Supporting Information

Rapid and reversible knockdown of endogenously tagged endosomal proteins via an optimized HaloPROTAC degrader.

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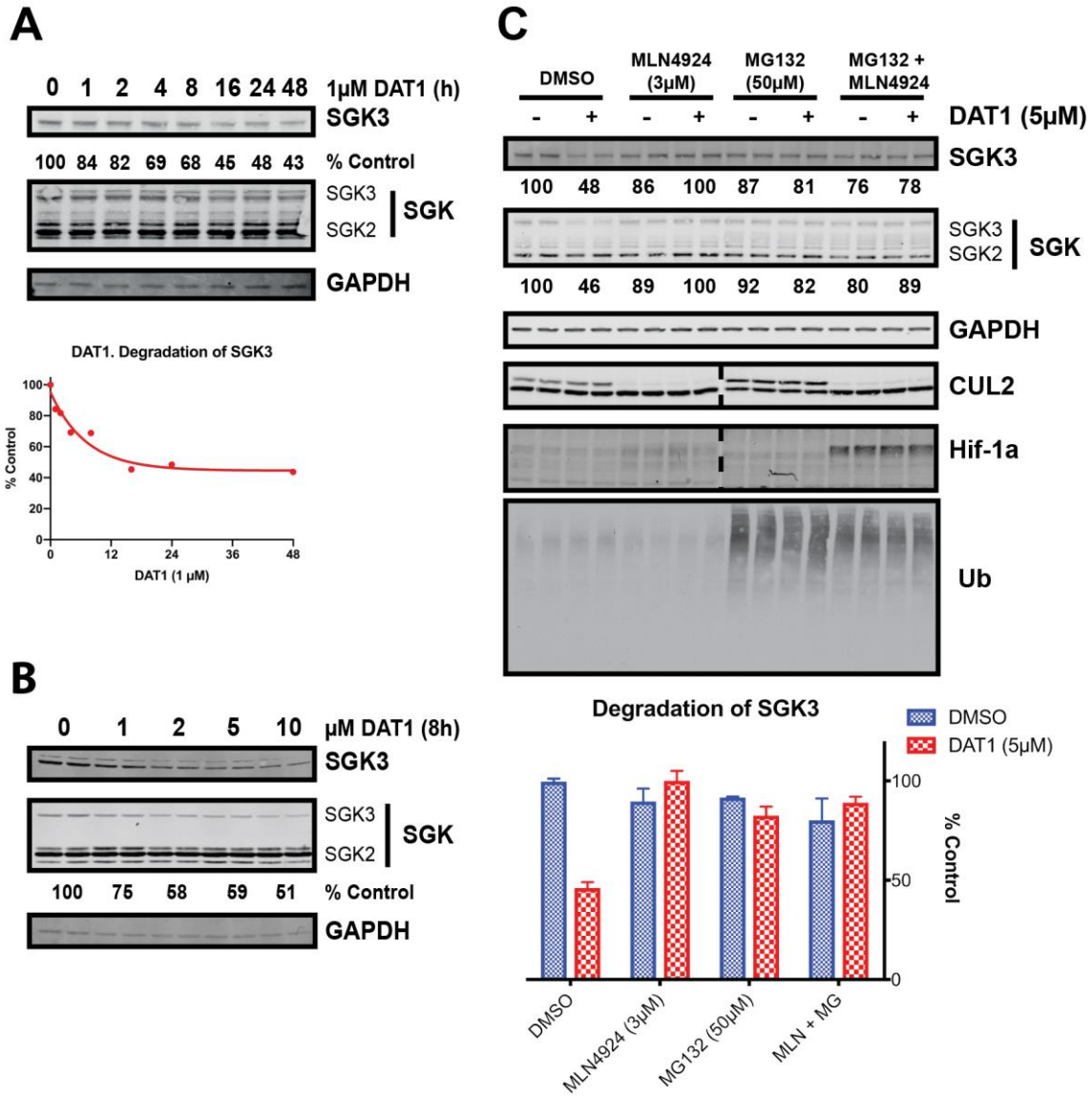
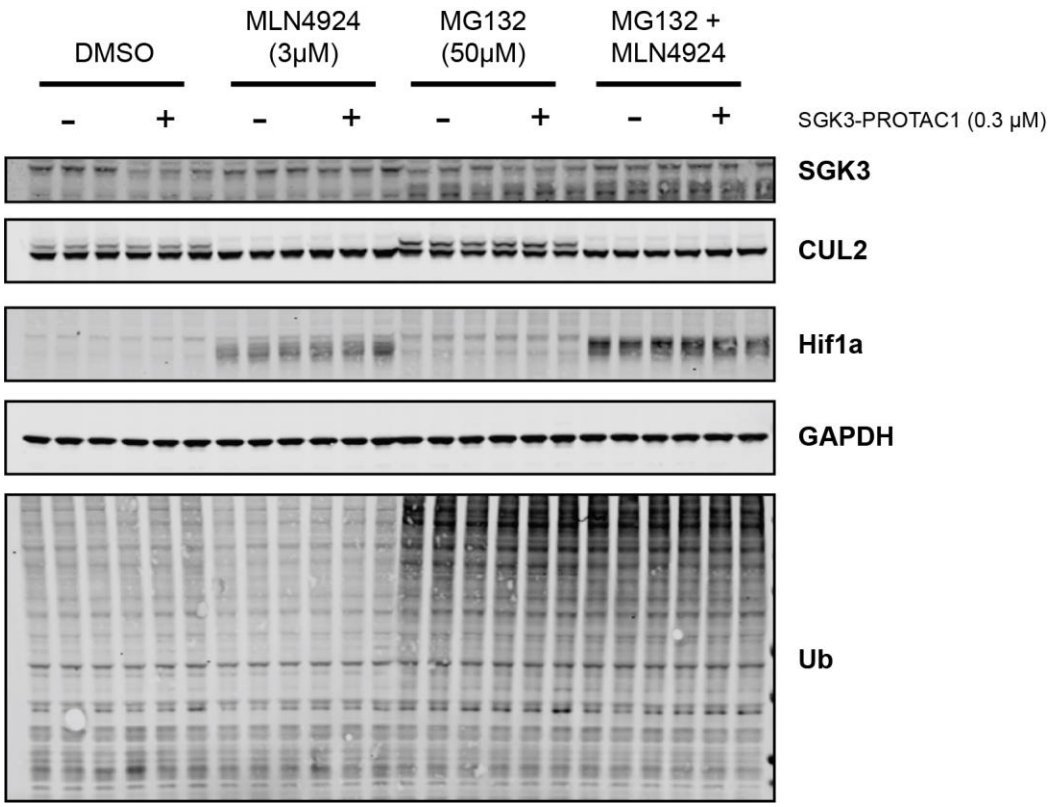
Contents

1. SUPPLEMENTARY FIGURES

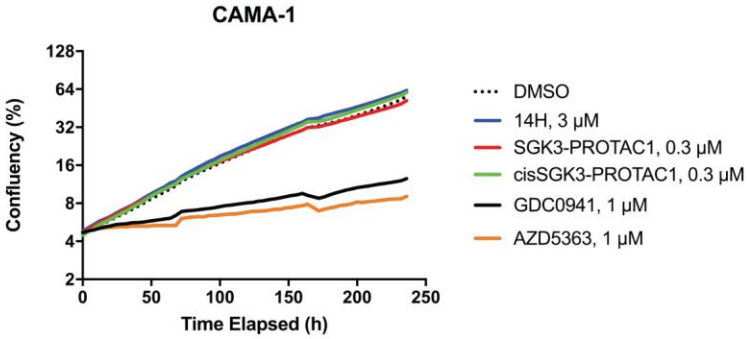


Supplementary Figure S1. Mechanistic characterization of DAT1. A. HEK293 cells were treated for up to 48h with 1 µM DAT1, and lysates analysed by western blot for SGK3 expression B. HEK293 cells were treated for 6 h with increasing doses of DAT1, and lysates analysed by western blot. C. HEK293 cells were pretreated with the indicated inhibitors before treatment for 6 h with 5 µM DAT1, and lysates analysed by western blot for SGK3 expression



Supplementary Figure S2. Mechanistic characterization of SGK3-PROTAC1. HEK293 cells were pretreated with the indicated inhibitors before treatment for 6 h with 0.3 μM SGK3PROTAC1, and lysates analysed by western blot for SGK3 expression

A



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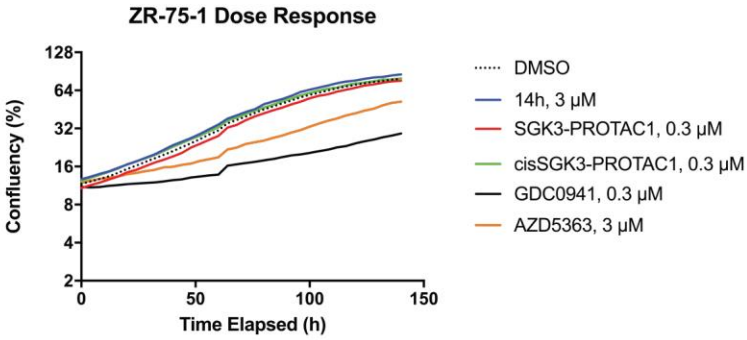


Supplementary Figure S3. SGK3-PROTAC compounds have no effect on cell growth. CAMA-1 and ZR-75-1 cells were seeded in 96 well plates, allowed to adhere overnight, and treated as indicated in the figure. Cell confluency was measured every 4 h on Incucyte S3.

2. MASS SPECTROMETRY METHODS

Cell culture and proteomic sample preparation

Cells pellets were lysed with 2 ml of lysis buffer (8 M Urea, 50 mM Ammonium bicarbonate containing protease inhibitor and phosphatase inhibitor). The sample were left on ice for 15 min and were then sonicated for 5 min in ice-cold water bath under high intensity with 30 sec on and off. After sonication, benzonase® endonuclease (Merck Millipore) with the volume ratio of 1: 100 were added into the lysate to degrade DNA and RNA. The lysate were then centrifuged at 12,000 rpm for 15 min at 4 °C, and supernatants were transferred into new eppendorf tubes. The protein concentration were measured with BCA assay (Pierce™ BCA Protein Assay Kit, Thermo). The lysate from each sample was reduced with 5 mM DTT at 45 °C for 30 min. Lysates were briefly centrifuged and were cool down to room temperature before alkylation in the dark with 10 mM iodoacetamide at room temperature. The alkylation was then quenched by the addition of 5 mM DTT. Samples were first digested by Lys-C with the weight ratio of 1:200 (w/w) for 4 h at 30 °c. Then, sample were diluted with 50 mM ammonium bicarbonate to 1.5 M Urea concentration. Samples were then digested with trypsin (Pierce trypsin, Thermo) at an enzyme to protein ratio of 1:50 (w/w) at room temperature with gentle shaking for overnight.

The digest was stopped by the addition of 1% TFA (v/v), centrifuged at 10,000 g for 10 min at room temperature. The supernatant were desalted on 200 mg SepPak tC18 cartridge (Waters). Desalted peptides were dried by vacuum centrifugation using Speedvac (Thermo).

TMT labelling and Basic C18 reverse phase (bRP) chromatography fractionation
Each sample (200 µg of peptides each) was re-suspended in 100 µL of 100 mM TEAB buffer. The TMT labelling reagents were equilibrated to room temperature and 41 µL anhydrous acetonitrile was added to each reagent channel and softly vortexed for 10 min. Peptides were transferred to the corresponding TMT channels and incubated for 1 h at room temperature. The reaction was quenched with 8 µL of 5% hydroxylamine. To ensure complete labelling, 1 µg of labelled samples from each channel were analyzed by LC-MS/MS prior to mix. After evaluation, the complete TMT labelled 9 samples were then combined, acidified and dried. Sep-Pak desalting was then performed and the elution was dried to completeness.
Ultimate 3000 high-pressure liquid chromatography (HPLC) system (Dionex) were used for basic C18 reverse phase chromatography fractionation operating at 569 µL/min with two buffers: buffer A (10 mM ammonium formate, pH 10) and buffer B (80% ACN, 10 mM ammonium formate, pH 10). The desalted mixture of TMT-labelled peptides were resuspended in 200 µL of buffer A (10 mM ammonium formate, pH10) and separated on a C18 reverse phase column (4.6 × 250 mm, 3.5 µm, Waters) with a gradient from 3% B to 12.5 % B in 10 min, 12.5% to 40% buffer B in 45 min, 40% B to 60% B in 25 min, 60% B to 80% B in 10 min, 80% B to 100% B in 2.5 min, 100% for 5 min, ramping to 3% B in 2.5 min and then 3% for 10 min. A total of 90 fractions (1 min per fraction) were collected before further concatenation into 30 final fractions. Each fraction was then dried and desalted over a C18 StageTip prior to analysis by mass spectrometry.

