Electronic Supplementary Material (ESI) for Chemical Science.

Oxime as a General Photocage for the Design of Visible Light Photoactivatable Fluorophores

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1. General experimental information

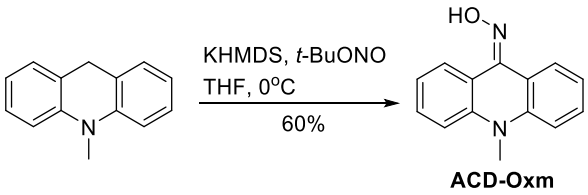
All solvents and chemicals for synthesis were purchased from Alfa Aesar and Sigma-Aldrich and used as received without further purification unless otherwise specified. CCK-8 kit was purchased from Dojindo Molecular Technologies, Inc. The 1H NMR spectroscopic measurements were carried out using a Bruker-600 NMR at 600 MHz with tetramethysilane (TMS) as the internal reference. Electrospray ionization (ESI) mass spectra were performed on a Bruker MicroToF ESI LC-MS System in positive-ion mode. The steady-state absorption spectra were obtained with a ThermoFisher Evolution 220 UV-Vis spectrophotometer in 1 cm path length quartz cells. Fluorescence spectra were recorded using spectrophotometer (SPEX FluoroLog-3). Quantum yield in DMSO was measured relative to the fluorescence of Rhodamine B ($\Phi = 0.65$) in ethanol or quinine sulfate ($\Phi = 0.55$) in 0.5 M H_2SO_4. Confocal fluorescent images of living cells were performed using Nikon A1R-si Laser Scanning Confocal Microscope (Japan) and Zeiss LSM 800 confocal laser microscope, equipped with lasers of 405/488/561/638 nm. The plate reader was from Infinite® 200 PRO (TECAN). Universal optical power meters were from MELLES GRIOT and Newport.

Fluorescence assays

Stock solutions of 5 mM in DMSO were diluted into 3 mL, 50 μM in the corresponding solvent in a 1 cm x 1 cm quartz cuvette. Measurements of emission spectra were recorded before light irradiation. Activation ratios were calculated from the peak emission intensity of the photolyzed oxime probes and the pre-irradiation intensity. Light irradiation was stopped at the corresponding time points to record emission spectra and continued until a plateau was reached.

2. Synthesis procedure

Synthesis of 10-Methylacridin-9(10*H*)-one oxime (ACD-Oxm, 1)[1]



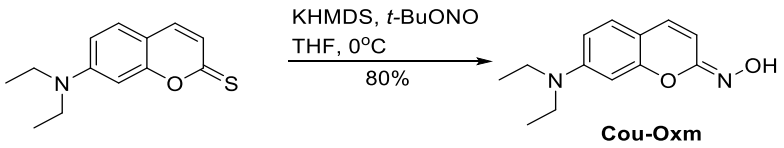
To the solution 10-methyl-9,10-dihydroacridine (500 mg, 2.6 mmol) of in THF (15 mL) was added *t*-BuONO (700 μL, 5.2 mmol) at 0 °C, followed by KHMDS (7.4 mL, 5.2 mmol, 0.7 M in hexane) dropwise at 0 °C, and the reaction was stirred at 0 °C for 0.5 h. Then the reaction was quenched with saturated aqueous NH_4Cl, and the aqueous layer was extracted three times with EtOAc (50 mL x 3). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated. Flash chromatography on silica gel (20% EtOAc/hexanes) yielded 349 mg (60%) of 10-Methylacridin-9(10*H*)-one oxime as a yellow solid.

R_f = 0.3 (ethyl acetate: hexane = 1: 4, v/v)

HRMS (ESI): calcd. for $C_{14}H_{13}N_2O^+$ $[M+H]^+$ 225.1022, found 225.1010.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.80 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.95 (dd, *J* = 7.8, 1.5 Hz, 1H), 7.46 (ddd, *J* = 8.6, 7.2, 1.6 Hz, 1H), 7.43 (ddd, *J* = 8.6, 7.1, 1.6 Hz, 1H), 7.17 (dd, *J* = 8.5, 1.0 Hz, 1H), 7.12 (d, *J* = 8.4 Hz, 1H), 7.09 (td, *J* = 7.4, 1.5 Hz, 2H), 3.58 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 145.77, 142.24, 140.65, 131.12, 130.76, 129.79, 124.66, 121.16, 120.59, 119.56, 116.66, 113.43, 113.03, 34.00.

Synthesis of 7- diethylaminocoumarin-oxime (Cou-Oxm, 3)



Cou-Oxm

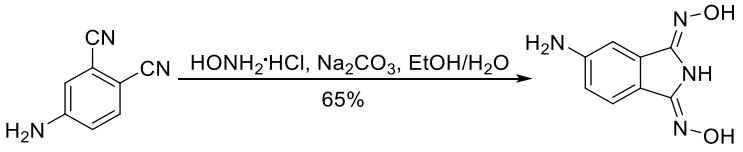
To a solution of SCou[2] (100 mg, 0.43 mmol) in MeOH (8 mL) was added Hydroxylamine hydrochloride (0.12 g, 1.7 mmol) and sodium acetate (0.14 g, 1.7 mmol), and the mixture was refluxed for 12 h and cooled to room temperature. The organic solvent was removed under reduced pressure. Flash chromatography on silica gel (20% EtOAc/hexanes) yielded 80 mg (80%) of **Cou-Oxm** as a yellow solid.

R_f = 0.2 (ethyl acetate: hexane = 1: 4, v/v)

HRMS (ESI): calcd. for $C_{13}H_{17}N_2O_2^+$ $[M+H]^+$ 233.1285, found 233.1270.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.01 (d, *J* = 8.6 Hz, 1H), 6.79 (d, *J* = 9.6 Hz, 1H), 6.51 (d, *J* = 2.4 Hz, 1H), 6.39 (dd, *J* = 8.6, 2.5 Hz, 1H), 6.00 (d, *J* = 9.7 Hz, 1H), 3.36 (dd, *J* = 7.1 Hz, 4H), 1.17 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 154.27, 152.44, 149.44, 130.85, 127.97, 110.11, 108.41, 107.23, 98.14, 44.59, 12.55.

Synthesis of 5-aminophthalimide dioxime (API-Oxm, 5)[3]



API-Oxm

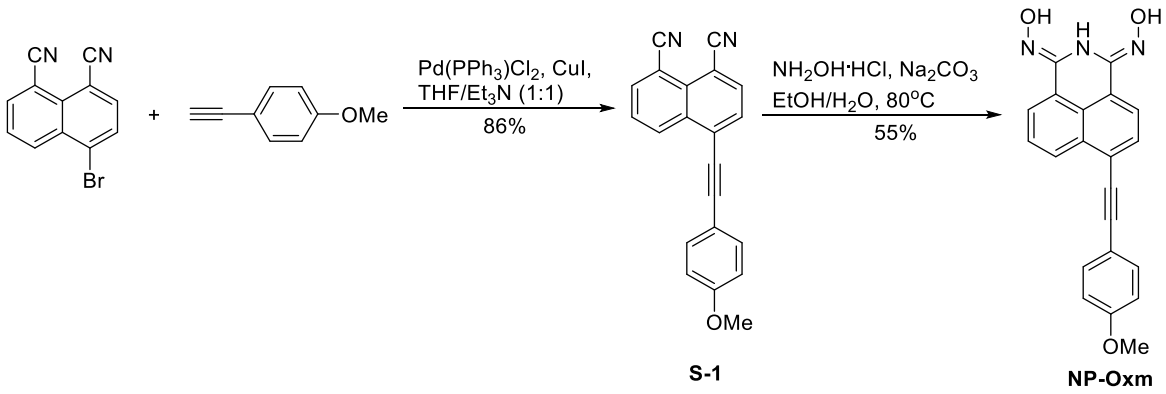
To a solution of 4-Aminophthalonitrile (0.5 g, 3.49 mmol) in water/ethanol (3:1 v/v, 20 mL) was added sodium carbonate (1.0 g, 9.43 mmol), and hydroxylamine hydrochloride (1.4 g, 20.1 mmol),

and the mixture was stirred at 75 °C for 8 h and cooled to room temperature. The product gradually precipitated as yellow crystals, filtered, and washed several times with ethanol yielded 0.44 g (65%) of **API-Oxm** as a yellow solid.

HRMS (ESI): calcd. for $C_8H_9N_4O_2^+$ $[M+H]^+$ 193.0720, found 193.0707.

^{1}H NMR (600 MHz, DMSO-d_6) δ 10.40 (s, 1H), 10.13 (s, 1H), 8.56 (s, 1H), 7.30 (d, J = 8.3 Hz, 1H), 6.79 (s, 1H), 6.69 (d, J = 8.4 Hz, 1H), 5.66 (s, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 150.59, 146.42, 132.71, 121.05, 118.46, 115.82, 103.40.

Synthesis of oxime caged 4-((4-Methoxyphenyl)ethynyl)-1,8-naphthalimide (NP-Oxm, 9)[4]



To a solution of aryl bromide (20 mg, 0.08 mmol), and 4-ethynylanisole (53.0 mg, 0.2 mmol) in THF (3 mL) in a 50 mL schlenk storage vessel was added Pd $(PPh_3)_2Cl_2$ (6.0 mg, 0.008 mmol), and cuprous iodide (3.0 mg, 0.016 mmol). Triethylamine (3 mL) was added via syringe and the vessel was capped. The reaction mixture was degassed via freeze-pump-thaw cycle three times before stirred at 45 °C for 18 h. After which time ^{1}H NMR indicated complete consumption of starting material. Then cooled to room temperature and diluted with water and extracted with EtOAc (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 (100% DCM) yielded 21 mg (86%) of **S-1**.[5]

R_f = 0.35 (DCM)

MS(ESI): calcd. for $C_{21}H_{13}N_2O^+$ $[M+H]^+$ 309.1, found 309.0.

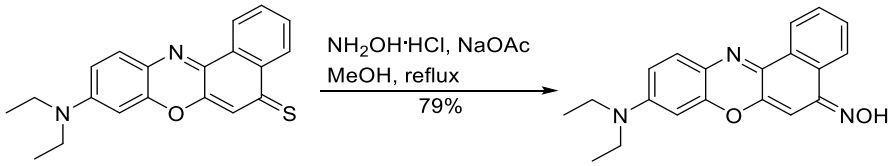
^{1}H NMR (600 MHz, $CDCl_3$) δ 8.81 (dd, *J* = 8.5, 1.3 Hz, 1H), 8.15 (dd, *J* = 7.2, 1.3 Hz, 1H), 8.06 (d, *J* = 7.6 Hz, 1H), 7.84 (d, *J* = 7.6 Hz, 1H), 7.75 (dd, *J* = 8.5, 7.2 Hz, 1H), 7.61 – 7.57 (m, 2H), 6.98 – 6.93 (m, 2H), 3.88 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 160.79, 138.13, 136.94, 133.58, 133.09, 132.87, 129.97, 128.87, 128.53, 127.07, 116.74, 116.65, 114.37, 113.68, 109.12, 107.55, 100.90, 84.50, 55.45.

To a solution of **S-1** (18 mg, 0.06 mmol) in water (6 mL) and EtOH (2mL) was added hydroxylamine hydrochloride (17.0 mg, 0.24 mmol) and sodium carbonate (13 mg, 0.12 mmol). The reaction was stirred at 80 ºC overnight and then cooled to room temperature, filtered, and washed with cold ethanol. Then recrystallized from boiling absolute ethanol and dried under vacuum to give the product 12 mg (55%) of **NP-Oxm** as yellow solid.

HRMS (ESI): calcd. for $C_{21}H_{16}N_3O_3^+$ $[M+H]^+$ 358.1186, found 358.1150.

^{1}H NMR (600 MHz, DMSO-d_6) δ 11.25 (s, 1H), 11.18 (s, 1H), 8.97 (s, 1H), 8.48 (dd, *J* = 8.3, 1.2 Hz, 1H), 8.24 (dd, *J* = 7.4, 1.2 Hz, 1H), 8.14 (d, *J* = 7.8 Hz, 1H), 7.87 (d, *J* = 7.8 Hz, 1H), 7.80 (d, *J* = 1.1 Hz, 1H), 7.74 – 7.66 (m, 2H), 7.09 – 7.04 (m, 2H), 3.83 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.46, 141.33, 141.28, 133.78, 132.38, 130.68, 128.09, 127.83, 126.01, 123.03, 122.53, 122.09, 121.82, 120.71, 115.03, 114.31, 97.25, 86.14, 55.84.

Synthesis of oxime caged Nile Red (NR-Oxm, 11)



NR-Oxm

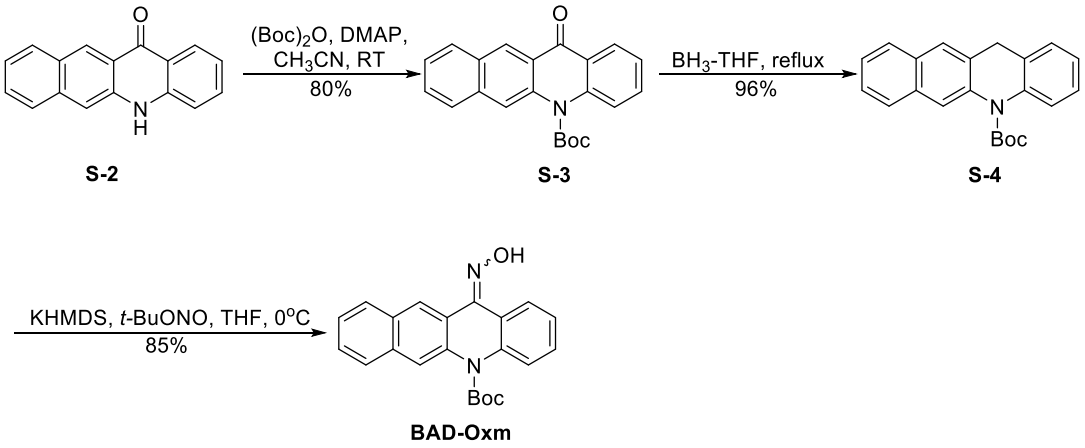
To a solution of SNile Red[2] (20 mg, 0.06 mmol) in MeOH (5 mL) was added Hydroxylamine hydrochloride (17 mg, 0.24 mmol) and sodium acetate (20 mg, 1.7 mmol), and the mixture was refluxed for 12 h and cooled to room temperature. The organic solvent was removed under reduced pressure. Flash chromatography on silica gel (30% EtOAc/hexanes) yielded 16 mg (79%) of **NR-Oxm** as an orange solid.

R_f = 0.2 (ethyl acetate: hexane = 1: 4, v/v)

HRMS (ESI): calcd. for $C_{20}H_{20}N_3O_2^+$ $[M+H]^+$ 334.1550, found 334.1528.

^{1}H NMR (600 MHz, DMSO-d_6) δ 11.82 (s, 1H), 8.43 – 8.37 (m, 1H), 8.20 – 8.13 (m, 1H), 7.61 – 7.54 (m, 2H), 7.36 (dd, J = 8.9, 1.1 Hz, 1H), 6.76 (d, J = 0.7 Hz, 1H), 6.59 – 6.54 (m, 1H), 6.42 (dd, J = 2.9, 1.3 Hz, 1H), 3.42 (q, J = 7.0 Hz, 4H), 1.13 (t, J = 7.0 Hz, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 149.68, 147.02, 146.47, 146.11, 142.75, 130.85, 130.38, 129.98, 129.15, 124.13, 124.02, 122.63, 108.55, 96.89, 94.42, 44.58, 12.97.

Synthesis of *tert*-butyl-12-(hydroxyimino) benzo[*b*]acridine-5(12*H*)-carboxylate (BAD-Oxm, 7)



To a solution of benzo[*b*]acridin-12(5*H*)-one[6] (65 mg, 0.27 mmol) in MeCN (5 mL) was added $(Boc)_2O$ (118 mg, 0.54 mmol) and DMAP (66 mg, 0.54 mmol), and the mixture was stirred overnight at room temperature. Then diluted with water and extracted with EtOAc (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 (10% EtOAc/hexanes) yielded 74 mg (80%) of **S-3** as a yellow solid.

R_f = 0.25 (ethyl acetate: hexane = 1: 9, v/v)

MS (ESI): calcd. for $C_{22}H_{20}NO_3^+$ $[M+H]^+$ 346.1, found 346.1

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.88 (s, 1H), 8.34 (dd, *J* = 7.9, 1.6 Hz, 1H), 8.22 (s, 1H), 8.01 (d, *J* = 8.3 Hz, 1H), 7.88 (d, *J* = 8.3 Hz, 1H), 7.73 (d, *J* = 8.2 Hz, 1H), 7.64 (ddd, *J* = 8.5, 7.1, 1.6 Hz, 1H), 7.58 (ddd, *J* = 8.1, 6.7, 1.2 Hz, 1H), 7.47 (ddd, *J* = 8.0, 6.7, 1.1 Hz, 1H), 7.34 (ddd, *J* = 7.9, 7.1, 1.0 Hz, 1H), 1.65 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 180.64, 152.23, 140.81, 136.02, 135.40, 133.13, 129.52, 129.48, 128.87, 128.10, 127.50, 126.93, 125.80, 124.57, 124.54, 123.83, 121.07, 117.88, 85.08, 77.30, 77.09, 76.87, 27.97.

To a solution of **S-3** (74 mg, 0.21 mmol) in THF (5 mL) was added BH_3-THF (1.0 M in THF, 0.42 mL, 0.42 mmol), and the mixture was refluxed for 45 minutes and cooled to room temperature. The reaction was quenched with saturated aqueous $NaHCO_3$ and the mixture was extracted with EtOAc (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 (5% EtOAc/hexanes) yielded 68 mg (96%) of **S-4** as a yellow solid.

R_f = 0.5 (ethyl acetate: hexane = 1: 9, v/v)

MS (ESI): calcd. for $C_{20}H_{22}NNaO_2^+$ $[M+Na]^+$ 354.1, found 354.1

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.74 – 7.70 (m, 1H), 7.67 – 7.63 (m, 1H), 7.55 (d, *J* = 9.4 Hz, 2H), 7.31 (ddd, *J* = 7.2, 5.2, 1.6 Hz, 2H), 7.18 – 7.12 (m, 2H), 7.04 (t, *J* = 7.4 Hz, 1H), 3.87 (s, 2H), 1.47 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 152.72, 138.91, 136.69, 133.01, 132.93, 132.12, 131.22, 127.79, 127.07, 126.97, 126.13, 125.65, 125.52, 125.27, 125.18, 125.04, 122.71, 81.95, 77.31, 77.10, 76.89, 34.31, 28.35.

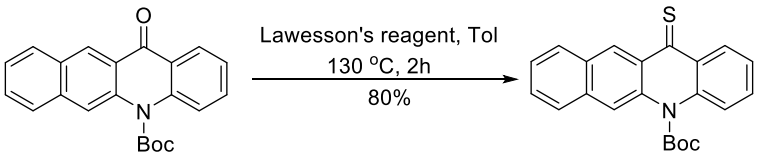
To the solution **S-4** (72 mg, 0.22 mmol) of in THF (5 mL) was added *t*-BuONO (36 μL, 0.26 mmol) at 0 °C, followed by KHMDS (0.7 M in hexane, 0.37 mL, 0.26 mmol) dropwise at 0 °C, and the reaction was stirred at 0 °C for 1 h. Then the reaction was quenched with saturated aqueous NH_4Cl, and the aqueous layer was extracted three times with EtOAc (50 mL x 3). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated. Flash chromatography on silica gel (15% EtOAc/hexanes) yielded the mixture of *Z/E* isomers of **BAD-Oxm** 68 mg (85 %) as a white solid.

R_f = 0.4 (ethyl acetate: hexane = 1: 4, v/v)

HRMS (ESI): calcd. for $C_{22}H_{21}N_2O_3^+$ $[M+H]^+$ 361.1547, found 361.1480.

Mixture of *Z/E* isomers. ^{1}H NMR (600 MHz, CD_3OD) δ 8.90 – 7.57 (m, 6H), 7.46 – 7.27 (m, 3H), 7.17 (dtd, *J* = 18.1, 7.6, 1.1 Hz, 1H), 1.42 (d, *J* = 4.3 Hz, 9H). ^{13}C NMR (150 MHz, CD_3OD) δ 152.51, 152.44, 145.06, 144.79, 138.27, 137.47, 134.87, 134.28, 132.95, 132.76, 130.97, 130.37, 129.37, 129.29, 128.93, 128.89, 128.71, 128.25, 127.83, 127.71, 127.30, 127.25, 127.13, 126.55, 125.69, 125.63, 125.55, 125.16, 125.12, 124.87, 124.41, 124.24, 123.97, 122.97, 122.93, 122.21, 82.54, 82.47, 26.98.

Synthesis of *tert*-butyl 12-thioxobenzo[*b*]acridine-5(12*H*)-carboxylate te (SBAD)



S-3 **SBAD**

To a mixture of compounds **S3** (20 mg, 0.06 mmol) and Lawesson's reagent (23 mg, 0.06) in Schlenk flask, 10 mL dry toluene was added. The mixture was heated at 130 °C for 2 h and then cooled to room temperature. The solvent was evaporated under reduced pressure. The residue was purified by flash silica gel column chromatography (11% EtOAc/hexanes) to afford the desired compound as solids in yield of 80% (17 mg).

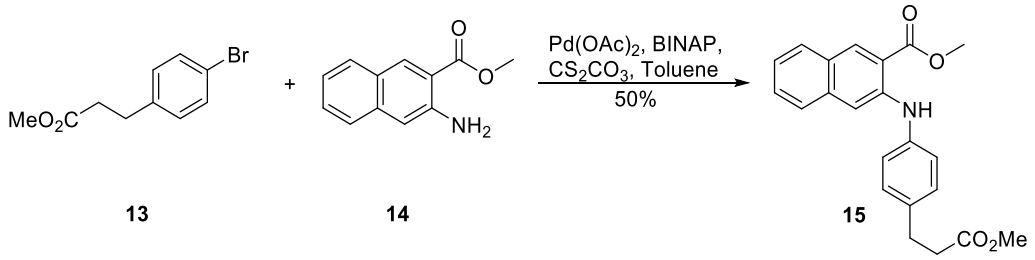
R_f = 0.6 (ethyl acetate: hexane = 1: 4, v/v)

HRMS (ESI): calcd. for $C_{22}H_{20}NO_2S^+$ $[M+H]^+$ 362.1209, found 362.1250.

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.13 (s, 1H), 8.61 (dd, *J* = 8.1, 1.6 Hz, 1H), 8.26 (s, 1H), 8.07 – 7.94 (m, 1H), 7.87 (d, *J* = 8.3 Hz, 1H), 7.75 (dd, *J* = 8.5, 1.1 Hz, 1H), 7.65 (ddd, *J* = 8.5, 7.0, 1.6 Hz, 1H), 7.59 (ddd, *J* = 8.2, 6.7, 1.2 Hz, 1H), 7.47 (ddd, *J* = 8.1, 6.7, 1.2 Hz, 1H), 7.31 (ddd, *J* = 8.2, 7.0, 1.1 Hz, 1H), 1.62 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 212.62, 151.99, 135.12, 135.04,

134.55, 134.26, 132.64, 131.43, 130.40, 129.85, 129.80, 128.96, 128.88, 127.42, 126.04, 124.67, 121.88, 118.82, 84.91, 27.97.

Synthesis of organelle-targeting BAD-Oxm probes



13 14 15

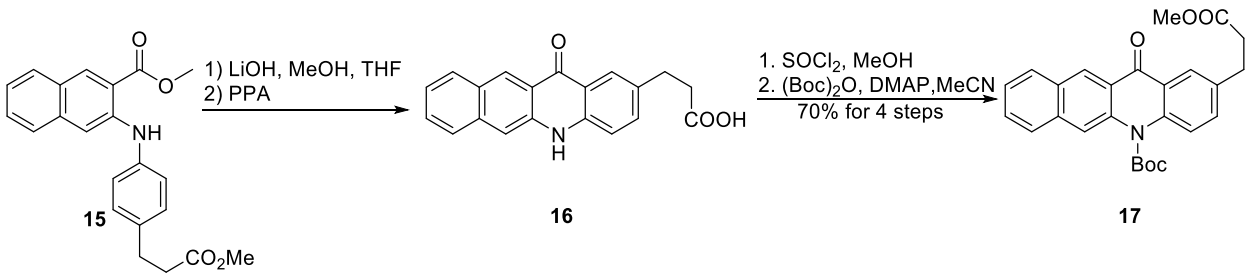
To a solution of methyl 3-(4-Bromophenyl) propionate (1.15 g, 4.7 mmol) and methyl 3-amino-2-naphthoate (1.0 g, 5.0 mmol) in toluene (30 mL) in a 100 mL schlenk storage vessel was added palladium (II) acetate (53 mg, 0.24 mmol), *rac*-BINAP (299 mg, 0.48 mmol) and Cesium carbonate (2.15 g, 6.58 mmol). Then the vessel was capped, and the reaction mixture was degassed via freeze-pump-thaw cycle three times before stirred at 120 ^{o}C for 24 h. Then cooled to room temperature, the contents were filtered through a short plug of celite using CH_2Cl_2 to transfer the material and then ethyl acetate (400 mL) was used to elute the product. The combined organic phase was washed with water and brine, dried over Na_2SO_4, filtered, and evaporated. Flash chromatography on silica gel (10% EtOAc/hexanes) yielded the di-ester **15** 853 mg (50 %) as a yellow oil.

R_f = 0.6 (ethyl acetate: hexane = 1: 4, v/v)

HRMS (ESI): calcd. for $C_{22}H_{22}NO_4^+$ $[M+H]^+$ 364.1543, found 364.1543.

^{1}H NMR (600 MHz, $CDCl_3$) δ 9.14 (s, 1H), 8.57 (d, *J* = 0.8 Hz, 1H), 7.73 (ddd, *J* = 8.1, 1.3, 0.6 Hz, 1H), 7.53 – 7.48 (m, 2H), 7.40 (ddd, *J* = 8.2, 6.7, 1.3 Hz, 1H), 7.29 – 7.25 (m, 2H), 7.23 – 7.18 (m, 3H), 3.98 (s, 3H), 3.70 (s, 3H), 2.96 (t, *J* = 7.8 Hz, 2H), 2.67 (dd, *J* = 8.4, 7.2 Hz, 2H). ^{13}C

NMR (150 MHz, $CDCl_3$) δ 173.44, 168.69, 143.32, 139.58, 137.07, 135.27, 133.84, 129.26, 129.10, 128.92, 126.13, 125.81, 123.01, 122.04, 115.25, 108.01, 52.18, 51.66, 35.82, 30.39.



To a solution of di-ester **15** (90 mg, 0.25 mmol) in THF (4 mL) and MeOH (2 mL) in a 25 mL flask was added a LiOH solution (18 mg in 2 mL H_2O), and the mixture was refluxed at 90 °C for 1 h and then cooled to room temperature. Then the organic solvent was evaporated, and the pH of the aqueous phase was adjusted to 6.0 with 6 M HCl. The precipitate was collected and dried under vacuum. The resulting di-carboxylic acid was put into the next step directly without further purification.

PPA (2.0 g) was added to a 25 mL round-bottom flask with a stir bar. The round-bottom flask was heated to 125 °C. The resulting 3-((4-(2-carboxyethyl)phenyl)amino)-2-naphthoic acid from the above step was added to the flask and stirred for 2 h. Then 10 mL water was slowly added, and the reaction was allowed to cool to 60 °C. After stirring for 1 h at 60 °C, the reaction was cooled to room temperature. Insoluble impurities were removed by vacuum filtration and the pH of the resulting solution was adjusted to 6.0 with 10 M NaOH. Then the aqueous layer was extracted four times with EtOAc (50 mL x 4). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated. The resulting benzo[*b*]acridin-12(5*H*)-one **16** was put into the next step directly without further purification.

To a solution of the above crude **16** in MeOH (10 mL), $SOCl_2$ (0.5 mL, 7 mmol) was added at 0 °C. After being stirred at room temperature for 12 h, the reaction mixture was concentrated in vacuo to provide the crude ester as a yellow solid, which was used directly in the next step without further purification.

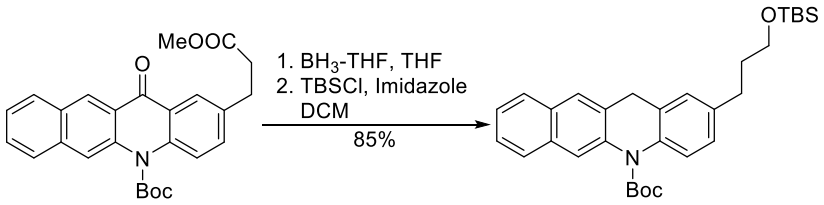
To a solution of the resulting ester in MeCN (5 mL) was added $(Boc)_2O$ (118 mg, 0.54 mmol) and DMAP (132 mg, 1.08 mmol), and the mixture was stirred overnight at room temperature. Then diluted with water and extracted with EtOAc (50 mL x 3). The combined organic phase was washed with water and brine, dried over Na_2SO_4, filtered, and evaporated. Flash chromatography on silica gel (20% EtOAc/hexanes) yielded 75 mg (70%) of **17** as a yellow solid.

R_f = 0.3 (ethyl acetate: hexane = 1: 4, v/v)

MS (ESI): calcd. for $C_{26}H_{26}NO_5^+$ $[M+H]^+$ 432.2, found 432.1.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.89 (s, 1H), 8.25 (s, 1H), 8.16 (d, *J* = 2.2 Hz, 1H), 8.03 (d, *J* = 8.3 Hz, 1H), 7.89 (d, *J* = 8.3 Hz, 1H), 7.71 (d, *J* = 8.6 Hz, 1H), 7.60 (ddd, *J* = 8.2, 6.7, 1.2 Hz, 1H), 7.54 – 7.47 (m, 2H), 3.69 (s, 3H), 3.07 (t, *J* = 7.8 Hz, 2H), 2.72 (t, *J* = 7.8 Hz, 2H), 1.65 (s, 9H).

^{13}C NMR (150 MHz, $CDCl_3$) δ 180.73, 173.12, 152.26, 139.37, 136.33, 136.03, 135.37, 133.56, 129.57, 129.51, 128.87, 128.08, 127.53, 125.87, 125.84, 124.75, 121.61, 118.27, 84.99, 51.76, 35.40, 30.16, 27.99.



17 **18**

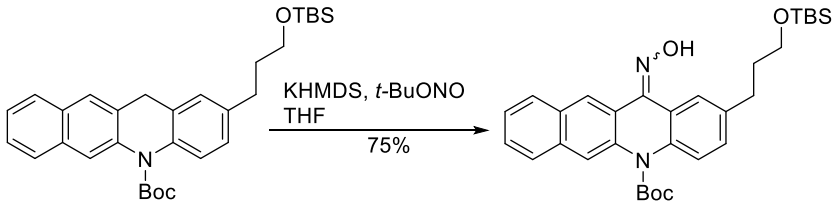
To a solution of **17** (185 mg, 0.43 mmol) in THF (6 mL) was added BH_3-THF (1.0 M in THF, 1.71 mL, 1.71 mmol), and the mixture was refluxed for 3 h and then cooled to room temperature. The reaction was quenched with saturated aqueous $NaHCO_3$ and the mixture was extracted with EtOAc (80 mL x 3). The combined organic phase was washed with water and brine, dried over Na_2SO_4, filtered, and evaporated. The resulting primary alcohol was put into the next step directly without further purification.

To the solution of the primary alcohol in DCM (5 mL) was added imidazole (88 mg, 1.29 mmol) and TBSCl (130 mg, 0.86 mmol) at 0 °C, and the reaction was stirred at room temperature for 2 h. Then the reaction was quenched with saturated aqueous NH_4Cl, and the aqueous layer was extracted three times with EtOAc (50 mL x 3). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated. Flash chromatography on silica gel (5% EtOAc/hexanes) yielded the TBS ether **18** (184 mg, 85 %).

R_f = 0.6 (ethyl acetate: hexane = 1: 9, v/v)

ESI-MS: calcd. for $C_{31}H_{42}N_2O_3Si^+$ $[M+H]^+$ 504.3, found 504.3.

^{1}H NMR (600 MHz, $CDCl_3$) δ 8.14 (s, 1H), 7.89 – 7.83 (m, 1H), 7.80 – 7.74 (m, 1H), 7.69 (s, 1H), 7.59 (d, *J* = 8.2 Hz, 1H), 7.47 – 7.40 (m, 2H), 7.15 – 7.09 (m, 2H), 3.98 (s, 2H), 3.67 (t, *J* = 6.3 Hz, 2H), 2.73 – 2.68 (m, 2H), 1.92 – 1.83 (m, 2H), 1.61 (s, 9H), 0.96 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 152.72, 139.23, 136.82, 136.55, 133.02, 132.62, 132.05, 131.12, 127.69, 126.93, 126.89, 126.19, 125.52, 125.39, 124.94, 124.82, 122.58, 81.72, 62.23, 34.44, 34.30, 31.52, 28.29, 25.96, 18.32, -5.27.



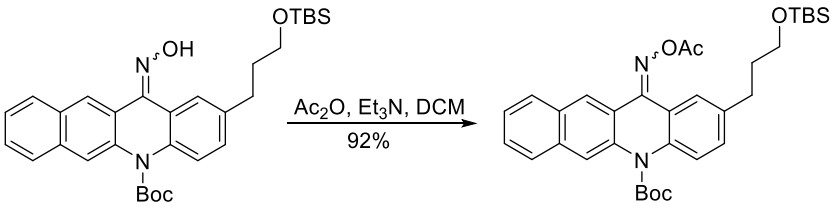
18 **19**

To the solution **18** (193 mg, 0.38 mmol) of in THF (5 mL) was added *t*-BuONO (70 μL, 0.58 mmol) at 0 °C, followed by KHMDS (0.7 M in hexane, 0.83 mL, 0.58 mmol) dropwise at 0 °C, and the reaction was stirred at 0 °C for 1 h. Then the reaction was quenched with saturated aqueous NH_4Cl, and the aqueous layer was extracted three times with EtOAc (50 mL x 3). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated. Flash chromatography on silica gel (10% EtOAc/hexanes) yielded a mixture of *Z/E* isomers of **19** (152 mg, 93 %).

R_f = 0.4 (ethyl acetate: hexane = 1: 4, v/v)

MS (ESI): calcd. for $C_{31}H_{41}N_2O_4Si^+$ $[M+H]^+$ 533.3, found 533.2.

Mixture of *Z/E* isomers. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.97 (s, 1H), 8.24 – 8.19 (m, 2H), 7.91 – 7.86 (m, 1H), 7.86 – 7.81 (m, 1H), 7.69 – 7.64 (m, 1H), 7.59 – 7.41 (m, 3H), 7.25 – 7.21 (m, 1H), 3.65 (3.64) (t, *J* = 6.2 Hz, 2H), 2.76 – 2.69 (m, 2H), 1.90 – 1.82 (m, 2H), 1.55 (1.54) (s, 9H), 0.90 (0.90) (s, 9H), 0.05 (0.05) (s, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 152.30, 152.26, 147.31, 147.10, 139.54, 138.70, 136.44, 135.60, 135.16, 134.53, 133.16, 132.96, 130.83, 130.20, 129.87, 129.70, 128.86, 128.71, 128.67, 128.31, 128.20, 128.13, 127.69, 127.60, 127.50, 126.91, 125.81, 125.80, 125.51, 124.84, 124.24, 124.04, 123.55, 123.18, 122.42, 82.66, 82.59, 62.28, 62.22, 34.33, 34.30, 31.70, 31.59, 28.21, 25.96, 18.34, -5.27.



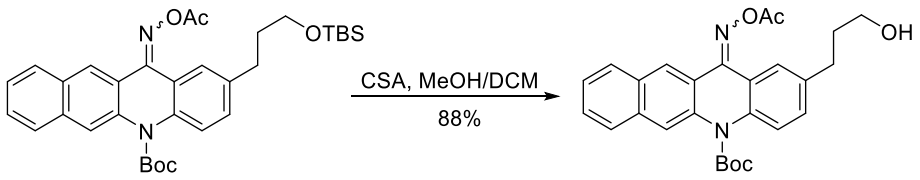
19 **20**

To the solution **19** (190 mg, 0.36 mmol) of in DCM (5 mL) was added Et_3N (250 μL, 1.8 mmol) at 0 °C, followed by Ac_2O (68 μL, 0.72 mmol) dropwise at 0 °C, and the reaction was stirred at room temperature for 12 h. Then the reaction was quenched with saturated aqueous NH_4Cl, and the aqueous layer was extracted three times with EtOAc (50 mL x 3). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated. Flash chromatography on silica gel (5% EtOAc/hexanes) yielded a mixture of *Z/E* isomers of **20** (190 mg, 92 %).

R_f = 0.4 (ethyl acetate: hexane = 1: 10, v/v)

MS (ESI): calcd. for $C_{33}H_{43}N_2O_5Si^+$ $[M+H]^+$ 575.3, found 575.2.

Mixture of *Z/E* isomers. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.69 (8.43) (s, 1H), 8.28 (8.22) (s, 1H), 8.02 – 7.64 (m, 4H), 7.58 – 7.44 (m, 2H), 7.33 (7.29) (dd, *J* = 8.5, 2.1 Hz, 1H), 3.69 – 3.62 (m, 2H), 2.79 – 2.72 (m, 2H), 2.34 (2.32) (s, 3H), 1.91 – 1.81 (m, 2H), 1.55 (1.54) (s, 9H), 0.92 (0.92) (s, 9H), 0.06 (s, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.58, 168.31, 153.05, 152.85, 152.01, 151.96, 139.78, 138.81, 136.78, 136.00, 134.87, 134.20, 133.44, 133.37, 130.70, 130.63, 130.24, 130.01, 129.96, 128.73, 128.67, 128.46, 128.24, 127.61, 127.54, 126.79, 126.61, 126.17, 126.02, 125.64, 124.99, 124.90, 124.71, 123.76, 123.33, 123.17, 122.38, 83.04, 82.96, 62.24, 61.97, 34.64, 34.49, 34.40, 34.26, 31.69, 31.57, 31.51, 28.16, 26.88, 25.96, 25.92, 25.25, 22.64, 20.69, 19.85, 19.81, 18.32, 18.28, 14.13, -5.27, -5.28.



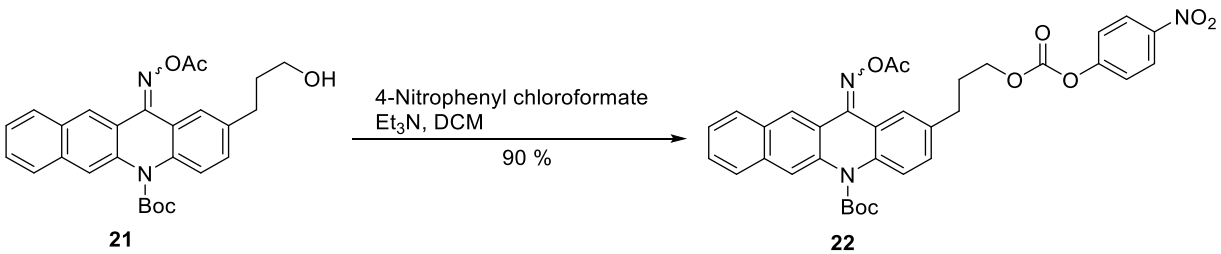
20 **21**

To the solution **20** (40 mg, 0.07 mmol) of in DCM (4 mL) was added CSA (3 mg, 0.014 mmol, dissolve in 2 mL MeOH) at 0 °C, the reaction was stirred at 0 °C for 12 h. Then diluted with EtOAc (150 mL) and washed with brine, dried over Na_2SO_4, filtered, and evaporated. Flash chromatography on silica gel (50% EtOAc/hexanes) yielded a mixture of *Z/E* isomers of **21** (28 mg, 88 %).

R_f = 0.25 (ethyl acetate: hexane = 1: 1, v/v)

MS (ESI): calcd. for $C_{27}H_{29}N_2O_5^+$ $[M+H]^+$ 461.2, found 461.2.

Mixture of *Z/E* isomers. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.71 (8.42) (s, 1H), 8.30 (8.22) (s, 1H), 8.04 – 7.66 (m, 4H), 7.62 – 7.45 (m, 2H), 7.33 (7.29) (dd, *J* = 8.5, 2.1 Hz, 1H), 3.71 (3.68) (t, *J* = 6.3 Hz, 2H), 2.80 (2.77) (t, *J* = 7.6 Hz, 2H), 2.34 (s, 1H), 1.96 – 1.87 (m, 0H), 1.55 (1.54) (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.84, 168.62, 152.95, 152.86, 152.03, 152.01, 139.36, 138.33, 136.91, 136.17, 134.88, 134.20, 133.48, 133.39, 130.65, 130.29, 130.04, 129.93, 128.95, 128.71, 128.49, 128.31, 127.66, 127.64, 127.61, 126.87, 126.60, 126.24, 126.09, 125.75, 125.00, 124.95, 124.85, 123.75, 123.40, 123.17, 122.47, 83.16, 83.08, 62.03, 61.60, 34.04, 33.91, 31.53, 31.37, 28.20, 28.18, 19.87.



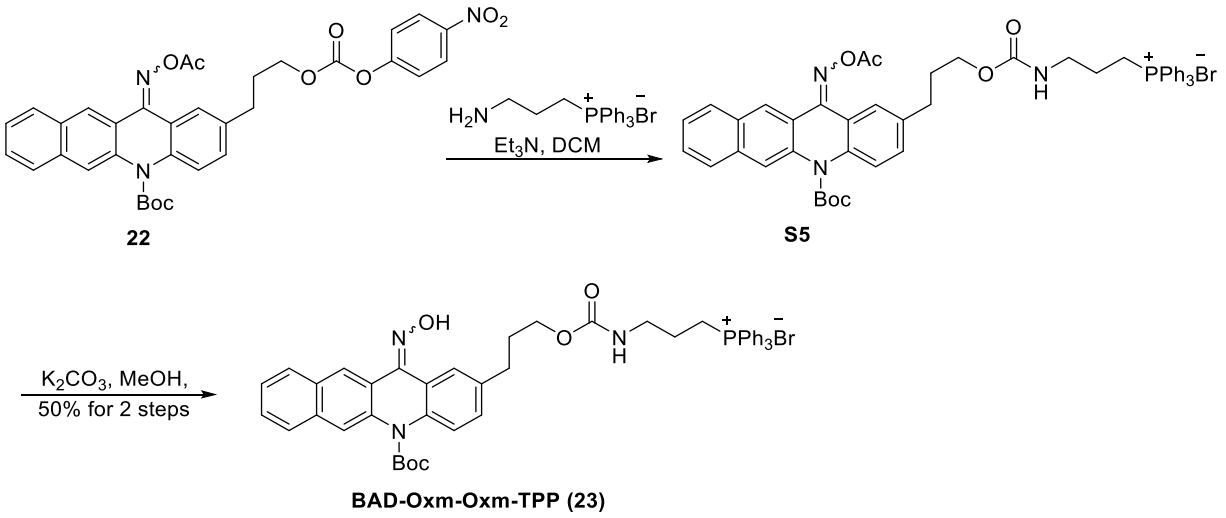
21 **22**

To the solution **21** (30 mg, 0.07 mmol) of in DCM (4 mL) was added 4-Nitrophenyl chloroformate (26 mg, 0.13 mmol) and Et_3N (49 μL, 0.35 mmol) at 0 °C, the reaction was stirred at room temperature for 12 h. Then the reaction was quenched with saturated aqueous NH_4Cl, and the aqueous layer was extracted three times with EtOAc (50 mL x 3). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated. Flash chromatography on silica gel (25% EtOAc/hexanes) yielded a mixture of *Z/E* isomers of **22** (39 mg, 90 %).

