.22.

10-amino-*N*-(2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)decanamide (**P6**, 52% yield over 2 steps). ^{1}H NMR (500 MHz, DMSO) δ 11.14 (s, 1H), 9.70 (s, 1H), 8.43 (d, *J* = 8.4 Hz, 1H), 7.82 (t, *J* = 7.9 Hz, 1H), 7.60 (d, *J* = 7.4 Hz, 1H), 5.12 (dd, *J* = 12.8, 5.2 Hz, 1H), 2.93 – 2.68 (m, 3H), 2.62–2.50 (m, 2H), 2.43 (t, *J* = 7.3 Hz, 2H), 2.09–1.98 (m, 1H), 1.62 – 1.47 (m, 4H), 1.34– 1.22 (m, 10H). LC/MS m/z calculated $[M+H]^+$ 443.23, found 443.28.

Preparation of PROTAC linkers with lenalidomide



General procedure to synthesize L1-L6 from lenalidomide.

DIPEA (1.5 mmol, 261 µL, 3 eq.) was added to a solution of lenalidomide (0.5 mmol, 129.6 mg), carboxylic acids **H1-H6** (0.5 mmol, 1 eq.), and HOAt (0.75 mmol, 102.1 mg, 1.5 eq.) in DMF, followed by the addition of HATU (0.75 mmol, 285.2 mg, 1.5 eq.), and the resulting mixture was stirred at room temperature for 1h. The reaction mixture was then directed subjected to reverse-phase flash chromatography to give the corresponding products (**B1-B7**).

The Boc protected linkers (**B1-B7**, 0.25 mmol) were dissolved in DCM (3 ml), and trifluoroacetic acid (0.3 ml) was added. The reaction was stirred for 12h at room temperature. The solvent and violates were evaporated, and the crude products were purified by HPLC (20-60% gradient of MeOH in 0.1% TFA/H_2O) to give the corresponding amines (**L1-L7**).

5-amino-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)pentanamide (**L1**, 61% yield over 2 steps). ^{1}H NMR (300 MHz, DMSO-*d*6) δ 11.02 (s, 1H), 9.76 (s, 1H), 7.91 - 7.60 (m, 4H), 7.54–7.46 (m, 2H), 5.14 (dd, *J* = 13.2, 5.0 Hz, 1H), 4.40 – 4.28 (m, 2H), 2.98–2.89 (m, 3H), 2.60 (d, *J* = 17.2 Hz, 1H), 2.40–2.31 (m, 3H), 2.02 (dd, *J* = 11.1, 5.9 Hz, 1H), 1.57 (dd, *J* = 14.6, 7.2 Hz, 2H), 1.46–1.40 (m, 2H). LC/MS m/z calculated $[M+H]^+$ 359.17, found 359.22.

6-amino-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)hexanamide (**L2**, 67% yield over 2 steps).^{1}H NMR (300 MHz, DMSO-d6) δ 11.04 (s, 1H), 9.78 (s, 1H), 7.89–7.64 (m, 3H), 7.55–7.48 (m, 2H), 5.15 (dd, *J* = 13.2, 5.0 Hz, 1H), 4.42 – 4.29 (m, 2H), 2.96–2.88 (m, 3H), 2.60 (d, *J* = 16.3 Hz, 1H), 2.39–2.30 (m, 3H), 2.08–1.96 (m, 1H), 1.62–1.54 (m, 2H), 1.45–1.37 (m, 2H), 1.35–1.28 (m, 2H). LC/MS m/z calculated $[M+H]^+$ 373.19, found 373.21.

7-amino-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)heptanamide (**L3**, 70% yield over 2 steps).^{1}H NMR (500 MHz, DMSO) δ 11.05 (s, 1H), 9.80 (s, 1H), 7.88–7.59 (m, 4H), 7.55–7.46 (m, 2H), 5.17 (dd, *J* = 13.2, 5.0 Hz, 1H), 4.43 – 4.30 (m, 2H), 3.02–2.86 (m, 3H), 2.64 (d, *J* = 16.3 Hz, 1H), 2.43–2.34 (m, 3H), 2.12–2.01 (m, 1H), 1.62–1.48 (m, 4H), 1.37–1.25 (m, 4H). LC/MS m/z calculated $[M+H]^+$ 387.20, found 387.20.

8-amino-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)octanamide (**L4**, 54% yield over 2 steps). ^{1}H NMR (500 MHz, DMSO) δ 11.03 (s, 1H), 9.79 (s, 1H), 7.88–7.60 (m, 4H), 7.55–7.44 (m, 2H), 5.15 (dd, *J* = 13.2, 5.0 Hz, 1H), 4.42 – 4.29 (m, 2H), 3.00–2.83 (m, 1H), 2.60 (d, *J* = 17.5 Hz, 1H), 2.41–2.30 (m, 3H), 2.13–1.94 (m, 1H), 1.64–1.48 (m, 4H), 1.35–1.24 (m, 6H). LC/MS m/z calculated $[M+H]^+$ 401.22, found 401.18.

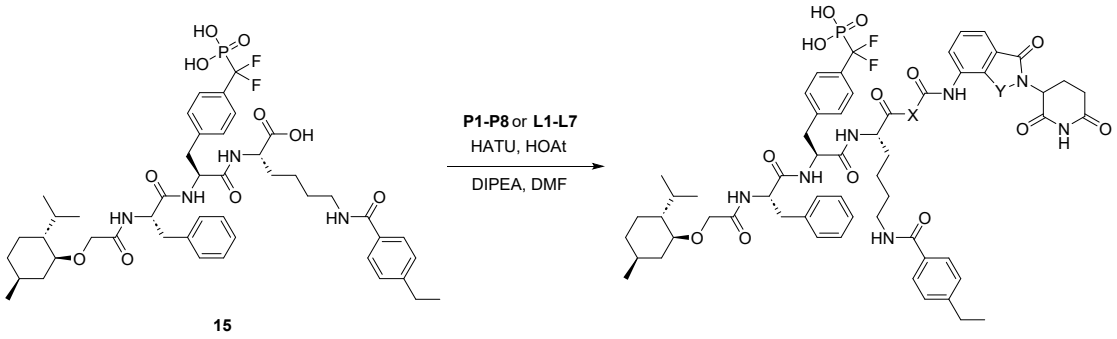
9-amino-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)nonanamide (**L5**, 51% yield over 2 steps). ^{1}H NMR (500 MHz, DMSO) δ 11.02 (s, 1H), 9.81 (s, 1H), 7.87–7.60 (m, 3H), 7.51–7.43 (m, 2H), 5.15 (dd, *J* = 13.2, 5.0 Hz, 1H), 4.41 – 4.29 (m, 2H), 3.00–2.83 (m, 1H), 2.60 (d, *J* = 17.4 Hz, 1H), 2.41–2.30 (m, 3H), 2.12–1.93 (m, 1H), 1.63–1.47 (m, 4H), 1.33–1.24 (m, 8H). LC/MS m/z calculated $[M+H]^+$ 415.23, found 415.20.

3-(2-aminoethoxy)-*N*-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)propenamide (**L6**, 62% yield over 2 steps). ^{1}H NMR (500 MHz, DMSO) δ 11.01 (s, 1H), 9.92 (s, 1H), 7.86 – 7.76 (m, 3H), 7.55 – 7.45 (m, 2H), 5.14 (dd, *J* = 13.4, 5.1 Hz, 1H), 4.42 – 4.28 (m, 2H), 3.75 (t, *J* = 6.3 Hz, 2H), 3.59 (t, *J* = 5.3 Hz, 2H), 3.01 – 2.86 (m, 3H), 2.68 – 2.55 (m, 3H), 2.36 – 2.25 (m, 1H), 2.05 – 1.98 (m, 1H). LC/MS m/z calculated $[M+H]^+$ 375.17, found 375.19.

N-(2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)-3-(piperazin-1-yl)propanamide (**L7**, 39% yield over 2 steps). ^{1}H NMR (500 MHz, DMSO) δ 11.01 (s, 1H), 10.16 (d, *J* = 18.9 Hz, 1H), 9.27 (brs, 1H), 7.85 – 7.79 (m, 1H), 7.55 – 7.45 (m, 2H), 5.15 (dd, *J* = 13.3, 5.2 Hz, 1H), 4.42 – 4.28 (m, 2H), 3.07 – 2.86 (m, 5H), 2.74 (t, *J* = 5.5 Hz, 2H), 2.68 – 2.49 (m, 7H), 2.35 – 2.23 (m, 1H), 2.08 – 1.98 (m, 1H). LC/MS m/z calculated $[M+H]^+$ 400.20, found 400.22.

Preparation of TC-PTP PROTACs

General method for synthesis of CRBN-based TC-PTP PROTACs



15

To a stirred solution of carboxylic acid **15** (29.9 mg, 0.05 mmol) and DIPEA (28 µL, 0.15 mmol) in DMF (2 mL) at ambient temperature was added the corresponding primary amine linkers (**P1-P6** or **L1-L8**, 0.075 mmol) and HATU (28 mg, 0.075 mmol). The reaction mixture was stirred for 1 hour. The residue was purified by HPLC (40-85% gradient of MeOH in 0.1% TFA/H_2O) to yield the corresponding product.