LC-MS/MS analysis
The LC separations were performed with a Thermo Dionex Ultimate 3000 RSLC Nano liquid chromatography instrument. Approximately 1 µg of concatenated peptides (Peptides quantitation by Nanodrop) from bRP chromatography were dissolved in 0.1% formic acid and then loaded on C18 trap column with 3 % ACN/0.1%TFA at a flow rate of 5 µL/min. Peptide separations were performed over EASY-Spray column (C18, 2 µm, 75 µm × 50 cm) with an integrated nano electrospray emitter at a flow rate of 300 nL/min. Peptides were separated with a 180 min segmented gradient as follows: the first 10 fractions starting from 5%~30% buffer B in 125 min (Note: the middle 10 fractions starting from 7% and the last 10 fractions starting from 10%), 30%~45% buffer B in 30 min, 45%~95% buffer B for 5 min, followed by a 5 min 95% B. Eluted peptides were analysed on an Orbitrap Fusion Lumos (Thermo Fisher Scientific, San Jose, CA) mass spectrometer. Spray voltage was set to 2 kV, RF lens level was set at 30%, and ion transfer tube temperature was set to 275 °C. The Orbitrap Fusion Lumos was operated in positive ion data dependent mode with synchronous precursor selection (SPS)-MS3 analysis for reporter ion quantitation. The mass spectrometer was operated in data-dependent Top speed mode with 3 seconds per cycle. The full scan was performed in the range of 350–1500 m/z at nominal resolution of 120 000 at 200 m/z and AGC set to 4 × 105 with maximal injection time 50 ms, followed by selection of the most intense ions above an intensity threshold of 5000 for collision-induced dissociation (CID)-MS2 fragmentation in the linear ion trap with 35% normalized collision energy. The isolation width was set to 0.7 m/z with no offset. Dynamic

exclusion was set to 60 seconds. Monoisotopic precursor selection was set to peptide, maximum injection time was set to 50 msec. Charge states between 2 to 7 were included for MS2 fragmentation. The top 5 fragment ions from each MS2 scan was notched out for MS3. The MS3 scan were performed with an isolation width of 2 m/z in the quadrupole, normalised HCD collision energy of 65% and analysis of fragment ions in the orbitrap using 50 000 resolving power with auto normal range scan from m/z 100 to 500 and AGC target of 5 × 104. The maximal injection time for MS3 scan was set to 86 ms.

Data Analysis

All the acquired LC-MS data were analysed using Proteome Discoverer software v.2.2 (Thermo Fisher Scientific) with Mascot search engine. A maximum missed cleavages for trypsin digestion was set to 2. Precursor mass tolerance was set to 20 ppm. Fragment ion tolerance was set to 0.6 Da. Carbamidomethylation on cysteine (+57.021 Da) and TMT-10plex tags on N termini as well as lysine (+229.163 Da) were set as static modifications. Variable modifications were set as oxidation on methionine (+15.995 Da) and phosphorylation on serine, threonine, and tyrosine (+79.966 Da). Data were searched against a complete UniProt Human (Reviewed 20,143 entry downloaded at Nov 2018). Peptide spectral match (PSM) error rates with a 1% FDR were determined using the target-decoy strategy coupled to Percolator modelling of true and false matches.

Both unique and razor peptides were used for quantitation. Reporter ion abundances were corrected for isotopic impurities based on the manufacturer's data sheets. Reporter ions were quantified from MS3 scans using an integration tolerance of 20 ppm with the most confident centroid setting. Signal-to-noise (S/N) values were used to represent the reporter ion abundance with a co-isolation threshold of 50% and an average reporter S/N threshold of 10 and above required for quantitation from each MS3 spectra to be used. The S/N value of each reporter ions from each PSM were used to represent the abundance of the localised phosphorylation sites. The precursor spectra with higher than 25% co-isolation were further manually checked. The total peptide amount was used for the normalisation. Protein ratios were calculated from medians of summed sample abundances of replicate groups. Standard deviation were calculated from three biological replicate values. The standard deviation of three biological replicates lower than 25% were used for further analyses. To determine the significant differences between different treatments, ANOVA model is used for statistical significance analysis.

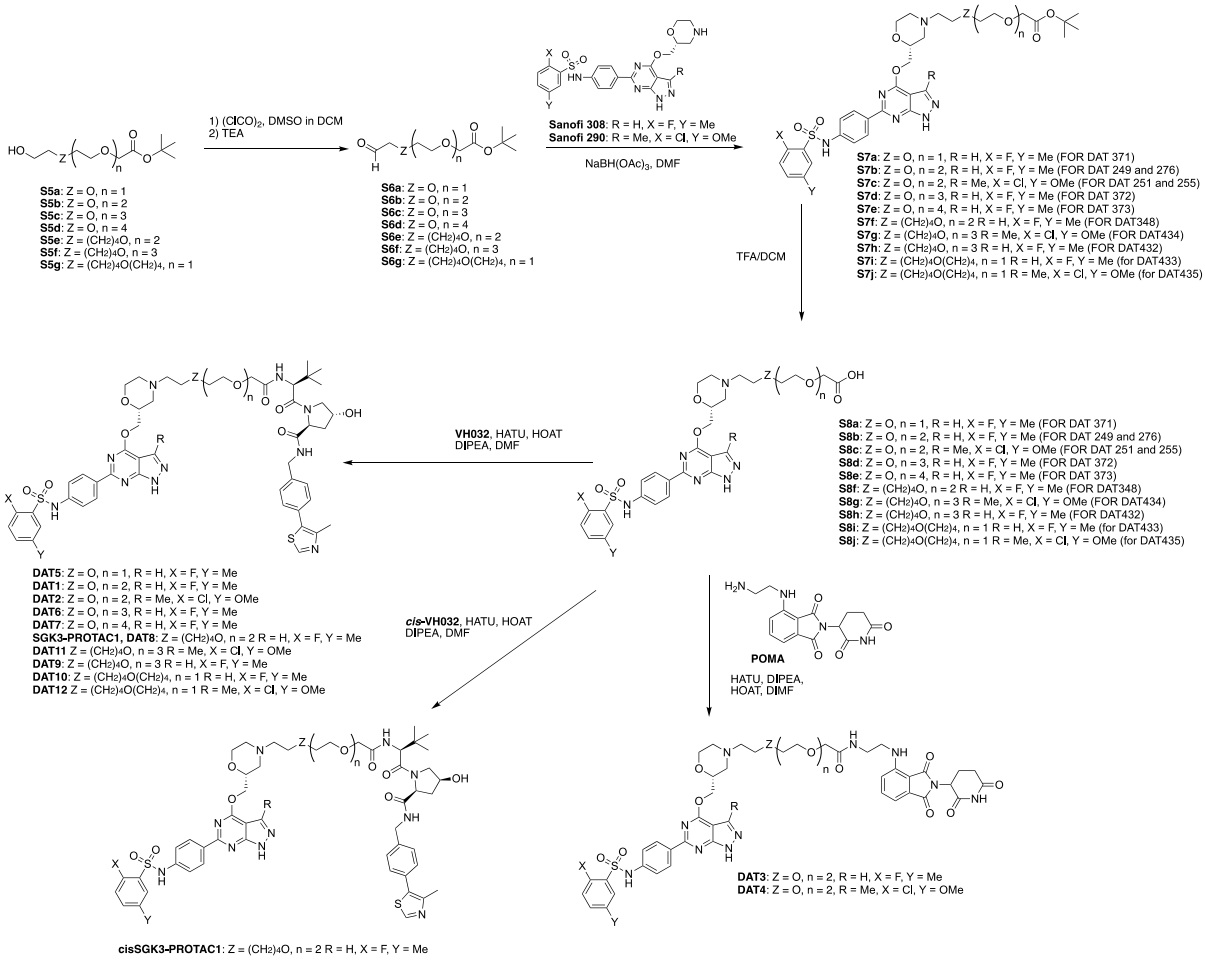
3. CHEMISTRY - GENERAL INFORMATION

All chemicals were purchased from commercial vendors and used without further purification, unless indicated otherwise. tert-butyl 2-(2-(2-hydroxyethoxy)ethoxy)acetate [1], tert-butyl 2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)acetate[2], tert-butyl 14-hydroxy-3,6,9,12-tetraoxatetradecanoat, tert-butyl 17-hydroxy-3,6,9,12,15-pentaoxaheptadecanoate[3] and 6-(benzyloxy)hexyl methanesulfonate[4] were prepared as previously reported. Sanofi290R and Sanofi308R were prepared as described in WO2014140065. 4-((2-aminoethyl)amino)-2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione (POMA) and (4R)-3-Methyl-L-valyl-4-hydroxy-N-[[4-(4-methyl-5-thiazolyl)phenyl]methyl]-L-prolinamide hydrochloride (VH032 amine) were prepared as previously described[5].

Reactions were magnetically stirred; commercially available anhydrous solvents were used. All reactions requiring anhydrous conditions were carried out under nitrogen atmosphere using oven-dried glassware. Normal phase TLC was carried out on pre-coated silica plates (Kieselgel 60 F254, BDH) with visualization via UV light (UV 254/365 nm) and/or basic potassium permanganate solution. Flash column chromatography (FCC) was performed using a Teledyne Isco Combiflash Rf or Rf200i, prepacked columns RediSep Rf Normal Phase. NMR spectra were recorded on a Bruker Ascend 400 or 500 MHz. Chemical shifts are reported in parts per million referenced to residual solvent peaks ($CDCl_3$= 7.26 ppm, CD_3OD = 3.32 ppm, DMSO = 2.50 ppm). The following abbreviations were used in reporting spectra, s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublets). Only major rotamer NMR spectra are reported. High Resolution Mass Spectra (HRMS) were recorded on a Bruker microTOF. Low resolution MS and analytical HPLC traces were recorded on an Agilent Technologies 1200 series HPLC connected to an Agilent Technologies 6130 quadrupole LC/MS, connected to an Agilent diode array detector. The column used was a Waters XBridge column (50 mm × 2.1 mm, 3.5 µm particle size), with a gradient from 5 % to 95% of acetonitrile in water (with 0.1 % of formic acid or aqueous ammonia solution) over 3 or 7 minutes. The flow rate was 0.7 mL/min. Preparative HPLC was performed on a Gilson Preparative HPLC System with a Waters X Bridge C18 column (100 mm x 19 mm; 5 µm particle size). The flow rate was 25 mL/min. Details about the conditions for preparative HPLC are provided in the experimental procedures.

4. SYNTHETIC SCHEMES

General synthetic scheme:



S5a: Z = O, n = 1
S5b: Z = O, n = 2
S5c: Z = O, n = 3
S5d: Z = O, n = 4
S5e: Z = $(CH_2)_4O$, n = 2
S5f: Z = $(CH_2)_4O$, n = 3
S5g: Z = $(CH_2)_4O(CH_2)_4$, n = 1

S6a: Z = O, n = 1
S6b: Z = O, n = 2
S6c: Z = O, n = 3
S6d: Z = O, n = 4
S6e: Z = $(CH_2)_4O$, n = 2
S6f: Z = $(CH_2)_4O$, n = 3
S6g: Z = $(CH_2)_4O(CH_2)_4$, n = 1

S7a: Z = O, n = 1, R = H, X = F, Y = Me (FOR DAT 371)
S7b: Z = O, n = 2, R = H, X = F, Y = Me (FOR DAT 249 and 276)
S7c: Z = O, n = 2, R = Me, X = Cl, Y = OMe (FOR DAT 251 and 255)
S7d: Z = O, n = 3, R = H, X = F, Y = Me (FOR DAT 372)
S7e: Z = O, n = 4, R = H, X = F, Y = Me (FOR DAT 373)
S7f: Z = $(CH_2)_4O$, n = 2 R = H, X = F, Y = Me (FOR DAT348)
S7g: Z = $(CH_2)_4O$, n = 3 R = Me, X = Cl, Y = OMe (FOR DAT434)
S7h: Z = $(CH_2)_4O$, n = 3 R = H, X = F, Y = Me (FOR DAT432)
S7i: Z = $(CH_2)_4O(CH_2)_4$, n = 1 R = H, X = F, Y = Me (for DAT433)
S7j: Z = $(CH_2)_4O(CH_2)_4$, n = 1 R = Me, X = Cl, Y = OMe (for DAT435)

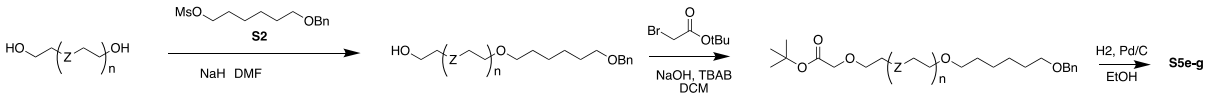
S8a: Z = O, n = 1, R = H, X = F, Y = Me (FOR DAT 371)
S8b: Z = O, n = 2, R = H, X = F, Y = Me (FOR DAT 249 and 276)
S8c: Z = O, n = 2, R = Me, X = Cl, Y = OMe (FOR DAT 251 and 255)
S8d: Z = O, n = 3, R = H, X = F, Y = Me (FOR DAT 372)
S8e: Z = O, n = 4, R = H, X = F, Y = Me (FOR DAT 373)
S8f: Z = $(CH_2)_4O$, n = 2 R = H, X = F, Y = Me (FOR DAT348)
S8g: Z = $(CH_2)_4O$, n = 3 R = Me, X = Cl, Y = OMe (FOR DAT434)
S8h: Z = $(CH_2)_4O$, n = 3 R = H, X = F, Y = Me (FOR DAT432)
S8i: Z = $(CH_2)_4O(CH_2)_4$, n = 1 R = H, X = F, Y = Me (for DAT433)
S8j: Z = $(CH_2)_4O(CH_2)_4$, n = 1 R = Me, X = Cl, Y = OMe (for DAT435)

