Supplementary Information

Near infrared imaging of Mer tyrosine kinase (MerTK) using MERi-SiR reveals tumor associated macrophage uptake in metastatic disease.

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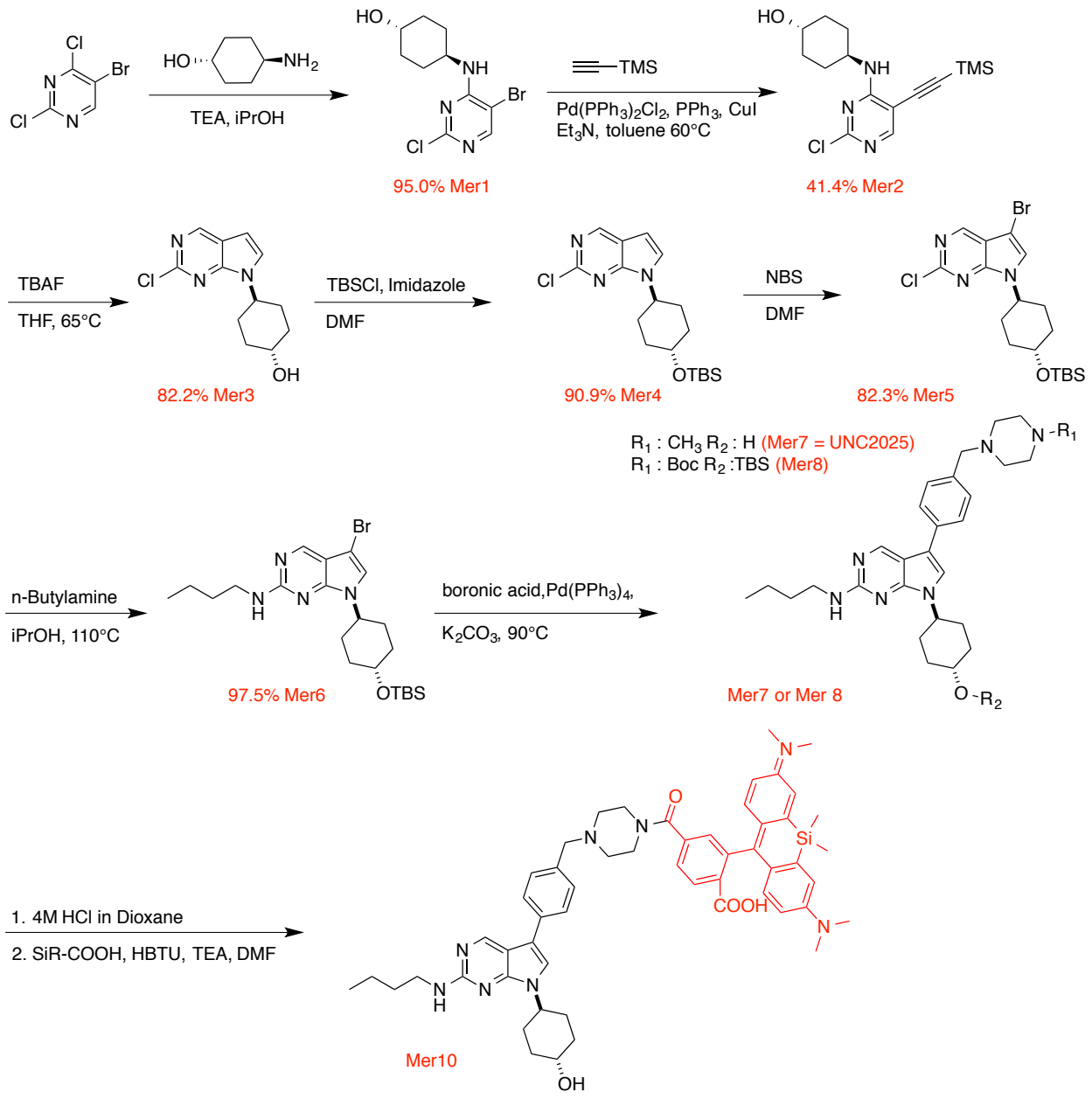
General Materials: Reagents were purchased from Sigma-Aldrich; deuterated solvents were from Cambridge Isotope Laboratories, Inc.; chemicals were used without further purification unless otherwise noted. SK-MEL-3, RAW 264.7, and CT26 cell lines were from ATCC and cultured according to manufacturer's guidelines in penicillin/ streptomycin; cells were routinely tested for mycoplasma and mouse antibody production (Bioreliance Corp.).

General Experimental procedures. Unless otherwise noted, reactions were carried out under an atmosphere of nitrogen or argon in air-dried glassware with magnetic stirring. Air- and/or moisture-sensitive liquids were transferred *via* syringe. Organic solutions were concentrated by rotary evaporation at 25 - 60 °C at 15-30 torr. Analytical thin layer chromatography (TLC) were performed using plates cut from glass sheets (silica gel 60 F-254 from Silicycle). Visualization was achieved under a 254 or 365 nm UV light and by immersion in an ethanolic solution of cerium sulfate, followed by treatment with a heat gun. Column chromatography was carried out as "Flash Chromatography" using silica gel G-25 (40-63 μm).

Chemical characterization: ^{1}H and ^{13}C NMR spectra were recored at 23 °C on a Bruker Avance III 400 MHz spectrometer. Chemical shifts were reported in parts per million (δ) and calibrated to the internal tetramethylsilane (TMS) standard or residual proton resonance and the natural abundance ^{13}C resonance of the solvent. Signal multiplicities are abbreviated as: s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), m (multiplet), br (broad). High performance liquid chromatography-mass spectrometry analysis (HPLC/MS) was conducted using a Waters instrument equipped with a Waters 2424 ELS Detector, Waters 2998 UV-Vis Diode array Detector, Waters 2475 Multi-wavelength Fluorescence Detector, and a Waters 3100 Mass Detector. Separations used Waters XTerra RP C18 5 μm, with a water/acetonitrile solvent gradient (0.1% formic acid).

Chemical synthesis



Supplementary Scheme 1. MERi-SiR synthetic scheme, which is based on the first 7-steps of the UNC2025 synthetic route described previously [Zhang et al., 2014, J Med Chem, 57, 7031-41], but using 4-(4-Boc-1-piperazinylmethyl)benzeneboronic acid pinacol ester (to yield Mer8), followed by amine deprotection and N,N,N',N'-Tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) coupling with SiR-COOH, to yield the Mer-inhibitor SiR-COOH conjugate (MERi-SiR).

Mer1: A solution of 2,4-dichloro-5-bromopyrimidine (1.8 g, 7.90 mmol), *trans*-4-aminocyclohexanol (937 mg, 6.18 mmol) and TEA (1.5 mL, 10.8 mmol) in iPrOH (30 mL) was stirred at ambient temperature for overnight. Next day, reaction mixture was concentrated in vacuo. Crude product was diluted with EtOAc and washed with water and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. Resulting crude product was diluted with 30 mL of EtOAc and 150 mL of hexane. Mer 1 compound (2.3 g, 7.54 mmol, yield : 95.0%) was obtained as a white solid after recrystallization at -20 °C. ^{1}H NMR (400 MHz, CHLOROFORM-d) δ 8.11 (s, 1H), 5.31 (d, *J* = 7.3 Hz, 1H), 4.02–4.00 (m, 1H), 3.74–3.63 (m, 1H), 2.20–2.10 (m, 2H), 2.09–2.00 (m, 2H), 1.80–1.74 (m, 1H), 1.57–1.43 (m, 2H), 1.41–1.28 (m, 2H). ^{13}C NMR (101 MHz, CHLOROFORM-d) δ 159.5, 158.9, 156.5, 103.1, 69.7, 49.4, 33.8, 30.5.

Mer2: A solution of Mer 1 (8.00 g, 26.1 mmol), ethynyl trimethylsilane (3.80 mL, 26.9 mmol) , $Pd(PPh_3)_2Cl_2$ (366 mg, 0.52 mmol), Cu(I)iodide (99.2 mg, 0.52 mmol) and TEA (18.2 mL, 130.5 mmol) in toluene (70 mL) was purged with nitrogen for 20 min. Reaction mixture was warmed and stirred at 60 °C for 2.5 h. The mixture was then diluted with EtOAc and water, and organic material was extracted (x3) with EtOAc. Combined organic phase was dried over Na_2SO_4 and concentrated in vacuo. Flash silica gel column chromatography afforded a product as a white solid (3.50 g, 10.8 mmol, yield : 41.4%). ^{1}H NMR (400 MHz, CHLOROFORM-d) δ 8.07 (s, 1H), 5.46 (d, *J* = 7.6 Hz, 1H), 4.01 (dtd, *J* = 11.1, 7.3, 7.3, 4.0 Hz, 1H), 3.70 (d, *J* = 2.0 Hz, 1H), 2.20–2.12 (m, 2H), 2.09–1.99 (m, 3H), 1.57–1.45 (m, 2H), 1.37–1.25 (m, 2H), 0.28 (s, 9H). ^{13}C NMR (101 MHz, CHLOROFORM-d) δ 162.1, 159.6, 158.1, 106.4, 101.1, 95.9, 69.5, 48.9, 33.6, 30.5, 0.2.

Mer3: To a transparent yellow solution of Mer2 (1.80 g, 5.56 mmol) in THF (24 mL) was added 1.0 M tetrabutylammonium fluoride (TBAF) solution in THF (14 mL) at r.t. Mixture was warmed and stirred at 65 °C for overnight. Mixture was then concentrated in vacuo, diluted with water and extracted with EtOAc (x3). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. Flash silica gel column chromatography afforded the desired product (1.15 g, 4.57 mmol, 82.2%) as a white solid. ^{1}H NMR (400 MHz, CHLOROFORM-d) δ 8.78 (s, 1H), 7.32 (d, *J* = 3.7 Hz, 1H), 6.59 (d, *J* = 3.7 Hz, 1H), 4.74 (tt, *J* = 12.1, 3.9 Hz, 1H), 3.87–3.78 (m, 1H), 3.16 (br. s., 1H), 2.25–2.16 (m, 2H), 2.16–2.07 (m, 2H), 1.88 (qd, *J* = 12.8, 3.3 Hz, 2H), 1.72–1.57 (m, 2H).

^{13}C NMR (101 MHz, CHLOROFORM-d) δ 152.9, 151.4, 150.7, 126.8, 117.9, 100.2, 69.3, 52.5, 34.3, 31.0.

