Supporting Information

Activity-Guided Design of HDAC11-Specific Inhibitors

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A. General Information

Reagents. Commercially available chemicals were purchased from Sigma, Aldrich, TCI Chemicals, Alfa Aesar, Fischer, Matrix Scientific, Combi-Blocks and Cayman. Unless otherwise noted, all reagents were used without further purification. FT895 (AG-CR1-3910-M010) from Adipogen was purchased and used for *in cell* SHMT2 fatty acylation assay and cell permeability assay using LC-MS, while all early in vitro assays were done with FT895 synthesized in-house. Anti-Flag affinity gel (#A2220) and anti-Flag antibody conjugated with horseradish peroxidase (#A8592) were purchased from Sigma-Aldrich. Acetyl-Histone H3 Antibody (#9675), acetyl α-tubulin (#9671) antibodies were purchased from Cell Signaling Technology. Trapoxin A (CAS 133155-89-2), SHMT2 (mSHMT Antibody (F-11): sc-390641- Non-conjugated), ß-actin antibody (SC-47778 HRP) and goat anti-mouse IgG-HRP (sc-2005) were purchased from Santa Cruz. Palmitic acid, 3× Flag peptide, and protease inhibitor cocktail were purchased from Sigma-Aldrich. ECL plus western blotting detection reagent and universal nuclease for cell lysis were purchased from Thermo Scientific Pierce. Acyl peptides (acetyl-H3K9, Free H3K9, myristoyl-H3K9, and Alk14) were synthesized according to known procedures.[1] The peptide sequence for the H3K9 peptide is KQTARK(modified)STGGWW with uncapped N-terminal and SHMT2 peptide is SDEVK(modified)AHLLAWW with capped acetyl N-terminal; K (lysine) with myristoyl group is the reported catalytic site of HDAC11.[7]

Instrumentation. High-resolution MS was obtained using DART-Orbitrap mass spectrometer. TLC plates were purchased from EDM Chemicals (TLC Silica gel 60 F254, 250 mm thickness). Flash column chromatography was performed over Silica gel 60 (particle size 0.04- 0.063 mm) from EDM Chemicals.

Proton nuclear magnetic resonance (^{1}H NMR) spectra and carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on INOVA 400 (500 MHz) and Bruker-500 (500 MHz). Chemical shif ts for protons are reported in parts per million downfield from tetramethylsilane and are referen ced to the NMR solvent residual peak (DMSO δ2.50 $CHCl_3$ δ 7.26). Chemical shifts for carbon s are reported in parts per million downfield from tetramethylsilane and are referenced to the ca rbon resonances of the NMR solvent (DMSO δ39.5 $CDCl_3$ δ 77.0). Data are represented as foll ows: chemical shift, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants in Hertz (Hz), and integration.

Analytical HPLC analysis was carried out on a SHIMADZU LC with Kinetex 5u EVO C18 100A column

(100 mm × 4.60 mm, 5 μm, Phenomenex) monitoring at 215 nm and 280 nm. Solvents for analytical HPLC were water with 0.1% (v/v) trifluoroacetic acid (TFA) as solvent A and acetonitrile with 0.1% (v/v) TFA as solvent B. Compounds were analyzed at a flow rate of 0.5 mL/min.

Cell culture. HEK293T MCF7 cells were cultured in DMEM with 10% (v/v) heat-inactivated FBS. The cell lines used for experiments had been passaged no more than 20 times and all cell lines were tested for and showed no mycoplasma contamination.

Abbreviations Used. NAD - Nicotinamide adenine dinucleotide, DTT – Dithiothreitol, NMR - Nuclear Magnetic Resonance, FBS- Fetal Bovine Serum, LC-MS - Liquid chromatography–mass spectrometry, HPLC - High-Performance Liquid Chromatography, MeOH–Methanol, EtOAc–ethyl acetate, THF–tetrahydrofuran, Et_2O–diethyl ether, CH_2Cl_2–dichloromethane, TEA–triethylamine, MeCN–acetonitrile, TLC–thin layer chromatography, TsOH–*p*-toluenesulfonic acid, DMEM (Dulbecco's Modified Eagle Medium), Alk14 (Palmitic Acid Alkyne) and BCA (Bicinchoninic acid).

B. Expression and Purification of HDACs and Sirtuins from HEK293T Cells

Flag-tagged HDAC and Sirtuin (SIRT) expression plasmids were previously described.[2] Full-length human HDACs and sirtuins were inserted into the pCMV-Tag 4a vector with a C-terminal Flag tag. All plasmids were transfected into HEK293T cells using FuGene 6 Transfection Reagent according to the manufacturer's protocol (from Promega). Eight 10-cm plates of cells were collected by centrifugation at 500 × g for 5 min and then lysed in 2 ml of Nonidet P-40 lysis buffer (25 mM Tris-HCl, pH 7.4, 150 mM NaCl, 10% (v/v) glycerol and 1% (v/v) Nonidet P-40) with protease inhibitor cocktail (1:100 dilution) at 4 °C for 30 min. After centrifugation at 15,000 × g for 15 min, the supernatant was collected and incubated with 20 μL anti-Flag affinity gel at 4 °C for 2 h. The affinity gel was washed three times with 1 ml of washing buffer (25 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.2% (v/v) Nonidet P-40) and then eluted with 100 μl of 300 μM 3× FLAG peptide (dissolved in 25 mM Tris-HCl, pH 7.4, 150 mM NaCl and 10% (v/v) glycerol) twice (total 200 μL) for 1 hr each time. The eluted proteins were checked by 12% SDS-PAGE to be at least 80% pure.

C. HDACs and Sirtuins Deacylase Activity Assay[3]

***In vitro* Deacylation Assay.** All inhibitors (SISs and FT895) were synthesized by our lab and they were dissolved in DMSO. For the deacylation assay using the zinc-dependent HDACs, HDACs (concentration

of each HDACs described in Supplementary Figure 4) were incubated in 40 μL reaction mixtures (25 mM Tris-HCl, pH 8.0, 50 mM NaCl, 25 μM H3K9 myristoyl peptide) at 37 °C for 20 min with 64 nM of HDAC11. To quench the reactions, 40 μL cold acetonitrile was mixed with the reaction mixture. After centrifuging at 15,000 × g for 15 min, the supernatant was collected and analyzed by HPLC using Kinetex 5u EVO C18 100A column (150 mm × 4.6 mm, Phenomenex). Solvents used for HPLC were water with 0.1% (v/v) trifluoroacetic acid (solvent A) and acetonitrile with 0.1% (v/v) trifluoroacetic acid (solvent B). The gradient for HPLC condition was 0% B for 2 min, 0–20% B in 2 min, 20–40% B in 13 min, 40–100% B in 2 min, and then 100% B for 5 min. The flow rate was 0.5 mL/min. Procedure for HPLC assay with SHMT2 myristoyl peptide is the same as H3K9 myristoyl procedure described above except 120 mins incubation time used instead of 20mins.

For the deacylation assay using sirtuins, sirtuins (concentration of each sirtuin is provided in Supplementary Figure 4) were incubated in 40 μL reaction mixtures (25 mM Tris-HCl, pH 8.0, 50 mM NaCl, 1 mM DTT, 1 mM NAD^+, 25 μM H3K9 acetyl or myristoyl peptide) at 37 °C for 5 min (SIRT1, 2 and 3) or 30 mins (for SIRT6). To quench the reactions, 40 μL of cold acetonitrile was mixed with the reaction mixture. After centrifuging at 15,000 g for 15 min, the supernatant was collected and analyzed by HPLC using Kinetex 5u EVO C18 100A column (150 mm × 4.6 mm, Phenomenex). Solvents used for HPLC were water with 0.1% (v/v) trifluoroacetic acid (solvent A) and acetonitrile with 0.1% (v/v) trifluoroacetic acid (solvent B). The gradient for HPLC condition was 0% B for 2 min, 0–20% B in 2 min, 20–40% B in 13 min, 40–100% B in 2 min, and then 100% B for 5 min. The flow rate was 0.5 mL/min.

The HPLC data was analyzed to get the conversation rate and inhibition rate according to the following equations:

$$\text{Conversion rate} = \frac{(Area\ of\ Free\ H3K9\ UV\ peak)}{(Area\ of\ Free\ H3K9\ UV\ peak)+(Area\ of\ Acyl\ H3K9)} X\ 100\ (\%)$$

$$\text{Inhibition rate} = \{100 - \frac{(Conversion\ rate\ of\ inhibitor-treated\ sample)}{(Conversion\ rate\ of\ sample\ with\ DMSO\ instead\ of\ inhibitor)} X100\}\ (\%)$$

In-cell Deacetylation Assay. SIS7, SIS17 and FT895 were synthesized by our lab for this assay. HEK293T cells cultured in 15-cm dishes were treated with inhibitors for 6 h. The cells were scraped off the plates and collected at 500 g for 5 min. Cells from each plate were then lysed in 200 μL of 4% SDS lysis buffer (50 mM triethanolamine at pH 7.4, 150 mM NaCl, 4% (w/v) SDS) with protease inhibitor cocktail (1:100 dilution) and nuclease (1:1000 dilution) at room temperature for 15 min. The protein

concentration in the total cell lysate was determined using a BCA assay and each amount of protein was loaded onto a 15% SDS-PAGE gel and resolved. The gel was used for western blot analysis with acetyl α-tubulin and acetyl histone H3 antibodies. The loading for each sample was checked by Coomassie blue-staining the polyvinylidene fluoride (PVDF) membranes.

Western Blot. Proteins were resolved by 12% or 15% SDS-PAGE and transferred to PVDF membranes. The membrane was blocked with 5% (w/v) BSA in PBS with 0.1% (v/v) Tween-20 (TPBS) at room temperature for 60 min. The primary antibody was diluted with fresh 5% (w/v) BSA in TPBS (1:5,000 dilution for antibodies to Flag, Ac-histone H3, Ac-α-tubulin and mSHMT2) and incubated with the membrane at room temperature for 1 h or at 4 °C for 12 h. After washing the membrane three times with TPBS, the secondary antibody (1:3,000 dilution with 5% (w/v) BSA in TPBS) was added and then incubated at room temperature for 1 h. The chemiluminescence signal in the membrane was recorded after developing in ECL Plus Western Blotting detection reagents using a Typhoon 9400 Variable Mode Imager (GE Healthcare Life Sciences).

Detection of Lysine Fatty Acylation on Endogenous SHMT2 by Western Blot. SIS7 and SIS17 were synthesized by our lab and FT895 (AG-CR1-3910-M010) was purchased from Adipogen for this assay. MCF7 cells were treated with 50 μM of Alk14 and inhibitor or DMSO for 6 hours. Two of 80-90% confluent 15-cm MCF7 cells (approximately $2*10^6$ cells per 15-cm dish) cells were collected by centrifugation at 1000 g for 5 min and lysed in 4% SDS lysis buffer (50 mM triethanolamine at pH 7.4, 150 mM NaCl, 4% (w/v) SDS) with protease inhibitor cocktail (1:100 dilution) and nuclease (1:1000 dilution) at room temperature for 15 min. The proteins were precipitated with cold methanol (200 μL per sample), cold chloroform (75 μL per sample), and cold water (150 μL per sample). After vortexing to mix well, samples were spun down at 17,000 g for 15 min. Proteins should be located between the upper layer and bottom layer. The upper layer of the solvents was gently removed. Then 1 mL of cold methanol was added to each sample to wash the proteins by vortexing them. Samples were centrifuged at 17,000g for 5 min. The washing was repeated once more. After methanol washing, methanol was removed from tubes and protein pellets in tubes were air-dried for 5-10 min. Proteins were dissolved in 100 μL of click chemistry buffer (25 mM HEPES, pH 7.4, 150 mM NaCl, and 4% (w/v) SDS). The proteins were sonicated for 30 min at room temperature to be resolubilized. The concentration of the resolubilized proteins was determined using a BCA assay. For each sample, 800 μg of proteins were used for click chemistry (for samples that have higher protein concentration, the volume was adjusted with click chemistry buffer).

