Supplementary information

VHL-independent degradation of Hepatitis B Virus e antigen (HBeAg) by VHL-binding chimeric small molecules

Liam T. Hales[1‡], Simon J. Mountford[1], Mina Takawy[2], Danni Colledge[2], Belinda Maher[3,4], Jake Shortt[3,4,5], Philip E. Thompson[1], Sam A. Greenall[3] and Nadia Warner[2]

[1]Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, Monash University; Parkville, 3052, Australia.

[2]Victorian Infectious Diseases Reference Laboratory, Royal Melbourne Hospital at The Peter Doherty Institute for Infection and Immunity, Melbourne, 3000, Australia

[3]Blood Cancer Therapeutics Laboratory, Department of Medicine, School of Clinical Sciences at Monash Health, Monash University, Clayton, 3168, Australia

[4]Monash Haematology, Monash Health, Clayton, 3168, Australia

[5]Sir Peter MacCallum Department of Oncology, The University of Melbourne, Parkville, 3010, Australia

[‡]Current address: MRC Protein Phosphorylation and Ubiquitylation Unit, Sir James Black Centre, School of Life Sciences, University of Dundee, Dundee, DD1 5EH United Kingdom

Table of Contents

Supplementary figures and data

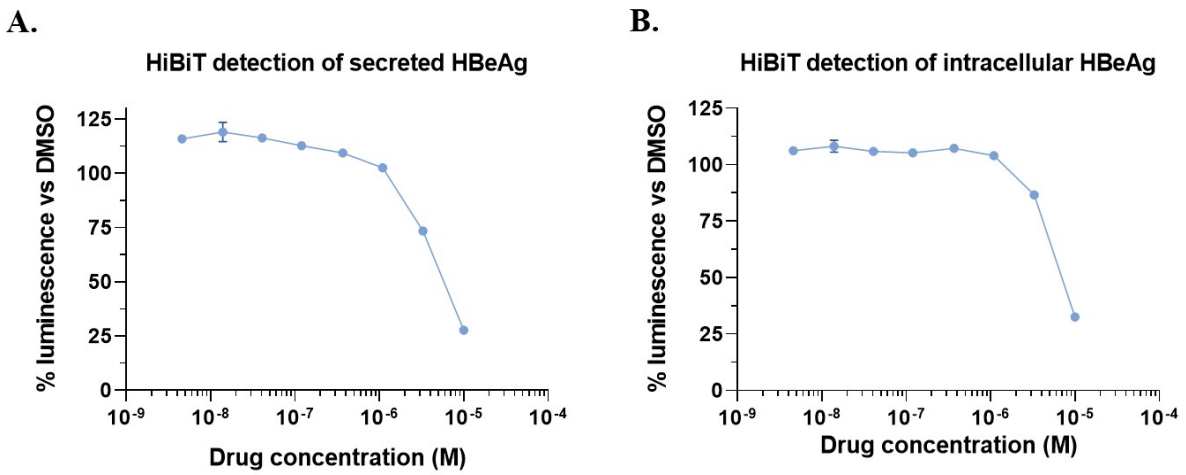


Figure S1 *HEK293T$^{HiBiT\text{-}HBeAg\text{-}S}$ cells were titrated with LH-1 for a 24 h treatment period.* ***A.*** *Luminescence related to the secreted levels of HiBiT-HBeAg was detected.* ***B.*** *The same treated cells were also assayed to determine the luminescence due to the intracellular levels of HiBiT-HBeAg.*

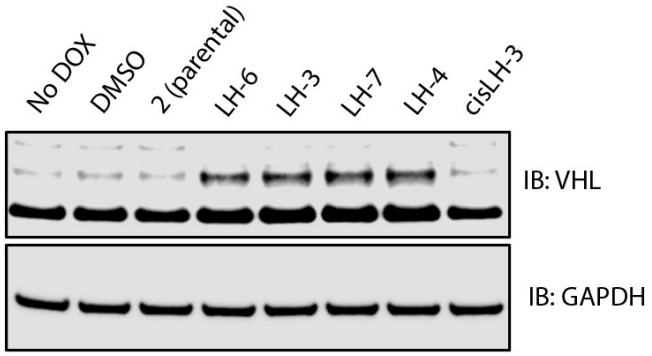


Figure S2: *HEK293T$^{HiBiT\text{-}HBeAg\text{-}S}$ cell lines were treated with compounds at 10 μM for 24 h then assessed via Western blot. All candidates containing trans-hydroxyproline within the VHL recruiting moiety stabilised VHL as previously reported.[1] The abrogated VHL binding of cisLH-3 was confirmed by a failure to stabilise VHL.*

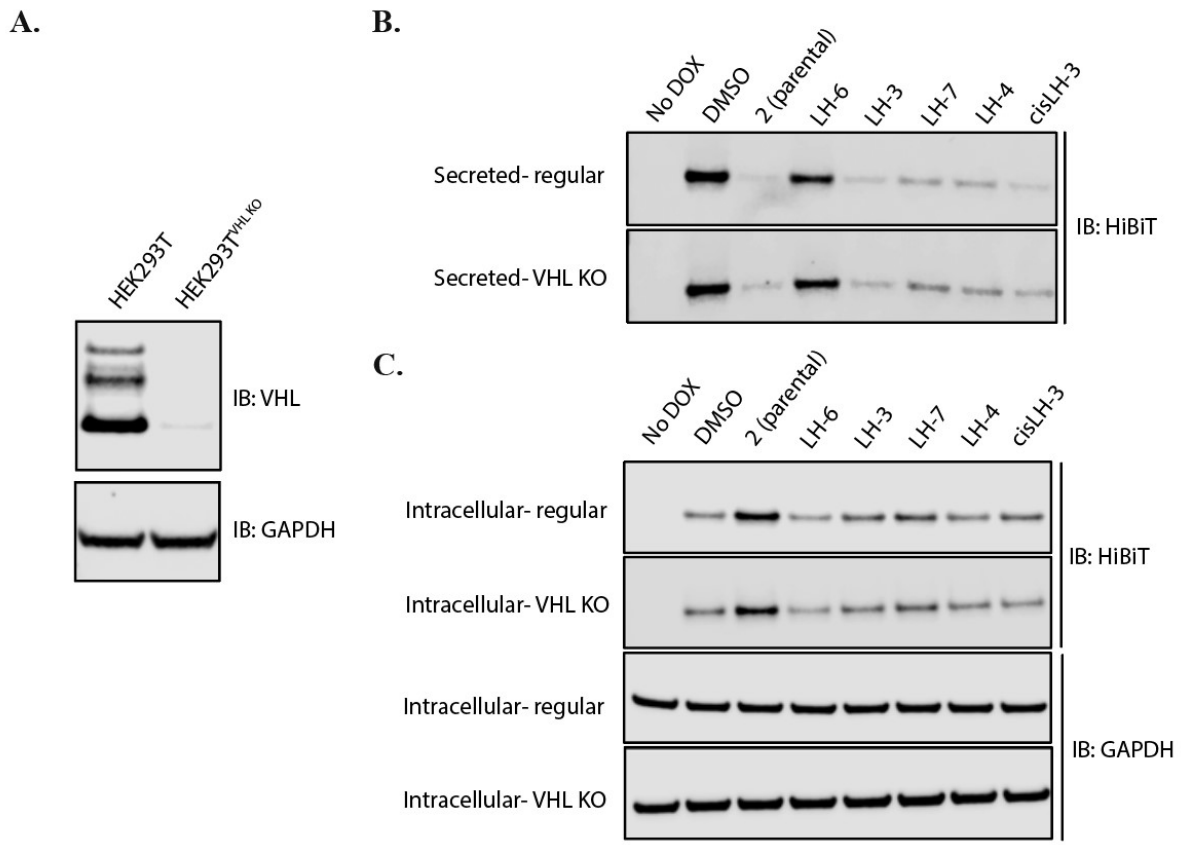


Figure S3 A. *HEK293T or HEK293T$^{VHL\ KO}$ were assessed via Western blot with immunoblotting for VHL, confirming near full knock-out of VHL.* ***B*** *and* ***C.*** *Regular or VHL KO HEK293T$^{HiBiT\text{-}HBeAg\text{-}S}$ cell lines were treated with the indicated compounds at 10 µM for 24 h. Secreted (B) and intracellular (C) HiBiT-HBeAg levels were assessed by immunoblotting the cell media and cell lysate respectively, using a HiBiT antibody.*

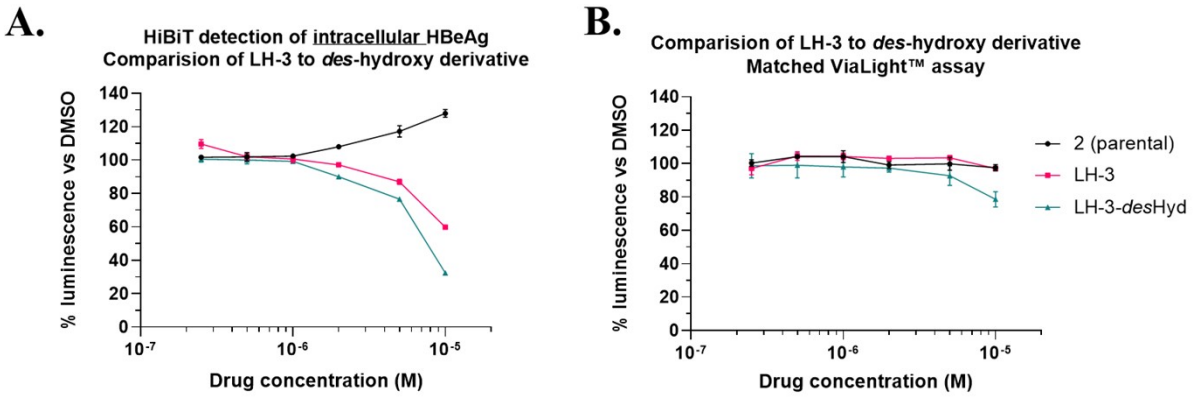


Figure S4 *HEK293T$^{HiBiT\text{-}HBeAg\text{-}S}$ cells were titrated with 2, LH-3 and LH-3-desHyd for 24 h.* ***A.*** *Luminescence related to the intracellular levels of HiBiT-HBeAg was detected.* ***B.*** *The same treated cells were also analysed with a ViaLight™ assay to determine cell viability. Cytotoxicity was evident at concentrations above 2 µM.*

Synthesis of HyT and truncated analogues

The chemistry utilised to prepare analogues of **LH-3** included both solution and solid phase approaches (**Figure S5**). Hydrophobic tagged analogues of **LH-3** were prepared in solution by addition of an aminohexanoic acid linker to a piperazinyl HAP intermediate. To generate **LH-3-fluor**, a terminally Fmoc protected linker was utilised. Alternatively, a terminally Boc protected linker was installed, then deprotected to facilitate addition of adamananeacetic acid or (-)-menthoxy acetic acid to generate **LH-3-adam** and **LH-3-menth,** respectively**.**

LH-3-T1 and **LH-3-*des*Hyd** were prepared using solid-phase methodology previously described by our group for the synthesis of BET-BRD targeting degraders.[2] Here, 4-(4-methylthiazoyl)benzylamine or benzylamine were anchored to backbone amide linker (BAL) resin, and the resultant secondary amines subject to a series of amide coupling/Fmoc deprotection cycles using the appropriate Fmoc protected amino acids. Introduction of the linker and HBeAg-recruiting motif completed the synthesis, yielding the desired compounds after a single purification step and no purification of intermediates. **LH-3-T2** lacked the benzylamine component, therefore was prepared on Rink amide resin by immobilsation of the *trans*-hydroxyproline carboxyl group. The simplest of the truncates **LH-3-T3** and **LH-3-T4** were assembled from intermediate **1** and the relevant piperzine-linker conjugates. **LH-3-Pom** was also prepared in solution phase via a series of amide couplings.
Full details can be found in the Synthethic chemistry section below.

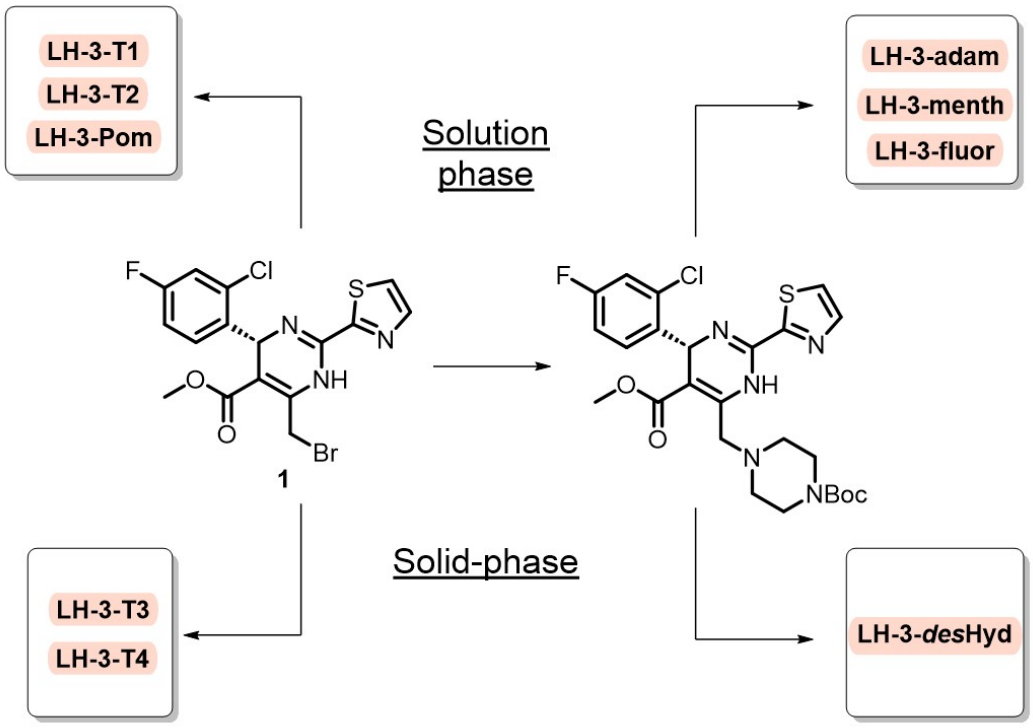


Figure S5 *Synthetic strategies for the preparation of LH-3 analogues.*

Synthetic chemistry

General chemistry

All solvents were purchased from standard suppliers, HAP starting material (methyl (*R*)-4-(2-chloro-4-fluorophenyl)-6-methyl-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate) was purchased from AstaTech (CAS: 1579251-94-7) and building blocks purchased from Combi-Blocks, Aaron Chemicals or Sigma-Aldrich unless otherwise indicated. All MSDSs were consulted before conducting each

experiment. All reactions were monitored by LC-MS and TLC (0.2 mm thick silica gel 60 GF_{254} plates). TLC plates were analysed by 254 nm UV light or by staining with either an aqueous solution of $KMnO_4$ (0.75% *w/w*)/K_2CO_3 (5% *w/w*)/10% NaOH (0.6% *v/v*) or a solution of ninhydrin (1.5% *w/v*)/AcOH (3% *v/v*) in *n*-BuOH, followed by heating. Preparative TLC was performed on Merck HPTLC plates (20 × 10 cm, silica gel 60 F_{254}) and following treatment with solvent the appropriate section of silica was removed and suspended in methanol before the removal of silica by filtration. Microwave reactions were performed in a CEM Discover/Explorer automated microwave synthesiser. DFPE resin was purchased from Sigma Aldrich (product no. 8.55035). Chloranil colourmetic analysis was performed by adding one drop each of 2% acetaldehyde in DMF and 2% chloranil in DMF to ~ 1 mg of resin, with blue beads indicating the presence of a secondary amine. The 2,4,6-trinitrobenzenesulphonic acid test was carried out by adding one drop each of 1% 2,4,6-trinitrobenzenesulphonic acid in DMF and 10% chloranil in DMF to ~1 mg of resin, with red beads indicating incomplete capping. Previously reported compounds are characterised by comparison to literature ^{1}H NMR and LC-MS data. Unreported compounds are characterised with ^{1}H NMR, ^{13}C NMR, ^{19}F NMR (if F is present in structure), LC-MS and HRMS. Compounds tested biologically are ≥ 95% pure by analytical HPLC analysis unless indicated otherwise.

NMR

^{1}H, ^{13}C and ^{19}F Nuclear Magnetic Resonance (NMR) spectra were obtained on a Bruker Advance III Nanobay 400 MHz spectrometer coupled to the BACS 60 automatic sample changer and obtained at 400.13 MHz, 100.62 MHz and 377.85 MHz respectively. All spectra were processed using MestReNova 11.0 software. The chemical shifts of spectra are reported in parts per million (ppm). The chemical shifts of all ^{1}H NMR were measured relative to the expected solvent peaks of the respective NMR solvents; $CDCl_3$, 7.26 ppm; CD_3OD, 3.31 ppm; DMSO-d6, 2.50 ppm. The data for all spectra are reported in the following format: chemical shift (multiplicity, coupling constant, integration). Multiplicity is defined as: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets, tt = triplet of triplets, ddd = doublet of doublet of doublets and m = multiplet. A broad resonance is denoted by the abbreviation b and the apparent splitting is denoted as the abbreviation app. Coupling constants are reported as *J* in Hertz (Hz). The chemical shifts of all ^{13}C NMR were measured relative to the expected solvent peaks of the respective NMR solvents; $CDCl_3$, 77.16 ppm; CD_3OD, 49.00 ppm; DMSO-*d*6, 39.52 ppm and multiplicity is assumed singlet unless indicated otherwise. The chemical shifts of all ^{19}F NMR were measured as reported following standard processing, unless CF_3COOH was present in the sample, in which case the CF_3COOH signal was referenced to -76.55 ppm.[1] Multiplicity is assumed to be a singlet unless indicated otherwise.

LC-MS

Method A: An Agilent Infinity II 6125B LC-MS SQ was used to perform reverse phase HPLC analysis. A Chromolith FastGradient RP-18e 50-2 column was used at a temperature of 35 °C. The sample injection volume was 2 µL, which was run in 0.1% formic acid in acetonitrile and 0.1% formic acid in water at a gradient of 5 – 100% over 3.8 min. Detection methods were 254 nm and 214 nm using a DAD detector. The low-resolution mass spectrum was acquired using electron spray ionization. Conditions: Quadrupole ion source with API-ES. The drying gas temperature was 350 °C. The capillary voltage in both positive and negative mode was 3000 V. The scan range was 100 –2000 *m/z* with a step size of 0.1 s over a 5 min acquisition time.

Method B: An Agilent UHPLC/MS (1260/6120) was coupled with a 1260 Infinity G1312B Binary pump, 1260 Infinity G1367E 1260 HiP ALS autosampler and 1290 Infinity G4212A1290 DAD detector. The liquid chromatography conditions were: Reverse phase HPLC analysis fitted with a Poroshell 120 EC-C18 3.0 × 50 mm 2.7-micron at a temperature of 35 °C. The sample injection volume was 1 µL, which was run in 0.1% formic acid in acetonitrile and 0.1% formic acid in water at a gradient of 5 – 100% over 3.8 min. Detection methods were either 254 nm or 214 nm. The low-resolution mass spectrum was performed using electron spray ionization. Conditions: Quadrupole ion source with API-ES. The drying gas temperature was 350 °C. The capillary voltage in both positive and negative mode was 3000 V. The scan range was 100 – 2000 *m/z* with a step size of 0.1 s over a 5 min acquisition time.

Method C (hydrophobic method): An Agilent Infinity II 6125B LC-MS SQ was used with a Chromolith FastGradient RP-18e 50-2 column was used at a temperature of 35 °C. The sample injection volume was 2 µL, which was run in 0.1% formic acid in acetonitrile and 0.1% formic acid in water at a gradient of 5 – 100% over 0.2 min, then isocratic at 100% for 2.8 min. Detection methods were 254 nm and 214 nm using a DAD detector. The low-resolution mass spectrum was performed using electron spray ionization. Conditions: Quadrupole ion source with API-ES. The drying gas temperature was 350 °C. The capillary voltage in both positive and negative mode was 3000 V. The scan range was 100 – 2000 *m/z* with a step size of 0.1 s over a 5 min acquisition time.

Analytical HPLC

Analytical HPLC was performed using two Agilent systems composed of the following:

Method A: a G7129C 1260 Vialsampler, a G7112B 1260 Bin pump, and a G7117C 1260 DAD HS. A ZORBAX Eclipse Plus C18 (100 × 4.6 mm, 3.5 µm) column was used. A flow rate of 1.0 mL/min was used with a gradient of 5 – 100% MeCN (0.1% formic acid) in H_2O (0.1% formic acid) over 9 mins, followed by 1 min at 5% MeCN (0.1% formic acid) in H_2O (0.1% formic acid).

Method B: a G1367E 1260 Autosampler, a G1312B 1260 Bin pump, and a G4212B 1260 DAD HS. A ZORBAX SB-C18 Rapid resolution (50 × 2.1 mm, 3.5 µm) column was used. A flow rate of 1.0 mL/min was used with a gradient of 5 – 100% MeCN (0.1% formic acid) in H_2O (0.1% formic acid) over 9 mins, followed by 1 min at 5% MeCN (0.1% formic acid) in H_2O (0.1% formic acid).

Data analysis and hardware operation was performed using Agilent OpenLab CDS Chemstation Edition Rev. C.01.10 [301]. Integration was performed manually and chromatograms compared against a blank. Purity at 254 nm was calculated using these integrations.

HRMS

HRMS analyses were performed using an Agilent 6224 or 6230 TOF LC/MS Mass Spectrometer coupled to an Agilent 1290 Infinity (Agilent, Palo Alto, CA). All data were acquired and reference mass corrected via a dual-spray electrospray ionization (ESI) source. Each scan or data point on the Total Ion Chromatogram (TIC) is an average of 13,700 transients, producing a spectrum every second. Mass spectra were created by averaging the scans across each peak and background subtracted against the first 10 seconds of the TIC. Acquisition was performed using the Agilent Mass Hunter Data Acquisition software version B.05.00 Build 5.0.5042.2 or version 10.14 Build 10.1.48 and analysis was performed using Mass Hunter Qualitative Analysis version B.05.00 Build 5.0.519.13.42 or version 10 Build 10.010305.0

Agilent 6224: Electrospray Ionisation; Drying gas flow: 11 L/min; Nebuliser: 45 psi; Drying gas temperature: 325 °C; Capillary Voltage (V_{cap}): 4000 V; Fragmentor: 160 V; Skimmer: 65 V; OCT RFV: 750 V; Scan range acquired: 100 – 1500 m/z; Internal Reference ions: Positive Ion Mode = m/z = 121.050873 & 922.009798.

Agilent 6230: Electrospray Ionisation; Drying gas flow: 10 L/min; Nebuliser: 20 psi; Drying gas temperature: 400 °C; Capillary Voltage (V_{cap}): 4000 V; Fragmentor: 260 V; Skimmer: 65 V; OCT RFV: 750 V; Scan range acquired: 100 – 1700 m/z; Internal Reference ions: Positive Ion Mode = *m/z* = 121.050873 & 922.009798.

Preparative HPLC purification

Preparative HPLC was performed on an Agilent system composed of a G7157A Prep Autosampler, a G7161A 1260 Prep Bin Pump, a G7114A 1260 VWD and a G7159B 1290 Prep FC. A Phenomex Luna Omega 5 µm Polar C18 (150 × 21.2 mm) column was used. Samples were prepared by dissolving the crude product in MeCN/H_2O (1 : 1). A gradient method was used composed of H_2O (0.1% TFA) and MeCN (0.1% TFA). The sample was injected in volumes between 100 and 900 µL. Data analysis and hardware operation was performed using Agilent OpenLab ChemStation Rev. LTS 01.11 [239]. Desired fractions were pooled and lyophilised to yield the product.

General methods

Solution phase methods:

General Method A- HATU mediated amide coupling: To a solution of amine in DMF (2 - 8 mL), the corresponding acid, HATU and DIPEA were added and the mixture was stirred at ambient temperature for 2 h. The reaction mixture was then diluted with water and extracted with EtOAc (4 × 10 - 50 mL). The combined organic layer was washed with brine (2 × 10 - 50 mL), dried over $MgSO_4$, filtered, and concentrated *in vacuo* to yield the crude product.

General method B- Substitution reaction: A solution of Boc-protected amine in DCM/TFA (1 : 1, 2 mL) was stirred at r.t for 30 min before removal of solvents under a stream of nitrogen. The residue was dissolved in DMF (0.5 - 2 mL) followed by the addition of desired amine, DIPEA, and KI (if indicated). The mixture was stirred at r.t before removal of solvents under a stream of nitrogen. A solution of 5% $NaHCO_3$ was added and the yellow precipitate collected by vacuum filtration. The crude product was purified as indicated.

Solid phase methods:

General method C- HATU or HCTU amide coupling: Amine containing resin was swollen in DCM for 30 min before filtration and resuspension in DMF. A solution of carboxylic acid (1 - 3 equiv.), HATU or HCTU (1 - 3 equiv.) and DIPEA (3 - 6 equiv.) in DMF was added and the mixture shaken at r.t for 1 h. The resin was collected by filtration and washed with DMF and DCM (5 × each). The 2,4,6-trinitrobenzenesulphonic acid test was used to determine if acylation was complete. If the reaction was incomplete, the procedure was repeated.

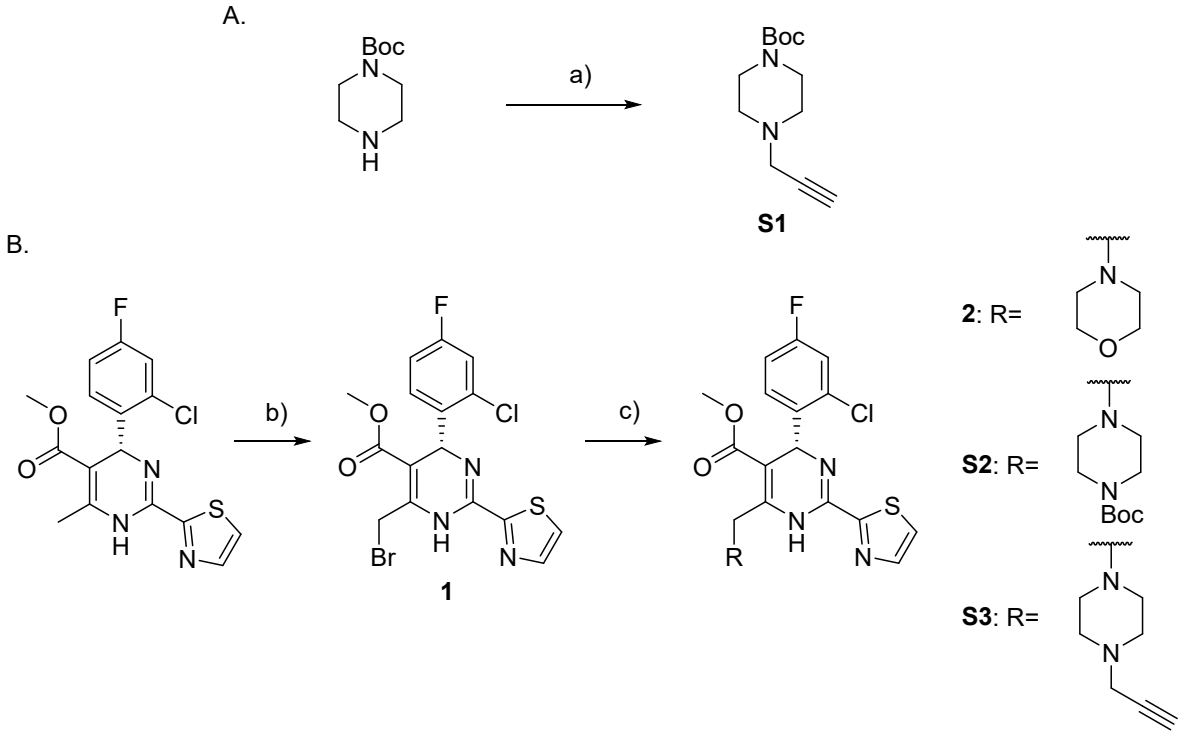
General method D- EDC.HCl/HOBt amide coupling: The resin was suspended in DCM/DMF (1 : 1) before the addition of EDC.HCl (2 equiv.), HOBt (2 equiv.) and DIPEA (6 equiv.). The resin was shaken at r.t for 5 min before the addition appropriate amine solution. The resin was shaken at r.t for 18 h, collected by filtration and washed with DMF and DCM (5 × each).

General method E- Fmoc deprotection: Fmoc containing resin was swollen in DCM for 30 min before filtration and resuspension in 20% piperidine in DMF and shaken at r.t. for 10 min before removal of the solvent by filtration. The process was repeated two further times before washing with DMF and DCM (5 × each).

General method F- analytical cleavage: A small amount of resin (~1 mg) was suspended in cleavage cocktail (0.5 mL) as indicated and shaken for 30 min. Solvents were blown off under nitrogen and the residue dissolved in MeCN. The resin was removed via filtration before analysis via LC-MS.

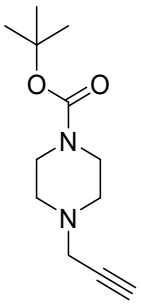
General method G- resin cleavage: Resin was thoroughly dried before being suspended in the indicated cleavage cocktail. The mixture was shaken at r.t for 1 h before filtration and collection of the filtrate. The process was repeated once. Combined filtrates were condensed under a stream of nitrogen and the product precipitated with diethyl ether (5 - 10 mL). The suspension was centrifuged and the supernatant carefully decanted to yield the crude product.

<u>Preparation of HAP HBV binders</u>



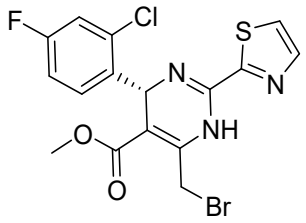
Scheme S1: A. Synthesis of modified piperazine- a) i) propargyl bromide, DIPEA, MeCN, r.t, 4 h, ii) 4 M HCl in dioxane, r.t, 2 h, 96% over 2 steps. B. Synthesis of HBc binders- b) N-bromosuccinimide, DCM, r.t, 1 h, 64%, c) aliphatic heterocycle, DMF or MeOH, 3 - 18 h, r.t, 40 - 74%.

S1: *tert*-butyl 4-(prop-2-yn-1-yl)piperazine-1-carboxylate[3]



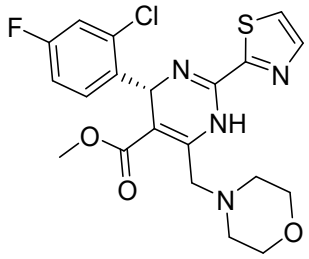
A solution of 1-Boc-piperazine (1.03 g, 5.55 mmol, 1.1 equiv.), propargyl bromide (80% in toluene, 562 µL, 5.04 mmol, 1 equiv.) and DIPEA (1.6 mL, 9.08 mmol, 1.8 equiv.) in MeCN (4 mL) was stirred at r.t for 4 h before the removal of solvents under a stream of nitrogen. The residue was suspended in water (30 mL) and extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated to yield a yellow oil (1.13 g, quant.) which was used without further purification. **^{1}H NMR ($CDCl_3$)** δ 3.48 (t, J = 5.1 Hz, 4H), 3.33 (d, J = 2.5 Hz, 2H), 2.53 (t, J = 5.1 Hz, 4H), 2.27 (t, J = 2.5 Hz, 1H), 1.46 (s, 9H). **LC-MS:** (Method A) t_R = 2.73 min, (ESI) *m/z* = 225.3, 100%). **TLC ($KMnO_4$ stain):** Petroleum ether : EtOAc (1 : 1), R_f = 0.46

1: Methyl (*R*)-6-(bromomethyl)-4-(2-chloro-4-fluorophenyl)-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate[4]



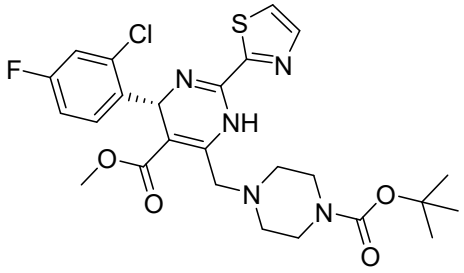
A solution of methyl (*R*)-4-(2-chloro-4-fluorophenyl)-6-methyl-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate (150 mg, 410 µmol, 1 equiv.) and NBS (80.3 mg, 451 µmol, 1.1 equiv.) in DCM (2 mL) was stirred at r.t for 1 h before the solvent was removed *in vacuo*. The crude product was purified via column chromatography (10 - 20% EtOAc/Petroleum Ether) to yield a yellow solid (116 mg, 64%) after trituration with petroleum ether. **^{1}H NMR ($CDCl_3$)** δ 8.27 (s, 0.3H, *rotamer*), 7.85 (d, *J* = 3.1 Hz, 1H), 7.57 – 7.44 (m, 1.7H, *rotamer*), 7.41 – 7.32 (m, 1H), 7.15 (dd, *J* = 8.3, 2.7 Hz, 1H), 7.01 – 6.88 (m, 1H), 6.18 (s, 0.3H, *rotamer*), 6.09 (d, *J* = 2.2 Hz, 0.7H, *rotamer*), 4.93 (d, *J* = 8.2 Hz, 1H), 4.75 (d, *J* = 11.4 Hz, 0.3H, *rotamer*), 4.58 (d, *J* = 8.5 Hz, 0.7H *rotamer*), 3.66 (s, 3H). **LC-MS:** (Method A) t_R = 3.97 min, (ESI) *m/z* = 443.9 and 445.9 ([M+H]$^+$, 100%). **TLC:** Petroleum ether : EtOAc (4 : 1), R_f = 0.34

2: Methyl (*R*)-4-(2-chloro-4-fluorophenyl)-6-(morpholinomethyl)-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate[5]



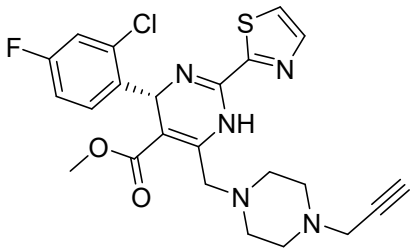
A solution of **H-2** (20 mg, 45 µmol, 1 equiv.) and morpholine (16 mg, 180 µmol, 4 equiv.) in anhydrous MeOH (2 mL) was stirred at r.t for 3 h. Solvents were removed *in vacuo* and the residue was dissolved in EtOAc (20 mL) and washed with water, sat. $NaHCO_3$ and brine (15 mL each). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*, yielding a yellow oil. The crude product was purified by flash column chromatography (0 - 2.5% MeOH/DCM) to yield a yellow solid (8 mg, 40%) after trituration with diethyl ether. **^{1}H NMR (CD_3OD)** δ 9.70 (s, 1H), 7.85 (d, *J* = 3.2 Hz, 1H), 7.44 (d, *J* = 3.1 Hz, 1H), 7.31 – 7.23 (m, 1H), 7.13 (dd, *J* = 8.7, 2.6 Hz, 1H), 6.90 (t, *J* = 8.2 Hz, 1H), 6.19 (s, 1H), 4.01 (d, *J* = 17.3 Hz, 1H), 3.90 – 3.78 (m, 5H), 3.60 (s, 3H), 2.62 (s, 4H). **^{13}C NMR (CD_3OD)** δ 167.7, 163.2, 163.1 (d, J_{CF}= 248.8 Hz), 149.3, 144.7, 139.2 (d, J_{CF}= 3.1 Hz), 134.8 (d, J_{CF}=10.7 Hz), 132.2 (d, J_{CF}= 8.8 Hz), 125.2, 117.8 (d, J_{CF}= 25.1 Hz), 115.6 (d, J_{CF}= 21.2 Hz), 68.0, 57.8, 55.8, 54.8, 51.7, 43.8. **^{19}F NMR (CD_3OD)** δ -114.67 **LC-MS:** (Method A) t_R = 3.25 min, (ESI) *m/z* = 451.1 ([M+H]$^+$, 100%). **HPLC:** (Method A) t_R = 4.54 min, 98% pure (254 nm). **HRMS:** (ESI+) calc'd for [$C_{20}H_{20}N_4O_3SClF$ + H]$^+$ 451.1001, found 451.1017. **TLC:** DCM : MeOH (39 : 1), R_f = 0.32

S2: Methyl (*R*)-6-((4-(tert-butoxycarbonyl)piperazin-1-yl)methyl)-4-(2-chloro-4-fluorophenyl)-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate[6]



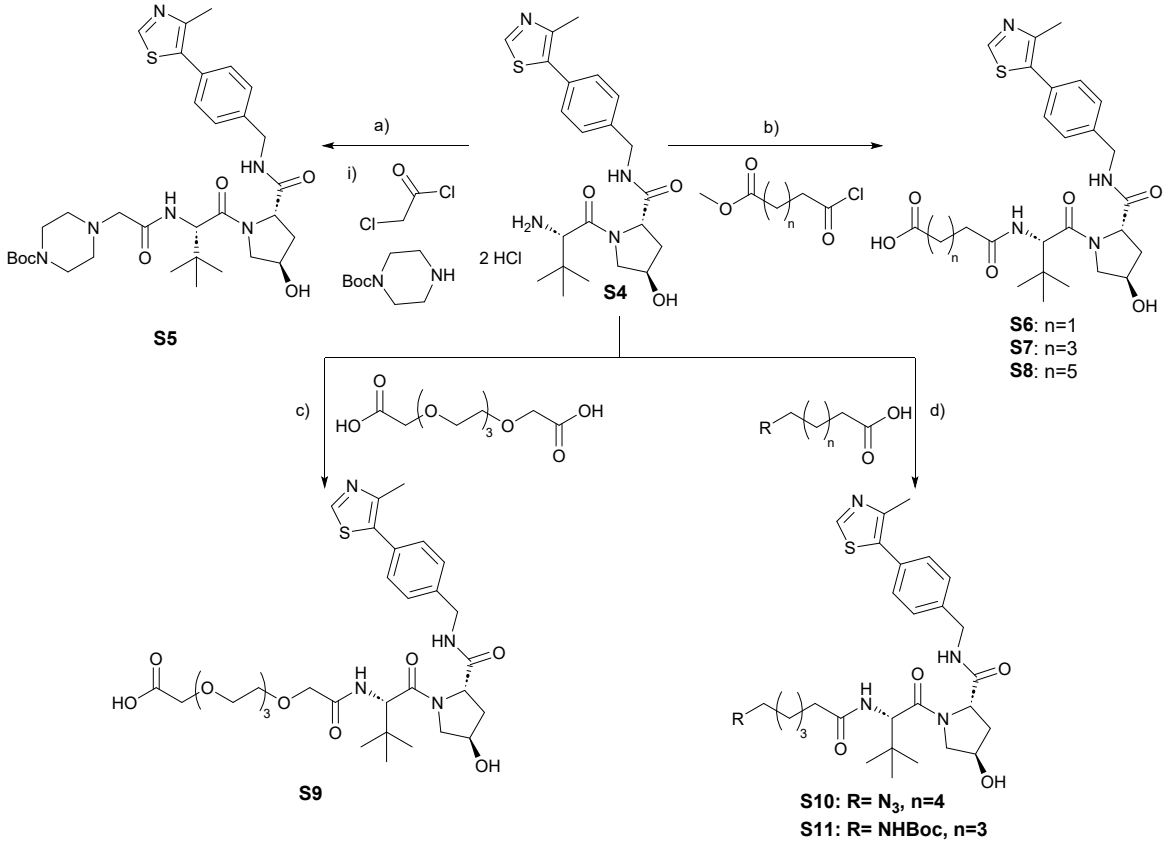
A solution of **1** (72 mg, 162 µmol, 1 equiv.) was treated according to General method B using 1-Boc-piperazine (151 mg, 810 µmol, 5 equiv.) and DIPEA ((21 mg, 162 µmol, 1 equiv.) for 18 h and purified via column chromatography (20 - 50% EtOAc/Petroleum Ether) to yield a yellow solid (66 mg, 74%) after trituration with petroleum ether. **^{1}H NMR ($CDCl_3$)** δ 9.68 (s, 1H), 7.83 (d, *J* = 3.1 Hz, 1H), 7.43 (d, *J* = 3.1 Hz, 1H), 7.31 – 7.22 (m, 1H), 7.12 (dd, *J* = 8.6, 2.6 Hz, 1H), 6.90 (td, *J* = 8.4, 2.6 Hz, 1H), 6.19 (s, 1H), 4.03 (d, *J* = 17.4 Hz, 1H), 3.85 (d, *J* = 17.4 Hz, 1H), 3.59 (s, 3H), 3.59 – 3.51 (m, 4H), 2.62 – 2.47 (m, 4H), 1.47 (s, 9H). **HPLC:** (Method B) t_R = 5.15 min, 99% pure (254 nm). **HRMS:** (ESI+) calc'd for $[C_{23}H_{23}ClFN_5O_2S + H]^+$ 488.1318, found 488.1330. **LC-MS:** (Method A) t_R = 3.51 min, (ESI) *m/z* = 550.2 ($[M+H]^+$, 100%). **TLC:** Petroleum ether : EtOAc (4 : 1), R_f = 0.15

S3: Methyl (*R*)-4-(2-chloro-4-fluorophenyl)-6-((4-(prop-2-yn-1-yl)piperazin-1-yl)methyl)-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate



A solution of **H-1** (67 mg, 297 µmol, 2.2 equiv.) was treated according to General method B using **S1** (60 mg, 135 µmol, 1 equiv.) and DIPEA (35 mg, 47 µmol, 4 equiv.) for 18 h and purified via column chromatography (20 - 50% EtOAc/Petroleum Ether) to yield a yellow solid (39 mg, 48%). after trituration with petroleum ether. **^{1}H NMR ($CDCl_3$)** δ 9.68 (s, 1H), 7.83 (d, *J* = 3.1 Hz, 1H), 7.43 (d, *J* = 3.1 Hz, 1H), 7.31 – 7.22 (m, 1H), 7.12 (dd, *J* = 8.6, 2.6 Hz, 1H), 6.90 (td, *J* = 8.4, 2.6 Hz, 1H), 6.19 (s, 1H), 4.03 (d, *J* = 17.4 Hz, 1H), 3.85 (d, *J* = 17.4 Hz, 1H), 3.59 (s, 3H), 3.59 – 3.51 (m, 4H), 2.62 – 2.47 (m, 4H), 1.47 (s, 9H). **^{13}C NMR ($CDCl_3$)** δ 166.8, 163.0, 161.6 (d, J_{CF}= 249.0 Hz), 146.7, 143.3, 130.7 (d, J_{CF}= 8.9 Hz), 123.1, 117.2 (d, J_{CF}= 24.6 Hz), 114.4 (d, J_{CF}= 21.1 Hz), 73.5, 56.5, 53.3, 52.5, 52.1, 51.3, 46.9, 29.4, 26.6, 17.7. One quaternary aromatic and two quaternary alkyl signals not observed. **^{19}F NMR ($CDCl_3$)** δ -114.49. **LC-MS:** (Method A) t_R = 3.65 min. (ESI) *m/z* = 488.1 ($[M+H]^+$, 100%). **HPLC:** (Method B) t_R =4.37 min, 95% pure (254 nm). **HRMS:** (ESI+) calc'd for $[C_{25}H_{29}ClFN_5O_4S + H]^+$ 550.1686, found 550.1705. **TLC:** Petroleum ether : EtOAc (1 : 1), R_f = 0.15

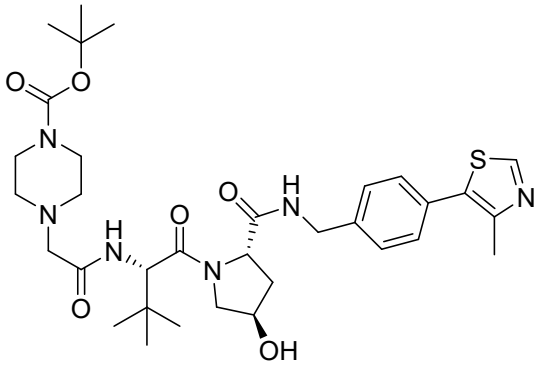
Preparation of VHL recruiter-linker conjugates



Scheme S2: Conjugation of linkers to VHL recruiter- a i) DIPEA, DCM, 0 °C - r.t, 2 h, ii) TEA, KI, DMF, 60 °C, 18 h, 26% (2 steps), b) i) TEA, dioxane, 80 °C, 20 h, 51 - 100%, ii) LiOH.H2O, 0 °C - r.t, 20 h, 58 - 85%, c) HATU, DIPEA, DMF, r.t, 2 h, 18%, d) HATU, DIPEA, DMF, r.t, 3 h, 80-81%.

VH032-amine (**S4**) was prepared according to previously reported procedures and spectroscopic characterisation consistent with literature vales.[7, 8]

S5: *tert*-Butyl 4-(2-(((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-2-oxoethyl)piperazine-1-carboxylate

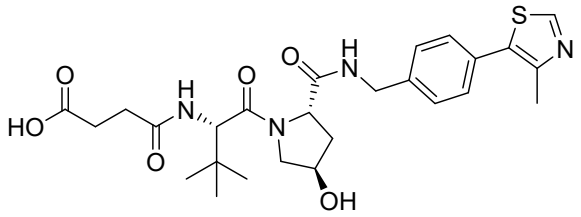


A solution of **S4** (71 mg, 141 µmol, 1 equiv.) and anhydrous DIPEA (73 mg, 564 µmol, 4 equiv.) in anhydrous DCM (2 mL) was cooled to 0 °C before the addition of chloroacetyl chloride (18 mg, 155 µmol, 1.1 equiv.). The mixture was allowed to warm to r.t and stirred for 2 h before dilution with DCM

(15 mL). The solution was washed with sat. $NaHCO_3$ and brine (10 mL each) then dried over $MgSO_4$ and concentrated *in vacuo*, yielding a yellow oil.