Mer4: To a solution of Mer3 (1.15 g, 4.57 mmol) and imidazole (1.00 g, 14.7 mmol) in DMF (90 mL), stirred at 0 °C, added a *tert*-Butylchlorodimethylsilane (1.20 g, 7.96 mmol). Resulting reaction mixture was stirred at 0 °C for 30 min. Mixture was then warmed to r.t. and stirred for overnight. The reaction was then quenched with water and organic material was extracted with DCM (x5). Organic material was dried over Na_2SO_4 and concentrated in vacuo. Flash silica gel column chromatography afforded the desired product (1.52 g, 4.15 mmol, 90.9%) as a white solid. ^{1}H NMR (400MHz, CHLOROFORM-d) δ 8.66 (s, 1 H), 7.19 (d, *J* = 3.7 Hz, 1H), 6.46 (d, *J* = 3.7 Hz, 1H), 4.60 (tt, *J* = 12.0, 3.8 Hz, 1H), 3.63 (tt, *J* = 10.6, 4.2 Hz, 1H), 2.02–1.87 (m, 4H), 1.81–1.67 (m, 2H), 1.59–1.44 (m, 2H), 0.82 (s, 9H), 0.00 (s, 6H). ^{13}C NMR (101 MHz, CHLOROFORM-d) δ 153.2, 151.5, 150.8, 126.7, 117.9, 100.0, 70.3, 52.7, 34.7, 31.1, 25.9, 18.1, -4.6.

Mer5: A solution of Mer4 (1.10 g, 3.01 mmol), N-bromosuccinimide(588 mg, 3.31 mmol) in DMF (10 mL) was stirred at r.t. With TLC monitoring, mixture was diluted with water and extracted with DCM (x4). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. Flash silica gel column chromatography afforded the desired product (1.10 g, 2.47 mmol, 82.3%) as a white solid. ^{1}H NMR (400 MHz, CHLOROFORM-d) δ 8.71 (s, 1 H), 7.28 (s, 1 H), 4.76–4.64 (m, 1 H), 3.76–3.64 (m, 1 H), 2.15–1.96 (m, 4 H), 1.88–1.72 (m, 2 H), 1.62–1.58 (m, 2 H), 0.90 (s, 9 H), 0.09 (s, 6 H). ^{13}C NMR (101 MHz, CHLOROFORM-d) δ 154.5, 151.0, 150.6, 126.0, 117.6, 88.6, 70.3, 53.3, 34.8, 31.3, 26.0, 18.3, -4.5.

Mer6: A solution of Mer5 (80 mg, 0.16 mmol) and butyl amine (33 mg, 0.45 mmol) in iPrOH (2.0 mL) was irradiated by microwave at 150 °C for 1.5 h. Mixture was diluted with EtOAc and extracted with water (x3). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. Flash column chromatography afforded the desired product (77 mg, 0.16 mmol, 97.5%) as a white solid. ^{1}H NMR (400 MHz, CHLOROFORM-d) δ 8.40 (s,1H), 6.84 (s, 1H), 5.19–5.08 (m, 1H), 4.53–4.42 (m, 1H), 3.74–3.63 (m, 1H), 3.46 (td, *J* = 7.0, 5.9 Hz, 2H), 2.03–2.01 (m, 4H), 1.85–1.70 (m, 2H), 1.68–1.51 (m, 4H), 1.50–1.38 (m, 2H), 0.97 (t, *J* = 7.3 Hz, 3H), 0.91 (s, 9H), 0.09 (s, 6H). ^{13}C NMR (101 MHz, CHLOROFORM-d) δ 159.6, 152.0, 149.8, 121.0, 111.5, 88.3, 70.6, 52.6, 41.6, 35.0, 31.9, 30.9, 26.0, 20.3, 18.3, 14.0, -4.5.

Mer7: A solution of Mer6 (100 mg, 0.21 mmol), 1-methyl-4-[4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl]piperazine (72 mg, 0.23 mmol), $Pd(PPh_3)_4$ (5 mg, 0.004 mmol) and K_2CO_3 (43 mg, 0.31 mmol) in dioxane and water mixture (v:v = 4:1, 0.7 mL) was stirred at 90 °C. The mixture was diluted with EtOAc and water, and extracted with EtOAc (x3). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. Resulting crude product was treated with 4N HCl in dioxane for 2 h stirring, and resulting crude mixture was concentrated in vacuo. Reversed phage flash column chromatography with Biotage® SNAP Ultra KP-C13-HS cartridge afforded the product (55 mg, 0.12 mmol, yield = 55.6 %) as white solid. ^{1}H NMR (400 MHz, CHLOROFORM-d) δ 8.78 (s, 1 H), 7.52 (d, *J* = 8.1 Hz, 2H), 7.36 (d, *J* = 8.1 Hz, 2H), 7.03 (s, 1H), 4.96 (t, *J* = 5.8 Hz, 1H), 4.54 (tt, *J* = 12.0, 3.8 Hz, 1H), 3.77 (tt, *J* = 10.9, 4.3 Hz, 1H), 3.53 (s, 2H), 3.52–3.45 (m, 2H), 2.48 (br.s., 6H), 2.29 (s, 3H), 2.22–2.07 (m, 4H), 1.97–1.83 (m, 2H), 1.77 (br. s., 2H), 1.70–1.52 (m, 4H), 1.46 (dq, *J* = 15.0, 7.3 Hz, 2H), 0.98 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (101 MHz, CHLOROFORM-d) δ 159.3, 153.4, 150.5, 136.4, 133.4, 130.0, 126.4, 118.6, 115.6, 110.3, 70.0, 62.9, 55.3, 53.3, 52.2, 46.2, 41.7, 34.8, 32.1, 30.8, 20.4, 14.1.

Mer8: A solution of Mer6 (100 mg, 0.21 mmol), 4-(4-Boc-1-piperazinylmethyl)benzeneboronic acid pinacol ester (92 mg, 0.23 mmol), $Pd(PPh_3)_4$ (5 mg, 0.004 mmol) and K_2CO_3 (43 mg, 0.31 mmol) in dioxane and water mixture (v:v = 4:1, 0.7 mL) was stirred at 90 °C. Reaction mixture was diluted with EtOAc and water, and extracted with EtOAc (x3). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. Flash silica gel column chromatography afforded the product (82 mg, 0.12 mmol, yield = 58.3 %) as a white solid. ^{1}H NMR (400 MHz, CHLOROFORM-d) δ 8.78 (s, 1H), 7.52 (d, *J* = 8.1 Hz, 2H), 7.35 (d, *J* = 8.1 Hz, 2H), 7.05 (s, 1H), 4.96 (t, *J* = 5.8 Hz, 1H), 4.53 (tt, *J* = 11.9, 3.6 Hz, 1H), 3.78–3.66 (m, 1H), 3.51–3.39 (m, 6H), 2.42 (t, *J* = 4.8 Hz, 4H), 2.14–1.98 (m, 4H), 1.95–1.78 (m, 2H), 1.71–1.54 (m, 5H), 1.51–1.40 (m, 11H), 1.01–0.95 (m, 3H), 0.92 (s, 9H), 0.10 (s, 6H). ^{13}C NMR (101 MHz, CHLOROFORM-d) δ 159.3, 154.9, 153.3, 150.4, 135.9, 133.7, 129.8, 126.4, 118.7, 115.7, 110.2, 79.6, 70.7, 62.9, 53.0, 52.3, 41.6, 41.5, 35.1, 32.0, 30.9, 28.5, 26.0, 20.3, 18.3, 14.0, -4.5.

Mer10: A solution of Mer8 (82 mg, 0.12 mmol) in 4M HCl dioxane solution (4.0 mL) was stirred at r.t. for 2 h. The mixture was concentrated in vacuo. Flash silica gel column chromatography afforded a product (50 mg, 0.10 mmol, yield = 81.2%) as yellow solid (Mer9). Without further characterization, purified product was used for next reaction. To a solution of Mer9 (0.045 M in

DMF, 305 µL) was added SiR-COOH (5 mg, 0.011 mmol), 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (4.4 mg, 0.012 mmol) and TEA (2 µL, 0.012 mmol), stirred at r.t. for 2 h, and purified using standard HPLC techniques performed on a Waters' liquid chromatography-mass spectrometry (LC-MS) system using a Waters' XTerra C18 5 µm column. The final compound (Mer10, MERi-SiR) was purified in 87.3% yield. ^{1}H NMR (400MHz, METHANOL-d4) δ 8.58 (s, 1H), 7.91 (d, *J* = 7.8 Hz, 1H), 7.54 (dd, *J* = 7.8, 1.0 Hz, 1H), 7.47 (s, 2H), 7.29 (s, 1H), 7.24(d, *J* = 8.1 Hz, 2H), 7.17 (s, 1H), 6.93 (d, *J* = 2.7 Hz, 2H), 6.64–6.57 (m, 2H), 6.50 (dd, *J* = 8.9, 2.8 Hz, 2H), 4.43 (t, *J* = 7.7 Hz, 1H), 3.76–3.54 (m, 4H), 3.46 (s, 2H), 3.35 (t, *J* = 7.1 Hz, 2H), 3.11 (q, *J* = 7.3 Hz, 1H), 2.83 (s, 10H), 2.52–2.23 (m, 4H), 2.08–1.86 (m, 6H), 1.56 (quin, *J*= 7.3 Hz, 2H), 1.48–1.30 (m, 4H), 1.24–1.17 (m, 2H), 0.93–0.87 (m, 3H), 0.52 (s, 2H), 0.43 (s, 2H). LRMS $[M]^{+}$ m/z calcd 917.49 for $C_{54}H_{65}N_8O_4Si$, found 917.96.

MERi-SiR characterization and modeling: Fluorescence spectra were measured with a Tecan Spark fluorescence plate reader, with 10 µM MERi-SiR dissolved in PBS. Docking and visualization of MERi-SiR in the Mer binding pocket was performed using UCSF Chimera and the Autodock Vina module. A crystal structure of Mer with the inhibitor UNC569 (PDB 3TCP) was used as the target. ChemAxon Marvin Sketch was used for energy minimization of MERi-SiR. A bounding box was placed around UNC569 followed by removal of the inhibitor. MERi-SiR was imported into the Chimera environment as a pdb file. Dock prep was performed on Mer and MERi-SiR to remove solvent and add hydrogens, and docking was conducted in Autodock Vina with 9 binding modes. The optimal binding configuration was colored such that Mer and surface are gray, and MERi with linked SiR fluorophore are indicated in cyan and red, respectively.

***In vitro* cell experiments:** SiRNA knockdown was performed using mouse and human SMARTpool ON-TARGET plus siRNA (referred to as "siMer") and ON-TARGET plus non-targeting siRNA #1 (referred to as "siControl"), all from GE Healthcare / Dharmacon (see Supplementary Table 1 for sequences). Transfection was performed using lipofectamine 3000 (Thermo) following manufacturer's guidelines. 500,000 cells were plated overnight in 10 cm dishes, transfected with 125 pmol siRNA, and assayed 72h later. Mer protein was quantified by sandwich ELISA, following lysis in NP-40 buffer and accounting for protein concentration using the micro-bicinchoninic acid (BCA) assay (Pierce). Sandwich ELISAs were conducted according to manufacturer guidelines using kits for human and mouse Mer (R&D Systems) and MaxiSorp

384-well plates (NUNC). After overnight seeding on 96-well plates (Ibidi), cells were treated with 1, 3, or 10 μM MERi-SiR for 1 hr, then rinsed 3x in warm PBS, provided fresh growth media, and imaged 30 min later using a DeltaVision (Applied Precision) modified Olympus BX63 microscopy system with a Neo sCMOS monochrome camera (Andor) and an environmental chamber, using the following excitation / emission wavelengths, with bandpasses in parentheses (all in nm): hoechst 33342 λ_{ex} / λ_{em} 360(40) / 455(50), MERi-SiR λ_{ex} / λ_{em} 645(30) / 705(72). RAW cells were treated with either 1 or 10 μM MERi-SiR, while SK-MEL-3 cells were treated with either 1 or 3 μM MERi-SiR; at each MERi-SiR concentration, fluorescence intensities of the siMer-treated cells were normalized to the corresponding fluorescence intensities of the siControl-treated cells, at the corresponding MERi-SiR dose.

