Supporting Information for

A silicon-rhodamine chemical-genetic hybrid for far red voltage imaging from defined neurons in brain slice

Gloria Ortiz,[‡] Pei Liu,[‡] Parker E. Deal,[‡] Ashley K. Nensel,[‡] Kayli N. Martinez,[‡] Kiarash Shamardani,[§] Hillel Adesnik,[§†] and Evan W. Miller[‡§†*]

Departments of [‡]Chemistry and [§]Molecular & Cell Biology and [†]Helen Wills Neuroscience Institute. University of California, Berkeley, California 94720, United States.

DOI: doi.org/10.1039/D1CB00156F

Table of Contents

Chemical Synthesis and characterization

Chemical reagents and solvents (dry) were purchased from commercial suppliers and used without further purification. Acid-dPEG$_{25}$-NHS ester was purchased from Quanta Biodesign. Compounds **1**[1], (*E*)-3-methoxy-*N*,*N*-dimethyl-4-(4-vinylstyryl)aniline[2], and HaloTag-amine[3] were prepared according to the literature procedures. All reactions were carried out in flame-dried flasks sealed with septa and conducted under a nitrogen atmosphere. Thin layer chromatography (TLC) (silica gel, F254, 250 μm) was performed on precoated TLC glass plates and were visualized by fluorescence quenching under UV light. Flash column chromatography was performed on Silicycle Silica Flash F60 (230–400 Mesh) using a forced flow of air at 0.5–1.0 bar. NMR spectra were recorded on a Bruker AVB-400 MHz and a Bruker AV-600 MHz spectrometer. Chemical shifts (δ) are expressed in parts per million (ppm) and are referenced to $CDCl_3$ (7.26 ppm, 77.0 ppm) or DMSO (2.50 ppm, 40 ppm). Coupling constants are reported as Hertz (Hz). Splitting patterns are indicated as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; m, multiplet. High-resolution mass spectra (ESI EI) were measured by the QB3/Chemistry mass spectrometry service at University of California, Berkeley. High performance liquid chromatography (HPLC) and low resolution ESI Mass Spectrometry were performed on an Agilent Infinity 1200 analytical instrument coupled to an Advion CMS-L ESI mass spectrometer. The column used for the analytical HPLC was Phenomenex Luna 5 μm C18(2) (4.6 mm I.D. × 150 mm) with a flow rate of 1.0 mL/min. Semi-preparative HPLC was performed on a Perkin Elmer Series 200 HPLC using a Phenomonex Luna 5 μm C18(2) (150 x 10 mm) column with a flow rate of 5.0 mL/min. In all cases, the mobile phases were MQ-H_2O with 0.05% trifluoroacetic acid (eluent A) and HPLC grade MeCN with 0.05% trifluoroacetic acid (eluent B). For analytical HPLC, signals were monitored at 254, 380, and 650 nm over 10 min, with a gradient of 10 to 100% eluent B for 6 min, then held at 100% B for 4 min. For semi-preparative HPLC, signals were monitored at 254 over 20 min with a gradient of 10 to 100% eluent B.

Spectroscopic Studies

UV-Vis absorbance and fluorescence spectra were recorded using a 2501 Spectrophotometer (Shimadzu) and a Quantamaster Master 4 L-format scanning spectrofluorometer (Photon Technologies International). The fluorometer is equipped with an LPS-220B 75-W xenon lamp and power supply, A-1010B lamp housing with integrated igniter, switchable 814 photon-counting/analog photomultiplier detection unit, and MD5020 motor driver. Samples were measured in 1-cm path length quartz cuvettes (Starna Cells).

The maximum absorption wavelength (λ_{max}), maximum emission wavelength (λ_{em}), and extinction coefficient (ε) were taken in PBS (100 mM $Na_2HPO_4 \cdot 7H_2O$, 150 mM NaCl, pH 7.4) solution containing 0.10 % (w/w) SDS using stock solutions of isoBeRSTs in DMSO (0.5-1 mM); the reported value for ε is an average (n = 3).

Quantum yields were determined by comparison to a rhodamine b standard in EtOH. Relative quantum yields (Φ_{fl}) were calculated by comparison to standard Cyanine5.5 carboxilic acid (Φ = 0.23 in PBS). Stock solutions of standards were prepared in DMSO (1 mM) and diluted with appropriate solvent (1:1000 dilution). Absorbance and emission (λ_{ex} = 625 nm) were taken at 4 concentrations. The absorbance value at the λ_{ex} was plotted against the integration of the area of fluorescence curve (635-800 nm). The λ_{ex} ensured the full fluorescence area of

the dyes excited at 625 nm was used for Φ_{fl} calculations. The slope of the linear best fit of the data was used to calculate the relative Φ_{fl} by the equation $\Phi_{fl(X)} = \Phi_{fl(R)}(S_R/S_X)(\eta_X/\eta_R)^2$, where S_R and S_X are the slopes of the standard and unknown, respectively, and η is the refractive index of the solution.[4]

Cell culture

All animal procedures were approved by the UC Berkeley Animal Care and Use Committees and conformed to the NIH Guide for the Care and Use and Laboratory Animals and the Public Health Policy.

HEK cell culture

Human embryonic kidney 293T (HEK) cells were obtained from the UC Berkeley Cell Culture Facility. Cell were maintained in Dulbecco's modified eagle medium (DMEM) supplemented with 4.5 g/L D-glucose, 10% fetal bovine serum (FBS; Thermo Scientific) and 1% GlutaMax (Invitrogen) at 37 °C in a humidified incubator with 5% CO_2. Cells were passaged and plated in DMEM (as above) at a density of 750,000 cells per well in a 6-well plate. Transfection of plasmids was carried out using Lipofectamine 3000 (Invitrogen) ~18-24 h after plating. The cells were split again 48 h after transfection and plated onto 12 mm glass coverslips pre-coated with Poly-D-Lysine (PDL; 1 mg/ml; Sigma-Aldrich) at a density of 75,000 cells per coverslip in DMEM supplemented with 1 g/L D-glucose, 10% FBS and 1% GlutaMax. Imaging was performed 12-18 h after plating.

Primary neuronal culture and transfection

Hippocampi were dissected from embryonic day 19 Sprague Dawley rats (Charles River Laboratory) in cold, sterile HBSS (zero Ca^{2+}, zero Mg^{2+}, phenol red). All dissection products were supplied by Invitrogen, unless otherwise stated. Hippocampal tissue was treated with trypsin (2.5%) for 15 min at 37 °C. The tissue was triturated using fire polished Pasteur pipettes, in minimum essential media (MEM) supplemented with 5% FBS, 2% B-27, 2% 1M dextrose (Fisher Scientific) and 1% GlutaMax. The dissociated cells were plated onto 12 mm diameter coverslips (Fisher Scientific) pre-treated with PDL (as above) at a density of 25-30,000 cells per coverslip in MEM supplemented media (as above). Neurons were maintained at 37 °C in a humidified incubator with 5% CO_2. At 1 day in vitro (DIV) half of the MEM supplemented media was removed and replaced with Neurobasal media containing 2% B-27 supplement and 1% GlutaMax. Transfection of plasmids was carried out using Lipofectamine 3000 (without P3000 reagent) at 6-7 DIV. Imaging was performed on mature neurons 13-16 DIV.

Unless stated otherwise, for loading of HEK cells and hippocampal neurons, DMSO stock solutions of isoBeRSTs (1 mM) were diluted directly into HBSS to working concentrations. For HEK cells and neurons, the typical working concentration was 500 nM for untargeted isoBeRSTs and 50 nM for isoBeRST-Halo. HEK cells were incubated for 30 mins with isoBeRSTs at 37 °C before exchanging dye/HBSS for HBSS without any dye. Neurons were treated identically, unless specified. All imaging was performed in HBSS at room temperature.

In utero electroporation

Pregnant mice at E15-16 were anaesthetized with 2.0% isoflurane, the abdomen was cleaned with 70% ethanol and swabbed with iodine, and a small vertical incision was made in the skin and abdominal wall and 8–12 embryos gently exposed. Each embryo was injected with 0.5–1 µl of DNA solution and 0.05% Fast Green dye. We used a pressure-controlled beveled glass pipette (Drummond, Custom Microbeveller) for injection. After each injection, the embryos were moistened with saline and voltage steps via tweezertrodes (BTX, 5 mm round, platinum, BTX electroporator) were applied with the positive electrode placed over the visual cortex and the negative electrode placed under the head of the embryo. Voltage was 40 V for 5 pulses at 1 Hz, each pulse lasting 50 ms. The embryos were returned to the abdomen, which was sutured, followed by suturing of the skin. The procedure typically lasted under 30 min.

Acute brain slice preparation

Mice were deeply anesthetized with isoflurane and quickly decapitated. After removing the scalp and skull, ice-cold artificial cerebrospinal fluid with sucrose (ACSF-sucrose) cutting solution (in mM: NaCl, 83; KCl, 2.5; $MgSO_4$, 3.3; NaH_2PO4, 1; $NaHCO_3$, 26.2; D-glucose, 22; sucrose, 72; and $CaCl_2$, 0.5) was applied to the brain. BFP fluorescence was checked with a hand-held laser before the brain was taken out. The brain was cut into 300 μm thick slices with a DTK-1000 slicer in ice-cold ACSF-sucrose cutting solution. The cut slices were incubated in sucrose cutting solution, bubbled with 95% O_2 and 5% CO_2, first at 31 °C for about 30 min and then at room temperature until further use.

Epifluorescence microscopy

Imaging was performed on an AxioExaminer Z-1 (Zeiss) equipped with a Spectra-X Light engine LED light (Lumencor), controlled with Slidebook (v6, Intelligent Imaging Innovations). Images were acquired with a W-Plan-Apo 20x/1.0 water objective (20x; Zeiss). Images were focused onto either an OrcaFlash4.0 sCMOS camera (sCMOS; Hamamatsu) or an eVolve 128 EMCCD camera (EMCCD; Photometrix). For isoBeRST images, the excitation light was delivered from a LED (6.72 W/cm^2; 20 ms exposure time) at 631/28 (bandpass) nm and emission was collected with a quadruple emission filter (430/32, 508/14, 586/30, 708/98 nm) after passing through a quadruple dichroic mirror (432/38, 509/22, 586/40, 654 nm LP). For EGFP images, the excitation light was delivered from a LED (5.77 W/cm^{-2}; 20 ms exposure time) at 475/34 nm and emission was collected with a quadruple emission filter (430/32, 508/14, 586/30, 708/98 nm) after passing through a quadruple dichroic mirror (432/38, 509/22, 586/40, 654 nm LP).

Brain slice staining, imaging, and electrophysiology

For bath application of the dye and cell staining, a slice was transferred to a 35 mm dish with 3mL ACSF-sucrose cutting solution (total volume) bubbled with 95% O_2 and 5% CO_2 to which dye stock solution was added (250 to 500 nM final concentration). The slice was incubated with the dye at room temperature for 15 min to 30 min with carbogen. For functional imaging, the slice was transferred to a fresh dish with ACSF recording solution (in mM: NaCl, 119; KCl, 2.5; $MgSO_4$, 1.3; NaH_2PO_4, 1.3; $NaHCO_3$, 26; D-glucose, 20 and $CaCl_2$, 2.5) using a plastic Pasteur pipette and washing is not necessary. Slices were imaged immediately or after a wait time of up to 2 hours (slices were in fresh ACSF). A small harp or staple is used to press down the slice to minimize disturbance on the slice during perfusion and to make sure slice is flat for even illumination.

Confocal imaging was performed with a Zeiss LSM 880 NLO AxioExaminer equipped with a Diode 405 nm laser line, Argon 458, 488, and 514 laser lines, a DPSS 561 nm laser line, a HeNe 633 laser line and a BiG-2 detector with a 690+ dichroic. Images were acquired using a W-Plan-Apo 20x/1.0 water objective and a Zeiss Airyscan detector.

For whole-cell, current clamp in slices, slices were transferred to the microscope perfusion chamber with ACSF recording solution. Patch pipettes were loaded with internal solution (as stated above) and had resistances of ~5 MOhm. A Multiclamp 700B amplifier (Molecular Devices) was used to amplify the signal, which is filtered at 2 kHz and digitized at 20 kHz (National Instruments). Samples were illuminated with spectra-X Light engine LED light (Lumencor). Images were acquired with a W-Plan-Apo 20x/1.0 water objective (20x; Zeiss) at a sampling rate of 1 kHz on a Zyla 4.2 sCMOS camera (Andor). A custom written MATLAB (Mathworks) script was used to control experiments.

Image analysis

For image intensity measurements, regions of interest were drawn around cells or neuronal cell bodies and the mean fluorescence was calculated in ImageJ (FIJI, NIH). Background fluorescence was subtracted by measuring the fluorescence where no cells grew. The fold turn-on was calculated by taking the ratio of transfected cells fluorescence and untransfected cells fluorescence, both background subtracted.

Analysis of voltage sensitivity in HEK cells was performed using ImageJ (FIJI). Briefly, a region of interest (ROI) was selected automatically based on fluorescence intensity and applied as a mask to all image frames. Fluorescence intensity values were calculated at known baseline and voltage step epochs. For analysis of voltage responses in neurons, regions of interest encompassing cell bodies (all of approximately the same size) were drawn in ImageJ and the mean fluorescence intensity for each frame extracted. ΔF/F values were calculated by first subtracting a mean background value from all raw fluorescence frames, to give a background subtracted trace (bkgsub). A baseline fluorescence value (Fbase) is calculated from the median of all the frames, and subtracted from each timepoint of the bkgsub trace to yield a ΔF trace. The ΔF was then divided by Fbase to give ΔF/F traces. No averaging has been applied to any voltage traces.

Electrophysiology and Imaging in HEK cells and primary cultured neurons

For electrophysiological experiments, pipettes were pulled from borosilicate glass (Sutter Instruments, BF150-86-10), with a resistance of 4–6 MΩ, and were filled with an internal solution; 115 mM potassium gluconate, 10 mM BAPTA tetrapotassium salt, 10 mM HEPES, 5 mM NaCl, 10 mM KCl, 2 mM ATP disodium salt, 0.3 mM GTP trisodium salt (pH 7.25, 275 mOsm). Recordings were obtained with an Axopatch 200B amplifier (Molecular Devices) at room temperature. The signals were digitized with a Digidata 1440A, sampled at 50 kHz and recorded with pCLAMP 10 software (Molecular Devices) on a PC. Fast capacitance was compensated in the on-cell configuration. For all electrophysiology experiments, recordings were only pursued if series resistance in voltage clamp was less than 30 MΩ. For whole-cell, voltage clamp recordings in HEK 293T cells, cells were held at -60 mV and hyper- and depolarizing steps applied from -100 to +100 mV in 20 mV increments.

Extracellular field stimulation was delivered by a SD9 Grass Stimulator connected to a recording chamber containing two platinum electrodes (Warner), with triggering provided through the same Digidata 1332A digitizer and pCLAMP 9 software (Molecular Devices) that ran the electrophysiology. Action potentials were triggered by 1 ms 60 V field potentials delivered at 5 Hz. To prevent recurrent activity, the HBBS bath solution was supplemented with synaptic blockers; 10 μM 2,3-Dioxo-6-nitro-1,2,3,4- tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX; Santa Cruz Biotechnology) and 25 μM DL-2-Amino-5-phosphonopentanoic acid (APV; Sigma-Aldrich). For both evoked action potentials and spontaneous activity, images were binned 4x4 to allow sampling rates of 0.5 kHz and 2500 frames (5 s) were acquired for each recording. For spontaneous and evoked activity recordings of isoBeRST, the excitation light was delivered from a LED (1.94 W/cm^2) at 631/28 (bandpass) nm and emission was collected with a quadruple emission filter (430/32, 508/14, 586/30, 708/98 nm) after passing through a quadruple dichroic mirror (432/38, 509/22, 586/40, 654 nm LP).