((4-((2S)-3-(((2S)-1-((5-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-5-oxopentyl)amino)-6-(4-ethylbenzamido)-1-oxohexan-2-yl)amino)-2-((S)-2-(2-(((1S,2R,5S)-2-isopropyl-5-methylcyclohexyl)oxy)acetamido)-3-phenylpropanamido)-3-oxopropyl)phenyl)difluoromethyl)phosphonic acid (**TC4P**) was prepared with **P1** using the general method, yield 49%, yellow solid. ^{1}H NMR (500 MHz, DMSO) δ 11.12 (s, 1H), 9.71 (s, 1H), 8.45 – 8.26 (m, 3H), 8.13 (dd, *J* = 12.9, 7.8 Hz, 1 H), 7.83 (t, *J* = 7.8 Hz, 1H), 7.76 – 7.60 (m, 3H), 7.54 – 7.29 (m, 4H), 7.29 – 7.04 (m, 7H), 5.13 (dd, *J* = 12.7, 5.4 Hz, 1H), 4.63 – 4.52 (m, 2H), 4.28 – 4.15 (m, 1H), 3.91 – 3.84 (m, 1H), 3.65 – 3.58 (m, 1H), 3.28 – 3.15 (m, 2H), 3.13 – 2.72 (m, 8H), 2.65 – 2.44 (m, 6H), 2.10 – 1.91 (m, 3H), 1.70 – 1.34 (m, 12H), 1.19 –1.05 (m, 5H), 0.88 – 0.65 (m, 9H), 0.64 – 0.56 (m, 3H). LC/MS m/z calculated $[M+H]^+$ 1253.55, found 1253.52.

((4-((2S)-3-(((2S)-1-((6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-6-oxohexyl)amino)-6-(4-ethylbenzamido)-1-oxohexan-2-yl)amino)-2-((S)-2-(2-(((1S,2R,5S)-2-isopropyl-5-

methylcyclohexyl)oxy)acetamido)-3-phenylpropanamido)-3-oxopropyl)phenyl)difluoromethyl)phosphonic acid (**TC5P**) was prepared with **P2** using the general method, yield 59%, yellow solid. ^{1}H NMR (500 MHz, DMSO δ 11.10 (s, 1H), 9.69 (s, 1H), 8.47 – 8.26 (m, 3H), 8.13 (dd, *J* = 12.9, 7.8 Hz, 1 H), 7.83 (t, *J* = 7.9 Hz, 1H), 7.76 – 7.67 (m, 2H), 7.60 (d, *J* = 7.4 Hz, 1H), 7.54 – 7.28 (m, 4H), 7.29 – 7.04 (m, 7H), 5.12 (dd, *J* = 12.9, 5.4 Hz, 1H), 4.63 – 4.52 (m, 2H), 4.28 – 4.15 (m, 1H), 3.91 – 3.84 (m, 1H), 3.65 – 3.58 (m, 1H), 3.28 – 3.16 (m, 2H), 3.15 – 2.73 (m, 8H), 2.65 – 2.45 (m, 6H), 2.10 – 1.91 (m, 3H), 1.69 – 1.31 (m, 14H), 1.29 –1.05 (m, 5H), 0.88 – 0.65 (m, 9H), 0.64 – 0.56 (m, 3H). LC/MS m/z calculated $[M+H]^+$ 1267.57, found 1267.64.

((4-((2S)-3-(((2S)-1-((7-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-7-oxoheptyl)amino)-6-(4-ethylbenzamido)-1-oxohexan-2-yl)amino)-2-((S)-2-(2-(((1S,2R,5S)-2-isopropyl-5-methylcyclohexyl)oxy)acetamido)-3-phenylpropanamido)-3-oxopropyl)phenyl)difluoromethyl)phosphonic acid (**TC6P**) was prepared with **P3** using the general method, yield 55%, yellow solid. ^{1}H NMR (500 MHz, DMSO δ 11.10 (s, 1H), 9.70 (s, 1H), 8.48 – 8.26 (m, 3H), 8.13 (dd, *J* = 12.8, 7.6 Hz, 1 H), 7.82 (t, *J* = 7.8 Hz, 1H), 7.76 – 7.67 (m, 2H), 7.59 (d, *J* = 7.4 Hz, 1H), 7.54 – 7.28 (m, 4H), 7.29 – 7.04 (m, 7H), 5.12 (dd, *J* = 12.8, 5.4 Hz, 1H), 4.63 – 4.52 (m, 2H), 4.28 – 4.15 (m, 1H), 3.91 – 3.84 (m, 1H), 3.65 – 3.58 (m, 1H), 3.28 – 3.16 (m, 2H), 3.15 – 2.74 (m, 8H), 2.65 – 2.51 (m, 4H), 2.44 (t, J = 7.2 Hz, 2H), 2.10 – 1.90 (m, 3H), 1.69 – 1.06 (m, 21H), 0.88 – 0.65 (m, 9H), 0.64 – 0.56 (m, 3H). LC/MS m/z calculated $[M+H]^+$ 1281.58, found 1281.68.

((4-((2S)-3-(((2S)-1-((8-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-8-oxooctyl)amino)-6-(4-ethylbenzamido)-1-oxohexan-2-yl)amino)-2-((S)-2-(2-(((1S,2R,5S)-2-isopropyl-5-methylcyclohexyl)oxy)acetamido)-3-phenylpropanamido)-3-oxopropyl)phenyl)difluoromethyl)phosphonic acid (**TC7P**) was prepared with **P4** using the general method, yield 51%, yellow solid. ^{1}H NMR (500 MHz, DMSO δ 11.10 (s, 1H), 9.71 (s, 1H), 8.48 – 8.26 (m, 3H), 8.13 (dd, *J* = 12.8, 7.6 Hz, 1 H), 7.83 (t, *J* = 7.9 Hz, 1H), 7.76 – 7.67 (m, 2H), 7.59 (d, *J* = 7.4 Hz, 1H), 7.54 – 7.28 (m, 4H), 7.29 – 7.04 (m, 7H), 5.12 (dd, *J* = 12.9, 5.3 Hz, 1H), 4.63 – 4.52 (m, 2H), 4.28 – 4.15 (m, 1H), 3.91 – 3.84 (m, 1H), 3.65 – 3.58 (m, 1H), 3.28 – 3.16 (m, 2H), 3.15 – 2.70 (m, 8H), 2.64 – 2.51 (m, 4H), 2.44 (t, J = 7.3 Hz, 2H), 2.10 – 1.90 (m, 3H), 1.69 – 1.06 (m, 23H), 0.88 – 0.65 (m, 9H), 0.64 – 0.56 (m, 3H). LC/MS m/z calculated $[M+H]^+$ 1295.60, found 1295.54.

((4-((2S)-3-(((2S)-1-((9-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-9-oxononyl)amino)-6-(4-ethylbenzamido)-1-oxohexan-2-yl)amino)-2-((S)-2-(2-(((1S,2R,5S)-2-isopropyl-5-methylcyclohexyl)oxy)acetamido)-3-phenylpropanamido)-3-

oxopropyl)phenyl)difluoromethyl)phosphonic acid (**TC8P**) was prepared with **P5** using the general method, yield 48%, yellow solid. ^{1}H NMR (500 MHz, DMSO δ 11.10 (s, 1H), 9.71 (s, 1H), 8.48 – 8.26 (m, 3H), 8.13 (dd, *J* = 12.8, 7.6 Hz, 1 H), 7.83 (t, *J* = 7.9 Hz, 1H), 7.76 – 7.67 (m, 2H), 7.59 (d, *J* = 7.4 Hz, 1H), 7.54 – 7.28 (m, 4H), 7.29 – 7.04 (m, 7H), 5.12 (dd, *J* = 12.9, 5.3 Hz, 1H), 4.63 – 4.52 (m, 2H), 4.28 – 4.15 (m, 1H), 3.91 – 3.84 (m, 1H), 3.65 – 3.58 (m, 1H), 3.28 – 3.16 (m, 2H), 3.15 – 2.70 (m, 8H), 2.62 – 2.51 (m, 4H), 2.43 (t, J = 7.3 Hz, 2H), 2.10 – 1.90 (m, 3H), 1.69 – 1.06 (m, 25H), 0.88 – 0.65 (m, 9H), 0.64 – 0.56 (m, 3H). LC/MS m/z calculated $[M+H]^+$ 1295.60, found 1295.54.

((4-((2S)-3-(((2S)-1-((10-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-10-oxodecyl)amino)-6-(4-ethylbenzamido)-1-oxohexan-2-yl)amino)-2-((S)-2-(2-(((1S,2R,5S)-2-isopropyl-5-methylcyclohexyl)oxy)acetamido)-3-phenylpropanamido)-3-oxopropyl)phenyl)difluoromethyl)phosphonic acid (**TC9P**) was prepared with **P6** using the general method, yield 48%, yellow solid. ^{1}H NMR (500 MHz, DMSO δ 11.10 (s, 1H), 9.71 (s, 1H), 8.48 – 8.26 (m, 3H), 8.13 (dd, *J* = 12.8, 7.6 Hz, 1 H), 7.83 (t, *J* = 7.9 Hz, 1H), 7.76 – 7.67 (m, 2H), 7.59 (d, *J* = 7.4 Hz, 1H), 7.54 – 7.28 (m, 4H), 7.29 – 7.04 (m, 7H), 5.12 (dd, *J* = 12.9, 5.3 Hz, 1H), 4.63 – 4.52 (m, 2H), 4.28 – 4.15 (m, 1H), 3.91 – 3.84 (m, 1H), 3.65 – 3.58 (m, 1H), 3.28 – 3.16 (m, 2H), 3.15 – 2.68 (m, 8H), 2.62 – 2.50 (m, 4H), 2.43 (t, J = 7.3 Hz, 2H), 2.10 – 1.90 (m, 3H), 1.69 – 1.06 (m, 25H), 0.88 – 0.65 (m, 9H), 0.64 – 0.56 (m, 3H). LC/MS m/z calculated $[M+H]^+$ 1323.63, found 1323.71.