DAT5: Z = O, n = 1, R = H, X = F, Y = Me
DAT1: Z = O, n = 2, R = H, X = F, Y = Me
DAT2: Z = O, n = 2, R = Me, X = Cl, Y = OMe
DAT6: Z = O, n = 3, R = H, X = F, Y = Me
DAT7: Z = O, n = 4, R = H, X = F, Y = Me
SGK3-PROTAC1, DAT8: Z = $(CH_2)_4O$, n = 2 R = H, X = F, Y = Me
DAT11 Z = $(CH_2)_4O$, n = 3 R = Me, X = Cl, Y = OMe
DAT9: Z = $(CH_2)_4O$, n = 3 R = H, X = F, Y = Me
DAT10: Z = $(CH_2)_4O(CH_2)_4$, n = 1 R = H, X = F, Y = Me
DAT12 Z = $(CH_2)_4O(CH_2)_4$, n = 1 R = Me, X = Cl, Y = OMe

DAT3: Z = O, n = 2, R = H, X = F, Y = Me
DAT4: Z = O, n = 2, R = Me, X = Cl, Y = OMe

cisSGK3-PROTAC1: Z = $(CH_2)_4O$, n = 2 R = H, X = F, Y = Me

Synthesis of linkers:



S1a: Z = O, n = 1
S1b: Z = O, n = 2
S1c: Z = $(CH_2)_2$, n = 1

S3a: Z = O, n = 1
S3b: Z = O, n = 2
S3c: Z = $(CH_2)_2$, n = 1

S4a: Z = O, n = 1
S4b: Z = O, n = 2
S4c: Z = $(CH_2)_2$, n = 1

5. CHEMISTRY - EXPERIMENTAL DATA

2-(2-((6-(benzyloxy)hexyl)oxy)ethoxy)ethan-1-ol (S3a)

To a solution of diethylene glycol (**S1a**) (26.2 mmol) in DMF (15 ml), NaH (60% dispersion in mineral oil, 314 mg, 7.8 mmol) was added at 0 C. The reaction mixture was stirred at 0 C for 1 hour, then 6-(benzyloxy)hexyl methanesulfonate (**S2)** was added dropwise. The reaction mixture was stirred for 24 h at room temperature. The solvents were removed under reduced pressure, then the excess of sodium hydride was quenched with a saturated solution of NH_4Cl and the reaction mixture was extracted with ethyl acetate. The organic layer was dried over $MgSO_4$, solvents were removed under reduced pressure and the desired product was purified by flash column chromatography (FCC) using a gradient from 20% to 40 of AcOEt in Heptane. Obtained: 4.2 g, 55%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.28 - 7.19 (m, 5H), 4.43 (s, 2H), 3.68 - 3.63 (m, 2H), 3.62 - 3.59 (m, 2H), 3.56 - 3.50 (m, 4H), 3.41 - 3.37 (m, 4H), 2.45 (t, *J*=6.2 Hz, 1H), 1.59 - 1.49 (m, 4H), 1.37 - 1.25 (m, 4H). ^{13}C NMR ($CDCl_3$) δ: 138.7, 128.4, 127.6, 127.5, 72.9, 72.5, 71.5, 70.5, 70.4, 70.2, 61.9, 29.7, 29.5, 26.1, 25.9.

1-phenyl-2,9,12,15-tetraoxaheptadecan-17-ol (S3b)

Prepared following the procedure described for **S3a**, yield 42%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.27 - 7.19 (m, 5H), 4.43 (s, 2H), 3.67 - 3.49 (m, 12H), 3.41 - 3.36 (m, 4H), 2.47 (s, 1H), 1.59 - 1.47 (m, 4H), 1.36 - 1.24 (m, 4H). ^{13}C NMR ($CDCl_3$) δ: 138.7, 128.4, 127.6, 127.5, 72.9, 72.5, 71.5, 70.6, 70.4, 70.0, 61.8, 29.7, 29.5, 26.1, 26.0.

6-((6-(benzyloxy)hexyl)oxy)hexan-1-ol (S3c)

Prepared following the procedure described for **S3a**, yield 82%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.30 - 7.17 (m, 5H), 4.43 (s, 2H), 3.56 (t, *J*=6.8 Hz, 2H), 3.40 (t, *J*=7.0 Hz, 2H), 3.32 (t, *J*=6.6 Hz, 4H), 3.32 (t, *J*=6.7 Hz, 4H), 1.60 - 1.47 (m, 8H), 1.34 - 1.27 (m, 8H). ^{13}C NMR ($CDCl_3$) δ: 138.7, 128.4, 127.6, 127.5, 72.9, 70.9, 70.8, 70.4, 62.9, 32.7, 29.7, 26.1, 26.0, 25.6.

***tert*-butyl 1-phenyl-2,9,12,15-tetraoxaheptadecan-17-oate (S4a)**

To a mixture of DCM and 37 % aqueous NaOH (1:1, 9 mL), compound **S3a** (2.08 mmol), TBAB (868 mg, 2.13 mmol) and tert-butyl bromoacetate (1.23 mL, 8.34 mmol) were added. The reaction mixture was vigorously stirred at room temperature overnight before being diluted with DCM. The organic layer was separated, washed with water and dried over $MgSO_4$. Solvents were removed under reduced pressure and the desired product was purified by FCC using a gradient from 30% to 40% of AcOEt in Heptane. Obtained 665 mg, 78% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.28 - 7.18 (m, 5H), 4.43 (s, 2H), 3.95 (s, 2H), 3.67 - 3.60 (m, 4H), 3.60 - 3.57 (m, 2H), 3.53 - 3.50 (m, 2H), 3.41 - 3.36 (m, 4H), 1.58 - 1.50 (m, 4H), 1.40 (s, 9H), 1.33 - 1.25 (m, 4H).^{13}C NMR ($CDCl_3$) δ: 169.7, 138.7, 128.4, 127.6, 81.5, 72.9, 71.4, 70.8, 70.7, 70.7, 70.4, 70.1, 69.1, 29.7, 29.6, 28.1, 26.1, 26.0.

***tert*-butyl 1-phenyl-2,9,12,15,18-pentaoxaicosan-20-oate (S4b)**

Prepared following the procedure described for **S4a**, yield 81%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.27 - 7.18 (m, 5H), 4.43 (s, 2H), 3.95 (s, 2H), 3.67 - 3.55 (m, 10H), 3.52 - 3.48 (m, 2H), 3.41 - 3.35 (m, 4H), 1.58 - 1.47 (m, 4H), 1.41 (s, 9H), 1.35 - 1.24 (m, 4H). ^{13}C NMR ($CDCl_3$) δ: 169.7, 138.7, 128.3, 127.6, 81.5, 72.9, 71.4, 70.7, 70.6, 70.4, 70.1, 69.1, 29.7, 29.6, 28.1, 26.1, 26.0.

***tert*-butyl 2-((6-((6-(benzyloxy)hexyl)oxy)hexyl)oxy)acetate (S4c)**

Prepared following the procedure described for **S4a**, yield 55%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.43 (s, 2H), 3.87 (s, 2H), 3.43 (t, *J*=6.6 Hz, 2H), 3.39 (t, *J*=6.6 Hz, 2H), 3.34 - 3.29 (m, 4H), 1.60 - 1.45 (m, 8H), 1.41 (s, 9H), 1.33 - 1.24 (m, 8H). ^{13}C NMR ($CDCl_3$) δ: 169.9, 138.7, 128.4, 127.6, 127.5, 81.4, 72.8, 71.5, 70.9, 70.4, 68.8, 68.4, 29.7, 29.4, 28.1, 26.1, 25.9.

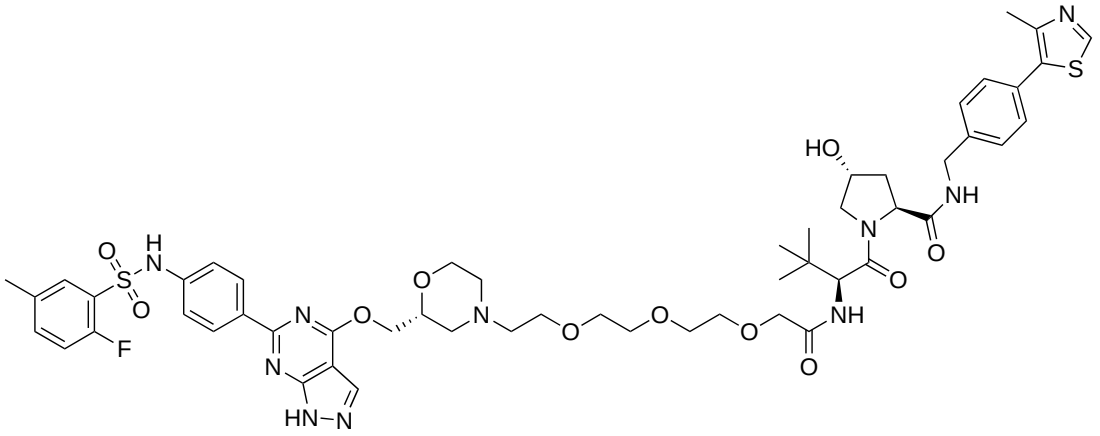
Procedure for the debenzylation of S3a-c to obtain S5e-g

A solution of **S4a-c** in EtOH (0.05 M) was hydrogenated in an H-cube at a flow rate of 0.5 ml/min at 70 °C and 10 atm. TLC analysis (50% AcOEt in Heptane, permanganate stain) showed complete conversion of the starting

material. The solvent was removed under reduced pressure and the desired product was used without any further purification. Yield: quantitative.

Representative procedure for the synthesis of SGK3 PROTACs:

(2*S*,4*R*)-1-((*S*)-2-(*tert*-butyl)-14-((*R*)-2-(((6-(4-((2-fluoro-5-methylphenyl)sulfonamido)phenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)oxy)methyl)morpholino)-4-oxo-6,9,12-trioxa-3-azatetradecanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (DAT1)

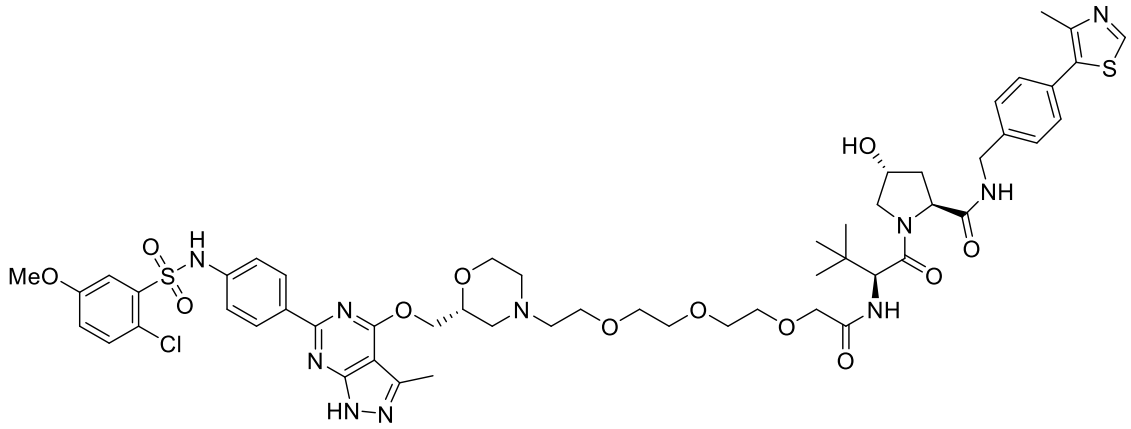


Oxalyl chloride (19 μL, 0.225 mmol) in DCM (0.2 mL) was cooled at -78 °C, then DMSO (22μL, 0.33 mmol) was added. After 10 minutes at -78 °C, a solution of *tert*-butyl 2-(2-(2-(2hydroxyethoxy)ethoxy)ethoxy)acetate (**S5b**, 40 mg, 0.15 mmol) in DCM was added. The reaction was left at -78 °C for 1 hour, then TEA (209 μL, 1.5 mmol) was added dropwise and the reaction mixture was left to reach room temperature over 30 minutes. TLC analysis (50% AcOEt in Heptane, permanganate stain) showed complete conversion of the starting material. DCM was added to dilute the reaction mixture which was then washed with a 5% solution of $NaHCO_3$. The organic layer was separated, dried over $MgSO_4$ and evaporated under reduced pressure to leave a thick yellow oil as the crude aldehyde which was dissolved in DMF (1 ml). Sanofi 308R (25 mg, 0.05 mmol) was added to the crude aldehyde, the mixture was stirred for 5 minutes, then $NaBH(OAc)_3$ (16 mg, 0.075 mmol) was added. The mixture was vigorously stirred overnight, quenched with a saturated solution of $NaHCO_3$ and extracted with DCM. Preparative HPLC purification gave the desired product **S7b**, tert-butyl (R)-2-(2-(2-(2-(2-(((6-(4-((2-fluoro-5-methylphenyl)sulfonamido)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl)oxy)methyl)morpholino)ethoxy)ethoxy)ethoxy)acetate as a white solid in 55% yield (20 mg). ^{1}H NMR (500 MHz, MeOD) δ: 8.35 (d, *J*=9.6 Hz, 2H), 8.10 (s, 1H), 7.73 (dd, *J*=2.2, 6.8 Hz, 1H), 7.43 - 7.39 (m, 1H), 7.25 (d, *J*=9.0 Hz, 2H), 7.14 (dd, *J*=8.5, 9.9 Hz, 1H), 4.73 - 4.66 (m, 2H), 4.10 - 4.04 (m, 2H), 3.99 (s, 2H), 3.95 - 3.91 (m, 1H), 3.77 - 3.57 (m, 19H), 3.10 (d, *J*=12.4 Hz, 1H), 2.84 (d, *J*=11.8 Hz, 1H), 2.67 - 2.64 (m, 2H), 2.35 - 2.29 (m, 4H), 2.20 (t, *J*=10.9 Hz, 1H), 1.45 (s, 9H). m/z = 745.3 $(M + H)^+$, expected 744.3 for $C_{35}H_{45}FN_6O_9S$.