Biotin-N_3 (5 µL of 5 mM solution in DMF), Tris[(1-benzyl-1H-1,2,3-triazole-4-yl)methyl]amine (5 µL of 2 mM solution in DMF), $CuSO_4$ (5 µL of 50 mM solution in H_2O) and Tris(2-carboxyethyl)phosphine (5 µL of 50 mM solution in H_2O) were added into the reaction mixture. The click chemistry reaction was allowed to proceed at 30°C for 60 min. Proteins were then precipitated using cold methanol, chloroform and and water as described above. After washing two times with cold methanol, the proteins were resolubilized in 120 µL of 4% SDS lysis buffer. Again, the concentrations of proteins were measured by BCA, and 10 µg of proteins for each sample were set aside as input. Then, 400 µg of proteins for sample was added into 10 mL of IP washing buffer (25 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.2% (v/v) NP-40) (this was to make sure that SDS was lower than 0.1% in IP washing buffer for efficient affinity purification with streptavidin beads). Streptavidin agarose beads (Thermo Fisher, 20 µl) were added into each sample. The mixture was agitated for 1 h at room temperature. After washing the beads three times with 1 mL of IP washing buffer, 24 µL of 4% (w/v) SDS lysis buffer and 6 µL of 6× loading dye were added to the beads and boiled for 10 min. The IP samples and inputs were resolved using 12% SDS-PAGE gel, transferred to nitrocellulose membrane, and analyzed by western blot for SHMT2 (samples; IP) and (input). The signal was recorded using a Typhoon 9400 Variable Mode Imager (GE Healthcare Life Sciences).

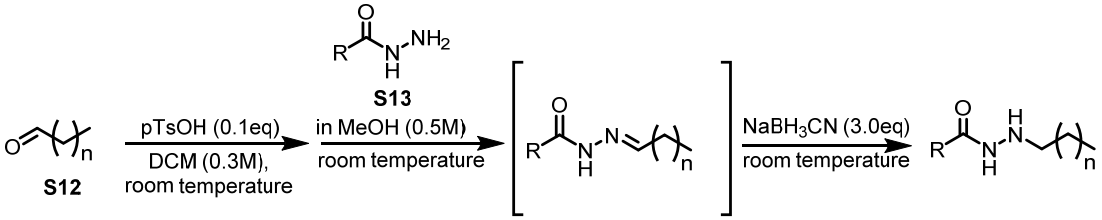
Quantification of Western Blot Signal and Statistical Analysis. The SHMT2 band intensity in the western blot was quantified with ImageJ. All signals were normalized using the control signal. Statistical evaluation of imaging data was done using two-way ANOVA. Differences between two groups were examined using unpaired two-tailed Student's t test. The P values were indicated (***$p<0.0005$). No statistical tool was used to pre-determine sample size. No blinding was done, no randomization was used, and no sample was excluded from analysis.

Cell permeability assay with MCF7 cell (Supplementary Figure 3). SIS7 and SIS17 were synthesized by our lab and FT895 (AG-CR1-3910-M010) was purchased from Adipogen for this assay. For reference traces of LC-MS, 5 mM stocks of SIS7, SIS17 and FT895 were prepared in DMSO. One µL of each stock was dissolved in 99 µl of acetonitrile. After checking there was no precipitation in acetonitrile (1 µL of 5 mM SIS7, SIS17 and FT895 are all soluble in 99 µL of acetonitrile), each diluted sample was injected into LC-MS. UV (280 nm) intensity was measured as a reference value indicating the amount of each compound (A_{ref}). For sample traces of LC-MS, 50 mM stocks of SIS7, SIS17 and FT895 were prepared in DMSO. In 80% confluent MCF7 cells in 15-cm dish in 8 mL DMEM media, 8 µL of each compound was added to each dish. The cells were incubated for 6 hrs at 37°C. After 6 hrs, the cells were collected,

washed with 1X PBS three times and lysed with 200 µL of cold water and 200 µL of dichloromethane by vortexing at maximum power for 6 mins. All samples were centrifuged at 17,000 g for 20 mins at 4ºC. The water layer was gently removed using a pipette and the dichloromethane layer was transferred into a new 1.5 ml tube to prevent cell pellets interrupting the flow of LC-MS. Dichloromethane was evaporated in a fume hood, and white residues left in the tubes were dissolved in 100 µL of acetonitrile. The samples were injected in LC-MS and their UV (280 nm) intensity was measured (A_{sample}). For both reference and samples, 50 µL was injected into LC-MS.

D. Synthetic Procedures of HDAC11-specific Inhibitors (SIS-compounds) and FT895

Procedure for the Synthesis of SIS4.[4]



Hexadecanal **S12** and substituted benzoic hydrazide **S13** are commercially available from Sigma-Aldrich, VWR chemical and Combi-block and they were used directly without further purification.

In a 10 mL round bottom flask equipped with a stir bar, hexadecanal **S12** (56 mg, 0.233 mmol, 1.0 equiv) and p-TsOH•H_2O (5 mg, 0.025 mmol, 0.1 equiv) were dissolved in CH_2Cl_2 (0.8 mL) at room temperature. After stirring for 30 min at room temperature, 4-bromobenzoic hydrazide **S13** (50 mg, 0.232 mmol, 1.0 equiv) in methanol (0.47 mL) was added into hexadecanal and p-TsOH reaction mixture. This mixture was stirred for 1 h at room temperature and then $NaCNBH_3$ (44 mg, 0.699 mmol, 3.0 equiv) in MeOH (0.8 mL) was added. After further stirring for 1 hr at room temperature, the reaction was quenched by $NaHCO_3$ (1M, aq) until there is no hydrogen gas generated from remaining $NaCNBH_3$. The organic phase was separated from the aqueous phase, and the aqueous phase was extracted with CH_2Cl_2 (5 mL × 2). The combined organic phase was dried over Na_2SO_4 and concentrated *in vacuo* to afford the crude product **SIS4**. After concentration in vacuo, the residue was subsequently purified through column chromatography (hexane/EtOAc: 2:8) to afford SIS4 as white powder (58mg, 57% yield from **S12**).

Compounds **SIS2, 5, 7, 10, 17, 21, 39, 40, 49, 50 and 51** were synthesized following the same procedure

as that for **SIS4**.

SIS4

4-bromo-N'-hexadecylbenzohydrazide (SIS4): yield: 43%, white powder; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62 (d, *J* = 8.3 Hz, 2H), 7.58 (d, *J* = 8.2 Hz, 2H), 3.31 (s, 1H), 2.92 (t, *J* = 7.3 Hz, 2H), 1.62 – 1.51 (m, 2H), 1.25 (s, 34H), 0.88 (t, *J* = 6.8 Hz, 4H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.3, 132.0, 131.7, 128.5, 126.6, 104.6, 77.3, 77.0, 76.8, 52.6, 52.3, 34.3, 32.5, 31.9, 29.71, 29.70, 29.68, 29.62, 29.58, 25.08, 24.62, 29.5, 29.5, 29.4, 29.2, 28.0, 27.3, 27.1, 25.1, 24.6, 22.7, 14.1; HRMS (DART Orbitrap, m/z): calcd for $C_9H_9Br_2O_2^+$ ($[M + H]^+$) 439.2312, found 439.2307.

SIS2

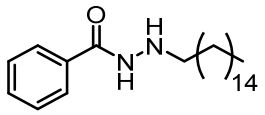
4-(tert-butyl)-N'-hexadecyl benzohydrazide (**SIS2**): yield: 48%, white powder; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81 (s, 1H), 7.69 (d, J = 8.4 Hz, 2H), 7.45 (d, J = 8.4 Hz, 2H), 2.92 (t, J = 7.3 Hz, 2H), 1.59 – 1.48 (m, 2H), 1.33 (s, 9H), 1.31 – 1.17 (m, 26H), 0.88 (t, J = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.2, 155.4, 130.0, 126.7, 125.6, 52.4, 35.0, 31.9, 31.2, 31.15, 29.71, 29.68, 29.67, 29.63, 29.60, 29.57, 29.4, 28.1, 27.1, 22.7, 14.1; HRMS (DART Orbitrap, m/z): calcd for $C_{27}H_{48}N_2O^+$ ($[M+H]^+$) 417.3839, found. 417.3828.

SIS5

4-fluoro-N'-hexadecylbenzohydrazide yield (**SIS5**): yield: 36%, white powder; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 – 7.72 (m, 2H), 7.64 (s, 1H), 7.16 – 7.08 (m, 2H), 2.92 (t, *J* = 7.3 Hz, 2H), 1.57 – 1.46 (m, 2H), 1.32 – 1.21 (m, 26H), 0.88 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.3, 165.9, 163.9, 129.3, 115.8, 52.4, 31.9, 29.71, 29.90, 29.67, 29.59, 29.5, 29.4, 28.1, 27.1, 22.7, 14.1. HRMS (DART Orbitrap, m/z): calcd for $C_{23}H_{40}FN_2O^+$($[M+H]^+$) 379.3119, found 379.3110.

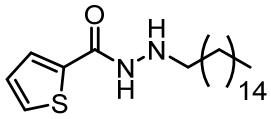
SIS7

4-(dimethylamino)-N'-hexadecylbenzohydrazide (**SIS7**):yield: 64%, white powder; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (d, J = 8.8 Hz, 2H), 6.66 (d, J = 8.8 Hz, 2H), 3.02 (s, 6H), 2.90 (t, J = 7.2 Hz, 2H), 1.38-1.25 (m, 29H), 0.87 (t, J = 6.5 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.4, 152.7, 128.3, 119.5, 111.1, 52.5, 40.1, 31.9, 29.71, 29.69, 29.67, 29.63, 29.60, 29.58, 29.38, 28.1, 27.2, 22.7, 14.1; HRMS (DART Orbitrap, m/z): calcd for $C_{25}H_{46}N_3O^+$ ([M + H]$^+$) 404.3635, found 404.3626.



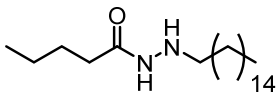
SIS10

N'-hexadecylbenzohydrazide (**SIS10**): yield: 39%, white powder; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.4 Hz, 2H), 7.52 (d, *J* = 7.4 Hz, 1H), 7.44 (t, *J* = 7.6 Hz, 2H), 2.93 (t, *J* = 7.3 Hz, 2H), 1.53 (dd, *J* = 14.8, Hz, 2H), 1.42 – 1.20 (m, 28H), 0.88 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.2, 132.9, 131.8, 128.7, 126.8, 52.4, 31.9, 29.71, 29.70, 29.67, 29.62, 29.59, 29.4, 28.1, 27.1, 22.7, 14.1; HRMS (DART Orbitrap, m/z): calcd for $C_{23}H_{41}N_2O^+$ ([M + H]$^+$) 361.3213, found 361.3205.



SIS17

N'-hexadecylthiophene-2-carbohydrazide (**SIS17**): yield: 51%, white powder; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53 (dd, *J* = 18.0, 4.4 Hz, 2H), 7.11 (t, *J* = 4.4 Hz, 1H), 2.95 (t, *J* = 7.3 Hz, 2H), 1.67 – 1.48 (m, 2H), 1.46 – 1.07 (m, 28H), 0.90 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.0, 136.6, 130.1, 128.4, 127.7, 77.3, 77.0, 76.8, 52.4, 31.9, 31.9, 29.71, 29.68, 29.62, 29.53, 29.47, 29.4, 29.2, 28.0, 28.0, 27.6, 27.3, 27.1, 26.4, 22.7, 14.1; HRMS (DART Orbitrap, m/z): calcd for $C_{21}H_{39}N_2OS^+$ ([M + H]$^+$) 367.2778, found 367.2768.

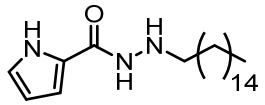


SIS18

N'-hexadecylpentanehydrazide (**SIS18**): yield 42%, white powder; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.91 (s, 1H), 2.80 (t, J = 7.1 Hz, 2H), 2.13 (t, J = 7.6 Hz, 2H), 1.76 – 1.54 (m, 2H), 1.51 – 1.41 (m, 2H), 1.37 – 1.16 (m, 26H), 0.95 – 0.81 (m, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.5, 52.3, 34.5, 31.9, 29.71, 29.69, 29.67, 29.62, 29.58, 29.54, 29.5, 29.4, 28.0, 27.6, 27.1, 22.7, 22.4, 14.14, 13.76; HRMS (DART Orbitrap, m/z): calcd for $C_{21}H_{45}N_2O^+$ ([M + H]$^+$) 341.3526, found 341.3518.

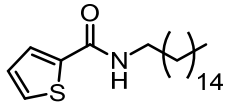
SIS21

N'-hexadecyl-4-(trifluoromethyl)benzohydrazide (**SIS21**): yield 55%, white powder; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 (d, *J* = 8.1 Hz, 2H), 7.72 (d, *J* = 8.2 Hz, 2H), 2.95 (t, *J* = 7.2 Hz, 2H), 1.59 – 1.50 (m, 2H), 1.38 – 1.21 (m, 26H), 0.88 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.9, 136.15, 133.6, 128.2, 127.3, 125.8, 123.6, 52.3, 31.9, 29.71, 29.67, 29.61, 29.57, 29.52, 29.4, 28.0, 27.1, 22.7, 14.1; ^{19}F NMR (376 MHz, $CDCl_3$) δ -63.1; HRMS (DART Orbitrap, m/z): calcd for $C_{24}H_{40}F_3N_2O^+$ ($[M + H]^+$) 429.3087, found 429.3025.



