Supporting Information

Light-Activatable Photochemically Targeting Chimeras (PHOTACs) Enable the Optical Control of Targeted Protein Degradation of HDAC6

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1. Supplemental Figures

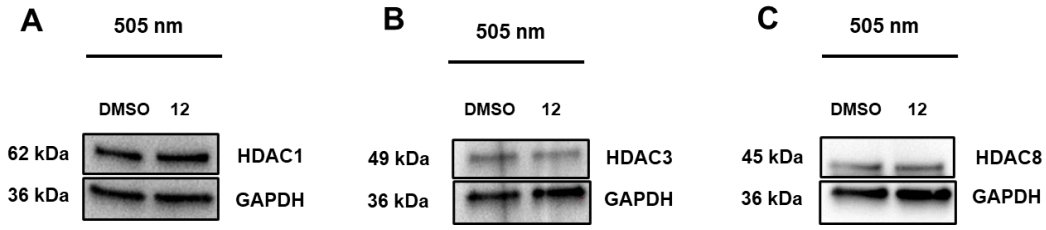


Figure S1. Immunoblot analysis of HDAC isoform levels in MM1.S after initial irradiation with 505 nm. MM.1S cells were treated with **12** (1 µM) or vehicle (DMSO) for 6 h. HDAC1 (A), HDAC3 (B), and HDAC8 (C) levels were analyzed by immunoblotting. GAPDH was used as loading control. Representative images from a total of n = 2 replicates.

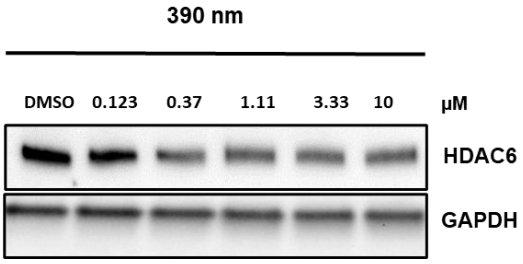


Figure S2. Immunoblot analysis of HDAC6 levels in MM1.S after treatment with compound **12** (1 µM) or vehicle (DMSO) for 6 h after irradiation with light of 390 nm. Representative immunoblot of HDAC6 and GAPDH protein levels in MM1.S cells treated with the indicated concentrations. Representative images from a total of n = 2 replicates.

2. Experimental Section

2.1 Docking

The crystal structures of HDAC6 (PDB ID: 5EEI) and CRBN (PDB ID: 4CI2) were obtained from the Protein Data Bank. The isolated CRBN was obtain by deleting the DDB1 part in the CRBN-DDB1 complex. Ternary complex modeling was performed in MOE software (version 2022). Briefly, the chemical structures of modeled molecules were prepared and optimized based on the MMFF94X force field. The receptors (HDAC6 and CRBN crystal complexes) were processed as follows: removal of water molecules, addition of hydrogen atoms and partial charges, protonation based on the Amber10: EHT force field. The method 4B was conducted in the software by submitting the prepared HDAC6, CRBN as well as the degraders. The best pose was kept based on the modeling score and the result from

ligand interactions, followed by visual inspection. The figures were generated using the PyMOL software (https://pymol.org/2/).

2.2 Biology

2.2.1 Cell Line and Cell Culture

The human multiple myeloma cell line MM.1S (ATCC, Manassas, VA, USA, CRL-2974) was cultivated in RPMI 1640 medium (Life Technologies, Darmstadt, Germany) supplemented with 10% fetal bovine serum (PAN Biotech GmbH, Aidenbach, Germany), 100 IU/mL penicillin and 0.1 mg/mL streptomycin (PAN Biotech GmbH, Aidenbach, Germany) and 1 mM sodium pyruvate (ThermoFisher Scientific Inc.; Waltham, MA, USA) at 37 °C and 5% CO_2 under humidified air.

2.2.2 Immunoblot

MM.1S cells (1×10^6 cells/mL) were plated into clear, sterile 6-well or 12-well plates (CytoOne, Hamburg, Germany, CC7682-7506, CC7682-7512) in RPMI 1640 medium without phenol red, supplemented with 10% fetal bovine serum (PAN Biotech GmbH, Aidenbach, Germany), 100 IU/mL penicillin and 0.1 mg/mL streptomycin (PAN Biotech GmbH, Aidenbach, Germany) and 1 mM sodium pyruvate (ThermoFisher Scientific Inc.; Waltham, MA, USA). The plates were incubated overnight at 37 °C and 5% CO_2 under humidified air. The following steps were performed under red light conditions. Compounds were first irradiated at 505 nm for 1 minute. Then, compounds or vehicle (DMSO) were added to the cells in the respective concentrations. The final DMSO concentration was 0,1%. The cells were then irradiated at 390 nm for 1 minute or kept in the dark. Thereafter, the cells were irradiated every hour for 30 seconds at 390 nm or kept in the dark and incubated for 6 h at 37 °C under humidified air with 5% CO_2. For rescue experiments, compound **12** was co-treated with the respective compounds at the indicated concentrations, with irradiation performed as indicated above.

Cell lysis was performed with Cell Extraction Buffer (Catalog# FNN0011, Thermo Fisher Scientific Inc., Waltham, MA, USA) supplemented with Halt Protease Inhibitor Cocktail (100X) (Catalog# 78429, Life-Technologies GmbH, Carlsbad, CA, USA) and phenylmethansulfonyl fluoride (Catalog# 10837091001, Sigma-Aldrich, St. Louis, MO, USA) according to the manufacturer`s protocol. Protein content was quantified using the Pierce™ BCA Protein Assay Kit (Catalog# 23225, Thermo Fisher Scientific Inc., Waltham, MA, USA) according to the manufacturer`s protocol. For the SDS-Page, proteins were denatured with Laemmli 2X Concentrate (Catalog# S3401-10VL, Sigma-Aldrich, St. Louis, MO, USA). Precision Plus Protein Unstained Standard (Catalog# 1610375, Bio-Rad, Hercules, CA, USA) was used as standard marker. SDS-Page was performed with 4-20% Mini-PROTEAN® TGX Stain-Free Protein Gel (Catalog# 4568095, Bio-Rad, Hercules, CA, USA) at 200 V for 40 minutes. Proteins were transferred to Immobilion-FL PVDF membranes (Catalog# IPFL00005, Millipore Merck, Burlinton, MA, USA) with the Trans-Blot Turbo Blot Transfer System (Catalog# 1704150, Bio-Rad, Hercules, CA, USA) at 1.0 A for 30

minutes. Membranes were then incubated with 5% milk-powder solution for 1 h at room temperature. Next, membranes were incubated with anti-HDAC1 (Catalog# 5356S, Cell Signaling Technology, Denver, MA, USA), anti-HDAC3 (Catalog# 85057S, Cell Signaling Technology, Denver, MA, USA), anti-HDAC6 (Catalog# 7558S, Cell Signaling Technology, Denver, MA, USA), anti-HDAC8 (Catalog# 66042S, Cell Signaling Technology, Denver, MA, USA), and anti-GAPDH (Catalog# 2118, Cell Signaling Technology, Denver, MA, USA) solutions at dilutions of 1:1000 to 1:8000 at 4 °C overnight. After washing, the membranes were incubated with HRP-conjugated secondary anti-rabbit antibody solution (Catalog# HAF008, R&D Systems Inc., Minneapolis, MN, USA) for 1.5 hours. The membranes were developed using Clarity western ECL substrate (Catalog# 1705061, Bio-Rad, Hercules, CA, USA). Detection was performed using ChemiDox XRS+ System (Catalog# 1708265, Bio-Rad, Hercules, CA, USA). Data quantification was performed using Image Lab Software 6.1 (Bio-Rad, Hercules, CA, USA). Further analysis was performed with GraphPad Prism *(*Graph Pad Prism 9.0, San Diego, CA, USA).