The crude intermediate was dissolved in DMF (2 mL) followed by the addition of 1-Boc-piperazine (131 mg, 705 µmol, 5 equiv.), triethylamine (43 mg, 423 µmol, 3 equiv.) and KI (12 mg, 71 µmol, 0.5 equiv.). The mixture was stirred at 60 °C for 18 h before removal of solvents under a stream of nitrogen. The residue was dissolved in 19% NH_4Cl (30 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with sat. $NaHCO_3$ and brine (20 mL each) then dried over $MgSO_4$ and concentrated *in vacuo*, yielding a brown oil. The crude product was purified via column chromatography (0 - 5% MeOH/DCM) to yield an off-white solid (26 mg, 28% over two steps). **^{1}H NMR ($CDCl_3$)** δ 8.67 (s, 1H), 7.80 (d, J = 8.4 Hz, 1H), 7.41 – 7.29 (m, 5H), 4.73 (t, J = 7.9 Hz, 1H), 4.57 – 4.48 (m, 2H), 4.41 (d, J = 8.5 Hz, 1H), 4.34 (dd, J = 14.9, 5.3 Hz, 1H), 4.09 (d, J = 11.4 Hz, 1H), 3.60 (dd, J = 11.3, 3.7 Hz, 1H), 3.49 – 3.42 (m, 4H), 3.01 (s, 2H), 2.57 – 2.53 (m, 1H), 2.51 (s, 3H), 2.50 – 2.45 (m, 4H), 2.11 (dd, J = 13.5, 8.2 Hz, 1H), 1.44 (s, 9H), 0.93 (s, 9H). **LC-MS:** (Method A) t_R = 3.22 min, (ESI) m/z = 657.3 ($[M+H]^+$, 100%). **TLC:** DCM : MeOH (95 : 5), R_f = 0.29

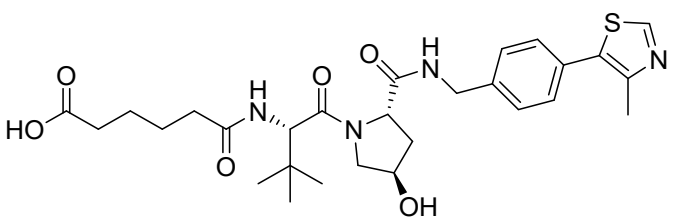
S6: 4-(((*S*)-1-((2*S*,4*R*)-4-Hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-4-oxobutanoic acid[9]



A solution of **S-4** (80 mg, 160 µmol, 1 equiv.) in anhydrous dioxane (2 mL) was treated with triethylamine (63 mg, 640 µmol, 4 equiv.) and methyl-4-chloro-4-oxobutanotate (50 mg, 320 µmol, 2 equiv.) and stirred for at 80 °C for 20 h. Solvents were removed *in vacuo* and the residue dissolved in DCM (20 mL), washed with water (15 mL) and brine (15 mL), dried over $MgSO_4$ and concentrated, yielding a brown oil. The crude product was purified by column chromatography (2 - 5% MeOH/DCM) to yield a white solid (88 mg, quant.).

The purified product (88 mg, 160 µmol, 1 equiv.) was dissolved in MeOH/H_2O (2 : 1, 3 mL) and cooled to 0 °C before the addition of $LiOH.H_2O$ (20 mg, 320 µmol, 2 equiv.). The mixture was allowed to warm to r.t and stirred for 20 h before solvents were removed *in vacuo*. The remaining residue was dissolved in water (30 mL) and acidified to pH 3 with 1 M HCl before extraction with EtOAc (3 × 30 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated, yielding a white solid (50 mg, 58%, 58% over two steps). **^{1}H NMR (CD_3OD)** δ 8.88 (s, 1H), 8.66 (t, J = 6.0 Hz, 1H), 7.93 (d, J = 8.8 Hz, 1H), 7.50 – 7.37 (m, 4H), 4.64 – 4.47 (m, 4H), 4.37 (dd, J = 15.5, 5.1 Hz, 1H), 3.89 (d, J = 11.1 Hz, 1H), 3.80 (dd, J = 11.0, 3.9 Hz, 1H), 2.57 (tdd, J = 16.6, 12.7, 4.5 Hz, 4H), 2.47 (s, 3H), 2.23 (dd, J = 13.3, 7.7 Hz, 1H), 2.08 (ddd, J = 13.2, 9.0, 4.4 Hz, 1H), 1.04 (s, 9H). **LC-MS:** (Method A) t_R = 3.09 min, (ESI) m/z = 529.2 ($[M-H]^-$, 100%), m/z = 554.2 ($[M+Na]^+$, 20%). **TLC:** DCM : MeOH (9 : 1), R_f = 0.15

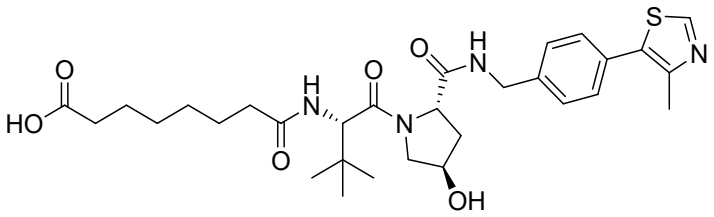
S7: 6-(((*S*)-1-((2*S*,4*R*)-4-Hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-6-oxohexanoic acid[9]



A solution of **S-4** (101 mg, 201 µmol, 1 equiv.) in anhydrous dioxane (2 mL) was treated with triethylamine (72 mg, 720 µmol, 4 equiv.) and methyl-6-chloro-6-oxohexanoate (49 mg, 262 µmol, 1.3 equiv.) and stirred for 80 °C for 20 h. Solvents were removed *in vacuo* and the residue dissolved in DCM (20 mL), washed with water (15 mL) and brine (15 mL), dried over $MgSO_4$ and concentrated, yielding a brown oil. The crude product was purified by column chromatography (2 - 5% MeOH/DCM) to yield a white solid (63 mg, 55%.).

The purified product (63 mg, 110 µmol, 1 equiv.) was dissolved in MeOH/H_2O (2 : 1, 3 mL) and cooled to 0 °C before the addition of LiOH.H_2O (9 mg, 220 µmol, 2 equiv.). The mixture was allowed to warm to r.t and stirred for 20 h before solvents were removed *in vacuo*. The remaining residue was dissolved in water (30 mL) and acidified to pH 3 with 1 M HCl before extraction with EtOAc (3 × 30 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated, yielding a white solid (48 mg, 79%, 43% over two steps). **^{1}H NMR (CD_3OD)** δ 8.87 (s, 1H), 8.65 (bt, J = 6.0 Hz, 1H), 7.86 (d, J = 8.9 Hz, 1H), 7.49 – 7.38 (m, 4H), 4.66 – 4.47 (m, 4H), 4.40 – 4.31 (m, 1H), 3.91 (d, J = 11.0 Hz, 1H), 3.80 (dd, J = 11.0, 3.9 Hz, 1H), 2.47 (s, 3H), 2.37 – 2.24 (m, 4H), 2.25 – 2.18 (m, 1H), 2.08 (ddd, J = 13.3, 9.0, 4.5 Hz, 1H), 1.71 – 1.55 (m, 4H), 1.04 (s, 9H). **LC-MS:** (Method A) t_R = 3.15 min, (ESI) *m/z* = 557.2 ([M-H]$^-$, 100%), *m/z* = 582.1 ([M+Na]$^+$, 20%). **TLC:** DCM : MeOH (9 : 1), R_f = 0.20

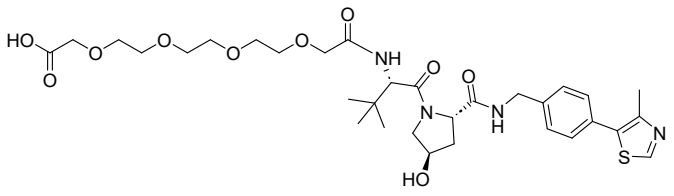
S8: 8-(((*S*)-1-((2*S*,4*R*)-4-Hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-8-oxooctanoic acid[10]



A solution of **S-4** (84 mg, 180 µmol, 1 equiv.) in anhydrous dioxane (2 mL) was treated with triethylamine (64 mg, 645 µmol, 4 equiv.) and methyl 8-chloro-8-oxooctanoate (42 mg, 198 µmol, 1.1 equiv.) and stirred for at 80 °C for 20 h. Solvents were removed *in vacuo* and the residue dissolved in DCM (20 mL), washed with water (15 mL) and brine (15 mL), dried over $MgSO_4$ and concentrated, yielding a brown oil. The crude product was purified by column chromatography (2 - 5% MeOH/DCM) to yield a white solid (55 mg, 51%.).

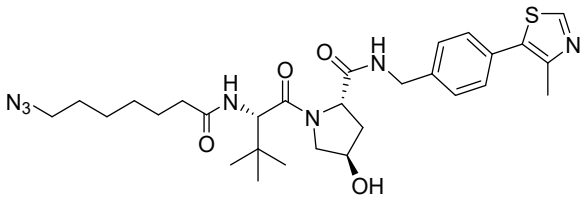
The purified product (55 mg, 92 µmol, 1 equiv.) was dissolved in MeOH/H_2O (2 : 1, 5 mL) and cooled to 0 °C before the addition of LiOH.H_2O (8 mg, 183 µmol, 2 equiv.). The mixture was allowed to warm to r.t and stirred for 20 h before solvents were removed *in vacuo*. The remaining residue was dissolved in water (20 mL) and acidified to pH 3 with 1 M HCl before extraction with EtOAc (3 × 30 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated, yielding a white solid (45 mg, 85%, 43% over two steps). **^{1}H NMR (CD_3OD)** δ 8.88 (s, 1H), 8.67 (bt, J = 6.0 Hz, 1H), 7.84 (bd, J = 8.9 Hz, 1H), 7.49 – 7.38 (m, 4H), 4.67 – 4.47 (m, 4H), 4.36 (dd, J = 15.5, 4.3 Hz, 1H), 3.91 (d, J = 11.0 Hz, 1H), 3.80 (dd, J = 11.0, 3.9 Hz, 1H), 2.48 (s, 3H), 2.34 – 2.18 (m, 5H), 2.08 (ddd, J = 13.2, 9.1, 4.5 Hz, 1H), 1.65 – 1.55 (m, 4H), 1.40 – 1.31 (m, 4H), 1.04 (s, 9H). **LC-MS:** (Method A) t_R = 3.23 min, (ESI) *m/z* = 585.3 ([M-H]$^-$, 100%), *m/z* = 609.3 ([M+Na]$^+$, 10%). **TLC:** DCM : MeOH (9 : 1), R_f = 0.36

S9: (S)-16-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carbonyl)-17,17-dimethyl-14-oxo-3,6,9,12-tetraoxa-15-azaoctadecanoic acid[11]



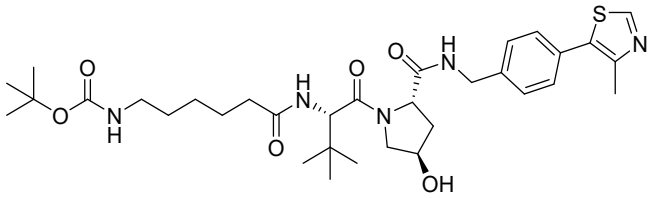
S-4 (50 mg, 99 µmol, 1 equiv) and 3,6,9,12-tetraoxatetradecane-1,14-dioic acid (40 mg, 149 µmol, 1.5 equiv.) were coupled according to General Method A using HATU (57 mg, 149 µmol, 1.5 equiv.) and DIPEA (51 mg, 397 µmol, 4 equiv.). The title compound was obtained as a white solid (12 mg, 18%) after column chromatography (0 - 15% MeOH/DCM). **^{1}H NMR (CD_3OD)** δ 8.88 (s, 1H), 7.49 – 7.38 (m, 4H), 4.71 (s, 1H), 4.65 – 4.48 (m, 3H), 4.35 (d, *J* = 15.4 Hz, 1H), 4.11 (d, *J* = 2.6 Hz, 2H), 4.01 – 3.82 (m, 3H), 3.81 – 3.75 (m, 1H), 3.74 – 3.59 (m, 12H), 2.47 (s, 3H), 2.30 – 2.20 (m, 1H), 2.08 (ddd, *J* = 13.4, 9.2, 4.5 Hz, 1H), 1.04 (s, 9H). **LC-MS:** (Method A) t_R = 3.19 min, (ESI) *m/z* = 677.3 ([M-H]$^-$, 100%), *m/z* = 679.3 ([M+H]$^+$, 100%). **TLC:** DCM : MeOH (85 : 15), R_f = 0.33

S10: (2S,4R)-1-((S)-2-(7-Azidoheptanamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide[12]



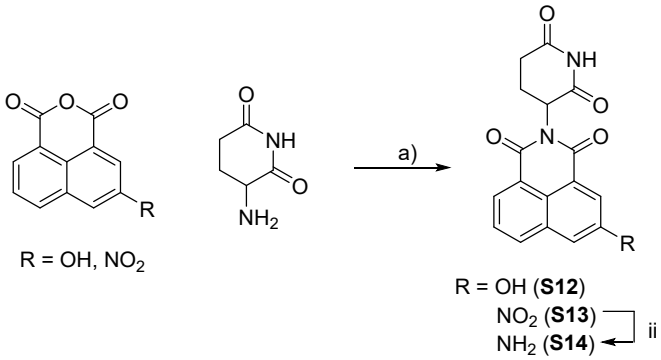
S4 (100 mg, 235 µmol, 1 equiv.) and 7-azidoheptanoic acid (48 mg, 282 µmol, 1.2 equiv.) were coupled according to General Method A. The title compound was obtained as a white solid (108 mg, 80%) after column chromatography (2 - 5% MeOH/DCM). **^{1}H NMR (CD_3OD)** δ 8.87 (s, 1H), 8.65 (t, *J* = 6.1 Hz, 1H), 7.82 (d, *J* = 9.0 Hz, 1H), 7.49 – 7.40 (m, 4H), 4.68 – 4.47 (m, 4H), 4.36 (dd, *J* = 15.4, 4.4 Hz, 1H), 3.91 (d, *J* = 11.0 Hz, 1H), 3.80 (dd, *J* = 11.0, 3.9 Hz, 1H), 3.30 – 3.26 (m, 2H), 2.47 (s, 3H), 2.35 – 2.17 (m, 3H), 2.08 (ddd, *J* = 13.3, 9.1, 4.5 Hz, 1H), 1.69 – 1.52 (m, 4H), 1.45 – 1.38 (m, 4H), 1.04 (s, 9H). **LC-MS:** (Method A) t_R = 3.32 min, (ESI) *m/z* = 578.3 ([M+Na]$^+$, 20%), *m/z* = 554.2 ([M-H]$^-$), 100%. **TLC:** DCM : MeOH (95 : 5), R_f = 0.20

S11: *tert*-Butyl (6-(((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-6-oxohexyl)carbamate[12]



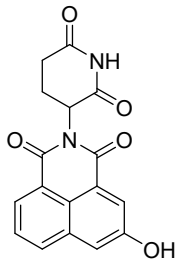
S4 (76 mg, 151 µmol, 1 equiv.) and Boc-6-aminohexanoic acid-OH (40 mg, 166 µmol, 1.1 equiv.) were coupled according to General Method 3A. The title compound was obtained as a white solid (79 mg, 81%) after column chromatography (2 - 5% MeOH/DCM). **^{1}H NMR (CD_3OD)** δ 8.87 (s, 1H), 8.64 (dt, 1H), 7.81 (d, *J* = 8.9 Hz, 1H), 7.49 – 7.40 (m, 4H), 4.66 – 4.45 (m, 1H), 4.36 (dd, *J* = 15.5, 5.2 Hz, 1H), 3.91 (d, *J* = 11.0 Hz, 1H), 3.80 (dd, *J* = 11.0, 3.9 Hz, 1H), 3.05 – 3.00 (m, 2H), 2.47 (s, 3H), 2.38 – 2.14 (m, 4H), 2.09 (ddd, *J* = 13.3, 9.1, 4.5 Hz, 1H), 1.66 – 1.57 (m, 2H), 1.52 – 1.43 (m, 2H), 1.42 (s, 9H), 1.37 – 1.29 (m, 4H), 1.04 (s, 9H). **LC-MS:** (Method A) t_R = 3.43 min, (ESI) *m/z* = 544.3 ([M-Boc]$^+$, 100%), *m/z* = 667.4 ([M+Na]$^+$), 20%. **TLC:** DCM : MeOH (95 : 5), R_f = 0.33

Synthesis of DGY cereblon recruiters[13]



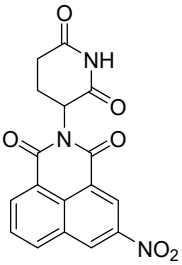
Scheme S3: Synthesis of CRBN recruiters S12 and S14 a) Et_3N, THF, reflux, 20 h, b) H_2, Pd/C, DMF, r.t, 4 h.

S12: 2-(2,6-Dioxopiperidin-3-yl)-5-hydroxy-1*H*-benzo[de]isoquinoline-1,3(2*H*)-dione



3-Hydroxy-1,8-naphthalic anhydride was suspended in THF (35 mL) and 3-aminopiperidine-2,6-dione hydrochloride and Et_3N were added. The suspension was heated to reflux for 24 h. The reaction mixture was reduced *in vacuo* and the resulting residue was treated with H_2O (50 mL) and then acidified with 1M HCl (5 mL). The suspension was stirred at r.t for 1 h and then collected by filtration to give a green solid (3.06 g) which was used without further purification. ^{1}H NMR (401 MHz, DMSO-d_6) δ 11.01 (s, 1H), 10.61 (s, 1H), 8.29 (d, J = 8.3 Hz, 1H), 8.28 (dd, J = 46.9, 7.2 Hz, 1H), 8.04 (dd, J = 44.5, 2.4 Hz, 1H), 7.77 (q, J = 7.8 Hz, 1H), 7.71 (t, J = 3.0 Hz, 1H), 5.88 – 5.75 (m, 1H), 3.00 – 2.85 (m, 1H), 2.65 – 2.53 (m, 2H), 2.09 – 1.96 (m, 1H). LC-MS (ESI$^+$) *m/z*: 325.1 $(M + H)^+$ (100%).

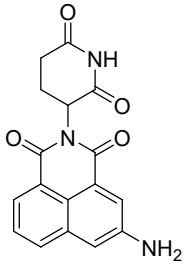
S13: 2-(2,6-Dioxopiperidin-3-yl)-5-nitro-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione



3-Nitro-1,8-naphthalic anhydride was suspended in THF (5 mL) and 3-aminopiperidine-2,6-dione hydrochloride and Et_3N were added. The suspension was heated to reflux for 20 h. The reaction mixture was allowed to cool to r.t. The resulting precipitate was collected and the precipitate was washed with a small amount of THF and then H_2O and dried to give the product as a grey solid (240 mg, 83%). ^{1}H NMR (401 MHz, DMSO) δ 11.06 (s, 1H), 9.57 – 9.52 (m, 1H), 8.99 (dd, J = 42.1, 2.3 Hz, 1H), 8.84 (d, J = 8.3 Hz, 1H), 8.72 (dd, J = 44.2, 7.3 Hz, 1H), 8.10 (q, J = 7.6 Hz, 1H), 5.87 (dd, J = 12.0, 5.7 Hz,

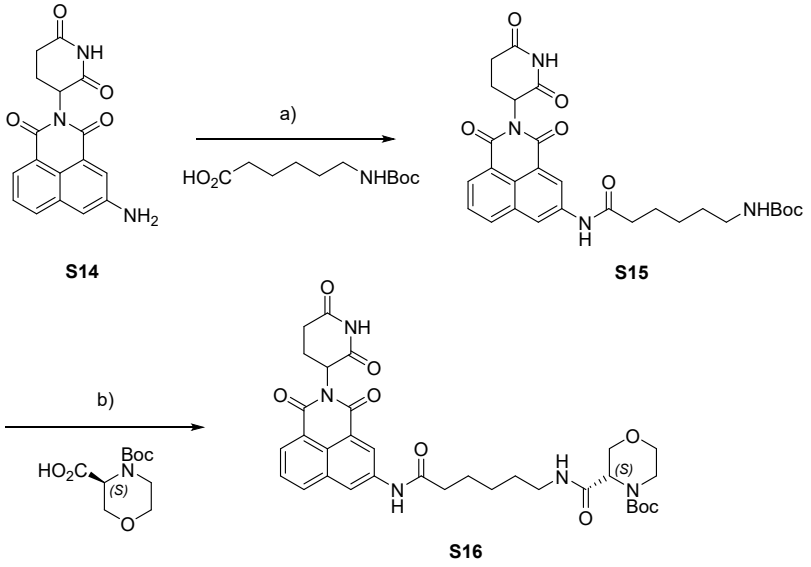
1H), 3.02 – 2.88 (m, 1H), 2.69 – 2.56 (m, 2H), 2.16 – 1.99 (m, 1H). LC-MS (ESI^+) *m/z*: 354.1 $(M + H)^+$ (100%).

S14: 5-Amino-2-(2,6-dioxopiperidin-3-yl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione



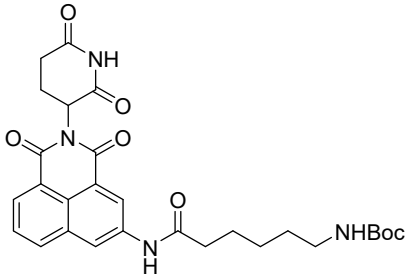
S13 was dissolved in DMF (10 mL) and treated with 10% Pd/C (40 mg) and H_2 at r.t for 4 h. The reaction mixture was filtered and solvent removed *in vacuo*. The residue was treated with EtOAc and filtered to give a mustard solid (87 mg) which was used without further purification. ^1H NMR (401 MHz, DMSO) δ 10.98 (s, 1H), 8.18 – 7.88 (m, 3H), 7.64 (q, *J* = 7.8 Hz, 1H), 7.33 (t, *J* = 2.5 Hz, 1H), 6.05 (s, 2H), 5.80 (dd, *J* = 11.9, 5.7 Hz, 1H), 2.98 – 2.86 (m, 1H), 2.65 – 2.53 (m, 2H), 2.07 – 1.97 (m, 1H). LC-MS (ESI^+) *m/z*: 324.1 $(M + H)^+$ (100%).

Synthesis of cereblon recruiter-linker conjugates



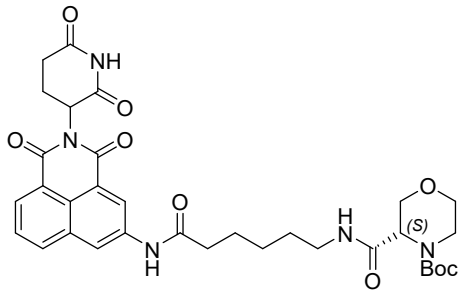
Scheme S4: Synthesis of CRBN recruiter-linker conjugates S16 a) HATU, DIPEA, DMF, r.t, 24 h, 83%, b) i) TFA/DCM (1 : 1), r.t, 1 h, ii) HATU, DIPEA, DMF, r.t, 40 min, 63%,

S15: *tert*-Butyl (6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-5-yl)amino)-6-oxohexyl)carbamate

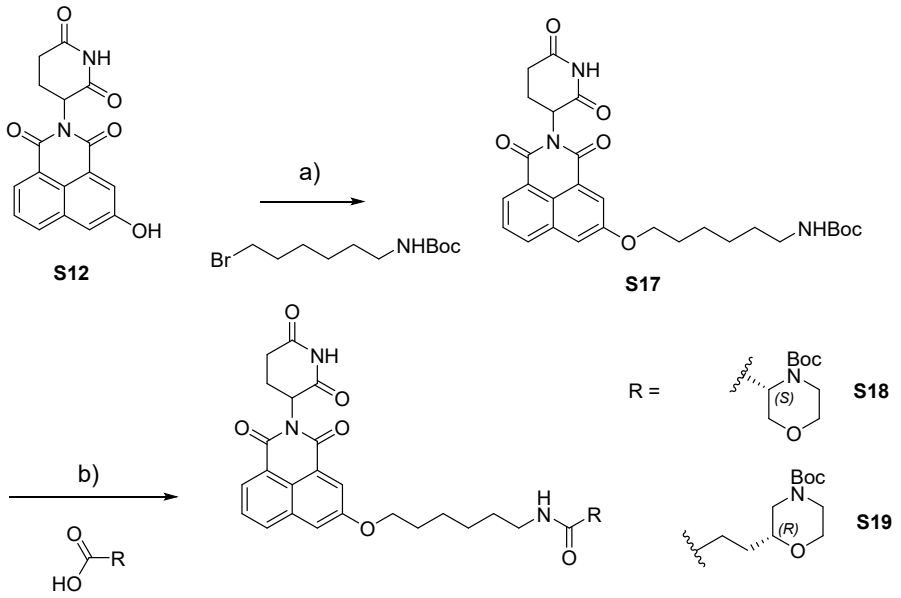


N-(*tert*-butoxycarbonyl)-6-aminocaproic acid and HATU were dissolved in DMF (1 mL) and DIPEA (6 eq) was added. After 2 min the mixture was added to a solution of **S14** in DMF (1 mL) and stirred at RT for 24 h. A 5% $NaHCO_3$ solution (15 mL) was added and the resulting suspension was filtered and the precipitate washed with H_2O and dried *in vacuo* to give a mustard solid (69 mg, 83%). ^{1}H NMR (401 MHz, DMSO-d_6) δ 11.01 (s, 1H), 10.53 (s, 1H), 8.85 (d, J = 8.2 Hz, 1H), 8.64 (dd, J = 49.7, 2.0 Hz, 1H), 8.47 – 8.27 (m, 2H), 7.84 (q, J = 7.8 Hz, 1H), 6.78 (s, 1H), 5.91 – 5.78 (m, 1H), 2.98 – 2.84 (m, 3H), 2.66 – 2.55 (m, 2H), 2.41 (t, J = 7.4 Hz, 2H), 2.09 – 1.99 (m, 1H), 1.72 – 1.59 (m, 2H), 1.46 – 1.25 (m, 13H). LC-MS (ESI$^+$) *m/z*: 437.1 (M – Boc + H)$^+$ (100%).

S16: *tert*-Butyl (3*S*)-3-((6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-5-yl)amino)-6-oxohexyl)carbamoyl)morpholine-4-carboxylate

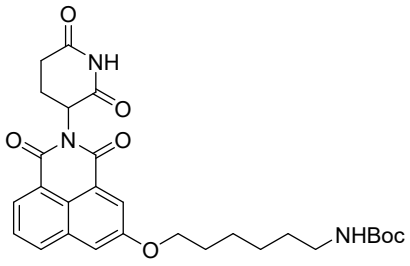


S15 was dissolved in DCM:TFA (1:1) (1 mL). The solution was stirred at r.t for 1 h. The solution was reduced and dried *in vacuo*. The residue was dissolved in DMF (1 mL) and (*S*)-4-(*tert*-butoxycarbonyl)morpholine-3-carboxylic acid was added and the solution cooled in an ice bath. HATU and DIPEA were added and stirring continued for 10 min before removal of the ice bath and allowing the reaction to come up to r.t. The reaction was stirred for 30 min and then 5% $NaHCO_3$ (10 mL) was added. The resulting precipitate was collected by filtration, washed with water and dried *in vacuo*. Purification by column chromatography (SiO_2, DCM:EtOAc 1:1 to 1:5) gave the product as a yellow solid (46 mg, 63%). ^{1}H NMR (401 MHz, DMSO) δ 11.02 (d, J = 3.2 Hz, 1H), 10.54 (d, J = 3.2 Hz, 1H), 8.85 (d, J = 7.2 Hz, 1H), 8.75 – 8.54 (m, 1H), 8.47 – 8.29 (m, 2H), 8.00 – 7.77 (m, 2H), 5.87 – 5.79 (m, 1H), 4.27 – 4.04 (m, 2H), 3.85 – 3.65 (m, 1H), 3.56 – 3.48 (m, 2H), 3.31 – 3.02 (m, 4H), 3.00 – 2.85 (m, 1H), 2.66 – 2.54 (m, 2H), 2.41 (t, J = 7.4 Hz, 2H), 2.11 – 2.00 (m, 1H), 1.71 – 1.60 (m, 2H), 1.52 – 1.42 (m, 2H), 1.41 – 1.30 (m, 11H). LC-MS (ESI$^+$) *m/z*: 550.2 (M – Boc + H)$^+$ (100%).



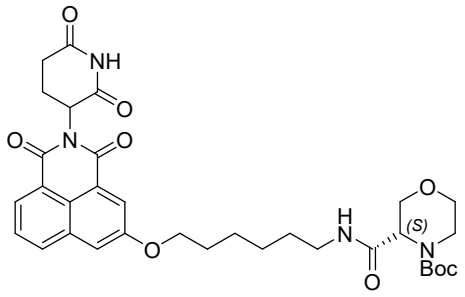
Scheme S5: Synthesis of CRBN recruiter-linker conjugates S18 and S19 a) K_2CO_3, DMF, 35 °C, 48 h, 47%, b) i) TFA/DCM (1 : 1), r.t, 1 h, ii) HATU, DIPEA, DMF, r.t, 40 min, 73-89%.

S17: *tert*-Butyl (6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-5-yl)oxy)hexyl)carbamate



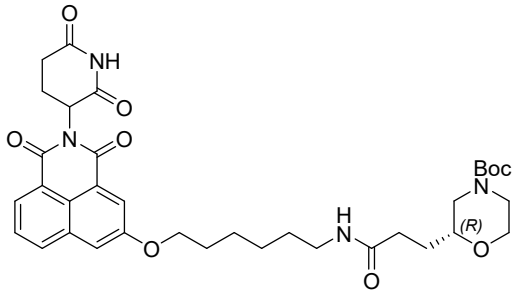
S12 was suspended in DMF (2 mL) and K_2CO_3 and N-(*tert*-Butoxycarbonyl)-6-bromohexylamine were added and the reaction mixture heated to 35 °C for 2 d. The reaction was quenched with 5% $NaHCO_3$ (20 mL) and the resulting precipitate was collected, washed with H_2O and dried *in vacuo* to give a yellow precipitate. Purification by column chromatography (SiO_2, DCM : EA 4 : 1) gave the product as a yellow solid (153 mg, 47%). ^{1}H NMR (401 MHz, DMSO-d_6) δ 11.01 (s, 1H), 8.37 (d, J = 8.6 Hz, 1H), 8.34 (dd, J = 47.2, 7.2 Hz, 1H), 8.13 – 7.95 (m, 2H), 7.84 (q, J = 7.9 Hz, 1H), 6.77 (s, 1H), 5.87 – 5.78 (m, 1H), 4.27 – 4.16 (m, 2H), 2.98 – 2.84 (m, 3H), 2.65 – 2.54 (m, 2H), 2.10 – 1.97 (m, 1H), 1.88 – 1.74 (m, 2H), 1.53 – 1.29 (m, 15H). LC-MS (ESI$^+$) *m/z*: 424.2 (M – Boc + H)$^+$ (100%).

S18: *tert*-Butyl (3*S*)-3-((6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-5-yl)oxy)hexyl)carbamoyl)morpholine-4-carboxylate

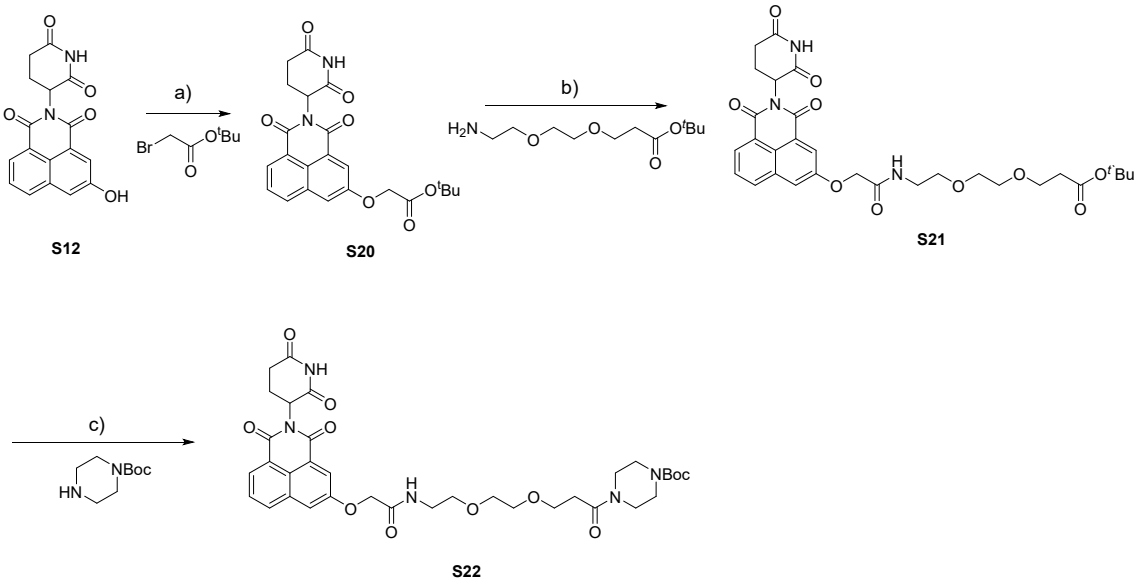


S17 was dissolved in DCM:TFA (1:1) (1 mL). The solution was stirred at r.t for 1 h. The solution was reduced under a stream of nitrogen and the residue dried *in vacuo*. The residue was dissolved in dry DMF (2 mL) and (*S*)-4-(*tert*-butoxycarbonyl)morpholine-3-carboxylic acid, DIPEA and HATU were added and the mixture stirred at r.t for 1 h. A 5% $NaHCO_3$ solution (30 mL) was added. The resulting precipitate was collected by filtration and washed with H_2O and dried *in vacuo* to give a yellow solid (92 mg). Purification by column chromatography (SiO_2) DCM:EtOAc, 1 : 1 gave the product as a pale yellow solid (62 mg, 73%). ^{1}H NMR (401 MHz, DMSO) δ 11.02 (s, 1H), 8.44 – 8.23 (m, 2H), 8.15 – 7.94 (m, 2H), 7.92 – 7.79 (m, 2H), 5.92 – 5.80 (m, 1H), 4.37 – 4.05 (m, 4H), 3.86 – 3.69 (m, 1H), 3.54 (d, *J* = 11.7 Hz, 2H), 3.26 – 3.02 (m, 3H), 3.01 – 2.85 (m, 1H), 2.64 – 2.55 (m, 2H), 2.12 – 2.01 (m, 1H), 1.86 – 1.76 (m, 2H), 1.55 – 1.28 (m, 15H). [NB: 1H missing under solvent] LC-MS (ESI$^+$) *m/z*: 537.2 (M – Boc + H)$^+$ (100%).

S19: *tert*-Butyl (2*R*)-2-(3-((6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-5-yl)oxy)hexyl)amino)-3-oxopropyl)morpholine-4-carboxylate

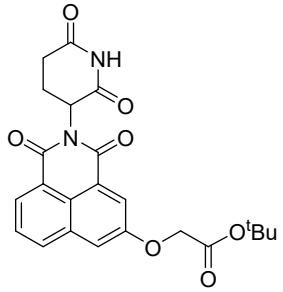


S17 was dissolved in DCM:TFA (1:1) (1 mL). The solution was stirred at r.t for 1 h. The solution was reduced under a stream of nitrogen and dried *in vacuo*. (*R*)-3-(4-(*tert*-butoxycarbonyl)morpholin-2-yl)propanoic acid in dry DMF (2 mL) was added along and DIPEA. HATU was then added and the mixture stirred at r.t for 1 h. A 5% $NaHCO_3$ solution (30 mL) was added. The resulting precipitate was collected by filtration and washed with H_2O and dried *in vacuo* to give a pale yellow solid (98 mg). Purification by column chromatography (SiO_2), DCM : EtOAc 1 : 2 to 1 : 3 gave the product as a pale yellow solid (79 mg, 89%). ^{1}H NMR (401 MHz, CD_3CN) δ 8.87 (s, 1H), 8.46 – 8.30 (m, 1H), 8.23 (d, *J* = 8.3 Hz, 1H), 8.20 – 8.07 (m, 1H), 7.78 – 7.70 (m, 2H), 6.34 (s, 1H), 5.81 (dd, *J* = 11.4, 5.4 Hz, 1H), 4.19 (t, *J* = 6.5 Hz, 2H), 3.92 – 3.67 (m, 3H), 3.37 (td, *J* = 11.7, 2.9 Hz, 1H), 3.30 – 3.20 (m, 1H), 3.14 (q, *J* = 6.5 Hz, 2H), 2.89 – 2.75 (m, 2H), 2.75 – 2.64 (m, 2H), 2.58 – 2.45 (m, 1H), 2.25 – 2.16 (m, 2H), 2.12 – 2.09 (m, 1H), 1.90 – 1.80 (m, 2H), 1.75 – 1.59 (m, 2H), 1.58 – 1.45 (m, 4H), 1.45 – 1.35 (m, 11H). LC-MS (ESI$^+$) *m/z*: 565.2 (M – Boc + H)$^+$ (100%).



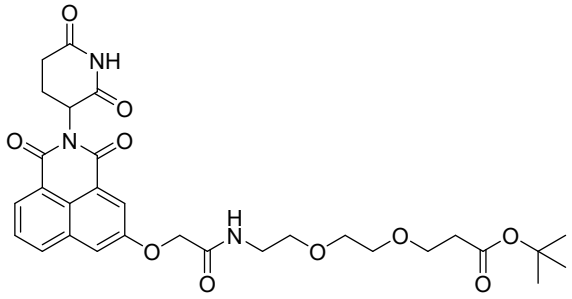
Scheme S6: Synthesis of CRBN recruiter-linker conjugate S22 a) K_2CO_3, DMF, r.t, 18 h, 58%, b) i) TFA/DCM (1 : 2), r.t, 3 h, ii) EDC.HCl, HOBt, DMF, r.t, 40 min, 54%, c) i) TFA/DCM (1 : 2), r.t, 1 h, ii) EDC.HCl, HOBt, DMF, r.t, 40 min, 69%,

S20: *tert*-Butyl 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-5-yl)oxy)acetate



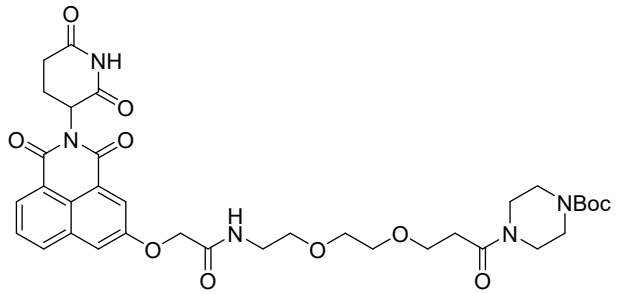
S12 was suspended in DMF (10 mL) and K_2CO_3 and *t*-butyl bromoacetate were added. The mixture was stirred at r.t for 1 h and further *t*-butyl bromoacetate (1 eq) was added. The suspension was stirred at r.t for 18 h. H_2O was added to the reaction mixture and the resulting precipitate was collected by filtration, washed with water and dried *in vacuo* to give a pale green solid. Trituration with DCM gave the product as a white solid (1.32 g, 58%). ^{1}H NMR (401 MHz, DMSO-d_6) δ 11.01 (s, 1H), 8.36 (dd, J = 46.4, 7.3 Hz, 1H), 8.36 (d, J = 8.3 Hz, 1H), 8.07 (dd, J = 42.3, 2.5 Hz, 1H), 7.97 (s, 1H), 7.85 (q, J = 7.7 Hz, 1H), 5.83 (dd, J = 12.1, 5.7 Hz, 1H), 4.95 (d, J = 4.0 Hz, 2H), 3.01 – 2.85 (m, 1H), 2.66 – 2.54 (m, 2H), 2.12 – 1.96 (m, 1H), 1.44 (d, J = 3.5 Hz, 9H). LC-MS (ESI$^+$) *m/z*: 383.0 (M – ^{t}Bu + H)$^+$ (100%)

S21: *tert*-Butyl 3-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-benzo[de]isoquinolin-5-yl)oxy)acetamido)ethoxy)ethoxy)propanoate

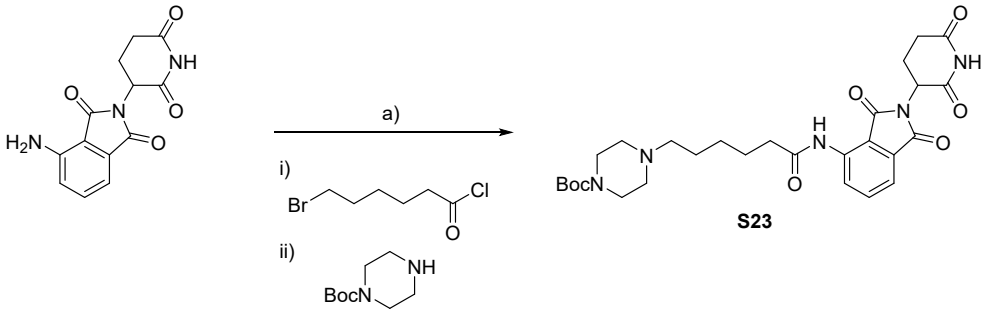


S20 was suspended in DCM (10 mL) and then TFA (5 mL) was added. The solution was stirred at r.t for 3 h. The solvent was then removed *in vacuo*. The resulting solid was triturated with Et_2O to give the deprotected carboxylic acid as a yellow solid (quant.). The yellow solid was suspended in dry DMF (3 mL) and tert-butyl 9-Amino-4,7-dioxanonanoate was added followed by HOBt and then EDC.HCl. The reaction was stirred at r.t for 4 h. The reaction was quenched with 5% $NaHCO_3$ solution (40 mL) and the resulting precipitate was collected and washed with water to give yellow solid (393 mg). Purification by column chromatography (SiO_2, EtOAc : DCM 4 : 1 (+ 2% MeOH)) gave the product as an off-white solid (252 mg, 54%). ^{1}H NMR (401 MHz, DMSO) δ 11.01 (s, 1H), 8.45 – 8.13 (m, 4H), 7.96 (dd, *J* = 6.8, 2.6 Hz, 1H), 7.85 (q, *J* = 7.5 Hz, 1H), 5.88 – 5.78 (m, 1H), 4.75 (s, 2H), 3.55 (q, *J* = 6.0 Hz, 2H), 3.51 – 3.41 (m, 6H), 3.32 – 3.27 (m, 2H), 3.00 – 2.87 (m, 1H), 2.69 – 2.53 (m, 2H), 2.39 (td, *J* = 6.2, 2.2 Hz, 2H), 2.11 – 2.00 (m, 1H), 1.37 (s, 9H). LC-MS (ESI$^+$) *m/z*: 542.1 (M - ^{t}Bu + H)$^+$ (100%).

S22: *tert*-Butyl 4-(3-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-5-yl)oxy)acetamido)ethoxy)ethoxy)propanoyl)piperazine-1-carboxylate

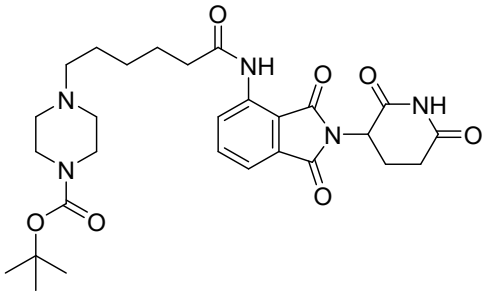


S21 was treated with TFA:DCM (1 : 2) (3 mL) and stirred at r.t for 1 h. The mixture was reduced *in vacuo* and the resulting residue was triturated with Et_2O. HATU, DMF and DIPEA were then added, stirred for 1 min, then Boc-piperazine was added and the mixture stirred at r.t for 3 h. A sat. solution of NH_4Cl was added and extracted with DCM (3 × 10 mL). The combined organic layer was washed with brine (2 × 10 mL), dried ($MgSO_4$), filtered and solvent removed *in vacuo* to give a yellow residue. Purification by column chromatography (SiO_2, DCM : EtOAc, 1 : 1 (+ 7% MeOH) gave the product as a beige solid (37 mg, 69%). ^{1}H NMR (401 MHz, DMSO-d_6) δ 11.02 (s, 1H), 8.49 – 8.12 (m, 4H), 7.96 (dd, *J* = 6.5, 2.6 Hz, 1H), 7.86 (q, *J* = 7.6 Hz, 1H), 5.89 – 5.78 (m, 1H), 4.76 (s, 2H), 3.60 (td, *J* = 6.5, 3.9 Hz, 2H), 3.53 – 3.20 (m, 16H), 3.01 – 2.86 (m, 1H), 2.66 – 2.54 (m, 4H), 2.10 – 1.99 (m, 1H), 1.39 (s, 9H). LC-MS (ESI$^+$) *m/z*: 710.3 (M + H)$^+$ (100%).



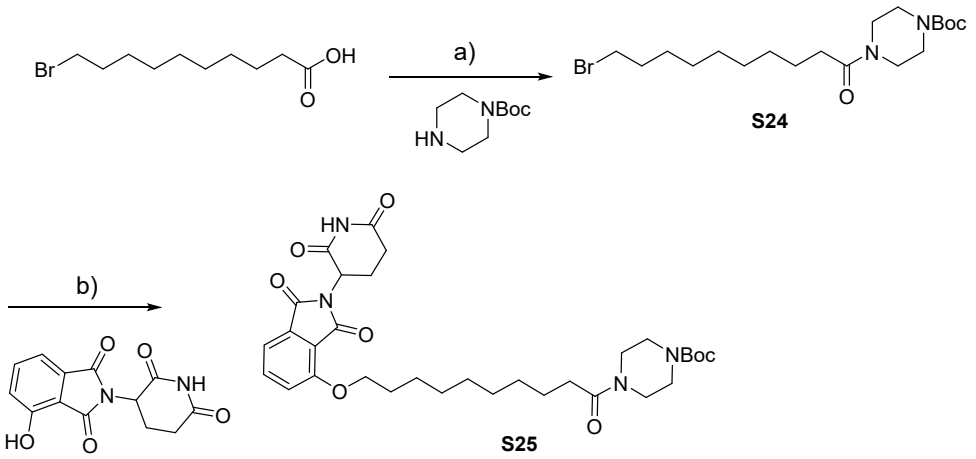
Scheme S7: Synthesis of CRBN recruiter-linker conjugate S23 a) i) dioxane, 0 - 100 °C, ii) TEA, KI, DMF, reflux, 24 h, 33% (2 steps).