R_f = 0.2 (ethyl acetate: hexane = 1: 3, v/v)

MS (ESI): calcd. for $C_{34}H_{32}N_3O_9^+$ $[M+H]^+$ 626.2, found 626.2.

Mixture of *Z/E* isomers. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.72 (8.29) (s, 1H), 8.33 – 8.19 (m, 3H), 8.05 – 7.67 (m, 4H), 7.61 – 7.45 (m, 2H), 7.39 – 7.29 (m, 3H), 4.35 (4.32) (t, *J* = 6.4 Hz, 2H), 2.85 (m, 2H), 2.35 (2.34) (s, 3H), 2.18 – 2.09 (m, 2H), 1.55 (1.55) (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 168.55, 168.46, 155.50, 155.41, 152.77, 152.75, 152.44, 151.97, 151.95, 145.35, 145.30, 138.10, 137.31, 137.18, 136.51, 134.82, 134.11, 133.50, 133.41, 130.66, 130.54, 130.31, 130.05, 129.82, 128.80, 128.70, 128.48, 128.41, 127.70, 127.65, 127.64, 127.07, 126.53, 126.33, 126.18, 126.03, 125.30, 125.27, 125.04, 124.98, 124.02, 123.44, 123.07, 122.48, 121.76, 121.70, 83.28, 83.18, 68.62, 68.43, 31.56, 31.30, 29.95, 29.92, 28.17, 19.88, 19.86.



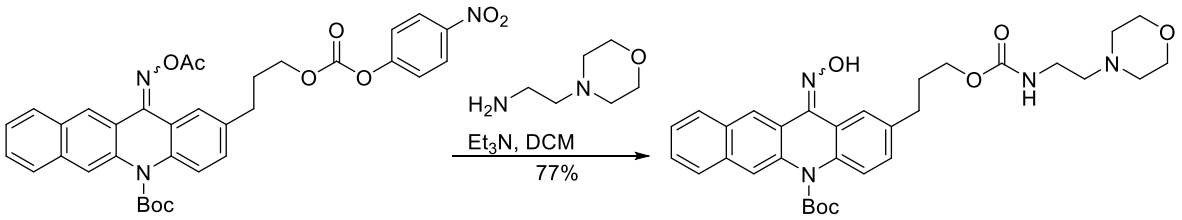
To the solution **22** (11 mg, 0.018 mmol) of in DCM (5 mL) was added (3-aminopropyl) triphenylphosphonium bromide (17 mg, 0.043 mmol) and Et_3N (15 μL, 0.10 mmol), the reaction was stirred at 40 °C for 24 h. Then the reaction was quenched with saturated aqueous NH_4Cl, and the aqueous layer was extracted three times with EtOAc (50 mL x 3). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated. The resulting residue was dissolved in MeOH (4 mL), then anhydrous potassium carbonate (5 mg, 0.04 mmol) was added to the solution at 0 °C. The mixture was stirred for 3 h at room temperature and then concentrated in *vacuo*. Flash chromatography on silica gel (5% MeOH/DCM) yielded a mixture of *Z/E* isomers of **23** (8 mg, 50 %).

R_f = 0.3 (MeOH: DCM = 1: 5, v/v)

HRMS (ESI): calcd. for $C_{47}H_{47}N_3O_5P^+$ $[M]^+$ 764.3248, found 764.2957.

Mixture of *Z/E* isomers. ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.14 (s, 1H), 8.54 (s, 1H), 8.21 – 8.05 (m, 2H), 7.88 – 7.75 (m, 3H), 7.72 – 7.52 (m, 9H), 7.52 – 7.37 (m, 5H), 7.23 – 7.04 (m, 3H), 4.10 – 3.96 (m, 2H), 3.84 – 3.68 (m, 2H), 3.63 – 3.41 (m, 2H), 2.87 – 2.69 (m, 2H), 2.03 – 1.92 (m, 2H), 1.92 – 1.77 (m, 2H), 1.48 (1.44) (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.39, 157.26, 152.34,

152.21, 145.19, 144.72, 138.80, 137.72, 136.28, 135.29, 135.18, 134.94, 134.92, 134.85, 134.83, 134.68, 133.47, 133.41, 133.34, 132.81, 132.64, 130.88, 130.46, 130.41, 130.38, 130.36, 130.28, 130.04, 128.98, 128.89, 128.81, 128.30, 128.17, 127.54, 127.27, 127.25, 126.49, 125.64, 125.55, 125.31, 124.90, 124.46, 124.13, 123.80, 122.74, 122.11, 118.54, 118.42, 117.97, 117.85, 82.24, 82.08, 64.37, 63.45, 40.35, 40.23, 32.23, 31.58, 29.67, 29.13, 28.23, 28.21, 22.80, 22.60, 20.08, 19.74.



22 **BAD-Oxm-MOR (24)**

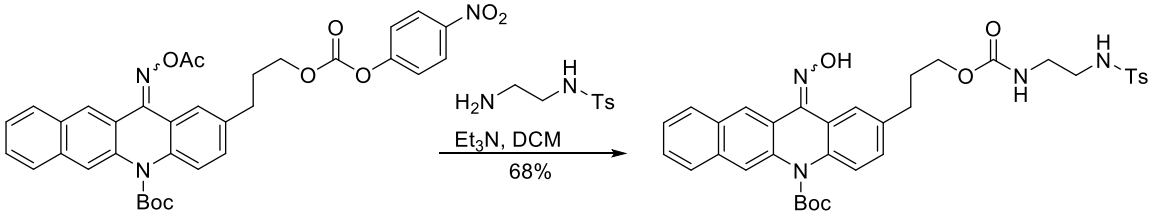
To the solution **22** (11 mg, 0.018 mmol) of in DCM (5 mL) was added 4-(2-Aminoethyl)morpholine (6 mg, 0.043 mmol) and Et_3N (15 μL, 0.10 mmol), the reaction was stirred at 40 °C for 24 h. Then the reaction was quenched with saturated aqueous NH_4Cl, and the aqueous layer was extracted three times with EtOAc (50 mL x 3). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated. Flash chromatography on silica gel (5% MeOH/DCM) yielded a mixture of *Z/E* isomers of **24** (8 mg, 77 %).

R_f = 0.3 (MeOH: DCM = 1: 9, v/v)

HRMS (ESI): calcd. for $C_{32}H_{39}N_4O_6^+$ $[M+H]^+$ 575.2864, found 575.2686.

Mixture of *Z/E* isomers. ^1H NMR (600 MHz, $CDCl_3$) δ 9.03 (s, 1H), 8.47 – 8.12 (m, 2H), 7.92 – 7.80 (m, 3H), 7.67 (dd, *J* = 18.5, 8.4 Hz, 1H), 7.55 – 7.41 (m, 2H), 7.18 (td, *J* = 6.4, 5.8, 3.0 Hz, 1H), 6.70 – 6.56 (5.51-5.49) (m, 1H), 4.25 – 4.04 (m, 2H), 4.05 – 3.94 (m, 2H), 3.87 – 3.78 (m, 2H), 3.48 – 3.39 (m, 2H), 2.84 – 2.77 (m, 2H), 2.70 – 2.52 (m, 6H), 2.01 – 1.95 (m, 2H), 1.56

(1.55) (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.01, 156.91, 152.39, 146.29, 145.99, 138.98, 137.64, 136.35, 135.39, 135.29, 134.73, 133.08, 132.76, 130.86, 130.26, 130.19, 129.62, 129.26, 129.09, 128.91, 128.69, 128.11, 127.92, 127.65, 127.54, 127.51, 126.65, 125.83, 125.69, 125.37, 124.46, 124.36, 124.03, 123.31, 123.28, 122.48, 82.62, 82.58, 66.21, 66.10, 66.05, 64.48, 59.07, 58.74, 53.83, 53.51, 37.18, 36.34, 33.21, 32.26, 29.50, 29.32, 28.23.



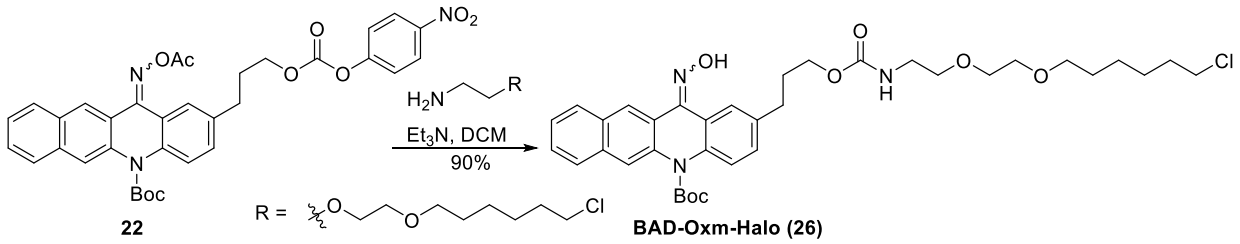
To the solution **10** (11 mg, 0.018 mmol) of in DCM (5 mL) was added 4-(2- N-(2-Aminoethyl)-4-methylbenzenesulfonamide (9 mg, 0.043 mmol) and Et_3N (15 μL, 0.10 mmol), the reaction was stirred at 40 °C for 24 h. Then the reaction was quenched with saturated aqueous NH_4Cl, and the aqueous layer was extracted three times with EtOAc (50 mL x 3). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated. Flash chromatography on silica gel (5% MeOH/DCM) yielded a mixture of *Z/E* isomers of **25** (8 mg, 68 %).

R_f = 0.3 (ethyl acetate: hexane = 1: 1, v/v)

HRMS (ESI): calcd. for $C_{35}H_{39}N_4O_7S^+$ $[M+H]^+$ 659.2534, found 659.2471.

Mixture of *Z/E* isomers. ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.01 (s, 1H), 8.34 – 8.12 (m, 2H), 7.91 – 7.80 (m, 2H), 7.78 – 7.71 (m, 2H), 7.68 (d, *J* = 8.4 Hz, 1H), 7.61 – 7.40 (m, 3H), 7.32 – 7.26 (m, 2H), 7.25 – 7.17 (m, 1H), 5.50 – 5.23 (m, 1H), 5.23 – 5.13 (m, 1H), 4.14 – 4.04 (m, 2H), 3.29 – 3.23 (m, 2H), 3.07 (s, 2H), 2.73 (t, *J* = 7.4 Hz, 2H), 2.39 (s, 3H), 1.99 – 1.93 (m, 2H), 1.54 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.22, 157.15, 152.29, 147.04, 146.78, 143.64, 143.60, 138.60,

137.72, 136.64, 136.62, 136.60, 135.83, 135.11, 134.49, 133.15, 132.93, 130.85, 130.25, 129.96, 129.81, 129.79, 129.56, 128.91, 128.74, 128.36, 128.32, 128.15, 127.68, 127.45, 127.08, 127.05, 126.94, 125.85, 125.67, 125.06, 124.43, 124.33, 123.61, 123.20, 122.47, 82.76, 82.71, 64.42, 64.37, 43.41, 43.29, 40.65, 31.91, 31.77, 31.64, 30.35, 30.09, 29.33, 28.22, 21.52.



To the solution **22** (11 mg, 0.018 mmol) of in DCM (5 mL) was added 2-[2-[(6-chlorohexyl)oxy] ethoxy]-ethanamine (10 mg, 0.045 mmol) and Et_3N (15 μL, 0.10 mmol), the reaction was stirred at 40 °C for 24 h. Then the reaction was quenched with saturated aqueous NH_4Cl, and the aqueous layer was extracted three times with EtOAc (50 mL x 3). The combined organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated. Flash chromatography on silica gel (5% MeOH/DCM) yielded a mixture of *Z/E* isomers of **26** (11 mg, 90 %).

R_f = 0.3 (ethyl acetate: hexane = 1: 1, v/v)

HRMS (ESI): calcd. for $C_{36}H_{47}ClN_3O_7^+$ $[M+H]^+$ 668.3097, found 668.2869.

Mixture of *Z/E* isomers. ^1H NMR (600 MHz, $CDCl_3$) δ 8.98 (s, 1H), 8.32 – 8.17 (m, 2H), 7.89 – 7.81 (m, 2H), 7.77 – 7.63 (m, 2H), 7.55 – 7.40 (m, 2H), 7.25 – 7.17 (m, 1H), 6.11 (5.36) (s, 1H), 4.34 – 4.02 (m, 2H), 3.72 – 3.60 (m, 4H), 3.60 – 3.51 (m, 2H), 3.51 – 3.33 (m, 5H), 2.81 – 2.73 (m, 2H), 2.00 – 1.94 (m, 2H), 1.78 – 1.69 (m, 2H), 1.69 – 1.58 (m, 3H), 1.54 (s, 9H), 1.48 – 1.33 (m, 4H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 156.82, 156.72, 152.34, 152.28, 146.80, 146.66, 139.10, 137.89, 136.52, 135.51, 135.22, 134.59, 133.10, 132.89, 130.83, 130.22, 129.70, 129.43, 129.27,

128.93, 128.58, 128.07, 127.97, 127.69, 127.55, 127.53, 126.82, 125.81, 125.76, 125.57, 125.12, 124.58, 124.41, 123.80, 123.54, 123.25, 122.40, 82.63, 82.62, 71.49, 71.35, 71.11, 70.43, 70.23, 70.19, 70.04, 69.82, 65.36, 64.06, 45.04, 40.74, 40.71, 32.74, 32.49, 31.94, 30.10, 29.91, 29.42, 29.29, 28.22, 26.67, 25.40, 25.38.

3. Mechanism study

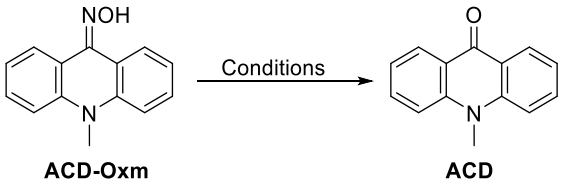
3.1 Solvent effect study

The solution of ACD-Oxm (8.0 mg, 0.036 mmol) in different solvents (10 mL) was added to a vessel and irradiated in open air under blue LEDs (430 nm, 62.2 μW cm^{-2}) at room temperature for 40-120 min. Then the reaction mixture was concentrated in vacuo. Flash chromatography on silica gel (20% EtOAc/hexanes) yielded the product ACD.

Entry	Conditions	Yield
1	Toluene, air, 120 min	78%[a]
2	Acetonitrile, air, 40 min	71%[a]
3	*t*-BuOH, air, 40 min	80%[a]
4	Acetonitrile/water (v/v 2:1), air, 40 min	65%[a]
5	DMSO, air, 50 min	85%[b]

[a] Isolated yield. [b] Yield determined by LC-Ms Assay.

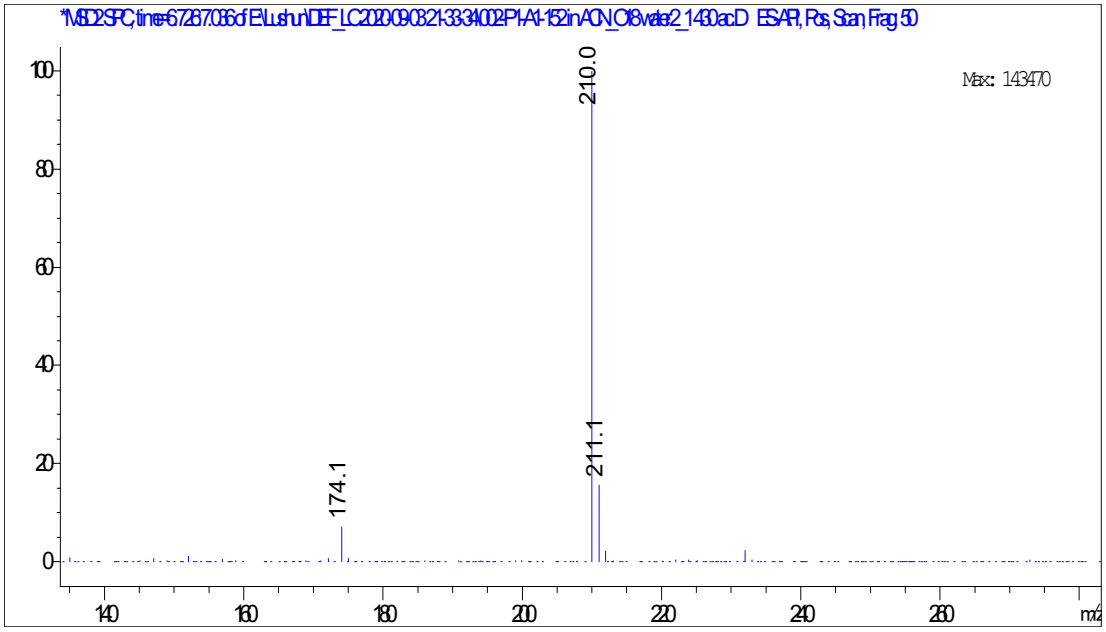
3.2 Control experiments



1) The solution of ACD-Oxm (5.0 mg, 0.022 mmol) in MeCN (5 mL) was added to a schlenk storage vessel, and then stirred at room temperature in the dark for 24 h, and no reaction occurred.

2) The solution of ACD-Oxm (5.0 mg, 0.022 mmol) in dry toluene (5 mL) was added to a schlenk storage vessel. Then the vessel was capped and degassed via freeze-pump-thaw cycle three times before irradiated under blue LEDs (430 nm, 62.2 μW cm^{-2}) for 40 min. No reaction occurred.

3) The solution of ACD-Oxm (4 mM) in MeCN/O^{18}-water (v/v = 2:1) in cuvette was irradiated under blue LEDs (430 nm, 62.2 μW cm^{-2}) for 20 min. we only observed m/z 210.0 in the LC-Ms assay, which was attributed to O^{16}-ACD.



4) The solution of ACD-Oxm (1.3 mg, 0.006 mmol) in MeCN (2 mL) in a schlenk storage vessel was added TEMPO (1.0 mg, 0.007 mmol). The mixture was stirred under irradiation of blue LEDs (430 nm, 62.2 μW cm^{-2}) in open air for 40 min. No reaction occurred.

5) The solution of ACD-Oxm (10.0 mg, 0.045 mmol) in MeCN (5 mL) in a schlenk storage vessel was added AIBN (7.4 mg, 0.045 mmol). The mixture was heated in the dark at 80 °C in open air for 2 h. Then the reaction mixture was concentrated in vacuo. Flash chromatography on silica gel (20% EtOAc/hexanes) yielded 7.6 mg ACD (80%).

6) The solution of ACD-Oxm (10.0 mg, 0.045 mmol) in MeCN (5 mL) was added to a schlenk storage vessel, and then stirred in open air at 80 ℃ in the dark for 2 h, and no reaction occurred.

4. Fluorescence quantum yield determination

Quantum yields measurements were determined using Rhodamine B (fluorescence quantum yield of 0.65 in ethanol); Quinine sulfate (fluorescence quantum yield of 0.55 in H_2SO_4 0.5 M) as a standard.

The fluorescence quantum yield, Φ_f (sample), was calculated according to the equation as following:

$$\frac{\Phi_{f,sample}}{\Phi_{f,ref}} = \frac{OD_{ref} \cdot I_{sample} \cdot d^2_{sample}}{OD_{sample} \cdot I_{ref} \cdot d^2_{ref}}$$

Φ_f: quantum yield of fluorescence;

I: integrated emission intensity;

OD: optical density at the excitation wavelength;

d: refractive index of solvents, d_{DMSO}=1.478; $d_{ethanol}$=1.36; d_{water}=1.33; $d_{DMSO/PBS}$ (v/v 3/7) =1.375; [7] $d_{DMSO/PBS}$ (v/v 7/3) =1.439. [7] Note: The effect of phosphate in the PBS buffer was not considered.

5. Photochemical quantum yield determination [8]

A 3.0 mL DMSO solution of oxime compounds (ACD-Oxm and NP-Oxm are 20 μM, Cou-Oxm, API-Oxm, BAD-Oxm, and NR-Oxm are 50 μM) in a quartz cuvette was exposed to corresponded irradiation (API-Oxm and BAD-Oxm by 405 nm, ACD-Oxm, Cou-Oxm, NP-Oxm and NR-Oxm by 430 nm) under ambient conditions. A 5 μL portion of this solution was

then subjected to LC-MS analysis. The areas of peaks from the LC trace recorded at 254 nm were integrated and the concentrations of the product were quantified by the corresponded pure oxidized compounds as reference. A total of 5 time points in an interval of 10 min were taken during the irradiation to build a trend line for appearance of photoproducts. The incident light intensity (~9.0 mW for 430 nm, ~8.0 mW for 405 nm) was measured before every measurement with a power-meter (Newport PMKIT-06-01) equipped with a Si photodiode detector (818-ST2/DB). The differences in concentration Δc were calculated from the difference in areas ΔS by

$$\Delta c = \Delta S \cdot \alpha \qquad \text{eq. 1}$$

where α is the slopes of the fitting curves, that correspond to the appearance rates of the photoproducts in μmol L^{-1} s^{-1}. The differences in molecules ΔM was obtained by

$$\Delta M = \Delta c \cdot V \cdot N_a \qquad \text{eq.2}$$

where V is the irradiated volume and N_a is Avogadro's number. The number of absorbed photons per time interval $N_{Photons}$ can be evaluated from the measured power incident intensity I_o and the energy of a photon of 430 nm and 405 nm wavelength E_p according to

$$N_{photons} = \frac{I_o(1-10^{-A})\times\Delta T}{E_{photons}} \qquad \text{eq.3}$$

where $(1\text{-}10^{-A})$ is the fraction of light absorbed (neglecting reflection of light off the cuvette surface) by the oxime solution while A is the corresponding absorbance of oxime solution at the irradiation wavelength.

The energy of a photon E_p is calculated by

$$E_{photons} = \frac{hc}{\lambda} \qquad \text{eq.4}$$

where h is Planck's constant, c, the speed of light and λ, the wavelength. Finally, the quantum yield of photoactivation Φ_o is then given by equation 5.

$$\emptyset_o = \frac{\Delta M}{N_{photons}} \qquad \text{eq.5}$$

6. DFT calculation

The ground and excited geometries of ACD/ACD-Oxm and NR/NR-Oxm were calculated with TD-DFT using the Gaussian 16 software package at the B3LYP/6-31G level. The electrostatic potential surfaces map in the ground state and excited state were calculated with the same TD-DFT at the B3LYP/6-31G level based on their optimized S1 geometries. The population analysis was performed within the Mulliken formalisms.

7. Cell Culture

HeLa and A431 cells were incubated in Dulbecco's modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C in the atmosphere containing 5% CO2. CHO-K1 cells were incubated in F-12K supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C in the atmosphere containing 5% CO2.

8. CCK-8 Assay

For the dark cytotoxicity: HeLa cells were seeded in flat-bottomed 96-well plates with the number of 1×10^4 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 BAD-Oxm. 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. For the phototoxicity comparison between oxime-caged and thio-caged probes, HeLa cells were seeded in flat-bottomed 96-well plates, 1×10^4 cells per well, with 200 μL complete culture media for 24 h. After washed with PBS for three times, the cells were incubated with

different concentrations of photosensitizers for 2 h. All stock solutions were prepared in DMSO (2 mM) and diluted with complete medium. Then the cells were washed with PBS (pH 7.4) three times, incubated in the phenol red-free culture media, and then get irradiated with white light (Prior Lumen200, 0.4 μW·cm^{-2}) or green light (Prior Lumen200, filter 500/20, 0.4 μW·cm^{-2}) or red light (Prior Lumen200, filter 615/30, 0.4 μW·cm^{-2}) for 30 minutes. Then the cell culture media is changed back by complete culture media. After cultured for 24 h, 10 μL Cell Counting Kit-8 (CCK-8) solution and 90 μl PBS (pH 7.4) were added per well simultaneously. After 1-2 hours, the absorbance at 450 nm was read by 96-well plates reader.

The viability of HeLa cells was calculated by the following equation:

$$CV = (As-Ab) / (Ac-Ab) \times 100\%$$

For the dark cytotoxicity, CV represents the viability of cells. As, Ac and Ab stand for the absorbance of cells containing BAD-Oxm cell control (0 μM) and blank control (wells containing neither cells nor BAD-Oxm).

For the phototoxicity, CV stands for the viability of cells, As, Ac and Ab stand for the absorbance of cells containing photosensitizer, cell control (0 μM photosensitizer), and blank control (wells containing neither cells nor photosensitizer).

9. Co-localization assay

A stock solution of BAD-Oxm and organelle targeting derivatives in chromatographic grade, anhydrous DMSO was prepared to a concentration of 2 mM. The solution was diluted to a final concentration of 2-10 μM by complete growth medium. Commercial MitoView™ 633, ERTracker™ Red (BODIPY™ TR Glibenclamide, LysoView™ 633, DRAQ5, and Nile Red were prepared to a concentration of 1 mM, and the stock solution was diluted to the working concentration in complete medium (100 nM). After incubation of BAD-Oxm-MOR, BAD-Oxm-Ts, and BAD-Oxm-TPP for 30 minutes under dark conditions, cells were washed with PBS (pH 7.4) twice and turned to confocal laser scanning microscope (CLSM). Cell imaging was performed on Zeiss LSM 800 confocal laser microscope with 63× immersion lens. BAD-Oxm dyes

were activated using 405 nm light at 70% laser intensity. Differential interference contrast (DIC) and fluorescent images were processed and analyzed using ImageJ. The Pearson's Coefficient was calculated by ImageJ.

10. H2B-HaloTag expression

Plasmids were purchased from addgene (Plasmid #91564). Transfection of plasmid was done in CHO-K1 cells by X-tremeGENE™ Transfection Reagents (Sigma) according to their protocol.

11. PALM image acquisition and analysis

Prior to PALM imaging, cells were washed and incubated with 5 μM BAD-Oxm-Halo in DMEM complete media for 1 hour in cell incubator before washing and subsequent imaging in 1x PBS (pH 7.4). Imaging was performed at room temperature on the Nikon n-STORM system, featuring a CFI HP Apo TIRF AC 100x oil objective (NA 1.49) on an inverted Nikon Ti Eclipse microscope with a quad cube filter (Chroma, zt405/488/561/640 m-TRF), piezo stage, and Perfect Focus System (Nikon) for Z-stability. Lasers used in this study: 50 mW 405 nm diode laser and 200 mW 561 nm solid-state lasers within an agilent MLC400B laser combiner with AOTF modulation.

PALM imaging was controlled with NIS-Elements Ar software and captured by an Andor iXON DU 897 EMCCD camera (EM gain setting=100, pixel size = 160 nm, 512 × 512 pixel field) with a cylindrical lens inserted in the light path (to introduce astigmatism and improve signal-to-noise). For BAD-Oxm-Halo, maximum 405/488 nm laser power (simultaneous) was used to photobleach fluorescence, and acquisition frames were collected in the 488 nm channel upon observing spontaneous reactivation fluorescent events within the nucleus. Imaging frames were collected for a total of 20,000 – 30,000 frames.

12. Supplementary Figures and Tables

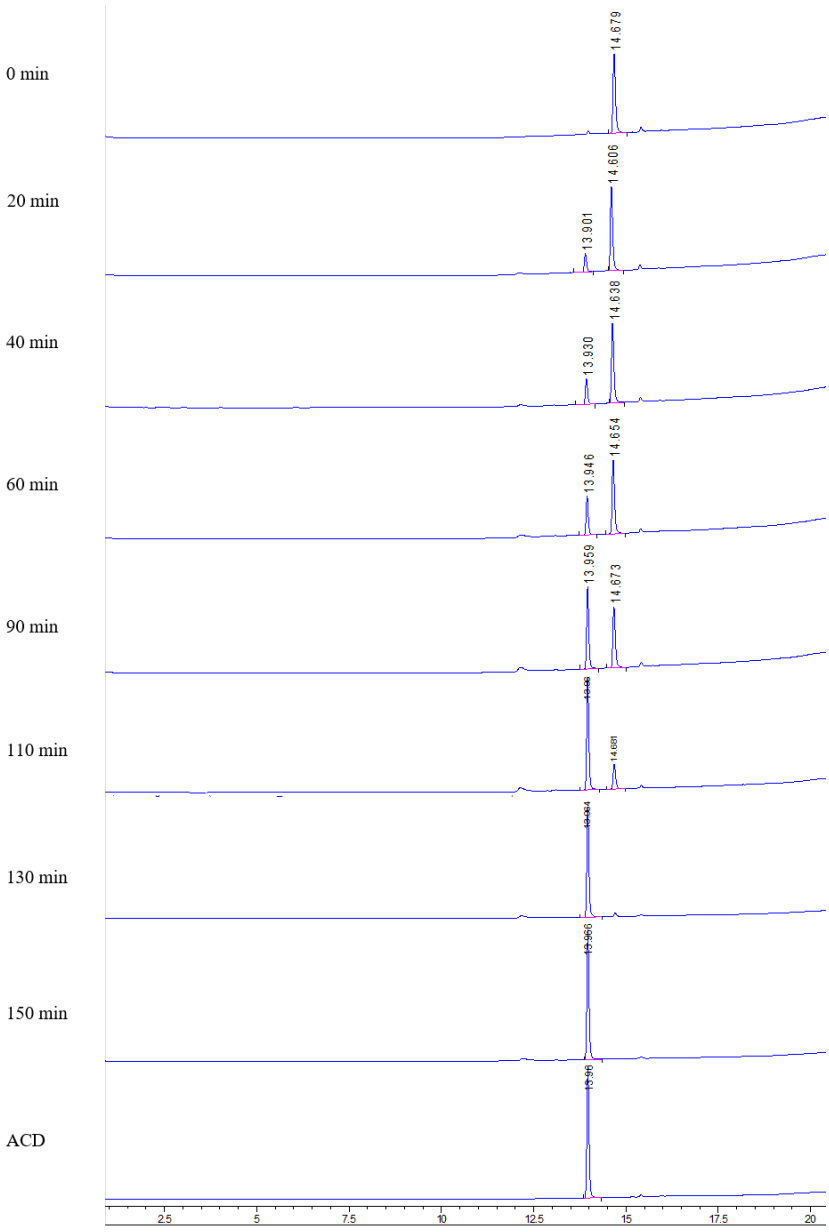
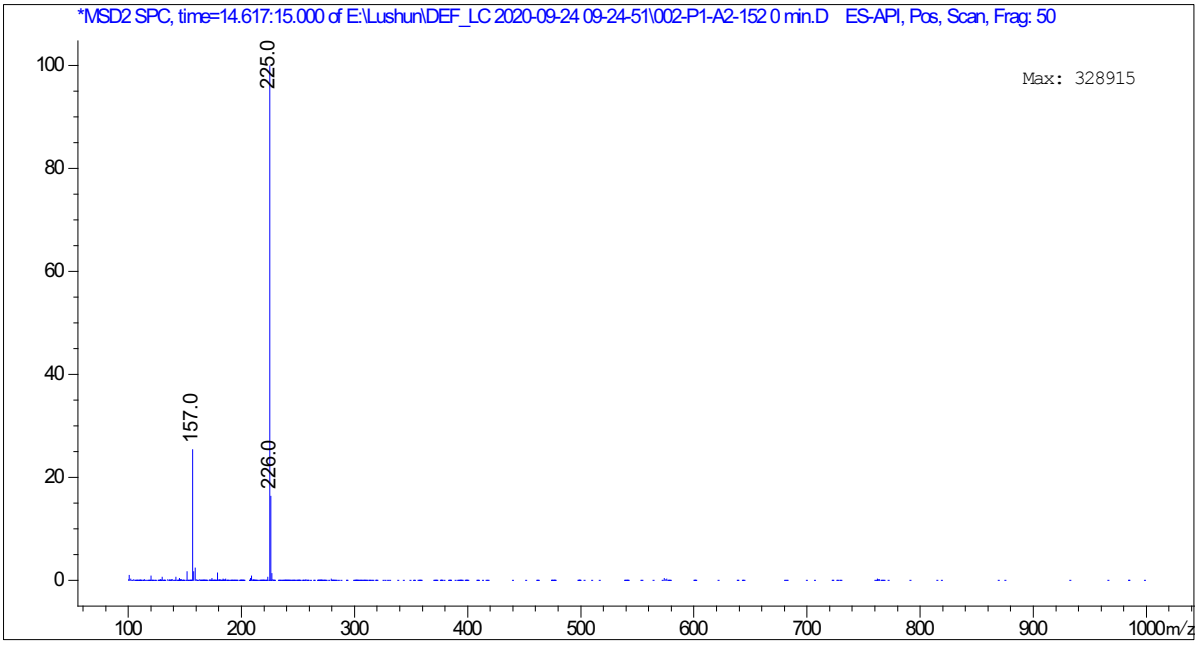
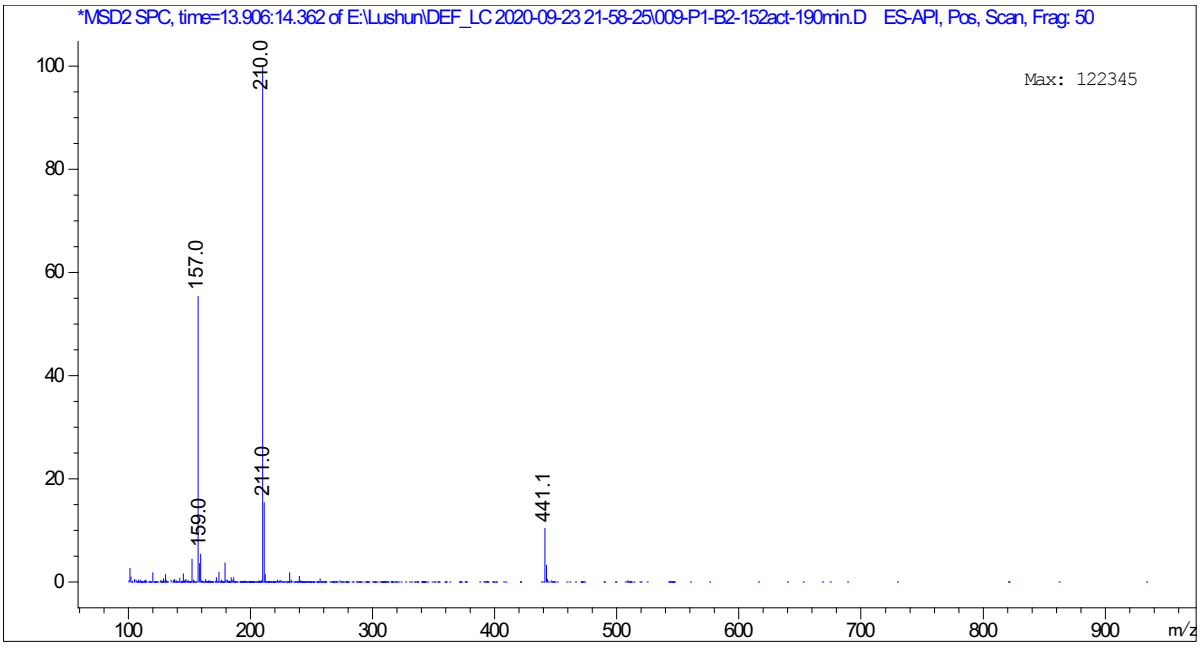


Figure S1. Overlay of HPLC spectra of ACD-Oxm (20 μM in DMSO) taken at the indicated light irradiation times (430 nm, 62.2 μW cm^{-2})



(a)



(b)

Figure S2. ESI-MS of ACD-Oxm before (a) and after (b) irradiation.

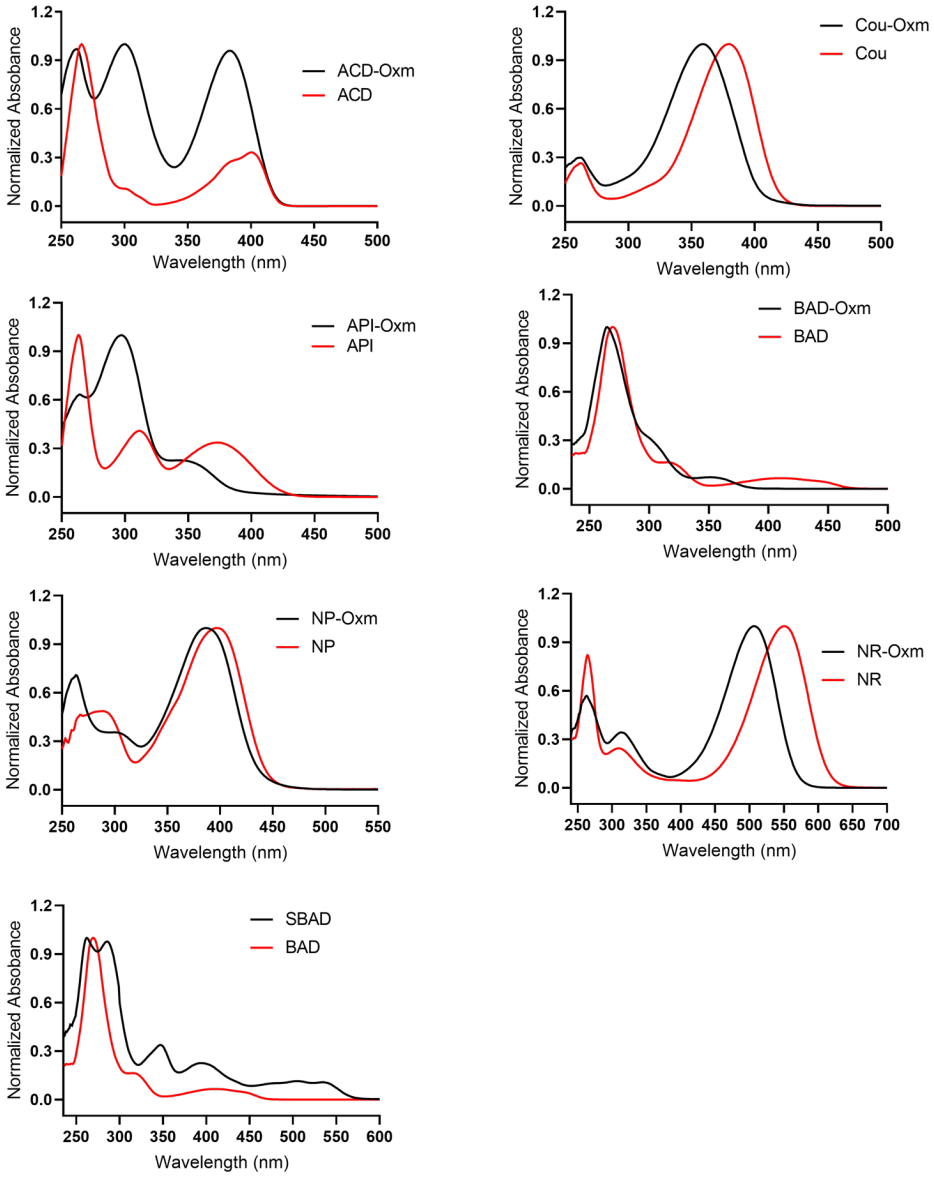


Figure S3. UV-vis absorption spectra of the oxime-caged fluorophores in DMSO.

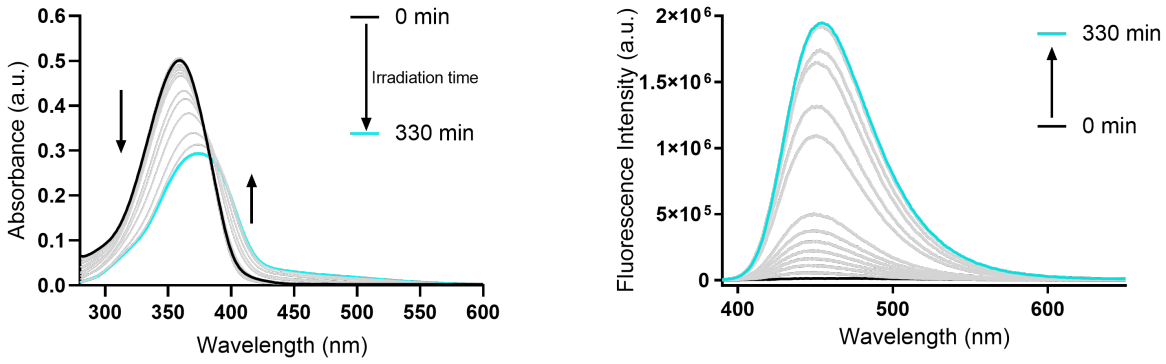
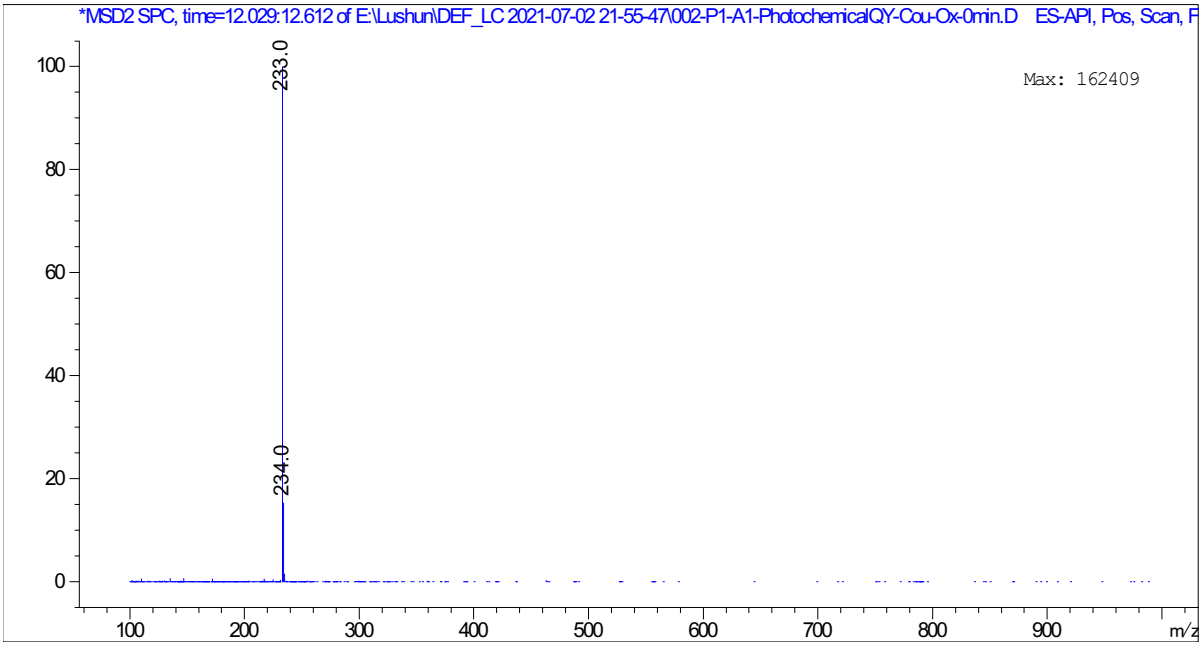
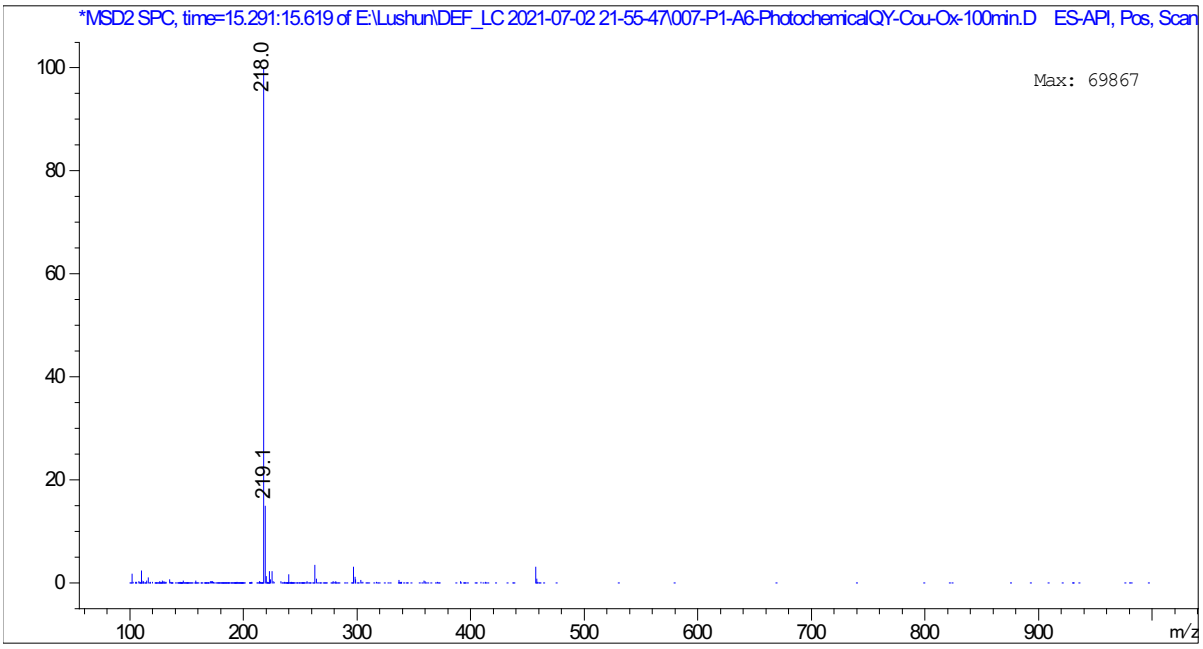


Figure S4. Photoactivation of Cou-Oxm (50 μM in DMSO, 430 nm, 62.2 μW cm^{-2}).



