Electronic Supplementary Material (ESI) for ChemComm.

PROTAC-Mediated Degradation of Class I Histone Deacetylase Enzymes in Corepressor Complexes

Joshua P. Smalley, Grace E. Adams, Christopher J. Millard, Yun Song, James K.S. Norris, John W.R. Schwabe*, Shaun M. Cowley* and James T. Hodgkinson*

Supplementary Information

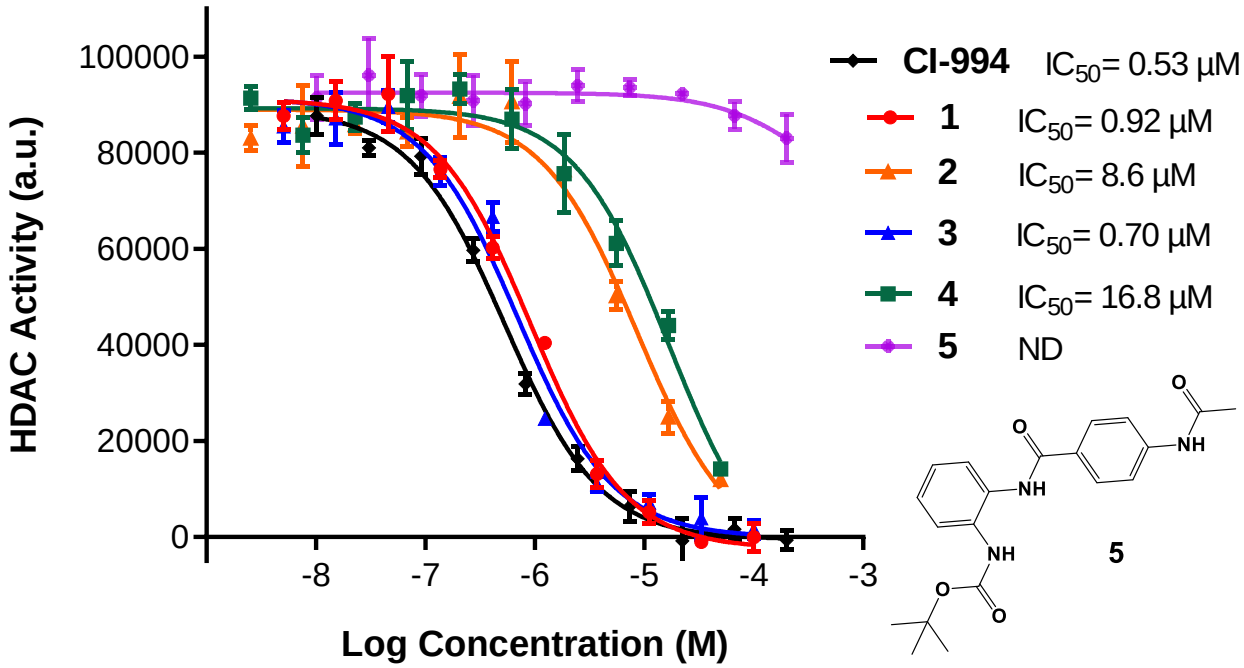
Table of Contents

Supplementary Information: Biology

1. *In vitro* HDAC Assay with CoREST Complex

Inhibition tests against LSD1-HDAC1-CoREST complex were performed using an enzyme fluorescence-based HDAC assay.[1] The inhibitor/PROTACs were dissolved at 50 mM in DMSO, then 1:2 serial dilutions performed using HDAC assay buffer (50 mM Tris pH 7.5, 150 mM NaCl) to afford range of concentrations. 10 uL of these solutions were added to individual wells, followed by 20 µL of HDAC complex dissolved in HDAC assay buffer (18 nM) and 20 µL of the substrate Boc-(Ac)Lys-AMC dissolved in HDAC assay buffer. The assays were performed in black 96-well plates with a reaction volume of 50 µL per well. All determinations were performed in triplicate. After an incubation of 20 minutes at 37 ˚C and 150 rpm, deacetylation was stopped by the addition of 50 µL of a developing solution containing trypsin (50 mM Tris pH 7.5, 100 mM NaCl, 10 mg/mL trypsin). Fluorescence intensity was measured with a plate reader (PerkinElmer, 2030 multilabel reader, VICTOR X5, λ_{ex} 335 nm, λ_{em} 460 nm). HDAC Activity was calculated by subtracting the average blank fluorescence from the well fluorescence. Graphpad Prism software was utilised to determine IC_{50} values.

Figure S1: AMC-Fluorescence Histone Deacetylase inhibition assay with the LSD1-CoREST-HDAC1 complex, including error bars.



2. Cell Lines and Materials

E14 wild type (WT) mouse embryonic stem (mES) cells were maintained on gelatinised plates in standard mES media consisting of Knockout Dulbecco's Modified Eagle Medium (KO DMEM) (GIBCO, 10829-018) supplemented with 15% Fetal Bovine Serum (FBS) (Sigma, F9665), 1X glutamine/penicillin/streptomycin (GIBCO, 10378-016), 100 μM β-mercaptoethanol (Sigma) and Leukaemia Inhibitory Factor (synthesised in house). HCT116 human colon carcinoma cells were grown in Dulbecco's Modified Eagle Medium (DMEM) (GIBCO, 41965-039) supplemented with 10% Fetal Bovine Serum (FBS) (Sigma) and 1X glutamine/ penicillin/streptomycin (GIBCO, 10378-016). Both cell lines were incubated at 37 ˚C with 5% CO_2. Cells were treated with PROTACs (0.01-40 μM) alongside HDAC inhibitors CI-994 (10/40 μM) and Panobinostat (30 nM) as controls.

3. Western Blotting

HCT116 or mES cells were treated 24 hours after seeding. 24 hours post treatment, cells were harvested, lysed in lysis buffer (50mM Tris-HCl, 150 mM NaCl, 0.5% NP-40, 0.5% Triton X-100) with protease inhibitor (Sigma, P8340), then incubated on ice for 30 minutes, before being centrifuged (18,000 rcf, 15 minutes, 4 ˚C). The supernatant was collected, and protein concentrations quantified via Bradford Assay using Protein Assay Dye Reagent Concentrate (BIO-RAD). For histone extraction, an equal volume of 0.4 N H_2SO_4 was added to the pellets and the extracts placed at 4 ˚C overnight. Following overnight incubation, the tubes were centrifuged (18,000 rcf, 15 minutes, 4 ˚C) and then the supernatant (histone extract) collected.

Western blots were run on NuPAGE™ 4-12% Bis-Tris gels with 20-30 μg of protein or 10 μL of acid-extracted histone loaded per lane, using NuPAGE™ LDS Sample Buffer (4X). PageRuler™ Plus Prestained Ladder was used for size standards. After gel electrophoresis at 140V for 75-90 minutes the separated proteins were transferrred onto nitrocellulose membrane at 30V for 60 minutes. The membranes were probed with primary antibodies (listed below) for 60-90 minutes. Blots were developed with complimentary IRDye conjugated secondary antibodies and the bands visualised using the Odyssey Infrared Imaging System. Image processing and band intensity quantification was performed using Image Studio Lite.

<u>Antibody Information</u>

Primary Antibodies;

α-tubulin - Sigma, t5168 (1:10,000 dilution)

HDAC1 - Abcam, 109411 (1:2,000 dilution)

HDAC2 - Merck Millipore, 05-814 (1:2,000 dilution)

HDAC3 - Abcam, 32369 (1:2,000 dilution)

H3 - Merck Millipore, 05-499 (1:1,000 dilution)

H3K9Ac - Upstate, 06-942 (1:1,000 dilution)

H3K27Ac - Merck Millipore, 07-360 (1:1,000 dilution)

H3K56Ac - Active Motif, 39281 (1:1,000 dilution)

Secondary Antibodies;

IRDye® 680LT - LI-COR Biosciences, 926-68023 (1:10,000 dilution)

IRDye® 800CW - LI-COR Biosciences, 926-32210 (1:10,000 dilution)

3.1. Western Blots for Histone 3 Lysine 9/27/56 Acetylation (H3KxAc)

Figure S2: B) Histone 3 Lysine 56 Acetylation (H3K56Ac) levels in E14 mouse embryonic stem cells after 24h; CI-994 = 40 µM, Panobinostat = 30 nM. Fold change in H3K56Ac levels shown.

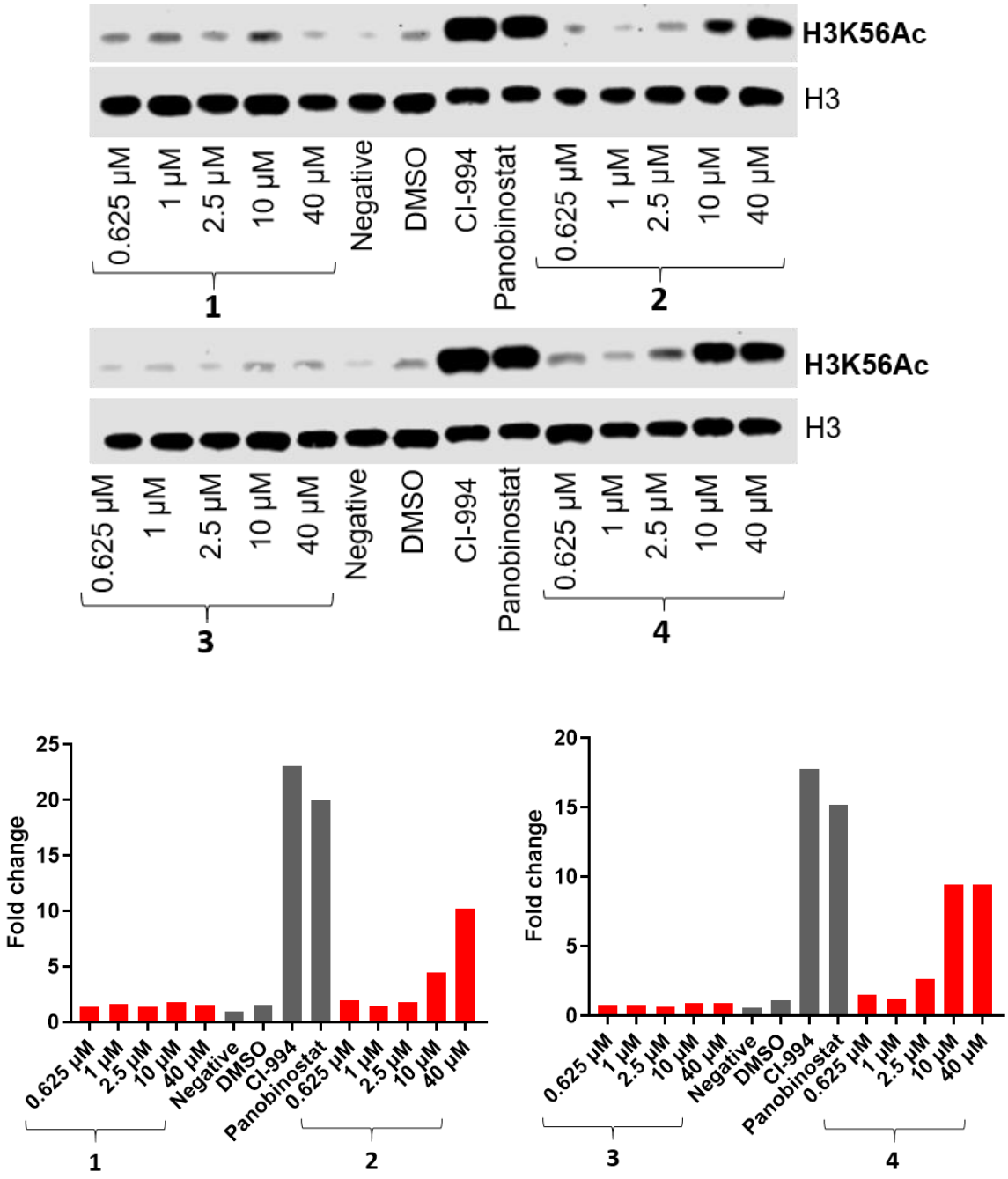
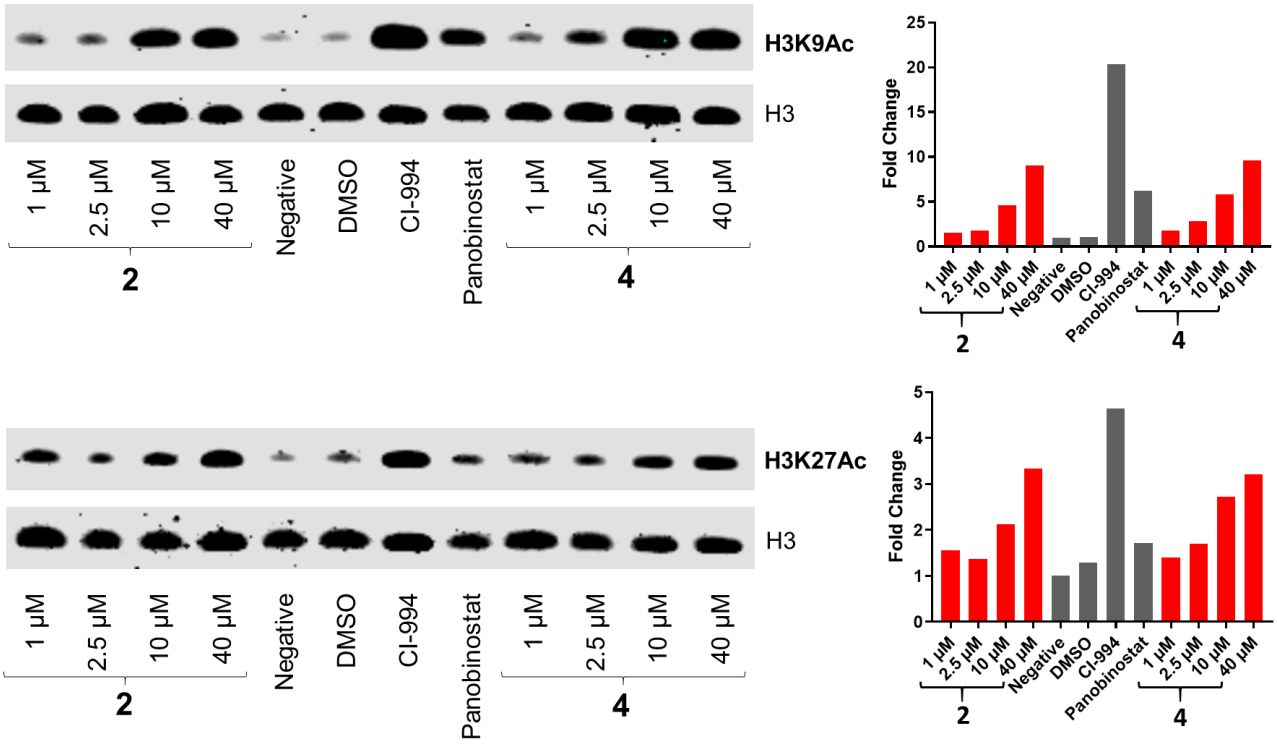


Figure S3: Histone 3 lysine 9 acetylation (H3K9Ac) and histone 3 lysine 27 acetylation (H3K27Ac) levels in HCT116 cells after 24h with **2** and **4**; CI-994 = 40 µM, Panobinostat = 30 nM. Fold change in H3K9/27Ac levels shown.



3.2. Western Blots for HDAC1/2&3 Degradation

Figure S4: HDAC 1,2 & 3 degradation occurs in a dose dependent manner with **4**. A) Immunoblot with HDAC 1,2 & 3 antibodies after 24h in HTC116 cell line. Numerical value represents percentage of protein compared to DMSO control = 100%. B) Histone 3 Lysine 9 acetylation levels after 24 hours in HTC116 cell line. C) **6** with the inactive VHL diastereoisomer does not induce degradation. CI-994 = 40μM, Panobinostat = 30 nM.

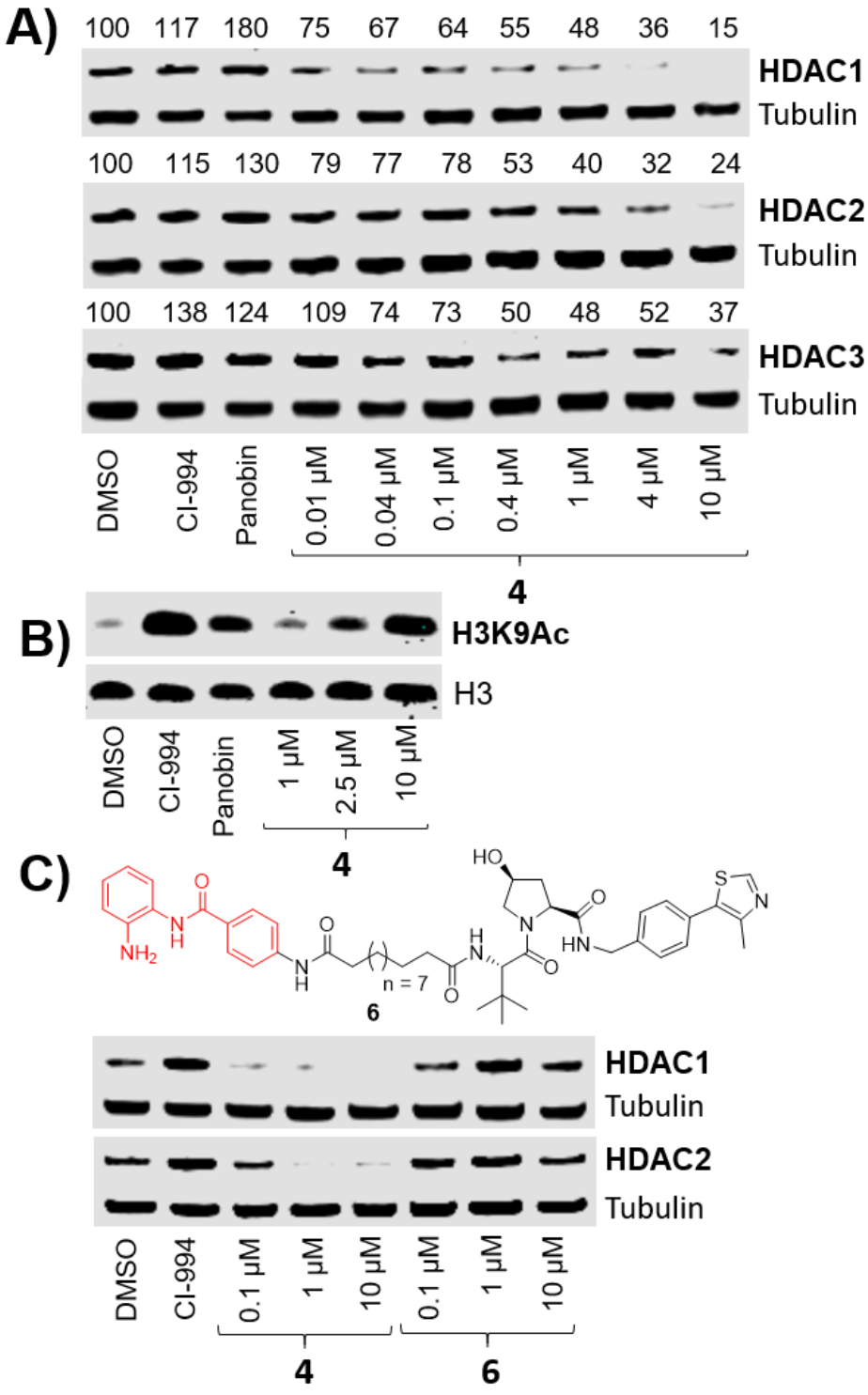
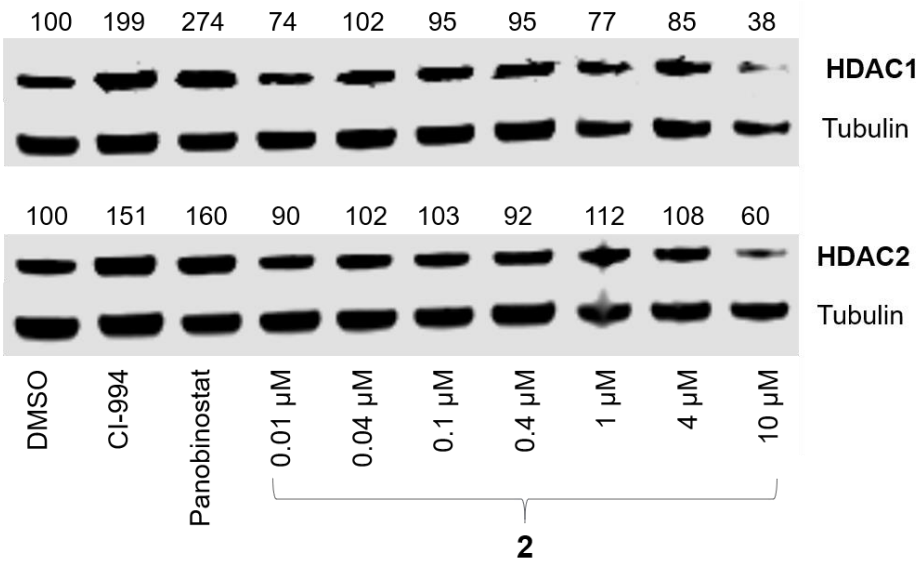


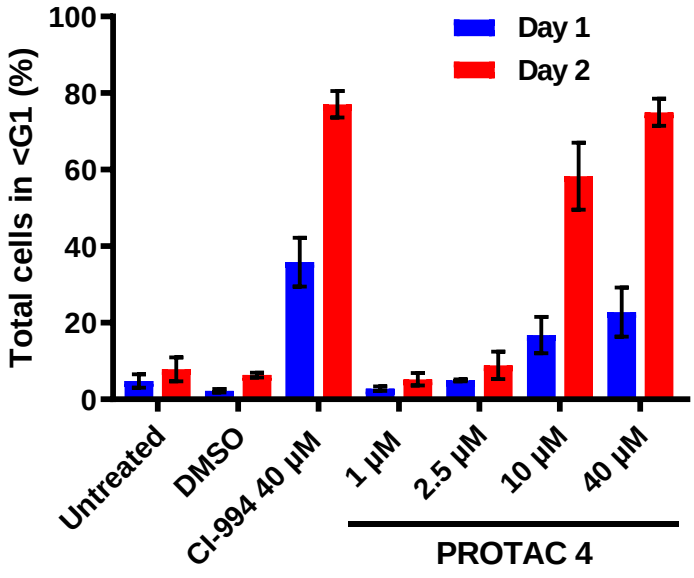
Figure S5: Effect of **2** on the levels of HDAC 1/2 in HCT116 cells following 24h treatment; CI-994 = 40 µM, Panobinostat = 30 nM.



4. Cell Viability Assay

To analyse cell death, cells were treated with DMSO, CI-994 (40 µM), or PROTAC **4** (1-40 µM) 24 hours after seeding. 24 hours post treatment, cells were harvested and fixed with 70% (vol/vol) ethanol at -20°C overnight. Cells were washed in PBS prior to incubation with 50 µg of propidium iodide and RNase A (10 µg/mL) for 30 min at room temperature in the dark. Samples were analysed using the BD FACSCanto II flow cytometer (BD Biosciences) in the PE_A channel with BD FACSDiva software.

Figure S5. FACS data of compound **4** and CI-994 in HCT116 colon cancer cell line.



Supplementary Information: Chemistry

5. General Methods

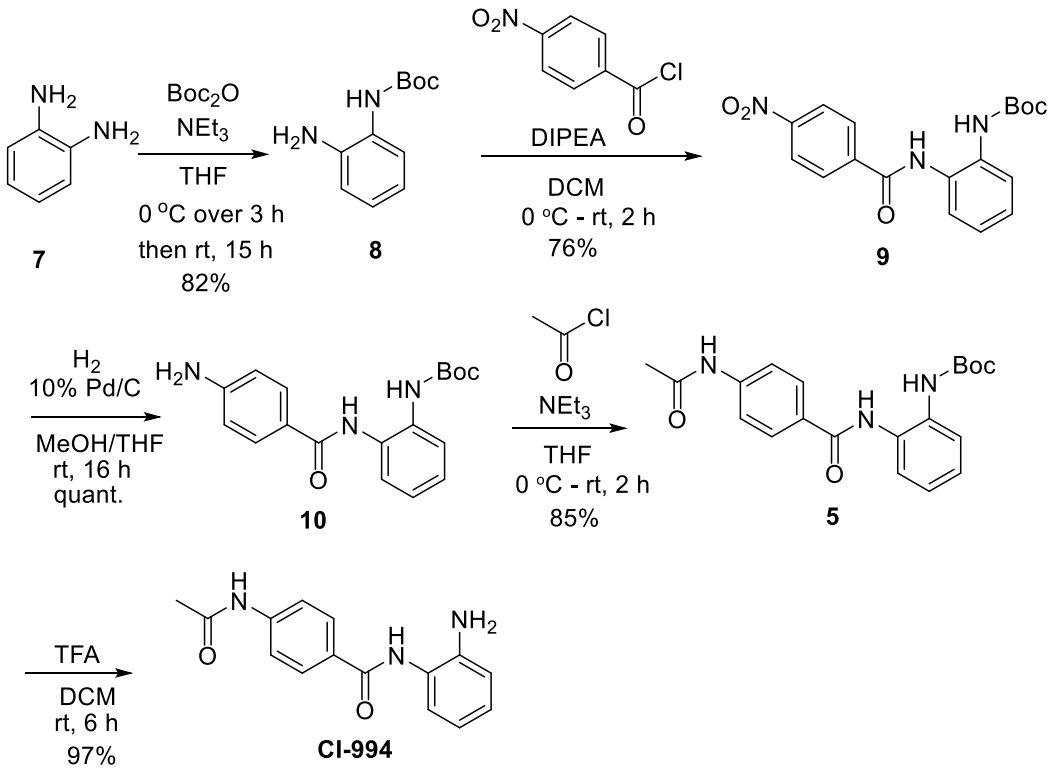
All reagents and solvents were obtained from Sigma Aldrich, Acros Organics, Fisher Scientific and were used as supplied unless stated otherwise. The active VHL ligand (4R)-3-Methyl-L-valyl-4-hydroxy-N-[[4-(4-methyl-5-thiazolyl)phenyl]methyl]-L-prolinamide hydrochloride and inactive (negative control) VHL ligand (2S,4S)-1-[(2S)-2-Amino-3,3-dimethyl-butanoyl]-4-hydroxy-N-[[4-(4-methylthiazol-5-yl)phenyl]methyl]pyrrolidine-2-carboxamide dihydrochloride were purchased from TOCRIS®. Biotage® Macroporous polystyrene-co-divinylbenzene (MP) carbonate resin (3.02 mmol/g loading capacity) was used for neutralizing amine TFA salts and scavenging excess TFA during tert-butoxycarbonyl deprotection reactions. Glassware was dried in oven at 100 °C for 12 hours for moisture sensitive reactions. Unless otherwise stated reactions were performed under nitrogen using anhydrous solvents. Dried THF and DCM were dried using an Innovative Technology inc. PureSolv solvent purification system. Room temperature refers to ambient temperature. Temperatures of 0 °C were maintained using an ice-water bath. The reactions were monitored by thin-layer chromatography (TLC) on aluminium backed silica gel. Unless otherwise stated Flash column chromatography was carried out with Silica Gel 60 using commercial solvents. All evaporations *in vacuo* were performed under reduced pressure using a Büchi rotary evaporator. All chemical names have been generated using ChemDraw Professional. Preparative column chromatography and flash column chromatography using a Biotage Isolera purification system was perfomed using silica gel 60 (230-400 mesh).