S23: *tert*-Butyl 4-(6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)-6-oxohexyl)piperazine-1-carboxylate



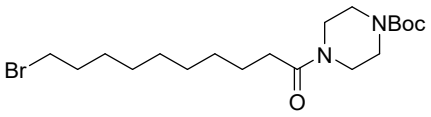
A solution of pomalidomide (75 mg, 275 µmol, 1 equiv.) in anhydrous dioxane (2 mL) was cooled to 0 °C before the addition of 6-bromohexanoyl chloride (117 mg, 549 µmol, 2 equiv.). The mixture was heated to 100 °C and stirred for 2 h. The solution as allowed to return to room temperature before concentration *in vacuo* to yield a yellow solid.

The crude intermediate was dissolved in DMF (1 mL) followed by the addition of 1-Boc-piperazine (37 mg, 199 µmol, 5 equiv.), triethylamine (139 mg, 1.37 mmol, 5 equiv.) and KI (5 mg, 27 µmol, 0.1 equiv.). The mixture was heated to reflux for 24 h before removal of solvents under a stream of nitrogen. The residue was dissolved in DCM (40 mL) and washed with 19% NH_4Cl (2 × 30 mL), water (30 mL) and brine (30 mL). The organic layer dried over $MgSO_4$ and concentrated *in vacuo*, yielding a yellow oil. The crude product was purified via column chromatography (0 - 100% EtOAc/Petroleum ether) to yield a pale yellow flaky solid (50 mg, 33% over two steps). **^{1}H NMR ($CDCl_3$)** δ 9.40 (s, 1H), 8.98 (bs, 1H), 8.80 (d, J = 8.5 Hz, 1H), 7.69 (d, J = 7.1 Hz, 1H), 7.53 (d, J = 7.3 Hz, 1H), 4.99 – 4.86 (m, 1H), 3.49 – 3.38 (m, 4H), 2.93 – 2.81 (m, 1H), 2.81 – 2.70 (m, 2H), 2.45 (t, J = 7.5 Hz, 2H), 2.42 – 2.32 (m, 6H), 2.18 – 2.09 (m, 1H), 1.76 (p, J = 7.5 Hz, 2H), 1.55 (p, J = 8.2, 7.7 Hz, 2H), 1.44 (s, 9H), 1.43 – 1.36 (m, 2H). **^{13}C NMR ($CDCl_3$)** δ 172.3, 171.1, 169.3, 168.2, 166.8, 154.9, 137.9, 136.5, 131.2, 125.4, 118.6, 79.8, 58.4, 53.0, 49.4, 37.9, 31.5, 28.5, 27.1, 26.3, 25.2, 22.8. **LC-MS:** (Method A) t_R = 3.22 min, (ESI) *m/z* = 556.3 ([M+H]$^+$, 100%). **HPLC:** (Method B) t_R = 4.25 min, 98% pure (254 nm). **HRMS:** (ESI+) calc'd for $[C_{28}H_{37}N_5O_7 + H]^+$ 556.2766, found 556.2785. **TLC:** DCM : MeOH (95 : 5), R_f = 0.24



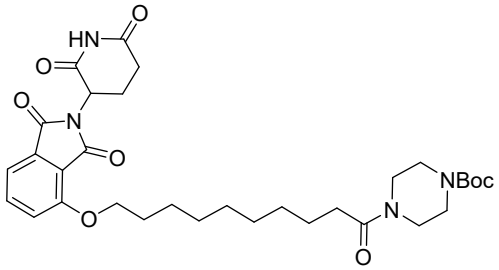
Scheme S8: Synthesis of CRBN recruiter-linker conjugate S25 a) EDC.HCl, HOBt, DMF, r.t, 4 h, 93%, b) $NaHCO_3$, KI, DMF, 50 °C, 18 h, 34%.

S24: *tert*-Butyl 4-(10-bromodecanoyl)piperazine-1-carboxylate



10-Bromodecanoic acid was dissolved in DCM (5 mL) and Boc-piperazine was added followed by HOBt and then EDC.HCl. The reaction was stirred at r.t for 4 h. The reaction mixture was diluted with DCM (25 mL) and washed with 0.5 M HCl (2 × 15 mL), brine (15 mL), 10% Na_2CO_3 (2 × 15 mL), brine (15 mL), dried ($MgSO_4$), filtered and reduced *in vacuo* to give a beige waxy solid (335mg). Purification by column chromatography (SiO_2, EtOAc: Petroleum ether, 1 : 2) gave the product as a white solid (310 mg, 93%). ^{1}H NMR (401 MHz, Chloroform-*d*) δ 3.64 – 3.53 (m, 2H), 3.47 – 3.33 (m, 8H), 2.37 – 2.27 (m, 2H), 1.84 (p, *J* = 7.0 Hz, 2H), 1.69 – 1.56 (m, 2H), 1.47 (s, 9H), 1.44 – 1.39 (m, 2H), 1.37 – 1.27 (m, 8H). LC-MS (ESI$^+$) *m/z*: 363.1 (M(^{79}Br) - ^{t}Bu + H)$^+$ (100%) and 365.1 (M(^{81}Br) - ^{t}Bu + H)$^+$.

S25: *tert*-Butyl 4-(10-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)decanoyl)piperazine-1-carboxylate (SJM-0038-03)



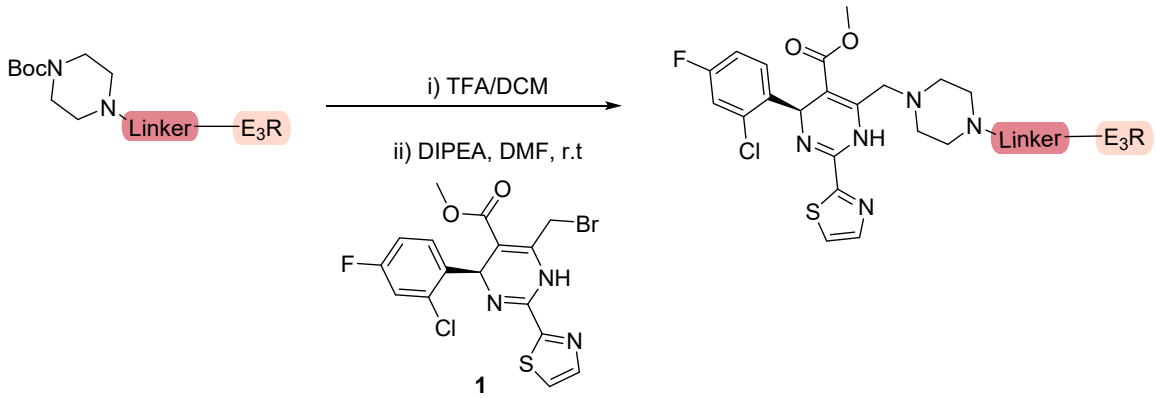
4-Hydroxythalidomide was dissolved in DMF (2 mL) and KI, $NaHCO_3$ and **S24** were added. The reaction mixture was heated to 50 °C for 18 h. The solution was allowed to cool to RT then quenched with a 5% $NaHCO_3$ solution (20 mL). The resulting suspension was centrifuged, and the supernatant was decanted and the pellet dried *in vacuo* to give a yellow residue (46 mg). Purification by column chromatography (SiO_2) DCM : EtOAc, 2 : 1, gave the product as a colourless residue (23 mg, 34%). ^{1}H NMR (401 MHz, Acetonitrile-d_3) δ 9.06 (s, 1H), 7.73 (dd, *J* = 8.5, 7.3 Hz, 1H), 7.39 (d, *J* = 7.2 Hz, 1H), 7.36 (d, *J* = 8.5 Hz, 1H), 4.96 (dd, *J* = 12.2, 5.2 Hz, 1H), 4.18 (t, *J* = 6.4 Hz, 2H), 3.52 – 3.26 (m,

8H), 2.88 – 2.57 (m, 3H), 2.34 – 2.25 (m, 2H), 2.15 – 2.04 (m, 1H), 1.86 – 1.74 (m, 2H), 1.60 – 1.45 (m, 4H), 1.43 (s, 9H), 1.41 – 1.24 (m, 8H). LC-MS (ESI^+) *m/z*: 557.2 (M - ^{t}Bu + H)$^+$ (100%).

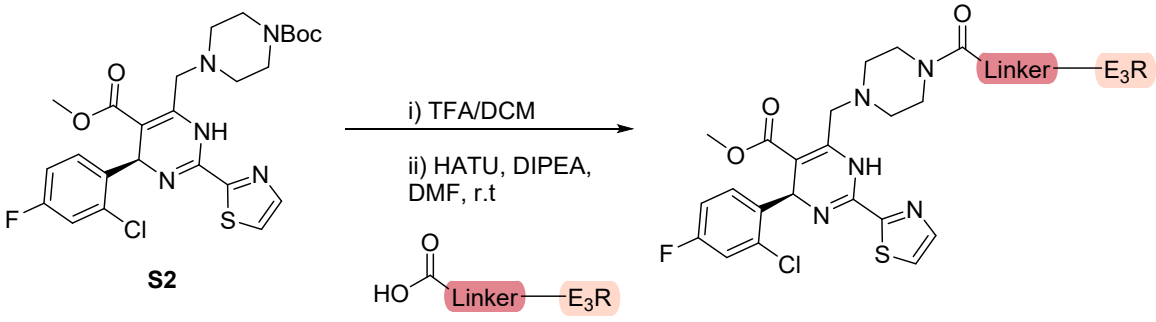
Synthesis of degrader candidates

LH series

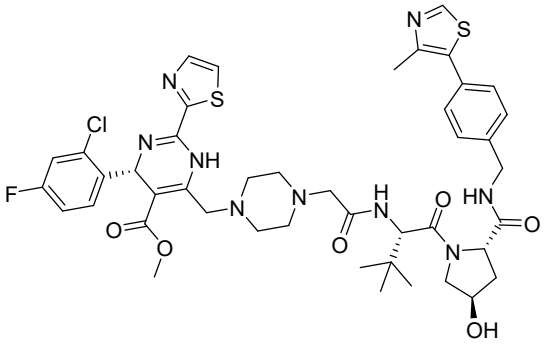
General scheme A:



General scheme B:



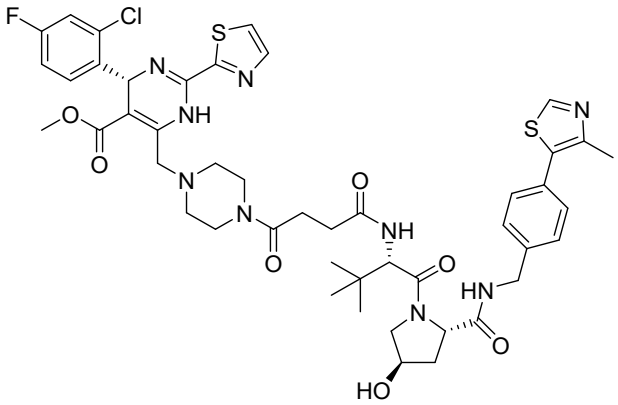
LH-1 (General scheme A): Methyl (*R*)-4-(2-chloro-4-fluorophenyl)-6-((4-(2-(((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-2-oxoethyl)piperazin-1-yl)methyl)-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate



A solution of **S5** (26 mg, 40 µmol, 1 equiv.) was treated according to General method B using **1** (18 mg, 40 µmol, 1 equiv.), DIPEA (20 mg, 160 µmol, 4 equiv.) and KI (6 mg, 20 µmol, 0.5 equiv.) for 2 h and purified via column chromatography (0 - 5% MeOH/DCM) to yield a yellow solid (13 mg, 36%). **^{1}H NMR (CD_3OD)** δ 8.86 (s, 1H), 7.93 (d, *J* = 3.1 Hz, 1H), 7.72 (d, *J* = 3.1 Hz, 1H), 7.51 – 7.40 (m,

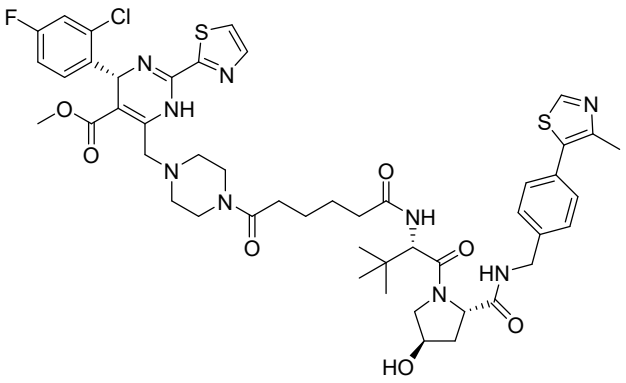
4H), 7.37 (dd, J = 6.0, 2.2 Hz, 1H), 7.21 (dd, J = 8.7, 2.6 Hz, 1H), 7.01 (td, J = 8.4, 2.8 Hz, 1H), 6.15 (s, 1H), 4.67 – 4.48 (m, 4H), 4.41 – 4.34 (m, 1H), 4.04 (d, J = 17.1 Hz, 1H), 3.95 – 3.85 (m, 2H), 3.81 (dd, J = 11.0, 3.8 Hz, 1H), 3.58 (s, 3H), 3.13 (d, J = 2.5 Hz, 2H), 2.72 (bs, 8H), 2.48 (s, 3H), 2.28 – 2.19 (m, 1H), 2.09 (ddd, J = 13.3, 9.3, 4.4 Hz, 1H), 1.05 (s, 9H).**^{13}C NMR (CD_3OD)** δ 174.3, 172.3, 171.9, 167.8, 163.3, 163.1 (d, J_{CF}= 248.1 Hz), 152.8, 149.0, 148.0, 146.7, 144.6, 140.2, 139.3 (d, J_{CF}= 3.6 Hz), 134.8 (d, J_{CF}= 10.4 Hz), 133.4, 132.1 (d, J_{CF}= 9.1 Hz), 131.6, 130.4, 129.0, 125.1, 117.8 (d, J_{CF}= 24.9 Hz), 115.5 (d, J_{CF}= 21.2 Hz), 98.9, 71.1, 61.8, 60.8, 58.4, 58.1, 57.3, 54.6, 54.5, 51.6, 43.8, 38.9, 37.0, 27.0, 15.9. **LC-MS:** (Method A) t_R = 3.39 min, (ESI) m/z = 920.3 ([M+H]$^+$, 100%). **HPLC:** (Method A) t_R = 5.69 min, ≥ 99% pure (254 nm). **^{19}F NMR (CD_3OD)** δ -114.50 **HRMS:** (ESI+) calc'd for $[C_{44}H_{51}N_9O_6S_2ClF + H]^+$ 920.3149, found 920.3157. **TLC:** DCM : MeOH (92.5 : 7.5), R_f= 0.28

LH-2 (General scheme B): Methyl (*R*)-4-(2-chloro-4-fluorophenyl)-6-((4-(4-(((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-4-oxobutanoyl)piperazin-1-yl)methyl)-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate



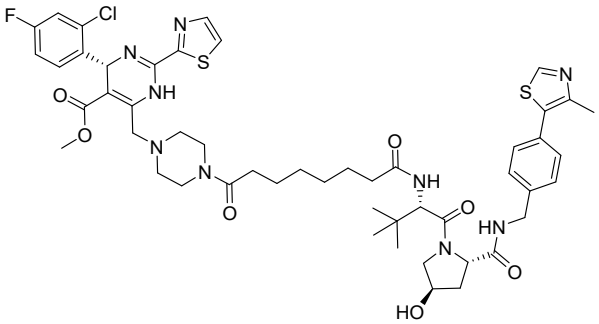
A solution of **S2** (9 mg, 16 µmol, 1 equiv.) in DCM : TFA (1 : 1, 2 mL) was stirred for 30 min, before the removal of DCM and TFA under a stream of nitrogen, giving a brown oil. The resultant amine was treated with **S6** (10 mg, 20 µmol, 1.2 equiv.), HATU (8 mg, 20 µmol, 1.2 equiv.) and DIPEA (9 mg, 65 µmol, 4 equiv.) according to General Method A. The crude product was purified via column chromatography (0 - 5% MeOH/DCM) to yield a yellow solid (6 mg, 40%). **^{1}H NMR (CD_3OD)** δ 8.87 (s, 1H), 7.92 (d, J = 3.1 Hz, 1H), 7.73 (d, J = 3.2 Hz, 1H), 7.50 – 7.44 (m, 2H), 7.44 – 7.37 (m, 3H), 7.22 (dd, J = 8.7, 2.6 Hz, 1H), 7.04 (td, J = 8.4, 2.6 Hz, 1H), 6.16 (s, 1H), 4.65 – 4.48 (m, 5H), 4.36 (d, J = 15.5 Hz, 1H), 4.07 (d, J = 17.1 Hz, 1H), 3.95 – 3.85 (m, 2H), 3.80 (dd, J = 11.0, 3.9 Hz, 1H), 3.69 (bs, 4H), 3.58 (s, 3H), 2.75 – 2.53 (m, 8H), 2.47 (s, 3H), 2.22 (dt, J = 13.2, 7.7 Hz, 1H), 2.08 (ddd, J = 13.3, 9.1, 4.4 Hz, 1H), 1.05 (s, 9H). **^{13}C NMR (CD_3OD)** δ 174.6, 174.5, 172.7, 172.3, 152.8, 144.6, 140.3, 132.1 (d, J_{CF}= 8.9 Hz), 131.5, 130.4, 129.0, 125.2, 117.8 (d, J_{CF}= 25.0 Hz), 115.5 (d, J_{CF}= 21.3 Hz), 71.1, 60.8, 59.2, 57.9, 57.5, 57.1, 54.3, 54.0, 51.6, 46.8, 43.7, 43.2, 38.9, 36.7, 31.6, 29.3, 27.1, 15.8. Six aromatic quaternary carbons not observed. **^{19}F NMR (CD_3OD)** δ -112.53 **LC-MS:** (Method A) t_R = 3.49 min, (ESI) m/z = 962.1 ([M+H]$^+$, 100%). **HPLC:** (Method A) t_R = 5.69 min, 96% pure (254 nm). **HRMS:** (ESI+) calc'd for $[C_{46}H_{53}N_9O_7S_2ClF + H]^+$, 962.3255 found 962.3259. **TLC:** DCM : MeOH (95 : 5), R_f= 0.39

LH-3 (General scheme B): Methyl (*R*)-4-(2-chloro-4-fluorophenyl)-6-((4-(6-(((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-6-oxohexanoyl)piperazin-1-yl)methyl)-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate



A solution of **S2** (13 mg, 24 µmol, 1 equiv.) in DCM : TFA (1 : 1, 2 mL) was stirred for 30 min, before the removal of DCM and TFA under a stream of nitrogen giving a brown oil. The resultant amine was treated with **S7** (16 mg, 28 µmol, 1.1 equiv.), HATU (11 mg, 23 µmol, 1.2 equiv.) and DIPEA (12 mg, 95 µmol, 4 equiv.) according to General Method A. The crude product was purified via column chromatography (0 - 7.5% MeOH/DCM) and preparative RP-HPLC (45–65% MeCN/H_2O, 15 min) to yield a yellow solid (8 mg, 28%, as the doubly protonated TFA salt). **^{1}H NMR (CD_3OD)** δ 9.04 (s, 1H), 7.99 (d, *J* = 3.1 Hz, 1H), 7.88 (d, *J* = 3.1 Hz, 1H), 7.52 (dd, *J* = 8.7, 5.9 Hz, 1H), 7.50 – 7.40 (m, 4H), 7.27 (dd, *J* = 8.6, 2.6 Hz, 1H), 7.10 (td, *J* = 8.3, 2.6 Hz, 1H), 6.18 (s, 1H), 4.82 – 4.46 (m, 7H), 4.38 (d, *J* = 15.5 Hz, 1H), 3.97 (bs, 3H), 3.91 (d, *J* = 11.1 Hz, 1H), 3.81 (dd, *J* = 11.0, 3.9 Hz, 1H), 3.62 (s, 3H), 3.61 – 3.43 (m, 4H), 2.52 – 2.46 (m, 5H), 2.41 – 2.16 (m, 3H), 2.09 (ddd, *J* = 13.3, 9.1, 4.5 Hz, 1H), 1.74 – 1.60 (m, 4H), 1.04 (s, 9H). **^{13}C NMR** δ 175.6, 174.5, 173.9, 172.3, 166.8, 163.4 (d, J_{CF}= 246.9 Hz), 162.4, 161.3, 153.2, 152.5, 149.4, 145.9, 140.5, 138.5 (d, J_{CF}= 3.6 Hz), 134.3, (d, J_{CF}= 10.4 Hz), 133.8, 133.0 (d, J_{CF}= 9.2 Hz), 131.1, 130.3, 129.0, 126.7, 118.1 (d, J_{CF}= 25.0 Hz), 116.0 (d, J_{CF}= 21.4 Hz), 107.3, 71.1, 60.9, 59.3, 59.0, 58.0, 54.0, 53.9, 52.2, 51.7, 43.7, 39.0, 36.6, 36.1, 33.3, 27.0, 26.4, 25.7, 15.5. **^{19}F NMR (CD_3OD)** δ -112.00, -76.55 (CF_3COOH) **LC-MS:** (Method A) t_R = 3.41 min, (ESI) *m/z* = 990.3 ($[M+H]^+$, 100%). **HPLC:** (Method A) t_R = 5.63 min, 95% pure (254 nm). **HRMS:** (ESI+) calc'd for $[C_{48}H_{57}N_9O_8S_2ClF + H]^+$ 990.3568, found 990.3582. **TLC:** DCM : MeOH (92.5 : 7.5), R_f= 0.25

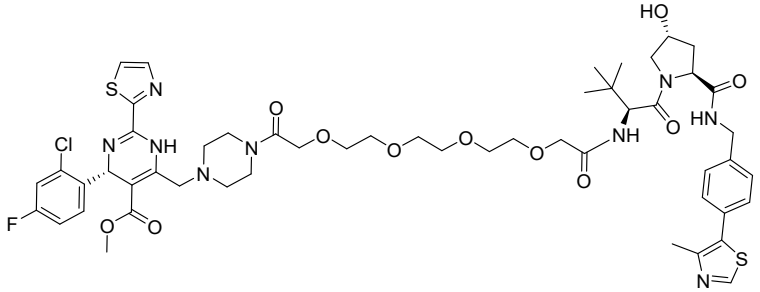
LH-4 (General scheme B): Methyl (*R*)-4-(2-chloro-4-fluorophenyl)-6-((4-(8-(((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-8-oxooctanoyl)piperazin-1-yl)methyl)-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate



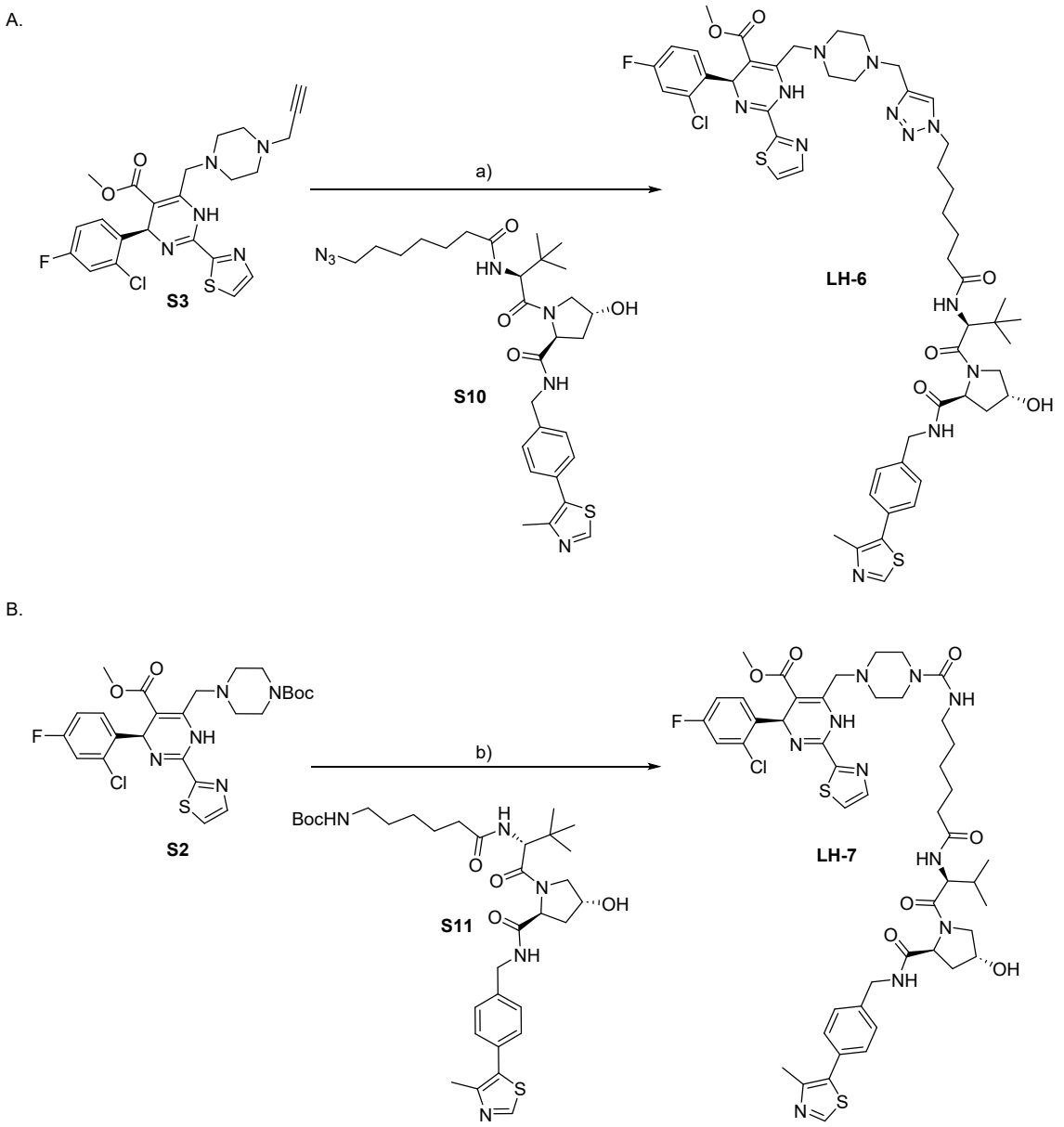
A solution of **S2** (11 mg, 19 µmol, 1 equiv.) in DCM : TFA (1 : 1, 4 mL) was stirred for 30 min, before the removal of DCM and TFA under a stream of nitrogen giving a brown oil. The resultant amine was treated with **S8** (13 mg, 23 µmol, 1.1 equiv.), HATU (9 mg, 23 µmol, 1.2 equiv.) and DIPEA (10 mg, 77 µmol, 4 equiv.) according to General Method A. The title compound was obtained as a yellow solid (11 mg, 54%) after preparative TLC (7.5% MeOH/DCM). **^{1}H NMR (CD_3OD)** δ 8.87 (s, 1H), 8.63 (bt, *J* = 6.0 Hz, 1H), 7.97 (d, *J* = 3.1 Hz, 1H), 7.88 – 7.78 (m, 2H), 7.52 – 7.37 (m, 6H), 7.26 (dd, *J* = 8.7, 2.7 Hz, 1H), 7.08 (td, *J* = 8.4, 2.7 Hz, 1H), 6.17 (s, 1H), 4.68 – 4.47 (m, 6H), 4.40 – 4.32 (m, 2H), 3.95 – 3.75 (m, 5H), 3.62 (s, 2H), 3.61 (s, 3H), 2.47 (s, 3H), 2.46 – 2.40 (m, 2H), 2.36 – 2.15 (m, 4H), 2.08 (ddd, *J* = 13.3, 9.1, 4.5 Hz, 1H), 1.63 (t, *J* = 7.2 Hz, 4H), 1.40 – 1.35 (m, 4H), 1.04 (s, 9H). **^{13}C NMR**

(CD_3OD) δ 176.0, 174.5, 174.3, 172.3, 152.9, 145.5, 140.4, 138.7 (d, J_{CF}= 3.8 Hz) 134.5 (d, J_{CF}= 8.2 Hz), 133.4, 132.7 (d, J_{CF}= 9.7 Hz), 131.5, 130.3, 129.0, 126.1, 118.0 (d, J_{CF}= 25.5 Hz), 115.9 (d, J_{CF}= 22.3 Hz) 71.1, 60.8, 59.0, 58.0, 54.1, 53.9, 52.0, 49.4, 43.7, 43.3, 38.8, 36.5, 33.7, 30.0, 29.9, 27.0, 26.8, 26.1, 15.8. One aliphatic CH, four aliphatic CH_2, one aromatic CH, three aromatic quaternary and one carbonyl carbon not observed. **^{19}F NMR (CD_3OD)** δ -113.29 **LC-MS:** (Method A) t_R = 3.51 min, (ESI) *m/z* = 1018.3 ($[M+H]^+$, 100%). **HPLC:** (Method A) t_R = 5.90 min, 95% pure (254 nm). **HRMS:** (ESI+) calc'd for $[C_{50}H_{61}N_9O_7S_2ClF + H]^+$ 1018.3881, found 1018.3877. **TLC:** DCM : MeOH (92.5 : 7.5), R_f = 0.41

LH-5 (General scheme B): Methyl (*R*)-4-(2-chloro-4-fluorophenyl)-6-((4-((*S*)-16-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidine-1-carbonyl)-17,17-dimethyl-14-oxo-3,6,9,12-tetraoxa-15-azaoctadecanoyl)piperazin-1-yl)methyl)-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate

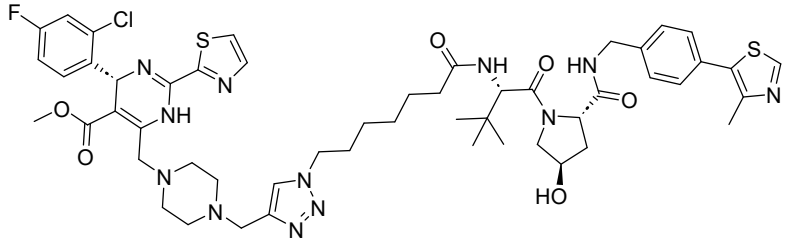


A solution of **S2** (10 mg, 18 µmol, 1 equiv.) in DCM : TFA (1 : 1, 2 mL) was stirred for 30 min, before the removal of DCM and TFA under a stream of nitrogen giving a brown oil. The resultant amine was treated with **S9** (12 mg, 18 µmol, 1 equiv.), HATU (8 mg, 18 µmol, 1.2 equiv.) and DIPEA (9 mg, 71 µmol, 4 equiv.) according to General Method A. The crude product was purified via column chromatography (0 - 10% MeOH/DCM) to yield a yellow solid (9 mg, 47%). **^{1}H NMR (CD_3OD)** δ 8.86 (s, 1H), 7.92 (d, *J* = 3.1 Hz, 1H), 7.74 (d, *J* = 3.2 Hz, 1H), 7.49 – 7.36 (m, 5H), 7.22 (dd, *J* = 8.8, 2.6 Hz, 1H), 7.03 (td, *J* = 8.4, 2.6 Hz, 1H), 6.15 (s, 1H), 4.70 (s, 1H), 4.63 – 4.47 (m, 3H), 4.36 (d, *J* = 15.5 Hz, 1H), 4.26 (s, 2H), 4.10 – 4.03 (m, 3H), 3.95 – 3.84 (m, 2H), 3.80 (dd, *J* = 11.0, 3.8 Hz, 1H), 3.76 – 3.60 (m, 16H), 3.58 (s, 3H), 2.69 – 2.54 (m, 4H), 2.47 (s, 3H), 2.23 (dd, *J* = 13.4, 7.6 Hz, 1H), 2.09 (ddd, *J* = 13.3, 9.3, 4.4 Hz, 1H), 1.04 (s, 9H). **^{13}C NMR (CD_3OD)** δ 174.4, 172.1, 171.6, 170.2, 167.8, 163.3, 163.1 (d, J_{CF}= 248.2 Hz), 161.9, 152.8, 149.1, 147.7, 146.6, 144.6, 140.3, 139.2 (d, J_{CF}= 3.6 Hz), 134.0, 133.4, 132.1 (d, J_{CF}= 8.5 Hz), 131.5, 130.4, 129.0, 125.2, 117.8 (d, J_{CF}= 24.9 Hz), 115.6 (d, J_{CF}= 21.1 Hz), 99.2, 72.2, 71.6, 71.5, 71.4, 71.4, 71.1, 71.1, 70.9, 60.8, 58.2, 58.1, 57.5, 57.1, 54.3, 54.0, 51.7, 49.2, 48.4, 46.3, 43.7, 43.2, 39.0, 37.1, 27.0, 15.9. **^{19}F NMR (CD_3OD)** δ -114.40 **LC-MS:** (Method A) t_R = 3.54 min, (ESI) *m/z* = 1110.3 ($[M+H]^+$, 100%). **HPLC:** (Method A) t_R = 5.68 min, ≥ 99% pure (254 nm). **HRMS:** (ESI+) calc'd for $[C_{52}H_{65}N_9O_{11}S_2ClF + H]^+$ 1110.399, found 1110.3967. **TLC:** DCM : MeOH (92.5 : 7.5), R_f= 0.31



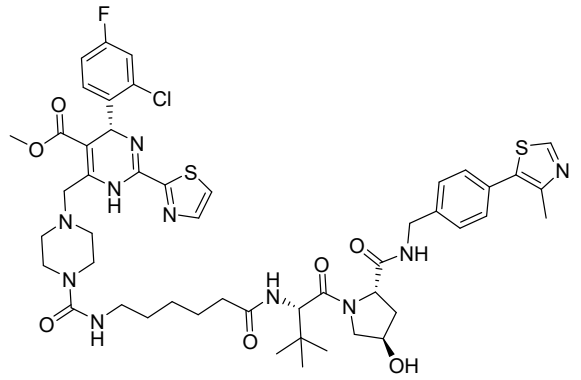
Scheme S9: Preparation of degrader candidates using triazole and urea linkage strategies. A. Synthesis of H-24 via CuAAC chemistry- a) CuSO4, sodium ascorbate, r.t, 20 h, 15%, B. Synthesis of H-25 using CDI coupling agent- b) i) P-13, 4 M HCl/dioxane, r.t, 30 min, ii) CDI, TEA, DCM, iii) H-4, TFA/DCM (1 : 1), r.t 30 min, iv) combine solutions, TEA, DMF, r.t, 26 h, 47%.

LH-6: Methyl (*R*)-4-(2-chloro-4-fluorophenyl)-6-((4-((1-(7-(((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-7-oxoheptyl)-1*H*-1,2,3-triazol-4-yl)methyl)piperazin-1-yl)methyl)-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate



To a solution of **S10** (15 mg, 26 µmol, 1 equiv.) and **S3** (15 mg, 29 µmol, 1.1 equiv.) in water : *tert*-butanol (1 : 1, 3 mL) was added freshly prepared aqueous solutions of $CuSO_4$ (100 mM, 77 µL, 8 µmol, 0.3 equiv.) and sodium ascorbate (100 mM, 26 µL, 3 µmol, 0.1 equiv.). The reaction mixture was then stirred at r.t for 20 h before removal of solvents *in vacuo* and suspension of the residue in water (20 mL). The suspension was extracted with DCM (2 × 15 mL) and the combined organic layers washed with brine (15 mL), dried over $MgSO_4$ and concentrated *in vacuo* to yield a yellow oil. The crude product was purified via column chromatography (0 - 15% MeOH/DCM) (4 mg, 15%). **^{1}H NMR ($CDCl_3$)** δ 8.80 (s, 1H), 8.21 (s, 1H), 7.87 (d, *J* = 3.1 Hz, 1H), 7.58 (d, *J* = 3.1 Hz, 1H), 7.51 – 7.42 (m, 1H), 7.41 – 7.31 (m, 5H), 7.14 (dd, *J* = 8.4, 2.6 Hz, 1H), 7.03 – 6.95 (m, 1H), 6.36 (bd, *J* = 8.5 Hz, 1H), 6.16 (s, 1H), 4.73 (t, *J* = 8.3 Hz, 1H), 4.61 – 4.48 (m, 3H), 4.46 – 4.30 (m, 6H), 4.10 (d, *J* = 11.2 Hz, 1H), 3.80 – 3.61 (m, 8H), 3.60 (s, 3H), 2.53 (s, 3H), 2.47 – 2.36 (m, 1H), 2.30 – 2.05 (m, 3H), 1.96 – 1.85 (m, 2H), 1.70 – 1.48 (m, 2H), 1.34 – 1.19 (m, 6H), 0.96 (s, 9H). **^{13}C NMR** Insufficient material to obtain a spectrum. **^{19}F NMR (CD_3OD)** δ -114.31 **LC-MS:** (Method A) t_R = 3.52 min, (ESI) *m/z* = 1071.3 ($[M+H]^+$, 60%), *m/z* = 536.3 ($[M+2H]^{2+}$, 100%). **HPLC:** (Method A) t_R = 5.87 min, 95% pure (254 nm). **HRMS:** (ESI+) calc'd for $[C_{52}H_{64}N_{12}O_6S_2ClF + H]^+$ 1071.4259, found 1071.4258. **TLC:** DCM : MeOH (9 : 1), R_f = 0.65

LH-7: Methyl (*R*)-4-(2-chloro-4-fluorophenyl)-6-((4-(((6-(((*S*)-1-((2*S*,4*R*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-6-oxohexyl)carbamoyl)piperazin-1-yl)methyl)-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate



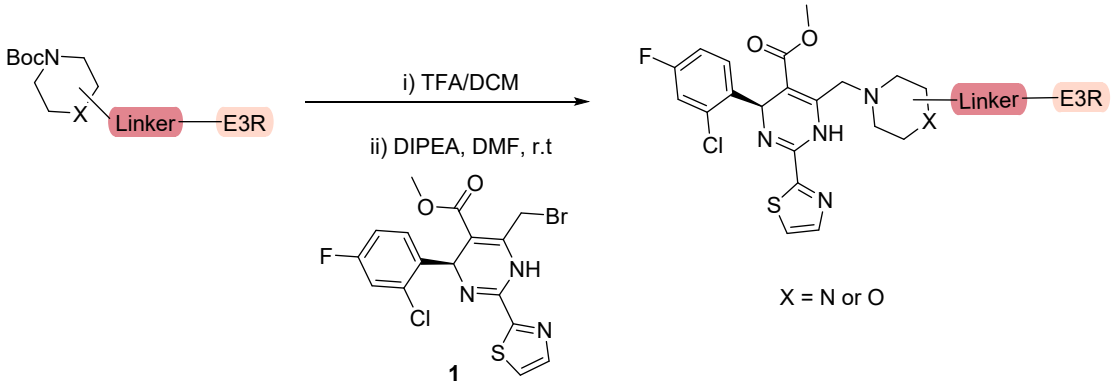
S11 (12 mg, 19 µmol, 1 equiv.) was stirred in 4 M HCl/dioxane at r.t for 30 min before removal of excess HCl by bubbling nitrogen through the suspension. Excess solvent was removed *in vacuo* and the residue suspended in DCM, followed by the addition of triethylamine until full dissolution of the hydrochloride salt. CDI (5 mg, 28 µmol, 1.5 equiv.) and triethylamine (6 mg, 57 µmol, 3 equiv.) were added and the solution stirred at r.t for 22 h at which point LC-MS analysis indicated the consumption of the starting material and formation of the activated carboxamide intermediate. Solvents were removed under a stream of nitrogen and the residue redissolved in DMF (500 µL).

A solution of **S2** (12 mg, 21 µmol, 1.1 equiv.) in DCM : TFA (1 : 1, 4 mL) was stirred for 30 min, before the removal of DCM and TFA under a stream of nitrogen. The residue was dissolved in DMF (500 µL) followed by the addition of triethylamine (6 mg, 57 µmol, 3 equiv.). This solution was combined with the activated carboxamide solution and stirred at r.t for 26 h. Solvents were removed under a stream of nitrogen and the residue redissolved in EtOAc (20 mL) and washed with water, sat. NH_4Cl, sat. $NaHCO_3$ and brine, dried over $MgSO_4$ and concentrated *in vacuo* to yield a yellow oil. The crude product was purified via preparative TLC (7.5% MeOH/DCM) to yield a yellow solid (9 mg, 47%). **^{1}H NMR ($CDCl_3$)** δ 8.98 (s, 1H), 7.99 (d, *J* = 3.1 Hz, 1H), 7.89 (d, *J* = 3.1 Hz, 1H), 7.52 (dd, *J* = 8.8, 6.0 Hz, 1H), 7.49 – 7.40 (m, 5H), 7.28 (dd, *J* = 8.6, 2.6 Hz, 1H), 7.11 (td, *J* = 8.3, 2.6 Hz, 1H), 6.18 (s, 1H), 4.73 (d, *J* = 16.2 Hz, 1H), 4.65 – 4.61 (m, 1H), 4.60 – 4.47 (m, 5H), 4.37 (d, *J* = 15.5 Hz, 1H), 3.90 (d, *J* = 10.8 Hz, 1H), 3.81 (dd, *J* = 10.9, 3.9 Hz, 1H), 3.63 (s, 3H), 3.57 – 3.43 (m, 4H), 3.22 – 3.14 (m, 3H), 2.49 (s, 3H), 2.35 – 2.17 (m, 4H), 2.08 (ddd, *J* = 13.3, 9.1, 4.5 Hz, 1H), 1.68 – 1.58 (m,

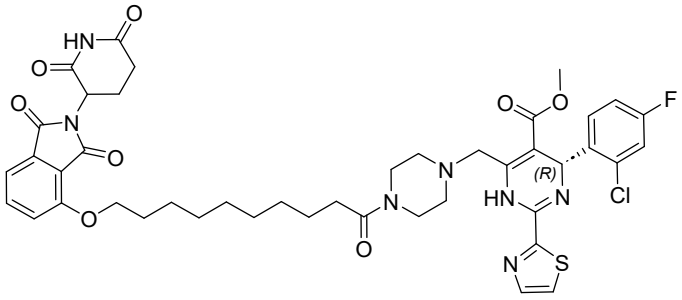
2H), 1.59 – 1.49 (m, 2H), 1.43 – 1.30 (m, 2H), 1.04 (s, 9H). **^{13}C NMR ($CDCl_3$)** δ 176.0, 174.4, 172.4, 168.8, 162.5, 152.9, 145.8, 142.2, 140.2, 138.4, 132.9 (d, J_{CF}= 8.8 Hz), 131.4, 130.3, 129.0, 126.7, 118.1 (d, J_{CF}= 24.9 Hz), 116.0 (d, J_{CF}= 21.6 Hz), 71.1, 60.8, 59.1, 59.0, 58.0, 53.9, 52.2, 49.6, 49.4, 43.7, 42.4, 42.0, 41.8, 38.9, 36.5, 30.7, 27.5, 27.0, 26.7, 15.8. Five quaternary aromatic carbon signals not observed. **^{19}F NMR (CD_3OD)** δ -112.82 **LC-MS:** (Method A) t_R = min, (ESI) *m/z* = ([M+H]$^+$, 100%). **HPLC:** (Method A) t_R = 5.74 min, 89% pure (254 nm). **HRMS:** (ESI+) calc'd for [$C_{49}H_{60}N_{10}O_7S_2ClF$ + H]$^+$ 1019.3833, found 1019.3812. **TLC:** DCM : MeOH (9 : 1), R_f= 0.62

SM series

General scheme:



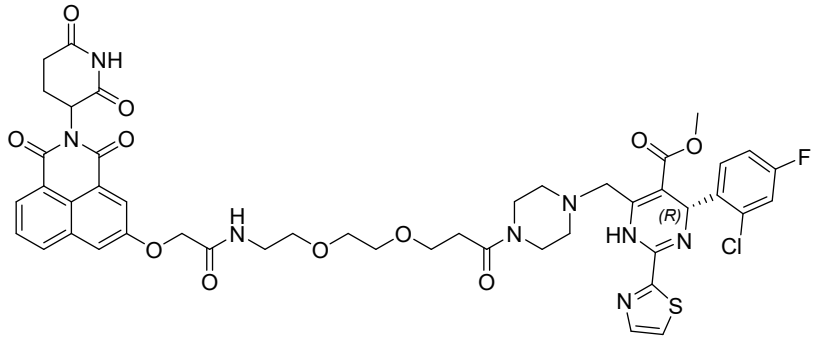
SM-1: Methyl (4*R*)-4-(2-chloro-4-fluorophenyl)-6-((4-(10-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)decanoyl)piperazin-1-yl)methyl)-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate



A solution of **S25** (23 mg, 38 µmol, 1 equiv.) was treated according to General method B using **1** (17 mg, 38 µmol, 1 equiv.), DIPEA (20 mg, 152 µmol, 4 equiv.) and KI (5 mg, 19 µmol, 0.5 equiv.) for 2.5 h and purified by column chromatography (DCM:EtOAc, 3: 1 [+ 3% MeOH]) to yield a pale yellow solid (28 mg, 89%). HRMS (ESI$^+$): found m/z 876.2953 (M + H)$^+$; ([$C_{43}H_{47}ClFN_7O_8S$] + H)$^+$ requires m/z 876.2952. ^{1}H NMR (401 MHz, Acetonitrile-d_3) δ 9.67 (s, 1H), 9.02 (s, 1H), 7.88 (d, *J* = 3.1 Hz, 1H), 7.73 (dd, *J* = 8.5, 7.3 Hz, 1H), 7.62 (d, *J* = 3.2 Hz, 1H), 7.45 – 7.34 (m, 3H), 7.25 (dd, *J* = 8.9, 2.7 Hz, 1H), 7.04 (td, *J* = 8.5, 2.7 Hz, 1H), 6.11 (s, 1H), 4.96 (dd, *J* = 12.0, 5.4 Hz, 1H), 4.19 (t, *J* = 6.4 Hz, 2H), 3.97 (d, *J* = 17.1 Hz, 1H), 3.85 (d, *J* = 17.0 Hz, 1H), 3.69 – 3.49 (m, 7H), 2.83 – 2.61 (m, 3H), 2.60 – 2.46 (m, 4H), 2.36 – 2.27 (m, 2H), 2.10 – 2.05 (m, 1H), 1.87 – 1.75 (m, 2H), 1.61 – 1.45 (m, 4H), 1.44 – 1.23 (m, 8H). HPLC: t_R= 5.58 min, >95% purity at 254 nm; LC-MS (ESI$^+$) *m/z*: 876.1 (M + H)$^+$ (100%).