To image competition of MERi-SiR with UNC2025, SK-MEL-3 were seeded overnight on 96-well plates (Ibidi), treated with UNC2025 or vehicle for 1.5 hr, then treated with the addition of MERi-SiR at the indicated concentration, rinsed 3x in warm PBS 30 min later, and imaged 30 min later as described above using the BX63 microscopy system (Olympus).

In the above studies, cell fluorescence intensities were quantified by first segmenting regions of interest around individual cells (using ImageJ and CellProfiler software), calculating the average fluorescence intensities within each of these single-cell regions, and then averaging all cells together for each experiment. Vehicle controls were used to measure and subtract background autofluorescence. These background-subtracted data were routinely normalized to average values observed in the experimental conditions with the highest fluorescence, to yield “fraction max” measurements as in Fig. 2B, or to average values observed in the control (e.g., siControl) experimental conditions, to yield “fraction control” measurements as in Fig. 2C.

To image accumulation of MERi-SiR in comparison to α-Mer-Alexa488 antibody-dye conjugate (R&D Systems clone 125518), antibody buffer was first exchanged for PBS using a Zeba Spin Desalting Column (Thermo/Fisher). SK-MEL-3 were seeded overnight on 96-well plates (Ibidi), treated with antibody at 1:100 dilution and 5μM MERi-SiR for 1h at 37°C in full growth media, rinsed and treated with fresh media, and immediately imaged as described above using the BX63 microscopy system. To image total Mer, including intracellular cytoplasmic Mer, cells were fixed and permeabilized in 4% paraformaldehyde and 0.5% Triton-X 100 for 15 min at 37°C, rinsed, blocked in full serum media, stained using 1:100 antibody dilution, rinsed, and

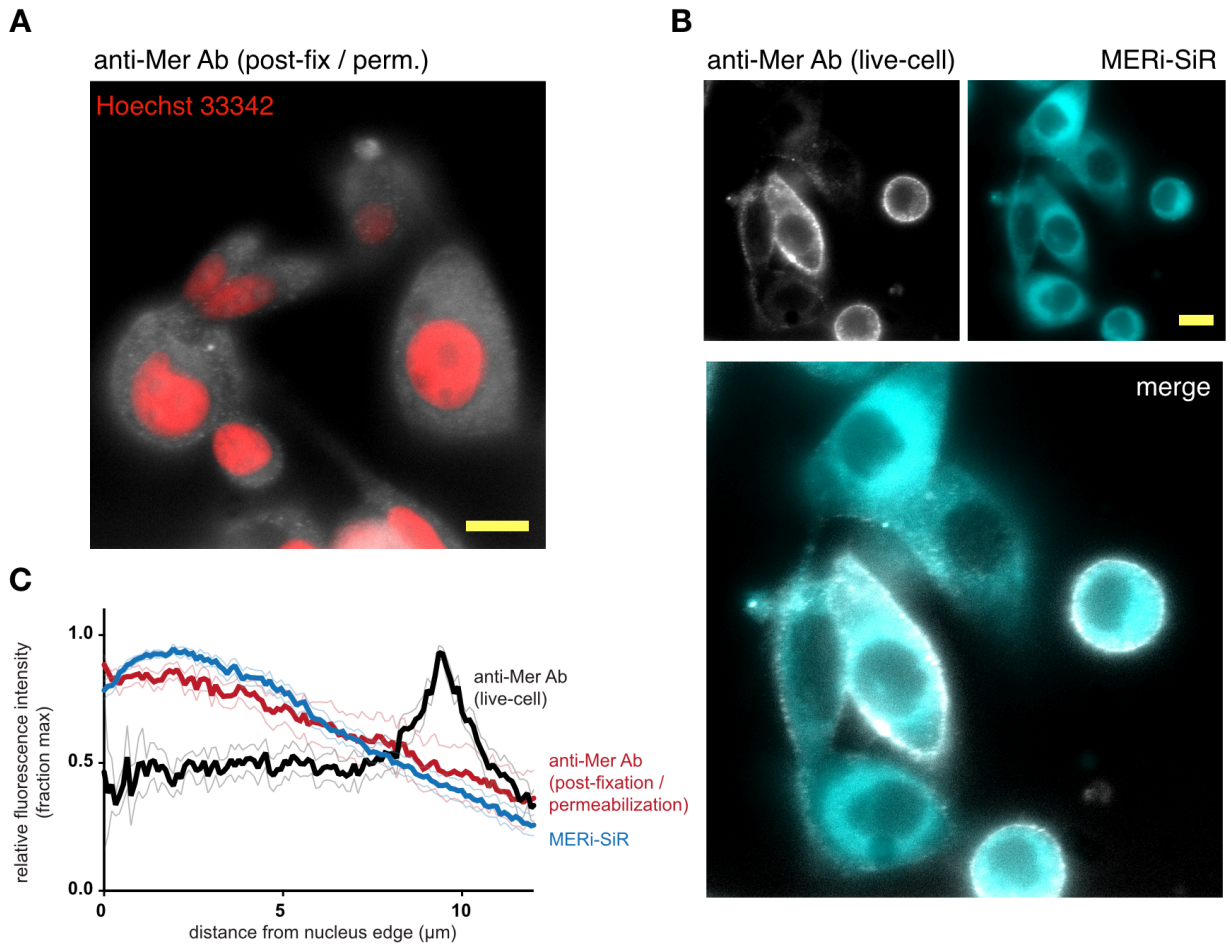
immediately imaged with Hoechst 33342 counterstaining. Radial profiles of MERi-SiR and α-Mer-Alexa488 were calculated in ImageJ using linear fluorescence intensity profile measurements drawn by hand, emanating radially from the edge of cell nuclei. Profiles were averaged across a cohort of cellular profiles (n>10) as a function of distance from cell nuclei. Before averaging together, each fluorescence profile was normalized by subtracting the background and dividing the profile by the maximum pixel intensity observed on that profile.

For western blots, 90% confluent 10 cm plates of SK-MEL-3 melanoma cells were treated with 200 nmol recombinant hGas6 (R&D Systems) or vehicle control. 30 min later, cells were treated with 20 μM UNC2025, MERi-SiR, or vehicle control. Cells were rinsed in cold PBS, lysed in cold NP-40 lysis buffer with HALT protease and phosphatase inhibitors (Thermo), and immediately transferred to Eppendorf tubes using a cell scraper. Cells were shaken for 30 min on ice and clarified by centrifugation at 16,000 x g. Lysate was loaded into NuPAGE Novex 4-12% Bis-Tris protein gels (Life Technologies) along with Magic Mark XP Western Protein Standard (Thermo). The following antibodies were used: Rb anti-p-Mer (pY681 & pY749), Abcam ab192649, 1:500; Rb anti-p-p44/42 MAPK (Erk1/2) (pT202/pY204) Cell Signaling Technology D13.14.4E, 1:2000; and Rb anti-GAPDH Cell Signaling Technology 14C10, 1:1000. Gt anti-Rb IgG HRP secondary Ab was used (Thermo Cat. 31460, 1:10,000) with SuperSignal West Pico Chemiluminescent Substrate (Thermo).

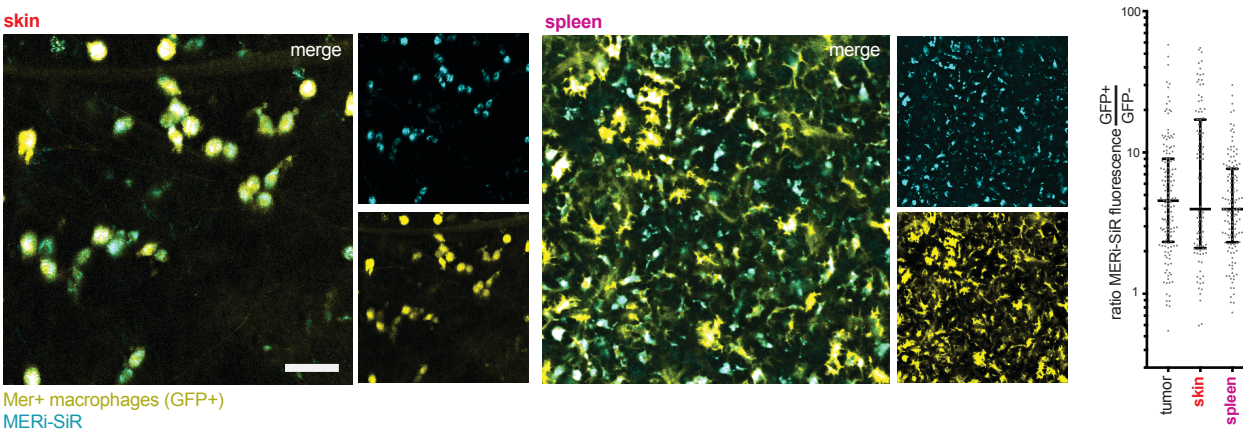
***In vivo* experiments and *ex vivo* imaging:** All animal research was performed in accordance with the guidelines from the Institutional Subcommittee on Research Animal Care. Experiments were performed 6-12 week old mice. Motivated by a previously published model of intraperitoneal carcinomatosis [Ripoll et al., 2010, Anticancer Res, 30, 5049-54], 2 x 10^6 murine colorectal carcinoma cells (CT26; ATCC) stably expressing tdTomato transgene were intraperitoneally (i.p.) injected into *MerTK*$^{GFP/+}$ NOD.CB17-Prkdc^{scid}/J mice; males were used, as they may exhibit more hepatic metastases in this model [Sorski et al., 2014, Surg Today, 44, 1925-34]. Roughly 10 days post-inoculation, 3 mg kg^{-1} MERi-SiR was i.p. injected from a 10 mM DMSO stock with 7 μl DMAC/solutol and 50 μl PBS. 24 h later animals were euthanized by CO_2 chamber and metastases were immediately imaged after surgical excision. Animals were monitored daily and were euthanized once ascites developed or health deteriorated (body condition score 2 or less). Microscopy was performed on an Olympus FV1000 confocal-multiphoton imaging system using a XLUMPLFLN 20x water immersion objective (NA 1.0;

Olympus America); 2x digital zoom; sequential scanning using 405-nm, 473-nm, 559-nm, and 635-nm diode lasers and a DM405/473/559/635-nm dichroic beam splitter; and collection of emitted light using beam splitters (SDM473, SDM560, and/or SDM 640) and emission filters BA430-455, BA490-540, BA575-620, and BA655-755 (all Olympus America).

