Supporting Information

Development of hematopoietic prostaglandin D synthase-degradation inducer

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[#]These authors contributed equally to this study.

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General

All chemicals were purchased from Sigma-Aldrich, FUJIFILM Wako Pure Chemicals, and Tokyo Chemical Industry, and were used without further purification. TLC analysis was conducted using Merck silica gel 60 F254 pre-coated plates and visualized using a 254 nm/365 nm UV lamp, and iodine stain. Column chromatography was performed using silica gel (spherical, neutral) purchased from Kanto Chemical. NH silica gels were purchased from Fuji Silysia Chemical, Ltd. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL ECZ600 spectrometer. High-resolution mass spectra were measured using a Shimadzu IT-TOF MS equipped with an electrospray ionization source.

Compound **1**[S1] and **12** [S2] was prepared according to reported method.

Synthesis of compound 2.

A mixture of **1**[S1] (1.51 g, 5.99 mmol) in dimethylformamide (8.0 mL), 1-Boc-piperazine (1.69 g, 8.99 mmol), EDCI (1.40 g, 7.19 mmol), and HOBt•H_2O (1.02 g, 6.59 mmol) was stirred at room temperature for 6 h. The reaction mixture was poured into water, and the precipitate was collected by filtration, and concentrated in vacuo. The residue and palladium on carbon (91.2 mg) in ethanol (60 ml) was stirred under hydrogen atmosphere at room temperature for overnight. The reaction mixture was filtrated, and concentrated, giving **2** (2.14 g, 92%) as a purple solid.

^{1}H NMR (600 MHz, DMSO-d_6) δ 6.67 (d, J = 6.6 Hz, 2H), 6.47 (d, J = 6.0 Hz, 2H), 4.54 (brs, 2H), 3.49-3.27 (m, 11H), 2.65-2.51 (m, 2H), 1.68-1.65 (m, 4H), 1.40 (s, 9H). ^{13}C NMR (600 MHz, DMSO-d_6) δ 172.32, 153.20, 142.29, 141.54, 117.97, 114.12, 78.54, 50.00, 43.93, 40.29, 36.60, 27.97, 27.45. ESI-MS m/z calculated for $C_{21}H_{33}N_4O_3$ $[M+H]^+$ 389.2547; Found. 389.2546.

Synthesis of compound 4.

A mixture of **2** (2.10 g, 5.40 mmol), **3** (1.17 g, 5.40 mmol), EDCI (1.25 g, 6.49 mmol) and HOBt•H_2O (0.922 g, 6.02 mmol) in dimethylformamide (20 mL) was stirred at room temperature for 6 h. The reaction mixture was poured into water, and the precipitate was collected by filtration, giving **4** (1.14 g, 36%) as a gray solid.

^{1}H NMR (600 MHz, DMSO-d_6) δ 10.23 (s, 1H), 9.08 (s, 2H), 7.55 (d, J = 9.0 Hz, 2H), 7.46 (t, J = 7.8 Hz, 2H), 7.29 (t, J = 7.8 Hz, 1H), 7.24 (d, J = 7.8 Hz, 2H), 6.93 (d, J = 9.0 Hz, 2H), 3.68-3.28 (m, 10H), 2.74-2.65 (m, 3H), 1.70-1.64 (m, 4H), 1.40 (s, 9H). ^{13}C NMR (600 MHz, DMSO-d_6) δ 172.75, 165.64, 161.03, 159.63, 153.78, 152.58, 147.93, 130.06, 129.79, 125.59, 124.19, 121.63, 121.46, 115.86, 79.14, 48.41, 44.52, 40.91, 37.13, 30.70, 28.03, 27.94. ESI-MS m/z calculated for $C_{32}H_{39}N_6O_5$ $[M+H]^+$ 587.2976; Found. 586.2985.

Synthesis of compound 5.

A mixture of **4** (20.7 mg, 0.0352 mmol) and TFA (40.2 mg, 0.352 mmol) in CH_2Cl_2 (1.0 mL) was stirred at room temperature for 2 h. The reaction mixture was concentrated, and the residue was purified by column chromatography (NH silica gel, CH_2Cl_2/MeOH = 10/1), giving **5** (19.3 mg, quant.) as colorless oil.

^{1}H NMR (600 MHz, MeOH-d_4) δ 9.06 (s, 2H), 7.92 (d, J = 9.0 Hz, 2H), 7.63 (d, J = 9.0 Hz, 2H), 7.41 (d, J = 7.2 Hz, 2H), 7.25 (t, J = 7.2 Hz, 1H), 7.17 (d, J = 7.2 Hz, 2H), 3.91-3.68 (m, 8H), 3.30-3.19 (m, 5H), 2.20-2.12 (m, 4H). ^{13}C NMR (600 MHz, MeOH-d_4) δ 175.61, 167.54, 163.78, 160.81, 154.18, 148.55, 133.17, 130.80, 126.87, 125.43, 123.39, 122.63, 118.87, 51.59, 44.74, 44.43, 43.44, 39.67, 38.53, 29.19. ESI-MS m/z calculated for $C_{27}H_{31}N_6O_3$ $[M+H]^+$ 487.2452; Found. 487.2468.

Synthesis of PROTAC(H-PGDS)-1.

A mixture of **5** (21.2 mg, 0.0436 mmol), **6** (21.9 mg, 0.0371 mmol), EDCI (12.0 mg, 0.0653 mmol), DIPEA (11.0 mg, 0.0872 mmol), and HOBt•H_2O (10.0 mg, 0.0653 mmol) in dimethylformamide (2.5 mL) at room temperature for two days. The reaction mixture was purified by preparative RP-HPLC (gradient: 15-50% MeCN-H_2O containing 0.1% TFA, 40 min), giving **PROTAC(H-PGDS)-1** (2.9 mg, 8%) as a white solid.

^{1}H NMR (600 MHz, DMSO-d_6) δ 11.09 (s, 1H), 9.09 (s, 2H), 7.64 (brs, 1H), 7.56 (d, J = 9.0 Hz, 2H), 7.47 (d, J = 9.0 Hz, 2H), 7.29 (d, J = 7.2 Hz, 2H), 7.25 (d, J = 7.2 Hz, 2H), 7.15 (d, J = 7.2 Hz, 1H), 7.03 (d, J = 7.2 Hz, 1H), 6.60 (brs, 1H), 5.04 (dd, J = 13.2, 5.4 Hz, 1H), 3.68-3.42 (m, 32H), 2.90-2.00 (m, 13H). ESI-MS m/z calculated for $C_{53}H_{64}N_9O_{13}$ $[M+H]^+$ 1034.4618; Found. 1034.4600.

HPLC: Inertsil WP300 C18, 5 μm, 4.6 mm × 250 mm, solvent A: 0.1% TFA/water, solvent B: 0.1% TFA/MeCN, gradient: 10-90% gradient of solvent B over 30 min, flow rate: 2 mL/min, 40°C; HPLC purity: >99% (t_R = 13.6 min).

Synthesis of compound 8.

A mixture of the **5** (56.7 mg, 0.119 mmol), **7** (36.2 mg, 0.108 mmol), EDCI (64.2 mg, 0.335 mmol), and DIPEA (49.2 mg, 0.324 mmol) in dimethylformamide (2.0 ml) was stirred at room temperature for 1.5 h. The reaction mixture was neutralized with saturated aq. $NaHCO_3$, and extracted with EtOAc. The organic layer was separated, washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography (silica gel, MeOH/CH_2Cl_2 = 0/100 to 9/91), giving **8** (23.7 mg, 25%) as a white solid.

^{1}H NMR (600 MHz, MeOH-d_4) δ 9.00 (s, 2H), 7.48 (d, J = 8.4 Hz, 2H), 7.40 (t, J = 8.4 Hz, 2H), 7.24 (t, J = 8.4 Hz, 1H), 7.15 (d, J = 8.4 Hz, 2H), 6.93 (d, J = 8.4 Hz, 2H), 5.44 (s, 1H), 3.70-3.53 (m, 28H), 3.29 (m, 2H), 2.80-2.63 (m, 5H), 1.83-1.76 (m, 4H), 1.23-1.18 (m, 2H). ESI-MS m/z calculated for

$C_{40}H_{54}N_9O_9$ $[M+H]^+$ 804.4039; Found. 804.4030.

Synthesis of compound 9.

The mixture of **8** (23.7 mg, 0.0294 mmol) and palladium on carbon (6.9 mg) in MeOH (4.0 mL) was stirred under the hydrogen atmosphere at room temperature for 24 h. The reaction mixture was filtrated, and concentrated, giving **9** (20.3 mg, 66%) as a white solid.