((4-((2S)-3-(((2S)-1-((5-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)amino)-5-oxopentyl)amino)-6-(4-ethylbenzamido)-1-oxohexan-2-yl)amino)-2-((S)-2-(2-(((1S,2R,5S)-2-isopropyl-5-methylcyclohexyl)oxy)acetamido)-3-phenylpropanamido)-3-oxopropyl)phenyl)difluoromethyl)phosphonic acid (**TC4L**) was prepared with **L1** using the general method, yield 46%, white solid. ^{1}H NMR (500 MHz, DMSO) δ ^{1}H NMR (500 MHz, DMSO) δ 10.99 (s, 1H), 9.84 (s, 1H), 8.38 – 8.26 (m, 2H), 8.17 – 8.08 (m, 1H), 8.00 – 7.86 (m, 1H), 7.82 (d, *J* = 8.0 Hz, 1H), 7.76 – 7.70 (m, 2H), 7.52 – 7.30 (m, 6H), 7.29 – 7.06 (m, 7H), 5.12 (dd, *J* = 13.3, 5.2 Hz, 1H), 4.64 – 4.56 (m, 2H), 4.40 – 4.28 (m, 2H), 4.28 – 4.15 (m, 1H), 3.92 – 3.84 (m, 1H), 3.68 (t, *J* = 6.4 Hz, 2H), 3.64 – 3.57 (m, 1H), 3.40 (td, *J* = 6.4, 3.2 Hz, 2H), 3.28 – 3.12 (m, 4H), 3.11 – 2.94 (m, 3H), 2.94 – 2.73 (m, 3H), 2.64 – 2.54 (m, 5H), 2.37 – 2.26 (m, 1H), 2.04 – 1.90 (m, 3H), 1.70 – 1.60 (m, 1H), 1.59 – 1.34 (m, 7H), 1.19 –1.07 (m, 5H), 0.89 – 0.68 (m, 9H), 0.64 – 0.58 (m, 3H). LC/MS m/z calculated $[M+H]^+$ 1239.57, found 1239.60.

((4-((2S)-3-(((2S)-1-((6-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)amino)-6-oxohexyl)amino)-6-(4-ethylbenzamido)-1-oxohexan-2-yl)amino)-2-((S)-2-(2-(((1S,2R,5S)-2-isopropyl-5-methylcyclohexyl)oxy)acetamido)-3-phenylpropanamido)-3-

oxopropyl)phenyl)difluoromethyl)phosphonic acid (**TC5L**) was prepared with **L2** using the general method, yield 52%, white solid. ^{1}H NMR (500 MHz, DMSO) δ 11.01 (s, 1H), 9.74 (s, 1H), 8.38 – 8.26 (m, 2H), 8.13 (dd, *J* = 12.9, 7.8 Hz, 1 H), 7.80 (d, *J* = 8.0 Hz, 1H), 7.77 – 7.70 (m, 2H), 7.53 – 7.29 (m, 6H), 7.29 – 7.05 (m, 7H), 5.15 (dd, *J* = 13.4, 5.2 Hz, 1H), 4.63 – 4.52 (m, 2H), 4.42 – 4.29 (m, 2H), 4.28 – 4.15 (m, 1H), 3.91 – 3.84 (m, 1H), 3.65 – 3.58 (m, 1H), 3.28 – 3.16 (m, 2H), 3.07 – 2.73 (m, 8H), 2.66 – 2.55 (m, 3H), 2.40 – 2.30 (m, 3H), 2.07 – 1.91 (m, 3H), 1.69 – 1.28 (m, 14H), 1.28– 1.07 (m, 5H), 0.90 – 0.65 (m, 9H), 0.64 – 0.57 (m, 3H). LC/MS m/z calculated $[M+H]^+$ 1253.59, found 1253.60.

((4-((2S)-3-(((2S)-1-((7-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)amino)-7-oxoheptyl)amino)-6-(4-ethylbenzamido)-1-oxohexan-2-yl)amino)-2-((S)-2-(2-(((1S,2R,5S)-2-isopropyl-5-methylcyclohexyl)oxy)acetamido)-3-phenylpropanamido)-3-oxopropyl)phenyl)difluoromethyl)phosphonic acid (**TC6L**) was prepared with **L3** using the general method, yield 50%, white solid. ^{1}H NMR (500 MHz, DMSO) δ 11.03 (s, 1H), 9.79 (s, 1H), 8.38 – 8.26 (m, 2H), 8.13 (dd, *J* = 12.9, 7.8 Hz, 1 H), 7.80 (d, *J* = 8.0 Hz, 1H), 7.77 – 7.66 (m, 2H), 7.53 – 7.29 (m, 6H), 7.29 – 7.05 (m, 7H), 5.17 (dd, *J* = 13.2, 5.0 Hz, 1H), 4.63 – 4.52 (m, 2H), 4.42 – 4.30 (m, 2H), 4.28 – 4.15 (m, 1H), 3.91 – 3.84 (m, 1H), 3.65 – 3.58 (m, 1H), 3.28 – 3.15 (m, 4H), 3.06 – 2.73 (m, 8H), 2.70 – 2.56 (m, 3H), 2.42 – 2.33 (m, 3H), 2.12 – 1.91 (m, 3H), 1.68 1.07 (m, 21H), 0.90 – 0.65 (m, 9H), 0.64 – 0.57 (m, 3H). LC/MS m/z calculated $[M+H]^+$ 1267.60, found 1267.60.

((4-((2S)-3-(((2S)-1-((8-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)amino)-8-oxooctyl)amino)-6-(4-ethylbenzamido)-1-oxohexan-2-yl)amino)-2-((S)-2-(2-(((1S,2R,5S)-2-isopropyl-5-methylcyclohexyl)oxy)acetamido)-3-phenylpropanamido)-3-oxopropyl)phenyl)difluoromethyl)phosphonic acid (**TC7L**) was prepared with **L4** using the general method, yield 42%, white solid. ^{1}H NMR (500 MHz, DMSO) δ 11.01 (s, 1H), 9.79 (s, 1H), 8.37 – 8.26 (m, 2H), 8.13 (dd, *J* = 12.9, 7.8 Hz, 1 H), 7.81 (d, *J* = 8.0 Hz, 1H), 7.76 – 7.70 (m, 2H), 7.55 – 7.29 (m, 6H), 7.29 – 7.04 (m, 7H), 5.16 (dd, *J* = 13.2, 5.0 Hz, 1H), 4.63 – 4.52 (m, 2H), 4.42 – 4.29 (m, 2H), 4.28 – 4.15 (m, 1H), 3.91 – 3.84 (m, 1H), 3.65 – 3.58 (m, 1H), 3.28 – 3.14 (m, 2H), 3.06 – 2.73 (m, 8H), 2.70 – 2.55 (m, 3H), 2.41 – 2.31 (m, 3H), 2.13 – 1.91 (m, 3H), 1.68 – 1.07 (m, 23H), 0.90 – 0.64 (m, 9H), 0.64 – 0.56 (m, 3H). LC/MS m/z calculated $[M+H]^+$ 1281.62, found 1281.60.

((4-((2S)-3-(((2S)-1-((9-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)amino)-9-oxononyl)amino)-6-(4-ethylbenzamido)-1-oxohexan-2-yl)amino)-2-((S)-2-(2-(((1S,2R,5S)-2-isopropyl-5-methylcyclohexyl)oxy)acetamido)-3-phenylpropanamido)-3-oxopropyl)phenyl)difluoromethyl)phosphonic acid (**TC8L**) was prepared with **L5** using the general method,

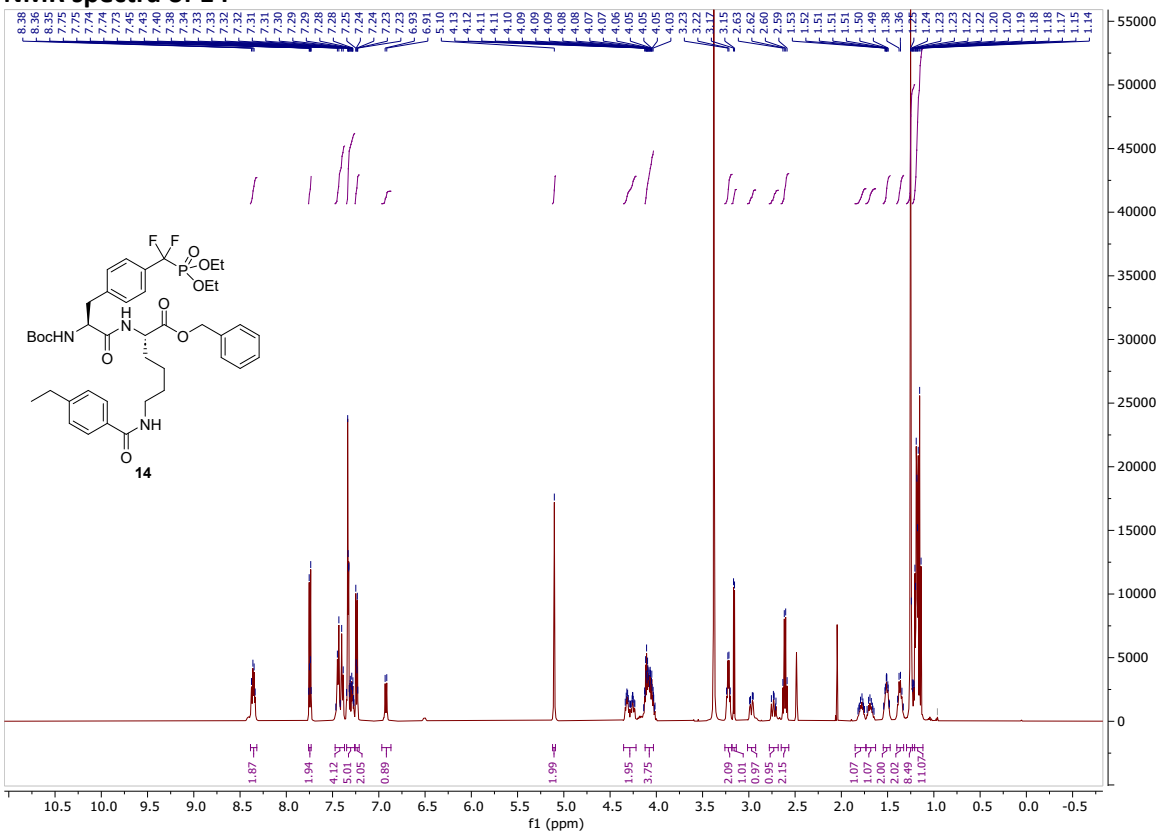
yield 37%, white solid. ^{1}H NMR (500 MHz, DMSO) δ 11.00 (s, 1H), 9.79 (s, 1H), 8.37 – 8.26 (m, 2H), 8.13 (dd, *J* = 12.9, 7.8 Hz, 1 H), 7.80 (d, *J* = 8.0 Hz, 1H), 7.76 – 7.60 (m, 2H), 7.56 – 7.29 (m, 6H), 7.29 – 7.03 (m, 7H), 5.15 (dd, *J* = 13.2, 5.1 Hz, 1H), 4.63 – 4.52 (m, 2H), 4.41 – 4.28 (m, 2H), 4.28 – 4.15 (m, 1H), 3.91 – 3.84 (m, 1H), 3.65 – 3.58 (m, 1H), 3.28 – 3.14 (m, 4H), 3.05 – 2.73 (m, 8H), 2.70 – 2.55 (m, 3H), 2.41 – 2.31 (m, 3H), 2.13 – 1.91 (m, 3H), 1.68 – 1.07 (m, 25H), 0.90 – 0.64 (m, 9H), 0.64 – 0.55 (m, 3H). LC/MS m/z calculated $[M+H]^+$ 1295.63, found 1295.70.