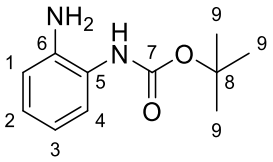
Analytical and semi-preparative HPLC were performed on a ThermoFisher Ultimate 3000 system with Chromeleon software on a Phenomenex Luna C18 column. Method 1, A= H_2O, B= CH_3CN, 5-100% B, 10 mL/min flow, 45 min gradient. Method 2, A= 0.1% TFA in H_2O, B= 0.1% TFA in CH_3CN, 5-100% B, 10 mL/min flow, 45 min gradient. Solutions were either made up in HPLC Grade acetonitrile (MeCN) and deionised water (1:1) or HPLC grade methanol

Nuclear magnetic resonance (NMR) spectra were acquired using a Bruker 500 (^{1}H, 500 MHz; ^{13}C 125 MHz) or Bruker 400 (^{1}H, 400 MHz; ^{13}C 100 MHz) instrument at ambient temperature using deuterated solvent as reference - $CDCl_3$ (δ_H = 7.26 ppm, δ_C = 77.00 ppm), DMSO-d_6 (δ_H = 2.50 ppm, δ_C = 39.51 ppm), CD_3OD (δ_H = 3.31 ppm, δ_C = 49.15 ppm), or CD_3CN (δ_H = 1.94 ppm, δ_C =1.39, 118.69 ppm). 1H NMR data are reported as: chemical shift, multiplicity [b, broad; s, singlet; d, doublet; t, triplet; q, quartet; quin, m for multiplet; or as a combination (e.g., dd, dt, etc.)], coupling constant(s) and integration. ^{13}C NMR spectra were recorded by broadband proton decoupling. 13C NMR chemical shifts (δ) are quoted to the nearest 0.1 ppm and referenced to the residual non-deuterated solvent peak. Where ^{1}H and ^{13}C NMR's have been fully assigned, 2D NMR including ^{1}H-^{1}H COSY (correlated spectroscopy), ^{1}H-^{13}C HSQC (heteronuclear single quantum coherence) and ^{1}H-^{13}C HMBC (heteronuclear multiple bond coherence) were used to aid assignment. ACDLabs software (Chemsketch and Spectrus Processor) was used for peak picking, integration and calculating coupling

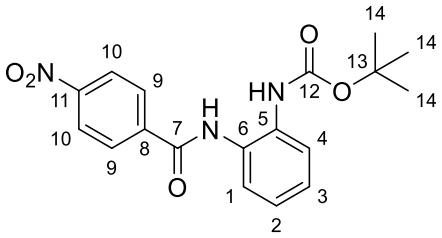
constants. High resolution mass spectra (HRMS) were recorded on a Water Aquity XEVO Q ToF machine and measured in m/z.

6. Synthesis of CI-994

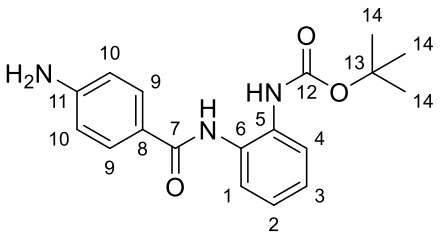




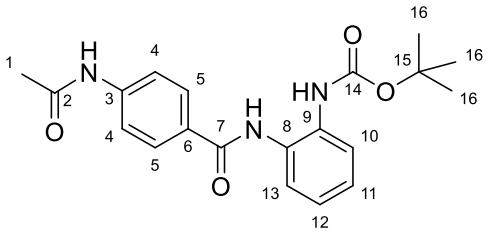
Tert-butyl (2-aminophenyl)carbamate, (8): A solution of Boc_2O (6.05 g, 27.7 mmol) in THF (50 mL) was added dropwise over 3 hours to a solution of **7** (3.00 g, 27.7 mmol) and triethylamine (4.64 mL, 33.3 mmol) in THF (25 mL) at 0 °C, then the mixture was stirred at room temperature for 15 hours. The reaction was mixture concentrated *in vacuo* to afford a grey crystalline solid and then re-dissolved in EtOAc (50 mL). This solution was washed with water (2 x 30 mL) and sat. brine (2 x 30 mL), filtered over Na_2SO_4, then concentrated *in vacuo* to afford a yellow/grey solid. The crude solid was purified by column chromatography (solid load, 10-25% EtOAc in hexane) to afford **8** (4.72 g, 22.5 mmol, 82% yield) as a yellow/grey solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ_H ppm 7.19 (d, *J*=7.7 Hz, 1 H, 1-CH), 6.92 (app. td, *J*=7.7, 1.3 Hz, 1 H, 2-CH), 6.70 (app. td, *J*=7.7, 1.3 Hz, 1 H, 3-CH), 6.68 (dd, *J*=7.7, 1.3 Hz, 1 H, 4-CH), 6.18 (br s, 1 H, NH), 3.64 (br s, 2 H, NH_2), 1.44 (s, 9 H, 9-CH_3). ^{13}C NMR (101 MHz, Chloroform-*d*) δ_C ppm 153.9 (C7), 140.0 (C6), 126.2 (double intensity: C3, C5), 124.8 (C4), 119.6 (C2), 117.6 (C1), 80.5 (C8), 28.4 (C9). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{11}H_{16}N_2O_2Na$: 231.1109, found 231.1112.



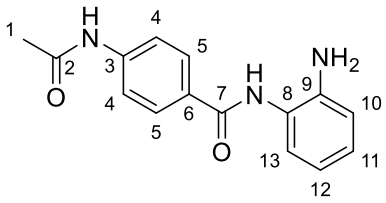
Tert-butyl (2-(4-nitrobenzamido)phenyl)carbamate, (9): DIPEA (5.23 mL, 30.0 mmol) was added to a solution of **8** (4.17 g, 20.0 mmol) in dry DCM (90 mL) at 0 °C, followed by the dropwise addition of 4-nitrobenzoyl chloride (4.09 g, 22.0 mmol) as a solution in dry DCM (10 mL). The mixture was stirred at 0 °C for 30 minutes, then at room temperature overnight. The reaction mixture was diluted with DCM and then washed with sat. $NaHCO_4$ (100 mL), 1M HCl (100 mL) and sat. brine (100 mL). The organic layer was then dried over Na_2SO_4 and concentrated *in vacuo* to afford a yellow solid (7.64 g). The crude product was triturated in EtOH and then filtered to afford **9** (5.52 g, 15.3 mmol, 76% yield) as a pale yellow solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ_H ppm 9.79 (s, 1 H, NH), 8.30 (d, *J*=8.9 Hz, 2 H, 10-CH), 8.14 (d, *J*=8.9 Hz, 2 H, 9-CH), 7.84 (d, *J*=7.7 Hz, 1 H, 1-CH), 7.20 - 7.26 (m, 1 H, 3-CH), 7.14 - 7.18 (m, 2 H, 2-CH,4-CH), 6.86 (s, 1 H, NH), 1.52 (s, 9 H, 14-CH_3). ^{13}C NMR (101 MHz, Chloroform-*d*) δ_C ppm 163.3 (C7), 155.0 (C12), 149.8 (C8), 140.0 (C11), 130.5 (C5), 129.6 (C6), 128.6 (C9), 126.3 (C2), 126.2 (C3), 125.9 (C1), 124.4 (C4), 123.7 (C10), 82.0 (C13), 28.3 (C14). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{18}H_{19}N_3O_5Na$: 380.1222, found 380.1223.



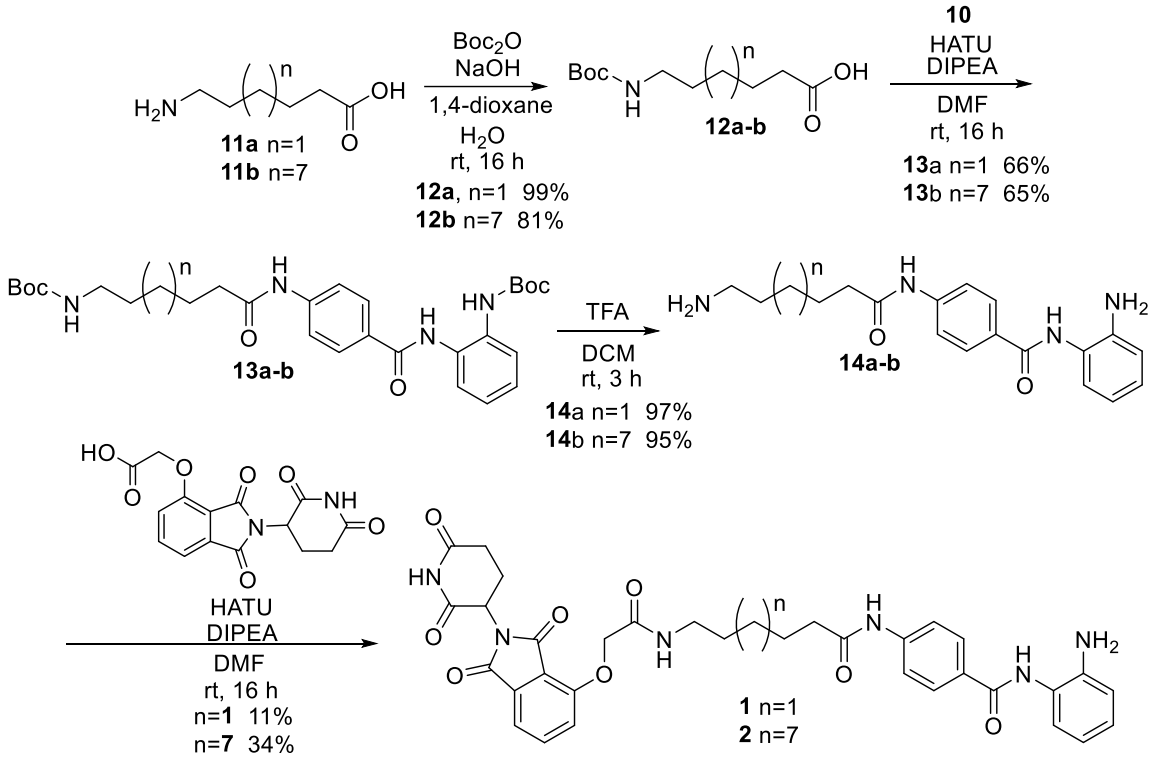
Tert-butyl (2-(4-aminobenzamido)phenyl)carbamate, (10): To a solution of **9** (5.52 g, 15.3 mmol) in MeOH/THF (1:1, 100 mL), 10% Pd/C (0.55 g) was added. The reaction flask was filled with nitrogen and evacuated 3 times using a Shlenk line, before a balloon of hydrogen was added and the resultant mixture stirred vigorously for 18 hours. The balloon of hydrogen was removed and the flask was flushed with nitrogen. The reaction mixture was filtered through celite, then the celite was washed with more MeOH (3 x 50 mL) and the filtrate concentrated *in vacuo* to afford **10** (5.23 g, 15.3 mmol, 100% yield) as a fluffy white crystalline solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ_H ppm 8.87 (s, 1 H, NH), 7.78 (d, *J*=8.7 Hz, 2 H, 9-CH), 7.64 (dd, *J*=7.7, 1.7 Hz, 1 H, 1-CH), 7.29 (dd, *J*=7.7, 1.7 Hz, 1 H, 4-CH), 7.14 (app. td, *J*=7.7, 1.7 Hz, 1 H, 2-CH), 7.12 (app. td, *J*=7.7, 1.7 Hz, 1 H, 3-CH), 7.06 (s, 1 H, NH), 6.66 (d, *J*=8.7 Hz, 2 H, 10-CH), 4.05 (s, 2 H, 11-NH_2), 1.51 (s, 9 H, 14-CH_3). ^{13}C NMR (101 MHz, Chloroform-*d*) δ_C ppm 165.7 (C7), 154.6 (C12), 150.1 (C8), 131.0 (C6), 130.3 (C5), 129.3 (C9), 125.7 (C1), 125.6 (C2,C3), 124.5 (C4), 123.5 (C11), 114.1 (C10), 81.0 (C13), 28.3 (C14). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{18}H_{21}N_3O_3Na$: 350.1481, found 350.1486.

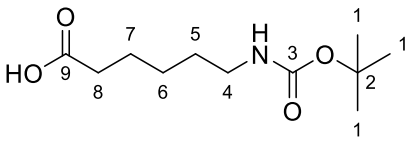


Tert-butyl (2-(4-acetamidobenzamido)phenyl)carbamate, (5): Triethylamine (0.255 mL, 1.833 mmol) was added to a solution of **10** (200 mg, 0.611 mmol) in dry THF (5 mL) at 0 °C, followed by the dropwise addition of acetyl chloride (0.052 mL, 0.733 mmol). The mixture was stirred at 0 °C for 30 minutes, then at room temperature for 2 hours. The reaction mixture was concentrated *in vacuo* to afford a white solid (440 mg). The crude product was purified by column chromatography (dry load, 100% EtOAc) to afford **5** (193 mg, 0.517 mmol, 85% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H ppm 10.24 (s, 1 H, NH), 9.74 (s, 1 H, NH), 8.67 (br s, 1 H, NH), 7.91 (d, *J*=8.7 Hz, 2 H, 5-CH), 7.73 (d, *J*=8.7 Hz, 2 H, 4-CH), 7.48 - 7.58 (m, 2 H, 10-CH,13-CH), 7.11 - 7.22 (m, 2 H, 11-CH,12-CH), 2.09 (s, 3 H, 1-CH_3), 1.45 (s, 9 H, 16-CH_3).
^{13}C NMR (101 MHz, DMSO-d_6) δ_C ppm 168.8 (C2), 164.8 (C7), 153.5 (C14), 142.5 (C3), 131.6 (C8), 129.9 (C9), 128.5 (C5), 128.3 (C10), 126.0 (C13), 125.5 (C11), 124.1 (C12), 123.9 (C10), 118.2 (C4), 79.7 (C15), 28.0 (C16), 24.1 (C1). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{20}H_{23}N_3O_4Na$: 392.1586, found 392.1586.

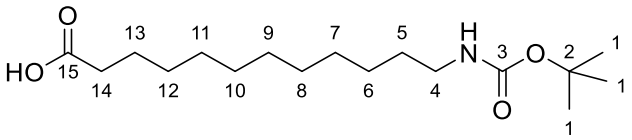


(4-acetamido-N-(2-aminophenyl)benzamide) CI-994: TFA (0.2 mL) was added to a stirring solution of **5** (47.5 mg, 0.129 mmol) in DCM (1 mL) and the resulting reaction mixture stirred at room temperature for 6 hours. The reaction mixture was concentrated *in vacuo* to afford a brown oil (51.7 mg). The crude oil was dissolved in MeOH (1 mL), agitated in MP-carbonate resin (3.02 mmol/g loading capacity, 150 mg) for 2.5 hours and then filtered. The filtrate was concentrated *in vacuo* to afford **CI-994** (35.6 mg, 0.126 mmol, 97 % yield) as a yellow/white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H ppm 10.19 (s, 1 H, NH), 9.54 (s, 1 H, NH), 7.93 (d, *J*=8.7 Hz, 2 H, 5-CH), 7.69 (d, *J*=8.7 Hz, 2 H, 4-CH), 7.15 (dd, *J*=7.7, 1.4 Hz, 1 H, 13-CH), 6.96 (app. td, *J*=7.7, 1.4 Hz, 1 H, 11-CH), 6.77 (dd, *J*=7.7, 1.4 Hz, 1 H, 10-CH), 6.59 (app. td, *J*=7.7, 1.4 Hz, 1 H, 12-CH), 4.87 (s, 2 H, 9-NH_2), 2.08 (s, 3 H, 1-CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ_C ppm 168.7 (C2), 164.7 (C7), 143.1 (C9), 142.1 (C3), 128.8 (C6), 128.7 (C5), 126.7 (C13), 126.4 (C11), 123.5 (C8), 118.0 (C4), 116.3 (C10), 116.1 (C12), 24.1 (C1). HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{15}H_{16}N_3O_2$: 270.1243, found 270.1247. Matches literature data.[2]

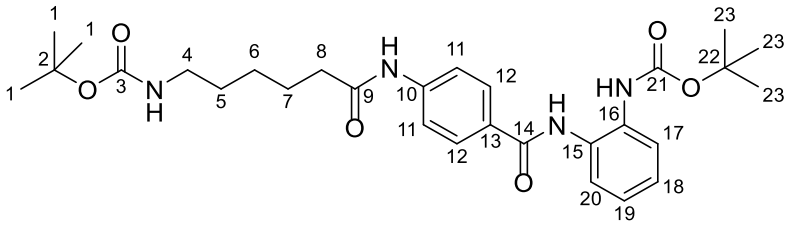




6-((Tert-butoxycarbonyl)amino)hexanoic acid, (12a): A solution of Boc_2O (3.66 g, 16.8 mmol) in 1,4-dioxane/water (2:1, 10 mL) was added slowly to a solution of **11a** (2.00 g, 15.2 mmol) and NaOH (0.61 g, 15.2 mmol) in 1,4-dioxane/water (2:1, 50 mL) at 0 °C, and then the mixture was stirred at room temperature for 18 hours. The reaction mixture was concentrated *in vacuo*, then the basic residue redissolved in water (100 mL) and washed with EtOAc (2 x 50 mL). The aqueous phase was then acidified with 1 M HCl to pH 1 and extracted with EtOAc (3 x 100 mL). The organic phases were combined, dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford **12a** (3.54 g, 15.0 mmol, 98% yield) as a colourless oil, which slowly crystallised to a white solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ_H ppm 10.95 (br s, 1 H, 9-CO_2H), 4.57 (br s, 1 H, NH), 3.03 - 3.17 (m, 2 H, 4-CH_2), 2.35 (t, *J*=7.5 Hz, 2 H, 8-CH_2), 1.65 (quin, *J*=7.5 Hz, 2 H, 7-CH_2), 1.46 - 1.54 (m, 2 H, 5-CH_2), 1.44 (s, 9 H, 1-CH_3), 1.33 - 1.41 (m, 2 H, 6-CH_2). ^{13}C NMR (101 MHz, Chloroform-*d*) δ_C ppm 179.0 (C9), 156.0 (C3), 79.2 (C2), 40.4 (C4), 33.9 (C8), 29.7 (C5), 28.4 (C1), 26.2 (C6), 24.3 (C7). MS (ESI) m/z 254 $[M+Na]^+$, 203 $[M-H]^-$.

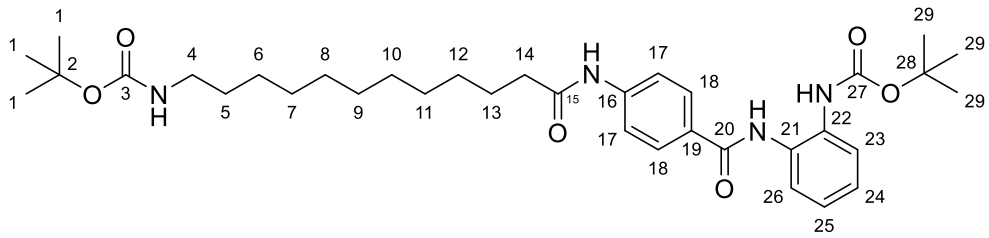


12-((Tert-butoxycarbonyl)amino)dodecanoic acid, (12b): A solution of Boc_2O (2.23 g, 10.22 mmol) in 1,4-dioxane/water (2:1, 10 mL) was added slowly to a solution of **11b** (2.00 g, 9.29 mmol) and NaOH (0.37 g, 9.29 mmol) in 1,4-dioxane/water (2:1, 50 mL) at 0 °C, and then the mixture was stirred at room temperature for 18 hours. The reaction mixture was concentrated *in vacuo*, then the basic residue redissolved in water (100 mL) and washed with EtOAc (2 x 50 mL). The aqueous phase was then acidified with 1 M HCl (ca. 15 mL) to pH 1 and extracted with EtOAc (3 x 100 mL). The organic phases were combined, dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford **12b** (2.41 g, 7.56 mmol, 81% yield) as a fluffy white solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ_H ppm 10.95 (br s, 1 H, CO_2H), 4.54 (br s, 1 H, NH), 3.02 - 3.15 (m, 2 H, 4-CH_2), 2.34 (t, *J*=7.4 Hz, 2 H, 14-CH_2), 1.63 (quin, *J*=7.4 Hz, 2 H, 13-CH_2), 1.41 - 1.50 (m, 11 H, 1-CH_3, 5-CH_2), 1.24 - 1.36 (m, 14 H, (6-12)-CH_2). ^{13}C NMR (101 MHz, Chloroform-*d*) δ_C ppm 179.2 (C15), 156.0 (C3), 79.1 (C2), 40.6 (C4), 34.0 (C14), 30.0 (C5), 29.5 (alkyl CH_2), 29.4 (alkyl CH_2), 29.3 (alkyl CH_2), 29.2 (alkyl CH_2), 29.1 (alkyl CH_2), 29.0 (alkyl CH_2), 28.4 (C1), 26.8 (alkyl CH_2), 24.7 (C13). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{17}H_{33}NO_4Na$: 338.2307, found 338.2307.

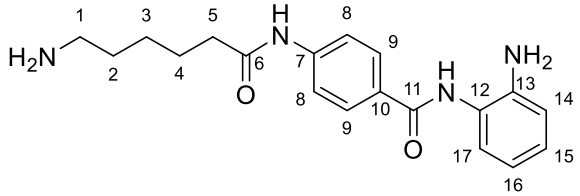


Tert-butyl (2-(4-(6-((tert-butoxycarbonyl)amino)hexanamido)benzamido)phenyl)carbamate, (13a): To a solution of **12a** (233 mg, 1.01 mmol) in dry DMF (7 mL) at 0 °C, DIPEA (0.48 mL, 2.75 mmol) and HATU (453 mg, 1.19 mmol) were added. The reaction mixture was stirred for 15 minutes, after which a solution of **10** (300 mg, 0.92 mmol) in DMF (3 mL) was added slowly and the resultant solution stirred at room temperature for 16 hours. The reaction mixture was diluted in EtOAc (20 mL), then washed with sat. $NaHCO_3$ (2 x 10 mL) and sat. brine (2 x 10 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford a crude brown oil (816 mg). The crude product was purified by column chromatography (50% EtOAc in hexane) to afford **13a** (328 mg, 0.60 mmol, 66% yield) as a yellow crystalline solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ_H ppm 9.35 (br s, 1 H, NH), 8.53 (br s, 1 H, NH), 7.88 (d, *J*=8.7 Hz, 2 H, 12-CH), 7.66 - 7.70 (m, 1 H, 20-CH), 7.63 (d, *J*=8.7 Hz, 2 H, 11-CH), 7.32 - 7.36 (m, 1 H, 17-CH), 7.29 (br s, 1 H, NH), 7.10 - 7.18 (m, 2 H, 18-CH,19-CH), 4.73 (br s, 1 H, NH), 3.06 (q, *J*=6.8 Hz, 2 H, 4-CH_2), 2.30 (br t, *J*=7.3 Hz, 2 H, 8-CH_2), 1.60 - 1.71 (m, 2 H, 7-CH_2), 1.49 (s, 9 H, 23-CH_3), 1.44 (s, 9 H, 1-CH_3), 1.39 - 1.47 (m, 2 H, 5-CH_2), 1.23-1.30 (m, 2 H, 6-CH_2). ^{13}C NMR (101 MHz, Chloroform-*d*) δ_C ppm 172.1 (C9), 165.5 (C14), 156.2 (C3), 154.6 (C21), 141.8 (C10), 130.6 (C15), 130.5 (C16), 129.0 (C13), 128.4 (C12), 126.0 (C18/19), 125.8 (C20), 125.6 (C18/19), 124.4 (C17), 119.1 (C11), 81.2 (C22), 79.2 (C2), 40.3 (C4), 37.3 (C8), 29.7 (C5), 28.4

(C1), 28.3 (C23), 26.2 (C6), 25.0 (C7). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{29}H_{40}N_4O_6Na$: 563.2846, found 563.2840.

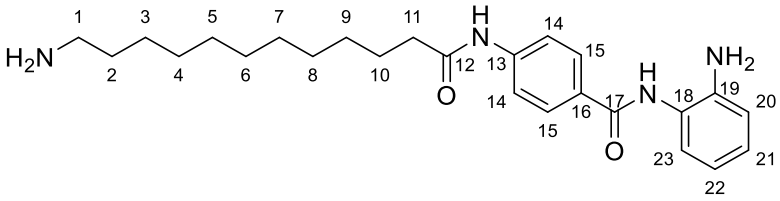


Tert-butyl (2-(4-(12-((tert-butoxycarbonyl)amino)dodecanamido)benzamido)phenyl)carbamate, (13b): To a solution of **12b** (318 mg, 1.01 mmol) in dry DMF (7 mL) at 0 °C, DIPEA (0.48 mL, 2.75 mmol) and HATU (454 mg, 1.19 mmol) were added. The reaction mixture was stirred for 15 minutes, after which a solution of **10** (300 mg, 0.92 mmol) in DMF (3 mL) was added slowly and the resultant solution stirred at room temperature for 16 hours. The reaction mixture was diluted in EtOAc (20 mL), then washed with sat. $NaHCO_3$ (2 x 10 mL) and sat. brine (2 x 10 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford a crude brown oil (0.92 g). The crude product was purified by column chromatography (50% EtOAc in hexane) to afford **13b** (374 mg, 0.59 mmol, 65% yield) as a pale yellow crystalline solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ_H ppm 9.21 (br s, 1 H, NH), 7.94 (s, 1 H, NH), 7.90 (d, *J*=8.7 Hz, 2 H, 18-CH), 7.73 (dd, *J*=7.7, 1.7 Hz, 1 H, 26-CH), 7.62 (d, *J*=8.7 Hz, 2 H, 17-CH), 7.29 (dd, *J*=7.7, 1.7 Hz, 1 H, 23-CH), 7.11 - 7.21 (m, 2 H, 24-CH,25-CH), 7.03 (s, 1 H, NH), 4.56 (br s, 1 H, NH), 3.09 (q, *J*=6.6 Hz, 2 H, 4-CH_2), 2.36 (t, *J*=7.5 Hz, 2 H, 14-CH_2), 1.67 - 1.75 (m, 2 H, 13-CH_2), 1.51 (s, 9 H, 29-CH_3), 1.41 - 1.48 (m, 11 H, 1-CH_3,5-CH_2), 1.23 - 1.34 (m, 14 H, (6-12)-CH_2). ^{13}C NMR (101 MHz, Chloroform-*d*) δ_C ppm 172.0 (C15), 165.2 (C20), 156.1 (C3), 154.6 (C27), 141.6 (C16), 130.8 (C21), 130.1 (C22), 129.2 (C19), 128.4 (C18), 125.9 (C24/25), 125.8 (C24/25), 125.7 (C26), 124.4 (C23), 119.1 (C17), 81.3 (C28), 79.1 (C2), 40.6 (C4), 37.7 (C14), 30.0 (alkyl CH_2), 29.4 (2x alkyl CH_2), 29.3 (alkyl CH_2), 29.2 (alkyl CH_2), 29.1 (2x alkyl CH_2), 28.4 (C1), 28.3 (C29), 26.7 (alkyl CH_2), 25.4 (C13). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{35}H_{52}N_4O_6Na$: 647.3785, found 647.3792.

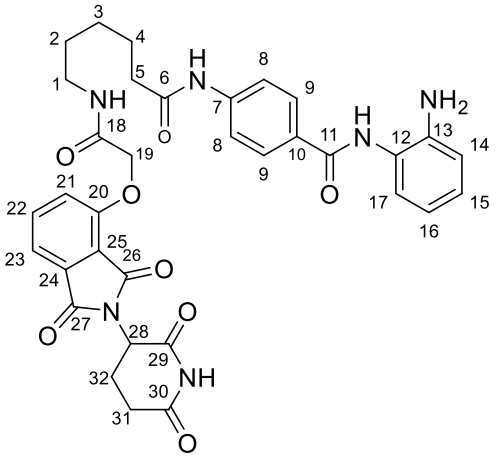


4-(6-Aminohexanamido)-N-(2-aminophenyl)benzamide, (14a): TFA (0.52 mL, 6.82 mmol) was added to a stirring solution of **13a** (185 mg, 0.341 mmol) in DCM (10 mL) and the resulting reaction mixture stirred at room temperature for 2.5 hours. The reaction mixture was concentrated *in vacuo* to afford an orange oil (280 mg). The crude oil was dissolved in MeOH (10 mL), agitated in MP-carbonate resin (3.02 mmol/g loading capacity, 0.677 g) for 2.5 hours and then filtered. The filtrate was concentrated *in vacuo* to afford **14a** (114 mg, 0.334 mmol, 97% yield) as a yellow/brown solid. ^{1}H NMR (400 MHz, Methanol-d_4) δ_H ppm 7.95 (d, *J*=8.7 Hz, 2 H, 9-CH), 7.73 (d, *J*=8.7 Hz, 2 H, 8-CH), 7.18 (dd, *J*=8.0, 1.3 Hz, 1 H, 17-CH), 7.07 (app. td, *J*=8.0, 1.3

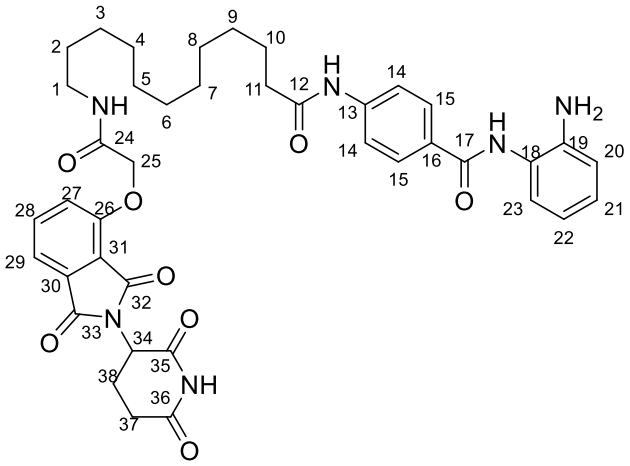
Hz, 1 H, 15-CH), 6.90 (dd, *J*=8.0, 1.3 Hz, 1 H, 14-CH), 6.76 (app. td, *J*=8.0, 1.3 Hz, 1 H, 16-CH), 2.79 (t, *J*=7.2 Hz, 2 H, 1-CH_2), 2.43 (t, *J*=7.2 Hz, 2 H, 5-CH_2), 1.73 (quin, *J*=7.2 Hz, 2 H, 4-CH_2), 1.61 (quin, *J*=7.2 Hz, 2 H, 2-CH_2), 1.44 (quin, *J*=7.2 Hz, 2 H, 3-CH_2). ^{13}C NMR (101 MHz, Methanol-*d*$_4$) δ_C ppm 174.8 (C6), 168.4 (C11), 144.0 (C13), 143.6 (C7), 130.5 (C10), 129.9 (C9), 128.7 (C17), 127.9 (C15), 125.4 (C12), 120.4 (C8), 119.8 (C16), 118.8 (C14), 41.5 (C1), 37.9 (C5), 30.7 (C2), 27.4 (C3), 26.4 (C4). HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{19}H_{25}N_4O_2$: 341.1978, found 341.1977.