Terbutyl ester deprotection was achieved by treating the obtained product (20 mg, 0.026 mmol) with a solution of TFA in DCM (50% v/v, 1 mL) for 2 hours. Volatile components were removed and the crude mixture was left under vacuum to remove any excess of TFA. VH032-amine (HCl salt, 12 mg, 0.026 mmol) and DMF were added and DIPEA was added until pH > 9 (~10 eq) HATU (10 mg, 0.026 mmol) and HOAT (3.5 mg, 0.026 mmol) were added to the reaction mixture and left to react for 1 hour at room temperature. HPLC-MS analysis of the reaction mixture showed the formation of the desired product (using a gradient from 5% to 95% of ACN in water with 0.01% of ammonia) m/z = 1101.4 $(M + H)^+$, expected 1100.4 for $C_{53}H_{65}FN_{10}O_{11}S_2$. Preparative HPLC (using a gradient from 5% to 95% of ACN in water with 0.01% ammonia) following by freeze drying, gave the desired product, 6.0 mg, 21% yield.

^{1}H NMR (400 MHz, MeOD) δ: 8.84 (1H, s), 8.32 (2H, d, *J*=8.3 Hz), 8.07 (1H, s), 7.72 (1H, dd, *J*=2.0, 6.9 Hz), 7.47 - 7.36 (6H, m), 7.23 (2H, d, *J*=9.1 Hz), 7.11 (1H, dd, *J*=8.5, 10.2 Hz), 4.70 - 4.66 (3H, m), 4.60 - 4.56 (2H, m), 4.52 - 4.49 (2H, m), 4.32 (1H, d, *J*=14.8 Hz), 4.07 - 4.00 (3H, m), 3.92 - 3.56 (16H, m), 3.05 (1H, d, *J*=10.6 Hz), 2.80 (1H, d, *J*=11.6 Hz), 2.66 - 2.57 (2H, m), 2.48 (1H, d, *J*=1.1 Hz), 2.46 - 2.44 (2H, m), 2.35 - 2.04 (8H, m), 1.03 (9H, s); ^{13}C NMR (MeOD) δ: 173.0, 170.7, 170.3, 161.3, 158.3, 155.8, 153.4, 147.6, 138.8, 130.4, 129.3, 128.9, 127.5, 119.1, 116.3, 100.4, 73.6, 70.9, 70.3, 70.1, 69.7, 68.2, 67.1, 66.1, 59.4, 57.7, 56.7, 55.1, 53.0, 42.3, 37.5, 35.7, 25.6, 19.2, 14.4.

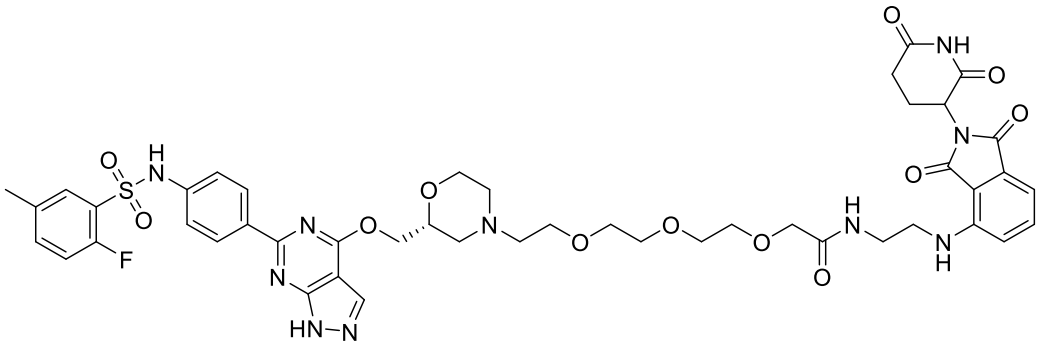
(2*S*,4*R*)-1-((*S*)-2-(*tert*-butyl)-14-((*R*)-2-(((6-(4-((2-chloro-5-methoxyphenyl)sulfonamido)phenyl)-3-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)oxy)methyl)morpholino)-4-oxo-6,9,12-trioxa-3-azatetradecanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (DAT2)



Prepared as reported above for **DAT1**, starting from **S5b,** Sanofi 290R and VH032-amine in 19% yield.

^{1}H NMR (400 MHz, MeOD) δ: 8.85 (1H, s), 8.29 (2H, d, *J*=9.8 Hz), 7.66 (1H, d, *J*=2.7 Hz), 7.49 - 7.36 (6H, m), 7.21 (2H, d, *J*=8.5 Hz), 7.06 (1H, dd, *J*=3.0, 8.8 Hz), 4.70 - 4.65 (2H, m), 4.62 - 4.56 (2H, m), 4.54 - 4.51 (2H, m), 4.38 - 4.31 (1H, m), 4.06 - 4.00 (3H, m), 3.94 - 3.87 (2H, m), 3.83 - 3.58 (16H, m), 3.08 (1H, d, *J*=11.4 Hz), 2.91 - 2.86 (2H, m), 2.84 - 2.80 (2H, m), 2.68 - 2.58 (4H, m), 2.50 - 2.45 (2H, m), 2.32 - 2.17 (3H, m), 2.14 - 2.05 (2H, m), 1.05 (9H, s); ^{13}C NMR (MeOD) δ: 173.0, 170.7, 170.3, 158.2, 151.4, 147.6, 138.8, 132.3, 132.0, 130.0, 129.2, 129.0, 128.9, 127.5, 122.4, 119.2, 118.5, 116.9, 99.1, 73.6, 70.9, 70.2, 70.1, 69.7, 68.2, 66.9, 66.1, 59.4, 57.7, 56.7, 55.2, 55.0, 53.1, 42.3, 37.5, 35.7, 25.6, 25.4, 17.9, 14.5, 12.7; m/z = 1147.5 $(M + H)^+$, expected 1146.4 for $C_{54}H_{67}ClN_{10}O_{12}S_2$.

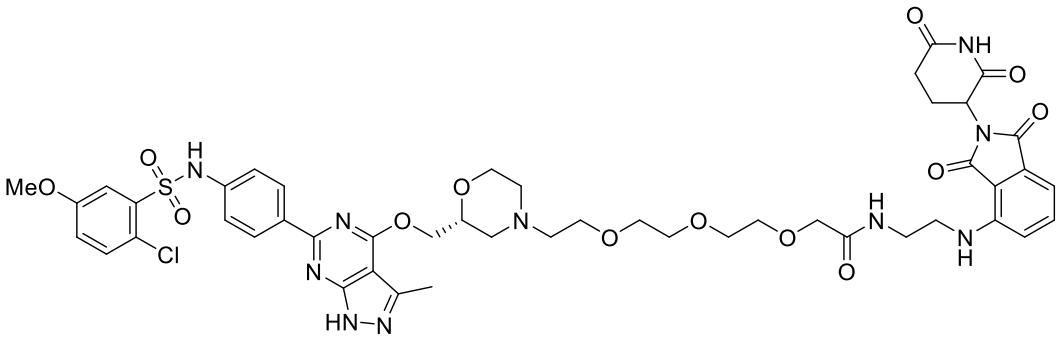
***N*-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethyl)-2-(2-(2-(2-((*R*)-2-(((6-(4-((2-fluoro-5-methylphenyl)sulfonamido)phenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)oxy)methyl)morpholino)ethoxy)ethoxy)ethoxy)acetamide (DAT3)**



Prepared as reported above for **DAT1**, starting from **S5b,** Sanofi 308R and POMA in 13% yield.

^{1}H NMR (400 MHz, MeOD) δ: 8.48 (s, 1H), 8.35 (d, *J*=9.6 Hz, 2H), 7.74 (dd, *J*=1.6, 6.7 Hz, 1H), 7.51 - 7.47 (m, 1H), 7.44 - 7.40 (m, 1H), 7.26 (d, *J*=9.0 Hz, 2H), 7.17 - 7.12 (m, 1H), 7.06 (d, *J*=9.0 Hz, 1H), 7.00 (d, *J*=6.3 Hz, 1H), 5.02 (dd, *J*=5.4, 12.3 Hz, 1H), 4.69 - 4.67 (m, 2H), 4.09 - 4.05 (m, 1H), 3.96 - 3.91 (m, 2H), 3.67-3.41 (m, 16H), 2.75 - 2.67 (m, 6H), 2.39 -2.03 (m, 9H); m/z = 987.4 $(M + H)^+$, expected 986.3 for $C_{46}H_{51}FN_{10}O_{12}S$.

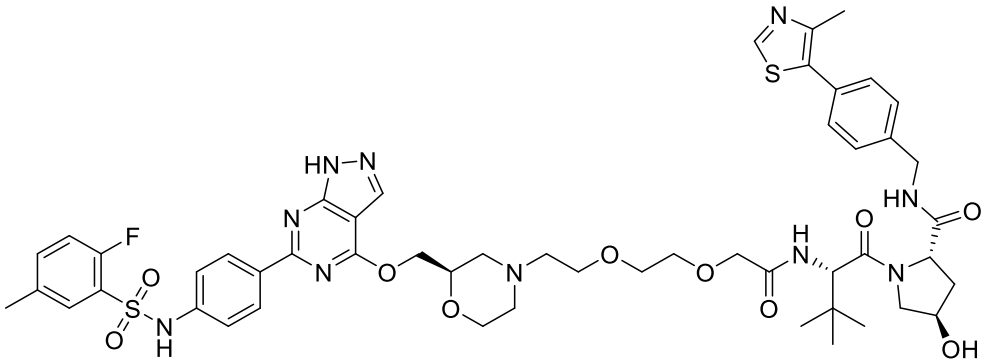
2-(2-(2-(2-((*R*)-2-(((6-(4-((2-chloro-5-methoxyphenyl)sulfonamido)phenyl)-3-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)oxy)methyl)morpholino)ethoxy)ethoxy)ethoxy)-*N*-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)amino)ethyl)acetamide (DAT4)



Prepared as reported above for **DAT1**, starting from **S5b,** Sanofi 290R and POMA in 16% yield.

^{1}H NMR (400 MHz, MeOD) δ: 8.29 (s, 1H), 8.21 (d, *J*=8.8 Hz, 2H), 7.53 (d, *J*=3.4 Hz, 1H), 7.39 - 7.30 (m, 3H), 7.15 (d, *J*=9.2 Hz, 3H), 6.99 (dd, *J*=3.2, 8.8 Hz, 1H), 6.93 (d, *J*=8.7 Hz, 1H), 6.88 - 6.86 (m, 1H), 4.89 (dd, *J*=5.4, 12.7 Hz, 1H), 4.59 - 3.94 (m, 5H), 3.84-3.30 (m, 19H), 2.79 - 2.56 (m, 6H), 2.51 - 2.43 (m, 2H), 2.27 - 2.08 (m, 7H); m/z = 1033.3 $(M + H)^+$, expected 1032.3 for $C_{47}H_{53}ClN_{10}O_{13}S$.

(2*S*,4*R*)-1-((*S*)-2-(2-(2-(2-((*R*)-2-(((6-(4-((2-fluoro-5-methylphenyl)sulfonamido)phenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)oxy)methyl)morpholino)ethoxy)ethoxy)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (DAT5)



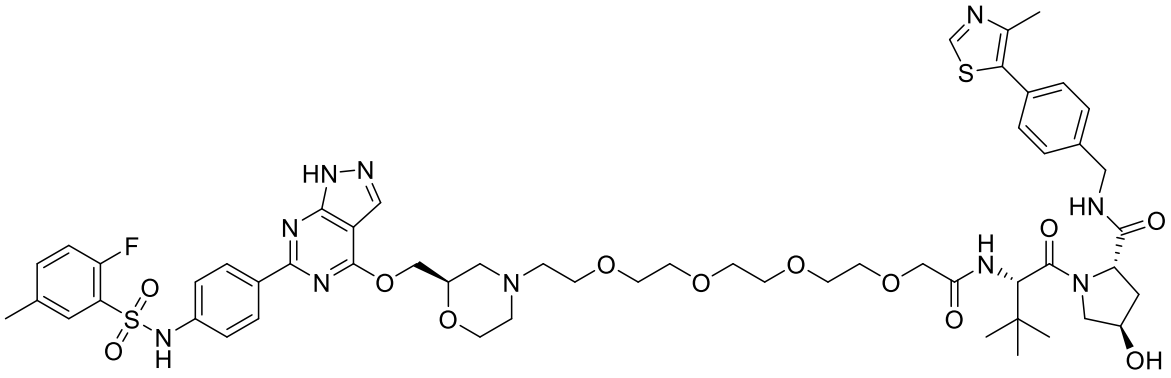
Prepared as reported above for **DAT1**, starting from **S5a,** Sanofi308R and VH032-amine in 23% yield.