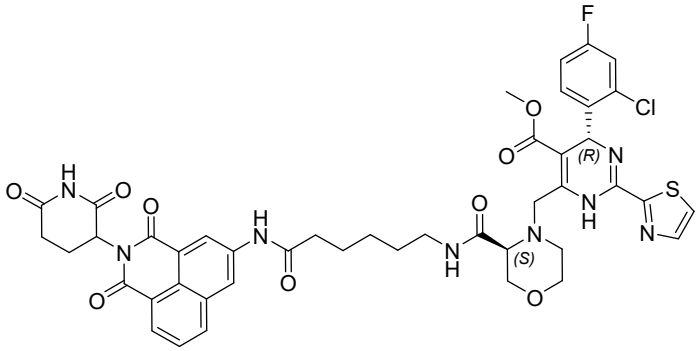
SM-2: Methyl (4*R*)-4-(2-chloro-4-fluorophenyl)-6-((4-(3-(2-(2-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-5-

yl)oxy)acetamido)ethoxy)ethoxy)propanoyl)piperazin-1-yl)methyl)-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate



A solution of **S22** (37 mg, 42 µmol, 1 equiv.) was treated according to General method B using **1** (19 mg, 42 µmol, 1 equiv.), DIPEA (22 mg, 168 µmol, 4 equiv.) and KI (6 mg, 21 µmol, 0.5 equiv.) for 2 h and purified by column chromatography (DCM:MeOH, 95:5) to yield a yellow solid (49 mg, 94%). HRMS (ESI$^+$): found m/z 973.2773 (M + H)$^+$; ([$C_{46}H_{46}ClFN_8O_{11}S$] + H)$^+$ requires m/z 973.2752. ^{1}H NMR (401 MHz, CD_3CN) δ 9.61 (s, 1H), 8.99 – 8.87 (m, 1H), 8.47 – 8.31 (m, 1H), 8.31 – 8.17 (m, 2H), 7.84 (d, *J* = 3.2 Hz, 1H), 7.79 – 7.70 (m, 2H), 7.59 (d, *J* = 3.2 Hz, 1H), 7.40 (dd, *J* = 8.7, 6.2 Hz, 1H), 7.34 – 7.28 (m, 1H), 7.23 (dd, *J* = 8.9, 2.7 Hz, 1H), 7.03 (td, *J* = 8.4, 2.7 Hz, 1H), 6.08 (s, 1H), 5.81 (dd, *J* = 11.2, 5.6 Hz, 1H), 4.68 (s, 2H), 3.92 (d, *J* = 17.1 Hz, 1H), 3.80 (d, *J* = 17.2 Hz, 1H), 3.67 (t, *J* = 6.4 Hz, 2H), 3.64 – 3.57 (m, 1H), 3.57 – 3.47 (m, 11H), 3.47 – 3.39 (m, 2H), 2.85 – 2.65 (m, 3H), 2.60 – 2.40 (m, 6H), 2.13 – 2.08 (m, 1H). [NB: 1H missing under solvent] HPLC: *tR* 5.01 min, >95% purity at 254 nm; LC-MS (ESI$^+$) *m/z*: 973.2 (M + H)$^+$ (100%).

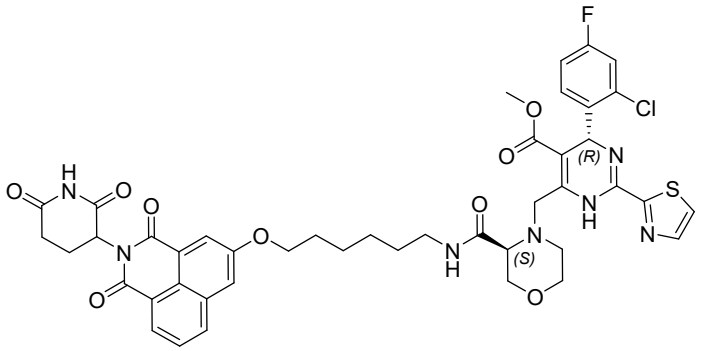
SM-3: Methyl (4*R*)-4-(2-chloro-4-fluorophenyl)-6-(((3*S*)-3-((6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-5-yl)amino)-6-oxohexyl)carbamoyl)morpholino)methyl)-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate



A solution of **S16** (46 mg, 62 µmol, 1 equiv.) was treated according to General method B using **1** (32 mg, 38 µmol, 1 equiv.), DIPEA (37 mg, 284 µmol, 4 equiv.) and KI (8 mg, 36 µmol, 0.5 equiv.) for 2 h and purified by column chromatography (DCM:MeOH, 95: 5) to yield a pale yellow solid (39 mg, 69%). HRMS (ESI$^+$):` found m/z 913.2543 (M + H)$^+$; $C_{44}H_{42}ClFN_8O_9S$ requires m/z 913.2541. ^{1}H NMR (401 MHz, DMSO) δ 11.02 (s, 1H), 10.53 (s, 1H), 10.02 (s, 1H), 8.85 (d, *J* = 6.9 Hz, 1H), 8.63 (d, *J* = 5.0 Hz, 1H), 8.47 – 8.28 (m, 2H), 8.23 (t, *J* = 5.7 Hz, 1H), 8.02 (d, *J* = 3.1 Hz, 1H), 7.93 (d, *J* = 3.1 Hz, 1H), 7.83 (q, *J* = 7.8 Hz, 1H), 7.44 – 7.32 (m, 2H), 7.15 (td, *J* = 8.4, 2.7 Hz, 1H), 6.01 (s, 1H), 5.89 – 5.78 (m, 1H), 4.05 (d, *J* = 16.9 Hz, 1H), 3.86 – 3.76 (m, 1H), 3.72 (d, *J* = 11.4 Hz, 1H), 3.64 (d, *J* = 16.9 Hz, 1H), 3.57 – 3.45 (m, 5H), 3.25 (dd, *J* = 8.8, 3.5 Hz, 1H), 3.19 – 3.03 (m, 2H), 3.01 – 2.86 (m, 1H), 2.84 – 2.75 (m, 1H), 2.65 – 2.54 (m, 2H), 2.47 – 2.34 (m, 3H), 2.10 – 1.96 (m, 1H), 1.71 – 1.55

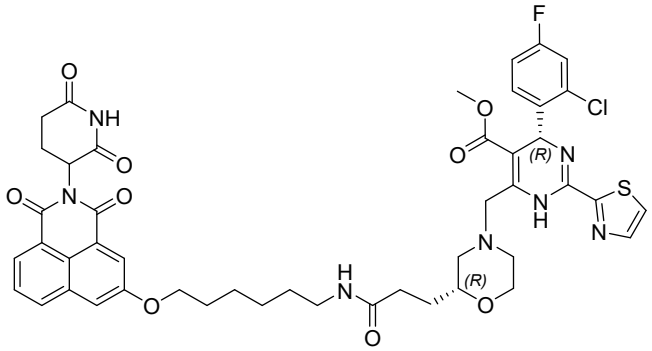
(m, 2H), 1.53 – 1.39 (m, 2H), 1.38 – 1.25 (m, 2H). HPLC: t_R 5.18 min, >95% purity at 254 nm; LC-MS (ESI^+) *m/z*: 913.2 $(M + H)^+$ (100%).

SM-4: Methyl (4*R*)-4-(2-chloro-4-fluorophenyl)-6-(((3*S*)-3-((6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-5-yl)oxy)hexyl)carbamoyl)morpholino)methyl)-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate



A solution of **S18** (30 mg, 47 µmol, 1 equiv.) was treated according to General method B using **1** (21 mg, 47 µmol, 1 equiv.), DIPEA (25 mg, 188 µmol, 4 equiv.) and KI (6 mg, 24 µmol, 0.5 equiv.) for 2 h and purified by column chromatography (DCM:MeOH, 97: 3) then triturated with Et_2O to yield a pale yellow solid (26 mg, 61%). HRMS (ESI^+): found m/z 900.2610 $(M + H)^+$; ($[C_{44}H_{43}ClFN_7O_9S] + H)^+$ requires m/z 900.2588. ^1H NMR (401 MHz, CD_3CN) δ 9.86 (s, 1H), 8.85 (s, 1H), 8.48 – 8.29 (m, 1H), 8.21 (d, *J* = 8.5 Hz, 1H), 8.16 – 8.02 (m, 1H), 7.88 (d, *J* = 3.1 Hz, 1H), 7.80 – 7.71 (m, 1H), 7.68 (d, *J* = 2.5 Hz, 1H), 7.60 (d, *J* = 2.5 Hz, 1H), 7.42 (dd, *J* = 8.8, 6.2 Hz, 1H), 7.15 (dd, *J* = 8.9, 2.7 Hz, 1H), 7.00 (td, *J* = 8.5, 2.6 Hz, 1H), 6.75 (s, 1H), 6.08 (s, 1H), 5.88 – 5.76 (m, 1H), 4.21 – 4.06 (m, 3H), 3.89 (dd, *J* = 11.2, 3.4 Hz, 1H), 3.78 – 3.72 (m, 1H), 3.69 (d, *J* = 17.0 Hz, 1H), 3.65 – 3.53 (m, 2H), 3.52 (s, 3H), 3.31 – 3.15 (m, 3H), 2.87 – 2.74 (m, 2H), 2.75 – 2.66 (m, 2H), 2.52 – 2.40 (m, 1H), 2.12 – 2.09 (m, 1H), 1.82 – 1.73 (m, 2H), 1.58 – 1.35 (m, 6H). HPLC: t_R 5.59 min, >95% purity at 254 nm; LC-MS (ESI^+) *m/z*: 900.2 $(M + H)^+$ (100%).

SM-5: Methyl (4*R*)-4-(2-chloro-4-fluorophenyl)-6-(((2*R*)-2-(3-((6-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxo-2,3-dihydro-1*H*-benzo[*de*]isoquinolin-5-yl)oxy)hexyl)amino)-3-oxopropyl)morpholino)methyl)-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate

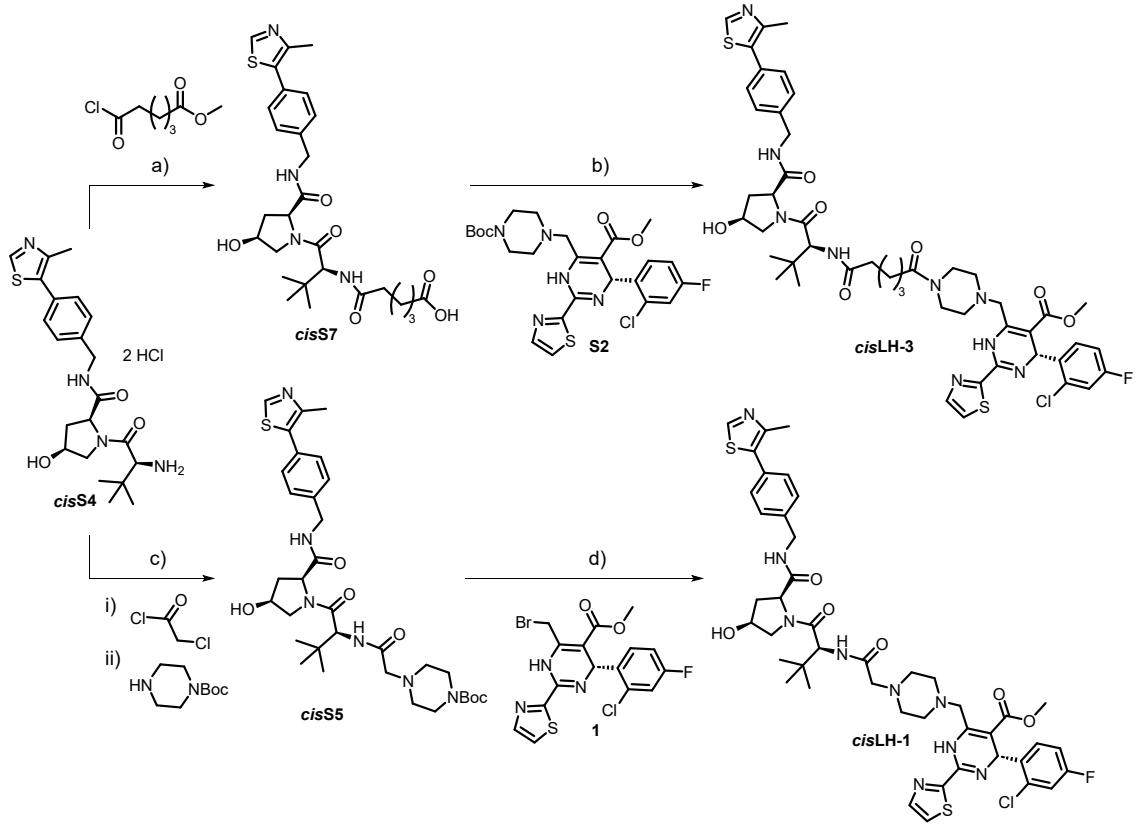


A solution of **S19** (30 mg, 45 µmol, 1 equiv.) was treated according to General method B using **1** (20 mg, 45 µmol, 1 equiv.), DIPEA (25 mg, 180 µmol, 4 equiv.) and KI (4 mg, 24 µmol, 0.5 equiv.) for 2 h and purified by column chromatography (DCM : EtOAc : MeOH, 65:30:5) to yield a pale yellow solid (35 mg, 84%). HRMS (ESI^+): found m/z 928.2922 $(M + H)^+$; ($[C_{46}H_{47}ClFN_7O_9S] + H)^+$ requires m/z 928.2901. ^1H NMR (401 MHz, CD_3CN) δ 9.62 (s, 1H), 8.88 (s, 1H), 8.35 (dd, *J* = 33.9, 7.3 Hz, 1H), 8.20 (d, *J* = 8.3 Hz, 1H), 8.17 – 8.02 (m, 1H), 7.88 (d, *J* = 3.2 Hz, 1H), 7.78 – 7.67 (m, 2H), 7.60 (d, *J* = 3.2 Hz, 1H), 7.38 (dd, *J* = 8.7, 6.2 Hz, 1H), 7.21 (dd, *J* = 8.9, 2.7 Hz, 1H), 7.01 (td, *J* = 8.5, 2.6

Hz, 1H), 6.31 (s, 1H), 6.04 (s, 1H), 5.85 – 5.76 (m, 1H), 4.15 (t, *J* = 6.6 Hz, 2H), 3.91 – 3.78 (m, 3H), 3.64 (td, *J* = 11.3, 2.4 Hz, 1H), 3.56 – 3.46 (m, 4H), 3.22 – 3.01 (m, 2H), 2.85 – 2.65 (m, 5H), 2.39 (td, *J* = 11.3, 3.2 Hz, 1H), 2.07 – 1.99 (m, 1H), 1.88 – 1.78 (m, 2H), 1.66 (q, *J* = 7.2 Hz, 2H), 1.56 – 1.44 (m, 4H), 1.43 – 1.33 (m, 2H) [NB. 3H under solvent]. HPLC: *tR* 5.46 min, >95% purity at 254 nm; LC-MS (ESI$^+$) *m/z*: 928.1 $(M + H)^+$ (100%).

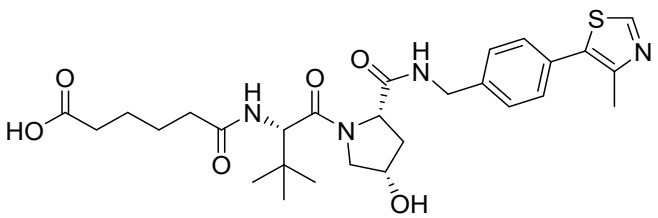
Synthesis of negative controls, cisLH-1 and cisLH-3

*cis*VH032-amine (*cis***S4**) was prepared according to previously reported procedures and spectroscopic characterisation consistent with literature vales.[14]



Scheme S10: Synthesis of *cis* epimers of *cis*LH-3 and *cis*LH-1 as chemicals controls- a) TEA, dioxane, 80 °C, 20 h, 24%, ii) $LiOH.H_2O$, 0 °C - r.t, 20 h, 75%, b) i) H-4, DCM/TFA, r.t, 30 min, ii) HATU, DIPEA, DMF, r.t, 2 h 50%, c) i) DIPEA, DCM, 0 °C - r.t, 2 h, ii) TEA, KI, DMF, 60 °C, 18 h, 58% (2 steps), d) DIPEA, KI, DMF, r.t, 18 h, 71%.

***cis*S7: 6-(((*S*)-1-((2*S*,4*S*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-6-oxohexanoic acid[9]**

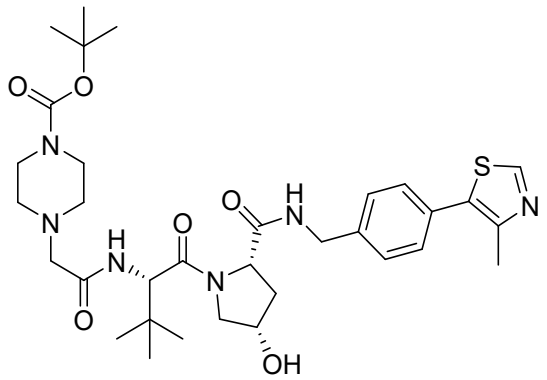


A solution of ***cis*S4** (40 mg, 79 µmol, 1 equiv.) in anhydrous dioxane (2 mL) was treated with triethylamine (24 mg, 238 µmol, 3 equiv.) and methyl-6-chloro-6-oxohexanoate (19 mg, 103 µmol, 1.3 equiv.) and stirred for 20 h at 80 °C. Solvents were removed *in vacuo* and the residue dissolved in DCM (10 mL), washed with water (10 mL) and brine (10 mL), dried over $MgSO_4$ and concentrated, yielding

a brown oil. The crude product was purified by column chromatography (2 - 5% MeOH/DCM) to yield a white solid (11 mg, 24%.).

The purified product (10 mg, 17 µmol, 1 equiv.) was dissolved in MeOH/H_2O (2 : 1, 1.5 mL) and cooled to 0 °C before the addition of LiOH.H_2O (2 mg, 35 µmol, 2 equiv.). The mixture was allowed to warm to r.t and stirred for 20 h before solvents were removed *in vacuo*. The remaining residue was dissolved in water (10 mL) and acidified to pH 3 with 1 M HCl before extraction with EtOAc (3 × 10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated, yielding a white solid (7 mg, 75%, 18% over two steps). **^{1}H NMR (CD_3OD)** δ 8.88 (s, 1H), 7.88 (d, *J* = 7.9 Hz, 1H), 7.49 – 7.36 (m, 5H), 4.61 – 4.49 (m, 4H), 4.39 (d, *J* = 4.7 Hz, 1H), 4.04 (dd, *J* = 10.6, 5.1 Hz, 1H), 3.70 (dd, *J* = 10.5, 3.8 Hz, 1H), 2.48 (s, 3H), 2.46 – 2.38 (m, 1H), 2.38 – 2.23 (m, 4H), 2.02 – 1.95 (m, 1H), 1.62 (dq, *J* = 6.7, 3.5, 3.0 Hz, 4H), 1.04 (s, 9H). **LC-MS:** (Method A) t_R = 3.15 min, (ESI) *m/z* = 557.2 ([M-H]$^-$, 100%), *m/z* = 582.1 ([M+Na]$^+$, 20%). **TLC:** DCM : MeOH (9 : 1), R_f = 0.20

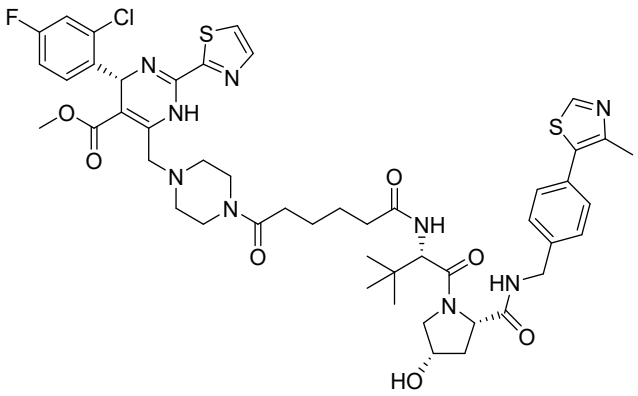
***cis*S5: *tert*-Butyl 4-(2-(((*S*)-1-((2*S*,4*s*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-2-oxoethyl)piperazine-1-carboxylate**



A solution of ***cis*S4** (20 mg, 40 µmol, 1 equiv.) and anhydrous DIPEA (21 mg, 159 µmol, 4 equiv.) in anhydrous DCM (2 mL) was cooled to 0 °C before the addition of chloroacetyl chloride (7 mg, 60 µmol, 1.1 equiv.). The mixture was allowed to warm to r.t and stirred for 2 h before dilution with DCM (15 mL). The solution was washed with sat. $NaHCO_3$ and brine (10 mL each) then dried over MgSO4 and concentrated *in vacuo*, yielding a yellow oil.

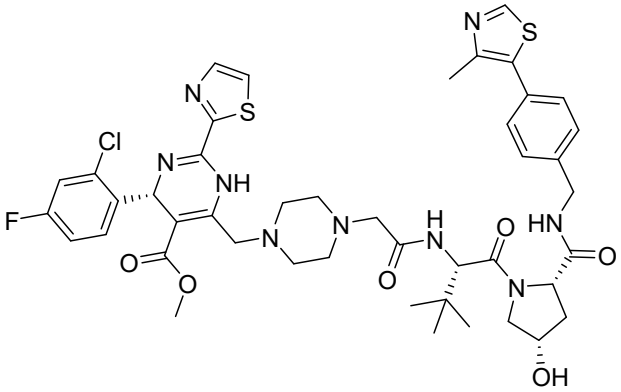
The crude intermediate was dissolved in DMF (1 mL) followed by the addition of 1-Boc-piperazine (37 mg, 199 µmol, 5 equiv.), triethylamine (12 mg, 119 µmol, 3 equiv.) and KI (3 mg, 20 µmol, 0.5 equiv.). The mixture was stirred at 60 °C for 18 h before removal of solvents under a stream of nitrogen. The residue was dissolved in 19% NH_4Cl (15 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with sat. $NaHCO_3$ and brine (15 mL each) then dried over $MgSO_4$ and concentrated *in vacuo*, yielding a brown oil. The crude product was purified via column chromatography (0 - 5% MeOH/DCM) to yield an off-white solid (15 mg, 58% over two steps). **^{1}H NMR ($CDCl_3$)** δ 8.69 (s, 1H), 7.68 (d, *J* = 9.0 Hz, 1H), 7.45 (d, *J* = 6.3 Hz, 1H), 7.37 (q, *J* = 8.2 Hz, 4H), 5.49 (d, *J* = 9.8 Hz, 1H), 4.74 (d, *J* = 8.9 Hz, 1H), 4.63 (dd, *J* = 14.9, 7.0 Hz, 1H), 4.53 – 4.39 (m, 2H), 4.32 (dd, *J* = 14.9, 5.1 Hz, 1H), 3.96 (dd, *J* = 10.9, 4.3 Hz, 1H), 3.79 (d, *J* = 10.9 Hz, 1H), 3.55 – 3.37 (m, 4H), 3.02 (s, 2H), 2.52 (s, 3H), 2.48 (s, 4H), 2.38 (d, *J* = 14.2 Hz, 1H), 2.18 (ddd, *J* = 13.9, 9.1, 4.9 Hz, 1H), 1.45 (s, 9H), 0.92 (s, 9H). **^{13}C NMR ($CDCl_3$)** δ 173.3, 172.6, 172.1, 169.9, 154.8, 150.5, 137.4, 131.5, 129.8, 128.4, 71.2, 61.5, 60.0, 58.9, 56.8, 55.4, 53.4, 43.7, 35.1, 34.9, 28.5, 26.5, 16.2. One quaternary aromatic signal not observed. **HPLC:** t_R = 5.00 min, 97% pure (254 nm). **HRMS:** (ESI+) calc'd for [$C_{33}H_{48}N_6O_6S$ + H]$^+$, 657.3429 found 657.3429. **LC-MS:** (Method A) t_R = 3.22 min, (ESI) *m/z* = 657.3 ([M+H]$^+$, 100%).**TLC:** DCM : MeOH (95 : 5), R_f = 0.29

***cis*LH-3: Methyl (*R*)-4-(2-chloro-4-fluorophenyl)-6-((4-(6-(((*S*)-1-((2*S*,4*S*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-6-oxohexanoyl)piperazin-1-yl)methyl)-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate**



A solution of ***cis*S7** (5 mg, 9 µmol, 1 equiv.) in DCM : TFA (1 : 1, 2 mL) was stirred for 30 min before the removal of DCM and TFA under a stream of nitrogen, giving a brown oil. The resultant amine was treated with **S2** (6 mg, 11 µmol, 1.2 equiv.), HATU (4 mg, 11 µmol, 1.2 equiv.) and DIPEA (5 mg, 36 µmol, 4 equiv.) according to General Method 4A. The crude product was purified via column chromatography (0 - 7.5% MeOH/DCM) to yield a yellow solid (4.4 mg, 50%). **^{1}H NMR (CD_3OD)** δ 8.87 (s, 1H), 7.92 (d, *J* = 3.1 Hz, 1H), 7.73 (d, *J* = 3.2 Hz, 1H), 7.49 – 7.36 (m, 5H), 7.22 (dd, *J* = 8.8, 2.6 Hz, 1H), 7.06 – 6.98 (m, 1H), 6.16 (s, 1H), 4.59 – 4.47 (m, 3H), 4.42 – 4.34 (m, 2H), 4.10 – 3.99 (m, 2H), 3.90 (d, *J* = 17.1 Hz, 1H), 3.77 – 3.63 (m, 5H), 3.58 (s, 3H), 2.68 – 2.54 (m, 4H), 2.47 (s, 3H), 2.47 – 2.40 (m, 3H), 2.40 – 2.23 (m, 2H), 1.98 (dt, *J* = 13.3, 4.7 Hz, 1H), 1.70 – 1.59 (m, 4H), 1.04 (s, 9H). **^{13}C NMR (CD_3OD)** δ 176.0, 174.9, 173.9, 172.6, 167.8, 152.9, 147.7, 144.6, 140.0, 132.1 (d, J_{CF}= 8.6 Hz), 131.6, 130.4, 129.1, 125.17, 123.0, 117.8 (d, J_{CF}= 24.7 Hz), 117.2, 115.5 (d, J_{CF}= 21.4 Hz), 71.5, 69.1, 61.0, 59.4, 57.6, 57.5, 57.1, 54.5, 54.0, 51.6, 47.1, 43.8, 43.0, 37.9, 36.1, 35.9, 33.7, 27.0, 26.6, 26.0, 15.8. Four quaternary aromatic signals not observed. **^{19}F NMR (CD_3OD)** δ -112.53 **LC-MS:** (Method A) t_R = 3.53 min, (ESI) *m/z* = 990.1 ($[M+H]^+$, 100%). **HPLC:** (Method A) t_R = 5.80 min, 96% pure (254 nm). **HRMS:** (ESI+) calc'd for $[C_{48}H_{57}ClFN_9O_7S_2 + H]^+$ 990.3568, found 990.3569. **TLC:** DCM : MeOH (92.5 : 7.5), R_f= 0.25

***cis*LH-1: Methyl (*R*)-4-(2-chloro-4-fluorophenyl)-6-((4-(2-(((*S*)-1-((2*S*,4*S*)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-2-oxoethyl)piperazin-1-yl)methyl)-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate**

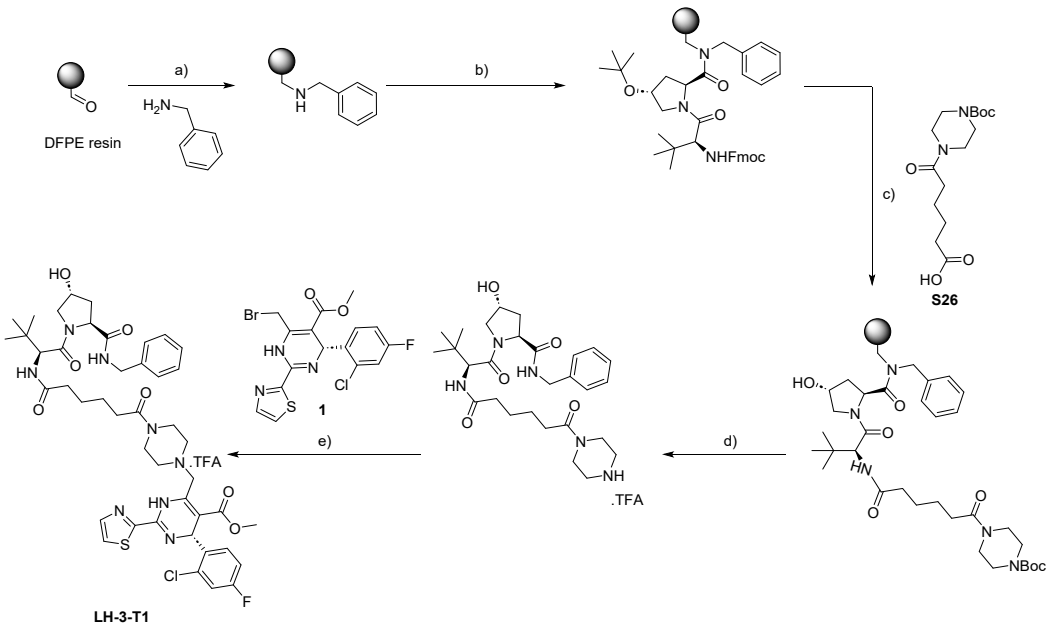


A solution of ***cis*S5** (10 mg, 15 µmol, 1 equiv.) was treated according to General method 4B using **1** (7 mg, 15 µmol, 1 equiv.), DIPEA (8 mg, 160 µmol, 4 equiv.) and KI (1 mg, 8 µmol, 0.5 equiv.) for 18 h and purified via column chromatography (0 - 5% MeOH/DCM) to yield a yellow solid (10 mg, 71%). **^{1}H NMR (CD_3OD)** δ 8.86 (s, 1H), 7.93 (d, *J* = 3.1 Hz, 1H), 7.73 (d, *J* = 3.1 Hz, 1H), 7.49 – 7.42 (m, 4H), 7.38 (dd, *J* = 8.7, 6.1 Hz, 1H), 7.21 (dd, *J* = 8.7, 2.6 Hz, 1H), 7.01 (td, *J* = 8.4, 2.6 Hz, 1H), 6.14 (s, 1H), 4.58 – 4.48 (m, 3H), 4.45 – 4.33 (m, 2H), 4.11 – 3.99 (m, 2H), 3.90 (d, *J* = 17.0 Hz, 1H), 3.72 (dd, *J* = 10.6, 3.9 Hz, 1H), 3.58 (s, 3H), 3.12 (d, *J* = 6.8 Hz, 2H), 2.71 (bs, 8H), 2.48 (s, 3H), 2.45 – 2.39 (m, 1H), 1.99 (dt, *J* = 13.3, 4.6 Hz, 1H), 1.05 (s, 9H). **^{13}C NMR (CD_3OD)** δ 174.7, 172.6, 172.2, 167.8, 152.9, 144.6, 140.0, 132.1 (d, J_{CF}= 9.7 Hz), 131.7, 130.4, 129.3, 129.1, 125.1, 117.8 (d, J_{CF}= 24.9 Hz), 115.5 (d, J_{CF}= 21.5 Hz), 71.5, 61.8, 61.0, 58.5, 57.6, 57.5, 57.2, 54.5, 51.6, 43.9, 37.9, 36.4, 27.0, 15.9.

Four quaternary aromatic signals not observed. **^{19}F NMR (CD_3OD)** δ -114.49 **LC-MS:** (Method B) t_R = 3.18 min, (ESI) *m/z* = 920.3 ([M+H]$^+$, 60%), *m/z* = 460.8 ([M+2H]$^{2+}$, 100%). **HPLC:** (Method A) t_R = 5.75 min, 99% pure (254 nm). **HRMS:** (ESI+) calc'd for [$C_{44}H_{51}ClFN_9O_6S_2$ + H]$^+$ 920.3149, found 920.3154. **TLC:** DCM : MeOH (92.5 : 7.5), R_f= 0.28

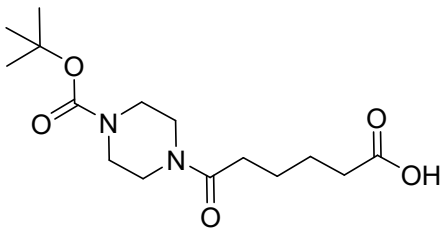
Synthesis of LH-3 and LH-1 analogues bearing altered E3 ligase recruiters

LH-3-T1



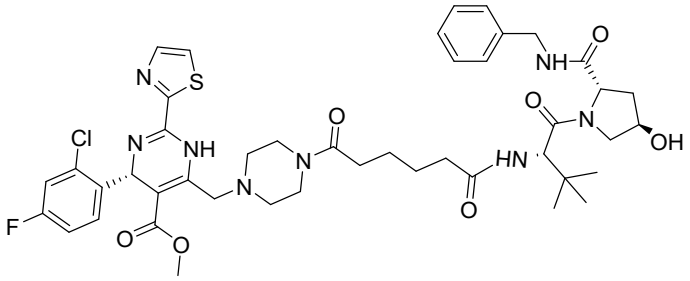
Scheme S11: Preparation of LH-3-T1 on DFPE resin- a) $NaCNBH_3$, DMF/MeOH/AcOH, 60 °C, 18 h, b) i) Fmoc-Hyp(O*t*Bu)-OH, HATU, DIPEA, DMF, r.t, 1 h, ii) 20% piperidine in DMF, iii) repeat with Fmoc-Tle-OH, c) i) 20% piperidine in DMF, r.t, 30 min, ii) HATU, DIPEA, DMF, r.t, 1 h, d) TFA : DCM (95 : 5), r.t, 1 h, 30% (crude, over 3 steps), e) DIPEA, KI, DMF, r.t, 18 h, 8%.

S26: 6-(4-(*tert*-Butoxycarbonyl)piperazin-1-yl)-6-oxohexanoic acid



A solution of 1-Boc-piperazine (200 mg, 1.07 mmol, 1 equiv.) and 2,7-oxepandione (90% pure, 170 mg, 1.18 mmol, 1.1 equiv.) in anhydrous DCM (2 mL) was heated to reflux for 2 h before the removal of solvent *in vacuo* to yield a white solid (339 mg). The crude product was a mixture of the desired product and a 1-Boc-piperazine dimer (approximately 50% pure, ~50% yield based on crude purity). The crude product was utilised in subsequent reactions without further purification. **LC-MS:** (Method A) t_R = 3.13 min, (ESI) *m/z* = 315.2 ([M+H]$^+$, 30%), *m/z* = 313.2 ([M-H]$^-$, 100%)

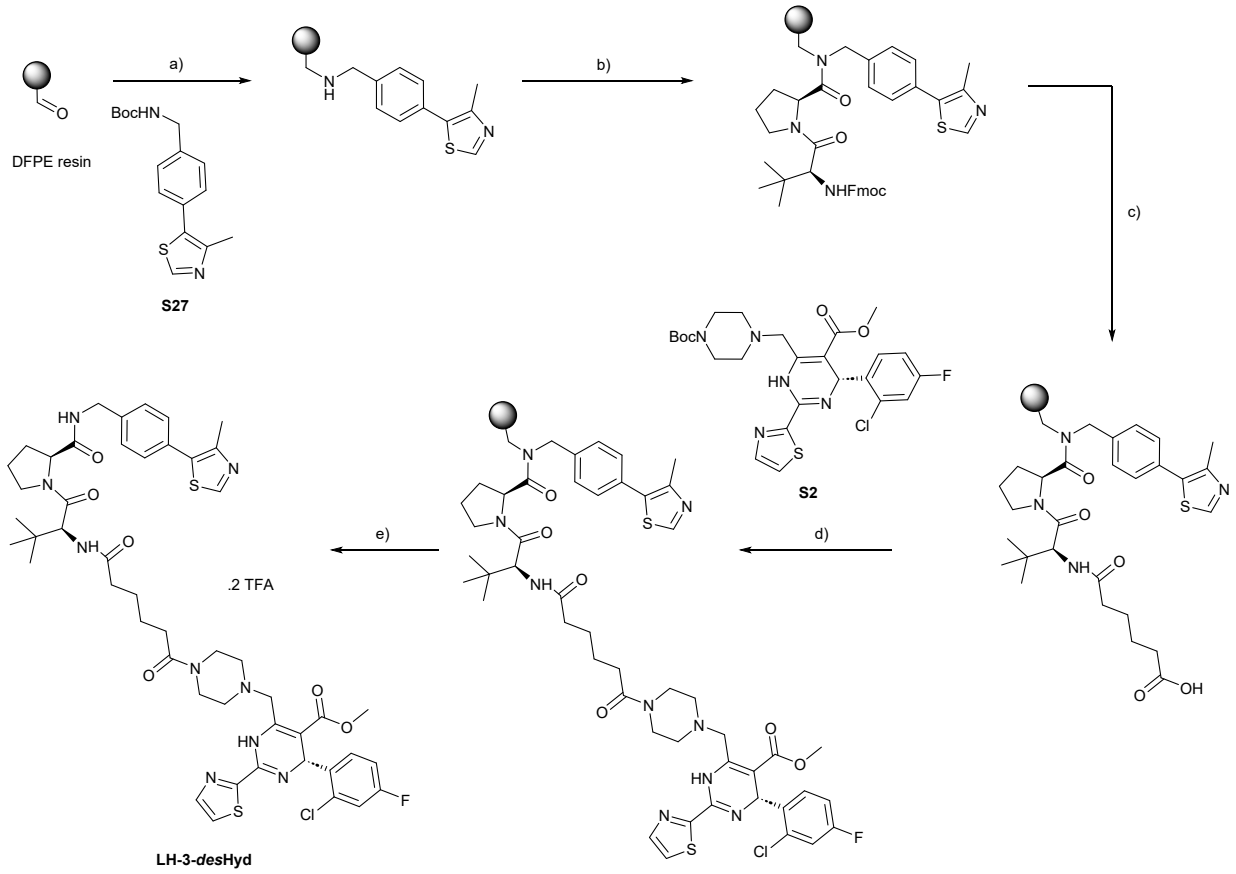
LH-3-T1: Methyl (*R*)-6-((4-(6-(((*S*)-1-((2*S*,4*R*)-2-(benzylcarbamoyl)-4-hydroxypyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-6-oxohexanoyl)piperazin-1-yl)methyl)-4-(2-chloro-4-fluorophenyl)-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate



DPFE resin (500 µmol, 1 equiv.) was swollen in DCM for 30 min then filtered and suspended in DMF/MeOH/AcOH (8 mL, 80 : 19 : 1) in a closed vessel. A solution of benzylamine (268 mg, 250 µmol, 5 equiv.) dissolved in DMF/MeOH/AcOH (2 mL, 80 : 19 : 1) was added, followed by the portion-wise addition of $NaCNBH_3$ (315 mg, 500 µmol, 10 equiv.). The suspension was heated to 60 °C for 18 h before the collection of the resin by filtration through a fritted syringe and subsequent washings with MeOH, DMF and DCM (5 × each). Chloranil test confirmed the presence of secondary amine. The resin (0.25 mmol) was coupled with Fmoc-L-Hyp(O*t*Bu)-OH (307 mg, 750 µmol, 3 equiv.) according to General method C using HCTU (310 mg, 750 µmol, 3 equiv.) and DIPEA (194 mg, 1.5 mmol, 6 equiv.). The Fmoc protecting group was removed according to General method E and amide coupling repeated using Fmoc-L-Tle-OH (265 mg, 750 µmol, 3 equiv.). Analytical cleavage according to general method F using TFA/DCM (95 : 5) indicated the formation of the resin-bound product (*m/z* = 556.3). The resin (125 µmol, 1 equiv.) was swollen in DCM for 30 min then filtered and Fmoc deprotected according to General method E before coupling with **S26** (236 mg, 50% pure, 375 µmol, 3 equiv.), HCTU (155 mg, 375 µmol, 3 equiv.) and DIPEA (97 mg, 750 µmol, 6 equiv.) according to General method C. The resin was cleaved according to General method G using TFA/DCM (95 : 5, 3 mL) to yield the crude intermediate product as a brown oil (24 mg, TFA salt, 30% crude yield).

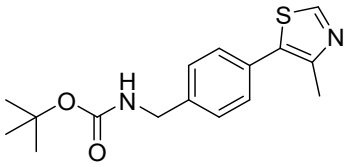
Compound **1** (4 mg, 9 µmol, 1 equiv.) was treated with the crude product from the previous step (7 mg, approx. 80% pure, 11 µmol, 1 equiv.) according to General method B. The crude product was purified by preparative RP-HPLC (40 - 60% MeCN/H_2O, 10 min) to yield the title compound as a yellow solid following lyophilisation (0.7 mg, 8%, as the singly protonated TFA salt). **^{1}H NMR (CD_3OD)** δ 8.49 (t, *J* = 6.1 Hz, 1H), 8.00 (d, *J* = 3.1 Hz, 1H), 7.89 (d, *J* = 3.1 Hz, 1H), 7.84 (d, *J* = 9.0 Hz, 1H), 7.51 (dd, *J* = 8.7, 6.0 Hz, 1H), 7.36 – 7.26 (m, 5H), 7.25 – 7.18 (m, 1H), 7.10 (td, *J* = 8.4, 2.6 Hz, 1H), 6.18 (s, 1H), 4.76 – 4.62 (m, 2H), 4.60 – 4.32 (m, 6H), 3.95 (s, 3H), 3.89 (d, *J* = 11.1 Hz, 1H), 3.80 (dd, *J* = 10.9, 4.0 Hz, 1H), 3.63 (s, 3H), 3.57 – 3.46 (m, 4H), 2.49 (t, *J* = 6.6 Hz, 2H), 2.33 (d, *J* = 6.5 Hz, 2H), 2.25 – 2.14 (m, 1H), 2.07 (ddd, *J* = 13.3, 9.0, 4.5 Hz, 1H), 1.70 – 1.63 (m, 4H), 1.02 (app d, *J* = 14.7 Hz, 9H, rotamers). **^{13}C NMR** Insufficient material to obtain a spectrum. **^{19}F NMR (CD_3OD)** δ -112.36, -76.55 (CF_3COOH). **LC-MS:** (Method A) t_R = 3.41 min, (ESI) *m/z* = 823.3 ($[M+H]^+$, 100%). **HPLC:** (Method A) t_R = 6.03 min, 96% pure (254 nm). **HRMS:** (ESI+) calc'd for $[C_{44}H_{54}N_8O_7SClF + H]^+$ 893.31581, found 893.3584. **TLC:** DCM : MeOH (92.5 : 7.5), R_f = 0.36

LH-3-desHyd



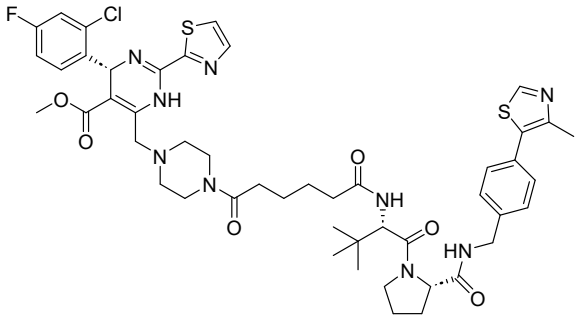
Scheme S12: Preparation of *des*-hydroxy derivative of LH-3 on DFPE resin a) i) 4M HCl/dioxane, r.t, 1 h, ii) $NaCNBH_3$, DMF/MeOH/AcOH, 60 °C, 18 h, b) i) HATU, DIPEA, DMF, r.t, 1 h, ii) 20% piperidine in DMF, r.t, 30 min, iii) repeat with next amino acid, c) i) 20% piperidine in DMF, r.t, 30 min, ii) succinic anhydride, TEA, DMF/DCM (1 : 1), r.t, 18 h, d) i) H-3, TFA : DCM (95 : 5), r.t, 30 min, ii) EDC.HCl, HOBt, DIPEA, DMF/DCM (1 : 1), r.t, 18 h, e) TFA : DCM (95 : 5), r.t, 1 h, 13% (4 steps).