(a)



(b)

Figure S5. ESI-MS of Cou-Oxm before (a) and after (b) irradiation.

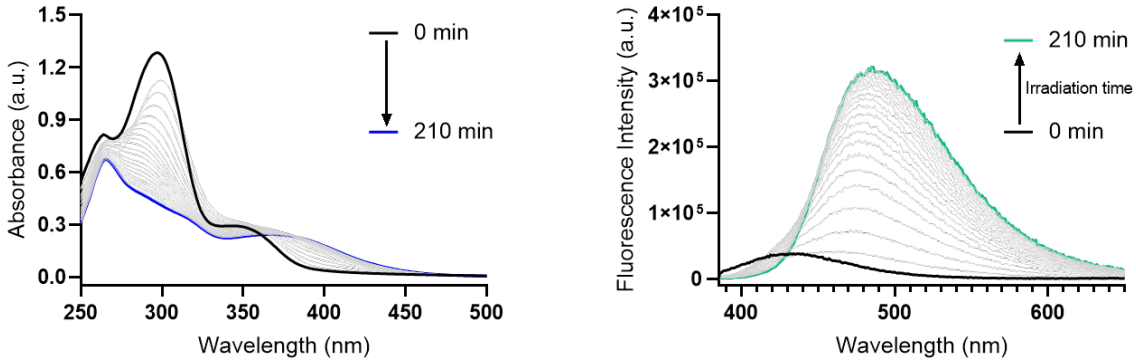


Figure S6. Photoactivation of API-Oxm (50 uM in DMSO, 405 nm, 70.0 μW cm^{-2}).

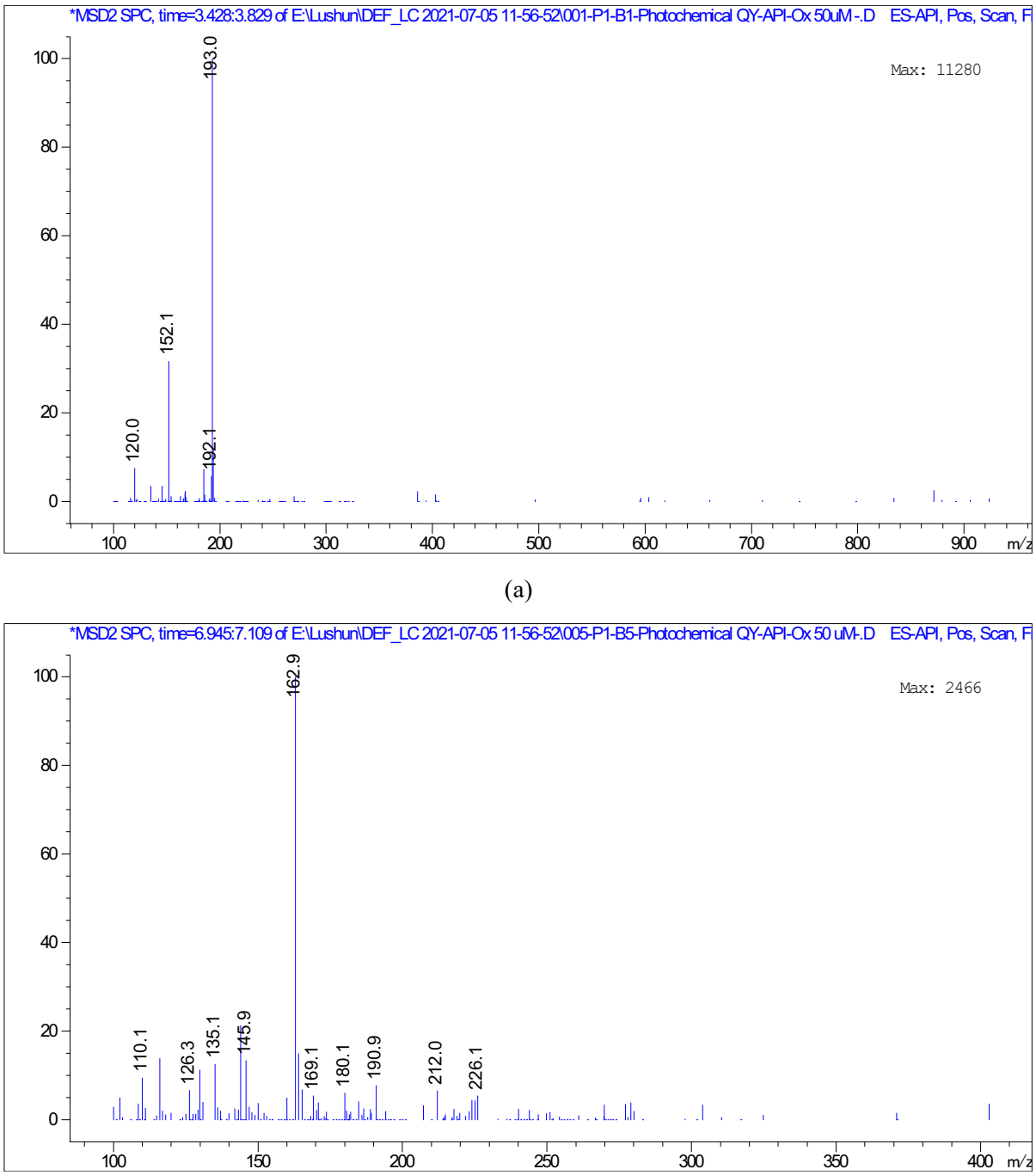


Figure S7. ESI-MS of API-Oxm before (a) and after (b) irradiation.

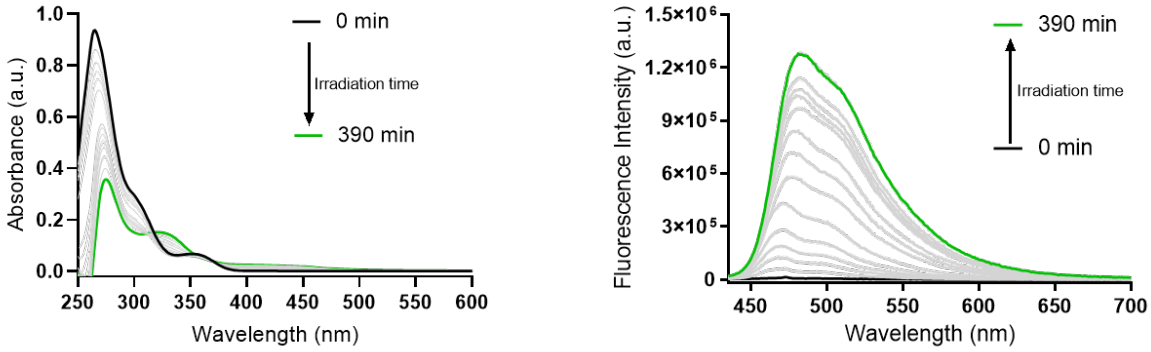
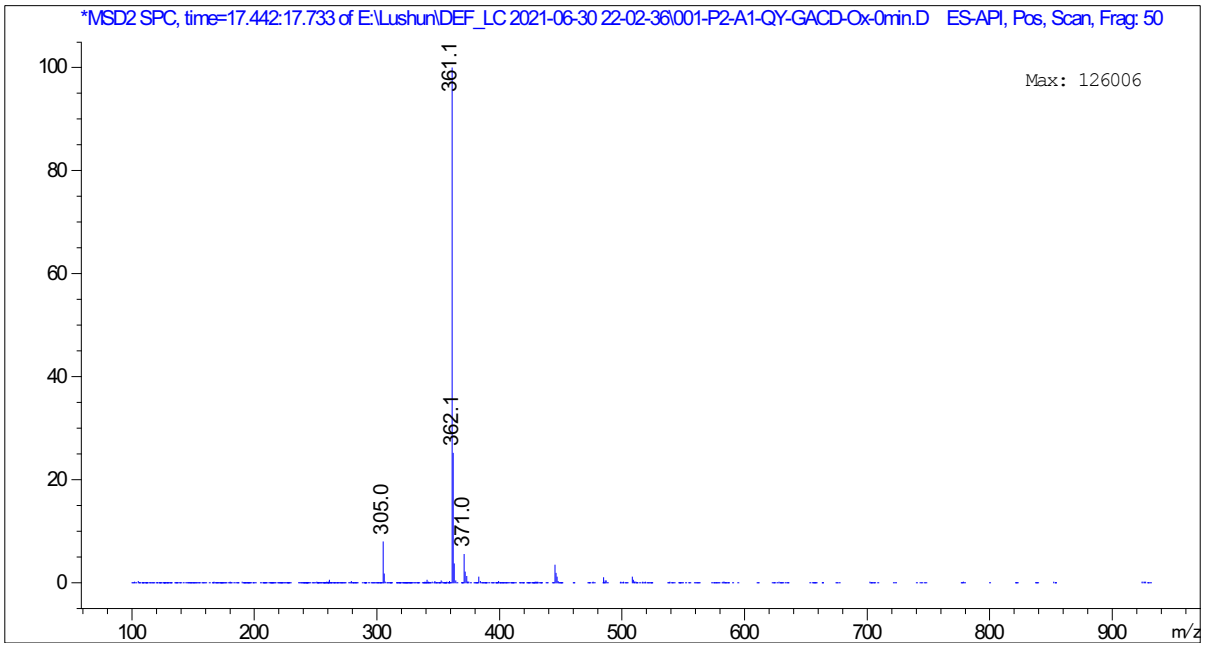
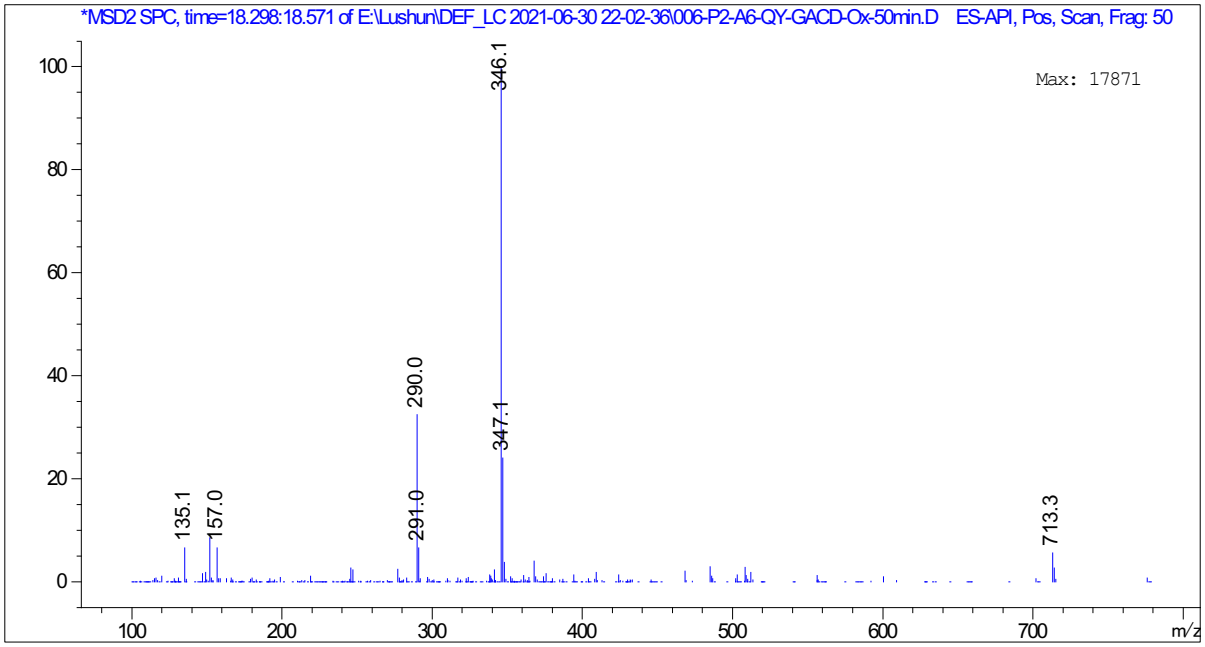


Figure S8. Photoactivation of BAD-Oxm (50 μM in DMSO, 430 nm, 62.2 μW cm^{-2}).



(a)



(b)

Figure S9. ESI-MS of BAD-Oxm before (a) and after (b) irradiation.

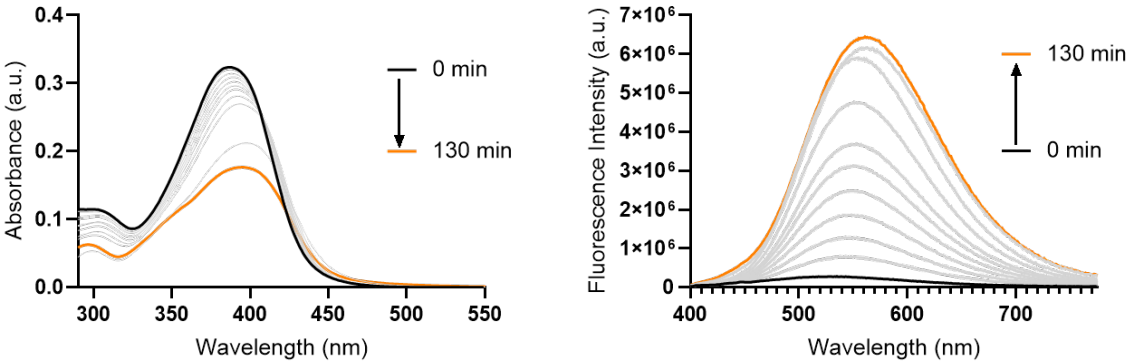
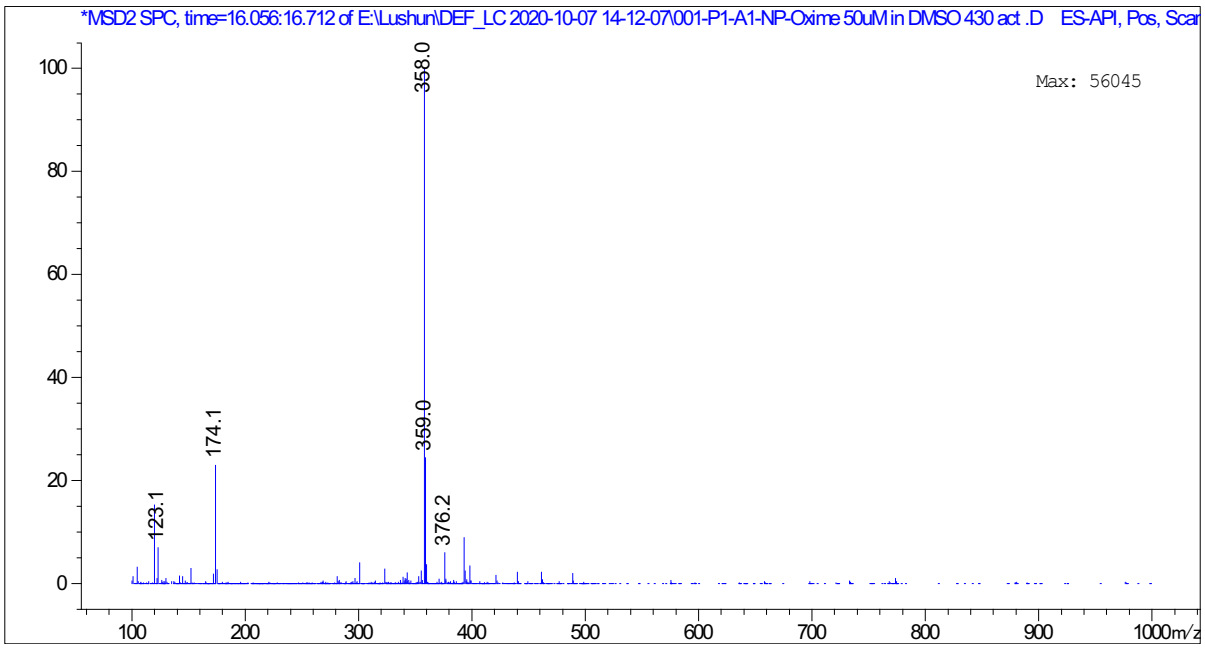
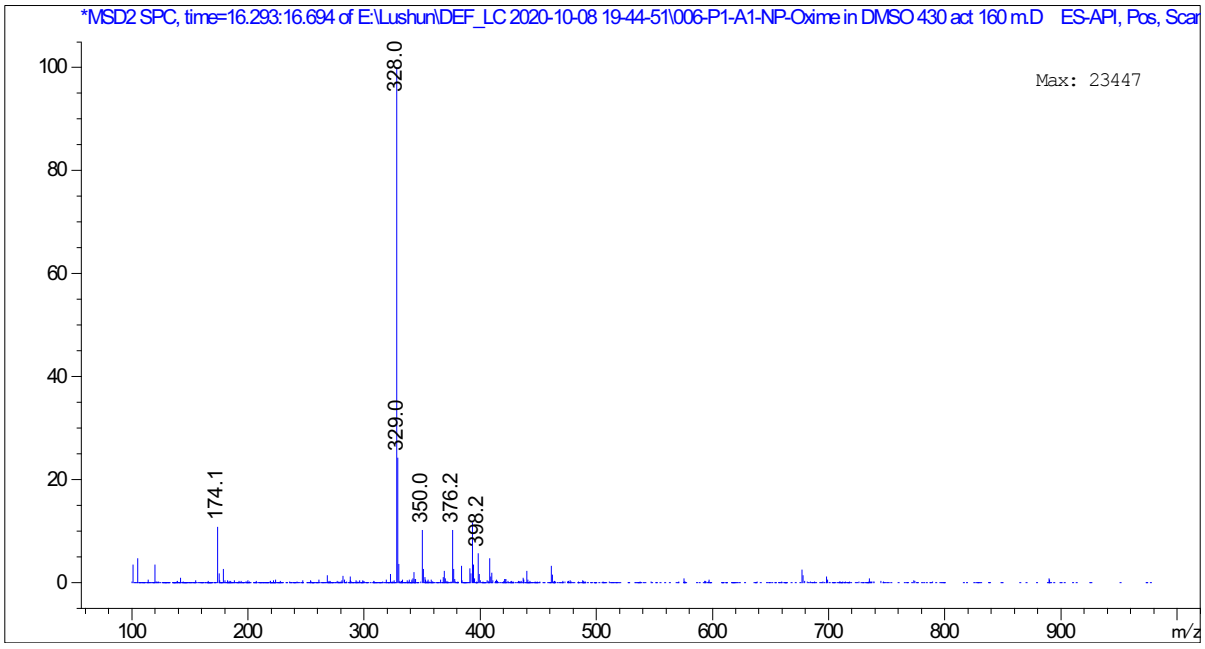


Figure S10. Photoactivation of NP-Oxm (50 μM in DMSO, 430 nm, 62.2 μW cm^{-2}).



(a)



(b)

Figure S11. ESI-MS of NP-Oxm before (a) and after (b) irradiation.

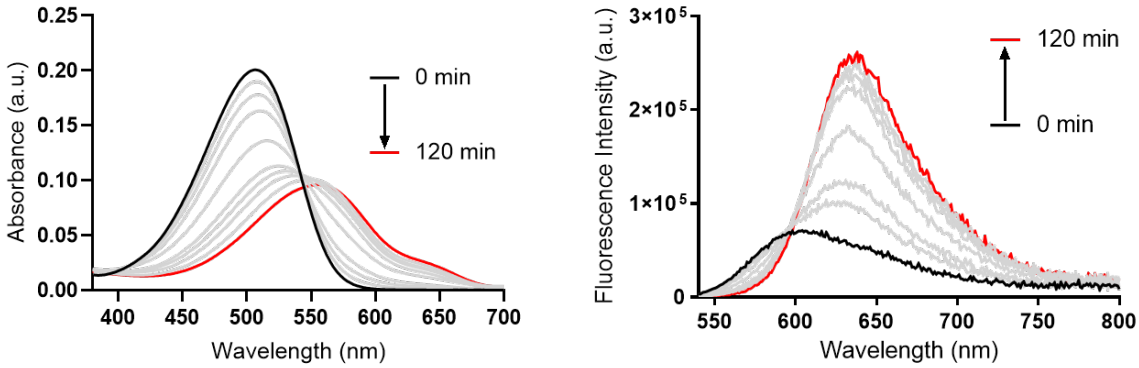
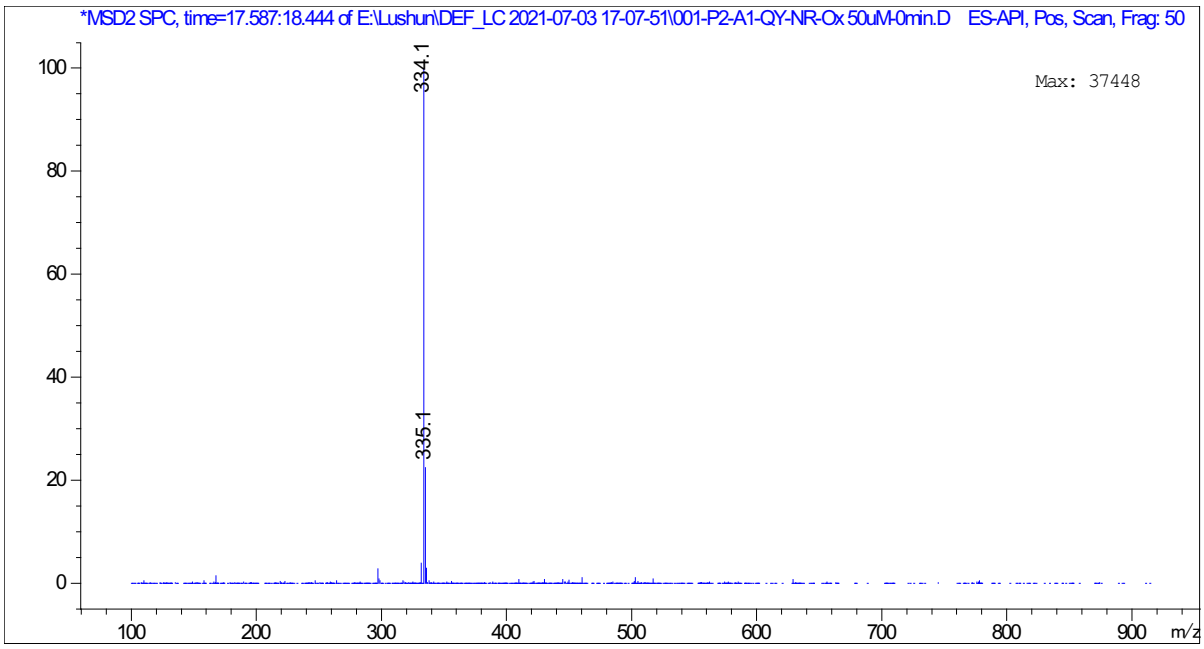
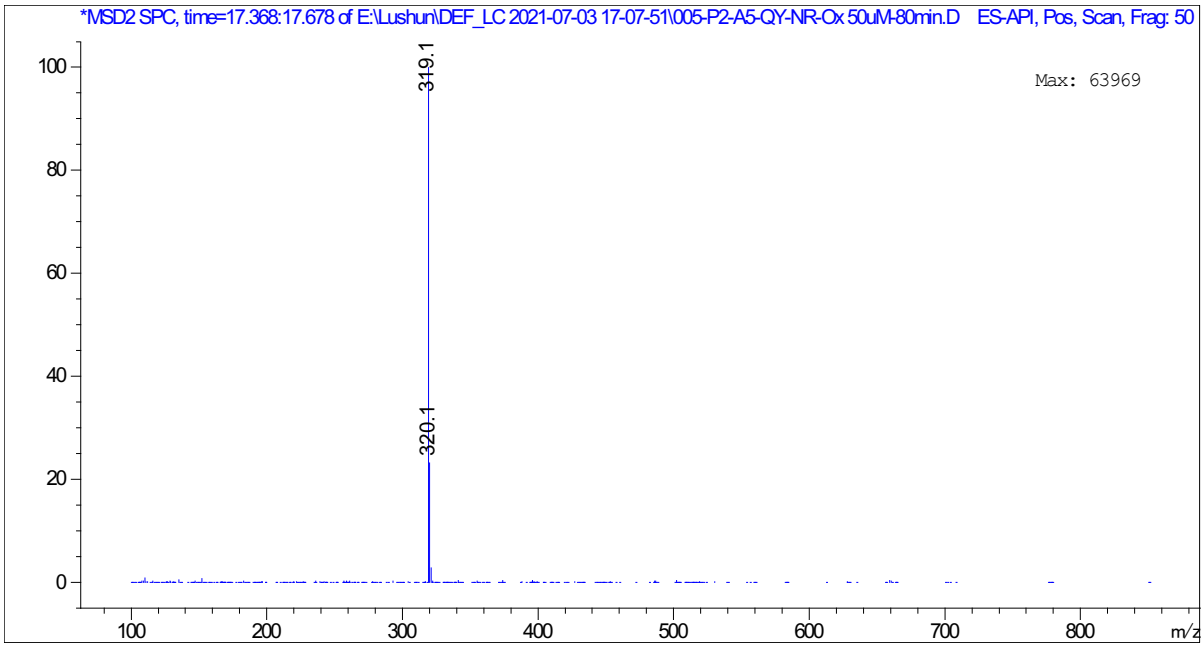


Figure S12. Photoactivation of NR-Oxm (50 μM in DMSO, 520-525 nm, 25.0 μW cm^{-2}).

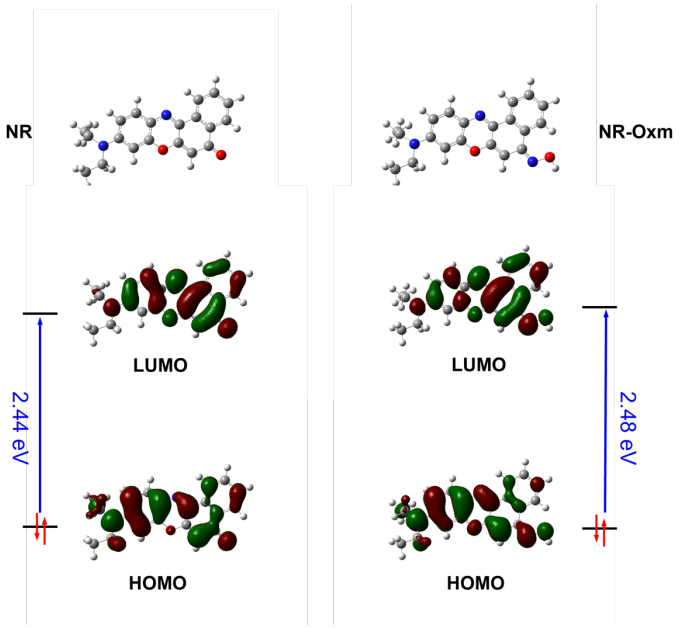


(a)



(b)

Figure S13. ESI-MS of NR-Oxm before (a) and after (b) irradiation.



Compound	ΔE_1(eV) [a] /λ_{max}(nm) [b]	Character	OI [c]	*f*
NR	2.44/507.91	ICT	H→L	0.9352
NR-Oxm	2.48/499.12	ICT	H→L	0.8695

[a] Peak position of the longest absorption band; [b] the energy gap of the absorption band;
[c] TD-DFT method with the orbitals Involved (OI).

Figure S14. Frontier molecular orbital energy levels and electron density of HOMO/LUMO of NR (left) and NR-Oxm (right).

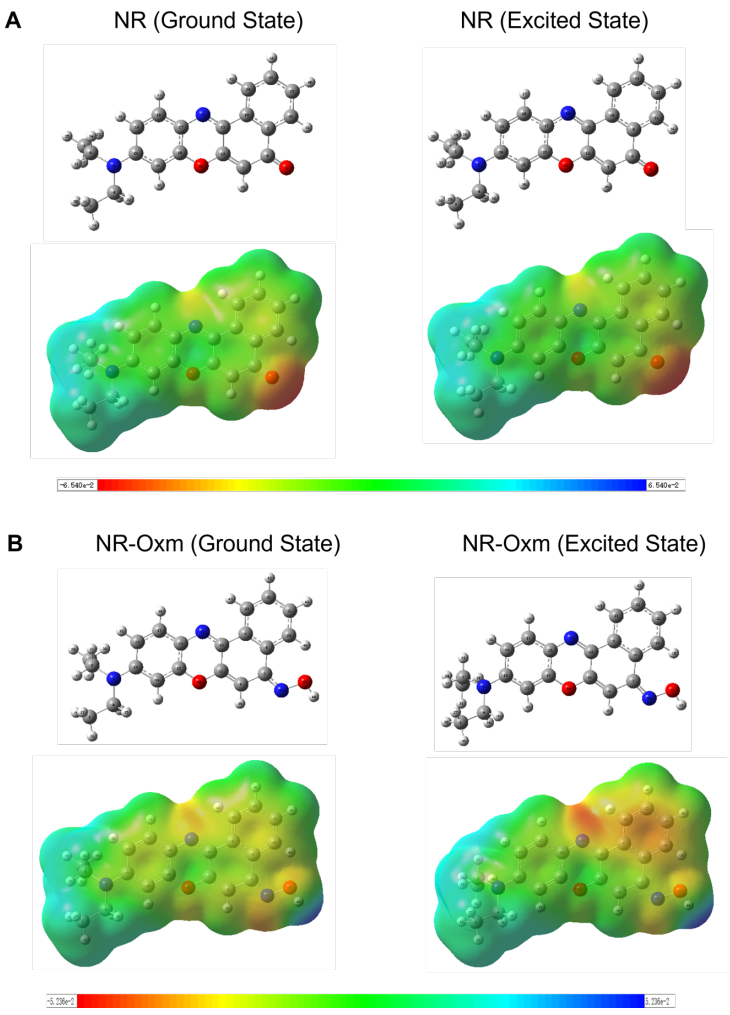
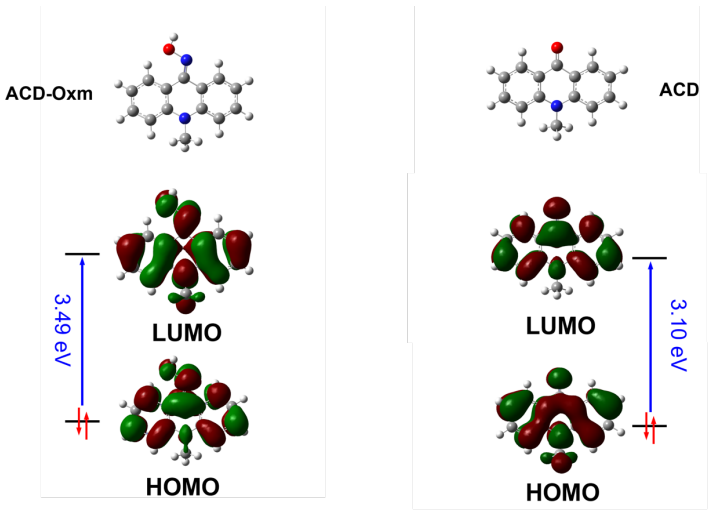


Figure S15. Electrostatic potential map of NR and NR-Oxm in the ground state (left), and in excited state (right).



Compound	ΔE_1(eV) [a] /λ_{max}(nm) [b]	Character	OI [c]	*f*
ACD-Oxm	3.49/354.8	--	H→L	0.1221
ACD	3.10/400.0	ICT	H→L	0.0791

[a] Peak position of the longest absorption band; [b] the energy gap of the absorption band;
[c] TD-DFT method with the orbitals Involved (OI).

Figure S16. Frontier molecular orbital energy levels and electron density of HOMO/LUMO of ACD-Oxm (left) and ACD (right)

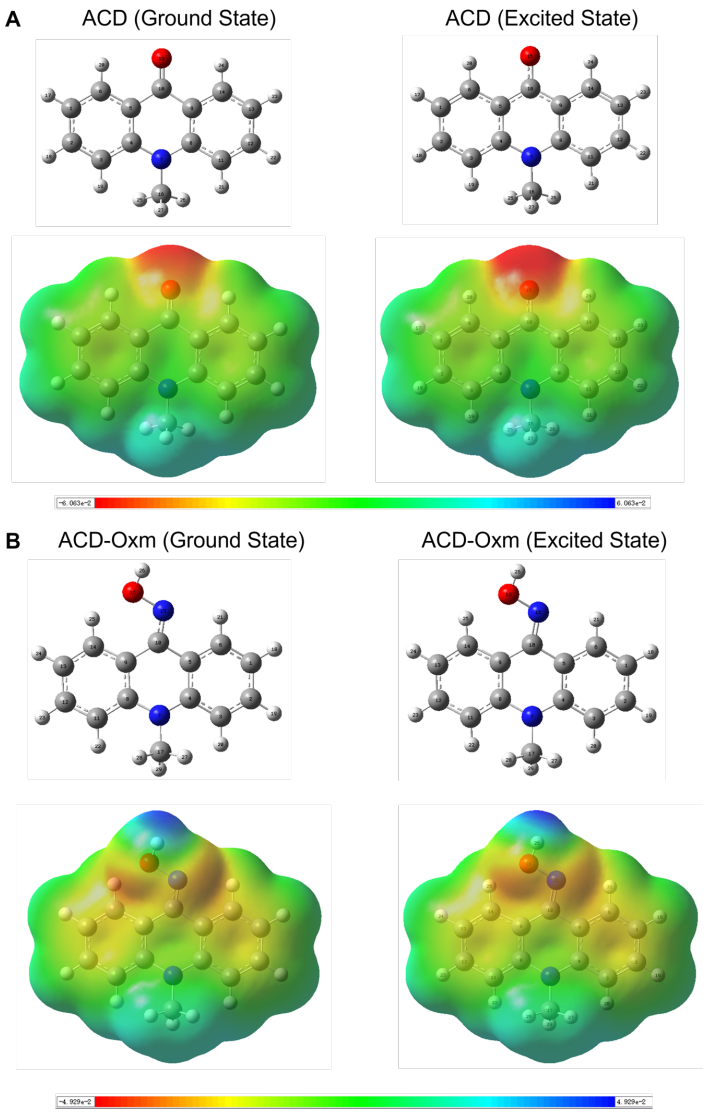


Figure S17. Electrostatic potential map of ACD and ACD-Oxm in the ground state (left), and in excited state (right).

Table S1. Atomic charges for optimized ground state and excited state of NR at B3LYP/6-31G (d, p) level.

Atom No.	Atomic charge (e)		Atom No.	Atomic charge (e)	
	S_0	S_1		S_0	S_1
1 O	-0.518893	-0.528054	**22 C**	-0.082956	-0.089843
2 C	-0.318318	-0.318165	**23 C**	-0.087718	-0.086654
3 C	-0.354912	-0.355172	**24 C**	-0.108411	-0.1088
4 C	-0.021213	-0.026964	**25 H**	0.110345	0.115091
5 C	-0.06615	-0.07211	**26 H**	0.124104	0.12744
6 N	-0.520107	-0.500461	**27 H**	0.112698	0.116132
7 C	-0.223832	-0.195932	**28 H**	0.119002	0.122993
8 C	0.393203	0.379813	**29 H**	0.125469	0.127829
9 C	0.007011	0.012604	**30 H**	0.120161	0.122134
10 C	0.055561	0.038782	**31 H**	0.109113	0.115208
11 O	-0.550721	-0.535938	**32 H**	0.124473	0.1307
12 C	0.375969	0.354616	**33 H**	0.113913	0.119242
13 C	0.237728	0.217854	**34 H**	0.116635	0.121015
14 N	-0.573442	-0.618086	**35 H**	0.106889	0.112443
15 C	-0.203364	-0.194948	**36 H**	0.102383	0.104274
16 C	0.324096	0.301124	**37 H**	0.105377	0.109276
17 C	0.258435	0.309277	**38 H**	0.086969	0.093679
18 C	-0.122591	-0.132395	**39 H**	0.117987	0.114026
19 C	-0.125894	-0.106255	**40 H**	0.090412	0.0847
20 C	0.358362	0.339329	**41 H**	0.089286	0.084378
21 C	-0.117857	-0.113816	**42 H**	0.110795	0.109631

Table S2. Atomic charges for optimized ground state and excited state of NR-Oxm at B3LYP/6-31G (d, p) level.

Atom No.	Atomic charge (e)		Atom No.	Atomic charge (e)	
	S_0	S_1		S_0	S_1
1 C	-0.352692	-0.343592	**23 C**	-0.107426	-0.119125
2 C	-0.318282	-0.321796	**24 N**	-0.317124	-0.314141
3 C	-0.064299	-0.067169	**25 O**	-0.410913	-0.3869
4 C	-0.018213	-0.027088	**26 H**	0.115159	0.114125
5 N	-0.519793	-0.495599	**27 H**	0.123656	0.124144
6 C	-0.173199	-0.141677	**28 H**	0.117838	0.115601
7 C	0.274432	0.268696	**29 H**	0.106705	0.110473
8 C	0.081953	0.077304	**30 H**	0.124418	0.124508
9 C	0.019183	0.015173	**31 H**	0.11023	0.111164
10 O	-0.566015	-0.515772	**32 H**	0.11005	0.113127
11 C	0.349867	0.345369	**33 H**	0.114391	0.113167
12 C	0.261541	0.209864	**34 H**	0.103767	0.098582
13 N	-0.59311	-0.616464	**35 H**	0.122205	0.121637
14 C	-0.208061	-0.187475	**36 H**	0.107182	0.121086
15 C	0.32552	0.285967	**37 H**	0.096389	0.099471
16 C	0.256826	0.306354	**38 H**	0.098451	0.104454
17 C	-0.127934	-0.127439	**39 H**	0.080792	0.094632
18 C	-0.128595	-0.103413	**40 H**	0.127758	0.113578
19 C	0.353561	0.305777	**41 H**	0.086474	0.073298
20 C	-0.126871	-0.129715	**42 H**	0.087679	0.077134
21 C	-0.087816	-0.106284	**43 H**	0.115109	0.104127
22 C	-0.08536	-0.082964	**44 H**	0.334569	0.337801

Table S3. Atomic charges for optimized ground state and excited state of ACD-Oxm at B3LYP/6-31G (d, p) level.

Atom No.	Atomic charge (e)		Atom No.	Atomic charge (e)	
	S_0	S_1		S_0	S_1
1 C	-0.092962	-0.083812	**16 O**	-0.41886	-0.393744
2 C	-0.094372	-0.096763	**17 C**	-0.184284	-0.195467
3 C	-0.123718	-0.125409	**18 H**	0.082938	0.09157
4 C	0.287698	0.281535	**19 H**	0.084538	0.089337
5 C	0.054825	0.066618	**20 H**	0.085066	0.090668
6 C	-0.11658	-0.129019	**21 H**	0.105951	0.116642
7 N	-0.640734	-0.59064	**22 H**	0.085786	0.077014
8 C	0.294084	0.268164	**23 H**	0.085513	0.072758
9 C	0.064043	0.075494	**24 H**	0.083102	0.077814
10 C	0.271454	0.274051	**25 H**	0.121429	0.105078
11 C	-0.127487	-0.135509	**26 H**	0.331099	0.33145
12 C	-0.089255	-0.124572	**27 H**	0.13008	0.140773
13 C	-0.099968	-0.075421	**28 H**	0.130254	0.149255
14 C	-0.113053	-0.146207	**29 H**	0.121103	0.143265
15 N	-0.317689	-0.35492			

Table S4. Atomic charges for optimized ground state and excited state of ACD at B3LYP/6-31G (d, p) level.

Atom No.	Atomic charge (e)		Atom No.	Atomic charge (e)	
	S_0	S_1		S_0	S_1
1 C	-0.094506	-0.094305	**15 O**	-0.52388	-0.580032
2 C	-0.087782	-0.083993	**16 C**	-0.186023	-0.188506
3 C	-0.129027	-0.13352	**17 H**	0.089764	0.09147
4 C	0.312035	0.317881	**18 H**	0.090582	0.093643
5 C	0.016058	0.038901	**19 H**	0.089426	0.089731
6 C	-0.111777	-0.101245	**20 H**	0.120573	0.126601
7 N	-0.657825	-0.656241	**21 H**	0.089428	0.089734
8 C	0.312042	0.317885	**22 H**	0.090582	0.093643
9 C	0.016057	0.038905	**23 H**	0.089764	0.09147
10 C	0.38049	0.335955	**24 H**	0.120573	0.126601
11 C	-0.129032	-0.133527	**25 H**	0.133384	0.134163
12 C	-0.087781	-0.083995	**26 H**	0.13338	0.134163
13 C	-0.094507	-0.094306	**27 H**	0.12978	0.130169
14 C	-0.111776	-0.101245			

Table S5. Mulliken atomic charges on donor and acceptor domains for ACD/ACD-Oxm, NR/NR-Oxm at B3LYP/6-31G (d, p) level.