^{1}H NMR (500 MHz, MeOD) δ: 8.81 (s, 1H), 8.37 - 8.32 (m, 3H), 8.06 (s, 1H), 7.72 (dd, *J*=2.3, 6.9 Hz, 1H), 7.42 - 7.35 (m, 6H), 7.25 (d, *J*=9.2 Hz, 2H), 7.15 - 7.10 (m, 2H), 4.72 - 4.66 (m, 3H), 4.59 - 4.55 (m, 1H), 4.52 - 4.48 (m, 2H), 4.36 - 4.33 (m, 1H), 4.11 - 4.07 (m, 1H), 4.03 (d, *J*=6.9 Hz, 2H), 3.97 - 3.93 (m, 1H), 3.86 (d, *J*=12.1 Hz, 1H), 3.81 - 3.63 (m, 11H), 3.15 (d, *J*=14.3 Hz, 1H), 2.96 (d, *J*=9.9 Hz, 1H), 2.80 (t, *J*=6.0 Hz, 2H), 2.46 - 2.42 (m, 4H), 2.36 - 2.33 (m, 5H), 2.25 - 2.20 (m, 1H), 2.12 - 2.06 (m, 1H), 1.02 (s, 9H).

^{13}C NMR (MeOD) δ: 172.9, 170.6, 170.3, 163.1, 161.1, 158.2, 155.7, 151.4, 147.6, 139.8, 138.7, 135.8, 135.7, 134.6, 133.2, 131.9, 130.5, 130.1, 129.3, 129.1, 128.9, 128.1, 126.8, 126.6, 118.9, 116.5, 116.3, 100.1, 73.2, 70.8, 69.9, 69.6, 67.6, 66.9, 65.6, 59.4, 57.5, 56.7, 54.7, 52.8, 42.3, 37.6, 35.8, 25.6, 19.1, 14.5; m/z = 1057.4 $(M + H)^+$, expected 1056.4 for

$C_{51}H_{61}FN_{10}O_{10}S_2$.

(2S,4R)-1-((S)-2-(tert-butyl)-17-((R)-2-(((6-(4-((2-fluoro-5-methylphenyl)sulfonamido)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl)oxy)methyl)morpholino)-4-oxo-6,9,12,15-tetraoxa-3-azaheptadecanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (DAT6)

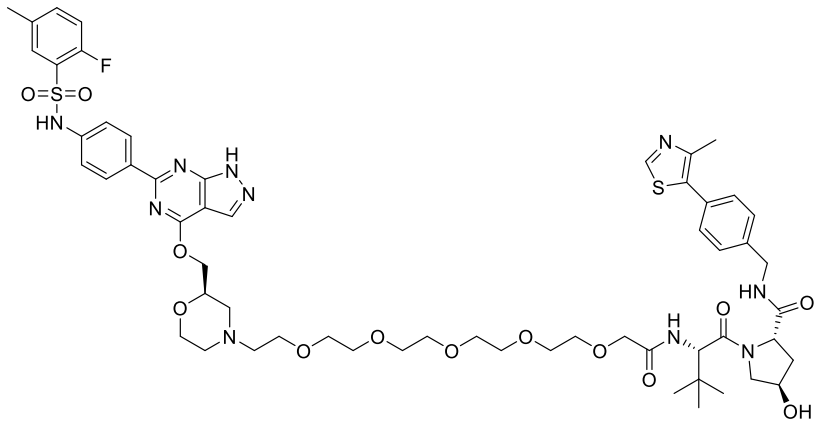


Prepared as reported above for **DAT1**, starting from **S5c,** Sanofi308R and VH032-amine in 24% yield.

^{1}H NMR (500 MHz, MeOD) δ: 8.81 (s, 1H), 8.31 (d, *J*=8.8 Hz, 2H), 8.05 (s, 1H), 7.70 (dd, *J*=2.2, 6.6 Hz, 1H), 7.42 - 7.33 (m, 4H), 7.23 (d, *J*=8.8 Hz, 2H), 7.09 (dd, *J*=7.9, 10.8 Hz, 1H), 4.68 - 4.65 (m, 3H), 4.57 - 4.48 (m, 3H), 4.32 (d, *J*=14.9 Hz, 1H), 4.09 - 4.05 (m, 1H), 4.00 - 3.99 (m, 2H), 3.96 - 3.92 (m, 1H), 3.85 (d, *J*=12.8 Hz, 1H), 3.80 - 3.54 (m, 16H), 3.18 (d, *J*=10.7 Hz, 1H), 2.93 (d, *J*=12.1 Hz, 1H), 2.78 - 2.73 (m, 2H), 2.46 - 2.29 (m, 7H), 2.23 - 2.17 (m, 1H), 2.09 - 2.03 (m, 1H), 1.00 (s, 9H).

^{13}C NMR (MeOD) δ: 173.0, 170.7, 170.3, 163.1, 161.0, 158.2, 155.7, 151.4, 147.6, 139.8, 138.8, 135.8, 135.7, 134.6, 133.2, 132.0, 130.5, 130.1, 129.3, 129.1, 128.9, 128.1, 127.5, 126.8, 126.6, 118.9, 116.5, 116.3, 100.1, 73.2, 70.8, 70.2, 70.1, 70.0, 69.7, 67.3, 66.9, 65.5, 59.4, 57.4, 56.8, 56.7, 54.6, 52.7, 42.3, 37.6, 35.7, 25.6, 19.2, 14.5; m/z = 1145.5 $(M + H)^+$, expected 1144.5 for $C_{55}H_{69}FN_{10}O_{12}S_2$.

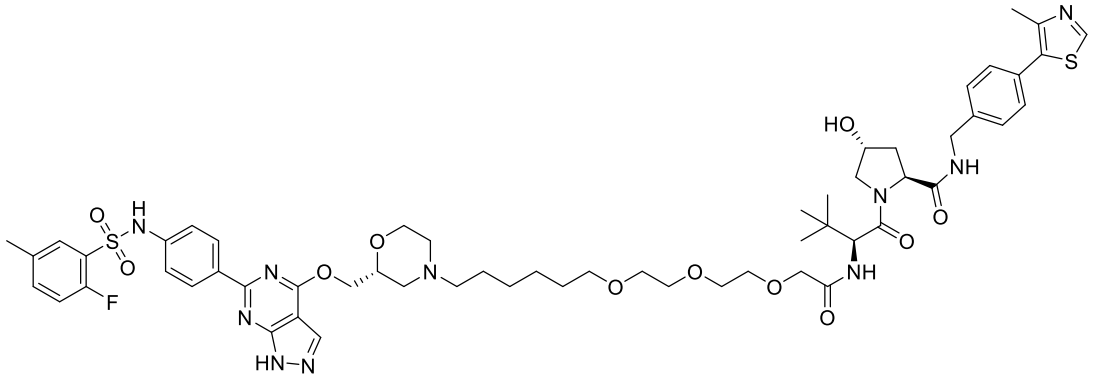
(2*S*,4*R*)-1-((*S*)-2-(*tert*-butyl)-20-((*R*)-2-(((6-(4-((2-fluoro-5-methylphenyl)sulfonamido)phenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)oxy)methyl)morpholino)-4-oxo-6,9,12,15,18-pentaoxa-3-azaicosanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (DAT7)



Prepared as reported above for **DAT1**, starting from **S5d,** Sanofi 308R and VH032-amine in 22% yield.

^{1}H NMR (500 MHz, MeOD) δ: 8.85 (s, 1H), 8.35 (d, *J*=8.9 Hz, 2H), 8.09 (s, 1H), 7.73 (dd, *J*=2.0, 6.7 Hz, 1H), 7.46 - 7.37 (m, 5H), 7.26 (d, *J*=9.2 Hz, 2H), 7.13 (dd, *J*=8.7, 10.4 Hz, 1H), 4.71 - 4.69 (m, 3H), 4.60 - 4.49 (m, 3H), 4.35 (d, *J*=15.0 Hz, 1H), 4.12 - 4.06 (m, 1H), 4.03 (d, *J*=5.6 Hz, 2H), 3.98 - 3.95 (m, 1H), 3.88 (d, *J*=11.7 Hz, 1H), 3.83 - 3.54 (m, 21H), 3.21 (d, *J*=9.9 Hz, 1H), 2.94 (d, *J*=12.7 Hz, 1H), 2.79 - 2.75 (m, 2H), 2.48 - 2.44 (m, 4H), 2.37 - 2.34 (m, 4H), 2.25 - 2.21 (m, 1H), 2.13 - 2.07 (m, 1H), 1.04 (s, 9H). ^{13}C NMR (MeOD) δ:174.4, 172.2, 171.8, 164.6, 162.6, 159.5, 157.5, 152.9, 149.1, 141.3, 140.3, 137.3, 137.2, 136.1, 136.0, 134.7, 133.5, 132.0, 131.6, 130.8, 130.4, 129.5, 129.0, 128.3, 128.1, 120.4, 118.0, 117.8, 101.6, 74.7, 72.4, 71.7, 71.6, 71.6, 71.5, 71.2, 71.1, 68.9, 68.4, 67.1, 60.9, 59.0, 58.2, 56.1, 54.3, 43.8, 39.0, 37.2, 27.1, 20.6, 15.9; m/z = 1189.5 $(M + H)^+$, expected 1188.5 for $C_{57}H_{73}FN_{10}O_{13}S_2$.

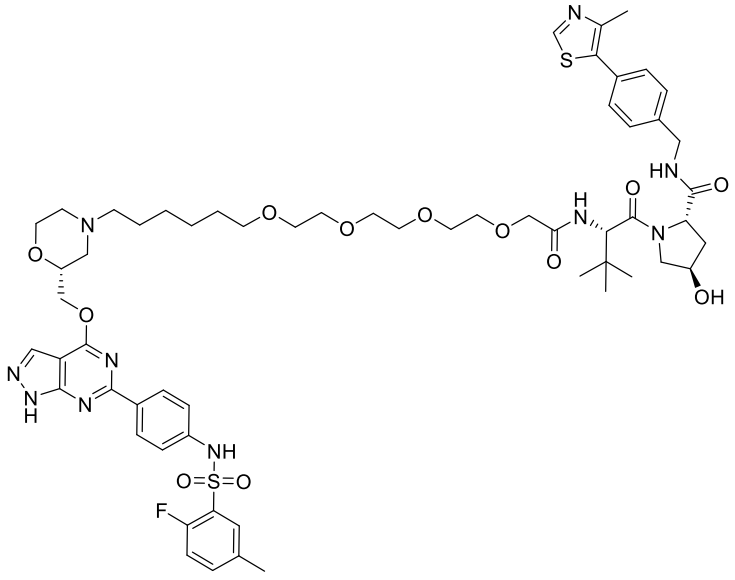
(2*S*,4*R*)-1-((*S*)-2-(*tert*-butyl)-18-((*R*)-2-(((6-(4-((2-fluoro-5-methylphenyl)sulfonamido)phenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)oxy)methyl)morpholino)-4-oxo-6,9,12-trioxa-3-azaoctadecanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (SGK3-PROTAC1, DAT8)



Prepared as reported above for **DAT1**, starting from **S5e,** Sanofi 308R and VH032-amine in 25% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.71 (s, 1H), 8.29 (d, *J*=8.3 Hz, 2H), 8.07 (s, 1H), 7.70 (dd, *J*=3.1, 7.5 Hz, 1H), 7.46 (t, *J*=5.5 Hz, 1H), 7.36 - 7.29 (m, 5H), 7.24 (d, *J*=8.1 Hz, 2H), 7.06 - 7.01 (m, 1H), 4.82 (t, *J*=7.9 Hz, 1H), 4.73 - 4.56 (m, 5H), 4.34 (dd, *J*=5.5, 14.4 Hz, 1H), 4.19 - 3.99 (m, 5H), 3.82 - 3.77 (m, 1H), 3.70 - 3.64 (m, 7H), 3.59 - 3.56 (m, 2H), 3.44 (t, *J*=6.6 Hz, 2H), 2.98 (d, *J*=11.3 Hz, 1H), 2.76 (d, *J*=11.5 Hz, 1H), 2.60 - 2.52 (m, 4H), 2.42 - 2.33 (m, 5H), 2.27 - 2.16 (m, 2H), 2.08 - 2.02 (m, 1H), 1.59 - 1.50 (m, 4H), 1.36 - 1.32 (m, 4H), 1.00 (s, 9H). ^{13}C NMR ($CDCl_3$) δ:171.5, 170.8, 170.7, 163.1, 161.1, 157.6, 155.6, 150.4, 148.5, 138.8, 138.0, 136.0, 135.9, 134.5, 134.0, 132.9, 131.6, 131.0, 130.9, 129.7, 129.5, 128.2, 120.1, 116.7, 116.5, 100.6, 73.7, 71.4, 71.2, 70.7, 70.5, 70.4, 70.2, 70.1, 67.5, 66.8, 58.9, 58.5, 57.2, 56.8, 55.0, 53.3, 43.3, 35.9, 34.9, 29.5, 27.2, 26.4, 26.2, 26.0, 20.6, 16.1; m/z = 1157.5 $(M + H)^+$, expected 1156.5 for $C_{57}H_{73}FN_{10}O_{11}S_2$.