DNA constructs

To express the HaloTag protein on the cell surface, an IgK leader sequence was fused to the N-terminal and a transmembrane domain (pDisplay) was added to the C-terminal of the HaloTag sequence. For the purpose of inmmunostaining, an HA tag was inserted. Mammalian expression vector pcDNA3 with either a cytomegalovirus (CMV) promoter or human synapsin promoter (Syn) was used for protein expression in HEK cells and cultured neurons, respectively. To increase expression in neurons, a regulatory element from the woodchuck hepatitis virus (WPRE) was used. In some constructs, nuclear-targeted EGFP was inserted down stream of HaloTag, separated by an internal ribosome entry site (IRES) sequence, in order to track the expression of HaloTag in live cells. The cloned

constructs were verified by sequencing. All the constructs were prepared using Qiagen Maxiprep kit, except those with CMV promoter. The following sequences were used (5' to 3'):

IgK

ATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCCAGGTTCCACTGGTGAC

HaloTag

GCAGAAATCGGTACTGGCTTTCCATTCGACCCCCATTATGTGGAAGTCCTGGGCGAGCGCATGCACT
ACGTCGATGTTGGTCCGCGCGATGGCACCCCTGTGCTGTTCCTGCACGGTAACCCGACCTCCTCCTA
CGTGTGGCGCAACATCATCCCGCATGTTGCACCGACCCATCGCTGCATTGCTCCAGACCTGATCGGT
ATGGGCAAATCCGACAAACCAGACCTGGGTTATTTCTTCGACGACCACGTCCGCTTCATGGATGCCT
TCATCGAAGCCCTGGGTCTGGAAGAGGTCGTCCTGGTCATTCACGACTGGGGCTCCGCTCTGGGTTT
CCACTGGGCCAAGCGCAATCCAGAGCGCGTCAAAGGTATTGCATTTATGGAGTTCATCCGCCCTATC
CCGACCTGGGACGAATGGCCAGAATTTGCCCGCGAGACCTTCCAGGCCTTCCGCACCACCGACGTC
GGCCGCAAGCTGATCATCGATCAGAACGTTTTTATCGAGGGTACGCTGCCGATGGGTGTCGTCCGCC
CGCTGACTGAAGTCGAGATGGACCATTACCGCGAGCCGTTCCTGAATCCTGTTGACCGCGAGCCACT
GTGGCGCTTCCCAAACGAGCTGCCAATCGCCGGTGAGCCAGCGAACATCGTCGCGCTGGTCGAAGA
ATACATGGACTGGCTGCACCAGTCCCCTGTCCCGAAGCTGCTGTTCTGGGGCACCCCAGGCGTTCTG
ATCCCACCGGCCGAAGCCGCTCGCCTGGCCAAAAGCCTGCCTAACTGCAAGGCTGTGGACATCGGC
CCGGGTCTGAATCTGCTGCAAGAAGACAACCCGGACCTGATCGGCAGCGAGATCGCGCGCTGGCTG
TCGACGCTCGAGATTTCCGGC

HA

TATCCATATGATGTTCCAGATTATGCT

pDisplay

GCTGTGGGCCAGGACACGCAGGAGGTCATCGTGGTGCCACACTCCTTGCCCTTTAAGGTGGTGGTGA
TCTCAGCCATCCTGGCCCTGGTGGTGCTCACCATCATCTCCCTTATCATCCTCATCATGCTTTGGCAG
AAGAAGCCACGT

IRES

GCCCCTCTCCCTCCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTT
TGTCTATATGTTATTTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTG
TCTTCTTGACGAGCATTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTC
GTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGG
CAGCGGAACCCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATACACCT
GCAAAGGCGGCACAACCCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTC
TCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGCACCCCATTGTATGGGATCTGAT
CTGGGGCCTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAAAACGTCTAGGCCCCCCGA
ACCACGGGGACGTGGTTTTCCTTTGAAAAACACGATGATAATATGGCCACA

Nuclear Localization Sequence

ATGGTGCCCAAGAAGAAGAGGAAAGTCGTGAGCAAGGGCGAGGAGGACAACATGGCCATCATCAA
GGAGTTCATGCGCTTCAAGGTGCAC

EGFP

AGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAAC
GGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAG
TTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCG
TGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGA
AGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGT
GAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGG
CAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAA
GCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCT
CGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTA
CCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGA
GTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAA

WPRE

GCTTATCGATAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTTG
CTCCTTTTACGCTATGTGGATACGCTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTT
TCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTCTTTATGAGGAGTTGTGGCCCGTTGTCAGG
CAACGTGGCGTGGTGTGCACTGTGTTTGCTGACGCAACCCCCACTGGTTGGGGCATTGCCACCACCT
GTCAGCTCCTTTCCGGGACTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACTCATCGCCGCCTGC
CTTGCCCGCTGCTGGACAGGGGCTCGGCTGTTGGGCACTGACAATTCCGTGGTGTTGTCGGGGAAAT
CATCGTCCTTTCCTTGGCTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCCTTCTGCTAC
GTCCCTTCGGCCCTCAATCCAGCGGACCTTCCTTCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCC
GCGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGCCGCCTCCCCGCATCGATACCG

CMV promoter

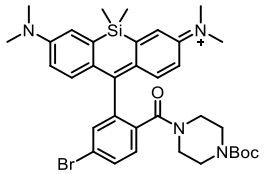
GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATAT
GGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCA
TTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGG
TGGACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCC
TATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTT
CCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACAT
CAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGG
AGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGC
AAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCT

Synapsin Promoter

GTGTCTAGACTGCAGAGGGCCCTGCGTATGAGTGCAAGTGGGTTTTAGGACCAGGATGAGGCGGGG
TGGGGGTGCCTACCTGACGACCGACCCCGACCCACTGGACAAGCACCCAACCCCCATTCCCCAAATT
GCGCATCCCCTATCAGAGAGGGGGAGGGGAAACAGGATGCGGCGAGGCGCGTGCGCACTGCCAGC
TTCAGCACCGCGGACAGTGCCTTCGCCCCCGCCTGGCGGCGCGCGCCACCGCCGCCTCAGCACTGAA
GGCGCGCTGACGTCACTCGCCGGTCCCCCGCAAACTCCCCTTCCCGGCCACCTTGGTCGCGTCCGCG

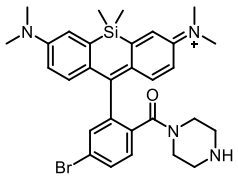
CCGCCGCCGGCCCAGCCGGACCGCACCACGCGAGGCGCGAGATAGGGGGGCACGGGCGCGACCAT
CTGCGCTGCGGCGCCGGCGACTCAGCGCTGCCTCAGTCTGCGGTGGGCAGCGGAGGAGTCGTGTCG
TGCCTGAGAGCGCAGTCGAGA

Synthetic Procedures



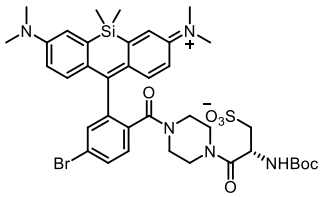
N-(10-(5-bromo-2-(4-(tert-butoxycarbonyl)piperazine-1-carbonyl)phenyl)-7-(dimethylamino)-5,5-dimethyldibenzo[b,e]silin-3(5H)-ylidene)-N-methylmethanaminium (2):

To a solution of silicon rhodamine **1**[1] (110 mg, 0.217 mmol, 1 eq) in CH_2Cl_2 (5.9 mL), oxalyl chloride (24.4 µL, 0.260 mmol, 1.2 eq) was added and stirred for 30 min. Triethylamine (7.68 µL, 0.056 mmol, 2 eq) and 1-Boc-Piperzine (31 mg, 0.168 mmol, 6 eq) were added in succession. The resulting mixture was stirred at room temperature for 4 h. Dilute HCl (~ 0.1 M) was added and the organics extracted with CH_2Cl_2 (3x). The combined organic extracts were dried with anhydrous sodium sulfate, filtered, and concentrated *in vacuo.* The crude residue was purified by preparative TLC to afford the chloride salt of **2** (90 mg, 55%) as a dark blue solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.86 (d, *J* = 8.2 Hz, 1H), 7.61 (s, 1H), 7.50 (d, *J* = 8.3 Hz, 1H), 7.37 (s, 2H), 7.17 (d, *J* = 9.5 Hz, 2H), 6.84 (d, *J* = 9.7 Hz, 2H), 3.35 – 3.01 (m, 8H), 1.44 (s, 9H), 0.67 (s, 3H), 0.52 (s, 3H); HRMS (ESI) calcd for $C_{35}H_{44}BrN_4O_3Si$ $[M]^+$ 675.2361, found 675.2349; LRMS (ESI) calcd for $C_{35}H_{44}BrN_4O_3Si$ $[M]^+$ 675.2, found 675.3.



N-(10-(5-bromo-2-(piperazine-1-carbonyl)phenyl)-7-(dimethylamino)-5,5-dimethyldibenzo[b,e]silin-3(5H)-ylidene)-N-methylmethanaminium (3):

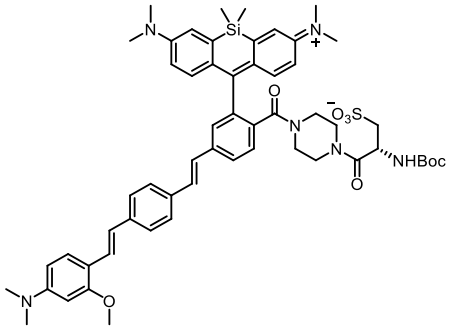
To **2** (48 mg, 0.12 mmol) in CH_2Cl_2 (1.5 mL) was added trifluoroacetic acid (1.5 mL). The reaction was stirred at room temperature for 1 h, then the solvent removed under a stream of nitrogen. Toluene was added and the reaction mixture was then concentrated *in vacuo* (1x). Methanol was added and the reaction mixture was concentrated *in vacuo* (3x). It was further dried under high vacuum to afford the TFA salt of **3** (52 mg, 91%) as a dark blue solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.87 (d, *J* = 8.1 Hz, 1H), 7.62 (s, 1H), 7.50 (d, *J* = 8.3 Hz, 1H), 7.37 (d, *J* = 2.6 Hz, 2H), 7.17 (d, *J* = 9.6 Hz, 2H), 6.84 (dd, *J* = 9.6, 2.5 Hz, 2H), 3.54 – 3.21 (m, 4H), 2.70 (bs, 2H), 2.54 (bs, 2H), 0.66 (s, 3H), 0.54 (s, 3H); HRMS (ESI) calcd for $C_{30}H_{36}BrN_4OSi$ $[M]^+$ 575.1836, found 575.1840; LRMS (ESI) calcd for $C_{30}H_{36}BrN_4OSi$ $[M]^+$ 575.2, found 575.2.



(R)-3-(4-(4-bromo-2-(7-(dimethylamino)-3-(dimethyliminio)-5,5-dimethyl-3,5-dihydrodibenzo[b,e]silin-10-yl)benzoyl)piperazin-1-yl)-2-((tert-butoxycarbonyl)amino)-3-oxopropane-1-sulfonate (4):

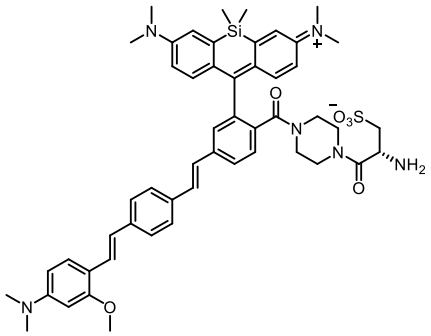
To **3** (67 mg, 0.083 mmol, 1 equiv), Boc-L-cysteic acid (33 mg, 0.12 mmol, 1.5 equiv), and HATU (47 mg, 0.12 mmol, 1.5 equiv) was added anhydrous DMF (2.2 mL) under nitrogen. Anhydrous diisopropylethylamine (54 µL, 0.31 mmol, 2.5 equiv) was added and the reaction stirred overnight at room temperature. The solvent was evaporated

under reduced pressure, the mixture diluted with CH_2Cl_2, and dilute HCl (~ 0.1 M) was added. The organics were extracted with CH_2Cl_2 (3x). The combined organic extracts were dried with anhydrous sodium sulfate, filtered, and concentrated *in vacuo.* The crude residue was purified by preparative TLC to yield **4** (56 mg, 82%) as a dark blue solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.70 (dd, *J* = 8.2, 1.9 Hz, 1H), 7.45 (d, *J* = 1.9 Hz, 1H), 7.28 (d, *J* = 8.3 Hz, 1H), 7.16 – 6.95 (m, 4H), 6.85 (d, *J* = 9.4 Hz, 1H), 6.62 (d, *J* = 8.9 Hz, 1H), 5.30 (s, 1H), 4.92 (s, 1H), 3.56 – 3.20 (m, 20H), 3.08 (dd, *J* = 13.1, 9.3 Hz, 1H), 3.01 – 2.95 (m, 1H), 2.32 (s, 1H), 1.35 (s, 9H), 0.54 (s, 3H), 0.47 (s, 3H); HRMS (ESI) calcd for $C_{38}H_{48}BrN_5NaO_7SSi$ $[M+Na]^+$ 848.2119, found 848.2131; LRMS (ESI) calcd for $C_{38}H_{49}BrN_5O_7SSi$ $[M+H]^+$ 826.2, found 826.3.



2-((tert-butoxycarbonyl)amino)-3-(4-(4-((E)-4-((E)-4-(dimethylamino)-2-methoxystyryl)styryl)-2-(7-(dimethylamino)-3-(dimethyliminio)-5,5-dimethyl-3,5-dihydrodibenzo[b,e]silin-10-yl)benzoyl)piperazin-1-yl)-3-oxopropane-1-sulfonate (5):

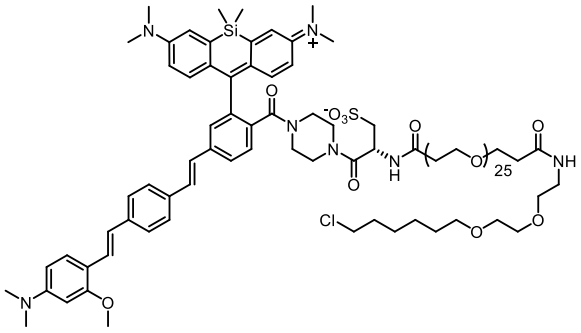
A vial was charged with **4** (47.0 mg, 56.9 μmol, 1.0 eq), (*E*)-3-methoxy-*N*,*N*-dimethyl-4-(4-vinylstyryl)aniline[2] (17.5 mg, 62.9 μmol, 1.1 eq), $Pd(OAc)_2$ (6.4 mg, 28.5 μmol, 0.5 eq), and P(o-tol)$_3$ (17.3 mg, 56.9 μmol, 1.0 eq). The vial was sealed and evacuated/backfilled with nitrogen (3x). Anhydrous DMF (0.68 mL) was added and the vial was evacuated/backfilled again with nitrogen (3x). Anhydrous Et_3N (0.24 mL) was added and the reaction was sealed and stirred at 75 °C for 2 h. The reaction was cooled, diluted with MeOH, filtered, and concentrated *in vacuo*. The crude residue was purified by preparative TLC (8% MeOH/CH_2Cl_2) to afford **5** (10 mg, 17%) as a green solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.57 – 7.41 (m, 8H), 7.30 – 7.11 (m, 5H), 7.06 (s, 1H), 6.98 (d, *J* = 16.4 Hz, 1H), 6.89 (d, *J* = 10.2 Hz, 1H), 6.67 (d, *J* = 9.8 Hz, 1H), 6.39 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.26 (d, *J* = 2.4 Hz, 1H), 5.37 (s, 1H), 5.00 (s, 1H), 3.94 (s, 3H), 3.59 – 3.29 (m, 20H), 3.14 (m 1H), 3.05 (s, 6H), 2.09 (s, 1H), 1.42 (s, 9H), 0.61 (s, 3H), 0.55 (s, 3H); HRMS (ESI) calcd for $C_{57}H_{68}N_6O_8SSi$ $[M+H]^+$ 1025.4661, found 1025.4659; LRMS (ESI) calcd for $C_{57}H_{68}N_6O_8SSi$ $[M+H]^+$ 1025.5, found 1025.5.



isoBeRST-pipcys (6):

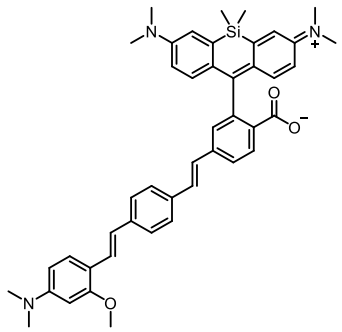
Trifluoroacetic acid (0.5 mL) was added to a solution of **6** (6.0 mg, 5.8 μmol) in CH_2Cl_2 (0.5 mL). The reaction was stirred at room temperature for 30 mins, then the solvent removed under a stream of nitrogen. Toluene was added

and the reaction mixture was concentrated *in vacuo* (1x). Methanol was added and the reaction mixture was concentrated *in vacuo* (3x). It was further dried under high vacuum to afford the TFA salt **6** (6.1 mg, quant) as a green solid. LRMS (ESI) calcd for $C_{52}H_{61}N_6O_6SSi$ $[M+H]^+$ 925.4, found 925.3.