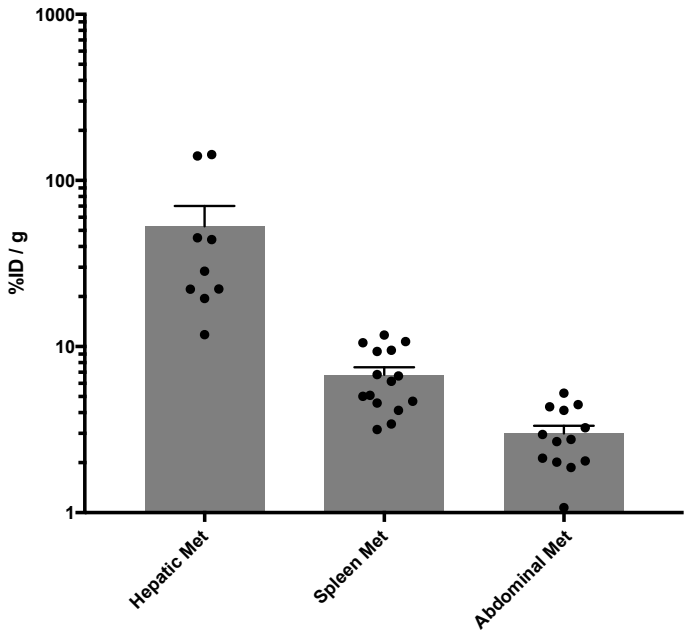
Gross quantification of MERi-SiR accumulation across metastatic nodules was performed using an Olympus OV100 imaging system. Freshly excised tumor-bearing organs (liver, spleen) and lesions found within the abdominal cavity, for instance adhered to the omentum, were imaged by OV110 fluorescence reflectance imaging for MERi-SiR accumulation. Fluorescence intensity values were calculated from regions of interest defined manually in ImageJ, after correcting for background autofluorescence.



Supplementary Fig. 1. A) Mer distribution in fixed and permeabilized SK-MEL-3 cells, using the fluorescent α-Mer-Alexa488 antibody conjugate. B) Distribution of co-administered MERi-SiR and α-Mer antibody in live SK-MEL-3 cell culture (λ_{ex} / λ_{em} for Ab and MERi-SiR were 490nm / 525nm and 645nm / 705nm, respectively). Scale bar = 15μm. C) From images similar to those in *A-B*, fluorescence intensity profiles were measured as radially extending from cell nuclei (n>10), normalized, averaged, and plotted as means (thick lines) ± S.E.M. (thin lines). MERi-SiR and post-fixation antibody treatment show highest staining near the cell nucleus that gradually decreases toward the cell edge, while live-cell antibody treatment produces distinct accumulation on the cell surface.



Supplementary Fig. 2. Mer imaging in healthy skin (left) and spleen (middle), along with corresponding quantification (right). MERi-SiR accumulates at roughly 4-fold higher levels in GFP+ cells in the skin and spleen compared to GFP- tissue, similar to as observed in CT26 tumor metastases (Fig. 4). At right, data shown as individual cells and their median ± interquartile range (n = 3 mice). Scale bar 50µm.

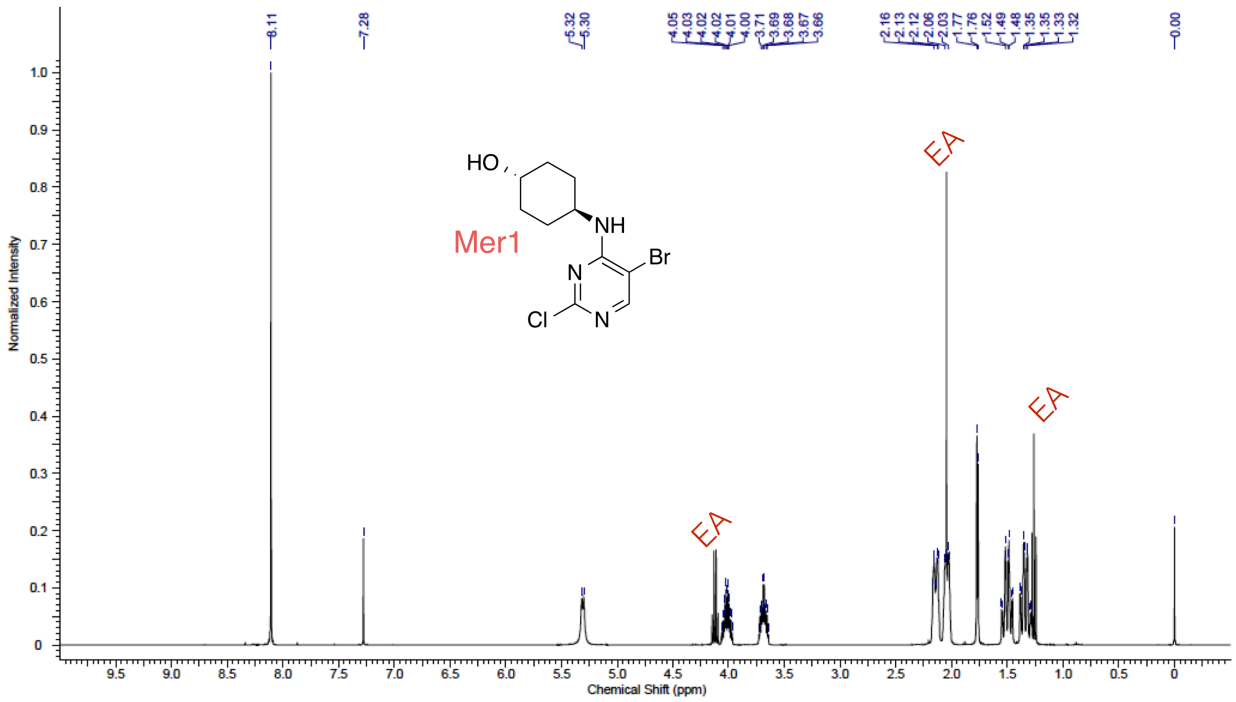


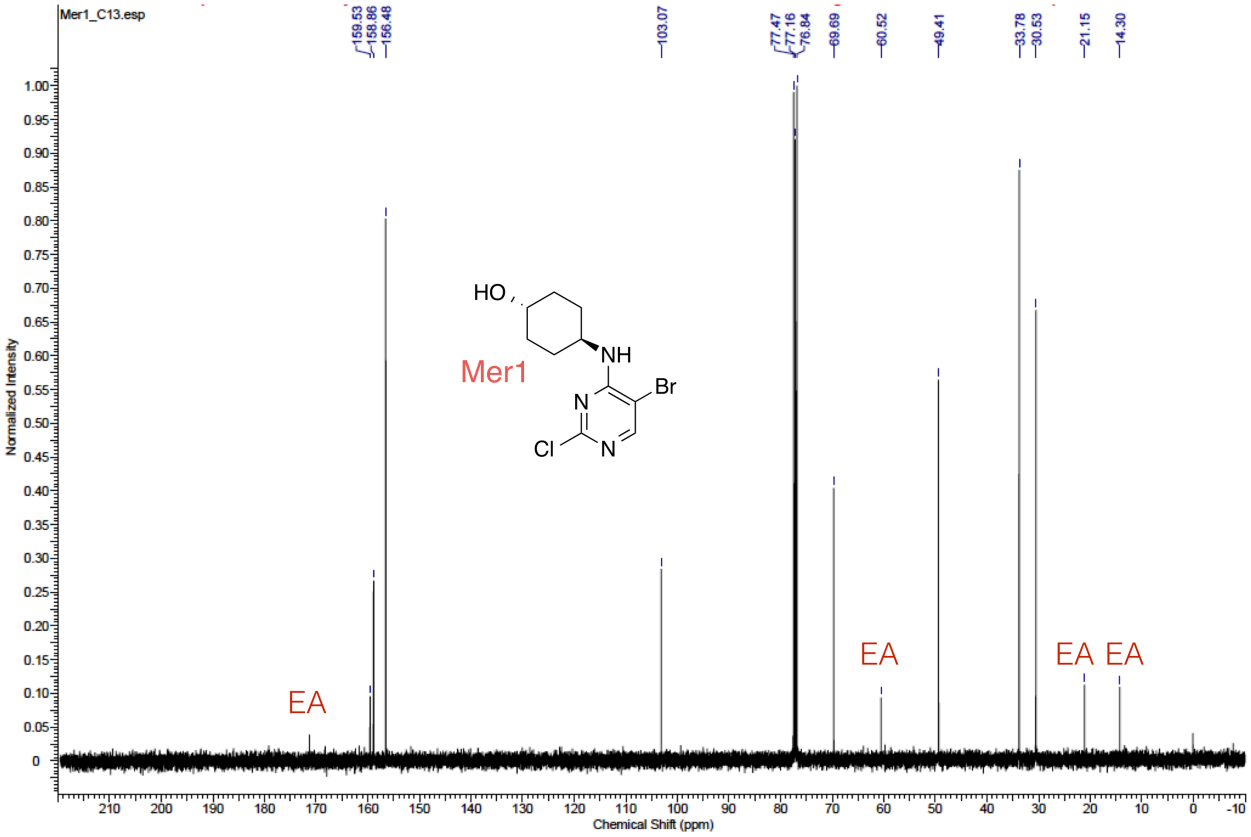
Supplementary Fig. 3. MERi-SiR accumulation depends on anatomical location of CT26 metastases (one-way ANOVA, $p<0.0001$, n=37 total tumor nodules across n = 3 mice). Metastases in tumor-bearing mice were identified 24 h post-treatment with 3 mg kg^{-1} MERi-SiR, using whole-organ fluorescence reflectance imaging to identify CT26-tdTomato lesions. MERi-SiR accumulation was measured by fluorescence intensity and converted to % injected dose per gram of tissue (%ID/g) using background correction and a standard dilution curve. Data are means ± standard error.

