Xanthone-Based Solvatochromic Fluorophores for Quantifying Micropolarity of Protein Aggregates

Lushun Wang [#] [a], Chia-Heng Hsiung [#] [b,c], Xiaojing Liu [b], Shichao Wang [a], Axel Loredo [a], Xin Zhang [*] [b,c], and Han Xiao [*] [a,d,e]

[a] Department of Chemistry, Rice University, 6100 Main Street, Houston, Texas, 77005, USA

[b] Department of Chemistry, Pennsylvania State University, University Park, PA 16802, USA

[c] Department of Biochemistry and Molecular Biology, University Park, PA 16802, USA

[d] Department of Biosciences, Rice University, 6100 Main Street, Houston, Texas, 77005, USA

[e] Department of Bioengineering, Rice University, 6100 Main Street, Houston, Texas, 77005, USA

[#] These authors contributed equally.

[*] To whom correspondence should be addressed. Email: han.xiao@rice.edu; xuz31@psu.edu

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1. Experimental Procedures

1.1 Absorbance spectra and molar extinction coefficient.

The absorption spectra were obtained with a ThermoFisher Evolution 220 UV-Vis spectrophotometer in 1 cm path length quartz cells. Fluorophores were prepared with the final concentration of 0.5, 1.0, 3.0, 5.0, and 10.0 μM in different solvents at room temperature and transferred in a quartz cuvette for measurements. The extinction coefficient was determined as the slope of the linear correlation by plotting absorbance maxima against the concentration of the fluorophore.

1.2 Fluorescence excitation and emission spectra.

Fluorescence spectra were recorded using spectrophotometer (SPEX FluoroLog-3). Each spectrum was normalized against its maxima to give the finalized spectra.

1.3 Empirical analysis of solvatochromism.

Empirical determination of solvatochromic properties of heteroatom xanthones derivatives were conducted by determination of emission maximum in a variety of solvents. Samples were analyzed at concentration of 20 μM, and the observed emission maxmium was plotted against the solvent polarity parameter $E_T(30)$ and the decimal logarithm of dielectric constants (ε).

1.4 Quantum yield measurements.

Quantum yield was measured according to a published guideline[1]. In brief, fluorophores were diluted from 2 mM DMSO stock solution into the desired solvent with corresponding absorbances at the excitation wavelength of 0.030, 0.045, 0.066, and 0.10 as the final concentration. For each individual concentration, the emission spectrum was collected. The integration of the emission spectrum was then plotted against the absorbance value at the excitation wavelength. The slope of the regression line was used to calculate the quantum yield by comparing against a reference fluorophore (Quinine sulfate, ϕ = 0.55 in H_2SO_4 0.5 M) according to the following equation:

$$\phi_x = \phi_{ref}\left(\frac{Gradient_x}{Gradient_{Ref}}\right)\left(\frac{n_x^2}{n_{ref}^2}\right)$$

Where the ϕ is the quantum yield, n is the refractive index of the solvent, and subscripts x and ref stand for fluorophore-of-interest and reference fluorophore, respectively.

1.5 Viscosity sensitivity measurements.

Viscosity sensitivity was measured according to a published protocol[2]. Fluorophores are diluted from 2 mM DMSO stock into a series of ethylene glycol and glycerol mixture at 20 ˚C with a final concentration of 20 μM. 2.5 mL of sample from each mixture was then transferred into a 1 cm path length quartz cell to collect the emission spectrum. Emission peak intensity was plotted as a function of solvent viscosity in a double logarithm manner and fitted by linear regression. The slope of the regression line represents the viscosity sensitivity value.

1.6 DFT calculation.

All calculations have been performed using the Gaussian 16 suite of quantum chemical packages[3]. The excited state related calculations are used with the Time-dependent DFT (TD-DFT) method. The B3LYP functional with the standard basis set 6-311++G (d, p) are employed for all elements. The solvent effect is taken into account by using the SMD solvent model in all solutions. All excited-state geometries in vacuum or solution are fully optimized without any symmetry constraints. In all cases, there was no vibrational mode with the imaginary frequency verified for all the stable stationary points.

1.7 Plasmid construction.

AgHalo, 110Q-SNAPf: The plasmids used in this Chapter were prepared as described in previous publications [2, 4]. E. coli DH5α competent cells (NEB, C2987I) were transformed with purified lab stock. A single transformant was collected and inoculated into 7 mL LB medium containing 50 ng/mL of kanamycin, grown for 16 h shaking at 37 ˚C. Cells were harvested and lysed for DNA extraction using a plasmid purification kit (Omgega Bio-Tek, E.Z.N.A. Plasmid Mini Kit). The accuracy of the amplified plasmid sequences was confirmed by sanger sequencing.

1.8 Mammalian Cell Culture and Transient Transfection.

Mammalian Cell Culture: Human Embryonic Kidney 293T (HEK293T) cells were (purchased from ATCC, CRL-3216) maintained and cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10 % fetal bovine serum (FBS) and 1% penicillin/streptomycin/glutamine (PSQ, Gibco) at 37 ˚C under 5% CO_2 in a HERAcell VIOS 160i CO_2 incubator (ThermoFisher Scientific). The cells are passaged using trypsin (TrypLE Express, Gibco) when confluency reached 90-95%. A new vial of cells will be revived after the current cells have been passage 20 times.

Transient Transfection: HEK293T cells were plated at 10 % (for 110Q-SNAPf) and 20% (for AgHalo) confluency in a 35mm Poly-D-Lysine-coated glass-bottomed MatTek dish (for confocal imaging) one

day prior to the experiment. The next day (20-25 % confluency for 110Q-SNAPf; and 40-50% confluency for AgHalo), cells were transiently transfected with 1 μg plasmids via lipofection (X-TremeGene 9 DNA Transfection Reagent, Roche). The optimal DNA to Lipofection reagent ratio has been optimized at 1 μg of DNA per 2 μL X-TremeGene 9 reagent in 100 μL Opti-MEM Reduced Serum Medium (Gibco). 1 μg of DNA was used for a single 35mm MatTek dish. The transfection mixture was incubated at room temperature for 30 min before being added dropwise to the culture medium. Proteins were transiently expressed for 24 h for AgHalo and 48 h for 110Q-SNAPf at 37 ˚C under 5% CO_2 before imaging.

In situ labeling of fluorophore: 0.5 μM Halo-SO_2X or SNAPf-SO_2X was added to the DMEM as the transfection take place. Neither of the fluorophores requires washing before imaging.

Small molecule Induced Aggregation, Disaggregation, and Degradation: In the experiment of AgHalo-SO_2X. At 24 hours post-transfection, stressors were added to respective dishes: 0.5 M sorbitol and 1 M NaCl were added to the 35mm MatTek dish 5 minutes prior to imaging. 50 μM Nilotinib was added to the MatTek dish at 24 hours post-transfection and incubated at 37 ºC in 5% CO_2 for 18 hours before imaging. In the experiment of 110Q-SNAPf-SO_2X. At 24 hours post-transfection, small molecules were added to respective dishes: 1 μM PD169316, 1μM YM1, and a combination of 1 μM PD169316 and 1 μM YM1.

1.9 CCK-8 Assay

HeLa cells were seeded in flat-bottomed 96-well plates with the number of $1x10^4$ cells per well in the presence of 200 μL complete culture media for 24 h. After washed with PBS three times, the HeLa cells were incubated with different concentrations (0, 2, 4, 6, 8, 10, 12 μM) of Halo-SO_2X and SNAP-SO_2X. All stock solutions were prepared in DMSO (2 mM) and diluted with complete medium. After cultured for 24 h, the cells were washed with PBS (pH 7.4) three times. 10 μL Cell Counting Kit-8 (CCK-8) solution and 90 μL PBS (pH 7.4) were added per well simultaneously. After another 1-hour culture, the absorbance at 450 nm was read by 96-well plate reader.

1.10 Lambda scan using Confocal Fluorescence Microscopy

The lambda scan microscopy was acquired on a Zeiss LSM 880 confocal microscope equipped with a Zeiss PlanApo 63x/1.4 oil immersion objective at PSU Huck Imaging Core Facility. When samples are ready to be imaged. Media was replaced with pre-warmed FluoroBrite DMEM (gibco) and cells were maintained at 37 ˚C and with 5% CO_2 throughout the experiment. Excitation for both Halo-SO_2X and SNAP-SO_2X are carried out at 405 nm diode laser using 20% laser power. The lambda scan from 410

nm to 600 nm with interval range of 8.9 nm is acquired simultaneously using 32-channel GaAsp (Airyscan) detector at 650 gain. For each independent experiment, at least 4 different fields of views were scanned for each sample. Data collected are saved as .czi file for later processing and analysis.

1.11 Analyses of Fluorescence Images

Image processing: All the confocal images were saved in their commercial extension files (.czi for Zeiss), and processed using NIH FIJI ImageJ. Based on the experimental conditions, the diffuse signal (in the absence of stressor) and punctate signal (in the presence of different stressors) for K73T-SO_2X, and the punctate signal for Htt-110Q-SO_2X were identified as the region of interests (ROIs). Emission spectra (410-600 nm) for these ROIs were extrapolated from images acquired using Lambda scan microscopy, and the wavelength of emission maximum was obtained for each sample. Emission maximum of the ROIs measured in each treatment group was then plotted with mean and standard deviation using GraphPad Prism. Images from wavelengths at 468, 512, 530, 539, 548, 575, and 584 nm were shown to indicate the emission shift in response to polarity changes in live cells. A script was written to automate the image processing procedure so that the large number of images acquired from the same experiment from the same instrument could be processed in bulk and have consistent adjustments through every image in an efficient manner. Script is available upon request.

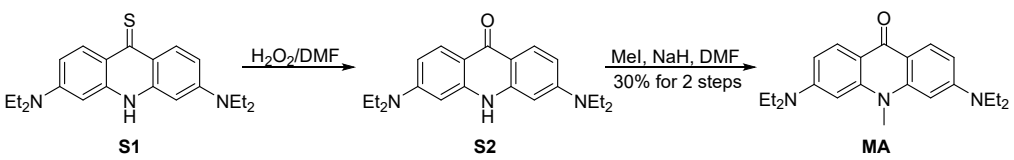
1.12 General synthetic and chromatographic methods

All solvents and chemicals for synthesis were purchased from Alfa Aesar and Sigma-Aldrich and used as received without further purification unless otherwise specified. Reactions were carried out in Synthware® round bottom flask and monitored via thin layer chromatography (Supelco, 60 Å F254). The ^{1}H NMR NMR and ^{13}C NMR spectroscopic measurements were carried out using a Bruker-600 NMR at 600 MHz with tetramethylsilane (TMS) as the internal reference. Electrospray ionization (ESI) mass spectra were performed on a Bruker MicroToF ESI LC-MS System in positive-ion mode.

2. Synthetic procedure

2.1 Synthesis of heteroatom xanthones derivatives

Synthesis of 3,6-bis(diethylamino)-10-methylacridin-9(10*H*)-one (**MA**)



To a solution of 3,6-bis(diethylamino)acridine-9(10*H*)-thione **S1**[5] (20 mg, 0.06 mmol) in DMF (4 mL) was add 30% H_2O_2 (0.5 mmol, 50 µL) at 0 °C. Then the reaction mixture was stirred at room

temperature and monitored by TLC. After the disappearance of the starting thione **S1**, the reaction mixture was poured into 20 ml water and extracted with dichloromethane (3 x 30 ml). The combined organic phase was washed with brine (2 x 30 ml)., dried over Na_2SO_4, filtered, and evaporated. The resulting 3,6-bis(diethylamino)acridin-9(10*H*)-one **S2** was put into the next step directly without further purification.

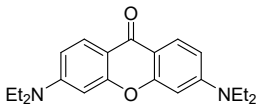
To a solution of the above crude **S2** in DMF (4 mL) was added NaH (12 mg, 0.3 mmol) at 0 °C and stirred at room temperature for 20 minutes before MeI (19 μL, 0.3 mmol) was added. After stirred at room temperature overnight, then the reaction mixture was poured into 20 ml saturated ammonium chloride and extracted with dichloromethane (3 x 30 ml). The combined organic phase was washed with brine (2 x 30 ml), dried over Na_2SO_4, filtered, and evaporated. Flash chromatography on silica gel (50% EtOAc/hexanes) yielded 6 mg (30% for 2 steps) of **MA** as a pale-yellow solid.

R_f = 0.20 (ethyl acetate: hexane = 1: 1, v/v)

MS (ESI): calcd. for $C_{22}H_{30}N_3O^+$ $[M+H]^+$ 352.2383, found 352.2352.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.34 (dd, *J* = 9.0, 2.9 Hz, 2H), 6.64 (dt, *J* = 9.0, 2.7 Hz, 2H), 6.36 (t, *J* = 2.8 Hz, 2H), 3.73 (d, *J* = 2.8 Hz, 3H), 3.49 (dd, *J* = 7.1, 2.8 Hz, 8H), 1.33 – 1.19 (m, 12H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 175.49, 151.27, 145.22, 129.14, 113.30, 107.03, 93.41, 44.72, 33.46, 12.65.

Synthesis of 3,6-bis(diethylamino)-9*H*-xanthen-9-one (**OX**)



ox

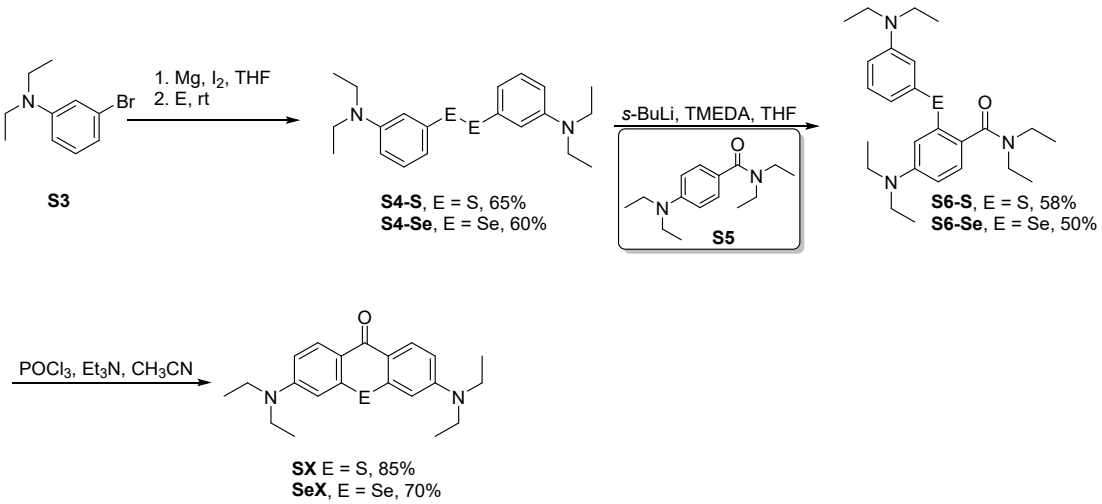
3,6-bis(diethylamino)-9*H*-xanthen-9-one (**OX**) was synthesized according to the literature[6].

R_f = 0.45 (ethyl acetate: hexane = 1: 1, v/v)

MS (ESI): calcd. for $C_{21}H_{27}N_2O_2^+$ $[M+H]^+$ 339.2067, found 339.2050.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.10 (d, *J* = 8.9 Hz, 2H), 6.64 (dd, *J* = 9.0, 2.5 Hz, 2H), 6.45 (d, *J* = 2.4 Hz, 2H), 3.45 (q, *J* = 7.1 Hz, 8H), 1.24 (t, *J* = 7.1 Hz, 12H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 174.84, 158.47, 151.99, 127.84, 111.47, 108.51, 96.19, 44.72, 12.56.

Synthesis of 3,6-bis(diethylamino)-9*H*-thioxanthen-9-one (**SX**) and 3,6-bis(diethylamino)-9*H*-selenoxanthen-9-one (**SeX**)



Synthesis of **S4-S**[7]

To a stirred solution of 3-bromo-*N, N*-diethylaniline (1.8 g, 7.9 mmol) in THF (20 mL) was added ground magnesium turnings (212 mg, 8.7 mmol). Then the catalytic amount of iodine was added, and the resulting mixture was refluxed for 3 hours. After cooling to room temperature, elemental sulfur (279 mg, 8.7 mmol) was added. The mixture was stirred at room temperature for 3 hours followed by the addition of 1 M aqueous HCl (10 mL). The reaction mixture was allowed to stir overnight under air, then extracted with diethyl ether (3 x 50 mL). The combined organic phase was washed with brine (2 x 30 ml), dried over Na_2SO_4, filtered, and evaporated. Flash chromatography on silica gel (20% CH_2Cl_2/hexanes) yielded 1.86 g (65%) of **S4-S** as a yellow oil.

R_f = 0.25 (CH_2Cl_2: hexane = 1: 4, v/v)

MS (ESI): calcd. for $C_{20}H_{29}N_2S_2^+$ $[M+H]^+$ 361.1767, found 361.1760.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.11 (t, *J* = 8.0 Hz, 2H), 6.82 (t, *J* = 2.1 Hz, 2H), 6.79 (dd, *J* = 7.7, 1.7 Hz, 2H), 6.51 (dd, *J* = 8.4, 2.5 Hz, 2H), 3.30 (q, *J* = 7.0 Hz, 8H), 1.10 (t, *J* = 7.0 Hz, 12H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 148.12, 138.28, 129.61, 114.14, 110.45, 110.22, 44.36, 12.47.

Synthesis of **S6-S**

To a solution of TMEDA (297 µL, 2.0 mmol) and amide **S5** (450 mg, 1.8 mmol) in THF (10 mL) at -78°C was added *sec*-Butyllithium (1.4 M in cyclohexane, 1.4 mL, 2.0 mmol). Then a solution of **S4-S** (720 mg, 2.0 mmol) was added to the solution dropwise. The resulting mixture was stirred at -78 °C for 3 h and then at room temperature for 18 h. A saturated NH_4Cl (25 mL) aqueous solution was added, then extracted with CH_2Cl_2 (3 x 50 mL). The combined organic phase was washed with brine (2 x 30 ml), dried over Na_2SO_4, filtered, and evaporated. Flash chromatography on silica gel (30% ethyl acetate/hexane) yielded 446 mg (58%) of **S6-S** as a colorless oil.

R_f = 0.30 (ethyl acetate: hexane = 1: 2, v/v)

MS (ESI): calcd. for $C_{25}H_{38}N_3OS^+$ $[M+H]^+$ 428.2730, found428.2733.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.12 (t, *J* = 8.0 Hz, 1H), 7.05 (d, *J* = 8.5 Hz, 1H), 6.75 (t, *J* = 2.0 Hz, 1H), 6.69 – 6.65 (m, 1H), 6.56 (dd, *J* = 8.4, 2.6 Hz, 1H), 6.47 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.40 (d, *J* = 2.5 Hz, 1H), 3.60 – 3.16 (m, 12H), 1.27 – 0.98 (m, 18H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.17, 148.26, 147.93, 135.17, 134.83, 129.70, 127.61, 125.04, 119.21, 115.63, 113.41, 110.90, 109.58, 77.23, 77.02, 76.81, 44.51, 44.39, 44.32, 12.47, 12.44.

Synthesis of **SX**

To a solution of **S6-S** (496 mg, 2.0 mmol) and triethylamine (1.1 mL, 7.9 mmol) in anhydrous acetonitrile (10 mL) was added phosphorus oxychloride (0.73 mL, 7.9 mmol). The resulting mixture was refluxed for 15 hours, then cooled to 0 °C. A NaOH aqueous solution (1 M, 40 mL) was added slowly, and the resulting mixture was stirred for 6 hours, then extracted with CH_2Cl_2 (3 x 50 mL). The combined organic phase was washed with brine (2 x 30 ml), dried over Na_2SO_4, filtered, and evaporated. Flash chromatography on silica gel (30% ethyl acetate/hexane) yielded 350 mg (85%) of **SX** as a pale-yellow solid.

R_f = 0.60 (ethyl acetate: hexane = 1: 1, v/v)

MS (ESI): calcd. for $C_{21}H_{27}N_2OS^+$ $[M+H]^+$ 355.1839, found 355.1838.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.40 (d, *J* = 9.2 Hz, 2H), 6.74 (dd, *J* = 9.1, 2.5 Hz, 2H), 6.52 (d, *J* = 2.5 Hz, 2H), 3.44 (q, *J* = 7.1 Hz, 8H), 1.23 (t, *J* = 7.1 Hz, 12H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 177.77, 149.57, 139.30, 131.17, 118.44, 110.78, 104.34, 44.49, 12.60.

Synthesis of **S4-Se**

To a stirred solution of 3-bromo-*N, N*-diethylaniline (1.8 g, 7.9 mmol) in THF (20 mL) was added ground magnesium turnings (212 mg, 8.7 mmol). Then the catalytic amount of iodine was added, and the resulting mixture was refluxed for 3 hours, then cooled to room temperature, and elemental Se (689 mg, 8.7 mmol) was added. The mixture was stirred at room temperature for 3 hours followed by the addition of 1 M aqueous HCl (10 mL). The reaction mixture was allowed to stir overnight under air, and then extracted with diethyl ether (3 x 50 mL). The combined organic phase was washed with brine (2 x 30 ml), dried over Na_2SO_4, filtered, and evaporated. Flash chromatography on silica gel (20% CH_2Cl_2/hexanes) yielded 2.16 g (60%) of **S4-Se** as a brown oil.

R_f = 0.30 (CH_2Cl_2: hexane = 1: 4, v/v)

MS (ESI): calcd. for $C_{20}H_{29}N_2Se_2^+$ $[M+H]^+$ 457.0656, found 457.0646.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.07 (dd, *J* = 8.4, 7.5 Hz, 2H), 6.93 (dd, *J* = 2.6, 1.6 Hz, 2H), 6.90 (dd, *J* = 7.5, 0.8 Hz, 2H), 6.53 (dd, *J* = 8.3, 1.8 Hz, 2H), 3.29 (q, *J* = 7.1 Hz, 8H), 1.11 (t, *J* = 7.1 Hz, 12H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 148.23, 132.08, 129.60, 117.72, 114.25, 110.96, 44.34, 12.50.

Synthesis of **S6-Se**

To a solution of TMEDA (318 µL, 2.14 mmol) and amide **S5** (483 mg, 1.95 mmol) in THF (15 mL) at -78°C was added *sec*-Butyllithium (1.4 M in cyclohexane, 1.53 mL, 2.14 mmol). After 0.5 h, a solution of **S4-Se** (977 mg, 2.14 mmol) was added dropwise. The resulting mixture was stirred at −78 °C for 3 h and then at room temperature for18 h. A saturated NH_4Cl (25 mL) aqueous solution was added, then extracted with CH_2Cl_2 (3 x 50 mL). The combined organic phase was washed with brine (2 x 30 ml), dried over Na_2SO_4, filtered, and evaporated. Flash chromatography on silica gel (20% ethyl acetate/hexane) yielded 463 mg (50%) of **S6-Se** as a colorless oil.

R_f = 0.50 (ethyl acetate: hexane = 1: 2, v/v)

MS (ESI): calcd. for $C_{25}H_{38}N_3OSe^+$ $[M+H]^+$ 476.2175, found 476.2171.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.11 (t, *J* = 7.9 Hz, 1H), 7.04 (d, *J* = 9.2 Hz, 1H), 6.94 – 6.91 (m, 1H), 6.84 (dd, *J* = 7.5, 1.2 Hz, 1H), 6.60 (dd, *J* = 8.4, 2.6 Hz, 1H), 6.46 – 6.42 (m, 2H), 3.56 – 3.34 (m, 4H), 3.31 (q, *J* = 7.0 Hz, 4H), 3.17 (q, *J* = 7.0 Hz, 4H), 1.26 – 1.14 (m, 6H), 1.11 (t, *J* = 7.0 Hz, 6H), 0.98 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.80, 148.38, 148.06, 132.34, 130.61, 129.82, 127.50, 125.17, 122.01, 121.98, 118.49, 118.45, 114.62, 111.36, 109.15, 44.40, 44.32, 13.56, 12.48, 12.44.

Synthesis of **SeX**

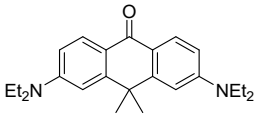
To a solution of **S6-Se** (309 mg, 0.65 mmol) and triethylamine (1.1 mL, 7.9 mmol) in anhydrous acetonitrile (10 mL) was added phosphorus oxychloride (0.73 mL, 7.9 mmol). The resulting mixture was refluxed for 15 hours, then cooled to 0 °C. A NaOH aqueous solution (1 M, 40 mL) was added slowly, and the resulting mixture was stirred for 6 hours, then extracted with CH_2Cl_2 (3 x 50 mL). The combined organic phase was washed with brine (2 x 30 ml), dried over Na_2SO_4, filtered, and evaporated. Flash chromatography on silica gel (30% ethyl acetate/hexane) yielded 183 mg (70%) of **SeX** as a pale-green solid.

R_f = 0.60 (ethyl acetate: hexane = 1: 1, v/v)

MS (ESI): calcd. for $C_{21}H_{27}N_2OSe^+$ $[M+H]^+$ 403.1283, found 403.1269.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.45 (d, *J* = 9.2 Hz, 2H), 6.72 (dd, *J* = 9.2, 2.6 Hz, 2H), 6.61 (d, *J* = 2.6 Hz, 2H), 3.43 (q, *J* = 7.1 Hz, 8H), 1.22 (t, *J* = 7.1 Hz, 12H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 179.60, 149.48, 136.77, 132.53, 119.78, 110.93, 107.03, 44.43, 12.62.

Synthesis of 3,6-bis(diethylamino)-10,10-dimethylanthracen-9(10*H*)-one (**CX**)



CX

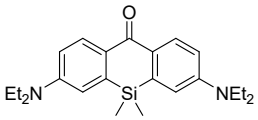
CX was synthesized according to the literature [8].

R_f = 0.70 (ethyl acetate: hexane = 1: 1, v/v)

MS (ESI): calcd. for $C_{24}H_{33}N_2O^+$ $[M+H]^+$ 365.2587, found 365.2556.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.23 (dd, *J* = 9.1, 1.4 Hz, 2H), 6.74 – 6.69 (m, 4H), 3.47 (q, *J* = 7.1 Hz, 8H), 1.70 (s, 6H), 1.25 (t, *J* = 7.1 Hz, 12H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 180.81, 152.34, 150.63, 129.36, 119.36, 110.34, 107.26, 44.60, 37.96, 33.62, 12.69.

Synthesis of 3,7-bis(diethylamino)-5,5-dimethyldibenzo[*b,e*]silin-10(5*H*)-one (**SiX**)



SiX

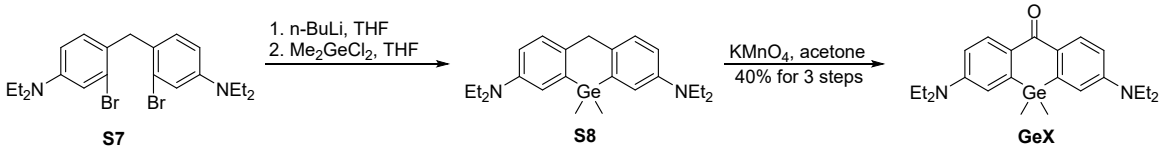
SiX was synthesized according to the literature[9].