^{1}H NMR (600 MHz, MeOH-d_4) δ 9.03 (s, 2H), 7.52 (d, J = 9.0 Hz, 2H), 7.43 (t, J = 8.4 Hz, 2H), 7.27 (t, J = 7.8 Hz, 1H), 7.19 (d, J = 7.2 Hz, 2H), 6.97 (d, J = 9.0 Hz, 2H), 3.75-3.51 (m, 32H), 2.79-2.61 (m, 5H), 1.85-1.81 (m, 4H). ESI-MS m/z calculated for $C_{40}H_{56}N_7O_9$ $[M+H]^+$ 778.4134; Found. 778.4098.

Synthesis of compound 11.

A mixture of **10** (4.50 g, 24.1 mmol) in dimethylformamide, phenol (2.49 g, 26.5 mmol), and K_2CO_3 (5.00 g, 36.2 mmol) was stirred at 60 °C for 2 h. The reaction mixture was quenched with ice water, and was extracted with EtOAc. The organic layer was washed with brine, dried over Na2SO4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (eluted with 2–25% EtOAc in hexane), giving **11** (5.09 g, 86%) as a white solid. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 9.10 (2H, s), 7.46 (2H, t, J = 9.0 Hz), 7.30 (1H, t, J = 9.0 Hz), 7.21 (2H, d, J = 9.0 Hz), 4.41 (2H, q, J = 7.8 Hz), 1.40 (3H, t, J = 7.8 Hz). ESI-MS m/z calculated. for $C_{13}H_{12}N_2O_3$ $[M+H]^+$; 245.0921 Found. 245.0920

Synthesis of compound 3.

A mixture of **11** (5.06 g, 20.7 mmol) in THF, and 1 M aq. NaOH (24.8 mL, 24.8 mmol) was stirred at room temperature overnight. The reaction mixture was concentrated THF in vacuo, and added 2 M aq.

HCl, and was extracted with EtOAc. The organic layer was washed with brine, dried over Na2SO4, and concentrated, giving **3** (3.57 g, 80%) as a white solid. ^{1}H-NMR (600 MHz, MeOH-d_4) δ: 9.06 (2H, s), 7.44 (2H, t, J = 7.8 Hz), 7.28 (1H, t, J = 7.8 Hz), 7.19 (2H, d, J = 7.8 Hz). ESI-MS m/z calculated. for $C_{11}H_8N_2O_3$ $[M+H]^+$; 217.0608 Found. 217.0615

Synthesis of PROTAC(H-PGDS)-2.

A mixture of the **9** (28.9 mg, 0.0276 mmol), **10** (9.7 mg, 0.0308 mmol) and DIPEA (15.6 mg, 0.0772 mmol) in dimethyl sulfoxide (1.0 mL) was stirred at 90 °C for 30 min. The mixture was then cooled to room temperature for 6 h. The reaction mixture was purified by preparative RP-HPLC (gradient: 10-90% MeCN-H_2O containing 0.1% TFA, 40 min), giving **PROTAC(H-PGDS)-2** (1.2 mg, 4%) as a white solid.

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.10 (s, 2H), 8.95-8.92 (m, 1H), 7.79 (m, 2H), 7.57 (m, 2H), 7.48-7.45 (m, 4H), 7.31 (t, J = 7.8 Hz, 1H), 7.22 (d, J = 7.8 Hz, 2H), 6.91 (d, J = 8.4 Hz, 1H), 4.96-4.89 (m, 1H), 3.93-3.44 (m, 32H), 3.20 (s, 3H), 3.09-2.06 (m, 13H). ESI-MS m/z calculated for $C_{54}H_{66}N_9O_{13}$ $[M+H]^+$ 1048.4775; Found. 1048.4807.

HPLC: Inertsil WP300 C18, 5 μm, 4.6 mm × 250 mm, solvent A: 0.1% TFA/water, solvent B: 0.1% TFA/MeCN, gradient: 10-90% gradient of solvent B over 30 min, flow rate: 2 mL/min, 40°C; HPLC purity: >95% (t_R = 14.5 min).

Synthesis of SNIPER(H-PGDS)-1.

A mixture of **5** (14.9 mg, 0.0295 mmol), **12** (20.0 mg, 0.0268 mmol), EDCI (7.7 mg, 0.0322 mmol), K_2CO_3 (8.9 mg, 0.0689 mmol), and HOBt•H_2O (4.5 mg, 0.0295 mmol) in dimethylformamide (1.0 mL) at room temperature for 3 h. Then, three drops of DIPEA (about 0.15 mL) was added and the mixture was stirred at room temperature overnight. The reaction mixture was concentrated, and the

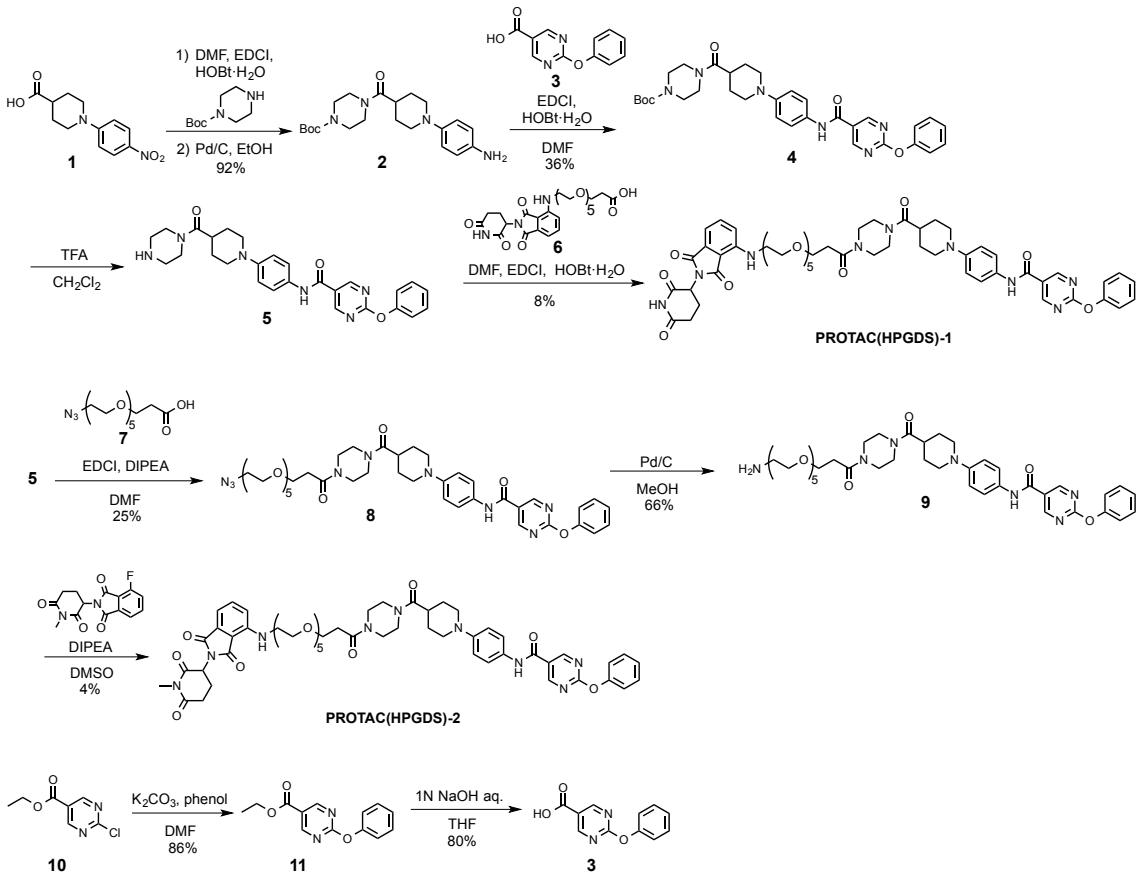
residue was washed with water. 4M HCl aq. was added to the residue, and the mixture stirred for 3h. The reaction mixture was concentrated, and residue was purified by preparative RP-HPLC (gradient: 30-50% MeCN-H_2O containing 0.1% TFA, 40 min), giving **SNIPER(H-PGDS)-1** (1.0 mg, 3%) as a white solid.

ESI-MS m/z calculated for $C_{59}H_{74}N_{10}O_{10}S$ $[M+2H]^+$ 557.2650; Found. 557.2647.

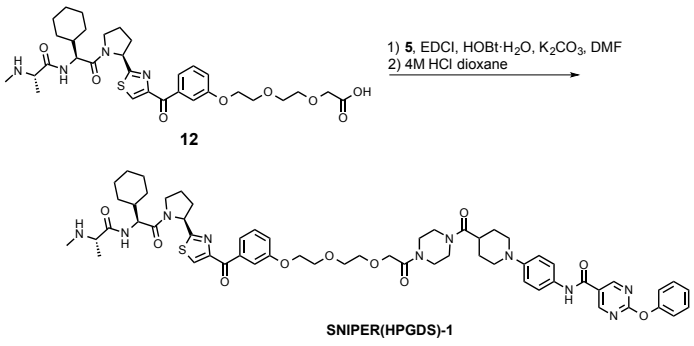
HPLC: Inertsil WP300 C18, 5 μm, 4.6 mm × 250 mm, solvent A: 0.1% TFA/water, solvent B: 0.1% TFA/MeCN, gradient: 10-90% gradient of solvent B over 30 min, flow rate: 2 mL/min, 40°C; HPLC purity: >98% (t_R = 13.7 min).