	RNA sequence	Catalog Number
Non-targeting siControl	UGGUUUACAUGUCGACUAA	D-001810-01-05
Pooled human siMerTK	GGAUGAAGCCUCCGACUAA	L-003155-00-0005
	GAACCAAGCAGACGUUAUU	
	GGAGAGACUUGUUAGGAAU	
	CUGAAUGAAUCUAGUGAUA	
Pooled mouse siMerTK	GGACGAAGCCUCCAAUUAA	L-040357-00-0005
	CCCAACUGGUCGUAAAUUA	
	CCUGUUAUAUUCCCGAUUA	
	CCAAAUGUCAUCCGACUUC	

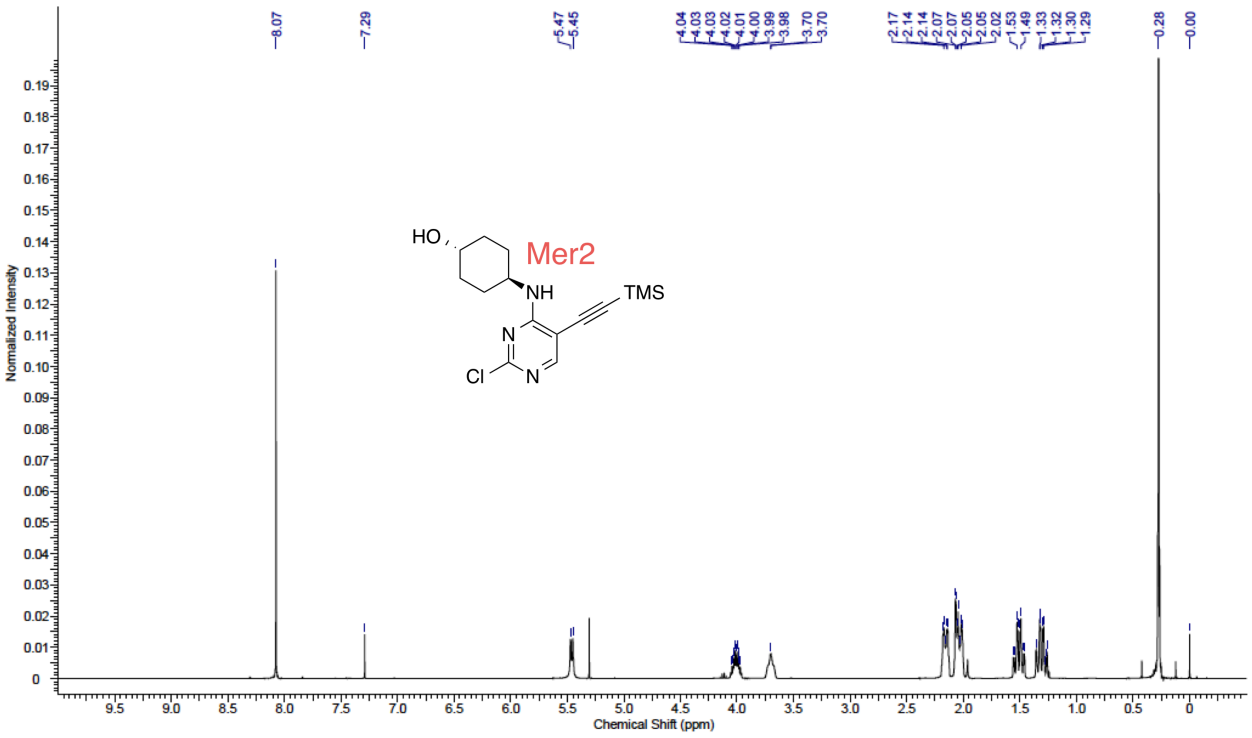
Supplementary Table 1. SiRNA sequences used for genetic knockdown experiments, performed with SMARTpool ON-TARGET plus siRNA reagents from GE Healthcare / Dharmacon.