R_f = 0.60 (ethyl acetate: hexane = 1: 2, v/v)

MS (ESI): calcd. for $C_{23}H_{33}N_2OSi^+$ $[M+H]^+$ 381.2357, found 381.2340.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.37 (d, *J* = 9.0 Hz, 2H), 6.80 (dd, *J* = 9.1, 2.8 Hz, 2H), 6.75 (d, *J* = 2.9 Hz, 2H), 3.46 (q, *J* = 7.1 Hz, 8H), 1.23 (t, *J* = 7.1 Hz, 12H), 0.46 (s, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 184.83, 148.99, 140.51, 131.84, 128.87, 113.67, 112.57, 44.39, 12.61, -1.09.

Synthesis of 3,7-bis(diethylamino)-5,5-dimethyldibenzo[*b,e*]germin-10(5*H*)-one (**GeX**)



S7 **S8** **GeX**

To a solution of 4,4'-methylenebis(3-bromo-*N, N*-diethylaniline) **S7** (988 mg, 2.22 mmol) in try THF (10 mL) at −78 °C was added *n*-Butyllithium (2.3 mL, 2.5 M in cyclohexane, 5.75 mmol). After stirred at

−78 °C for 30 minutes, then dichlorodimethylgermane (500 mg, 2.88 mmol) was added slowly to the reaction solution. The resulting mixture was stirred at −78 °C for 30 minutes and then at room temperature for 18 h. HCl aqueous solution (1 M, 4 mL) and subsequently saturated $NaHCO_3$ aqueous solution (6 mL) were added, then extracted with CH_2Cl_2 (3 x 80 mL). The combined organic phase was washed with aqueous $Na_2S_2O_3$ (2 x 20 ml) and brine (2 x 30 ml), dried over Na_2SO_4, filtered, and evaporated. The resulting blue viscous liquid **S8** was put into the next step without further purification.

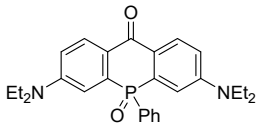
To the solution of crude **S8** in acetone (15 mL) was added potassium permanganate (700 mg, 4.43 mmol) in small portions over 3 hours at 0 °C. Then the reaction mixture was stirred overnight at room temperature. Manganese (IV) oxide and excess potassium permanganate were filtered off and washed with chloroform. The combined organic phase was evaporated. Then flash chromatography on silica gel (ethyl acetate/*n*-hexane/$CHCl_3$, 1:9:1, v/v) yielded 378 mg (40%) of **GeX** as a pale-yellow amorphous solid.

R_f = 0.45 (ethyl acetate: hexane = 1: 2, v/v)

MS (ESI): calcd. for $C_{23}H_{33}GeN_2O^+$ $[M+H]^+$ 427.1799, found 427.1791.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.39 (d, *J* = 9.1 Hz, 2H), 6.76 (dd, *J* = 9.1, 2.8 Hz, 2H), 6.68 (d, *J* = 2.9 Hz, 2H), 3.45 (q, *J* = 7.1 Hz, 8H), 1.23 (t, *J* = 7.1 Hz, 12H), 0.58 (s, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 184.72, 149.03, 143.33, 132.27, 128.88, 113.65, 112.10, 44.38, 12.62, -1.54.

Synthesis of 3,7-bis(diethylamino)-5-phenyl-10*H*-acridophosphin-10-one 5-oxide (**POX**)



POX

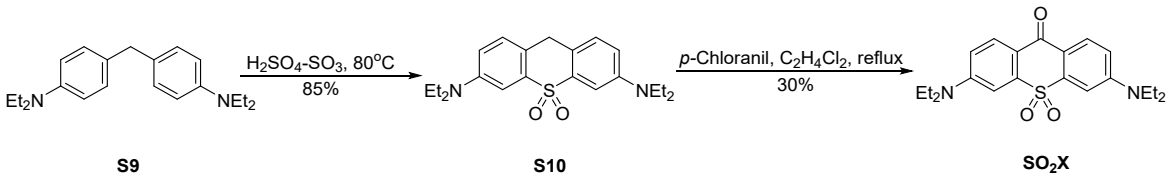
POX was synthesized according to the literature [10].

R_f = 0.13 (ethyl acetate: hexane = 2:1, v/v)

MS (ESI): calcd. for $C_{27}H_{32}N_2O_2P^+$ $[M+H]^+$ 447.2196, found 447.2196.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.28 (dd, *J* = 9.1, 6.0 Hz, 2H), 7.64 – 7.57 (m, 2H), 7.41 – 7.36 (m, 1H), 7.36 – 7.30 (m, 2H), 7.11 (dd, *J* = 14.9, 2.8 Hz, 2H), 6.83 (dd, *J* = 9.1, 2.8 Hz, 2H), 3.52 – 3.33 (m, 8H), 1.17 (t, *J* = 7.1 Hz, 12H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 180.11 (d, *J* = 8.5 Hz), 150.35 (d, *J* = 12.4 Hz), 135.44 (d, *J* = 76.4 Hz), 134.76 (d, *J* = 67.8 Hz), 131.44 (d, *J* = 10.4 Hz), 131.25 (d, *J* = 2.7 Hz), 130.38 (d, *J* = 10.2 Hz), 128.49 (d, *J* = 12.2 Hz), 123.87 (d, *J* = 6.9 Hz), 114.13 (d, *J* = 2.2 Hz), 111.40 (d, *J* = 7.7 Hz), 44.53, 12.46.

Synthesis of 3,6-bis(diethylamino)-9*H*-thioxanthen-9-one 10,10-dioxide (**SO_2X**) [11]



To fuming H_2SO_4-SO_3 (18-24% free SO_3, 5 mL) was added 4,4'-methylenebis (*N*, *N*-diethylaniline) **S9** (500 mg, 1.61 mmol). The resulting mixture was stirred at 80 °C for 15 hours. Then the reaction mixture was allowed to cool to 0 °C, and aq. NaOH solution (5.0 M) was added to adjust pH to 9.0. The resulting mixture was extracted with ethyl acetate (3 x 80 mL). The combined organic phase was washed with brine (2 x 30 ml), dried over Na_2SO_4, filtered, and evaporated. Then flash chromatography on silica gel (ethyl acetate/*n*-hexane, 1:3, v/v) yielded 509 mg (85%) of **S10** as a pale-yellow solid.

R_f = 0.35 (ethyl acetate: hexane = 1:3, v/v)

MS (ESI): calcd. for $C_{21}H_{29}N_2O_2S^+$ $[M+H]^+$ 373.1944, found 373.1958.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.31 (s, 2H), 7.20 (d, *J* = 8.6 Hz, 2H), 6.71 (d, *J* = 8.6 Hz, 2H), 3.98 (s, 2H), 3.38 (dd, *J* = 7.1, 2.6 Hz, 8H), 1.18 – 1.13 (m, 12H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 146.77, 138.25, 129.45, 125.05, 114.88, 106.09, 44.47, 32.07, 12.38.

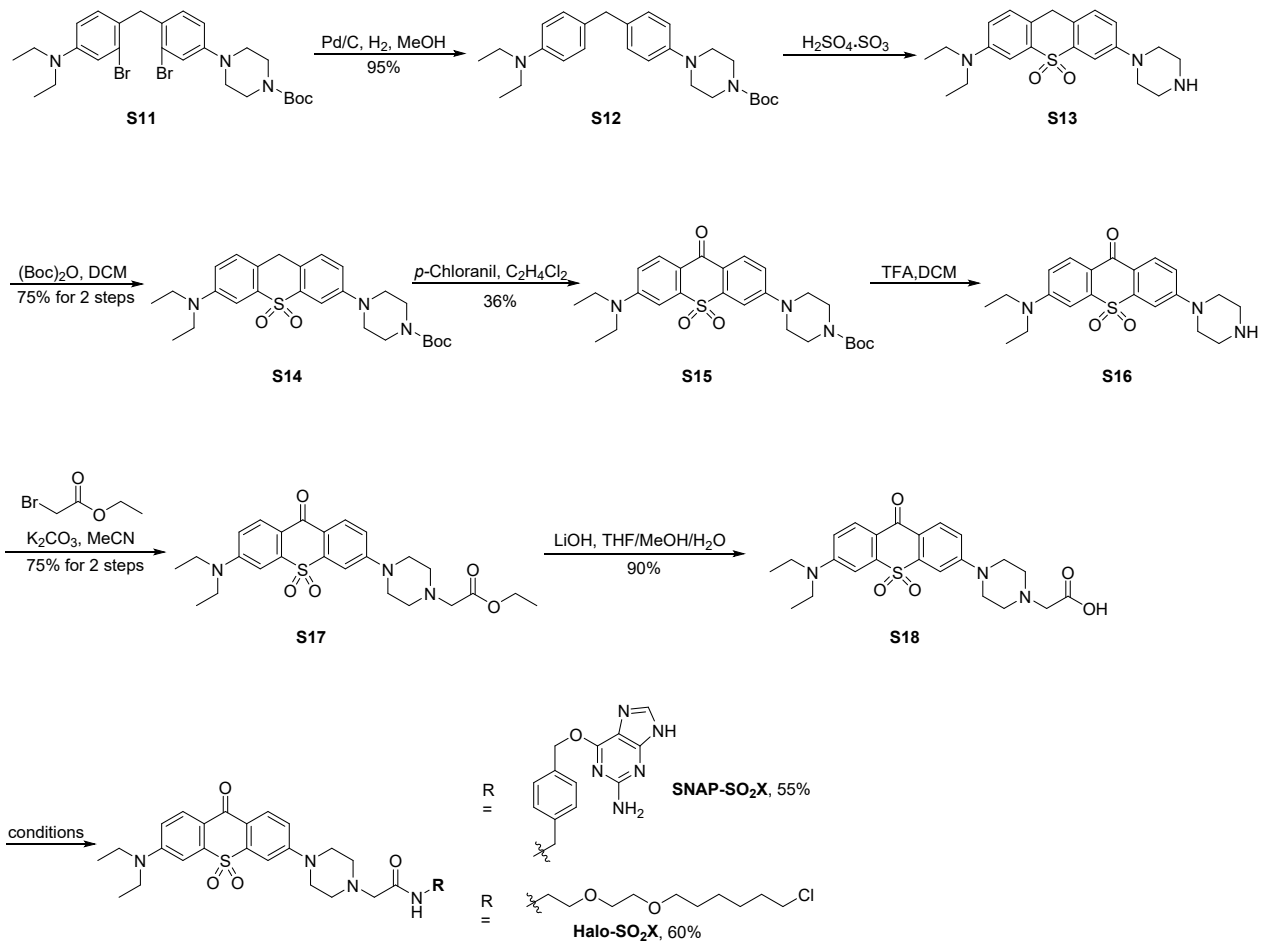
To a solution of **S10** (450 mg, 1.21 mmol) in $C_2H_4Cl_2$ was added *p*-Chloranil (893 mg, 3.63 mmol). After stirred at 90 °C for 15 hours, the reaction mixture was allowed to cool to room temperature and quenched by adding aq. 20% Na_2SO_3 (20 mL) and stirredfor 30 min. The resulting mixture was extracted with CH_2Cl_2 (3 x 80 mL). The combined organic phase was washed with brine (2 x 40 ml), dried over Na_2SO_4, filtered, and evaporated. Then flash chromatography on silica gel (CH_2Cl_2/*n*-hexane, 1:4, v/v) yielded 140 mg (30%) of **SO_2X** as a brown foam.

R_f = 0.60 (ethyl acetate: hexane = 1:1, v/v)

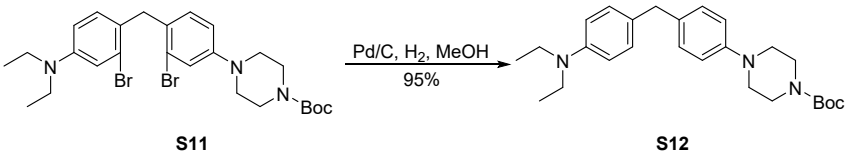
MS (ESI): calcd. for $C_{21}H_{27}N_2O_3S^+$ $[M+H]^+$ 387.1737, found 387.1580.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.18 (d, *J* = 9.0 Hz, 2H), 7.17 (d, *J* = 2.6 Hz, 2H), 6.84 (dd, *J* = 9.1, 2.7 Hz, 2H), 3.51 (q, *J* = 7.2 Hz, 8H), 1.26 (t, *J* = 7.1 Hz, 12H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 175.38, 151.04, 142.84, 131.25, 118.31, 114.31, 103.64, 44.81, 12.44.

2.2 Synthesis of SO_2X-targeting probes (Halo-SO_2X, SNAP-SO_2X)



tert-butyl 4-(4-(4-(diethylamino)benzyl)phenyl)piperazine-1-carboxylate (**S12**)



To a solution of compound **S11** [12](600 mg, 1.03 mmol) in MeOH (10 mL) was added Pd/C (catalytic amount) under N_2 atmosphere. Then the reaction flask was evacuated and purged with hydrogen three times. After stirred overnight under a H_2 atmosphere at ambient temperature, the catalyst was removed by filtration through a pad of celite. The filtrate was concentrated in *vacuo*. The residue was purified by flash chromatography on silica gel (eluent: 10% ethyl acetate in hexanes) to provide compound **S12** (414 mg, 95%) as a colorless oil.

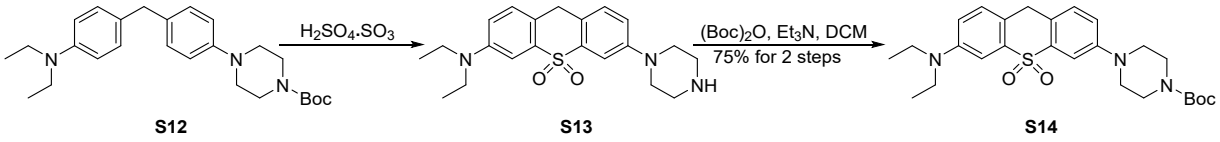
R_f = 0.30 (ethyl acetate: hexane = 1:8, v/v)

MS (ESI): calcd. for $C_{26}H_{38}N_3O_2^+$ $[M+H]^+$ 424.2959, found 424.2953.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.12 – 7.08 (m, 2H), 7.01 (dd, *J* = 8.7, 1.4 Hz, 2H), 6.87 – 6.83 (m, 2H), 6.66 – 6.57 (m, 2H), 3.81 (s, 2H), 3.56 (dd, *J* = 7.3, 3.3 Hz, 4H), 3.35 – 3.28 (m, 4H), 3.09 – 3.06 (m, 4H), 1.48

(d, *J* = 1.4 Hz, 9H), 1.13 (td, *J* = 7.1, 1.4 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 154.75, 149.43, 146.18, 134.24, 129.59, 129.51, 128.38, 116.88, 112.12, 79.81, 49.83, 44.39, 39.95, 32.48, 28.43, 12.59.

tert-butyl 4-(6-(diethylamino)-10,10-dioxido-9H-thioxanthen-3-yl) piperazine-1-carboxylate (**S14**)



S12 **S13** **S14**

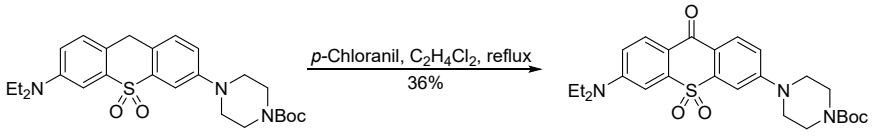
To fuming H_2SO_4-SO_3 (18-24% free SO_3, 5 mL) was added **S12** (300 mg, 0.71 mmol). After stirred at 80 °C for 15 hours, the reaction mixture was allowed to cool to 0 °C. Then aq. NaOH solution (5.0 M) was added to adjust pH to 9.0. The resulting mixture was extracted with ethyl acetate (3 x 80 mL). The combined organic phase was washed with brine (2 x 30 ml), dried over Na_2SO_4, filtered, and evaporated. The resulting blue viscous liquid **S13** was put into the next step without further purification.

To a solution of **S13** in CH_2Cl_2 (5 mL) was added $(Boc)_2O$ (310 mg, 1.42 mmol) and Et_3N (0.49 mL, 3.55 mmol). After stirred overnight at room temperature, the reaction mixture was diluted with water and extracted with CH_2Cl_2 (50 mL x 3). The combined organic phase was washed with water and brine, dried over Na_2SO_4, filtered, and evaporated. Flash chromatography on silica gel (30% EtOAc/hexanes) yielded 258 mg (75%) of **S14** as a yellow oil.

R_f = 0.15 (ethyl acetate: hexane = 1:3, v/v)

HRMS (ESI): calcd. for $C_{26}H_{36}N_3O_4S^+$ $[M+H]^+$ 486.2421, found 486.2426.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.57 (d, *J* = 2.6 Hz, 1H), 7.30 (d, *J* = 2.8 Hz, 1H), 7.28 (d, *J* = 8.4 Hz, 1H), 7.20 (d, *J* = 8.5 Hz, 1H), 6.99 (dd, *J* = 8.4, 2.6 Hz, 1H), 6.72 (dd, *J* = 8.5, 2.8 Hz, 1H), 4.02 (s, 2H), 3.58 (t, *J* = 5.2 Hz, 4H), 3.39 (q, *J* = 7.1 Hz, 4H), 3.18 (t, *J* = 5.1 Hz, 4H), 1.49 (s, 9H), 1.16 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 154.65, 150.29, 146.92, 138.38, 138.03, 130.23, 129.58, 129.43, 124.32, 119.79, 114.98, 110.88, 106.01, 80.06, 49.04, 44.47, 32.30, 28.41, 12.36.



S14 **S15**

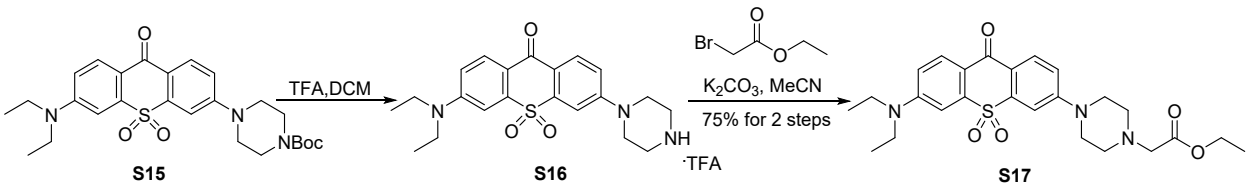
To a solution of **S14** (80 mg, 0.17 mmol) in $C_2H_4Cl_2$ was added *p*-Chloranil (122 mg, 0.50 mmol). After stirred at 90 °C for 15 hours, the reaction mixture was allowed to cool to room temperature and quenched by adding aq. 20% Na_2SO_3 (20 mL) and stirred for 30 min. The resulting mixture was

extracted with CH_2Cl_2 (3 x 80 mL). The combined organic phase was washed with brine (2 x 40 ml), dried over Na_2SO_4, filtered, and evaporated. Then flash chromatography on silica gel (ethyl acetate/*n*-hexane, 1:1, v/v) yielded 30 mg (36%) of **S15** as a brown foam.

R_f = 0.40 (ethyl acetate: hexane = 1:1, v/v)

MS (ESI): calcd. for $C_{26}H_{34}N_3O_5S^+$ $[M+H]^+$ 500.2214, found 500.2198.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.23 (d, *J* = 8.9 Hz, 1H), 8.18 (d, *J* = 9.1 Hz, 1H), 7.40 (d, *J* = 2.6 Hz, 1H), 7.17 (d, *J* = 2.7 Hz, 1H), 7.07 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.85 (dd, *J* = 9.1, 2.7 Hz, 1H), 3.62 (t, *J* = 5.2 Hz, 4H), 3.51 (q, *J* = 7.2 Hz, 4H), 3.48 (t, *J* = 5.5 Hz, 4H), 1.50 (s, 9H), 1.26 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 175.36, 154.56, 153.47, 151.27, 142.84, 142.52, 131.46, 131.04, 121.02, 117.84, 117.12, 114.39, 106.56, 103.66, 80.41, 46.81, 44.87, 28.40, 12.42.



To a stirred solution of **S15** (60 mg, 0.12 mmol) in CH_2Cl_2 (5 mL) was added TFA (2 mL) at 0 °C. The reaction mixture was gradually warmed to room temperature and stirred for 4 h. Solvents and volatiles were removed in vacuo. Then the resulting residue was dissolved in CH_2Cl_2 (5 mL) and concentrated again to afford the desired amine as the corresponding TFA salt, which was used directly in the next step without further purification.

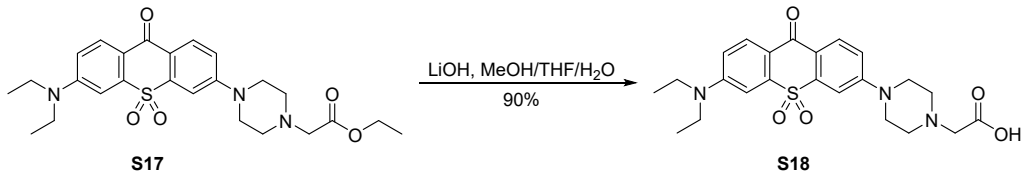
To a solution of **S16** in MeCN (5 mL) was added K_2CO_3 (33 mg, 0.24 mmol), followed by the addition of ethyl bromoacetate (40 mg, 0.24 mmol) at room temperature. After stirred at 50 °C for 12 h, solvents and volatiles were removed in vacuo. The residue was dissolved in CH_2Cl_2 (50 mL) and washed with water and brine, dried over Na_2SO_4, filtered, and evaporated. Flash chromatography on silica gel (50% EtOAc/hexanes) yielded 44 mg (75%) of **S17** as a yellow foam.

R_f = 0.10 (ethyl acetate: hexane = 1:1, v/v)

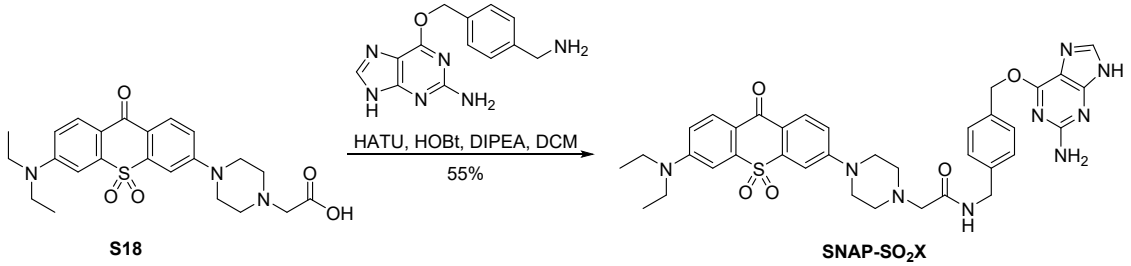
MS (ESI): calcd. for $C_{25}H_{32}N_3O_5S^+$ $[M+H]^+$ 486.2057, found 486.2010.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.21 (dd, *J* = 9.0, 2.0 Hz, 1H), 8.18 (dd, *J* = 9.1, 2.0 Hz, 1H), 7.41 (d, *J* = 2.4 Hz, 1H), 7.17 (t, *J* = 2.4 Hz, 1H), 7.07 (d, *J* = 9.1 Hz, 1H), 6.85 (d, *J* = 9.1 Hz, 1H), 4.22 (dd, *J* = 7.2, 2.0 Hz, 2H), 3.59 – 3.55 (m, 4H), 3.54 – 3.48 (m, 4H), 3.35 (d, *J* = 2.0 Hz, 2H), 2.84 (t, *J* = 5.1 Hz, 4H), 1.34 – 1.27 (m, 3H), 1.25 (t, *J* = 3.6 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 175.37, 169.50, 153.41, 151.27, 142.89,

142.53, 131.44, 131.03, 120.93, 117.95, 117.01, 114.39, 106.55, 103.70, 61.01, 58.81, 52.18, 46.63, 44.86, 14.23, 12.43.



To a solution of **S17** (49 mg, 0.10 mmol) in THF (2 mL) and MeOH (1 mL) at 0 °C was added LiOH (4.8 mg, 0.2 mmol in 2 mL water). The reaction mixture was gradually warmed to room temperature and stirred for 4 h. After the removal of THF and MeOH in vacuo, the residue was dissolved in CH_2Cl_2 (5 mL). Then aq. HCl solution (1.0 M) was added to adjust pH to 7.0. The resulting mixture was extracted with CH_2Cl_2 (3 x 30 mL). The combined organic phase was washed with brine (2 x 30 ml), dried over Na_2SO_4, filtered, and evaporated. The resulting acid **S18** (41 mg, 90%) was used directly in the next step without further purification.



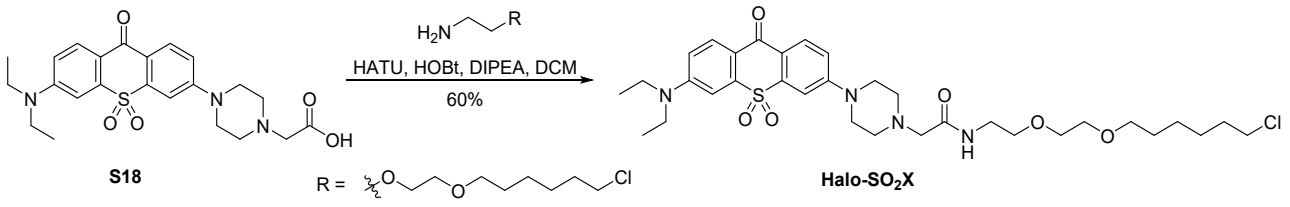
To a solution of acid **S18** (10 mg, 22 µmol) in DMF (4 mL) was added HATU (17 mg, 44 µmol), HOBt (3 mg, 22 µmol) and DIPEA (20 µL, 0.11 mmol) at 0 °C, followed by addition of O^6 -(4-Aminomethyl-benzyl)guanine[13] (18 mg, 66 µmol). After stirred at room temperature for 12 h, the reaction mixture was diluted with water and extracted with CH_2Cl_2 (30 mL x 3). The combined organic phase was washed with saturated sodium bicarbonate solution (20 mL x 2) and brine, dried over Na_2SO_4, filtered, and evaporated. Flash chromatography on silica gel (5% MeOH/ CH_2Cl_2) yielded 8.5 mg (55%) of **SNAP-SO_2X** as a yellow oil.

R_f = 0.45 (MeOH: CH_2Cl_2 = 1:9, v/v)

HRMS (ESI): calcd. for $C_{36}H_{40}N_9O_5S^+$ $[M+H]^+$ 710.2868, found 710.2796.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.16 (d, *J* = 9.0 Hz, 2H), 7.74 (s, 1H), 7.45 (d, *J* = 7.5 Hz, 2H), 7.36 (s, 1H), 7.29 (d, *J* = 7.7 Hz, 2H), 7.16 (d, *J* = 2.6 Hz, 1H), 6.97 (s, 1H), 6.86 – 6.79 (m, 1H), 5.48 (s, 2H), 5.01 (s, 2H), 4.52 (d, *J* = 5.8 Hz, 2H), 3.49 (q, *J* = 7.1 Hz, 4H), 3.40 – 3.37 (m, 4H), 3.20 – 3.17 (m, 2H), 2.69 – 2.66 (m, 4H), 1.24 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$_3mm tube) δ 175.40, 169.67, 160.76, 159.36, 154.29, 153.46, 151.29, 142.86, 142.39, 138.73, 137.38, 135.56, 131.44, 130.96, 129.33, 128.13,

120.81, 117.88, 116.98, 114.62, 114.43, 106.52, 103.68, 68.14, 61.45, 52.95, 46.79, 44.88, 42.99, 12.43.