HPLC Condition

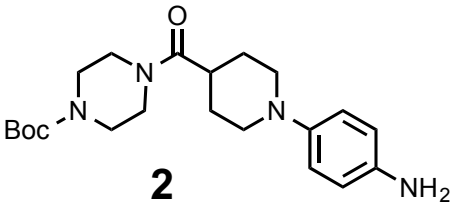
Preparative HPLC was performed using an Inertsil WP300 C18 column (5 μm, 20 mm × 250 mm, GL science) at a flow rate of 10 mL/min on a JASCO PU-4180 HPLC, and eluents were detected at 220 nm by a JASCO UV-2075. Analytical HPLC was performed using an Inertsil WP300 C18 column (5 μm, 4.6 mm × 250 mm, GL science) at a flow rate of 2.0 mL/min on a JASCO PU-4185 HPLC, and eluents were detected at 220 nm by a JASCO MD-4010.



Scheme S1. Synthesis of **PROTAC(H-PGDS)-1** and **PROTAC(H-PGDS)-2**.

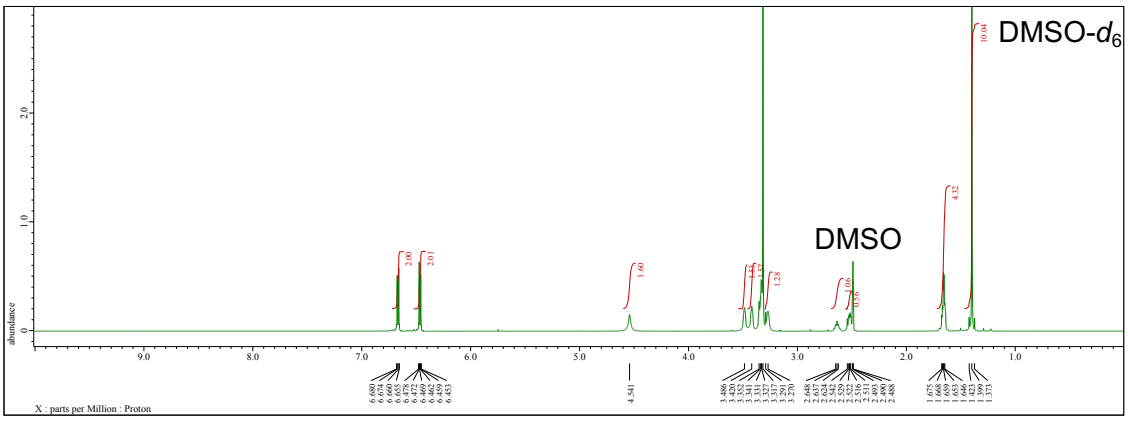


Scheme S2. Synthesis of **SNIPER(H-PGDS)-1**.



2

^{1}H-NMR of Compound **2**



^{13}C-NMR of Compound **2**

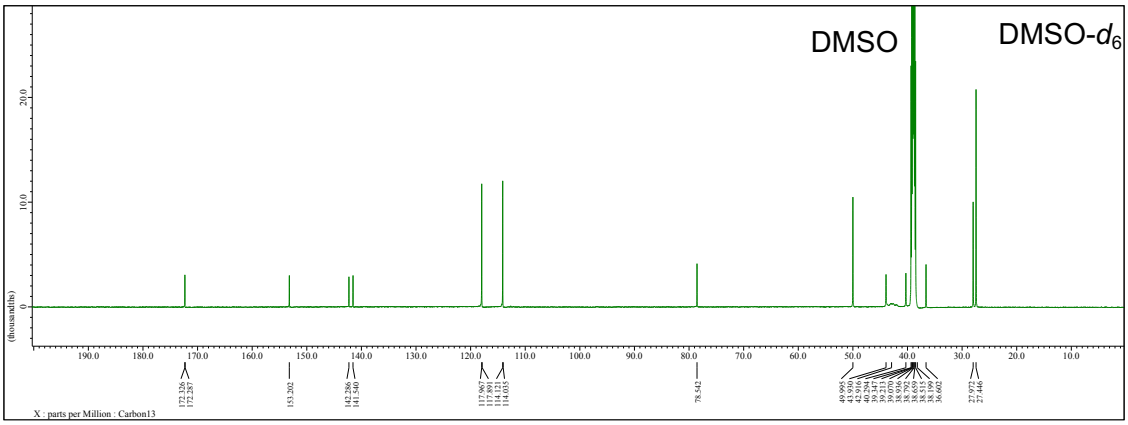


Figure S1. ^{1}H- and ^{13}C-NMR spectra of compound **2**.

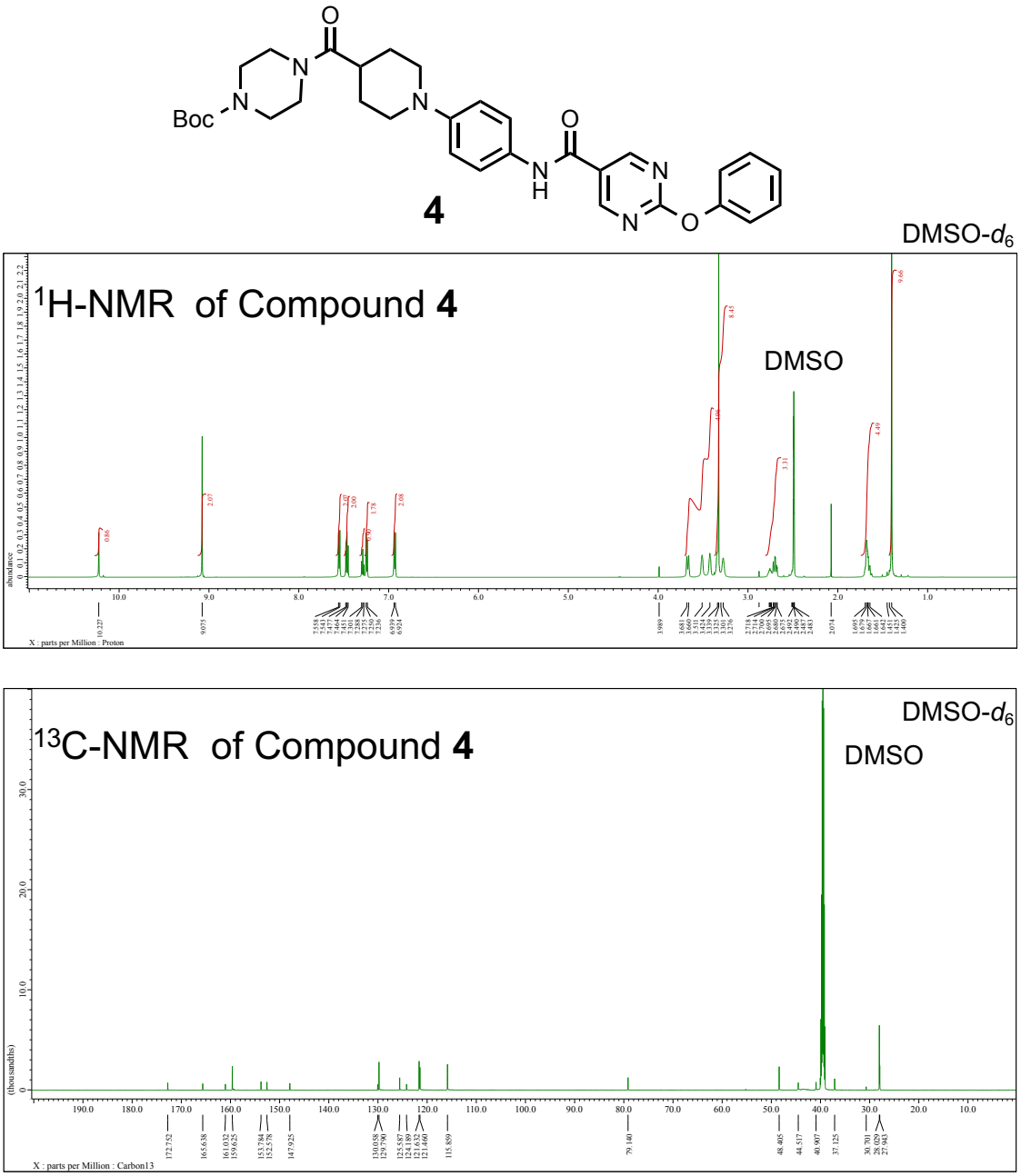
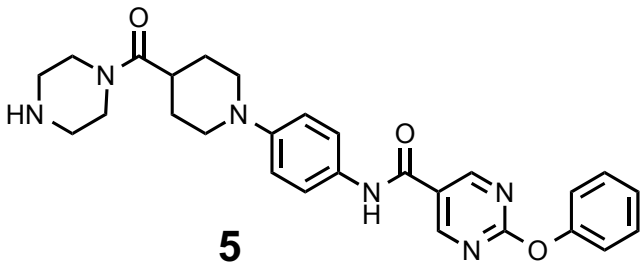
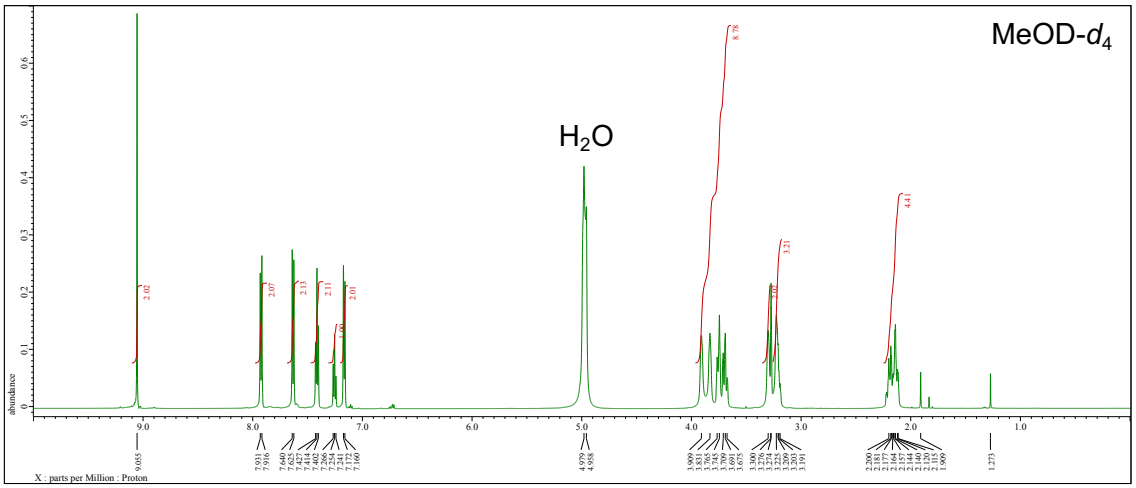