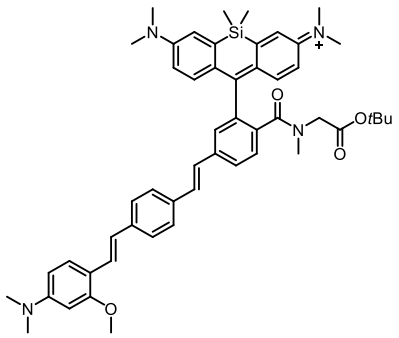
isoBeRST-Halo (7):

A vial was charged with **6** (6.0 mg, 5.8 μmol, 1.0 eq) and Acid-dPEG$_{25}$-NHS ester (7.7 mg, 5.8 μmol, 1.0 eq). Anhydrous DMF (0.6 mL) and anhydrous diisopropylethylamine (27 μL, 40 μmol, 6.9 eq) were added and the vial flushed with nitrogen. The reaction was stirred at room temperature for 7 h. Upon completion by LCMS, HaloTag-Amine[3] (5.8 mg, 25 μmol, 4.2 eq) and HATU (2.7 mg, 7.0 μmol, 1.2 eq) were added and the reaction stirred for 18 h. The reaction was then diluted with MeCN (1.0 mL). The diluted reaction was purified by semi-preparative HPLC to give **isoBeRST-Halo 7** (1.5 mg, 11%) as a green solid. HRMS (ESI) calcd for $C_{116}H_{185}ClKN_7O_{35}SSi$ $[M+H+K]^{2+}$ 1185.5875, found 1185.5829. LRMS (ESI) calcd for $C_{116}H_{187}ClN_7O_{35}SSi$ $[M+3H]^{3+}$ 778.1, found 778.3.



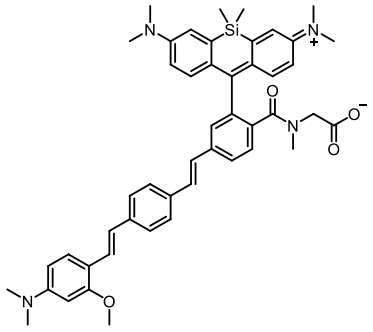
4-((E)-4-((E)-4-(dimethylamino)-2-methoxystyryl)styryl)-2-(7-(dimethylamino)-3-(dimethyliminio)-5,5-dimethyl-3,5-dihydrodibenzo[b,e]silin-10-yl)benzoate (8):

A vial was charged with **1**[1] (20 mg, 39 μmol, 1 eq), (*E*)-3-methoxy-*N*,*N*-dimethyl-4-(4-vinylstyryl)aniline[2] (11 mg, 39 μmol, 1 eq), $Pd(OAc)_2$ (4.4 mg, 22 μmol, 0.55 eq), and P(*o*-tol)$_3$ (12 mg, 39 μmol, 1 eq). The vial was evacuated/backfilled with nitrogen (3x). Anhydrous DMF (0.43 mL) was added and the mixture was evacuated/backfilled with nitrogen (3x). Anhydrous Et_3N (0.21 mL) was added and the vial was sealed and stirred overnight at 80 °C. The crude residue was purified by preparative TLC (30% EtOAc/hexanes) to afford **8** (13 mg, 47%) as a dark orange-red solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, *J* = 8.0 Hz, 1H), 7.71 (d, *J* = 8.1 Hz, 1H), 7.55 – 7.42 (m, 6H), 7.24 – 7.09 (m, 3H), 7.05 – 6.95 (m, 3H), 6.92 (d, *J* = 8.9 Hz, 2H), 6.63 (d, *J* = 8.4 Hz, 2H), 6.40 (d, *J* = 8.6 Hz, 1H), 6.27 (s, 1H), 3.94 (s, 3H), 3.05 (s, 6H), 3.02 (s, 12H), 0.73 (s, 3H), 0.66 (s, 3H); HRMS (ESI) calcd for $C_{45}H_{48}N_3O_3Si$ $[M+H]^+$ 706.3459, found 706.3450. LRMS (ESI) calcd for $C_{45}H_{48}N_3O_3Si$ $[M+H]^+$ 706.3, found 706.3.



N-(10-(2-((2-(tert-butoxy)-2-oxoethyl)(methyl)carbamoyl)-5-((E)-4-((E)-4-(dimethylamino)-2-methoxystyryl)styryl)phenyl)-7-(dimethylamino)-5,5-dimethyldibenzo[b,e]silin-3(5H)-ylidene)-N-methylmethanaminium (9):

To a solution of **8** (69 mg, 0.098 mmol, 1 eq) in CH_2Cl_2 (3.5 mL), oxalyl chloride (9.9 uL, 0.117 mmol, 1.2 eq) was added and stirred for 30 min under nitrogen. Anhydrous Et_3N (27 µL, 0.196 mmol, 2 eq) then a solution of sarcosine *tert*-butyl ester hydrochloride (31 mg, 0.168 mmol, 6 eq) and Et_3N (81 µL, 0.586 mmol, 6 eq) were added in succession. The resulting mixture was stirred at room temperature for 2 h. Dilute HCl (~ 0.1 M) was added and the organics extracted with CH_2Cl_2 (3x). The combined organic extracts were dried with anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. The crude residue was purified by preparative TLC to afford chloride salt of **9** (17 mg, 20%) as a dark green solid. HRMS (ESI) calcd for $C_{52}H_{61}N_4O_4Si$ $[M]^+$ 833.4457, found 833.4465. LRMS (ESI) calcd for $C_{52}H_{61}N_4O_4Si$ $[M]^+$ 833.4, found 832.8.



isoBeRST-sarcosine (10):

To **9** (17 mg, 0.02 mmol) in CH_2Cl_2 (2.0 mL) was added trifluoroacetic acid (1.5 mL). The reaction was stirred at room temperature for 3 h, then the solvent removed under a stream of nitrogen. Toluene was added and the reaction mixture was then concentrated *in vacuo* (1x). Methanol was added and the reaction mixture was concentrated *in vacuo* (3x). It was then dissolved in 1:6 DMSO/MeCN and purified by preparative HPLC to afford the TFA salt **10** (5.0 mg, 28%) as a green solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, *J* = 8.1 Hz, 1H), 7.64 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.54 – 7.44 (m, 7H), 7.36 – 7.33 (m, 2H), 7.20 – 7.05 (m, 4H), 7.01 – 6.95 (m, 1H), 6.80 (dd, *J* = 9.6, 2.9 Hz, 2H), 6.39 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.26 (d, *J* = 2.4 Hz, 1H), 3.93 (s, 3H), 3.92 (s, 2H) 3.35 (s, 12H), 3.05 (s, 6H), 2.90 (s, 3H), 0.60 (s, 3H), 0.55 (s, 3H). HRMS (ESI) calcd for $C_{48}H_{53}N_4O_4Si$ $[M+H]^+$ 777.3831, found 777.3839. LRMS (ESI) calcd for $C_{48}H_{53}N_4O_4Si$ $[M+H]^+$ 777.4, found 777.3.

Figures, Schemes, and Tables

Scheme S1. Synthesis of isoBeRST-sarcosine

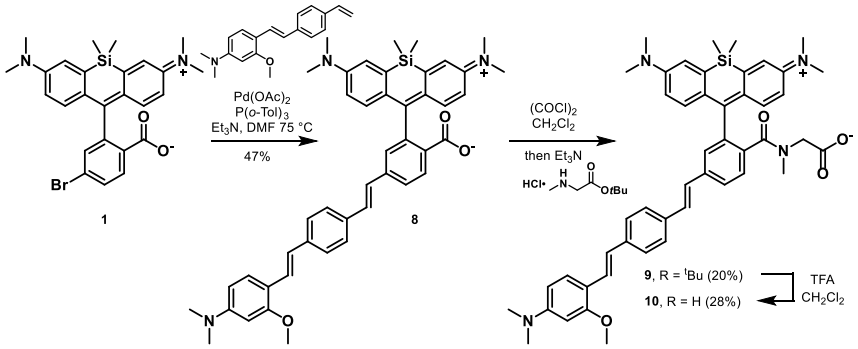


Table S1. Comparison of Rhodamine and Silicon-Rhodamine dyes.

	RhoVR-Halo[a]	isoBeRST-Halo (**7**)	RhoVR-pipcys[a]	isoBeRST-pipcys (**6**)
λ_{ex} (nm)	565[b]	662[c]	565[b]	662[c]
λ_{em} (nm)	585[b]	677[c]	585[b]	681[c]
ΔF/F (HEK)[d]	34%	21%	38%	24%
ΔF/F (neurons)[e]	10%	10%	---	---
ΔF/F (brain slice)[f]	4.3%	3.3%	---	---
SNR (brain slice)[f]	3.3	4.9	---	---
ε	---	---	82,000[b]	172,000[c]
Φ_{fl}	0.05[b]	0.034[c]	0.026[b]	0.05[c]
max selectivity[g]	9.5x	50x	---	---
photobleach rate (HEK)[h]	---	---	-4.2×10^{-5} s^{-1}	-8.0×10^{-5} s^{-1}
photobleach rate (brain slice)[i]	-3.6×10^{-5} ms^{-1}	5.8×10^{-7} ms^{-1}	---	---

[**a**] Characterization data for RhoVR-Halo and RhoVR-pipcys are from Deal, *et al. J Am Chem Soc*, **2020**, *142*, 614. RhoVR-Halo is Compound **15**; RhoVR-pipcys is Compound **7**. [**b**] In phosphate buffered saline, pH 7.2, 0.1% SDS. [**c**] In phosphate buffered saline, pH 7.4, 0.1% SDS. [**d**] per 100 mV in voltage-clamped HEK293T cells. [**e**] per spike in action potentials evoked by field stimulation of cultured rat hippocampal neurons. [**f**] per spike in action potentials evoked by whole-cell electrophysiology in brain slice. [**g**] Ratio of fluorescence in HaloTag-expressing cells compared to non-expressing cells. Summary of data from **Figure S5f**. [**h**] Linear slope of bleach curve for the first 30 s. Summary of data from **Figure S8a**. [**i**] Initial linear slope of bleach curve over first 250 ms Summary of data from **Figure S8b**.

Figure S1.

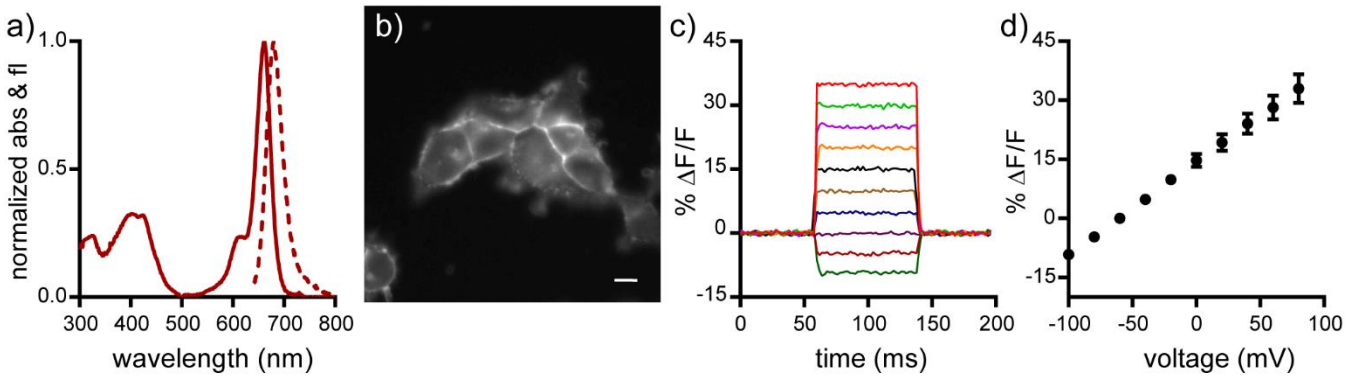


Figure S1. Cellular and *in vitro* characterization of isoBeRST-sarc **10**. a) Normalized absorbance (solid line) and emission (dashed line) spectra of isoBeRST-sarc in PBS, pH 7.4, 0.1% SDS. b) HEK cells stained with 500 nM isoBeRST-sarc. Scale bar is 10 μm. c) Plot of the fractional change in fluorescence of **10** vs time for 80 ms hyper- and depolarizing steps (-100 to +80 mV in 20 mV increments) from a holding potential of -60 mV for single HEK cells under whole-cell voltage-clamp mode. d) Plot of % ΔF/F vs final membrane potential summarizing data from five separate cells, revealing a voltage sensitivity of 24% ΔF/F per 100 mV. Error bars are ±S.D.

Figure S2.

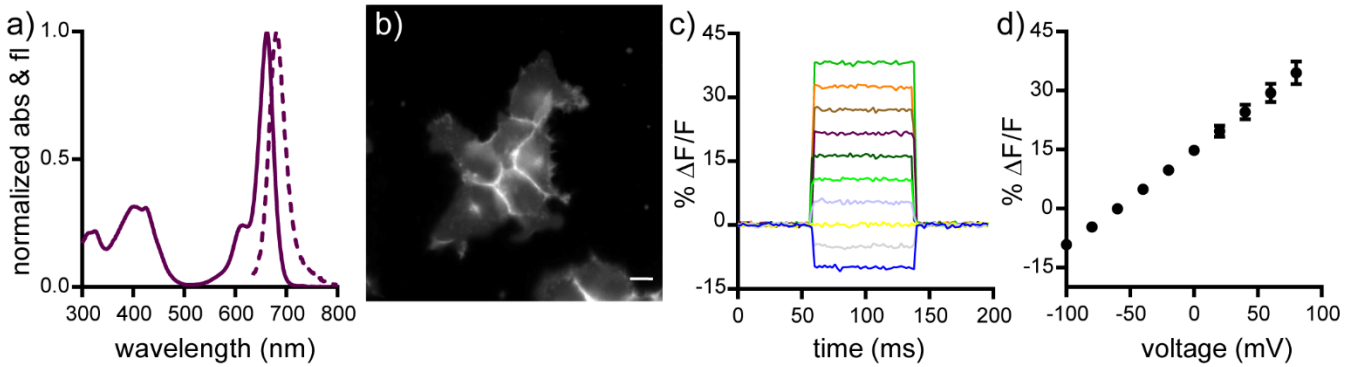


Figure S2. Cellular and *in vitro* characterization of isoBeRST-pipcys **6**. a) Normalized absorbance (solid line) and emission (dashed line) spectra of isoBeRST-pipcys in PBS, pH 7.4, 0.1% SDS. b) HEK cells stained with 500 nM isoBeRST-pipcys. Scale bar is 10 μm. c) Plot of the fractional change in fluorescence of **6** vs time for 80 ms hyper- and depolarizing steps (-100 to +80 mV in 20 mV increments) from a holding potential of -60 mV for single HEK cells under whole-cell voltage-clamp mode. d) Plot of % ΔF/F vs final membrane potential summarizing data from five separate cells, revealing a voltage sensitivity of 24% ΔF/F per 100 mV. Error bars are ±S.D.

Figure S3.