To a solution of acid **S18** (20 mg, 44 μmol) in DMF (4 mL) was added HATU (34 mg, 88 μmol), HOBt (6 mg, 22 μmol) and DIPEA (40 μL, 0.22 mmol) at 0 °C, followed by addition of 2-[2-[(6-chlorohexyl)oxy] ethoxy]-ethanamine (20 mg, 88 μmol). After stirred at room temperature for 12 h, the reaction mixture was diluted with water and extracted with CH_2Cl_2 (30 mL x 3). The combined organic phase was washed with saturated sodium bicarbonate solution (20 mL x 2) and brine, dried over Na_2SO_4, filtered, and evaporated. Flash chromatography on silica gel (5% MeOH/ CH_2Cl_2) yielded 17.5 mg (60%) of **Halo-SO_2X** as a pale-yellow oil.

R_f = 0.20 (MeOH: CH_2Cl_2 = 1:20, v/v)

HRMS (ESI): calcd. for $C_{33}H_{48}ClN_4O_6S^+$ $[M+H]^+$ 663.2978, found 663.2951.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.22 (d, *J* = 9.0 Hz, 1H), 8.18 (d, *J* = 9.1 Hz, 1H), 7.47 – 7.43 (m, 1H), 7.41 (d, *J* = 2.6 Hz, 1H), 7.17 (d, *J* = 2.7 Hz, 1H), 7.09 (dd, *J* = 9.0, 2.6 Hz, 1H), 6.86 (dd, *J* = 9.1, 2.7 Hz, 1H), 3.68 – 3.61 (m, 2H), 3.61 – 3.57 (m, 4H), 3.54 – 3.47 (m, 10H), 3.44 (t, *J* = 6.8 Hz, 2H), 3.11 (s, 2H), 2.71 (t, *J* = 5.2 Hz, 4H), 1.72 (dt, *J* = 14.6, 6.8 Hz, 2H), 1.59 – 1.53 (m, 2H), 1.44 – 1.36 (m, 2H), 1.35 – 1.30 (m, 2H), 1.26 (t, *J* = 7.2 Hz, 6H), 1.25 – 1.19 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$_3mm_tubes) δ 175.34, 169.56, 153.46, 151.26, 142.84, 142.50, 131.45, 130.98, 120.99, 117.82, 117.06, 114.39, 106.50, 103.64, 71.33, 70.36, 70.10, 69.91, 61.46, 52.89, 47.01, 45.04, 44.86, 38.66, 32.48, 29.43, 26.64, 25.35, 12.41.

3. Supplementary Figures and Tables

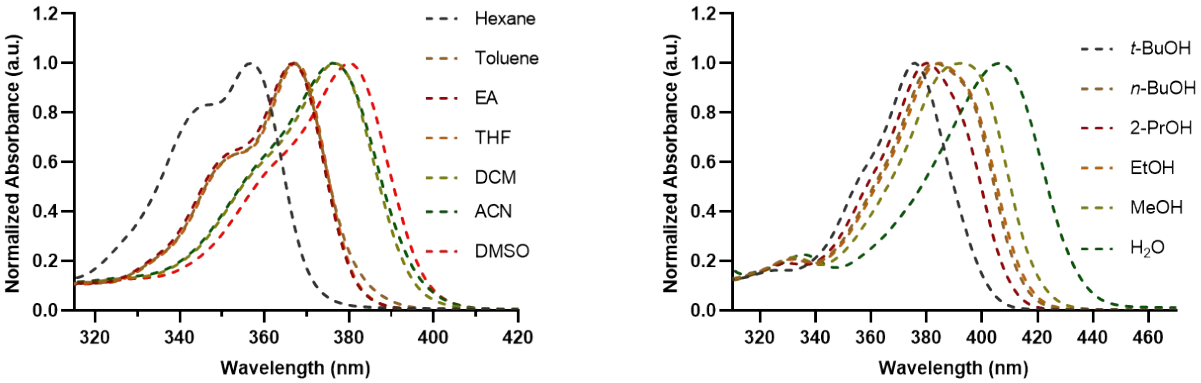


Figure S1. Normalized absorption spectra of **MA** in aprotic and protic solvents.

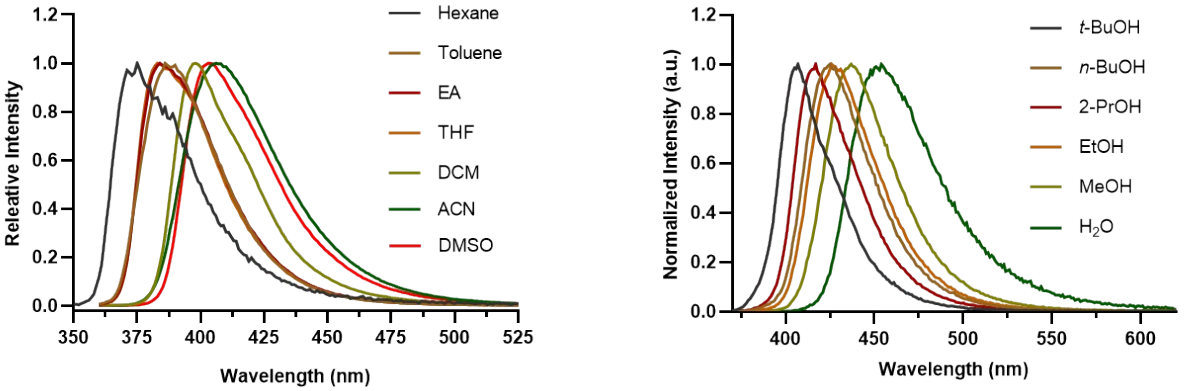


Figure S2. Normalized emission spectra of **MA** in aprotic and protic solvents.

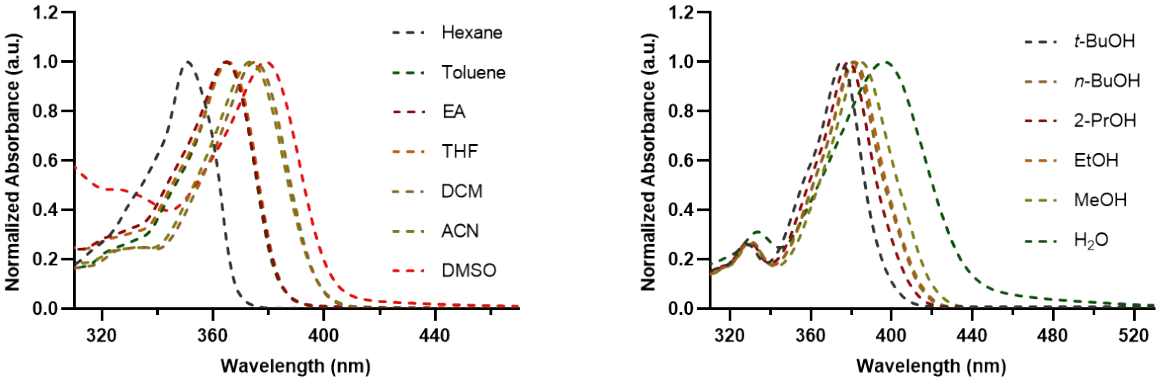


Figure S3. Normalized absorption spectra of **OX** in aprotic and protic solvents.

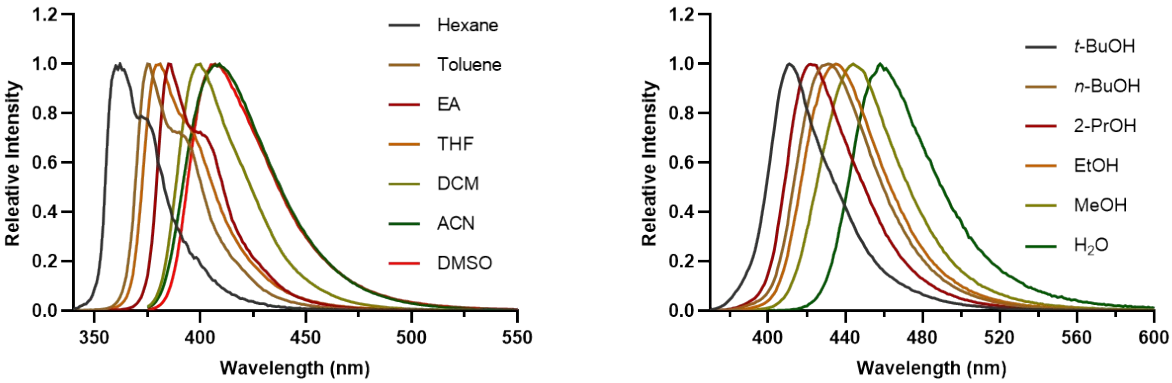


Figure S4. Normalized emission spectra of **OX** in aprotic and protic solvents.

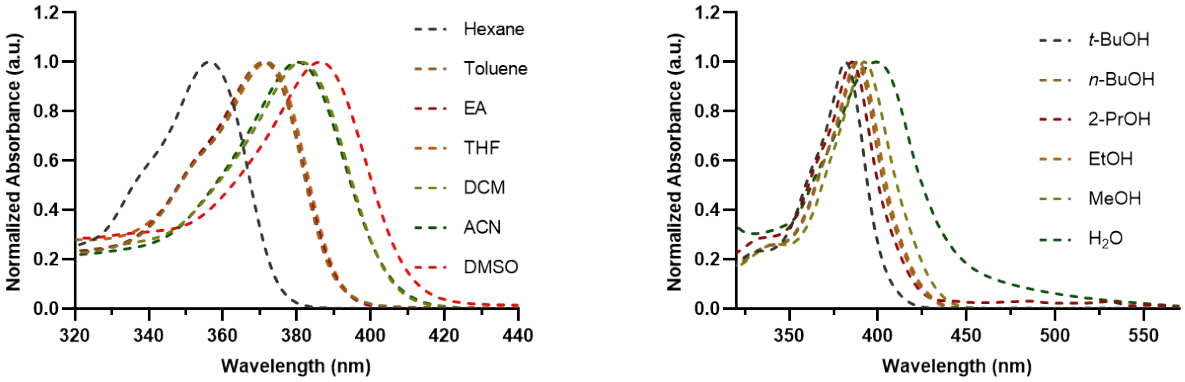


Figure S5. Normalized absorption spectra of **SX** in aprotic and protic solvents.

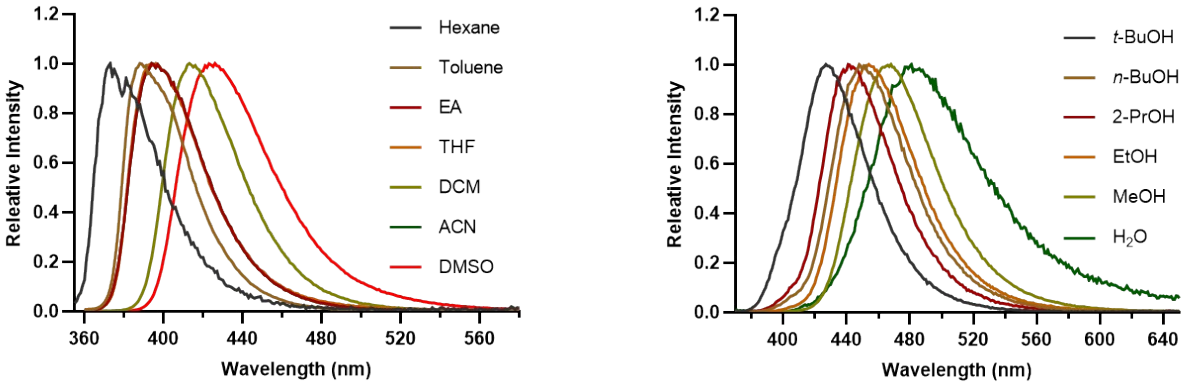


Figure S6. Normalized emission spectra of **SX** in aprotic and protic solvents.

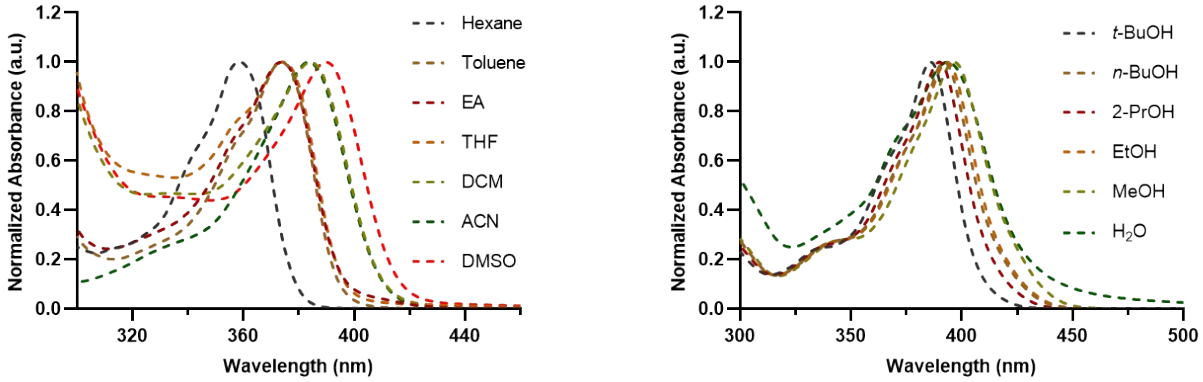


Figure S7. Normalized absorption spectra of **SeX** in aprotic and protic solvents.

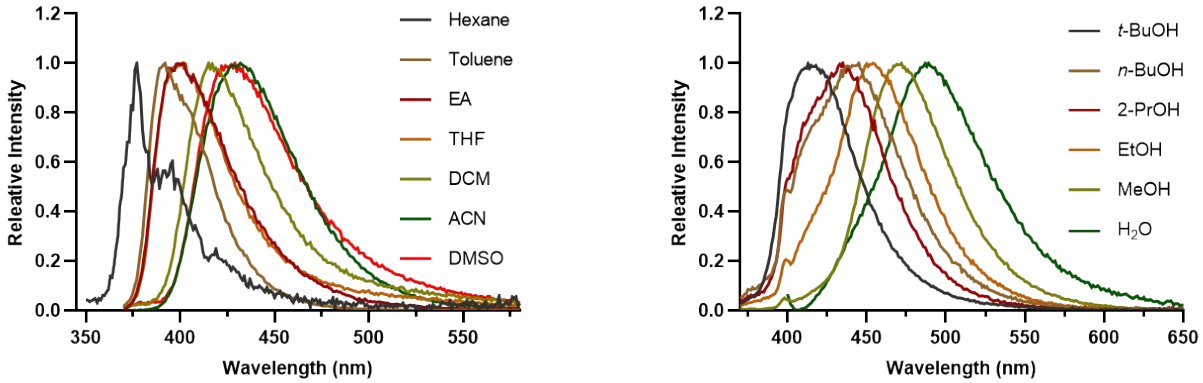


Figure S8. Normalized emission spectra of **SeX** in aprotic and protic solvents.

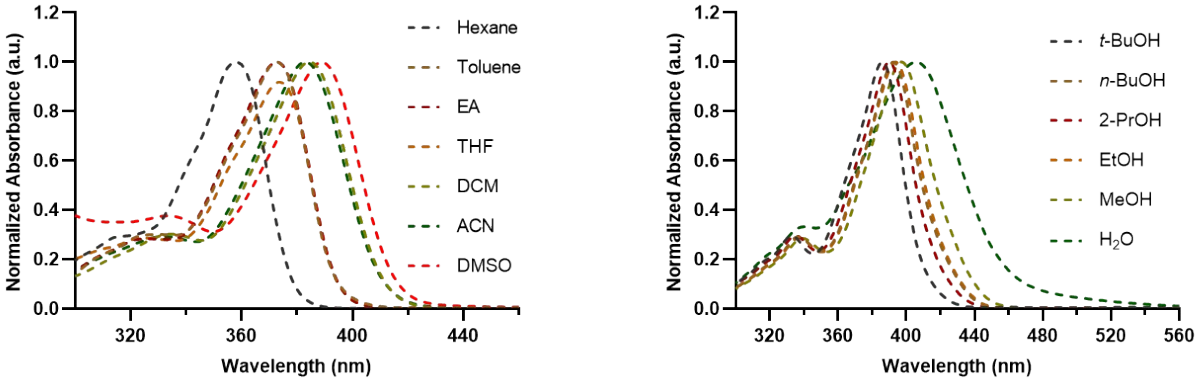


Figure S9. Normalized absorption spectra of **CX** in aprotic and protic solvents.

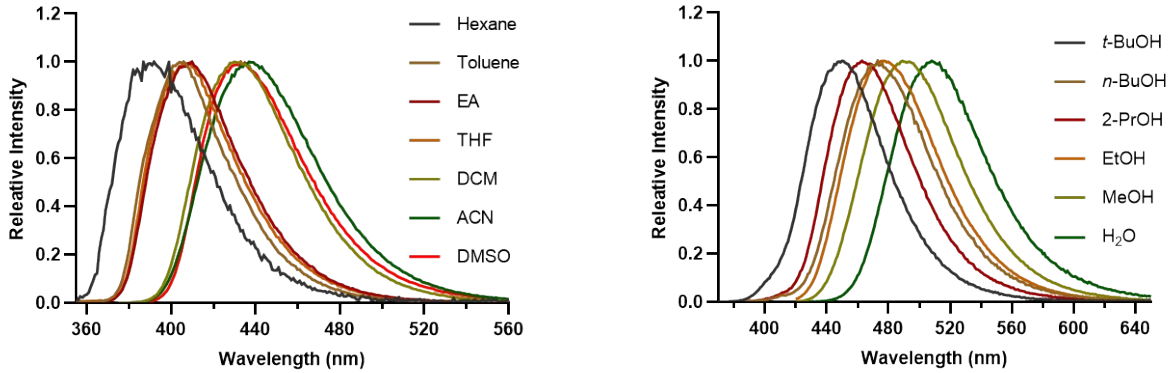


Figure S10. Normalized emission spectra of **CX** in aprotic and protic solvents.

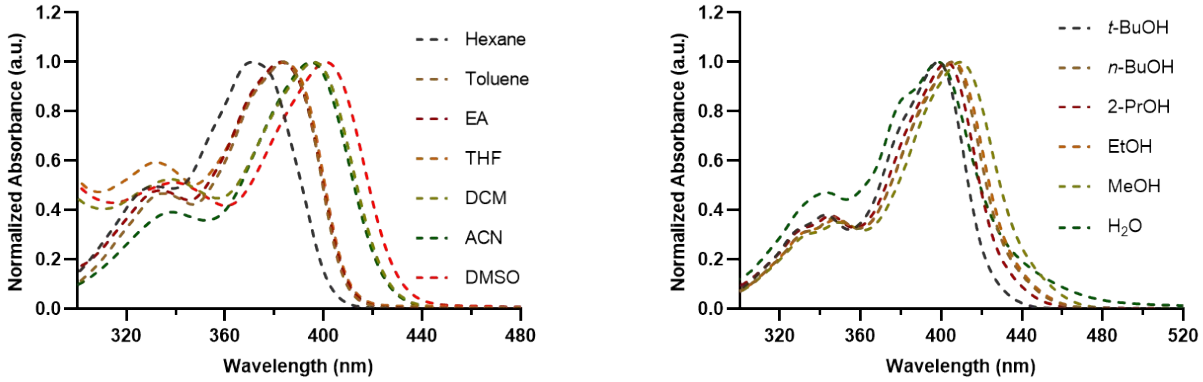


Figure S11. Normalized absorption spectra of **SiX** in aprotic and protic solvents.

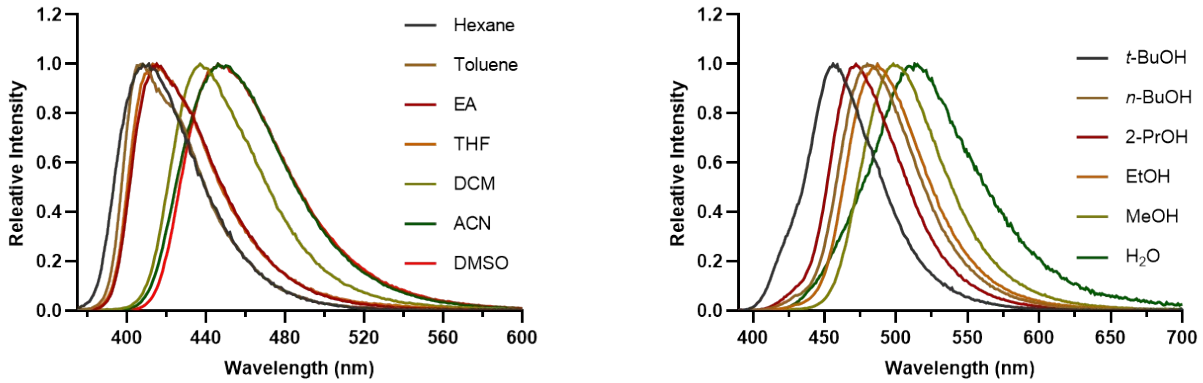


Figure S12. Normalized emission spectra of **SiX** in aprotic and protic solvents.

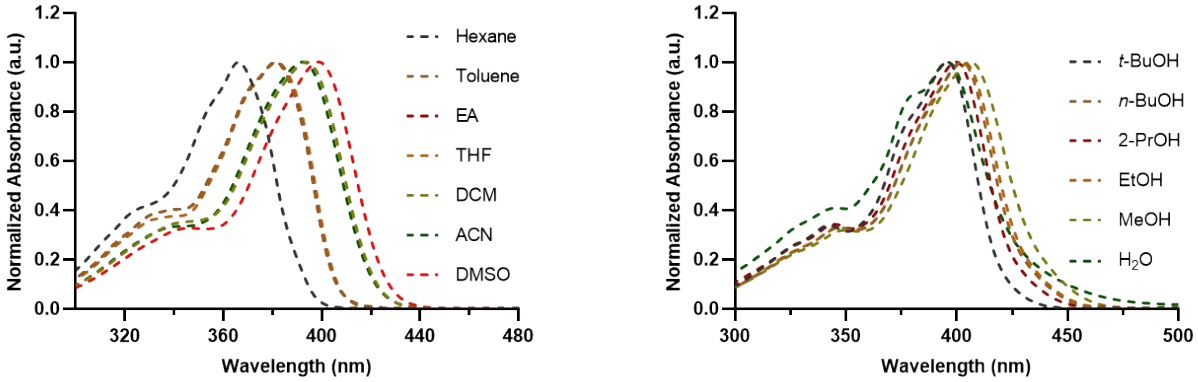


Figure S13. Normalized absorption spectra of **GeX** in aprotic and protic solvents.

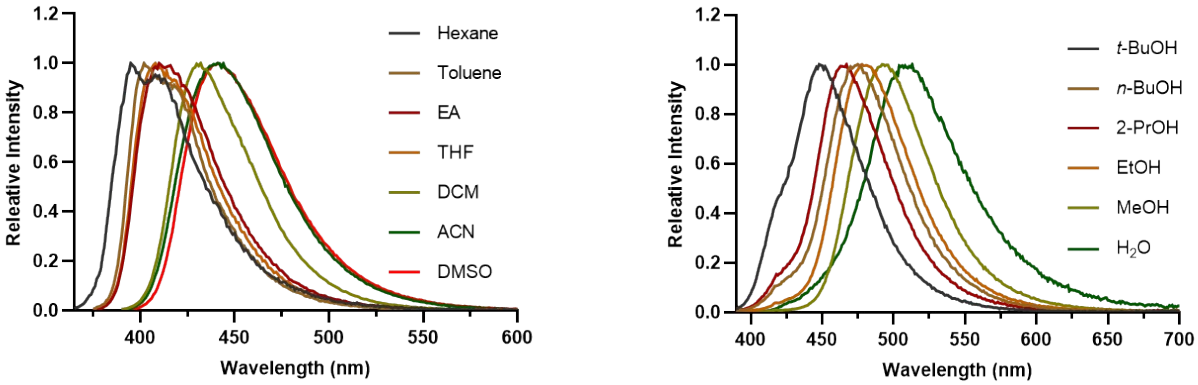


Figure S14. Normalized emission spectra of **GeX** in aprotic and protic solvents.

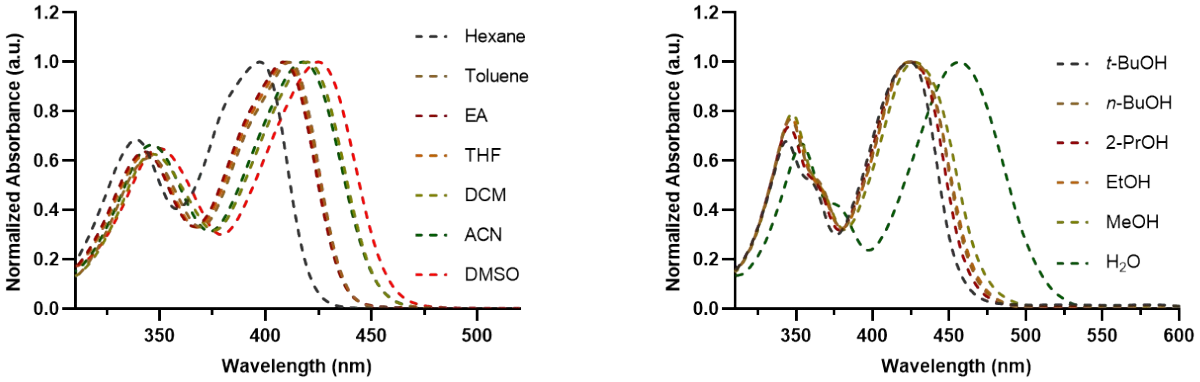


Figure S15. Normalized absorption spectra of **POX** in aprotic and protic solvents.

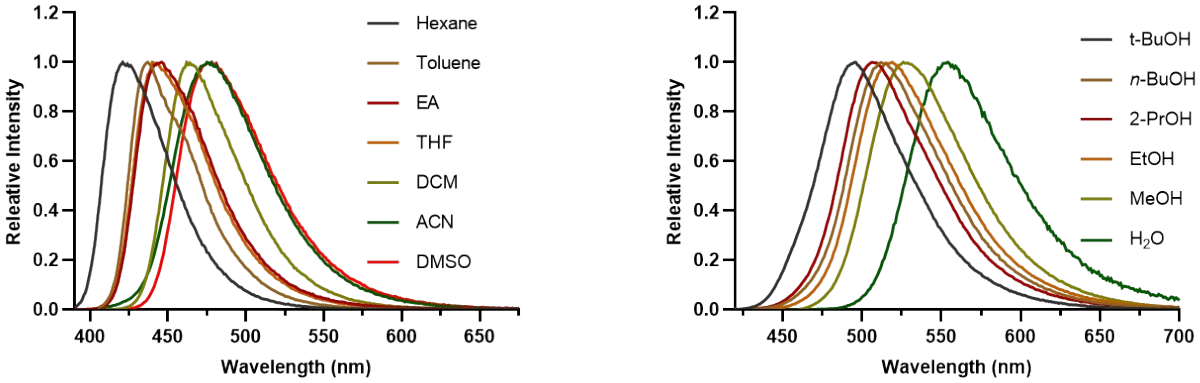


Figure S16. Normalized emission spectra of **POX** in aprotic and protic solvents.