Figure S2. ^{1}H- and ^{13}C-NMR spectra of compound **4**.



5

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



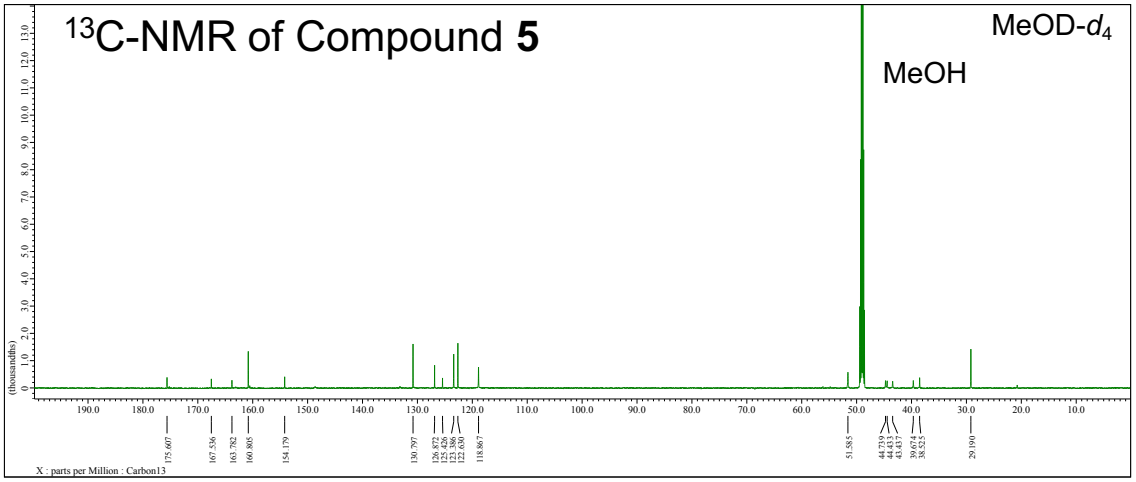
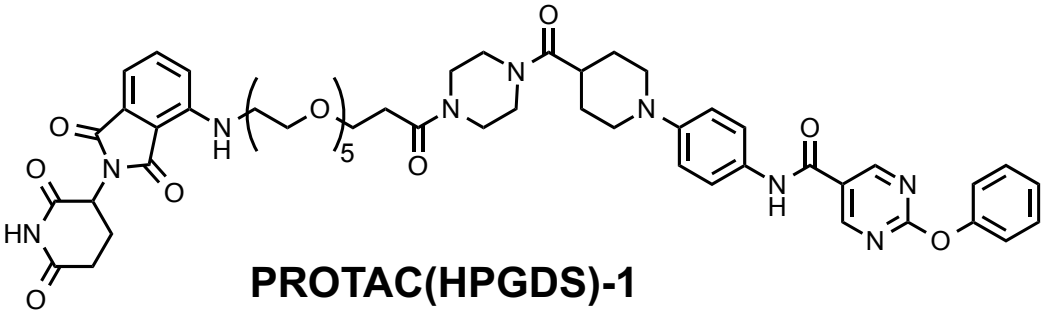
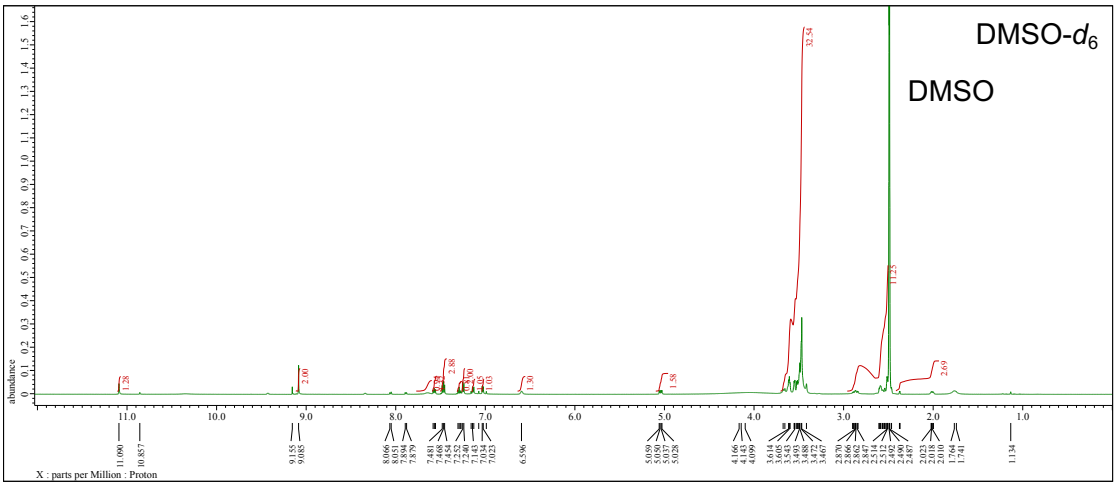


Figure S3. ^{1}H- and ^{13}C-NMR spectra of compound **5**.