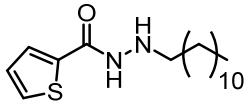
SIS39

N'-hexadecyl-^{1}H-pyrrole-2-carbohydrazide (**SIS39**): yield 29%, white powder; ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.31 (s, 1H, pyrrole NH), 7.24 (q, *J* = 1.8 Hz, 1H, amide NH), 7.02 – 6.86 (m, 1H), 6.27 (ddd, *J* = 11.9, 6.1, 3.0 Hz, 1H), 5.78 (s, 1H), 2.96 – 2.68 (m, 3H), 2.55 (ddd, *J* = 12.3, 9.9, 5.3 Hz, 1H), 1.67 – 1.44 (m, 10H), 1.45 – 1.11 (m, 57H), 0.90 (t, *J* = 6.8 Hz, 7H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.6, 161.3, 160.6, 124.6, 124.6, 124.3, 122.0, 121.1, 116.9, 109.9, 109.7, 109.7, 109.5, 108.7, 77.3, 77.1, 76.8, 59.8, 58.7, 31.9, 29.72, 29.68, 29.64, 29.61, 29.58, 29.56, 29.54, 29.46, 29.4, 28.0, 27.4, 27.3, 27.2, 27.1, 26.6, 22.7, 14.1; HRMS (DART Orbitrap, m/z): calcd for $C_{21}H_{40}N_3O^+$ ($[M + H]^+$) 350.3166, found 350.3159.



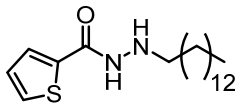
SIS40

N-hexadecylthiophene-2-carboxamide (**SIS40**): yield 48%, white powder; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 (dd, *J* = 12.5, 4.3 Hz, 2H), 7.09 (t, *J* = 4.3 Hz, 1H), 5.96 (s, 1H), 3.45 (q, *J* = 6.8 Hz, 2H), 1.73 – 1.51 (m, 5H), 1.28 (s, 25H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.8, 139.2, 129.6, 127.8, 127.6, 77.3, 77.0, 76.8, 40.1, 31.9, 29.71, 29.69, 29.67, 29.66, 29.60, 29.55, 29.4, 29.3, 27.0, 22.7, 14.1; HRMS (DART Orbitrap, m/z): calcd for $C_{21}H_{38}NOS^+$ ($[M + H]^+$) 352.2669, found 352.2663.



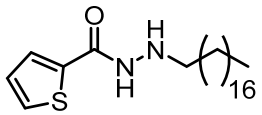
SIS49

N'-dodecylthiophene-2-carbohydrazide (**SIS49**): yield 56%, white powder; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64 – 7.42 (m, 2H), 7.19 – 7.04 (m, 1H), 3.47 – 3.25 (m, 3H), 1.68 – 1.49 (m, 2H), 1.43 – 1.08 (m, 22H), 0.90 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.1, 162.0, 136.6, 130.1, 128.4, 127.7, 125.9, 104.6, 77.3, 77.0, 76.8, 52.6, 52.4, 51.4, 34.1, 33.6, 32.5, 31.9, 30.0, 29.90, 29.71, 29.70, 29.67, 29.66, 29.63, 29.61, 29.58, 29.54, 29.52, 29.50, 29.4, 29.2, 28.4, 28.0, 27.5, 27.3, 27.1, 26.6, 26.4, 25.0, 24.7, 24.6, 22.7, 20.8, 18.5, 16.8, 14.1; HRMS (DART Orbitrap, m/z): calcd for $C_{17}H_{31}N_2OS^+$ ($[M + H]^+$) 311.2152, found 311.2143.



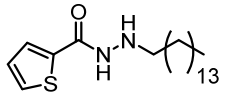
SIS50

N'-tetradecylthiophene-2-carbohydrazide (**SIS50**): yield 44%, white powder; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53 (dd, *J* = 14.8, 4.4 Hz, 2H), 7.11 (t, *J* = 4.3 Hz, 1H), 3.33 (s, 1H), 2.95 (t, *J* = 7.1 Hz, 1H), 1.58 (ddt, *J* = 34.1, 14.7, 6.8 Hz, 3H), 1.48 – 1.08 (m, 31H), 0.90 (t, *J* = 6.8 Hz, 5H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.0, 130.1, 128.4, 127.7, 104.6, 77.3, 77.0, 76.8, 52.6, 52.4, 32.5, 31.9, 30.4, 29.7, 29.62, 29.59, 29.4, 29.3, 29.2, 28.9, 28.7, 28.0, 27.4, 27.1, 26.0, 25.8, 25.1, 24.6, 23.8, 23.6, 23.0, 22.7, 14.1; HRMS (DART Orbitrap, m/z): calcd for $C_{19}H_{35}N_2OS^+$ ($[M + H]^+$) 339.2465, found 339.2456.



SIS52

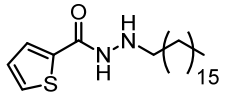
N'-octadecylthiophene-2-carbohydrazide (**SIS52**): yield 32%, white powder; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.61 – 7.45 (m, 2H), 7.17 – 7.10 (m, 1H), 4.38 (t, *J* = 5.8 Hz, 2H), 3.33 (s, 11H), 2.95 (t, *J* = 7.3 Hz, 1H), 2.74 (q, *J* = 7.2 Hz, 8H), 1.66 – 1.51 (m, 6H), 1.22 (d, *J* = 52.6 Hz, 89H), 0.91 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 130.2, 130.1, 128.5, 128.4, 127.8, 127.7, 104.6, 77.3, 77.0, 76.8, 52.6, 52.4, 45.8, 32.5, 31.9, 29.7, 29.6, 29.5, 29.4, 28.0, 27.1, 24.6, 22.7, 14.1, 8.6; HRMS (DART Orbitrap, m/z): calcd for $C_{23}H_{43}N_2OS^+$ ($[M + H]^+$) 395.3370, found. 395.3081.



SIS65

N'-pentadecylthiophene-2-carbohydrazide (SIS65): yield 62%, white powder; ^{1}H NMR (500 MHz, Chloroform-d) δ 7.62 – 7.45 (m, 2H), 7.11 (t, J = 4.3 Hz, 1H), 2.95 (t, J = 7.4 Hz, 2H), 1.55 (t, J = 7.4 Hz,

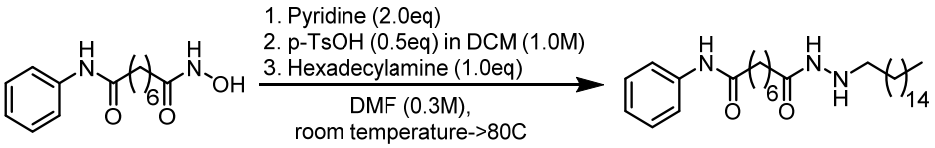
2H), 1.27 (s, 25H), 0.90 (t, J = 6.8 Hz, 4H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.98, 136.62, 133.69, 130.93, 130.10, 128.84, 128.36, 127.71, 77.29, 77.04, 76.78, 52.36, 34.38, 31.94, 29.71, 29.70, 29.67, 29.62, 29.59, 29.53, 29.38, 29.24, 28.00, 27.11, 22.71, 14.14; HRMS (DART Orbitrap, m/z): calcd for $C_{20}H_{36}N_2OS^+$ ($[M + H]^+$) 353.2621, found. 353.2613.



SIS66

N'-heptadecylthiophene-2-carbohydrazide (SIS66): yield 58%, white powder; ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.63 – 7.42 (m, 1H), 7.12 (dt, *J* = 11.9, 4.4 Hz, 1H), 4.14 (q, *J* = 7.2 Hz, 0H), 2.95 (t, *J* = 7.3 Hz, 1H), 1.27 (d, *J* = 2.3 Hz, 22H), 0.90 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.98, 161.57, 161.52, 137.34, 137.00, 136.62, 133.41, 130.23, 130.12, 128.38, 128.31, 128.14, 127.79, 127.76, 127.71, 126.49, 77.29, 77.04, 76.78, 65.25, 63.09, 60.42, 52.36, 32.84, 31.94, 30.25, 29.96, 29.74, 29.72, 29.68, 29.62, 29.59, 29.53, 29.46, 29.38, 27.96, 27.79, 27.25, 27.10, 22.71, 14.21, 14.14; HRMS (DART Orbitrap, m/z): calcd for $C_{22}H_{40}N_2OS^+$ ($[M + H]^+$) 381.2934, found. 381.2926.

Procedure for the Synthesis of SIS15.[5]

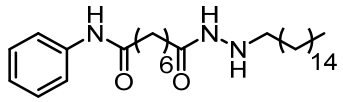


S14 **S14**

N^1-hydroxy-N^8-phenyloctanediamide (**S14)** is commercially available from Combi-Block and they were used directly without further purification.

To a stirred solution of N^1-hydroxy-N^8-phenyloctanediamide (**S14**) (100 mg, 0.38 mmol, 1.0 equiv) and pyridine (0.76 mL, 0.76 mmol, 2.0 equiv) in anhydrous DMF (0.76 mL, 0.5 M) was added dropwise a solution of p-TsOH (36 mg, 0.19 mmol, 0.5 equiv) in anhydrous dichloromethane (0.19 mL,) at room temperature. The mixture was refluxed for 1 h. Addition of water (5 mL) caused the intermediate to separate. Then, the crude was used for further reaction without purification. To a stirred solution of intermediate in anhydrous CH_2Cl_2 (1.3 ml) was gradually added a solution of hexadecylamine (92 mg, 0.38 mmol, 1.0 equiv) in anhydrous CH_2Cl_2 at room temperature under N_2. The mixture was refluxed for 12 hours, washed with 1% aqueous sodium hydrogen carbonate and water, dried with $MgSO_4$ and evaporated to dryness *in vacuo*. The residue was subsequently purified through column chromatography

with (hexane:EtOAc=8:2). (57 mg, 31% yield from S14).