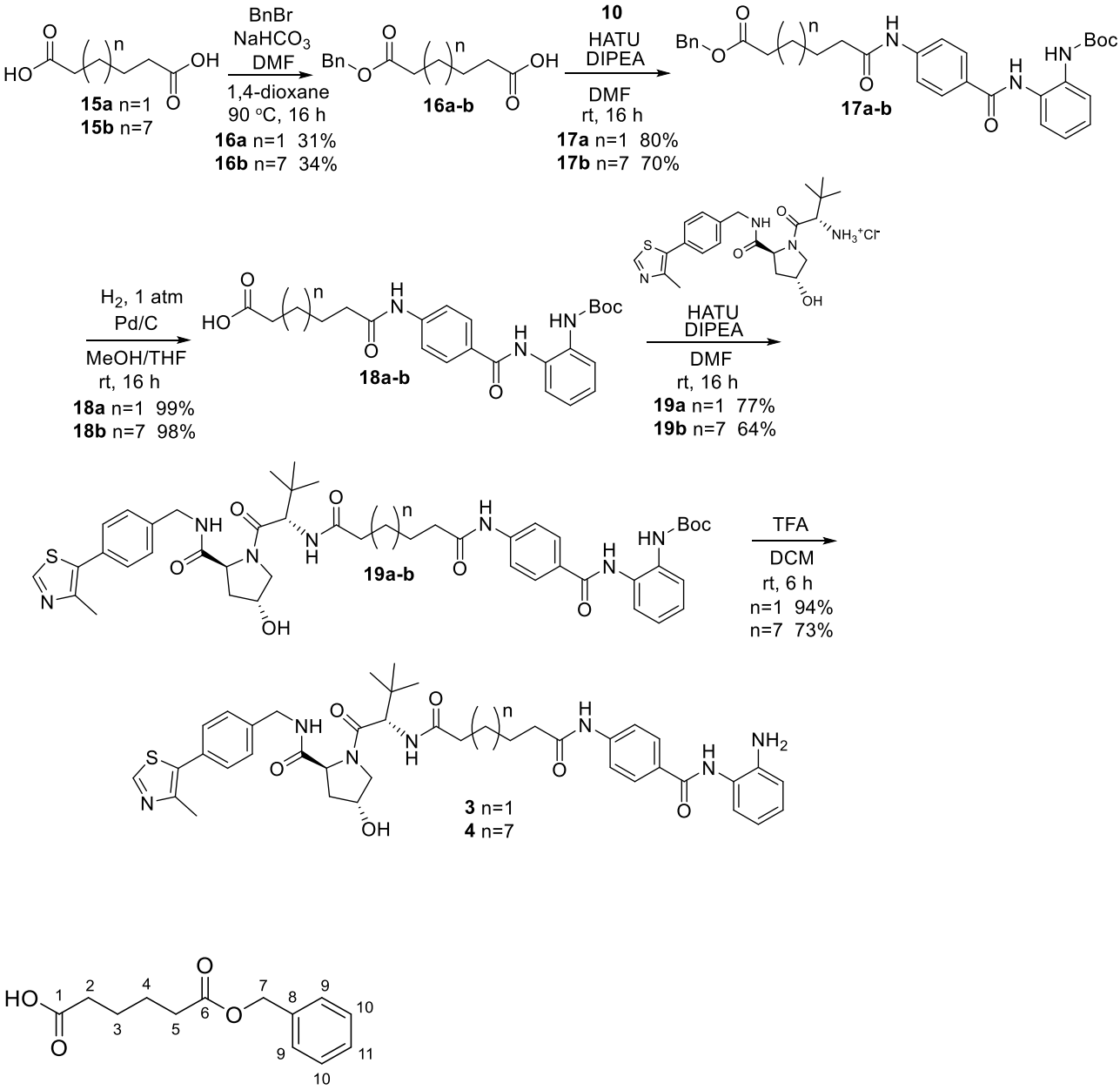
4-(12-Aminododecanamido)-N-(2-aminophenyl)benzamide, (14b): TFA (0.39 mL, 5.10 mmol) was added to a stirring solution of **13b** (159 mg, 0.255 mmol) in DCM (10 mL) and the resulting reaction mixture stirred at room temperature for 2.5 hours. The reaction mixture was concentrated *in vacuo* to afford an orange oil (322 mg). The crude oil was dissolved in MeOH (10 mL), agitated in MP-carbonate resin (3.02 mmol/g loading capacity, 0.80 g) for 2.5 hours and then filtered. The filtrate was concentrated *in vacuo* to afford **14b** (104 mg, 0.242 mmol, 95% yield) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ_H ppm 10.15 (s, 1 H, NH), 9.55 (br s, 1 H, NH), 7.94 (d, *J*=8.7 Hz, 2 H, 15-CH), 7.71 (m, *J*=8.7 Hz, 2 H, 14-CH), 7.16 (dd, *J*=7.7, 1.4 Hz, 1 H, 23-CH), 6.96 (app. td, *J*=7.7, 1.4 Hz, 1 H, 21-CH), 6.78 (dd, *J*=7.7, 1.4 Hz, 1 H, 20-CH), 6.59 (app. td, *J*=7.7, 1.4 Hz, 1 H, 22-CH), 4.87 (br s, 2 H, NH_2), 3.78 (br s, 2 H, NH_2), 2.58 (t, *J*=7.2 Hz, 2 H, 1-CH_2), 2.34 (t, *J*=7.2 Hz, 2 H, 11-CH_2), 1.60 (br t, *J*=7.2 Hz, 2 H, 10-CH_2), 1.33 - 1.42 (m, 2 H, 2-CH_2), 1.25 (br s, 14 H, (3-9)-CH_2). ^{13}C NMR (101 MHz, DMSO-*d*$_6$) δ_C ppm 171.7 (C12), 164.7 (C17), 143.1 (C19), 142.1 (C13), 128.7 (C16), 128.6 (C15), 126.6 (C23), 126.3 (C21), 123.5 (C18), 118.1 (C14), 116.3 (C22), 116.1 (C20), 40.8 (C1), 36.5 (C11), 31.3 (C2), 29.0 (alkyl CH_2), 28.95 (alkyl CH_2), 28.9 (alkyl CH_2), 28.85 (alkyl CH_2), 28.8 (alkyl CH_2), 28.7 (alkyl CH_2), 26.2 (alkyl CH_2), 25.0 (C10). HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{25}H_{37}N_4O_2$: 425.2917, found 425.2918.



***N*-(2-aminophenyl)-4-(6-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido) hexanamido)benzamide, (1):** The acetic acid functionalised thalidomide was prepared as previously reported in the literature.[3] To a solution of 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetic acid (41.5 mg, 0.125 mmol) in dry DMF (2 mL) at 0 °C, DIPEA (0.065 mL, 0.375 mmol) and HATU (57.0 mg, 0.150 mmol) were added. The reaction mixture was stirred for 15 minutes, after which a solution of **14a** (42.5 mg, 0.125 mmol) in DMF (1 mL) was added slowly and the resultant solution stirred at room temperature for 16 hours. The reaction mixture was diluted in EtOAc (10 mL), then washed with sat. $NaHCO_3$ (2 x 10 mL) and sat. brine (2 x 10 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford a pale yellow solid (45.8 mg). The crude product was purified by column chromatography (5% MeOH in DCM) to afford **1** (9.7 mg, 0.014 mmol, 11% yield) as a pale yellow solid. ^{1}H NMR (400 MHz, Methanol-d_4) δ_H ppm 7.89 (d, *J*=8.8 Hz, 2 H, 9-CH), 7.77 (dd, *J*=8.4, 7.3 Hz, 1 H, 22-CH), 7.67 (d, *J*=8.8 Hz, 2 H, 8-CH), 7.50 (dd, *J*=7.3, 0.5 Hz, 1 H, 23-CH), 7.39 (dd, *J*=8.4, 0.5 Hz, 1 H, 21-CH), 7.19 (dd, *J*=7.7, 1.5 Hz, 1 H, 17-CH), 7.07 (app. td, *J*=7.7, 1.5 Hz, 1 H, 15-CH), 6.90 (dd, *J*=7.7, 1.5 Hz, 1 H, 14-CH), 6.77 (td, *J*=7.7, 1.5 Hz, 1 H, 16-CH), 5.12 (dd, *J*=12.5, 5.5 Hz, 1 H, 28-CH), 4.72 (s, 2 H, 19-CH_2), 3.32 - 3.37 (m, 2 H, 1-CH_2), 2.80 – 2.92 (m, 1 H, 31-CH), 2.65 - 2.78 (m, 2 H, 31-CH,32-CH), 2.41 (t, *J*=7.4 Hz, 2 H, 5-CH_2), 2.08 - 2.18 (m, 1 H, 32-CH), 1.74 (quin, *J*=7.7 Hz, 2 H, 4-CH_2), 1.60 - 1.69 (m, 2 H, 2-CH_2), 1.41 - 1.52 (m, 2 H, 3-CH_2). ^{13}C NMR (101 MHz, Methanol-d_4) δ_C ppm 174.8 (C6), 174.7 (C30), 171.6 (C29), 170.0 (C18), 168.4 (C27), 168.3 (C11), 167.9 (C26), 156.3 (C20), 144.0 (C13), 143.6 (C7), 138.4 (C22), 135.0 (C24), 130.4 (C10), 129.9 (C9), 128.7 (C15), 127.9 (C17), 125.5 (C12), 121.8 (C21), 120.4 (C8), 119.8 (C16), 119.4 (C25), 118.9 (C14), 118.2 (C23), 69.5 (C19), 50.7 (C28), 40.1 (C1), 38.0 (C5), 32.3 (C31), 30.0 (C2), 27.5 (C3), 26.4 (C4), 23.8 (C32). HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{34}H_{35}N_6O_8$: 655.2516, found 655.2515.

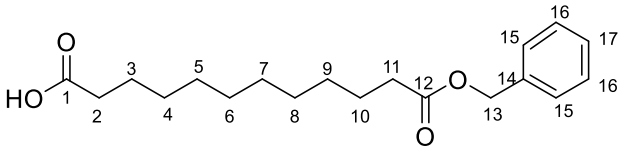


N-(2-aminophenyl)-4-(12-(2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetamido) dodecanamido)benzamide, (2): To a solution of 2-((2-(2,6-dioxopiperidin-3-yl)-1,3-dioxoisoindolin-4-yl)oxy)acetic acid (31.6 mg, 0.095 mmol) in dry DMF (2 mL) at 0 °C, DIPEA (0.050 mL, 0.285 mmol) and HATU (43.4 mg, 0.114 mmol) were added. The reaction mixture was stirred for 15 minutes, after which a solution of **14b** (40.4 mg, 0.095 mmol) in DMF (1 mL) was added slowly and the resultant solution stirred at room temperature for 18 hours. The reaction mixture was diluted in EtOAc (10 mL), then washed with sat. $NaHCO_3$ (2 x 10 mL) and sat. brine (2 x 10 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford a brown oil (74.2 mg). The crude product was purified by column chromatography (5% MeOH in DCM) to afford **2** (25.4 mg, 0.033 mmol, 34% yield) as a pale yellow solid**.** ^{1}H NMR (400 MHz, DMSO-d_6) δ_H ppm 11.11 (br s, 1 H, NH), 10.11 (s, 1 H, NH), 9.54 (s, 1 H, NH), 7.93 (d, *J*=8.8 Hz, 2 H, 15-CH), 7.91 (s, 1H, NH), 7.81 (dd, *J*=8.4, 7.2 Hz, 1 H, 28-CH), 7.70 (d, *J*=8.8 Hz, 2 H, 14-CH), 7.50 (d, *J*=7.2 Hz, 1 H, 29-CH), 7.39 (d, *J*=8.4 Hz, 1 H, 27-CH), 7.15 (dd, *J*=7.7, 1.4 Hz, 1 H, 23-CH), 6.96 (app. td, *J*=7.7, 1.4 Hz, 1 H, 21-CH), 6.78 (dd, *J*=7.7, 1.4 Hz, 1 H, 20-CH), 6.59 (app. td, *J*=7.7, 1.4 Hz, 1 H, 22-CH), 5.12 (dd, *J*=12.9, 5.4 Hz, 1 H, 34-CH), 4.87 (s, 2 H, NH_2), 4.76 (s, 2 H, 25-CH_2), 3.13 (q, *J*=6.5 Hz, 2 H, 1-CH_2), 2.85 - 2.95 (m, 1 H, 37-CH), 2.53 - 2.63 (m, 2 H, 37-CH,38-CH), 2.33 (t, *J*=7.4 Hz, 2 H, 11-CH_2), 2.00 - 2.07 (m, 1 H, 38-CH), 1.59 (quin, *J*=7.0 Hz, 2 H, 10-CH_2), 1.39 - 1.47 (m, 2 H, 2-CH_2), 1.23 - 1.31 (m, 14 H, (3-9)-CH_2). ^{13}C NMR (101 MHz, DMSO-d_6) δ_C ppm 172.8 (C36), 171.7 (C12), 169.9 (C35), 166.7 (C24), 166.6 (C33), 165.5 (C32), 164.7 (C17), 155.0 (C26), 143.1 (C19), 142.1 (C13), 136.9 (C28), 133.0 (C30), 128.7 (C16), 128.6 (C15), 126.6 (C23), 126.3 (C21), 123.5 (C18), 120.4 (C27), 118.1 (C14), 116.8 (C31), 116.3 (C22), 116.1 (C20), 116.0 (C29), 67.7 (C25), 48.8 (34), 38.3 (C1), 36.5 (C11), 31.0 (C37), 29.0 (alkyl CH_2), 28.9 (3x alkyl CH_2), 28.8 (alkyl CH_2), 28.7 (alkyl CH_2), 28.6 (alkyl CH_2), 26.3 (alkyl CH_2), 25.0 (C10), 22.0 (C38). HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{40}H_{47}N_6O_8$: 739.3455, found 739.3455.

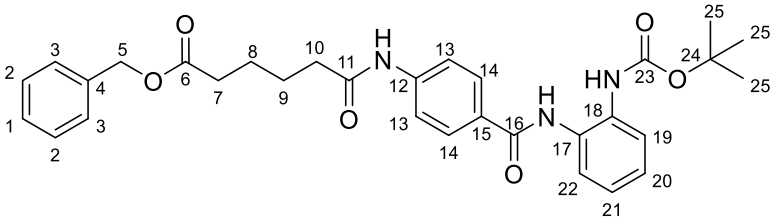


6-(benzyloxy)-6-oxohexanoic acid, (16a): To a solution of **15a** (1.00 g, 6.84 mmol) in 1,4-dioxane/DMF (1:1, 40 mL), was added BnBr (0.81 mL, 6.84 mmol), followed by the addition of $NaHCO_3$ (0.59 g, 7.02 mmol). The resulting suspension was heated at 90 °C for 16 hours. The reaction mixture was left to cool to room temperature and then concentrated *in vacuo* to afford an off-white oil. The crude residue was then suspended in EtOAc (50 mL) and washed with sat. NaCl (50 mL) and water (50 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford a cloudy white oil (0.965 g). The crude product was purified by column chromatography (50% EtOAc in hexane) to afford **16a** (0.499 g, 2.09 mmol, 31% yield) as a colourless oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ_H ppm 11.04 (br s, 1 H, CO_2H), 7.30 - 7.41 (m, 5 H, (9-11)-CH), 5.13 (s, 2 H, 7-CH_2), 2.34 - 2.42 (m, 4 H, 2-CH_2,5-CH_2), 1.64 - 1.77 (m, 4 H, 3-CH_2,4-CH_2).

^{13}C NMR (101 MHz, Chloroform-*d*) δ_C ppm 179.4 (C1), 173.2 (C6), 136.0 (C8), 128.6 (C10), 128.3 (C11), 128.2 (C9), 66.3 (C7), 33.9 (C5), 33.6 (C2), 24.3 (C3/4), 24.1 (C3/4). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{13}H_{16}O_4Na$: 259.0946, found 259.0946.

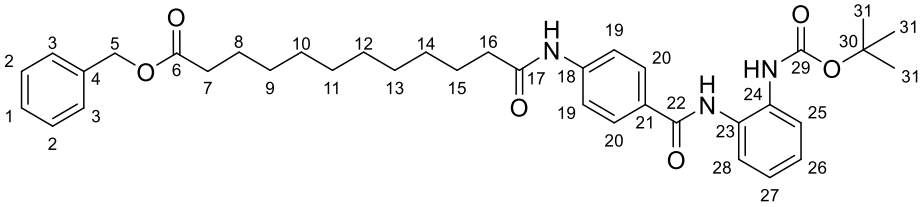


12-(benzyloxy)-12-oxododecanoic acid, (16b): To a solution of **15b** (2.00 g, 8.68 mmol) in 1,4-dioxane/DMF (1:1, 40 mL), was added BnBr (1.03 mL, 8.68 mmol), followed by the addition of $NaHCO_3$ (0.73 g, 8.68 mmol). The resulting suspension was heated at 90 °C for 16 hours. The reaction mixture was left to cool to room temperature and then concentrated *in vacuo* to afford a cloudy oil. The crude residue was then suspended in EtOAc (50 mL) and washed with sat. NaCl (50 mL) and water (50 mL). The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford an off-white solid (2.39 g). The crude product was purified by column chromatography (50% EtOAc in hexane) to afford **16b** (0.998g, 2.99 mmol, 34% yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ_H ppm 11.07 (br s, 1 H, 1-CO_2H), 7.32 - 7.40 (m, 5 H, (15-17)-CH), 5.13 (s, 2 H, 13-CH_2), 2.30 - 2.42 (m, 4 H, 2-CH_2,11-CH_2), 1.57 - 1.71 (m, 4 H, 3-CH_2,10-CH_2), 1.23 - 1.37 (m, 12 H, (4-9)-CH_2). ^{13}C NMR (101 MHz, Chloroform-*d*) δ_C ppm 179.9 (C1), 173.7 (C12), 136.1 (C14), 128.5 (C16), 128.2 (C15), 128.1 (C17), 66.1 (C13), 34.3 (C11), 34.0 (C2), 29.4 (alkyl CH_2), 29.3 (alkyl CH_2), 29.2 (2x alkyl CH_2), 29.1 (alkyl CH_2), 29.0 (alkyl CH_2), 24.9 (C10), 24.6 (C3). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{19}H_{28}O_4Na$: 343.1885, found 343.1887.

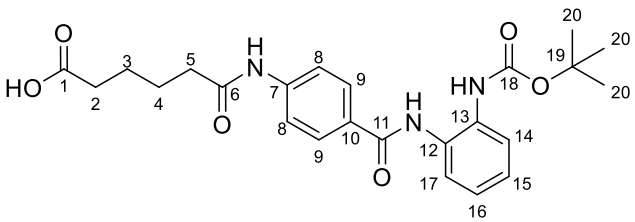


Benzyl 6-((4-((2-((tert-butoxycarbonyl)amino)phenyl)carbamoyl)phenyl)amino)-6-oxohexanoate, (17a) : To a solution of **16a** (164 mg, 0.693 mmol) in dry DMF (7 mL) at 0 °C, DIPEA (0.28 mL, 1.60 mmol) and HATU (304 mg, 0.799 mmol) were added. The reaction mixture was stirred for 15 minutes, after which a solution of **10** (174 mg, 0.533 mmol) in DMF (3 mL) was added slowly and the resultant solution stirred at room temperature for 64 hours. The reaction mixture was diluted in EtOAc (20 mL), then washed with sat. $NaHCO_3$ (2 x 10 mL) and sat. brine (2 x 10 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford a crude brown oil (510 mg). The crude product was purified by column chromatography (50% EtOAc in hexane), to give **17a** (234 mg, 0.424 mmol, 80% yield) as a pale yellow crystalline solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ_H ppm 9.24 (br s, 1 H, NH), 8.09 (s, 1 H, NH), 7.89 (d, *J*=8.8 Hz, 2 H, 14-CH), 7.71 (dd, *J*=7.7, 1.7 Hz, 1 H, 22-CH), 7.63 (d, *J*=8.8 Hz, 2 H, 13-CH), 7.32 - 7.38 (m, 5 H, (1-3)-CH), 7.27 (dd, *J*=7.7, 1.7 Hz, 1 H, 19-CH), 7.11 - 7.20 (m, 2 H, 20-CH,21-CH), 7.10 (s, 1 H, NH), 5.14 (s, 2 H, 5-CH_2), 2.40 (t, *J*=6.9 Hz, 2 H, 7-CH_2), 2.32 (t, *J*=6.9 Hz, 2 H, 10-CH_2), 1.65 - 1.75 (m, 4 H, 8-

CH_2,9-CH_2), 1.51 (s, 9 H, 25-CH_3). ^{13}C NMR (101 MHz, Chloroform-*d*) δ_C ppm 173.6 (C6), 171.3 (C11), 165.3 (C16), 154.6 (C23), 141.5 (C12), 135.8 (C4), 130.7 (C17), 130.2 (C18), 129.1 (C15), 128.6 (C2), 128.4 (C1), 128.3 (C14), 128.2 (C3), 126.0 (C21), 125.8 (C22), 125.7 (C20), 124.5 (C19), 119.1 (C13), 81.3 (C24), 66.4 (C5), 37.0 (C10), 33.8 (C7), 28.3 (C25), 24.6 (C9), 24.2 (C8). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{31}H_{35}N_3O_6Na$: 568.2424, found 568.2422.

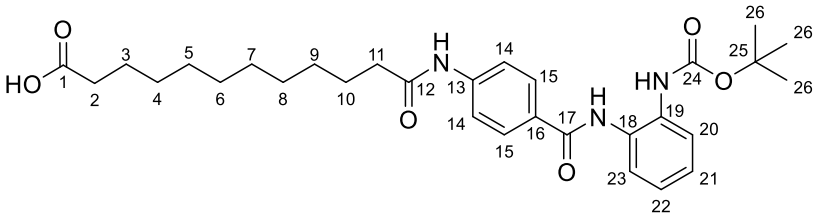


Benzyl 12-((4-((2-((tert-butoxycarbonyl)amino)phenyl)carbamoyl)phenyl)amino)-12-oxododeca-noate, (17b): To a solution of **16b** (257 mg, 0.802 mmol) in dry DMF (7 mL) at 0 °C, DIPEA (0.32 mL, 1.833 mmol) and HATU (356 mg, 0.936 mmol) were added. The reaction mixture was stirred for 15 minutes, after which a solution of **10** (200 mg, 0.611 mmol) in DMF (3 mL) was added slowly and the resultant solution stirred at room temperature for 18 hours. The reaction mixture was diluted in EtOAc (20 mL), then washed with sat. $NaHCO_3$ (2 x 10 mL) and sat. brine (2 x 10 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford a crude brown oil (632 mg). The crude product was purified by column chromatography (50% EtOAc in hexane) to give **17b** (274 mg, 0.430 mmol, 70%) as a pale yellow solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ_H ppm 9.20 (br s, 1 H, NH), 7.89 (d, *J*=8.7 Hz, 2 H, 20-CH), 7.78 (br s, 1 H, NH), 7.73 (dd, *J*=7.7, 1.6 Hz, 1 H, 28-CH), 7.60 (d, *J*=8.7 Hz, 2 H, 19-CH), 7.31 - 7.40 (m, 5 H, 1,2,3-CH), 7.28 (dd, *J*=7.7, 1.6 Hz, 1 H, 25-CH), 7.11 - 7.22 (m, 2 H, 26-CH,27-CH), 7.01 (s, 1 H, NH), 5.12 (s, 2 H, 5-CH_2), 2.30 - 2.40 (m, 4 H, 7-CH_2,16-CH_2), 1.68 - 1.74 (m, 2 H, 15-CH_2), 1.60 - 1.68 (m, 2 H, 8-CH_2), 1.51 (s, 9 H, 31-CH_3), 1.24 - 1.37 (m, 12 H, (9-14)-CH_2). ^{13}C NMR (101 MHz, Chloroform-*d*) δ_C ppm 173.8 (C6), 171.9 (C17), 165.2 (C22), 154.6 (C29), 141.5 (C18), 136.0 (C4), 130.8 (C23), 130.1 (C24), 129.2 (C21), 128.5 (C2), 128.4 (C20), 128.2 (C1), 128.1 (C3), 125.9 (C27), 125.8 (C26), 125.7 (C28), 124.5 (C25), 119.1 (C19), 81.3 (C30), 66.1 (C5), 37.7 (C16), 34.3 (C7), 29.3 (2x alkyl CH_2), 29.25 (alkyl CH_2), 29.2 (alkyl CH_2), 29.1 (alkyl CH_2), 29.0 (alkyl CH_2), 28.3 (C31), 25.4 (C15), 24.9 (C8). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{37}H_{47}N_3O_6Na$: 652.3363, found 652.3364.