Compounds		Mulliken atomic charges		
		S_0	S_1	△
ACD	Donor unit	0.143392	0.244077	0.100685
	Acceptor unit	-0.14339	-0.24408	-0.10069
ACD-Oxm	Donor unit	0.133997	0.143166	0.009169
	Acceptor unit	-0.134	-0.14316	-0.00917
NR	Donor unit	0.785875	0.880716	0.094841
	Acceptor unit	-0.78588	-0.88072	-0.09484
NR-Oxm	Donor unit	0.729271	0.790698	0.061427
	Acceptor unit	-0.729269	-0.7907	-0.06143

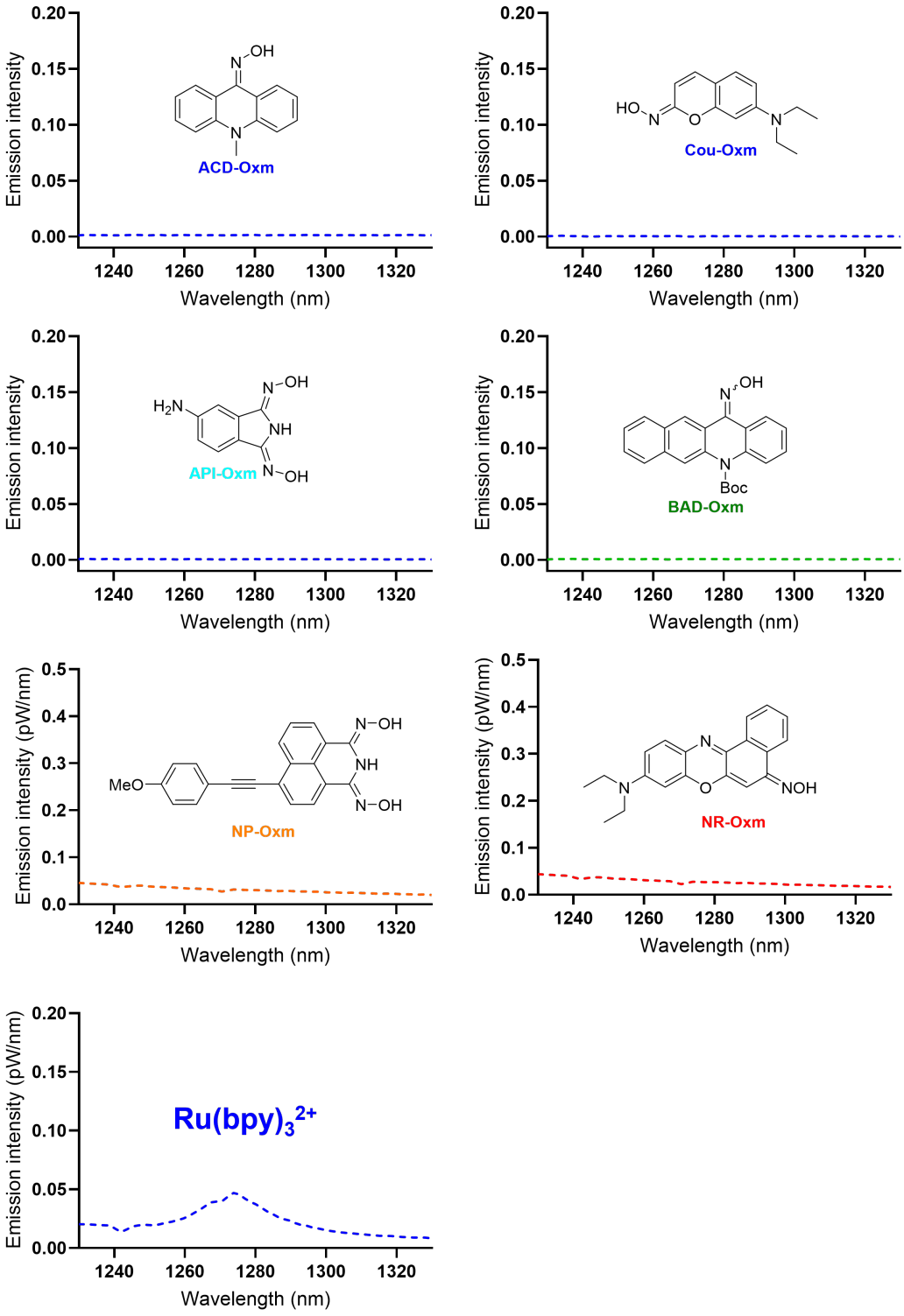


Figure S18. Near-IR phosphorescence spectra of singlet oxygen generated by excitation of oxime-caged probes and the reference ($Ru(bpy)_3^{2+}$) in oxygen-saturated methanol with 405 nm laser pulses at 25 °C. The sample solutions were prepared at a concentration of 50 μM.

Table S6. Photophysical Data of Oxime-Caged and Uncaged Fluorophores in buffer.

Compounds	λ_{abs} (nm)	ε^c ($*10^4$ M^{-1} cm^{-1})	λ_{em} (nm)	$\Phi_f{}^d$	Brightness
ACD-Oxm (**1**) [a]	291, 385	0.26, 0.23	450	0.002	5
ACD (**2**) [a]	406	1.40	429, 455	0.50	7,000
Cou-Oxm (**3**) [a]	363	2.46	477	0.005	123
Cou (**4**) [a]	390	2.13	478	0.13	2,769
API-Oxm (**5**) [a]	291, 348	2.29, 0.55	495	0.04	220
API (**6**) [a]	308, 374	0.79, 0.86	555	0.12	1,032
BAD-Oxm (**7**) [a]	302, 355	0.54, 0.14	471	0.003	4
BAD (**8**) [a]	325, 439	0.62, 0.33	514	0.29	957
NP-Oxm (**9**) [b]	303, 389	0.65, 1.60	505	0.009	144
NP (**10**) [b]	401	0.86	587	0.07	602
NR-Oxm (**11**) [b]	514	2.80	622	0.06	1680
NR (**12**) [b]	568	3.77	654	0.38	14326

[a] Compounds were dissolved in pH 7.4 buffer (DMSO/PBS V/V 30/70). [b] Compounds were dissolved in pH 7.4 buffer (DMSO/PBS V/V 70/30). [c] ε: molar extinction coefficients. [d] Fluorescence quantum yields were measured using rhodamine B in ethanol or quinine sulfate in 0.5 M H_2SO_4 as the reference

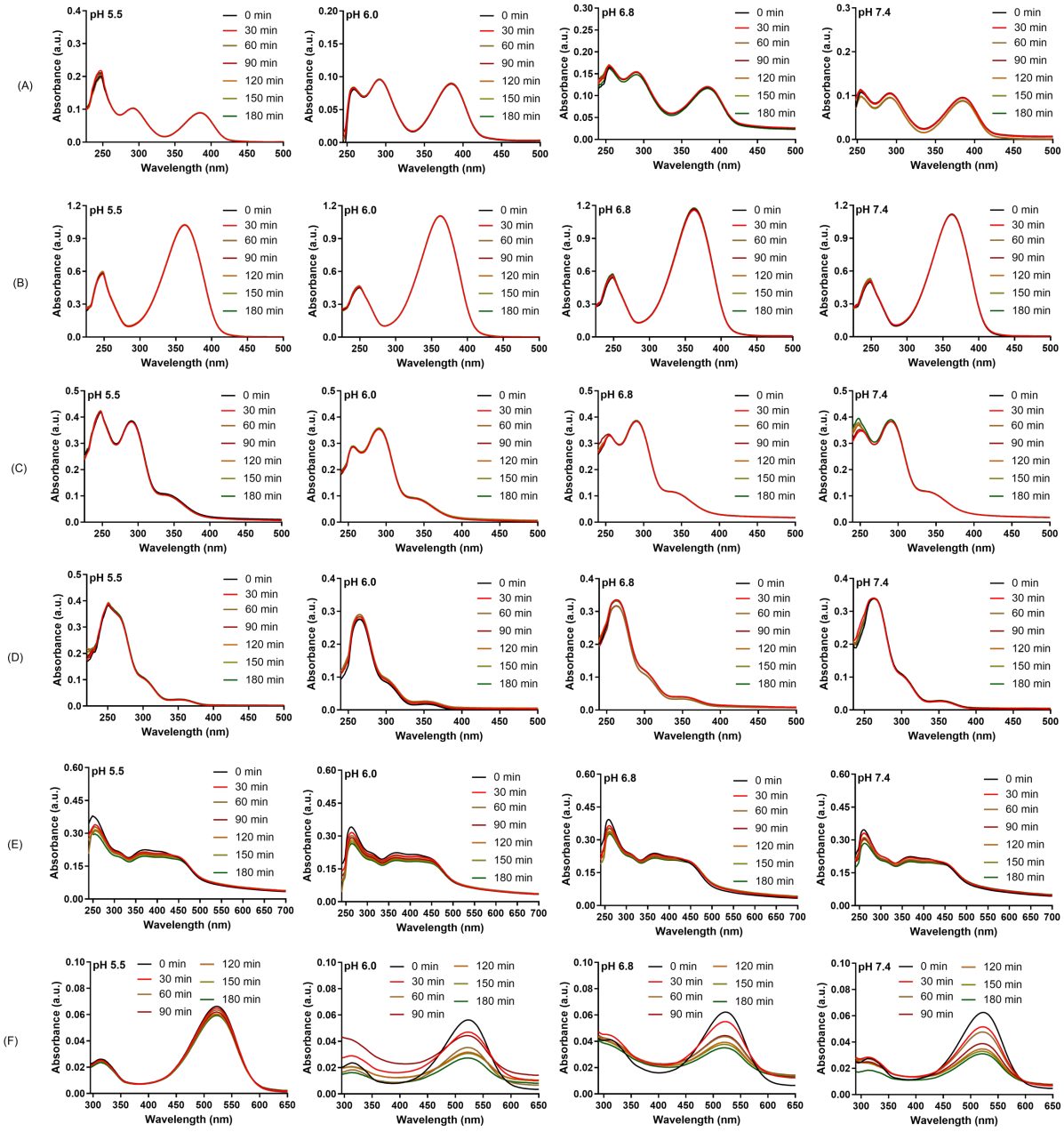


Figure S19. Dark stability of oxime-based probes in DMSO/PBS buffer (V/V 30/70) at different pH under ambient air atmosphere without light based on UV−vis absorption change. (A) ACD-Oxm; (B) Cou-Oxm; (C) API-Oxm; (D) BAD-Oxm; (E) NP-Oxm; (F) NR-Oxm.

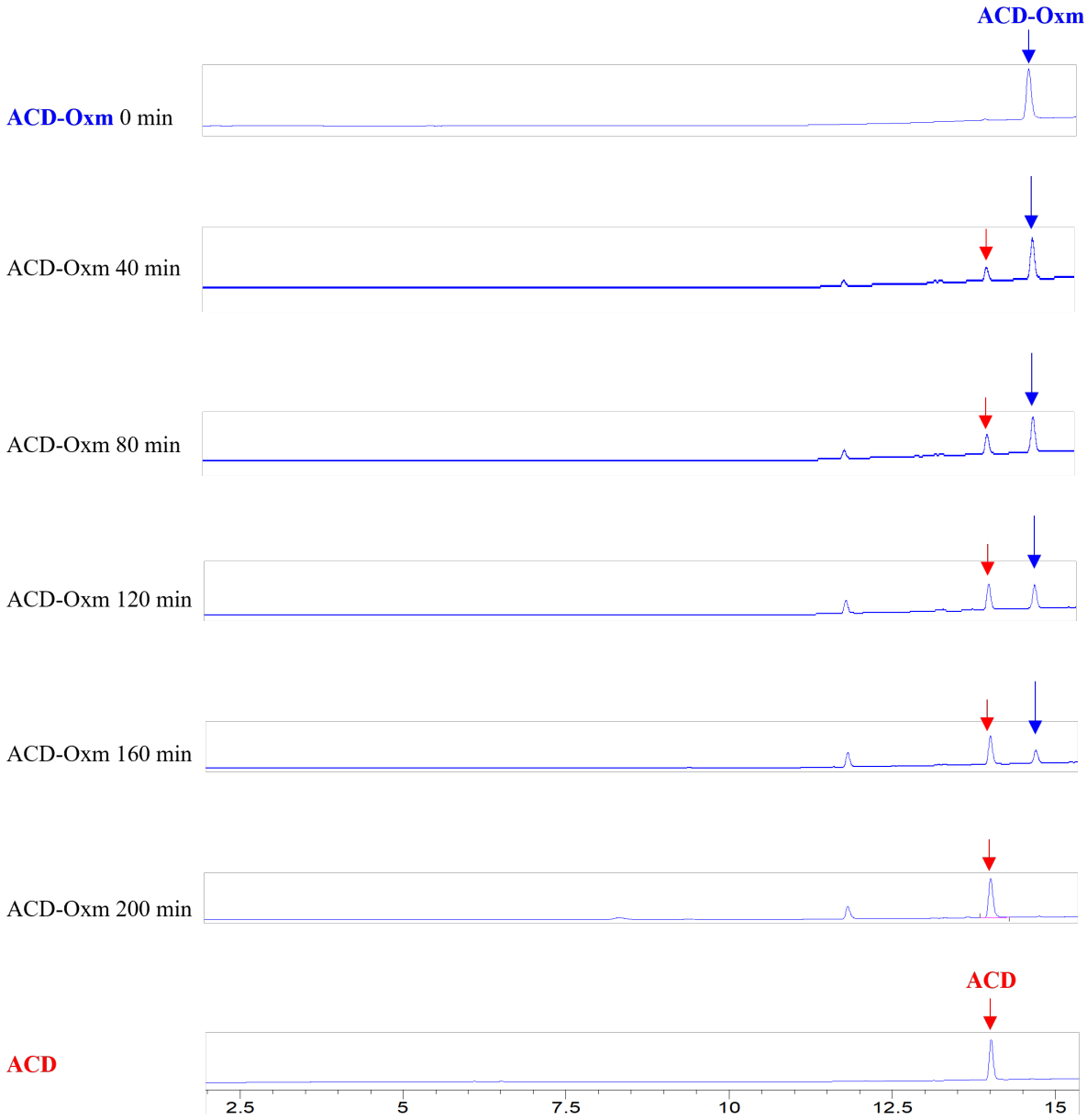


Figure S20. Overlay of HPLC spectra of ACD-Oxm (20 μM in pH 7.4 DMSO/PBS buffer (V/V 30/70) taken at the indicated light irradiation times (430 nm, 62.2 μW cm^{-2}).

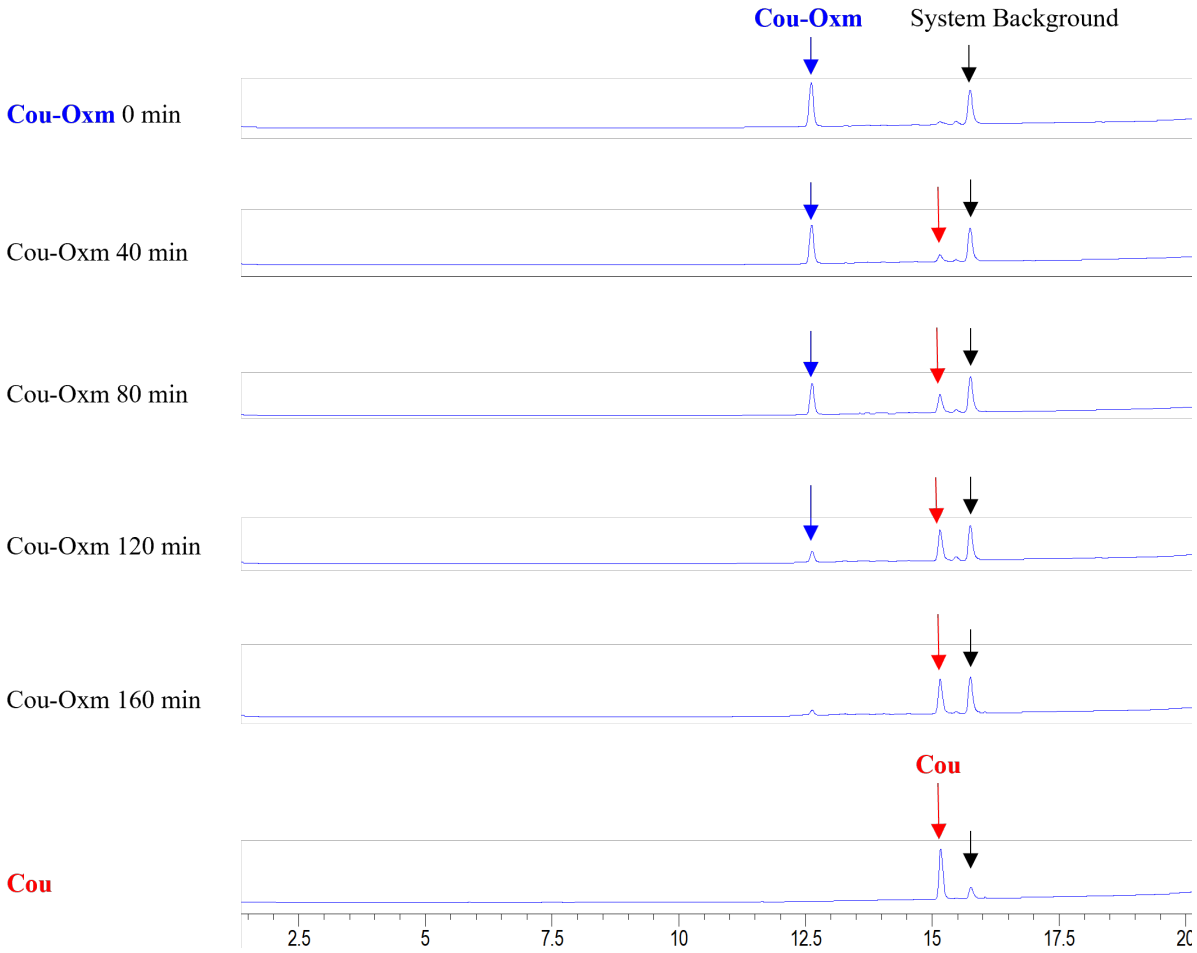


Figure S21. Overlay of HPLC spectra of Cou-Oxm (20 μM in pH 7.4 DMSO/PBS buffer (V/V 30/70) taken at the indicated light irradiation times (430 nm, 62.2 μW cm^{-2}).

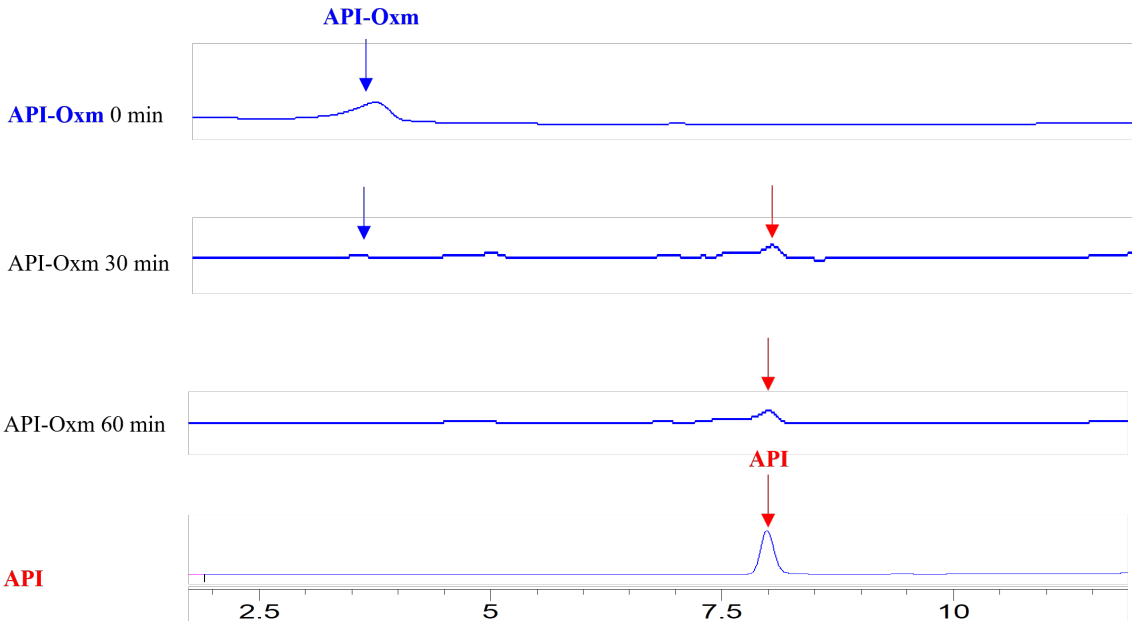


Figure S22. Overlay of HPLC spectra of Cou-Oxm (20 μM in pH 7.4 DMSO/PBS buffer (V/V 30/70) taken at the indicated light irradiation times (365 nm handheld lamp).

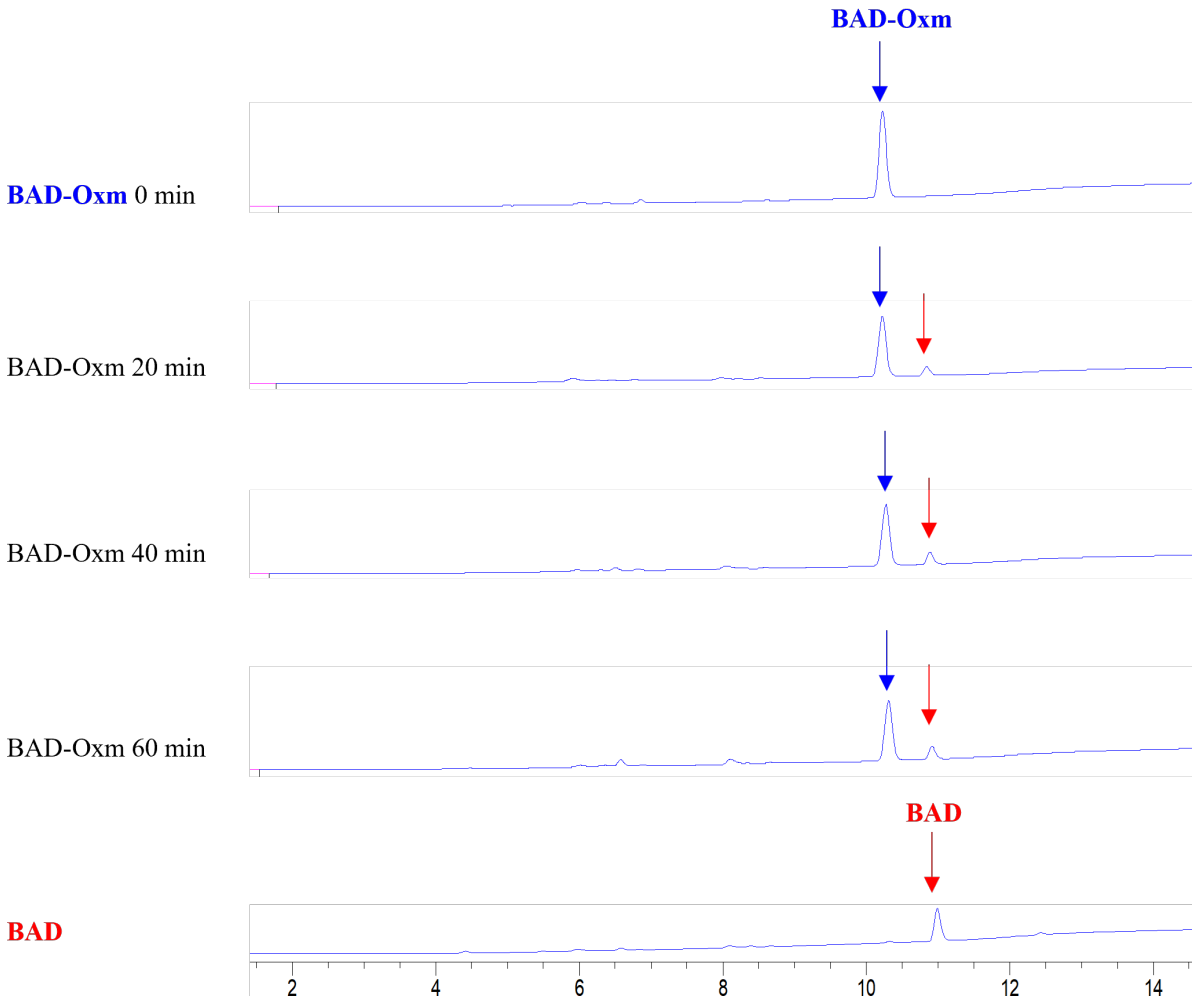


Figure S23. Overlay of HPLC spectra of BAD-Oxm (20 μM in pH 7.4 DMSO/PBS buffer (V/V 30/70) taken at the indicated light irradiation times (405 nm, 70.0 μW cm^{-2}).

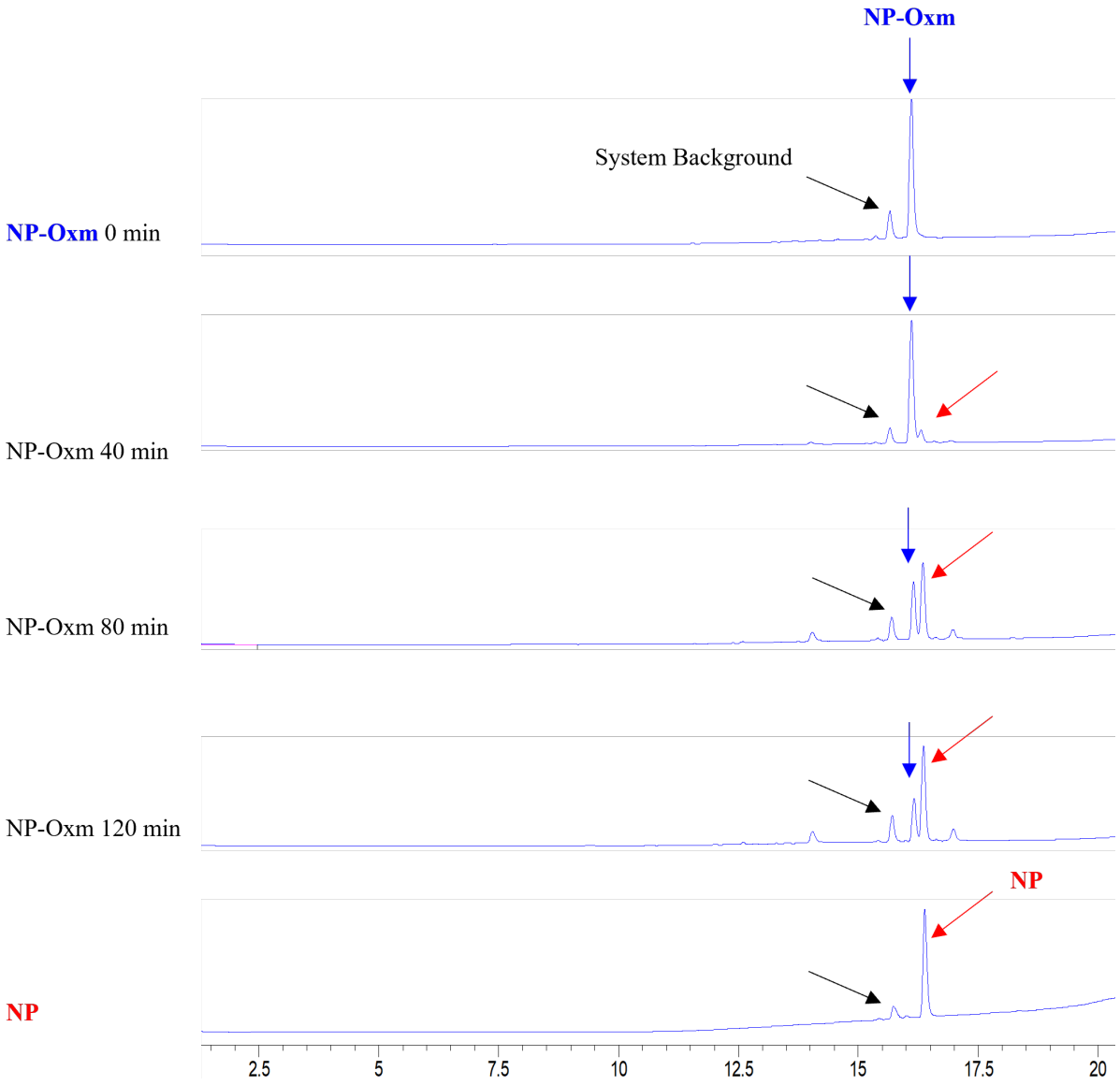


Figure S24. Overlay of HPLC spectra of NP-Oxm (20 μM in pH 7.4 DMSO/PBS buffer (V/V 70/30) taken at the indicated light irradiation times (430 nm, 62.2 μW cm^{-2}).

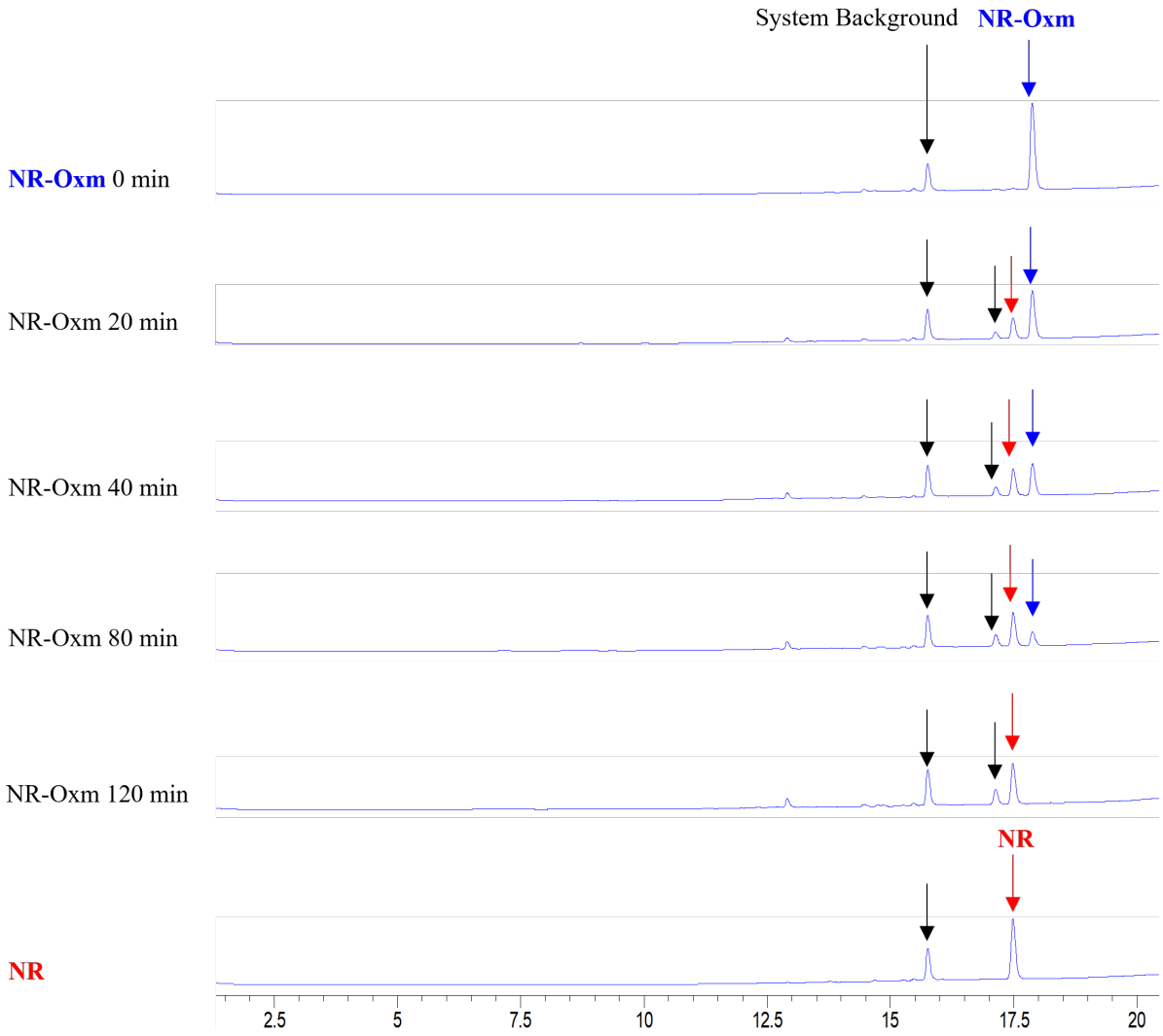


Figure S25. Overlay of HPLC spectra of NR-Oxm (20 μM in pH 7.4 DMSO/PBS buffer (V/V 70/30) taken at the indicated light irradiation times (430 nm, 62.2 μW cm^{-2}).

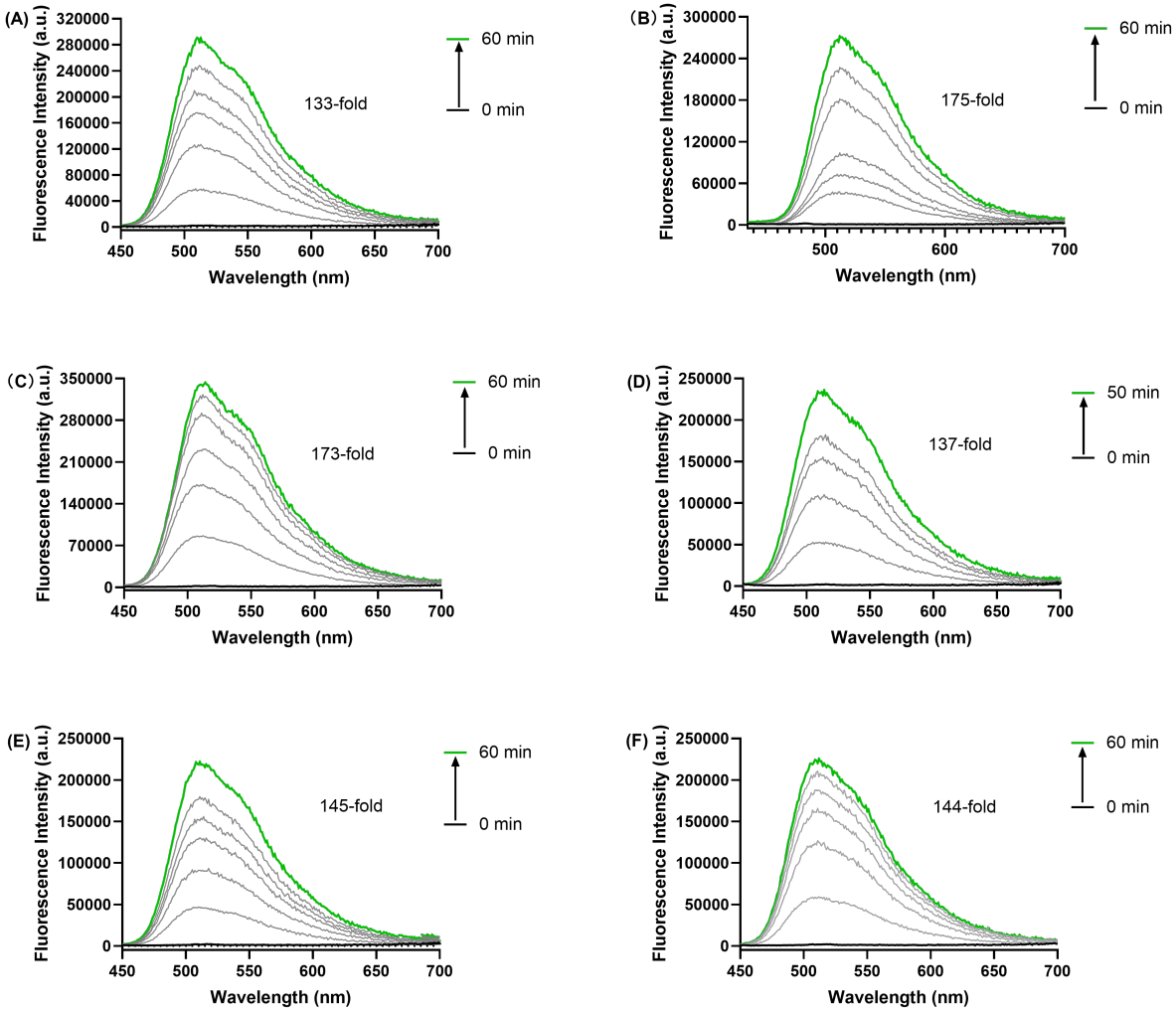


Figure S26. Emmision spectra of the photoactivation of BAD-Oxm at different pH, 20 μM in DMSO/PBS (V/V 30/70) (405 nm, 70.0 μW cm^{-2}). (A) pH 5.5; (B) pH 6.0; (C) pH 6.8; (D) pH 7.4; (E) pH 8.5; (F) pH 9.4.

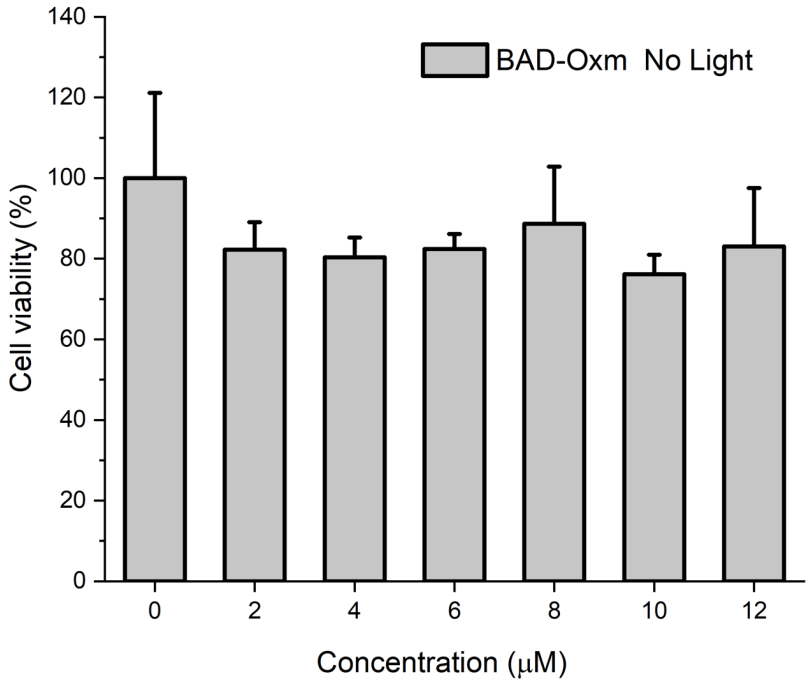


Figure S27. Cell viability of BAD-Oxm using HeLa cells with an increased concentration.

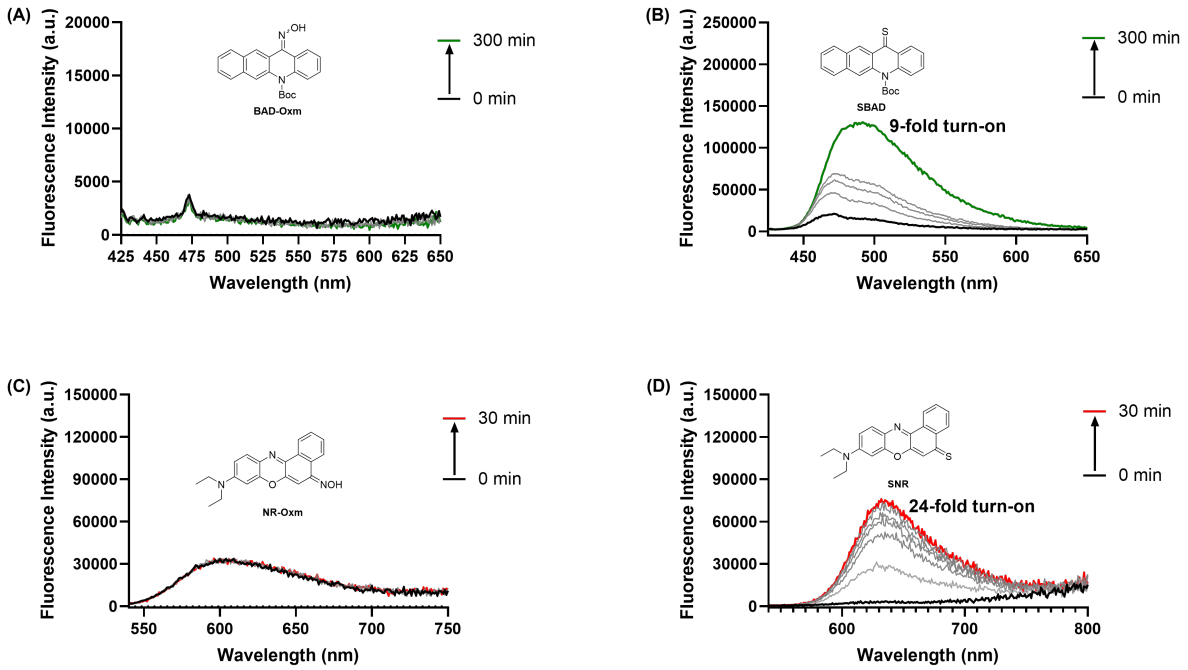


Figure S28. Stability of fluorophores in DMSO (20 μM) under ambient air atmosphere with indoor daily lighting based on fluorescence change. (A) BAD-Oxm; (B) SBAD; (C) NR-Oxm; (D) SNR.

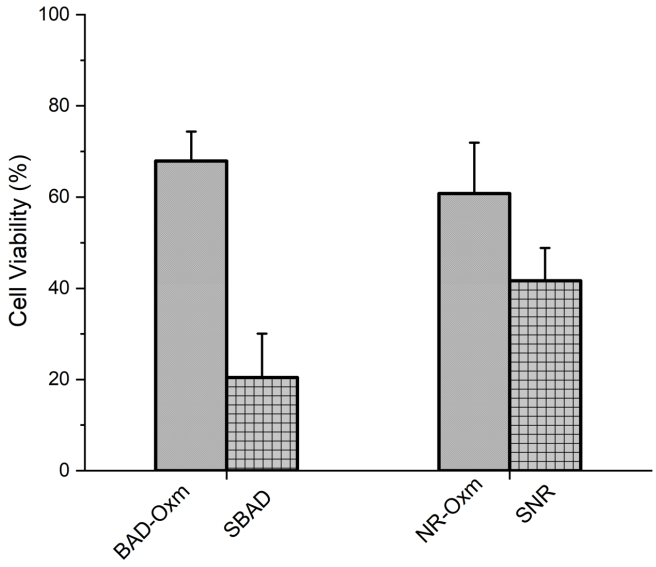


Figure S29. Cell viability of HeLa cells after treatment with 5 μM fluorophores in the presence of light. BAD-Oxm and SBAD (400-700 nm, 0.4 μW cm^{-2}); NR-Oxm (490-510 nm, 0.4 μW cm^{-2}); SNR (600-630 nm, 0.4 μW cm^{-2}).

