Electronic Supplementary Material (ESI) for Materials Advances.

Electronic Supporting Information

Carbonic anhydrase inhibitors-decorated benzothioxanthene-based photosensitizers for synergistic treatment of hypoxic tumours

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I. Experimental Section

Materials

All Reactions needing inert atmosphere were performed under nitrogen using oven-dried glassware and Schlenk techniques. All solvents were obtained from commercial suppliers and used as received. Sodium hydride (NaH, Aldrich, 60% dispersion in mineral oil), copper(II) sulfate pentahydrate ($CuSO_4.5H_2O$, Fluorochem, 98%), sodium ascorbate (Fluorochem, 99%), hexyne (Sigma-Aldrich, 98%) were used as received. Compounds **1**, **Sulfo-prop**, **Sulfocoum-prop** and **Coum-prop** were prepared according to a literature procedure.[1-2] TLC were carried out on Merck DC Kieselgel 60 F-254 aluminium sheets and spots were visualized with an UV-lamp (λ = 254/365 nm). Purifications were performed by silica gel column chromatography (Merck 40−60 µM).

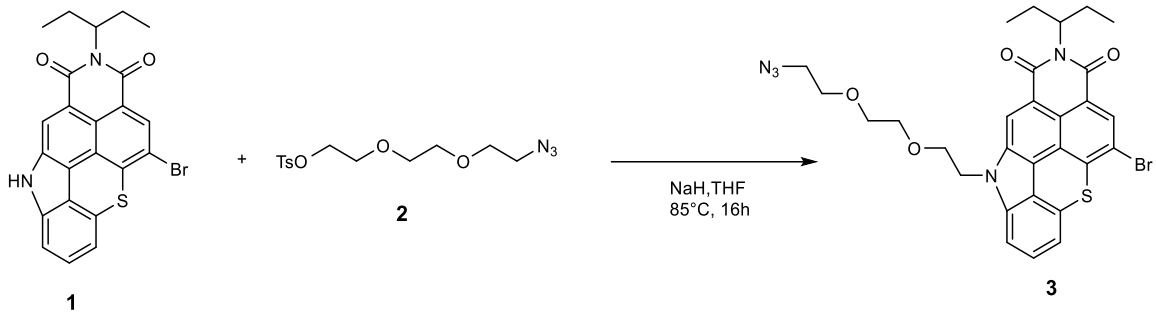
General instruments

IR spectra were carried out on a Perkin Elmer Spectrum 2 FT-IR instrument using the attenuated total reflectance (ATR) measurement mode (diamond crystal). The wavenumber range analyzed was 500-4000 cm^{-1} and the optical resolution of the instrument was 4 cm^{-1}. NMR spectroscopy and mass spectrometry were performed at the Laboratoire de Mesures Physiques (LMP) of the University of Montpellier (UM). ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were recorded either on Bruker 400 MHz Avance III HD or 500 MHz Avance III spectrometers at 298 K. Deuterated solvents $CDCl_3$ was used as received (purchased from Sigma-Aldrich). ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were calibrated to TMS based on the relative chemical shift of the residual non-deuterated solvent as an internal standard. Chemical shifts (δ) are expressed in ppm and coupling constants values (^{n}J) are expressed in Hz. Abbreviations used for NMR spectra are as follows: s: singlet; d: doublet, t: triplet, q: quadruplet; quint: quintuplet, sx: sextuplet and m: multiplet. High Resolution Mass spectra (HRMS) were recorded on a Bruker MicroTof QII instrument in positive modes (ESI). UV-Visible absorption spectra were recorded in DMF with a JASCO V-750 UV-Visible-NIR spectrophotometer in 10 mm quartz cells (Hellma); molar extinction coefficients ε ($L.mol^{-1}.cm^{-1}$) are expressed as log $\boldsymbol{\varepsilon}$. The extinction coefficients were determined by preparing solutions of BTXI derivatives at different concentrations in DMF. The concentration range was chosen to remain in the linear range of the Beer–Lambert relationship (*A ca*. 0.2– 0.8).

The fluorescence spectrum was measured with a Fluorolog FL3-spectrofluorimeter222 (Horiba Jobin Yvon, Palaisau, France) equipped with a 450 W xenon arc lamp, a thermostatically controlled cell holder compartment (25°C), a UV-visible photomultiplier tube R928 (HAMAMATSU Japan) and an InGa As infrared detector cooled with liquid nitrogen (DSS-IGA 020L Electro-Optical System Inc, Phoenixville, PA, USA). The excitation beam is separated by a monochromator double SPEX grating (1200 lines/mm blased at 330 nm). The fluorescence was measured by the UV-detector Visible *via* the SPEX dual grating emission monochromator (1200 lines/mm blased at 500 nm). The singlet oxygen production was measured by the infrared detector via the monochromator double SPEX grating emission line (600 lines/mm blased at 1 µm). All spectra were measured in using 4-sided quartz cells.