((4-((2*S*)-3-(((2*S*)-1-((2-(3-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)amino)-3-oxopropoxy)ethyl)amino)-6-(4-ethylbenzamido)-1-oxohexan-2-yl)amino)-2-((*S*)-2-(2-(((1*S*,2*R*,5*S*)-2-isopropyl-5-methylcyclohexyl)oxy)acetamido)-3-phenylpropanamido)-3-oxopropyl)phenyl)difluoromethyl)phosphonic acid (**TP1L**) was prepared with **L6** using the general method, yield 49%, white solid. ^{1}H NMR (500 MHz, DMSO) δ 10.99 (s, 1H), 9.84 (s, 1H), 8.38 – 8.26 (m, 2H), 8.17 – 8.08 (m, 1H), 8.00 – 7.86 (m, 1H), 7.82 (d, *J* = 8.0 Hz, 1H), 7.76 – 7.70 (m, 2H), 7.52 – 7.30 (m, 6H), 7.29 – 7.06 (m, 7H), 5.12 (dd, *J* = 13.3, 5.2 Hz, 1H), 4.64 – 4.56 (m, 2H), 4.40 – 4.28 (m, 2H), 4.28 – 4.15 (m, 1H), 3.92 – 3.84 (m, 1H), 3.68 (t, *J* = 6.4 Hz, 2H), 3.64 – 3.57 (m, 1H), 3.40 (td, *J* = 6.4, 3.2 Hz, 2H), 3.28 – 3.12 (m, 4H), 3.11 – 2.95 (m, 3H), 2.94 – 2.73 (m, 3H), 2.64 – 2.54 (m, 5H), 2.37 – 2.26 (m, 1H), 2.04 – 1.90 (m, 3H), 1.70 – 1.60 (m, 1H), 1.59 – 1.34 (m, 7H), 1.29 –1.07 (m, 5H), 0.89 – 0.68 (m, 9H), 0.64 – 0.58 (m, 3H). ^{13}C NMR (126 MHz, DMSO) δ 173.34, 171.99, 171.54, 171.06, 170.99, 169.77, 169.49, 168.29, 166.49, 147.43, 140.43, 137.45 (d, J = 25.7 Hz), 134.12, 133.14, 132.61, 129.75, 129.58, 129.51, 129.12, 128.36, 128.00, 127.69, 126.75, 126.29, 125.67, 121.21, 119.55, 79.79, 79.66, 69.20, 67.73, 66.85, 54.30, 54.00, 53.17, 53.04, 52.00, 47.89, 47.82, 46.93, 38.96, 38.10, 37.94, 36.94, 34.44, 32.55, 31.68, 31.24, 29.40, 28.45, 25.59, 23.29, 23.16, 23.11, 22.60, 21.34, 16.46, 15.83. LC/MS m/z calculated $[M+H]^+$ 1255.57, found 1255.60. HRMS m/z $[M+H]^+$ Calcd for $C_{59}H_{86}Cl_2N_{11}O_5S_2$ 1255.5651; found 1255.5646.

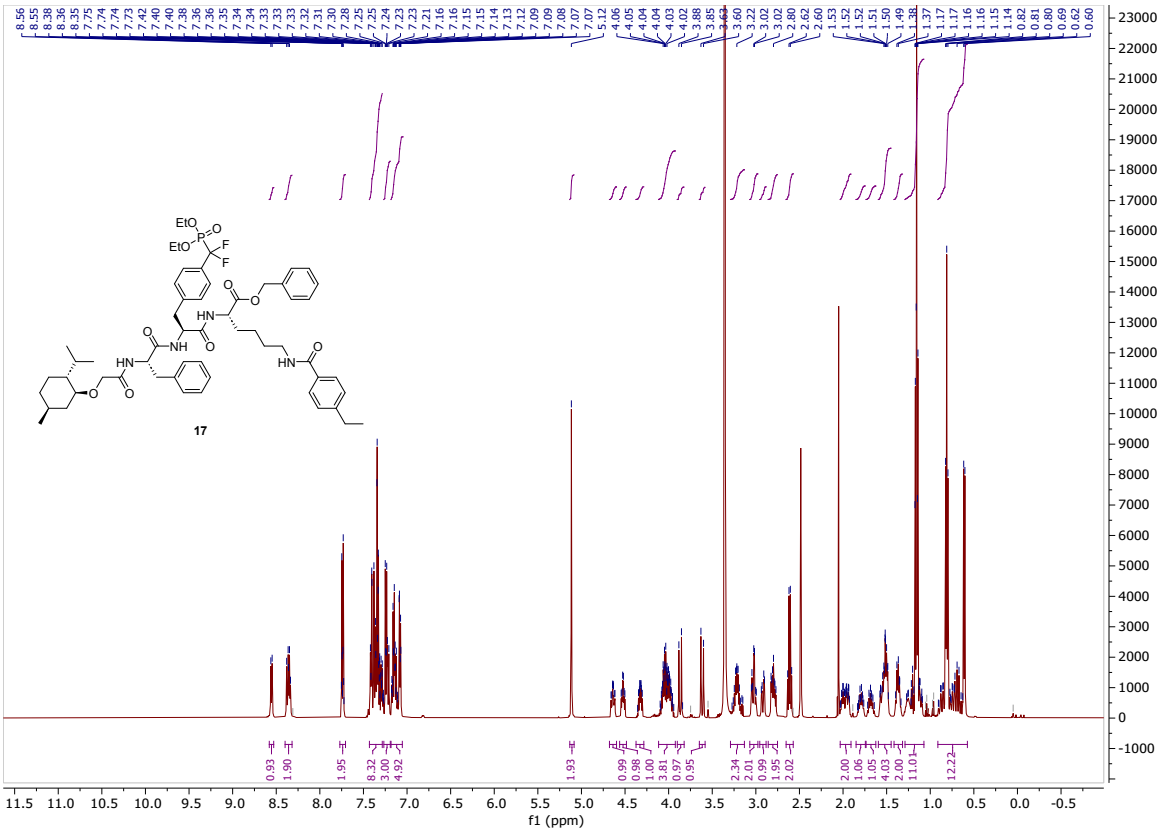
((4-((2S)-3-(((2S)-1-(4-(3-((2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl)amino)-3-oxopropyl)piperazin-1-yl)-6-(4-ethylbenzamido)-1-oxohexan-2-yl)amino)-2-((S)-2-(2-(((1S,2R,5S)-2-isopropyl-5-methylcyclohexyl)oxy)acetamido)-3-phenylpropanamido)-3-oxopropyl)phenyl)difluoromethyl)phosphonic acid (**TPiL**) was prepared with **L7** using the general method, yield 49%, white solid. ^{1}H NMR (500 MHz, DMSO) δ 10.98 (s, 1H), 9.28 (s, 1H), 8.38 – 8.26 (m, 2H), 8.17 – 8.08 (m, 1H), 8.00 – 7.86 (m, 1H), 7.85 – 7.79 (m, 1H),7.76 – 7.70 (m, 2H), 7.55 – 7.30 (m, 6H), 7.29 – 7.06 (m, 7H), 5.15 (dd, *J* = 13.3, 5.2 Hz, 1H), 4.64 – 4.56 (m, 2H), 4.43 – 4.28 (m, 2H), 4.28 – 4.15 (m, 1H), 3.92 – 3.84 (m, 1H), 3.64 – 3.57 (m, 1H), 3.50 – 3.12 (m, 8H), 3.11 – 2.73 (m, 8H), 2.64 – 2.53 (m, 7H), 2.37 –

2.26 (m, 1H), 2.04 – 1.90 (m, 3H), 1.77 – 1.60 (m, 1H), 1.59 – 1.33 (m, 7H), 1.28 –1.07 (m, 5H), 0.89 – 0.68 (m, 9H), 0.64 – 0.58 (m, 3H). LC/MS m/z calculated $[M+H]^+$ 1280.60, found 1280.60.