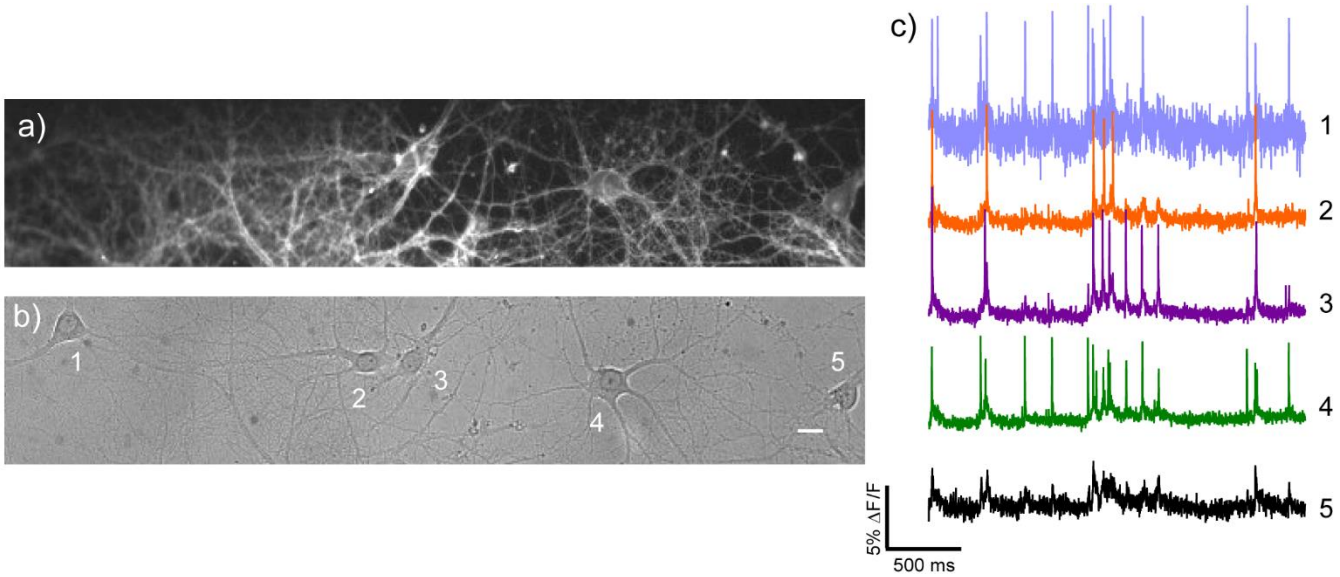


Figure S3. Voltage imaging of spontaneous neuronal activity with isoBeRST-pipcys. a) Wide-field fluorescence and b) differential interference contrast (DIC) images of cultured rat hippocampal neurons stained with isoBeRST-pipcys (**6**, 500 nM, 30 mins. Scale bar is 20 μm. c) Optical traces of spontaneous activity of the neurons in panels a-b) recorded at 500 Hz and shown as ΔF/F vs time.

Figure S4.

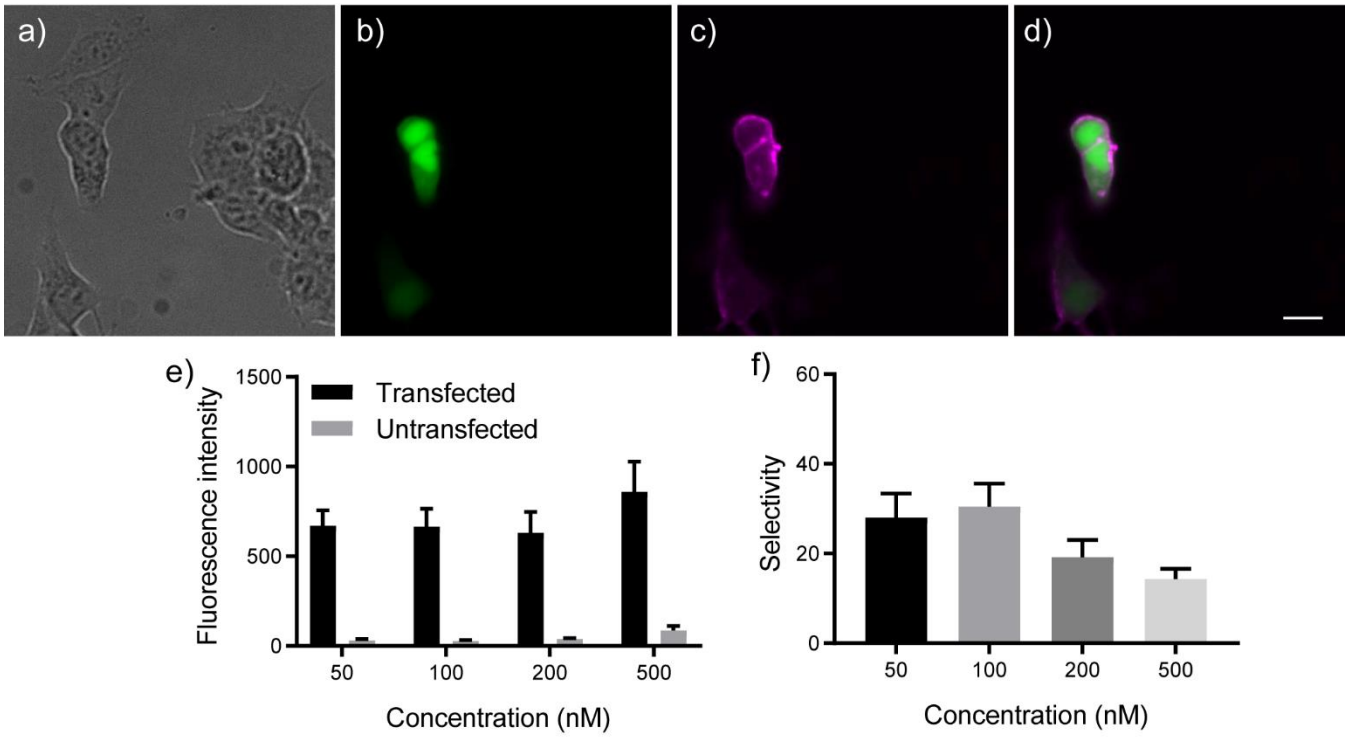


Figure S4. Loading condition screen of isoBeRST-Halo in HEK cells. a-d) Wide-field microscopy images of HEK cells transfected with CMV-HaloTag-pDisplay and stained with isoBeRST-Halo (50 nM, 30 mins). a) DIC image of HEK cells. b) Nuclear EGFP fluorescence indicates HaloTag expression. c) Membrane associated isoBeRST-Halo fluorescence. d) Merge of fluorescence from EGFP (green) and isoBeRST-Halo (magenta), demonstrating the selective labeling of HaloTag-expressing cells with **7**. Scale bar is 10 μm. e) Plot of isoBeRST-Halo fluorescence in HaloTag (+, transfected) and HaloTag(-, untransfected) HEK cells for isoBeRST-Halo at different concentrations. f) Plot of selectivity between transfected and untransfected HaloTag HEK cells labeled with isoBeRST-Halo. Data are mean fluorescence ± SEM from HaloTag-expressing and non-expressing HEK cells labeled with isoBeRST-Halo from 3 different coverslips and at least n = 6 fields of view, comprising 20 to 50 cells each.

Figure S5.

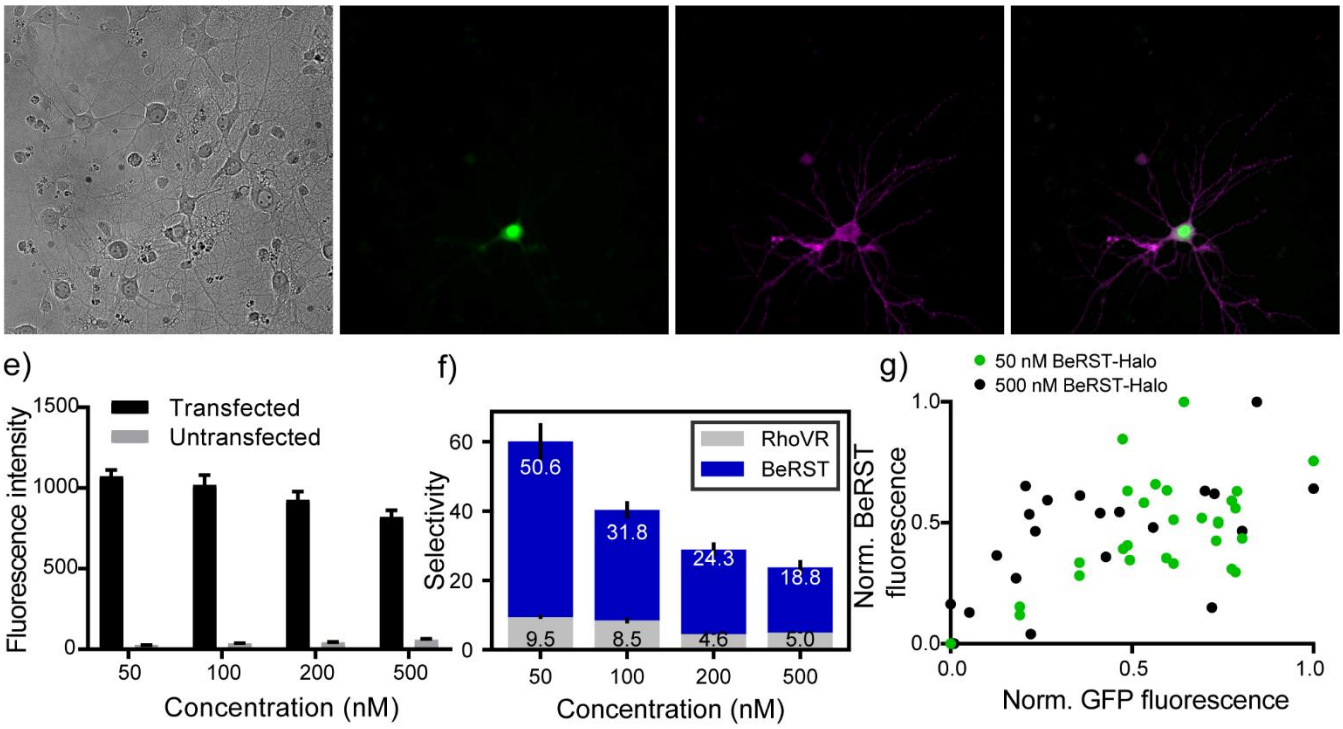


Figure S5. Quantification of the selective labeling in HaloTag-expressing neurons with isoBeRST-Halo at varying concentrations of **7**. a-d) Wide-field microscopy images of isoBeRST-Halo labeling in a HaloTag-expressing neuron. a) DIC image of neurons. b) Nuclear EGFP fluorescence indicates HaloTag expression. c) isoBeRST-Halo fluorescence is restricted to the membrane. d) Merge of EGFP (green) and isoBeRST-Halo (magenta) fluorescence, demonstrating selective labeling of HaloTag-expressing neuron with **7**. Scale bar is 20 μm. e) Plot of isoBeRST-Halo fluorescence in HaloTag (+, transfected) and HaloTag(-, untransfected) neurons for isoBeRST-Halo at different concentrations. f) Plot of selectivity between transfected and untransfected HaloTag neurons labeled with isoBeRST-Halo (blue) vs. RhoVR-Halo (grey). Data for isoBeRST-Halo are mean fluorescence ± SEM from HaloTag-expressing and non-expressing neurons labeled with isoBeRST-Halo from 4 different coverslips and at least n = 5 fields of view, comprising 1 to 4 cells each. Data for RhoVR-Halo are mean fluorescence ±SEM quantified from Deal, *et al. J Am Chem Soc*, **2020**, *142*, 614 and represent a similar number of neurons. g) Plot of **7** fluorescence vs nuclear EGFP fluorescence from HaloTag-expressing neurons at 50 nM or 500 nM **7**. Each point represents an individual neuron.

Figure S6.

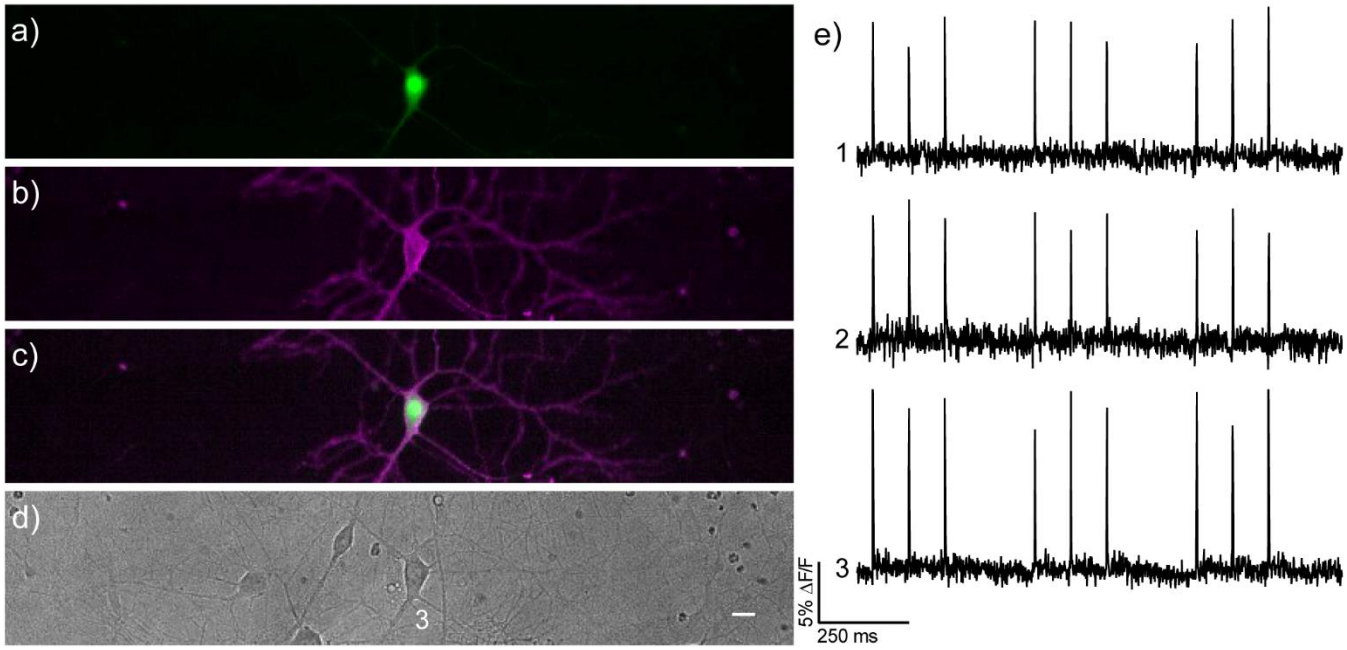


Figure S6. Field stimulation of neurons expressing HaloTag and stained with isoBeRST-Halo. a-d) Wide-field microscopy images of neurons sparsely transfected with HaloTag-pDisplay and stained with 50 nM isoBeRST-Halo **7**. a) EGFP fluorescence image of neuron expressing HaloTag. b) isoBeRST-Halo fluorescence image. c) Merge of EGFP (green) and isoBeRST-Halo (magenta) fluorescence, demonstrating selective labeling of HaloTag-expressing neuron with **7**. d) DIC image of neurons. Scale bar is 20 μm. e) Field stimulation-evoked action potentials shown as ΔF/F vs time of HaloTag-expressing neurons stained with 50 nM isoBeRST-Halo. Voltage recording labeled trace 3 was acquired from the labeled neuron in panels a-d). Images were acquired at 500 Hz and represent single-trial acquisitions.

Figure S7.