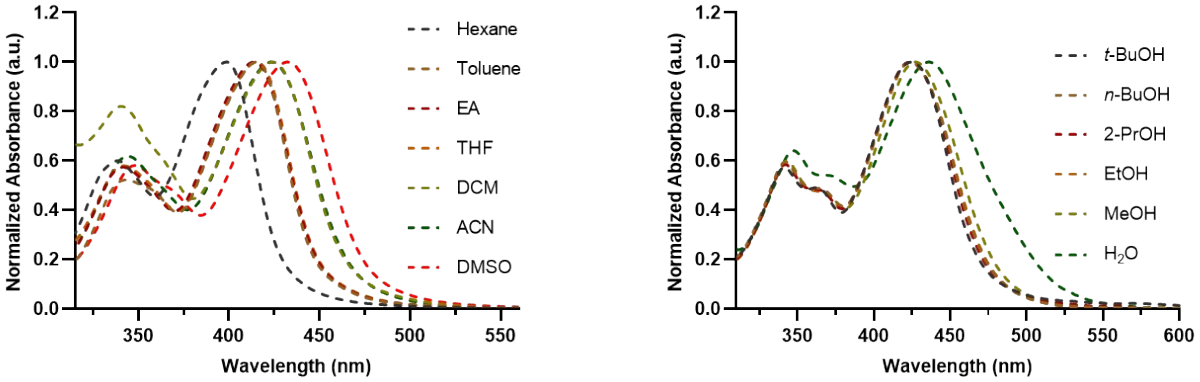


Figure S17. Normalized absorption spectra of **SO_2X** in aprotic and protic solvents.

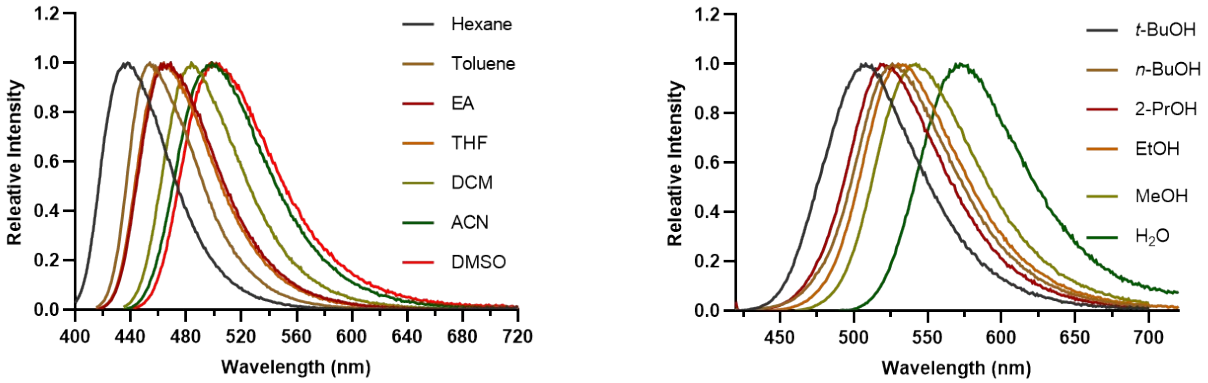


Figure S18. Normalized emission spectra of **SO_2X** in aprotic and protic solvents.

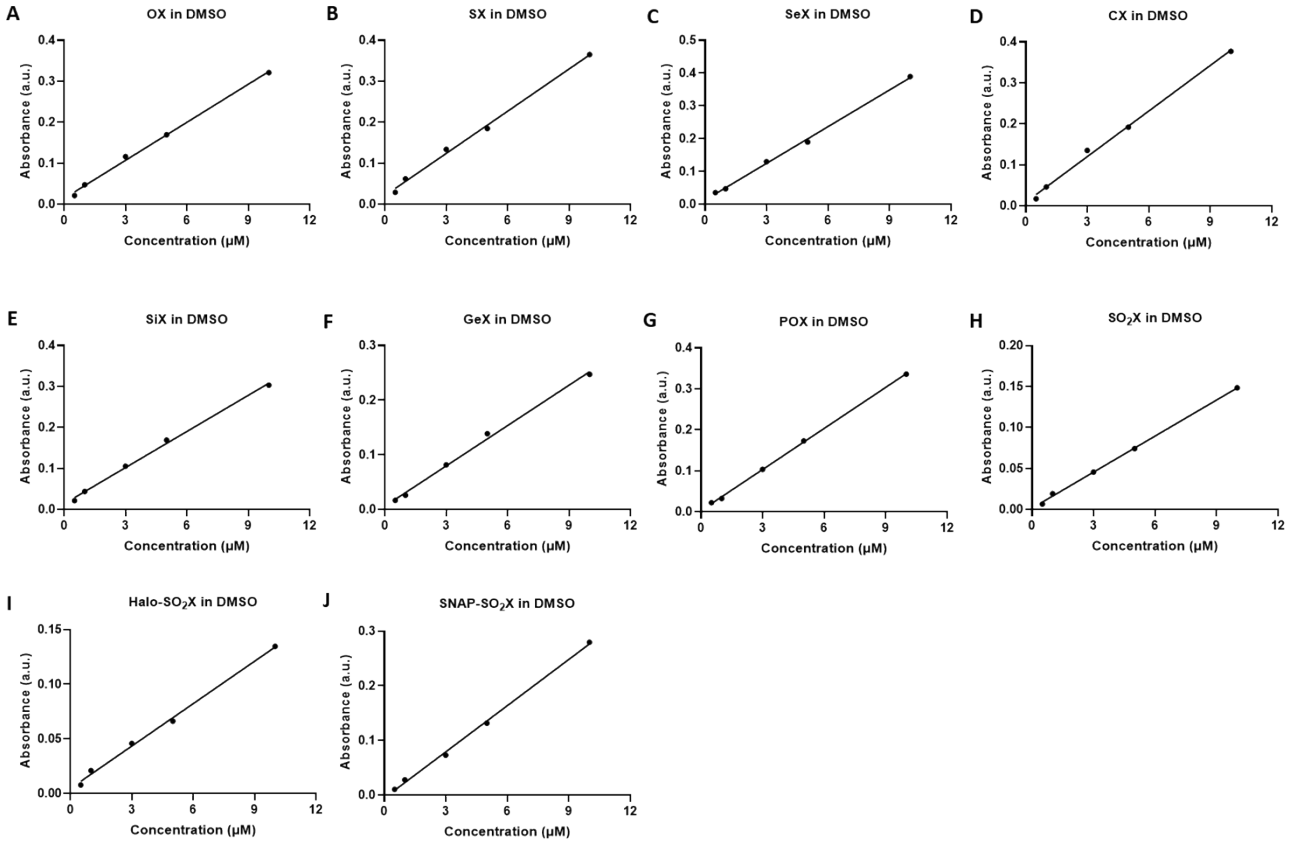


Figure S19. The Beer-Lambert plots of OX, SX, SeX, CX, SiX, GeX, POX, SO_2X, Halo-SO_2X and SNAP-SO_2X in DMSO at 0.5, 1.0, 3.0, 5.0 and 10.0 μM. (A) Absorbance at 376 nm; (B) Absorbance at 382 nm; (C) Absorbance at 386 nm; (D) Absorbance at 386 nm; (E) Absorbance at 398 nm; (F) Absorbance at 394 nm; (G) Absorbance at 422 nm; (H) Absorbance at 432 nm; (I-J) Absorbance at 430 nm.

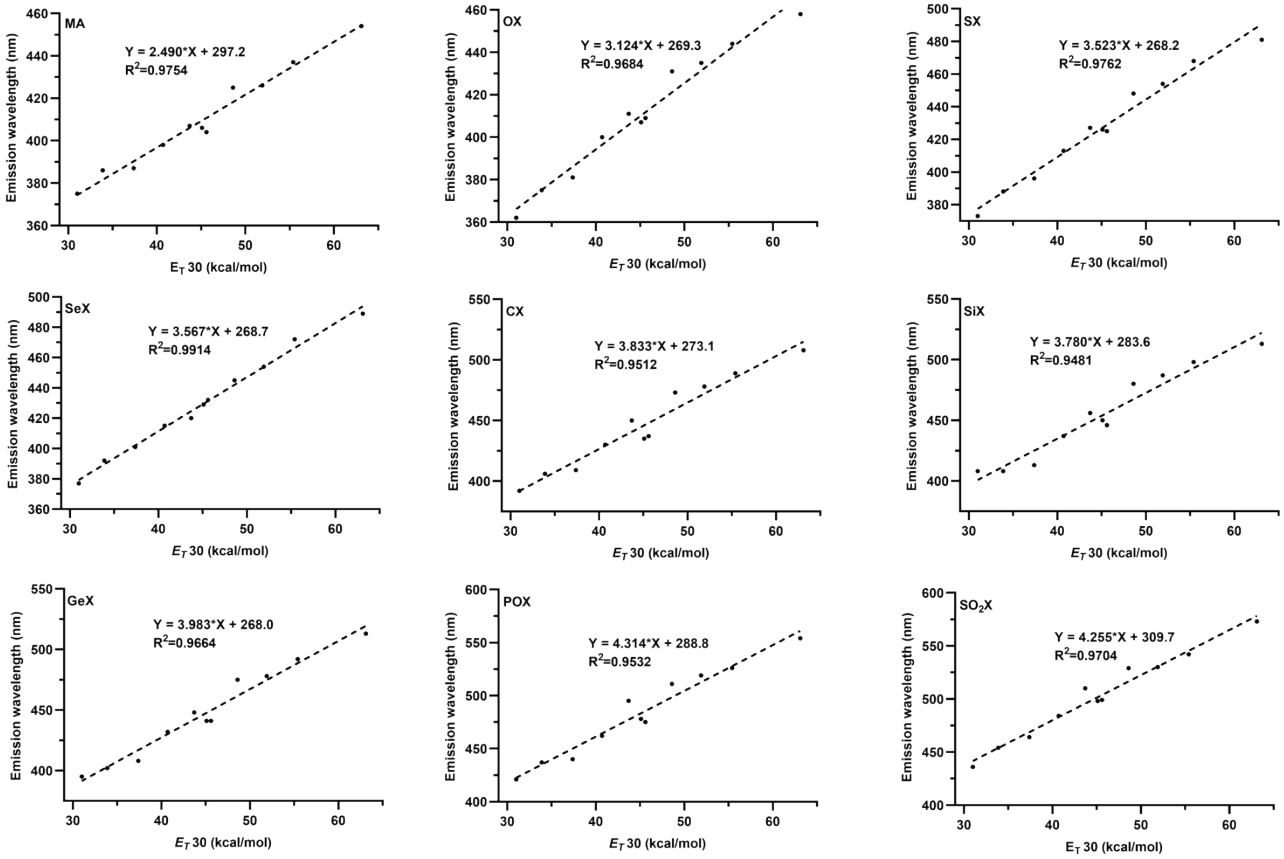


Figure S20. Polarity dependence of heteroatom xanthone probes. The polarity sensitivity was quantified via the slope of the linear correlation between emission maximum in different solvents (10 μM) *versus* the E_T30 parameter for xanthone probes.

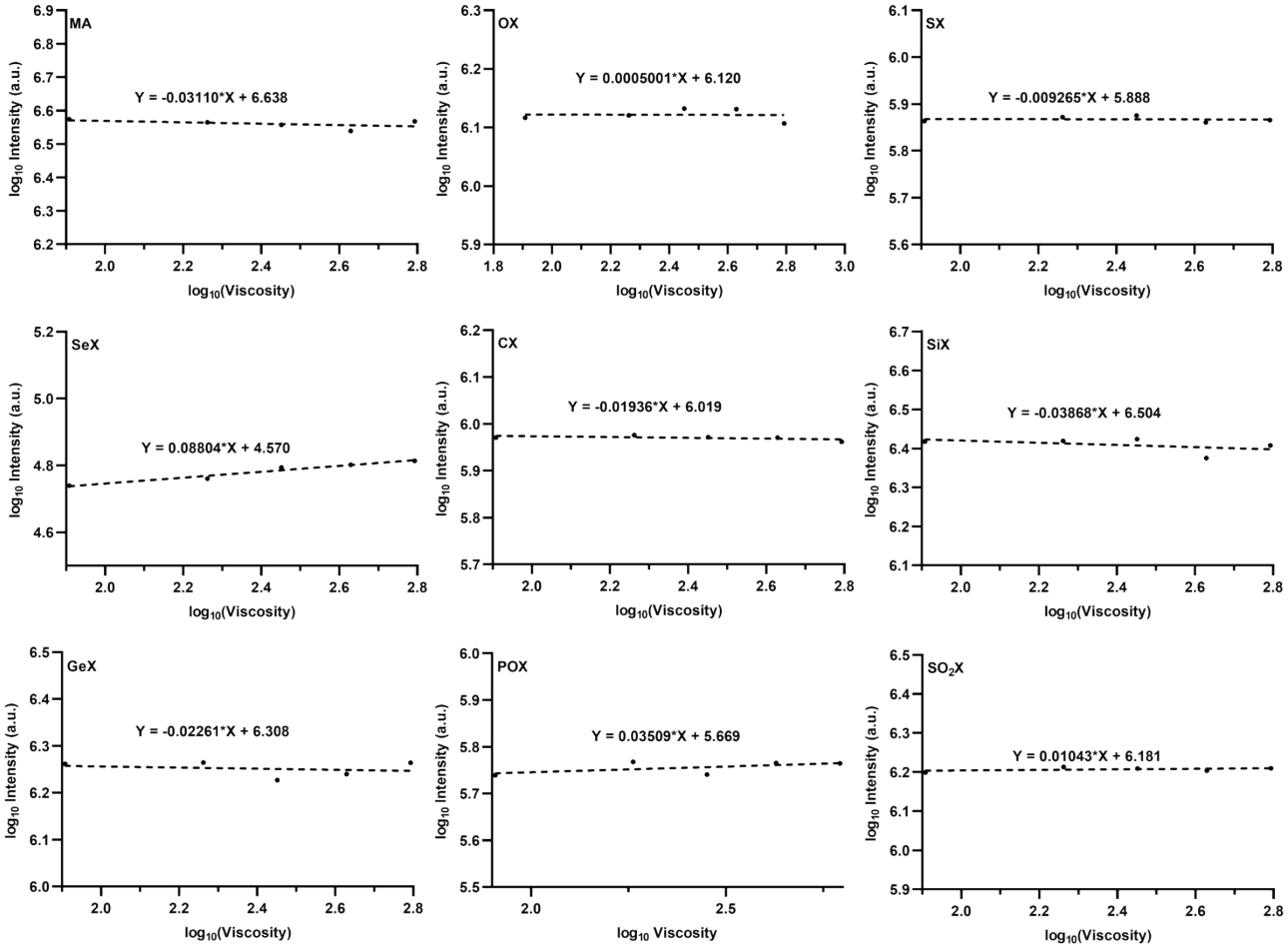


Figure S21. Logarithmic plot of fluorescent intensity as a function of solvent viscosity for heteroatom xanthone probes: Fluorescence measurement was carried out using 20 μM compounds in a series of ethylene glycol/glycerol (EG/G) solutions in the following mixing ratios: EG/G = 70/30 (81 cP), 50/50 (183 cP), 40/60 (283 cP), 30/70 (426 cP), 20/80 (621 cP).

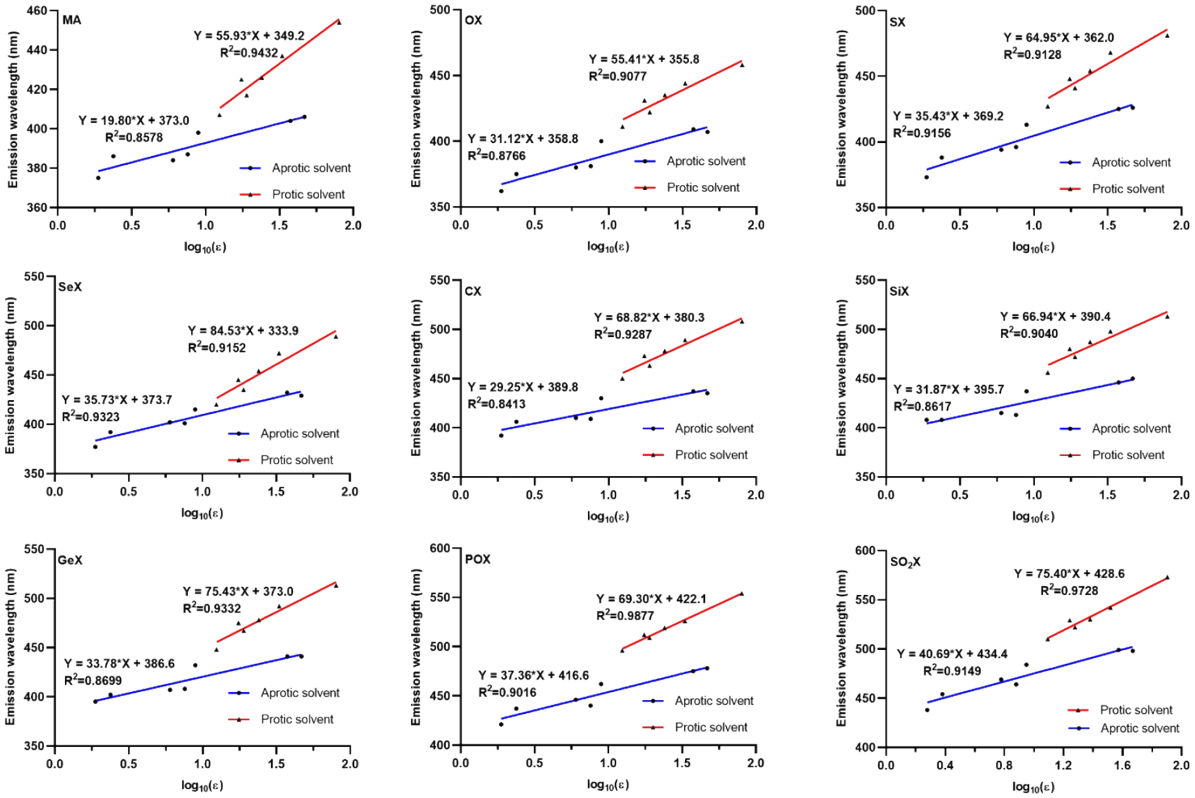


Figure S22. Polarity dependence of heteroatom xanthone probes. The polarity sensitivity was quantified via the slope of the linear correlation between emission maximum in different solvents (10 μM) and $\log_{10}$ (dielectric constant ε). Aprotic solvents: hexane (ε = 1.88), toluene (ε = 2.38), ethyl acetate (ε = 6.02), THF (ε = 7.58), DCM (ε = 8.93), acetonitrile (ε = 37.5), DMSO (ε = 46.68); Protic solvents: *t*-BuOH (ε = 12.4), *n*-BuOH (ε = 17.5), 2-PrOH (ε = 17.9), EtOH (ε = 24.0), MeOH (ε = 32.7), Water (ε = 80.1).

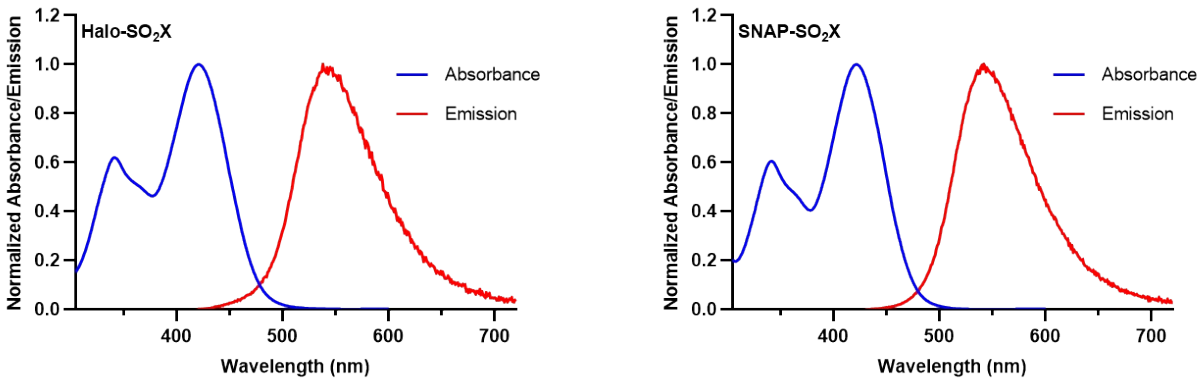


Figure S23. Normalized absorption and emission spectra of **SNAP-SO_2X** and **Halo-SO_2X** in methanol**.**

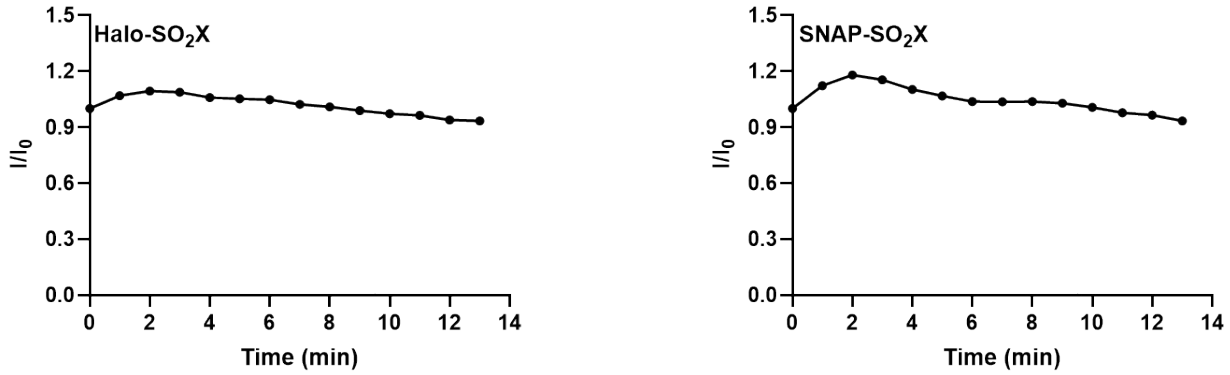


Figure S24. Photostability of **Halo-SO_2X** and **SNAP-SO_2X** (10 µM in pH 7.4 DMSO/PBS buffer (V/V 90/10) taken at the indicated light irradiation times (405 nm, 70.0 µW cm^{-2}).

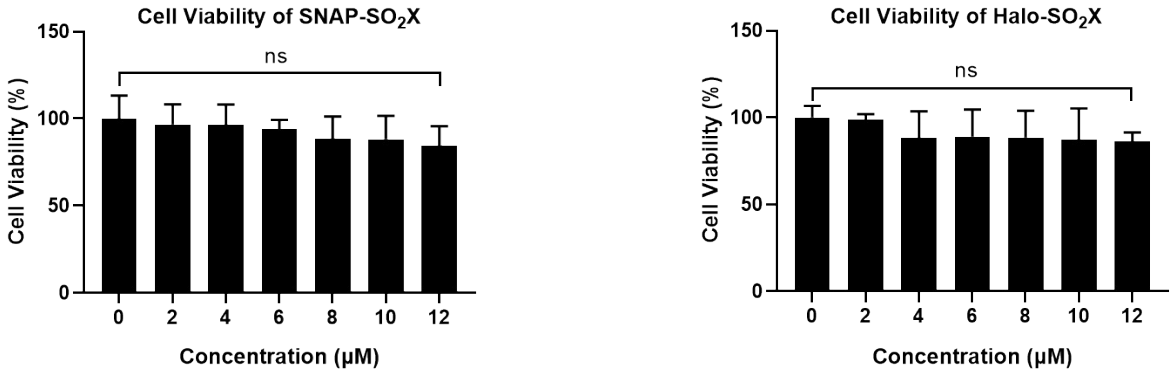


Figure S25. Cell viabilities of **Halo-SO_2X** and **SNAP-SO_2X** using HeLa cells with an increased concentration.

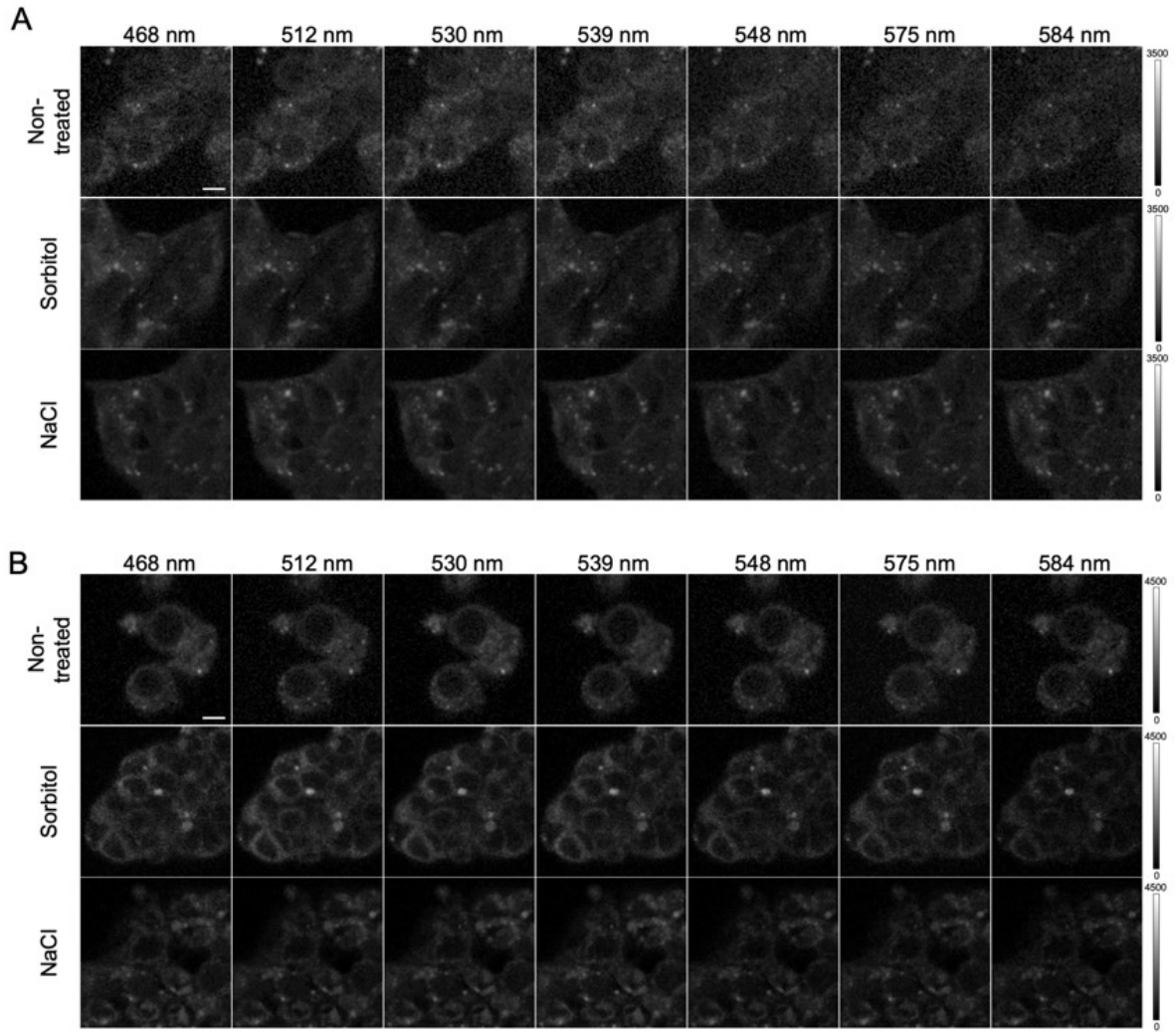


Figure S26. Emission spectrum for (A) Cellular autofluorescence caused by stress in the absence of SO_2X probes; (B) Non-specific signal caused by free SO_2X probe inside the cell.