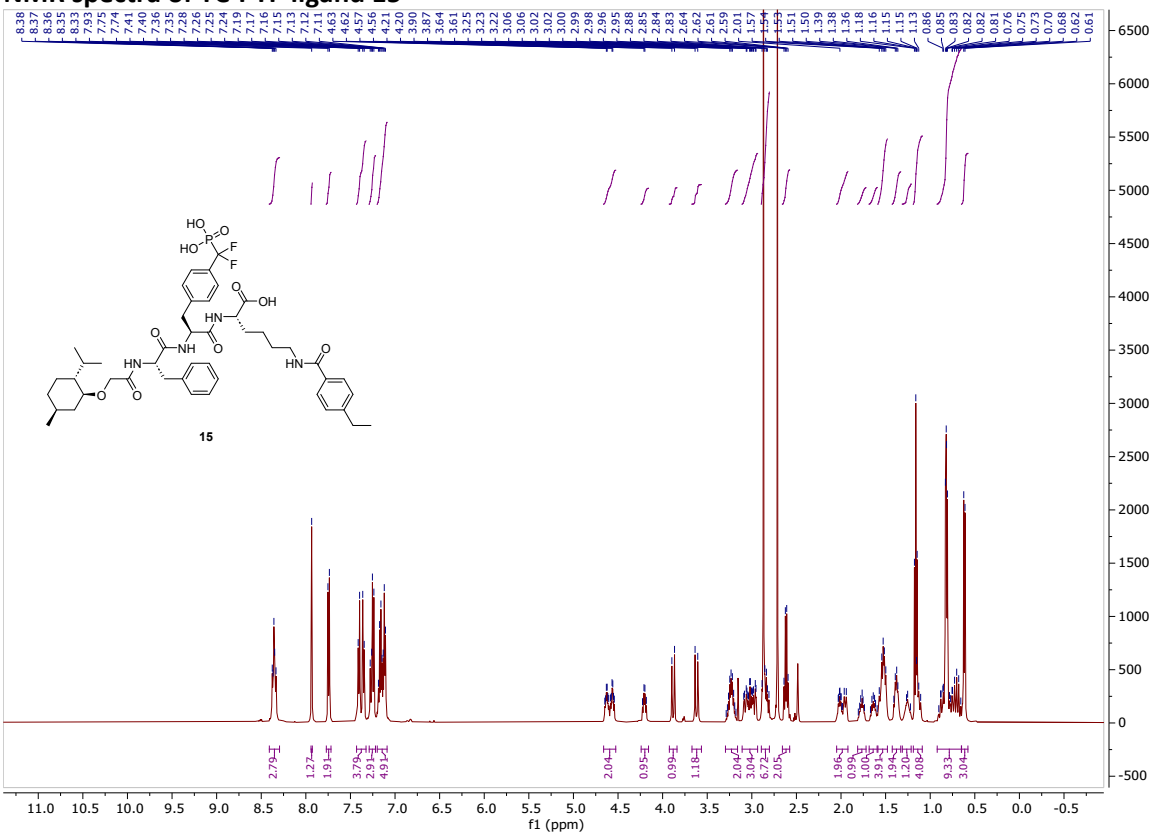
^{1}H NMR spectra of 14



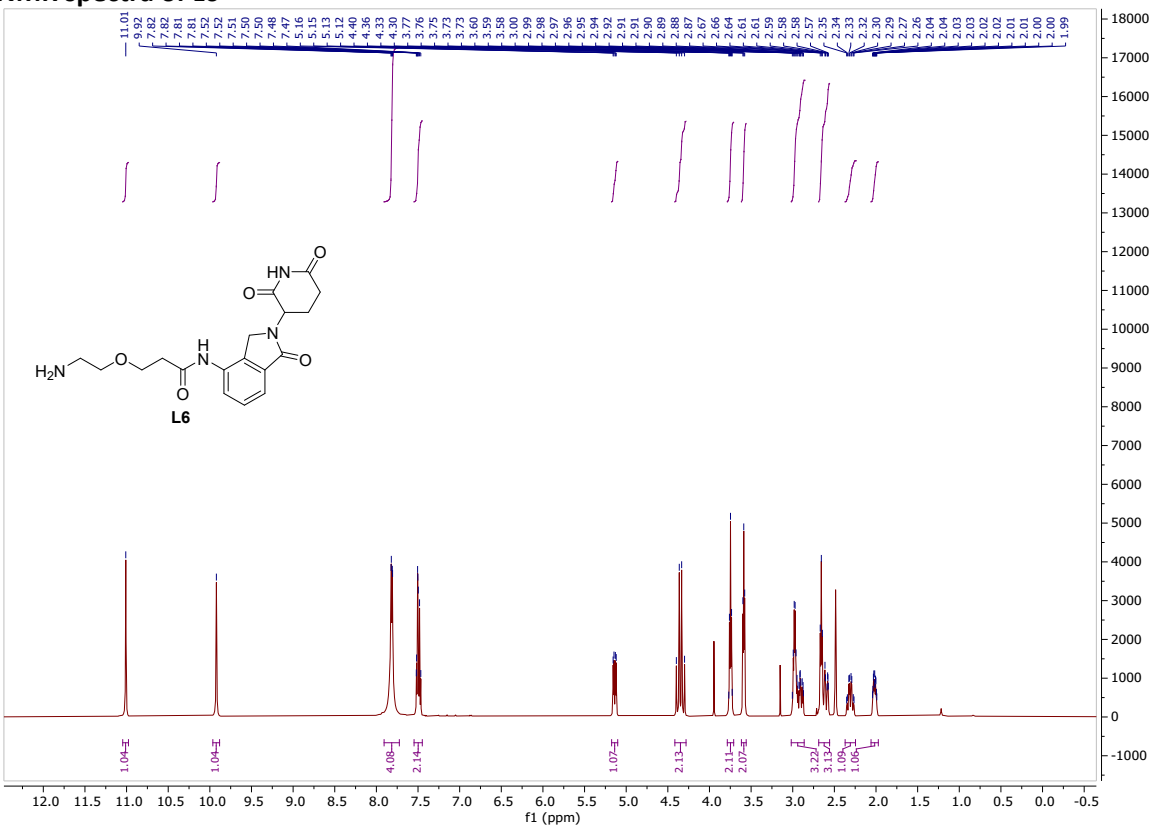
^{1}H NMR spectra of 17



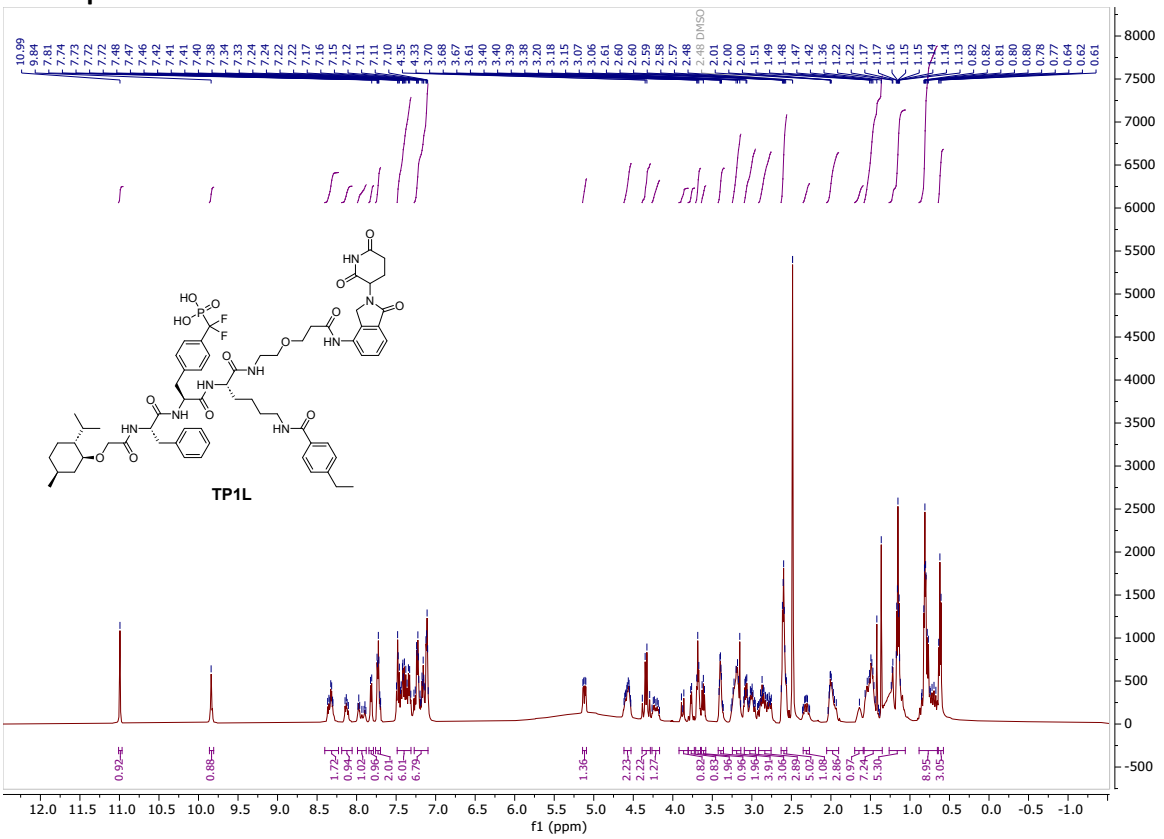
^{1}H NMR spectra of TC-PTP ligand 15



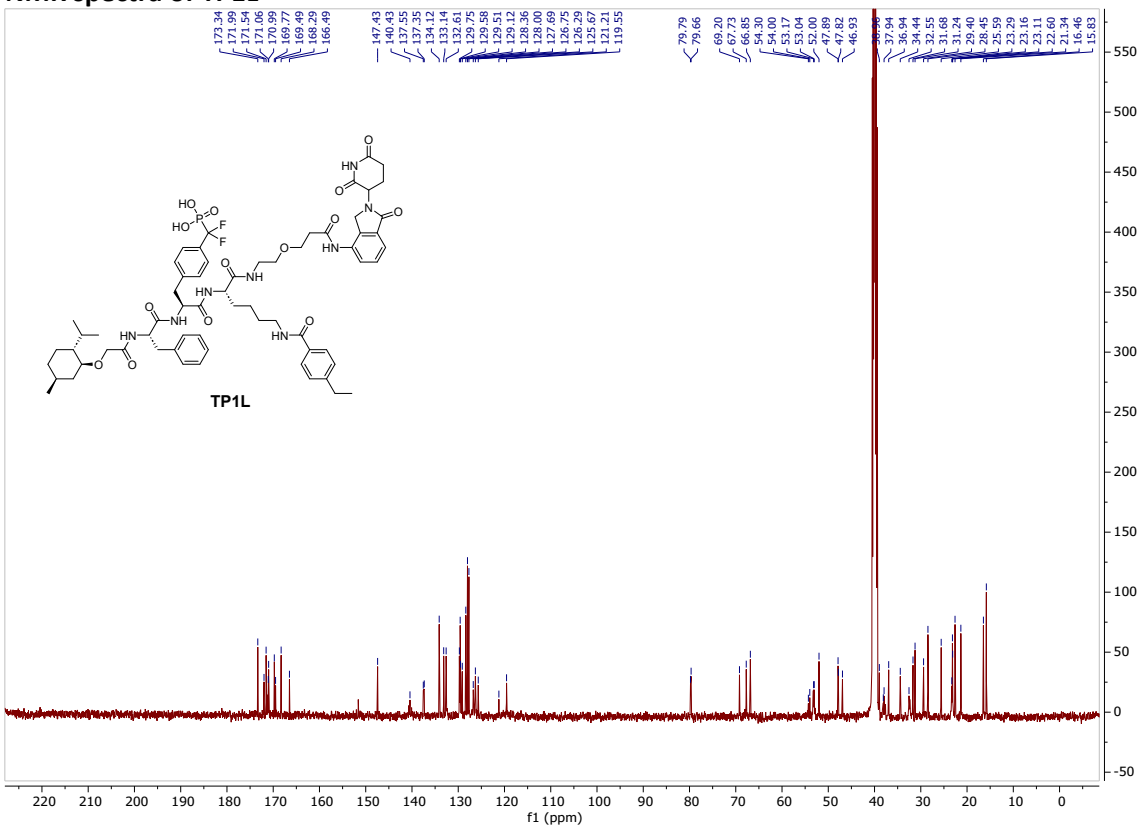
^{1}H NMR spectra of L6