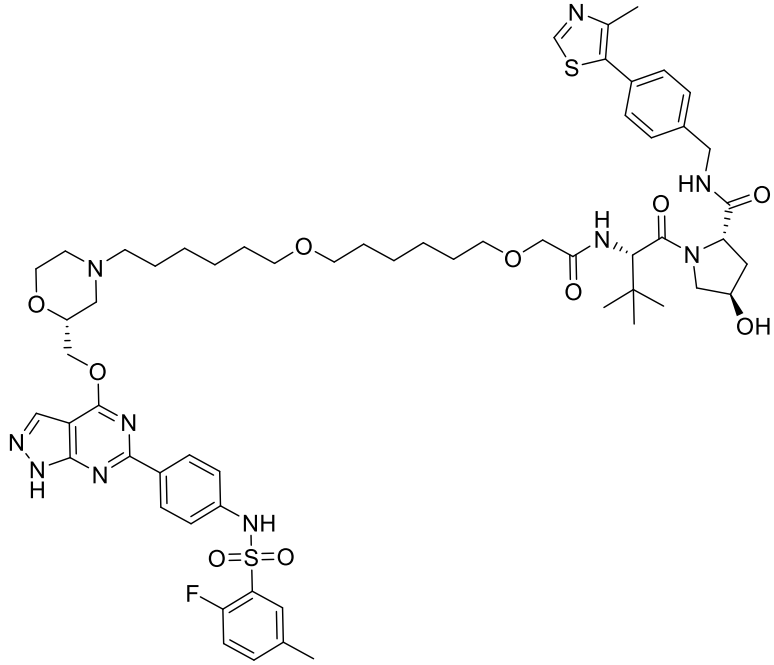
(2*S*,4*R*)-1-((*S*)-2-(*tert*-butyl)-21-((*R*)-2-(((6-(4-((2-fluoro-5-methylphenyl)sulfonamido)phenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)oxy)methyl)morpholino)-4-oxo-6,9,12,15-tetraoxa-3-azahenicosanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (DAT9)



Prepared as reported above for **DAT1**, starting from **S5f,** Sanofi 308R and VH032-amine in 26% yield.

^{1}H NMR (400 MHz, MeOD) δ: 8.85 (s, 1H), 8.34 (d, *J*=9.1 Hz, 2H), 8.08 (s, 1H), 7.72 (dd, *J*=2.2, 6.7 Hz, 1H), 7.46 - 7.38 (m, 5H), 7.25 (d, *J*=8.9 Hz, 2H), 7.12 (dd, *J*=8.4, 10.1 Hz, 1H), 4.72 - 4.69 (m, 3H), 4.61 - 4.48 (m, 3H), 4.35 (d, *J*=15.9 Hz, 1H), 4.15 - 4.08 (m, 1H), 4.06 - 3.99 (m, 3H), 3.88 (d, *J*=11.9 Hz, 1H), 3.83 - 3.76 (m, 2H), 3.72 - 3.52 (m, 13H), 3.44 (t, *J*=6.4 Hz, 2H), 3.26 (d, *J*=11.9 Hz, 1H), 3.03 (d, *J*=12.9 Hz, 1H), 2.69 - 2.63 (m, 2H), 2.56 - 2.42 (m, 5H), 2.34 (s, 3H), 2.26 - 2.21 (m, 1H), 2.12 - 2.05 (m, 1H), 1.65 - 1.51 (m, 4H), 1.42 - 1.32 (m, 4H), 1.04 (s, 9H). ^{13}C NMR (MeOD) δ:173.0, 170.7, 170.3, 166.6, 163.0, 161.0, 158.2, 157.7, 155.7, 151.4, 147.6, 139.8, 138.8, 135.8, 135.7, 134.6, 134.5, 133.2, 132.0, 131.6, 130.5, 130.1, 129.3, 129.1, 129.0, 128.1, 127.5, 126.8, 126.6, 118.9, 116.5, 116.3, 100.1, 73.0, 70.9, 70.8, 70.3, 70.2, 70.1, 69.8, 69.7, 66.7, 65.2, 59.4, 58.1, 56.8, 56.7, 54.0, 52.2, 42.3, 37.6, 35.7, 29.1, 26.6, 25.6, 24.9, 19.2, 14.5; m/z = 12001.5 $(M + H)^+$, expected 1200.5 for $C_{59}H_{77}FN_{10}O_{12}S_2$.

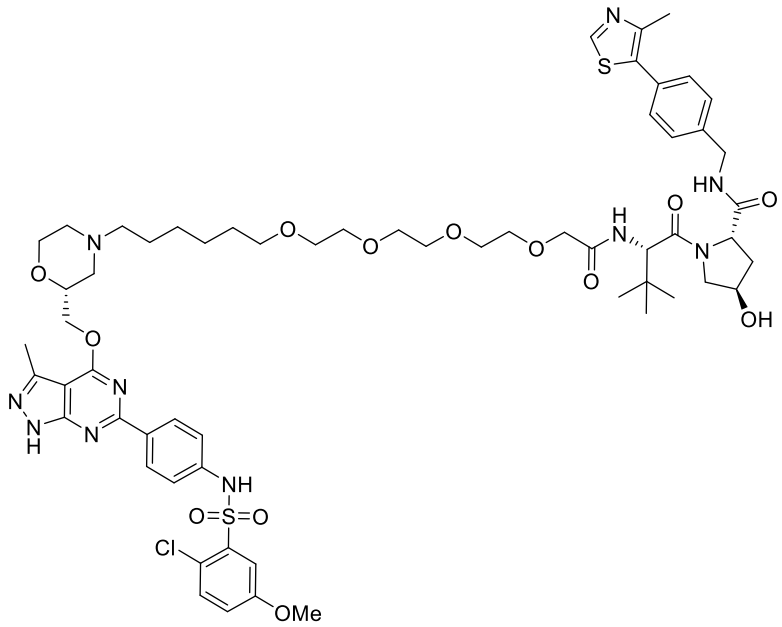
(2*S*,4*R*)-1-((*S*)-2-(2-((6-((6-((*R*)-2-(((6-(4-((2-fluoro-5-methylphenyl)sulfonamido)phenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)oxy)methyl)morpholino)hexyl)oxy)hexyl)oxy)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (DAT10)



Prepared as reported above for **DAT1**, starting from **S5g,** Sanofi 308R and VH032-amine in 27% yield.

^{1}H NMR (400 MHz, MeOD) δ: 8.86 (s, 1H), 8.36 (d, *J*=8.8 Hz, 2H), 8.24 (s, 1H), 8.10 (s, 1H), 7.73 (dd, *J*=2.0, 6.8 Hz, 1H), 7.47 - 7.39 (m, 5H), 7.26 (d, *J*=9.2 Hz, 2H), 7.13 (dd, *J*=8.1, 10.4 Hz, 1H), 4.78 - 4.75 (m, 2H), 4.72 - 4.68 (m, 1H), 4.61 - 4.50 (m, 3H), 4.35 (d, *J*=15.7 Hz, 1H), 4.19 - 4.15 (m, 1H), 4.09 (dd, *J*=3.7, 12.5 Hz, 1H), 3.96 (d, J=4.4 Hz, 2H), 3.90 - 3.78 (m, 3H), 3.55 (t, *J*=6.1 Hz, 2H), 3.47 - 3.38 (m, 5H), 3.22 (d, *J*=12.9 Hz, 1H), 2.90 - 2.86 (m, 2H), 2.81 - 2.71 (m, 2H), 2.46 (s, 3H), 2.35 (s, 3H), 2.27 - 2.21 (m, 1H), 2.13 - 2.06 (m, 2H), 1.69 - 1.53 (m, 8H), 1.44 - 1.35 (m, 8H), 1.05 (s, 9H). ^{13}C NMR (MeOD) δ: 172.9, 170.6, 170.3, 163.0, 161.1, 158.3, 155.7, 151.4, 147.6, 139.8, 138.8, 135.8, 135.7, 134.6, 133.2, 132.0, 130.5, 130.1, 129.3, 129.0, 128.1, 127.5, 118.9, 116.5, 116.3, 100.1, 72.6, 71.5, 70.4, 70.3, 69.7, 69.3, 66.3, 64.6, 59.5, 57.8, 56.7, 56.6, 53.4, 42.3, 37.6, 35.8, 29.3, 29.2, 29.1, 26.3, 25.7, 25.5, 24.4, 19.1, 14.5; m/z = 1169.6 $(M + H)^+$, expected 1168.5 for $C_{59}H_{77}FN_{10}O_{10}S_2$.

(2S,4R)-1-((S)-2-(tert-butyl)-21-((R)-2-(((6-(4-((2-chloro-5-methoxyphenyl)sulfonamido)phenyl)-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-4-yl)oxy)methyl)morpholino)-4-oxo-6,9,12,15-tetraoxa-3-azahenicosanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (DAT11)

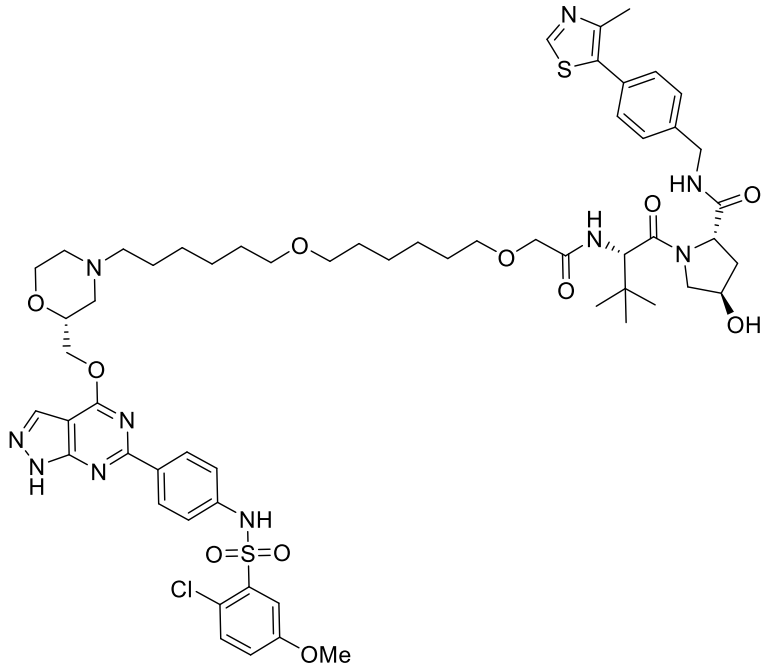


Prepared as reported above for **DAT1**, starting from **S5f,** Sanofi 290R and VH032-amine in 22% yield.

^{1}H NMR (400 MHz, MeOD) δ: 8.85 (s, 1H), 8.30 (d, *J*=9.2 Hz, 2H), 7.64 (d, *J*=3.3 Hz, 1H), 7.46 - 7.37 (m, 5H), 7.25 (d, *J*=9.0 Hz, 2H), 7.08 (dd, *J*=3.1, 9.1 Hz, 1H), 4.71 - 4.55 (m, 4H), 4.53 - 4.49 (m, 2H), 4.34 (d, *J*=15.3 Hz, 1H), 4.09 - 4.04 (m, 3H), 3.97 - 3.94 (m, 1H), 3.88 (d, *J*=11.0 Hz, 1H), 3.82 - 3.77 (m, 4H), 3.76 - 3.52 (m, 14H), 3.44 (t, *J*=6.4 Hz, 2H), 3.09 (d, *J*=10.4 Hz, 1H), 2.86 (d, *J*=12.6 Hz, 1H), 2.55 (s, 3H), 2.49 - 2.46 (m, 5H), 2.31 - 2.16 (m, 3H), 2.12 - 2.06 (m, 1H), 1.58 - 1.53 (m, 4H), 1.38 - 1.34 (m, 4H), 1.05 (s, 9H).

^{13}C NMR (MeOD) δ:173.0, 170.7, 170.3, 163.8, 161.2, 158.3, 151.4, 147.6, 139.5, 138.8, 137.3, 133.3, 132.5, 132.0, 130.1, 129.3, 129.1, 129.0, 128.1, 127.5, 122.2, 119.2, 118.8, 117.3, 99.2, 73.4, 70.9, 70.8, 70.3, 70.2, 70.2, 70.1, 69.8, 69.7, 66.8, 65.8, 59.4, 58.5, 56.7, 55.1, 54.7, 52.7, 42.3, 37.5, 35.7, 29.2, 26.9, 25.7, 25.6, 14.5, 12.7; m/z = 1247.6 $(M + H)^+$, expected 1246.5 for $C_{60}H_{79}ClN_{10}O_{13}S_2$.