Table S1. Photophysical properties of **MA** in different solvents

Solvent (Dielectric constant)	λ_{abs} **(nm)**	ε **(M^{-1} cm^{-1})**	λ_{em} **(nm)**
Hexane (1.88)	357	25200	378
Toluene (2.38)	367	30300	385
Ethyl Acetate (6.02)	367	30820	384
Tetrahydrofuran (7.58)	367	31213	384
Dichloromethane (8.93)	377	29834	398
Acetonitrile (37.5)	377	17555	405
DMSO (46.68)	380	29775	405
t-BuOH (12.4)	376	21111	407
n-BuOH (17.5)	384	18730	424
2-PrOH (17.9)	380	19802	418
EtOH (24.55)	385	18666	426
MeOH (32.7)	393	18662	437
Water (80.1)	406	17502	454

Table S2. Photophysical properties of **OX** in different solvents

Solvent (Dielectric constant)	**λ_{abs} (nm)**	**ε (M^{-1} cm^{-1})**	**λ_{em} (nm)**
Hexane (1.88)	344	31617	362
Toluene (2.38)	358	39164	375
Ethyl Acetate (6.02)	360	41709	384
Tetrahydrofuran (7.58)	360	40504	382
Dichloromethane (8.93)	372	47351	400
Acetonitrile (37.5)	370	36502	408
DMSO (46.68)	376	38916	408
t-BuOH (12.4)	370	41198	411
n-BuOH (17.5)	376	38524	431
2-PrOH (17.9)	376	35958	425
EtOH (24.55)	380	44219	435
MeOH (32.7)	384	33747	444
Water (80.1)	404	24811	458

Table S3. Photophysical properties of **SX** in different solvents

Solvent (Dielectric constant)	λ_{abs} **(nm)**	ε **(M^{-1} cm^{-1})**	λ_{em} **(nm)**
Hexane (1.88)	350	37878	373
Toluene (2.38)	364	41377	388
Ethyl Acetate (6.02)	366	41517	394
Tetrahydrofuran (7.58)	366	48335	395
Dichloromethane (8.93)	378	42141	412
Acetonitrile (37.5)	376	41629	397
DMSO (46.68)	382	47690	426
t-BuOH (12.4)	376	42728	427
n-BuOH (17.5)	382	39310	448
2-PrOH (17.9)	382	39953	442
EtOH (24.55)	386	34498	454
MeOH (32.7)	392	36049	468
Water (80.1)	406	25664	481

Table S4. Photophysical properties of **SeX** in different solvents

Solvent (Dielectric constant)	λ_{abs} **(nm)**	ε **(M^{-1} cm^{-1})**	λ_{em} **(nm)**
Hexane (1.88)	354	39800	377
Toluene (2.38)	368	41001	392
Ethyl Acetate (6.02)	370	36803	399
Tetrahydrofuran (7.58)	370	37199	401
Dichloromethane (8.93)	380	44120	415
Acetonitrile (37.5)	380	38949	432
DMSO (46.68)	386	47674	429
t-BuOH (12.4)	380	38928	413
n-BuOH (17.5)	384	37390	444
2-PrOH (17.9)	386	45431	436
EtOH (24.55)	390	34812	454
MeOH (32.7)	394	36760	472
Water (80.1)	402	22266	489

Table S5. Photophysical properties of **CX** in different solvents

Solvent (Dielectric constant)	**λ_{abs} (nm)**	**ε (M^{-1} cm^{-1})**	**λ_{em} (nm)**
Hexane (1.88)	354	42688	392
Toluene (2.38)	368	38946	404
Ethyl Acetate (6.02)	370	40787	409
Tetrahydrofuran (7.58)	368	49048	407
Dichloromethane (8.93)	382	47584	430
Acetonitrile (37.5)	380	46486	436
DMSO (46.68)	386	40360	434
t-BuOH (12.4)	382	41720	450
n-BuOH (17.5)	386	40716	472
2-PrOH (17.9)	386	39252	464
EtOH (24.55)	392	31755	477
MeOH (32.7)	396	37115	489
Water (80.1)	418	29783	508

Table S6. Photophysical properties of **SiX** in different solvents

Solvent (Dielectric constant)	**λ_{abs} (nm)**	**ε (M^{-1} cm^{-1})**	**λ_{em} (nm)**
Hexane (1.88)	364	38475	410
Toluene (2.38)	380	31543	409
Ethyl Acetate (6.02)	380	34516	415
Tetrahydrofuran (7.58)	382	37196	413
Dichloromethane (8.93)	392	34310	437
Acetonitrile (37.5)	392	35269	446
DMSO (46.68)	398	37275	446
t-BuOH (12.4)	392	35861	456
n-BuOH (17.5)	398	33361	479
2-PrOH (17.9)	398	39181	472
EtOH (24.55)	402	30617	487
MeOH (32.7)	406	31036	498
Water (80.1)	410	20429	513

Table S7. Photophysical properties of **GeX** in different solvents

Solvent (Dielectric constant)	**λ_{abs} (nm)**	**ε (M^{-1} cm^{-1})**	**λ_{em} (nm)**
Hexane (1.88)	360	29834	410
Toluene (2.38)	376	26739	409
Ethyl Acetate (6.02)	378	24734	415
Tetrahydrofuran (7.58)	378	31635	413
Dichloromethane (8.93)	390	26982	437
Acetonitrile (37.5)	388	27642	446
DMSO (46.68)	394	28190	446
t-BuOH (12.4)	390	30471	456
n-BuOH (17.5)	394	24859	479
2-PrOH (17.9)	394	22110	472
EtOH (24.55)	398	28138	487
MeOH (32.7)	402	21231	498
Water (80.1)	406	14680	513

Table S8. Photophysical properties of **POX** in different solvents

Solvent (Dielectric constant)	**λ_{abs} (nm)**	**ε (M^{-1} cm^{-1})**	**λ_{em} (nm)**
Hexane (1.88)	336, 390	20486, 32294	421
Toluene (2.38)	346, 406	23609, 35240	437
Ethyl Acetate (6.02)	343, 405	25321, 35580	446
Tetrahydrofuran (7.58)	343, 406	25321, 34540	440
Dichloromethane (8.93)	347, 416	25635, 34410	462
Acetonitrile (37.5)	346, 417	25216, 35590	475
DMSO (46.68)	350, 422	23588, 33370	478
t-BuOH (12.4)	350, 424	22304, 36084	495
n-BuOH (17.5)	352, 426	28478, 32994	511
2-PrOH (17.9)	352, 426	20766, 30060	508
EtOH (24.55)	347, 426	27043, 33960	519
MeOH (32.7)	347, 428	26973, 33450	526
Water (80.1)	354, 456	16603, 27890	554

Table S9. Photophysical properties of **SO_2X** in different solvents

Solvent (Dielectric constant)	**λ_{abs} (nm)**	**ε [a] (M^{-1} cm^{-1})**	**λ_{em} (nm)**	**Φ_f [b]**
Hexane (1.88)	334, 392	8850, 14800	436	0.03
Toluene (2.38)	350, 412	9100, 15684	458	0.06
Ethyl Acetate (6.02)	346, 412	9150, 15526	470	0.04
Tetrahydrofuran (7.58)	350, 412	10364, 20750	471	0.04
Dichloromethane (8.93)	352, 424	13802, 19634	488	0.05
Acetonitrile (37.5)	350, 424	11020, 17179	502	0.04
DMSO (46.68)	356, 432	9074, 15450	507	0.06
t-BuOH (12.4)	341, 424	9400, 15943	510	0.07
n-BuOH (17.5)	348, 424	9524, 15167	529	0.12
2-PrOH (17.9)	350, 424	12537, 17161	522	0.12
EtOH (24.55)	352, 426	8107, 13724	530	0.11
MeOH (32.7)	352, 430	8300, 13100	542	0.13
Water (80.1)	358, 462	7593, 11627	573	0.03

[a] ε: extinction coefficients.; [b] Quantum yields were measured using quinine sulfate in 0.5 M H_2SO_4 as the reference.

Table S10. Photophysical Data of SO_2X-targeting probes in methanol

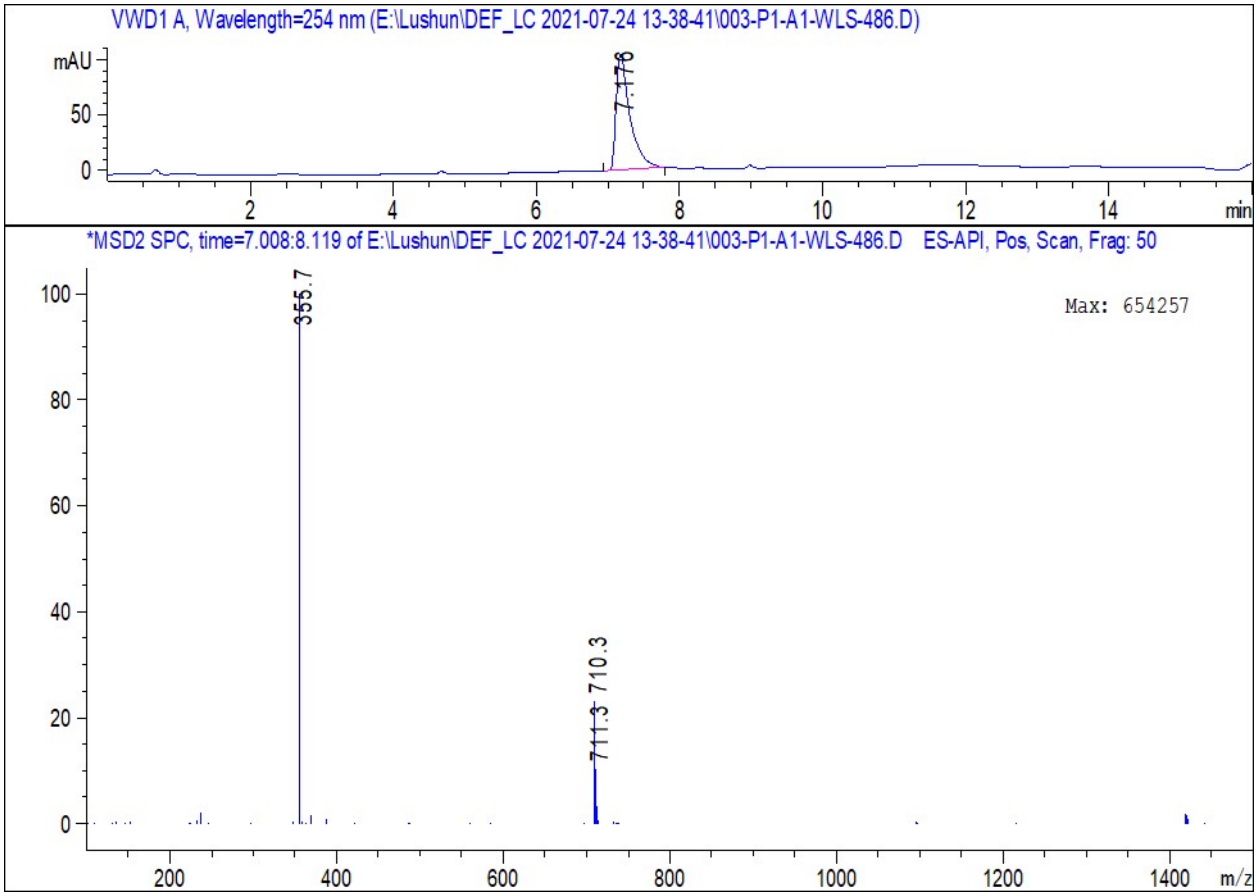
Compounds	**λ_{abs} (nm)**	**ε [a] (M^{-1} cm^{-1})**	**λ_{em} (nm)**	**Φ_f [b]**
SNAP-SO_2X	342, 422	8550, 26138	542	0.10
Halo-SO_2X	346, 421	6300, 13370	542	0.09

[a] ε: extinction coefficients.; [b] Quantum yields were measured using quinine sulfate in 0.5 M H_2SO_4 as the reference.