2.2.3 Expression and Purification of SpyCatcher S50C

The monocystein SpyCatcher S50C expression and purification were performed as previously described.[1] Briefly, SpyCatcher S50C was expressed in *Escherichia coli* Rosetta DE3. Therefore, cells were cultured in Luria-Bertani (LB) media supplemented with kanamycin (50 µg/mL) and chloramphenicol (100 µg/mL) at 37°C while shaking at 130 rpm. Cells were gown to OD_{600} of 0.87 and protein expression was induced with 1 mM IPTG at 18 °C overnight while shaking at 130 rpm. In the following, the cells were harvested by centrifugation at 4000 × g for 20 minutes at 4 °C and lysed in the presence of 50 mM Tris-HCl pH 8.0, 200 mM NaCl, 1 mM TCEP, and 1 mM PMSF by sonification.

Following ultracentrifugation (20000 × g for 40 minutes), the soluble fraction was passed over the Ni-NTA agarose column and SpyCatcher S50C was eluted with elution buffer (50 mM Tris-HCl pH 8.0, 200 mM NaCl, 400 mM imidazole, 1 mM TCEP). The affinity-purified protein was subjected to size exclusion chromatography (SEC), using a Superdex 75 16/600 GL column (Cytiva) equilibrated with SEC buffer (50 mM HEPES pH 7.5, 200 mM NaCl, 0.1 mM TCEP). Eluted protein was concentrated using an Amicon Ultra centrifugal filter (Millipore)and flash-frozen in liquid nitrogen.

2.2.4 Labeling of SpyCatcher S50C with BODIPY-FL-maleimide

Labeling of SpyCatcher with BODIPY-FL-maleimide was performed as previously described.[1] In brief, purified SpyCatcher S50C protein was incubated with DTT (8 mM) at 4 °C for 1 h. DTT was removed using a Superdex 75 16/600 GL size exclusion column (Cytiva) in a buffer containing 50 mM TRIS pH 7.5, 150 mM NaCl, and 0.1 mM TCEP. BODIPY-FL-maleimide (Thermo Fisher) was dissolved in 100% DMSO and mixed with SpyCatcher S50C to achieve a 1.1 molar excess of BODIPY-FL-maleimide. SpyCatcher S50C labeling was carried out overnight at 4 °C. The labeled SpyCatcher S50C was then purified on a Superdex 75 16/600 GL size exclusion column (Cytiva) in buffer containing 50 mM Tris pH

7.5, 150 mM NaCl, and 1 mM TCEP. The purified protein was concentrated using an Amicon Ultra centrifugal filter (Millipore), flash-frozen in liquid nitrogen, and stored at -80 °C.

2.2.5 Expression, purification and labeling with BODIPY-FL-SpyCatcher S50C of CRBN/DDB1 complex

Co-expression and purification of His_6-DDB1ΔB and Flag-Spy-CRBN was performed as previously described.[1,2] In brief, recombinant His_6-DDB1ΔB and Flag-Spy-CRBN were expressed in *Trichoplusia ni* High-Five insect cells using the baculovirus expression system (Invitrogen). The cells were collected by centrifugation (1500 × g for 15 minutes at 10°C) and lysed in the presence of 50 mM Tris pH 8.0, 200 mM NaCl, 1 mM TCEP, 1 mM PMSF and 1x protease inhibitor cocktail (Sigma) by sonification. Following ultracentrifugation (193400 × g, 1h, 4°C), the soluble fraction was supplemented with 250 U Benzonase (Sigma #70664-3) and stirred for 1 h at room temperature. Afterwards, the soluble fraction was passed over Flag-M2 sepharose (GenScript) and eluted with wash buffer (50 mM Tris-HCl pH 8.0, 200 mM NaCl, 1 mM TCEP) supplemented with 0.15 mg/ml Flag peptide (GenScript). The affinity-purified protein was further purified via ion exchange chromatography (ThermoScientific, Poros 50HQ) and directly covalently labelled with BODIPY-FL-SpyCatcher S50C. Therefore, Flag-Spy-CRBN-His_6-Spy-DDB1ΔB was incubated overnight at 4 °C with BODIPY-FL labeled SpyCatcher S50C protein at a stoichiometric ratio. Afterwards, BODIPY-FL-$SpyCatcher_{S50C}$(Flag-Spy-CRBN-His_6-Spy-DDB1ΔB) was further purified on a Superdex 200 10/300 GL size exclusion column (Cytiva) in buffer containing 25 mM HEPES pH 7.5, 200 mM NaCl, and 1 mM TCEP. Labeling was monitored with absorption at 280 and 490 nm. The protein peak corresponding to the labeled BODIPY-FL-$SpyCatcher_{S50C}$(Flag-Spy-CRBN-His_6-Spy-DDB1ΔB) was pooled, concentrated using ultrafiltration (Millipore) and flash frozen in liquid nitrogen at 6.25 µM and stored at −80°C.

2.3 TR-FRET ternary complex formation

A mixture of 100 nM GST-HDAC6 (BPS Bioscience #50006), 100 nM BODIPY-FL-$SpyCatcher_{S50C}$(Flag-Spy-CRBN-His_6-Spy-DDB1ΔB) complex and 1 x anti-GST-Tb antibody (Revvity # 61GSTTLF) was prepared in assay buffer (HEPES 25 mM HEPES pH7.5 200 mM NaCl + 0.1% Pluronic F-68 solution (Sigma) + 1 mM TCEP) and 15 µL/well were added to a black 384 well plate (Corning # 4514). Compound **12** was irradiated with light at the wavelength of 390 nm or 505 nm for 5 minutes and in the following dispensed using a D300 Dispenser (Tecan). The DMSO content was normalized to 0,2% (v/v) and the plate was sealed with aluminum foil. Compound irradiation and dispensing were performed in a dark room. After 30 minutes incubation in the dark, the TR-FRET signals at 490 nm (terbium) and 520 nm (BODIPY) were recorded with a 60 µs delay over 400 µs and over 10 cycles (cycle time: 42 s) for each data point using a PHERAstar FSX microplate reader (BMG Labtech). The TR-FRET signals were

extracted by calculating the 520 nm / 490 nm ratio and averaged over the 10 cycles for each data point Data from two independent replicates (n=2) were plotted using GraphPad PRISM 10.

2.4 Photophysical Evaluation

2.4.1 Determination of photophysical properties

For UV-vis spectroscopy measurements on a Tecan Spark Multimode Microplate Reader (Tecan Trading AG, Switzerland), all samples were prepared under red light to avoid the formation of the *cis*-isomers. Stock solutions (10 mM in DMSO) were prepared in the dark and diluted to a final concentration of 25 µM for measurements. UV-vis spectra were recorded either after irradiation with a wavelength of 505 nm or 390 nm to obtain the distinct spectra of the *trans*- and the *cis*-isomers. Thermal relaxation was measured by preirradiating the PHOTACs with a wavelength of 390 nm and observing the absorption at 370 nm over the 12 h at 37 °C. The photochemical stability was measured by switching the irradiation of the PHOTACs for 5 min with either 390 nm or 505 nm with subsequent absorption measurements at 370 nm. The measured absorption was then normalized at the isosbestic point. The photostationary states of the *cis*- and *trans*-isomers were calculated by measuring the absorption at 370 nm after illumination with wavelengths of 390 nm and 505 nm in comparison to the absorption measured in the dark.