^{1}H NMR spectra of TP1L



^{13}C NMR spectra of TP1L



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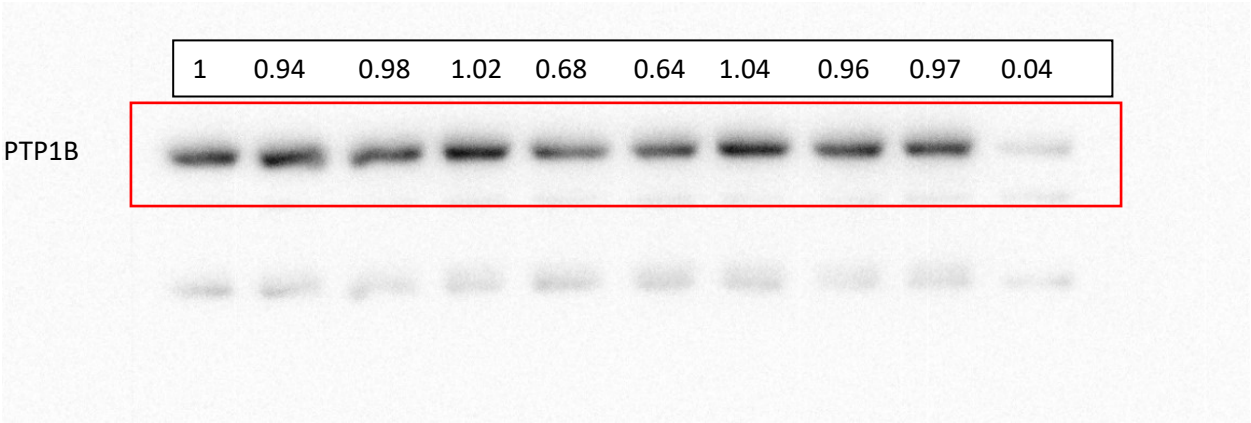
Original Unprocessed Western Blots

Fig. 2



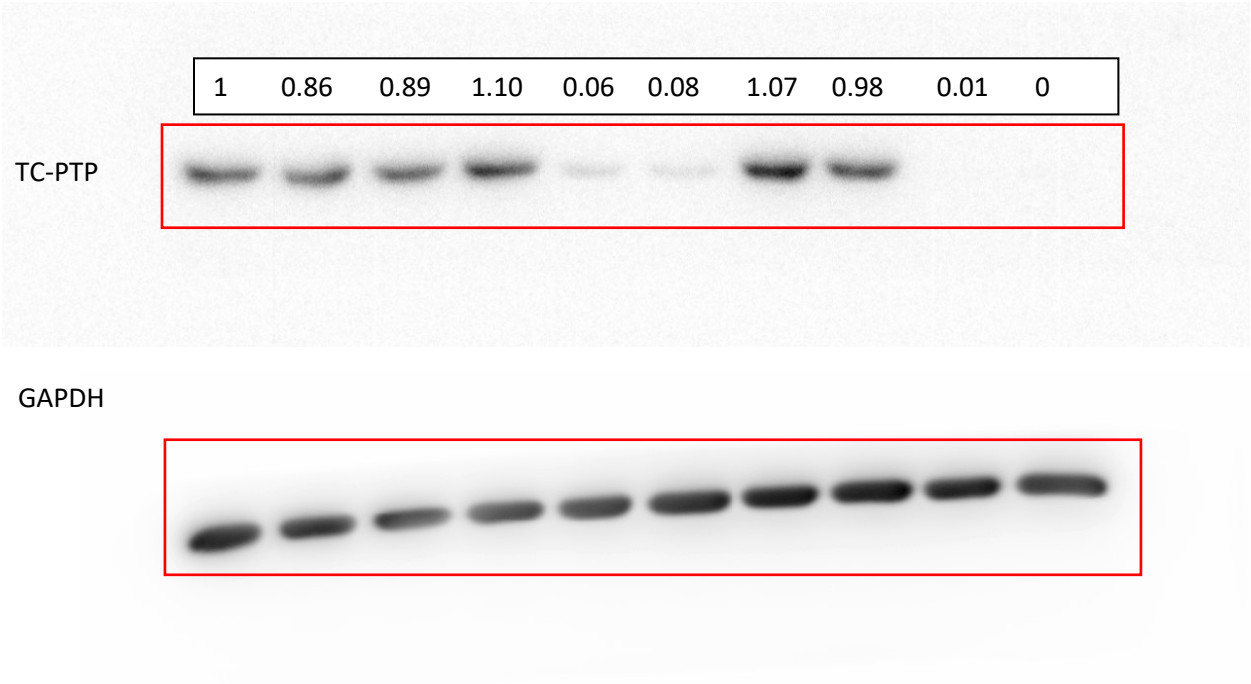
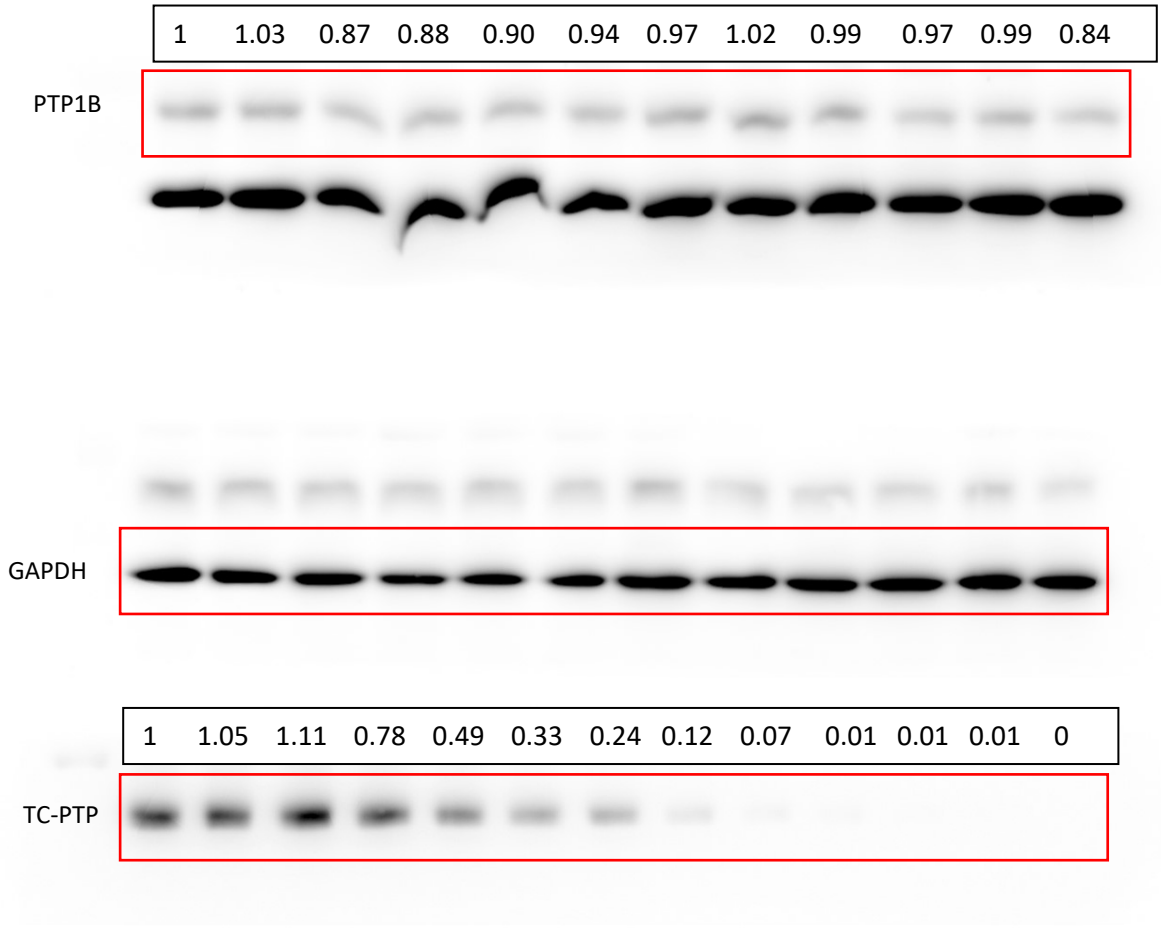