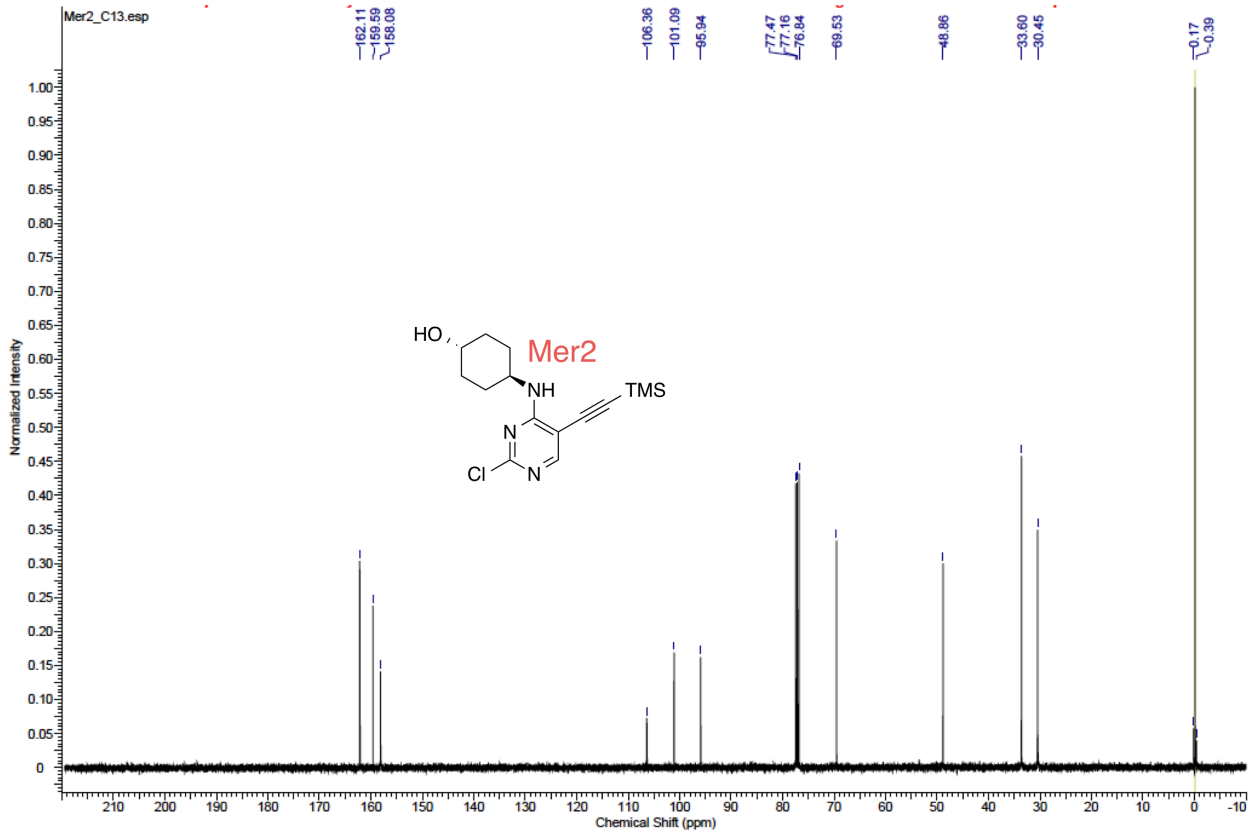
^{1}H and ^{13}C NMR spectra of Mer1



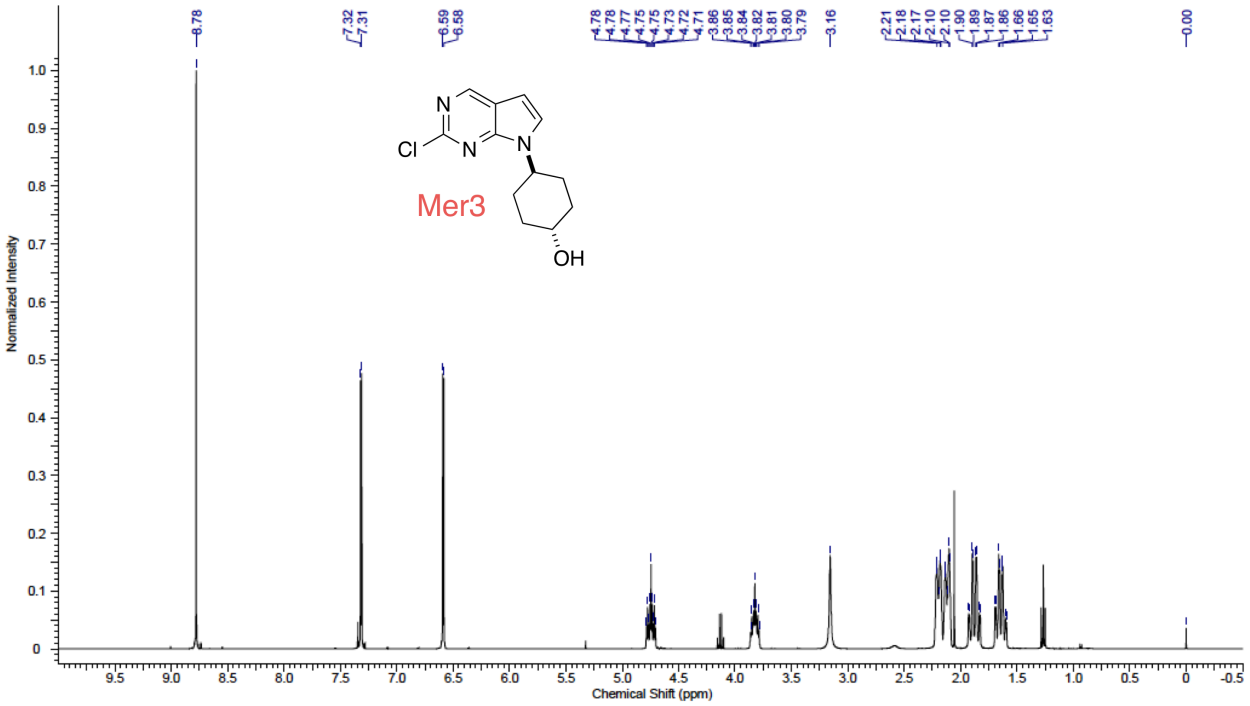


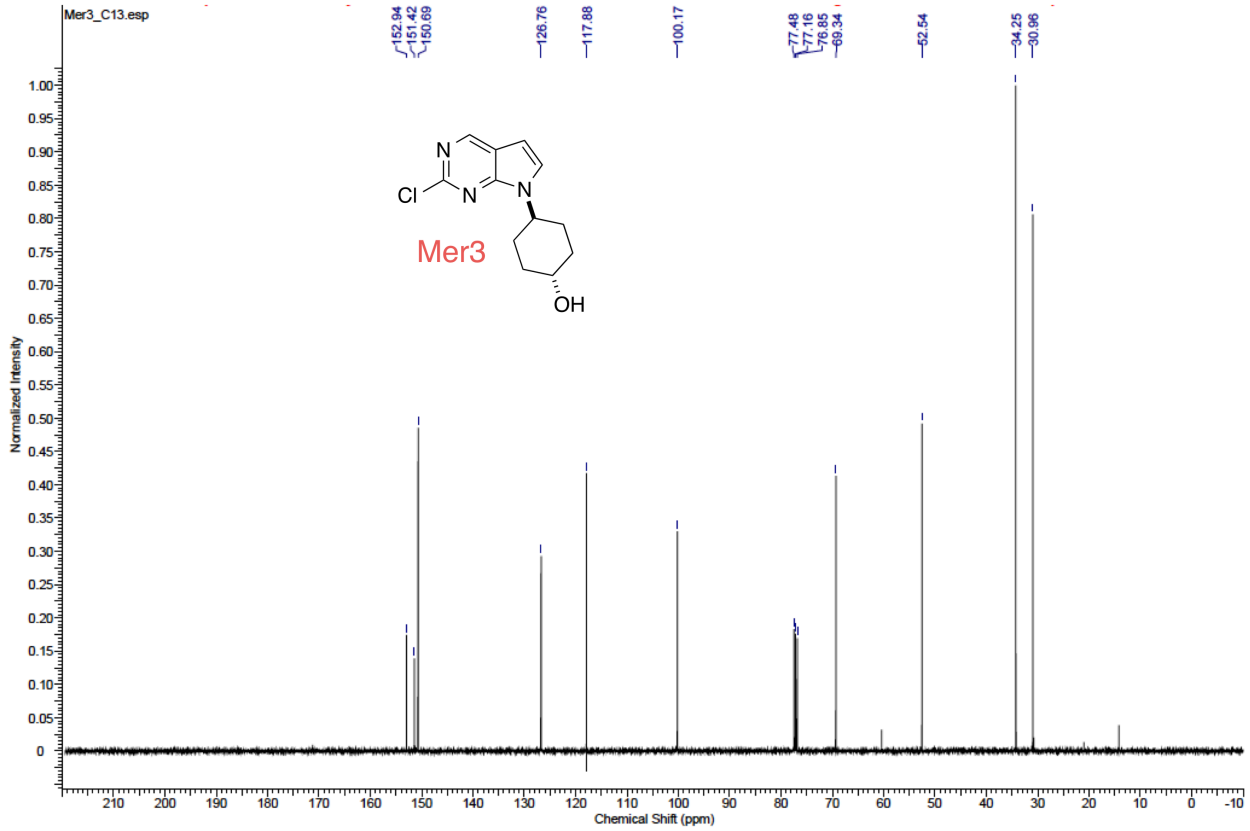
^{1}H and ^{13}C NMR spectra of Mer2



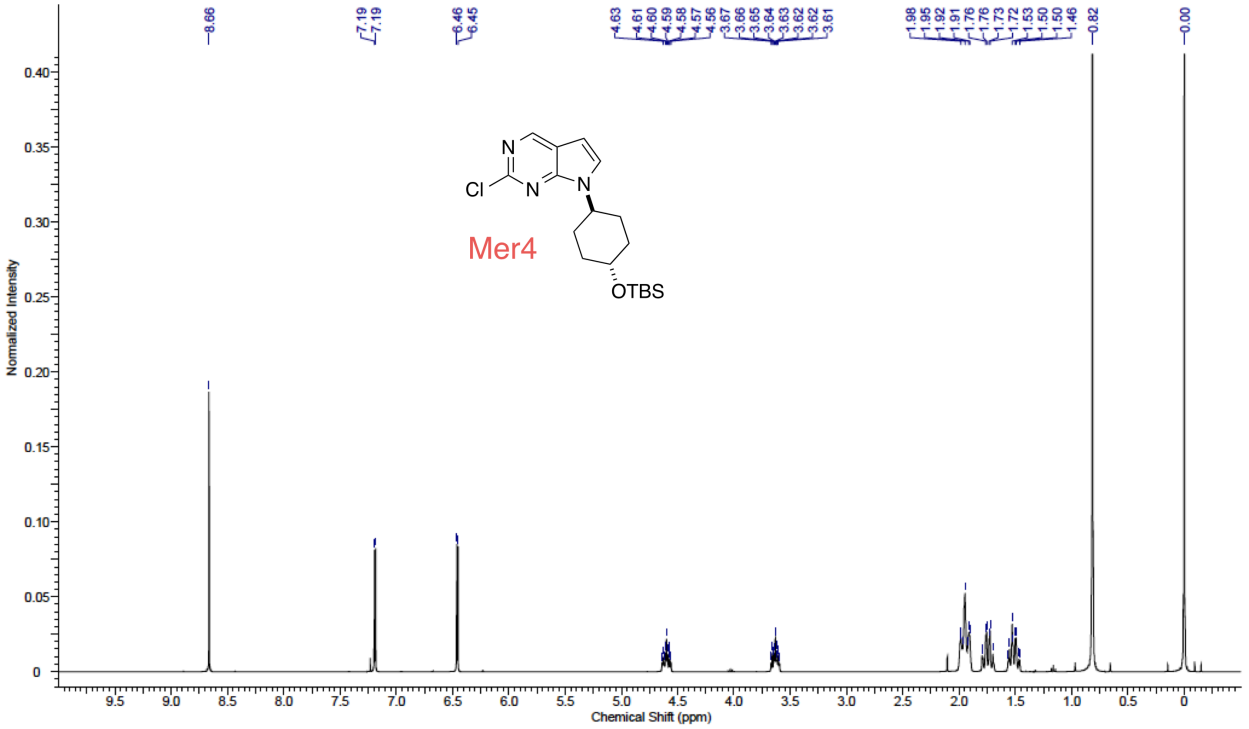


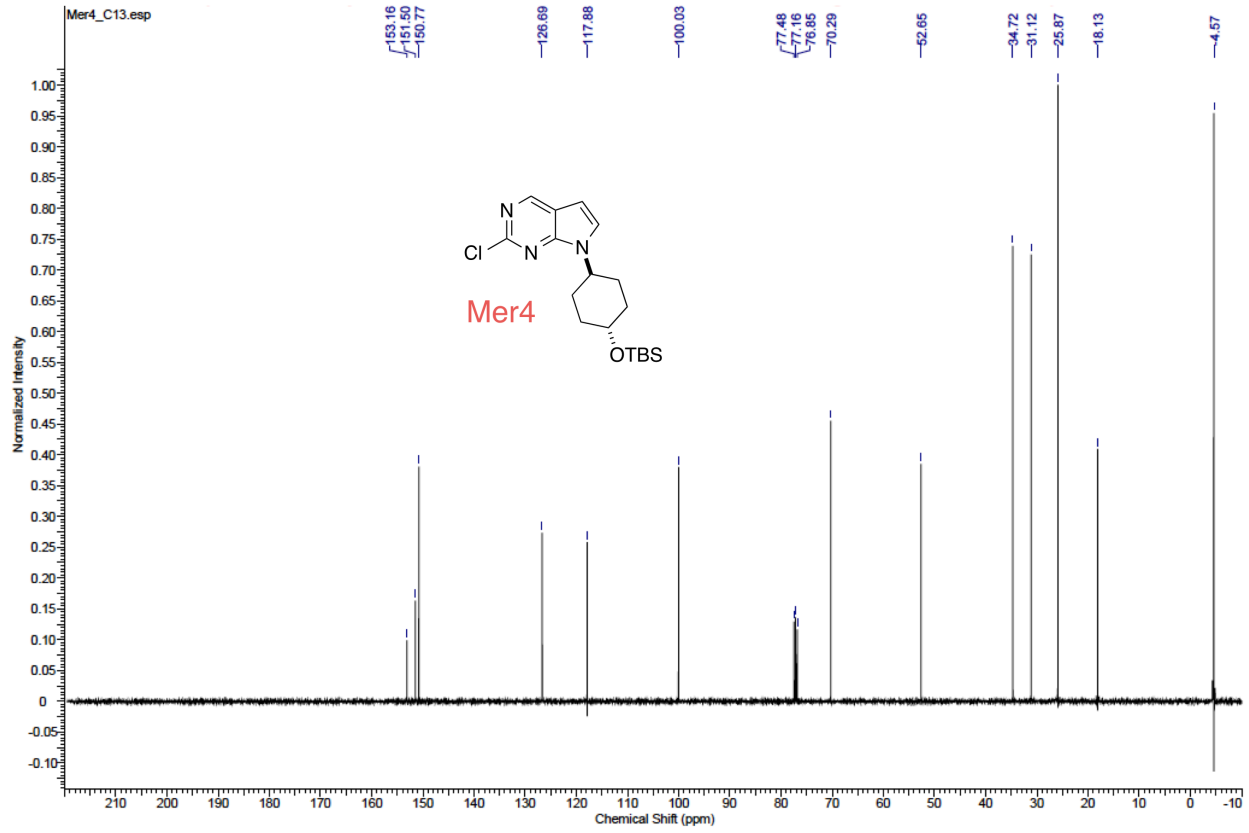