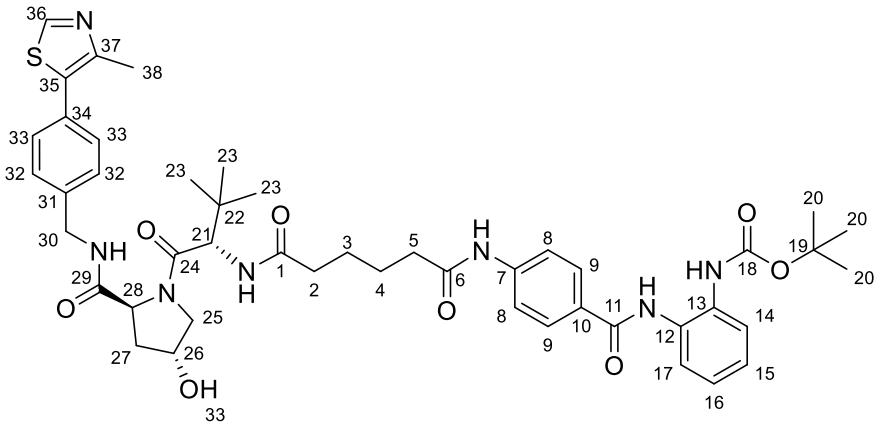


6-((4-((2-((Tert-butoxycarbonyl)amino)phenyl)carbamoyl)phenyl)amino)-6-oxohexanoic acid, (18a): To a solution of **17a** (190 mg, 0.348 mmol) in MeOH (20 mL), 10% Pd/C (20.0 mg) was added. The reaction flask was filled with nitrogen and evacuated 3 times using a Shlenk line, before a balloon of hydrogen was added and the resultant mixture stirred vigorously for 18 hours, after which the solid had crashed out as a white

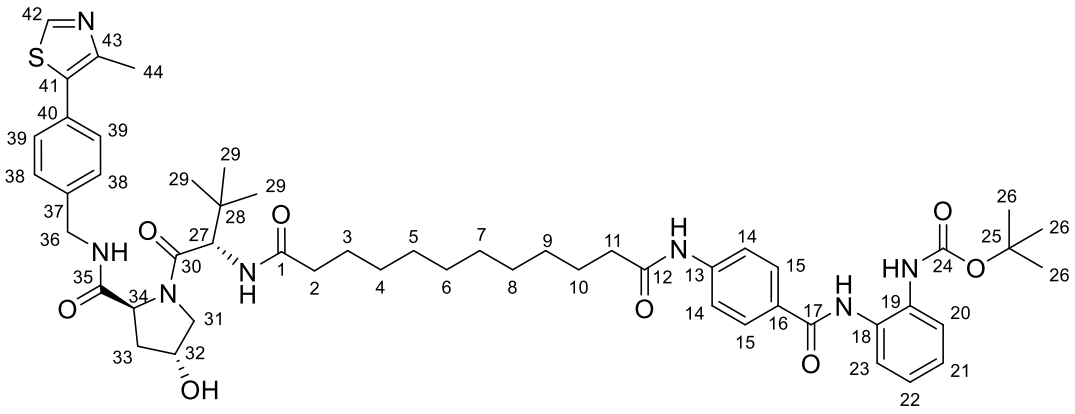
precipitate. The balloon of hydrogen was removed and the flask was flushed with nitrogen. The reaction mixture was diluted in more MeOH (20 mL) and THF (5 mL) to dissolve the precipitate, filtered through a glass microfiber filter paper, and the filtrate concentrated *in vacuo* to afford **18a** (159 mg, 0.345 mmol, 99% yield) as a white crystalline solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H ppm 11.97 (br s, 1 H, CO_2H), 10.21 (s, 1 H, NH), 9.75 (s, 1 H, NH), 8.68 (s, 1 H, NH), 7.91 (d, *J*=8.7 Hz, 2 H, 9-CH), 7.74 (d, *J*=8.7 Hz, 2 H, 8-CH), 7.48 – 7.58 (m, 2 H, 14-CH,17-CH), 7.11 - 7.22 (m, 2 H, 15-CH,16-CH), 2.36 (t, *J*=7.1 Hz, 2 H, 5-CH_2), 2.25 (t, *J*=7.1 Hz, 2 H, 2-CH_2), 1.52 - 1.68 (m, 4 H, 3-CH_2,4-CH_2), 1.45 (s, 9 H, 20-CH_3). ^{13}C NMR (101 MHz, DMSO-d_6) δ_C ppm 174.4 (C1), 171.6 (C6), 164.7 (C11), 153.5 (C18), 142.5 (C7), 131.6 (C13), 130.0 (C12), 128.5 (C9), 128.3 (C10), 126.0 (C17), 125.5 (C16), 124.2 (C15), 123.9 (C14), 118.2 (C8), 79.7 (C19), 36.2 (C5), 33.5 (C2), 28.0 (C20), 24.5 (C4), 24.2 (C3). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{24}H_{29}N_3O_6Na$: 478.1954: found 478.1955.



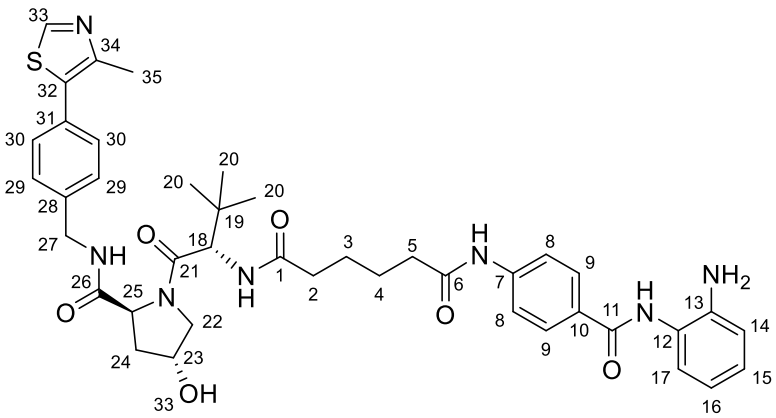
12-((4-((2-((Tert-butoxycarbonyl)amino)phenyl)carbamoyl)phenyl)amino)-12-oxododecanoic acid, (18b) : To a solution of **17b** (171 mg, 0.271 mmol) in MeOH (20 mL), 10% Pd/C (20.0 mg) was added. The reaction flask was filled with nitrogen and evacuated 3 times using a Shlenk line, before a balloon of hydrogen was added and the resultant mixture stirred vigorously for 18 hours, after which the solid had crashed out as a white precipitate. The balloon of hydrogen was removed and the flask was flushed with nitrogen. The reaction mixture was diluted in more MeOH (20 mL) and THF (5 mL) to dissolve the precipitate, filtered through a glass microfiber filter paper, and the filtrate concentrated *in vacuo* to afford **18b** (146 mg, 0.266 mmol, 98% yield) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H ppm 11.96 (br s, 1 H, CO_2H), 10.17 (s, 1 H, NH), 9.74 (s, 1 H, NH), 8.68 (br s, 1 H, NH), 7.91 (d, *J*=8.5 Hz, 2 H, 15-CH), 7.74 (d, *J*=8.5 Hz, 2 H, 14-CH), 7.48 - 7.59 (m, 2 H, 20-CH,23-CH), 7.09 - 7.24 (m, 2 H, 21-CH,22-CH), 2.34 (t, *J*=7.3 Hz, 2 H, 11-CH_2), 2.18 (t, *J*=7.3 Hz, 2 H, 2-CH_2), 1.55 - 1.65 (m, 2 H, 10-CH_2), 1.46 - 1.51 (m, 2 H, 3-CH_2), 1.45 (s, 9 H, 26-CH_3), 1.23 - 1.30 (m, 12 H, (4-9)-CH_2). ^{13}C NMR (101 MHz, DMSO-d_6) δ_C ppm 174.5 (C1), 171.8 (C12), 164.7 (C17), 153.5 (C24), 142.6 (C13), 131.6 (C19), 130.0 (C18), 128.5 (C15), 128.2 (C16), 126.0 (C23), 125.4 (C22), 124.1 (C21), 123.9 (C20), 118.2 (C14), 79.7 (C25), 36.5 (C11), 33.7 (C2), 28.9 (2x alkyl CH_2), 28.8 (alkyl CH_2), 28.7 (alkyl CH_2), 28.6 (alkyl CH_2), 28.5 (alkyl CH_2), 28.0 (C26), 25.0 (C10), 24.5 (C3). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{30}H_{41}N_3O_6Na$: 562.2893, found 562.2886.



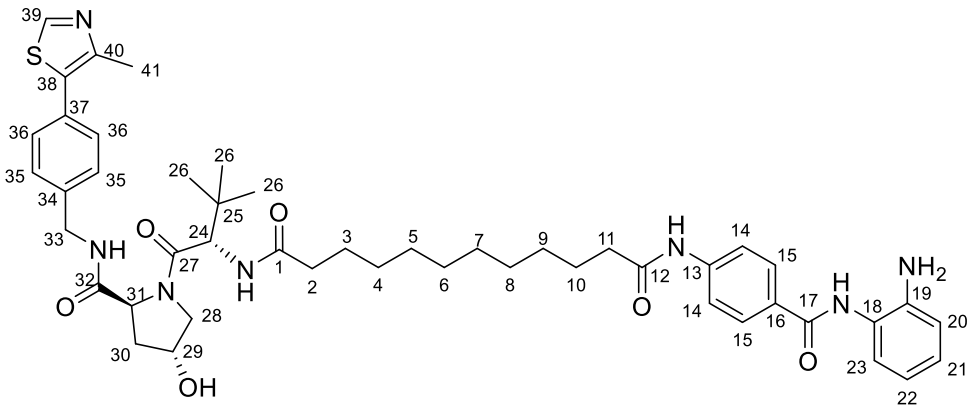
Tert-butyl(2-(4-(6-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-6-oxohexanamido)benzamido)phenyl)carbamate, (19a): To a solution of **18a** (54.3 mg, 0.119 mmol) in dry DMF (1 mL) at 0 °C, DIPEA (0.05 mL, 0.298 mmol) and HATU (45.4 mg, 0.119 mmol) were added. The reaction mixture was stirred for 15 minutes, after which a solution of (4R)-3-Methyl-L-valyl-4-hydroxy-N-[[4-(4-methyl-5-thiazolyl)phenyl]methyl]-L-prolinamide hydrochloride (50.0 mg, 0.099 mmol) in DMF (1 mL) was added slowly and the resultant solution stirred at room temperature for 16 hours. The reaction mixture was diluted in EtOAc (10 mL), then washed with sat. $NaHCO_3$ (2 x 5 mL) and sat. brine (2 x 5 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford a pale-yellow tar (125 mg). The crude product was purified by column chromatography (0-5% MeOH in DCM) to afford **19a** (70.0 mg, 0.077 mmol, 77% yield) as a white solid. ^{1}H NMR (400 MHz, Methanol-*d*$_4$) δ_H ppm 8.86 (s, 1 H, 36-CH), 7.92 (d, *J*=8.9 Hz, 2 H, 9-CH), 7.73 (d, *J*=8.9 Hz, 2 H, 8-CH), 7.56 - 7.62 (m, 1 H, 17-CH), 7.43 - 7.46 (m, 2 H, 33-CH), 7.41 - 7.43 (m, 1 H, 14-CH), 7.38 - 7.41 (m, 2 H, 32-CH), 7.16 - 7.26 (m, 2 H, 15-CH,16-CH), 4.61 - 4.66 (m, 1 H, 21-CH), 4.54 - 4.60 (m, 1 H, 28-CH), 4.47 - 4.54 (m, 2 H, 26-CH,30-CH), 4.32 - 4.38 (m, 1 H, 30-CH), 3.86 - 3.97 (m, 1 H, 25-CH), 3.77 - 3.84 (m, 1 H, 25-CH), 2.46 (s, 3 H, 38-CH_3), 2.42 (t, *J*=7.1 Hz, 2 H, 5-CH_2), 2.30 - 2.37 (m, 2 H, 2-CH_2), 2.17 - 2.26 (m, 1 H, 27-CH), 2.04 - 2.12 (m, 1 H, 27-CH), 1.66 - 1.77 (m, 4 H, 3-CH_2,4-CH_2), 1.48 (s, 9 H, 20-CH_3), 1.04 (s, 9 H, 23-CH_3). ^{13}C NMR (101 MHz, Methanol-*d*$_4$) δ_C ppm 175.8 (C1), 174.6 (C6), 174.5 (C29), 172.4 (C24), 167.9 (C11), 156.4 (C18), 153.0 (C36), 149.2 (C37), 143.9 (C7), 140.4 (C31), 133.6 (C35), 133.2 (C13), 131.8 (C12), 131.6 (C34), 130.6 (C10), 130.5 (C32), 129.7 (C9), 129.1 (C33), 127.5 (C15/16), 127.3 (C17), 126.4 (C15/16), 125.7 (C14), 120.5 (C8), 81.9 (C19), 71.2 (C26), 61.0 (C28), 59.2 (C21), 58.2 (C25), 43.8 (C30), 39.1 (C27), 37.9 (C5), 36.7 (C2), 36.5 (C22), 28.8 (C20), 27.2 (C23), 26.7 (C3), 26.5 (C4), 16.0 (C38). HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{46}H_{57}N_7O_8SNa$: 890.3887, found 890.3884.



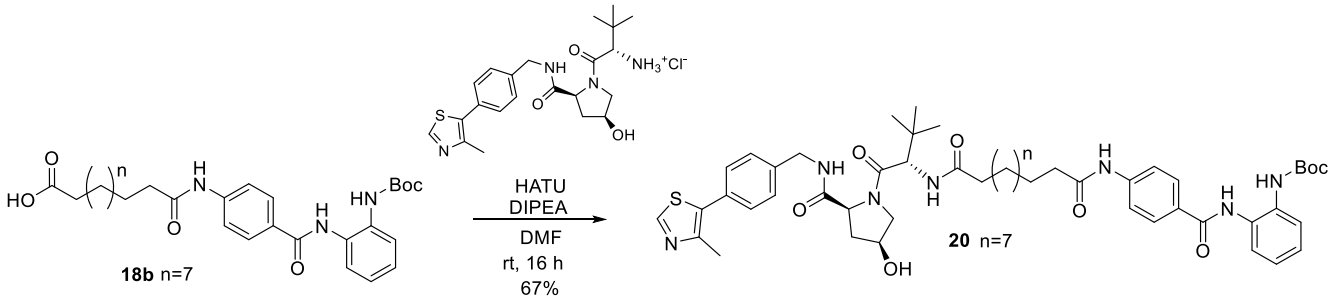
Tert-butyl(2-(4-(12-(((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrr olidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-12-oxododecanamido)benzamido)phenyl)carbamate, (19b): To a solution of **18b** (65.5 mg, 0.121 mmol) in dry DMF (1 mL) at 0 °C, DIPEA (0.05 mL, 0.298 mmol) and HATU (48.0 mg, 0.126 mmol) were added. The reaction mixture was stirred for 15 minutes, after which a solution of (4R)-3-Methyl-L-valyl-4-hydroxy-N-[[4-(4-methyl-5-thiazolyl)phenyl]methyl]-L-prolinamide hydrochloride (50.0 mg, 0.099 mmol) in DMF (1 mL) was added slowly and the resultant solution stirred at room temperature for 16 hours. The reaction mixture was diluted in EtOAc (10 mL), then washed with sat. $NaHCO_3$ (2 x 5 mL) and sat. brine (2 x 5 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford a dark yellow tar (147 mg). The crude product was purified by column chromatography (0-5% MeOH in DCM) to afford **19b** (61.9 mg, 0.064 mmol, 64% yield) as a white solid. ^{1}H NMR (400 MHz, Methanol-*d*$_4$) δ_H ppm 8.86 (s, 1 H, 42-CH), 7.93 (d, *J*=8.8 Hz, 2 H, 15-CH), 7.73 (d, *J*=8.8 Hz, 2 H, 14-CH), 7.56 - 7.63 (m, 1 H, 23-CH), 7.44 - 7.47 (m, 2 H, 39-CH), 7.42 (m, 1 H, 20-CH), 7.38 - 7.41 (m, 2 H, 38-CH_2), 7.15 - 7.27 (m, 2 H, 21-CH,22-CH), 4.61 - 4.66 (m, 1 H, 27-CH), 4.55 - 4.60 (m, 1 H, 34-CH), 4.50 - 4.55 (m, 1 H, 36-CH), 4.47 - 4.50 (m, 1 H, 32-CH), 4.30 - 4.39 (m, 1 H, 36-CH), 3.84 - 3.94 (m, 1 H, 31-CH), 3.76 - 3.82 (m, 1 H, 31-CH), 2.46 (s, 3 H, 44-CH_3), 2.39 (t, *J*=7.5 Hz, 2 H, 11-CH_2), 2.17 - 2.32 (m, 3 H, 2-CH_2,33-CH), 2.03 - 2.11 (m, 1 H, 33-CH), 1.65 - 1.75 (m, 2 H, 3-CH_2), 1.59 (m, 2 H, 10-CH_2), 1.49 (s, 9 H, 26-CH_3), 1.29 - 1.37 (m, 12 H, (4-9)-CH_2), 1.03 (s, 9 H, 29-CH_3). ^{13}C NMR (101 MHz, Methanol-*d*$_4$) δ_C ppm 176.2 (C1), 175.1 (C12), 174.6 (C35), 172.5 (C30), 167.9 (C17), 156.4 (C24), 153.0 (C42), 149.1 (C43), 143.9 (C13), 140.4 (C37), 133.6 (C41), 133.2 (C19), 131.8 (C18), 131.6 (C40), 130.6 (C16), 130.5 (C38), 129.7 (C15), 129.1 (C39), 127.5 (C21/22), 127.3 (C23), 126.4 (C21/22), 125.7 (C20), 120.4 (C14), 81.9 (C25), 71.2 (C32), 61.0 (C34), 59.1 (C27), 58.2 (C31), 43.8 (C36), 39.0 (C33), 38.2 (C11), 36.8 (C2), 36.7 (C28), 30.7 (alkyl CH_2), 30.6 (alkyl CH_2), 30.55 (alkyl CH_2), 30.5 (alkyl CH_2), 30.4 (2x alkyl CH_2), 28.8 (C26), 27.2 (C29), 27.1 (C10), 26.9 (C3), 16.0 (C44). HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{52}H_{70}N_7O_8S$: 952.5007, found 952.5009.

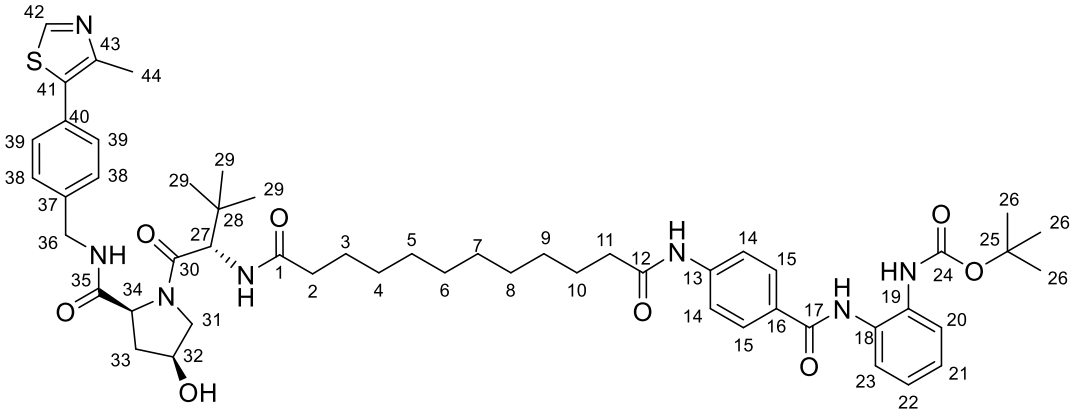


N-1-(4-((2-aminophenyl)carbamoyl)phenyl)-N6-((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)adipamide, (3): TFA (0.2 mL) was added to a stirring solution of **19a** (25.3 mg, 0.029 mmol) in DCM (1 mL) and the resulting reaction mixture stirred at room temperature overnight. The reaction mixture was concentrated *in vacuo* to afford an orange oil. The crude oil was dissolved in MeOH (2 mL), agitated in MP-carbonate resin (3.02 mmol/g loading capacity, 200 mg) for 2.5 hours and then filtered. The filtrate was concentrated *in vacuo* to afford **3** (21.0 mg, 0.027 mmol, 94% yield) as a pale yellow solid. Prior to biological evaluation the product was further purified by semi-preparative HPLC (5-95% MeCN in H_2O, 260 nm, 45 min gradient). ^{1}H NMR (400 MHz, Methanol-d_4) δ_H ppm 8.87 (s, 1 H, 33-CH), 7.95 (d, *J*=8.7 Hz, 2 H, 9-CH), 7.73 (d, *J*=8.7 Hz, 2 H, 8-CH), 7.43 - 7.48 (m, 2 H, 30-CH), 7.39 - 7.43 (m, 2 H, 29-CH), 7.18 (dd, *J*=8.0, 1.5 Hz, 1 H, 17-CH), 7.08 (app. td, *J*=8.0, 1.5 Hz, 1 H, 15-CH), 6.90 (dd, *J*=8.0, 1.4 Hz, 1 H, 14-CH), 6.77 (app. td, *J*=8.0, 1.4 Hz, 1 H, 16-CH), 4.63 (s, 1 H, 18-CH), 4.53 - 4.59 (m, 1 H, 25-CH), 4.43 - 4.52 (m, 2 H, 23-CH,27-CH), 4.33 - 4.39 (m, 1 H, 27-CH), 3.89 - 3.95 (m, 1 H, 22-CH), 3.77 - 3.84 (m, 1 H, 22-CH), 2.47 (s, 3 H, 35-CH_3), 2.43 (br t, *J*=6.9 Hz, 2 H, 5-CH_2), 2.30 - 2.37 (m, 2 H, 2-CH_2), 2.18 - 2.25 (m, 1 H, 24-CH), 2.05 - 2.12 (m, 1 H, 24-CH), 1.67 - 1.77 (m, 4 H, 3-CH_2,4-CH_2), 1.04 (s, 9 H, 20-CH_3). ^{13}C NMR (101 MHz, Methanol-d_4) δ_C ppm 175.8 (C1), 174.7 (C6), 174.6 (C26), 172.5 (C21), 168.4 (C11), 153.0 (C33), 149.2 (C34), 143.9 (C13), 143.6 (C7), 140.4 (C28), 133.6 (C32), 131.7 (C31), 130.6 (C10), 130.5 (C29), 129.9 (C9), 129.1 (C30), 128.6 (C15), 127.8 (C17), 125.6 (C12), 120.5 (C8), 119.8 (C16), 118.9 (C14), 71.2 (C23), 61.0 (C25), 59.2 (C18), 58.2 (C22), 43.9 (C27), 39.1 (C24), 37.9 (C5), 36.7 (C19), 36.5 (C2), 27.2 (C20), 26.7 (C3), 26.5 (C4), 16.0 (C35). HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{41}H_{50}N_7O_6S$: 768.3543, found 768.3543.



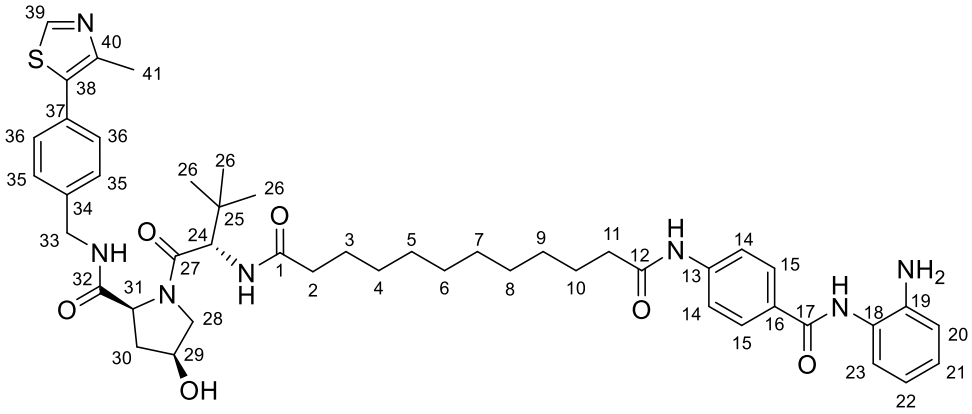
N1-(4-((2-aminophenyl)carbamoyl)phenyl)-N12-((S)-1-((2S,4R)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)dodecanediamide, (4): TFA (0.2 mL) was added to a stirring solution of **19b** (37.6 mg, 0.0395 mmol) in DCM (2 mL) and the resulting reaction mixture stirred at room temperature for 7 hours. The reaction mixture was concentrated *in vacuo* to afford an orange oil (54 mg). The crude oil was dissolved in MeOH (2 mL), agitated in MP-carbonate resin (3.02 mmol/g loading capacity, 210 mg) for 2.5 hours and then filtered. The filtrate was concentrated *in vacuo* to afford **4** (25.8 mg, 0.0288 mmol, 73% yield) as a pale yellow solid. Prior to biological evaluation the product was further purified by semi-preparative HPLC (5-95% MeCN in H_2O, 260 nm, 45 min gradient). ^{1}H NMR (400 MHz, Methanol-*d*$_4$) δ_H ppm 8.86 (s, 1 H, 39-CH), 7.95 (d, *J*=8.8 Hz, 2 H, 15-CH), 7.72 (d, *J*=8.8 Hz, 2 H, 14-CH), 7.43 - 7.48 (m, 2 H, 36-CH), 7.38 - 7.42 (m, 2 H, 35-CH), 7.18 (dd, *J*=7.8, 1.3 Hz, 1 H, 23-CH), 7.07 (app. td, *J*=7.8, 1.3 Hz, 1 H, 21-CH), 6.90 (dd, *J*=7.8, 1.3 Hz, 1 H, 20-CH), 6.76 (app. td, *J*=7.8, 1.3 Hz, 1 H, 22-CH), 4.60 - 4.66 (m, 1 H, 24-CH), 4.55 - 4.60 (m, 1 H, 31-CH), 4.50 - 4.55 (m, 1 H, 33-CH), 4.47 - 4.50 (m, 1 H, 29-CH), 4.31 - 4.39 (m, 1 H, 33-CH), 3.86 - 3.93 (m, 1 H, 28-CH), 3.76 - 3.83 (m, 1 H, 28-CH), 2.47 (s, 3 H, 41-CH_3), 2.40 (t, *J*=7.5 Hz, 2 H, 11-CH_2), 2.18 - 2.33 (m, 3 H, 2-CH_2,30-CH), 2.03 - 2.12 (m, 1 H, 30-CH), 1.70 (quin, *J*=7.2 Hz, 2 H, 3-CH_2), 1.53 - 1.64 (m, 2 H, 10-CH_2), 1.28 - 1.41 (m, 12 H, (4-9)-CH_2), 1.03 (s, 9 H, 26-CH_3). ^{13}C NMR (101 MHz, Methanol-*d*$_4$) δ_C ppm 176.2 (C1), 175.1 (C12), 174.6 (C32), 172.5 (C27), 168.4 (C17), 153.0 (C39), 149.2 (C40), 144.0 (C19), 143.7 (C13), 140.4 (C34), 133.6 (C38), 131.7 (C37), 130.6 (C16), 130.5 (C35), 129.9 (C15), 129.1 (C36), 128.6 (C21), 127.8 (C23), 125.6 (C18), 120.4 (C14), 119.8 (C22), 118.9 (C20), 71.2 (C29), 61.0 (C31), 59.1 (C24), 58.2 (C28), 43.8 (C33), 39.1 (C30), 38.2 (C11), 36.8 (C25), 36.7 (C2), 30.7 (alkyl CH_2), 30.6 (alkyl CH_2), 30.55 (alkyl CH_2), 30.5 (alkyl CH_2), 30.4 (2x alkyl CH_2), 27.2 (C26), 27.1 (C10), 26.9 (C3), 16.0 (C41). HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{47}H_{62}N_7O_6S$: 852.4476, found 852.4482.





Tert-butyl (2-(4-(12-(((S)-1-((2S,4S)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrroli din-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)amino)-12-oxododecanamido)benzamido)phenyl)carbamate, (20): To a solution of **18b** (51.4 mg, 0.095 mmol) in dry DMF (1 mL) at 0 °C, DIPEA (0.04 mL, 0.238 mmol) and HATU (39.3 mg, 0.103 mmol) were added. The reaction mixture was stirred for 15 minutes, after which a solution of (2S,4S)-1-[(2S)-2-Amino-3,3-dimethyl-butanoyl]-4-hydroxy-N-[[4-(4-methylthiazol-5-yl)phenyl]methyl]pyrrolidine-2-carboxamide dihydrochloride (40.0 mg, 0.079 mmol) in DMF (1 mL) was added slowly and the resultant solution stirred at room temperature for 16 hours. The reaction mixture was diluted in EtOAc (10 mL), then washed with sat. $NaHCO_3$ (2 x 5 mL) and sat. brine (2 x 5 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford a dark yellow tar (104 mg). The crude product was purified by column chromatography (0-5% MeOH in DCM) to afford **20** (50.8 mg, 0.053 mmol, 67% yield) as a white solid. ^{1}H NMR (400 MHz, Methanol-d_4) δ_H ppm 8.86 (s, 1 H, 42-CH), 7.93 (d, *J*=8.8 Hz, 2 H, 15-CH), 7.83 (d, *J*=8.3 Hz, 1 H, NH), 7.73 (d, *J*=8.8 Hz, 2 H, 14-CH), 7.56 - 7.63 (m, 1 H, 23-CH), 7.41 - 7.47 (m, 3 H, 20-CH, 39-CH_2), 7.38 - 7.41 (m, 2 H, 38-CH), 7.17 - 7.26 (m, 2 H, 21-CH,22-CH), 4.47 - 4.56 (m, 3 H, 27-CH,34-CH,36-CH), 4.32 - 4.41 (m, 2 H, 32-CH,36-CH), 4.03 (dd, *J*=10.6, 5.1 Hz, 1

H, 31-CH), 3.66 - 3.75 (m, 1 H, 31-CH), 2.46 (s, 3 H, 44-CH_3), 2.42 - 2.45 (m, 1 H, 33-CH), 2.39 (t, *J*=7.5 Hz, 2 H, 11-CH_2), 2.18 - 2.33 (m, 2 H, 2-CH_2), 1.93 - 2.02 (m, 1 H, 33-CH), 1.70 (quin, *J*=7.3 Hz, 2 H, 3-CH_2), 1.54 - 1.63 (m, 2 H, 10-CH_2), 1.49 (s, 9 H, 26-CH_3), 1.34 - 1.38 (m, 4 H, 4-CH_2,9-CH_2), 1.27 - 1.33 (m, 8 H, (5-8)-CH_2), 1.03 (s, 9 H, 29-CH_3). ^{13}C NMR (101 MHz, Methanol-*d*$_4$) δ_C ppm 176.6 (C1), 175.1 (C12), 175.0 (C35), 172.8 (C30), 167.9 (C17), 156.4 (C24), 153.0 (C42), 149.2 (C43), 143.9 (C13), 140.1 (C37), 133.5 (C41), 133.2 (C19), 131.8 (C18), 131.7 (C40), 130.5 (C38), 130.3 (C16), 129.7 (C15), 129.2 (C39), 127.5 (C21/22), 127.3 (C23), 126.4 (C21/22), 125.7 (C20), 120.4 (C14), 81.9 (C25), 71.6 (C32), 61.1 (C34), 59.4 (C27), 57.8 (C31), 44.0 (C36), 38.2 (C11), 38.0 (C33), 36.6 (C2), 36.1 (C28), 30.7 (alkyl CH_2), 30.6 (alkyl CH_2), 30.55 (alkyl CH_2), 30.5 (alkyl CH_2), 30.4 (2x alkyl CH_2), 28.8 (C26), 27.2 (C29), 27.1 (C10), 26.9 (C3), 16.0 (C44). HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{52}H_{70}N_7O_8S$: 952.5007, found 952.4999.