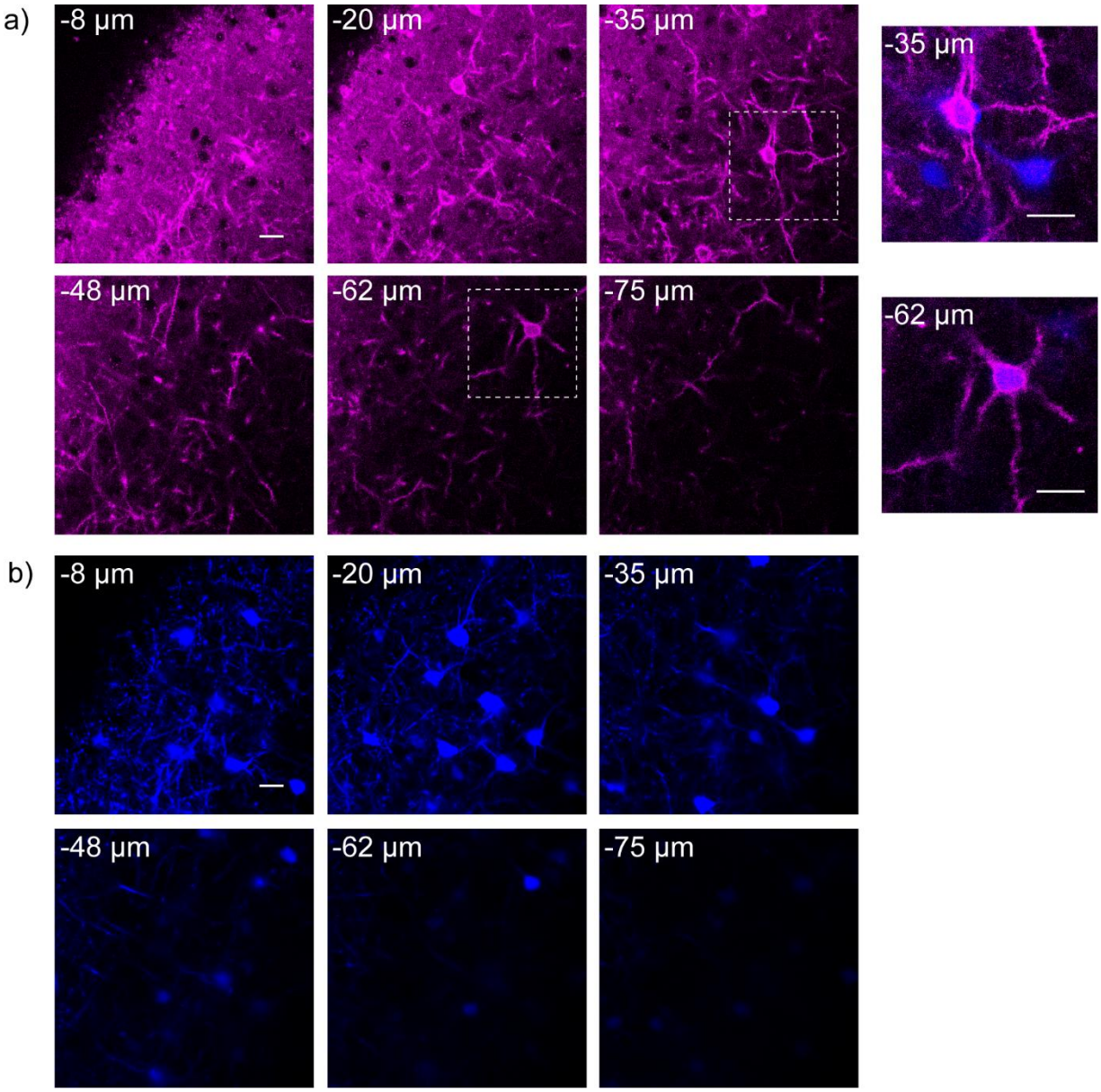


Figure S7. One-photon confocal microscopy imaging of isoBeRST-Halo in mouse brain slice expressing HaloTag-pDisplay and pCAG-BFP following *in utero* electroporation. Z-stack imaging was conducted from surface of the slice to deeper into the tissue and the depth relevant to the surface was indicated in each image. isoBeRST-Halo (**7**) was used at a concentration of 250 nM and applied in ACSF for 15 minutes before moving the slice into fresh ACSF and imaging. Imaging took place 90 minutes after transferring to fresh ACSF. a) Membrane-associated isoBeRST-Halo (**7**) fluorescence was observed in BFP-positive cells with clear visualization of neuronal processes and subcellular structures, including dendritic spines. b) BFP was used for screening positive pups and expressing slices. Scale bar is 20 µm. Insets labeled "-35 µm" and "-62 µm" are overlays of the isoBeRST-Halo (**7**) fluorescence (magenta) and BFP fluorescence (blue) from the respective boxed areas in panels (a) and (b).

Figure S8.

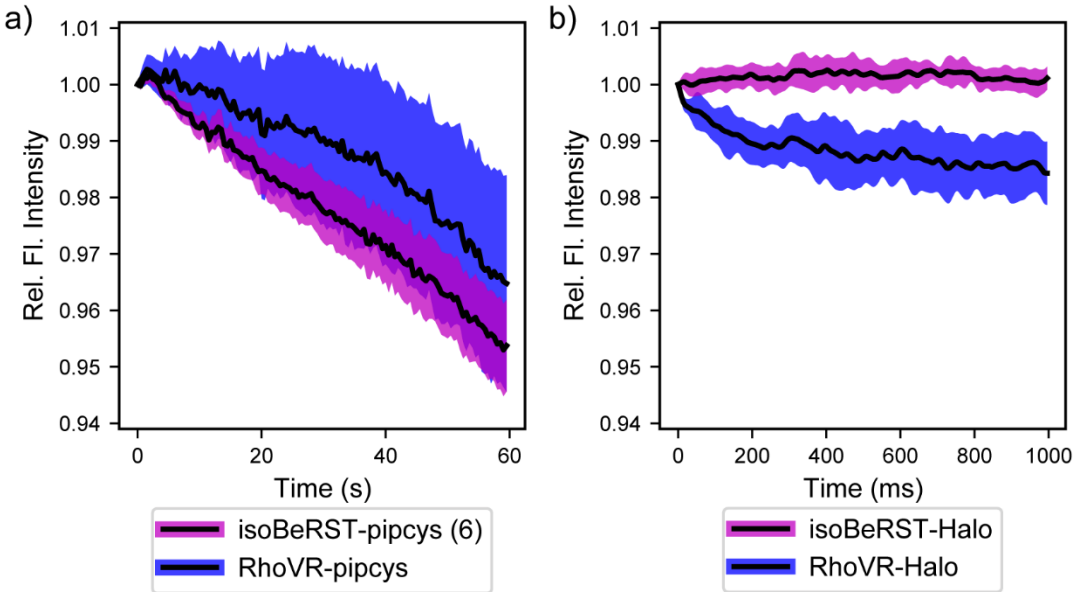


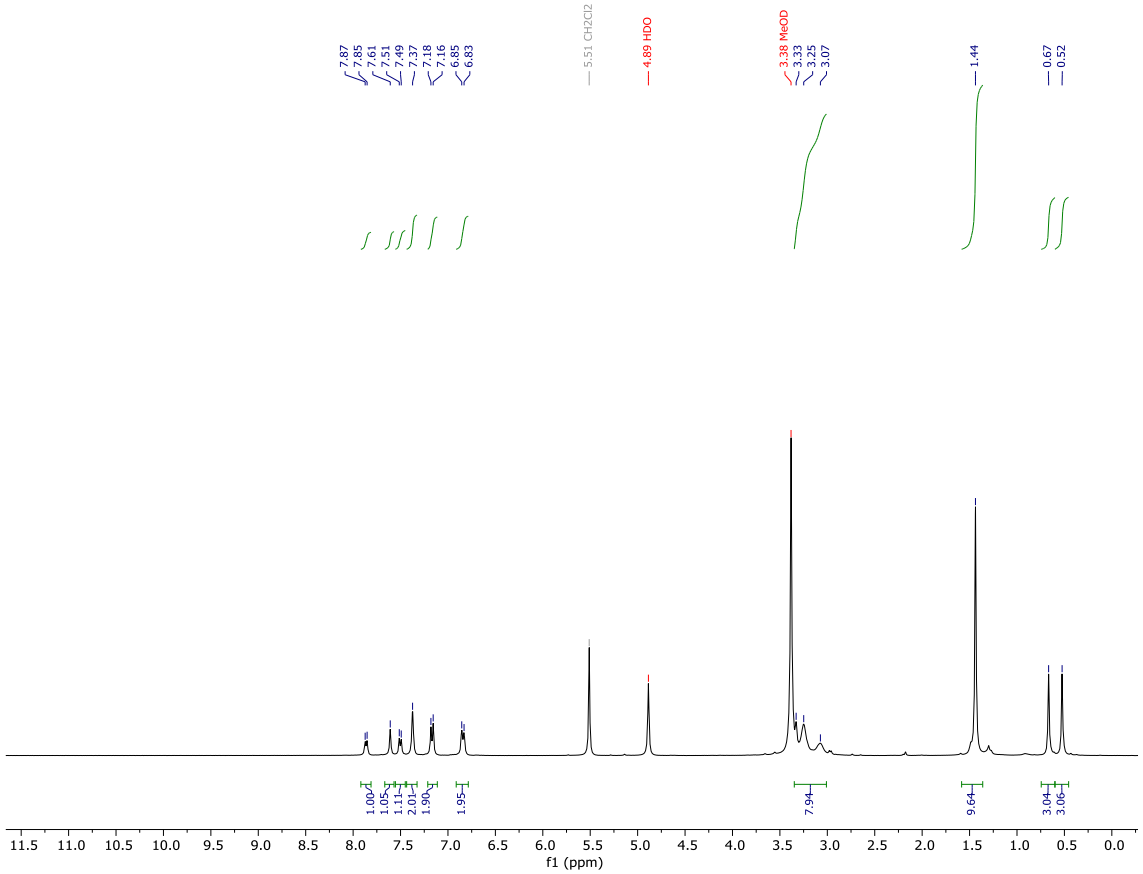
Figure S8. Comparison of bleach rates of RhoVR-Halo and isoBeRST-Halo 7 in brain slice.

a) Plots of bleach curves showing relative fluorescence intensity vs time for HEK cells loaded with RhoVR-pipcys (blue, compound **7** from Deal, *et al. J Am Chem Soc*, **2020**, *142*, 614) or with isoBeRST-pipcys (magenta, compound **6**, this manuscript). Data are mean fluorescence intensity (background subtracted) ± S.E.M. for n = 18 independent cell groupings across two different coverslips. Illumination intensities for both RhoVR-pipcys and isoBeRST-pipcys (**6**) were identical.

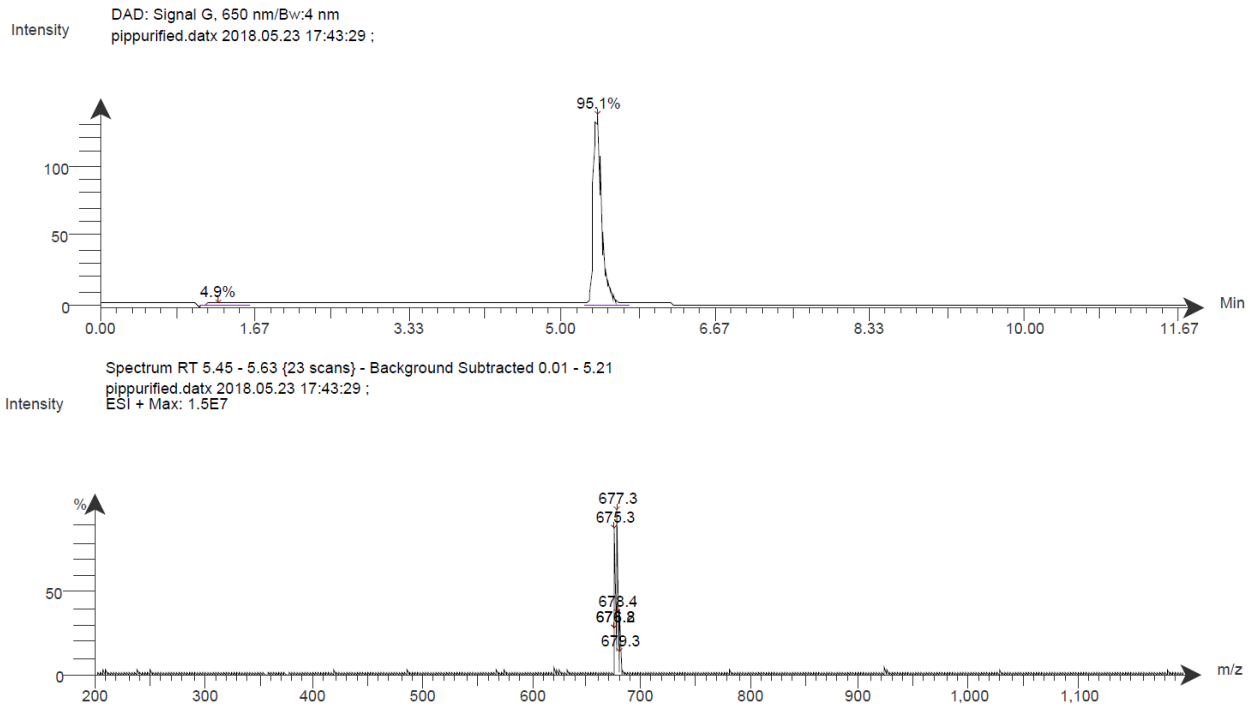
b) Plots of bleach curves showing relative fluorescence intensity vs time for neurons stained with RhoVR-Halo (blue) or isoBeRST-Halo **7** (magenta). Bleach curves are estimated by fitting an asymmetric least squares spline to the raw data. Data are the mean spline (black) ± the S.E.M. for n = 3 independent experiments. Illumination intensities for both RhoVR-Halo and isoBeRST-Halo (**7**) were matched at 10 mW. No bleach correction is applied to data in **Figure 3f** in the main text.