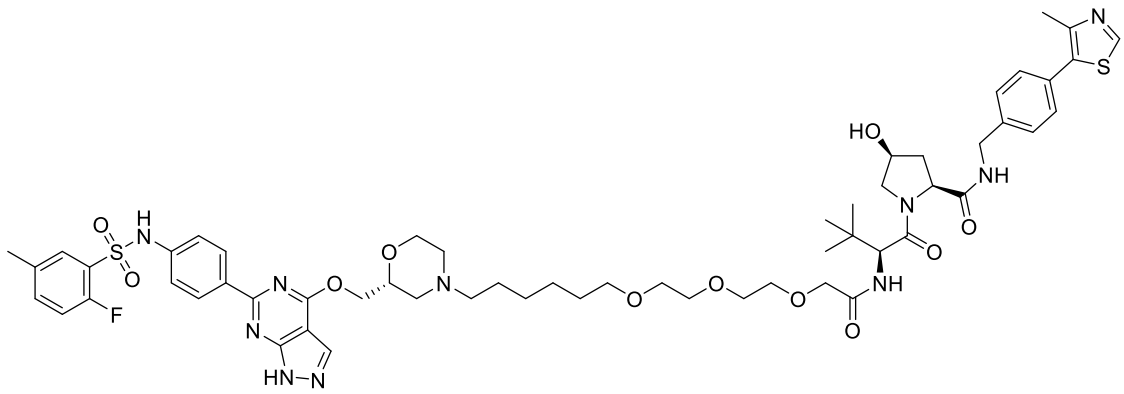
(2S,4R)-1-((S)-2-(2-((6-((6-((R)-2-(((6-(4-((2-chloro-5-methoxyphenyl)sulfonamido)phenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-yl)oxy)methyl)morpholino)hexyl)oxy)hexyl)oxy)acetamido)-3,3-dimethylbutanoyl)-4-hydroxy-N-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (DAT12)



Prepared as reported above for **DAT1**, starting from **S5g,** Sanofi 290R and VH032-amine in 26% yield.

^{1}H NMR (400 MHz, MeOD) δ: 8.85 (s, 1H), 8.33 - 8.28 (m, 3H), 7.64 (d, *J*=3.0 Hz, 1H), 7.46 - 7.38 (m, 5H), 7.25 (d, *J*=8.8 Hz, 2H), 7.09 (dd, *J*=3.0, 8.6 Hz, 1H), 4.71 - 4.68 (m, 3H), 4.61 - 4.50 (m, 3H), 4.35 (d, *J*=15.4 Hz, 1H), 4.16 - 4.11 (m, 1H), 4.08 - 4.03 (m, 1H), 3.97 - 3.95 (m, 2H), 3.88 (d, *J*=10.9 Hz, 1H), 3.83 - 3.76 (m, 5H), 3.53 (t, *J*=6.8 Hz, 2H), 3.41 - 3.37 (m, 5H), 3.12 (d, *J*=12.9 Hz, 1H), 2.78 - 2.75 (m, 2H), 2.66 - 2.52 (m, 6H), 2.46 (s, 3H), 2.23 - 2.20 (m, 1H), 2.12 - 2.08 (m, 1H), 1.66 - 1.51 (m, 8H), 1.43 - 1.35 (m, 8H), 1.05 (s, 9H). ^{13}C NMR (MeOD) δ: 172.9, 170.6, 170.3, 165.5, 163.7, 161.2, 158.3, 151.4, 147.6, 142.1, 139.5, 138.8, 137.3, 133.2, 132.5, 132.0, 130.1, 129.3, 129.1, 128.9, 128.1, 127.5, 122.2, 119.1, 118.7, 117.3, 99.2, 72.8, 71.5, 70.4, 70.3, 69.7, 69.3, 66.3, 64.9, 59.5, 58.0, 56.8, 56.6, 55.1, 53.9, 52.1, 42.3, 37.6, 35.8, 29.3, 29.2, 26.5, 25.7, 25.6, 25.5, 24.7, 14.5, 12.7; m/z = 1201.5 $(M + H)^+$, expected 1200.5 for $C_{59}H_{77}ClN_{10}O_{11}S_2$.

(2*S*,4*S*)-1-((*S*)-2-(*tert*-butyl)-18-((*R*)-2-(((6-(4-((2-fluoro-5-methylphenyl)sulfonamido)phenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)oxy)methyl)morpholino)-4-oxo-6,9,12-trioxa-3-azaoctadecanoyl)-4-hydroxy-*N*-(4-(4-methylthiazol-5-yl)benzyl)pyrrolidine-2-carboxamide (cisSGK3-PROTAC1)



Prepared as reported above for **DAT1**, starting from **S5e,** Sanofi 308R and cisVH032-amine in 23% yield.

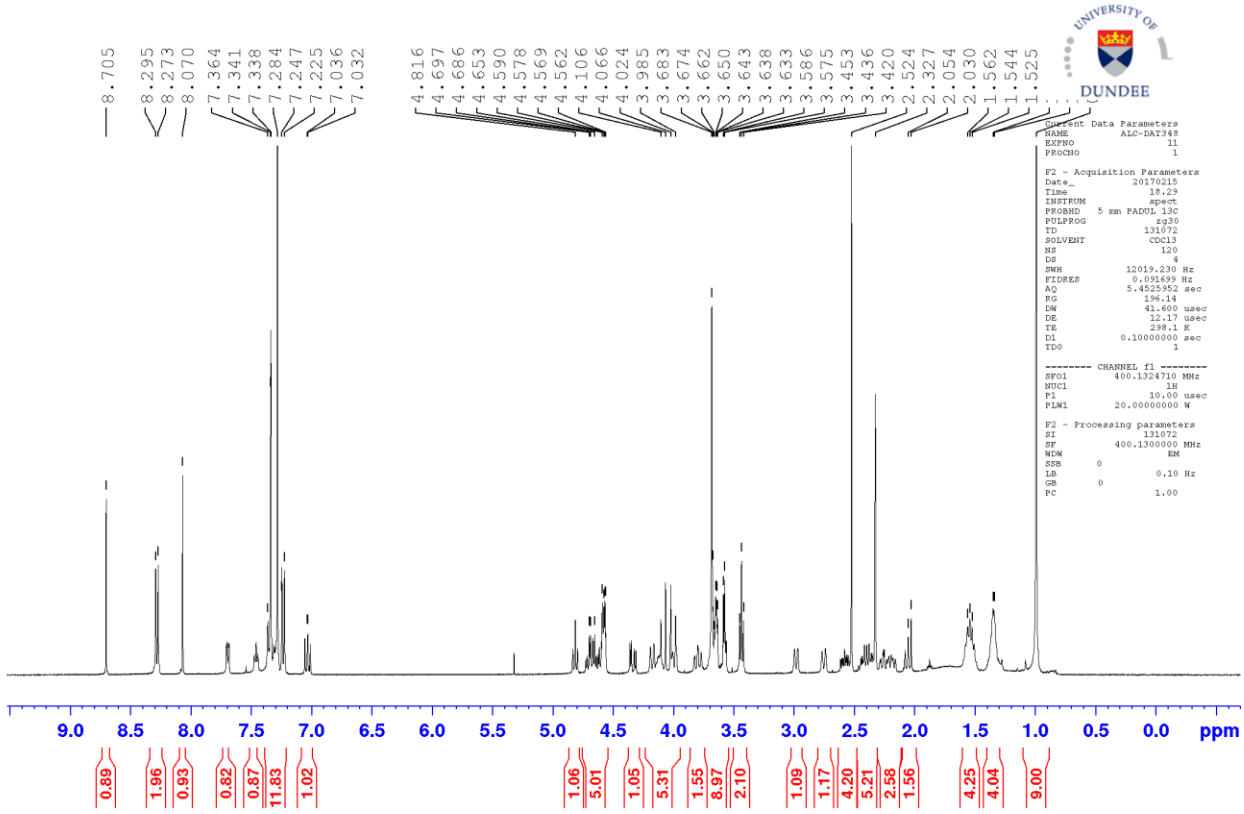
^{1}H NMR (500 MHz, MeOD) δ: 8.86 (s, 1H), 8.36 (d, *J*=9.1 Hz, 2H), 8.10 (s, 1H), 7.73 (dd, *J*=1.9, 6.8 Hz, 1H), 7.45 - 7.39 (m, 5H), 7.26 (d, *J*=8.3 Hz, 2H), 7.13 (dd, *J*=8.3, 10.5 Hz, 1H), 4.79 - 4.73 (m, 3H), 4.63 - 4.60 (m, 1H), 4.55 - 4.50 (m, 2H), 4.44 - 4.36 (m, 2H), 4.17 - 4.15 (m, 1H), 4.09 - 3.97 (m, 4H), 3.83 - 3.78 (m, 1H), 3.75 - 3.63 (m, 11H), 3.60 - 3.56 (m, 11H), 3.47 - 3.38 (m, 3H), 3.18 (d, J=14.2 Hz, 1H), 2.85 - 2.65 (m, 4H), 2.49 - 2.41 (m, 4H), 2.34 (s, 3H), 2.03 - 1.98 (m, 1H), 1.68 - 1.54 (m, 4H), 1.41 - 1.31 (m, 4H), 1.04 (s, 9H).

^{13}C NMR (MeOD) δ: 174.9, 172.4, 172.1, 164.5, 162.6, 159.5, 157.5, 152.9, 149.2, 141.3, 140.1, 137.3, 137.2, 136.1, 136.0, 134.7, 133.4, 131.9, 131.7, 130.8, 130.5, 129.3, 129.1, 128.3, 128.2, 120.5, 118.0, 117.8, 101.6, 74.2, 72.4, 72.2, 71.7, 71.6, 71.3, 71.2, 67.9, 66.2, 61.2, 59.4, 58.3, 57.7, 55.1, 53.4, 43.9, 37.9, 36.6, 30.6, 27.8, 27.1, 27.0, 26.0, 20.6, 15.9; m/z = 1157.5 $(M + H)^{+}$, expected 1156.5 for $C_{57}H_{73}FN_{10}O_{11}S_2$.

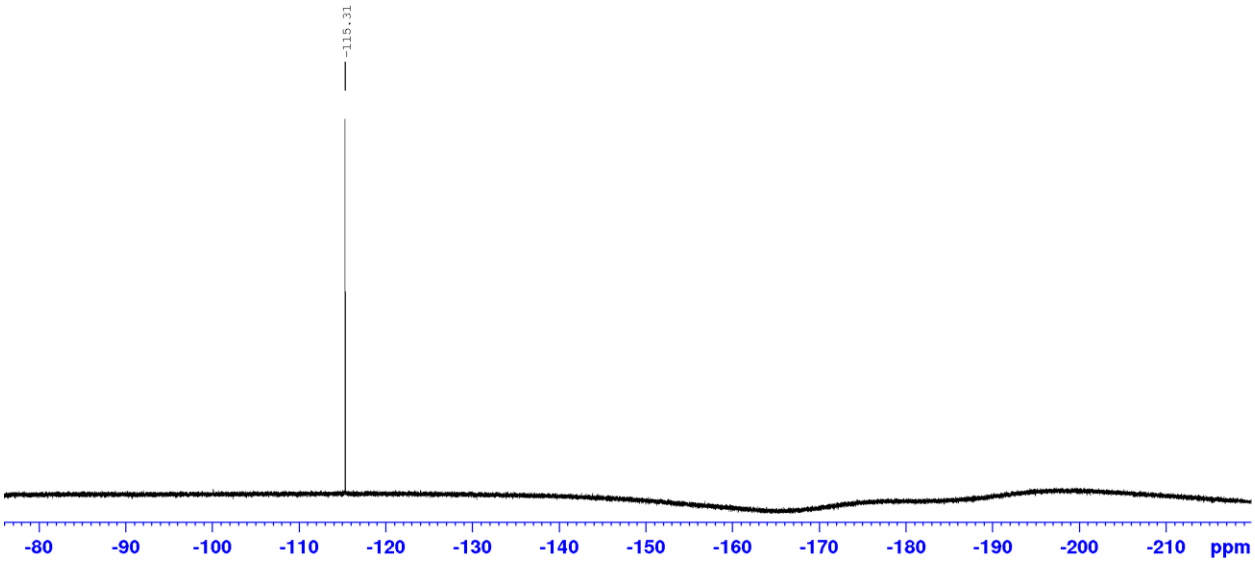
6. COMPOUND NMR SPECTRA

NMR spectra of SGK3-PROTAC1 and cisSGK3-PROTAC1

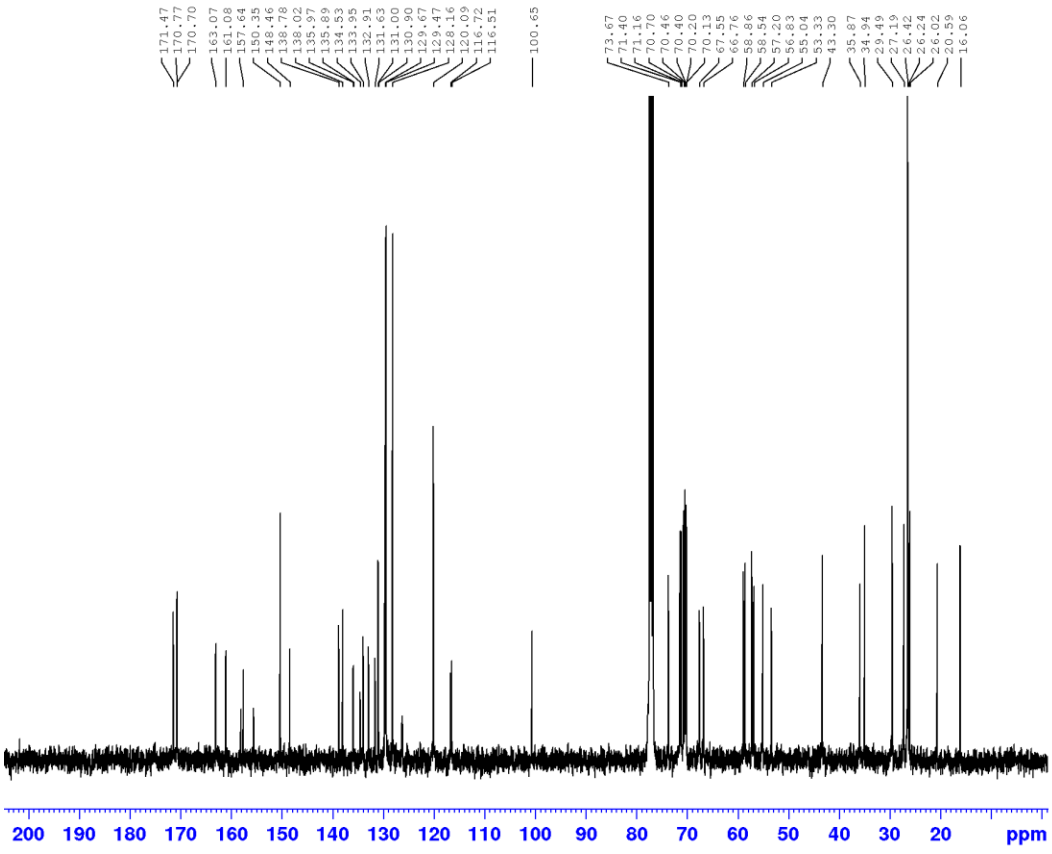
^{1}H-NMR (400 MHz, $CDCl_3$), SGK3-PROTAC1