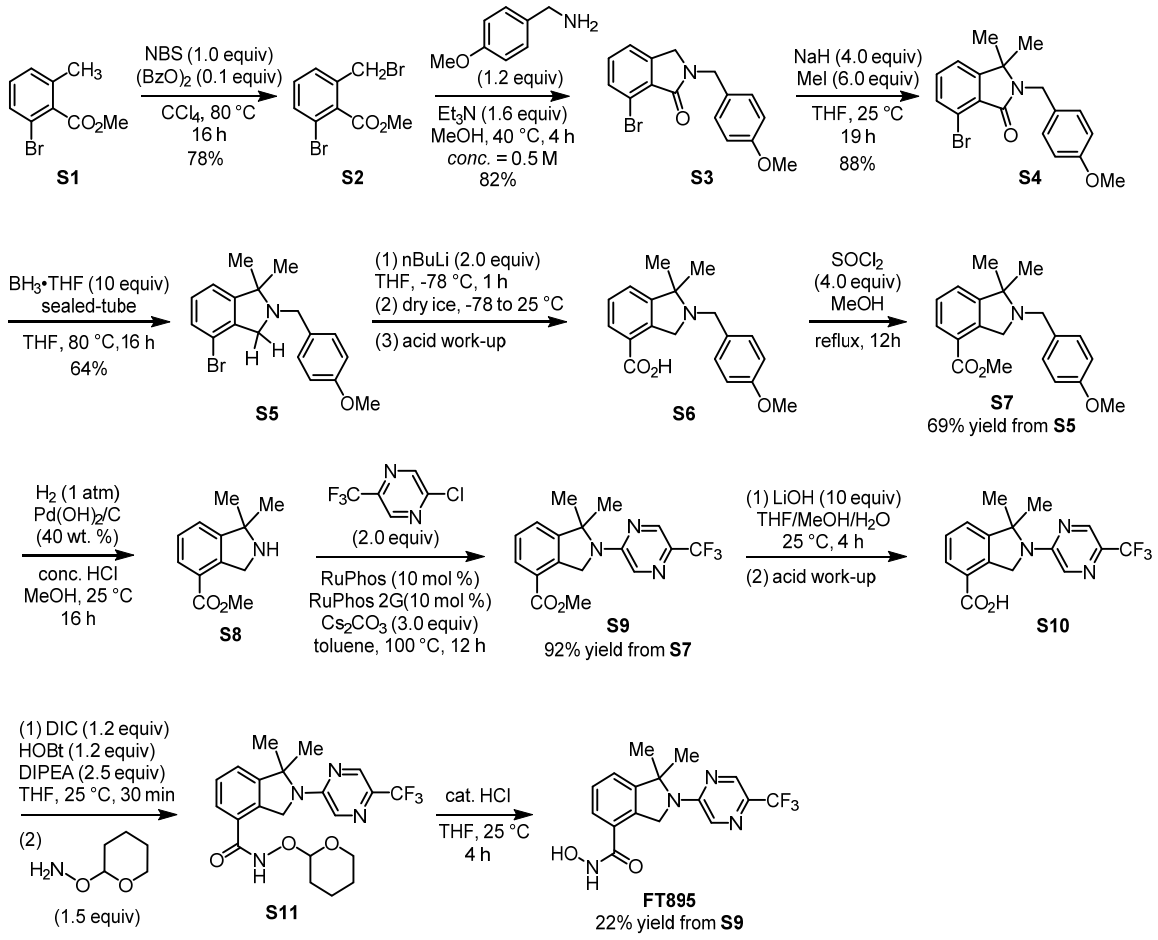


SIS15

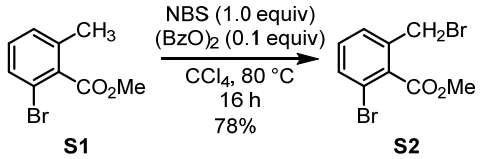
8-(2-hexadecylhydrazineyl)-8-oxo-N-phenyloctanamide (SIS15): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.55 (d, *J* = 7.9 Hz, 1H), 7.34 (t, *J* = 7.7 Hz, 2H), 7.12 (t, *J* = 7.4 Hz, 1H), 3.91 (d, *J* = 7.3 Hz, 1H), 2.38 (t, *J* = 7.4 Hz, 2H), 1.83 – 1.57 (m, 10H), 1.28 (s, 35H), 1.06 – 0.77 (m, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.4, 138.0, 129.0, 124.2, 119.7, 77.3, 77.0, 76.8, 32.8, 31.9, 30.4, 30.1, 29.71, 29.69, 29.67, 29.6, 29.5, 29.4, 29.4, 29.3, 28.9, 28.3, 28.0, 27.1, 22.7, 19.8, 14.1, 14.1; HRMS (DART Orbitrap, m/z): calcd for $C_{30}H_{54}N_3O_2^+$ ($[M + H]^+$) 488.4832, found 488.4279.

Procedure for the Synthesis of FT895. The compound **FT895** was synthesized according to a known procedure[6] but with several modifications as described below. All *in vitro* assays were performed by FT895 synthesized through below procedure.

Overall Synthetic Scheme of FT895.



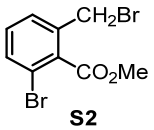
Detailed Procedures for FT895 Preparation.



Methyl 2-bromo-6-(bromomethyl)benzoate **S2** was synthesized from methyl 2-bromo-6-methylbenzoate **S1** through a known procedure.[6] **S1** is commercially available and it was used directly without further purification.

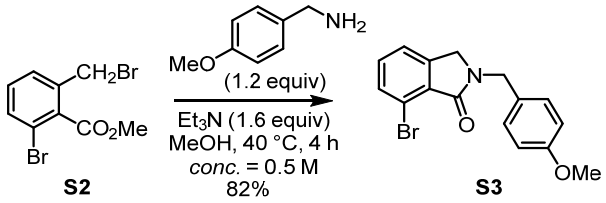
In a 250 mL round bottom flask equipped with a stir bar, methyl 2-bromo-6-methylbenzoate **S1** (6.7 g, 29.3 mmol, 1.0 equiv) was dissolved in CCl_4 (140 mL). Subsequently, NBS (5.21 g, 29.3 mmol, 1.0 equiv) and benzoyl peroxide (0.7 g, 2.9 mmol, 0.1 equiv) were added and the resulting mixture was vigorously stirred at 80 °C for 16 h. The reaction was cooled down to room temperature and filtered to remove solid. The filtrate was washed with brine (100 mL× 3) and dried over anhydrous Na_2SO_4. After

concentration *in vacuo*, the desired product methyl 2-bromo-6-(bromomethyl)benzoate **S2** was obtained as light yellow oil, which was used in the next step without further purification (7.0 g, 78% yield).



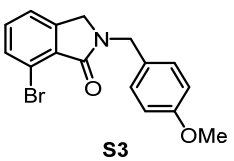
S2

Methyl 2-bromo-6-(bromomethyl)benzoate (**S2**): ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57 (d, J = 8.0 Hz, 1H), 7.41 (d, J = 7.7 Hz, 1H), 7.28 (dd, J = 8.7, 7.1 Hz, 1H), 4.51 (s, 2H), 4.02 (s, 3H); LC-MS (ESI, m/z): calcd for $C_9H_9Br_2O_2^+$([M + H]$^+$) 305.89, found 305.90.



7-Bromo-2-(4-methoxybenzyl)isoindolin-1-one **S3** was synthesized from **S2** through a known procedure with modifications.[6]

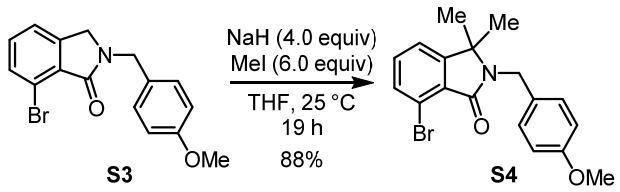
To an oven-dried 100 mL round bottom flask equipped with a stir bar was added **S2** (5.6 g, 18.1 mmol, 1.0 equiv). After the flask was evacuated and backfilled with N_2 twice, anhydrous MeOH (36 mL), 4-methoxybenzylamine (2.9 mL, 22 mmol, 1.2 equiv) and Et_3N (4.0 mL, 29 mmol, 1.6 equiv) were added via syringes. The reaction was warmed up to 40 °C and stirred at this temperature for 4 h. The mixture was cooled to room temperature and concentrated under reduced pressure. H_2O (50 mL) was added and the solution was extracted with CH_2Cl_2 (100 mL× 3). The organic phase was separated from the aqueous phase, and dried over Na_2SO_4. After concentration *in vacuo*, the residue was subsequently purified through column chromatography (hexanes/EtOAc: from 10:1 to 2:1) to afford the product 7-bromo-2-(4-methoxybenzyl)isoindolin-1-one **S3** as white solid (4.8 g, 82% yield).



S3

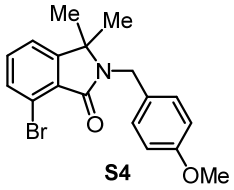
7-Bromo-2-(4-methoxybenzyl)isoindolin-1-one (**S3**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (d, J = 6.7 Hz, 1H), 7.39 – 7.22 (m, 5H), 6.86 (d, J = 8.2 Hz, 2H), 4.72 (s, 2H), 4.19 (s, 2H), 3.79 (s, 3H); LC-MS (ESI,

m/z): calcd for $C_{16}H_{15}BrNO_2^+$([M + H]$^+$) 332.02, found 332.03.

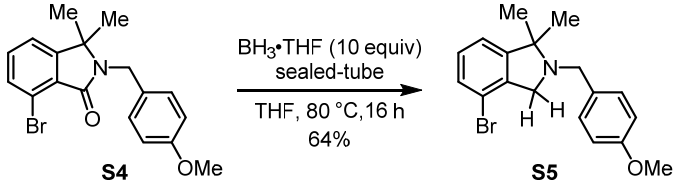


7-Bromo-2-(4-methoxybenzyl)-3,3-dimethylisoindolin-1-one **S4** was synthesized from **S3** through a known procedure with modifications.[6]

To a flame-dried 100 mL round bottom flask equipped with a stir bar was added NaH (60%, 576 mg, 14.4 mmol, 4.0 equiv). After the flask was evacuated and backfilled with N_2 twice, anhydrous THF (10 mL) was added via a syringe. Subsequently, **S3** (1.2 g, 3.6 mmol, 1.0 equiv) in THF (10 mL) was added dropwise and the mixture was stirred at room temperature for 3 h. MeI (1.34 mL, 21.6 mmol, 6.0 equiv) was added and the reaction mixture was kept stirring for additional 16 h until **S3** was fully consumed (monitored by TLC). The reaction was cooled down in ice-bath and quenched by adding H_2O (10 mL) slowly. EtOAc (50 mL) was added and the organic phase was separated from the aqueous phase, which was extracted with EtOAc (50 mL × 2). The combined organic layers were dried over anhydrous Na_2SO_4. After concentration *in vacuo*, the residue was subsequently purified through column chromatography (hexanes/EtOAc: from 10:1 to 4:1) to afford the product 7-bromo-2-(4-methoxybenzyl)-3,3-dimethylisoindolin-1-one **S4** as colorless oil (1.14 g, 88% yield).

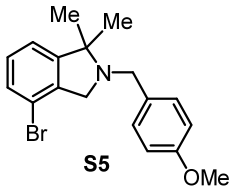


7-Bromo-2-(4-methoxybenzyl)-3,3-dimethylisoindolin-1-one (**S4**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 – 7.54 (m, 1H), 7.40 – 7.28 (m, 4H), 6.82 (d, J = 8.7 Hz, 2H), 4.67 (s, 2H), 3.77 (s, 3H), 1.35 (s, 6H); LC-MS (ESI, m/z): calcd for $C_{18}H_{19}BrNO_2^+$([M + H]$^+$) 360.05, found 360.07.



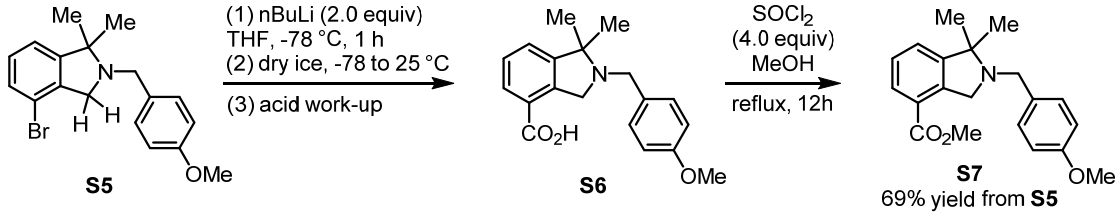
4-Bromo-2-(4-methoxybenzyl)-1,1-dimethylisoindoline **S5** was synthesized from **S4** through a known procedure.[6]

To a 500 mL sealed tube equipped with a stir bar were added substrate **S4** (1.2 g, 3.3 mmol, 1.0 equiv) and borane-THF complex solution (1.0 M, 33 mL, 33 mmol, 10 equiv). The reaction mixture was tightly sealed and stirred for 19 h at 80 °C. After cooling down to room temperature, MeOH (50 mL) was added to quench the reaction and the resulting mixture was stirred for additional 1 h at room temperature. After concentration *in vacuo*, the residue was subsequently purified through column chromatography (hexanes/EtOAc: from 50:1 to 10:1) to afford 4-bromo-2-(4-methoxybenzyl)-1,1-dimethylisoindoline **S5** as yellow oil (0.73 g, 64% yield).



S5

4-Bromo-2-(4-methoxybenzyl)-1,1-dimethylisoindoline (**S5**) ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 – 7.26 (m, 3H), 7.09 – 7.05 (m, 2H), 6.86 (d, J = 8.5 Hz, 2H), 3.80 (s, 3H), 3.75 (s, 2H), 3.73 (s, 2H), 1.35 (s, 6H); LC-MS (ESI, m/z): calcd for $C_{18}H_{21}BrNO^+$ ($[M + H]^+$) 346.07, found 346.07.

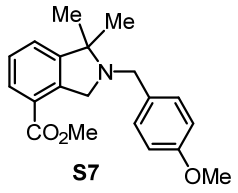


S5 **S6** **S7**
69% yield from **S5**

To a flame-dried 100 mL round bottom flask equipped with a stir bar was added **S5** (346 mg, 1.0 mmol, 1.0 equiv). After this flask was evacuated and backfilled with N_2 twice, anhydrous THF (20 mL) were added via a syringe and the mixture was cooled down to -78 °C. nBuLi (2.5 M in hexanes, 0.8 mL, 2.0 mmol, 2.0 equiv) was added dropwise and the mixture was stirred for 1 h at -78 °C. N_2 was removed and CO_2 (generated from dry-ice and dried over conc. H_2SO_4) was bubbled into the reaction mixture at -78 °C for 20 min. CO_2 was replaced by N_2 and the mixture was allowed to gradually warm up to room temperature and vigorously stirred for 8 h. 1.0 M HCl (10 mL) and EtOAc (40 mL) were added to quench the reaction. The organic phase was separated from the aqueous phase, and the aqueous phase was extracted with EtOAc (50 mL × 2). The combined organic phase was dried over Na_2SO_4 and

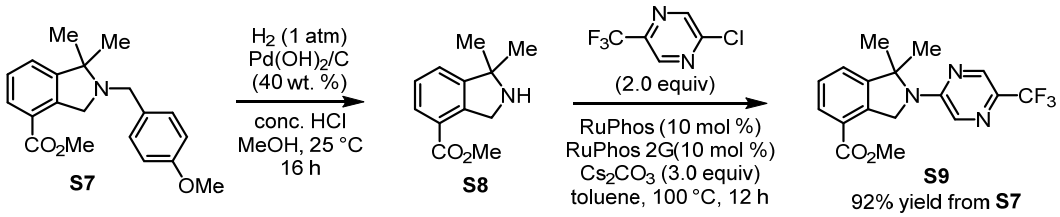
concentrated *in vacuo* to afford the crude product **S6**, which was directly used in the next step without further purification.