N1-(4-((2-aminophenyl)carbamoyl)phenyl)-N12-((S)-1-((2S,4S)-4-hydroxy-2-((4-(4-methylthiazol-5-yl)benzyl)carbamoyl)pyrrolidin-1-yl)-3,3-dimethyl-1-oxobutan-2-yl)dodecanediamide, (6): TFA (0.4 mL) was added to a stirring solution of **20** (29.0 mg, 0.030 mmol) in DCM (2 mL) and the resulting reaction mixture stirred at room temperature for 2.5 hours. The reaction mixture was concentrated *in vacuo* to afford an orange oil (33 mg). The crude oil was dissolved in MeOH (2 mL), agitated in MP-carbonate resin (3.02 mmol/g loading capacity, 100 mg) for 2.5 hours and then filtered. The filtrate was concentrated *in vacuo* to afford **6** (25.6 mg, 0.029 mmol, 98% yield) as a pale yellow solid. Prior to biological evaluation the product was further purified by semi-preparative HPLC (5-95% MeCN in H_2O, 260 nm, 45 min gradient). ^{1}H NMR (400 MHz, Methanol-*d*$_4$) δ_H ppm 8.86 (s, 1 H, 39-CH) 7.95 (d, *J*=8.7 Hz, 2 H, 15-CH) 7.72 (d, *J*=8.7 Hz, 2 H, 14-CH) 7.44 (d, *J*=8.5 Hz, 2 H, 36-CH) 7.40 (d, *J*=8.5 Hz, 2 H, 35-CH) 7.18 (dd, *J*=7.8, 1.3 Hz, 1 H, 23-CH) 7.07 (app. td, *J*=7.8, 1.3 Hz, 1 H, 21-CH) 6.90 (dd, *J*=7.8, 1.3 Hz, 1 H, 20-CH) 6.76 (app. td, *J*=7.8, 1.3 Hz, 1 H, 22-CH), 4.47 - 4.58 (m, 3 H, 24-CH,31-CH,33-CH) 4.32 - 4.40 (m, 2 H, 29-CH,33-CH) 4.03 (dd, *J*=10.5, 5.1 Hz, 1 H, 28-CH) 3.69 (dd, *J*=10.5, 3.5 Hz, 1 H, 28-CH) 2.47 (s, 3 H, 41-CH_3) 2.42 - 2.45 (m, 1 H, 30-CH) 2.40 (t, *J*=7.4 Hz, 2 H, 11-CH_2) 2.18 - 2.32 (m, 2 H, 2-CH_2) 1.97 (dt, *J*=13.3, 4.3 Hz, 1 H, 30-CH) 1.70 (quin, *J*=7.3 Hz, 2 H, 3-CH_2) 1.53 - 1.63 (m, 2 H, 10-CH_2) 1.33 - 1.39 (m, 4 H, 4-CH_2,9-CH_2) 1.29 - 1.33 (m, 8 H, (5-8)-CH_2) 1.03 (s, 9 H, 26-CH_3). ^{13}C NMR (101 MHz, Methanol-*d*$_4$) δ_C ppm 176.5 (C1), 175.1 (C12), 175.0 (C32), 172.8 (C27), 168.4 (C17), 153.0 (C39), 149.2 (C40), 143.9 (C19), 143.7 (C13), 140.2 (C34), 133.5 (C38), 131.7 (C37), 130.6 (C16), 130.5 (C35), 129.9 (C15), 129.2 (C36), 128.6 (C21), 127.8 (C23), 125.6 (C18), 120.4 (C14), 119.8 (C22), 118.9 (C20), 71.6 (C29), 61.1 (C31), 59.4 (C24), 57.8 (C28), 44.0 (C33),

38.2 (C11), 38.0 (C30), 36.6 (C2), 36.1 (C25), 30.7 (alkyl CH_2), 30.6 (alkyl CH_2), 30.55 (alkyl CH_2), 30.5 (alkyl CH_2), 30.4 (2x alkyl CH_2), 27.2 (C26), 27.1 (C10), 26.9 (C3), 16.0 (C41). HRMS (ESI) m/z: $[M+H]^+$ calculated for $C_{47}H_{62}N_7O_6S$: 852.4482, found 852.4483.

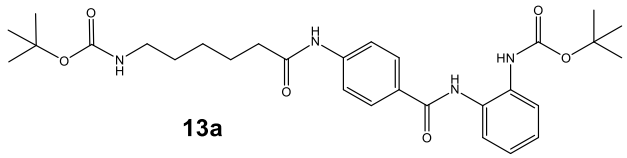
References

1 Active Motif Inc., HDAC Assay Kit (Fluorescent), 2013, viewed 4th February 2020, https://www.activemotif.jp/documents/ 1524.pdf.

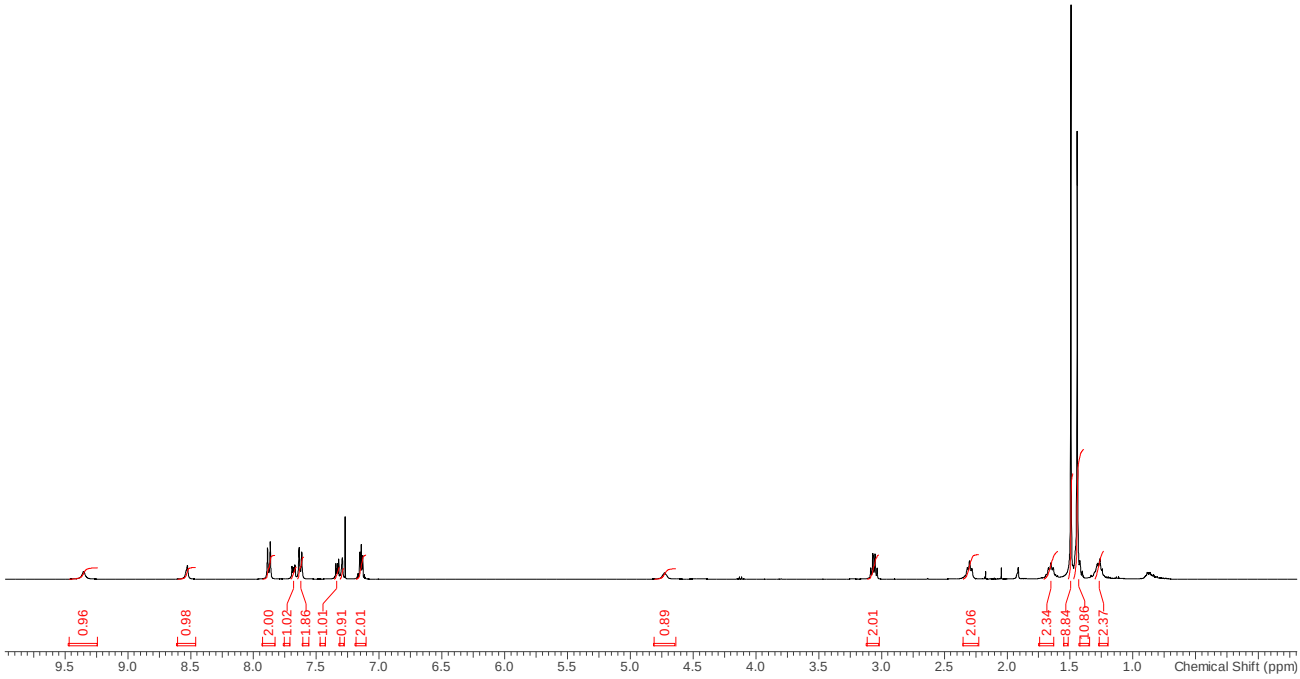
2 E. Holson, F. F. Wagner and G. P. Stahly, *U. S. Patent*, US20130102677A1, 2013.

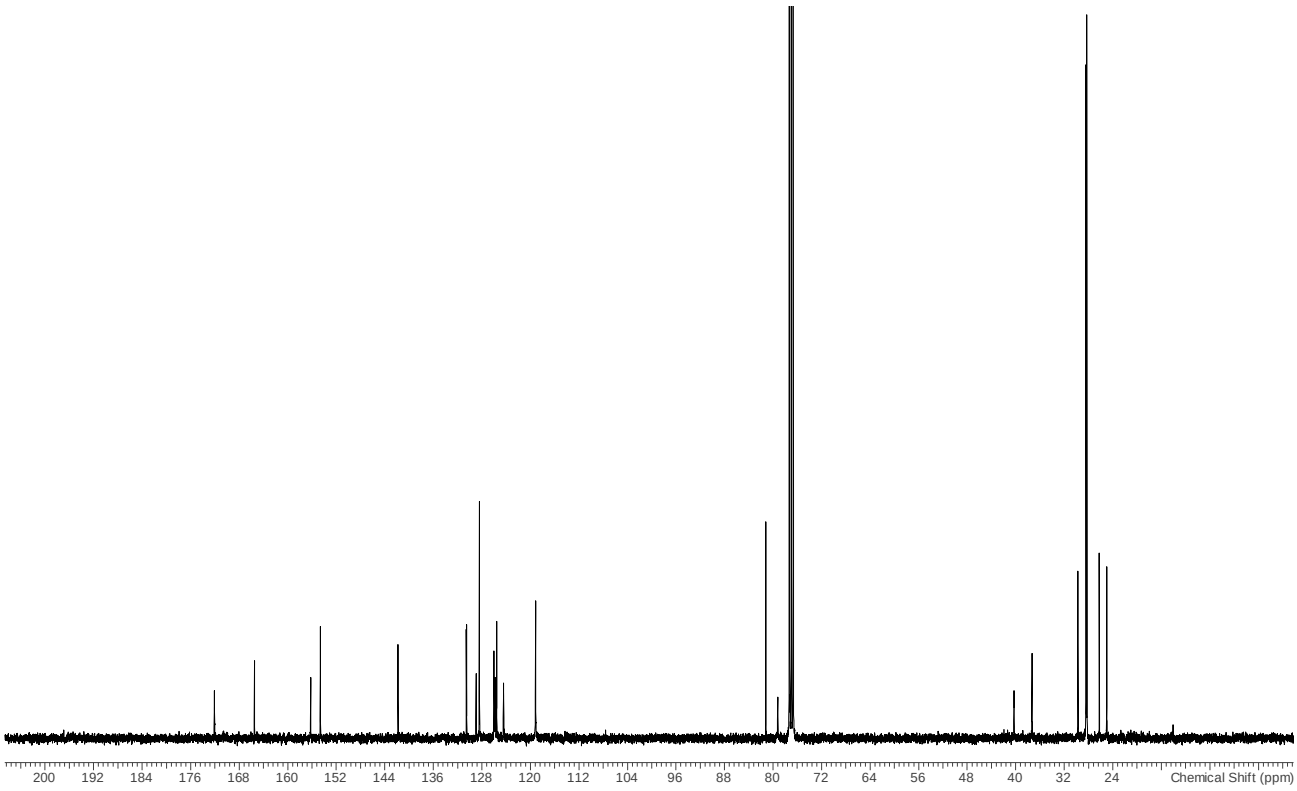
3 J. Lohbeck and A. K. Miller, *Bioorganic & Medicinal Chemistry Letters*, 2016, **26**, 5260-5262.

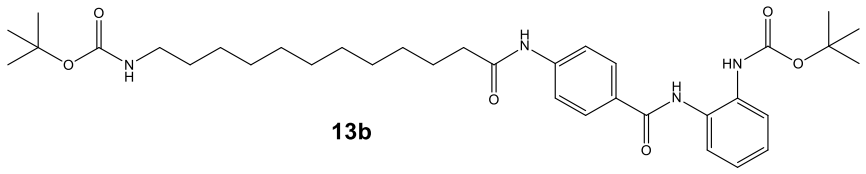
Appendix: ^{1}H NMR and ^{13}C NMR of Novel Compounds



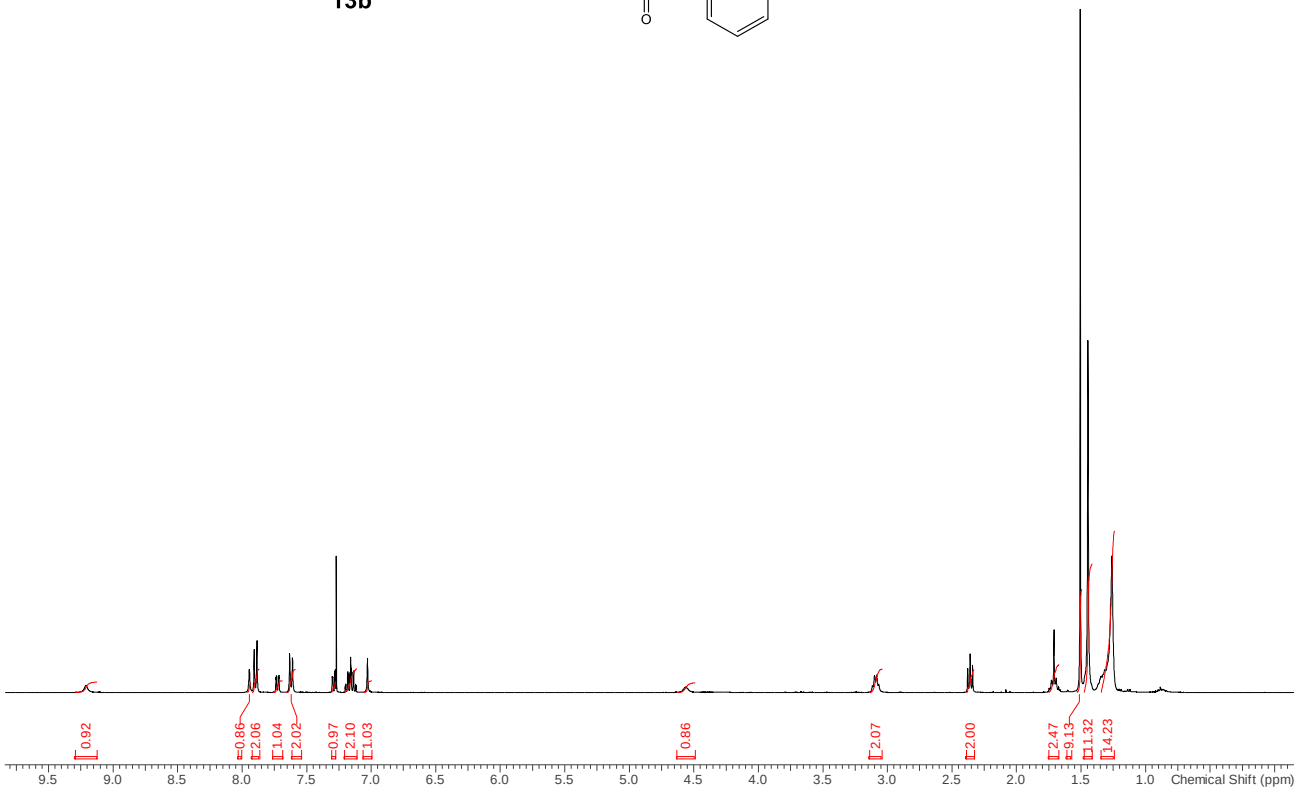
13a

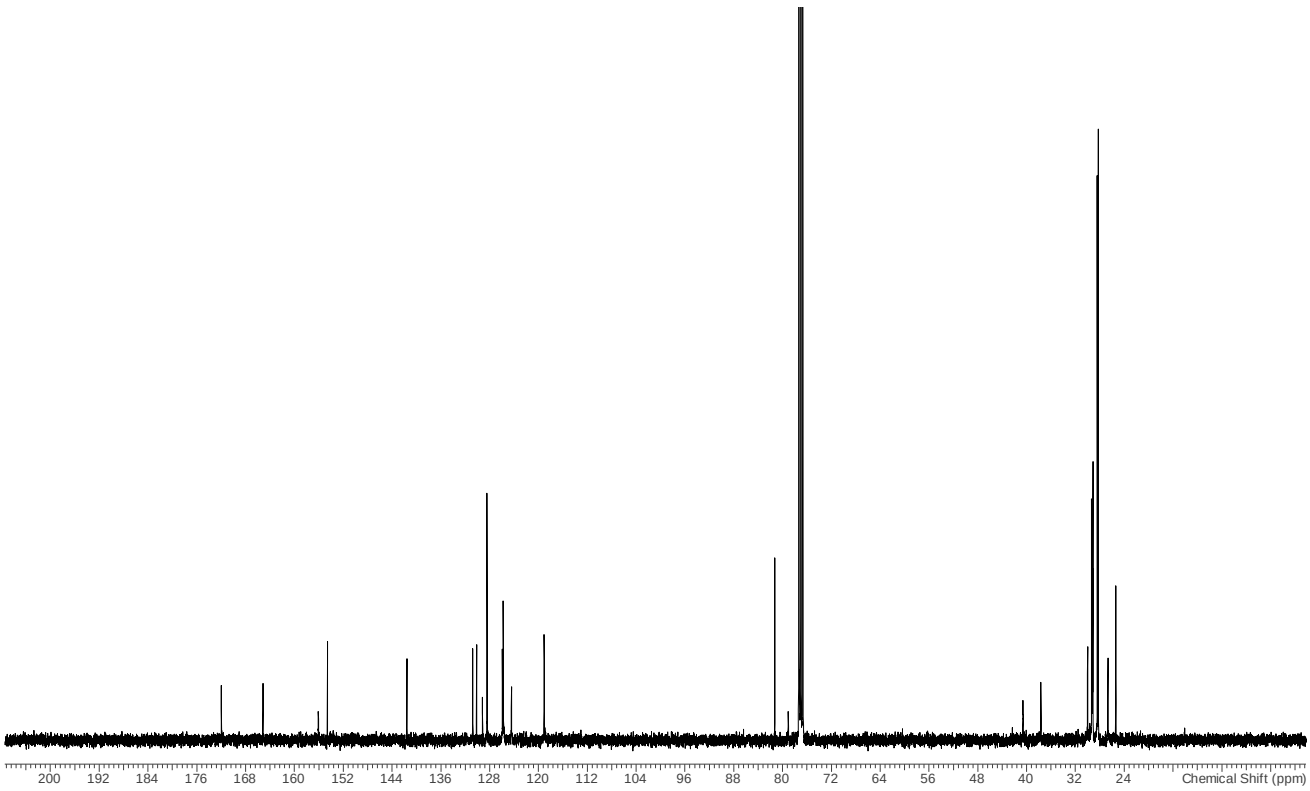


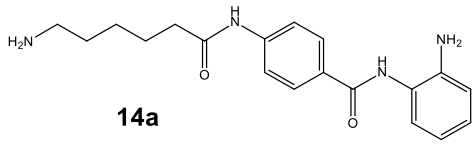




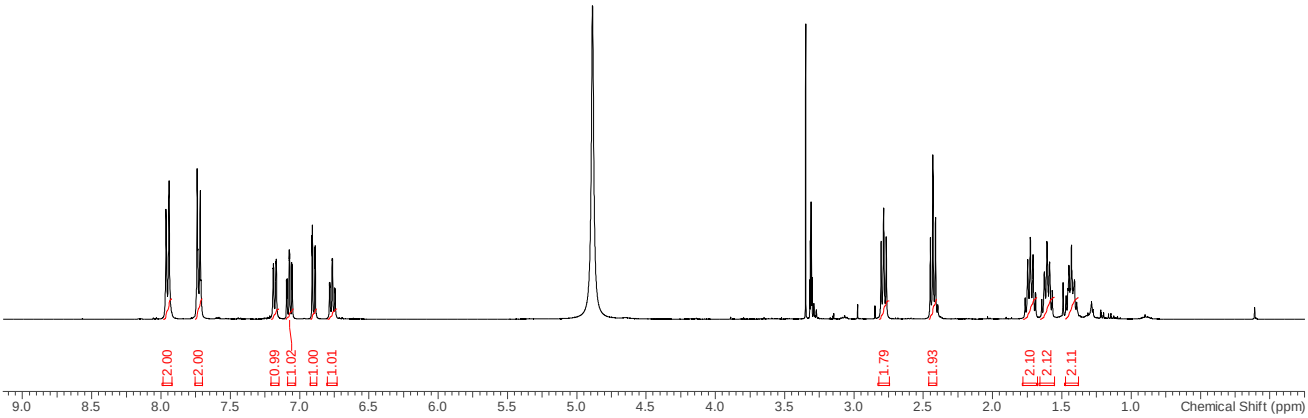
13b

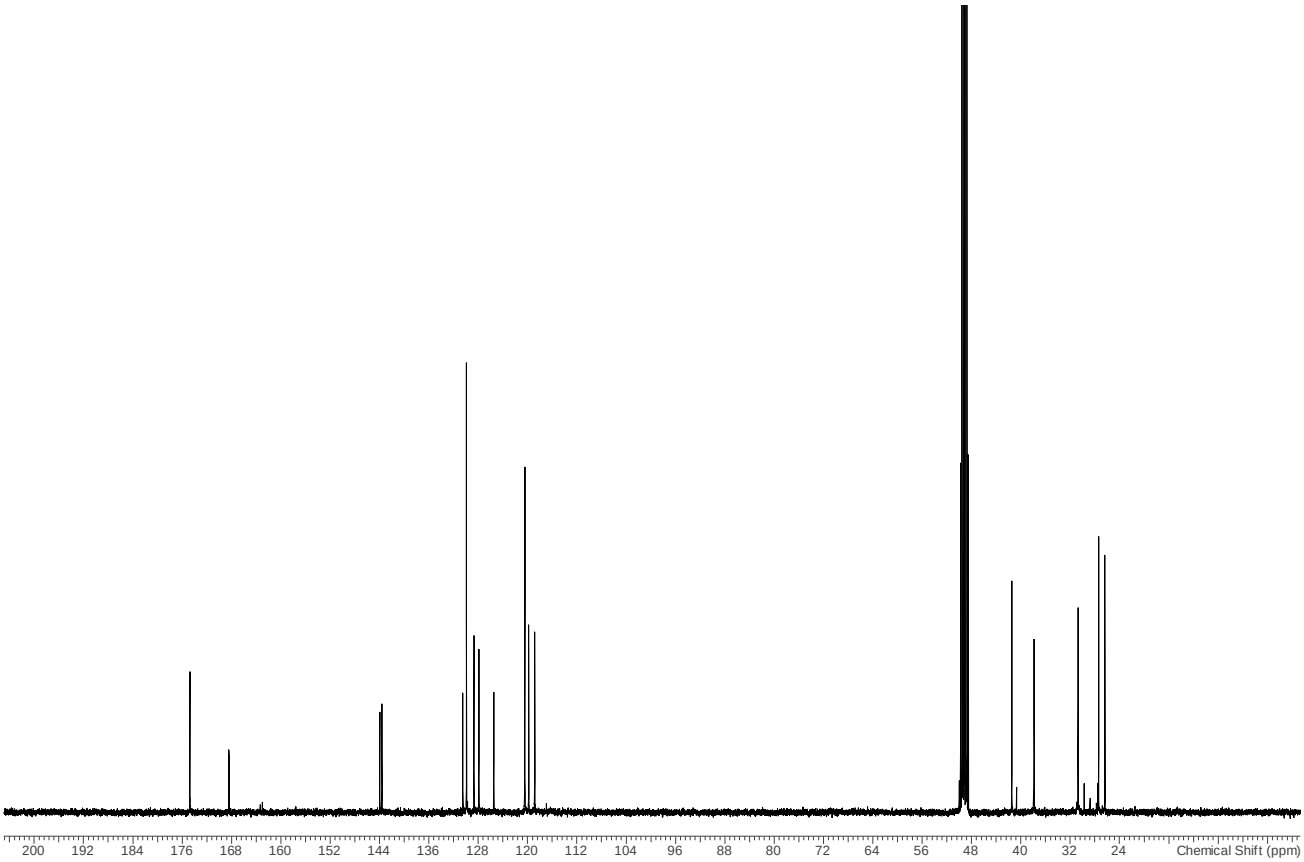

0.92
0.86
2.06
1.04
2.02
0.97
2.10
1.03
0.86
2.07
2.00
2.47
9.13
11.32
4.23
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
Chemical Shift (ppm)

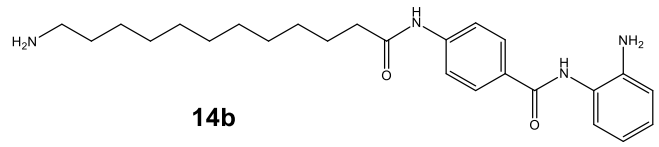

200 192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24
Chemical Shift (ppm)