2.4.2 LED Illumination

For illumination of the cells, we used the cell disco system described by the Trauner & Thorn-Seshold and co-workers.[3] Light-emitting diodes (LEDs) 390 nm (VL390-5-15) and 505 nm (B5-433-B505) were purchased from Roithner Lasertechnik.

2.5 Chemistry

2.5.1 General Information

Chemicals were obtained from abcr GmbH (Karlsruhe, Germany), Acros Organics (Geel, Belgien), Carbolution Chemicals (Sankt Ingberg, Germany), Sigma-Aldrich (Steinheim, Germany,) TCI Chemicals (Eschborn, Germany) or VWR (Langenfeld, Germany) and used without further purification. Technical-grade solvents were distilled prior to use. For all HPLC purposes, acetonitrile in HPLC-grade quality (HiPerSolv CHROMANORM, VWR, Langenfeld, Germany) was used. Water was purified with a PURELAB flex® (ELGA VEOLIA, Celle, Germany). Thin-layer chromatography (TLC) was carried out on prefabricated plates (silica gel 60, F254, Merck). Components were visualized either by irradiation with ultraviolet light (254 nm or 366 nm) or by appropriate staining. Column chromatography was carried out on silica gel (60 Å, 40–60 µm, Acros Organics, Geel, Belgien). If no solvent is stated, an aqueous solution was prepared with demineralized water. Mixtures of two or more solvents are specified as “solvent A”/“solvent B”, 3/1, *v*/*v*; meaning that 100 mL of the respective mixture consists of 75 mL of “solvent A” and 25 mL of “solvent B”. The uncorrected melting points were determined using a Büchi

(Essen, Germany) Melting Point M-560 apparatus. Diastereomeric ratios were determined by ^{1}H NMR spectroscopy.

2.5.2 Nuclear Magnetic Resonance Spectroscopy (NMR)

Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded either on a Bruker AVANCE 500 MHz at a frequency of 500 MHz (^{1}H) and 126 MHz (^{13}C) or a Bruker AVANCE III HD 600 MHz at a frequency of 600 MHz (^{1}H) and 151 MHz (^{13}C). The chemical shifts are given in parts per million (ppm). As solvents, deuterated chloroform ($CDCl_3$), deuterated methanol (methanol-d_4) and deuterated dimethyl sulfoxide (DMSO-d_6) were used. The residual solvent signal ($CDCl_3$: ^{1}H NMR: 7.26 ppm, ^{13}C NMR: 77.1 ppm; DMSO-d_6: ^{1}H NMR: 2.50 ppm, ^{13}C NMR: 39.52 ppm; methanol-d_4: ^{1}H NMR: 3.31 ppm, 4.87 ppm, ^{13}C NMR: 49.00 ppm) was used for calibration. The multiplicity of each signal is reported as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m) or combinations thereof. Multiplicities and coupling constants are reported as measured and might disagree with the expected values.

2.5.3 Mass spectrometry (MS)

High-resolution electrospray-ionization mass spectra (HRMS-ESI) were acquired with a Bruker Daltonik GmbH micrOTOF coupled to a an LC Packings Ultimate HPLC system and controlled by micrOTOFControl3.4 and HyStar 3.2-LC/MS, with a Bruker Daltonik GmbH ESI-qTOF Impact II coupled to a Dionex UltiMateTM 3000 UHPLC system and controlled by micrOTOFControl 4.0 and HyStar 3.2-LC/MS or with a micrOTOF-Q mass spectrometer (Bruker, Bremen, Germany) with ESI-source coupled with an HPLC Dionex UltiMate 3000 (Thermo Scientific, Heysham, United Kingdom). Low-resolution electrospray-ionization mass spectra (LRMS-ESI) were acquired with an Advion expression® compact mass spectrometer (CMS) coupled with an automated TLC plate reader Plate Express® (Advion, Ithaca, NY, USA).

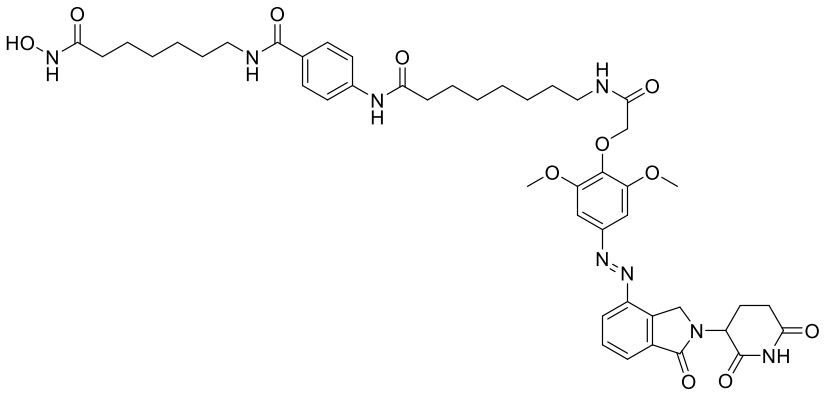
2.5.4 High Performance Liquid Chromatography (HPLC)

A Thermo Fisher Scientific (Heysham, United Kingdom) UltiMateTM 3000 UHPLC system with a Nucleodur 100-5 C18 (250 × 4.6 mm, Macherey Nagel, Düren, Germany) with a flow rate of 1 mL/min and a temperature of 25 °C, or a 100-5 C18 (100 × 3 mm, Macherey Nagel, Düren, Germany) with a flow rate of 0.5 mL/min and a temperature of 25 °C was used with an appropriate gradient. For preparative purposes, an AZURA Prep. 500/1000 gradient system with a Nucleodur 110-5 C18 HTec (150 × 32 mm, Macherey Nagel, Düren, Germany) column with 20 mL/min was used. Detection was implemented with UV absorption measurement at wavelengths of λ = 220 nm and λ = 250 nm. Bidest. H_2O (A) and MeCN (B) were used as eluents with an addition of 0.1% TFA in case of eluent A. Purity: The purity of all final compounds was 95% or higher. Purity was determined via HPLC with the Nucleodur 100-5 C18 (250 × 4.6 mm, Macherey Nagel, Düren, Germany) at 250 nm. After column equilibration for 5 min, a linear gradient from 5% A to 95% B in 7 min followed by an isocratic regime of 95% B for 10 min was used.

2.5.5 Synthesis and compound characterization

Compound **2**, **3**, **4**, **6**, **7**, **8**, **9**, and **10** were synthesized according to literature procedures.[4-6]

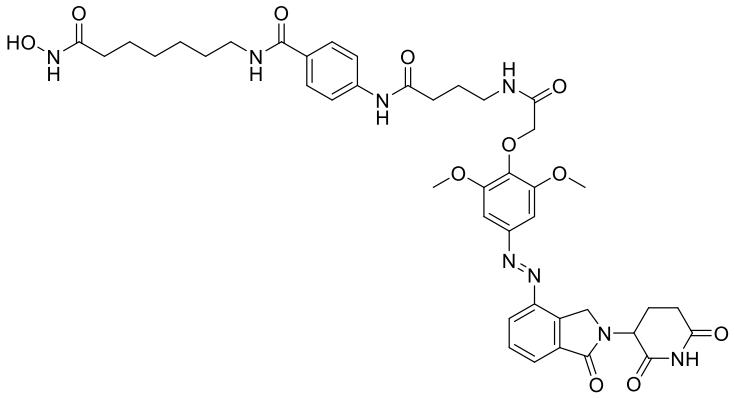
(*E*)-4-{8-[2-(4-{[2-(2,6-Dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl]diazenyl}-2,6-dimethoxyphenoxy)acetamido]octanamido}-*N*-[7-(hydroxyamino)-7-oxoheptyl]benzamide (11)