To a 50 mL round bottom flask equipped with a stir bar and reflux condenser was added the crude **S6** (1.0 mmol, 1.0 equiv) and anhydrous MeOH (20 mL). At 0 °C, $SOCl_2$ (0.29 mL, 4.0 mmol, 4.0 equiv) was added and the mixture was warmed up to reflux for 12 h. After concentration *in vacuo*, the residue was subsequently purified through column chromatography (hexanes/EtOAc: from 50:1 to 10:1) to afford methyl 2-(4-methoxybenzyl)-1,1-dimethylisoindoline-4-carboxylate **S7** as colorless oil (225 mg, 69% yield from **S5**).



S7

Methyl 2-(4-methoxybenzyl)-1,1-dimethylisoindoline-4-carboxylate (**S7**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (dd, *J* = 7.1, 1.1 Hz, 1H), 7.37 – 7.29 (m, 4H), 6.87 (d, *J* = 8.4 Hz, 2H), 4.11 (s, 2H), 3.82 (s, 3H), 3.81 (s, 3H), 3.78 (s, 2H), 1.37 (s, 6H); LC-MS (ESI, m/z): calcd for $C_{20}H_{24}NO_3^+$ ($[M + H]^+$) 326.16, found 326.14.



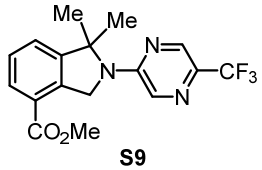
S7 **S8** **S9**
92% yield from **S7**

Methyl 1,1-dimethyl-2-(5-(trifluoromethyl)pyrazin-2-yl)isoindoline-4-carboxylate **S9** was synthesized from **S7** through a known procedure.[6]

To a 10 mL 2-neck round bottom flask equipped with a stir bar and a three-way adapter was added $Pd(OH)_2/C$ (80 mg, 40 wt. %). After the flask was evacuated and backfilled twice with N_2, a solution of **S7** (200 mg, 0.61 mmol, 1.0 equiv) in MeOH (5 mL) and conc. HCl (0.2 mL) were added via syringes. The mixture was degassed with brief evacuation and backfilled three times with H_2, and then vigorously stirred under H_2 balloon at 22 °C for 16 h (monitored by TLC until the substrate **S7** was fully consumed).

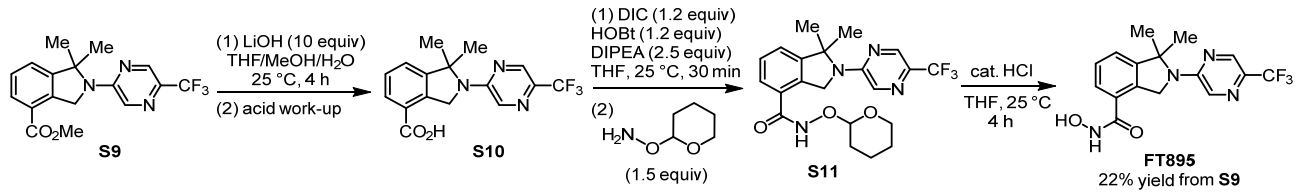
The solution was filtered through a Celite pad and washed with MeOH (10 mL). The filtrate was concentrated *in vacuo* and the residue was dissolved in CH_2Cl_2 (20 mL) and washed with 10% K_2CO_3 (3 mL). The organic phase was separated from the aqueous phase, and the aqueous phase was extracted with CH_2Cl_2 (10 mL × 3). The combined organic phase was dried over Na_2SO_4 and concentrated *in vacuo* to afford the crude product **S8**, which was directly used in the next step without further purification.

To a flame-dried sealable 3-dram vial equipped with a stir bar was added **S8** (120 mg, 0.583 mmol, 1.0 equiv), 2-chloro-5-(trifluoromethyl)pyrazine (215 mg, 1.18 mmol, 2.0 equiv), RuPhos Pd G2 (47 mg, 0.06 mmol, 10 mol%), RuPhos (28 mg, 0.06 mmol, 10 mol%) and $CsCO_3$ (570 mg, 1.75 mmol, 3.0 equiv). After this vial was evacuated and backfilled with N_2 twice, anhydrous toluene (3.0 mL) were added via syringe and the mixture was warmed up to 110 °C and stirred for 12 h. The reaction was cooled down to room temperature. EtOAc (4 mL) and H_2O (3 mL) were added to quench the reaction. The organic phase was separated from the aqueous phase, and the aqueous phase was extracted with EtOAc (10 mL × 3). The combined organic phase was dried over Na_2SO_4. After concentration *in vacuo*, the residue was subsequently purified through column chromatography (hexanes/EtOAc: from 20:1 to 5:1) to afford methyl 1,1-dimethyl-2-(5-(trifluoromethyl)pyrazin-2-yl)isoindoline-4-carboxylate **S9** as light yellow solid (186 mg, 92% yield form **S7**).



S9

Methyl 1,1-dimethyl-2-(5-(trifluoromethyl)pyrazin-2-yl)isoindoline-4-carboxylate (**S9**): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1H), 8.10 (s, 1H), 8.02 (d, J = 6.5 Hz, 1H), 7.52 – 7.42 (m, 2H), 5.13 (s, 2H), 3.97 (s, 3H), 1.84 (s, 6H); ^{19}F NMR (376 MHz, $CDCl_3$) δ -66.23 (s); LC-MS (ESI, m/z): calcd for $C_{17}H_{17}F_3N_3O_2^+$ ($[M + H]^+$) 352.12, found 352.13.



S9 **S10** **S11** **FT895**
22% yield from **S9**

In a 2-dram vial equipped with a stir bar, compound **S9** (35 mg, 0.1 mmol, 1.0 equiv) was dissolved in a

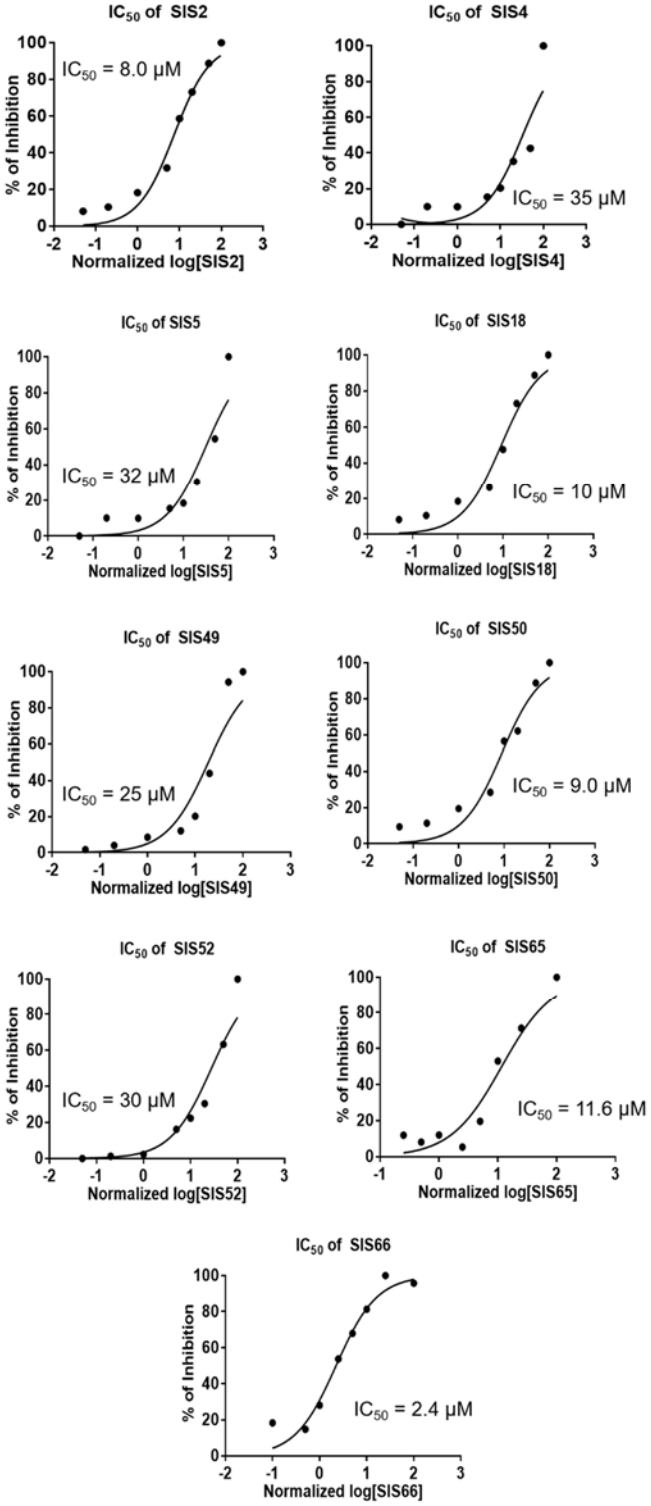
mixed solvent of MeOH/THF (1:4) (1.5 mL) and cooled to 0 °C. LiOH (42 mg, 1.0 mmol, 10 equiv) in 0.5 mL H_2O was added and the reaction was vigorously stirred for 4 h until the starting material was fully consumed (monitored by TLC). The organic solvent was removed *in vacuo* and the residue was diluted with H_2O (5 mL) and Et_2O (1 mL). The organic phase was separated from the aqueous phase, and the aqueous phase was acidified with aqueous 1.0 M HCl. EtOAc (5 mL) was added. The organic phase was separated from the aqueous phase, and the aqueous phase was extracted with EtOAc (10 mL × 3). The combined organic phase was dried over Na_2SO_4 and concentrated *in vacuo* to afford the crude product **S10**, which was used directly in the next step without purification.

To an oven-dried 3-dram vial equipped with a stir bar were added the crude compound **S10** (25 mg, 0.074 mmol, 1.0 equiv) obtained in last step and anhydrous THF (1.5 mL). Subsequently, N, N-diisopropylcarbodiimide (DIC, 14 μL, 0.09 mmol, 1.2 equiv), hydroxybenzotriazole (HOBt, 14 mg, 0.09 mmol, 1.2 equiv) and N, N-diisopropylethylamine (DIPEA, 32 μL, 0.185 mmol, 2.5 equiv) were added and the mixture was stirred at room temperature for 30 min. *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (11 mg, 0.09 mmol, 1.2 equiv) in THF (0.5 mL) was then added and reaction mixture was kept stirring at room temperature for 2 h. The reaction was diluted with EtOAc (4 mL) and H_2O (2 mL). The organic phase was separated from the aqueous phase, and it was washed with brine (2 mL) and dried over Na_2SO_4. After concentration *in vacuo*, the residue was re-dissolved in THF (2 mL) and concentrated HCl (50 μL) was added. The mixture was stirred at room temperature for 2 h until the starting material **S11** was fully consumed (monitored by TLC). EtOAc (5 mL) and saturated $NaHCO_3$ solution (1 mL) were added to quench the reaction and to remove any residual hydrochloric acid. The organic phase was separated from the aqueous phase, and the aqueous phase was extracted with EtOAc (10 mL × 3) and dried over Na_2SO_4. The combined organic phase was dried over Na_2SO_4. After concentration *in vacuo*, the residue was purified through column chromatography (hexanes/EtOAc: from 4:1 to 1:2) to afford N-hydroxy-1,1-dimethyl-2-(5-(trifluoromethyl)pyrazin-2-yl)isoindoline-4-carboxamide **FT895** as white solid (7.7 mg, 22% yield form **S9**), which is a known compound.[6]

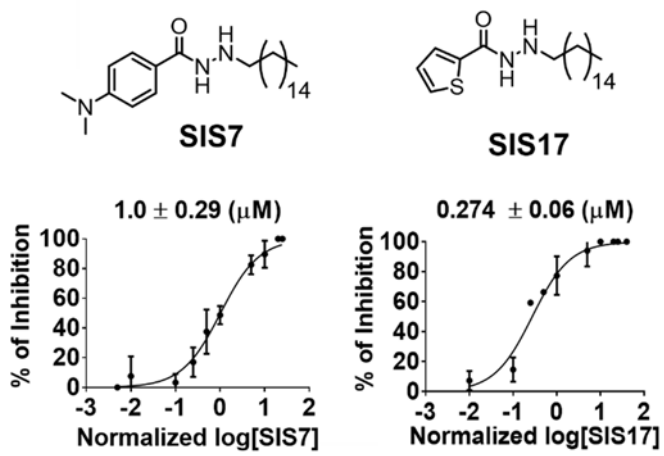
N-Hydroxy-1,1-dimethyl-2-(5-(trifluoromethyl)pyrazin-2-yl)isoindoline-4-carboxamide (FT895): ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.19 (s, 1H), 9.11 (s, 1H), 8.55 (s, 1H), 8.12 (s, 1H), 7.55 (d, J = 7.6 Hz, 2H), 7.43 (t, J = 7.7 Hz, 1H), 5.05 (s, 2H), 1.76 (s, 6H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.7, 153.4, 149.2, 139.8, 133.7, 131.7, 129.0 (q, J = 34.4 Hz), 128.7, 128.1, 126.3, 124.7, 123.3 (q, J = 271.3

Hz), 67.8, 53.3, 26.5; ^{19}F NMR (376 MHz, DMSO-d_6) δ -64.54 (s); LC-MS (ESI, m/z): calcd for $C_{16}H_{16}F_3N_4O_2^+$ ($[M + H]^+$) 353.11, found 353.07.