H2N
O
H
N
O
H
N
NH2

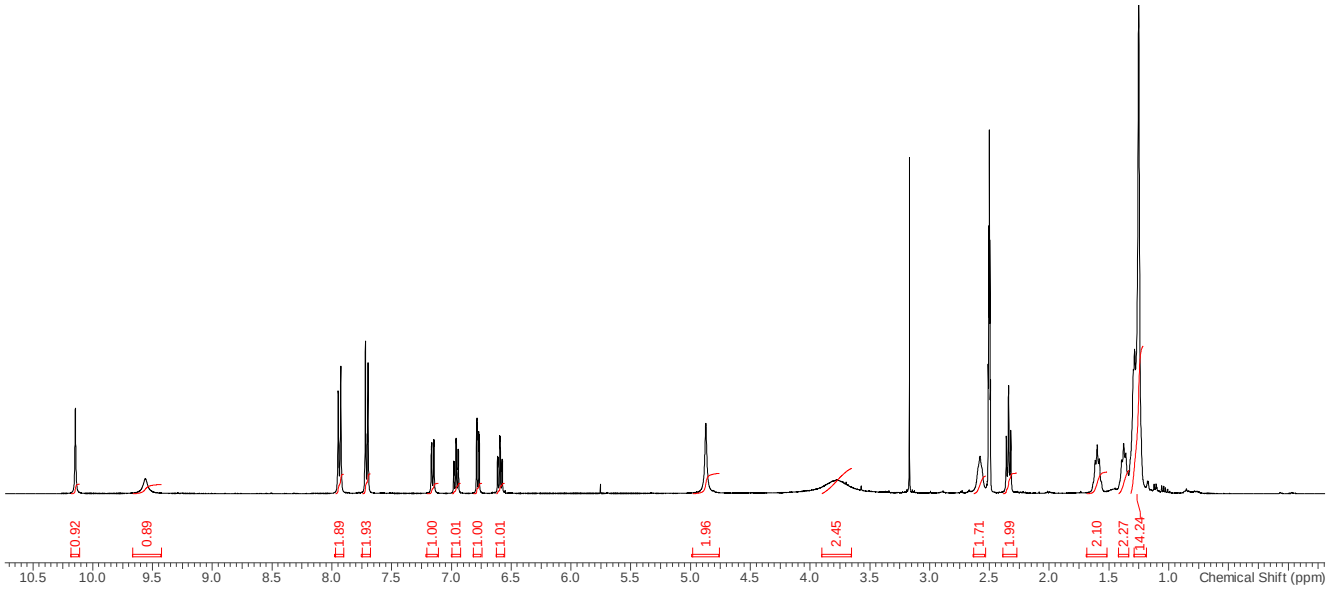
14a

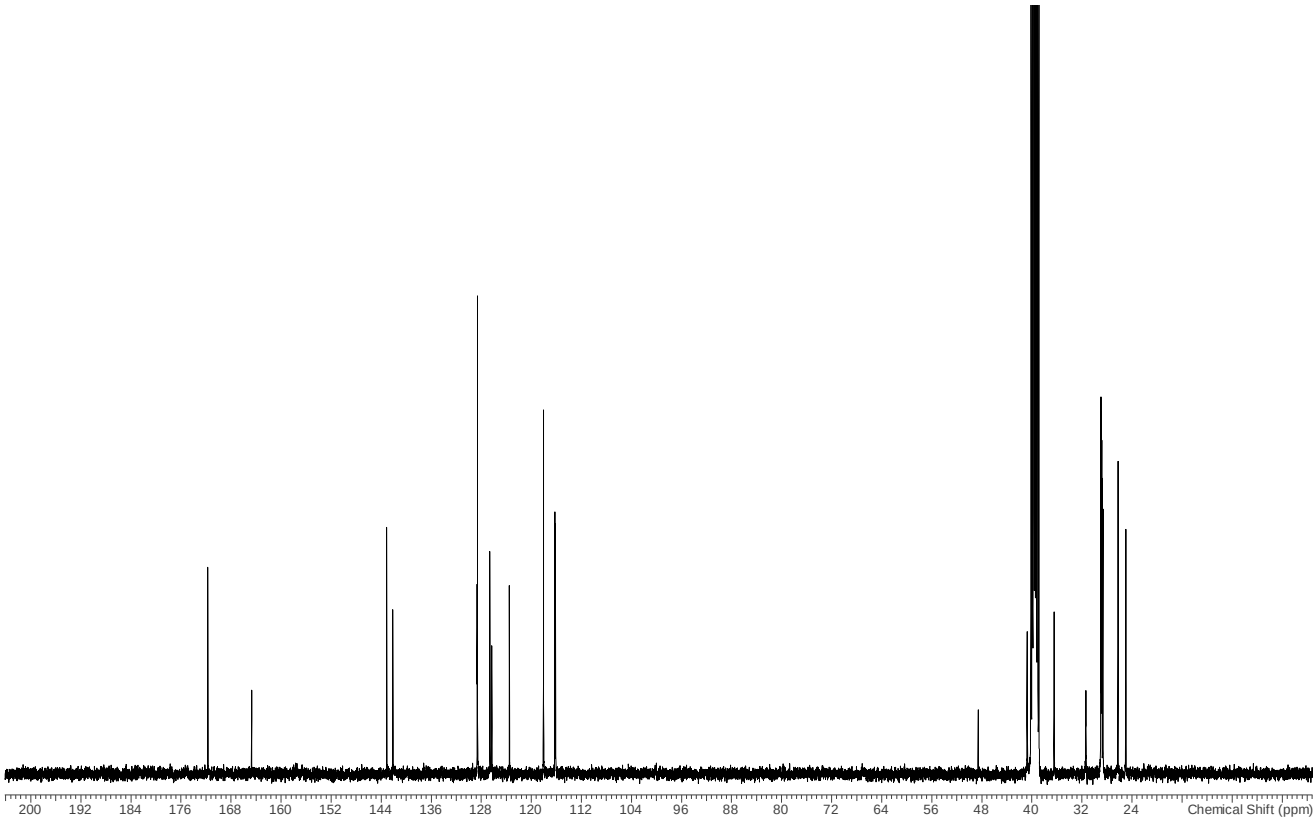

2.00
2.00
0.99
1.02
1.00
1.01
1.79
1.93
2.10
2.12
2.11
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Chemical Shift (ppm)

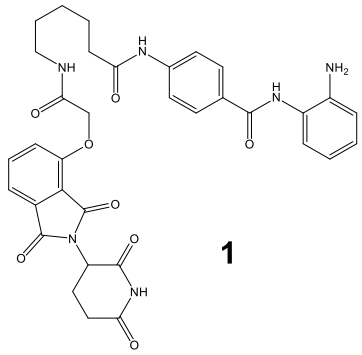

200
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
Chemical Shift (ppm)


H2N
O
H
N
H
N
O
NH2

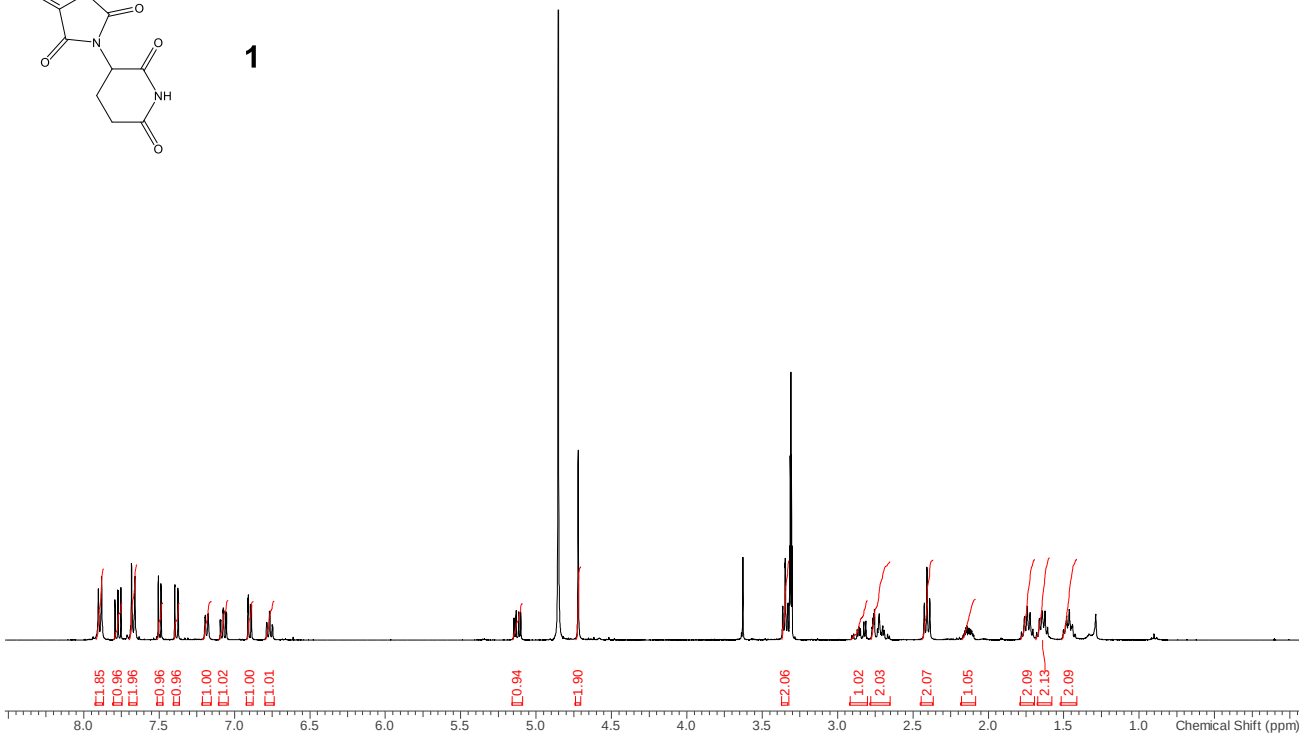
14b

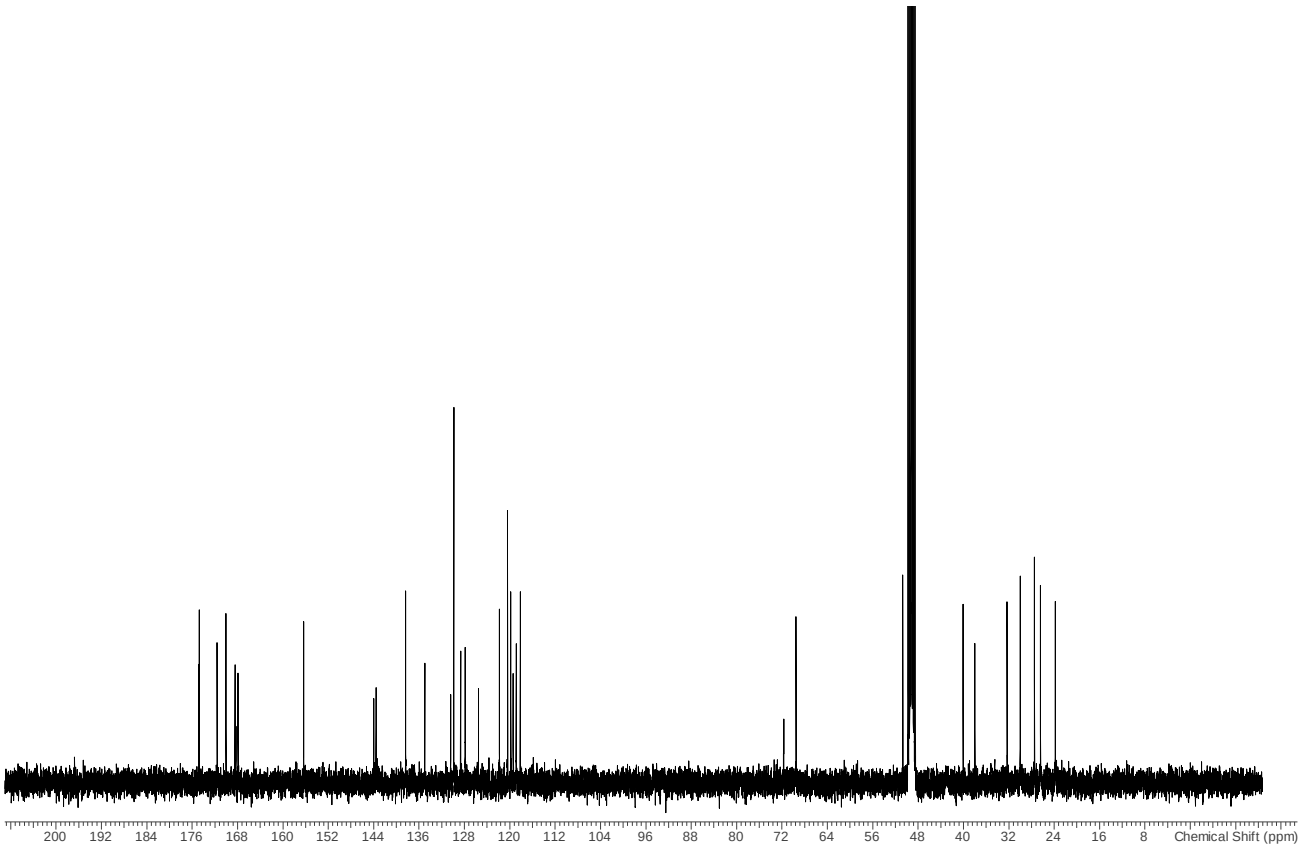

0.92
0.89
1.89
1.93
1.00
1.01
1.00
1.01
1.96
2.45
1.71
1.99
2.10
2.27
14.24
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
Chemical Shift (ppm)

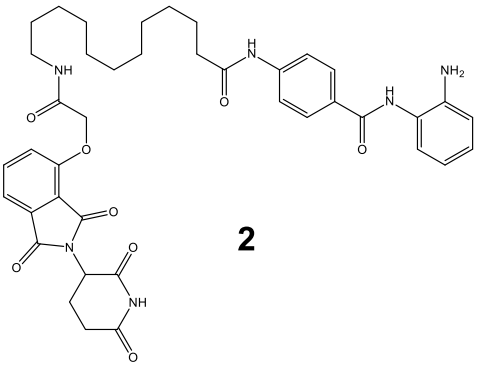

200 192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24
Chemical Shift (ppm)


NH
H
N
O
NH2
H
N
O
O
O
O
N
O
O
NH
O

1

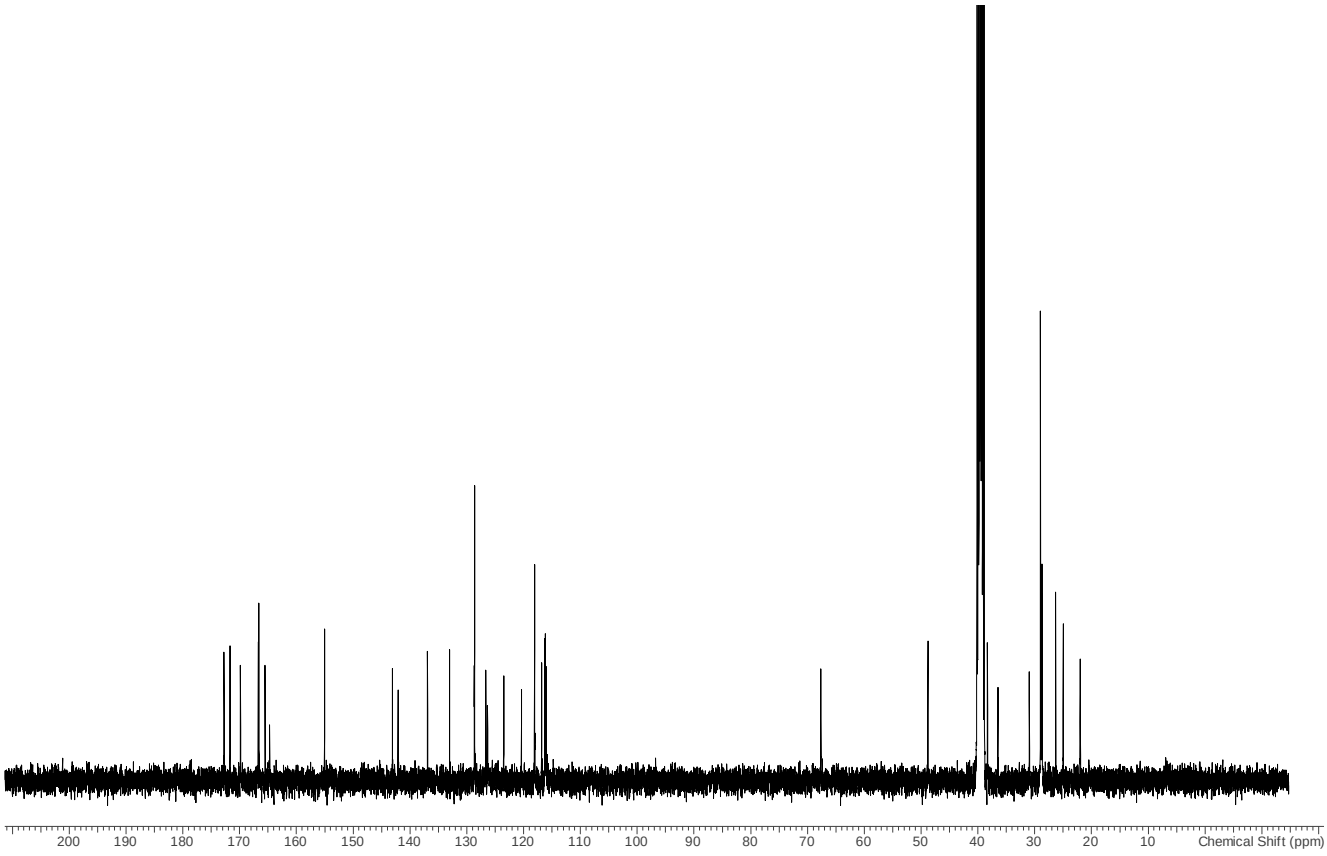

1.85
0.96
1.96
0.96
0.96
1.00
1.02
1.00
1.01
0.94
1.90
2.06
1.02
2.03
2.07
1.05
2.09
2.13
2.09
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Chemical Shift (ppm)

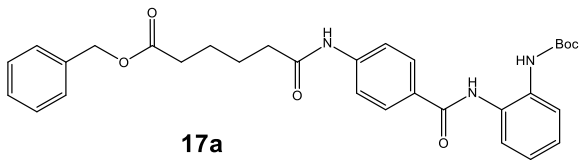

200
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
Chemical Shift (ppm)


NH
O
O
H
N
O
H
N
O
NH2
O
O
N
O
O
NH
O

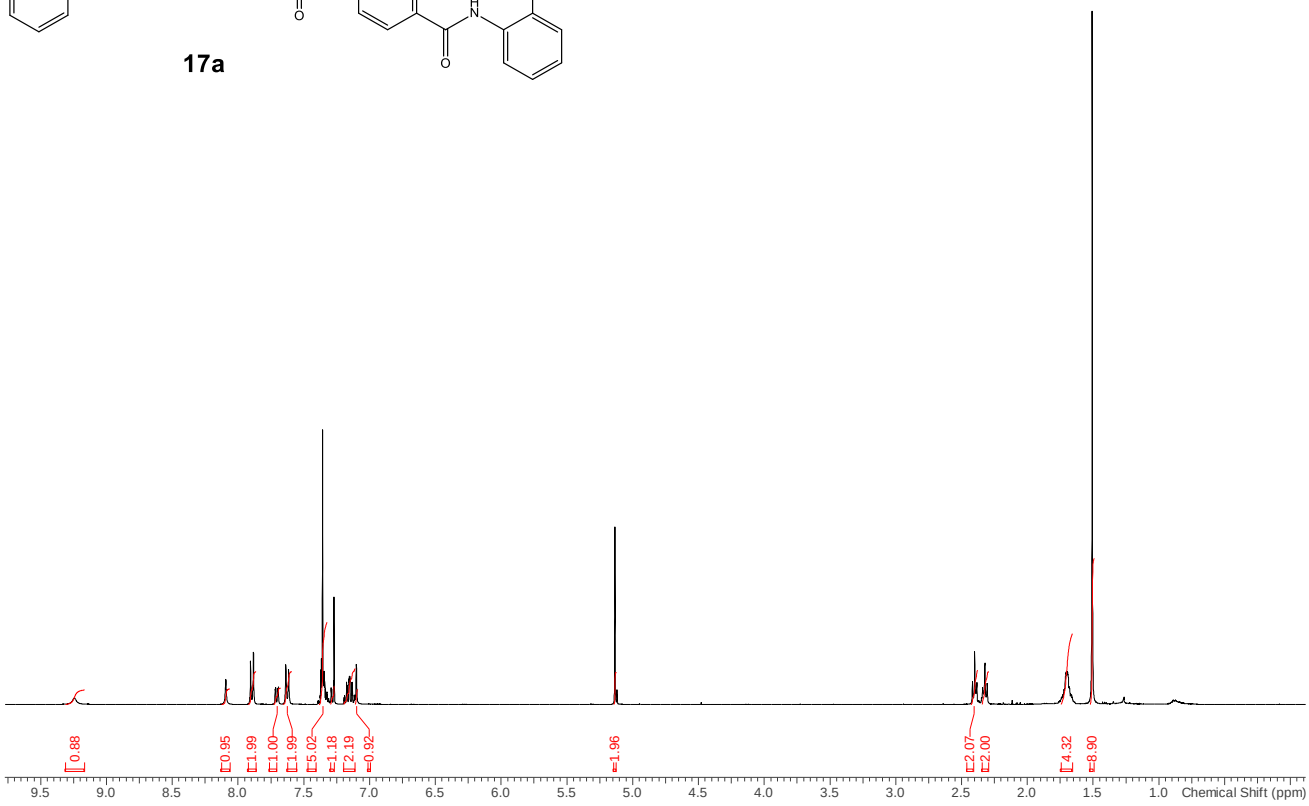
2

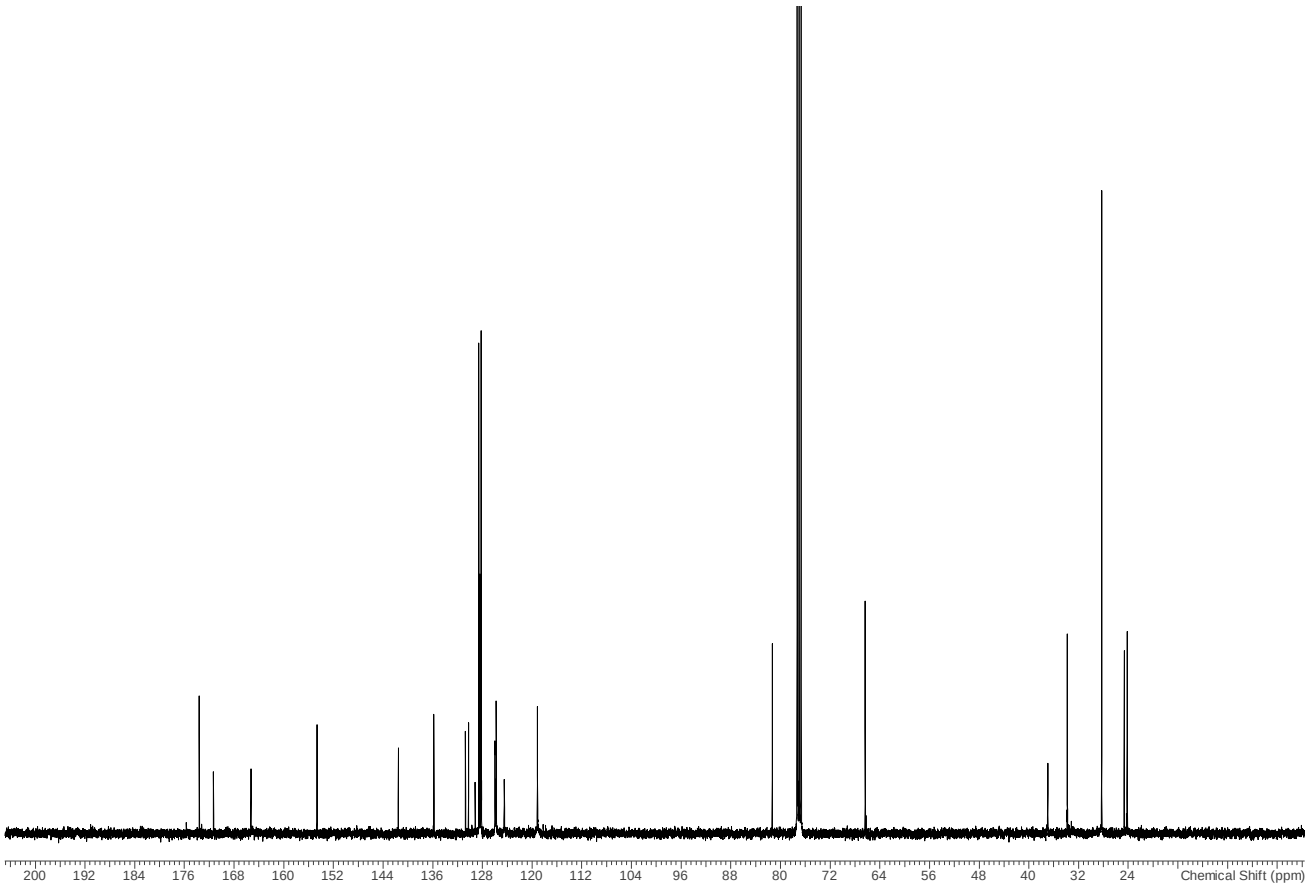
1.03
0.97
0.89
2.77
1.12
1.92
1.17
1.18
0.96
1.00
0.98
0.96
1.11
1.74
2.07
2.16
1.18
2.11
2.22
1.18
2.24
2.23
4.32
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Chemical Shift (ppm)

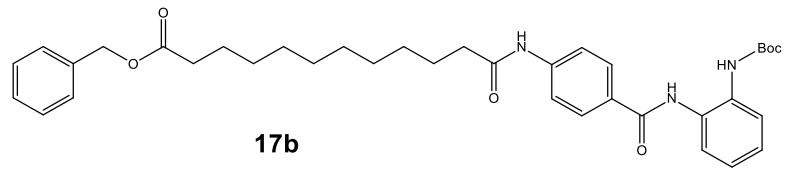

200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
Chemical Shift (ppm)


O
O
H
N
O
H
N
O
HN
Boc

17a

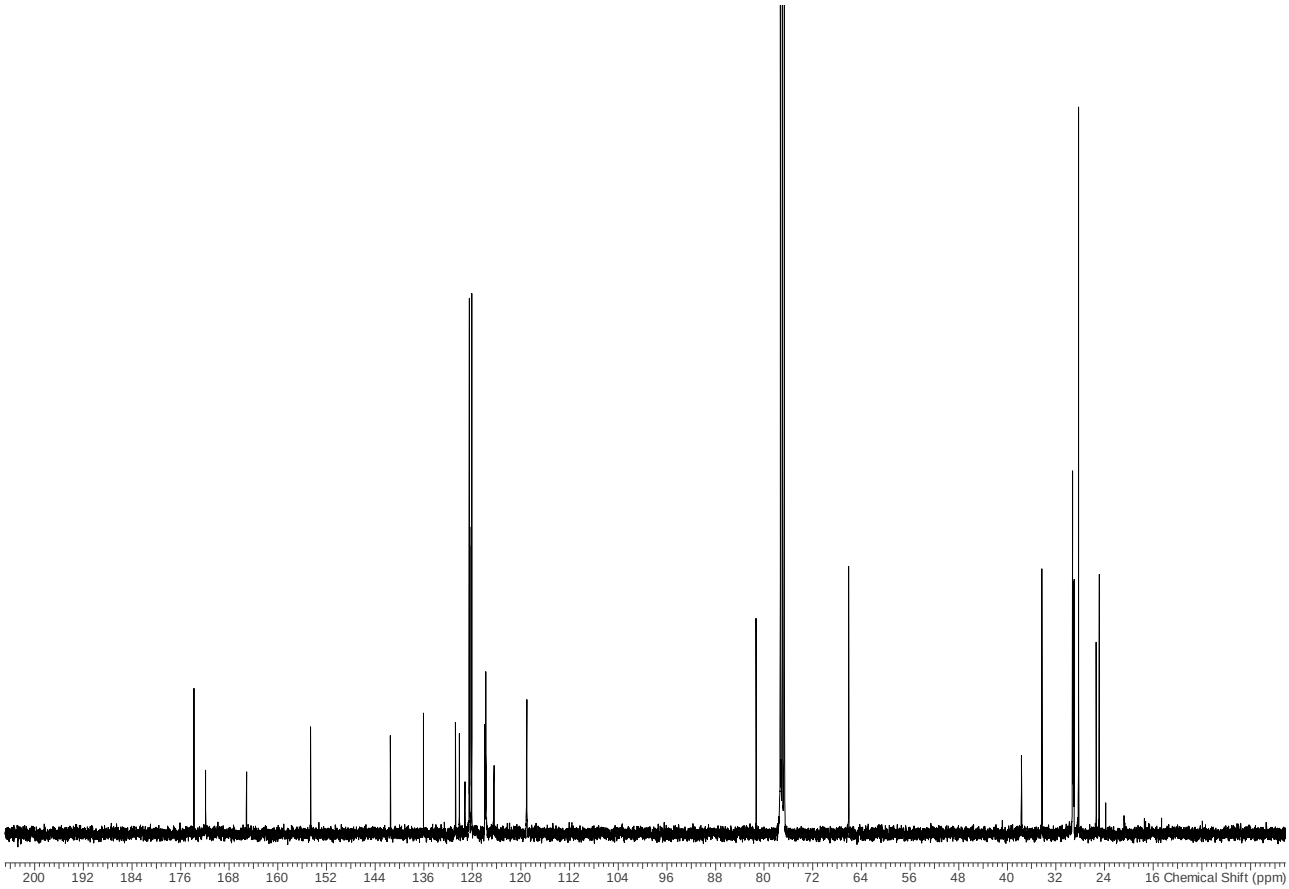

0.88
0.95
1.99
1.00
1.99
5.02
1.18
2.19
0.92
1.96
2.07
2.00
4.32
8.90
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
Chemical Shift (ppm)

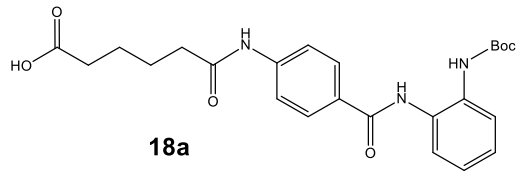

200 192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24
Chemical Shift (ppm)