^{1}H and ^{13}C NMR spectra of Mer3



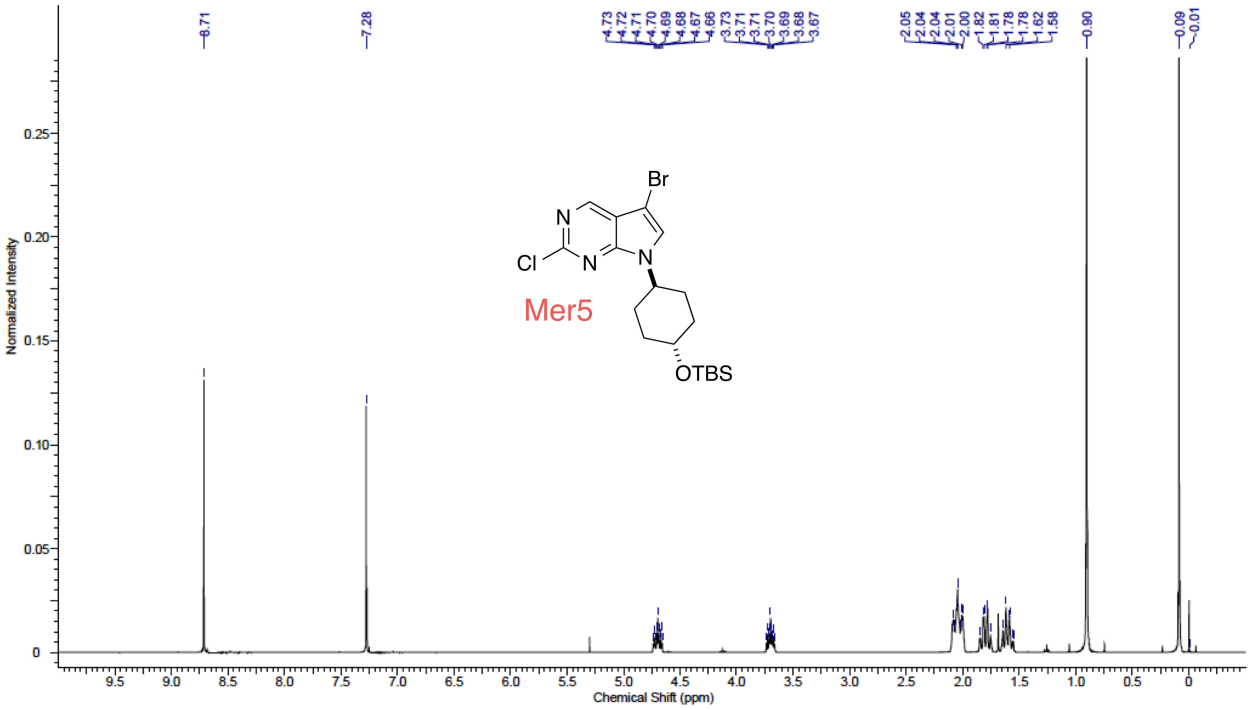


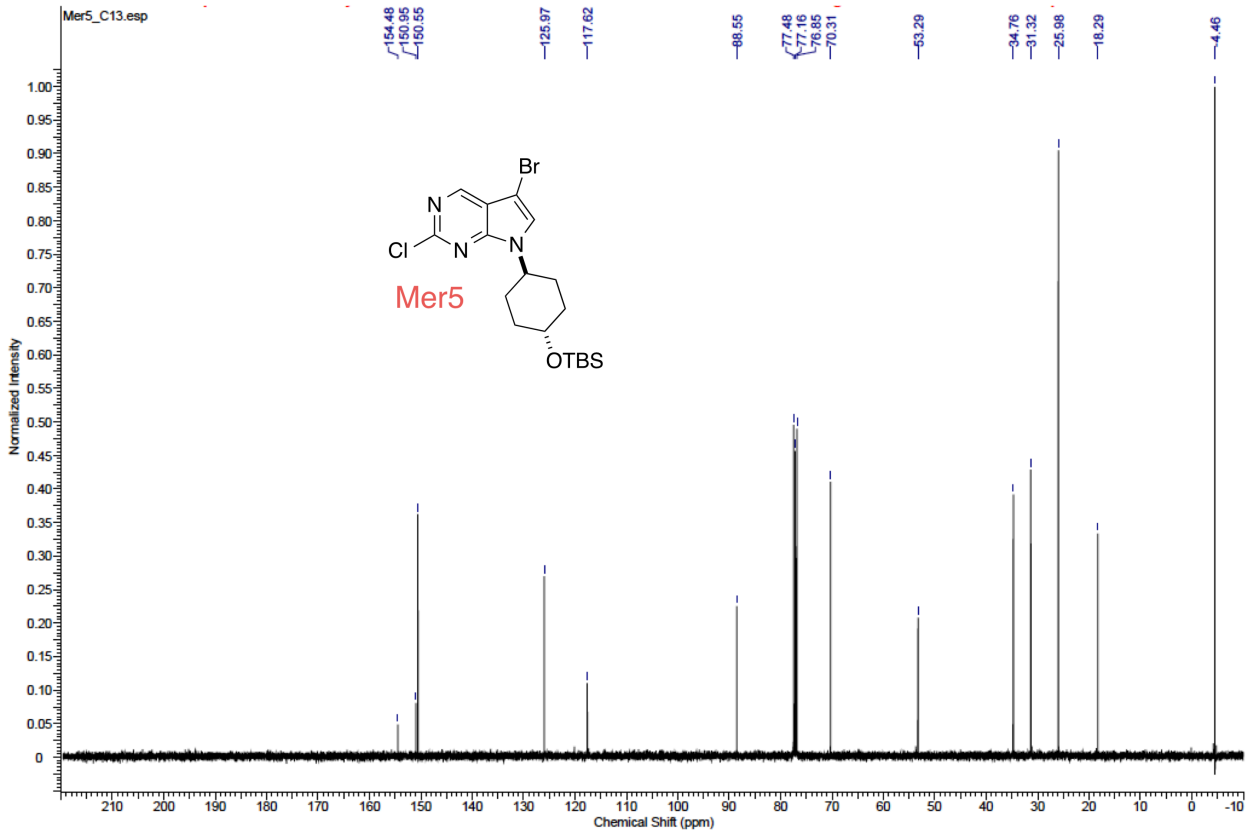
^{1}H and ^{13}C NMR spectra of Mer4



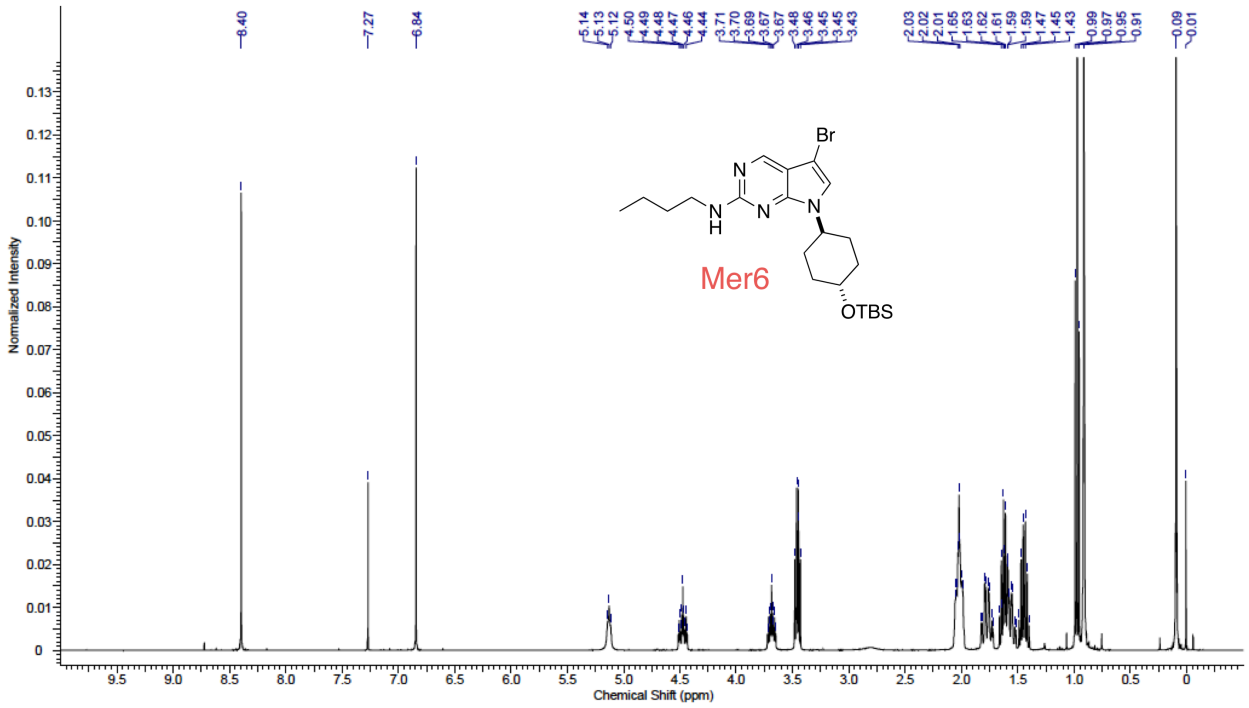


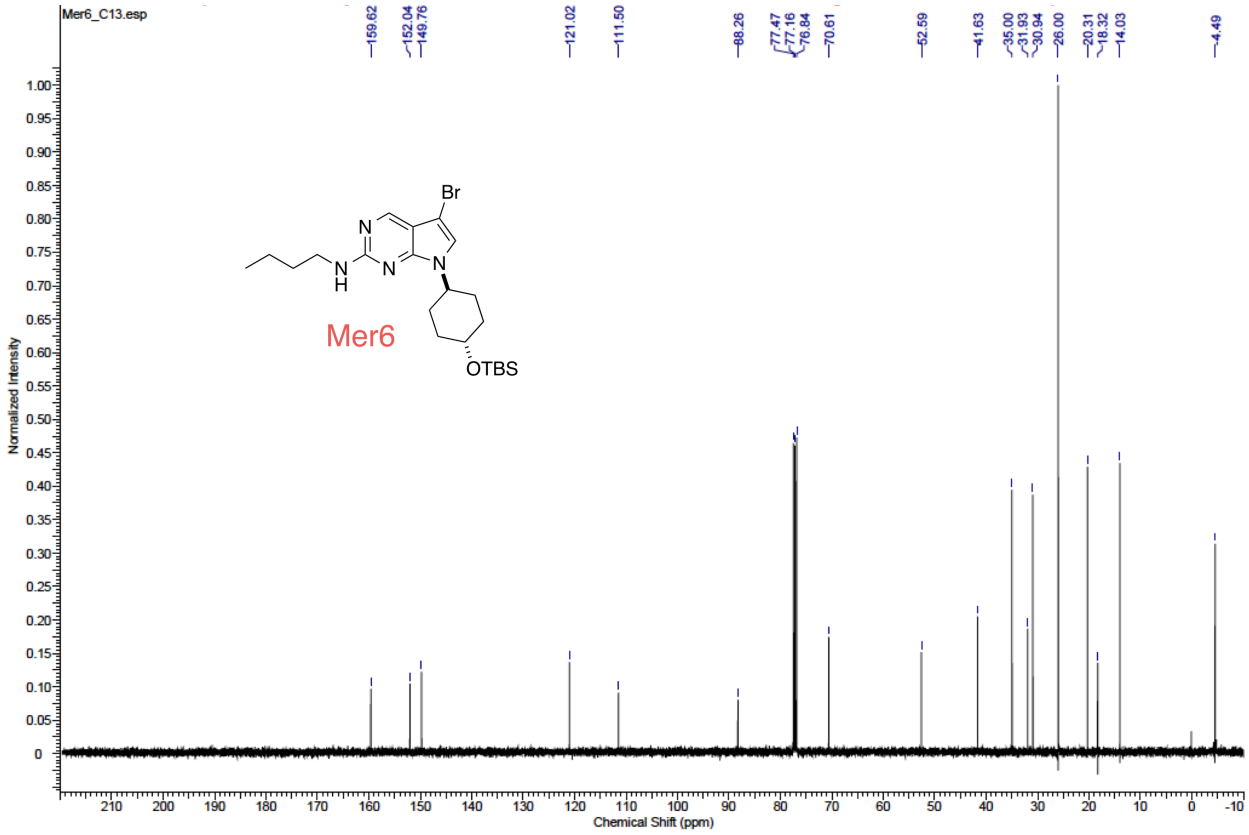
^{1}H and ^{13}C NMR spectra of Mer5



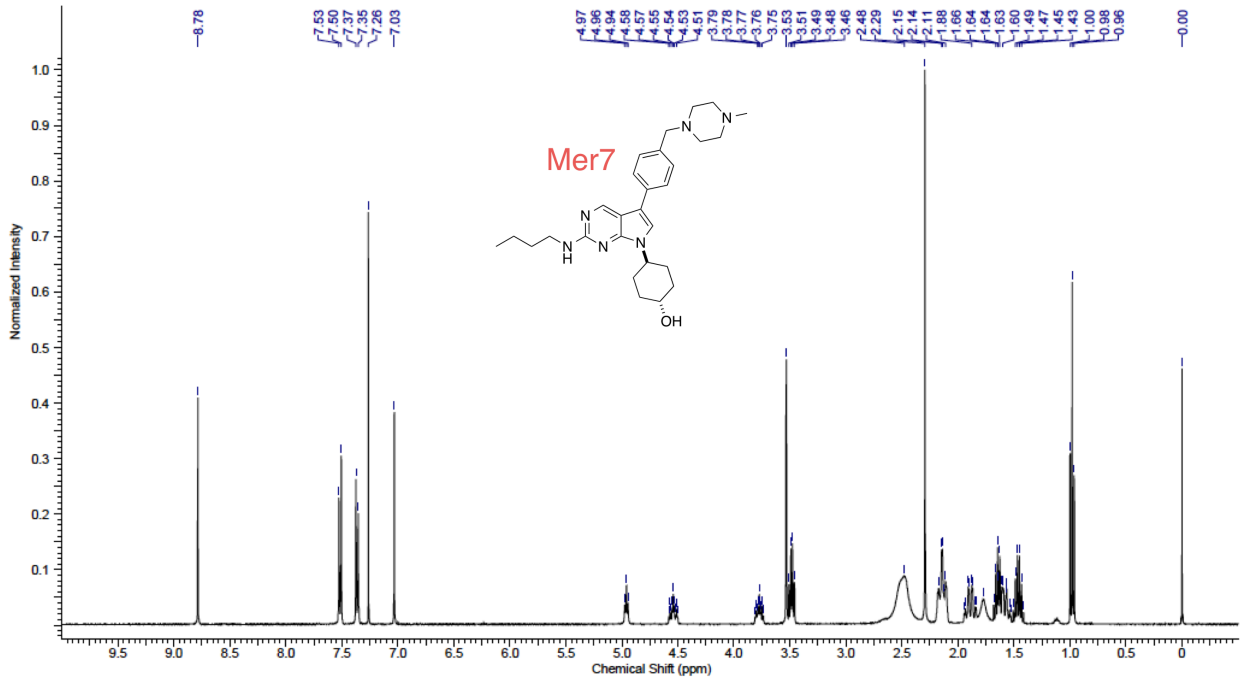