E. Supplementary Figures and Table.

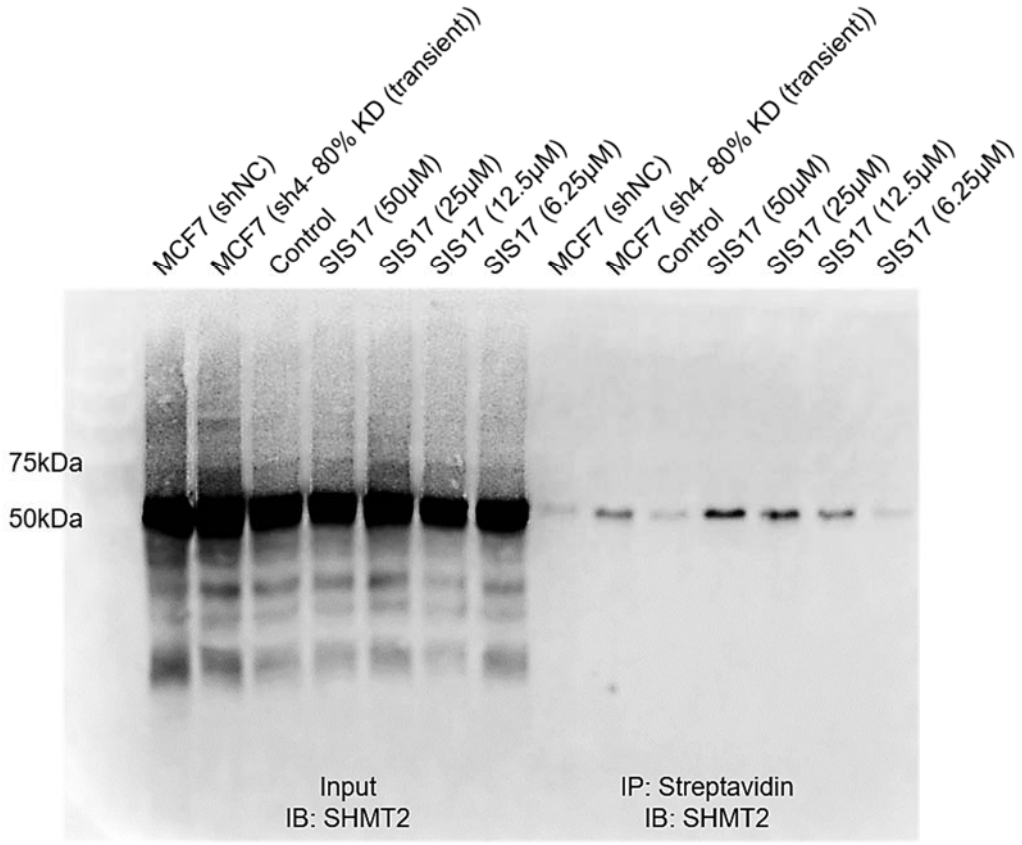


Supplementary Figure 1. IC_{50} curves of SIS2, SIS4, SIS5, SIS18, SIS49, SIS50, SIS52, SIS65 and SIS66 with myristoyl-H3K9.

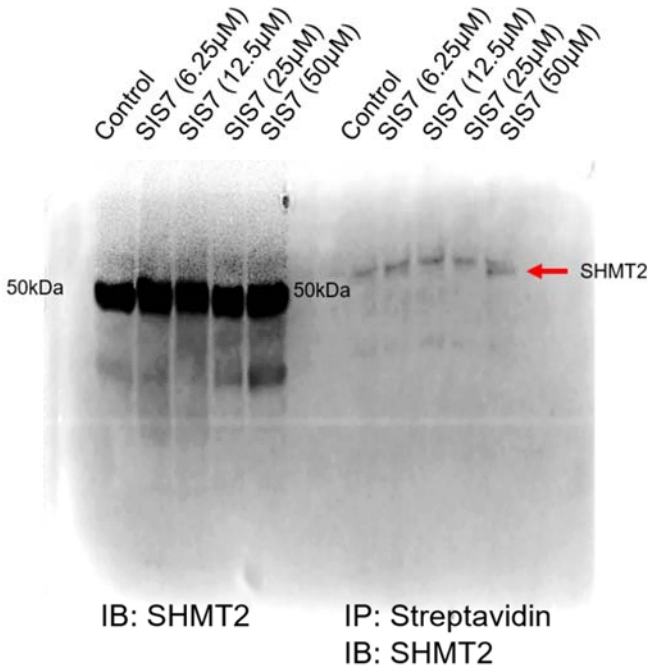


Supplementary Figure 2. IC_{50} curves of SIS7 and SIS17 with myristoyl-SHMT2.

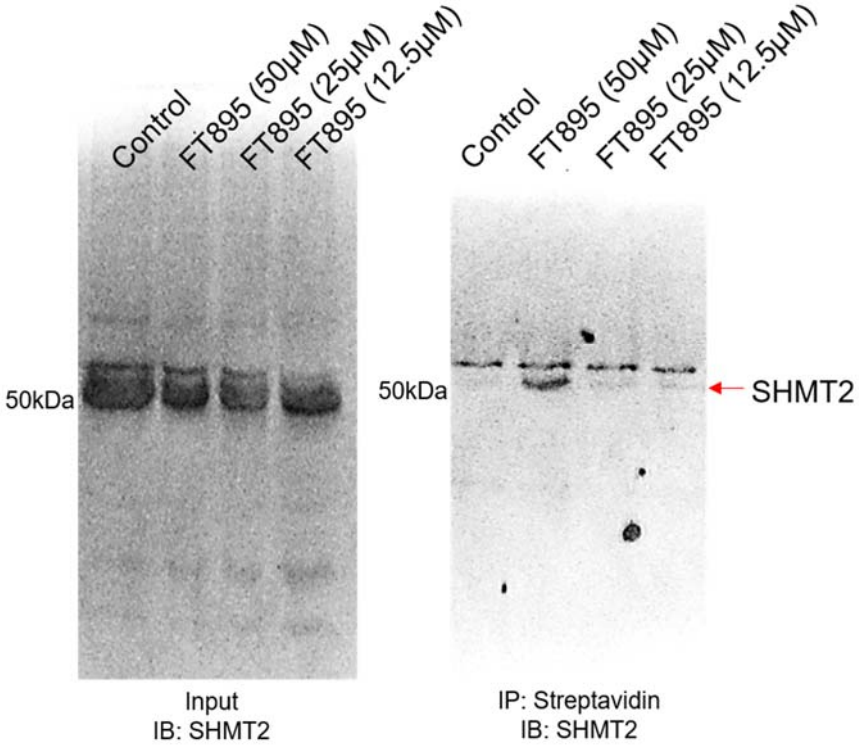
(A)



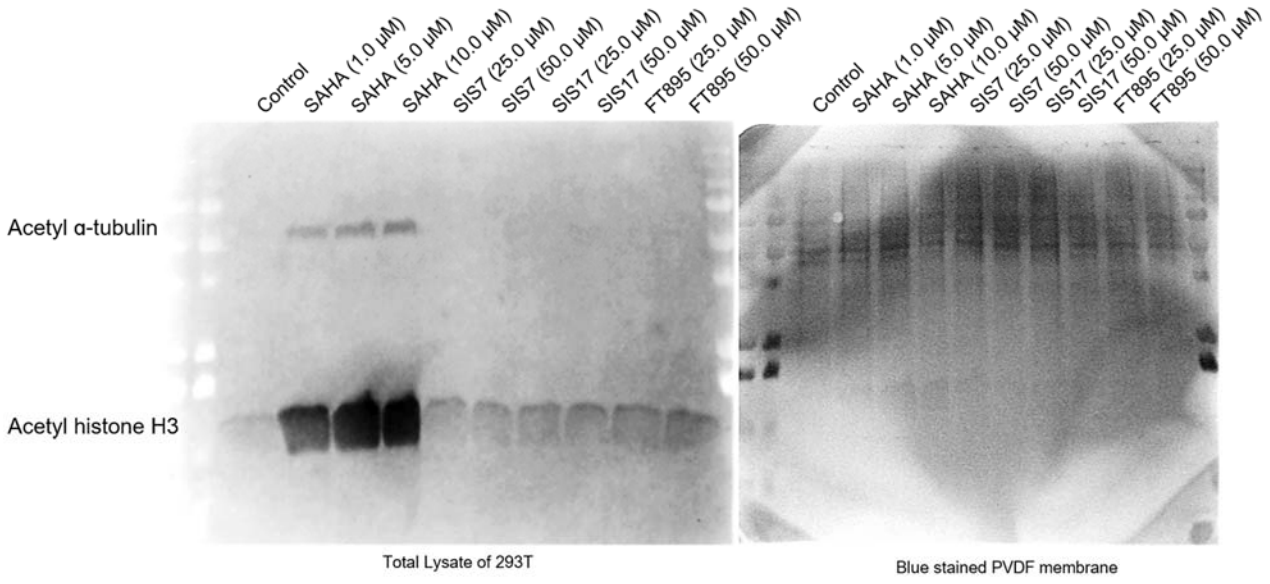
(B)


Control
SIS7 (6.25μM)
SIS7 (12.5μM)
SIS7 (25μM)
SIS7 (50μM)
Control
SIS7 (6.25μM)
SIS7 (12.5μM)
SIS7 (25μM)
SIS7 (50μM)
50kDa
50kDa
SHMT2
IB: SHMT2
IP: Streptavidin
IB: SHMT2

(C)


Control
FT895 (50μM)
FT895 (25μM)
FT895 (12.5μM)
Control
FT895 (50μM)
FT895 (25μM)
FT895 (12.5μM)
50kDa
50kDa
SHMT2
Input
IB: SHMT2
IP: Streptavidin
IB: SHMT2

(D)

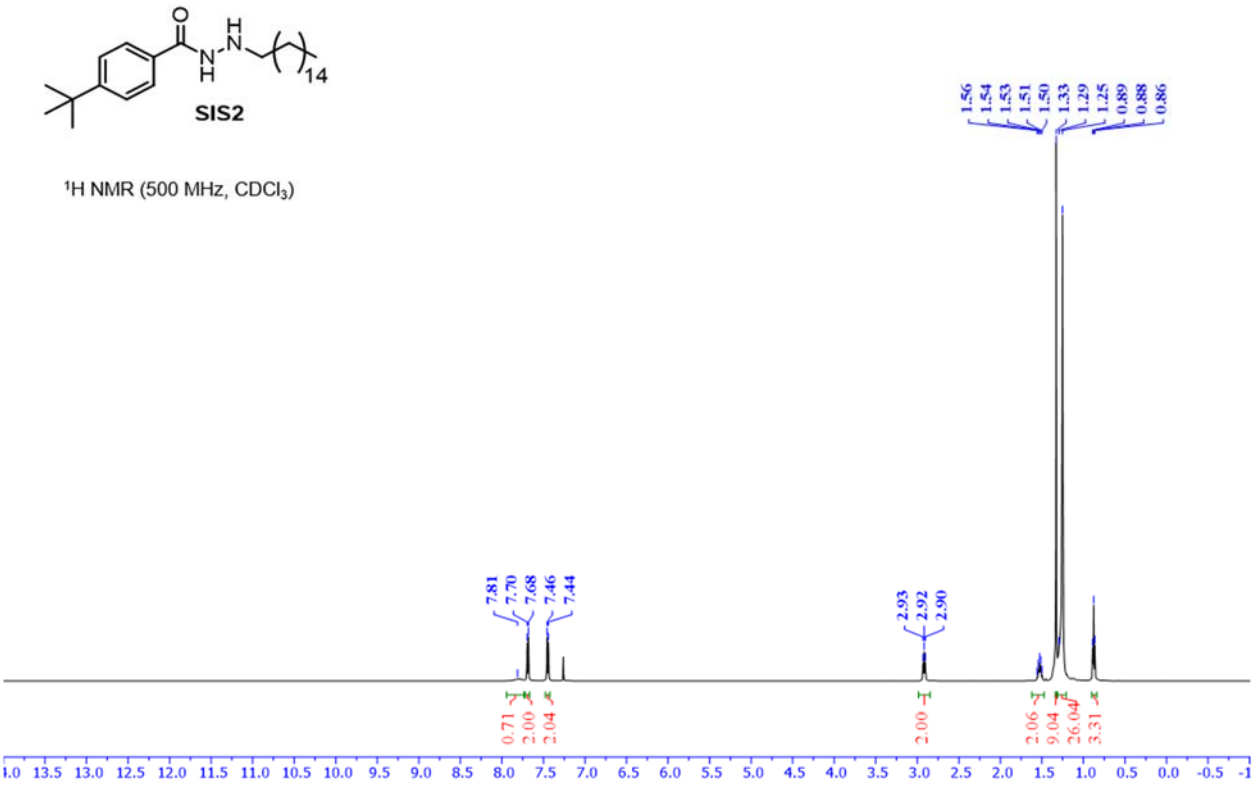


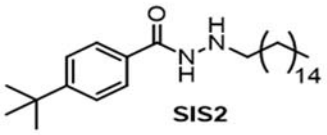
Supplementary Figure 3. Full gel images. (**A).** Endogenous fatty acylation level of SHMT2 with SIS17. **(B)** Endogenous fatty acylation level of SHMT2 with SIS7. **(C)** Endogenous fatty acylation level of SHMT2 with FT895. **(D)** Full gel image for Figure 4.