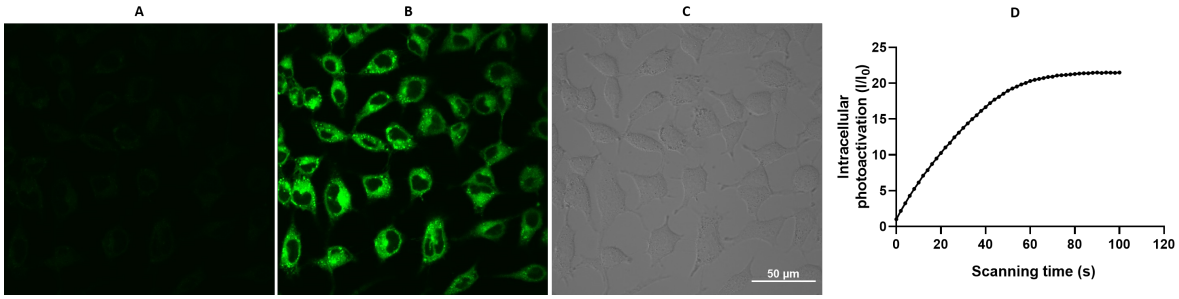


Figure S30. Cell permeability and intracellular photoactivation of BAD-Oxm in HeLa cells. (A) Intracellular fluorescence before light activation; (B) Intracellular fluorescence after light activation; (C) Bright filed; (D) Relative intracellular fluorescence intensity changes of BAD-Oxm during photoactivation in living HeLa cells using a 405 nm laser. Conditions: 10 µM for 1h; 405 nm activation and excitation wavelength with 515/30 detector. Scale bar = 50 µm.

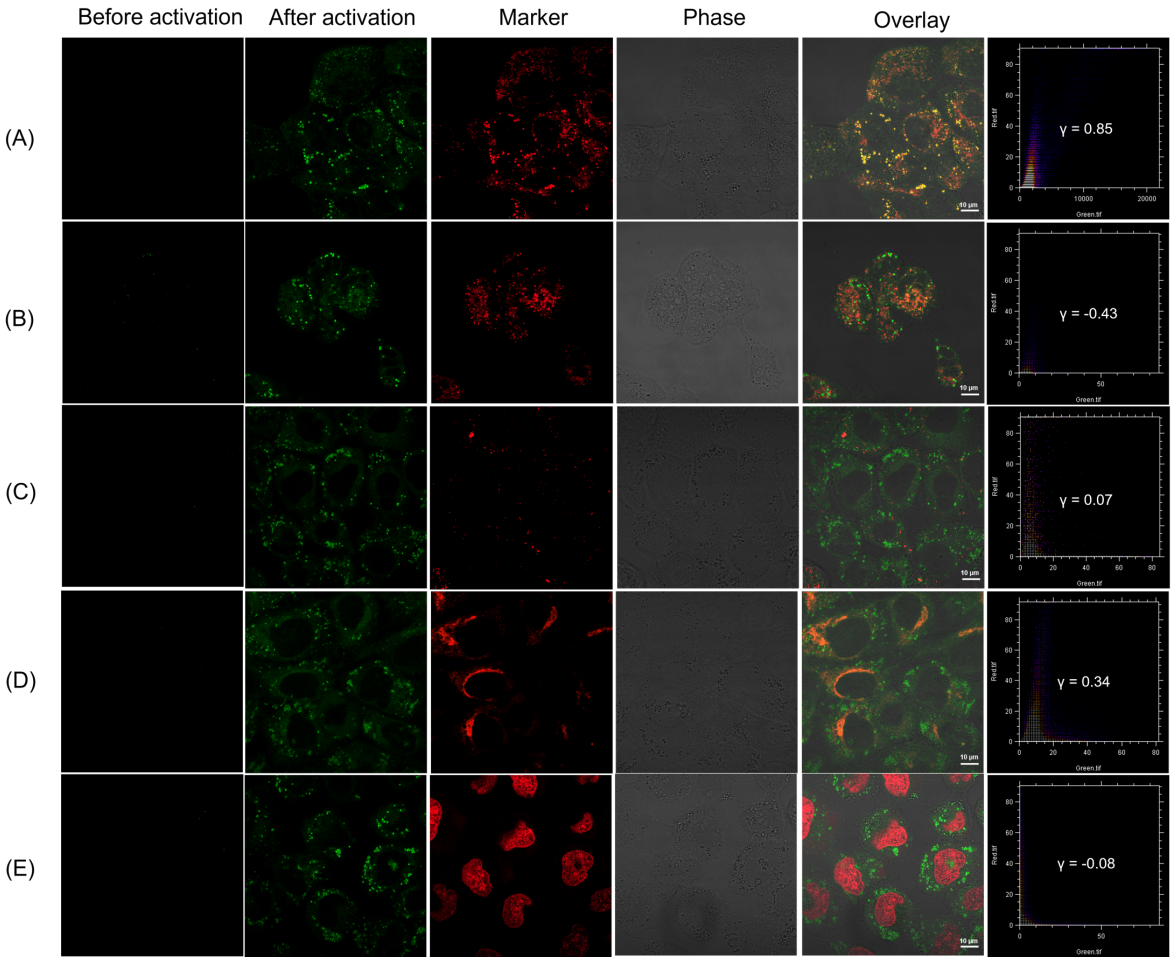


Figure S31. Co-localization between BAD-Oxm (7) and organelle-targeting commercial dyes. (A) BAD-Oxm (7) and lipid droplet marker (Nile red). (B) BAD-Oxm (7) and mitochondria marker (MitoView™ 633). (C) BAD-Oxm (7) and lysosomes marker (LysoView™ 633). (D) BAD-Oxm (7) and endoplasmic reticulum marker (ERTracker™ Red (BODIPY™ TR Glibenclamide). (E) BAD-Oxm (7) and nuclear marker (DRAQ5). Conditions: incubation of Hela cells with 5 μM BAD-Oxm for 1 h; 405 nm activation and excitation wavelength with 515/30 detector. Scale bar = 10 μm.

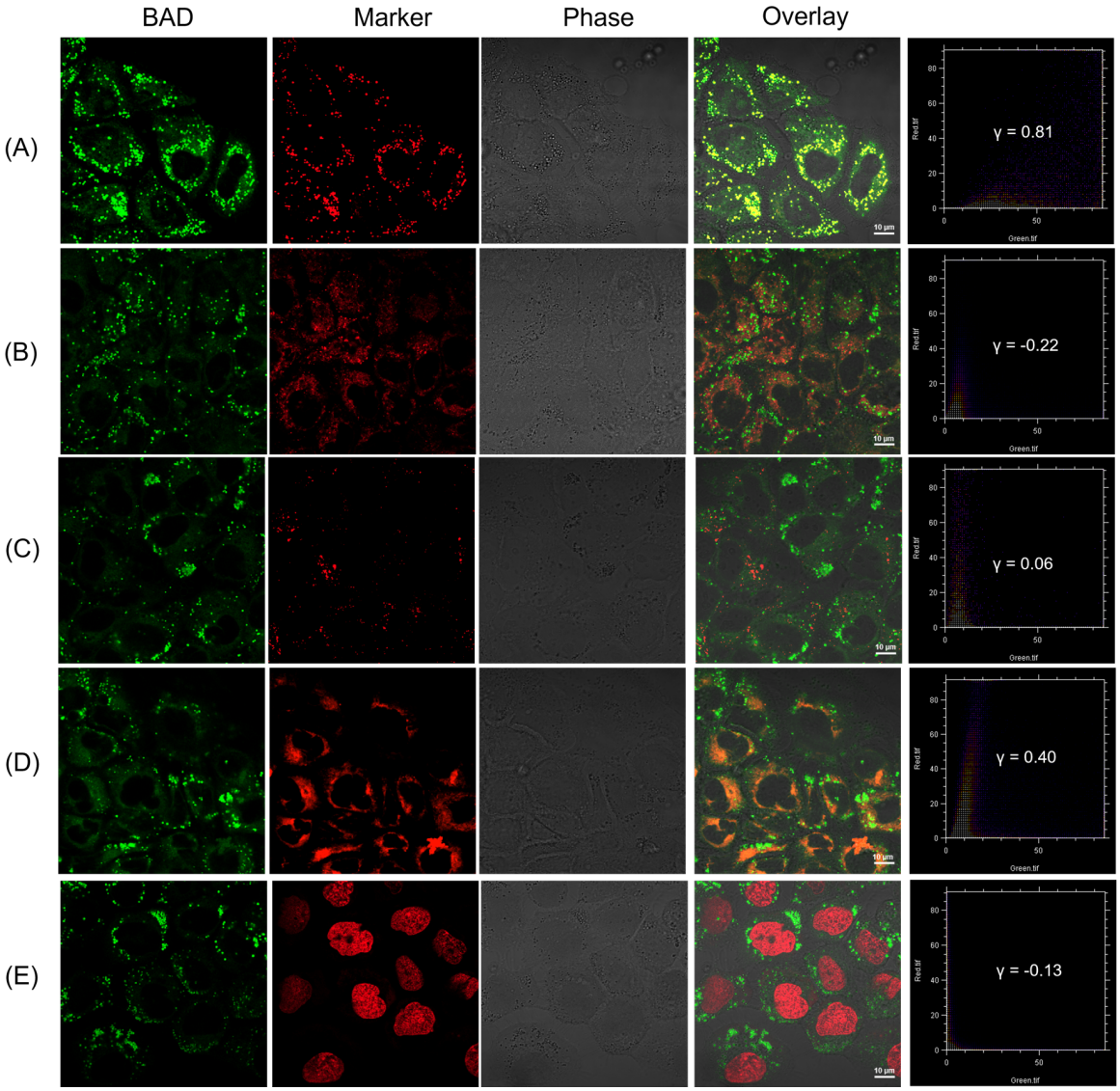


Figure S32. Co-localization between BAD and organelle-targeting commercial dyes. (A) BAD and lipid droplet marker (Nile Red). (B) BAD and mitochondria marker (MitoView™ 633). (C) BAD and lysosomes marker (LysoView™ 633). (D) BAD and endoplasmic reticulum marker (ERTracker™ Red (BODIPY™ TR Glibenclamide). (E) BAD and nuclear marker (DRAQ5). Conditions: incubation of Hela cells with 5 µM BAD for 1h; 405 nm activation and excitation wavelength with 515/30 detector. Scale bar = 10 µm.

Table S7. Photophysical Data of oxime-caged targeting probes

Compounds [a]	λ_{abs} (nm)	ε^b ($*10^4$ M^{-1} cm^{-1})	λ_{em} (nm)	Φ_f [c]	Turn-on fold
BAD-Oxm-TPP (**23**)	264, 306, 351	1.15, 0.25, 0.11	439	0.008	90[d]
BAD-Oxm-MOR (**24**)	264, 306, 351	1.52, 0.40, 0.14	452	0.006	108 [d]
BAD-Oxm-Ts (**25**)	265, 305, 359	2.63, 0.69, 0.20	453	0.005	184 [d]
BAD-Oxm-Halo (**26**)	265, 306, 356	2.63, 0.65, 0.24	455	0.007	103[d]

[a] Compounds were dissolved in DMSO (50 μM). [b] ε: molar extinction coefficients. [c] Quantum yields were measured using rhodamine B in ethanol or quinine sulfate in 0.5 M H_2SO_4 as the reference. [d] 20 μM in PBS (pH 7.4)/DMSO (V/V 50/50) (430 nm, 62.2 μW cm^{-2}).

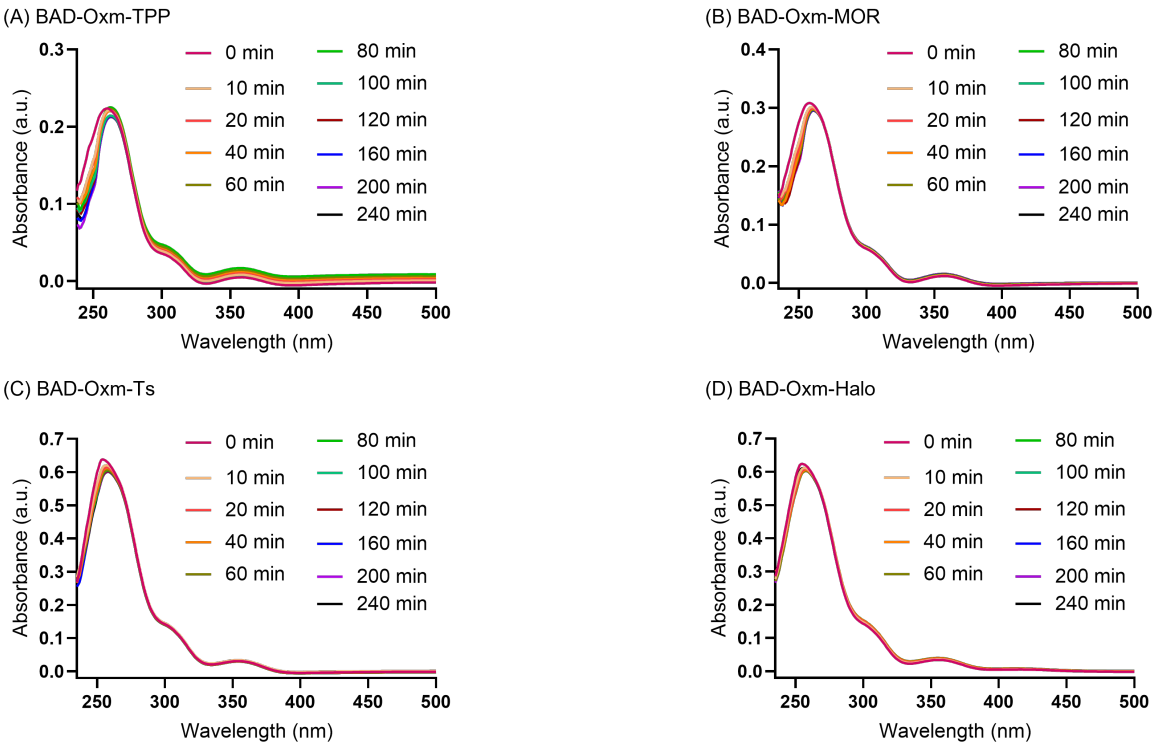


Figure S33. Stability of BAD-Oxm probes 20 μM in PBS (pH 7.4)/DMSO (V/V 50/50) mixed solvent under ambient air atmosphere without light. (A) BAD-Oxm-TPP; (B) BAD-Oxm-MOR; (C) BAD-Oxm-Ts; (D) BAD-Oxm-Halo.

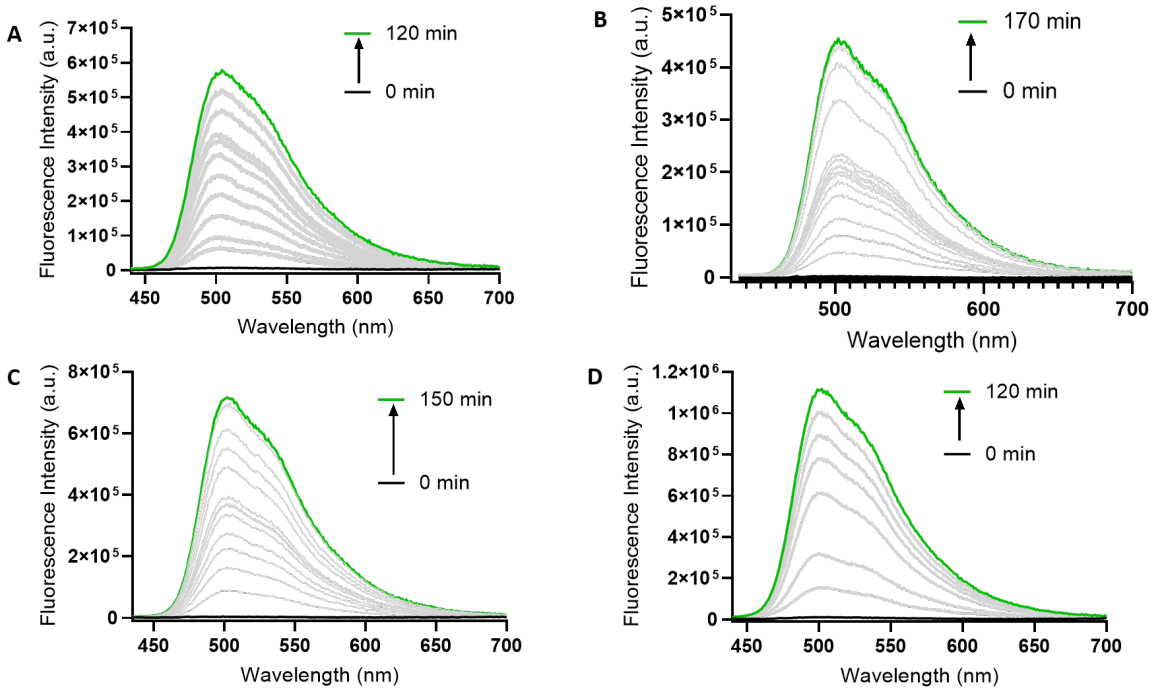


Figure S34. Emmision spectra of the photoactivation of BAD-Oxm targeting probes, 20 μM in PBS (pH 7.4)/DMSO (V/V 50/50) (430 nm, 62.2 μW cm^{-2}). (A)BAD-Oxm-TPP; (B)BAD-Oxm-MOR; (C) BAD-Oxm-Ts; (D) BAD-Oxm-Halo.

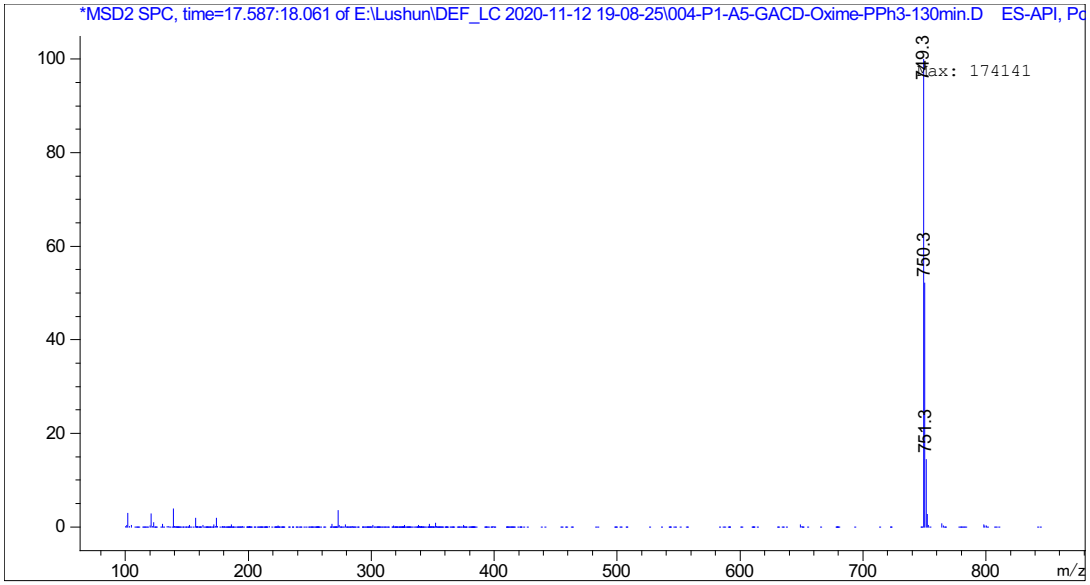


Figure S35. ESI-MS of BAD-Oxm-TPP after irradiation.

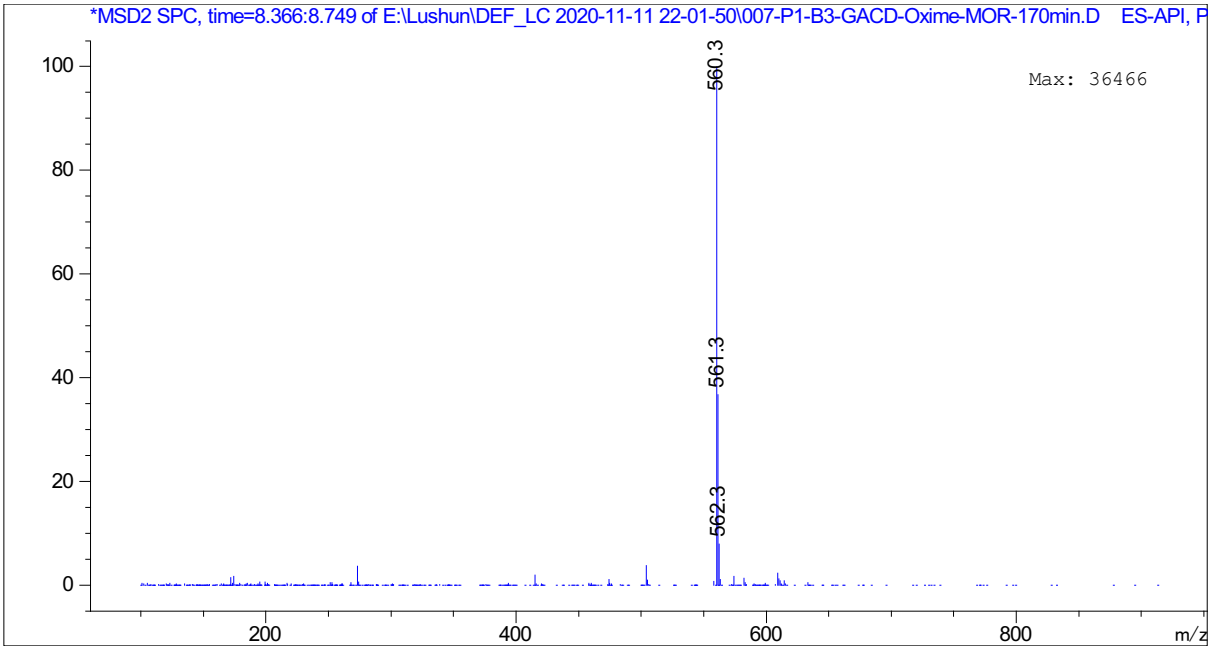


Figure S36. ESI-MS of BAD-Oxm-MOR after irradiation.

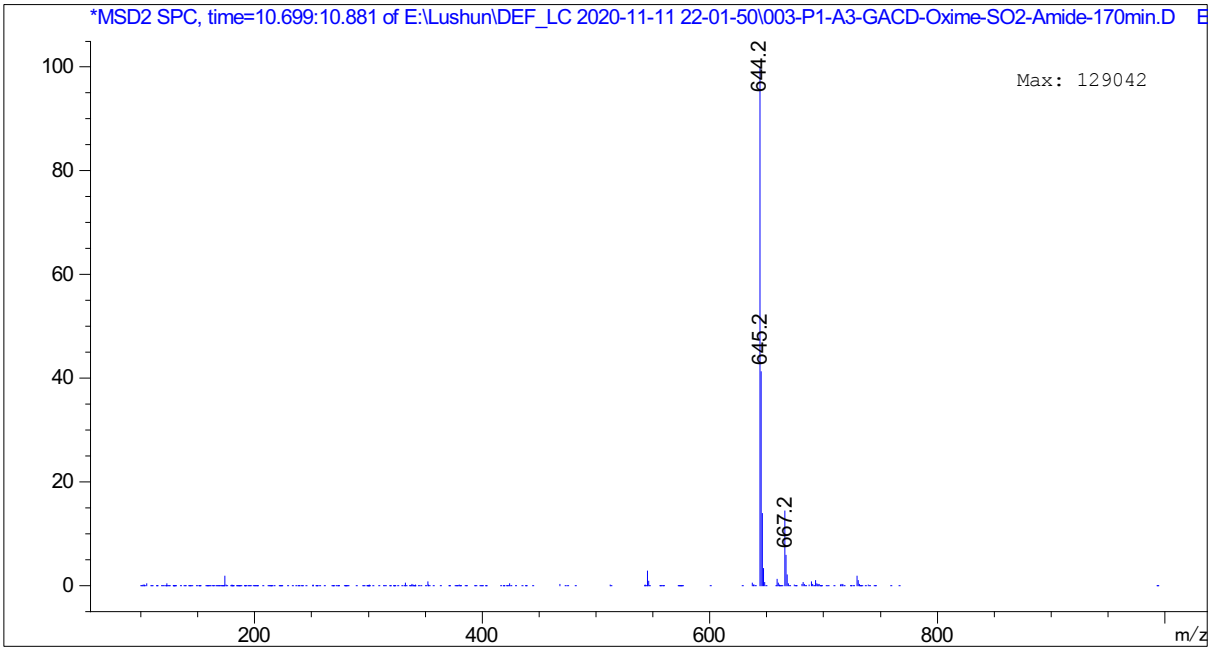


Figure S37. ESI-MS of BAD-Oxm-MOR after irradiation.

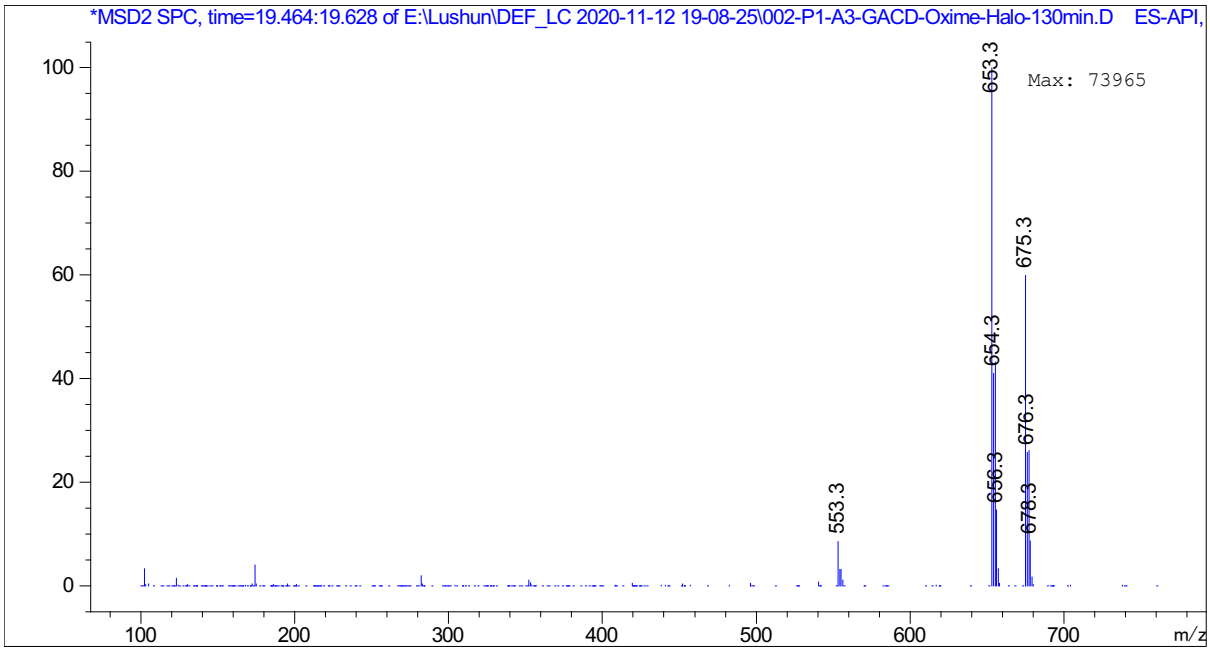


Figure S38. ESI-MS of BAD-Oxm-Halo after irradiation.

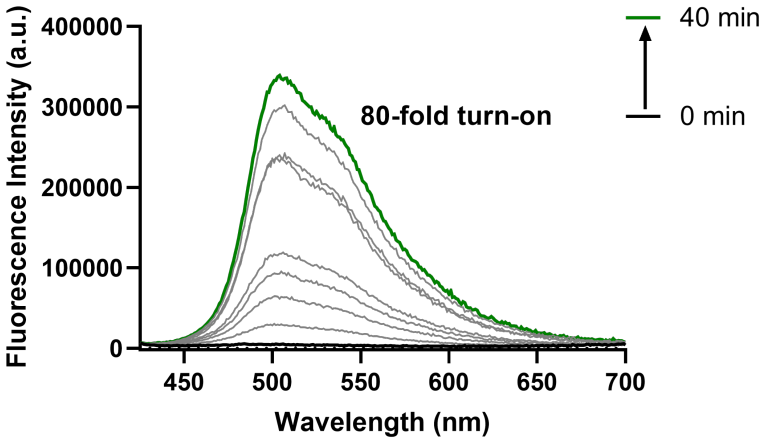


Figure S39. Emmision spectra of the photoactivation of BAD-Oxm-Halo labeled SOD1 protein (20 μM in 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 405 nm, 70.0 μW cm^{-2}). SOD1(A4V)-Halo: 20 μM SOD1[9] was labeled with 50 μM BAD-Oxm-Halo for 10 min on ice in stock solution (50 mM Tris-HCl, pH 7.5, 100 mM NaCl) to allow bio-orthogonal conjugation. The resulting SOD1(A4V)-Halo was purified by PD-10 Columns (Cytiva Sephadex ™ G-25 M) following the manufactures' instructions to remove the excess BAD-Oxm-Halo. Expected 52633, observed 52635.

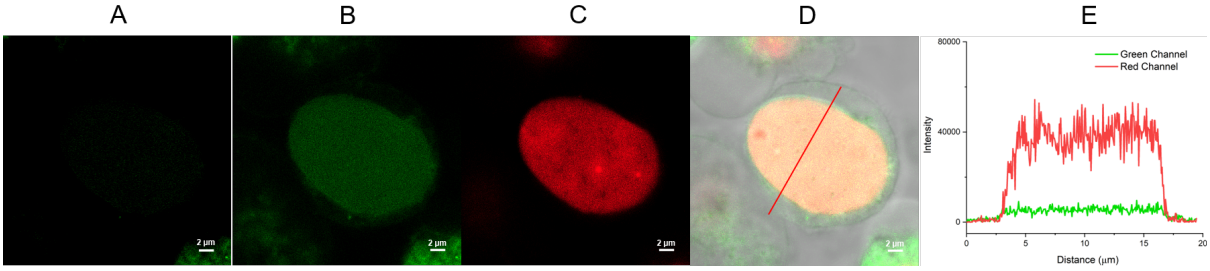
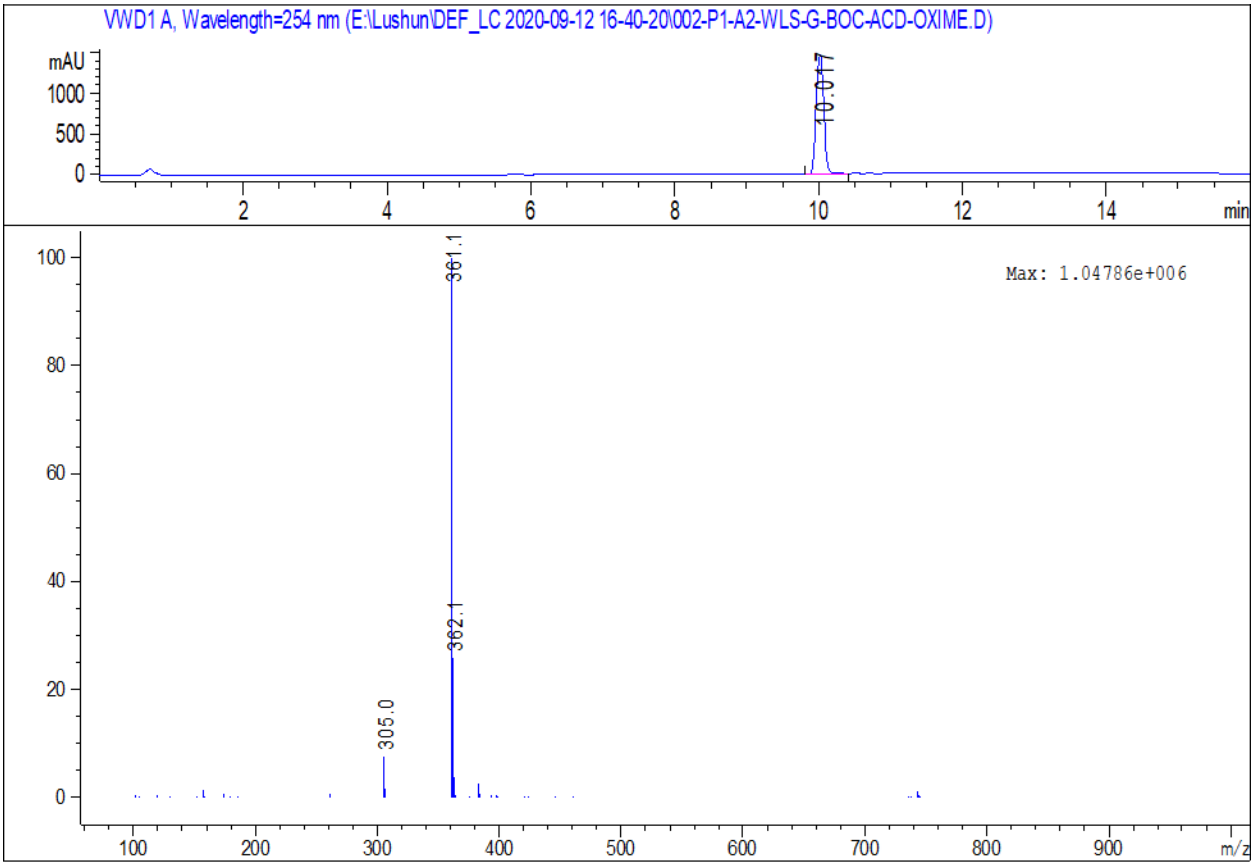


Figure S40. Confocal images of BAD-Oxm-Halo (5 μM) in H2B-HaloTag-expressed CHO-K1 cells . (A) Before light activation; (B) After light activation using 405 nm laser; (C) Fluorescence image of DRAQ5; (D) Merged image of activated BAD-Oxm-Halo, DRAQ5 and bright field; (E) Relative intracellular intensity profile of BAD-Oxm-Halo and DRAQ5. Scale bar = 2 μm.