Synthesis and characterization

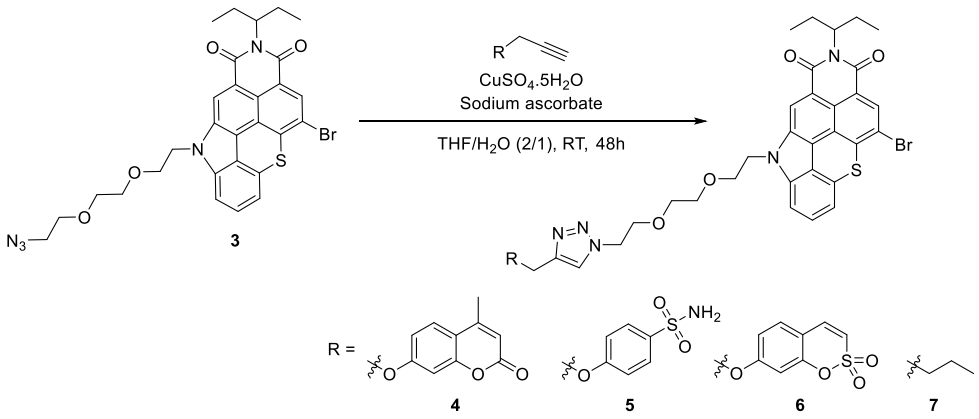
Synthesis of compound **3**:



Scheme 1: Synthesis of compound **3**[2]

A solution of compound **1** (50 mg, 0.107 mmol) and sodium hydride 60 % (22 mg, 0.535 mmol, 5 equiv.) in THF (10 mL) was stirred for 15 minutes at room temperature under inert atmosphere. Compound **2**[2] (44 mg, 0.133 mmol, 1.25 equiv) was then added to the resulting solution and the mixture was stirred under reflux overnight. The *N*-alkylation was followed by TLC (6/4, cyclohexane/ ethyl acetate). When it was judged finished, the solvent was evaporated under reduced pressure and the residue was diluted with CH_2Cl_2. The organic layer was washed several times with 200 mL of water, dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography on silica gel (6/4, cyclohexane/ ethyl acetate, R_F = 0.34) to obtain compound **3** as an orange solid (45 mg,

yield: 68 %). ATR-FTIR: $\tilde{\nu}$ (cm^{-1}) = 2969, 2872 (ν_{Csp3-H}), 2111 (ν_{N3}), 1681, 1630 (ν_{CO}), 1574, 1539 ($\nu_{C=C\ aromatic}$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.69 (s, 1H), 8.40 (s, 1H), 7.47 (t, $^3J_{H-H}$ = 7.9 Hz, 1H), 7.23 (d, $^3J_{H-H}$ = 7.5 Hz, 1H), 7.10 (d, $^3J_{H-H}$ = 7.5 Hz, 1H), 5.09 (m, 1H), 4.58 (t, $^3J_{H-H}$ = 5.3 Hz, 2H), 3.94 (t, $^3J_{H-H}$ = 5.3 Hz, 2H), 3.53 (m, 6H), 3.25 (t, 2H, $^3J_{H-H}$ = 5.0 Hz), 2.26 (m, 2H), 1.93 (m, 2H), 0.90 (t, $^3J_{H-H}$ = 7.5 Hz, 6H). **^{13}C{^{1}H} NMR** (126 MHz, $CDCl_3$): δ 144.9, 141.3, 134.6, 133.4, 130.0 , 129.8, 123.0, 122.0, 119.0, 114.1, 107.8, 71.2, 71.1, 70.3, 69.4, 69.0, 57.7, 50.8, 45.2, 25.2, 21.8, 11.5. HRMS (ESI^+) *m/z* calcd for $C_{29}H_{29}BrN_5O_4S$ $[M+H]^+$ = 622.1118, found: 622.1105.