After swelling of the modified resin **9** (250 mg, 0.104 mmol/g, 0.260 mmol, 1.00 *eq.*) in DMF (5 mL) for 30 min, Fmoc-deprotection was performed by treatment with 20% piperidine in DMF (2 x 5 mL). Afterwards, the resin was washed with DMF (3 × 5 mL), dichloromethane (3 × 5 mL), and DMF (3 × 5 mL). For the subsequent amide coupling reaction, a solution of (*E*)-2-(4-{[2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl]diazenyl}-2,6-dimethoxyphenoxy)acetic acid (288 mg, 0.468 mmol, 1.80 *eq*), HATU (312 g, 0.780 mmol, 3.00 *eq.*), and DIPEA (226 µL, 1.30 mmol, 5.00 *eq.*) in DMF (5 mL) was agitated for 5 min and then added to the resin. The amide coupling was performed overnight at room temperature. The resin was then washed with DMF (3 × 5 mL) and dichloromethane (3 × 5 mL) and dried *in vacuo*. Cleavage of the resin was performed by dissolving the resin in a mixture of 5% trifluoroacetic acid in dichloromethane (8 mL) for 1 h. The filtrate was then collected and purified by preparative HPLC (acetonitrile/water (0.1% TFA): gradient 5−95%) to obtain the title compound as a an orange solid (35 mg, 15%). ^{1}H NMR (600 MHz, DMSO-d_6, δ [ppm]): The hydroxamic acid –OH signal was not detectable due to proton exchange, 11.01 (s, 1H), 10.31 (s, 1H), 10.03 (s, 1H), 8.26 (t, *J* = 5.6 Hz, 1H), 8.21 (d, *J* = 7.8 Hz, 1H), 7.95 – 7.88 (m, 2H), 7.81 – 7.75 (m, 3H), 7.66 – 7.61 (m, 2H), 7.35 (s, 2H), 5.15 (dd, *J* = 13.3, 5.1 Hz, 1H), 4.82 (d, *J* = 19.0 Hz, 1H), 4.70 (d, *J* = 19.0 Hz, 1H), 4.43 (s, 2H), 3.94 (s, 6H), 3.24 – 3.15 (m, 4H), 2.97 - 2.91 (m, 1H), 2.67 – 2.59 (m, 1H), 2.58 – 2.52 (m, 1H), 2.31 (t, *J* = 7.5 Hz, 2H), 2.10 – 2.03 (m, 1H), 1.93 (t, *J* = 7.4 Hz, 2H), 1.61 - 1.56 (m, 2H), 1.49 - 1.46 (m, 6H), 1.36 – 1.21 (m, 10H); ^{13}C NMR (151 MHz, DMSO-d_6, δ [ppm]): 172.9, 171.6, 171.0, 169.1, 167.9, 167.1, 165.5, 152.6, 148.3, 146.4, 141.7, 139.3, 134.5, 133.8, 129.6, 128.9, 128.6, 127.9, 125.5, 118.1, 100.6, 71.9, 56.3, 51.8, 48.3, 40.1, 38.2, 36.4, 32.2, 31.2, 29.1, 29.0, 28.6, 28.5, 28.3, 26.2, 26.2, 25.1, 24.9, 22.3; HRMS

m/z (ESI$^+$) [found: 885.4082, $C_{45}H_{57}N_8O_{11}^+$ requires $[M+H]^+$ 885.4069]; HPLC retention time 11.64 min, purity: 95.1%.

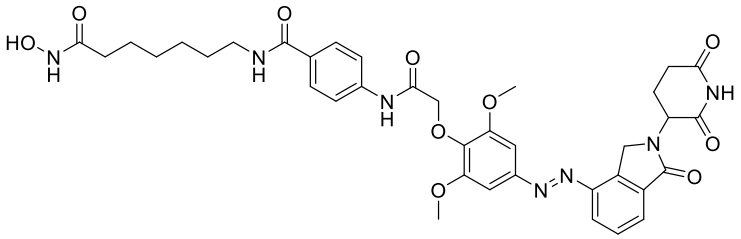
(*E*)-4-{4-[2-(4-{[2-(2,6-Dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl]diazenyl}-2,6-dimethoxyphenoxy)acetamido]butanamido}-*N*-[7-(hydroxyamino)-7-oxoheptyl]benzamide (12)



After swelling of the modified resin **10** (250 mg, 0.104 mmol/g, 0.260 mmol, 1.00 *eq.*) in DMF (5 mL) for 30 min, Fmoc-deprotection was performed by treatment with 20% piperidine in DMF (2 x 5 mL). Afterwards, the resin was washed with DMF (3 × 5 mL), dichloromethane (3 × 5 mL), and DMF (3 × 5 mL). For the subsequent amide coupling reaction, a solution of (*E*)-2-(4-{[2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl]diazenyl}-2,6-dimethoxyphenoxy)acetic acid (288 mg, 0.468 mmol, 1.80 *eq*), HATU (312 g, 0.780 mmol, 3.00 *eq.*), and DIPEA (226 µL, 1.30 mmol, 5.00 *eq.*) in DMF (5 mL) was agitated for 5 min and then added to the resin. The amide coupling was performed overnight at room temperature. The resin was then washed with DMF (3 × 5 mL) and dichloromethane (3 × 5 mL) and dried *in vacuo*. Cleavage of the resin was performed by dissolving the resin in a mixture of 5% trifluoroacetic acid in dichloromethane (8 mL) for 1 h. The filtrate was then collected and purified by preparative HPLC (acetonitrile/water (0.1% TFA): gradient 5−95%) to obtain the title compound as a an orange solid (20 mg, 9%). ^{1}H NMR (600 MHz, DMSO-d_6, δ [ppm]): The hydroxamic acid –OH signal was not detectable due to proton exchange, 11.01 (s, 1H), 10.31 (s, 1H), 10.10 (s, 1H), 8.26 (t, *J* = 5.6 Hz, 1H), 8.21 (d, *J* = 7.8 Hz, 1H), 8.02 (t, *J* = 5.9 Hz, 1H), 7.92 (d, *J* = 7.5 Hz, 1H), 7.80 (t, *J* = 7.7 Hz, 1H), 7.78 – 7.73 (m, 2H), 7.65 – 7.60 (m, 2H), 7.36 (s, 2H), 5.15 (dd, *J* = 13.3, 5.1 Hz, 1H), 4.81 (d, *J* = 19.0 Hz, 1H), 4.70 (d, *J* = 19.0 Hz, 1H), 4.43 (s, 2H), 3.95 (s, 6H), 3.26 (q, *J* = 6.6 Hz, 2H), 3.21 (q, *J* = 6.6 Hz, 2H), 2.97 - 2.91 (m, 1H), 2.67 – 2.59 (m, 1H), 2.59 – 2.50 (m, 1H), 2.38 (t, *J* = 7.5 Hz, 2H), 2.09 - 2.05 (m, 1H), 1.94 (t, *J* = 7.4 Hz, 2H), 1.84 - 1.80 (m, 2H), 1.51 - 1.46 (m, 4H), 1.31 - 1.23 (m, 4H); ^{13}C NMR (151 MHz, DMSO-d_6, δ [ppm]): 172.9, 171.1, 171.0, 169.1, 168.1, 167.1, 165.5, 152.6, 148.3, 146.4, 141.6, 139.3, 134.5, 133.8, 129.6, 129.0, 128.5, 127.9, 125.5, 118.1, 100.6, 71.9, 56.3, 51.8, 48.3, 40.4, 40.1, 37.8,