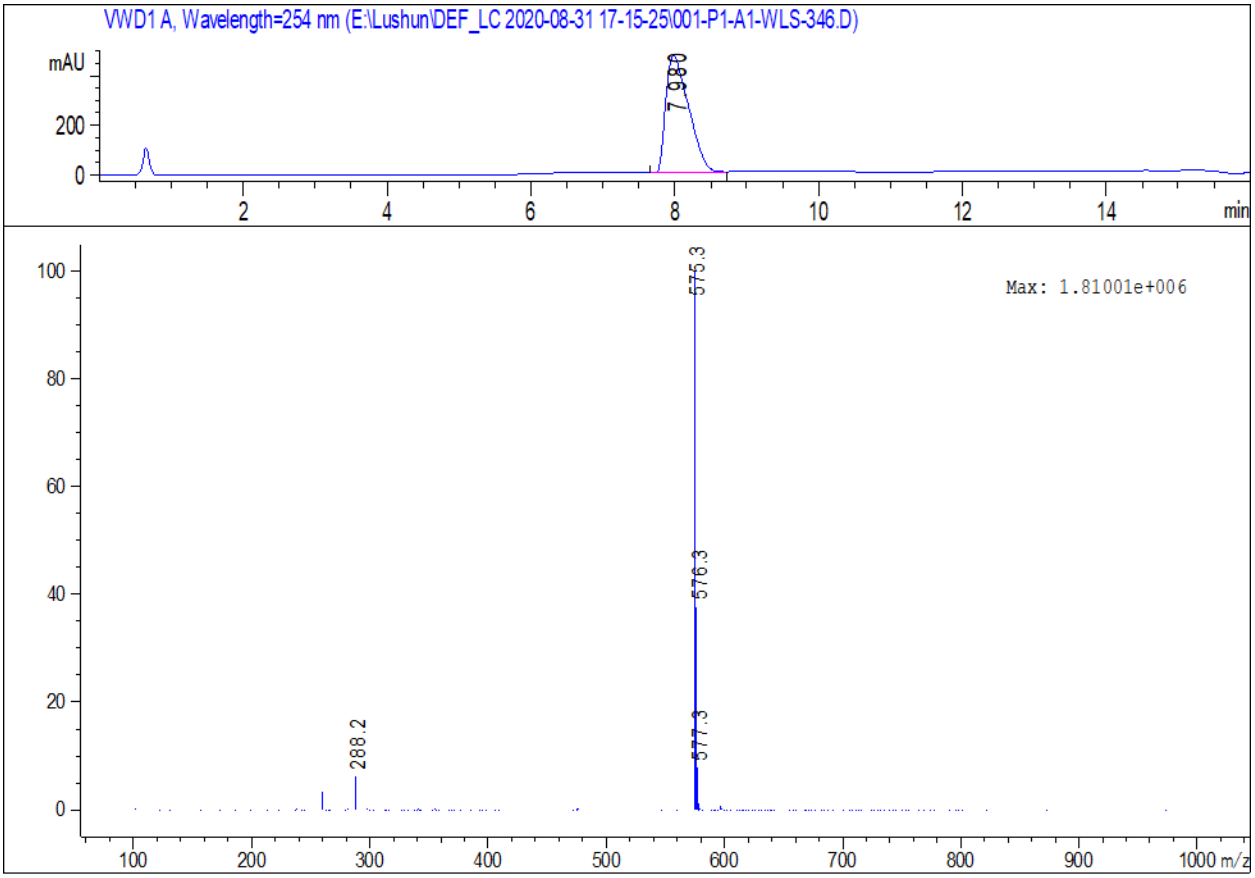
13. Characterization spectra

13.1 LC-MS spectra

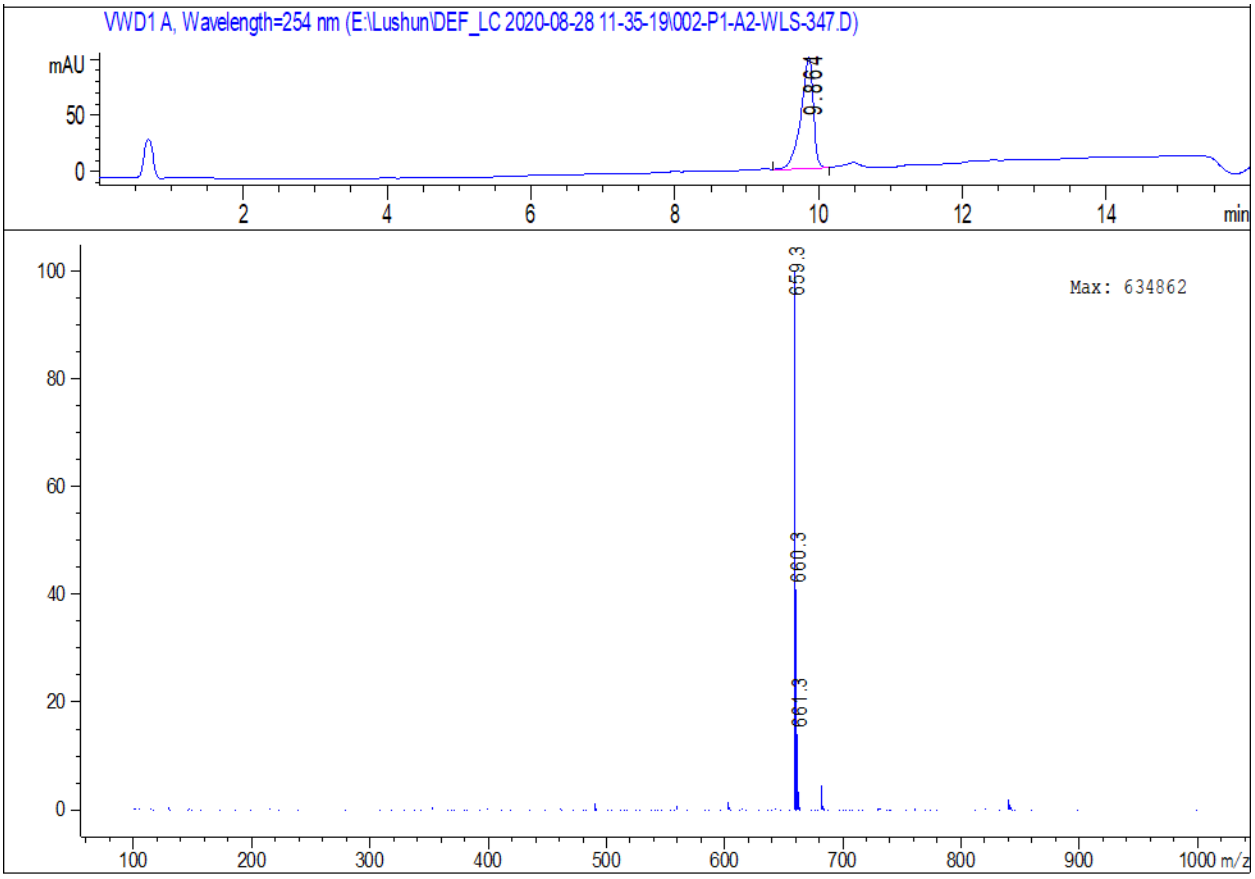
LC-MS spectrum of **BAD-Oxm**



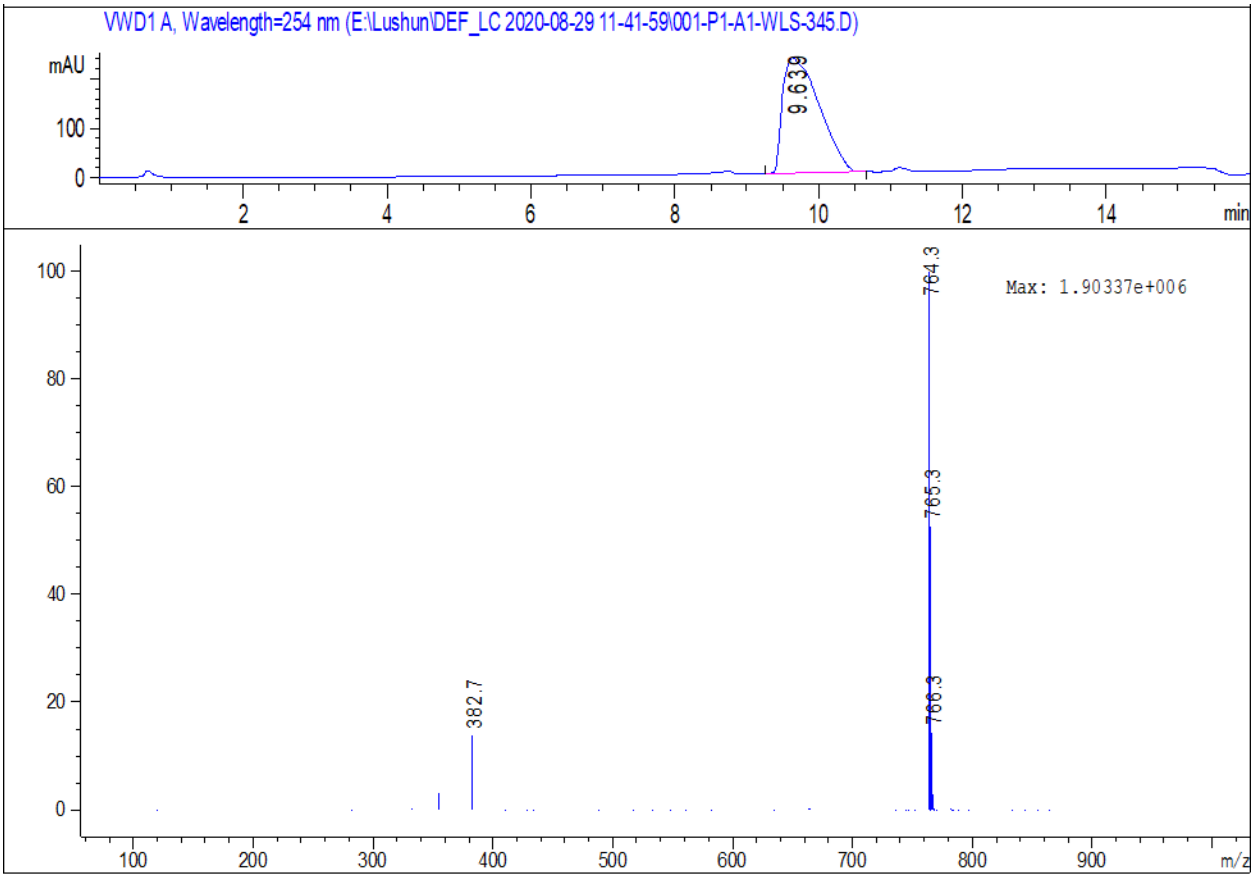
LC-MS spectrum of **BAD-Oxm-MOR**



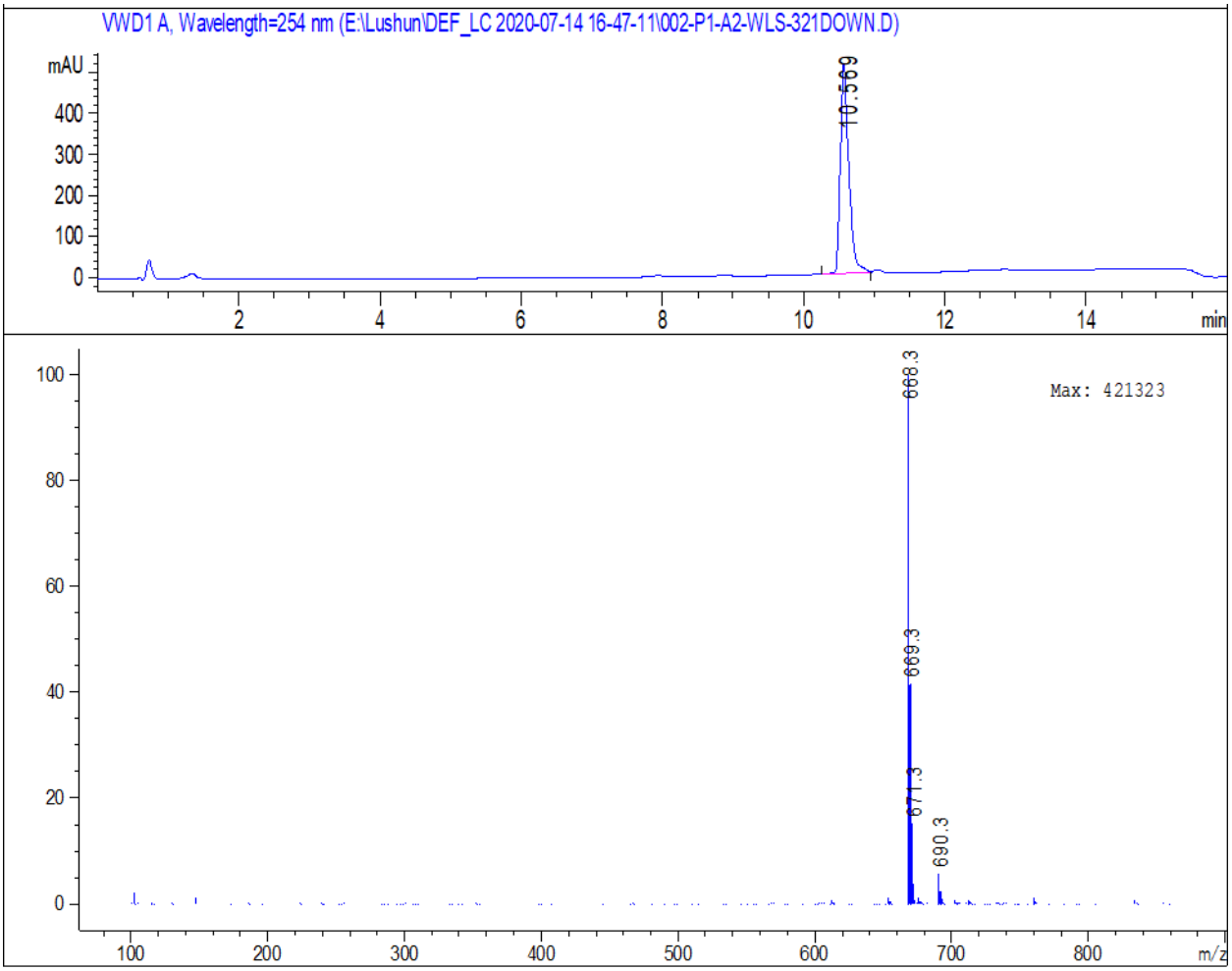
LC-MS spectrum of **BAD-Oxm-Ts**



LC-MS spectrum of **BAD-Oxm-TPP**

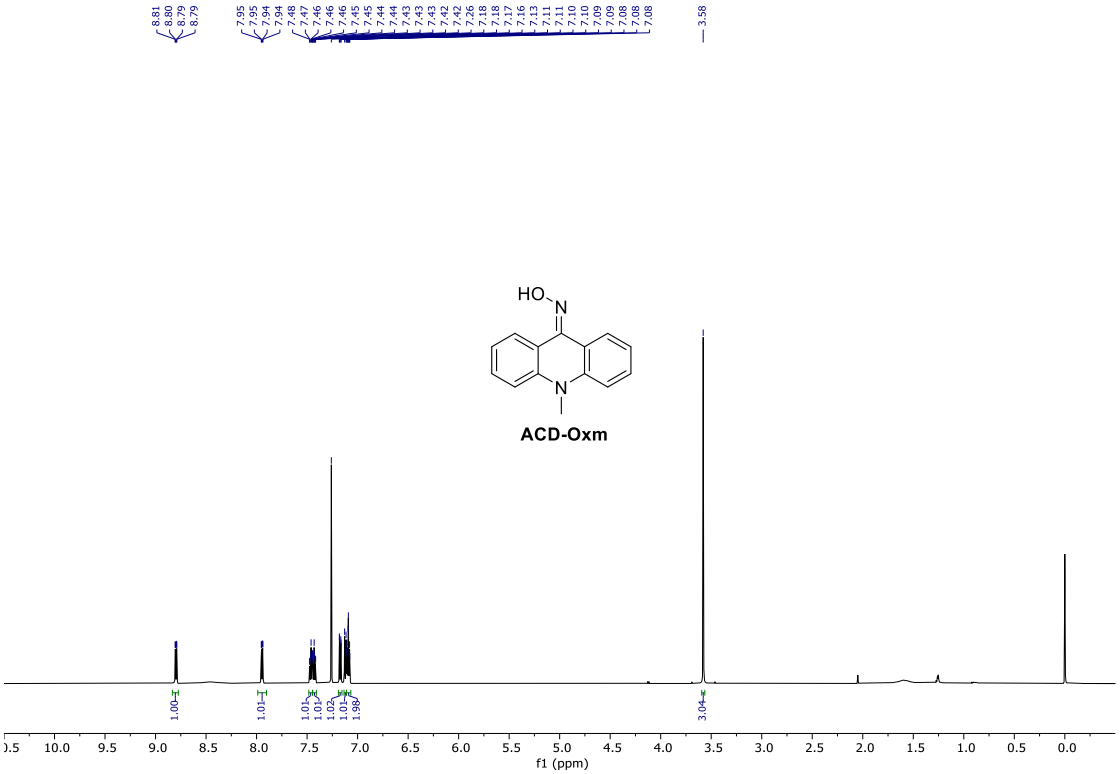


LC-MS spectrum of **BAD-Oxm-Halo**

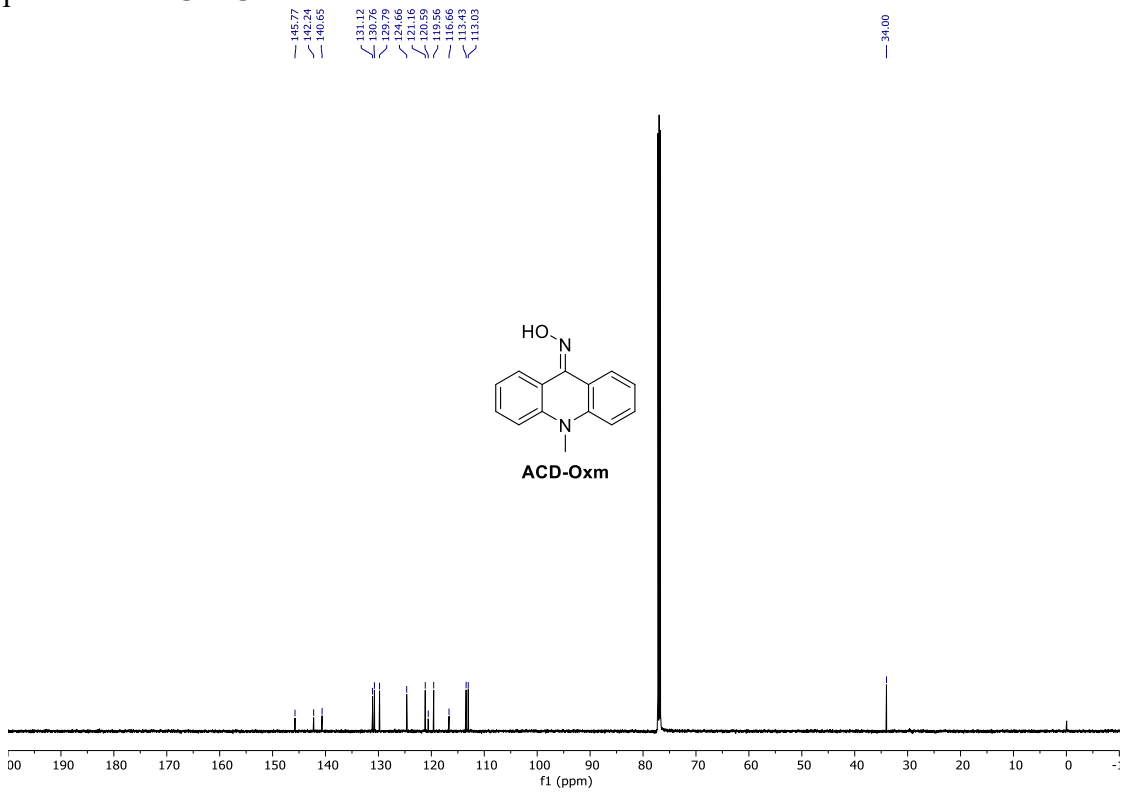


13.2 NMR spectra

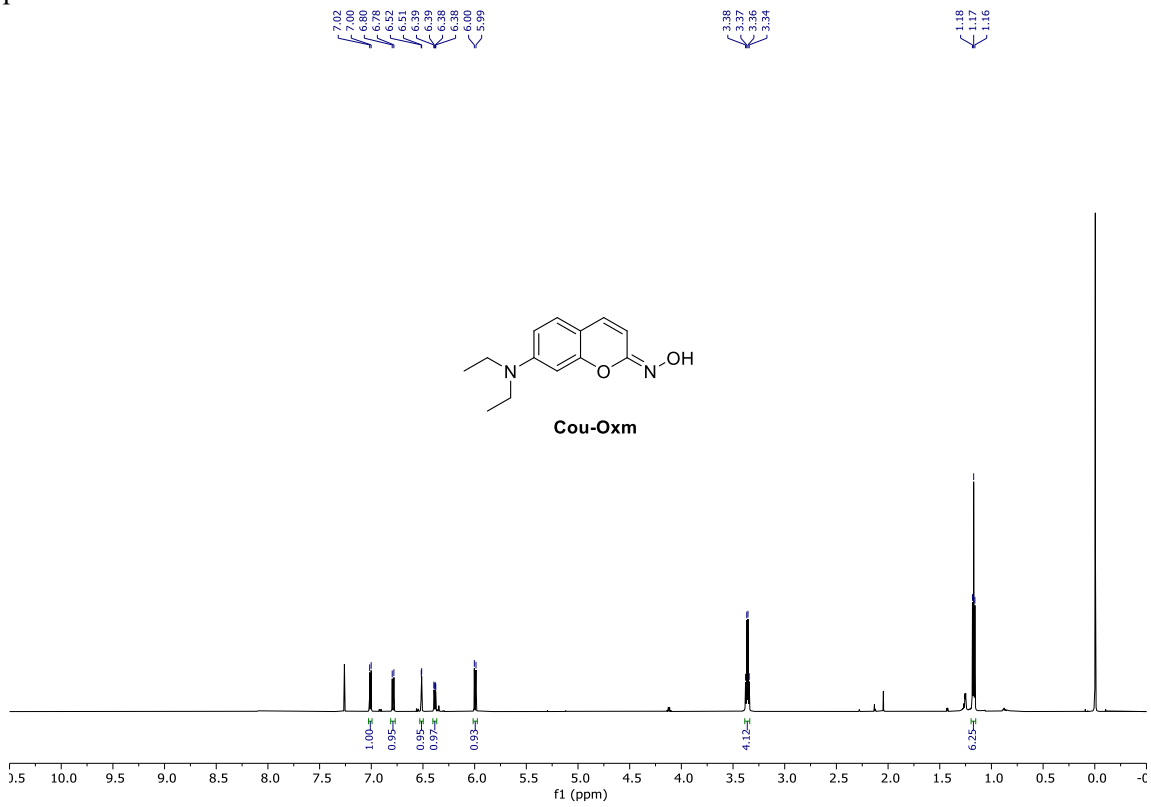
^{1}H spectrum of **ACD-Oxm**



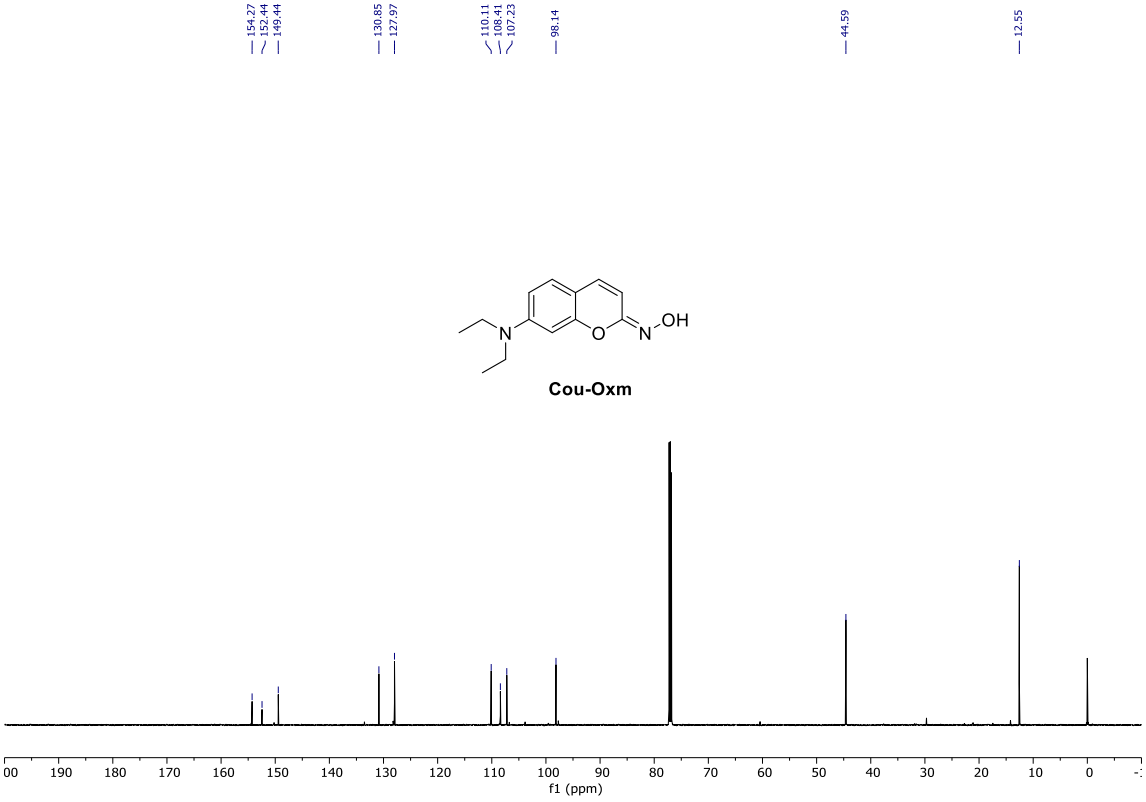
^{13}C spectrum of **ACD-Oxm**



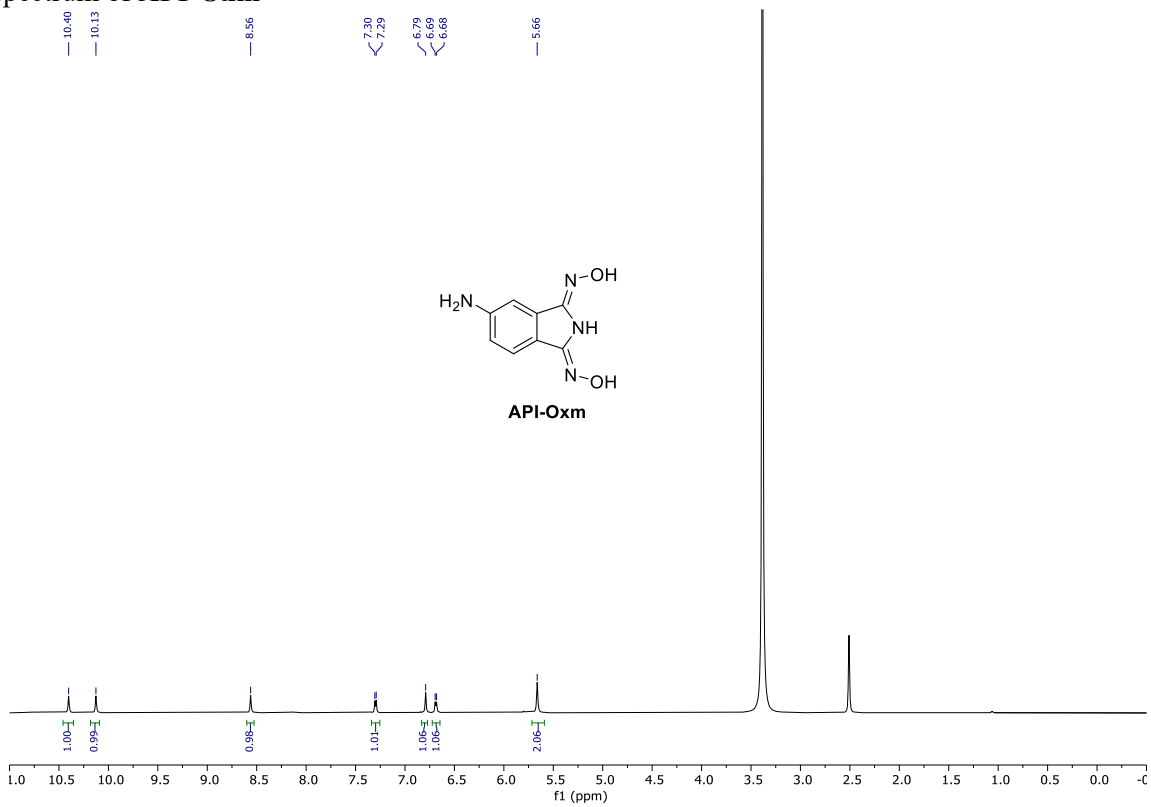
^{1}H spectrum of **Cou-Oxm**



^{13}C spectrum of **Cou-Oxm**

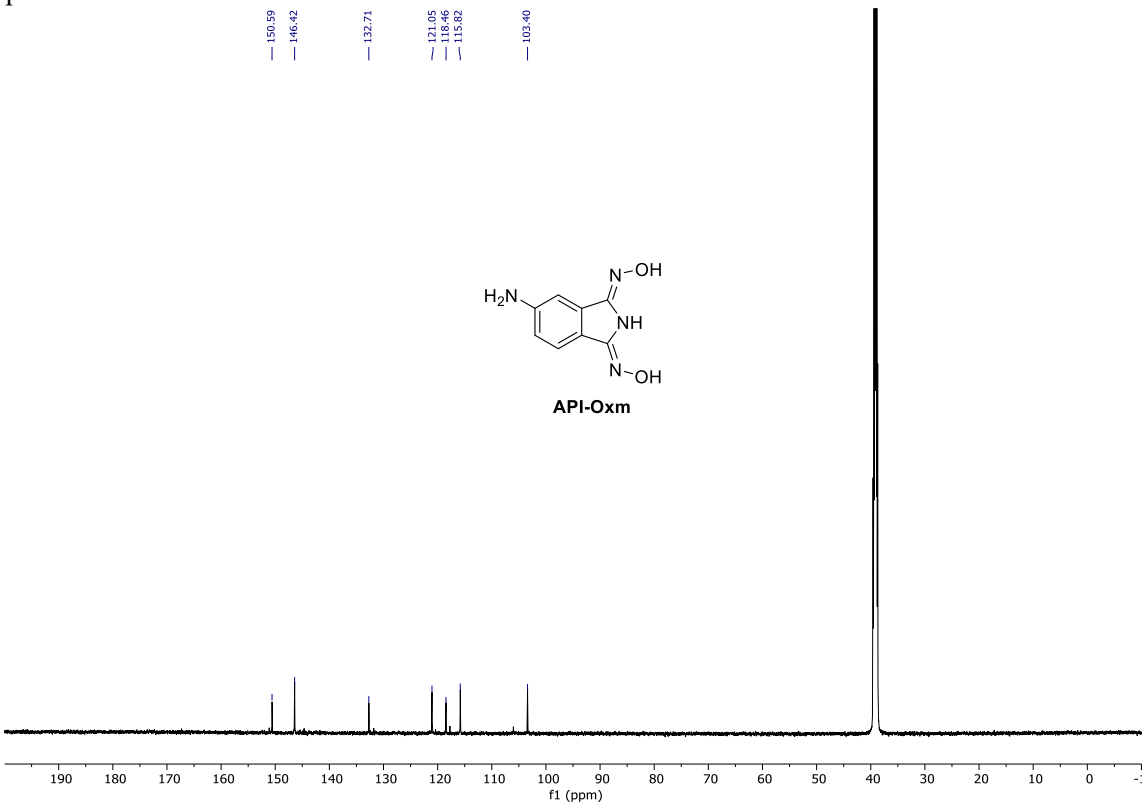


^{1}H spectrum of **API-Oxm**



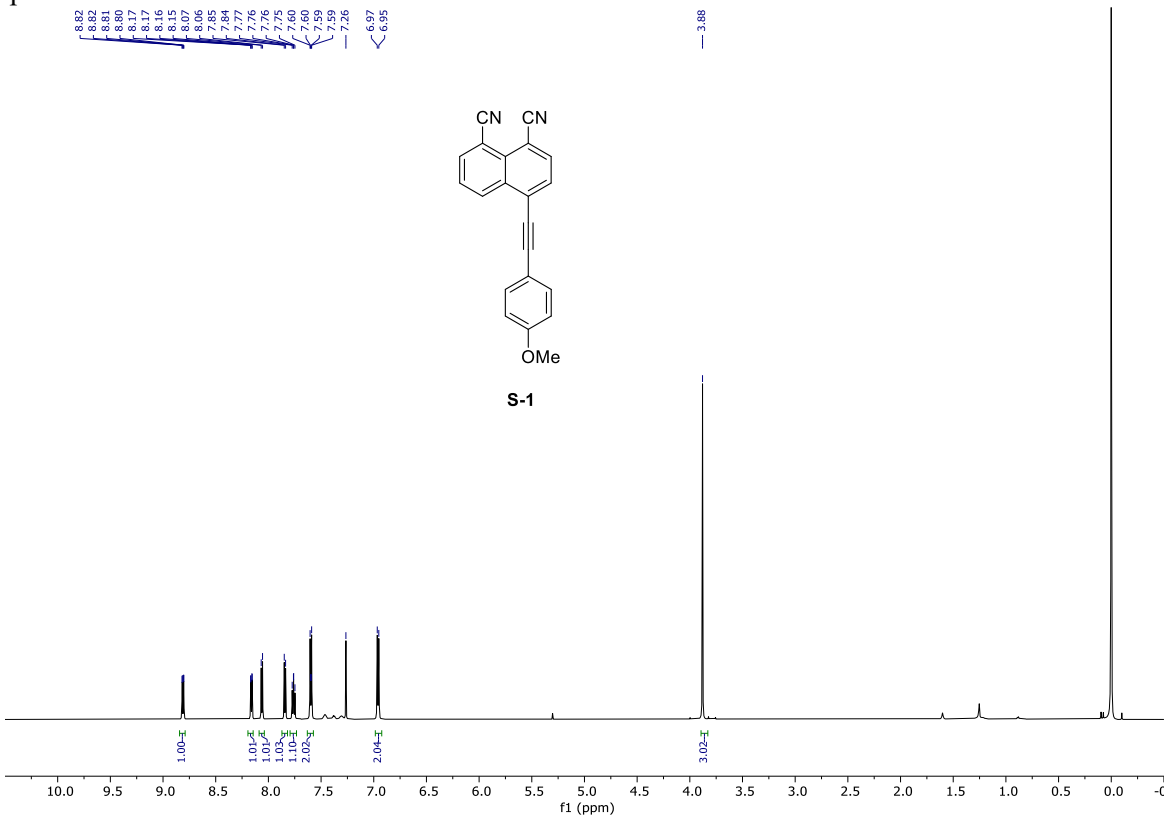
API-Oxm

^{13}C spectrum of **API-Oxm**

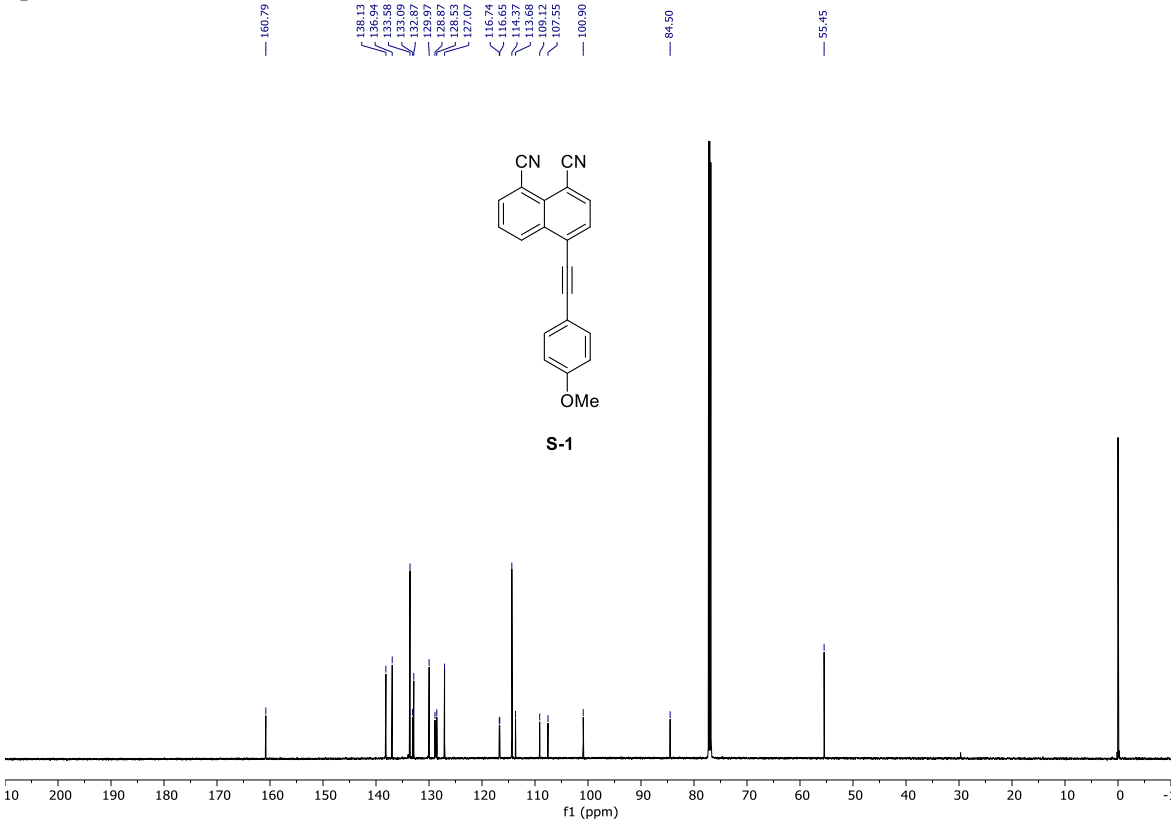


API-Oxm

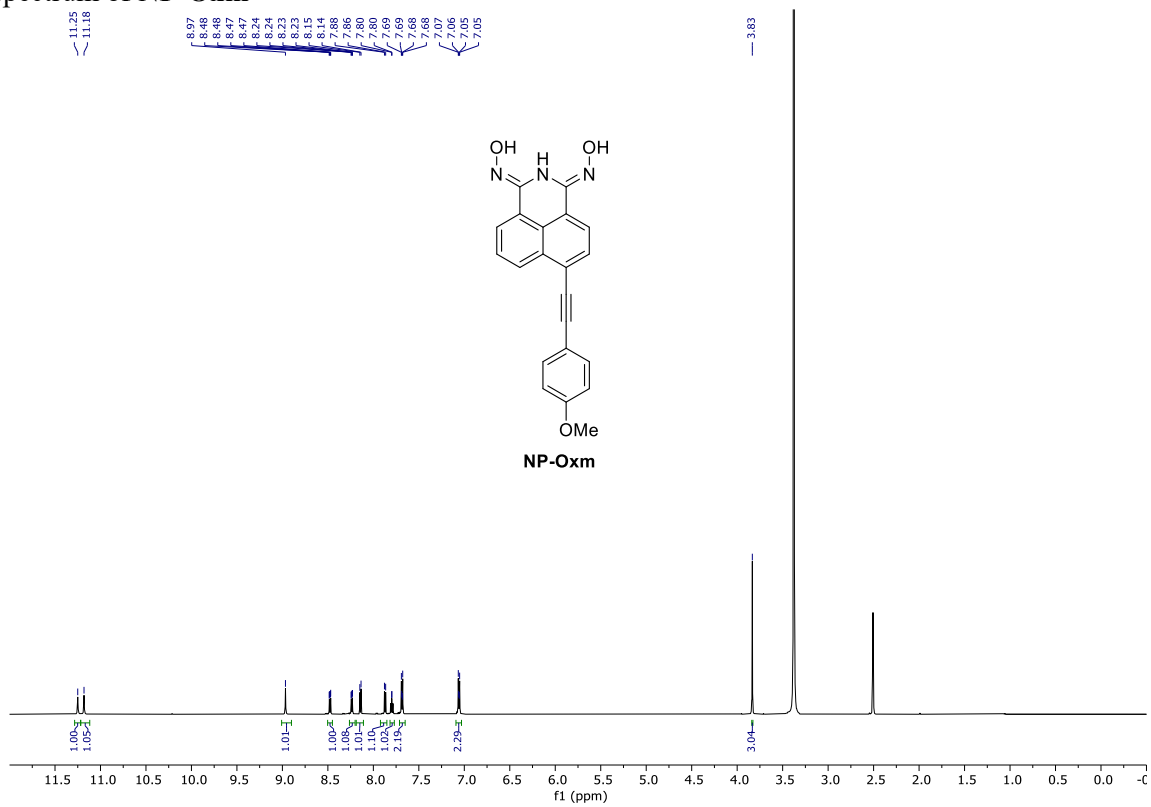
^{1}H spectrum of **S-1**



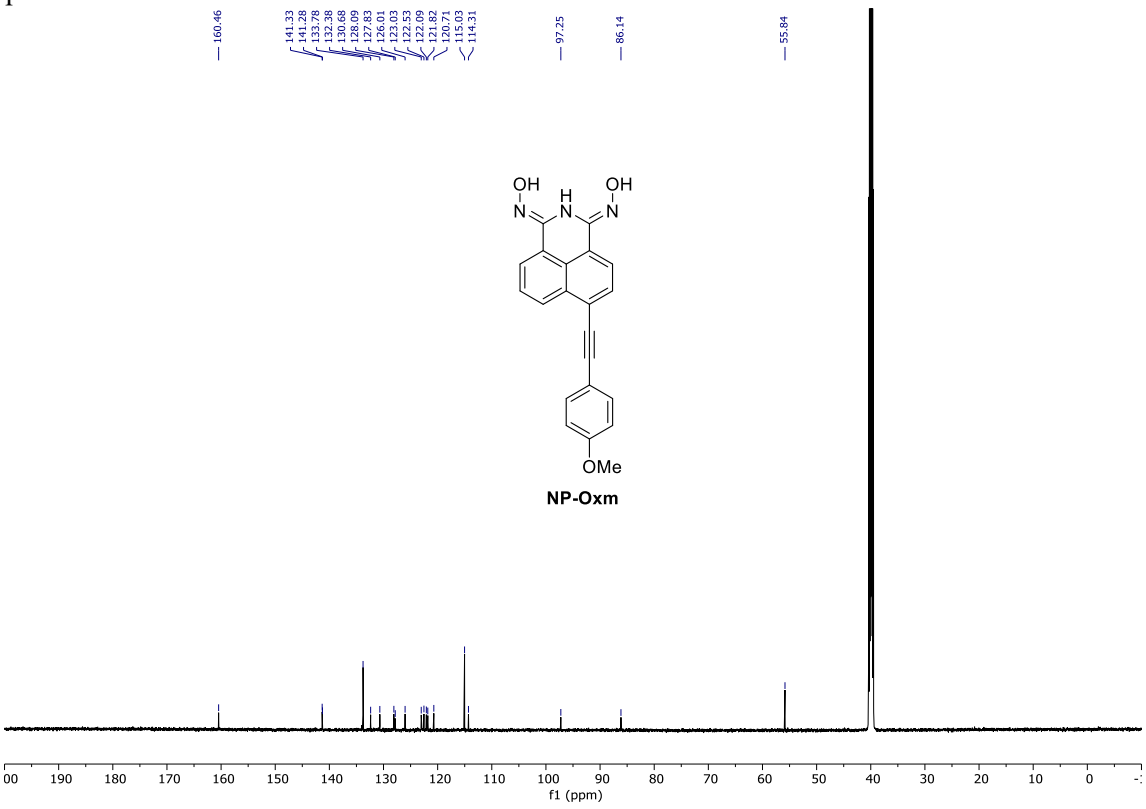
^{13}C spectrum of **S-1**



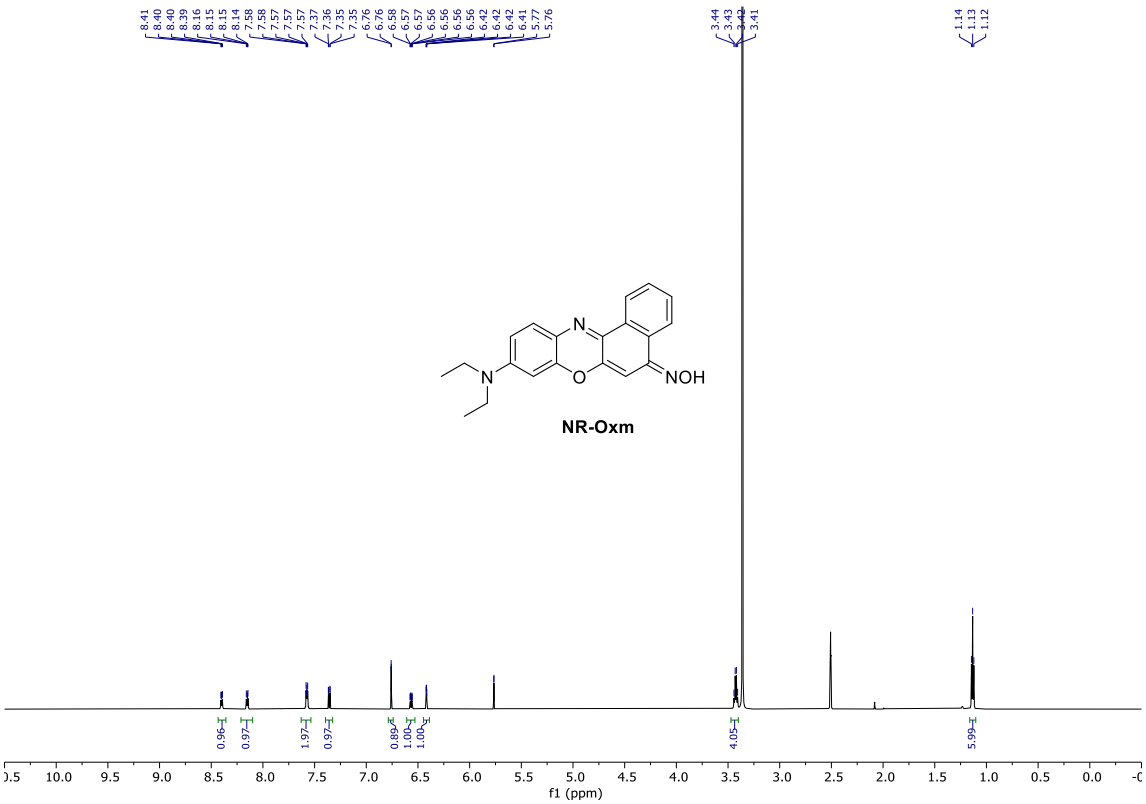
^{1}H spectrum of **NP-Oxm**



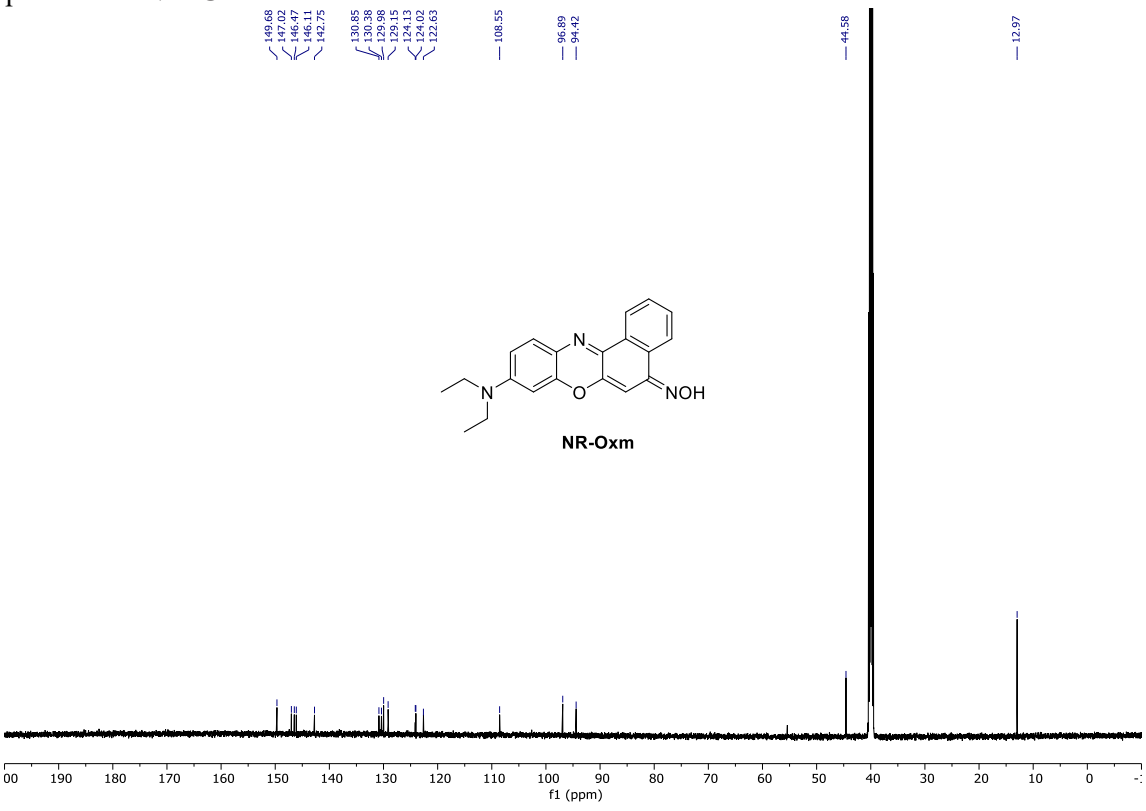