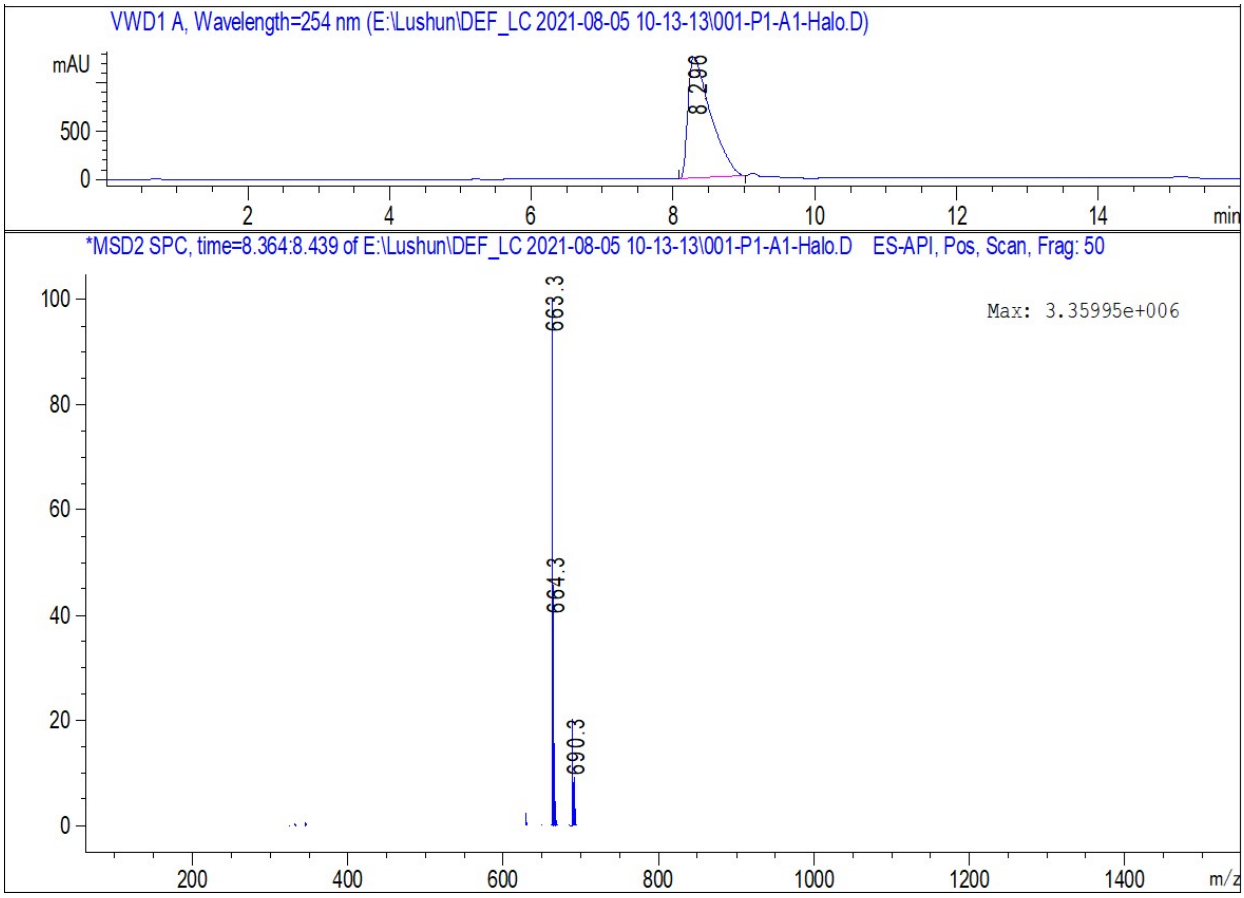
4. Characterization spectra

4.1 LC-MS spectra

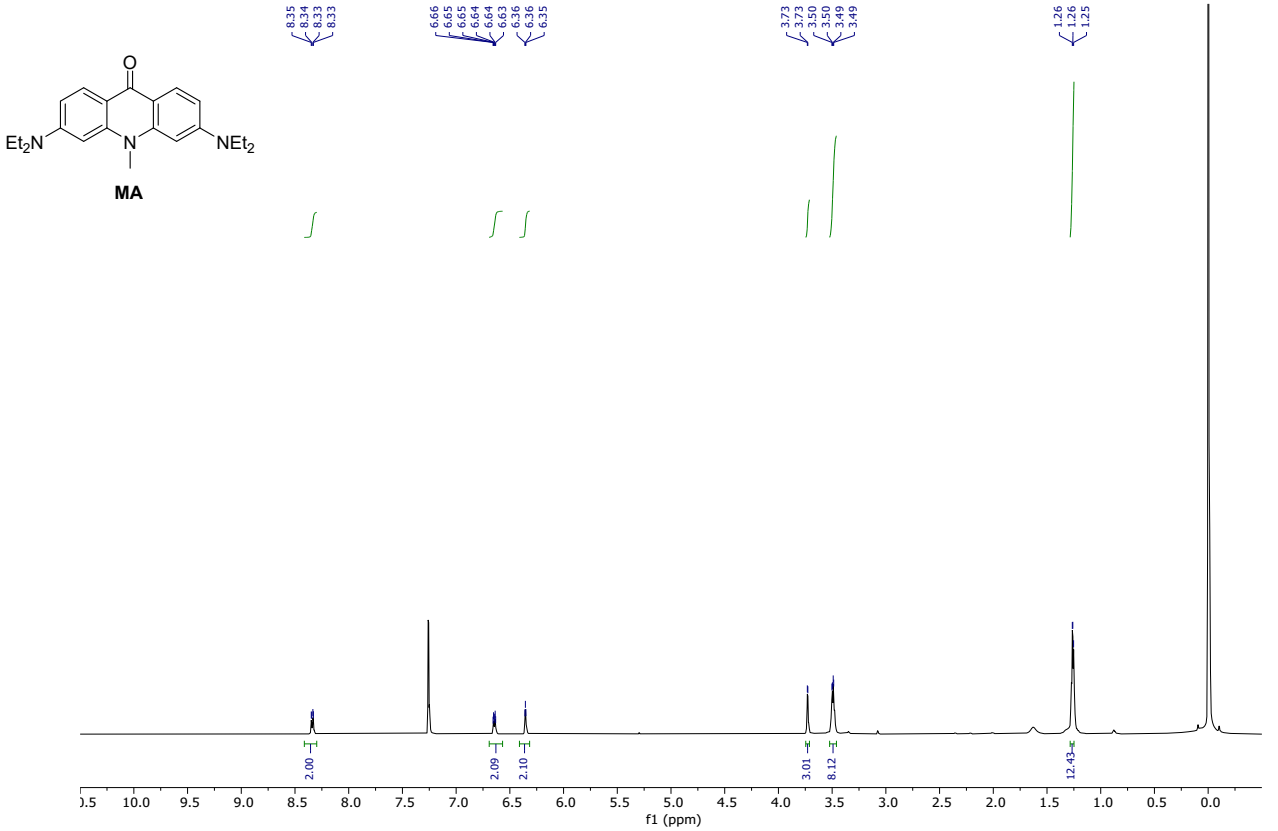
LC-MS spectrum of **SNAP-SO_2X**

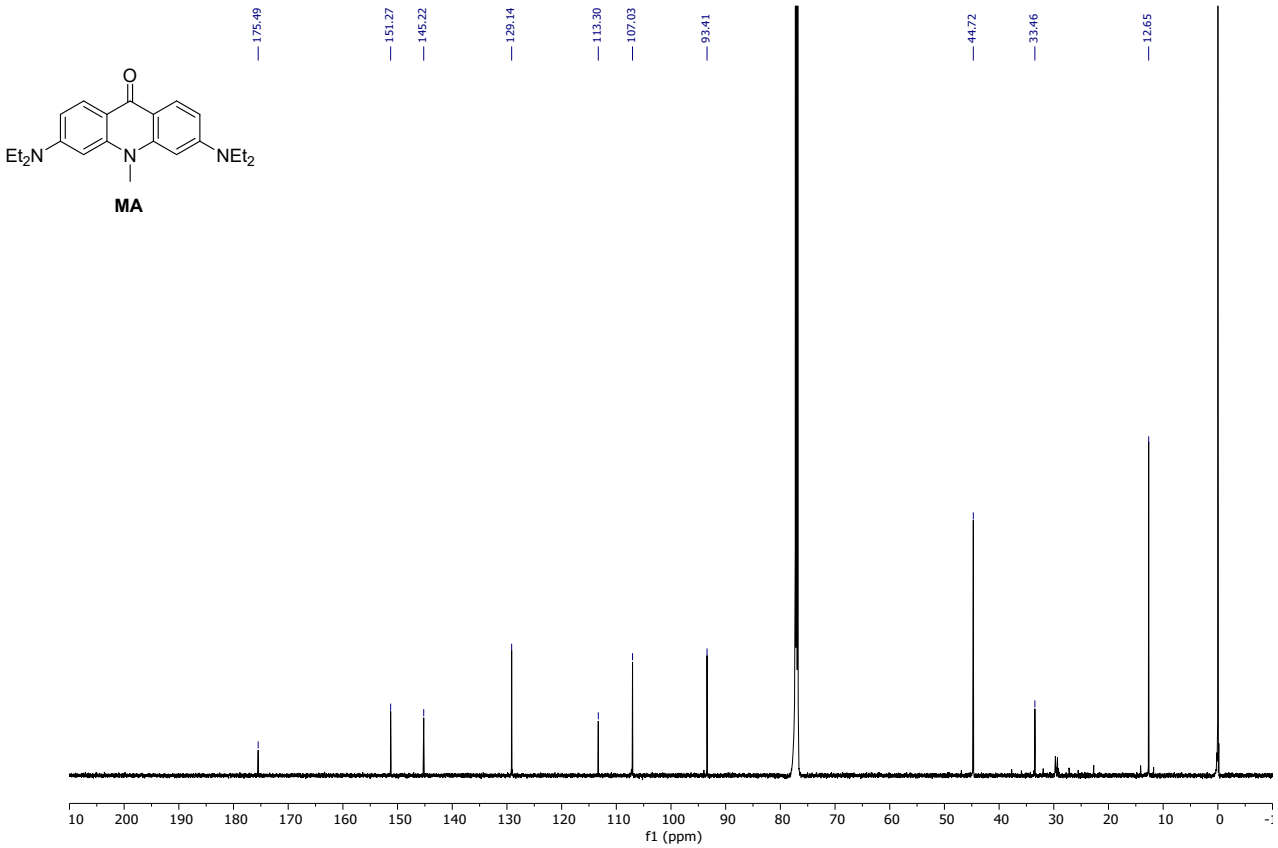


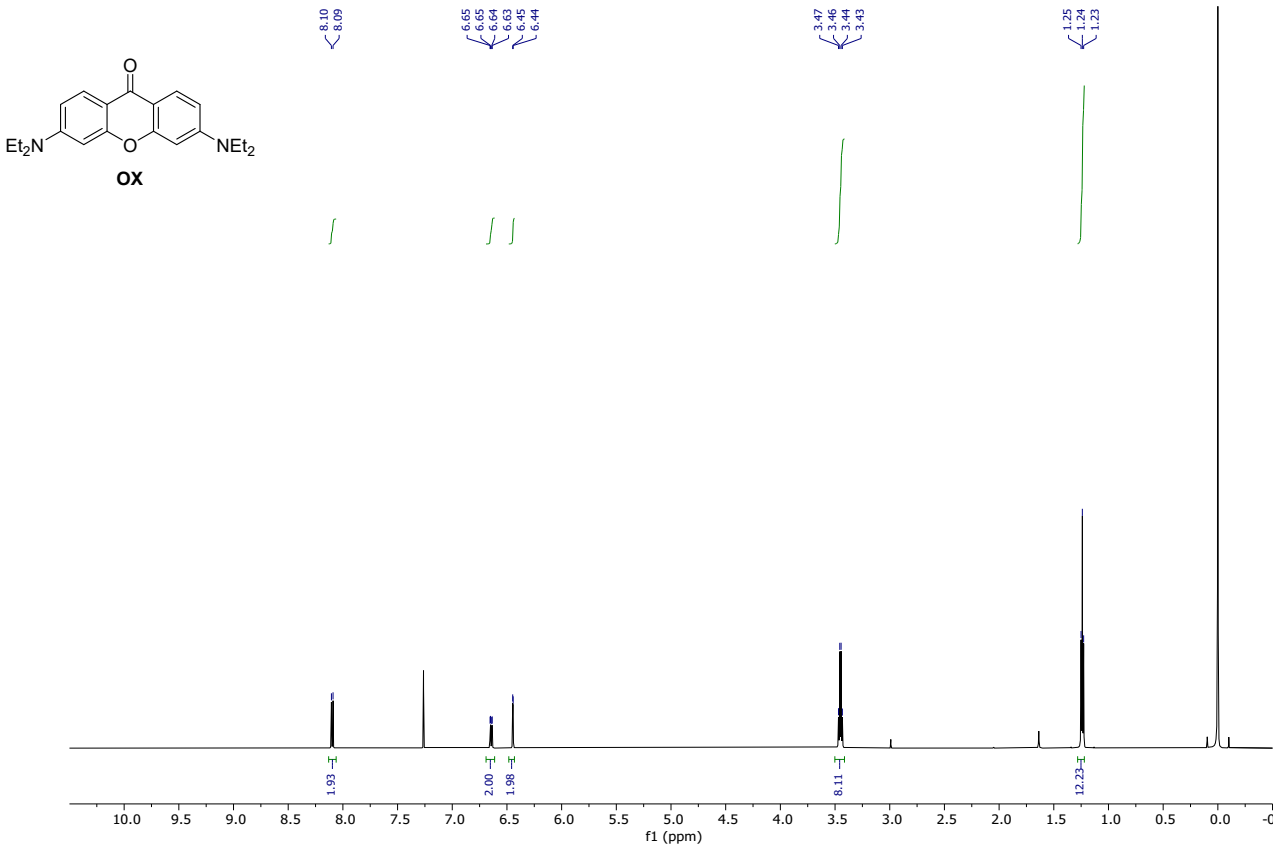
LC-MS spectrum of **Halo-SO_2X**

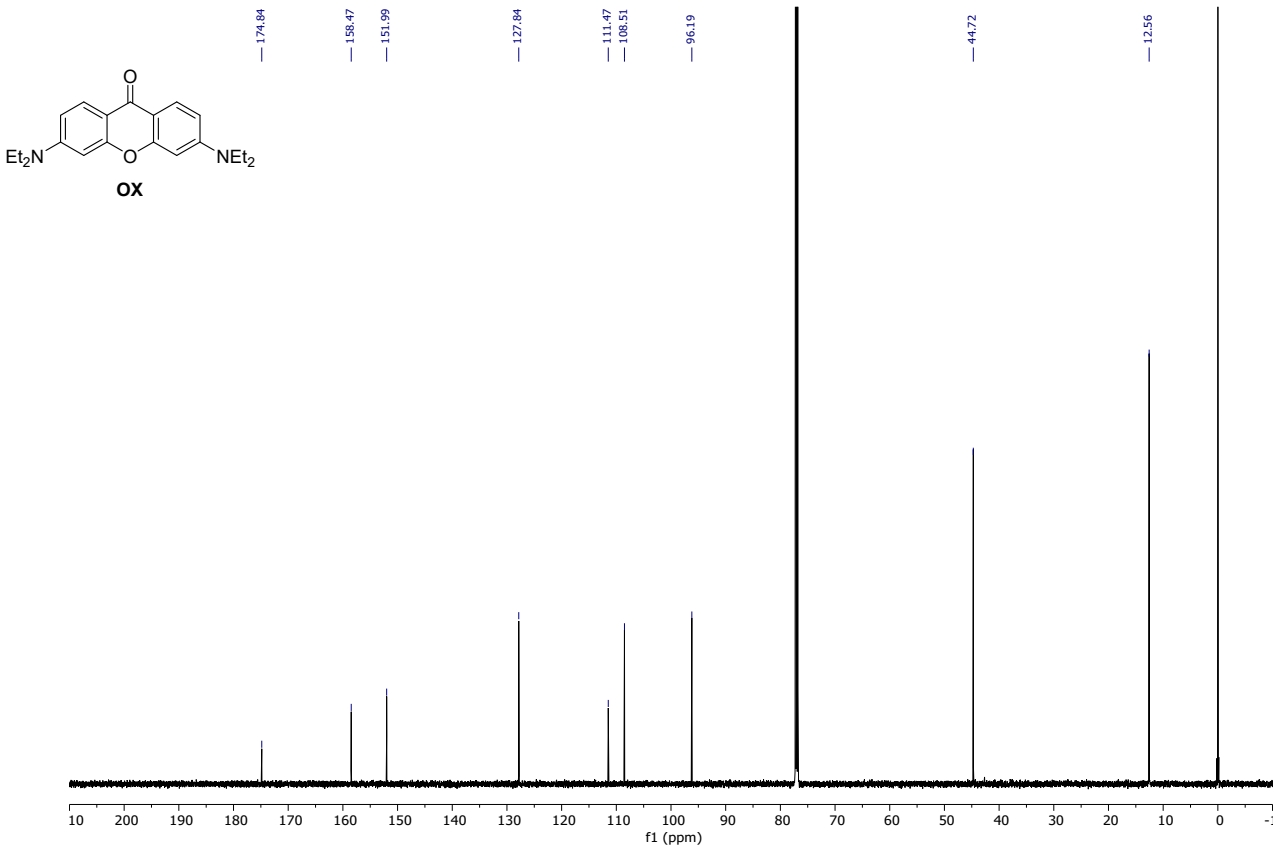


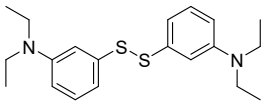
4.2 NMR spectra





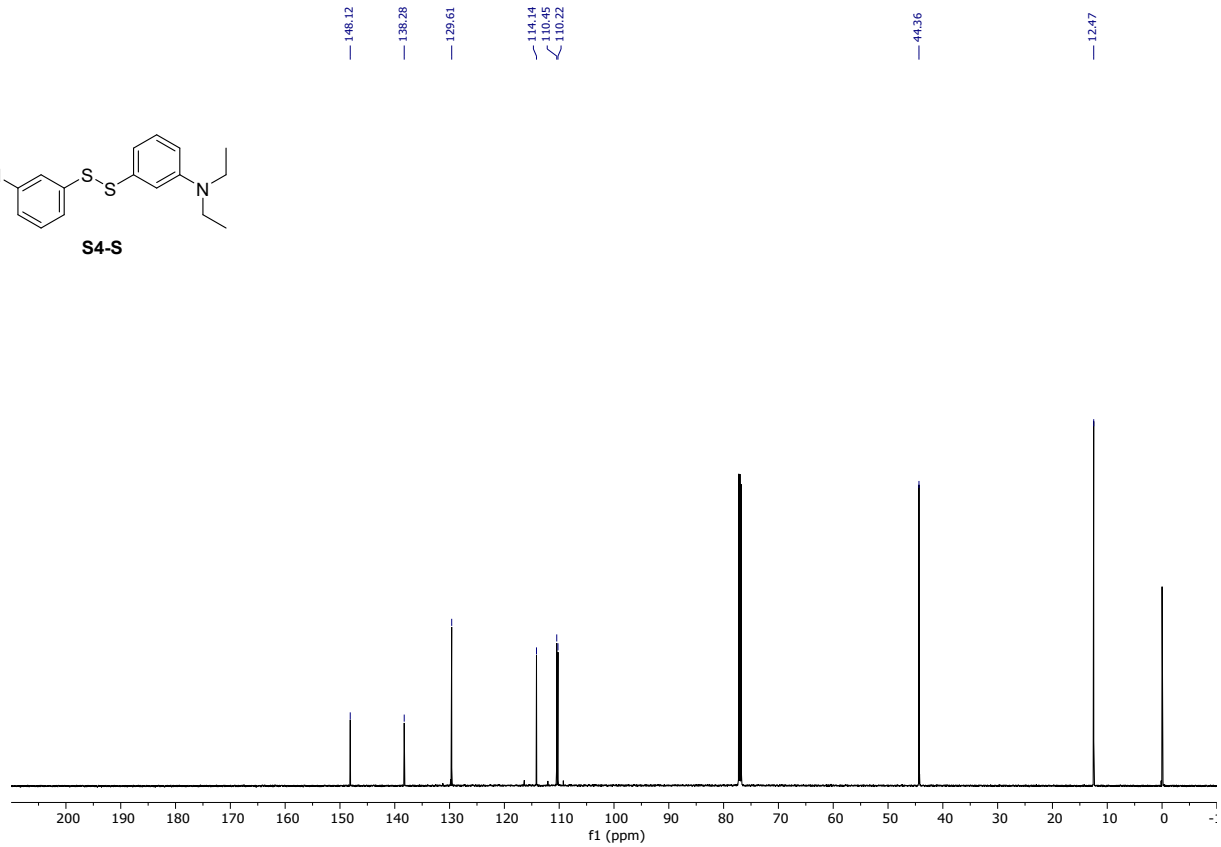

8.10
8.09
6.65
6.65
6.64
6.63
6.45
6.44
3.47
3.46
3.44
3.43
1.25
1.24
1.23
O
Et2N
O
NEt2
OX
1.93
2.00
1.98
8.11
12.23
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


174.84
158.47
151.99
127.84
111.47
108.51
96.19
44.72
12.56
O
Et2N
O
NEt2
OX
10
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

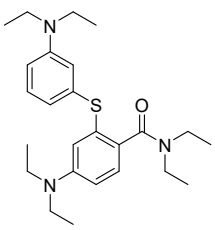


S4-S

7.26
7.12
7.11
7.10
6.82
6.82
6.82
6.80
6.80
6.79
6.78
6.52
6.52
6.51
6.50
3.32
3.30
3.29
3.28
1.11
1.10
1.09
2.00
1.99
1.99
2.05
8.92
12.06
f1 (ppm)

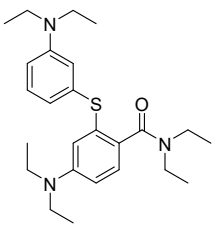

148.12
138.28
129.61
114.14
110.45
110.22
44.36
12.47
f1 (ppm)

S4-S



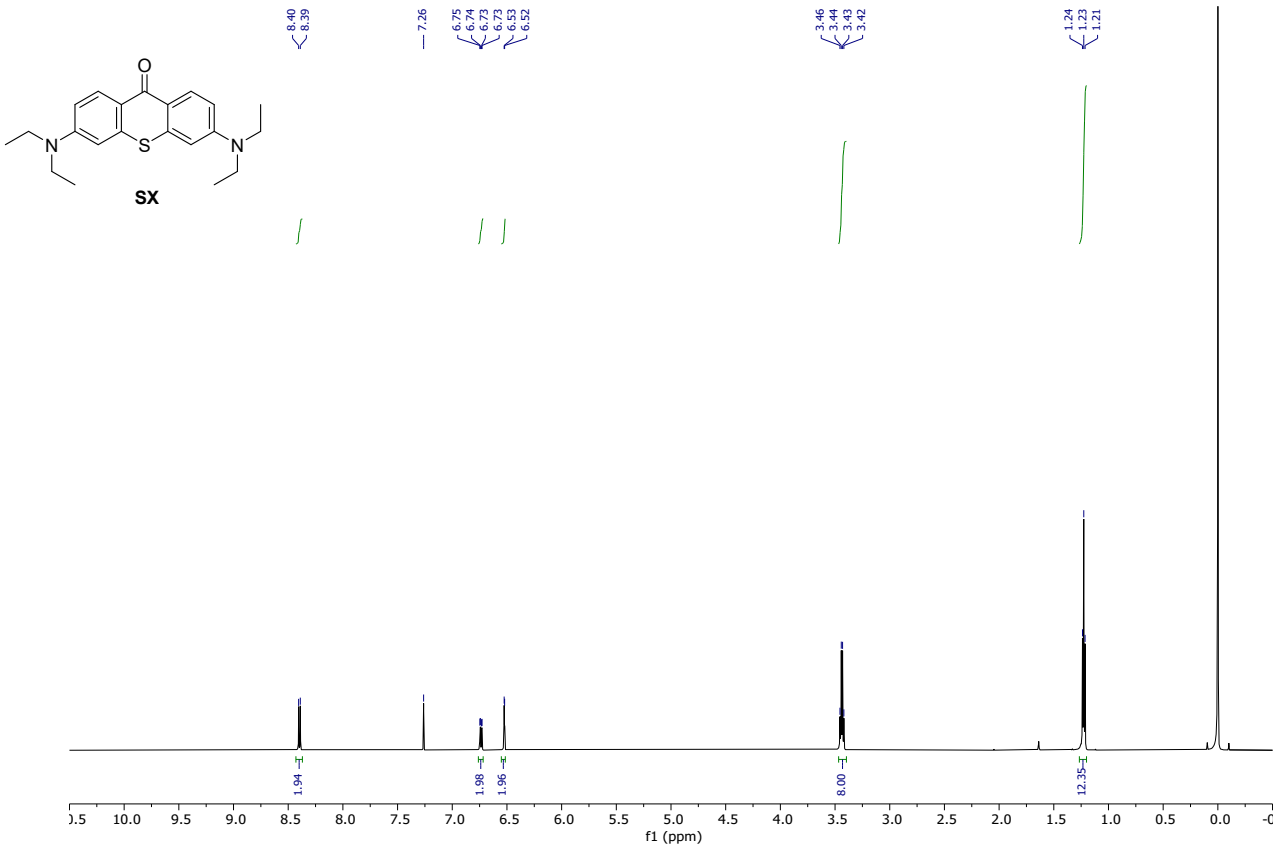
S6-S

7.13
7.12
7.10
7.06
7.05
6.76
6.75
6.75
6.68
6.68
6.67
6.67
6.66
6.57
6.56
6.55
6.55
6.48
6.48
6.47
6.46
6.40
6.40
3.54
3.44
3.43
3.41
3.40
3.32
3.31
3.30
3.28
3.25
3.22
3.21
3.20
3.19
1.26
1.25
1.22
1.21
1.21
1.20
1.19
1.18
1.17
1.16
1.14
1.12
1.10
1.09
1.07
1.03
1.02
1.01
1.00
1.00
0.99
1.01
0.99
0.93
1.03
1.03
12.44
18.69
f1 (ppm)

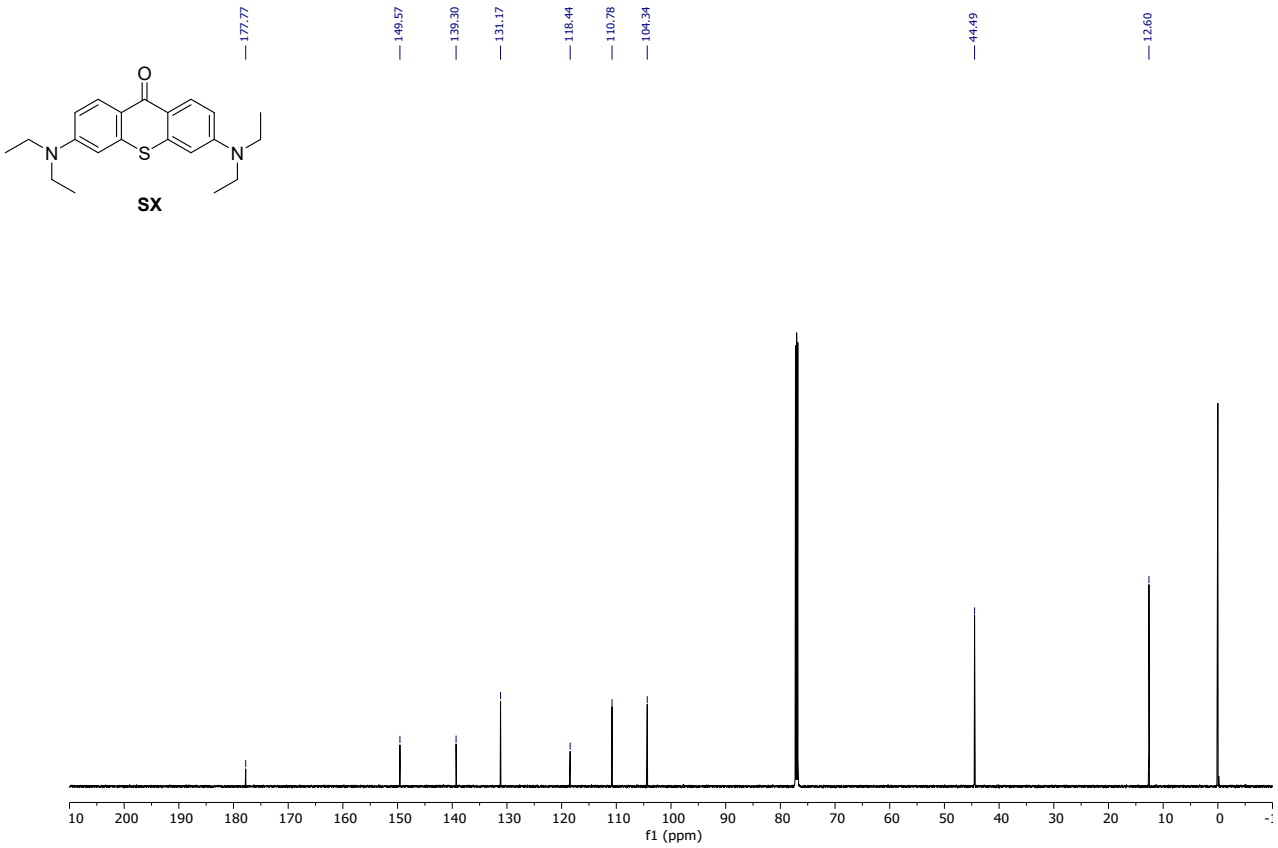


S6-S

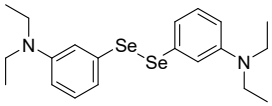
170.17
148.26
147.93
135.17
134.83
129.70
127.61
125.04
119.21
115.63
115.41
110.98
109.58
77.23
77.02
76.81
44.51
44.39
44.32
12.47
12.44
f1 (ppm)


8.40
8.39
7.26
6.75
6.74
6.73
6.73
6.53
6.52
3.46
3.44
3.43
3.42
1.24
1.23
1.21
1.94
1.98
1.96
8.00
12.35
f1 (ppm)

SX

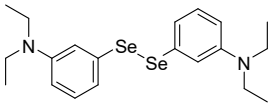

177.77
149.57
139.30
131.17
118.44
110.78
104.34
44.49
12.60
f1 (ppm)

SX


N
Se
Se
N

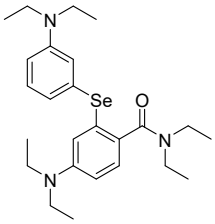
S4-Se

7.26
7.09
7.07
7.07
7.06
6.94
6.93
6.93
6.93
6.91
6.91
6.89
6.89
6.53
6.53
6.52
6.52
3.31
3.30
3.29
3.28
1.12
1.11
1.10
2.09
2.00
2.04
2.00
8.10
12.03
f1 (ppm)


N
Se
Se
N

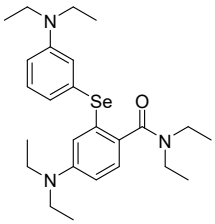
S4-Se

148.23
132.08
129.60
117.72
114.25
110.96
44.34
12.50
f1 (ppm)


N
Se
O
N
N

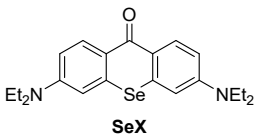
S6-Se

7.26
7.12
7.11
7.10
7.05
7.04
6.93
6.92
6.92
6.85
6.85
6.84
6.83
6.61
6.60
6.60
6.59
6.45
6.44
6.44
6.43
3.42
3.41
3.32
3.31
3.30
3.29
3.19
3.18
3.17
3.15
1.17
1.12
1.11
1.10
1.00
0.98
0.97
1.04
0.99
0.99
0.97
1.00
2.00
3.99
4.09
3.98
5.92
6.03
6.01
f1 (ppm)


N
Se
O
N
N

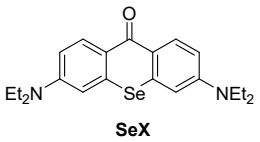
S6-Se

170.80
148.38
148.06
132.34
130.61
129.82
127.50
125.17
122.01
121.98
118.49
114.45
114.42
111.36
109.15
44.40
44.32
13.56
12.48
12.44
f1 (ppm)


O
Et2N
Se
NEt2

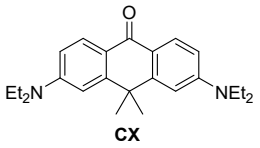
SeX

8.46
8.45
7.26
6.73
6.72
6.71
6.71
6.62
6.61
3.45
3.44
3.42
3.41
1.24
1.22
1.21
2.00
2.15
2.11
8.09
12.03
f1 (ppm)


O
Et2N
Se
NEt2

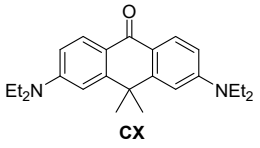
SeX

179.60
149.48
136.77
132.53
119.78
110.93
107.03
44.43
12.62
f1 (ppm)


O
Et2N
NEt2

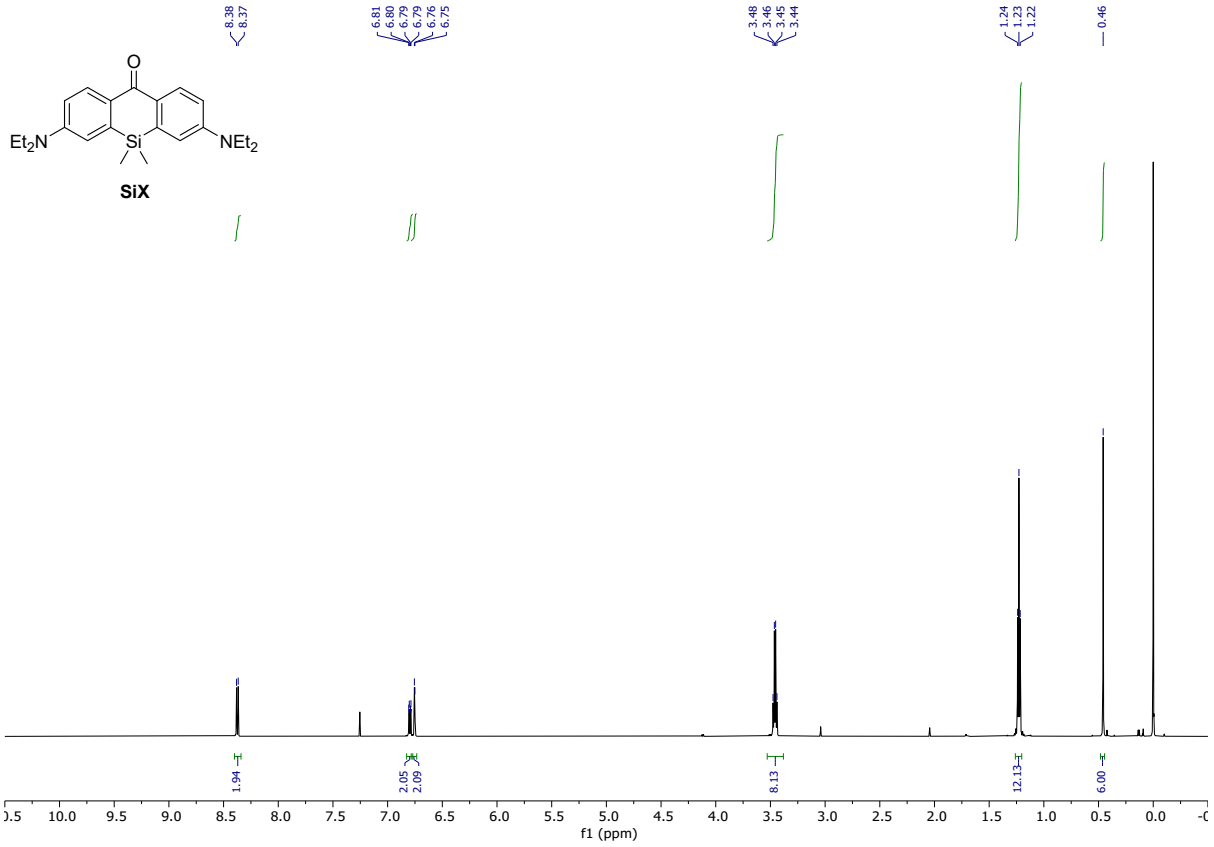
CX

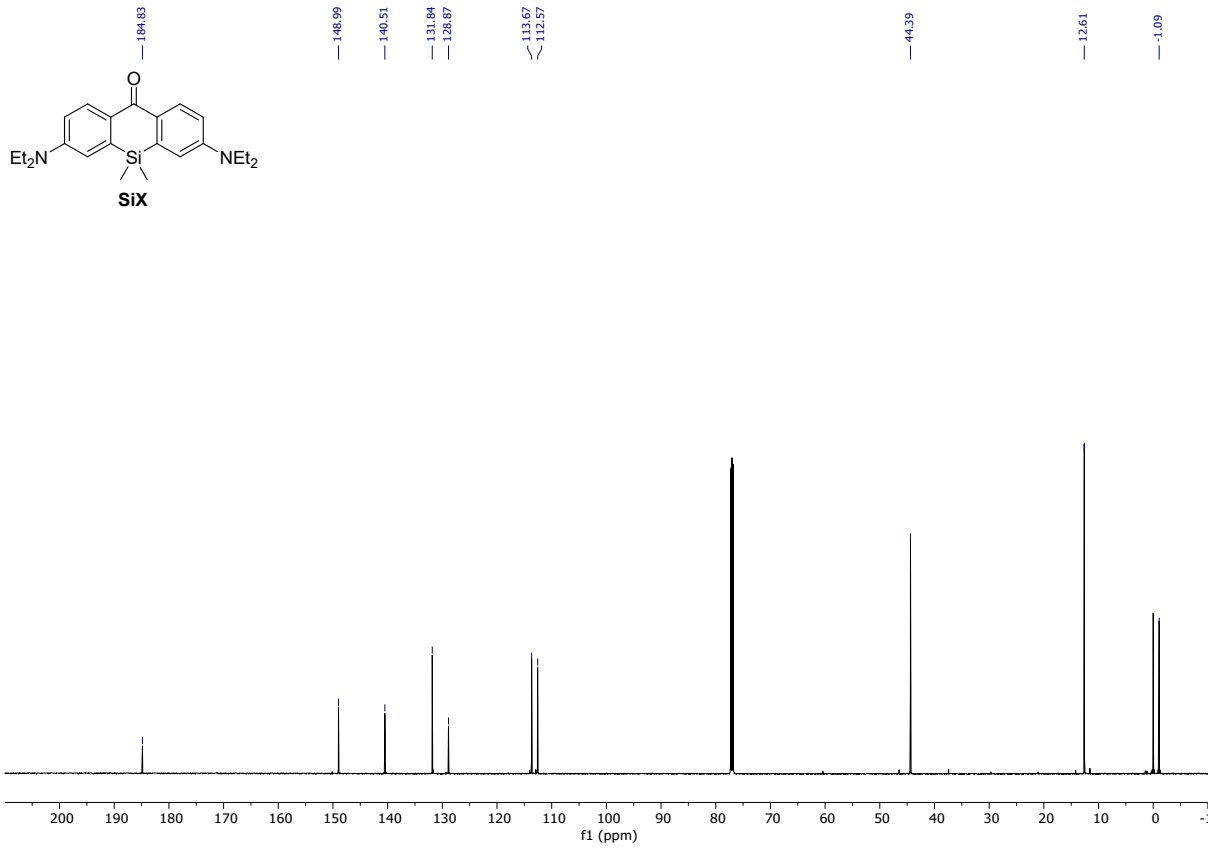
8.24
8.24
8.23
8.23
8.22
6.72
6.72
6.71
6.70
3.49
3.48
3.47
3.46
1.70
1.26
1.25
1.24
1.89
4.04
7.99
6.00
12.41
f1 (ppm)