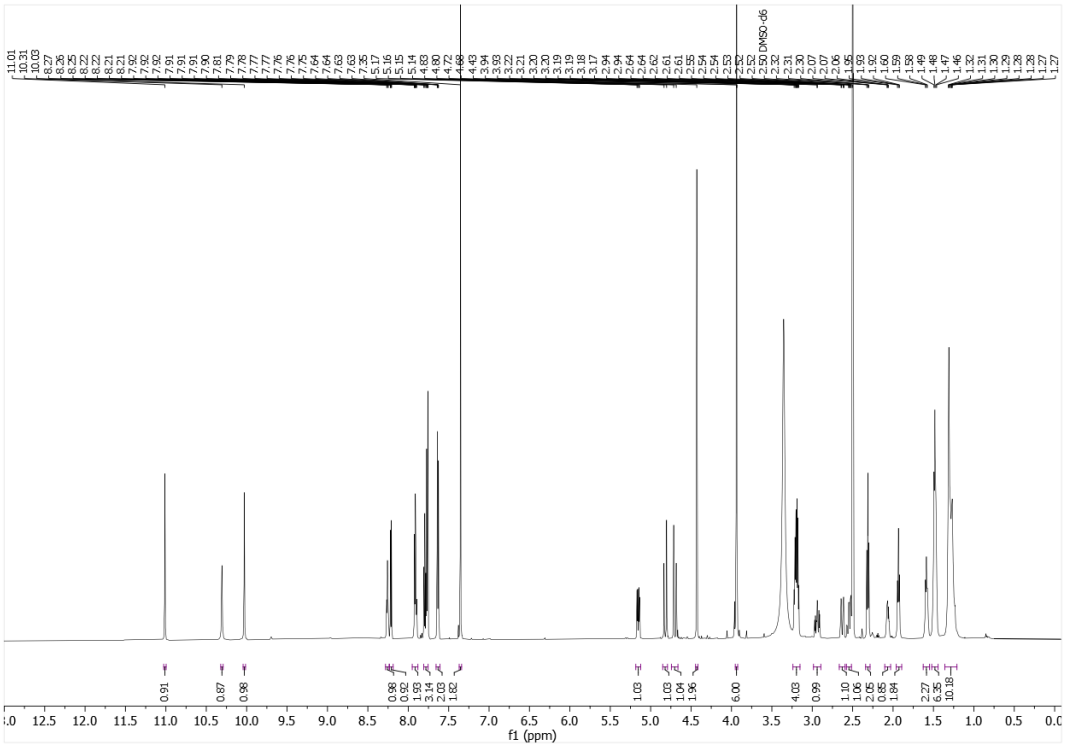
33.8, 32.2, 31.3, 29.1, 28.3, 26.2, 25.1, 22.3; HRMS m/z (ESI$^+$) [found: 829.3457, $C_{41}H_{49}N_8O_{11}^+$ requires [M+H]$^+$ 829.3443]; HPLC retention time 11.08 min, purity: 95.7%.

(*E*)-4-[2-(4-{[2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl]diazenyl}-2,6-dimethoxyphenoxy)acetamido]-*N*-[7-(hydroxyamino)-7-oxoheptyl]benzamide (13)

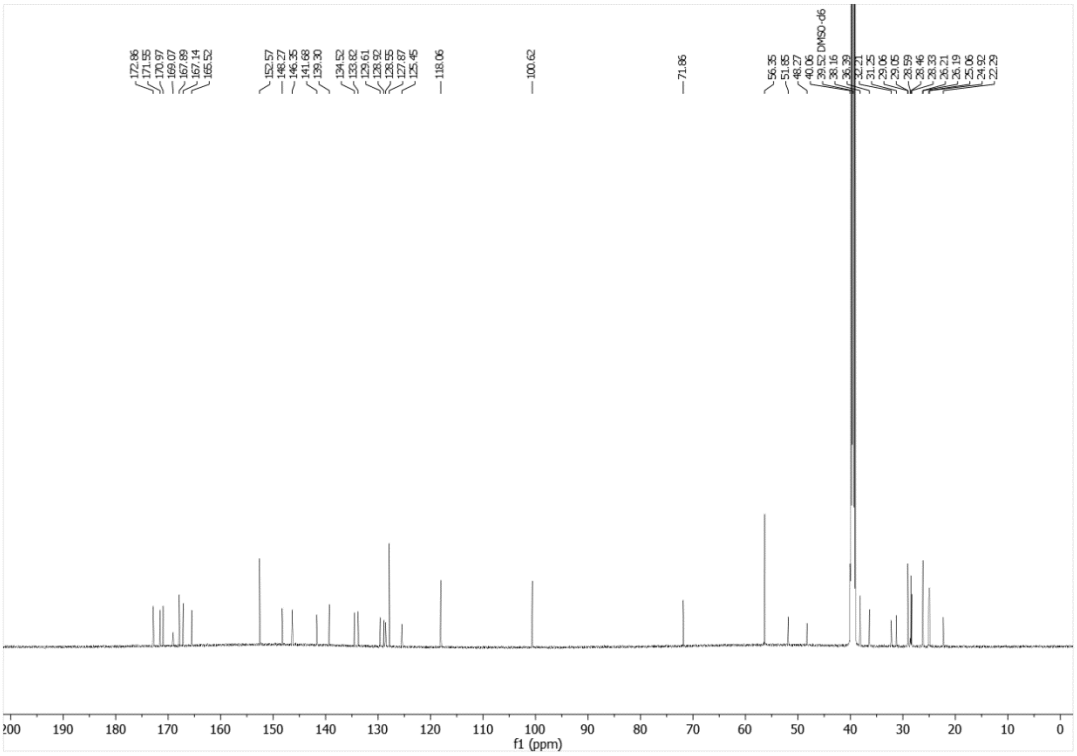


After swelling of the modified resin **8** (250 mg, 0.104 mmol/g, 0.260 mmol, 1.00 *eq.*) in DMF (5 mL) for 30 min, Fmoc-deprotection was performed by treatment with 20% piperidine in DMF (2 x 5 mL). Afterwards, the resin was washed with DMF (3 × 5 mL), dichloromethane (3 × 5 mL), and DMF (3 × 5 mL). For the subsequent amide coupling reaction, a solution of (*E*)-2-(4-{[2-(2,6-dioxopiperidin-3-yl)-1-oxoisoindolin-4-yl]diazenyl}-2,6-dimethoxyphenoxy)acetic acid (288 mg, 0.468 mmol, 1.80 *eq*), HATU (312 g, 0.780 mmol, 3.00 *eq.*), and DIPEA (226 µL, 1.30 mmol, 5.00 *eq.*) in DMF (5 mL) was agitated for 5 min and then added to the resin. The amide coupling was performed overnight at room temperature. The resin was then washed with DMF (3 × 5 mL) and dichloromethane (3 × 5 mL) and dried *in vacuo*. Cleavage of the resin was performed by dissolving the resin in a mixture of 5% trifluoroacetic acid in dichloromethane (8 mL) for 1 h. The filtrate was then collected and purified by preparative HPLC (acetonitrile/water (0.1% TFA): gradient 5−95%) to obtain the title compound as a an orange solid (30 mg, 16%). ^{1}H NMR (600 MHz, DMSO-d_6, δ [ppm]): The hydroxamic acid –OH and the phtalimide –NH signal were not detectable due to proton exchange, 11.01 (s, 1H), 10.32 (s, 1H), 10.04 (s, 1H), 8.34 (t, *J* = 5.6 Hz, 1H), 8.22 (d, *J* = 7.9 Hz, 1H), 7.92 (d, *J* = 7.5 Hz, 1H), 7.86 – 7.72 (m, 4H), 7.38 (s, 2H), 5.15 (dd, *J* = 13.3, 5.1 Hz, 1H), 4.82 (d, *J* = 19.0 Hz, 1H), 4.70 (d, *J* = 19.0 Hz, 1H), 4.67 (s, 2H), 3.96 (s, 6H), 3.23 (q, *J* = 6.7 Hz, 2H), 2.97 - 2.91 (m, 1H), 2.64 - 2.61 (m, 1H), 2.57 - 2.51 (m, 1H), 2.09 - 2,05 (m, 1H), 1.94 (t, *J* = 7.4 Hz, 2H), 1.51 - 1.48 (m, 4H), 1.30 - 1.26 (m, 4H); ^{13}C NMR (151 MHz, DMSO-d_6, δ [ppm]): 172.9, 171.0, 169.1, 167.3, 167.1, 165.5, 158.2, 158.0, 152.6, 148.3, 146.4, 140.4, 139.4, 134.5, 133.8, 129.9, 129.6, 128.6, 128.1, 125.5, 118.7, 100.7, 72.0, 56.5, 51.9, 48.3, 40.1, 32.2, 31.3, 29.1, 28.3, 26.2, 25.1, 22.3; HRMS m/z (ESI$^+$) [found: 744.2932, $C_{37}H_{42}N_7O_{10}^+$ requires [M+H]$^+$ 744.2915]; HPLC retention time 11.58 min, purity: 95.1%.