S27*: tert*-Butyl (4-(4-methylthiazol-5-yl)benzyl)carbamate[7]



To a solution of *N*-Boc-4-bromobenzylamine (3.53 g, 12.3 mmol, 1 equiv.) in DMF (10 mL) was added $Pd(OAc)_2$ (29 mg 123 µmol, 0.01 equiv.), KOAc (2.42 g, 24.7 mmol, 2 equiv.) and 4-methylthiazole (2.45 g, 24.7 mmol, 2 equiv.). The reaction mixture was stirred at 140 °C for 24 h. The reaction mixture was then diluted with water and DMF removed *in vacuo* before extraction with DCM (3 × 50 mL). The combined organic layers was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give the crude product as a brown solid. The crude product was purified via column chromatography (33% EtOAc/Petroleum ether) yielding the title compound as a white solid (1.58 mg, 47%) **^{1}H NMR (CD_3OD)** δ 8.89 (s, 1H), 7.46 (d, *J* = 8.3 Hz, 2H), 7.40 (d, *J* = 8.5 Hz, 2H), 4.30 (d, *J* = 5.5 Hz, 2H), 2.50 (s, 3H), 1.48 (s, 9H). **LC-MS:** (Method A) t_R = 3.65 min, (ESI) m/z = 305.1 ($[M+H]^+$, 100%). **TLC:** Petroleum ether : EtOAc (2 : 1), R_f = 0.37

LH-3-*des*Hyd: Methyl (*R*)-4-(2-chloro-4-fluorophenyl)-6-((4-(6-(((*S*)-3,3-dimethyl-1-((*S*)-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-1-oxobutan-2-yl)amino)-6-oxohexanoyl)piperazin-1-yl)methyl)-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate

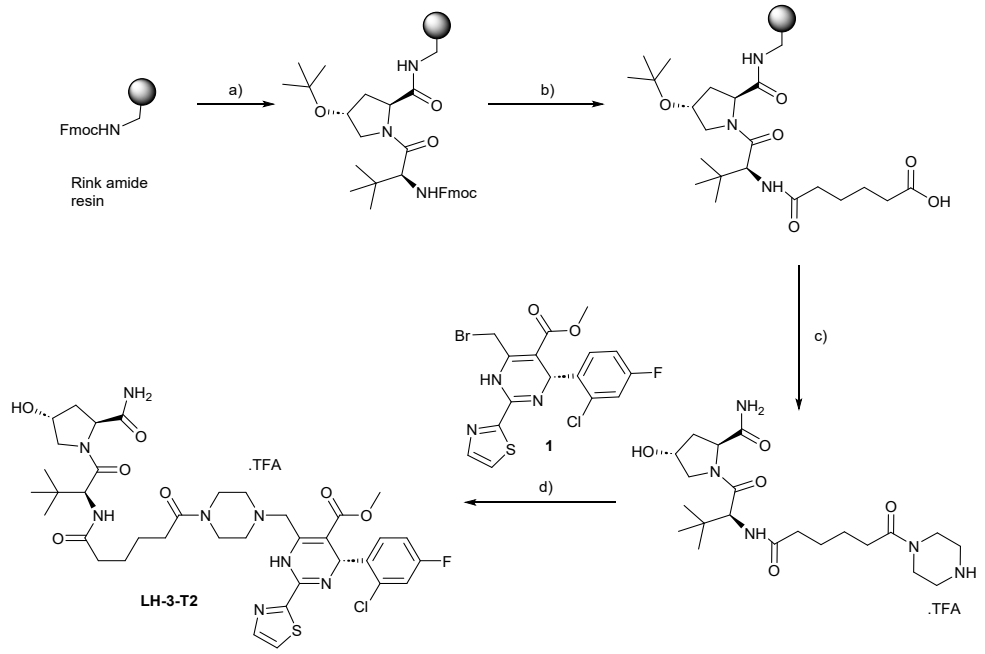


Intermediate resin was prepared from DFPE resin (200 µmol) in the same manner as described for LH-3-T1 using **S27** (treated with 4M HCl/dioxane for 1 h at r.t before use) in place of benzylamine, and Fmoc-L-Pro-OH in place of Fmoc-L-Hyp(O*t*Bu)-OH. A portion of the resin (50 µmol, 1 equiv.) was swollen in DCM for 30 min then filtered and Fmoc deprotected according to General method E. The resin was then suspended in DMF/DCM (1 : 1, 4 mL) before the addition of 2,7-oxepanedion (32 mg, 250 µmol, 5 equiv.) and triethylamine (30 mg, 300 µmol, 6 equiv.) and shaking at r.t for 4 h. The resin was collected by filtration and washed with DMF and DCM (5 × each).

Separately, **S2** (30 mg, 55 µmol, 1.1 equiv.) was dissolved in DCM/TFA (1 : 1, 2 mL) and stirred at r.t for 30 min before the removal of solvents under a stream of nitrogen. The resultant residue was dissolved in DCM/DMF (20 : 1, 1 mL) before the addition of DIPEA (39 mg, 300 µmol, 6 equiv.).

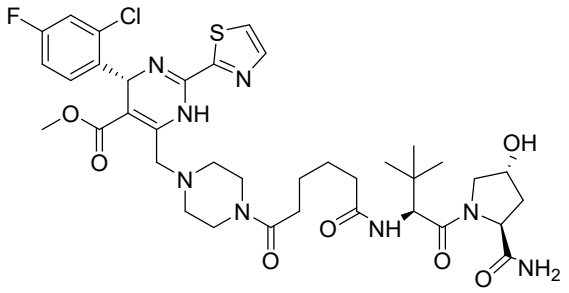
The resin was coupled with deprotected amine solution according to General method D. Cleavage according to General method G using TFA/DCM (95 : 5, 3 mL) gave the crude product as product as a yellow solid which was purified by preparative RP-HPLC (45 - 55% MeCN/H_2O, 15 min) to yield the title compound as a yellow solid following lyophilisation (7.7 mg, 13% over four steps, as the doubly protonated TFA salt). **^{1}H NMR (CD_3OD)** δ 8.95 (s, 1H), 8.01 (d, J = 3.1 Hz, 1H), 7.91 (d, J = 3.1 Hz, 1H), 7.86 (bd, J = 8.4 Hz, 1H), 7.53 (dd, J = 8.7, 6.0 Hz, 1H), 7.49 – 7.42 (m, 4H), 7.30 (dd, J = 8.7, 2.6 Hz, 1H), 7.12 (td, J = 8.3, 2.6 Hz, 1H), 6.19 (s, 1H), 4.78 – 4.35 (m, 7H), 4.03 – 3.94 (m, 4H), 3.78 – 3.70 (m, 1H), 3.64 (d, J = 1.6 Hz, 3H), 3.62 – 3.49 (m, 4H), 2.55 – 2.50 (m, 2H), 2.49 (s, 3H), 2.39 – 2.29 (m, 2H), 2.25 (dd, J = 8.2, 5.7 Hz, 1H), 2.21 – 2.08 (m, 2H), 2.04 – 1.93 (m, 2H), 1.73 – 1.63 (m, 4H), 1.07 (s, 9H). **^{13}C NMR** δ 175.9, 174.6, 173.9, 172.2, 170.2, 166.9, 163.6 (d, J_{CF}= 248.6 Hz), 152.9, 145.9, 140.3, 138.5 (d, J_{CF}= 1.9 Hz), 133.0 (d, J_{CF}= 9.7 Hz), 131.5, 130.4, 129.0, 127.0, 126.6, 118.1 (d, J_{CF}= 24.9 Hz), 116.0 (d, J_{CF}= 21.8 Hz), 62.0, 59.3, 59.3, 53.9, 52.2, 51.8, 49.6, 43.6, 36.05, 36.02 33.3, 30.7, 27.1, 26.4, 26.3, 25.6, 15.8. Four quaternary and three aromatic quaternary signals not observed. **^{19}F NMR (CD_3OD)** δ -112.38, -76.55 (CF_3COOH). **LC-MS:** (Method B) t_R = 3.29 min, (ESI) m/z = 974.3 ($[M+H]^+$, 100%). **HPLC:** (Method A) t_R = 6.10 min, ≥ 99% pure (254 nm). **HRMS:** (ESI+) calc'd for $[C_{48}H_{57}ClFN_9O_6S_2 + H]^+$ 974.3619, found 974.3634. **TLC:** DCM : MeOH (95 : 5), R_f= 0.36

LH-3-T2



Scheme S13: Preparation of LH-3-T2 on Rink amide resin i) 20% piperidine in DMF, r.t, 30 min, ii) Fmoc-Hyp-OH, HATU, DIPEA, DMF, r.t, 1 h, iii) repeat with Fmoc-Tle-OH, b) i) 20% piperidine in DMF, r.t, 30 min, ii) adipoyl chloride, DMF, r.t, 2 h, iii) H_2O wash, c) i) EDC.HCl, HOBt, DIPEA, DMF, r.t, 18 h, ii) TFA : DCM (95 : 5), r.t, 1 h, 71% (crude, over 3 steps), d) DIPEA, KI, DMF, r.t, 18 h, 27%.

LH-3-T2: Methyl (*R*)-6-((4-(6-(((*S*)-1-((2*S*,4*R*)-2-carbamoyl-4-hydroxypyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-6-oxohexanoyl)piperazin-1-yl)methyl)-4-(2-chloro-4-fluorophenyl)-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate

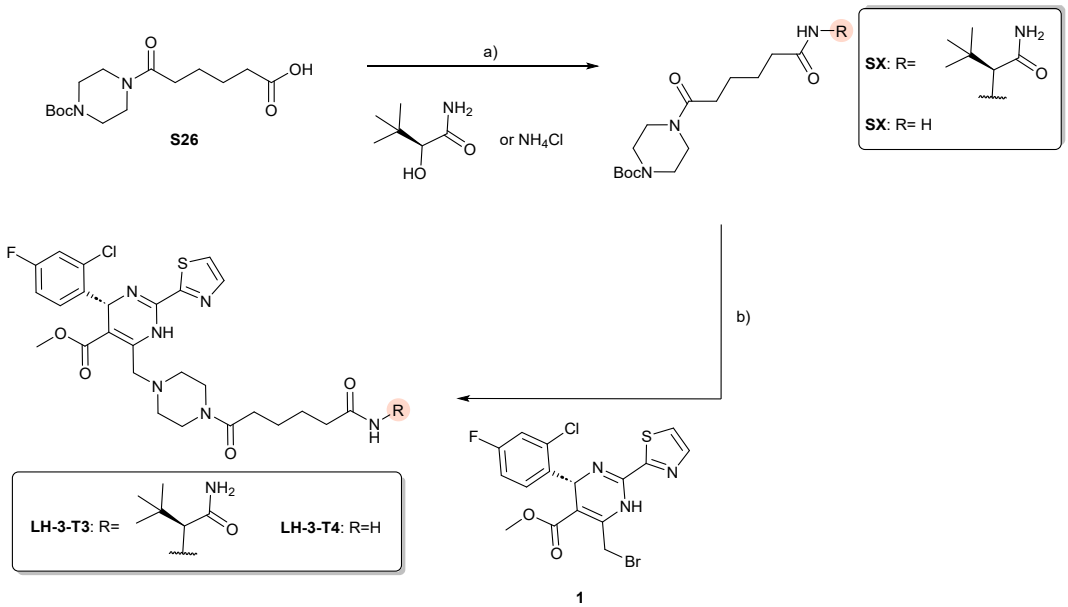


Rink amide AM resin (260 mg, 200 µmol, 0.7 mmol/g loading) was swollen in DCM for 30 min then filtered and suspended in DMF before removal of the Fmoc group by General method E. The resin was coupled with Fmoc-L-Hyp(O*t*Bu)-OH (246 mg, 600 µmol, 3 equiv.) according to General method C using HCTU (248 mg, 600 µmol, 3 equiv.) and DIPEA (155 mg, 1.2 mmol, 6 equiv.). The Fmoc protecting group was removed according to General method E and the cycle repeated using Fmoc-L-Tle-OH (212 mg, 600 µmol, 3 equiv.). To the resin (100 µmol) was added adipoyl chloride (128 µmol, 7 equiv.) in DMF (3 mL) and the suspension shaken at r.t for 2 h. The resin was collected by filtration and washed with water, DMF and DCM (5 × each). Analytical cleavage according to General method F using TFA indicated the formation of the resin-bound product (*m/z* = 370.2).

The resin was coupled with 1-Boc-piperazine (56 mg, 300 µmol, 3 equiv.) according to General method D and cleaved according to General method G using TFA (3 mL) to yield the crude intermediate product as a brown oil (55 mg, TFA salt, 71% crude yield). **1** (5 mg, 11 µmol, 1 equiv.) was treated with the crude product (6 mg, 17 µmol, 1.5 equiv.) according to General method B and the product was purified

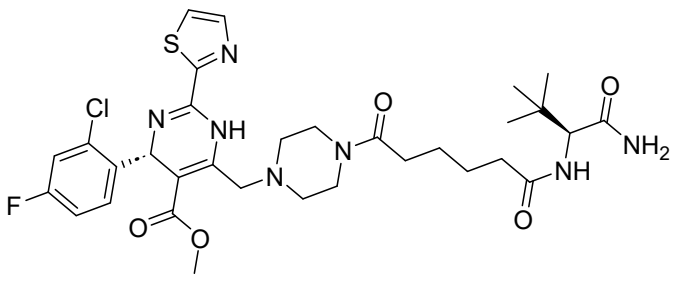
by preparative RP-HPLC (45 - 55% MeCN/H_2O, 10 min) to yield the title compound as a yellow solid following lyophilisation (3.9 mg, 27%, as the singly protonated TFA salt). **^{1}H NMR (CD_3OD)** δ 8.00 (d, *J* = 3.1 Hz, 1H), 7.90 (d, *J* = 3.1 Hz, 1H), 7.52 (dd, *J* = 8.7, 5.9 Hz, 1H), 7.28 (dd, *J* = 8.7, 2.6 Hz, 1H), 7.11 (td, *J* = 8.3, 2.6 Hz, 1H), 6.18 (s, 1H), 4.78 – 4.43 (m, 5H), 4.06 – 3.92 (m, 4H), 3.88 (dt, *J* = 11.0, 1.8 Hz, 1H), 3.78 (dd, *J* = 11.0, 4.0 Hz, 1H), 3.63 (s, 3H), 3.60 – 3.47 (m, 4H), 2.49 (t, *J* = 6.8 Hz, 2H), 2.38 – 2.27 (m, 2H), 2.26 – 2.16 (m, 1H), 2.04 (ddd, *J* = 13.3, 9.0, 4.5 Hz, 1H), 1.73 – 1.58 (m, 4H), 1.05 (s, 9H). **^{13}C NMR (CD_3OD)** δ 176.7, 175.6, 173.9, 172.3, 166.9, 163.7 (J_{CF}= 241.7 Hz), 152.5, 149.4, 145.9, 138.5 (d, J_{CF}= 3.7 Hz), 134.4 (d, J_{CF}= 10.5 Hz), 132.97 (d, J_{CF}= 9.1 Hz), 126.7, 118.14 (d, J_{CF}= 21.4 Hz), 116.02 (d, J_{CF}= 21.4 Hz), 107.3, 71.0, 60.2, 59.3, 59.0, 57.9, 52.2, 51.7, 43.7, 39.1, 36.5, 36.1, 33.3, 27.0, 26.4, 25.6. **^{19}F NMR (CD_3OD)** δ -112.43, -76.55 (CF_3COOH). **LC-MS:** (Method A) t_R = 3.14 min, (ESI) *m/z* = 803.3 ($[M+H]^+$, 100%). **HPLC:** (Method A) t_R = 5.28 min, 95% pure (254 nm). **HRMS:** (ESI+) calc'd for $[C_{37}H_{48}N_8O_7SClF + H]^+$ 803.3112, found 803.3117. **TLC:** DCM : MeOH (92.5 : 7.5), R_f = 0.19

LH-3-T3 and LH-3-T4



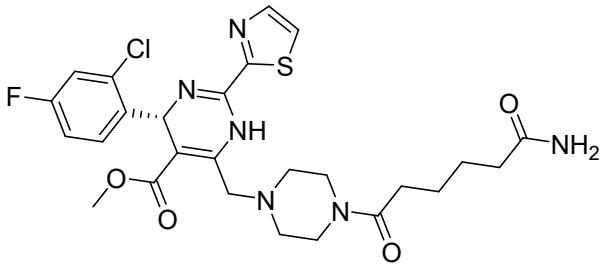
Scheme S14: Preparation of LH-3 and truncates, T3 and T4- a) HATU, DIPEA, DMF, r.t, 2 h, 30 - 65%, b) DIPEA, KI, DMF, r.t, 18 h, 28 - 82%.

LH-3-T3: Methyl (*R*)-6-((4-(6-(((*S*)-1-amino-3,3-dimethyl-1-oxobutan-2-yl)amino)-6-oxohexanoyl)piperazin-1-yl)methyl)-4-(2-chloro-4-fluorophenyl)-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate



S26 (100 mg, 50% pure, 159 µmol, 1 equiv.) and 2-amino-3,3-dimethylbutanamide hydrochloride (53 mg, 318 µmol, 2 equiv.) were coupled according to General Method A using HATU (121 mg, 318 µmol, 2 equiv.) and DIPEA (82 mg, 636 µmol, 4 equiv.). The crude product was purified via column chromatography (0 - 10% MeOH/DCM) to yield the title compound as a clear oil (44 mg, 65%). **^{1}H NMR (CD_3OD)** δ 7.76 (d, *J* = 9.1 Hz, 1H), 4.33 – 4.23 (m, 1H), 3.60 – 3.51 (m, 4H), 3.43 (app. dt, 4H), 2.43 (t, *J* = 7.0 Hz, 2H), 2.36 – 2.27 (m, 2H), 1.73 – 1.57 (m, 4H), 1.47 (s, 9H), 1.02 (s, 9H). **^{13}C NMR (CD_3OD)** δ 175.60, 175.52, 175.25, 173.96, 81.57, 61.91, 46.53, 42.55, 36.47, 34.83, 33.68, 28.62, 27.19, 26.59, 25.93. **LC-MS:** (Method B) t_R = 3.14 min, (ESI) *m/z* = 427.3 ([M+H]$^+$, 100%) **TLC:** DCM : MeOH (95 : 5), R_f = 0.08. This intermediate (11 mg, 26 µmol, 1.1 equiv.) was treated according to General method B using compound **1** (10 mg, 23 µmol, 1 equiv.) and DIPEA (12 mg, 94 µmol, 4 equiv.) for 18 h and purified via column chromatography (0 - 10% MeOH/DCM) to yield a yellow solid (13 mg, 82%). **^{1}H NMR (CD_3OD)** δ 7.93 (d, *J* = 3.2 Hz, 1H), 7.74 (d, *J* = 3.1 Hz, 1H), 7.40 (dd, *J* = 8.7, 6.0 Hz, 1H), 7.22 (dd, *J* = 8.8, 2.6 Hz, 1H), 7.04 (td, *J* = 8.4, 2.7 Hz, 1H), 6.16 (s, 1H), 4.28 (s, 1H), 4.07 (d, *J* = 17.0 Hz, 1H), 3.91 (d, *J* = 17.1 Hz, 1H), 3.76 – 3.64 (m, 4H), 3.59 (s, 3H), 2.67 – 2.55 (m, 4H), 2.45 (t, *J* = 7.0 Hz, 2H), 2.33 (td, *J* = 7.0, 2.8 Hz, 2H), 1.74 – 1.60 (m, 4H), 1.02 (s, 9H). **^{13}C NMR (CD_3OD)** δ 175.6, 175.3, 173.9, 167.8, 163.1 (d, J_{CF}= 247.9 Hz), 163.3, 147.7, 146.7, 144.6, 139.2 (d, J_{CF}= 3.6 Hz), 134.8 (d, J_{CF}= 10.3 Hz), 132.2 (d, J_{CF}= 9.0 Hz), 125.2, 117.8 (d, J_{CF}= 24.9 Hz), 115.6 (d, J_{CF}= 21.3 Hz), 61.9, 57.5, 57.1, 54.5, 54.1, 51.6, 47.1, 43.0, 36.5, 34.9, 33.7, 27.2, 26.6, 26.1. **^{19}F NMR (CD_3OD)** δ -114.45, -76.55 (CF_3COOH). **LC-MS:** (Method B) t_R = 3.13 min, (ESI) *m/z* = 690.3 ([M+H]$^+$, 100%). **HPLC:** (Method A) t_R = 5.55 min, 96% pure (254 nm). **HRMS:** (ESI+) calc'd for [$C_{32}H_{41}N_7O_5SClF$ + H]$^+$, 690.2650 found 690.2635. **TLC:** DCM : MeOH (9 : 1), R_f = 0.50

LH-3-T4: Methyl (*R*)-6-((4-(6-amino-6-oxohexanoyl)piperazin-1-yl)methyl)-4-(2-chloro-4-fluorophenyl)-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate



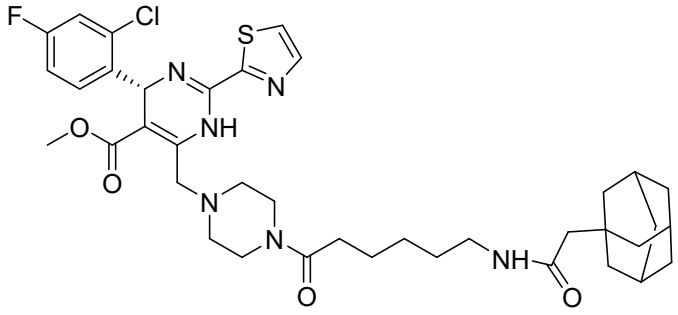
S26 (100 mg, 50% pure, 159 µmol, 1 equiv.) and NH_4Cl (26 mg, 477 µmol, 3 equiv.) were coupled according to General Method A using HATU (121 mg, 318 µmol, 2 equiv.) and DIPEA (82 mg, 636 µmol, 4 equiv.). The crude product was purified via column chromatography (0 - 5% MeOH/DCM) to yield the title compound as a white solid (19 mg, 38%). **^{1}H NMR (CD_3OD)** δ 3.59 – 3.51 (m, 4H), 3.49 – 3.38 (m, 4H), 2.44 (t, *J* = 7.2 Hz, 2H), 2.24 (t, *J* = 6.9 Hz, 2H), 1.73 – 1.59 (m, 4H), 1.47 (s, 9H). **^{13}C NMR (CD_3OD)** δ 178.79, 173.99, 156.25, 81.61, 46.53, 42.56, 36.11, 33.66, 28.60, 26.39, 25.90. **LC-MS:** (Method B) t_R = 3.02 min, (ESI) *m/z* = 314.3 ([M+H]$^+$, 100%) **TLC:** DCM : MeOH (95 : 5), R_f = 0.11. A solution of this intermediate (11 mg, 31 µmol, 1.1 equiv.) was treated according to General method B using **1** (12 mg, 28 µmol, 1 equiv.) and DIPEA (20 mg, 110 µmol, 4 equiv.) for 18 h and purified via column chromatography (0 - 10% MeOH/DCM) to yield a yellow solid (4.4 mg, 28%). **^{1}H NMR (CD_3OD)** δ 7.93 (d, *J* = 3.1 Hz, 1H), 7.74 (d, *J* = 3.2 Hz, 1H), 7.40 (dd, *J* = 8.7, 6.1 Hz, 1H), 7.22 (dd, *J* = 8.8, 2.6 Hz, 1H), 7.04 (td, *J* = 8.4, 2.7 Hz, 1H), 6.16 (s, 1H), 4.08 (d, *J* = 17.1 Hz, 1H), 3.91 (d, *J* = 17.1 Hz, 1H), 3.78 – 3.65 (m, 4H), 3.59 (s, 3H), 2.67 – 2.57 (m, 4H), 2.45 (t, *J* = 6.9 Hz, 2H), 2.25 (d, *J* = 6.8 Hz, 2H), 1.66 (p, *J* = 2.9 Hz, 4H). **^{13}C NMR (CD_3OD)** δ 173.9, 167.8, 163.1 (d, J_{CF}= 247.9 Hz), 163.3, 147.7, 146.7, 144.6, 139.2 (d, J_{CF}= 3.3 Hz), 134.9 (d, J_{CF}= 11.2 Hz), 132.1 (d, J_{CF}= 9.1 Hz), 125.1, 117.8 (d, J_{CF}= 25.1 Hz), 115.5 (d, J_{CF}= 21.3 Hz), 99.2, 57.5, 57.1, 54.5, 54.0, 51.6, 47.0, 43.0, 36.1, 33.7, 26.5, 26.0. One carbonyl carbon not observed. **^{19}F NMR (CD_3OD)** δ -114.69. **LC-MS:** (Method B) t_R = 3.06 min, (ESI) *m/z* = 577.2 ([M+H]$^+$, 100%). **HPLC:** (Method A) t_R = 5.19

min, ≥ 99% pure (254 nm). **HRMS:** (ESI+) calc'd for $[C_{26}H_{30}N_6O_4SClF + H]^+$, 577.1811 found 577.1795. **TLC:** DCM : MeOH (9 : 1), R_f = 0.42

Synthesis of hydrophobic LH-3 analogues

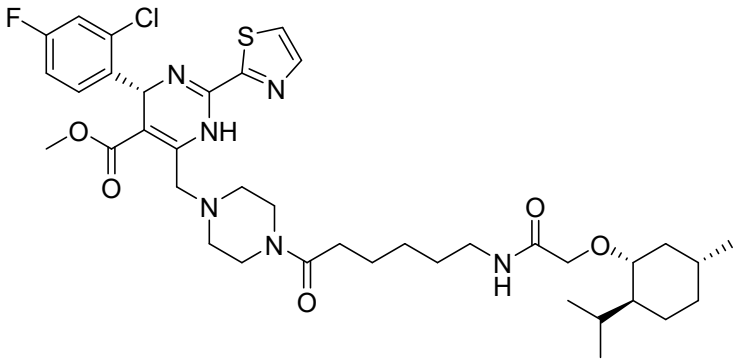
A solution of **S2** (25 mg, 45 µmol, 1 equiv.) in DCM : TFA (1 : 1, 2 mL) was stirred for 30 min before the removal of DCM and TFA under a stream of nitrogen, giving a brown oil. The resultant amine was coupled with Boc-aminohexanoic acid (13 mg, 55 µmol, 1.2 equiv.), HATU (35 mg, 91 µmol, 2 equiv.) and DIPEA (24 mg, 182 µmol, 4 equiv.) according to General Method A. The crude product was obtained as a yellow solid (25 mg, 84%) and used without further purification.

LH-3-adam: Methyl (*R*)-6-((4-(6-(2-(adamantan-1-yl)acetamido)hexanoyl)piperazin-1-yl)methyl)-4-(2-chloro-4-fluorophenyl)-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate



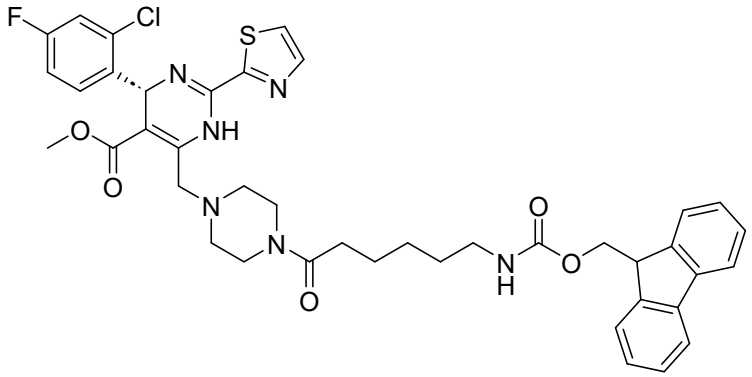
The crude product from the previous step (10 mg, 15 µmol, 1 equiv.) was dissolved in DCM/TFA (1 : 1, 2 mL) and stirred at r.t for 30 min. Solvents were removed under a stream of nitrogen and the residue was coupled with 1-adamantaneacetic acid (4 mg, 19 µmol, 1.2 equiv.), HATU (12 mg, 31 µmol, 2 equiv.) and DIPEA (8 mg, 60 µmol, 4 equiv.) according to General Method A. The crude product was purified by preparative RP-HPLC (45 - 75% MeCN/H_2O, 15 min) to yield the title compound as a yellow solid following lyophilisation (5 mg, 26%, as the singly protonated TFA salt). **^{1}H NMR (CD_3OD)** δ 8.00 (d, *J* = 3.1 Hz, 1H), 7.90 (d, *J* = 3.1 Hz, 1H), 7.52 (dd, *J* = 8.8, 6.0 Hz, 1H), 7.28 (dd, *J* = 8.6, 2.6 Hz, 1H), 7.11 (td, *J* = 8.3, 2.6 Hz, 1H), 6.18 (s, 1H), 4.73 (d, *J* = 16.0 Hz, 1H), 4.57 (d, *J* = 16.0 Hz, 1H), 3.97 (bs, 4H), 3.63 (s, 3H), 3.62 – 3.44 (m, 4H), 3.17 (t, *J* = 7.0 Hz, 2H), 2.48 (t, *J* = 7.5 Hz, 2H), 1.97 – 1.93 (m, 3H), 1.92 (s, 2H), 1.81 – 1.71 (m, 3H), 1.70 – 1.59 (m, 11H), 1.54 (q, *J* = 7.2 Hz, 2H), 1.48 – 1.33 (m, 2H). **^{13}C NMR (CD_3OD)** δ 174.1, 173.8, 166.9, 149.4, 145.9, 138.5 (d, J_{CF}= 3.6 Hz), 134.4 (d, J_{CF}= 10.9 Hz), 133.0 (d, J_{CF}= 9.3 Hz), 126.7, 118.2 (d, J_{CF}= 25.0 Hz), 116.0 (d, J_{CF}= 21.3 Hz), 107.4, 59.3, 54.0, 53.9, 52.2, 52.0, 51.7, 43.8, 40.0, 37.9, 33.8, 33.5, 30.3, 30.2, 27.6, 25.8. Four quaternary carbon and one CH not observed. **^{19}F NMR (CD_3OD)** δ -112.07, -76.55 (CF_3COOH). **LC-MS:** (Method A) t_R = 3.67 min, (ESI) *m/z* = 739.3 ($[M+H]^+$, 100%). **HPLC:** (Method A) t_R = 6.79 min, 96% pure (254 nm). **HRMS:** (ESI+) calc'd for $[C_{38}H_{48}N_6O_4SClF + H]^+$, 739.3203 found 739.3217. **TLC:** DCM : MeOH (9 : 1), R_f = 0.49

LH-3-menth: Methyl (*R*)-4-(2-chloro-4-fluorophenyl)-6-((4-(6-(2-(((1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl)oxy)acetamido)hexanoyl)piperazin-1-yl)methyl)-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate



The crude product from the previous step (6 mg, 9 µmol, 1 equiv.) was dissolved in DCM/TFA (1 : 1, 2 mL) and stirred at r.t for 30 min. Solvents were removed under a stream of nitrogen and the residue was coupled with (-)-menthoxyacetic acid (3 mg, 11 µmol, 1.2 equiv.), HATU (7 mg, 18 µmol, 2 equiv.) and DIPEA (5 mg, 36 µmol, 4 equiv.) according to General Method A. The crude product was purified by column chromatography (0 - 100% EtOAc/Petroleum ether) to yield the title compound as a yellow solid (1.0 mg, 41%). **^{1}H NMR (CD_3OD)** δ 7.93 (d, *J* = 3.1 Hz, 1H), 7.74 (d, *J* = 3.1 Hz, 1H), 7.40 (dd, *J* = 8.7, 6.1 Hz, 1H), 7.22 (dd, *J* = 8.8, 2.6 Hz, 1H), 7.04 (td, *J* = 8.4, 2.6 Hz, 1H), 6.16 (s, 1H), 4.58 (s, 1H), 4.11 – 4.00 (m, 2H), 3.95 – 3.83 (m, 2H), 3.80 – 3.64 (m, 4H), 3.59 (s, 3H), 3.26 (t, *J* = 6.9 Hz, 2H), 3.20 (dd, *J* = 10.6, 4.1 Hz, 1H), 2.68 – 2.57 (m, 4H), 2.43 (t, *J* = 7.5 Hz, 2H), 2.24 – 2.16 (m, 1H), 2.12 – 2.04 (m, 1H), 1.72 – 1.61 (m, 4H), 1.59 – 1.50 (m, 2H), 1.44 – 1.27 (m, 6H), 0.93 (dd, *J* = 6.8, 2.2 Hz, 6H), 0.81 (d, *J* = 7.0 Hz, 3H). **^{13}C NMR (CD_3OD)** Insufficient material to obtain spectra **^{19}F NMR (CD_3OD)** δ -114.87. **LC-MS:** (Method A) t_R = 3.78 min, (ESI) *m/z* = 759.3 ($[M+H]^+$, 100%). **HPLC:** (Method A) t_R = 7.44 min, 99% pure (254 nm). **HRMS:** (ESI+) calc'd for $[C_{38}H_{52}N_6O_5SClF + H]^+$, 759.3465 found 759.3465. **TLC:** EtOAc, R_f = 0.27

LH-3-fluor: Methyl (*R*)-6-((4-(6-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)hexanoyl)piperazin-1-yl)methyl)-4-(2-chloro-4-fluorophenyl)-2-(thiazol-2-yl)-1,4-dihydropyrimidine-5-carboxylate

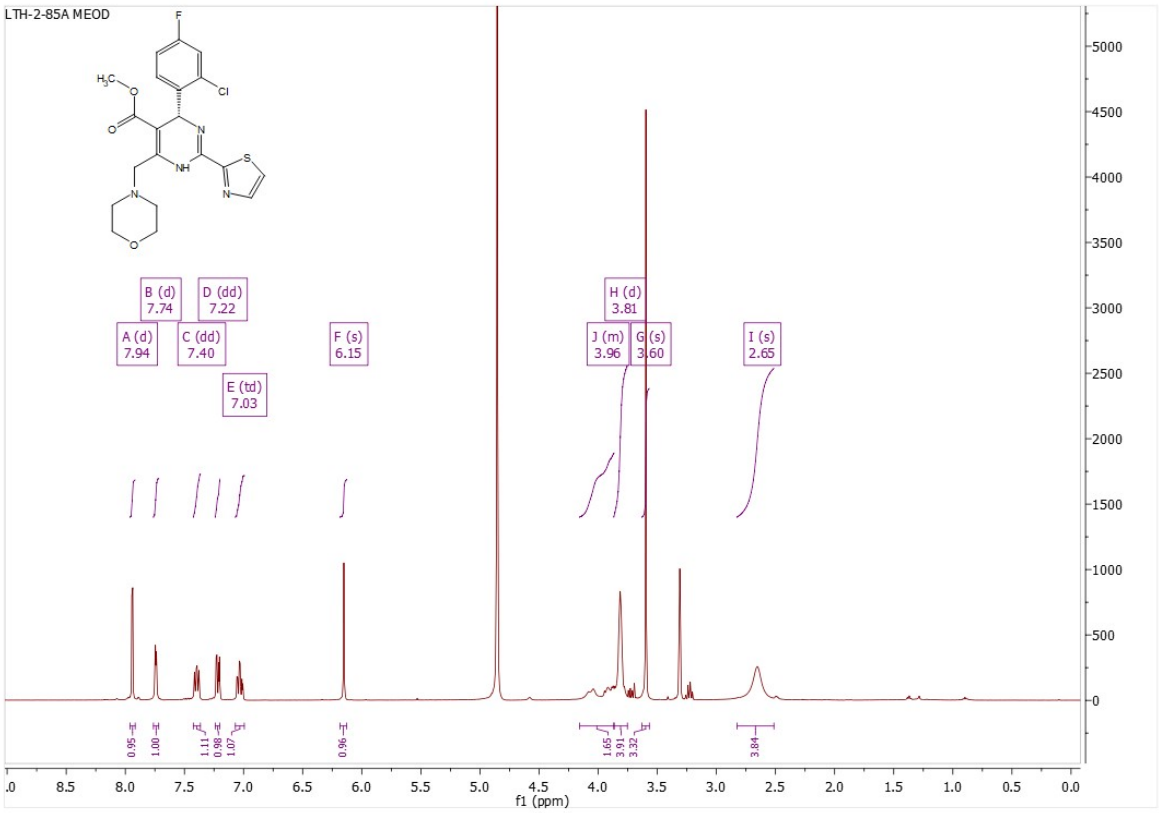


A solution of **S2** (25 mg, 45 µmol, 1 equiv.) in DCM : TFA (1 : 1, 2 mL) was stirred for 30 min before the removal of DCM and TFA under a stream of nitrogen, giving a brown oil. The resultant amine was coupled with Fmoc-aminohexanoic acid (8 mg, 22 µmol, 1.2 equiv.), HATU (14 mg, 36 µmol, 2 equiv.) and DIPEA (10 mg, 73 µmol, 4 equiv.) according to General Method A. The crude product was purified by column chromatography (0 - 50% EtOAc/Petroleum ether) to yield the title compound as a yellow solid (1.1 mg, 8%).

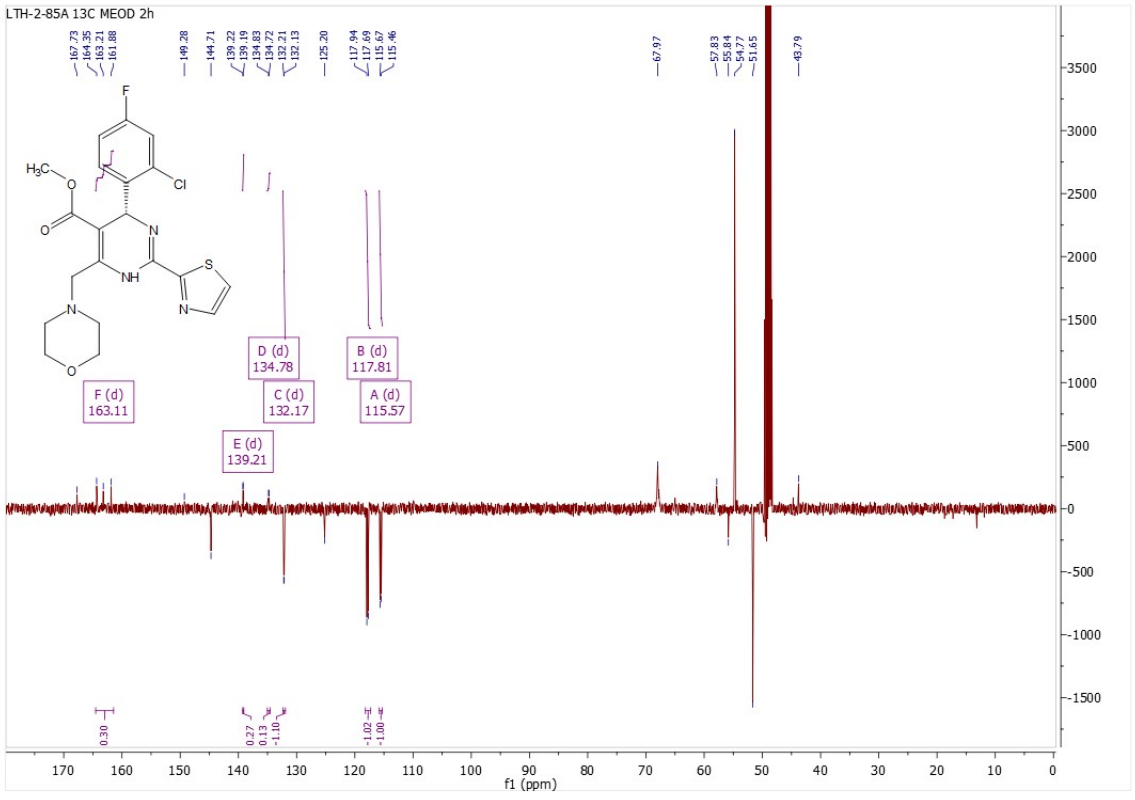
^{1}H NMR (CD_3OD) δ 7.92 (d, *J* = 3.2 Hz, 1H), 7.78 (dd, *J* = 7.5, 3.1 Hz, 2H), 7.73 (d, *J* = 3.1 Hz, 1H), 7.64 (d, *J* = 7.5 Hz, 2H), 7.41 – 7.34 (m, 3H), 7.30 (d, *J* = 7.3 Hz, 2H), 7.22 (dd, *J* = 8.7, 2.6 Hz, 1H), 7.03 (td, *J* = 8.4, 2.6 Hz, 1H), 6.15 (s, 1H), 4.34 (d, *J* = 6.9 Hz, 2H), 4.20 (t, *J* = 6.9 Hz, 1H), 4.04 (d, *J* = 17.1 Hz, 1H), 3.87 (d, *J* = 17.1 Hz, 1H), 3.79 – 3.60 (m, 4H), 3.57 (s, 3H), 3.13 (t, *J* = 6.8 Hz, 2H), 2.58 (t, *J* = 5.0 Hz, 3H), 2.41 (t, *J* = 7.5 Hz, 2H), 1.63 (p, *J* = 7.4 Hz, 2H), 1.53 (p, *J* = 7.0 Hz, 2H), 1.44 – 1.27 (m, 3H).**^{13}C NMR (CD_3OD)** δ 174.1, 173.2, 173.1, 144.6, 132.1, 125.2, 117.8 (d, J_{CF}= 25.2 Hz),

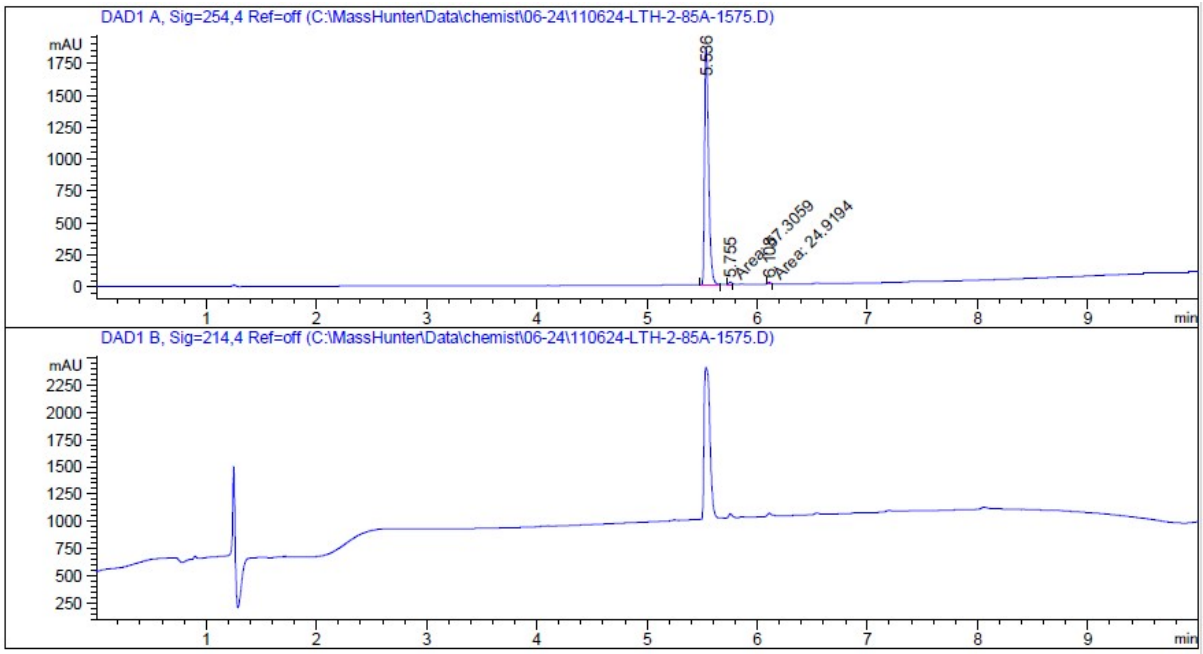
115.5 (d, J_{CF}= 21.1 Hz), 81.8, 68.8, 57.5, 57.1, 54.5, 54.1, 51.6, 49.6, 47.1, 43.0, 41.3, 39.7, 35.6, 33.9, 32.7, 30.3, 27.6, 27.0, 26.1, 24.4, 22.7, 21.4, 16.6. Four quaternary aromatic signals not observed. **^{19}F NMR (CD_3OD)** δ -114.87. **LC-MS:** (Method A) t_R = 3.88 min, (ESI) *m/z* = 785.2 ($[M+H]^+$, 100%). **HPLC:** (Method A) t_R = 7.26 min, 98% pure (254 nm). **HRMS:** (ESI+) calc'd for $[C_{41}H_{42}N_6O_5SClF + H]^+$, 785.2683 found 785.2680. **TLC:** EtOAc, R_f= 0.45

Spectroscopic data for key compounds used in testing



2 *^{1}H NMR spectrum of **2***

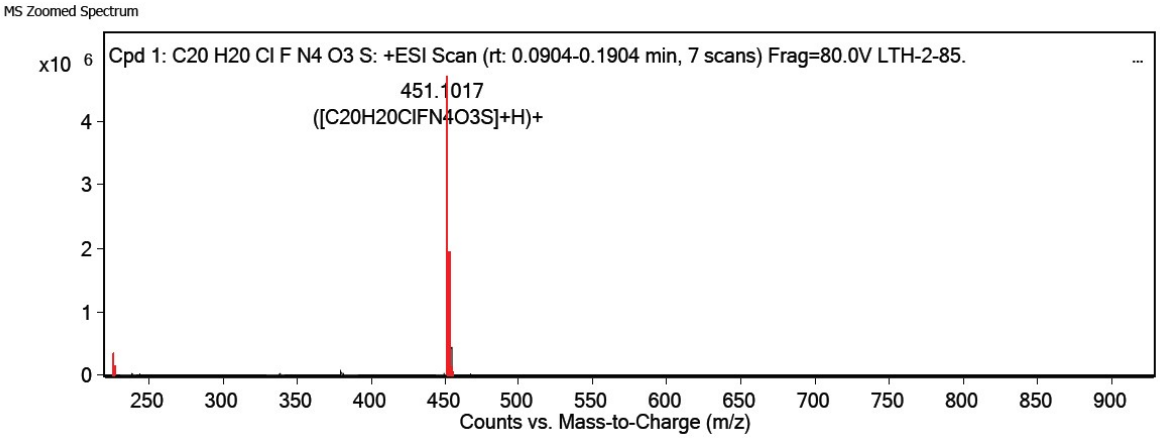




Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.536	BV	0.0398	4743.03955	1859.09607	98.2959
2	5.755	MM	0.0350	57.30587	27.26673	1.1876
3	6.108	MM	0.0324	24.91944	12.82584	0.5164
Totals :				4825.26486	1899.18864	