^{13}C spectrum of **NP-Oxm**



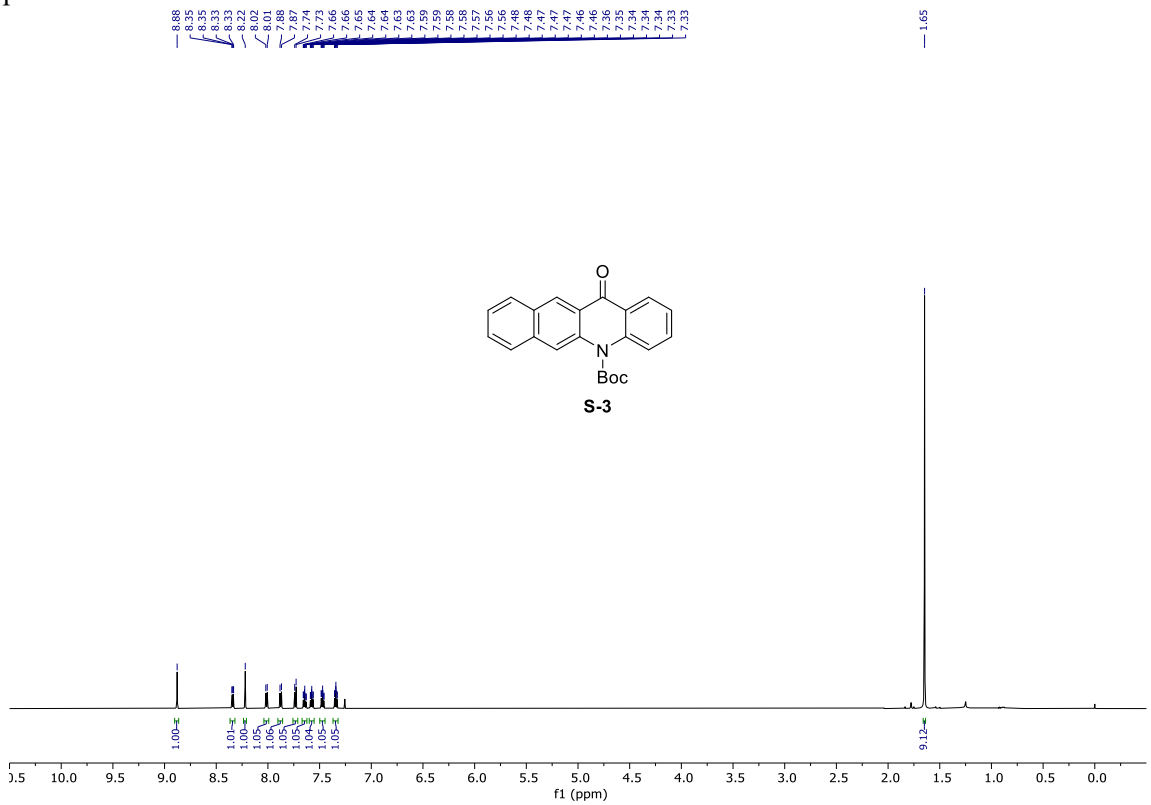
^{1}H spectrum of **NR-Oxm**



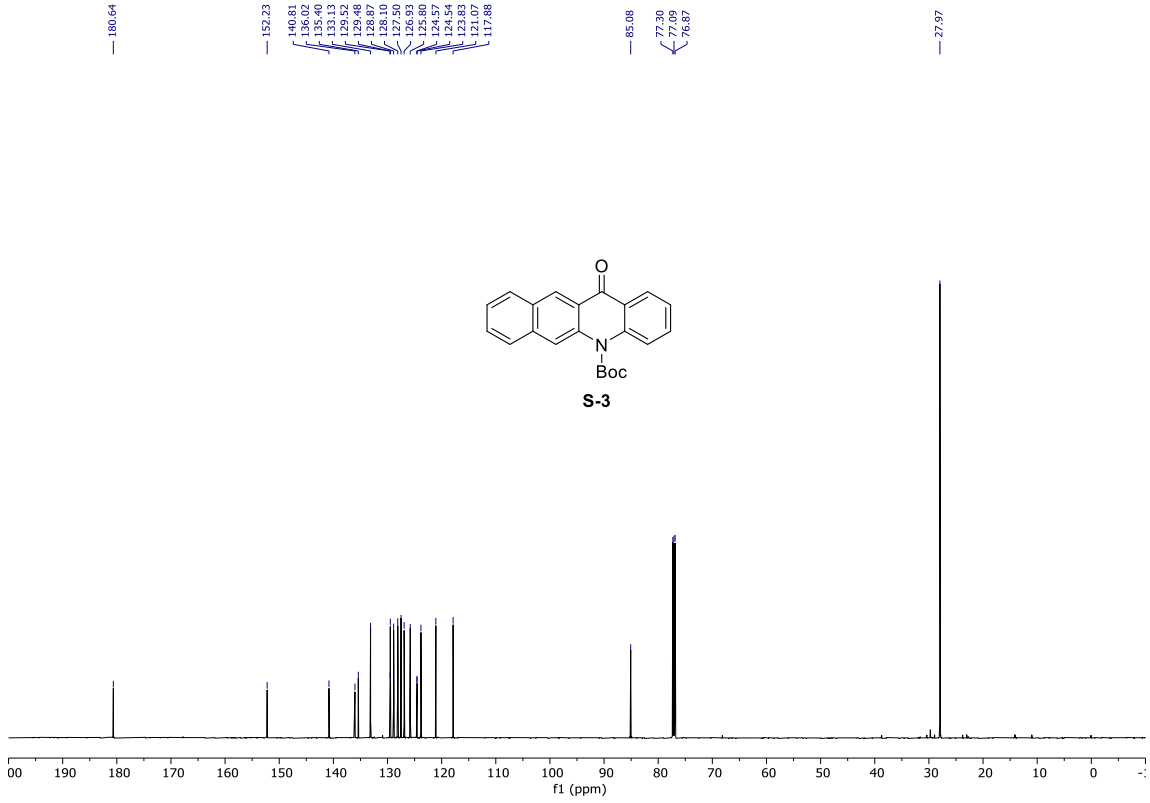
^{13}C spectrum of **NR-Oxm**



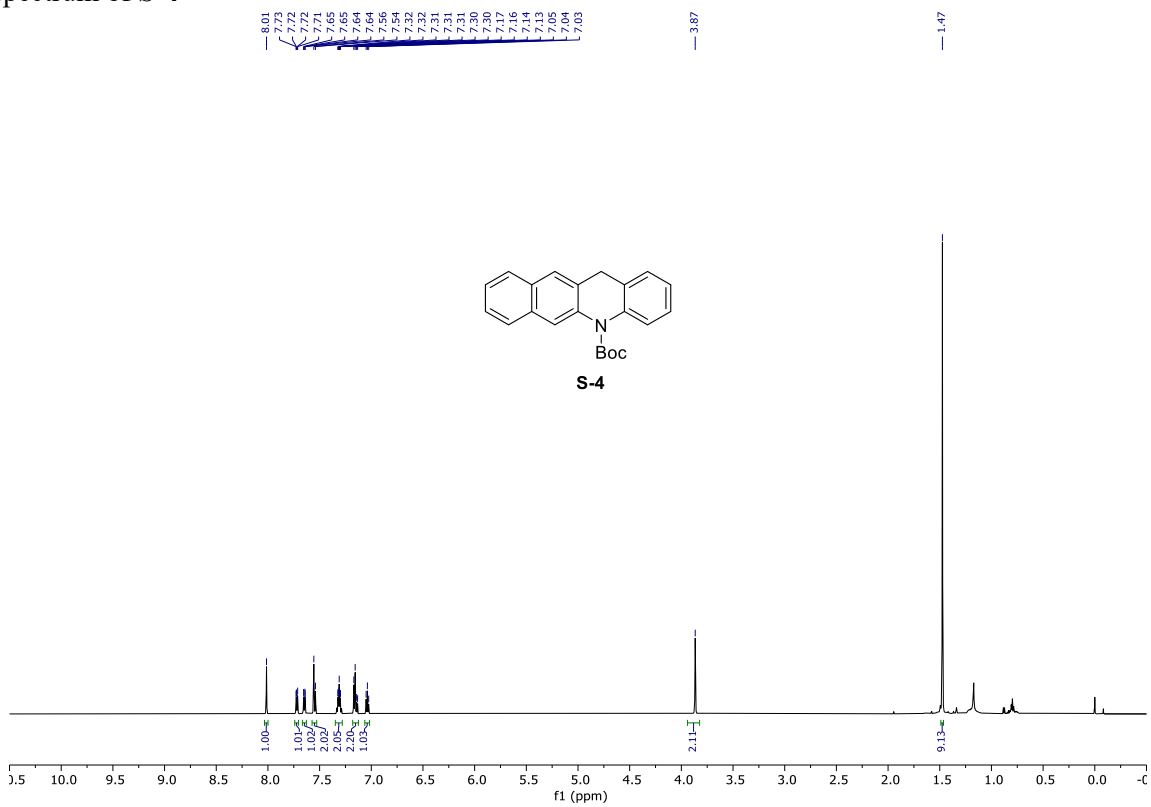
^{1}H spectrum of **S-3**



^{13}C spectrum of **S-3**

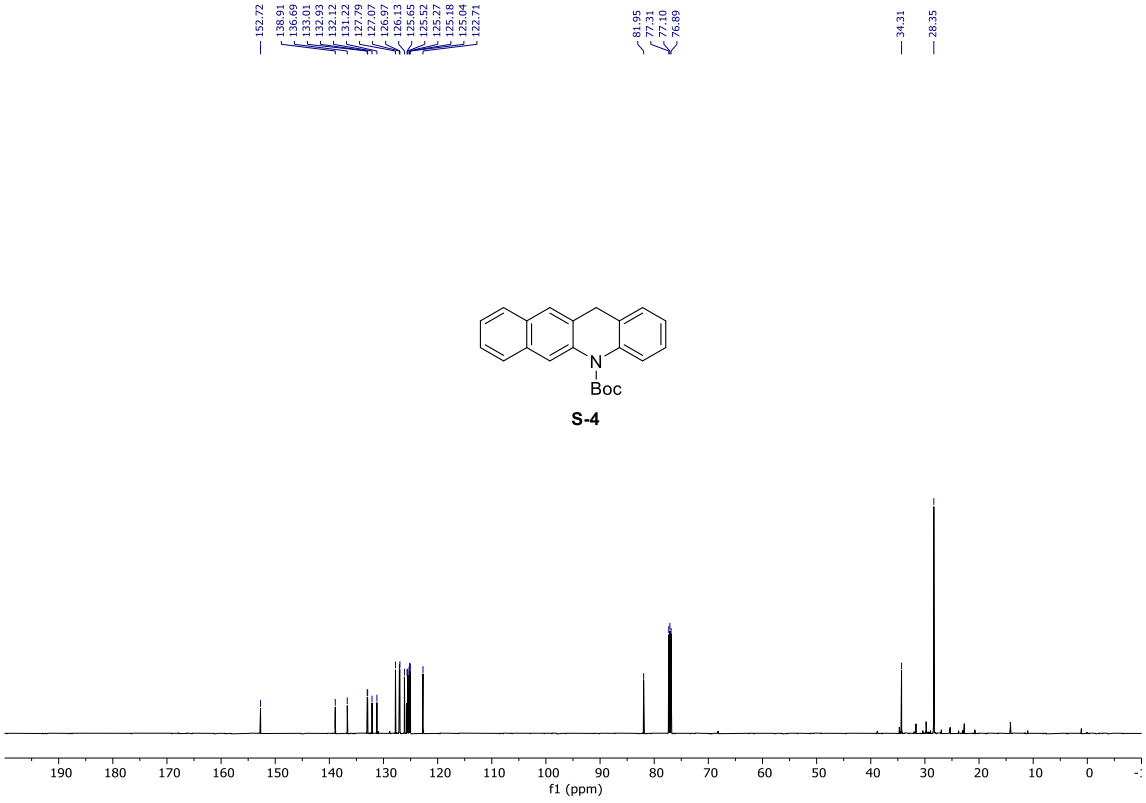


^{1}H spectrum of **S-4**



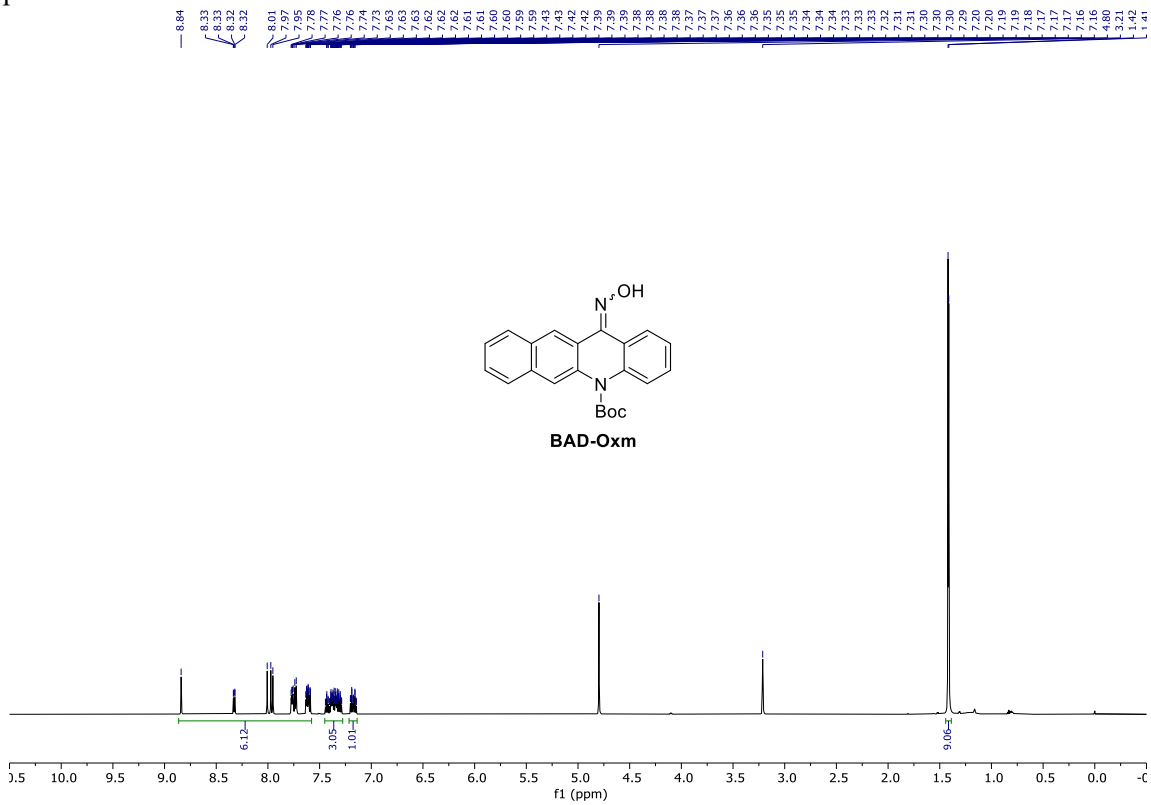
S-4

^{13}C spectrum of **S-4**

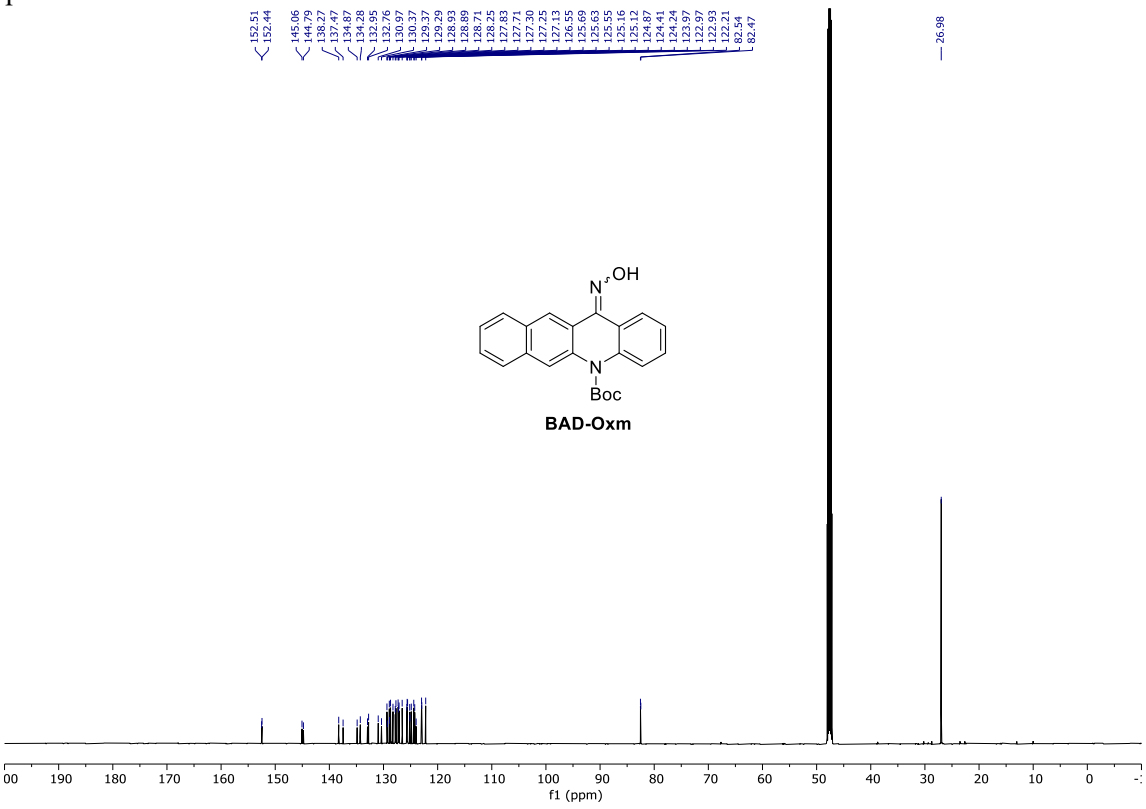


S-4

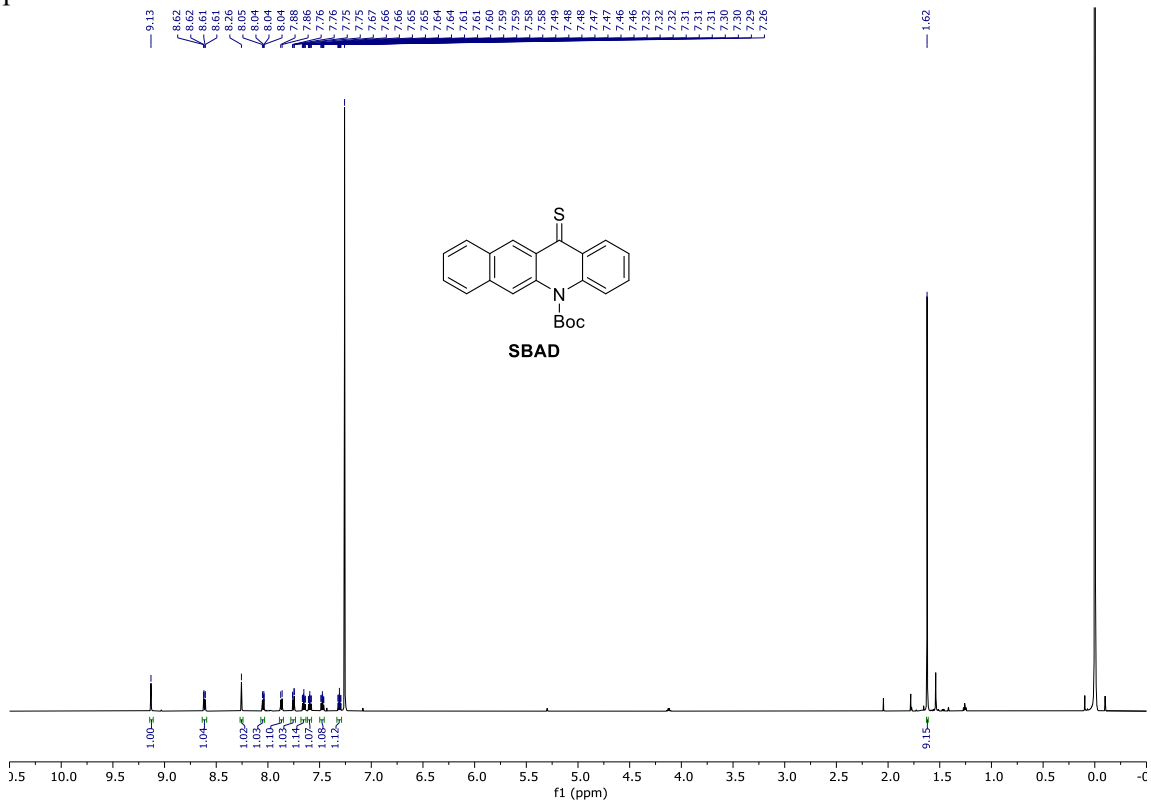
^{1}H spectrum of **BAD-Oxm**



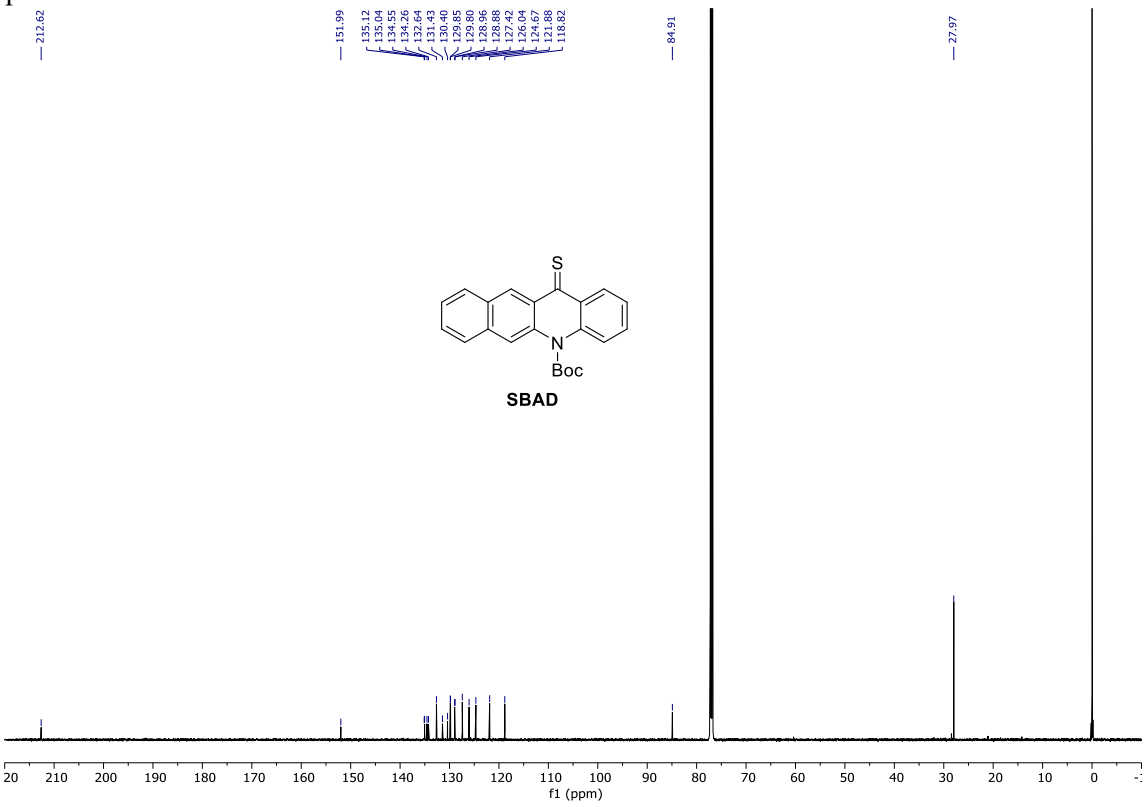
^{13}C spectrum of **BAD-Oxm**



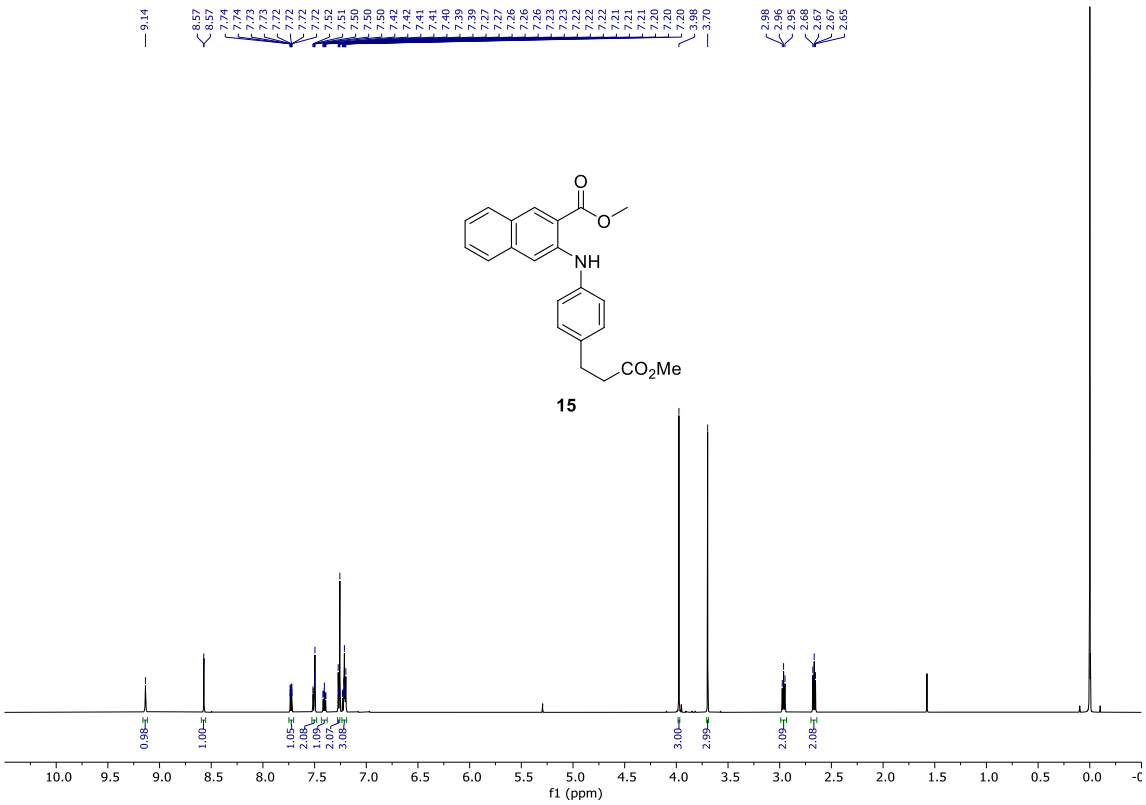
^{1}H spectrum of **SBAD**



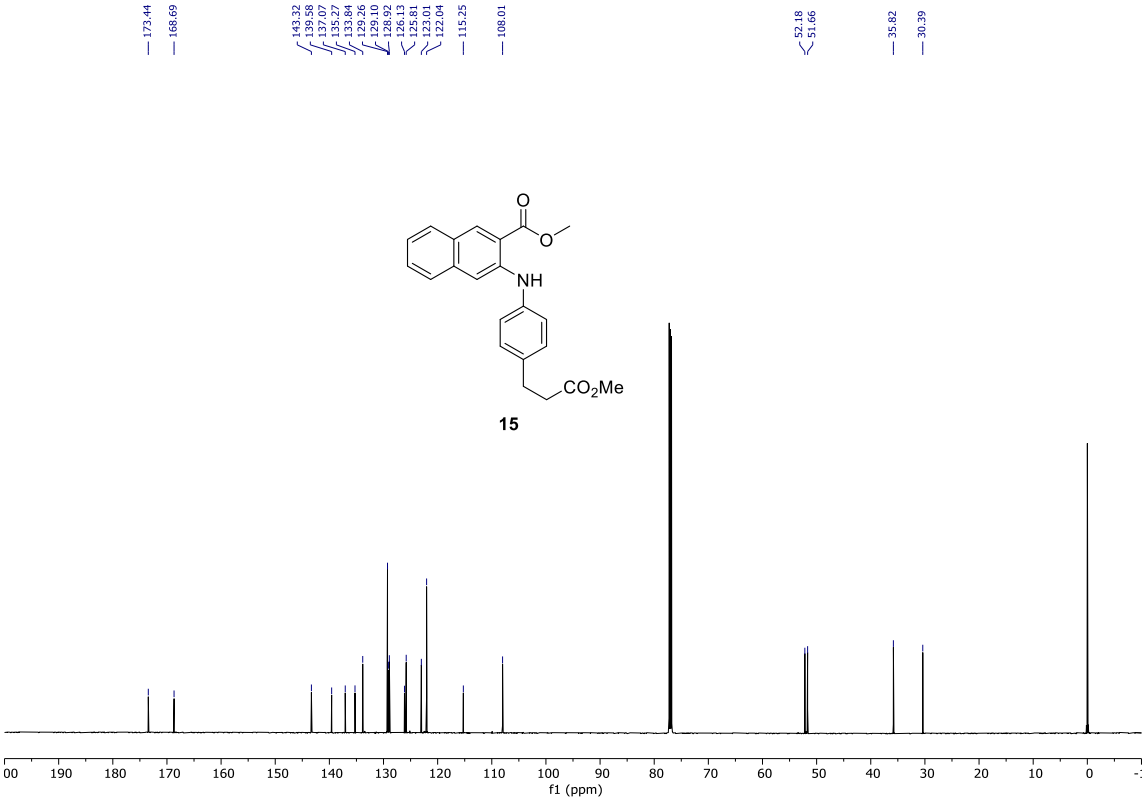
^{13}C spectrum of **SBAD**



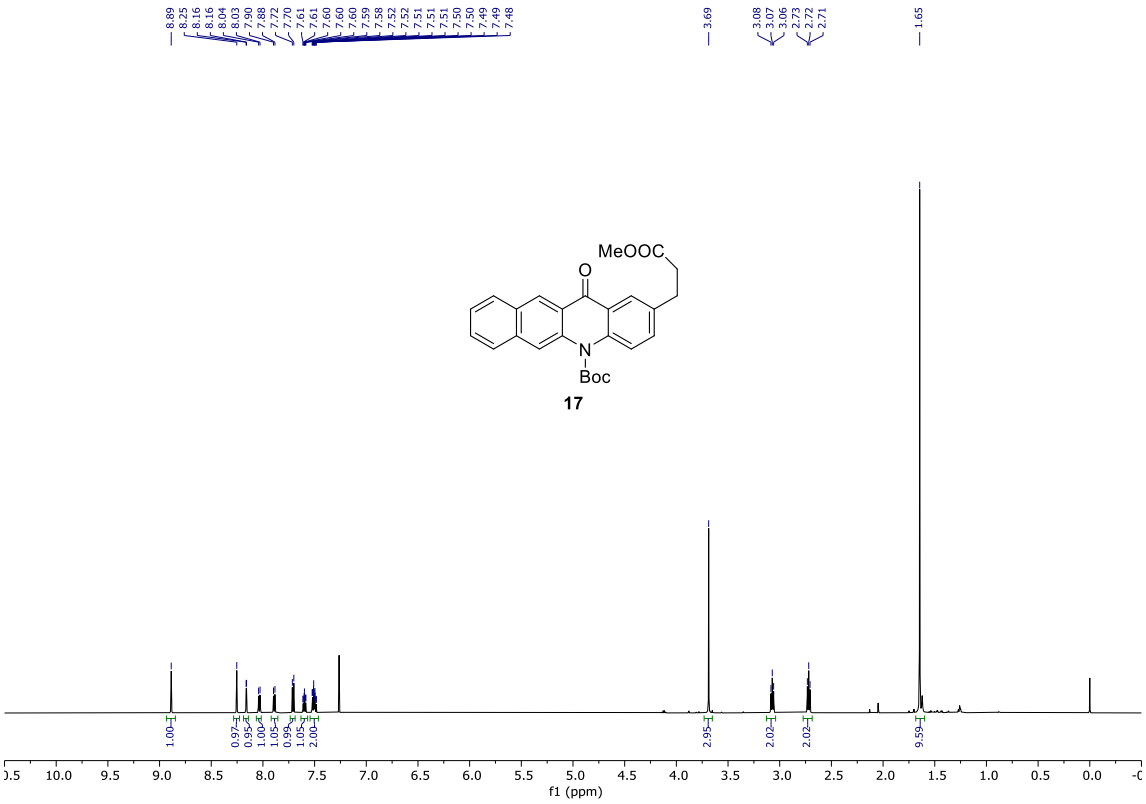
^{1}H spectrum of compound **15**



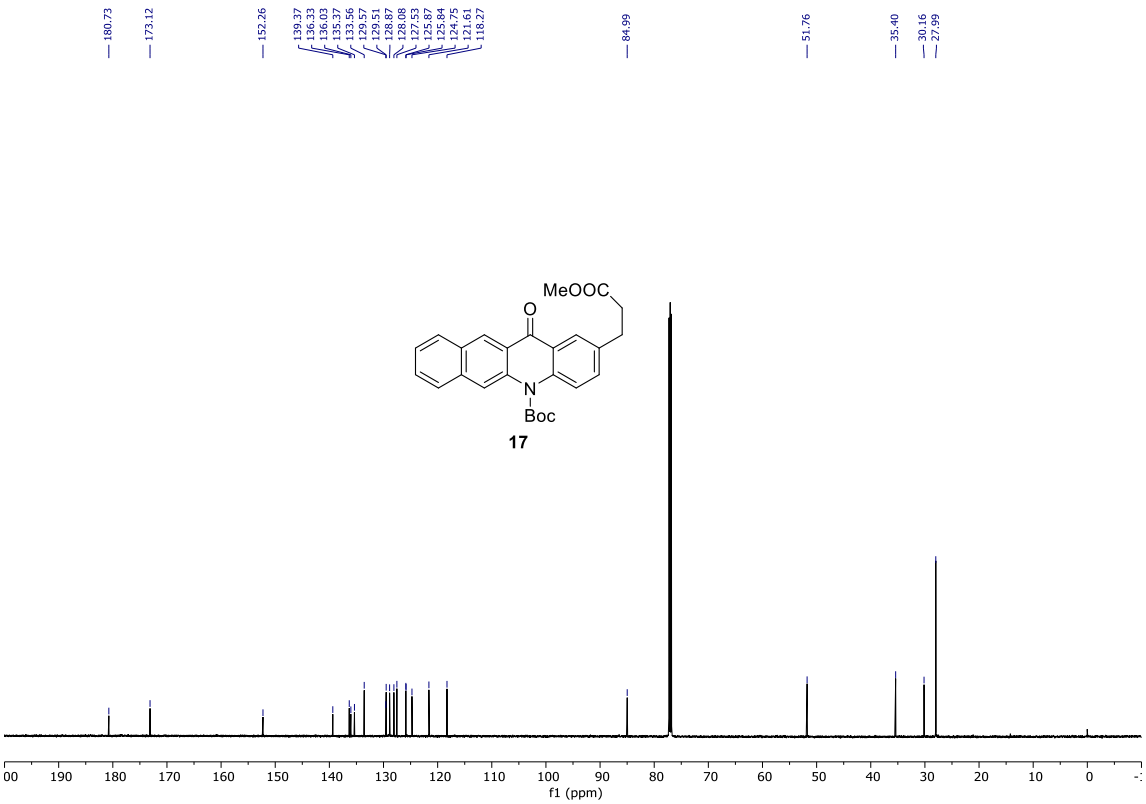
^{13}C spectrum of compound **15**



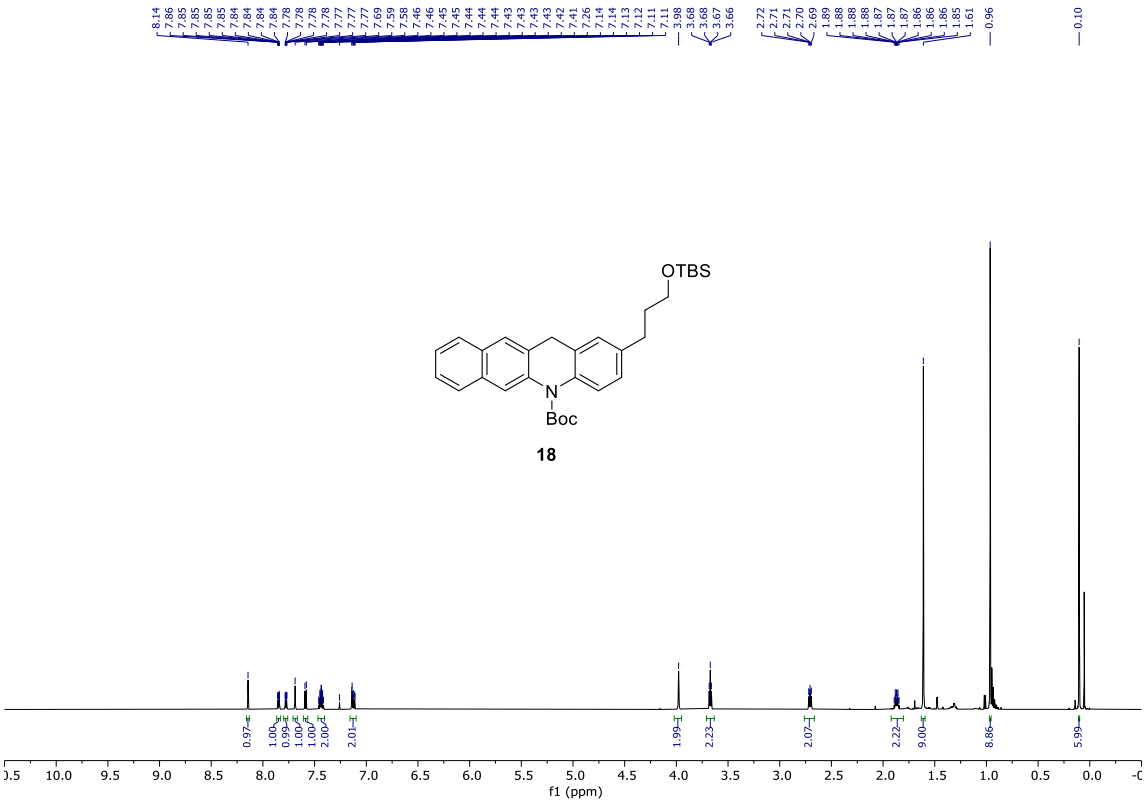
^{1}H spectrum of compound **17**



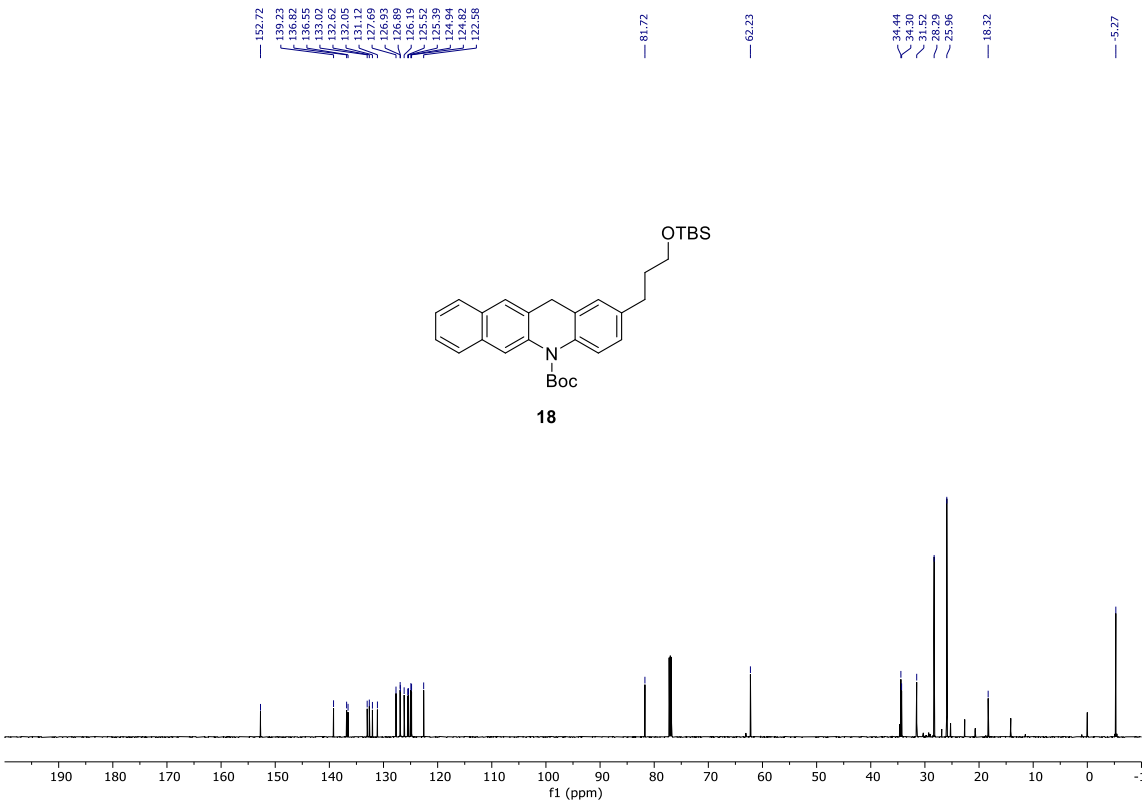
^{13}C spectrum of compound **17**



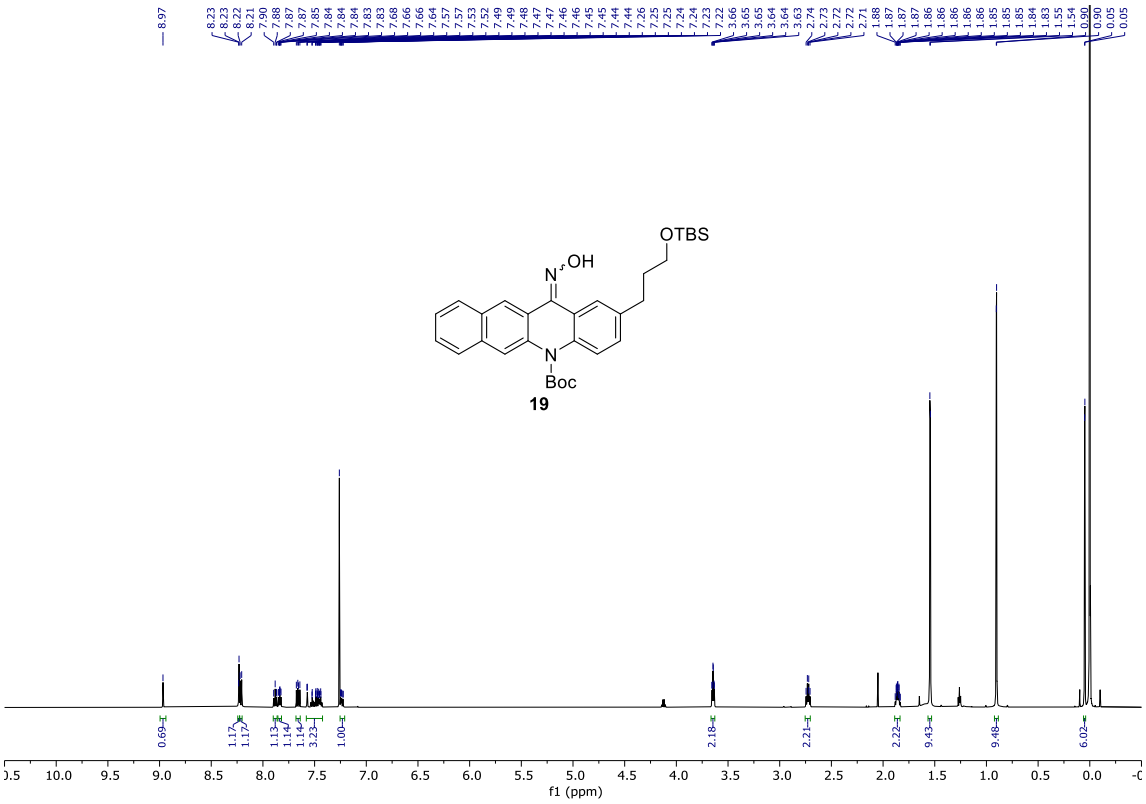
^{1}H spectrum of compound **18**



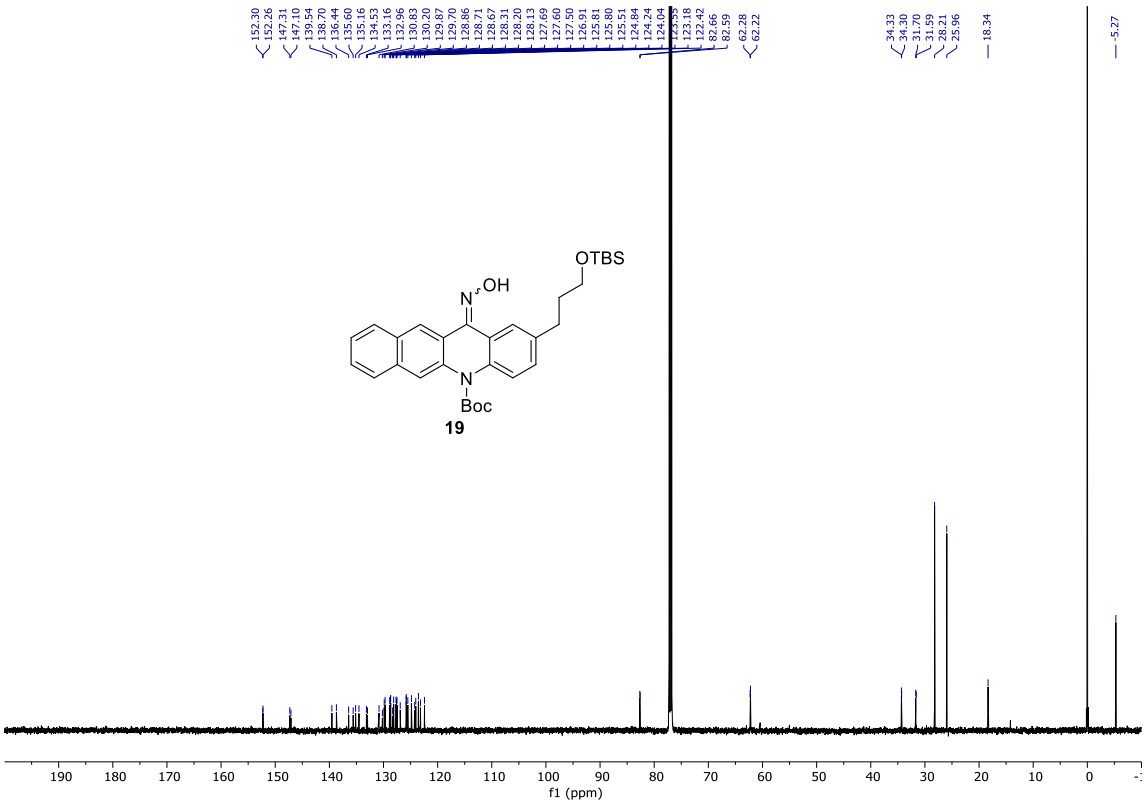
^{13}C spectrum of compound **18**