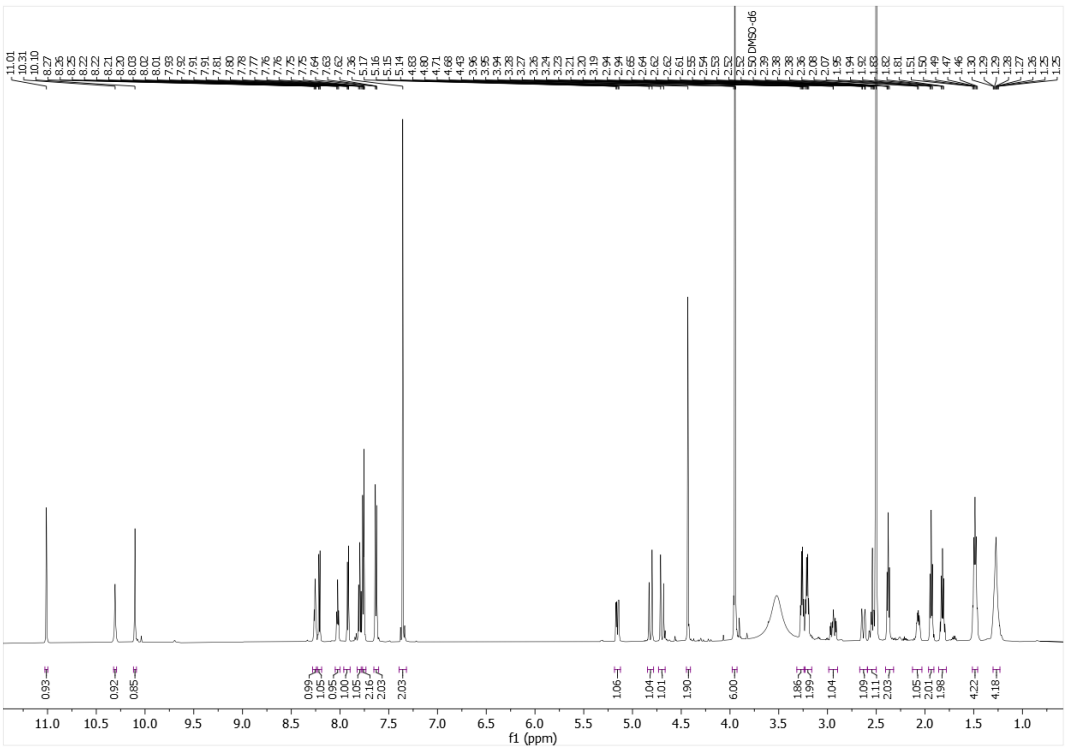
3. NMR spectra



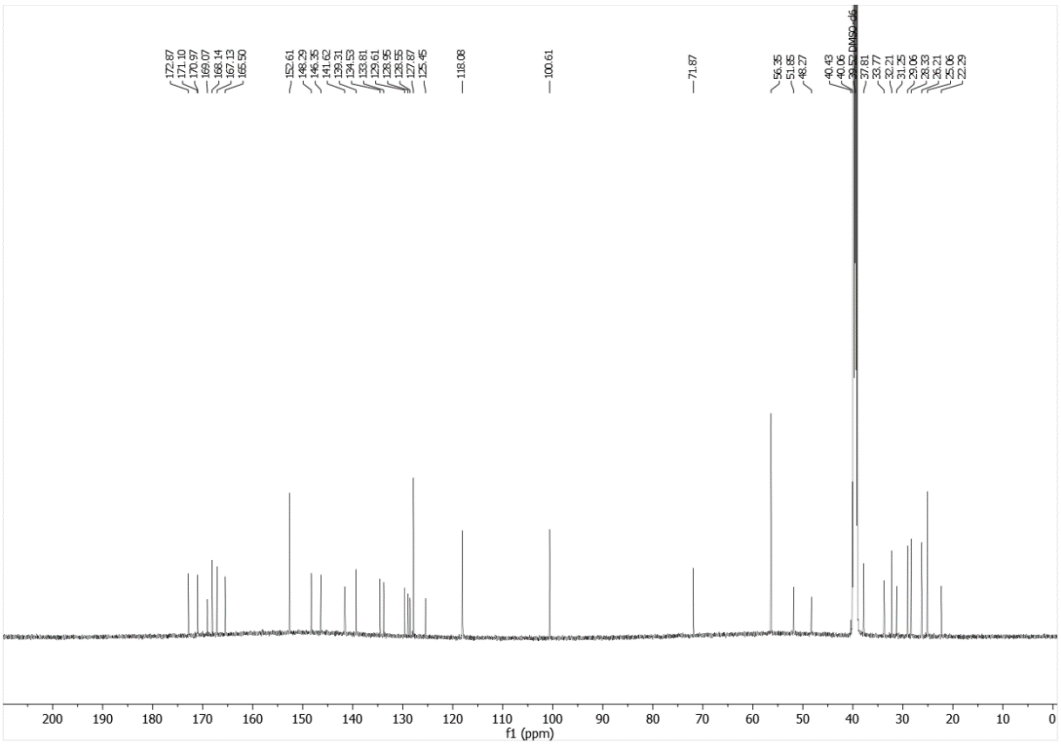
^{1}H-NMR spectrum of **11** (600 MHz, DMSO-d_6).