HPLC trace of ***2***

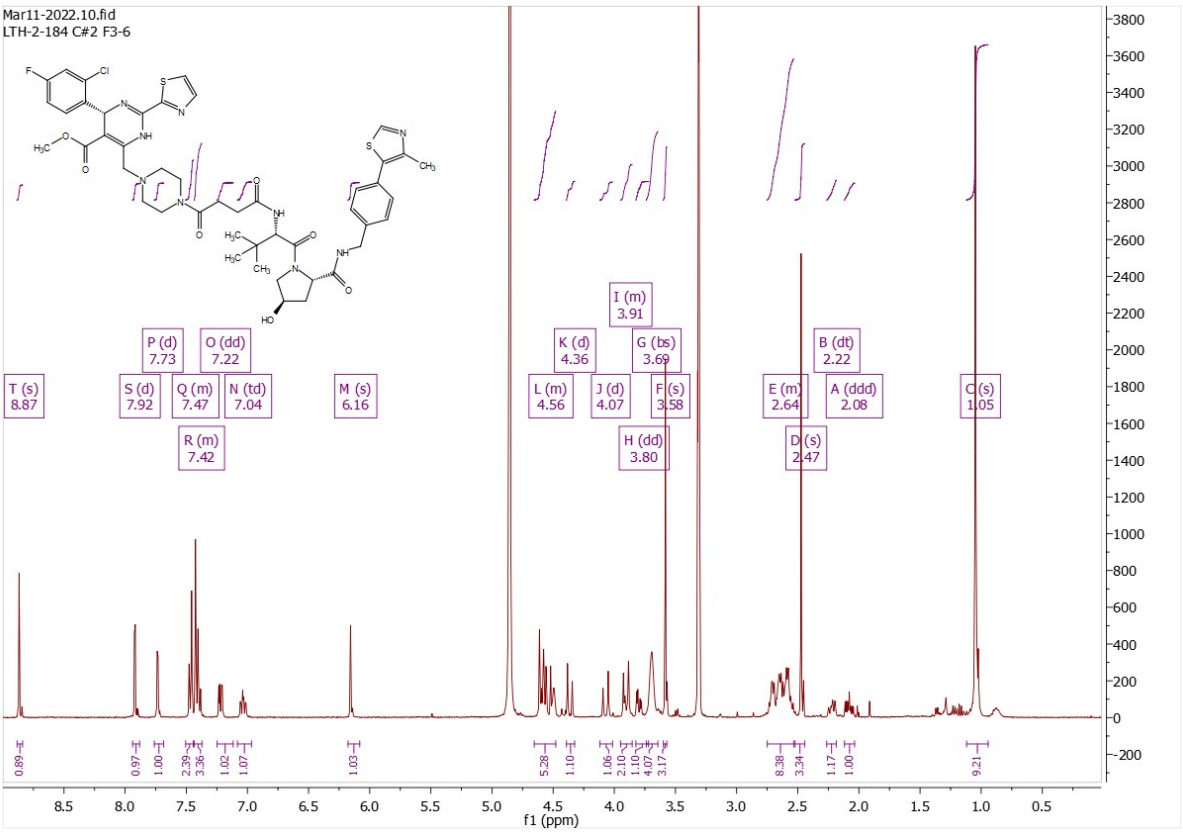


MS Spectrum Peak List

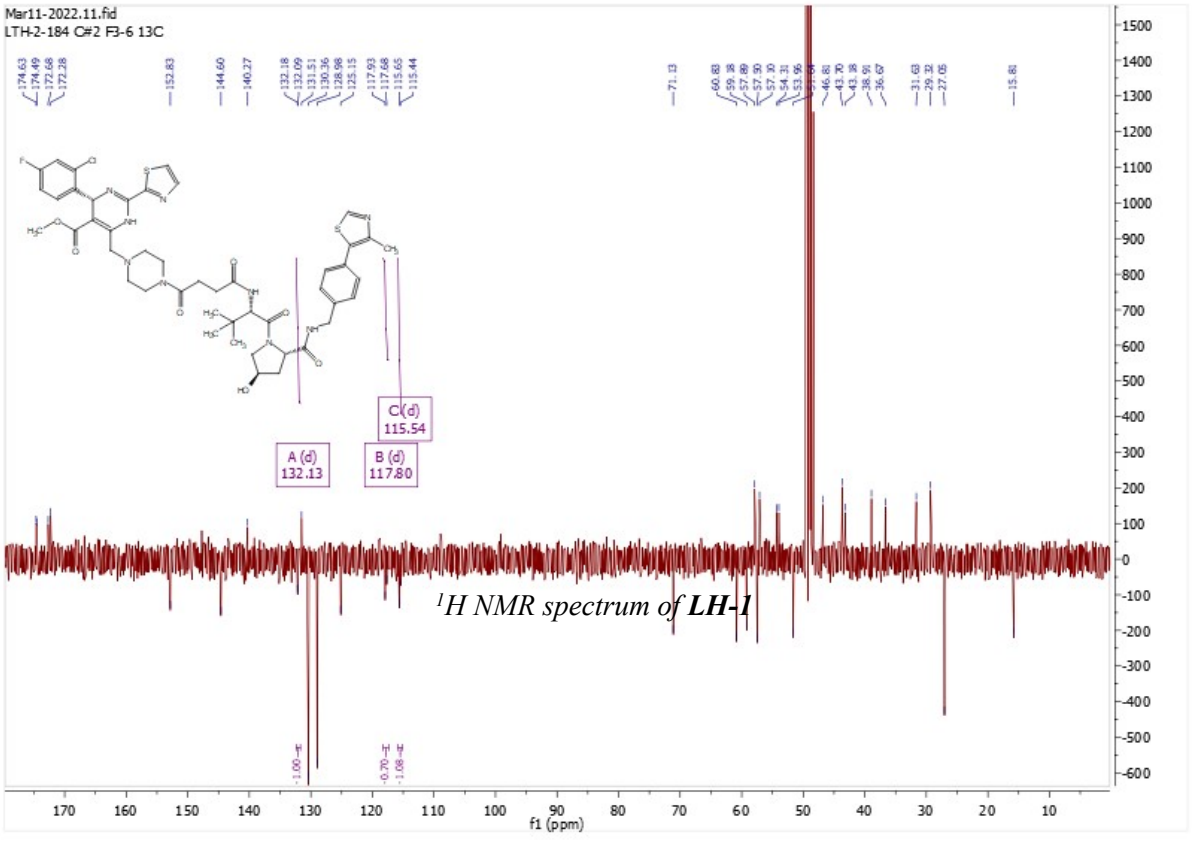
m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
226.0547	226.0537	-4.42	2	338110.28	C20H20ClFN4O3S	(M+2H)+2
451.1017	451.1001	-3.46	1	4880292.72	C20H20ClFN4O3S	(M+H)+
473.0817	473.0821	0.81	1	9190.81	C20H20ClFN4O3S	(M+Na)+

--- End Of Report ---

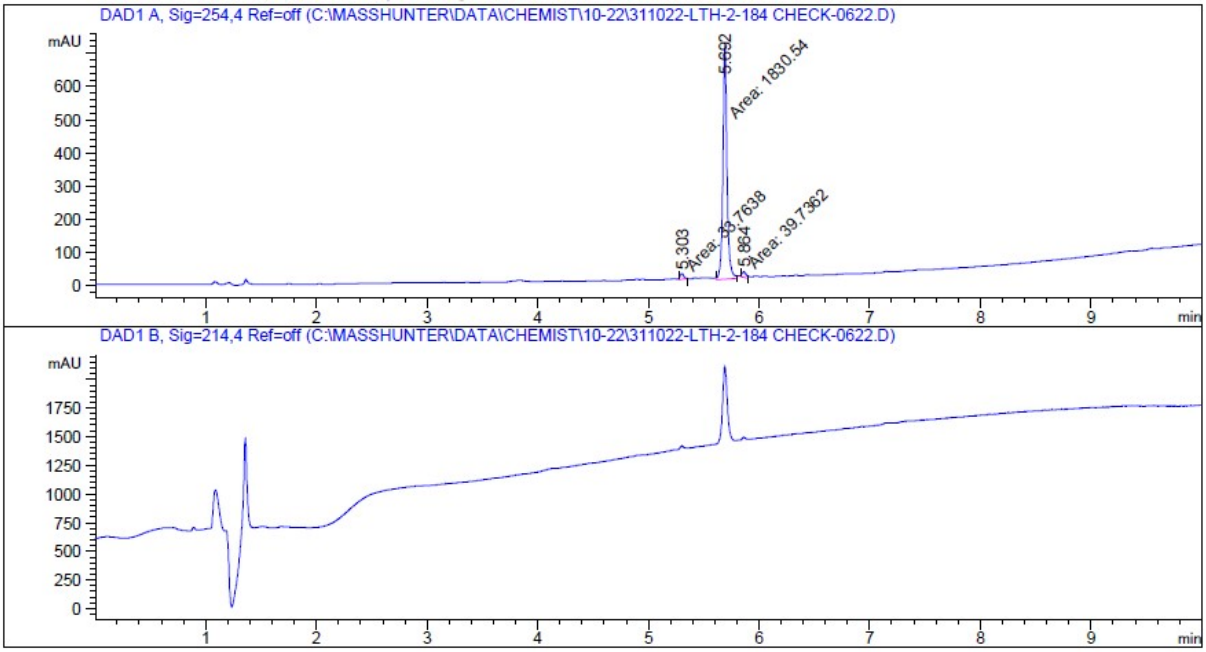
HRMS of ***2***



LH-1



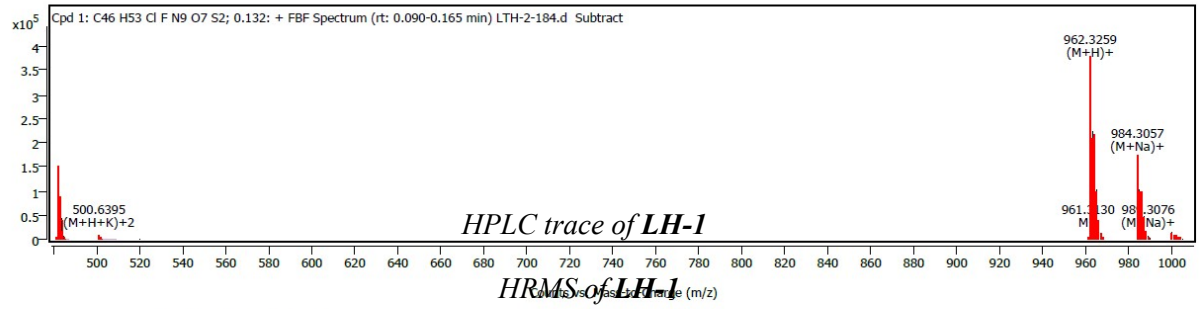
*^{1}H NMR spectrum of **LH-1***



Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.303	MM	0.0345	33.76379	16.32655	1.7733
2	5.692	MM	0.0432	1830.53613	706.41370	96.1398
3	5.864	MM	0.0396	39.73615	16.72925	2.0869
Totals :				1904.03608	739.46950	

Compound Spectra



HPLC trace of ***LH-1***

HRMS of ***LH-1***

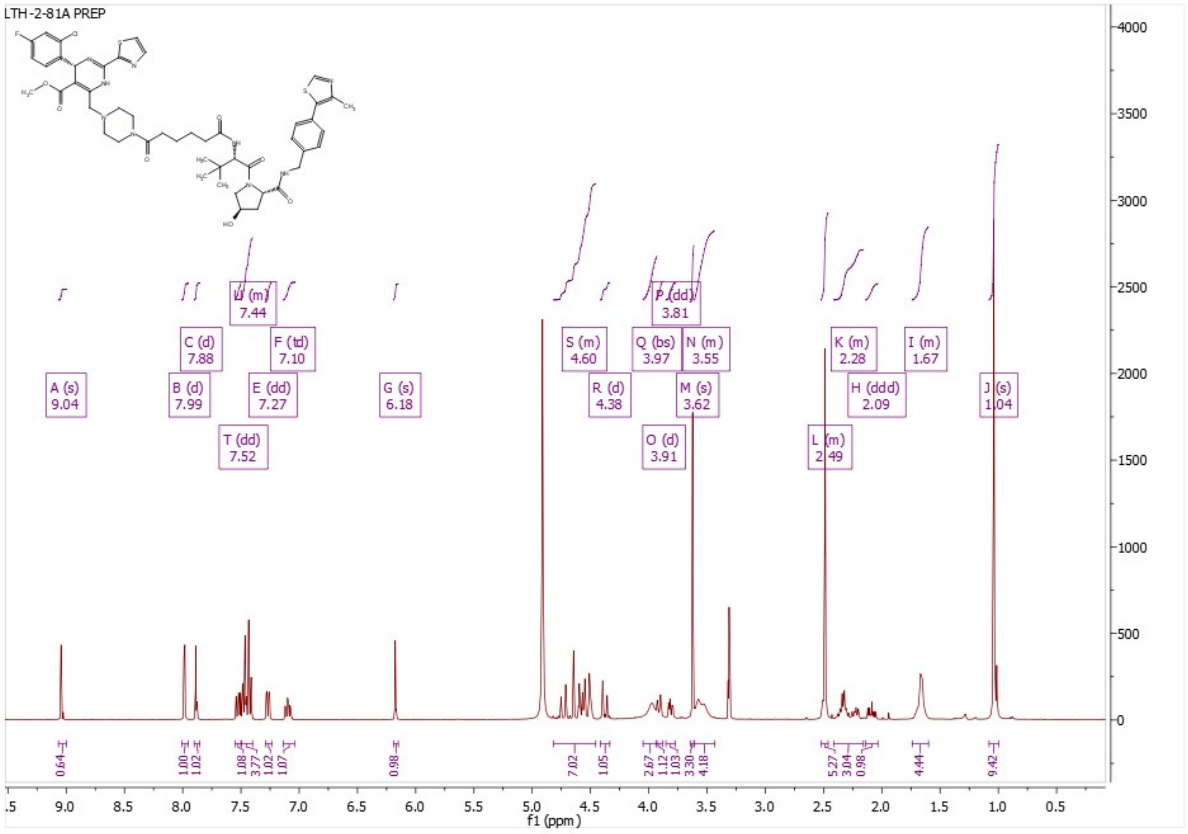
Spectrum Peaks (Max. 3)

m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
962.3259	962.3255	0.45	356949	100.00	100.00	(M+H)+	1
963.3291	963.3284	0.73	224345	62.85	55.51	(M+H)+	1
964.3259	964.3248	1.17	215054	60.25	57.51	(M+H)+	1

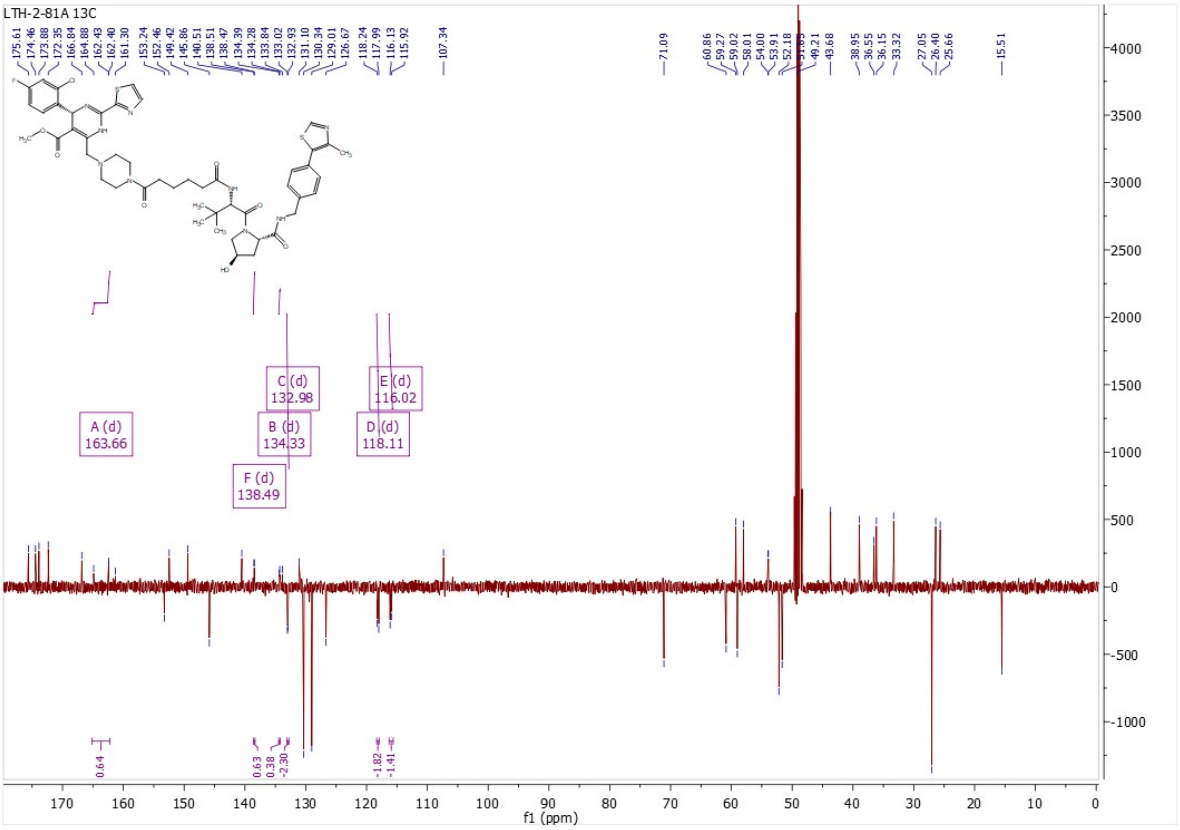
Spectrum Peaks (Max. 3)

m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
962.3259	962.3255	0.45	356949	100.00	100.00	(M+H)+	1
963.3291	963.3284	0.73	224345	62.85	55.51	(M+H)+	1
964.3259	964.3248	1.17	215054	60.25	57.51	(M+H)+	1

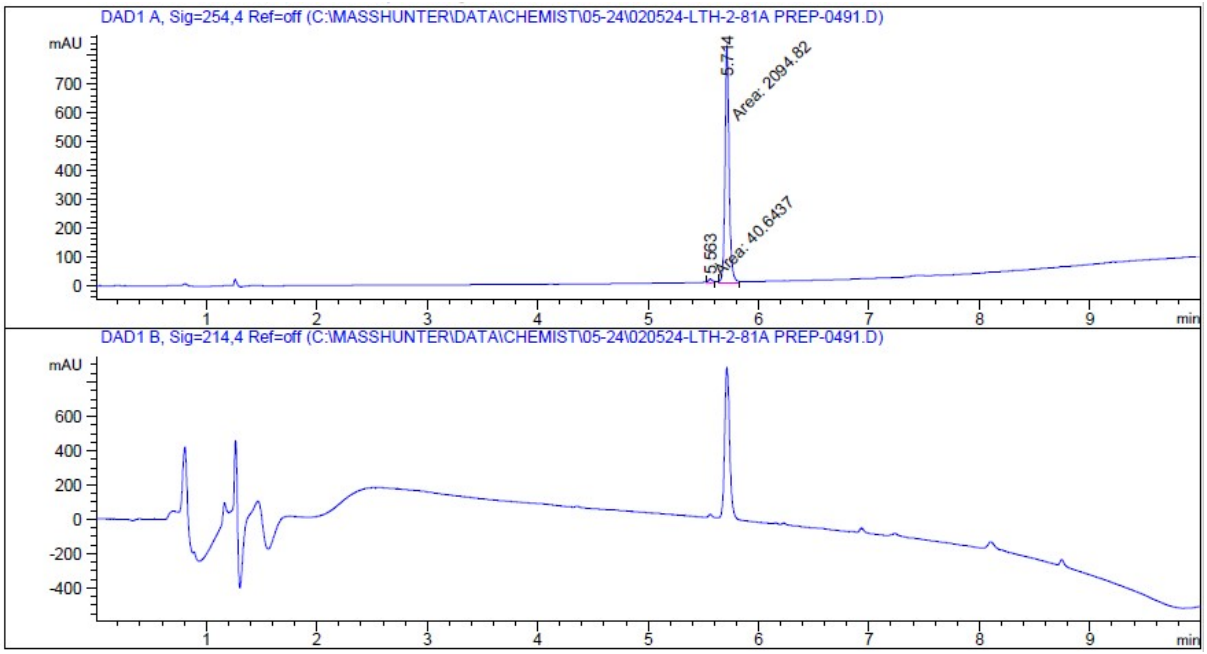
LH-3



[1]H NMR spectrum of ***LH-3***



[13]C NMR spectrum of ***LH-3***

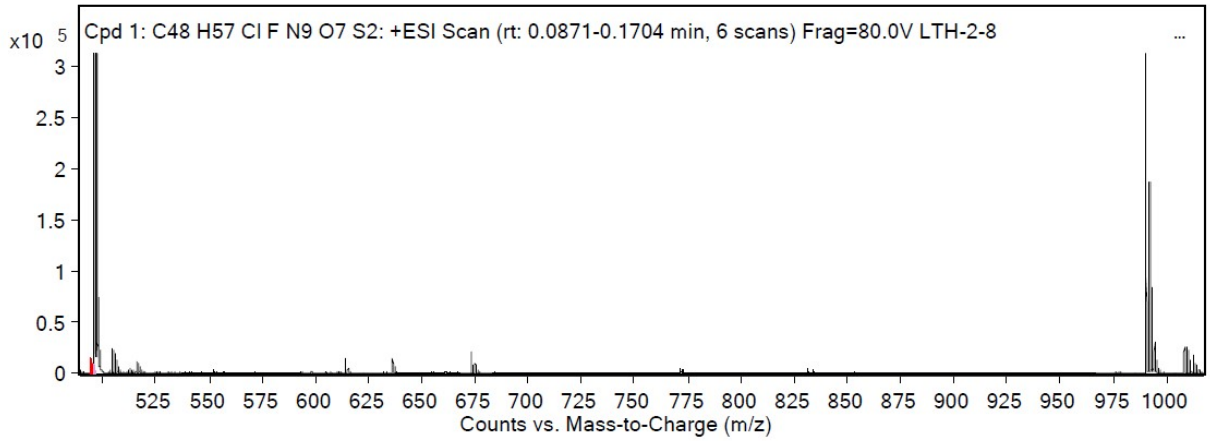


Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.563	MM	0.0463	40.64372	14.63263	1.9033
2	5.714	MM	0.0427	2094.82495	818.52057	98.0967
Totals :				2135.46867	833.15320	

HPLC trace of ***LH-3***

MS Zoomed Spectrum



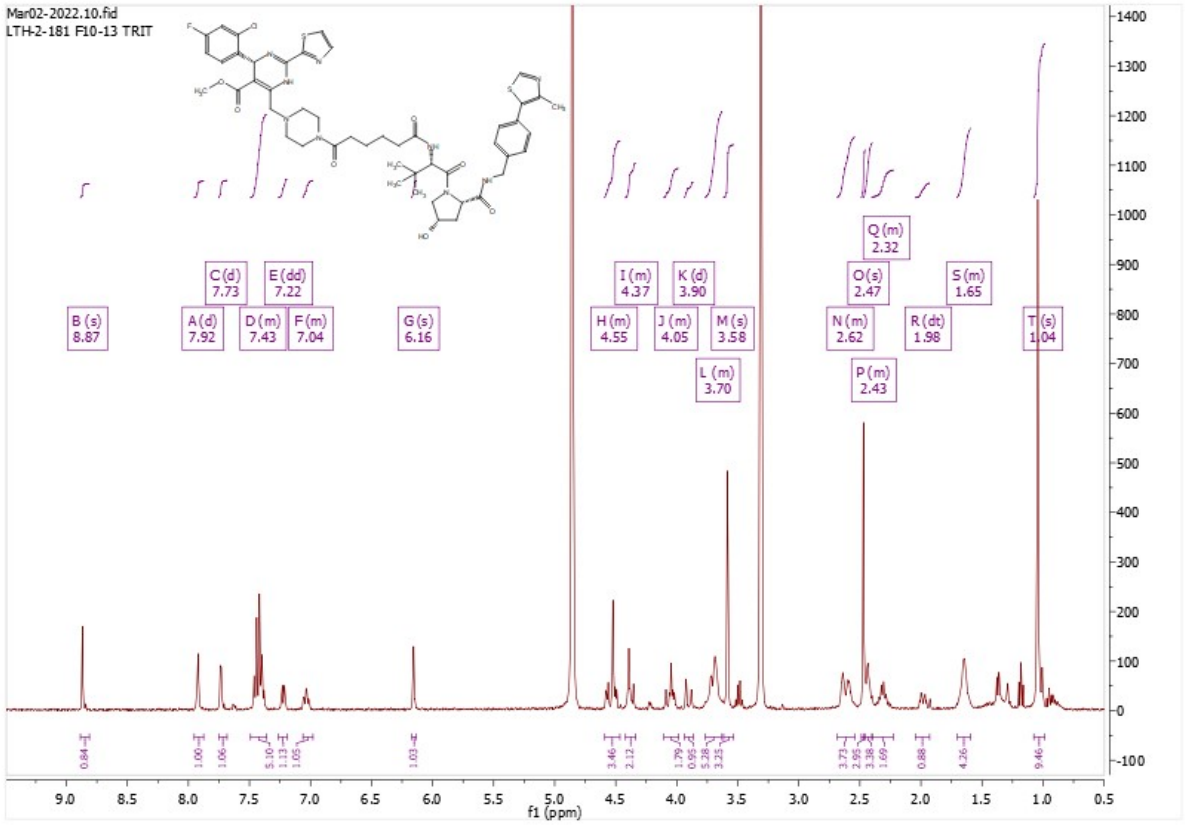
MS Spectrum Peak List

m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
494.6748	494.6742	-1.19	2	14382.55	C48H57ClFN9O7S2	M+2
990.3582	990.3568	-1.44	1	314602.32	C48H57ClFN9O7S2	(M+H)+
1012.3447	1012.3387	-5.93	1	17676.92	C48H57ClFN9O7S2	(M+Na)+

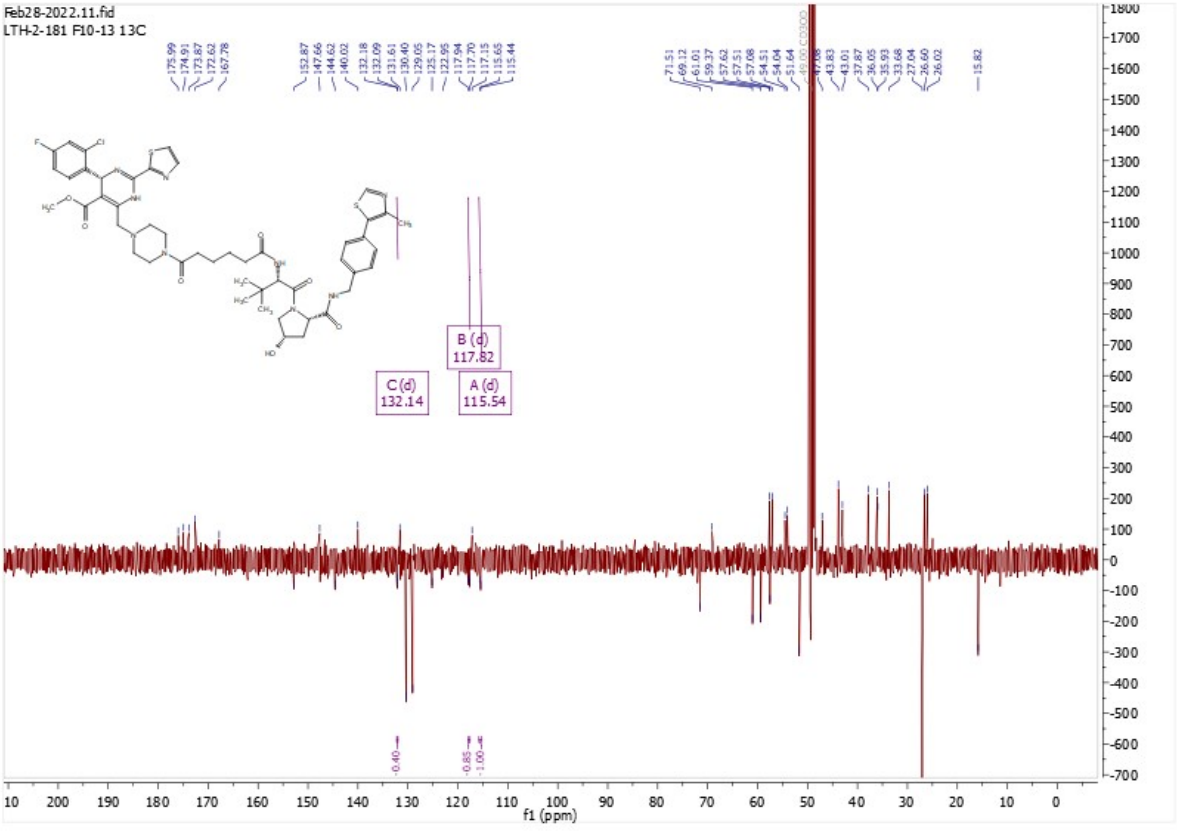
--- End Of Report ---

HRMS of ***LH-3***

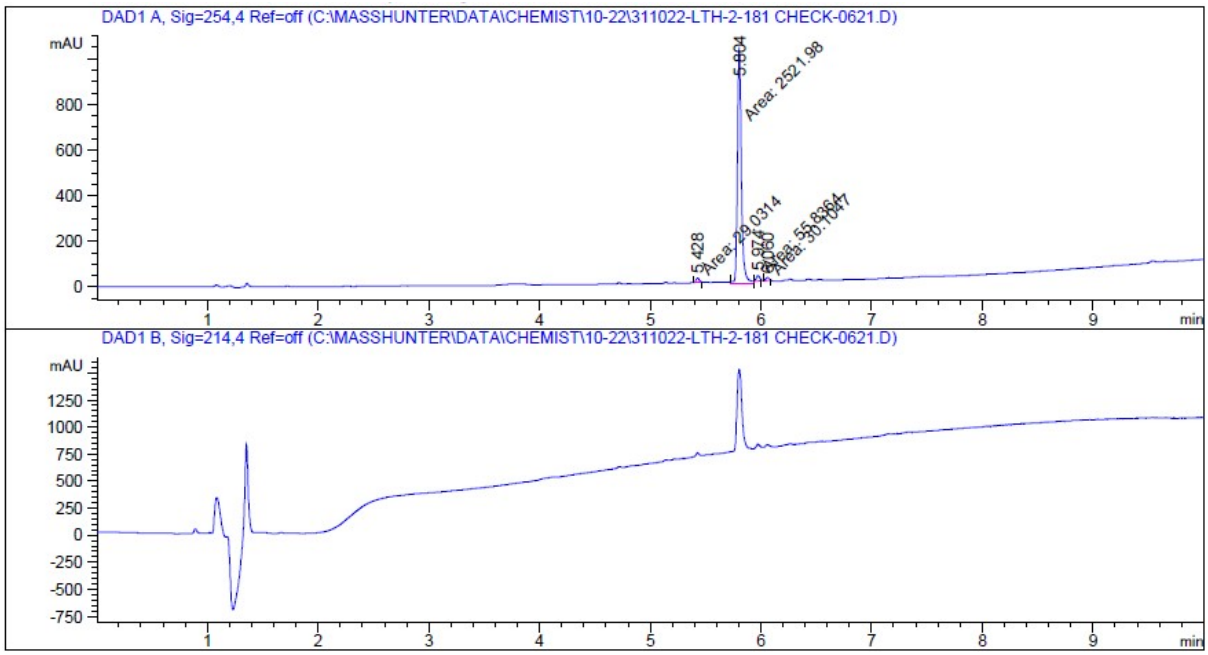
cisLH-3



[1]H NMR spectrum of ***cisLH-3***



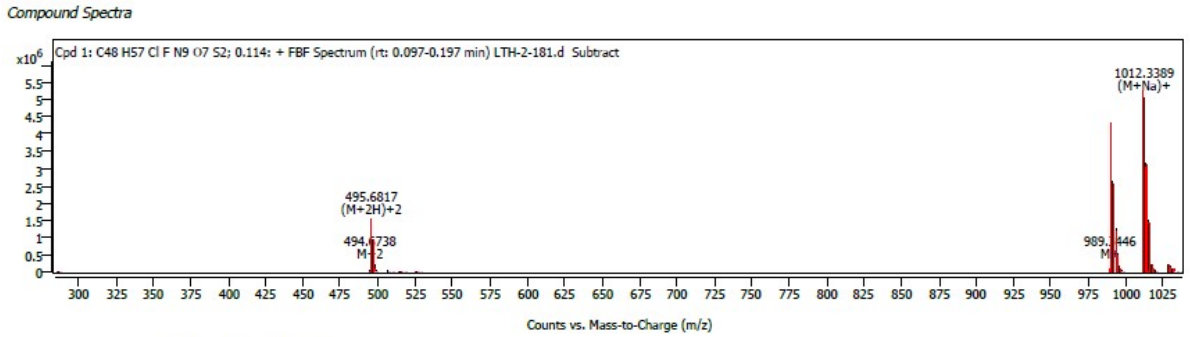
[13]C NMR spectrum of ***cisLH-3***



Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.428	MM	0.0284	29.03141	17.05242	1.1009
2	5.804	MM	0.0405	2521.97559	1037.92297	95.6399
3	5.974	MM	0.0369	55.83640	25.23281	2.1175
4	6.060	MM	0.0351	30.10468	14.30583	1.1416
Totals :				2636.94807	1094.51404	

*HPLC trace of **cisLH-3***



Spectrum Peaks (Max. 3)

m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
990.3569	990.3568	0.17	4172122	100.00	100.00	(M+H)+	1
1012.3389	1012.3387	0.14	5055870	100.00	100.00	(M+Na)+	1
1013.3421	1013.3416	0.42	3153374	62.37	57.70	(M+Na)+	1

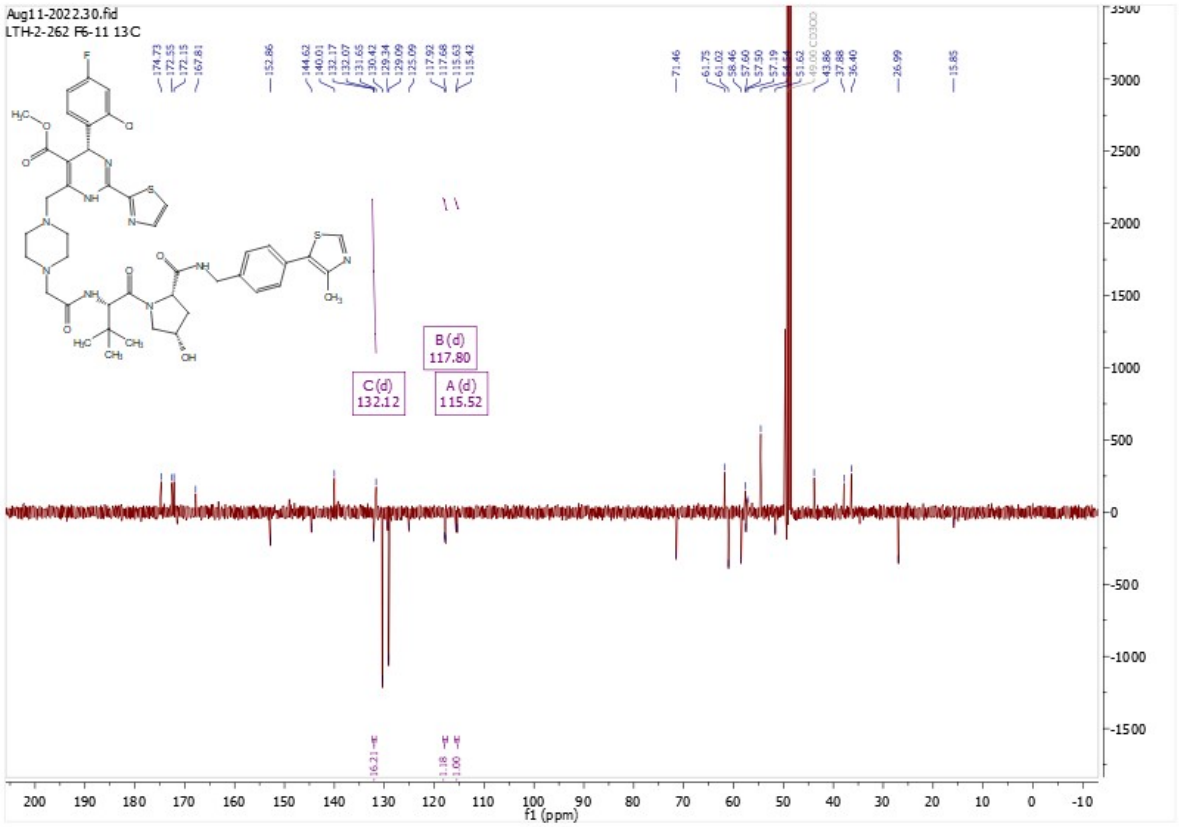
Spectrum Peaks (Max. 3)

m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
990.3569	990.3568	0.17	4172122	100.00	100.00	(M+H)+	1
1012.3389	1012.3387	0.14	5055870	100.00	100.00	(M+Na)+	1
1013.3421	1013.3416	0.42	3153374	62.37	57.70	(M+Na)+	1

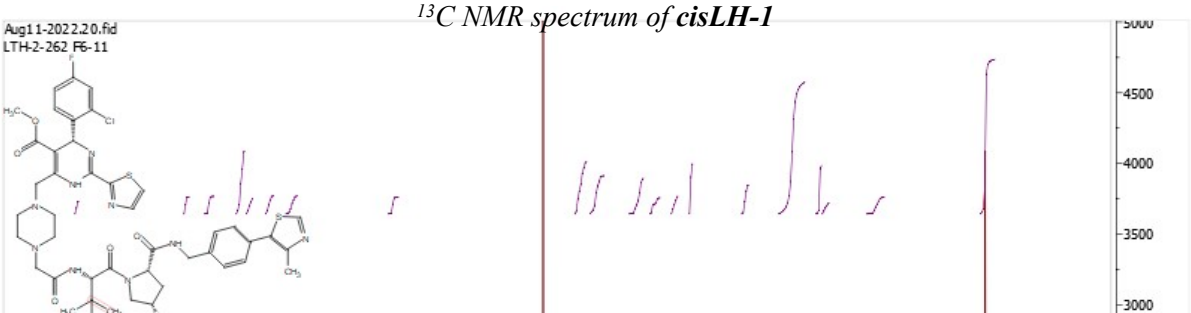
*HRMS of **cisLH-3***

cisLH-1

[1]H NMR spectrum of ***cisLH-1***

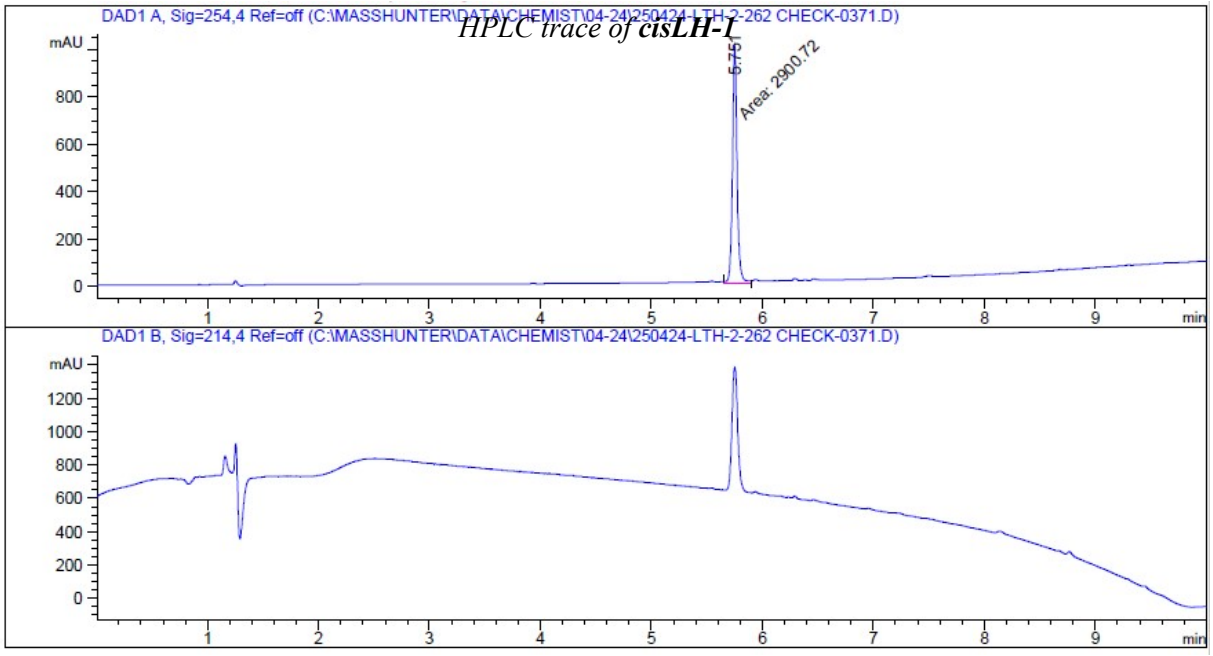


[13]C NMR spectrum of ***cisLH-1***



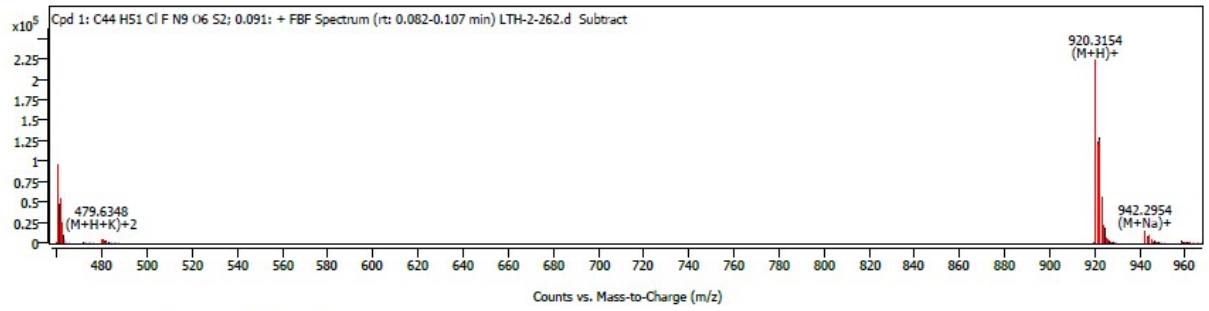
Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.751	MM	0.0482	2900.71509	1003.81793	100.0000
Totals :				2900.71509	1003.81793	



HPLC trace of ***cisLH-1***

Compound Spectra



Spectrum Peaks (Max. 3)

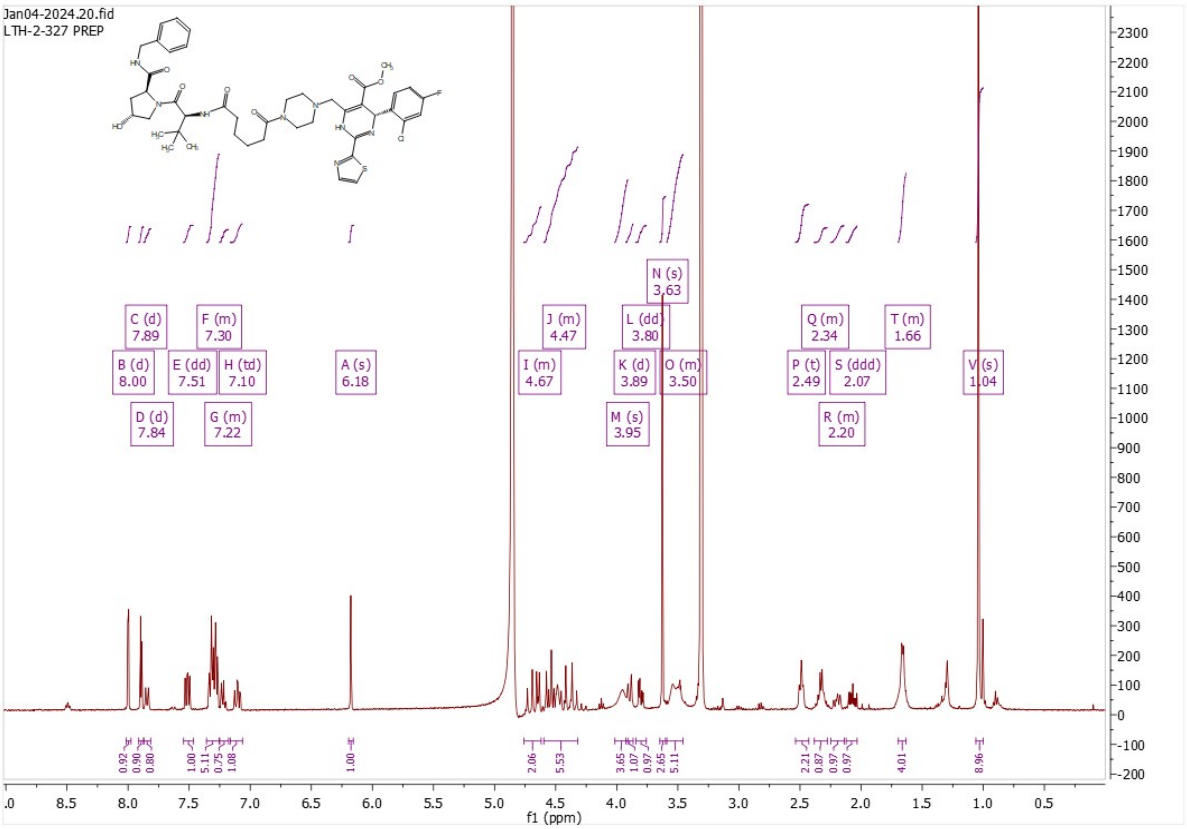
m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
920.3154	920.3149	0.50	224194	100.00	100.00	(M+H)+	1
921.3178	921.3178	0.01	123623	55.14	53.28	(M+H)+	1
922.3143	922.3141	0.19	128491	57.31	56.10	(M+H)+	1