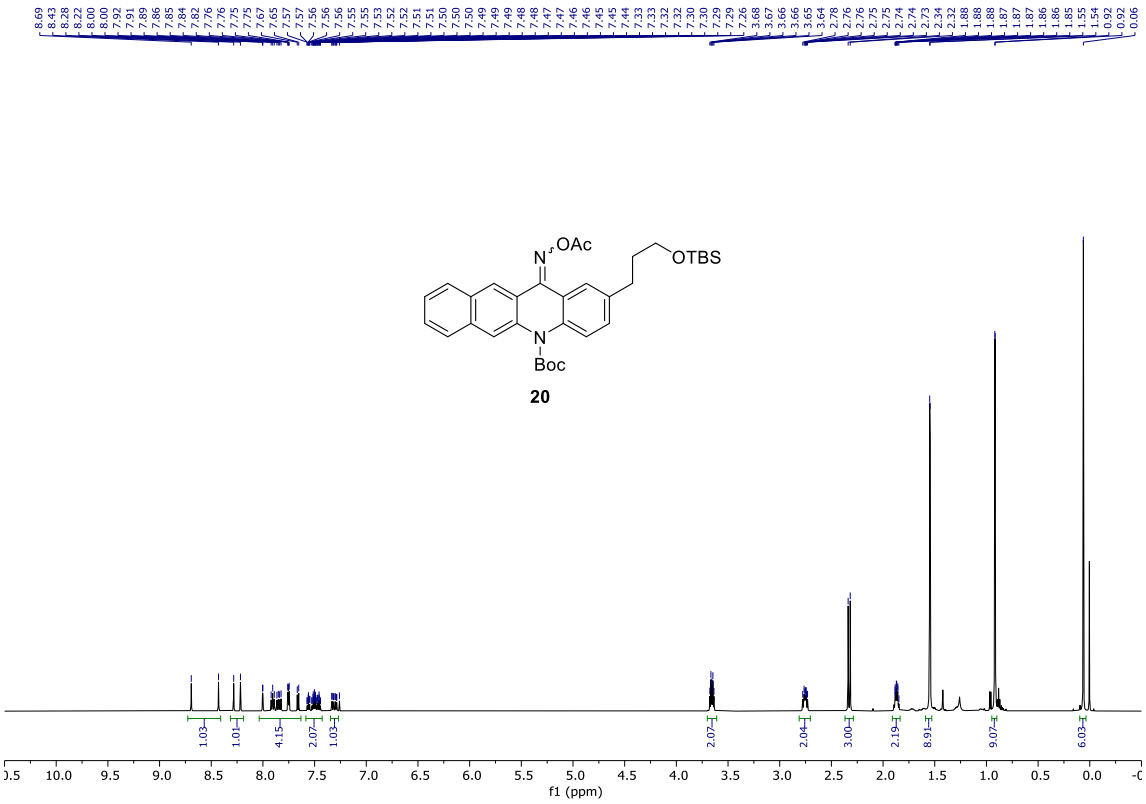
^{1}H spectrum of compound **19**



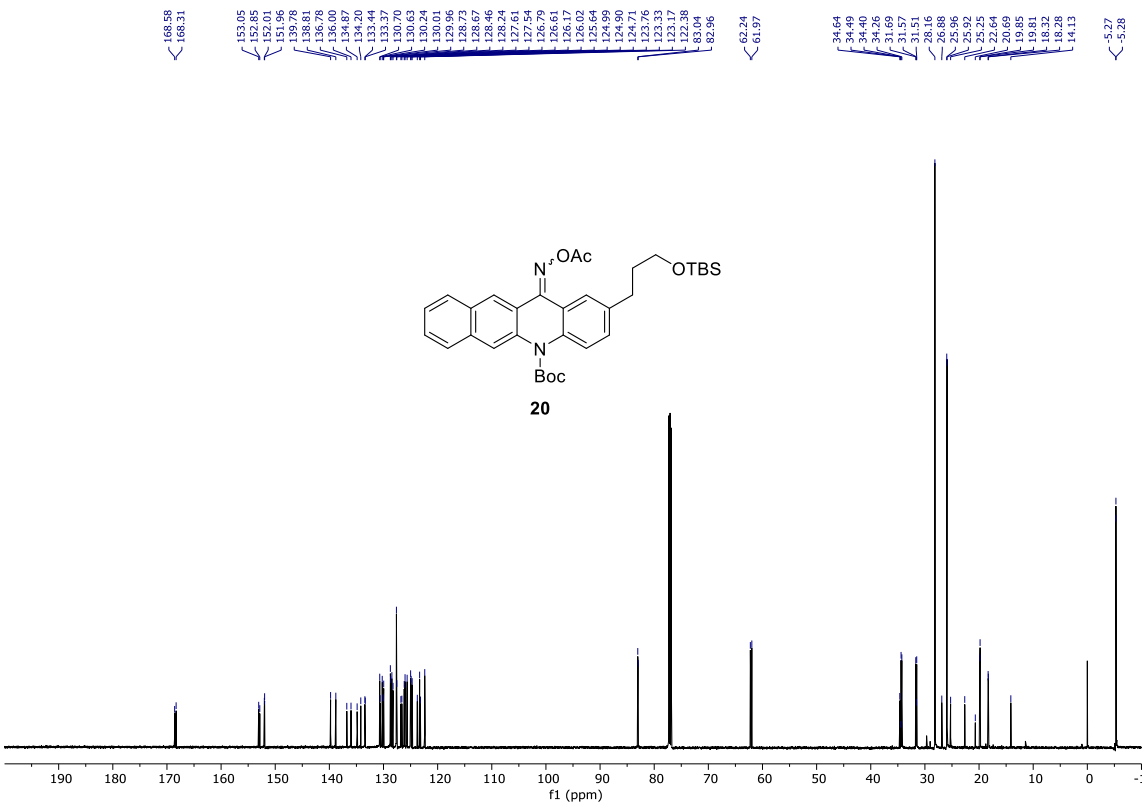
^{13}C spectrum of compound **19**



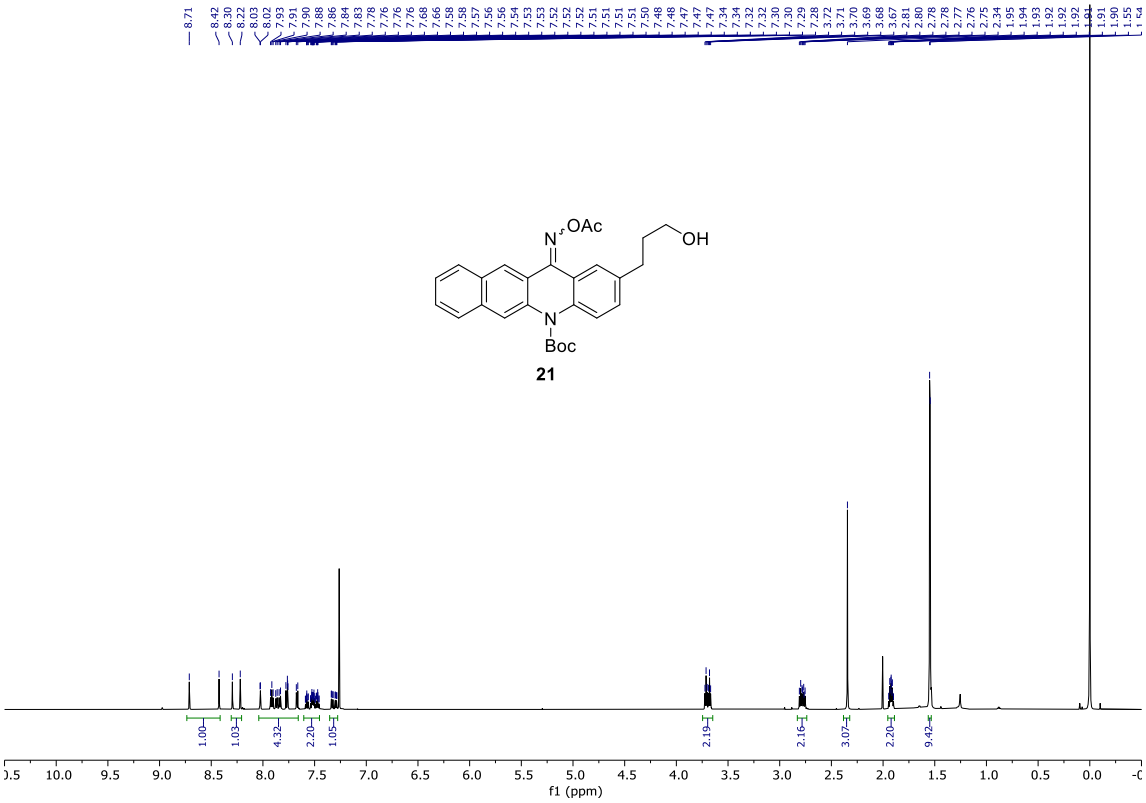
^{1}H spectrum of compound **20**



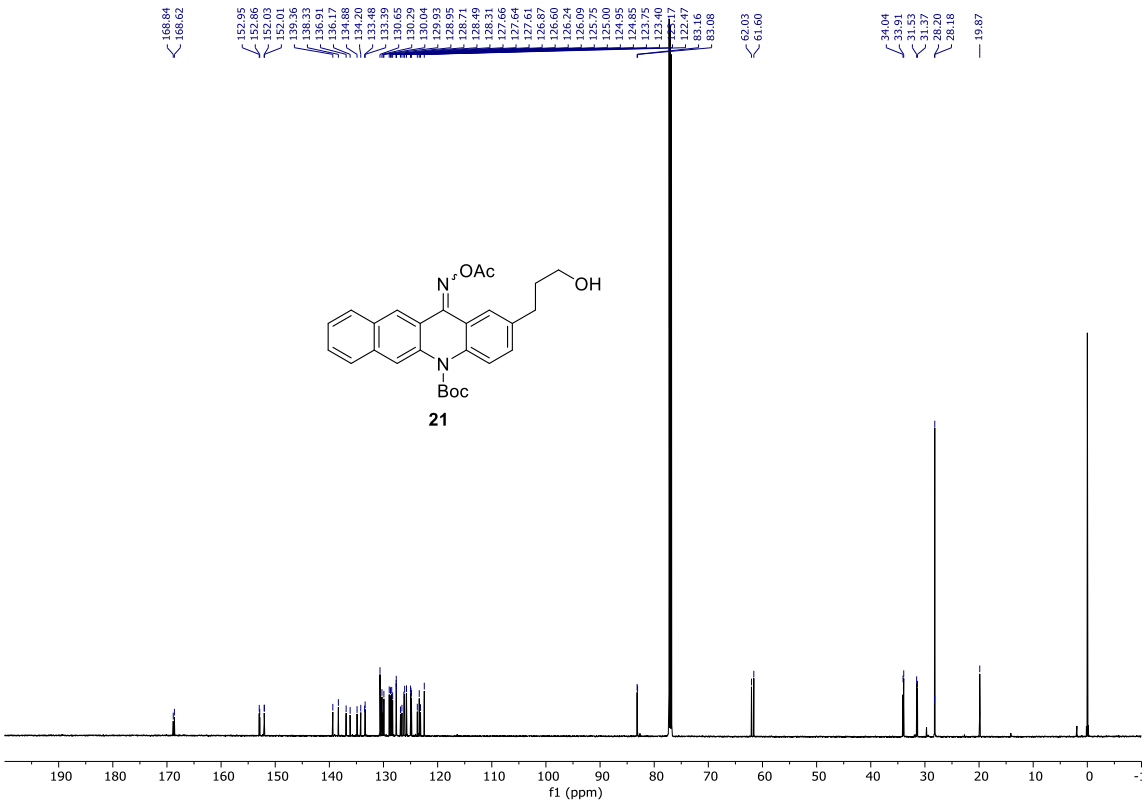
^{13}C spectrum of compound **20**



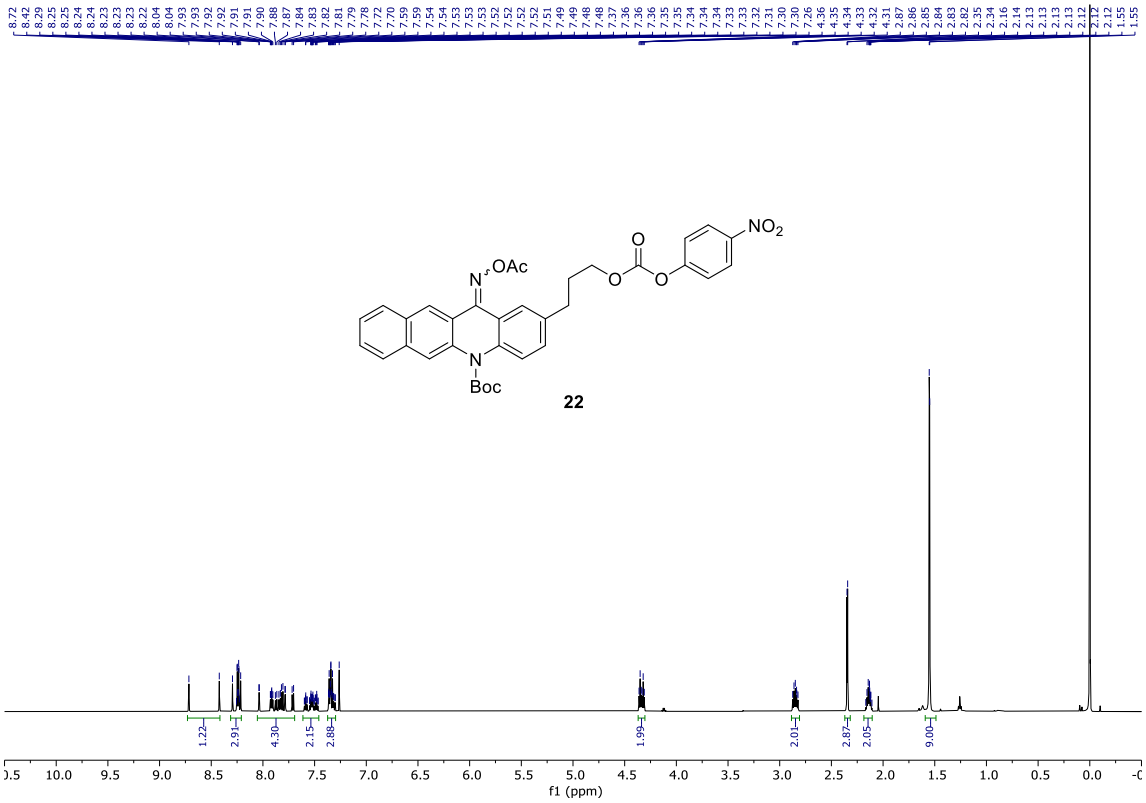
^{1}H spectrum of compound **21**



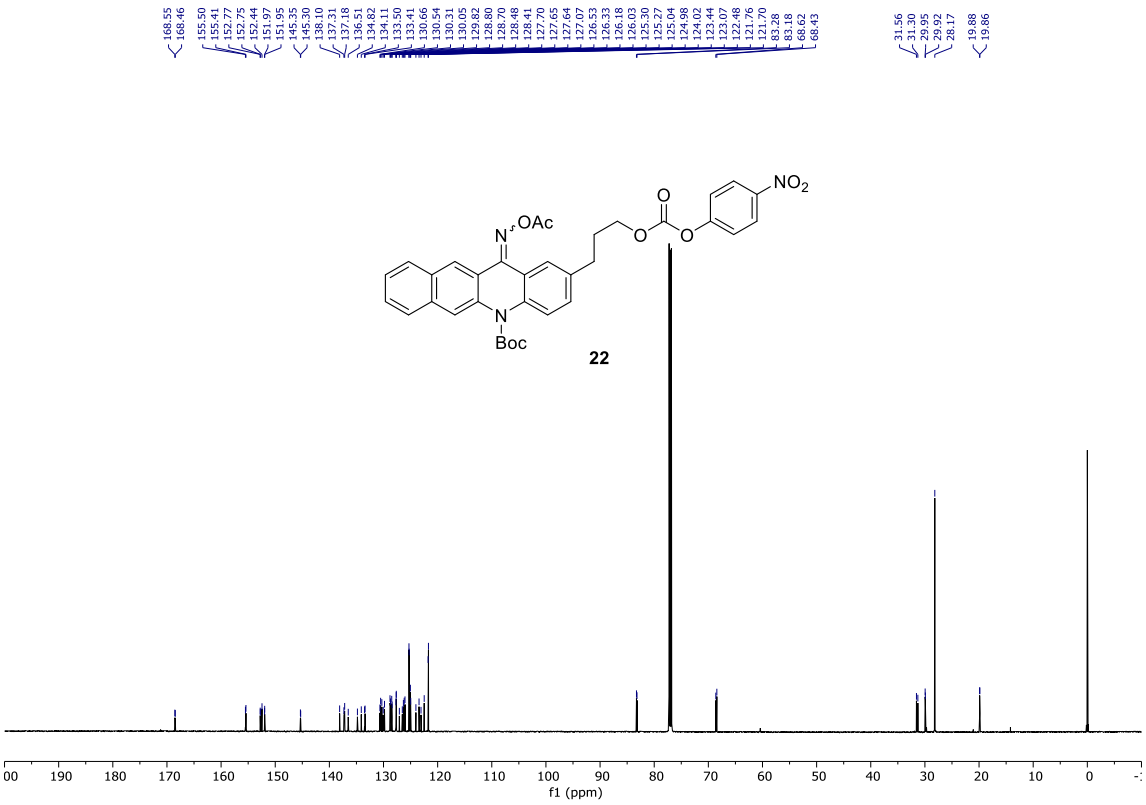
^{13}C spectrum of compound **21**



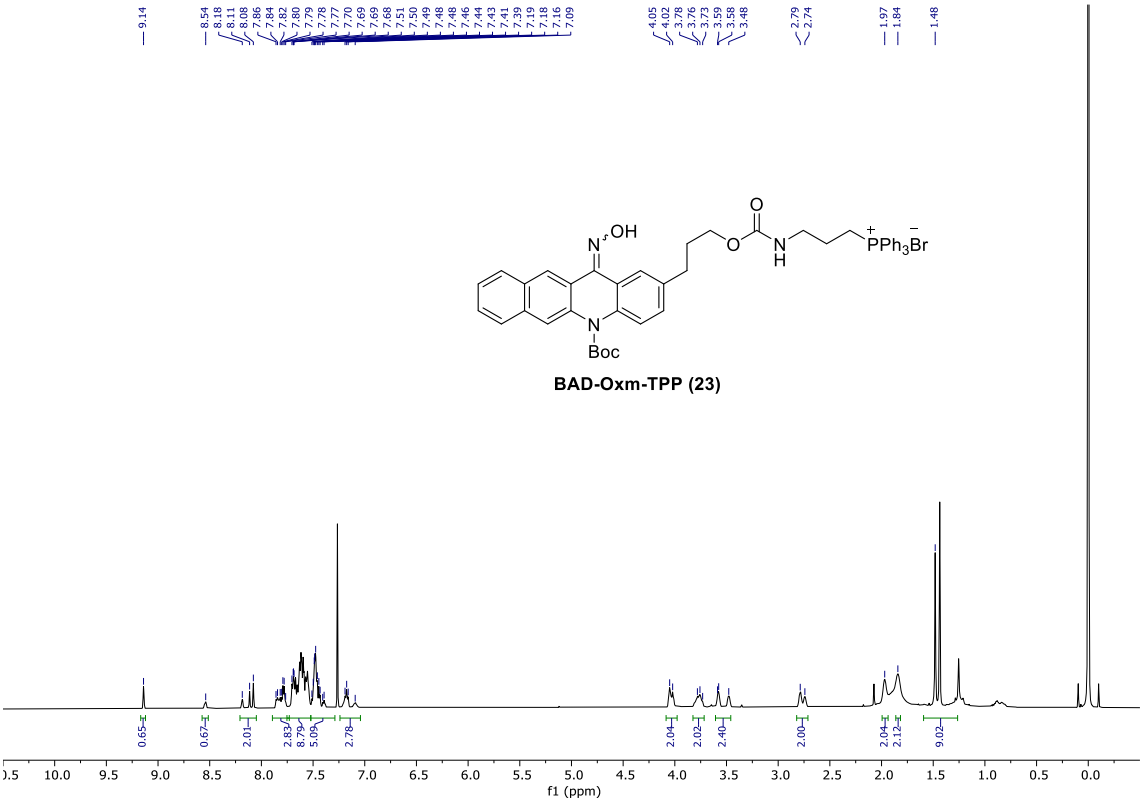
^{1}H spectrum of compound **22**



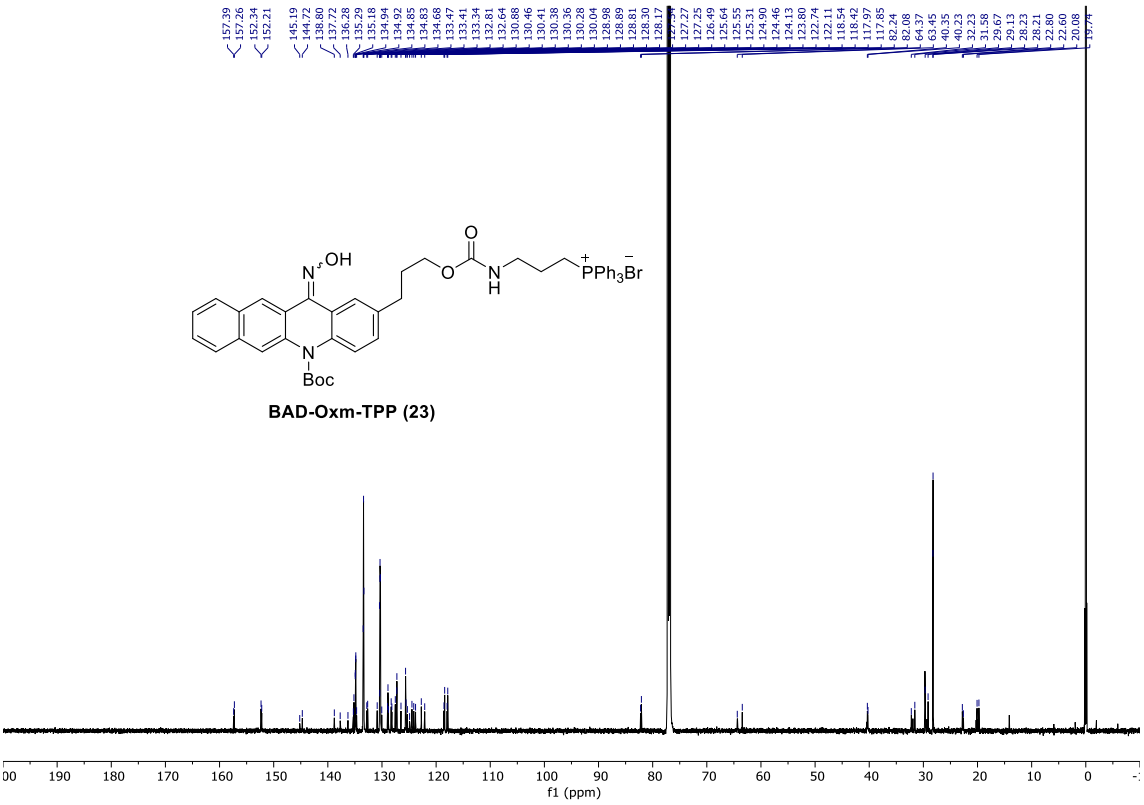
^{13}C spectrum of compound **22**



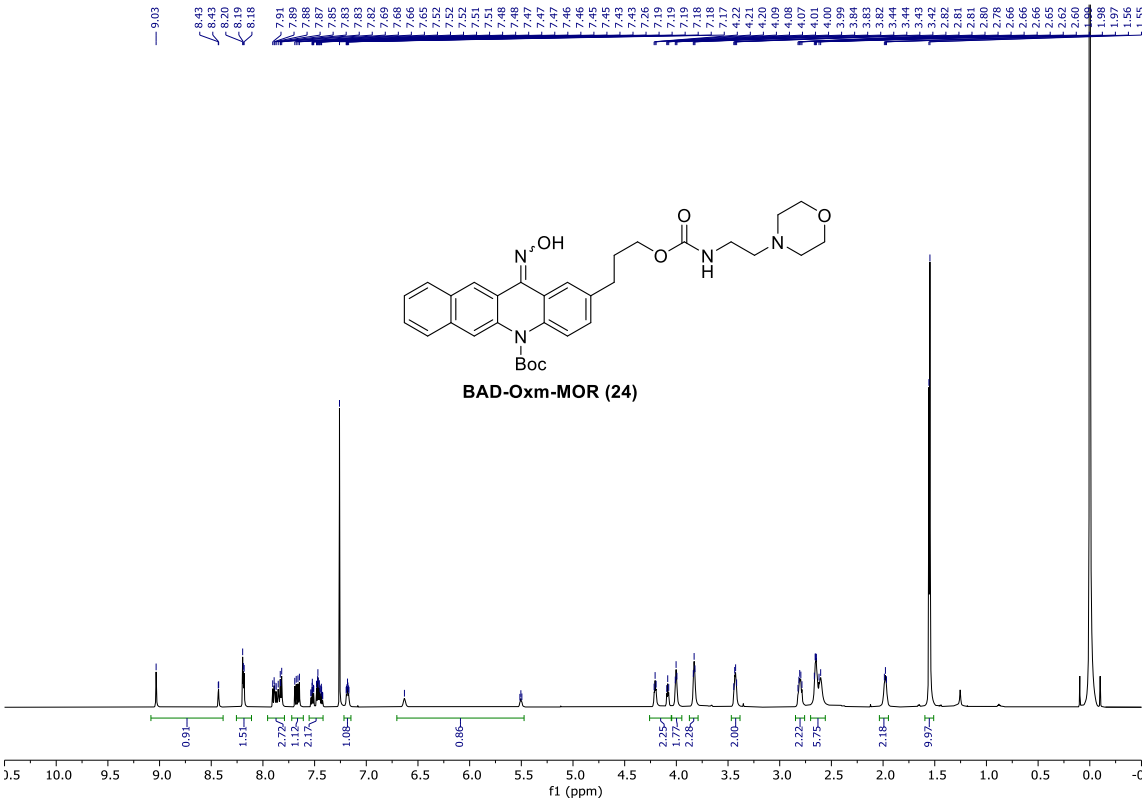
^{1}H spectrum of **BAD-Oxm-TPP**



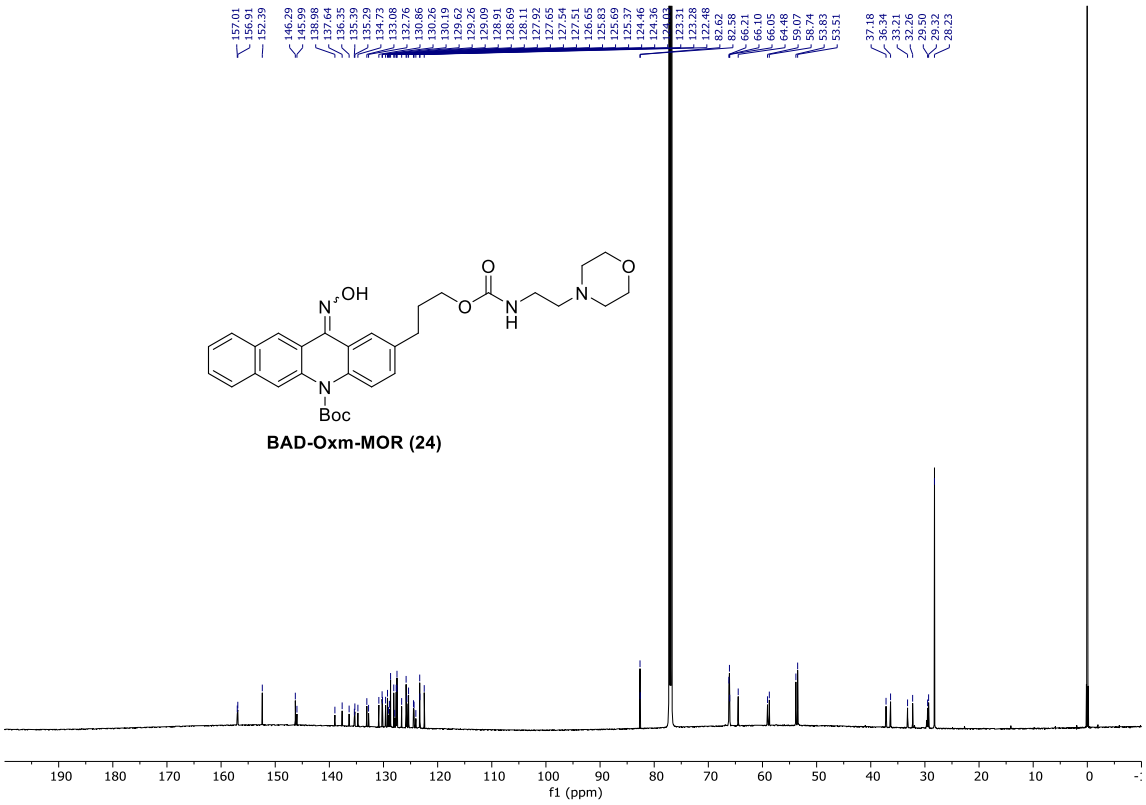
^{13}C spectrum of **BAD-Oxm-TPP**



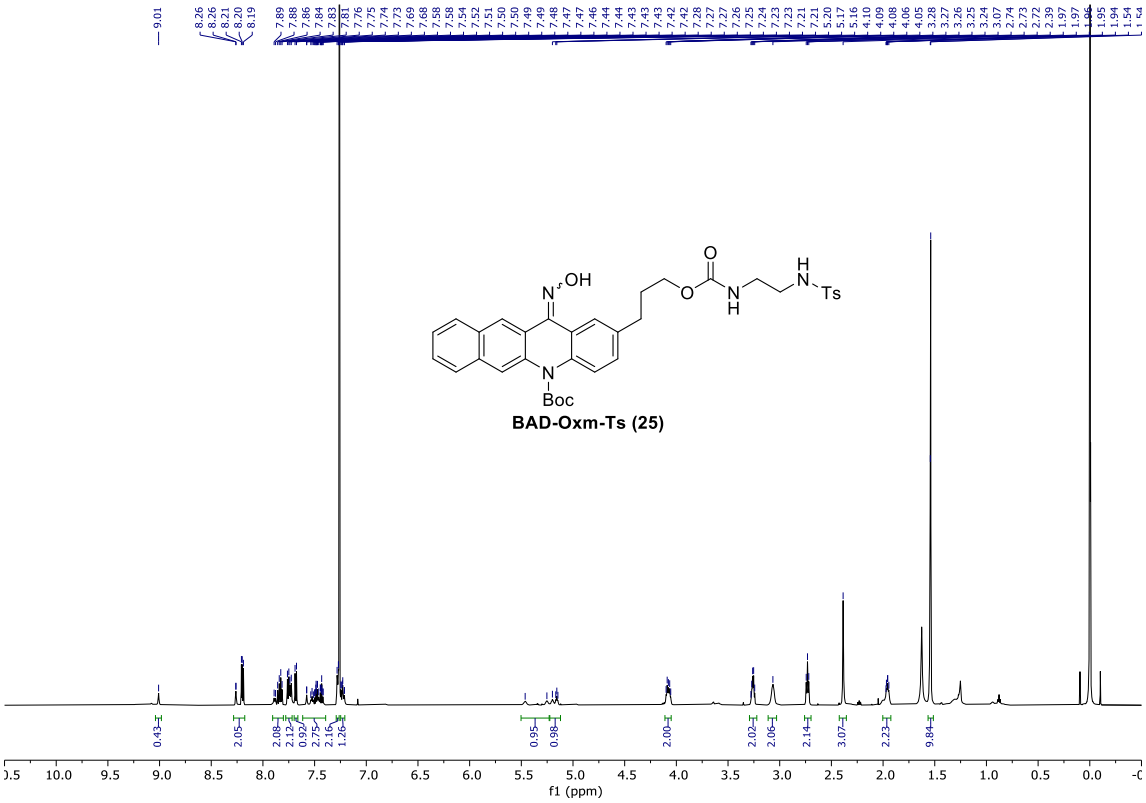
^{1}H spectrum of **BAD-Oxm-MOR**



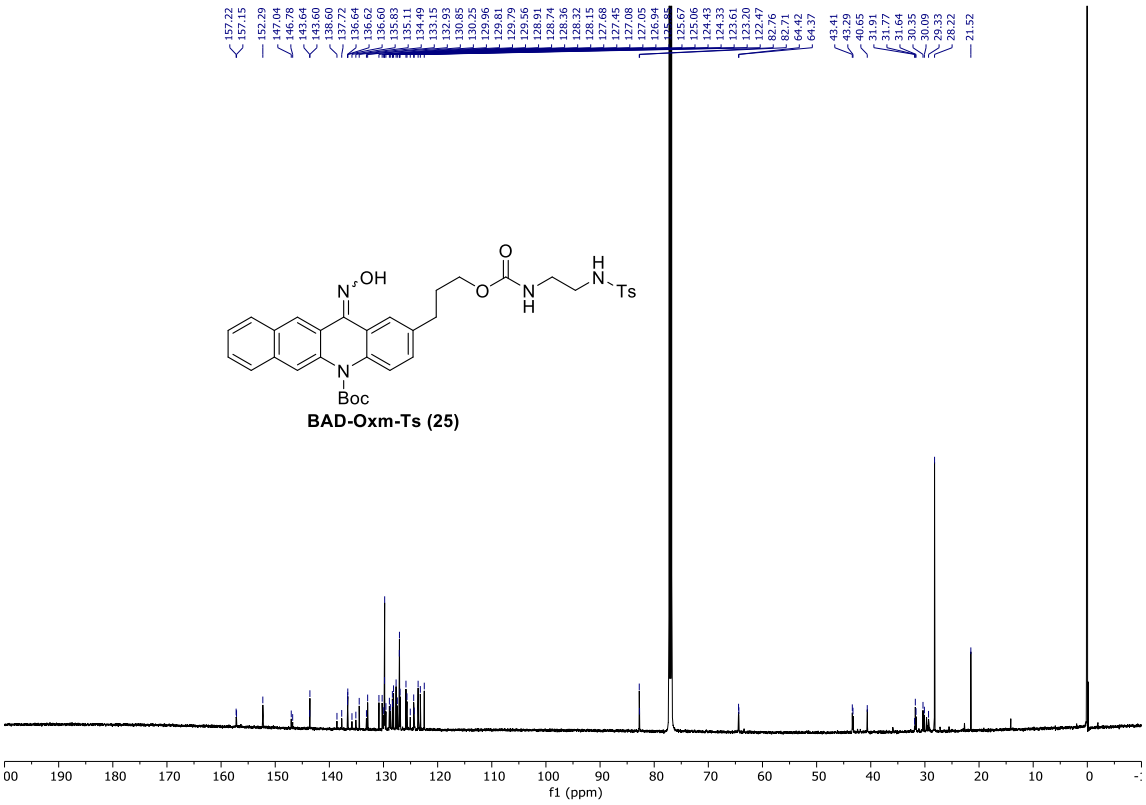
^{13}C spectrum of **BAD-Oxm-MOR**



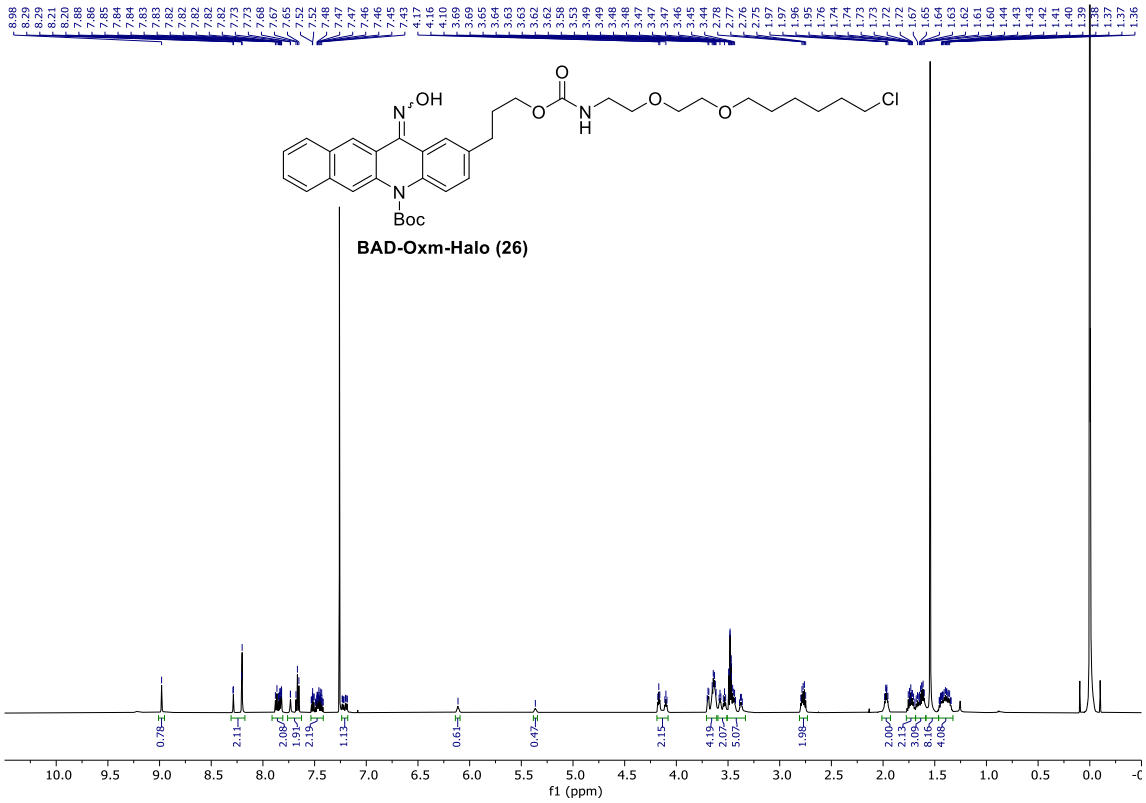
^{1}H spectrum of **BAD-Oxm-Ts**



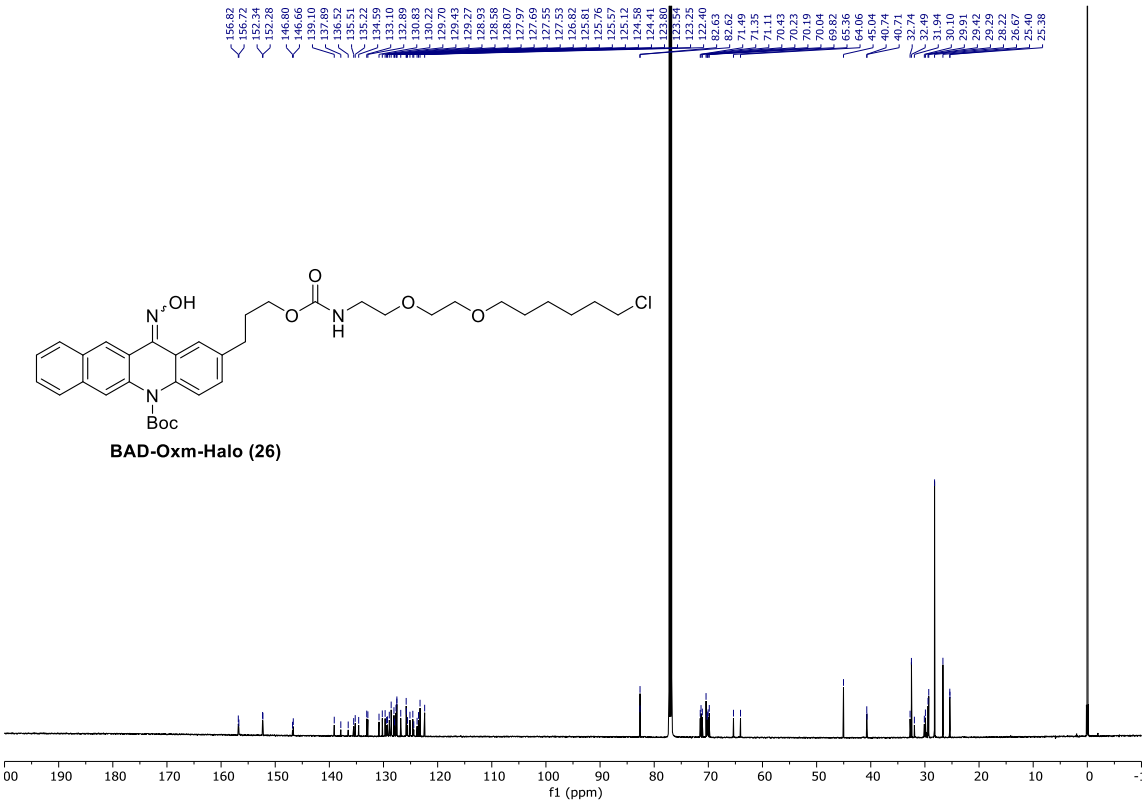
^{13}C spectrum of **BAD-Oxm-Ts**



^{1}H spectrum of **BAD-Oxm-Halo**



^{13}C spectrum of **BAD-Oxm-Halo**



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