PROTAC(HPGDS)-1

^{1}H-NMR of Compound **PROTAC(HPGDS)-1**



HPLC spectrum of Compound **PROTAC(HPGDS)-1**

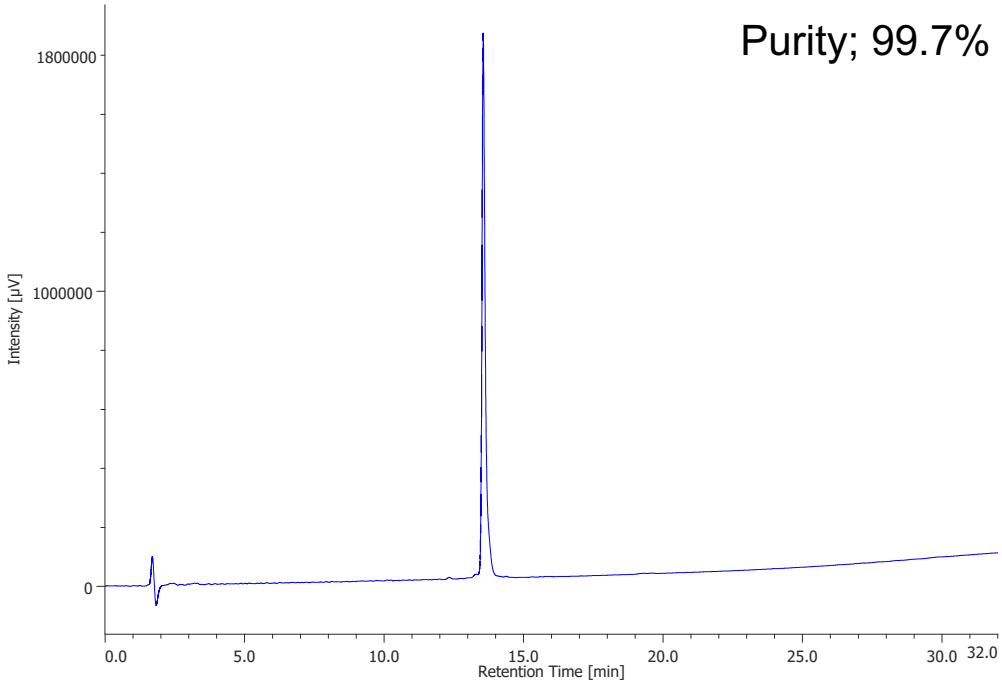
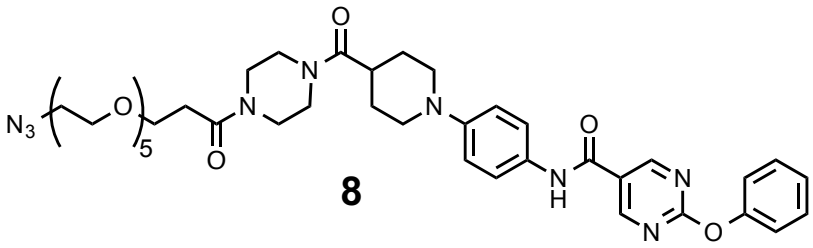


Figure S4. ^{1}H-NMR spectrum and HPLC chromatogram of **PROTAC(HPGDS)-1**.



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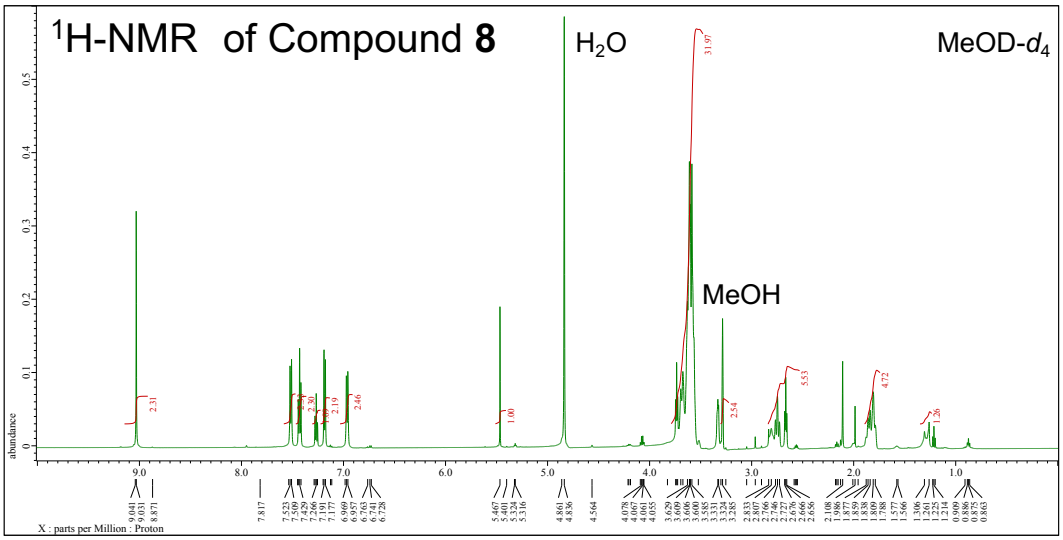

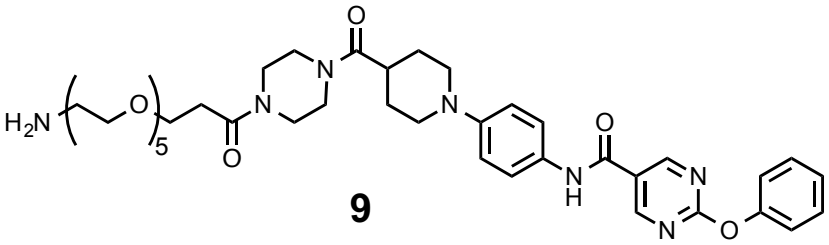

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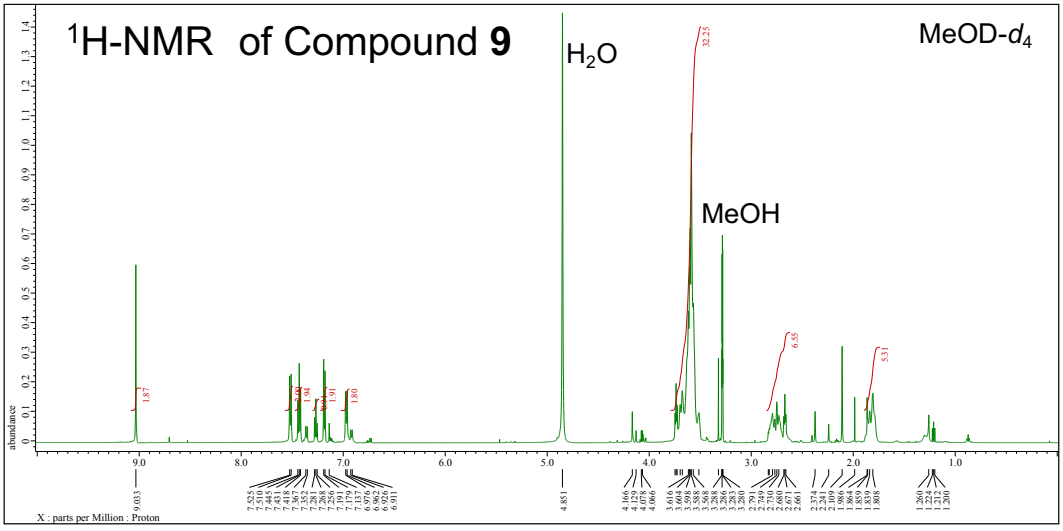
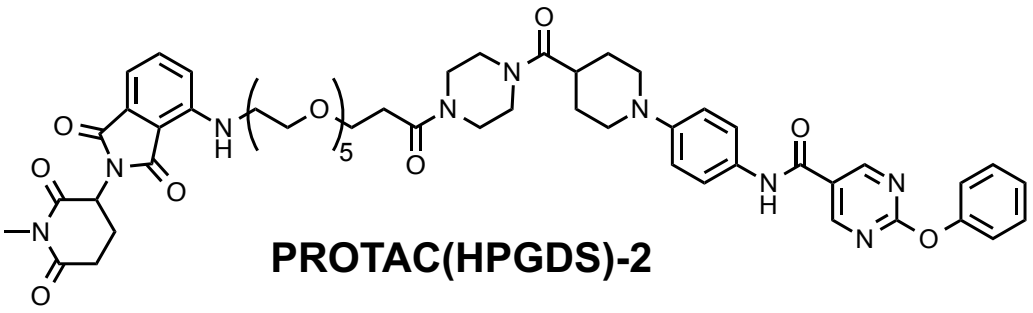
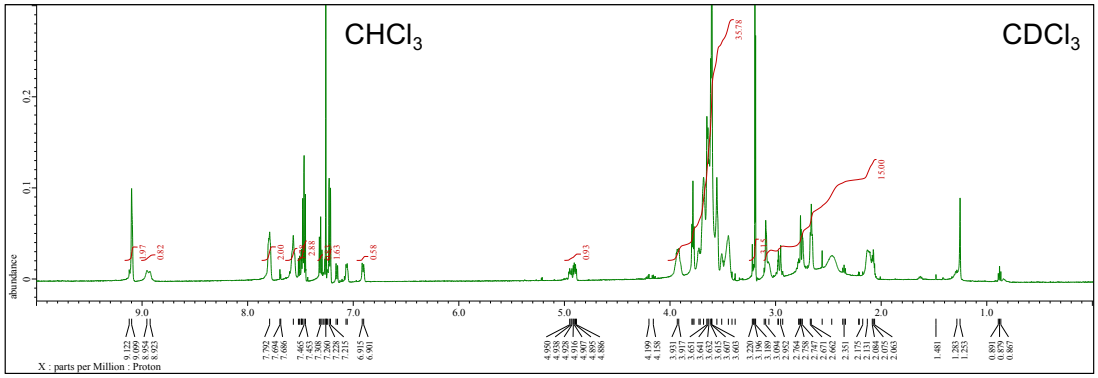


Figure S5. ^{1}H-NMR spectra of compounds **8** and **9**.