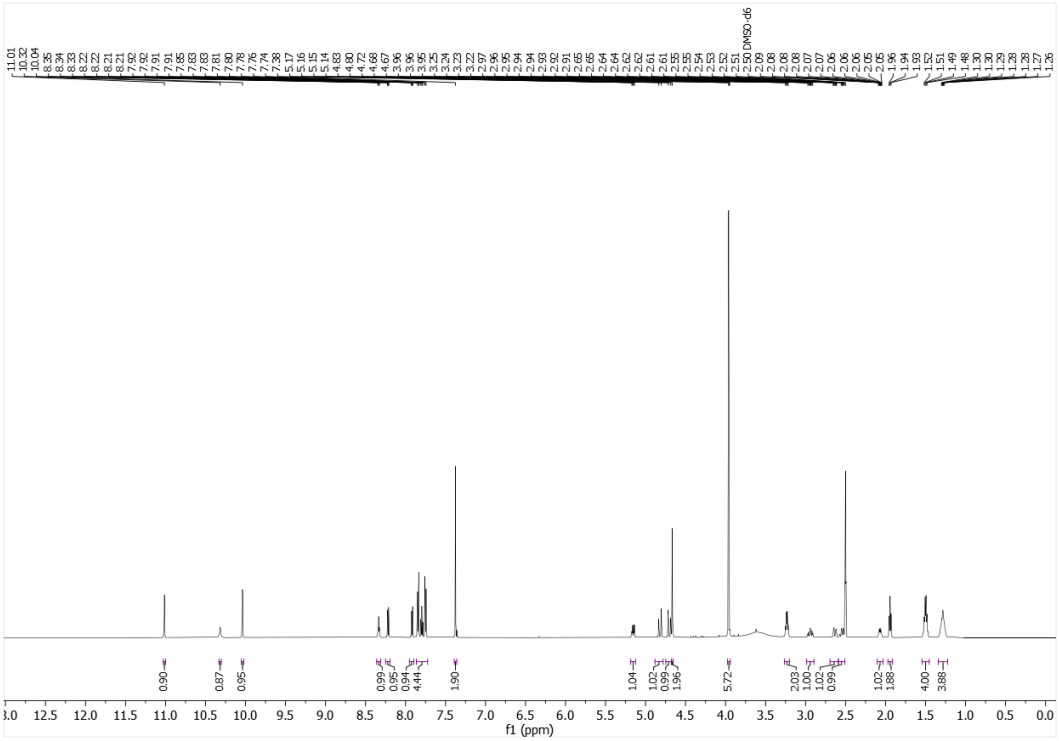
^{13}C-NMR spectrum of **11** (151 MHz, DMSO-d_6).



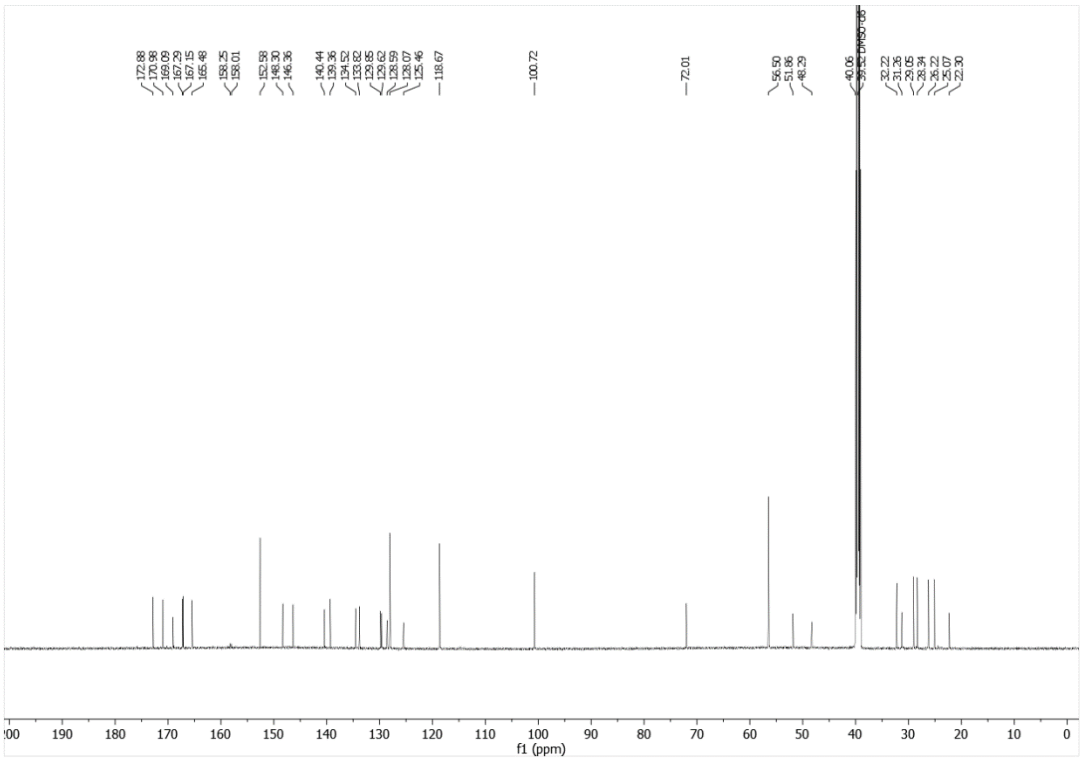
^{1}H-NMR spectrum of **12** (600 MHz, DMSO-d_6).



^{13}C-NMR spectrum of **12** (151 MHz, DMSO-d_6).

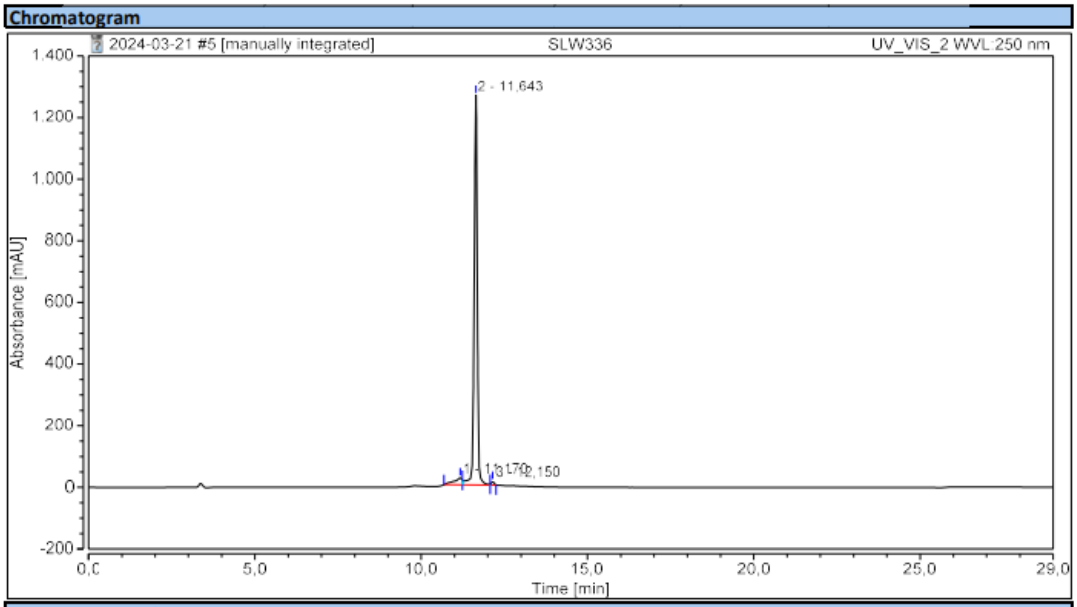


^{1}H-NMR spectrum of **13** (600 MHz, DMSO-d_6).



^{13}C-NMR spectrum of **13** (151 MHz, DMSO-d_6).