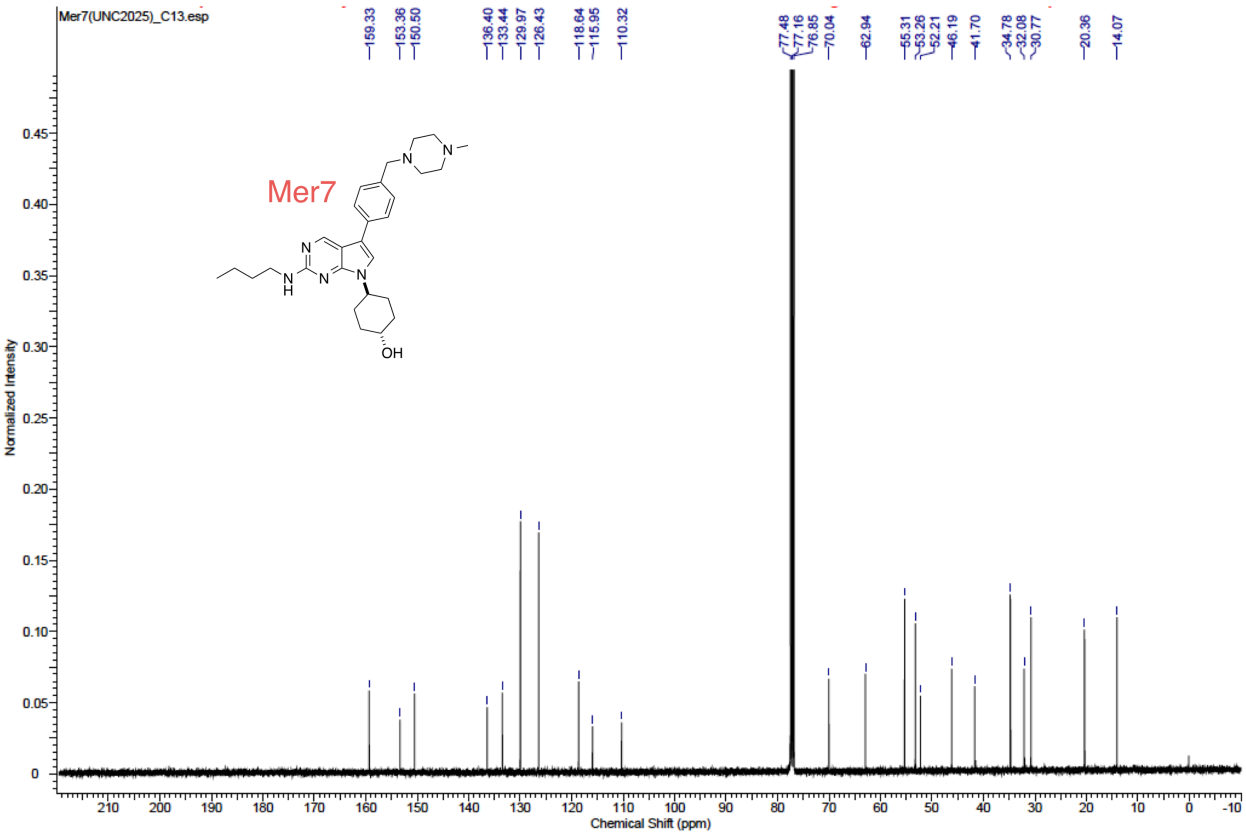
^{1}H and ^{13}C NMR spectra of Mer6



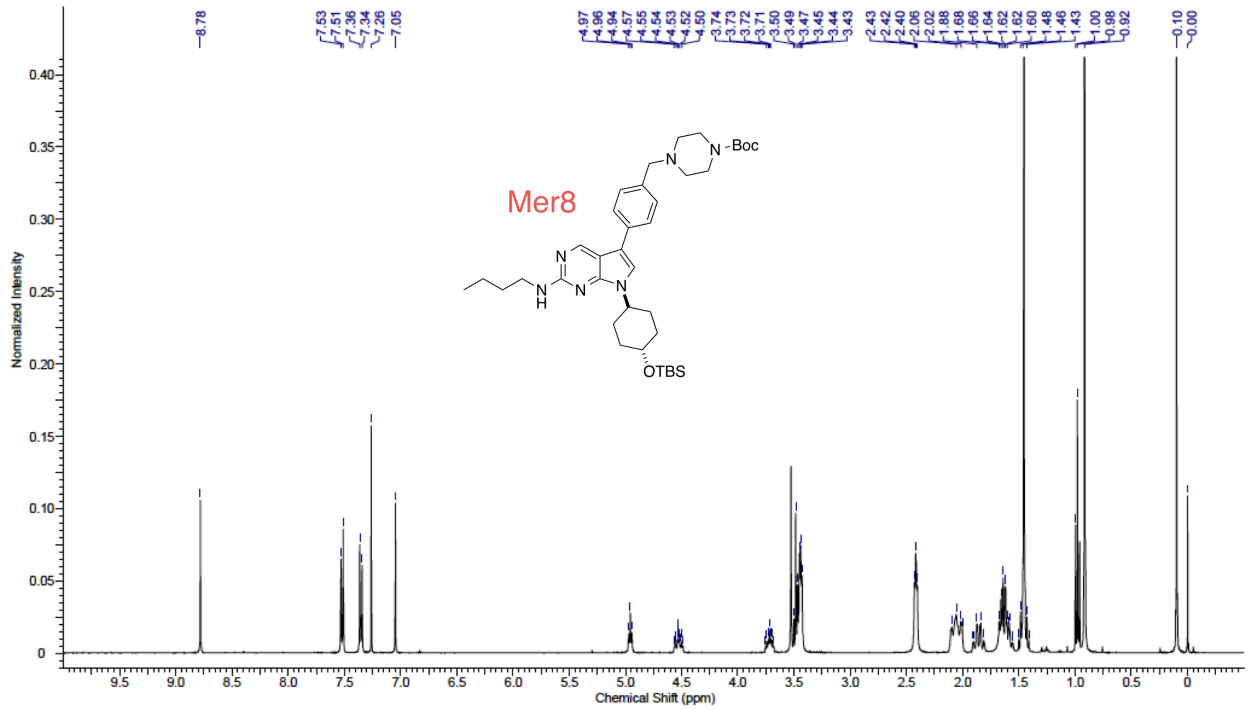


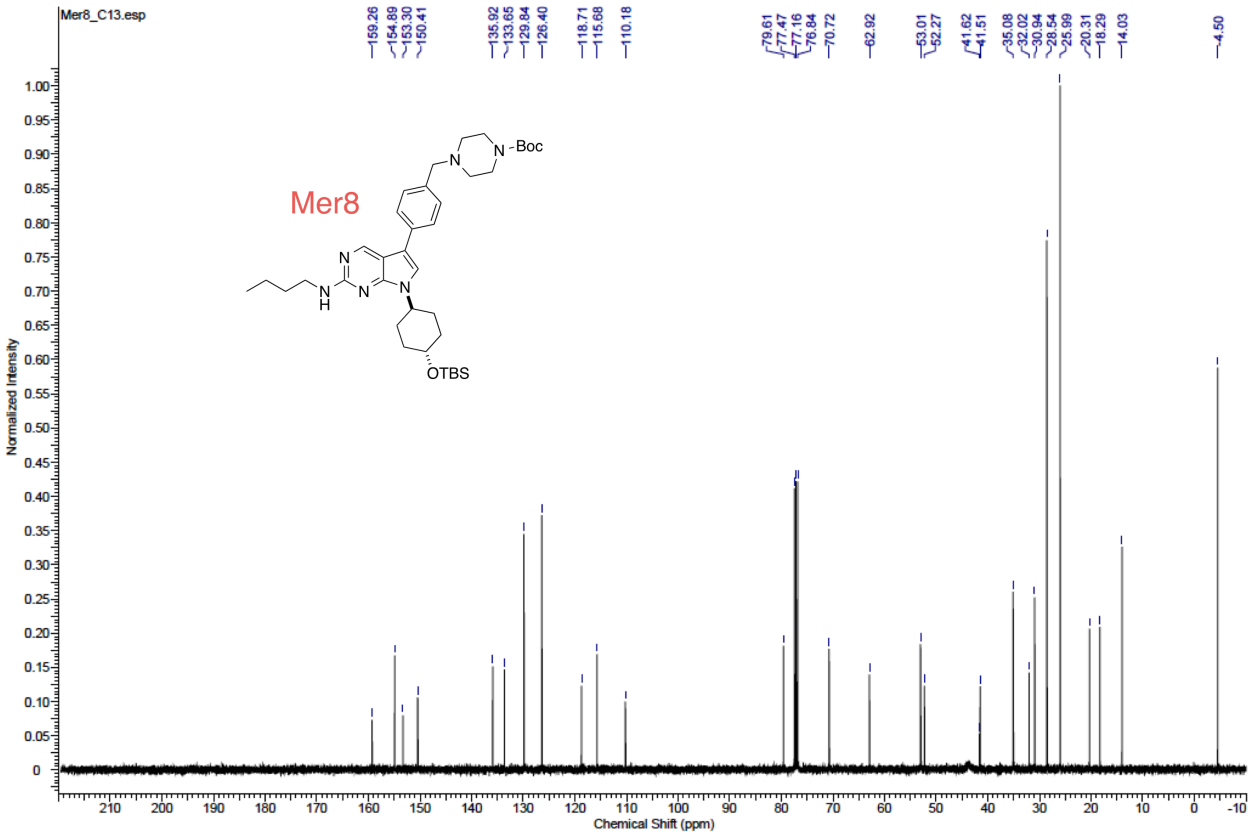
^{1}H and ^{13}C NMR spectra of Mer7





^{1}H and ^{13}C NMR spectra of Mer8





^{1}H NMR spectra of Mer10 (MERi-SiR)