Fig. 3a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PTP1B
GAPDH
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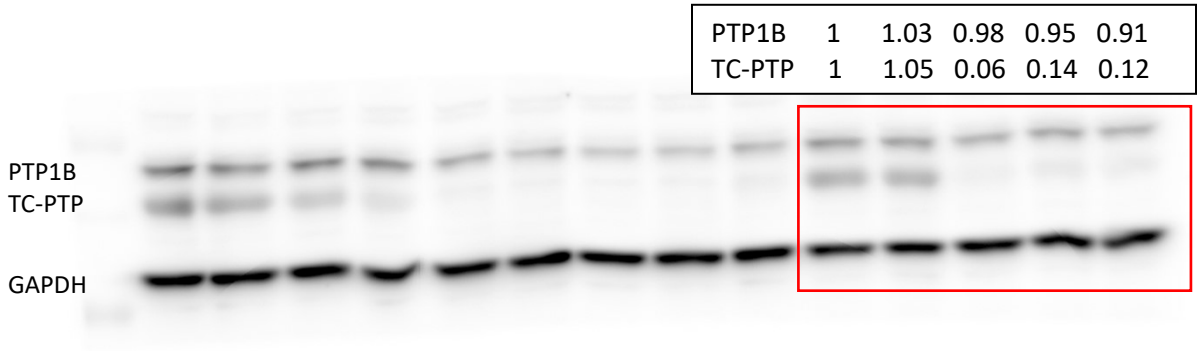
TC-PTP

Fig. 3c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TC-PTP 1 1.05 0.06 0.14 0.12
PTP1B
TC-PTP
GAPDH

Fig. 3d

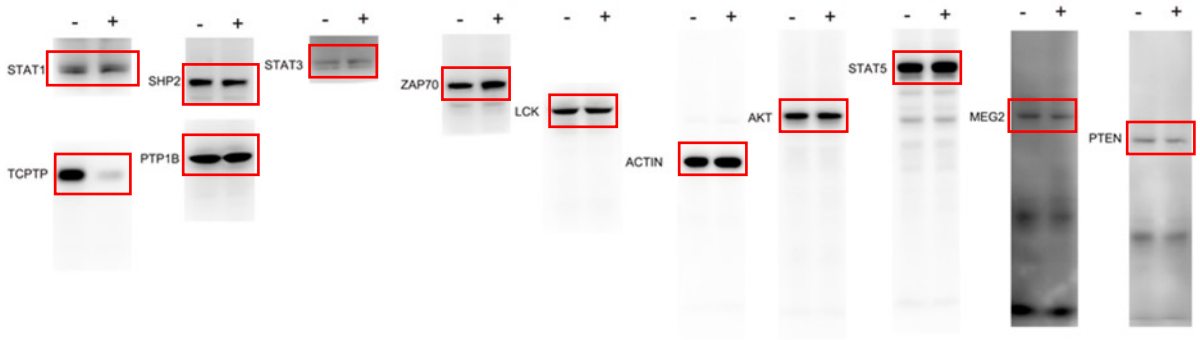


Fig. 3e

PTP1B	1	0.92	0.98	1.04	0.40	0.42	0.99	1.02
TC-PTP	1	0.20	0.91	1.12	0.43	0.45	0.78	0.82

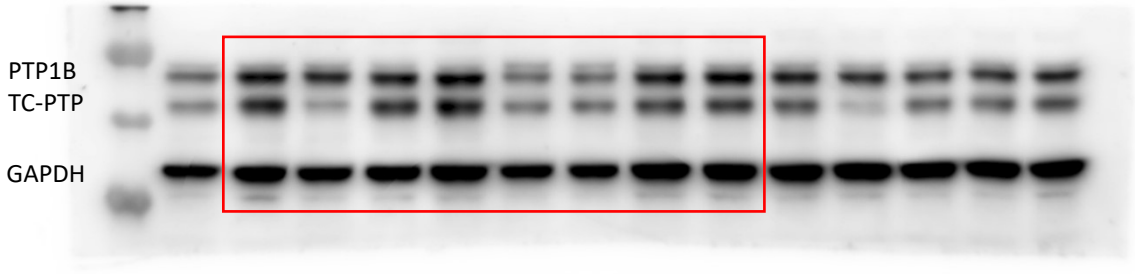


Fig. 4a

1	0.87	0.87	1.33	1.15	1.60	1.82	1.84	2.77	2.99	3.65	3.24

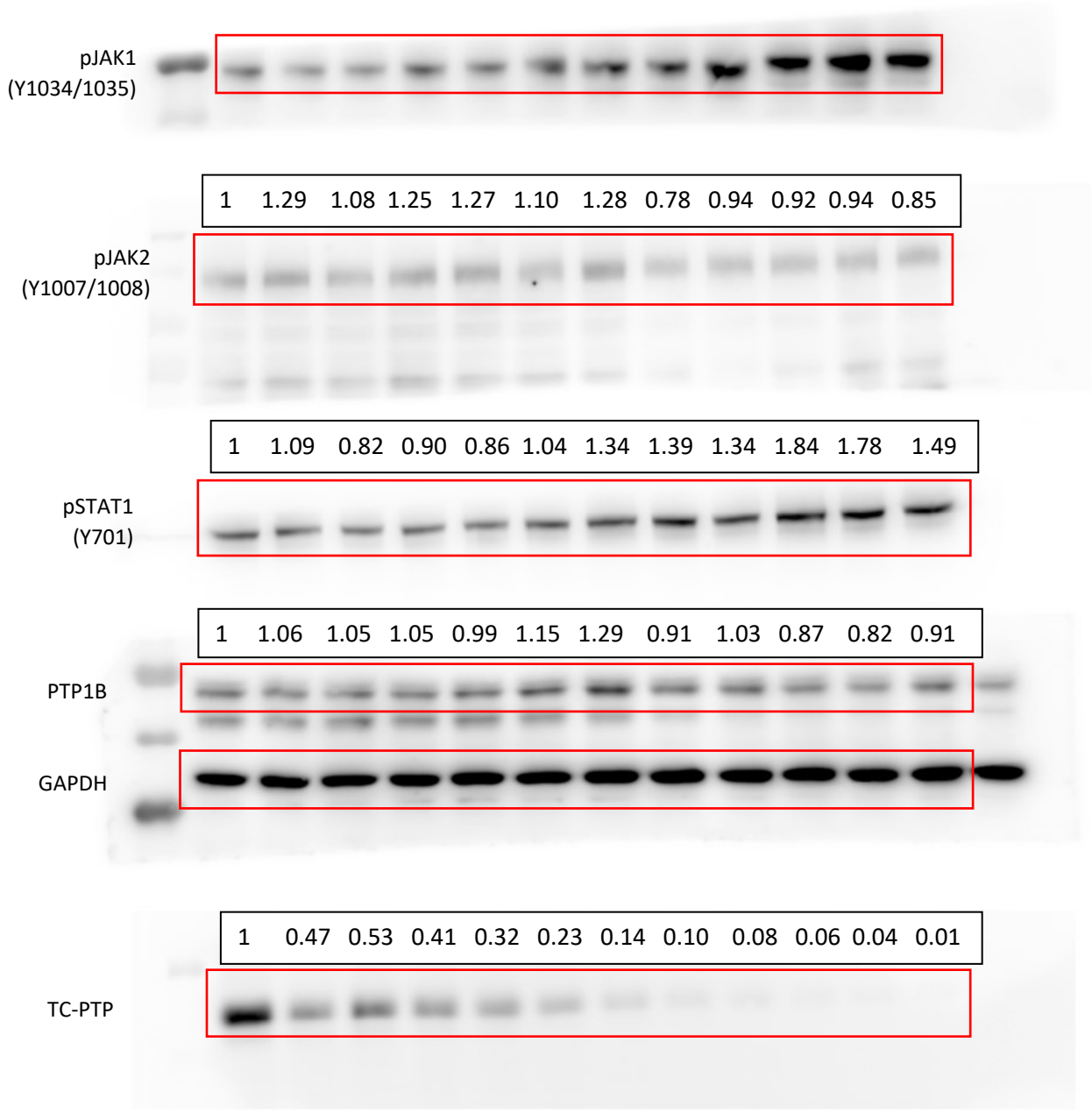
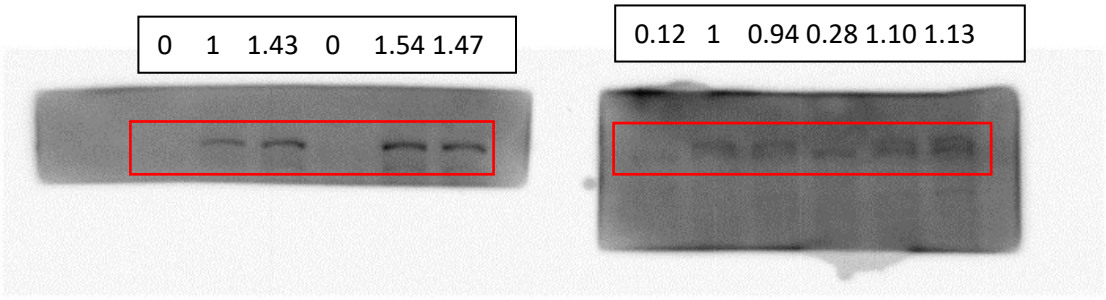
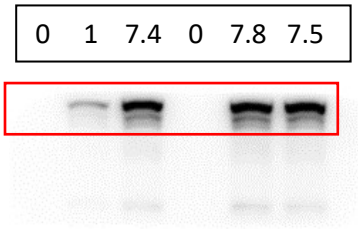