PROTAC(HPGDS)-2

^{1}H-NMR of Compound **PROTAC(HPGDS)-2**



HPLC spectrum of Compound **PROTAC(HPGDS)-2**

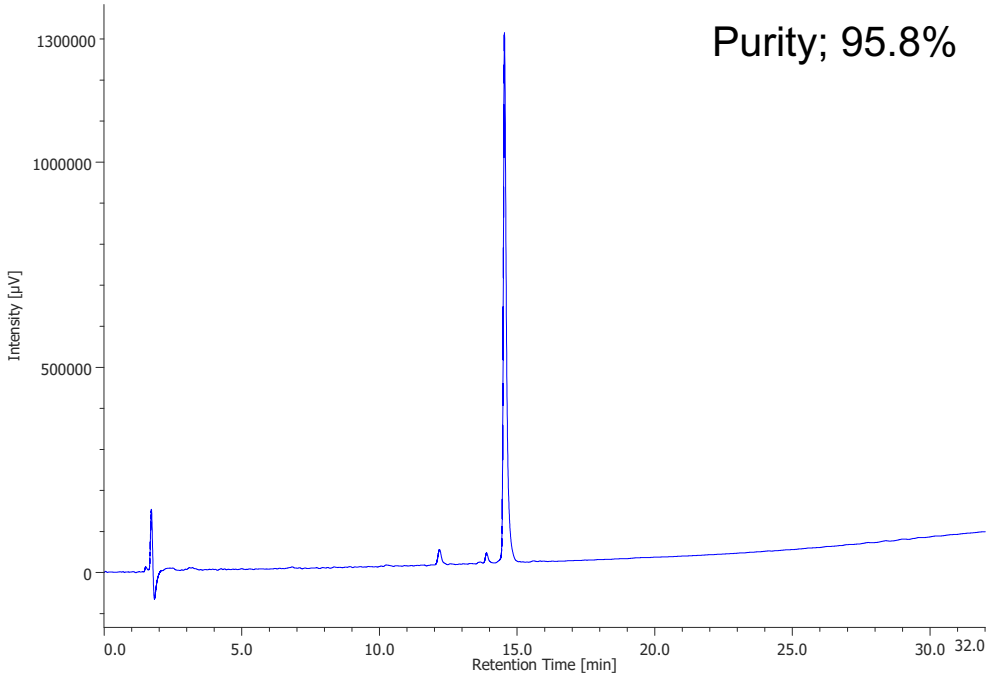
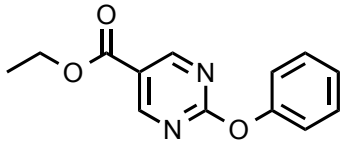


Figure S6. ^{1}H-NMR spectrum and HPLC chromatogram of **PROTAC(HPGDS)-2**.



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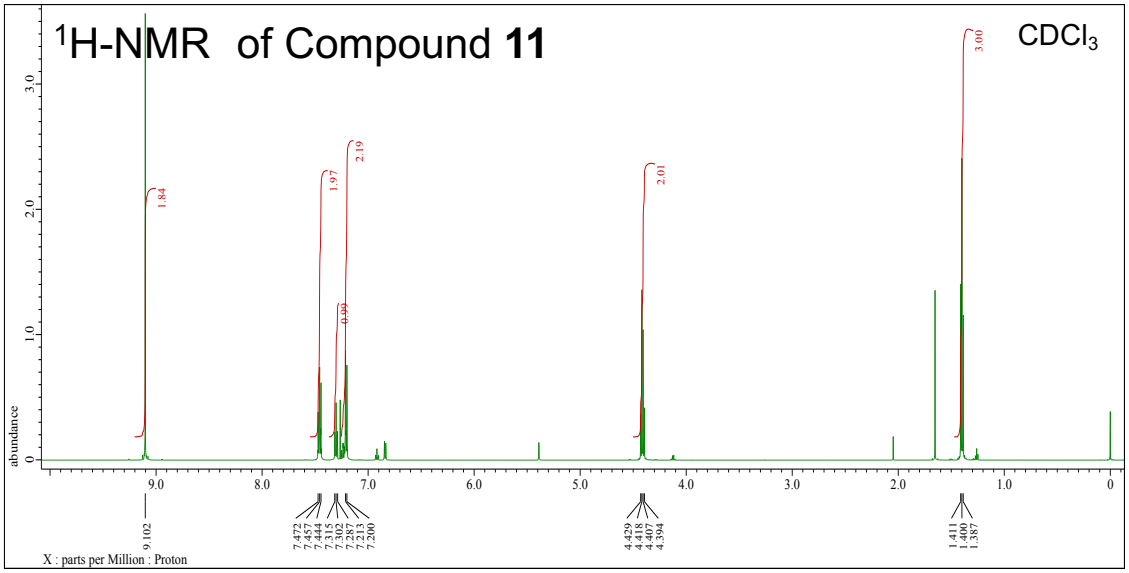

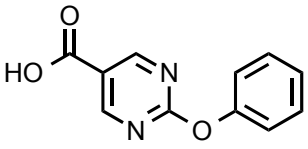

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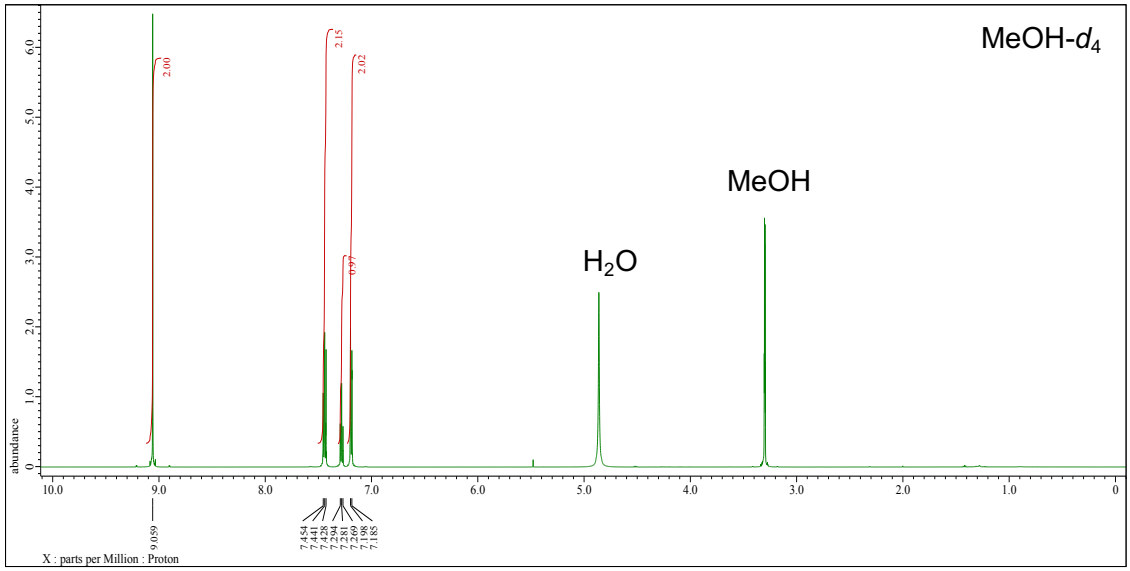
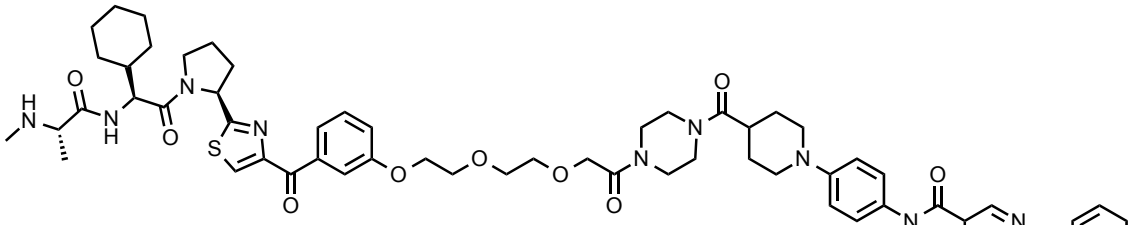
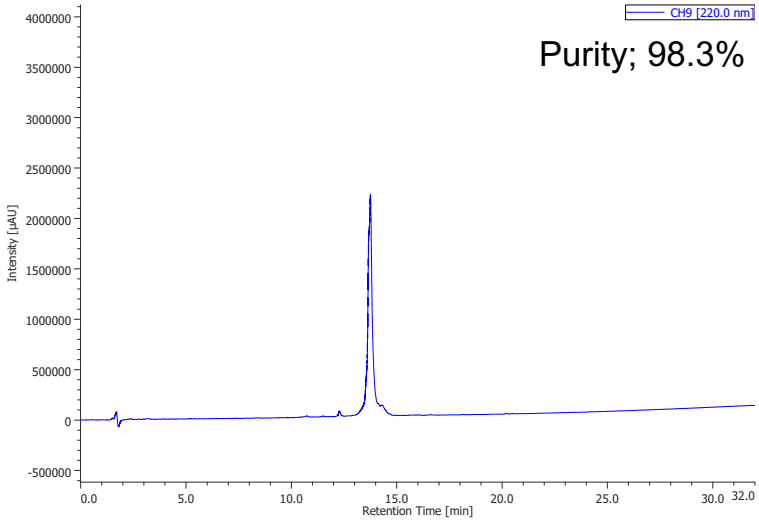


Figure S7. ^{1}H-NMR spectra of compounds **11** and **3**.