Scheme 1: Synthesis of CAi-based BTIs

General procedure for the azide-alkyne Huisgen cycloaddition. In a 50 mL two-necked round-bottom flask, compound **3** (40 mg, 0.064 mmol, 1 equiv.) and the corresponding alkyne (1 equiv.) were dissolved in THF (10 mL). In a Schlenk tube, $CuSO_4.5H_2O$ (2 mg, 0.019 mmol, 0.3 equiv.) and sodium ascorbate (15 mg, 0.081 mmol, 1 equiv.) were dissolved in H_2O (5 mL) and this mixture was added to the solution of compound **3** and alkyne in THF. The reaction was allowed to proceed for 48 hours at room temperature. The reaction was monitored by TLC (6/4, cyclohexane/ ethyl acetate). After reaction completion, the mixture was then diluted with dichloromethane (50 mL) and washed with water (50 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated under vacuum. The crude product was purified by column chromatography on silica gel using cyclohexane/ethyl acetate mixture (8/2 to 6/4) as eluents to give the expected compound.

Compound 4: Yield 60%, R_F = 0.25. ATR-FTIR: $\tilde{\nu}$ (cm^{-1}) = 2922, 2857 (ν_{Csp3-H}), 1716, 1686, 1611 (ν_{CO}), 1576, 1459 ($\nu_{C=C}$ aromatic) cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.67 (s, 1H), 8.39 (s, 1H), 7.83 (s, 1H), 7.53 (d, $^3J_{H-H}$ = 8.8 Hz, 2H), 7.46 (t, $^3J_{H-H}$ = 7.9 Hz, 1H), 7.19 (d, $^3J_{H-H}$

= 8.2 Hz, 1H), 7.09 (d, $^3J_{H\text{-}H}$ = 7.5 Hz, 1H), 6.93 (m, 2H), 6.18 (d, $^3J_{H\text{-}H}$ = 7.9 Hz, 1H), 5.12 (m, 3H), 4.76 (d, $^3J_{H\text{-}H}$ = 3.6 Hz, 3H), 4.52 (m, 3H), 3.87 (m, 2H), 3.81 (m, 2H), 3.52 (s, 4H), 2.41 (d, $^3J_{H\text{-}H}$ = 1.0 Hz, 3H), 2.26 (m, 2H), 1.91 (m, 2H), 0.89 (t, $^3J_{H\text{-}H}$ = 7.5 Hz, 6H).**^{13}C{^{1}H} NMR** (126 MHz, $CDCl_3$): δ 161.0, 155.1, 152.2 , 142.9, 141.2, 134.7 , 129.9, 125.6, 124.4, 123.0, 122.0, 119.0, 114.3, 114.2, 114.1, 112.4, 112.1, 107.7, 102.2, 71.1, 70.6, 69.5, 62.4, 57.8, 50.6 , 45.3, 25.2, 18.7, 11.5. **HRMS (ESI$^+$)** ***m/z*** calcd for $C_{42}H_{39}BrN_5O_7S$ $[M+H]^+$: 836.1748, found: 836.1748. UV-Vis (DMF): λ_{max}(log ε): 410(4.63), 481(4.68).

Compound 5: Yield 84 % R_F = 0.25. ATR-FTIR: $\tilde{\nu}$ (cm^{-1}) = 2924, 2855 ($\nu_{Csp3\text{-}H}$), 1686, 1632 (ν_{CO}), 1579, 1498 ($\nu_{C=C}$ aromatic), 1379, 1155 ($\nu_{S=O}$) cm^{-1}. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.72 (s, 1H), 8.36 (s, 1H), 7.79 (d, $^3J_{H\text{-}H}$ = 8.4 Hz, 2H), 7.58 (s, 1H), 7.46 (t, $^3J_{H\text{-}H}$ = 7.8 Hz, 1H), 7.22 (d, $^3J_{H\text{-}H}$ = 8.3 Hz, 1H), 7.09 (d, $^3J_{H\text{-}H}$ = 8.0 Hz, 1H), 6.95 (d, $^3J_{H\text{-}H}$ = 8.5 Hz, 2H), 5.15 (s, 2H), 5.09 (m, 1H), 4.86 (s, 2H), 4.54 (t, $^3J_{H\text{-}H}$ = 5.1 Hz, 2H), 4.43 (t, $^3J_{H\text{-}H}$ = 5.0 Hz, 2H), 3.85 (t, $^3J_{H\text{-}H}$ = 5.1 Hz, 2H), 3.73 (t, $^3J_{H\text{-}H}$ = 5.0 Hz, 2H), 3.43 (s, 4H), 2.25 (m, , 2H), 1.91 (m, 2H), 0.89 (t, $^3J_{H\text{-}H}$ = 7.4 Hz, 6H).**^{13}C{^{1}H} NMR**(126 MHz, $CDCl_3$) δ 161.3, 160.8, 141.1, 134.3, 129.9, 128.7, 125.6, 122.7, 121.8, 118.6, 115.3, 114.1, 114.0, 107.9, 77.1, 76.9, 71.0, 70.6, 70.2, 69.4, 57.7, 56.1, 45.3, 34.2, 30.4, 29.8, 25.1, 22.4, 14.2, 11.5. **HRMS (ESI$^+$)** ***m/z*** calcd for $C_{38}H_{38}BrN_6O_7S_2$ $[M+H]^+$: 833.1421, found: $[M+H]^+$= 833.1413. UV-Vis (DMF): λ_{max}(log ε): 412(4.65), 483(4.69)