^{19}F-NMR (471 MHz, $CDCl_3$), SGK3-PROTAC1



^{13}C-NMR (101 MHz, $CDCl_3$), SGK3-PROTAC1



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PROCNO                   1

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DE                    8.66 usec
TE                   298.2 K
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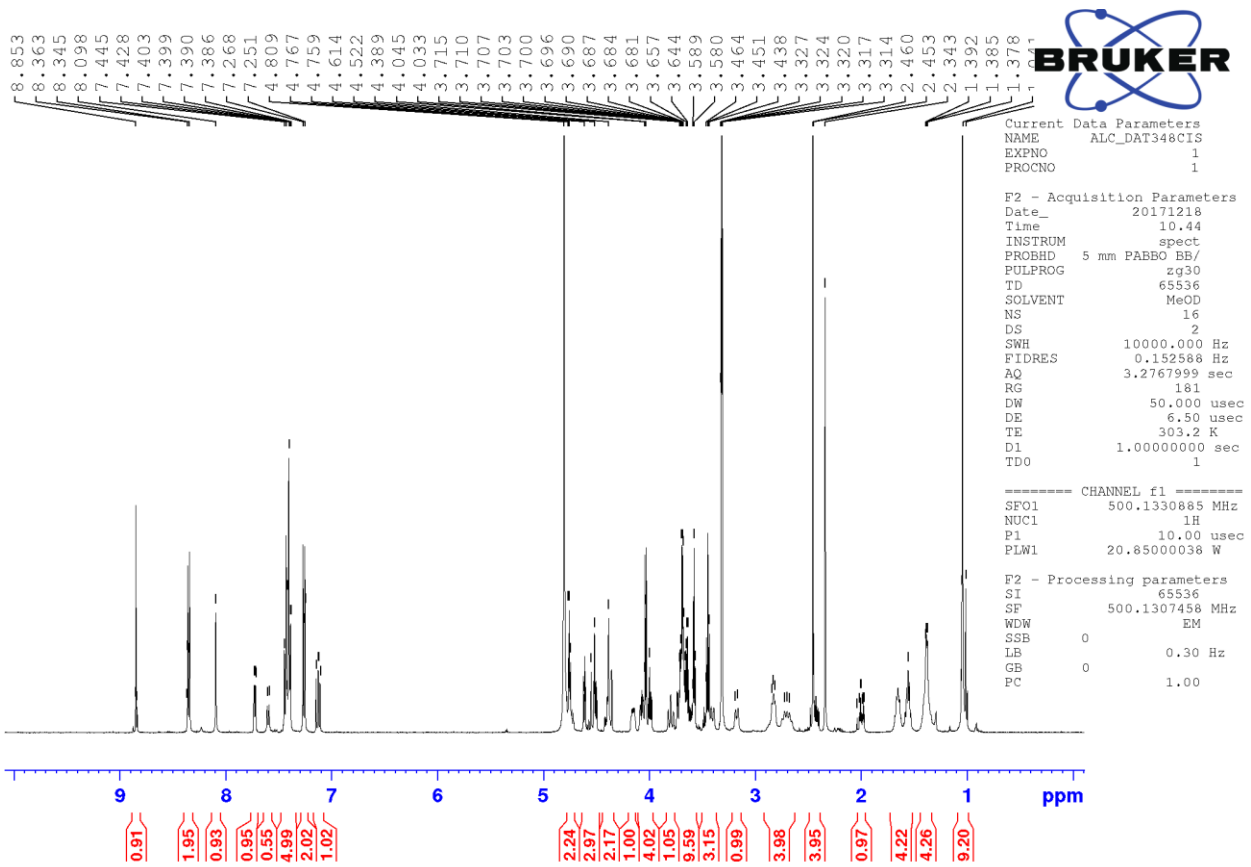
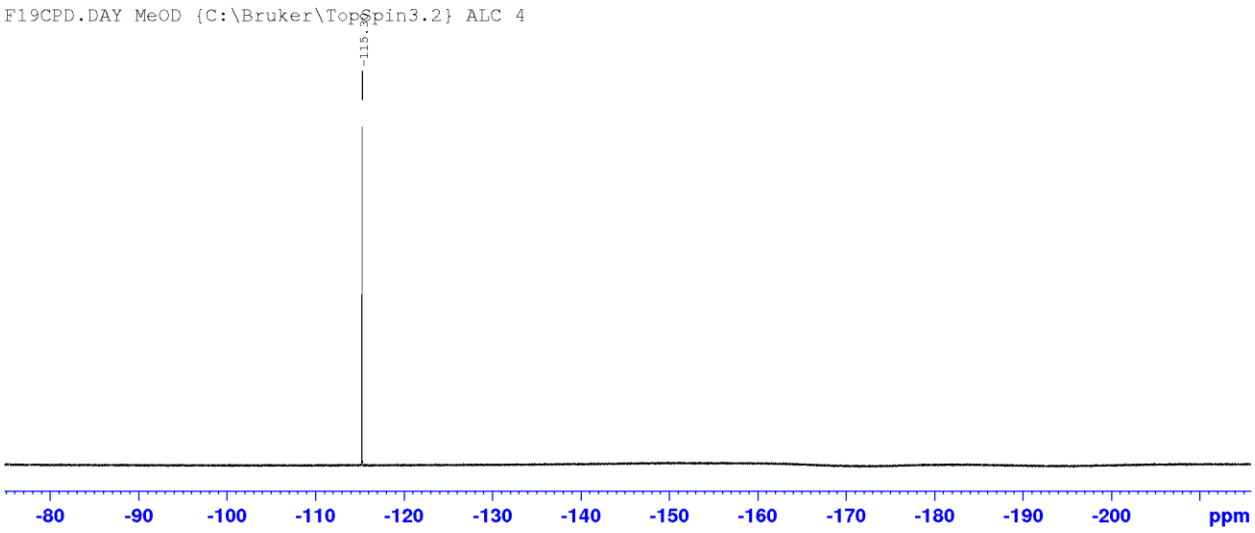
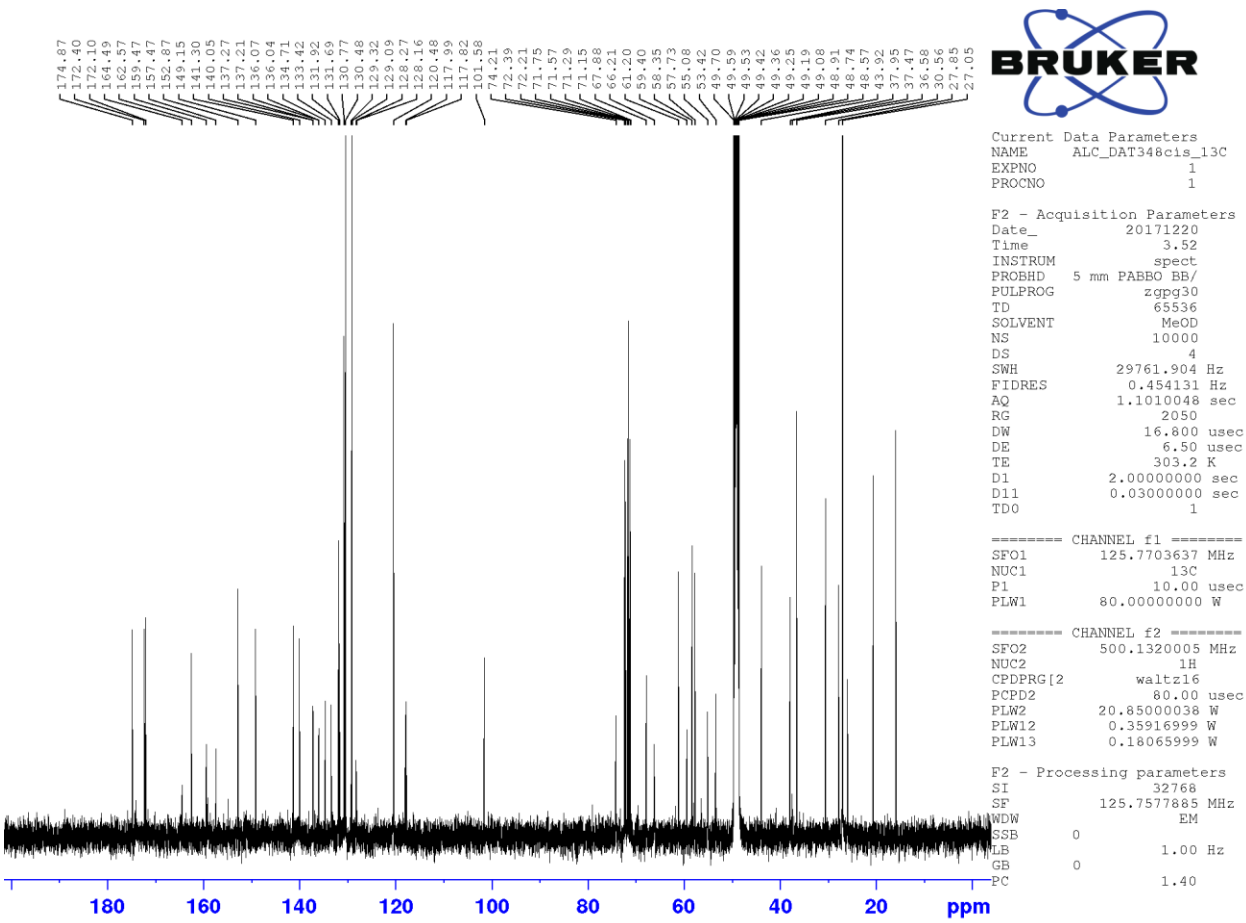
^{1}H-NMR (500 MHz, MeOD), cisSGK3-PROTAC1



^{19}F-NMR (471 MHz, MeOD), cisSGK3-PROTAC1



^{13}C-NMR (126 MHz, MeOD), cisSGK3-PROTAC1



7. REFERENCES

(1) Crew, A. P.; Raina, K.; Dong, H.; Qian, Y.; Wang, J.; Vigil, D.; Serebrenik, Y. V; Hamman, B. D.; Morgan, A.; Ferraro, C.; Siu, K.; Neklesa, T. K.; Winkler, J. D.; Coleman, K. G.; Crews, C. M. Identification and Characterization of Von Hippel-Lindau-Recruiting Proteolysis Targeting Chimeras (PROTACs) of TANK-Binding Kinase 1. *J. Med. Chem.* **2017**.

(2) Girardini, M.; Maniaci, C.; Hughes, S. J.; Testa, A.; Ciulli, A. Cereblon versus VHL: Hijacking E3 Ligases against Each Other Using PROTACs. *Bioorg. Med. Chem.* **2019**, *27*, 2466–2479.

(3) Maniaci, C.; Hughes, S. J.; Testa, A.; Chen, W.; Lamont, D. J.; Rocha, S.; Alessi, D. R.; Romeo, R.; Ciulli, A. Homo-PROTACs: Bivalent Small-Molecule Dimerizers of the VHL E3 Ubiquitin Ligase to Induce Self-Degradation. *Nat. Commun.* **2017**, *8*, 830.

(4) Quici, S.; Manfredi, A.; Raimondi, L.; Sironi, A. Synthesis and Properties of New Lipophilic Macrotricyclic Cylindrical Cryptands. *J. Org. Chem.* **1995**, *60*, 6379–6388.

(5) Zoppi, V.; Hughes, S. J.; Maniaci, C.; Testa, A.; Gmaschitz, T.; Wieshofer, C.; Koegl, M.; Riching, K. M.; Daniels, D. L.; Spallarossa, A.; Ciulli, A. Iterative Design and Optimization of Initially Inactive Proteolysis Targeting Chimeras (PROTACs) Identify VZ185 as a Potent, Fast, and Selective von Hippel–Lindau (VHL) Based Dual Degrader Probe of BRD9 and BRD7. *J. Med. Chem.* **2019**, *62*, 699–726.