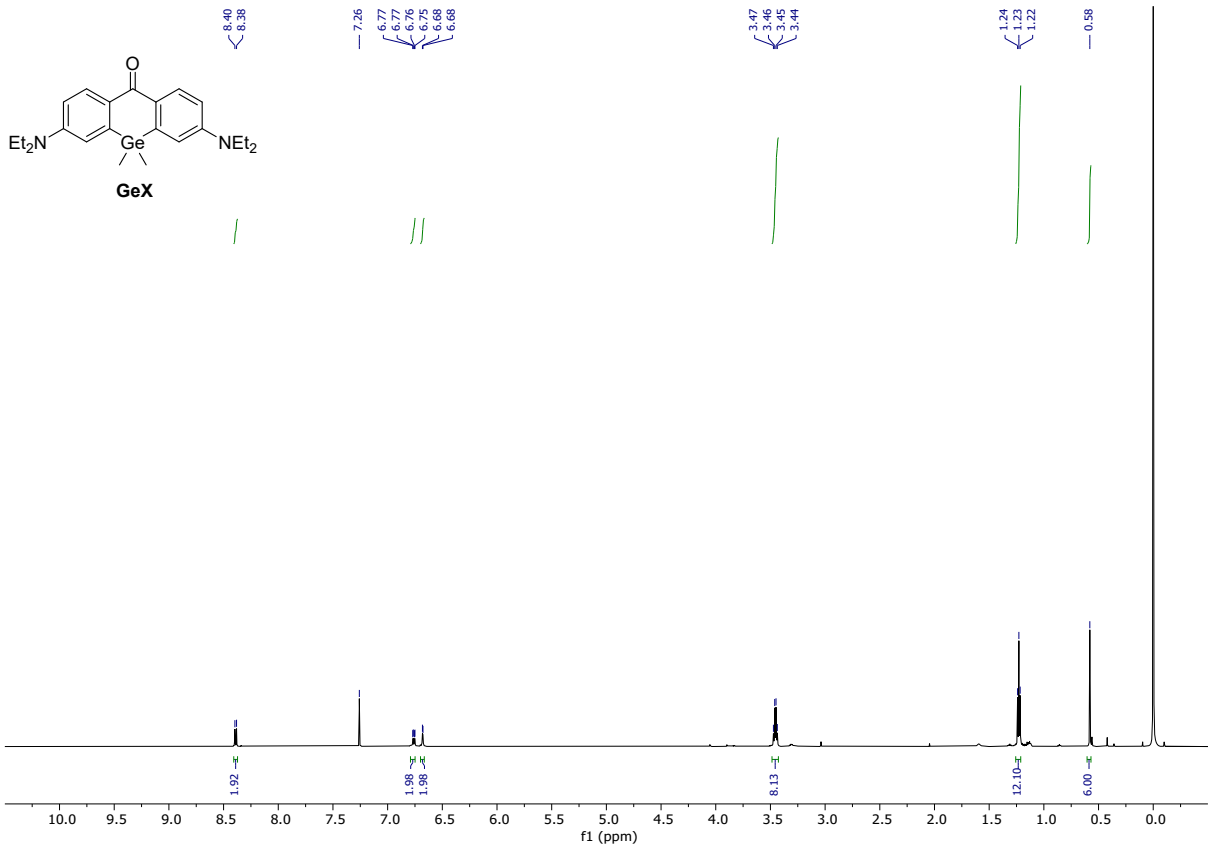

O
Et2N
NEt2

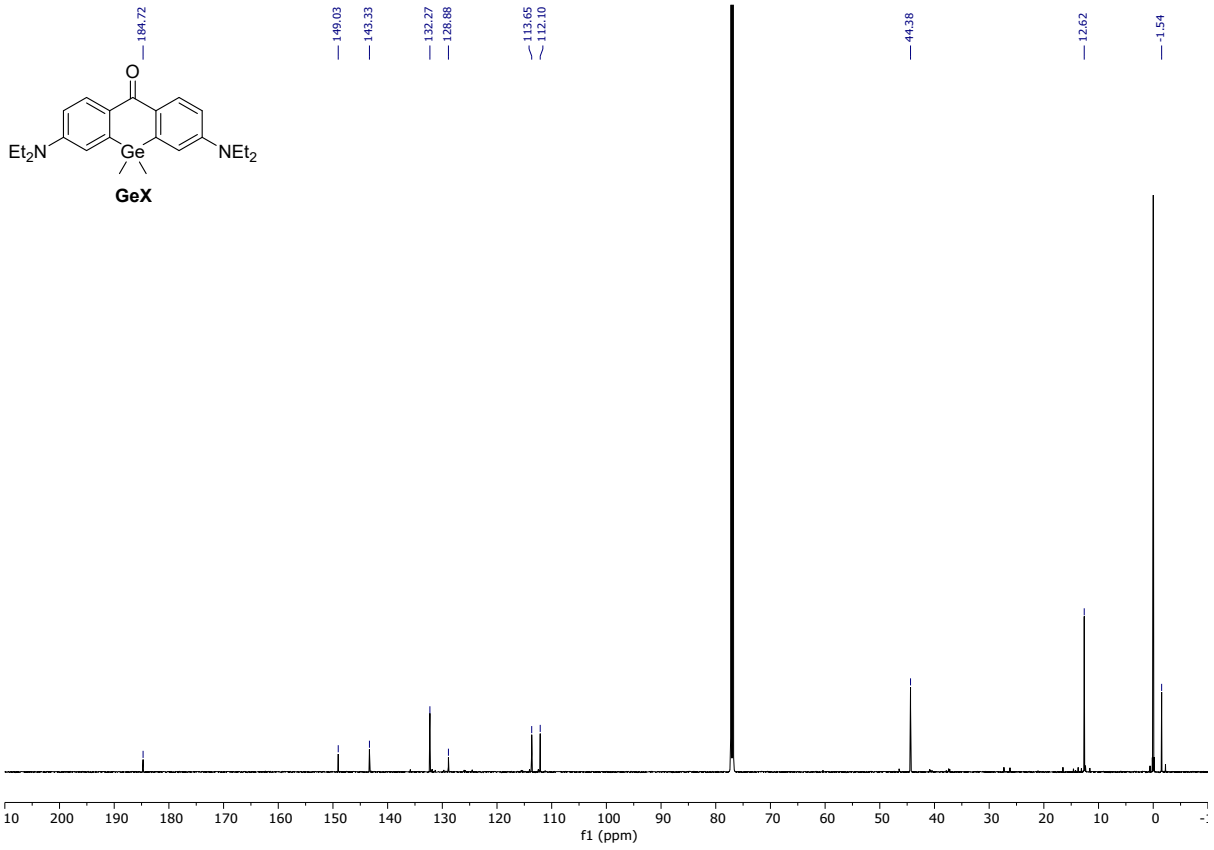
CX

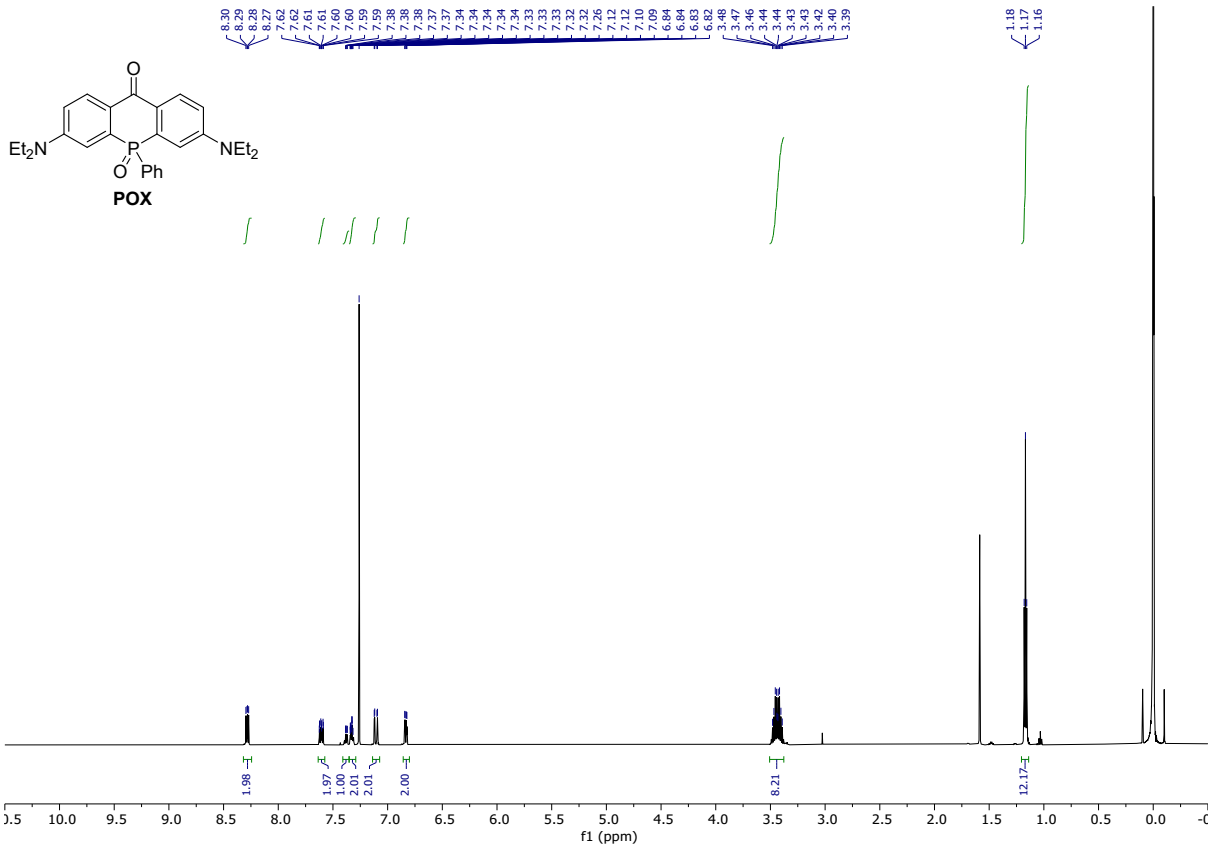
180.81
152.34
150.63
129.36
119.36
110.34
107.26
44.60
37.96
33.62
12.69
f1 (ppm)

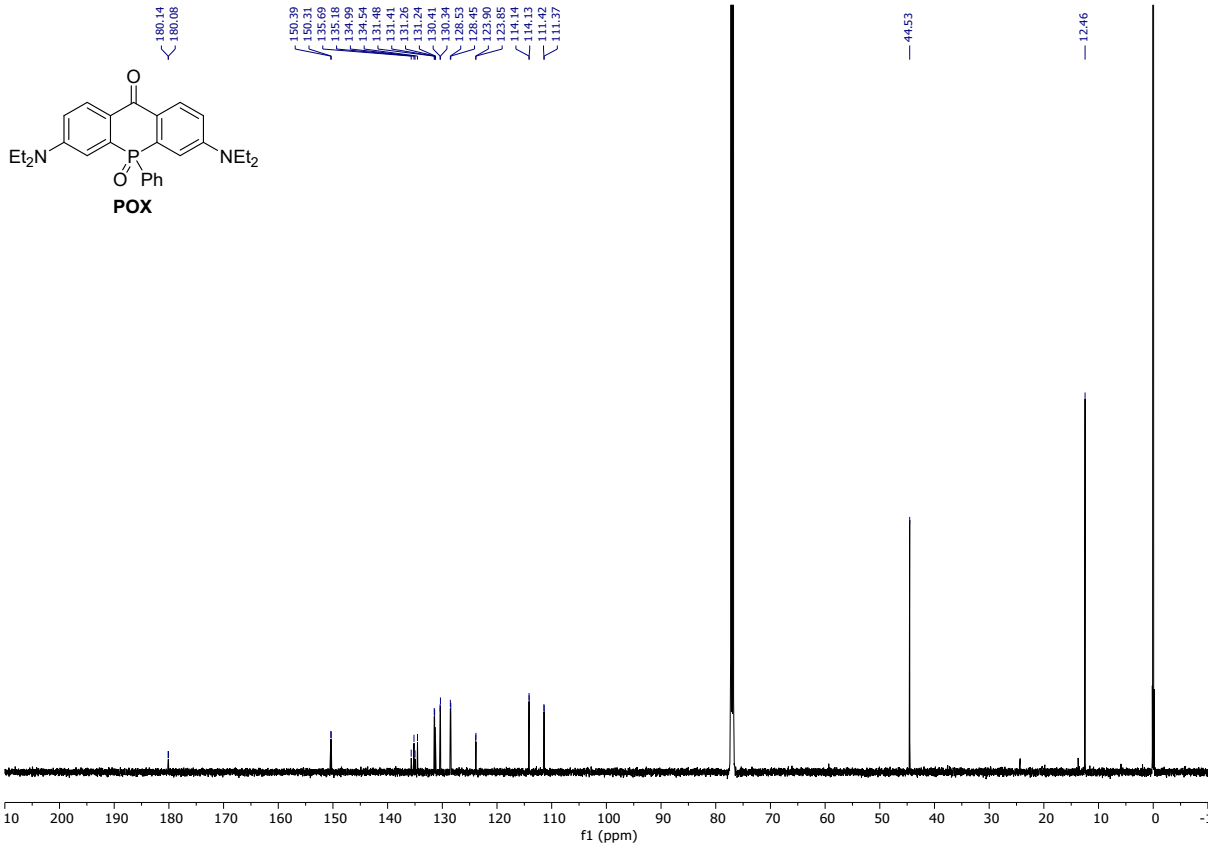

8.38
8.37
6.81
6.80
6.79
6.79
6.76
6.75
3.48
3.46
3.45
3.44
1.24
1.23
1.22
0.46
O
Et2N
Si
NEt2
SiX
1.94
2.05
2.09
8.13
12.13
6.00
f1 (ppm)

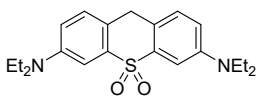

184.83
148.99
140.51
131.84
128.87
113.67
112.57
44.39
12.61
-1.09
O
Et2N
Si
NEt2
SiX
f1 (ppm)


8.40
8.38
7.26
6.77
6.77
6.76
6.75
6.68
6.68
3.47
3.46
3.45
3.44
1.24
1.23
1.22
0.58
O
Et2N
Ge
NEt2
GeX
1.92
1.98
1.98
8.13
12.10
6.00
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


184.72
149.03
143.33
132.27
128.88
113.65
112.10
44.38
12.62
-1.54
O
Et2N
Ge
NEt2
GeX
10 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

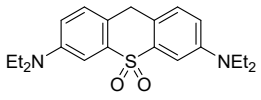

POX
f1 (ppm)


POX
f1 (ppm)


Et2N
NEt2
S
O
O

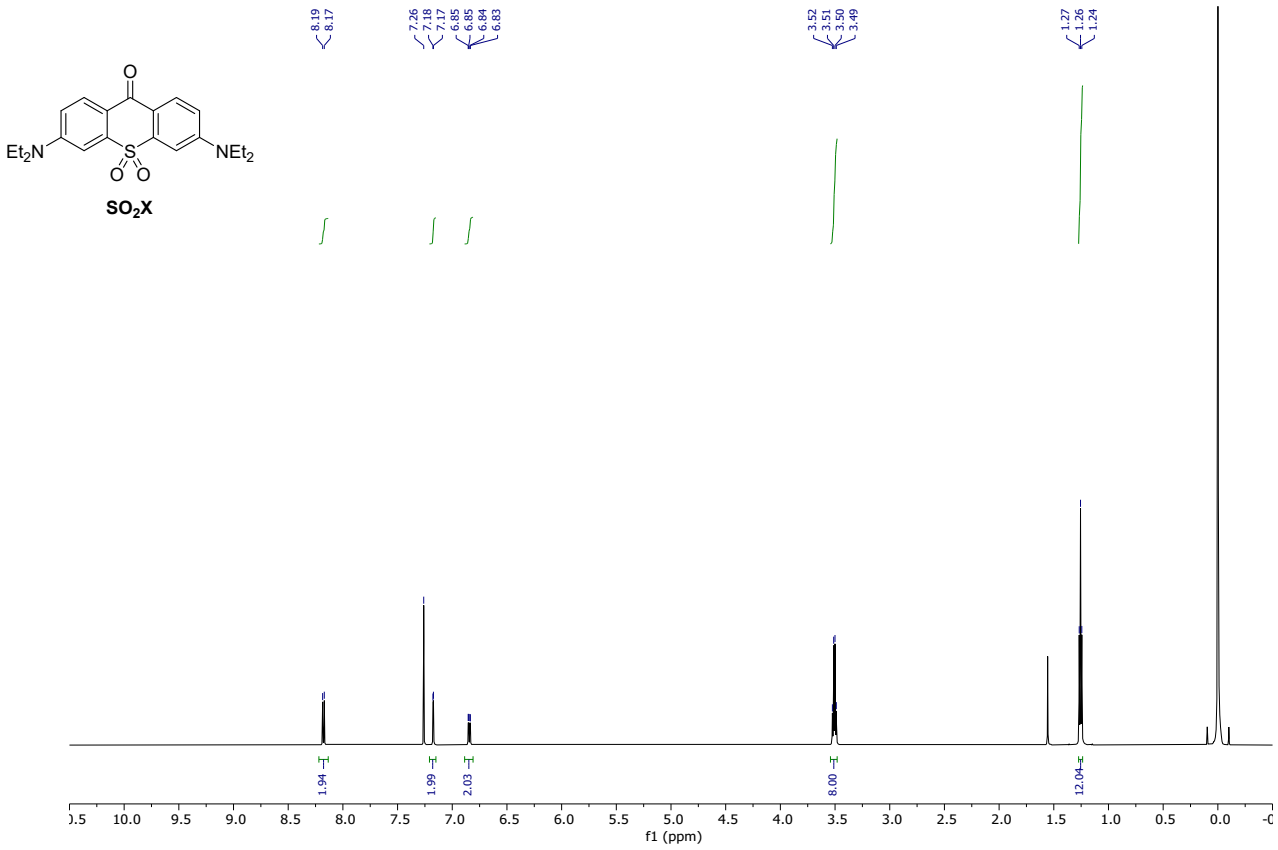
S10

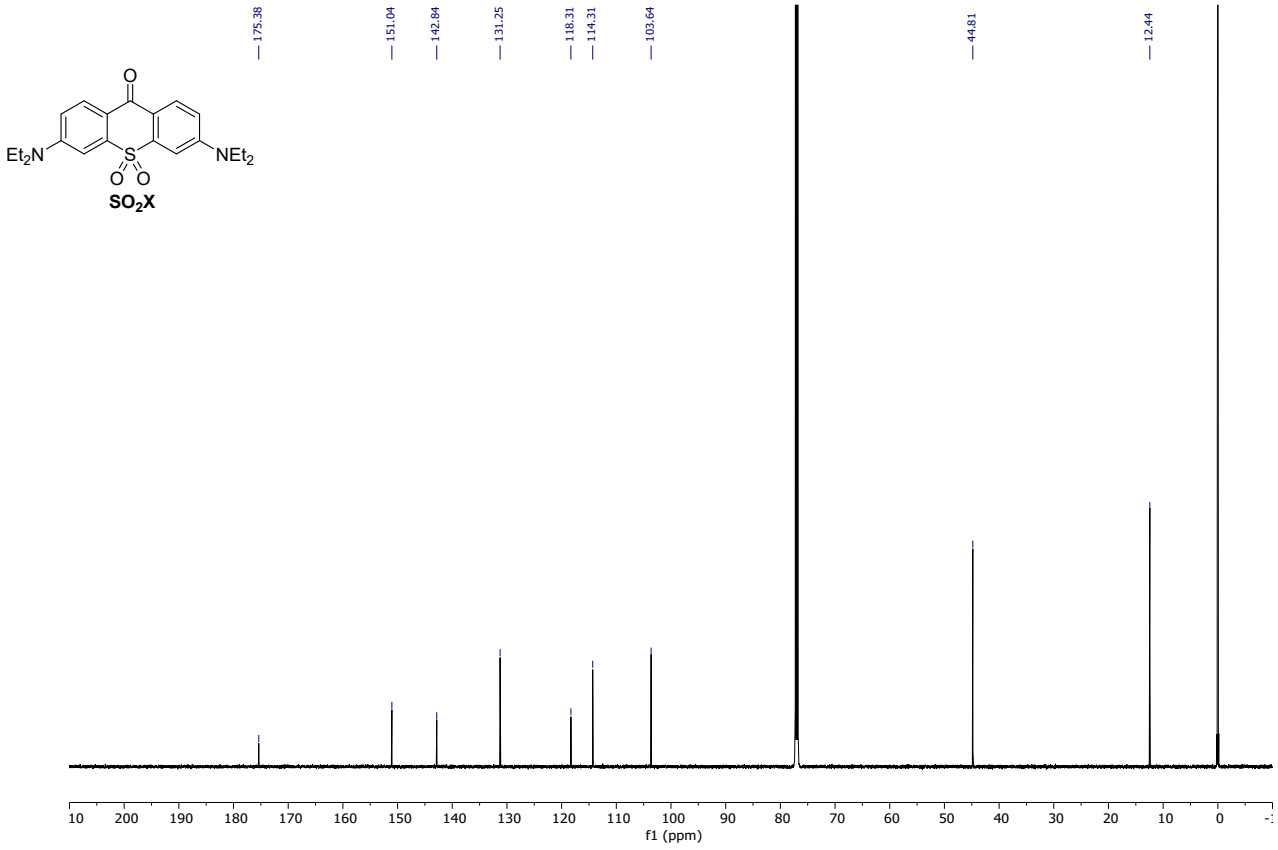
7.31
7.26
7.20
7.19
6.72
6.71
3.98
3.39
3.39
3.38
3.38
3.37
1.17
1.17
1.16
1.16
1.15
2.01
2.10
2.00
2.03
8.17
12.05
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

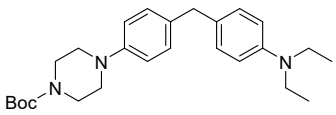

Et2N
NEt2
S
O
O

S10

146.77
138.25
129.45
125.05
114.88
106.09
44.47
32.07
12.38
10 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

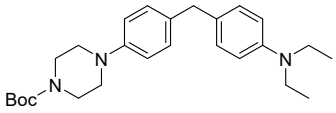

8.19
8.17
7.26
7.18
7.17
6.85
6.85
6.84
6.83
3.52
3.51
3.50
3.49
1.27
1.26
1.24
1.94
1.99
2.03
8.00
12.04
f1 (ppm)


175.38
151.04
142.84
131.25
118.31
114.31
103.64
44.81
12.44
f1 (ppm)


Boc
N
N
N

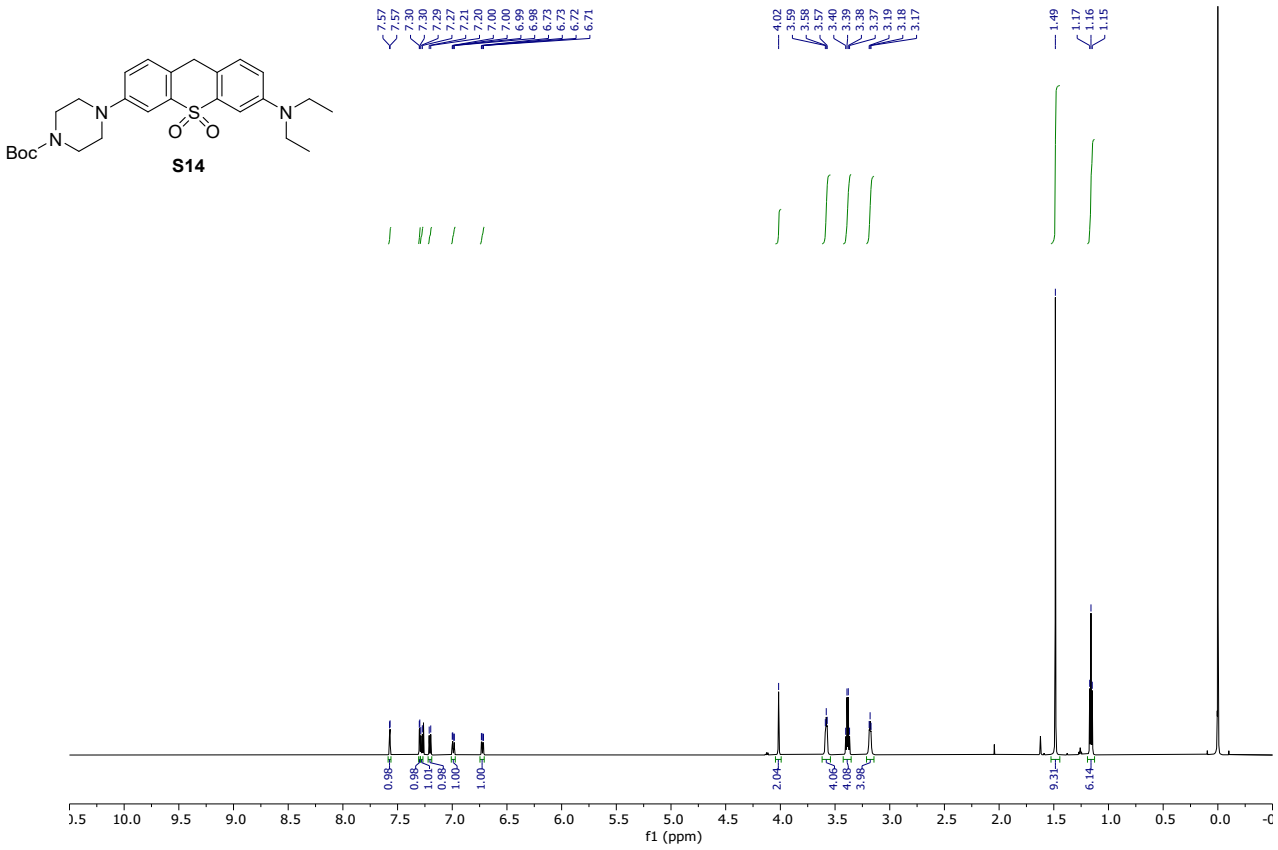
S12

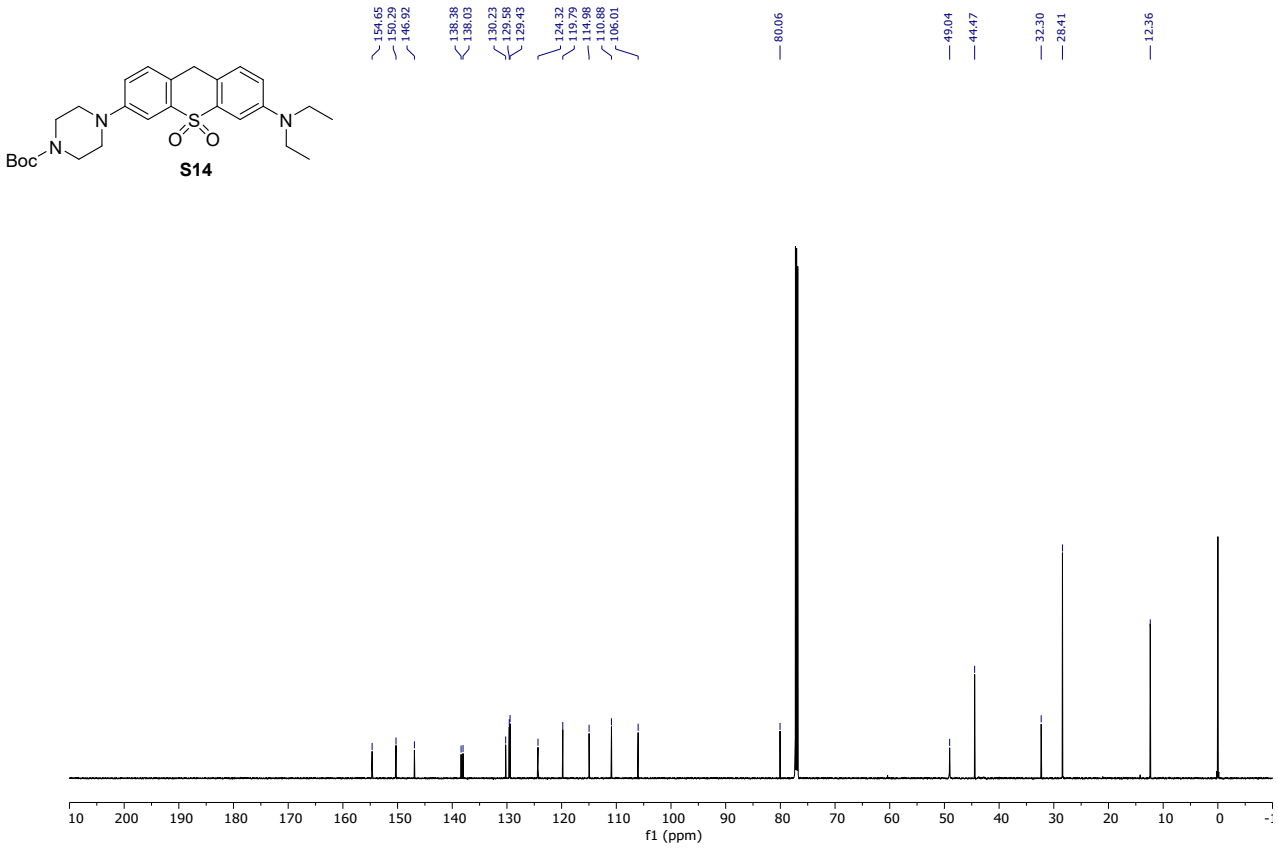
7.26
7.11
7.11
7.10
7.09
7.02
7.02
7.01
7.01
6.86
6.86
6.85
6.84
6.63
6.62
6.61
6.61
3.81
3.57
3.57
3.56
3.56
3.33
3.33
3.32
3.32
3.31
3.31
3.30
3.08
3.08
3.07
1.49
1.49
1.48
1.15
1.14
1.14
1.13
1.12
1.12
1.93
2.15
1.90
2.00
2.01
4.01
4.01
4.04
9.00
6.00
f1 (ppm)