O
O
H
N
O
HN
Boc
H
N
O

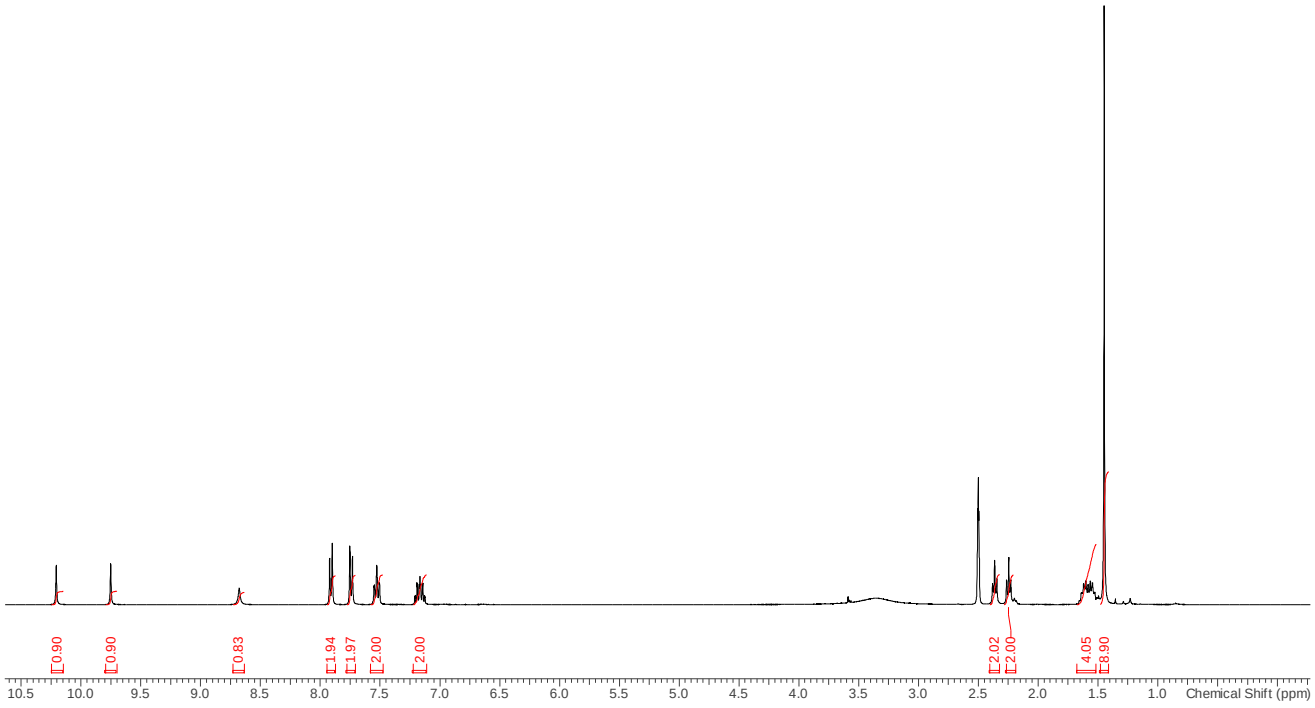
17b

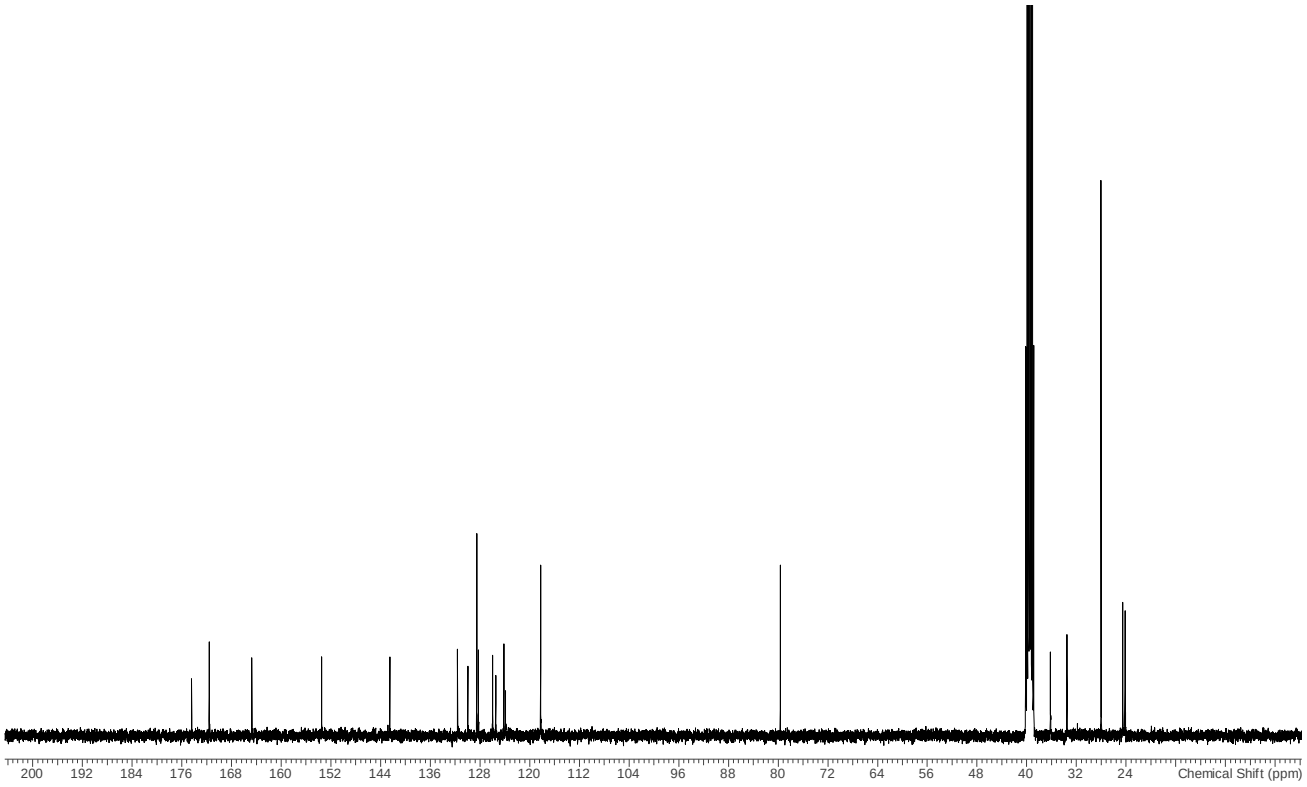
0.91
1.96
0.97
1.00
2.02
4.76
1.05
2.10
1.05
1.93
3.98
2.47
2.17
8.88
12.12
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Chemical Shift (ppm)

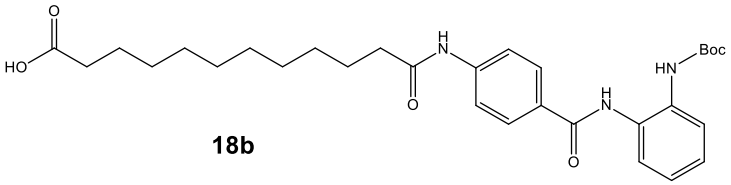

200
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16 Chemical Shift (ppm)


O
HO
H
N
O
HN
Boc
H
N
O

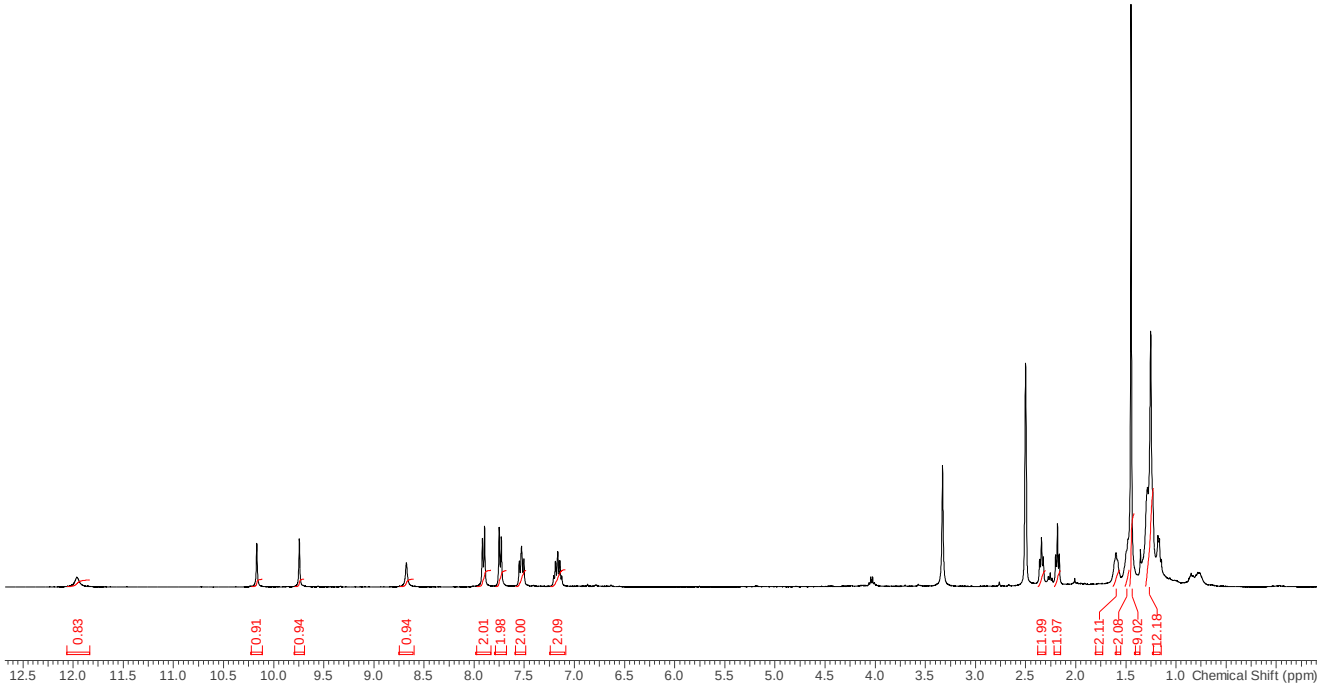
18a

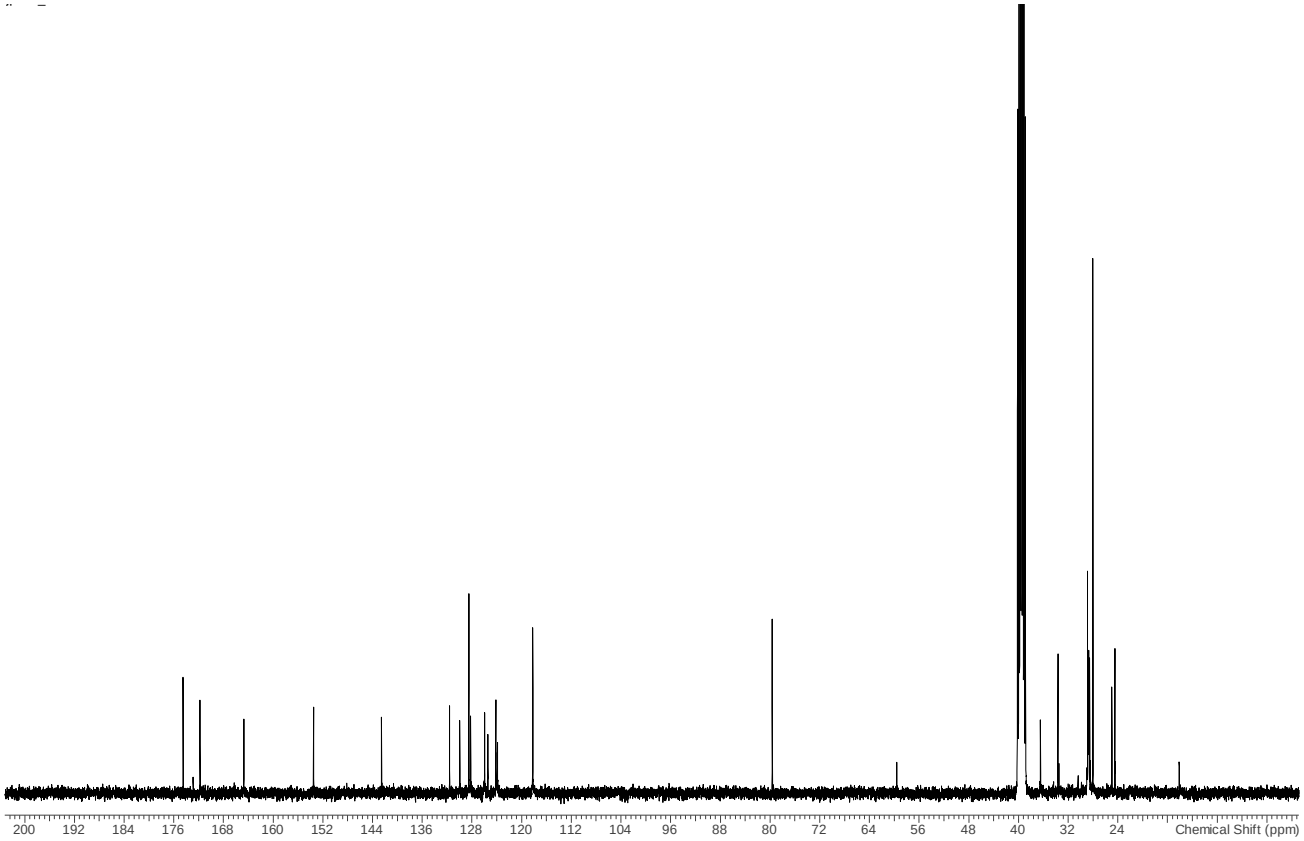

0.90
0.90
0.83
1.94
1.97
2.00
2.00
2.02
2.00
4.05
8.90
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
Chemical Shift (ppm)

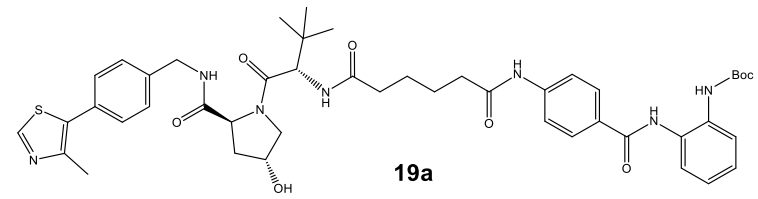

200 192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24
Chemical Shift (ppm)


O
HO
H
N
O
H
N
HN
Boc
O

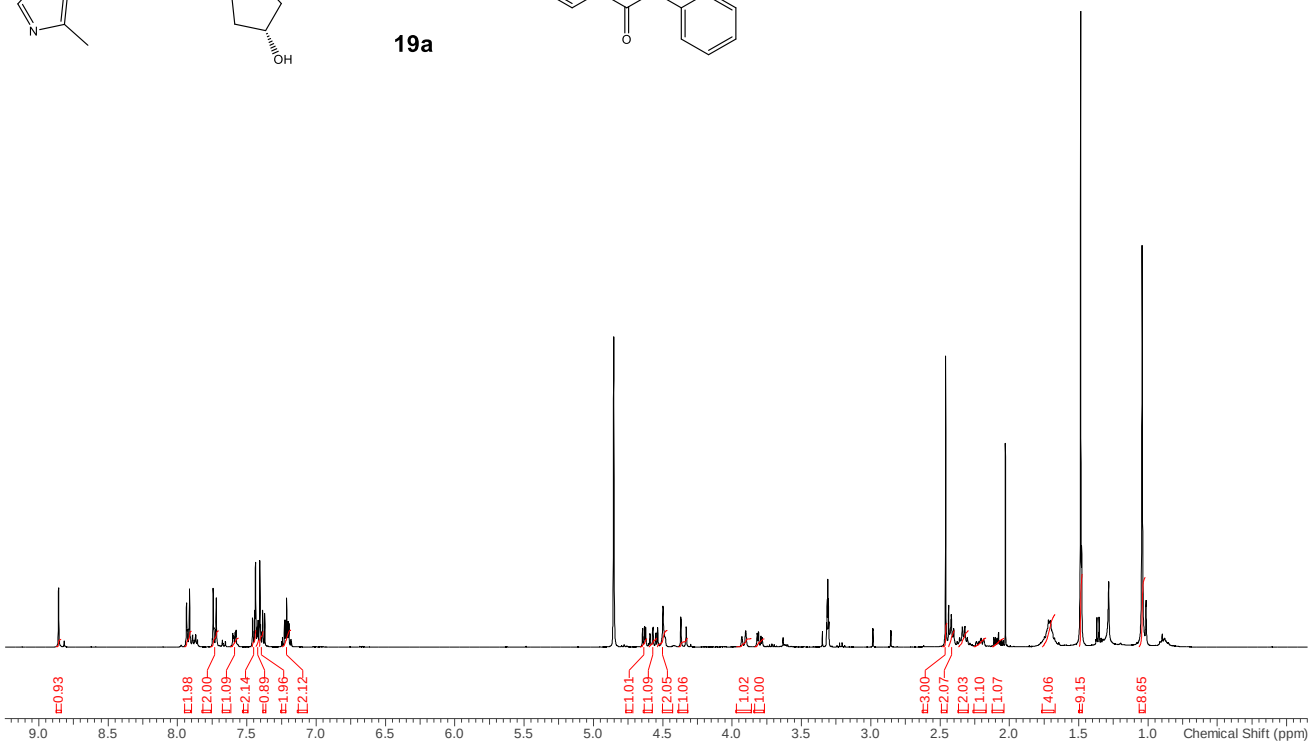
18b

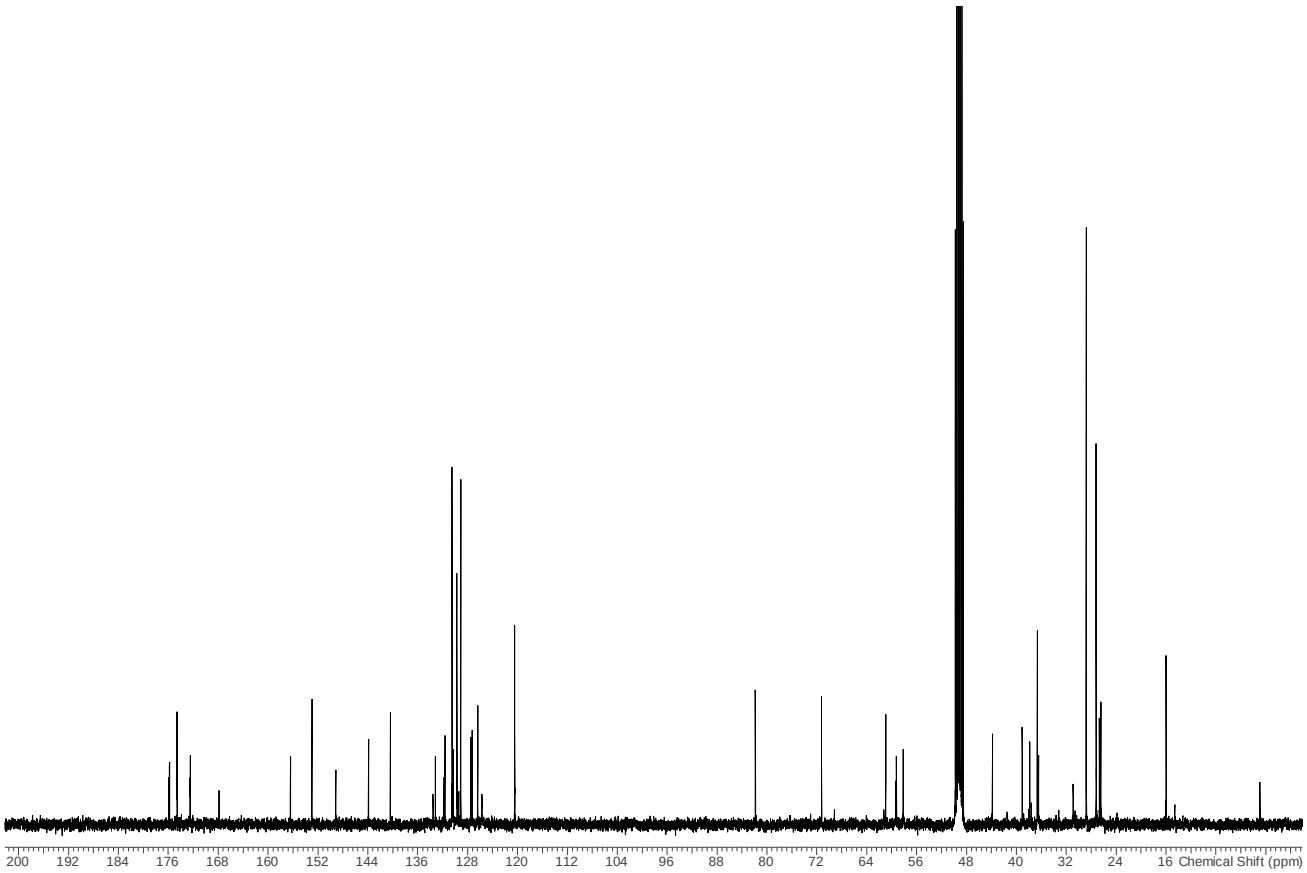

0.83
0.91
0.94
0.94
2.01
1.98
2.00
2.09
1.99
1.97
2.11
2.08
9.02
12.18
12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 Chemical Shift (ppm)

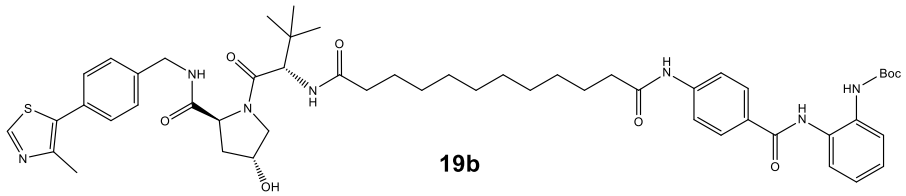

200 192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 Chemical Shift (ppm)


S
N
NH
O
N
O
OH
O
N
H
O
H
N
O
HN
Boc

19a

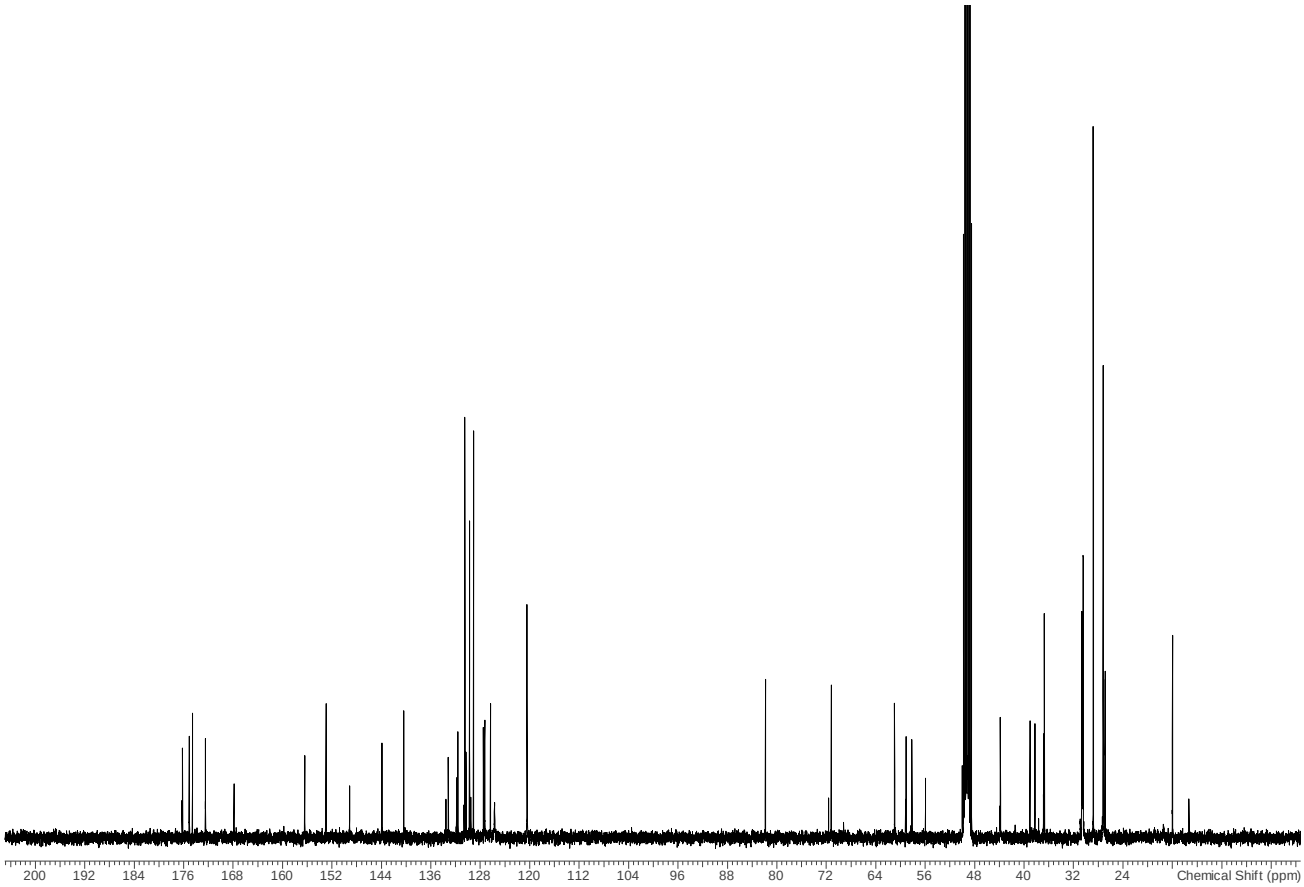

0.93
1.98
2.00
1.09
2.14
0.89
1.96
2.12
1.01
1.09
2.05
1.06
1.02
1.00
3.00
2.07
2.03
1.10
1.07
4.06
9.15
8.65
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
Chemical Shift (ppm)

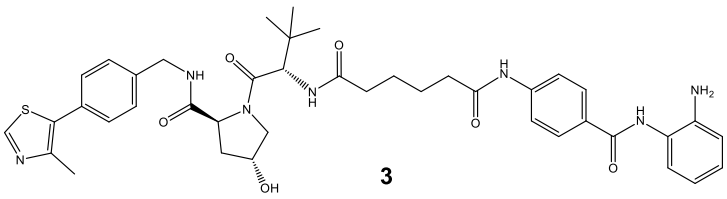

200 192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 16
Chemical Shift (ppm)