Spectrum Peaks (Max. 3)

m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
920.3154	920.3149	0.50	224194	100.00	100.00	(M+H)+	1
921.3178	921.3178	0.01	123623	55.14	53.28	(M+H)+	1
922.3143	922.3141	0.19	128491	57.31	56.10	(M+H)+	1

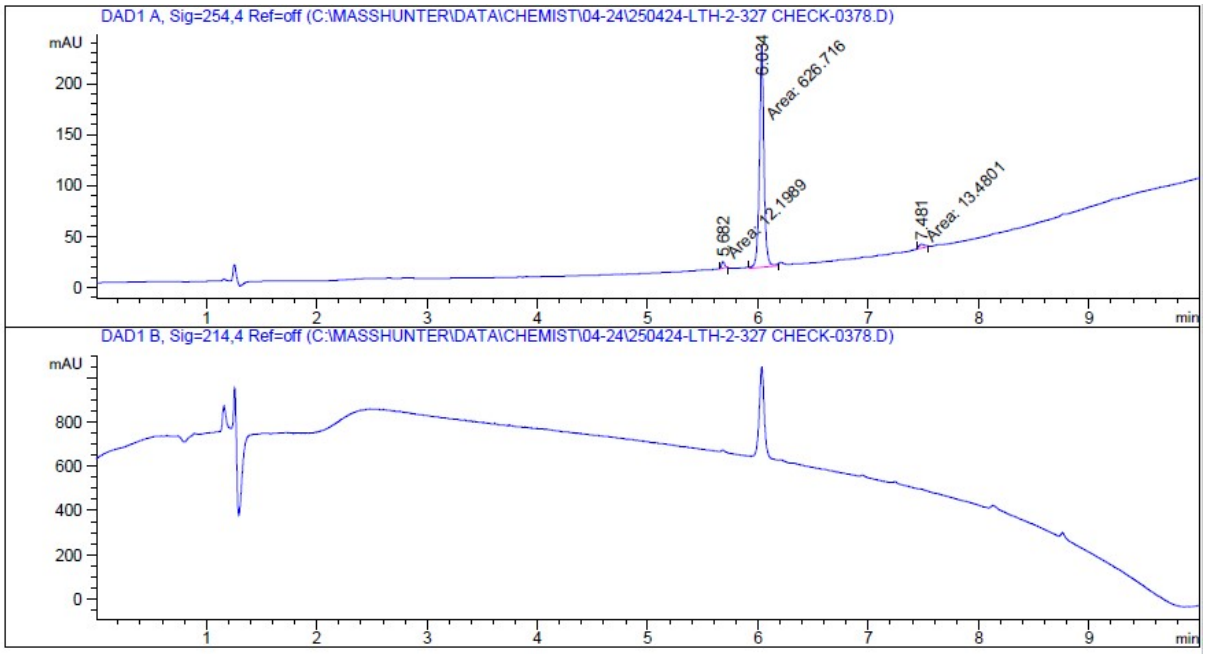
*HRMS of **cisLH-1***

LH-3-T1



[1]H NMR spectrum of ***LH-3-T1***

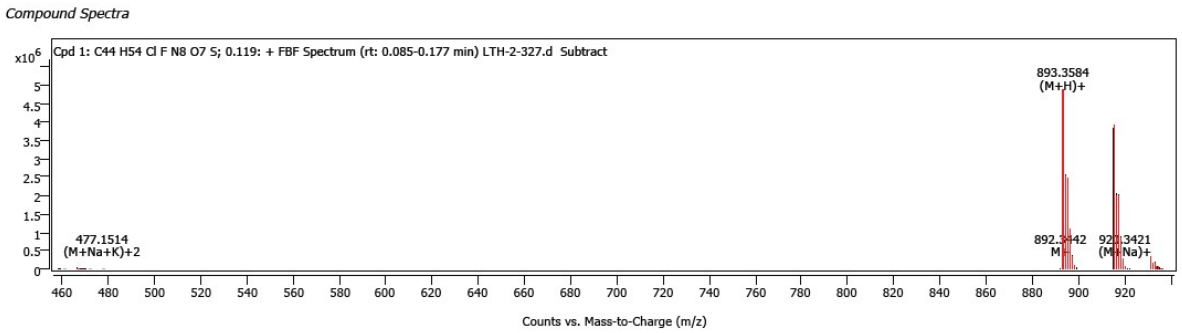
Insufficient material to obtain a ^{13}C NMR spectrum



Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.682	MM	0.0311	12.19886	6.53600	1.8699
2	6.034	MM	0.0481	626.71606	217.08002	96.0639
3	7.481	MM	0.0610	13.48014	3.68232	2.0663
Totals :				652.39506	227.29834	

*HPLC trace of **LH-3-T1***



Spectrum Peaks (Max. 3)

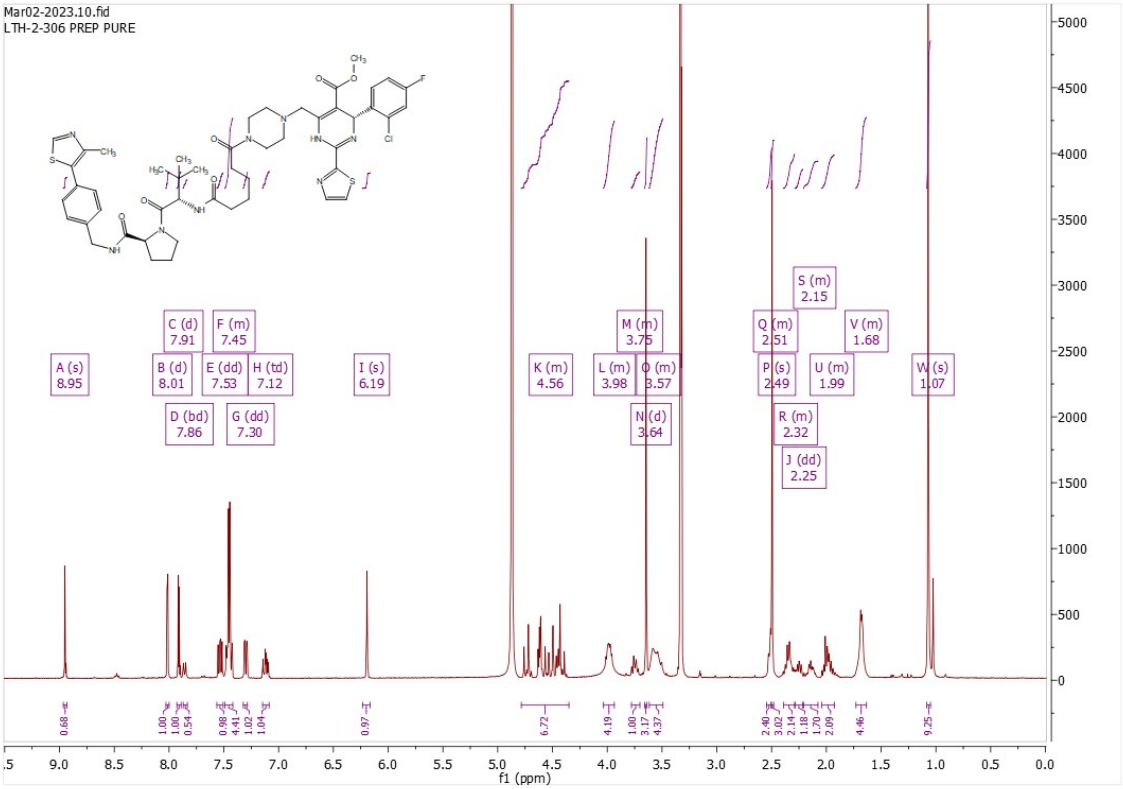
m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
893.3584	893.3581	0.33	4758555	100.00	100.00	(M+H)+	1
894.3615	894.3611	0.42	2571581	54.04	52.20	(M+H)+	1
915.3403	915.3401	0.25	3839215	100.00	100.00	(M+Na)+	1

Spectrum Peaks (Max. 3)

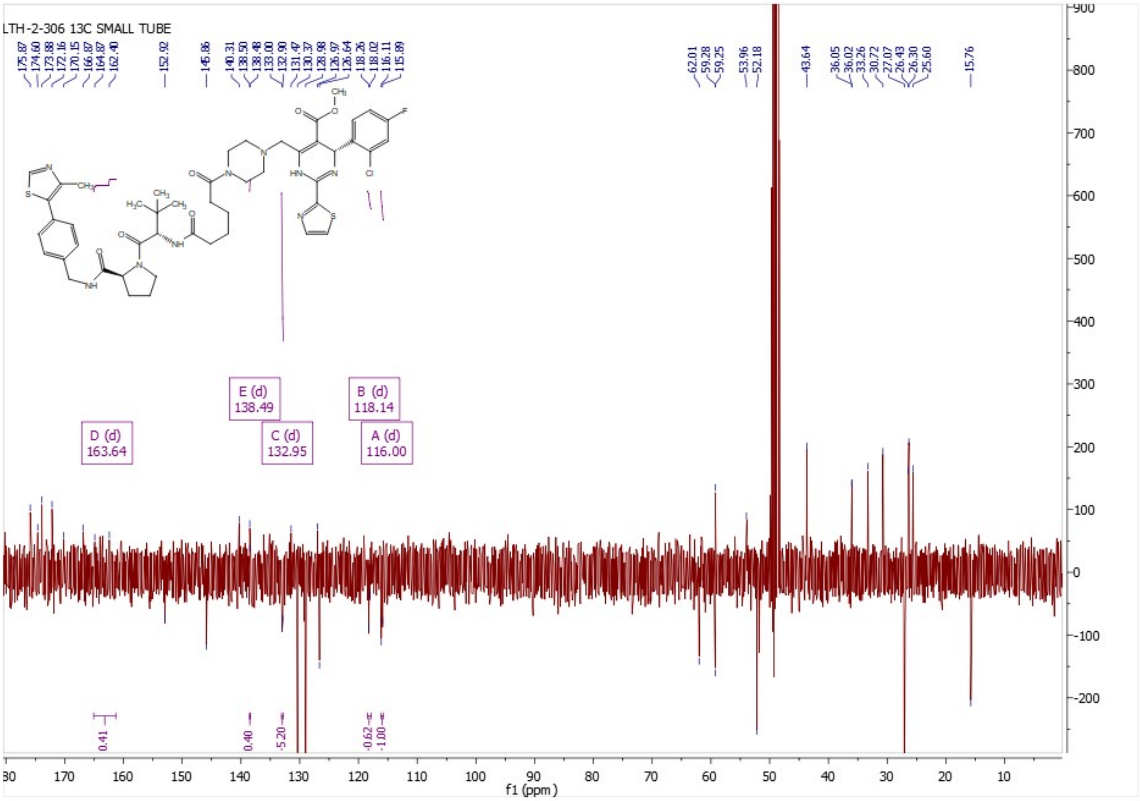
m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
893.3584	893.3581	0.33	4758555	100.00	100.00	(M+H)+	1
894.3615	894.3611	0.42	2571581	54.04	52.20	(M+H)+	1
915.3403	915.3401	0.25	3839215	100.00	100.00	(M+Na)+	1

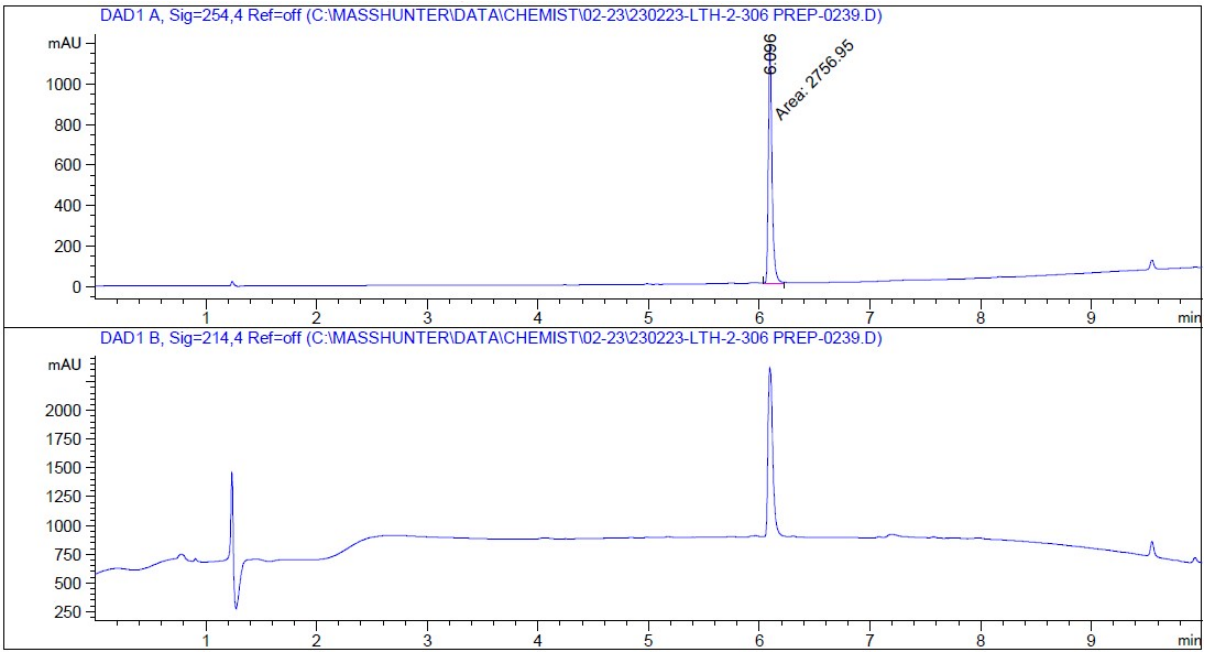
*HRMS of **LH-3-T1***

LH-3-desHyd



[1]H NMR spectrum of ***LH-3-desHyd***

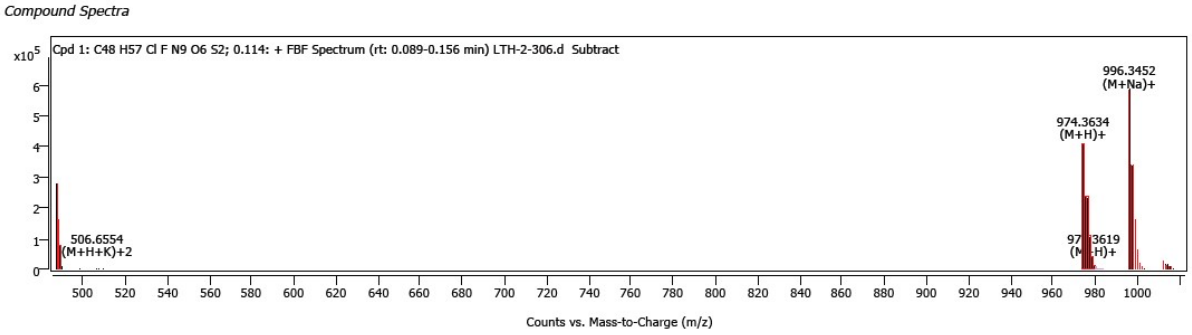




Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.096	MM	0.0392	2756.94849	1173.63953	100.0000
Totals :				2756.94849	1173.63953	

HPLC trace of ***LH-3-desHyd***



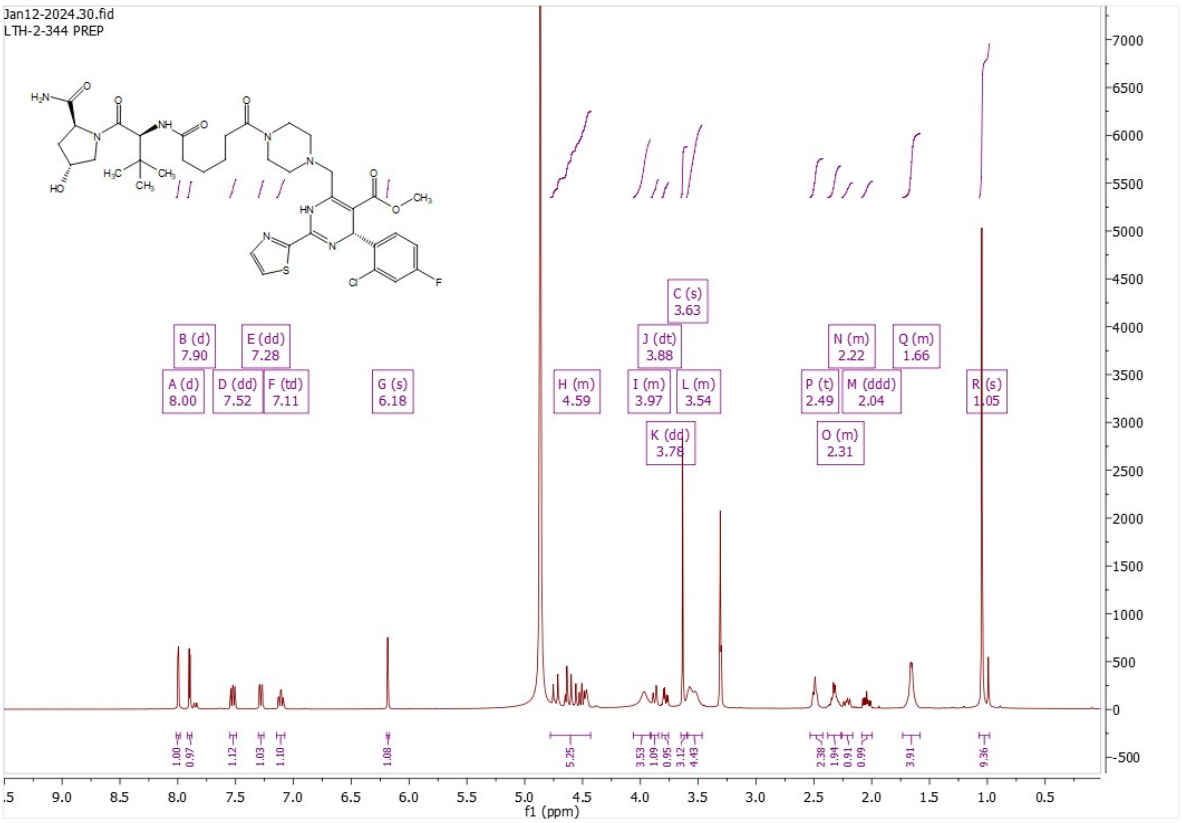
Spectrum Peaks (Max. 3)

m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
974.3634	974.3619	1.58	409712	100.00	100.00	(M+H)+	1
996.3452	996.3438	1.44	587282	100.00	100.00	(M+Na)+	1
998.3447	998.3433	1.47	340490	57.98	58.51	(M+Na)+	1

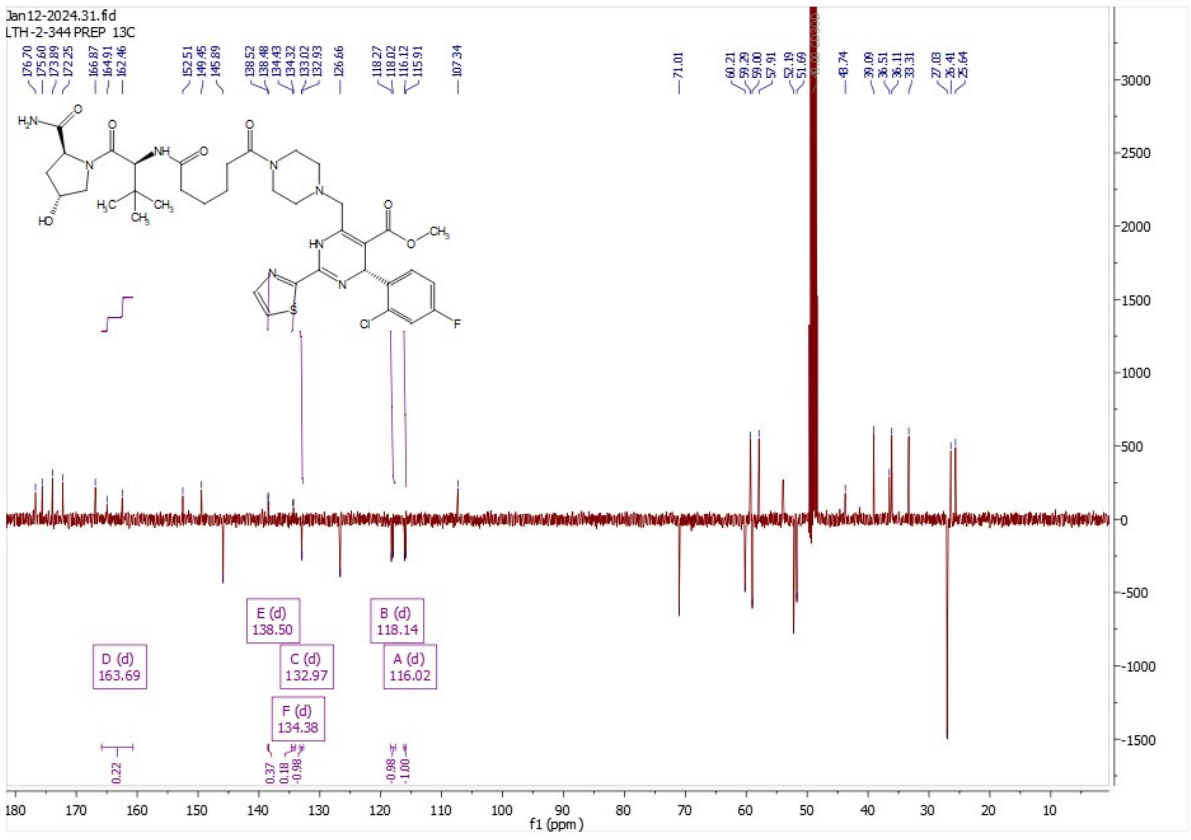
Spectrum Peaks (Max. 3)

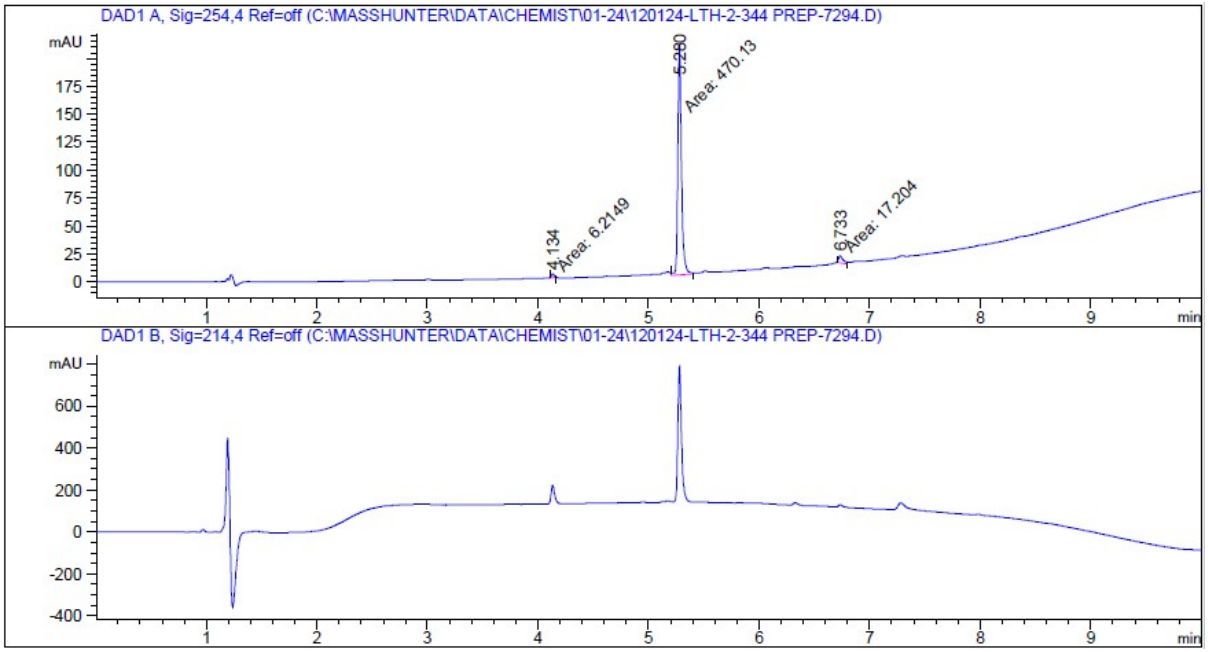
m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
974.3634	974.3619	1.58	409712	100.00	100.00	(M+H)+	1
996.3452	996.3438	1.44	587282	100.00	100.00	(M+Na)+	1
998.3447	998.3433	1.47	340490	57.98	58.51	(M+Na)+	1

HRMS of ***LH-3-desHyd***



LH-3-T2 *^{1}H NMR spectrum of **LH-3-T2***

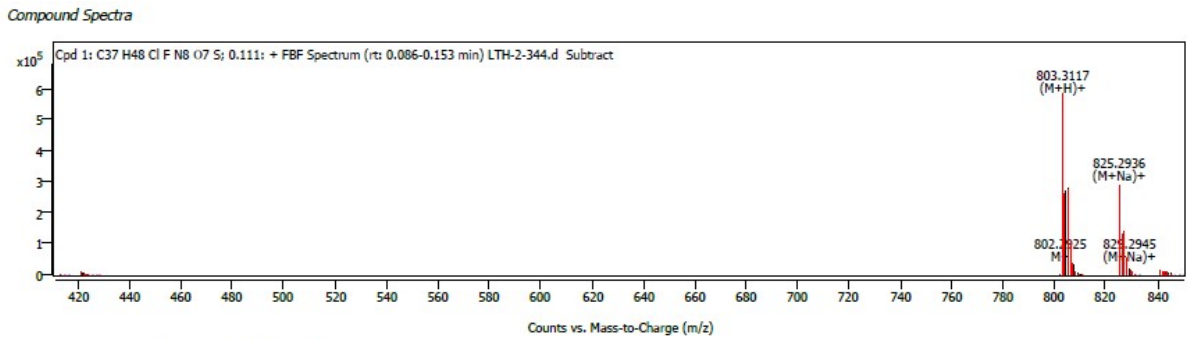




Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.134	MM	0.0303	6.21490	3.42327	1.2592
2	5.280	MM	0.0382	470.13043	205.35364	95.2550
3	6.733	MM	0.0472	17.20398	6.07183	3.4858
Totals :				493.54931	214.84873	

*HPLC trace of **LH-3-T2***



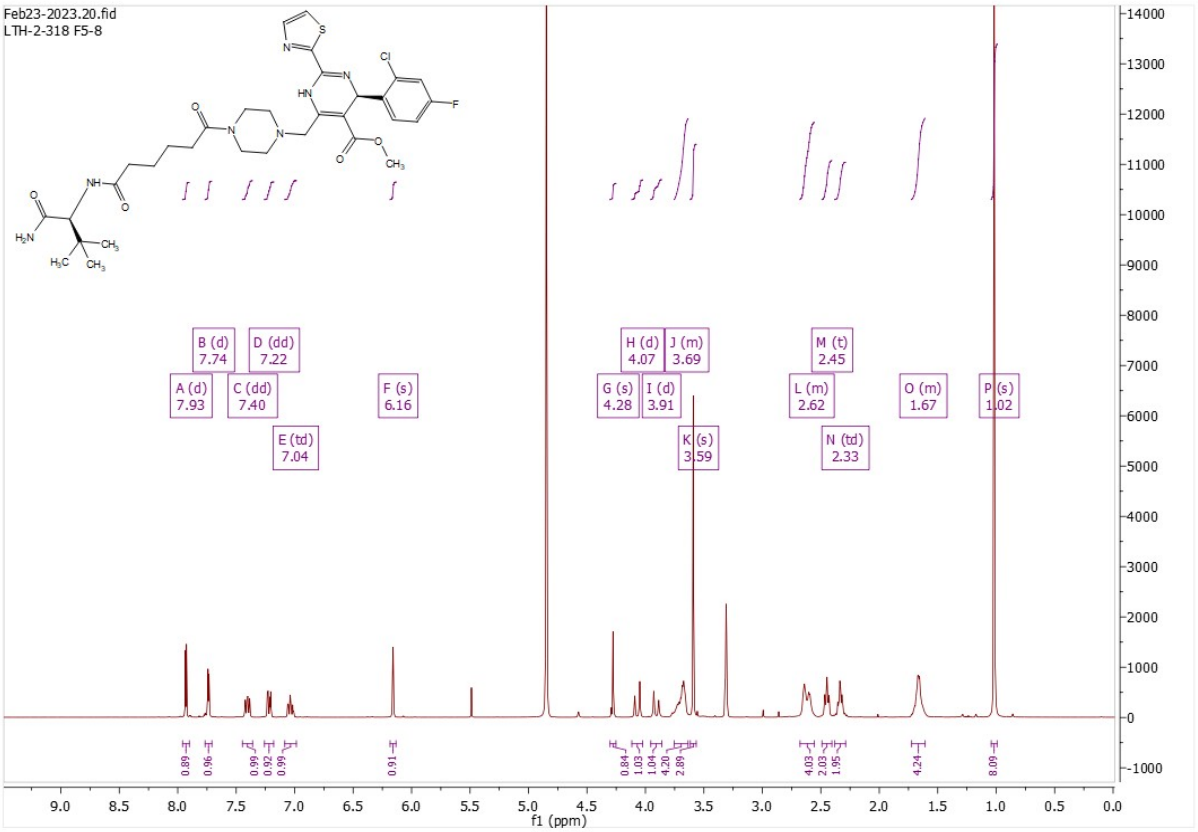
Spectrum Peaks (Max. 3)

m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
803.3117	803.3112	0.66	580260	100.00	100.00	(M+H)+	1
805.3108	805.3101	0.79	278879	48.06	47.61	(M+H)+	1
825.2936	825.2931	0.55	288921	100.00	100.00	(M+Na)+	1

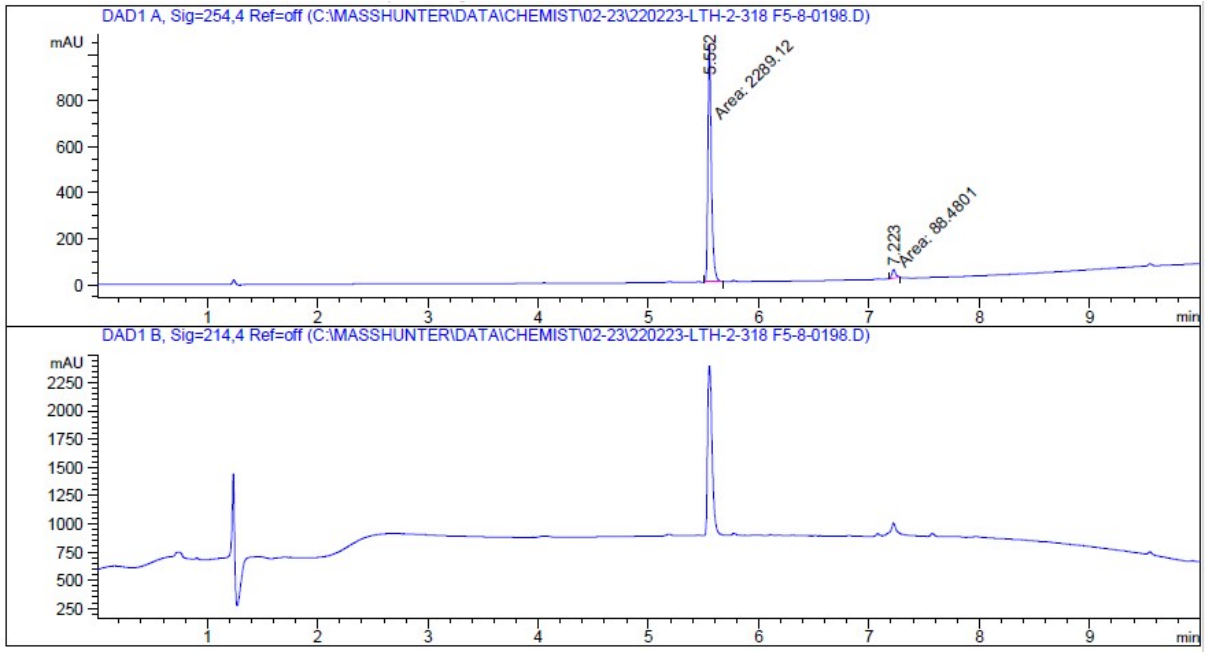
Spectrum Peaks (Max. 3)

m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
803.3117	803.3112	0.66	580260	100.00	100.00	(M+H)+	1
805.3108	805.3101	0.79	278879	48.06	47.61	(M+H)+	1
825.2936	825.2931	0.55	288921	100.00	100.00	(M+Na)+	1

*HRMS of **LH-3-T2***

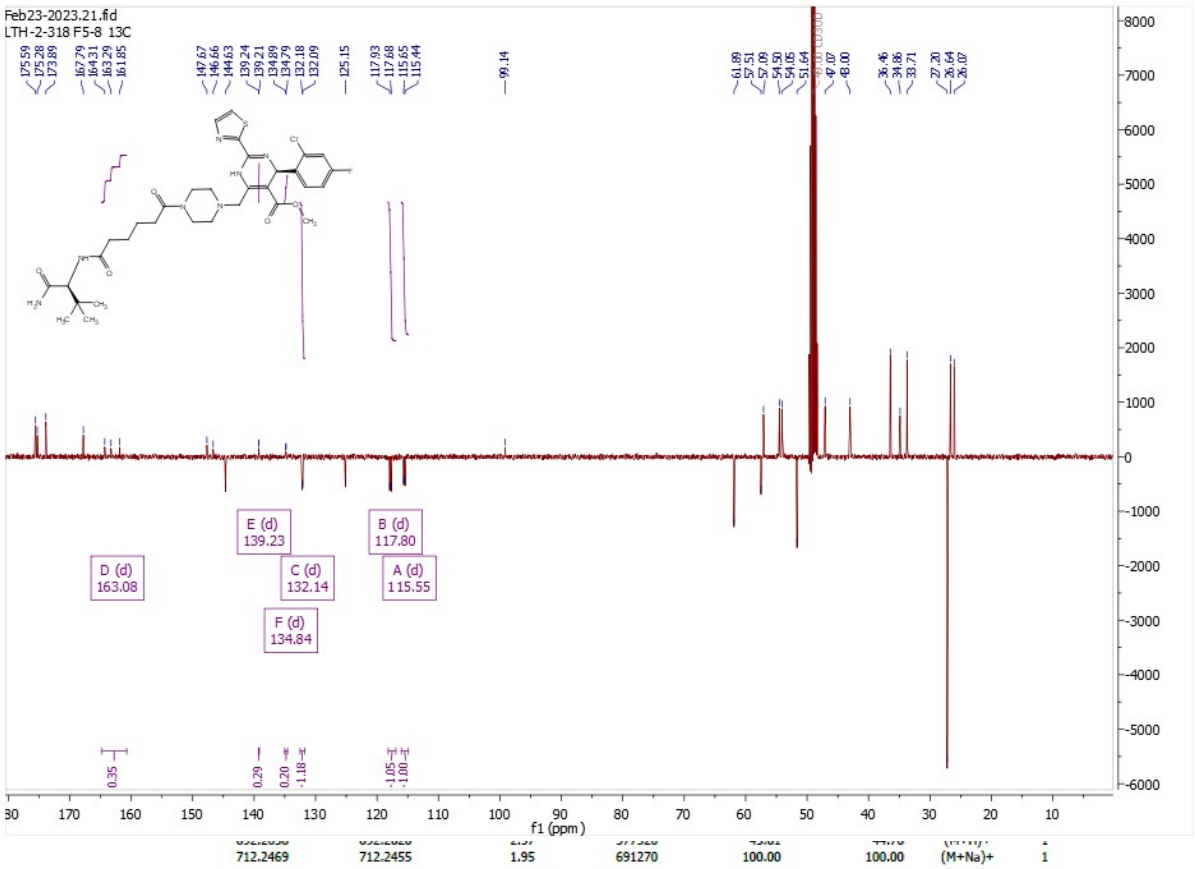


LH-3-T3 *^{1}H NMR spectrum of **LH-3-T3***



Signal 1: DAD1 A, Sig=254,4 Ref=off

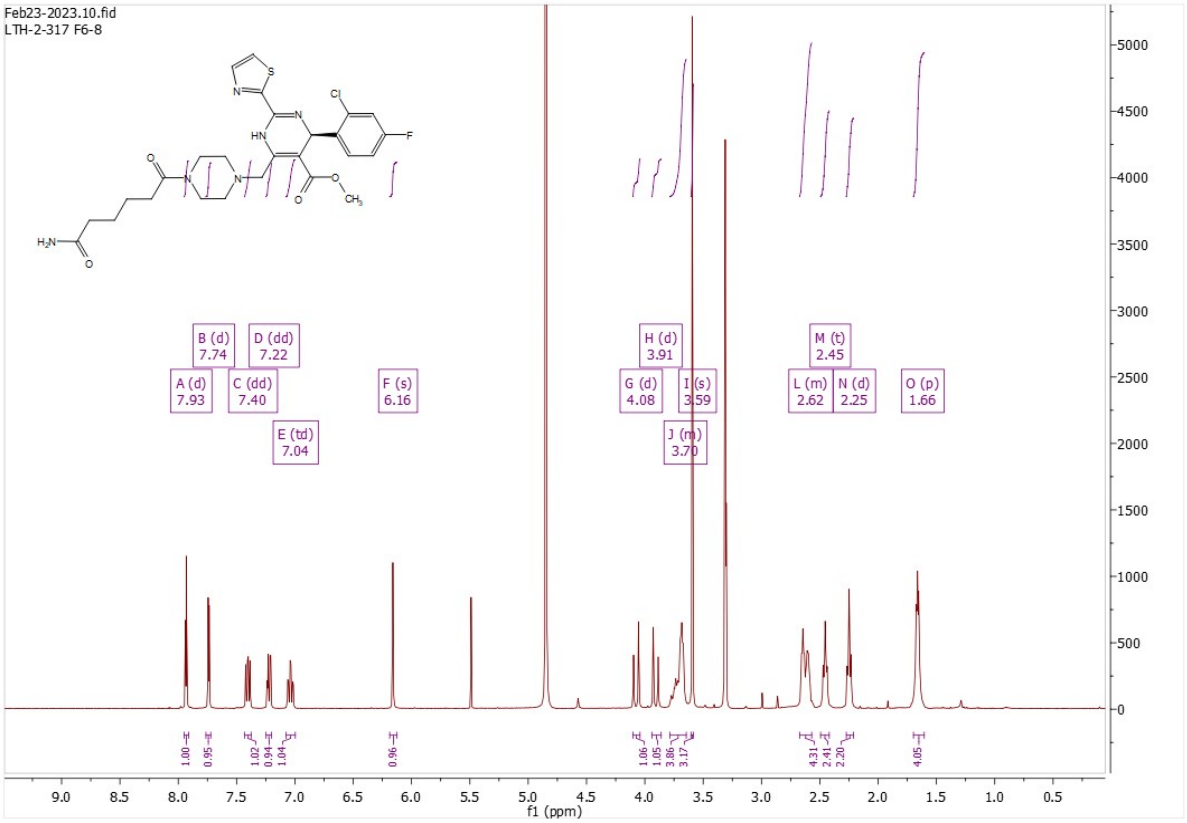
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %



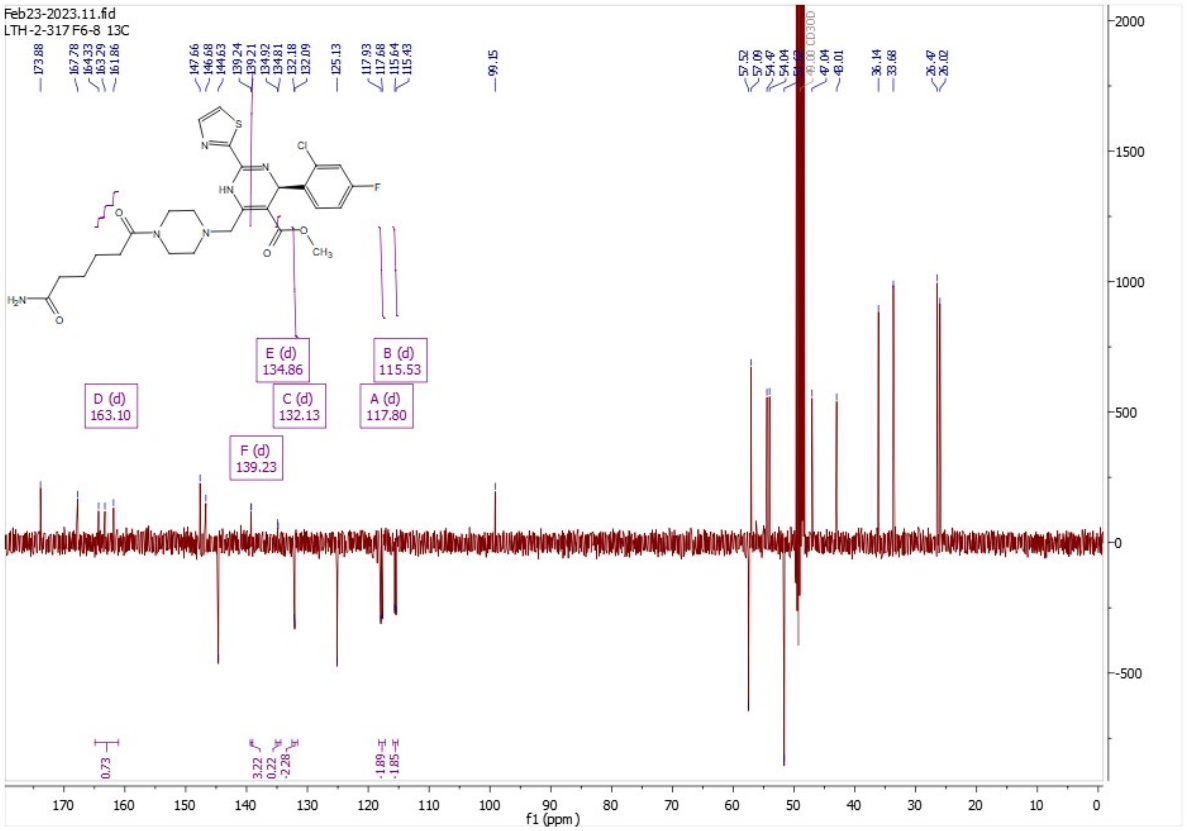
712.2469	712.2455	1.95	691270	100.00	100.00 (M+Na)+ 1

HRMS of ***LH-3-T3***

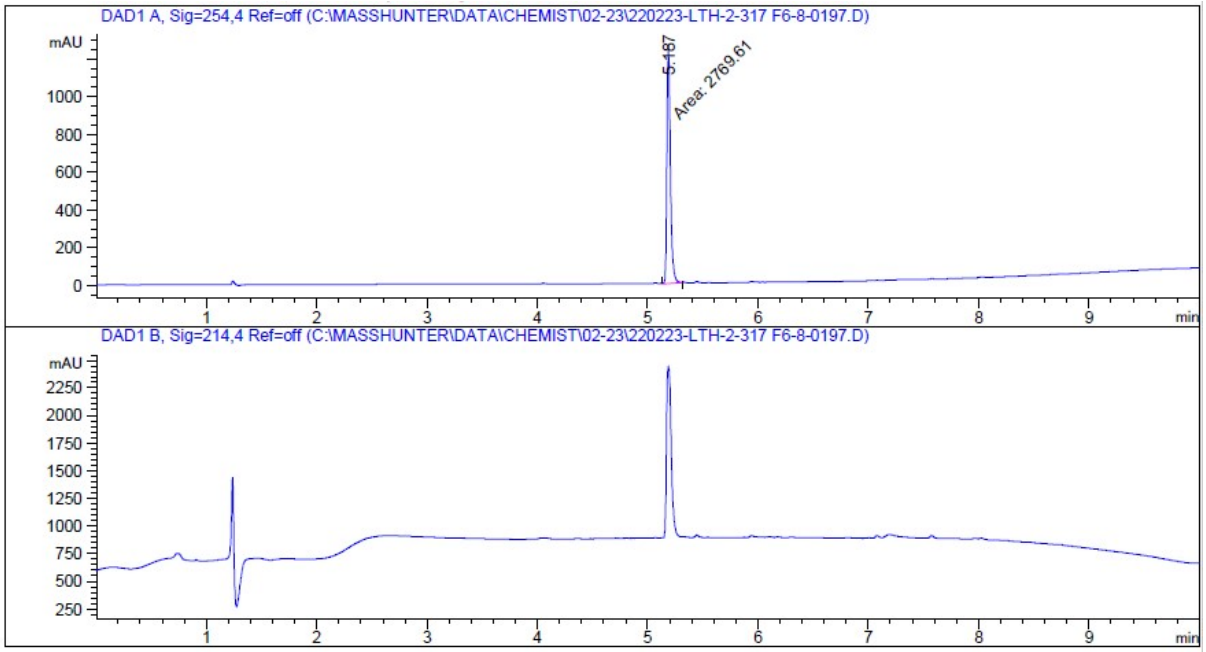
LH-3-T4



[1]H NMR spectrum of ***LH-3-T4***



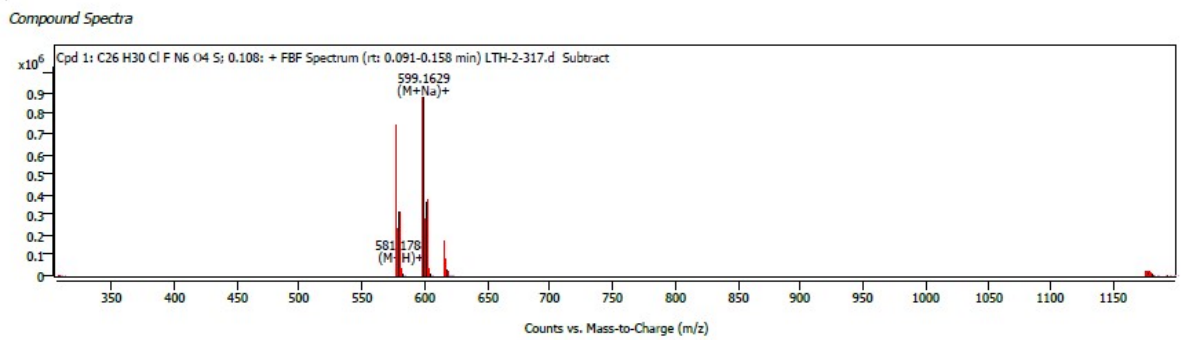
[13]C NMR spectrum of ***LH-3-T4***



Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.187	MM	0.0366	2769.61499	1262.33496	100.0000
Totals :				2769.61499	1262.33496	