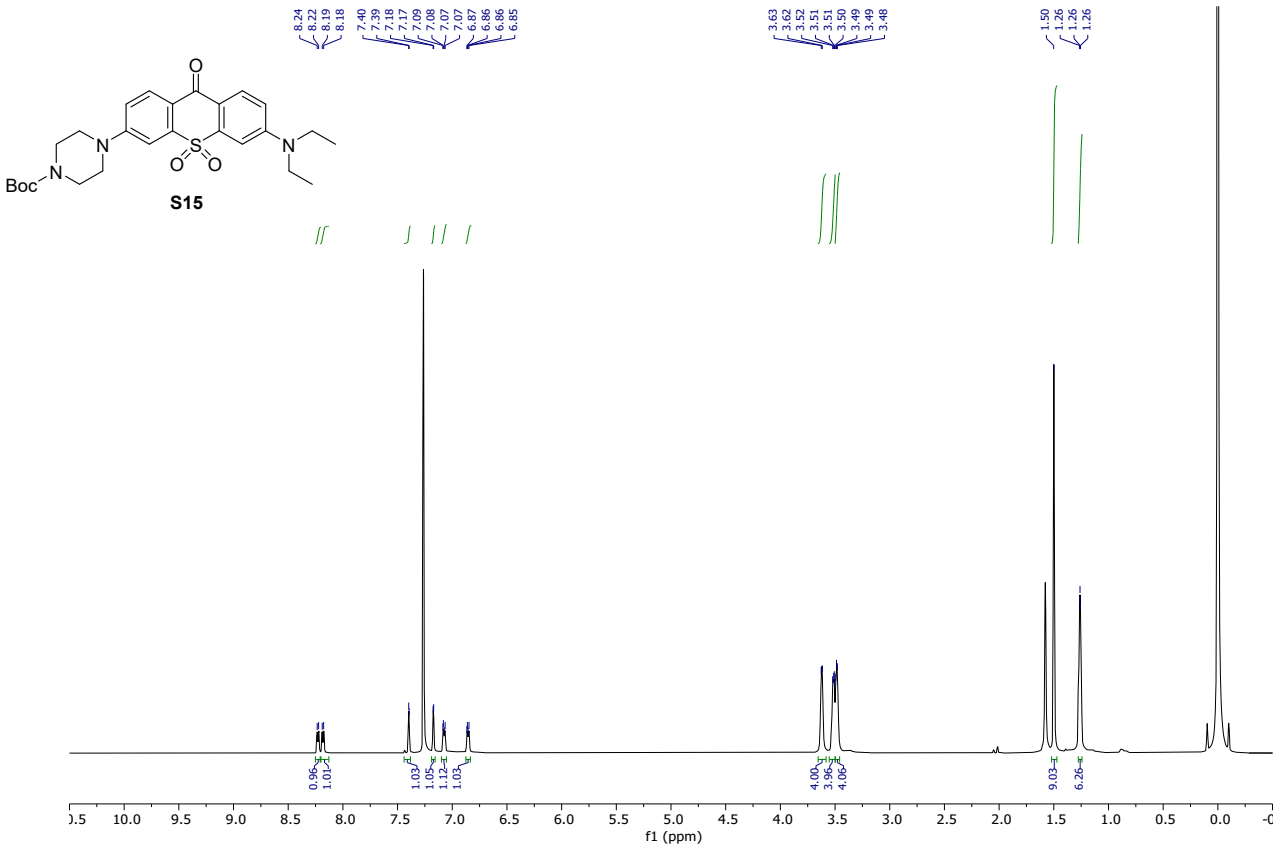

Boc
N
N
N

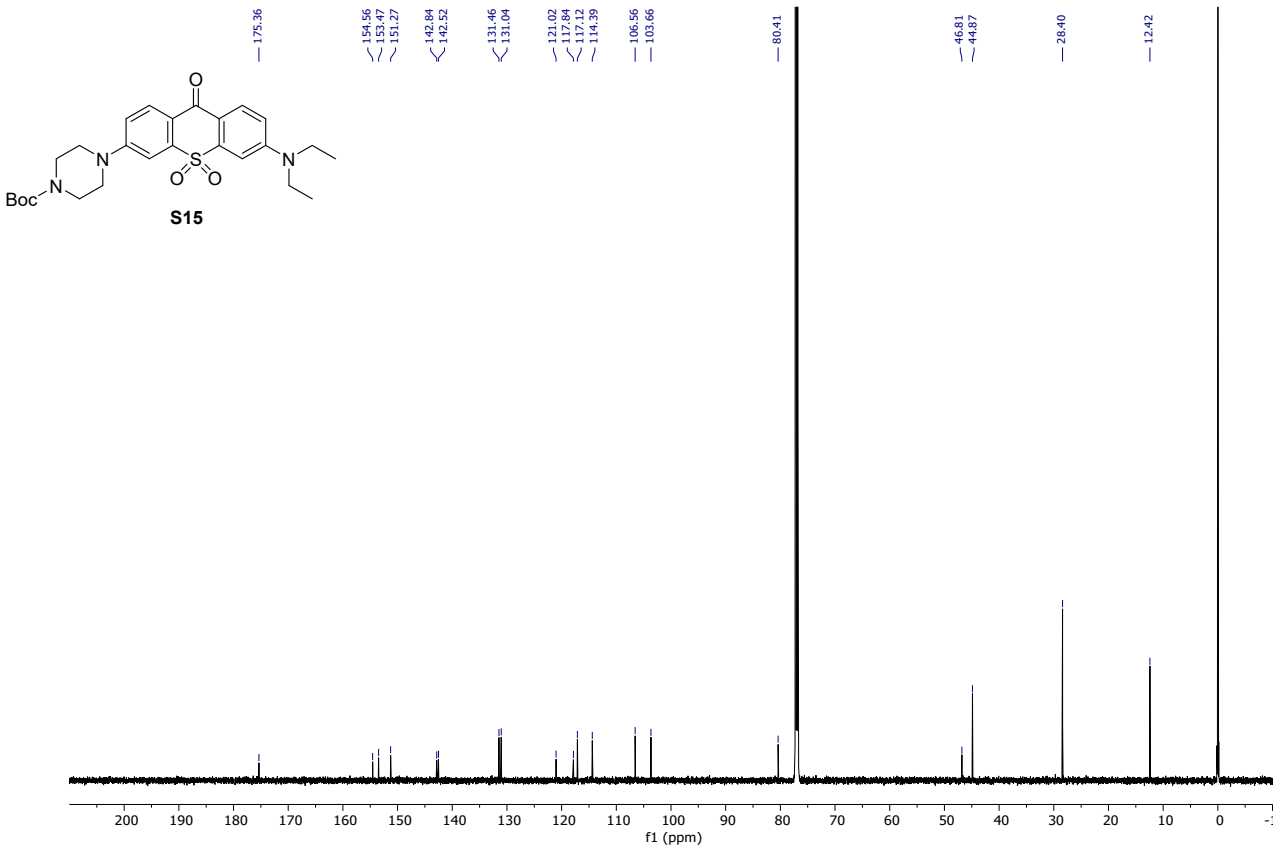
S12

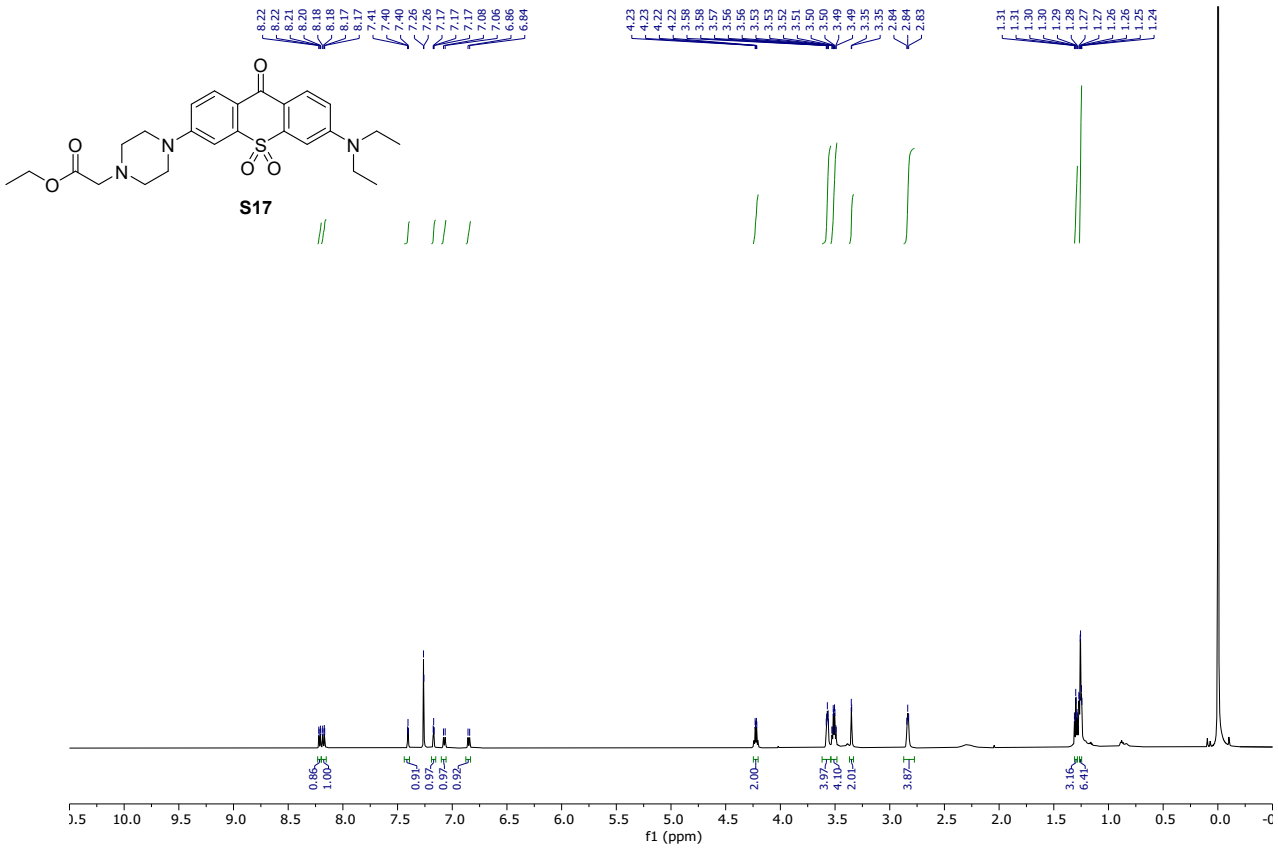
154.75
149.43
146.18
134.24
129.59
129.51
128.38
116.88
112.12
79.81
49.83
44.39
39.95
32.48
28.43
12.59
f1 (ppm)

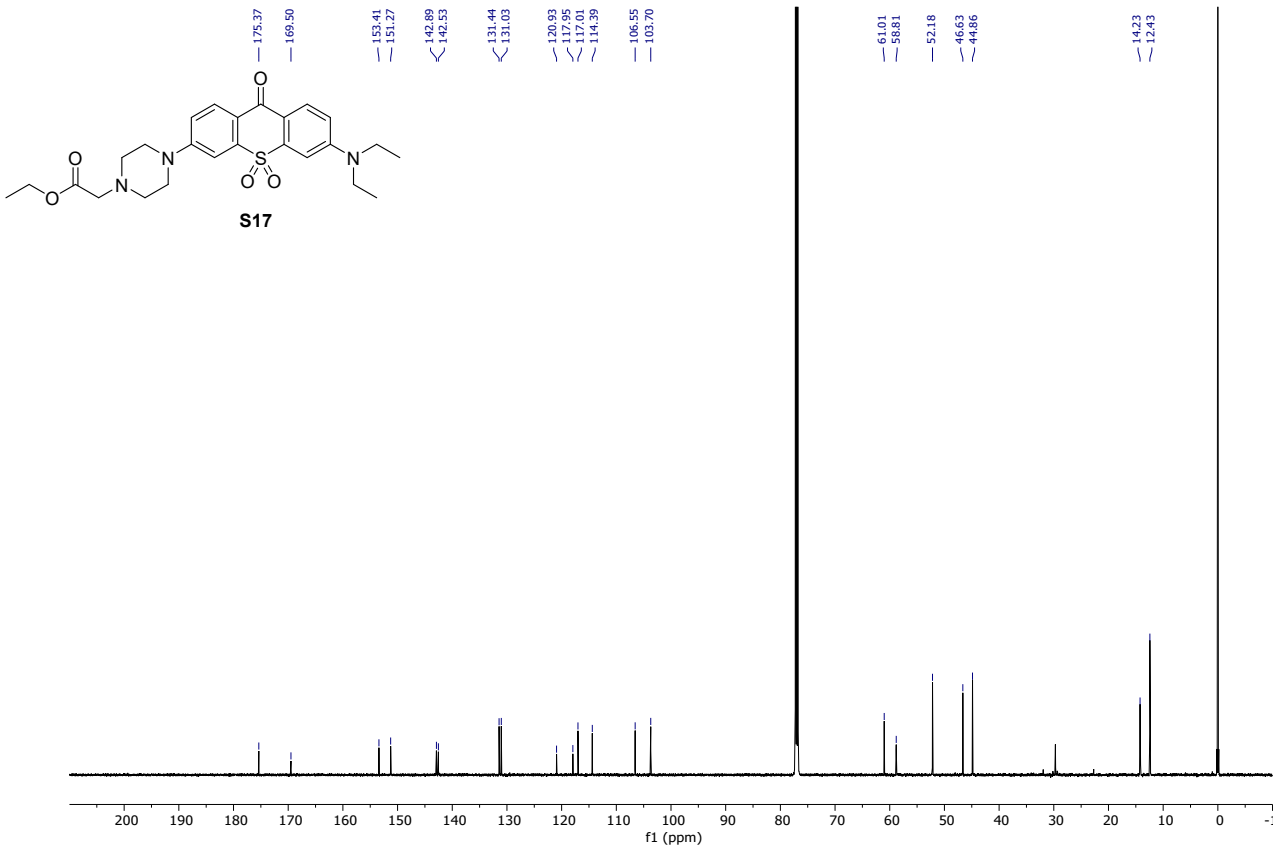

S14
7.57
7.57
7.30
7.30
7.29
7.27
7.21
7.20
7.00
7.00
6.99
6.98
6.73
6.73
6.72
6.71
4.02
3.59
3.58
3.57
3.40
3.39
3.38
3.37
3.19
3.18
3.17
1.49
1.17
1.16
1.15
0.98
0.98
1.01
0.98
1.00
1.00
2.04
4.06
4.08
3.98
9.31
6.14
f1 (ppm)

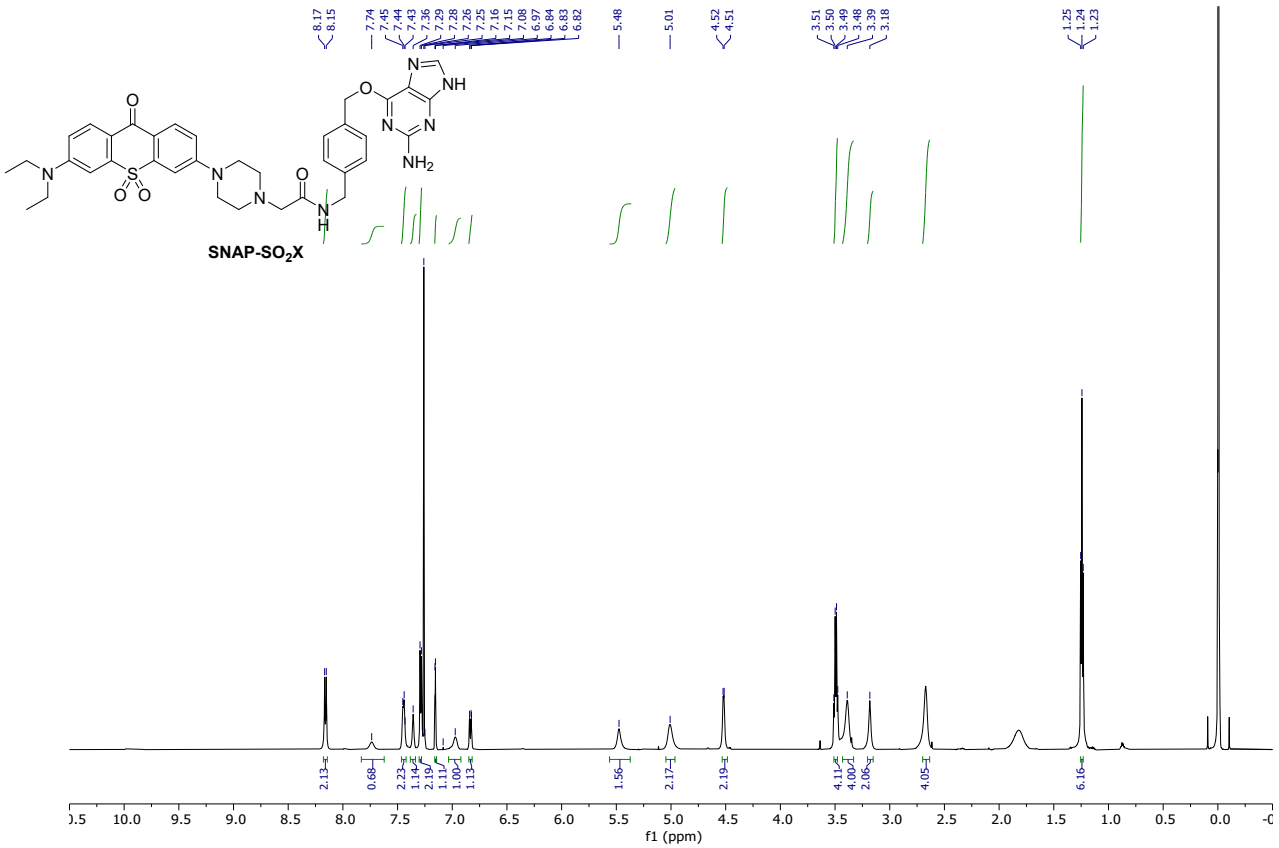

S14
154.65
150.29
146.92
138.38
138.03
130.23
129.58
129.43
124.32
119.79
114.98
110.88
106.01
80.06
49.04
44.47
32.30
28.41
12.36
f1 (ppm)

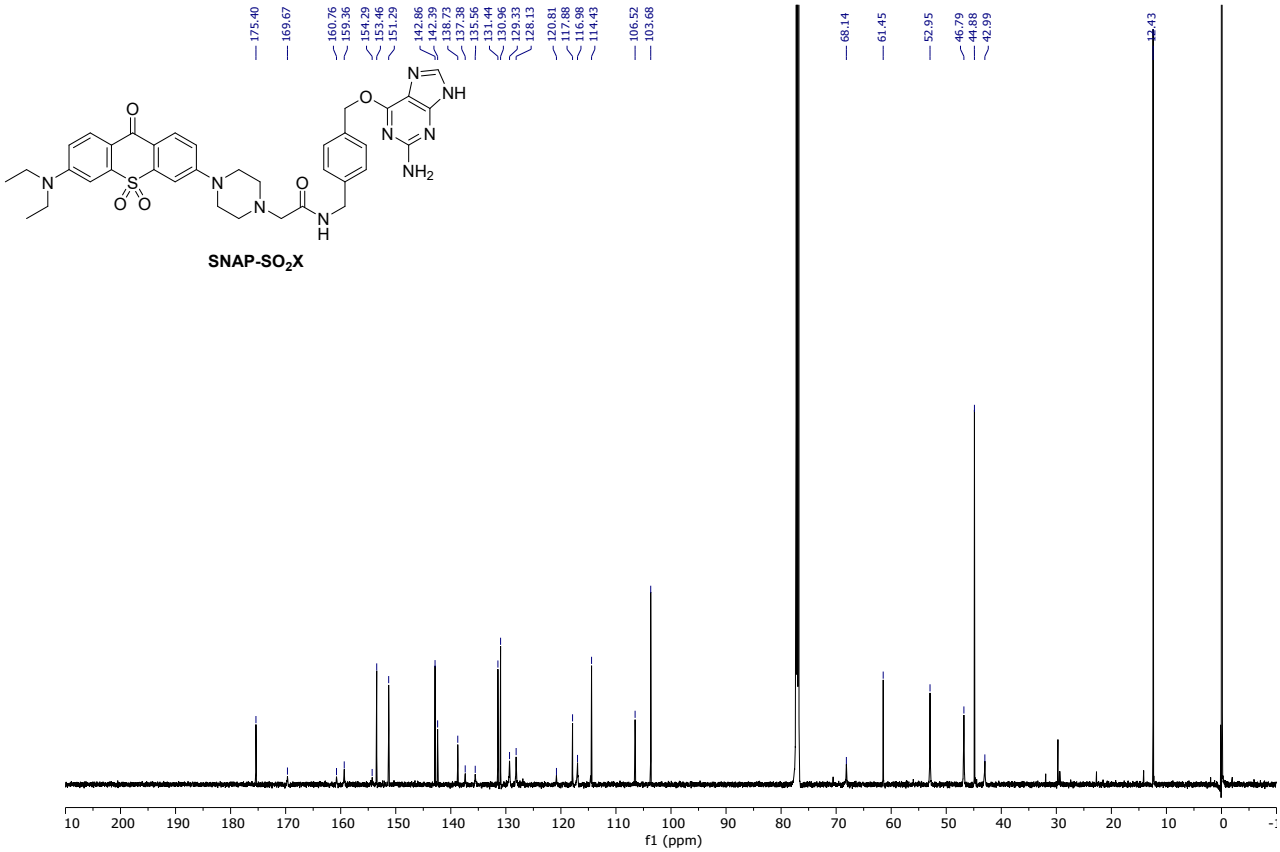

S15
f1 (ppm)

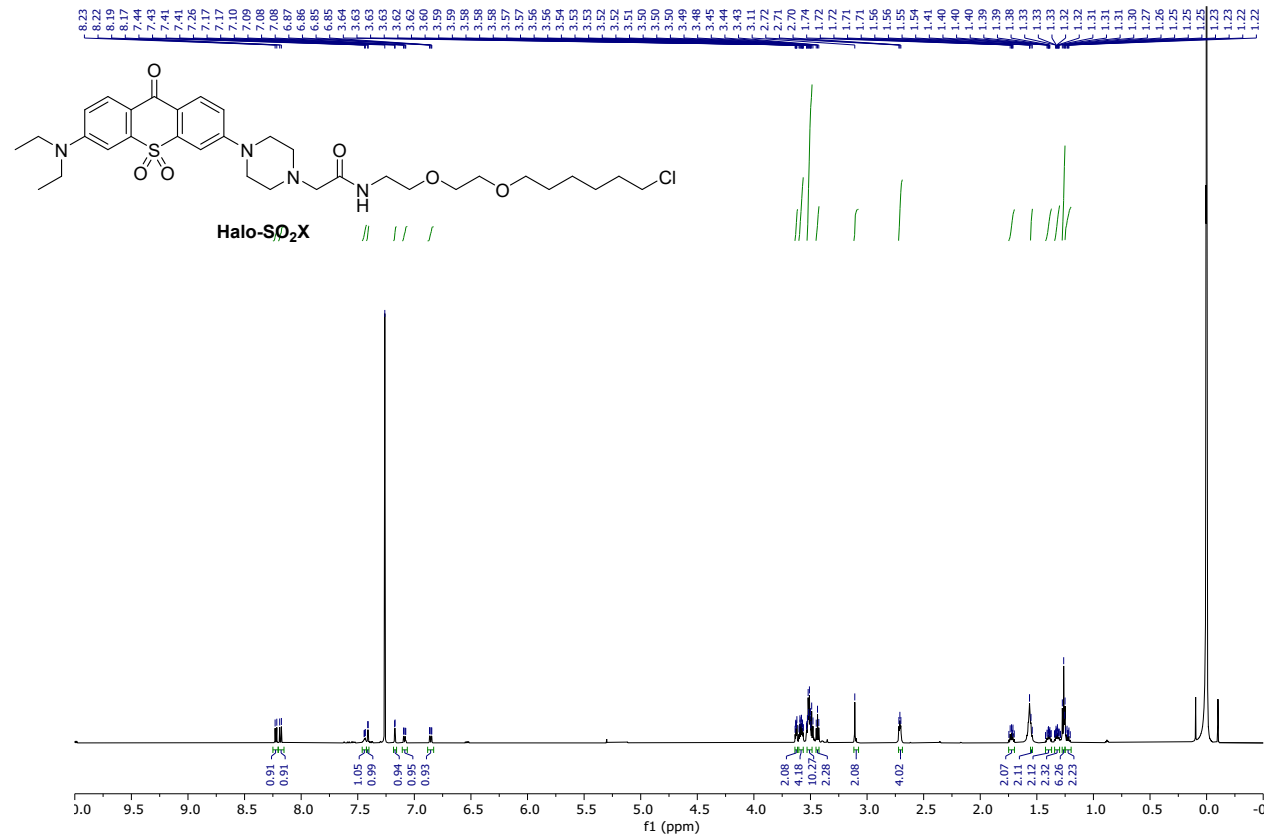

S15
f1 (ppm)

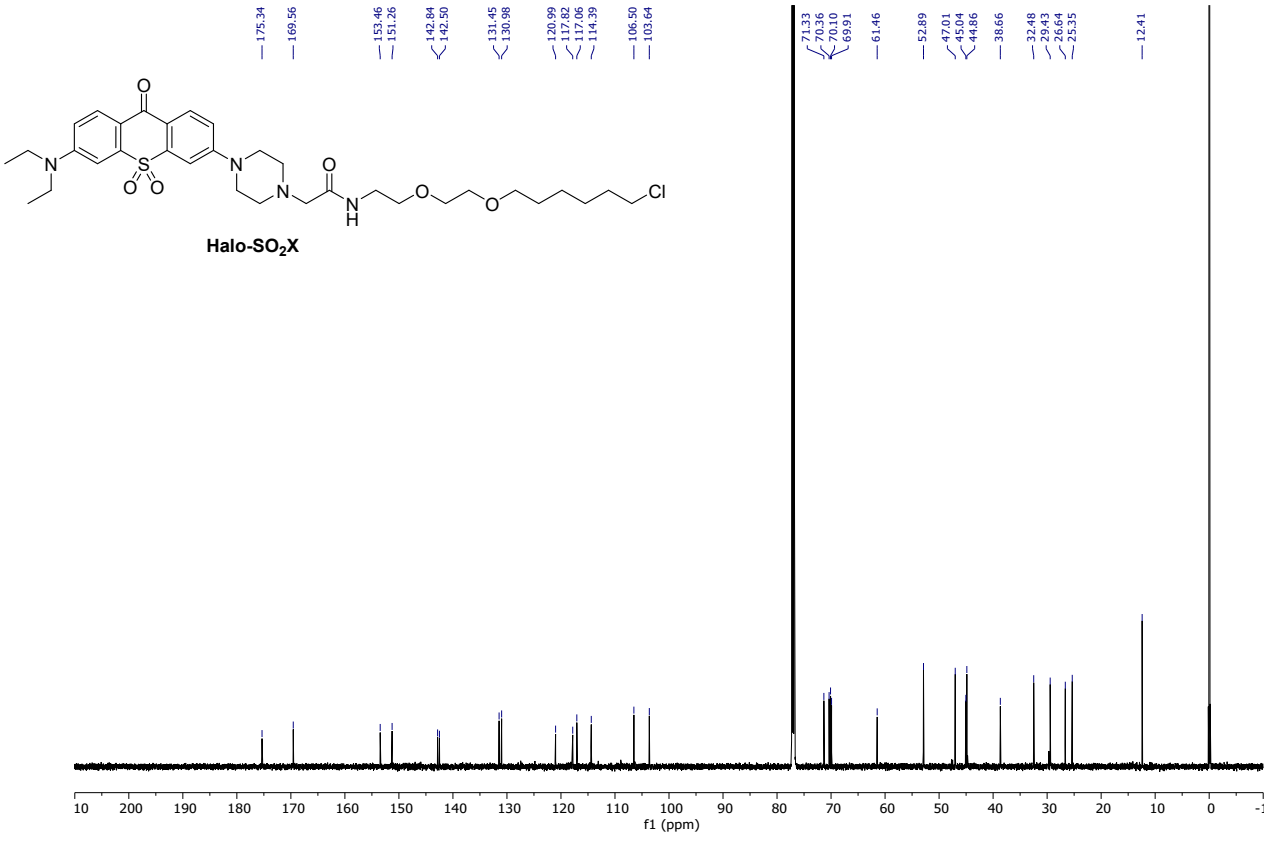

S17
f1 (ppm)


S17
f1 (ppm)


SNAP-SO2X
f1 (ppm)


SNAP-SO2X
f1 (ppm)


Halo-SO2X
f1 (ppm)


Halo-SO2X
f1 (ppm)

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