Supplementary Table 1. Measuring the cellular concentration of SIS7, SIS17, and FT895 using LC-MS.

Compounds	**Peak area of compounds extracted from cells**	**Peak area of 2.5 nmol of standard compounds**	**Amount of compounds in 10^6 cells (μg)**
SIS7	0	216099	0
SIS17	4728572	5188898	0.84
FT895	201191	10089691	0.03

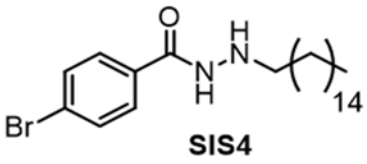
F. NMR Spectra





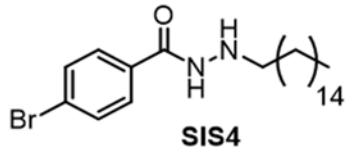
SIS2

^{13}C NMR (126 MHz, $CDCl_3$)



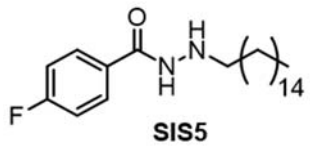
SIS4

^{1}H NMR (500 MHz, $CDCl_3$)



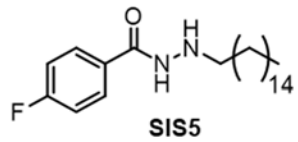
SIS4

^{13}C NMR (126 MHz, $CDCl_3$)



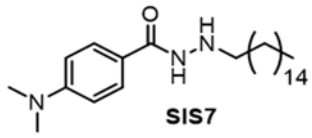
SIS5

^{1}H NMR (500 MHz, $CDCl_3$)



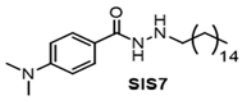
SIS5

^{13}C NMR (126 MHz, $CDCl_3$)



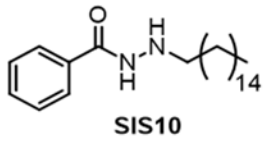
SIS7

^{1}H NMR (500 MHz, $CDCl_3$)



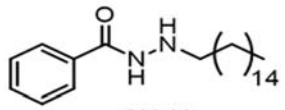
SIS7

^{13}C NMR (126 MHz, $CDCl_3$)



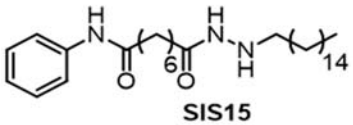
SIS10

^{1}H NMR (500 MHz, $CDCl_3$)



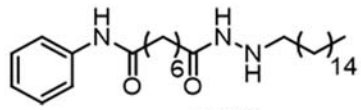
SIS10

^{13}C NMR (126 MHz, $CDCl_3$)



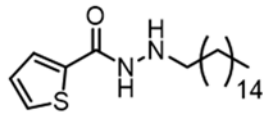
SIS15

^{1}H NMR (500 MHz, $CDCl_3$)



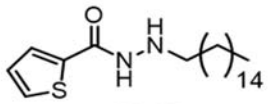
SIS15

^{13}C NMR (126 MHz, $CDCl_3$)



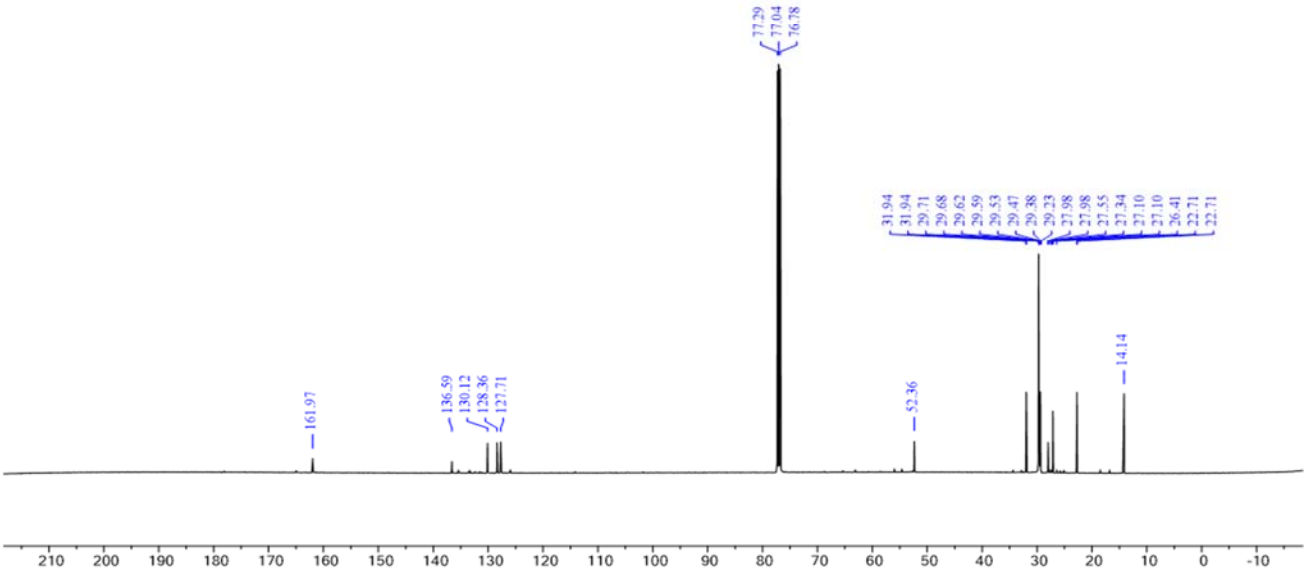
SIS17

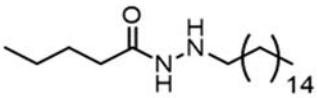
^{1}H NMR (500 MHz, $CDCl_3$)



SIS17

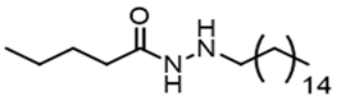
^{13}C NMR (126 MHz, $CDCl_3$)





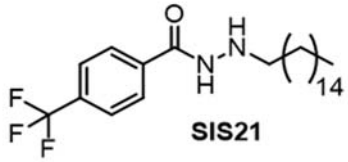
SIS18

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



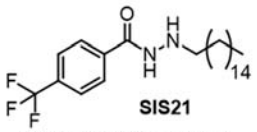
SIS18

^{13}C NMR (126 MHz, $CDCl_3$)



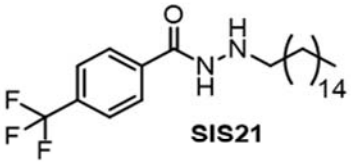
SIS21

^{1}H NMR (500 MHz, $CDCl_3$)



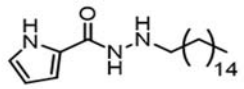
SIS21

^{13}C NMR (126 MHz, $CDCl_3$)



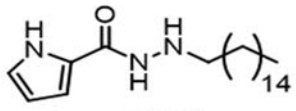
SIS21

^{19}F NMR (376 MHz, $CDCl_3$)



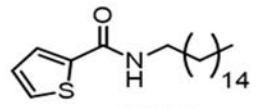
SIS39

^{1}H NMR (500 MHz, $CDCl_3$)



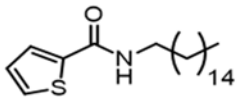
SIS39

^{13}C NMR (126 MHz, $CDCl_3$)



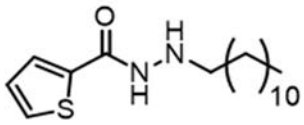
SIS40

^{1}H NMR (500 MHz, $CDCl_3$)



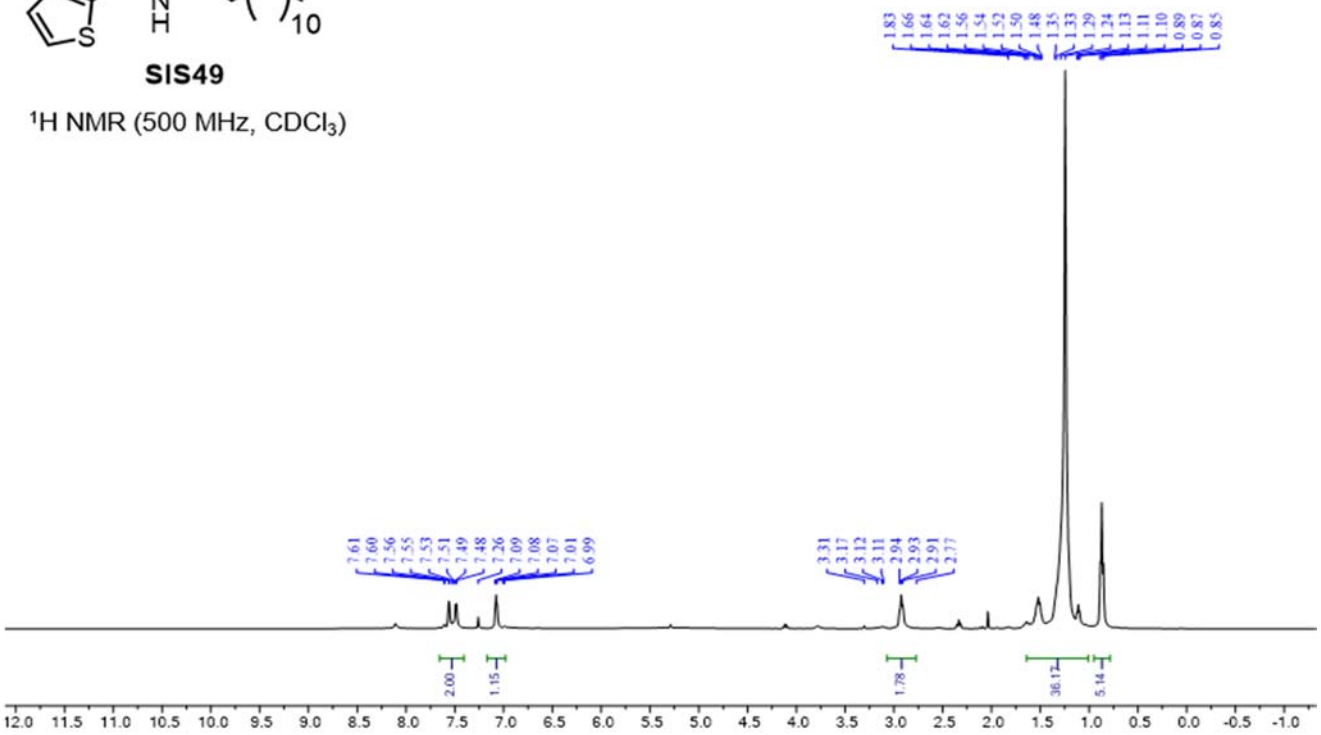
SIS40

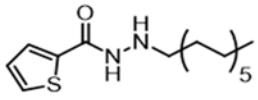
^{13}C NMR (126 MHz, $CDCl_3$)



SIS49

^{1}H NMR (500 MHz, $CDCl_3$)