Spectra of Compounds

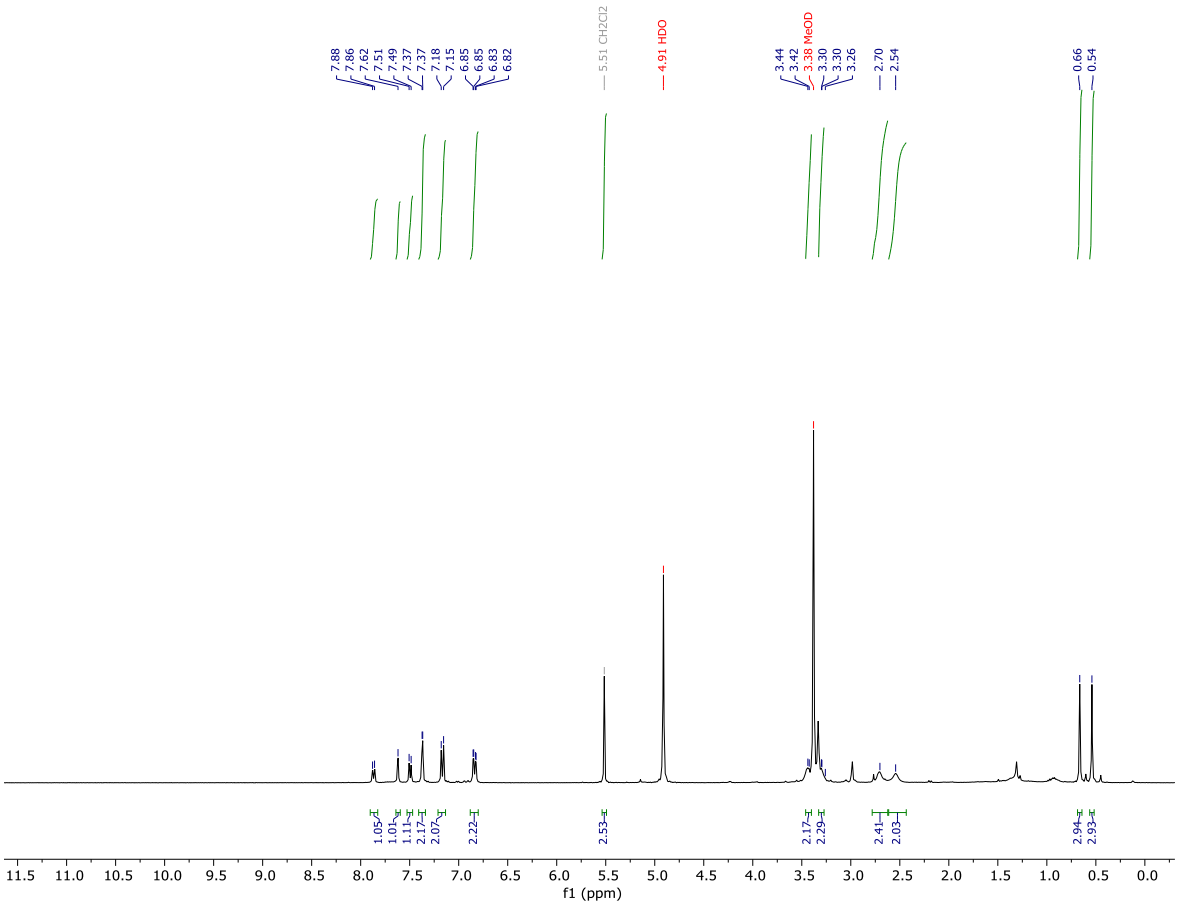
*^{1}H NMR of compound **2***



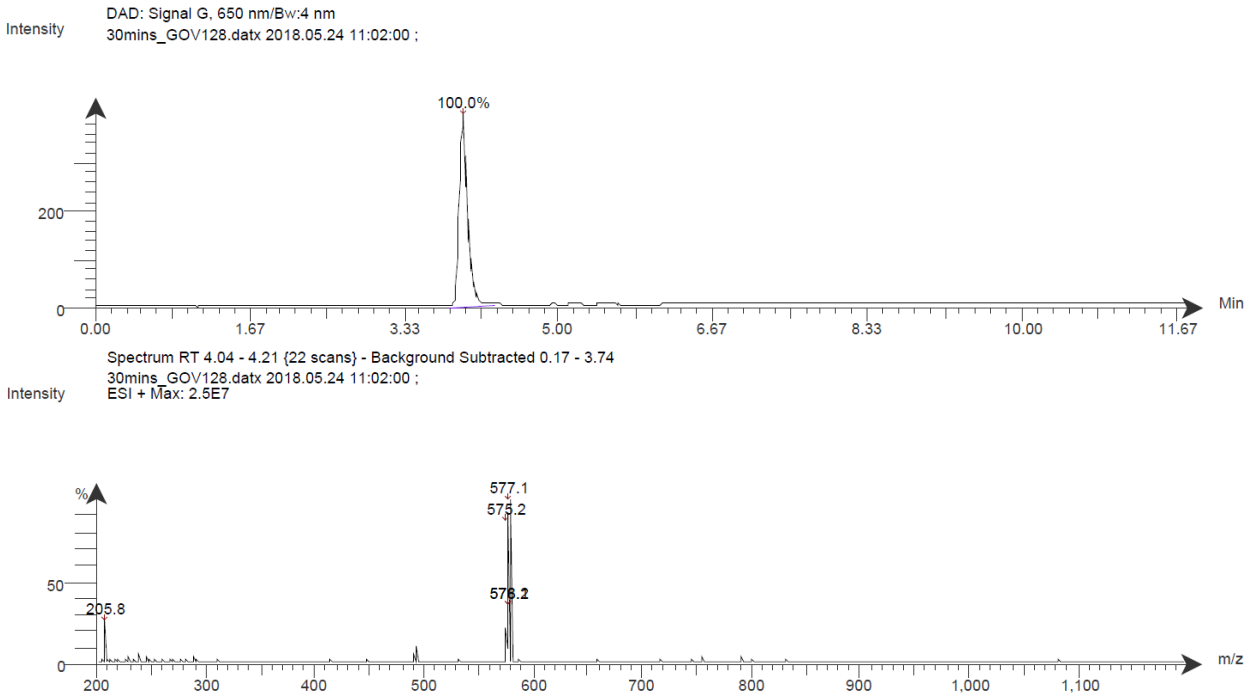
*LCMS of compound **2***



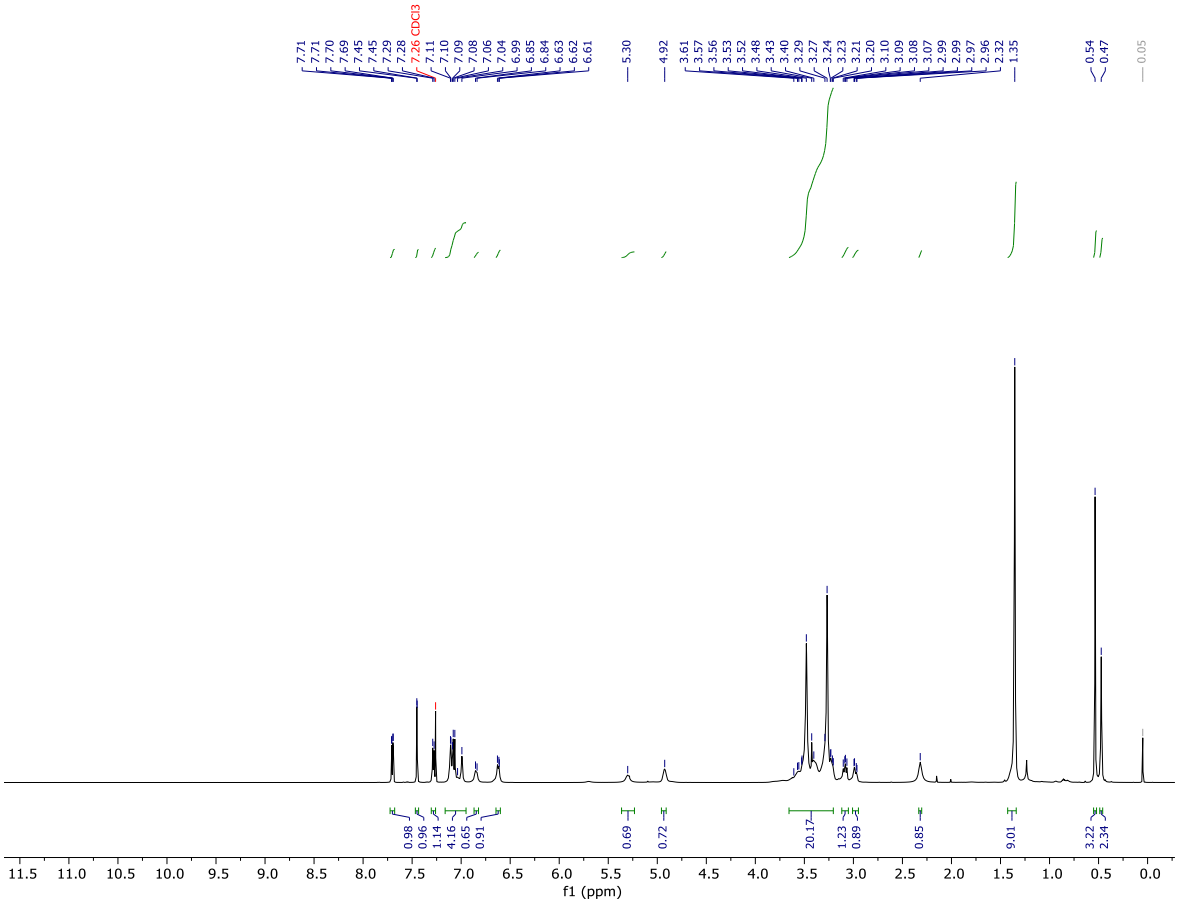
*^{1}H NMR of compound **3***



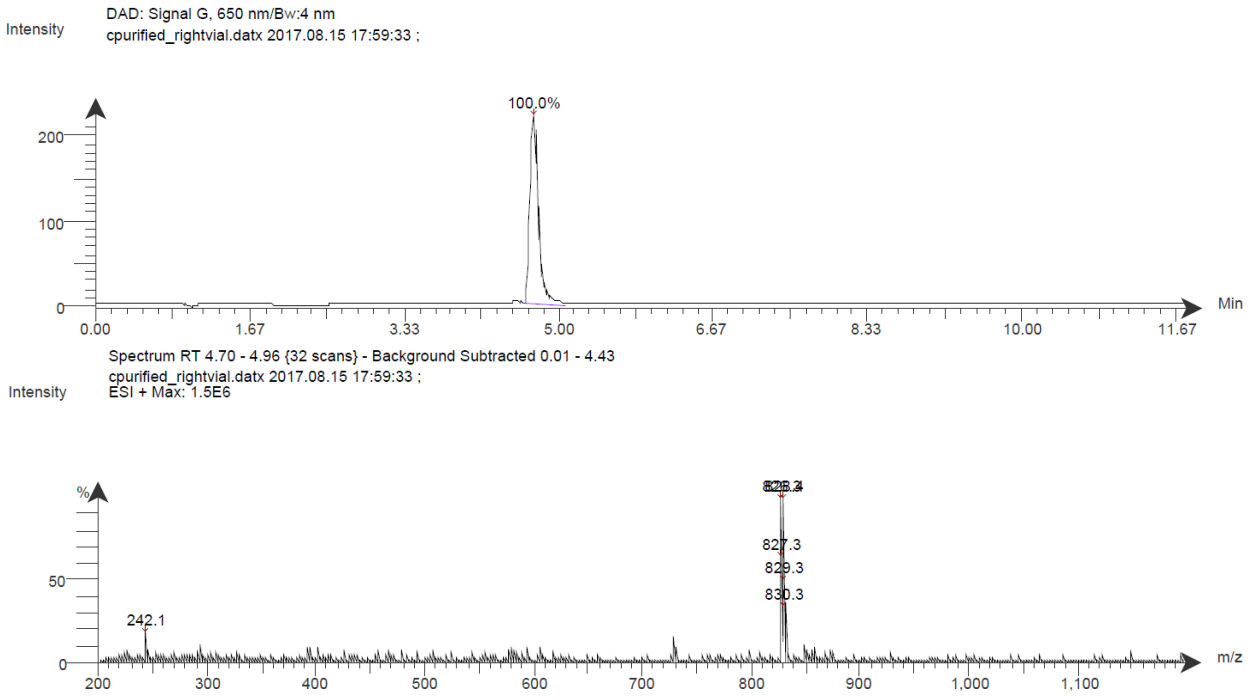
*LCMS of compound **3***



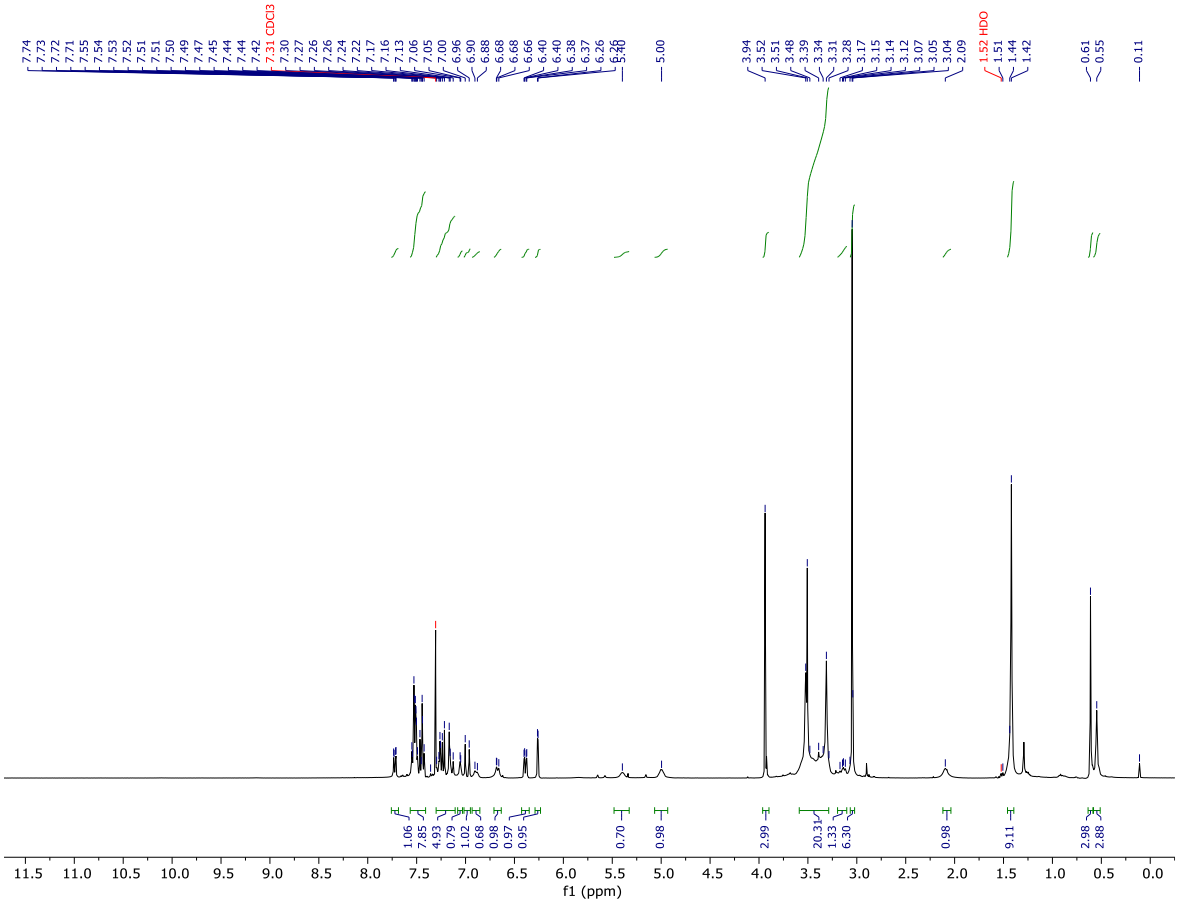
*^{1}H NMR of compound **4***



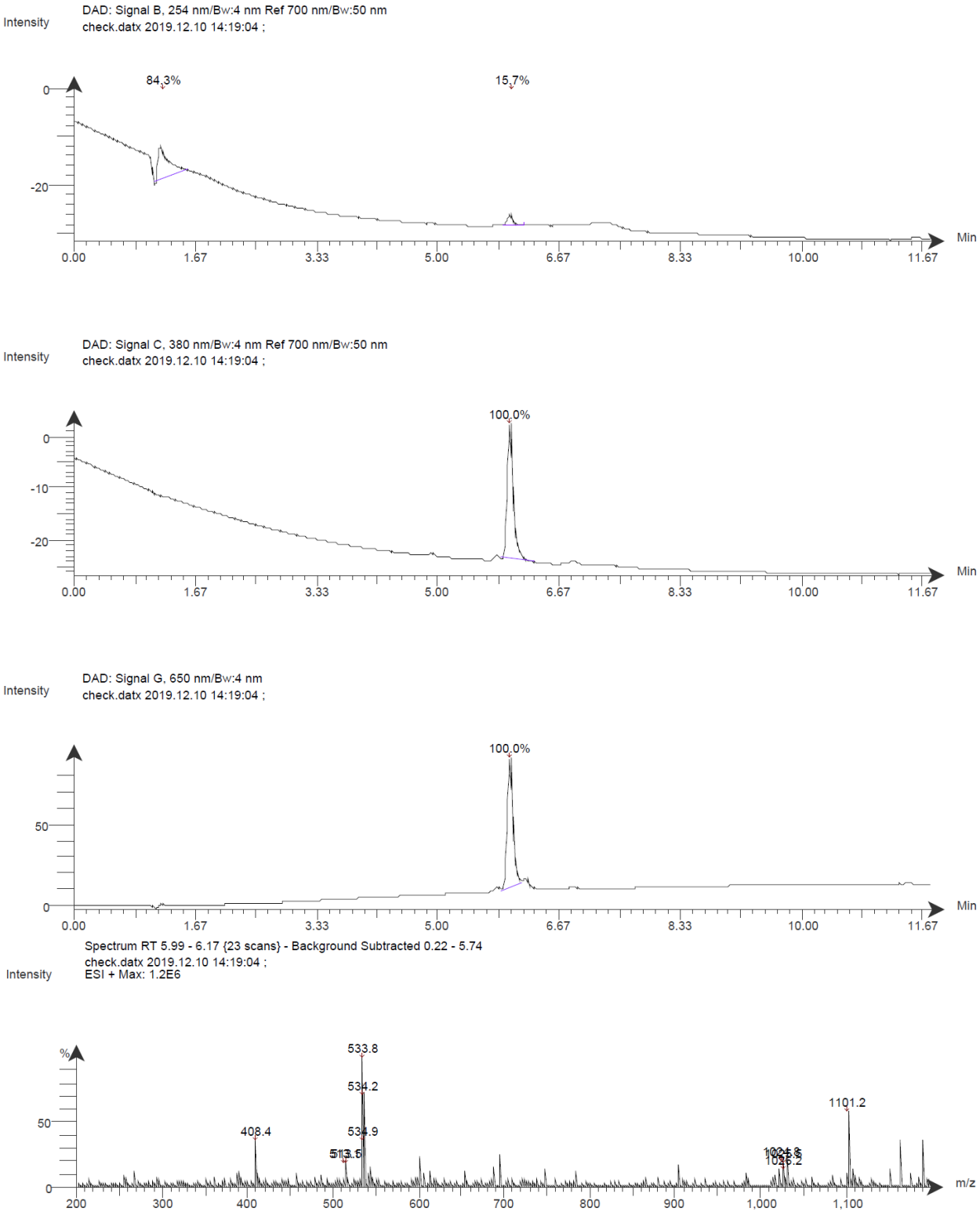
*LCMS of compound **4***



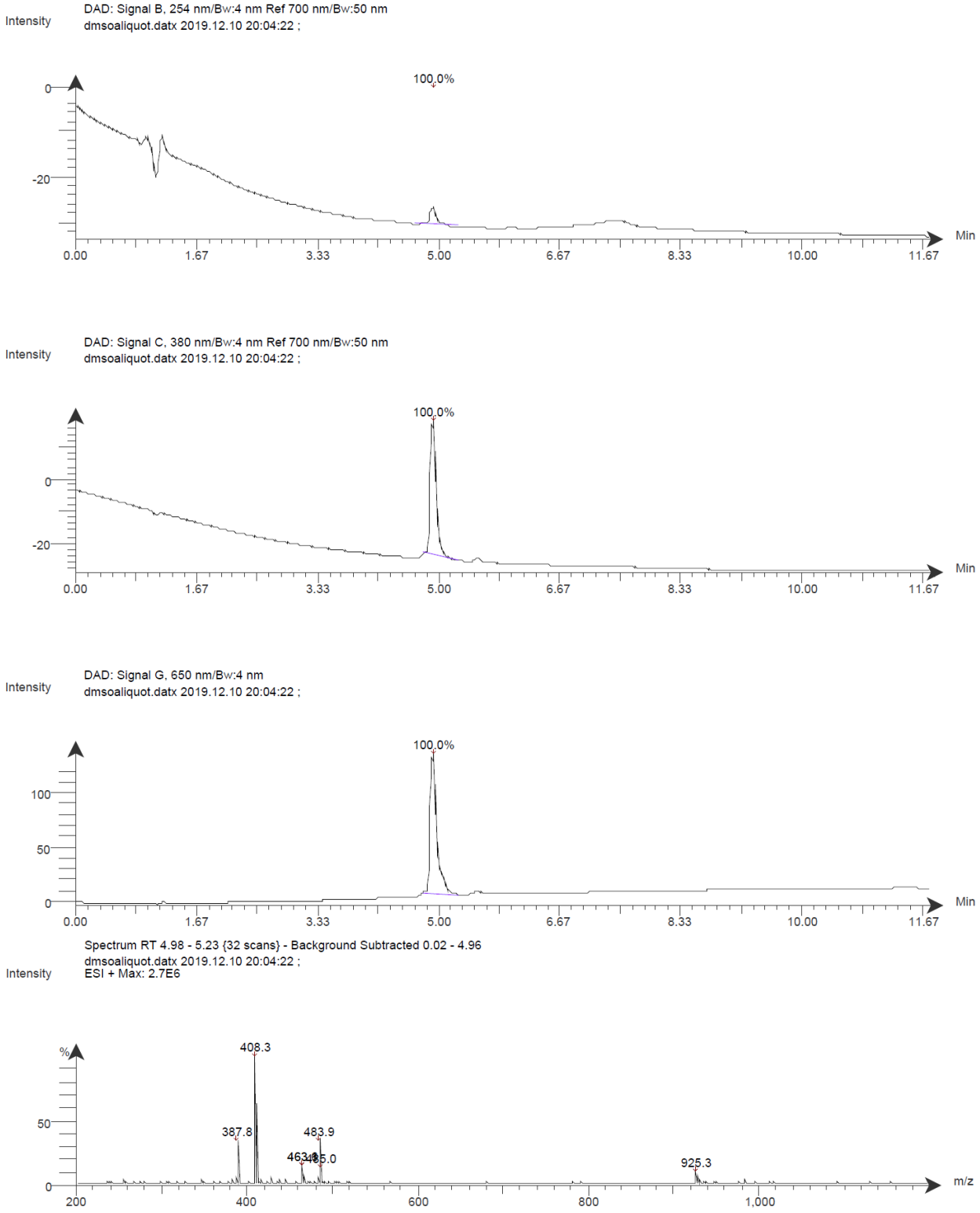
*^{1}H NMR of compound **5***



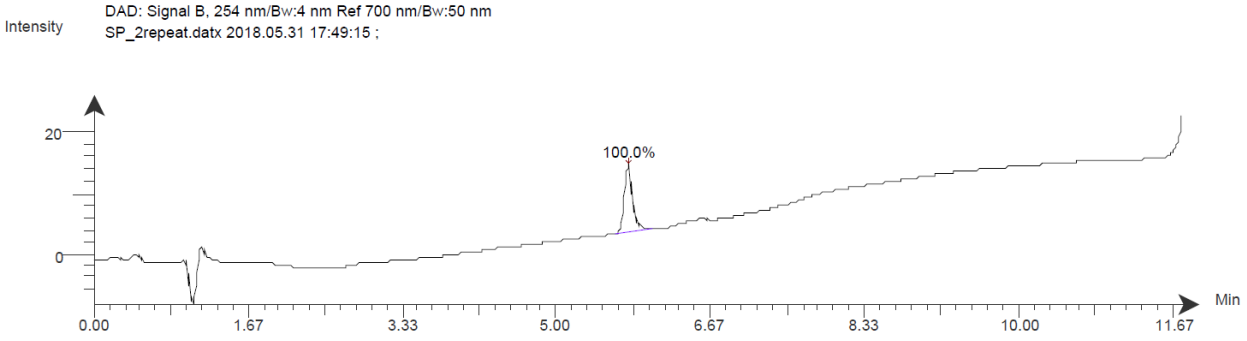
*LCMS of compound **5***

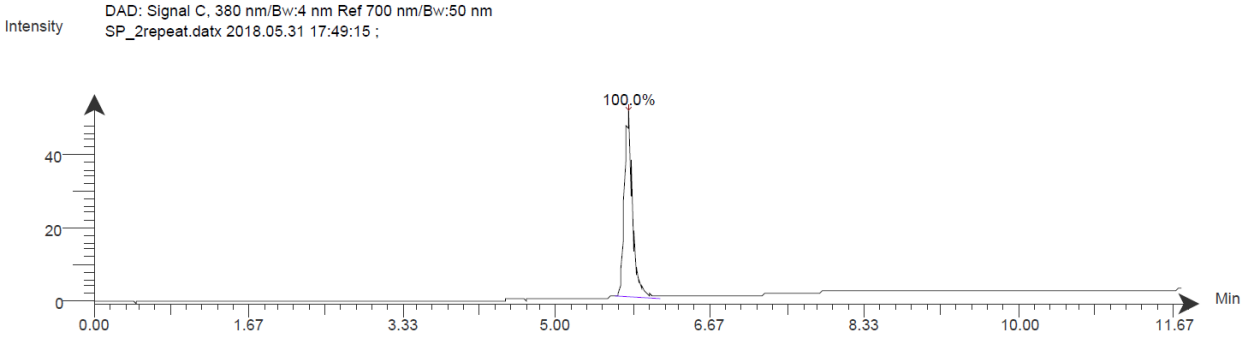


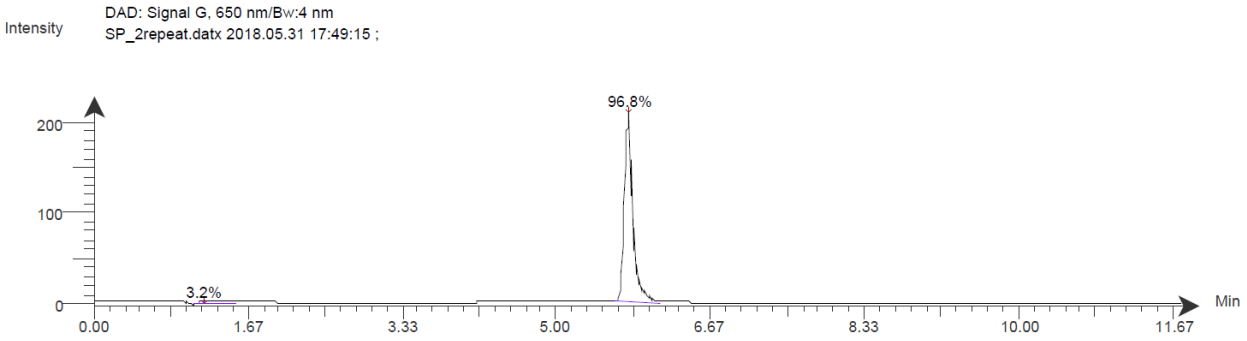
*LCMS of compound **6***

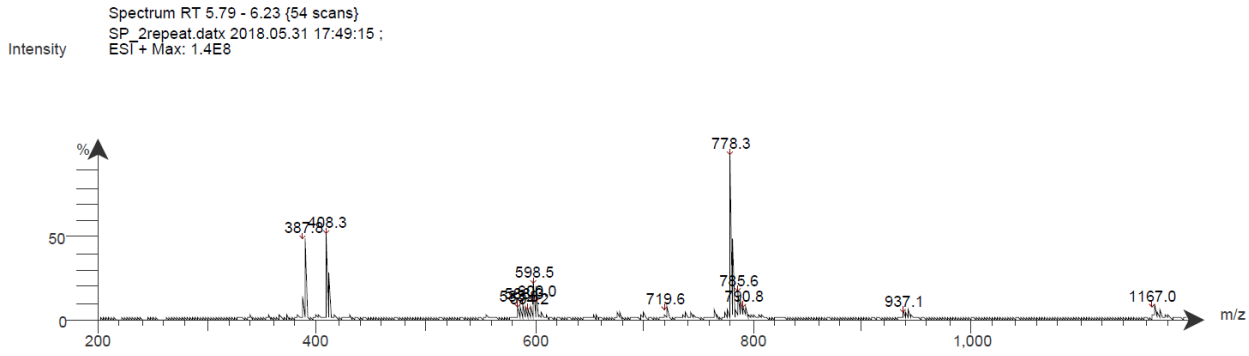


*LCMS of compound **7***

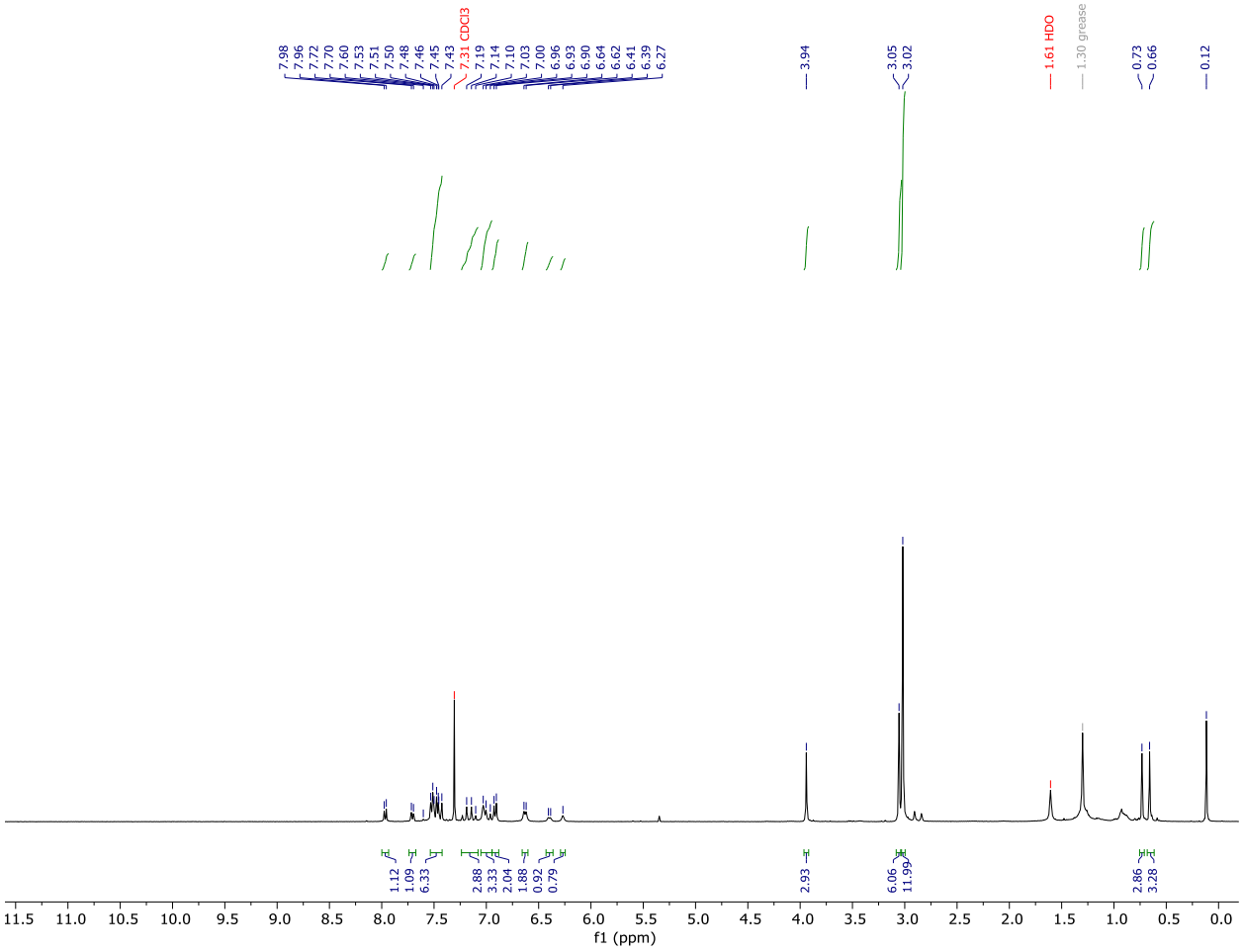