SNIPER(HPGDS)-1

HPLC spectrum of Compound **SNIPER(HPGDS)-1**



MS spectrum of Compound **SNIPER(HPGDS)-1**

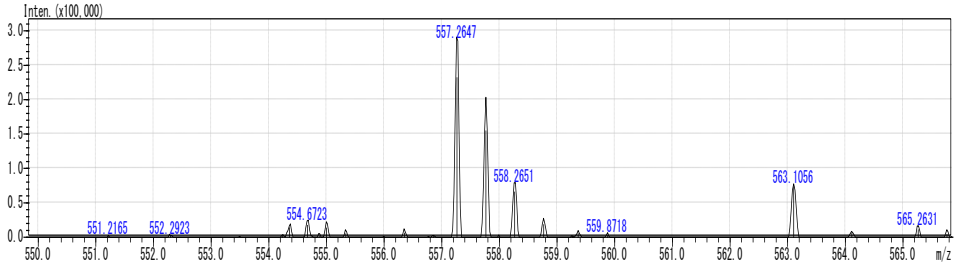


Figure S8. HPLC chromatogram and IT-TOF MS spectrum of **SNIPER(HPGDS)-1**.

Biological assessment methods

Reagents

Tissue culture plastics were purchased from Greiner Bio-One (Frickenhausen, Germany). Cycloheximide was purchased from Sigma-Aldrich (St. Louis, MO, USA). MG132 was purchased from Peptide Institute (Osaka, Japan). MLN7243 was purchased from Active Biochem (Maplewood, NJ, USA). Pomalidomide was purchased from Cayman Chemical (Ann Arbor, MI, USA). A23187 was purchased from Merck KGaA (Darmstadt, Germany).

Cell culture

Human chronic myelogenous leukemia KU812 cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS) (Thermo Fisher Scientific, Waltham, MA, USA), and 100 μg/mL kanamycin (Sigma-Aldrich). Human megakaryoblastic leukemia MEG-01s cells were cultured in Dulbecco's Modified Eagle's medium (Sigma-Aldrich) supplemented with 10% FBS and 100 μg/mL kanamycin. KU812 cells were obtained from the Japanese Collection of Research Bioresources (Osaka, Japan) Cell Bank (JCRB0104).[S3]

Western blot analysis

Cells were lysed with SDS lysis buffer (0.1 M Tris-HCl at pH 8.0, 10% glycerol, and 1% SDS). Protein concentration was measured by the BCA method (Thermo Fischer Scientific), and an equal amount of protein lysate was separated by SDS-PAGE, transferred to PVDF membranes (Millipore, County Cork, Ireland), and analyzed by western blot using the appropriate antibodies. The immunoreactive proteins were visualized using Clarity Western ECL substrate (Bio-Rad, Hercules, CA, USA), and their light emission was quantified with a LAS-3000 lumino-image analyzer (Fuji, Tokyo, Japan). The following

antibodies were used: anti-H-PGDS rabbit polyclonal antibody (pAb);[S4] anti-mPGES-1 mouse monoclonal antibody (mAb);[S5] anti-CRBN rabbit pAb (#7180) (Cell Signaling Technology, Danvers, MA, USA); anti-β-Actin mouse mAb (A2228) (Sigma-Aldrich); anti-Cyclin B1 mouse mAb (sc-245 HRP) (Santa Cruz, Dallas, TX, USA); anti-cIAP1 goat pAb (AF8181) (R&D Systems, Minneapolis, MN, USA); anti-AKR-1B1 rabbit pAb (15439-1-AP) (Proteintech, Rosemont, IL, USA); and anti-β-Tubulin rabbit pAb (ab6046) (Abcam, Cambridge, UK).

RNA isolation and quantitative PCR

Total RNA was prepared from cells using a RNeasy kit (Qiagen, Hilden, Germany). First-strand cDNA was synthesized from 1 μg of total RNA with an oligo-dT primer using the SuperScript First-Strand Synthesis System (Invitrogen, Carsbad, CA, USA). Quantitative real-time PCR was performed with an ABI Prism 7300 sequence detection system (Applied Biosystems, Foster City, CA, USA) using SYBR GreenER (Invitrogen) with gene-specific primers. Human *36B4* mRNA was used as an invariant control. The following PCR primers were used (5′ to 3′): *36B4*, GGCCCGAGAAGACCTCCTT and CCAGTCTTGATCAGCTGCACA; *H-PGDS*, TGCCGTCGCTAACTGGATAA and GAGATGCCCCCGAGAAAAAC.

Binding affinity to H-PGDS

The fluorescence polarization-based (FP-based) binding assay was performed using Prostaglandin D Synthase (hematopoietic-type) FP-Based Inhibitor Screening Assay Kit - Green (600007) (Cayman Chemical). In brief, the binding assays were performed in non-binding black 384-well and used a recombinant human H-PGDS protein, glutathione and fluorescent probe in assay buffer to produce a final volume of 47.5 μl. Then, 2.5 μl of test compounds made up as stocks in DMSO was added and the plate was incubated for 1 h at room temperature. Each was tested against H-PGDS in triplicate at

final test compound concentrations (2.50 μM, 1.25 μM, 625 nM, 313 nM, 156 nM, 78.1 nM, 39.0 nM, 19.5 nM, 9.77 nM, 4.88 nM and 2.44 nM). Plates were then read with excitation wavelengths (470 nm) and emission wavelengths (530 nm) on a EnVision Multilabel Plate Reader (PerkinElmer, Waltham, MA, USA). The measurements of fluorescent polarization of a molecule (mP) are taken in the fluorescent polarization mode. The percentage of inhibition of test compounds was calculated according to the following equation:

$$\text{Percentage of inhibition} = \left(\frac{\text{mP}_{100\%} - \text{mP}_{\text{sample}}}{\text{mP}_{100\%}}\right) \times 100$$

where $\text{mP}_{\text{sample}}$ is the value of the wells containing test compounds, and $\text{mP}_{100\%}$ is the value of the maximum binding well. The concentration of test compounds that reduces the mP value by 50% (IC_{50}) was estimated from a graph plotted the mP value versus the concentration of the compounds on semi-log axis.

Expression and Purification of recombinant H-PGDS

Human H-PGDS were expressed and purified as described previously.[S6] In brief, *E. coli* BL21 (DE3) cells (Thermo Fisher Scientific) were transformed with the prepared plasmids. The cells were grown in LB medium (Nacalai Tesque, Kyoto, Japan) at 37 ºC, induced with 600 μM isopropyl β-D-1-thiogalactopyranoside (Nakalai Tesque), and then cultured further for 4 h at 37 ºC. The cells were collected and disrupted by sonication in PBS containing a protease inhibitor cocktail (Roche, Basel, Switzerland). After removal of cell debris by centrifugation, the supernatant was filtered and applied onto a GSH-Sepharose 4B column (GE Healthcare UK Ltd., Buckinghamshire, England). After the resin had been washed with PBS, the protein adsorbed on the GSH-Sepharose 4B was eluted with 50 mM Tris-HCl (pH 9.0) containing 10 mM GSH. Protein concentration was determined by the BCA method (Thermo Fischer Scientific).

Inhibitory activity against H-PGDS

The activity of recombinant H-PGDS was measured by the spectrophotometric method with 1 mM 1-chloro-2,4-dinitrobenzene (Sigma-Aldrich), 1 mM glutathione (Sigma-Aldrich), and 2.8 μg/mL recombinant human H-PGDS in 100 mM Tris-HCl (pH 8.0) in the presence 0.1 mg/ml IgG (rabbit γ-goblin, Sigma-Aldrich). The reactions were monitored in a 96 well microplate, and the enzymatic product was followed at 340 nm over 3 min at 25 °C using a microplate spectrophotometer (Multiskan FC, Thermo Fisher Scientific). The IC_{50} values were calculated using GraphPad Prism software (version 5 for Windows).

Measurement of PGD_2 levels

PGD_2 levels in the medium were measured by using a PGD_2 MOX ELISA Kit (512011) (Cayman Chemical) according to the manufacturer's instructions. The absorbance at 405 nm was measured using an EnVision Multilabel Plate Reader (PerkinElmer).

Statistical analysis

Two-tailed Student's t tests were used to determine the significance of differences between experimental groups.