SIS49

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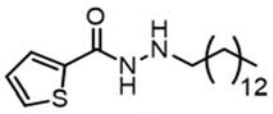
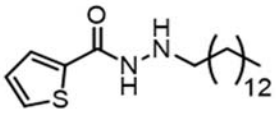


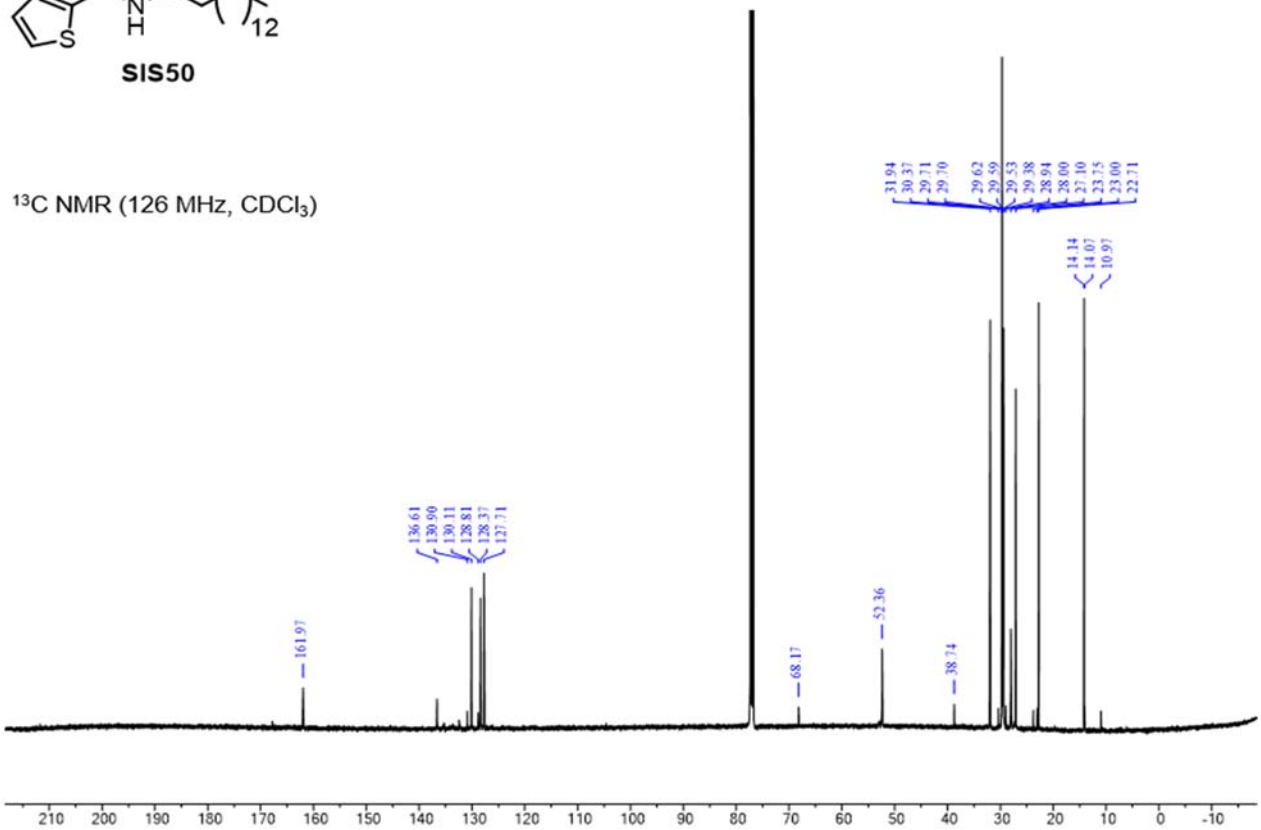
SIS50

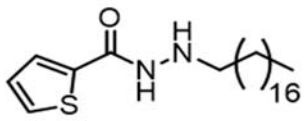
^{1}H NMR (500 MHz, $CDCl_3$)



SIS50

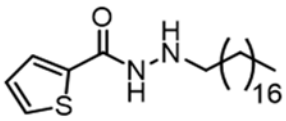
^{13}C NMR (126 MHz, $CDCl_3$)





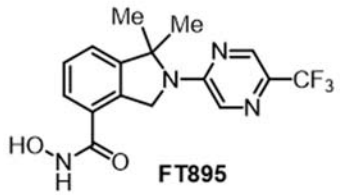
SIS52

^{1}H NMR (500 MHz, $CDCl_3$)



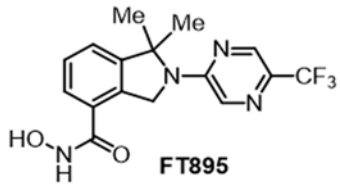
SIS52

^{13}C NMR (126 MHz, $CDCl_3$)



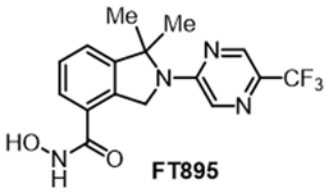
FT895

^{1}H NMR (500 MHz, DMSO-d6)



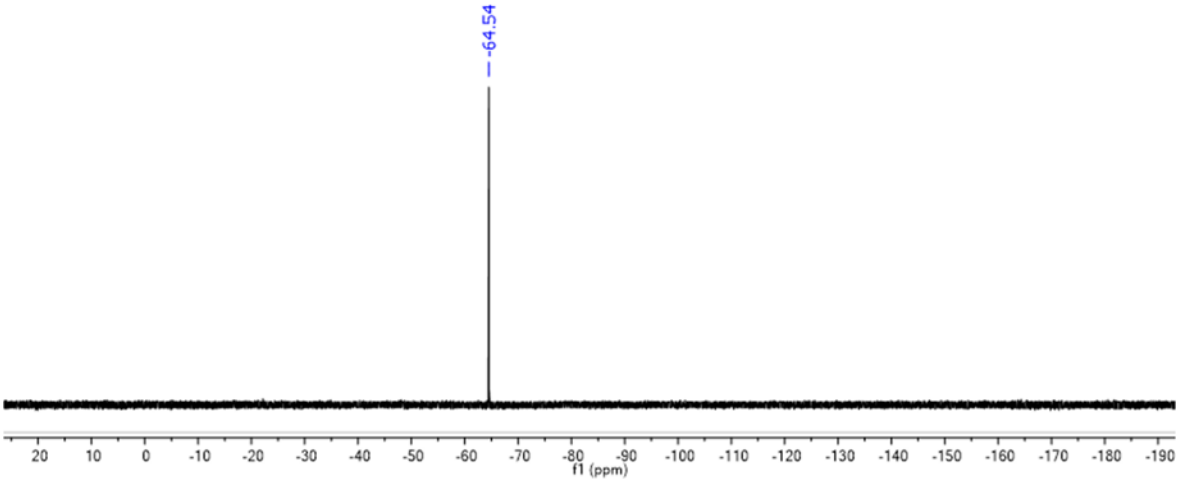
FT895

^{13}C NMR (126 MHz, DMSO-d6)

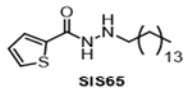


FT895

^{19}F NMR (376 MHz, DMSO-d6)



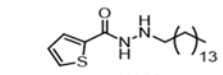
1H NMR (500 MHz, Chloroform-d) δ 7.62 – 7.45 (m, 2H), 7.11 (t, *J* = 4.3 Hz, 1H), 2.95 (t, *J* = 7.4 Hz, 2H), 1.55 (t, *J* = 7.4 Hz, 2H), 1.27 (s, 25H), 0.90 (t, *J* = 6.8 Hz, 4H).



SIS65

^{1}H NMR (500 MHz, CDCl3)

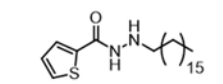
^{13}C NMR (126 MHz, CDCl$_3$) δ 161.98, 136.62, 133.69, 130.93, 130.10, 128.84, 128.36, 127.71, 77.29, 77.04, 76.78, 52.36, 34.38, 31.94, 29.71, 29.70, 29.67, 29.62, 29.59, 29.53, 29.38, 29.24, 28.00, 27.11, 22.71, 14.14.



SIS65

^{13}C NMR (126 MHz, CDCl3)

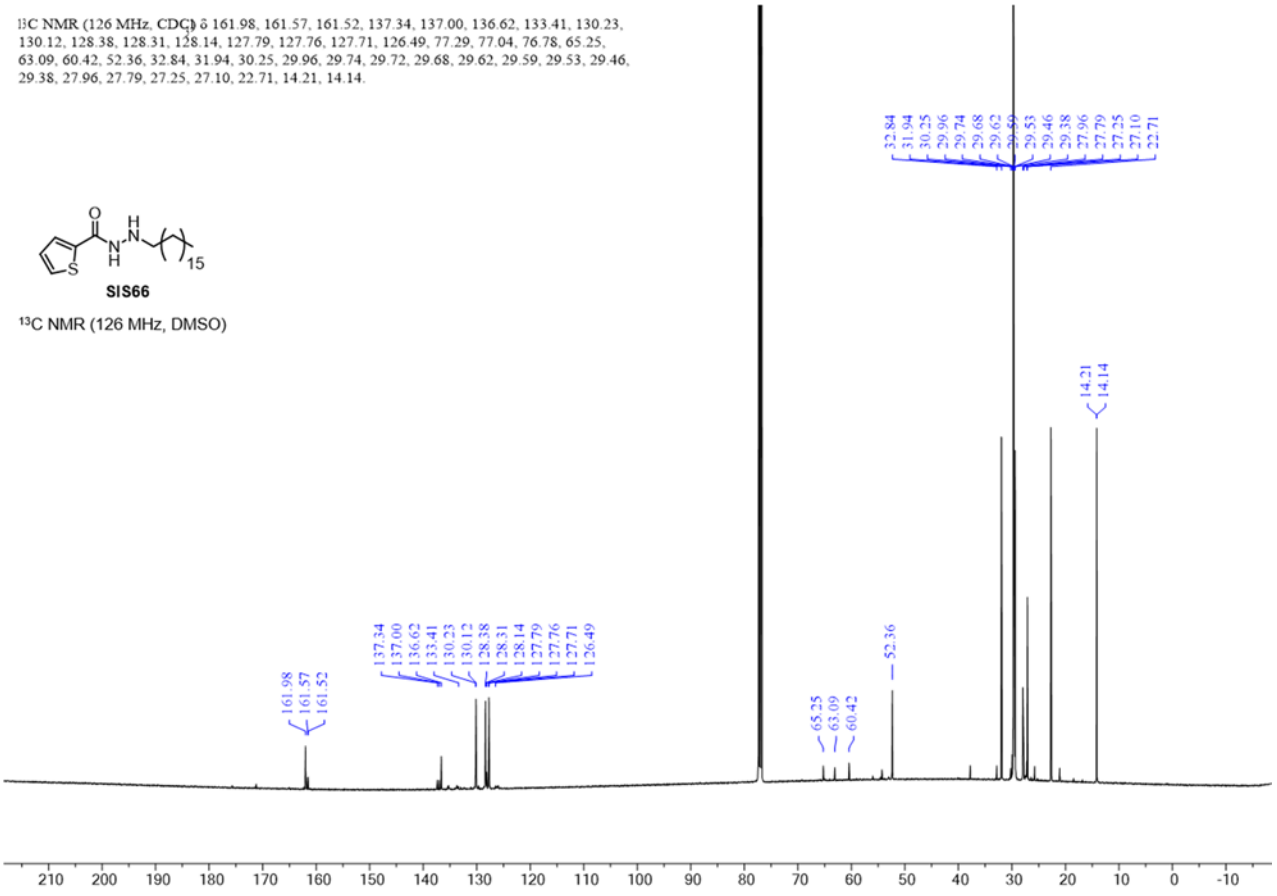
^{1}H NMR (500 MHz, Chloroform-d) δ 7.63 – 7.42 (m, 1H), 7.12 (dt, J = 11.9, 4.4 Hz, 1H), 4.14 (q, J = 7.2 Hz, 0H), 2.95 (t, J = 7.3 Hz, 1H), 1.27 (d, J = 2.3 Hz, 22H), 0.90 (t, J = 6.9 Hz, 2H).



SIS66

^{1}H NMR (500 MHz, CDCl3)

^{13}C NMR (126 MHz, CDCl$_3$) δ 161.98, 161.57, 161.52, 137.34, 137.00, 136.62, 133.41, 130.23, 130.12, 128.38, 128.31, 128.14, 127.79, 127.76, 127.71, 126.49, 77.29, 77.04, 76.78, 65.25, 63.09, 60.42, 52.36, 32.84, 31.94, 30.25, 29.96, 29.74, 29.72, 29.68, 29.62, 29.59, 29.53, 29.46, 29.38, 27.96, 27.79, 27.25, 27.10, 22.71, 14.21, 14.14.



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