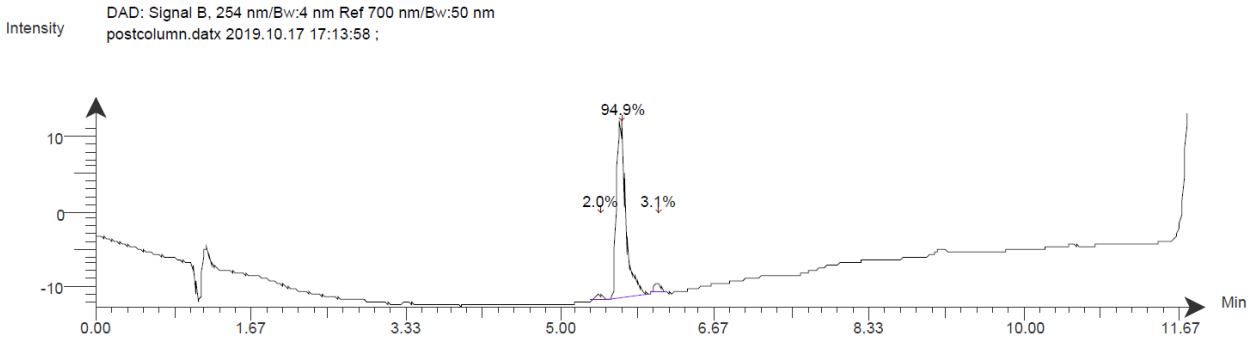


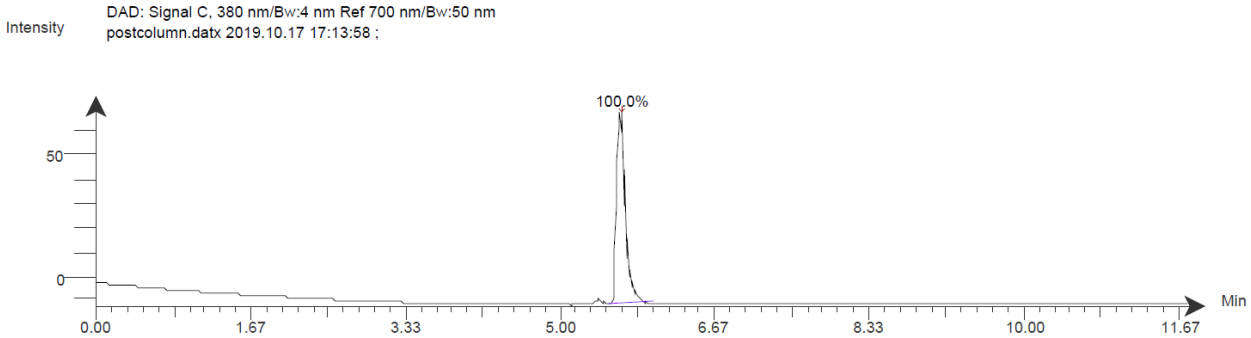


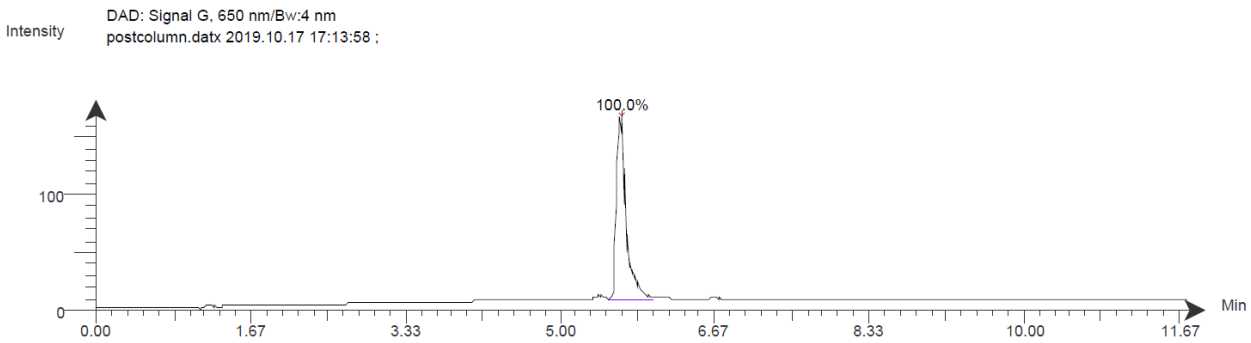
*^{1}H NMR of compound **8***

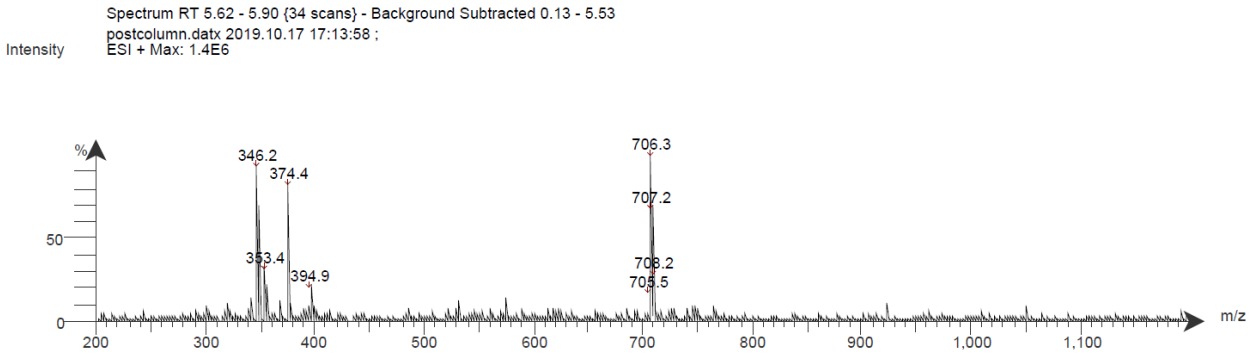


*LCMS of compound **8***

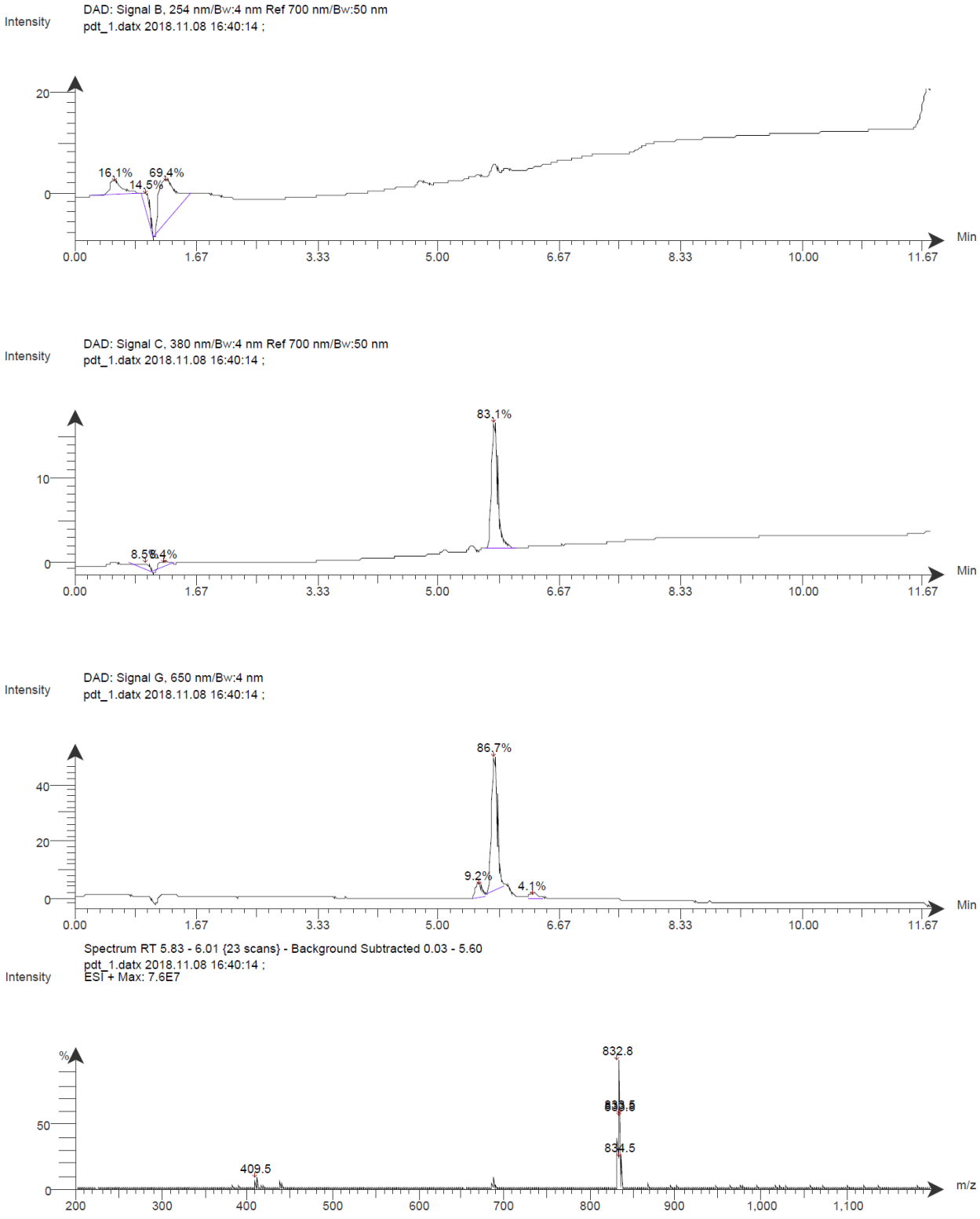




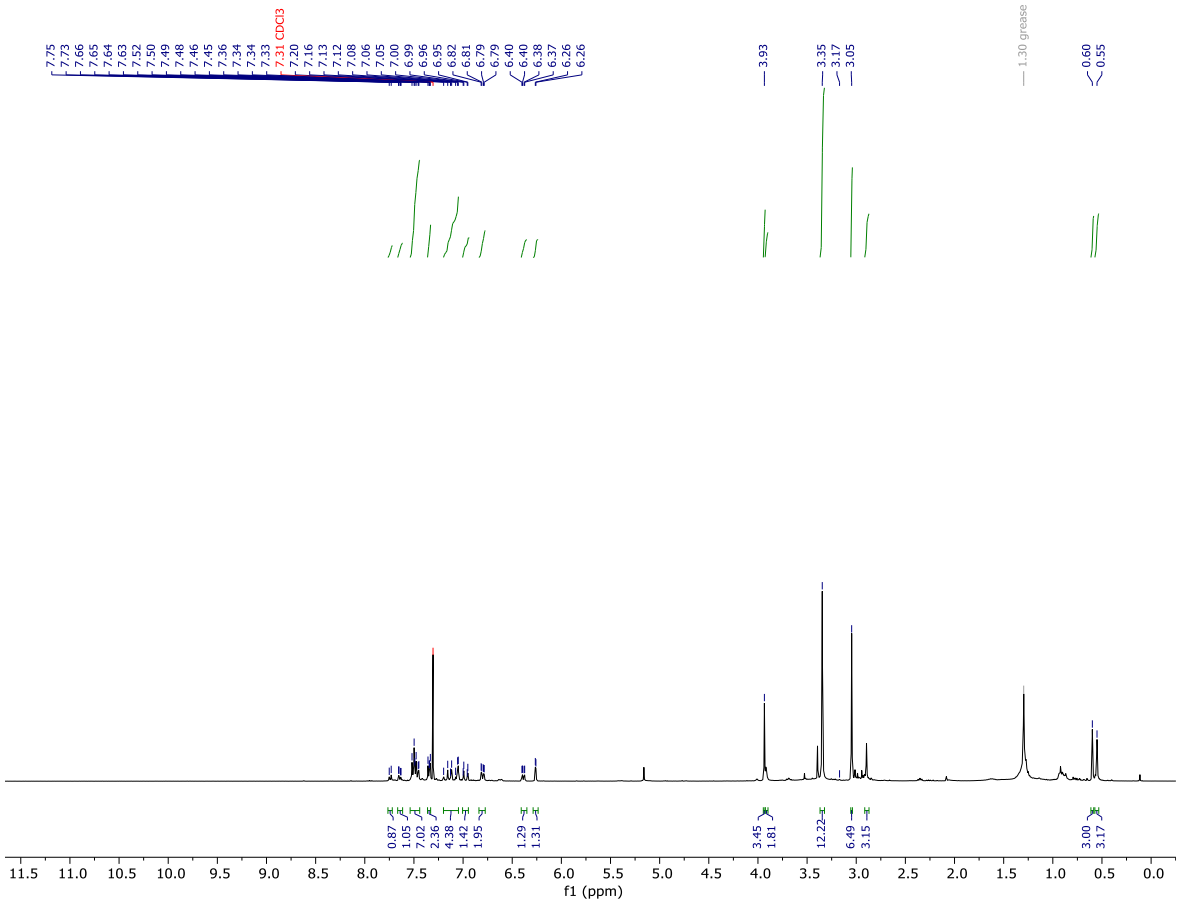




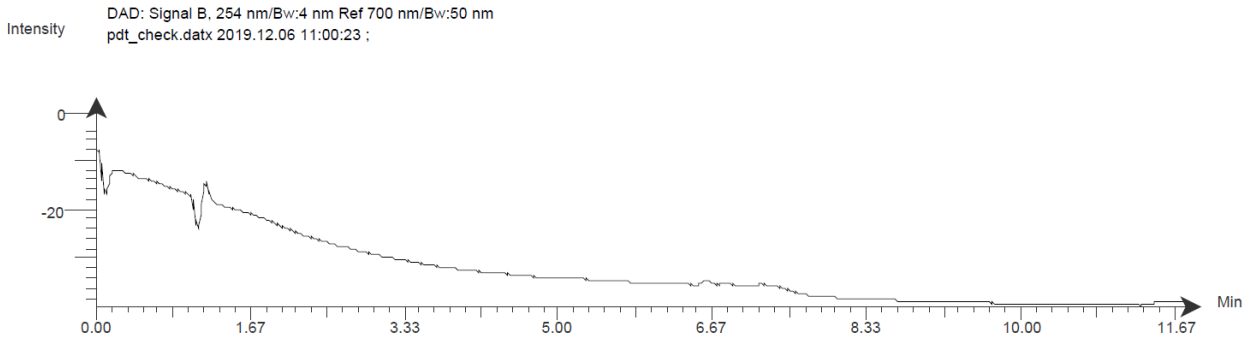
*LCMS of compound **9***

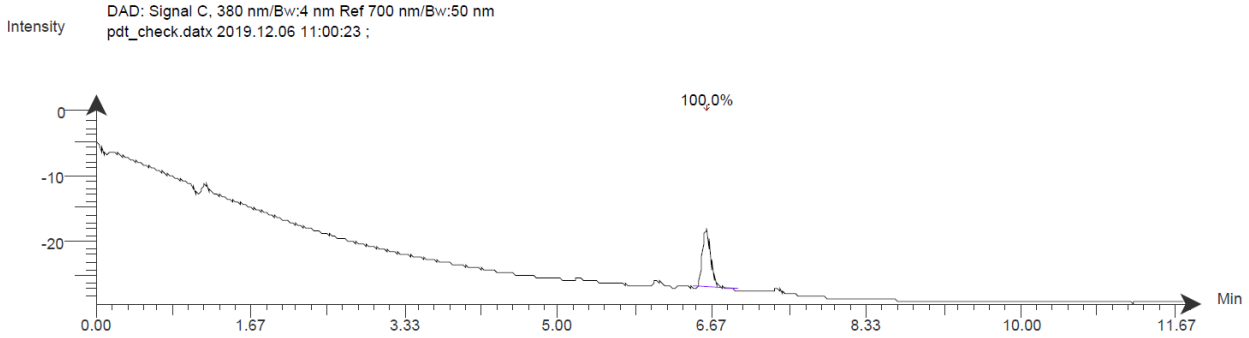


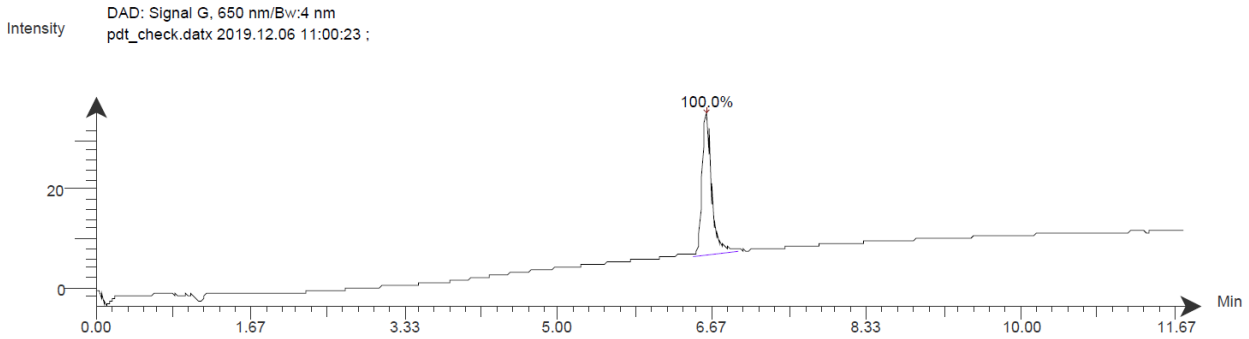
*^{1}H NMR of compound **10***

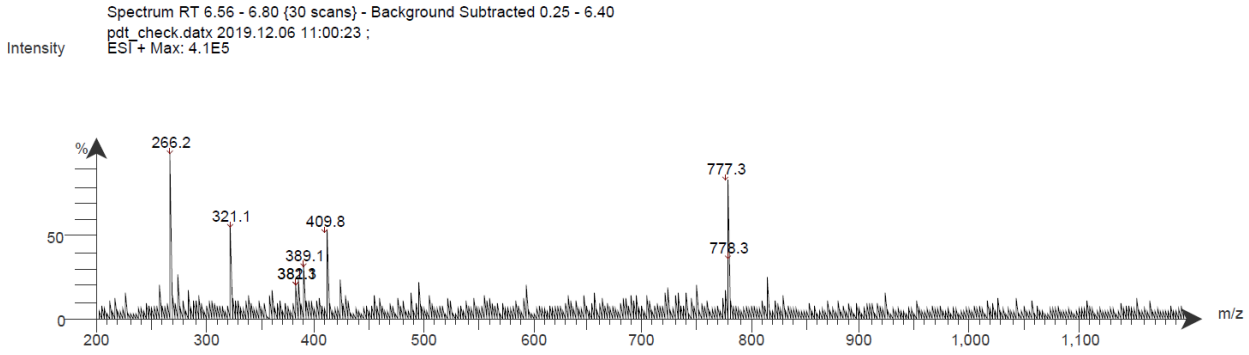


*LCMS of compound **10***









References

(1) Kozma, E.; Estrada Girona, G.; Paci, G.; Lemke, E. A.; Kele, P. Bioorthogonal Double-Fluorogenic Siliconrhodamine Probes for Intracellular Super-Resolution Microscopy. *Chem. Commun.* **2017**, *53* (50), 6696–6699. https://doi.org/10.1039/c7cc02212c.

(2) Woodford, C. R.; Frady, E. P.; Smith, R. S.; Morey, B.; Canzi, G.; Palida, S. F.; Araneda, R. C.; Kristan, W. B.; Kubiak, C. P.; Miller, E. W.; Tsien, R. Y. Improved PeT Molecules for Optically Sensing Voltage in Neurons. *J. Am. Chem. Soc.* **2015**, *137* (5), 1817–1824. https://doi.org/10.1021/ja510602z.

(3) Neklesa, T. K.; Tae, H. S.; Schneekloth, A. R.; Stulberg, M. J.; Corson, T. W.; Sundberg, T. B.; Raina, K.; Holley, S. A.; Crews, C. M. Small-Molecule Hydrophobic Tagging-Induced Degradation of HaloTag Fusion Proteins. *Nat. Chem. Biol.* **2011**, *7* (8), 538–543. https://doi.org/10.1038/nchembio.597.

(4) Fery-Forgues, S.; Lavabre, D. Are Fluorescence Quantum Yields So Tricky to Measure? A Demonstration Using Familiar Stationery Products. *J. Chem. Educ.* **1999**, *76* (9), 1260. https://doi.org/10.1021/ed076p1260.