S
N
NH
O
O
N
OH
O
N
H
O
H
N
O
H
N
O
HN
Boc

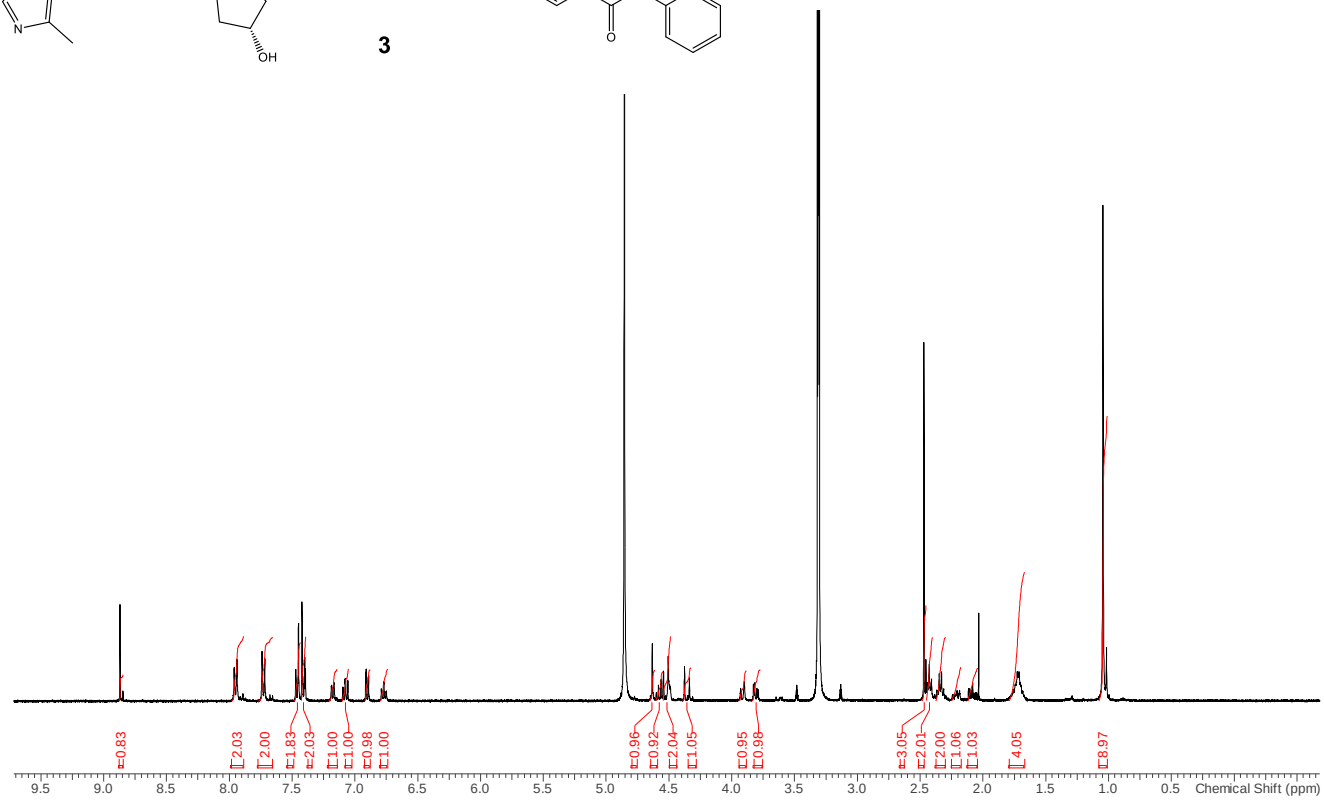
19b

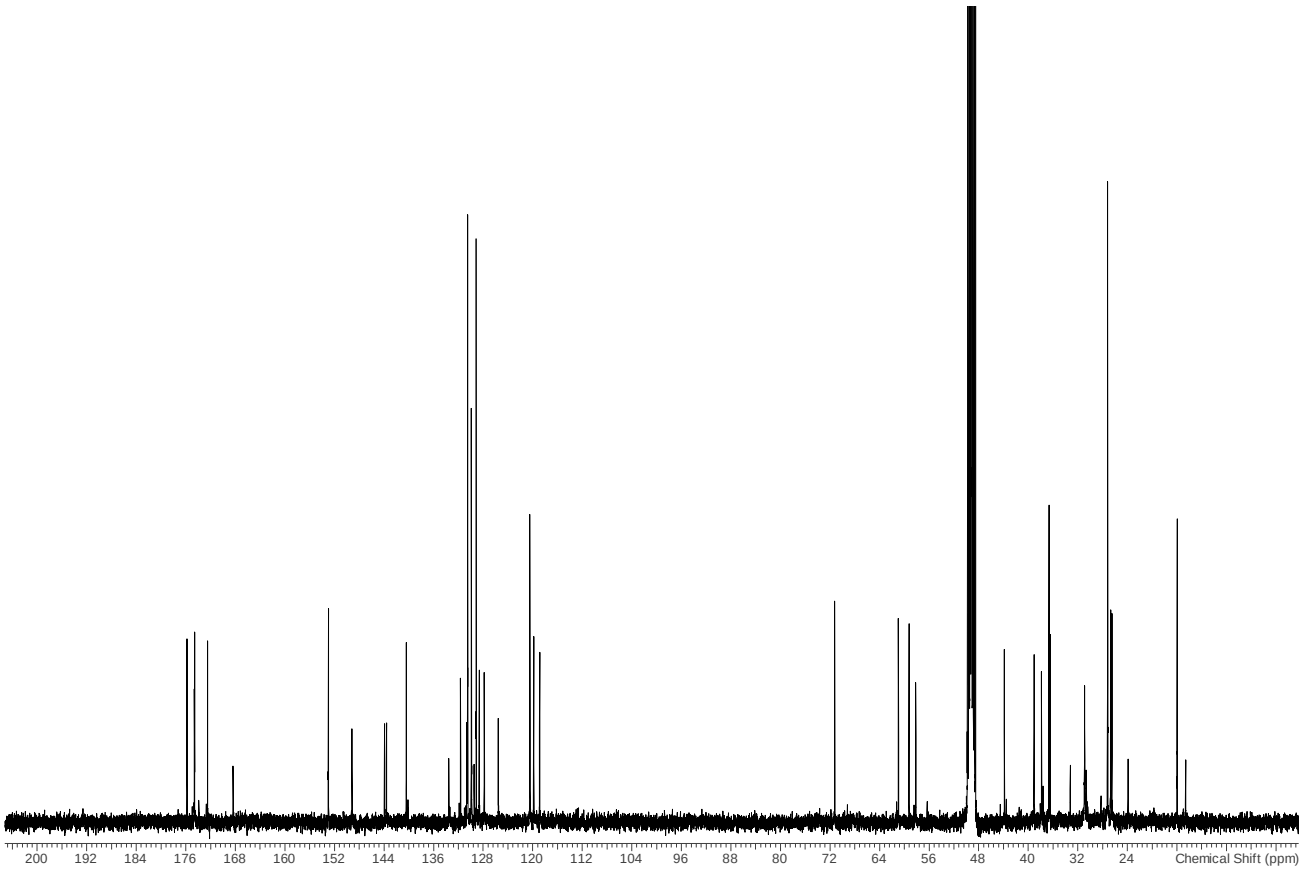
0.92
1.94
1.92
1.00
2.02
0.92
1.86
2.00
0.90
1.04
1.02
0.78
1.01
0.94
0.91
2.94
2.00
3.00
1.00
2.00
2.00
8.93
2.39
8.80
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Chemical Shift (ppm)

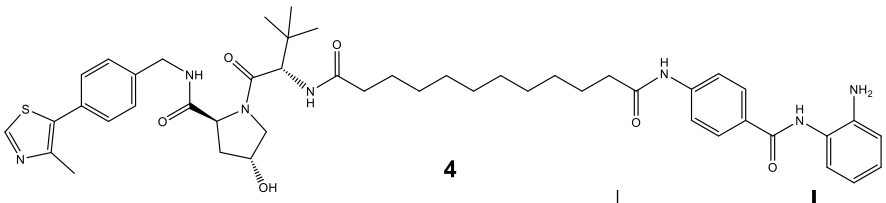

200
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
Chemical Shift (ppm)


S
N
NH
O
N
O
OH
O
N
H
O
H
N
O
H
N
O
NH2

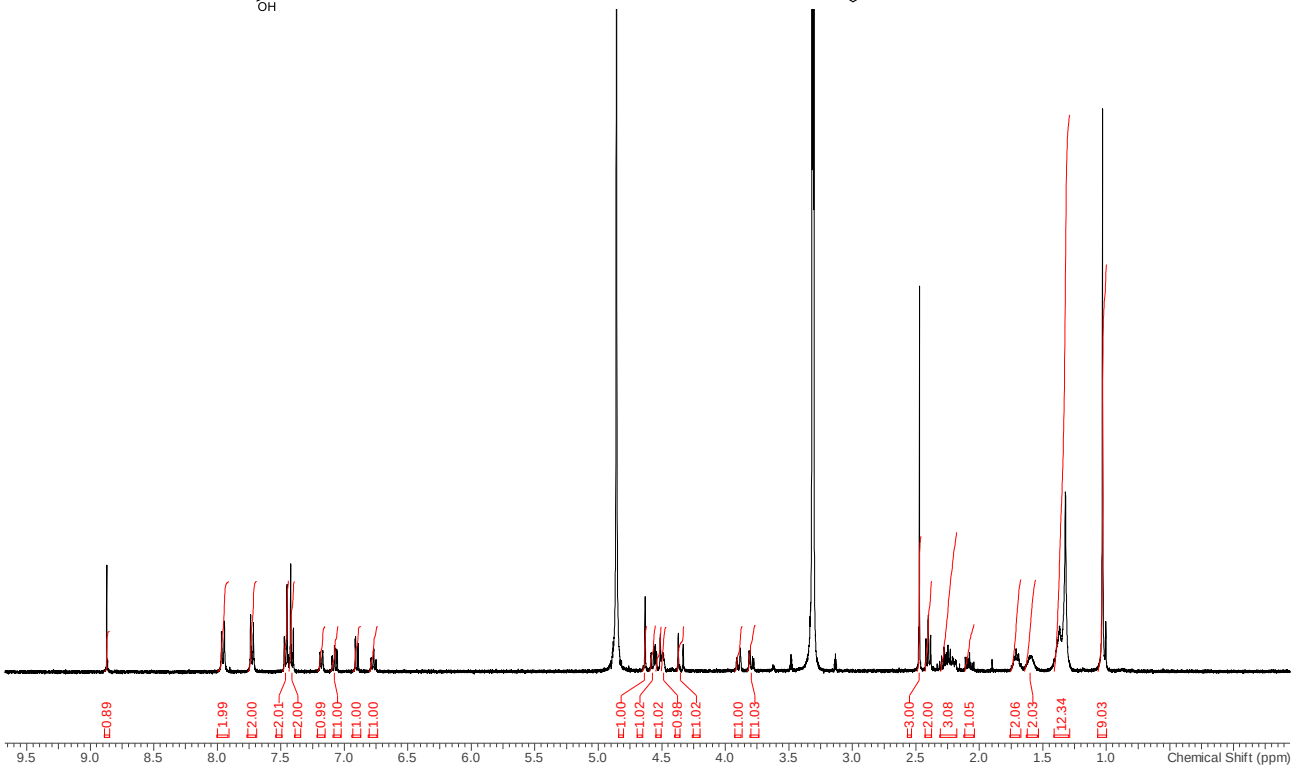
3

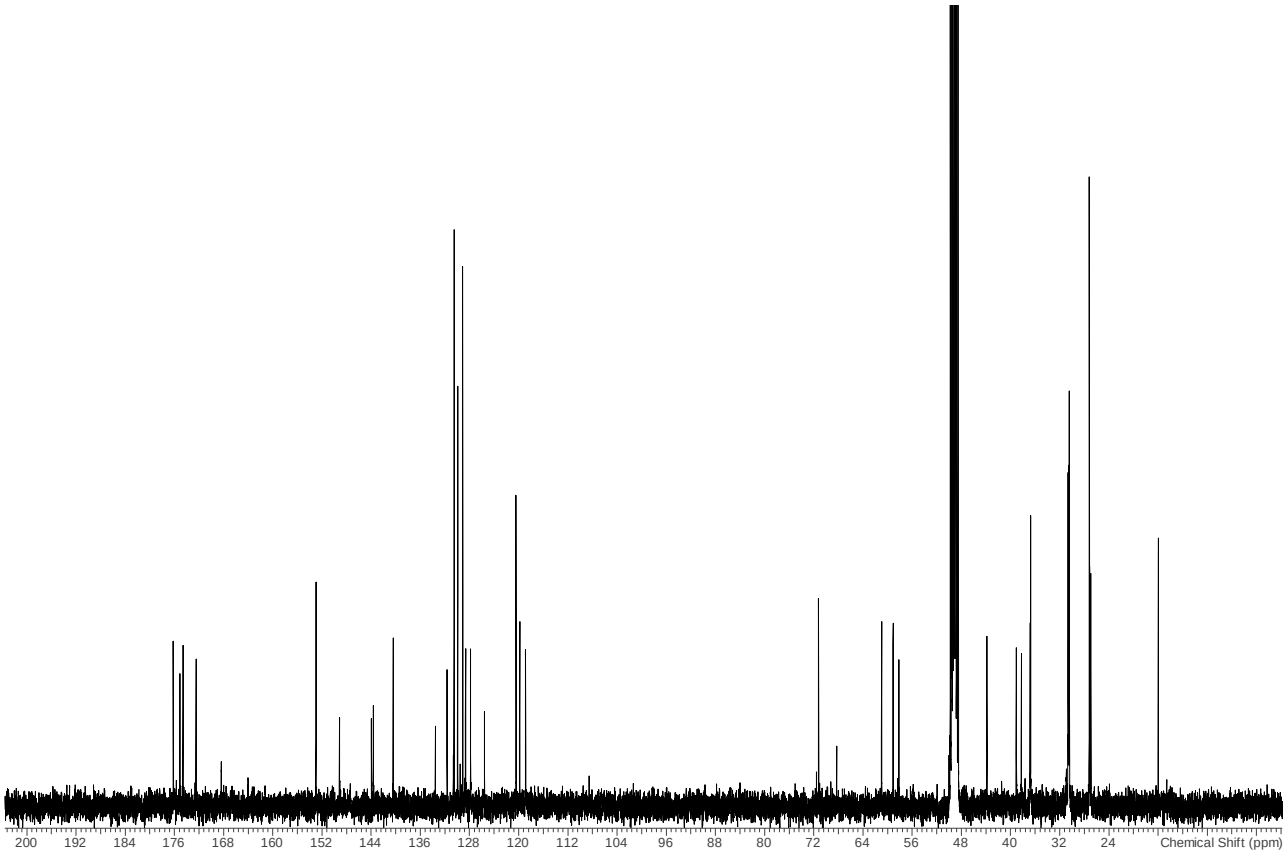

∫=0.83
∫=2.03
∫=2.00
∫=1.83
∫=2.03
∫=1.00
∫=1.00
∫=0.98
∫=1.00
∫=0.96
∫=0.92
∫=2.04
∫=1.05
∫=0.95
∫=0.98
∫=3.05
∫=2.01
∫=2.00
∫=1.06
∫=1.03
∫=4.05
∫=8.97
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 Chemical Shift (ppm)

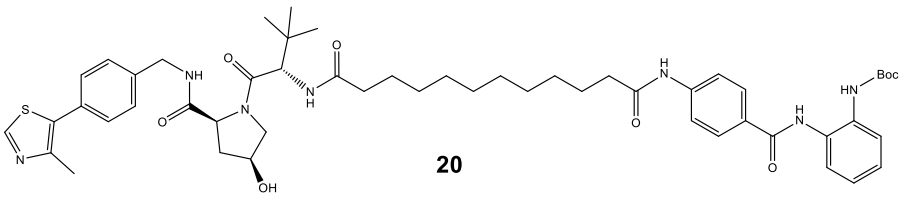

200 192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 Chemical Shift (ppm)



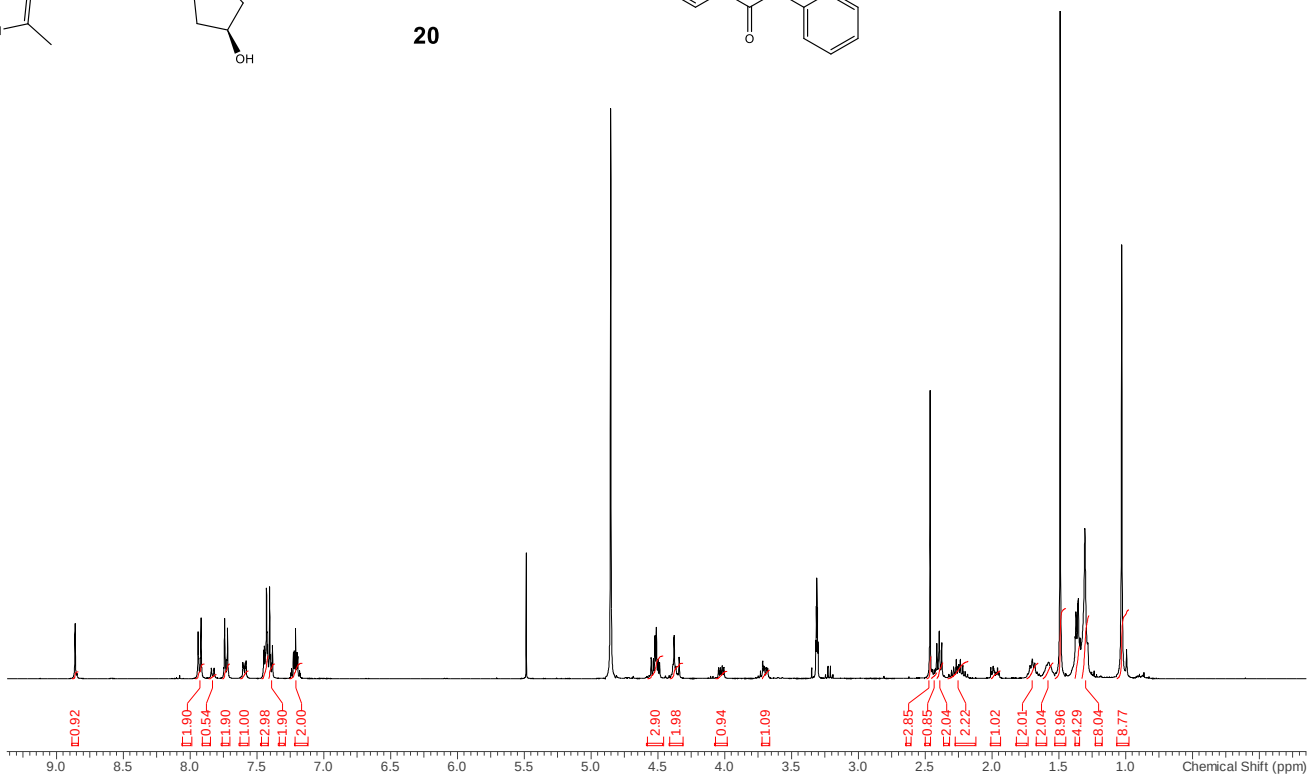
4

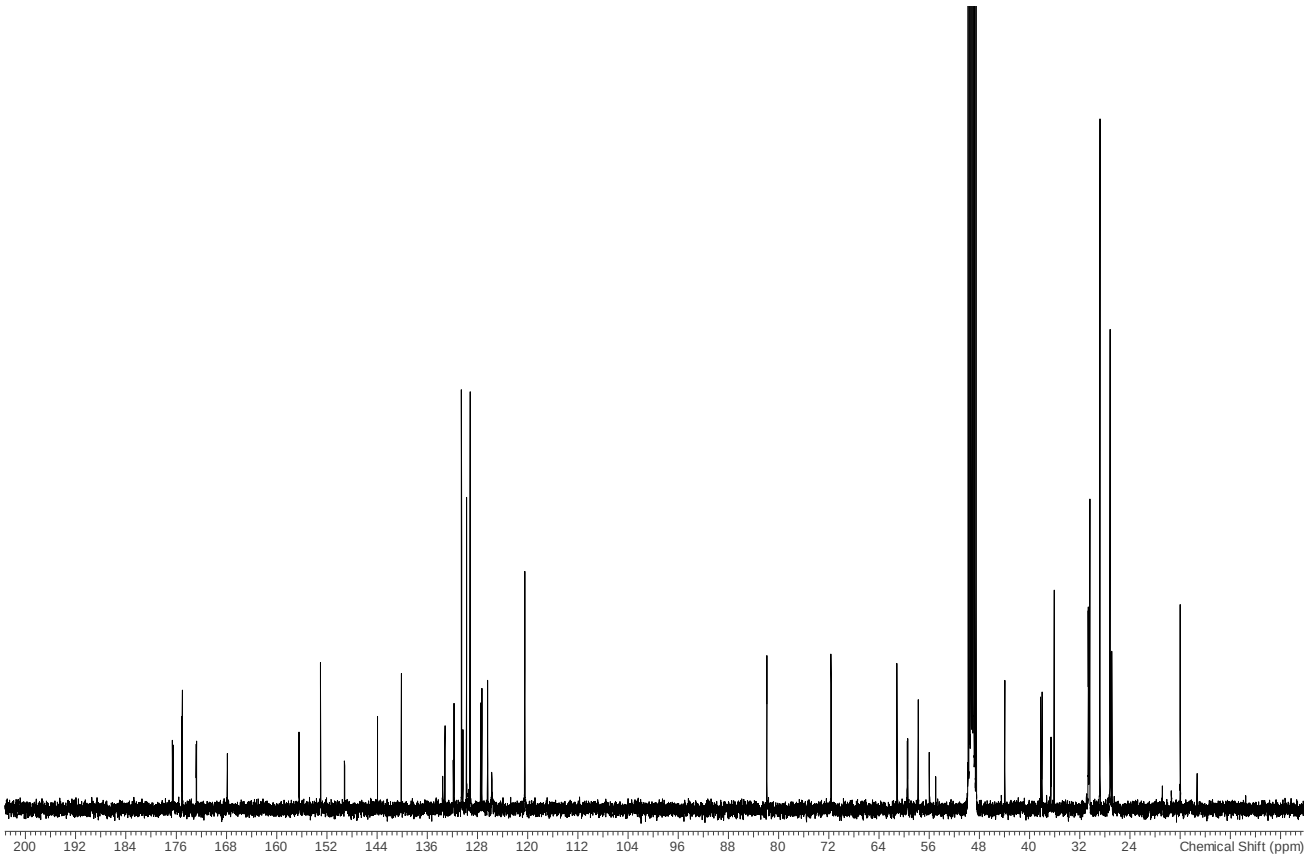

0.89
1.99
2.00
2.01
2.00
0.99
1.00
1.00
1.00
1.00
1.02
1.02
0.98
1.02
1.00
1.03
3.00
2.00
3.08
1.05
2.06
2.03
12.34
9.03
Chemical Shift (ppm)

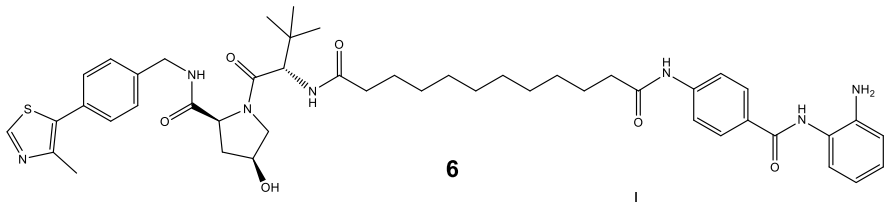

Chemical Shift (ppm)



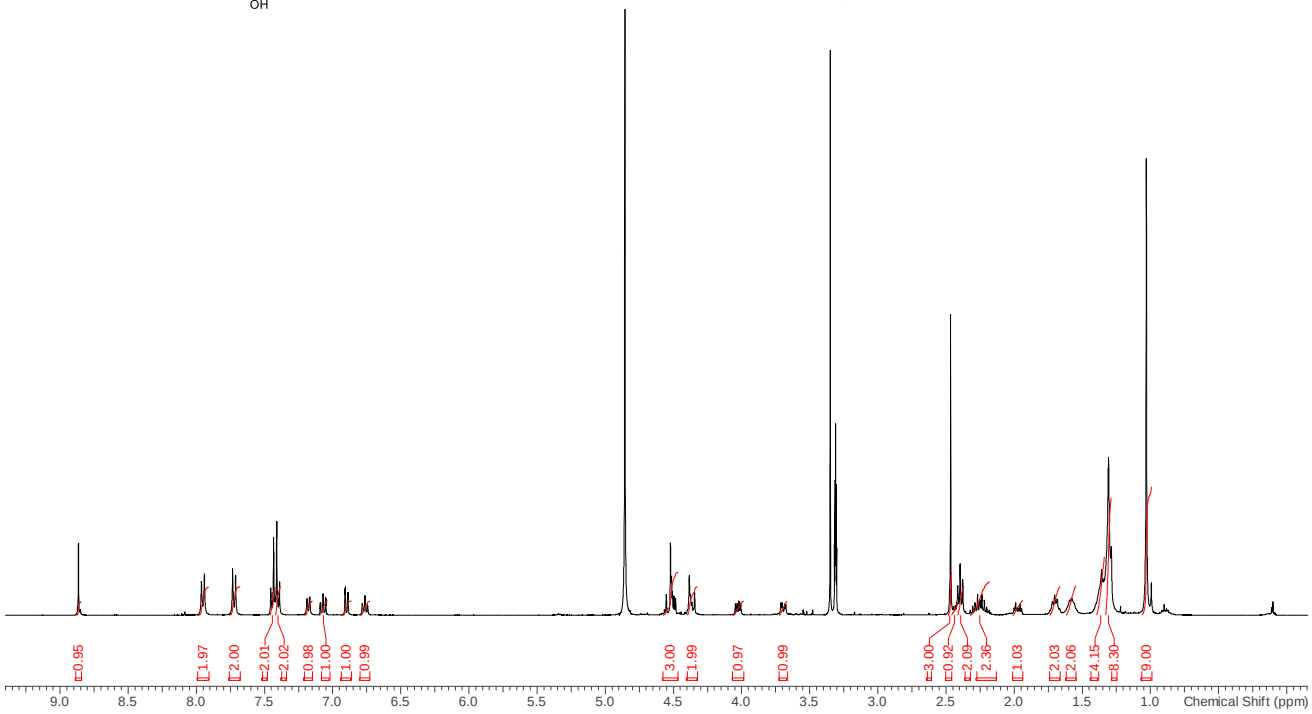
20

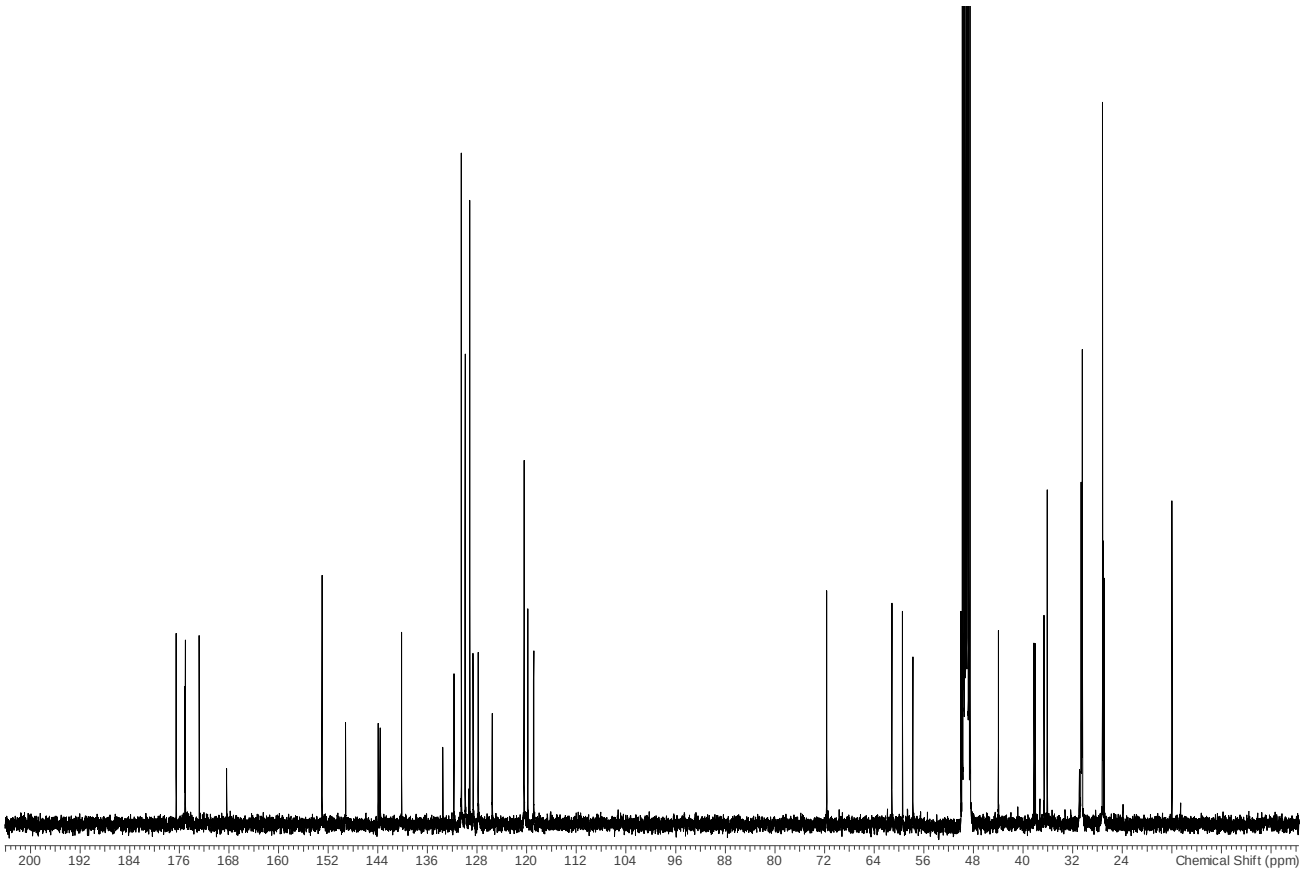

0.92
1.90
0.54
1.90
1.00
2.98
1.90
2.00
2.90
1.98
0.94
1.09
2.85
0.85
2.04
2.22
1.02
2.01
2.04
8.96
4.29
8.04
8.77
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
Chemical Shift (ppm)


200 192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24
Chemical Shift (ppm)


S
N
NH
O
N
O
O
N
H
O
H
N
O
H
N
NH2
O
OH

6


0.95
1.97
2.00
2.01
2.02
0.98
1.00
1.00
0.99
3.00
1.99
0.97
0.99
3.00
0.92
2.09
2.36
1.03
2.03
2.06
4.15
8.30
9.00
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Chemical Shift (ppm)


200
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
Chemical Shift (ppm)