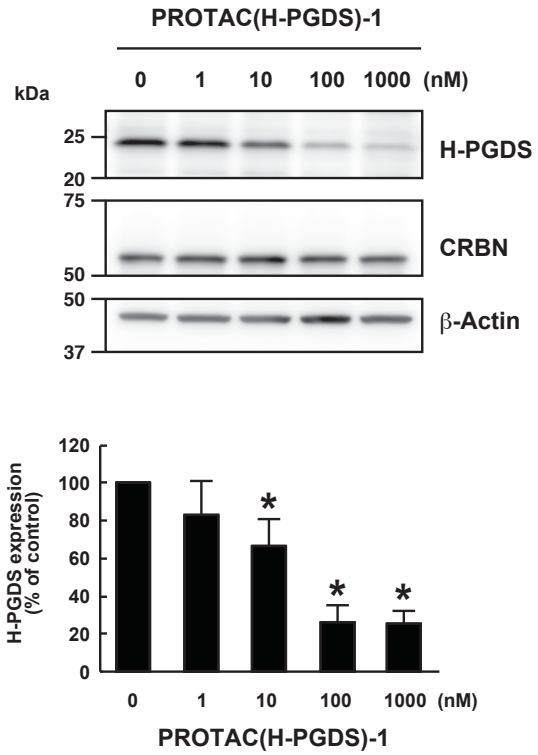


Figure S9. PROTAC(H-PGDS)-1 reduced H-PGDS protein levels in MEG-01s cells. Cells were incubated with the indicated concentration of **PROTAC(H-PGDS)-1** for 6 h. H-PGDS/β-Actin ratio was normalized by vehicle control as 100. Data in the bar graph are mean ± SD (n = 3). *P < 0.05 compared with vehicle-treated control in a two-tailed Student's t test.

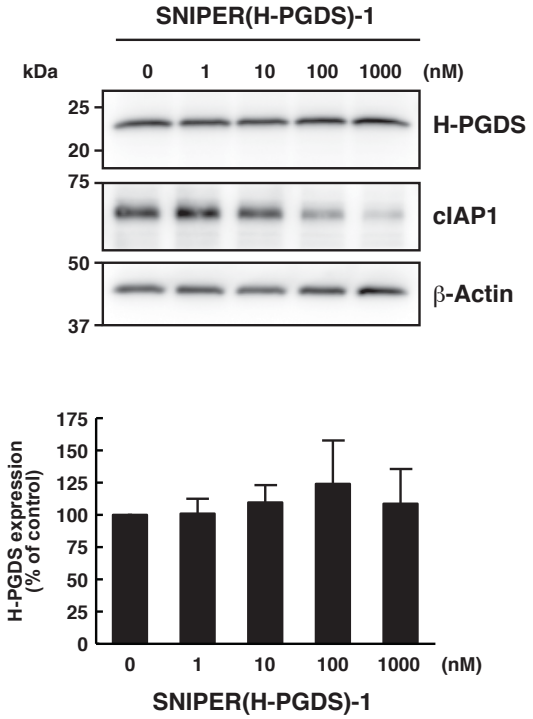


Figure S10. SNIPER(H-PGDS)-1 did not affect H-PGDS protein levels in KU812 cells. Cells were incubated with the indicated concentration of **SNIPER(H-PGDS)-1** for 6 h. H-PGDS/β-Actin ratio was normalized by vehicle control as 100. Data in the bar graph are mean ± SD (n = 3).

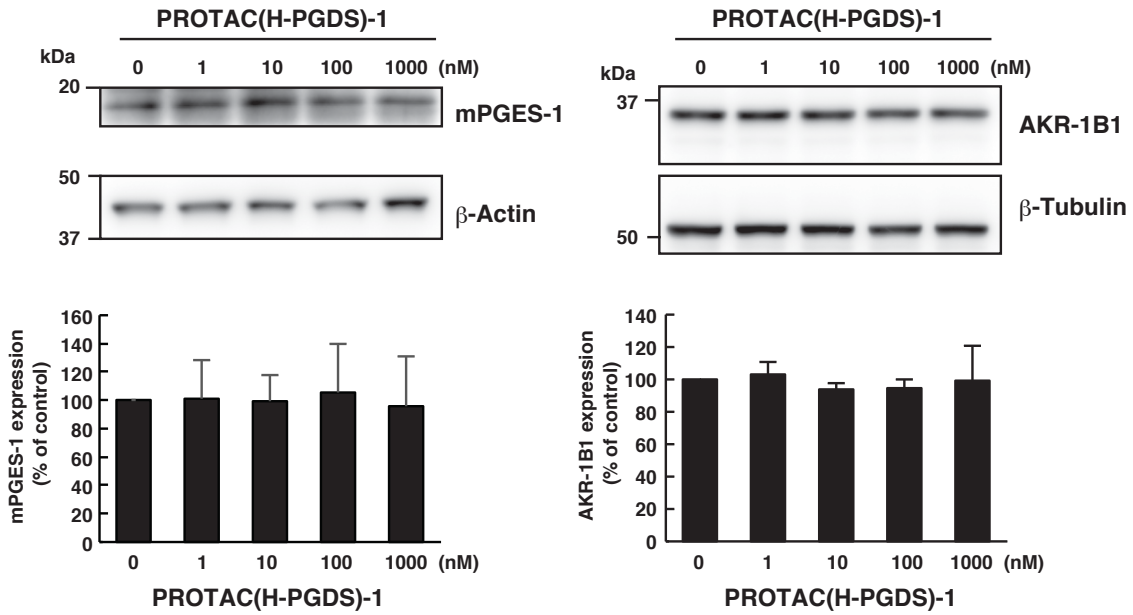


Figure S11. PROTAC(H-PGDS)-1 did not affect mPGES-1 and AKR-1B1 protein levels in KU812 cells. Cells were incubated with the indicated concentration of **PROTAC(H-PGDS)-1** for 24 h. mPGES-1/β-Actin and AKR-1B1/β-Tubulin ratios were normalized by vehicle control as 100. Data in the bar graphs are mean ± SD (n = 3).

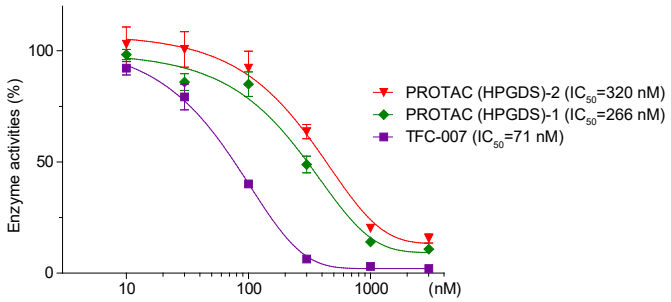


Figure S12. Inhibition of H-PGDS enzyme activity by **PROTAC(H-PDGS)-1**, **PROTAC(H-PDGS)-2** and TFC-007. Recombinant H-PDGS enzyme activity was measured as described in Methods.

References

S1) de Candia, M.; Fiorella, F.; Lopopolo, G.; Carotti, A.; Romano, M. R;, Lograno, M. D.; Martel, S.; Carrupt, P. A.; Belviso, B. D.; Caliandro, R.; Altomare, C.; Synthesis and biological evaluation of direct thrombin inhibitors bearing 4-(piperidin-1-yl)pyridine at the P1 position with potent anticoagulant activity. T. *J. Med. Chem.* **2013**, *56*, 8696–8711

S2) Ohoka, N.; Okuhira, K.; Ito, M.; Nagai, K.; Shibata, N.; Hattori, T.; Ujikawa, O.; Shimokawa, K.; Sano, O.; Koyama, R.; Fujita, H.; Teratani, M.; Matsumoto, H.; Imaeda, Y.; Nara, H.; Cho, N.; Naito, M.; *In Vivo* Knockdown of Pathogenic Proteins via Specific and Nongenetic Inhibitor of Apoptosis Protein (IAP)-dependent Protein Erasers (SNIPERs). *J Biol Chem.* **2017**, *292*, 4556–4570.

S3) Kishi, K. A New Leukemia Cell Line with Philadelphia Chromosome Characterized as Basophil Precursors. Leuk. Res. 1985, 9, 381–390.

S4) Mohri, I.; Eguchi, N.; Suzuki, K.; Urade, Y.; Taniike, M. Hematopoietic Prostaglandin D Synthase is Expressed in Microglia in the Developing Postnatal Mouse Brain. *Glia* **2003**, *42*, 263–274.

S5) Lazarus, M.; Eguchi, N.; Matsumoto, S.; Nagata, N.; Yano, T.; Killian, G. J.; Urade, Y. Species-specific Expression of Microsomal Prostaglandin E Synthase-1 and Cyclooxygenases in Male Monkey Reproductive Organs. Prostaglandins Leukot. Essent. *Fatty Acids* **2004**, *71*, 233–240.

S6) Aritake, K.; Kado, Y.; Inoue, T.; Miyano, M.; Urade, Y. Structural and Functional Characterization of HQL-79, an Orally Selective Inhibitor of Human Hematopoietic Prostaglandin D Synthase. *J. Biol. Chem.* **2006**, *281*, 15277–15286.