Fig. 4b

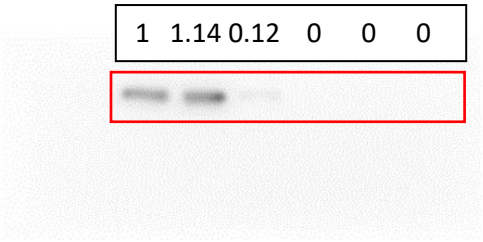
pJAK1 (Y1034/1035) pJAK2 (Y1007/1008)



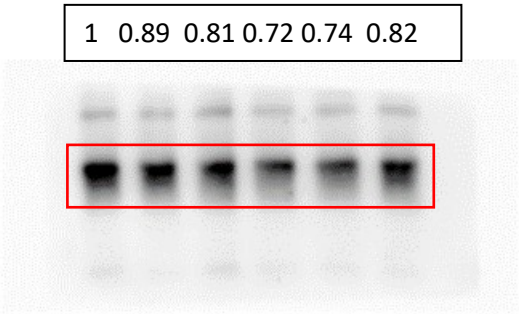
pSTAT1 (Y701)



TC-PTP

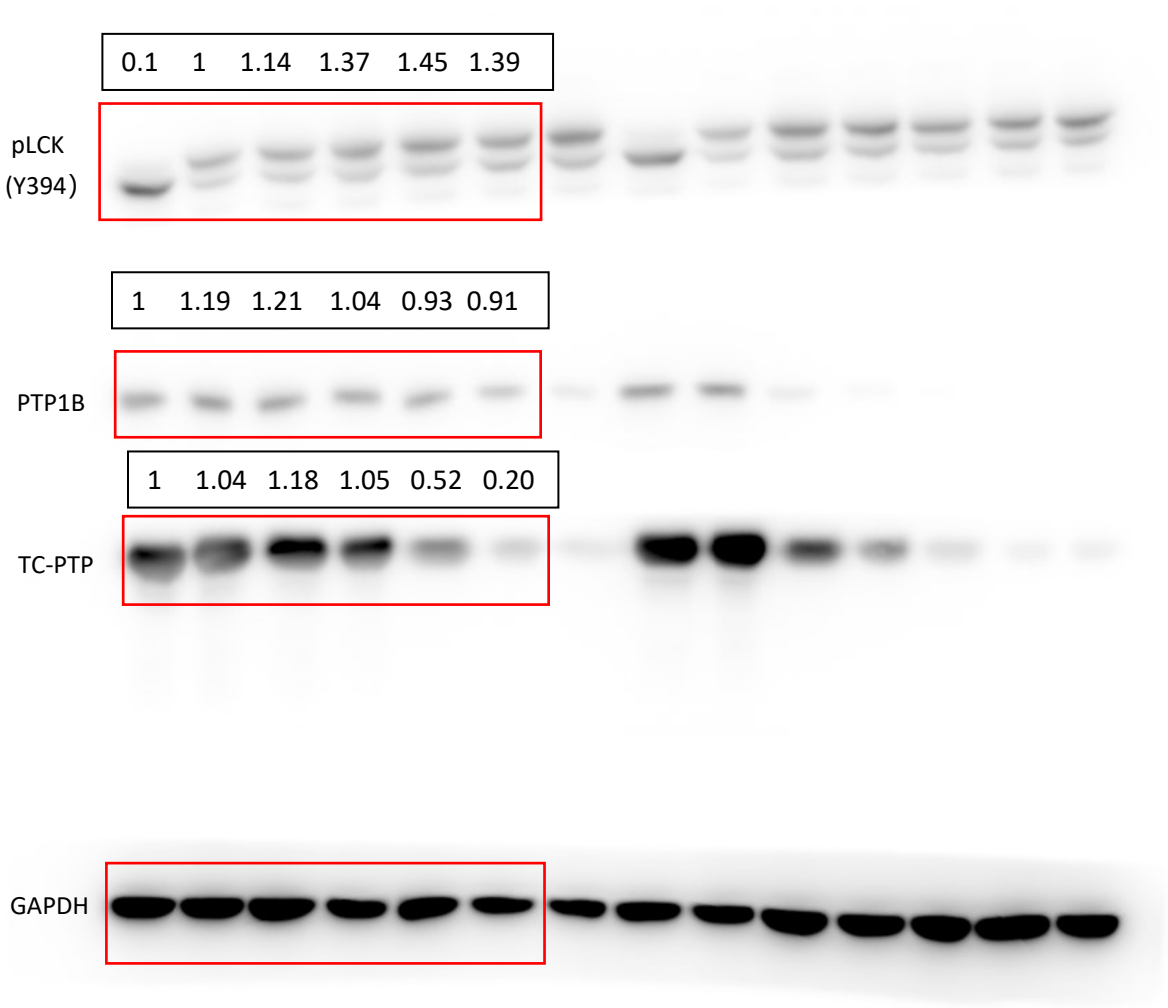


PTP1B



GAPDH

Fig. 5a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pLCK
(Y394)
1 1.19 1.21 1.04 0.93 0.91
PTP1B
1 1.04 1.18 1.05 0.52 0.20
TC-PTP
GAPDH

Fig. 5b

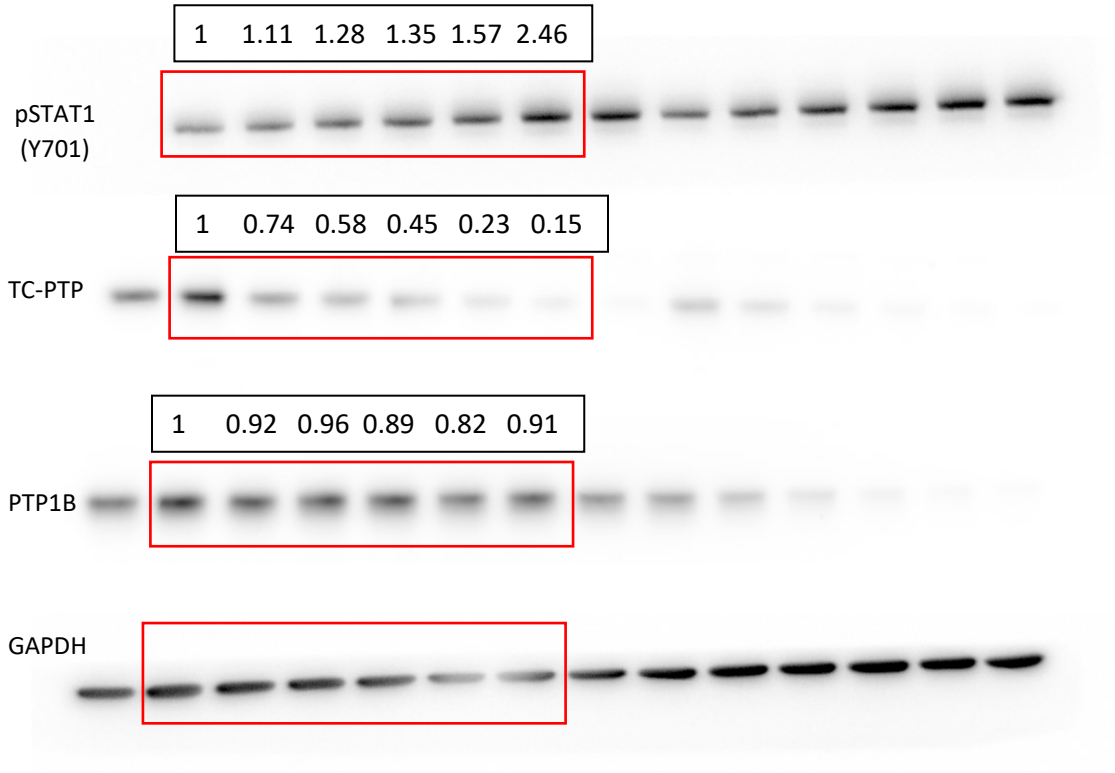

Fig. S1

	DMSO	TC5P	TC7P	TC8P	TC9P	TC4P	TC6P	DU-14	3
PTP1B	1	0.93	1.02	1.08	0.99	0.96	0.98	0.01	0.96
TC-PTP	1	0.52	0.91	1.35	1.12	0.75	0.71	0.01	1.09