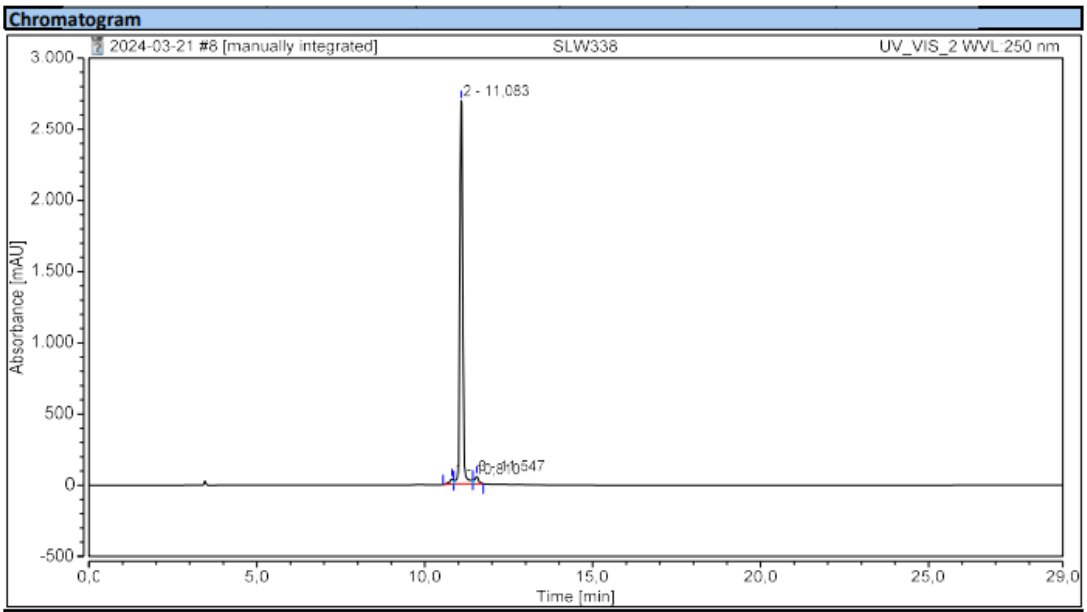
4. HPLC chromatograms



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount n.a.
1		11,170	6,018	22,169	4,20	1,71	n.a.
2		11,643	136,317	1264,185	95,08	97,41	n.a.
3		12,150	1,034	11,396	0,72	0,88	n.a.
Total:			**143,369**	**1297,750**	**100,00**	**100,00**	

HPLC chromatogram of **11**.

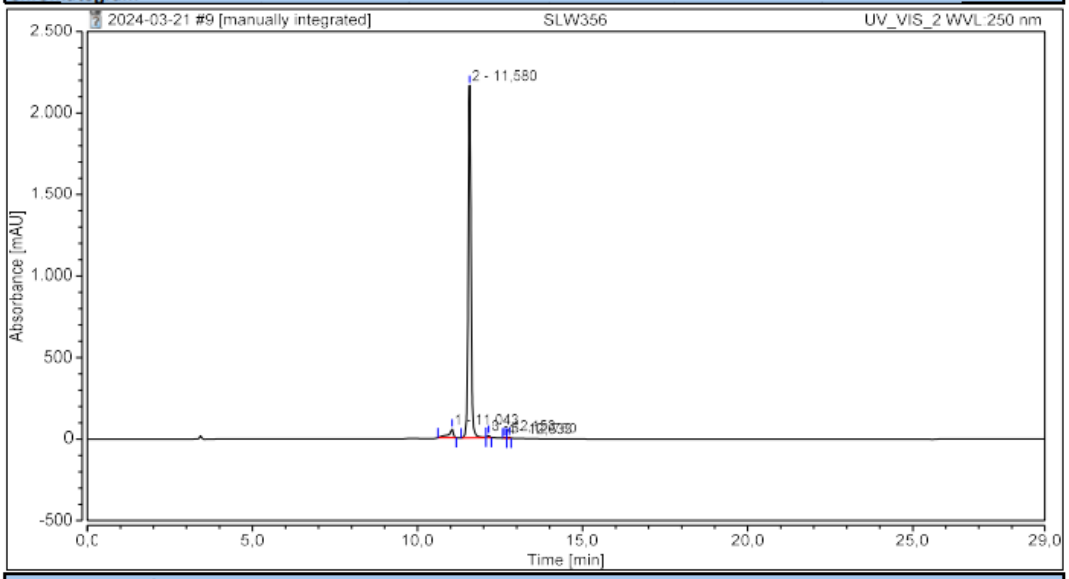


Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount n.a.
1		10,810	4,541	36,450	1,61	1,31	n.a.
2		11,083	270,169	2692,427	95,74	96,66	n.a.
3		11,547	7,471	56,647	2,65	2,03	n.a.
Total:			**282,181**	**2785,524**	**100,00**	**100,00**	

HPLC chromatogram of **12**.

Chromatogram



Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount n.a.
1		11,043	9,290	54,155	4,18	2,42	n.a.
2		11,580	211,426	2162,972	95,10	96,60	n.a.
3		12,153	1,149	14,077	0,52	0,63	n.a.
4		12,633	0,144	2,485	0,06	0,11	n.a.
5		12,780	0,310	5,386	0,14	0,24	n.a.
Total:			**222,318**	**2239,074**	**100,00**	**100,00**	

HPLC chromatogram of **13**.

5. References

1 R. P. Nowak, S. L. DeAngelo, D. Buckley, Z. He, K. A. Donovan, J. An, N. Safaee, M. P. Jedrychowski, C. M. Ponthier, M. Ishoey, T. Zhang, J. D. Mancias, N. S. Gray, J. E. Bradner and E. S. Fischer, Plasticity in binding confers selectivity in ligand-induced protein degradation, *Nat. Chem. Biol.*, 2018, **14**, 706–714.

2 E. S. Wang, A. L. Verano, R. P. Nowak, J. C. Yuan, K. A. Donovan, N. A. Eleuteri, H. Yue, K. H. Ngo, P. H. Lizotte, P. C. Gokhale, N. S. Gray and E. S. Fischer, Acute pharmacological degradation of Helios destabilizes regulatory T cells, *Nat. Chem. Biol.*, 2021, **17**, 711–717.

3 M. Borowiak, W. Nahaboo, M. Reynders, K. Nekolla, P. Jalinot, J. Hasserodt, M. Rehberg, M. Delattre, S. Zahler, A. Vollmar, D. Trauner and O. Thorn-Seshold, Photoswitchable Inhibitors of Microtubule Dynamics Optically Control Mitosis and Cell Death, *Cell*, 2015, **162**, 403–411.

4 M. Reynders, B. S. Matsuura, M. Bérouti, D. Simoneschi, A. Marzio, M. Pagano and D. Trauner, PHOTACs enable optical control of protein degradation, *Sci. Adv.*, 2020, **6**, eaay5064.

5 L. Sinatra, J. Yang, J. Schliehe-Diecks, N. Dienstbier, M. Vogt, P. Gebing, L. M. Bachmann, M. Sönnichsen, T. Lenz, K. Stühler, A. Schöler, A. Borkhardt, S. Bhatia and F. K. Hansen, Solid-Phase Synthesis of Cereblon-Recruiting Selective Histone Deacetylase 6 Degraders (HDAC6 PROTACs) with Antileukemic Activity, *J. Med. Chem.*, 2022, **65**, 16860–16878.

6 L. Sinatra, J. J. Bandolik, M. Roatsch, M. Sönnichsen, C. T. Schoeder, A. Hamacher, A. Schöler, A. Borkhardt, J. Meiler, S. Bhatia, M. U. Kassack and F. K. Hansen, Hydroxamic Acids Immobilized on Resins (HAIRs): Synthesis of Dual-Targeting HDAC Inhibitors and HDAC Degraders (PROTACs), *Angew. Chem. Int. Ed.*, 2020, **59**, 22494–22499.