*HPLC trace of **LH-3-T4***



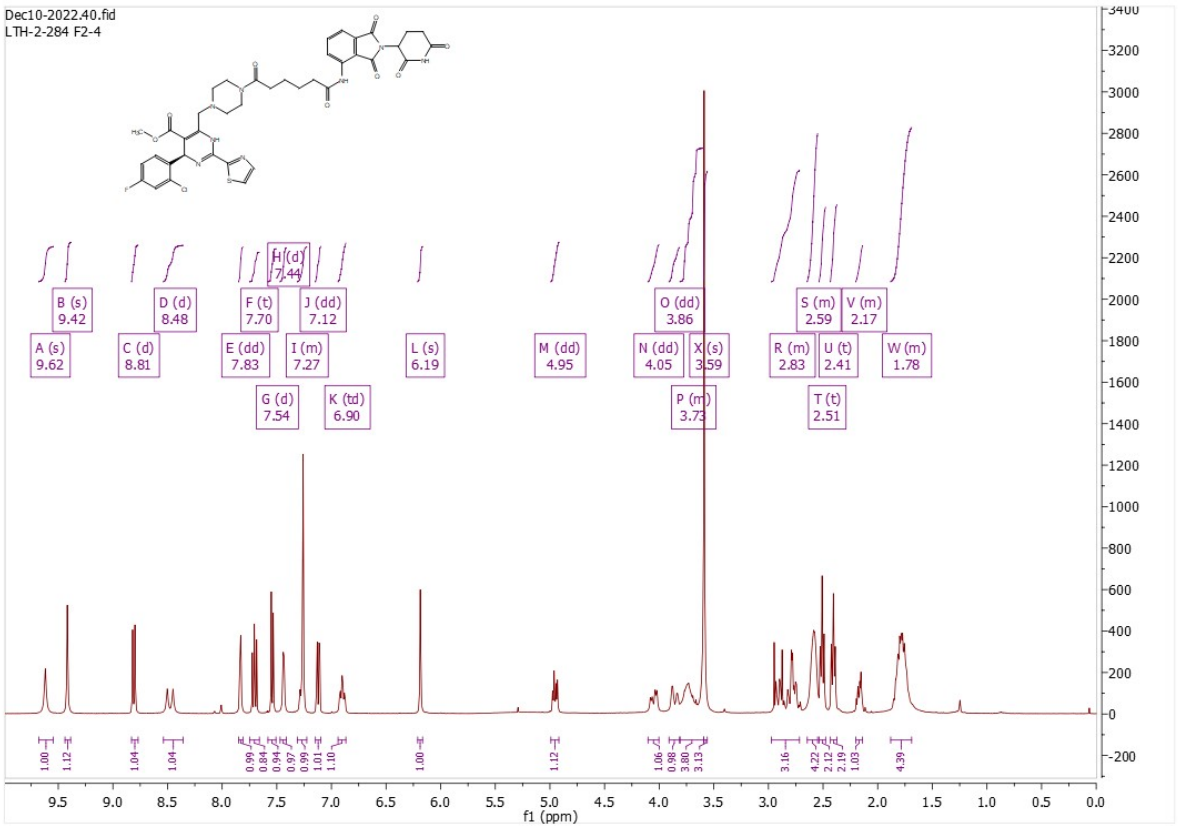
Spectrum Peaks (Max. 3)

m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
577.1811	577.1795	2.78	743466	100.00	100.00	(M+H)+	1
599.1629	599.1614	2.53	882953	100.00	100.00	(M+Na)+	1
601.1611	601.1594	2.70	360308	40.81	42.13	(M+Na)+	1

Spectrum Peaks (Max. 3)

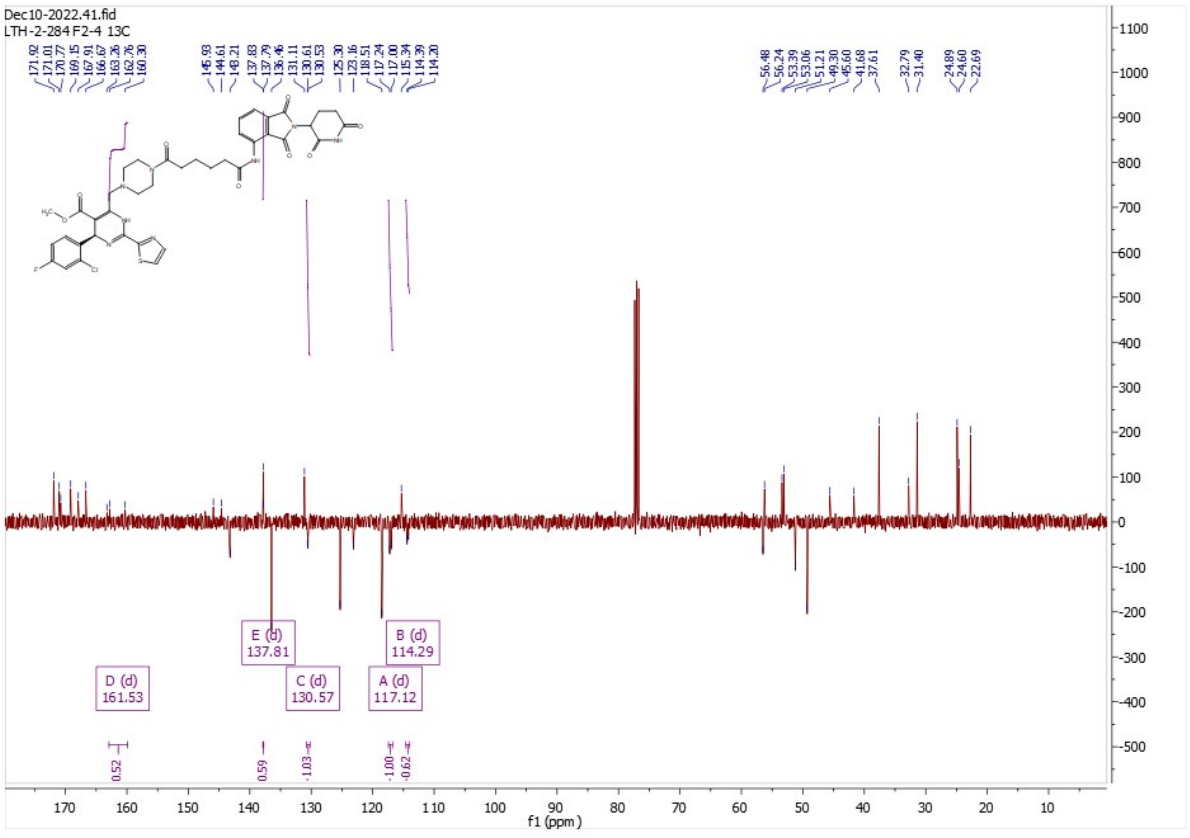
m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
577.1811	577.1795	2.78	743466	100.00	100.00	(M+H)+	1
599.1629	599.1614	2.53	882953	100.00	100.00	(M+Na)+	1
601.1611	601.1594	2.70	360308	40.81	42.13	(M+Na)+	1

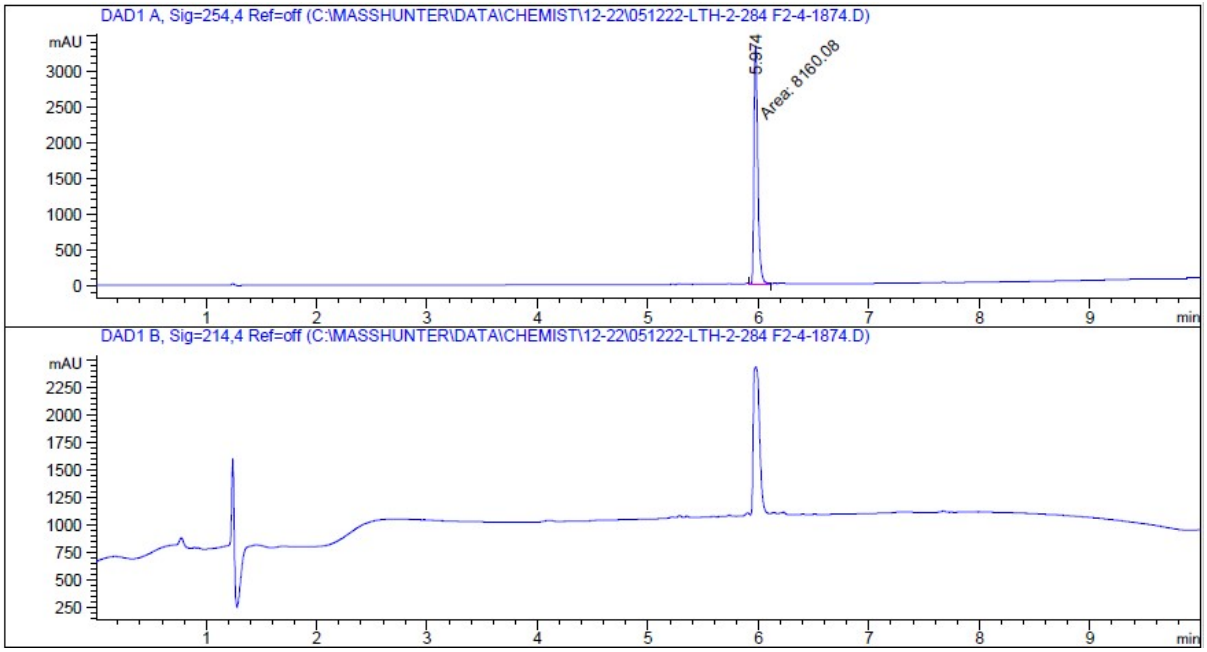
*HRMS of **LH-3-T4***



LH-3-Pom

[1]H NMR spectrum of ***LH-3-Pom***

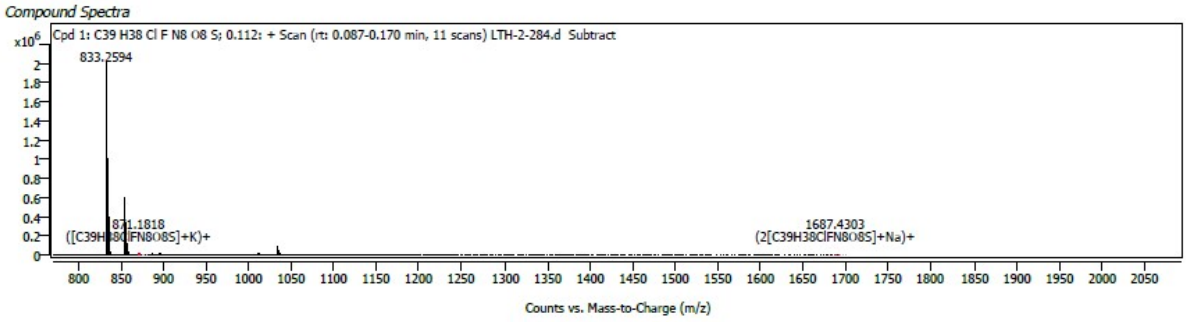




Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.974	MM	0.0407	8160.08301	3338.14160	100.0000
Totals :				8160.08301	3338.14160	

*HPLC trace of **LH-3-Pom***



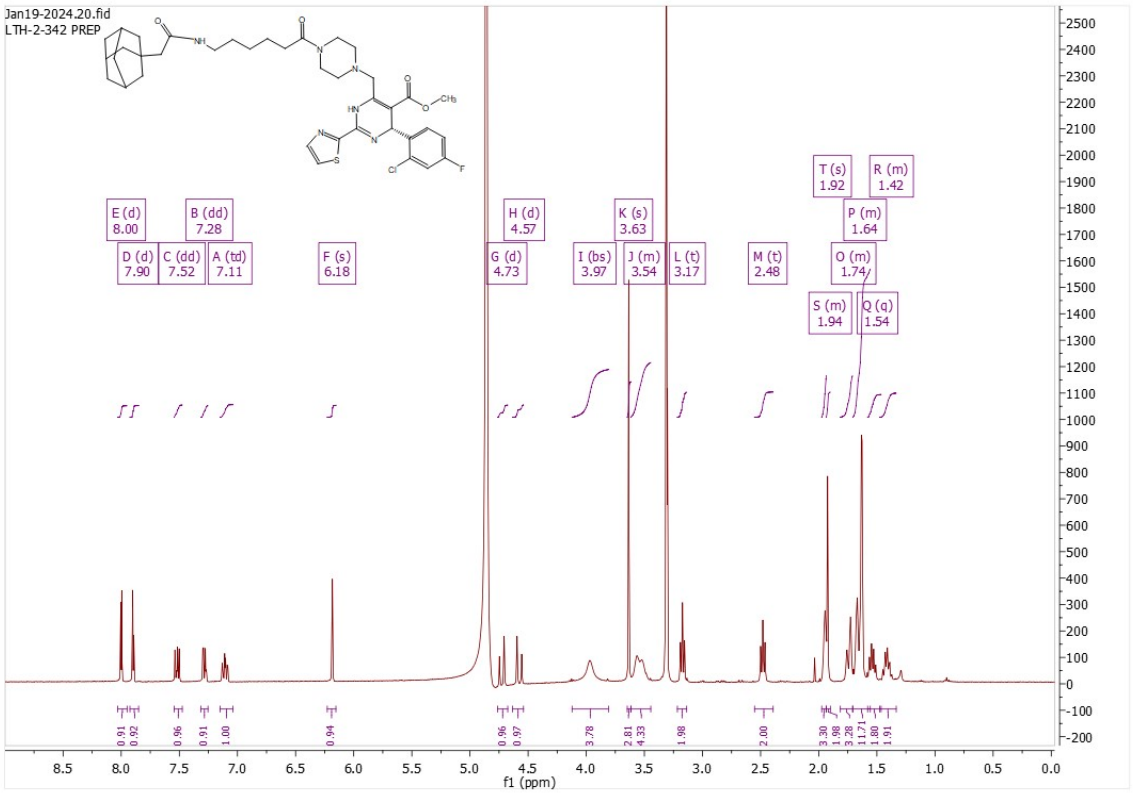
Spectrum Peaks (Max. 3)

m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
871.1818	871.1837	-2.26	23821	100.00	100.00	(M+K)+	1
872.1853	872.1867	-1.54	10695	44.90	46.65	(M+K)+	1
873.1820	873.1827	-0.76	12233	51.35	55.97	(M+K)+	1

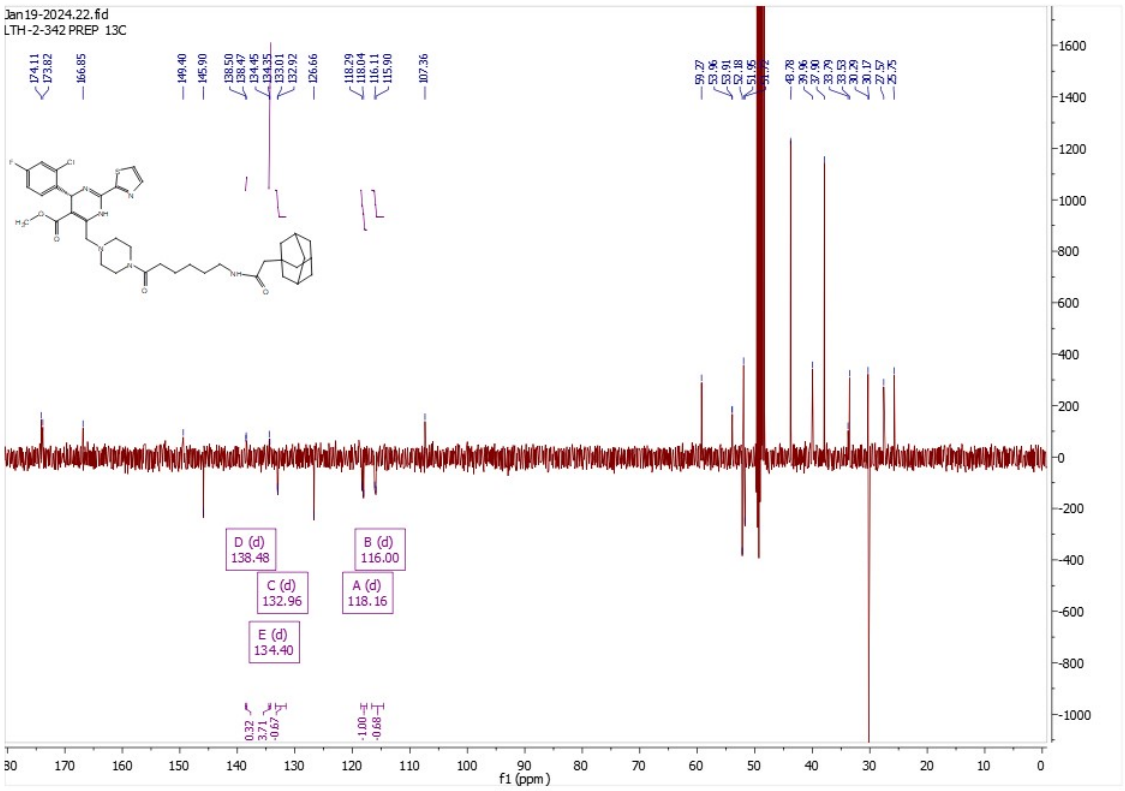
Spectrum Peaks (Max. 3)

m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
871.1818	871.1837	-2.26	23821	100.00	100.00	(M+K)+	1
872.1853	872.1867	-1.54	10695	44.90	46.65	(M+K)+	1
873.1820	873.1827	-0.76	12233	51.35	55.97	(M+K)+	1

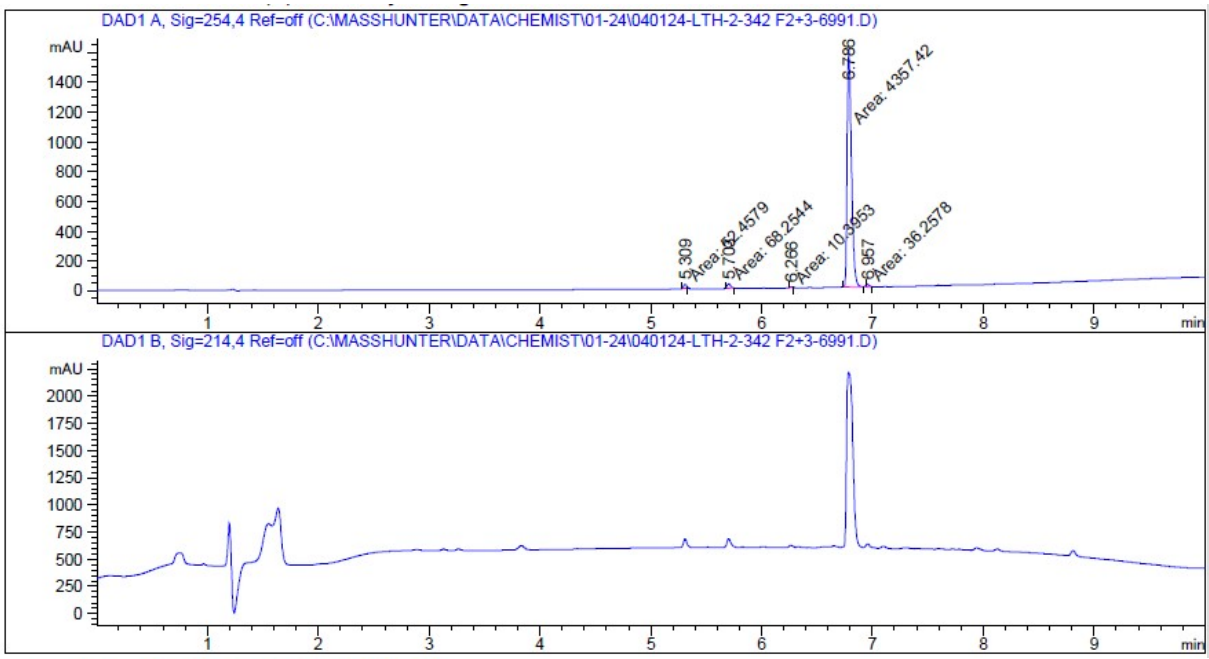
*HRMS of **LH-3-Pom***



LH-3-adam



^{13}C NMR spectrum of ***LH-3-adam***

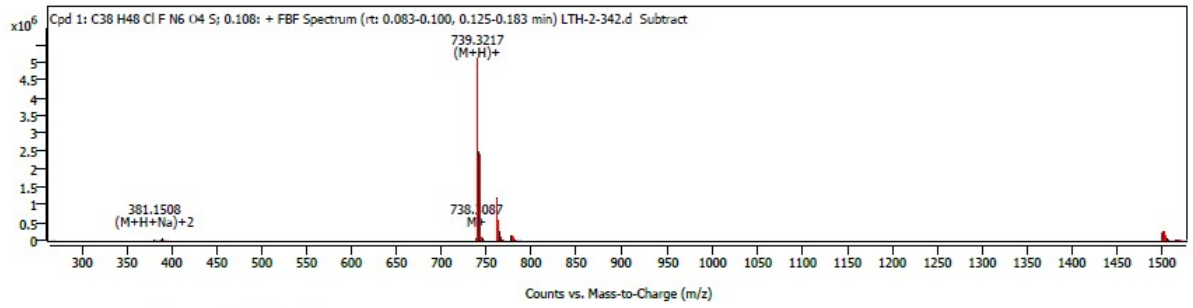


Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.309	MM	0.0298	52.45792	29.33819	1.1593
2	5.703	MM	0.0363	68.25436	31.34859	1.5085
3	6.266	MM	0.0257	10.39526	6.73329	0.2297
4	6.786	MM	0.0456	4357.41895	1592.46521	96.3011
5	6.957	MM	0.0376	36.25781	16.07712	0.8013
Totals :				4524.78429	1675.96240	

*HPLC trace of **LH-3-adam***

Compound Spectra

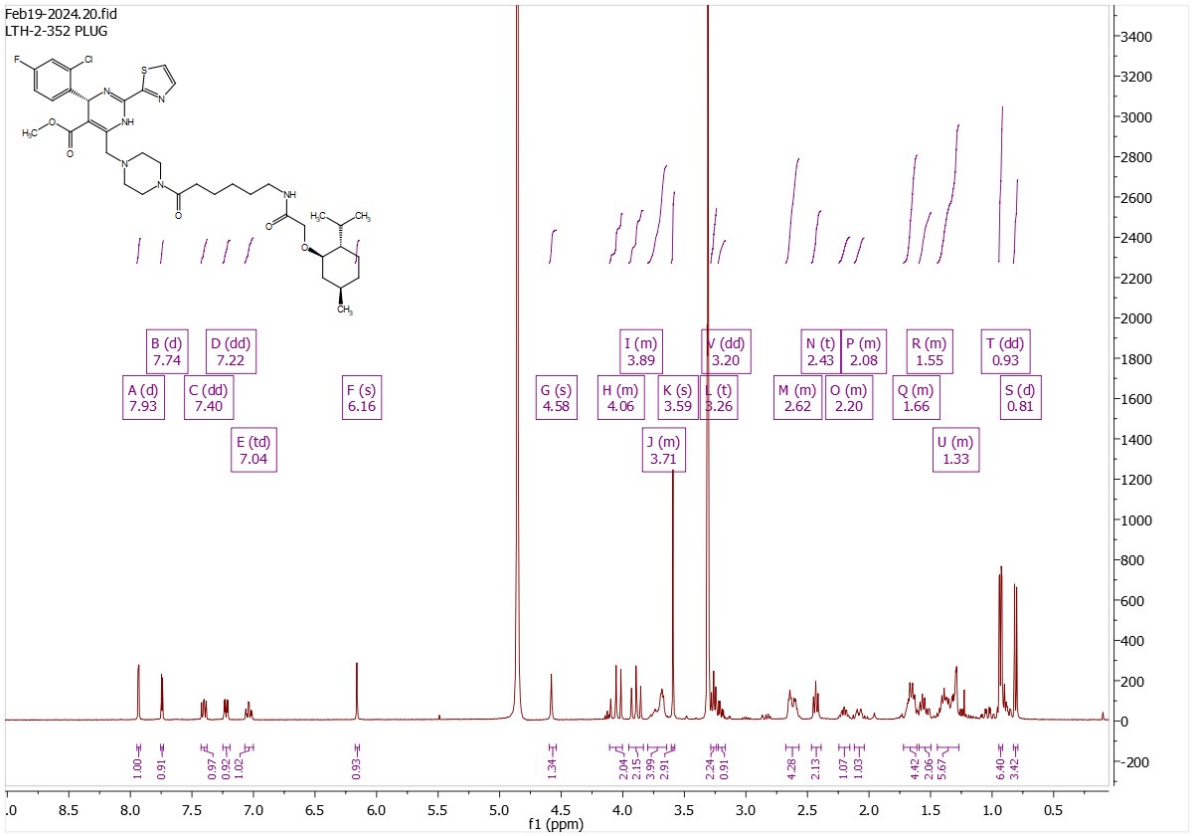


Spectrum Peaks (Max. 3)

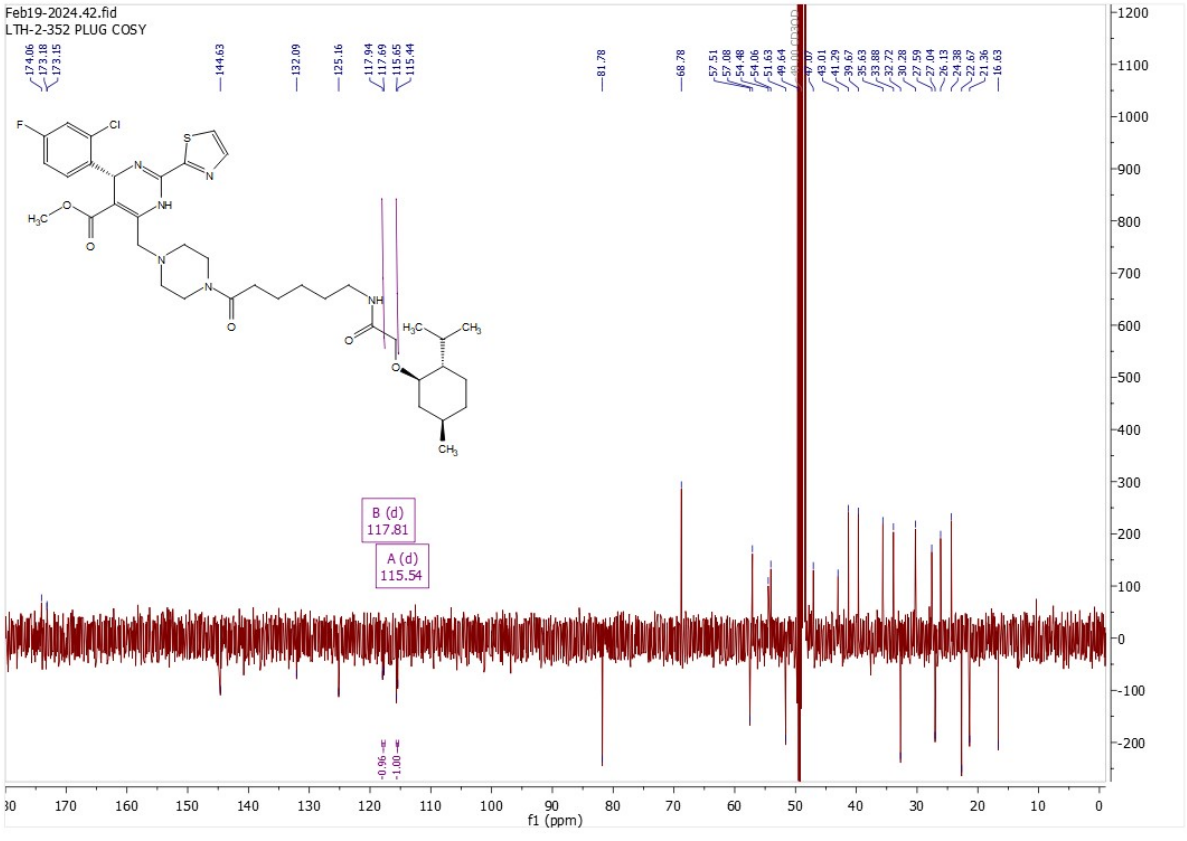
m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
739.3217	739.3203	1.87	4903664	100.00	100.00	(M+H)+	1
740.3248	740.3233	1.99	2336845	47.66	44.80	(M+H)+	1
741.3208	741.3192	2.06	2456420	50.09	47.10	(M+H)+	1

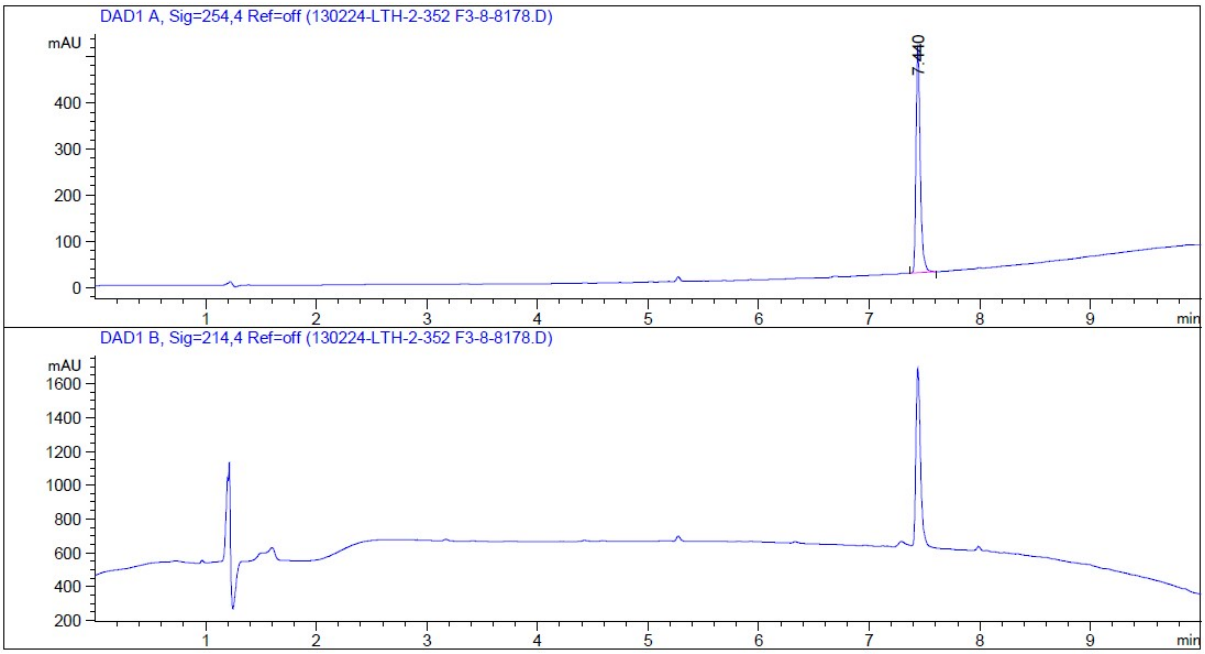
Spectrum Peaks (Max. 3)

m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
739.3217	739.3203	1.87	4903664	100.00	100.00	(M+H)+	1
740.3248	740.3233	1.99	2336845	47.66	44.80	(M+H)+	1
741.3208	741.3192	2.06	2456420	50.09	47.10	(M+H)+	1


Feb19-2024.20.fid
LTH-2-352 PLUG
B (d) 7.74
D (dd) 7.22
A (d) 7.93
C (dd) 7.40
E (td) 7.04
F (s) 6.16
G (s) 4.58
I (m) 3.89
H (m) 4.06
K (s) 3.59
J (m) 3.71
V (dd) 3.20
L (t) 3.26
N (t) 2.43
P (m) 2.08
M (m) 2.62
O (m) 2.20
R (m) 1.55
Q (m) 1.66
U (m) 1.33
T (dd) 0.93
S (d) 0.81
f1 (ppm)

LH-3-menth


Feb19-2024.42.fid
LTH-2-352 PLUG COSY
B (d) 117.81
A (d) 115.54
f1 (ppm)

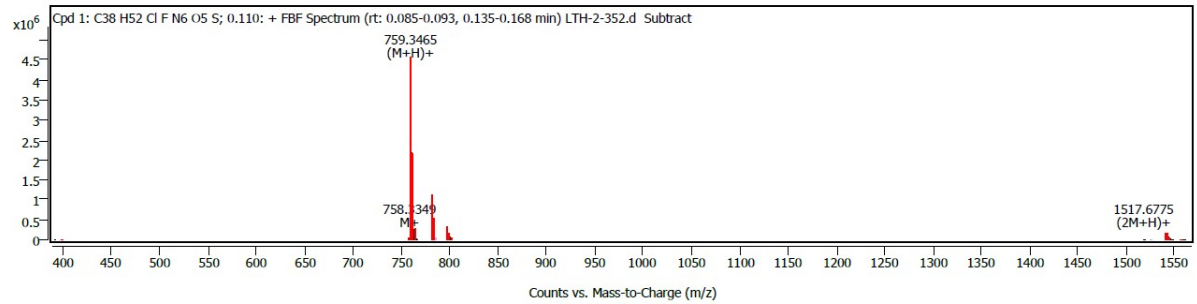


Signal 1: DAD1 A, Sig=254,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.440	VB	0.0405	1308.15222	491.70953	100.0000
Totals :				1308.15222	491.70953	

HPLC trace of ***LH-3-menth***

Compound Spectra

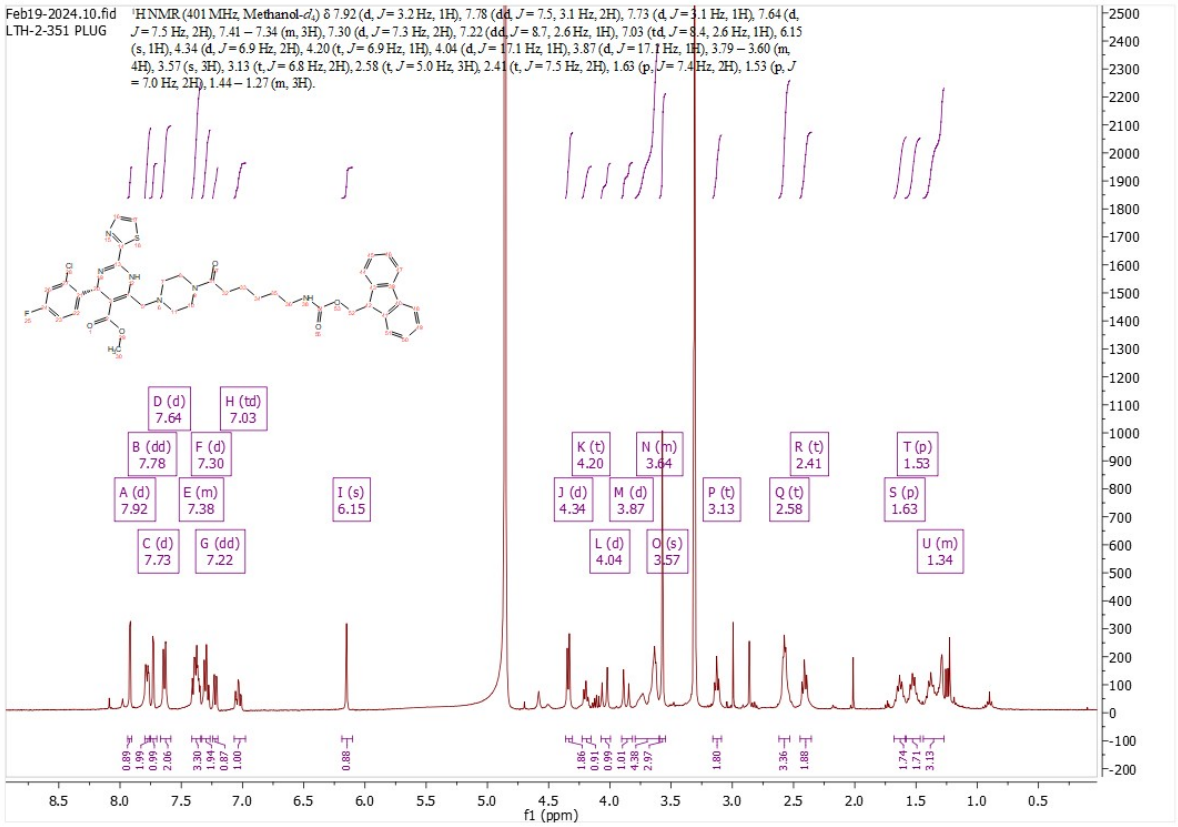


Spectrum Peaks (Max. 3)

m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
759.3465	759.3465	-0.06	4466485	100.00	100.00	(M+H)+	1
760.3497	760.3495	0.17	2070855	46.36	44.88	(M+H)+	1
761.3457	761.3455	0.28	2176074	48.72	47.34	(M+H)+	1

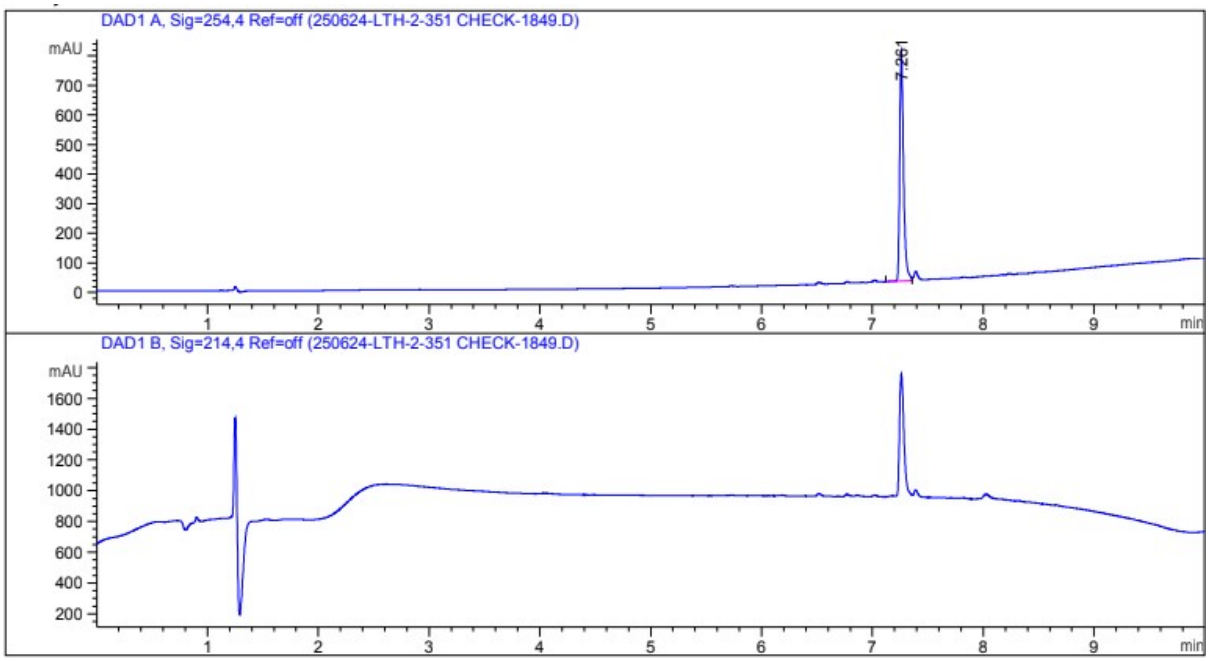
Spectrum Peaks (Max. 3)

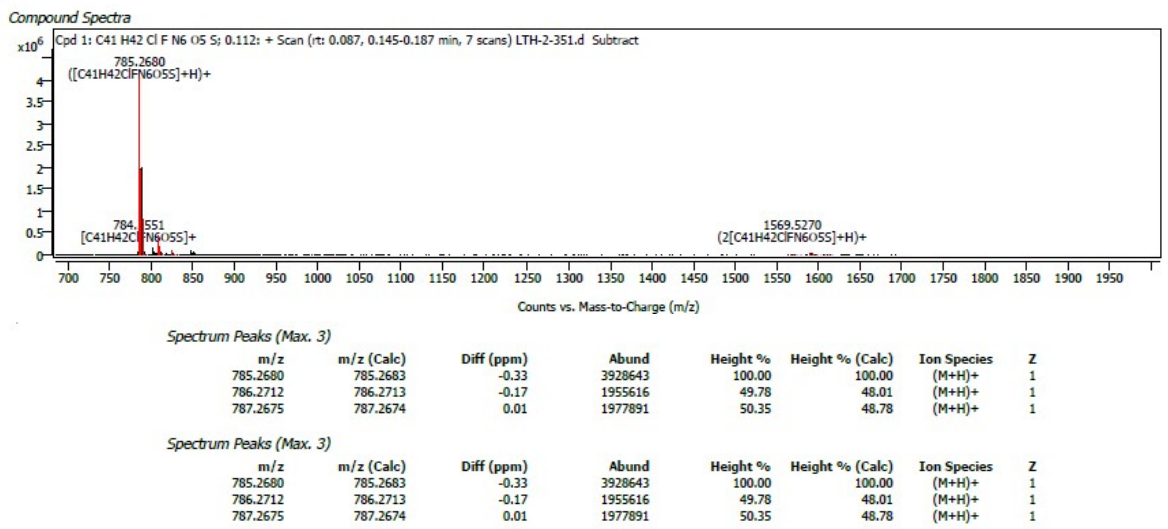
m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
759.3465	759.3465	-0.06	4466485	100.00	100.00	(M+H)+	1
760.3497	760.3495	0.17	2070855	46.36	44.88	(M+H)+	1
761.3457	761.3455	0.28	2176074	48.72	47.34	(M+H)+	1



LH-3-fluor *^{1}H NMR spectrum of **LH-3-fluor***

HPLC trace of ***LH-3-fluor***





Spectrum Peaks (Max. 3)

m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
785.2680	785.2683	-0.33	3928643	100.00	100.00	(M+H)+	1
786.2712	786.2713	-0.17	1955616	49.78	48.01	(M+H)+	1
787.2675	787.2674	0.01	1977891	50.35	48.78	(M+H)+	1

Spectrum Peaks (Max. 3)

m/z	m/z (Calc)	Diff (ppm)	Abund	Height %	Height % (Calc)	Ion Species	Z
785.2680	785.2683	-0.33	3928643	100.00	100.00	(M+H)+	1
786.2712	786.2713	-0.17	1955616	49.78	48.01	(M+H)+	1
787.2675	787.2674	0.01	1977891	50.35	48.78	(M+H)+	1

*HRMS of **LH-3-fluor***

References

1. J. Frost, S. Rocha and A. Ciulli, *J. Biol. Chem.*, 2021, **297**.
2. L. T. Hales and P. E. Thompson, *Chem. Eur. J.*, 2023, **29**, e202301975.
3. N. B. Yapici, S. Mandalapu, K. Michael Gibson and L. Bi, *Bioorg. Med. Chem. Lett.*, 2015, **25**, 3476-3480.
4. WO 2020/125730 A1, 2020.
5. *WO Pat.,* WO 2015/078391 A1, 2015.
6. *WO Pat.,* WO 2014/153459 A2, 2014.
7. C. Galdeano, M. S. Gadd, P. Soares, S. Scaffidi, I. Van Molle, I. Birced, S. Hewitt, D. M. Dias and A. Ciulli, *J. Med. Chem.*, 2014, **57**, 8657-8663.
8. X. Han, C. Wang, C. Qin, W. Xiang, E. Fernandez-Salas, C.-Y. Yang, M. Wang, L. Zhao, T. Xu, K. Chinnaswamy, J. Delproposto, J. Stuckey and S. Wang, *J. Med. Chem.*, 2019, **62**, 941-964.
9. *USA Pat.,* 2019.
10. *WO Pat.,* WO 2018/013559 A1, 2018.
11. *WO Pat.,* WO 2019/123367 A1, 2019.
12. C. Cao, J. Yang, Y. Chen, P. Zhou, Y. Wang, W. Du, L. Zhao and Y. Chen, *J. Med. Chem.*, 2020, **63**, 11012-11033.
13. M. de Wispelaere, G. Du, K. A. Donovan, T. Zhang, N. A. Eleuteri, J. C. Yuan, J. Kalabathula, R. P. Nowak, E. S. Fischer, N. S. Gray and P. L. Yang, *Nat. Commun.*, 2019, **10**, 3468.
14. M. Zengerle, K.-H. Chan and A. Ciulli, *ACS Chem. Biol.*, 2015, **10**, 1770-1777.