Compound 6: Yield 73% R_F = 0.25. ATR-FTIR: $\tilde{\nu}$ (cm^{-1}) = 2969, 2880 ($\nu_{Csp3\text{-}H}$), 1683, 1634 (ν_{CO}), 1573, 1490 ($\nu_{C=C}$ aromatic), 1352, 1167 ($\nu_{S=O}$) cm^{-1}. **^{1}H NMR** (400MHz, $CDCl_3$): δ 8.69 (s, 1H), 8.41 (s, 1H), 7.69 (s, 1H), 7.46 (t, $^3J_{H\text{-}H}$ = 7.8 Hz, 1H), 7.20 (m, 2H), 7.10 (m, 2H), 6.99 (m, 2H), 6.77 (m, 1H), 5.11 (s, 2H), 4.72 (s, 1H), 4.53 (m, 2H), 4.46 (m, 2H), 3.86 (m, 2H), 3.77 (m, 2H), 3.47 (s, 4H), 2.26 (m, 2H), 1.92 (m, 2H), 0.89 (t, $^3J_{H\text{-}H}$ = 7.3 Hz, 6H). **^{13}C{^{1}H}** NMR (126 MHz, $CDCl_3$): δ 155.7, 145.6, 141.1, 135.7, 134.5, 130.1, 129.8, 123.0, 122.8, 121.9, 120.1, 119.9, 119.6, 119.2, 119.0, 118.8, 114.7, 114.2, 107.6, 77.4, 77.1, 76.9, 71.0, 70.6, 70.1, 69.4, 62.6, 57.7, 56.5, 50.5, 45.1, 31.7, 30.4, 29.8, 25.1, 14.2, 11.5. **HRMS (ESI$^+$)** ***m/z*** calcd for $C_{40}H_{37}BrN_5O_8S$ $[M+H]^+$: 858.1261, found: 858.1260. UV-Vis (DMF): λ_{max}(log ε): 410(4.61), 480(4.65)

Compound 7: Yield 44 %. R_F = 0.3. ATR-FTIR: $\tilde{\nu}$ (cm^{-1}) =2917 (ν_{CH3}). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.63 (s, 1H), 8.35 (s, 1H), 7.42 (t, $^3J_{H\text{-}H}$ = 7.9 Hz, 1H), 7.28 (s, 1H), 7.17 (d, $^3J_{H\text{-}H}$ = 8.1 Hz, 1H), 7.03 (d, $^3J_{H\text{-}H}$ = 7.5 Hz, 1H), 5.09 (m, 1H), 4.52 (t, $^3J_{H\text{-}H}$ = 5.3 Hz, 2H), 4.38 (t, $^3J_{H\text{-}H}$ = 5.1 Hz, 2H), 3.86 (t, $^3J_{H\text{-}H}$ = 5.2 Hz, 2H), 3.72 (t, $^3J_{H\text{-}H}$ = 5.2 Hz, 2H), 3.46 (m, 4H), 2.64 (m, 2H), 2.26 (m, 2H), 1.92 (m, 2H), 1.58 (m, 2H), 1.33 (m, 2H), 0.90 (t, $^3J_{H\text{-}H}$ = 7.4, Hz,

6H), 0.88 (t, $^{3}J_{H\text{-}H}$ = 7.3 Hz, 3H).**^{13}C{^{1}H} NMR** (126 MHz, $CDCl_3$) : δ 148.3, 141.1, 134.4, 132.8, 131.0, 130.0, 129.8, 128.1, 122.9, 122.0 (x2), 121.7, 118.8, 114.2, 114.0, 107.7, 77.1, 71.1, 70.6, 70.1, 69.8, 57.7, 50.1, 45.1, 31.7, 29.8, 25.5, 25.1, 22.4, 14.0, 11.5. **HRMS (ESI^{+})** ***m/z*** calcd for $C_{35}H_{39}BrN_5O_4S$ $[M+H]^+$ = 704.1901. found: $[M+H]^+$ = 704.1905. UV-Vis (DMF): λ_{max}(log ε): 410(4.61), 480(4.65).

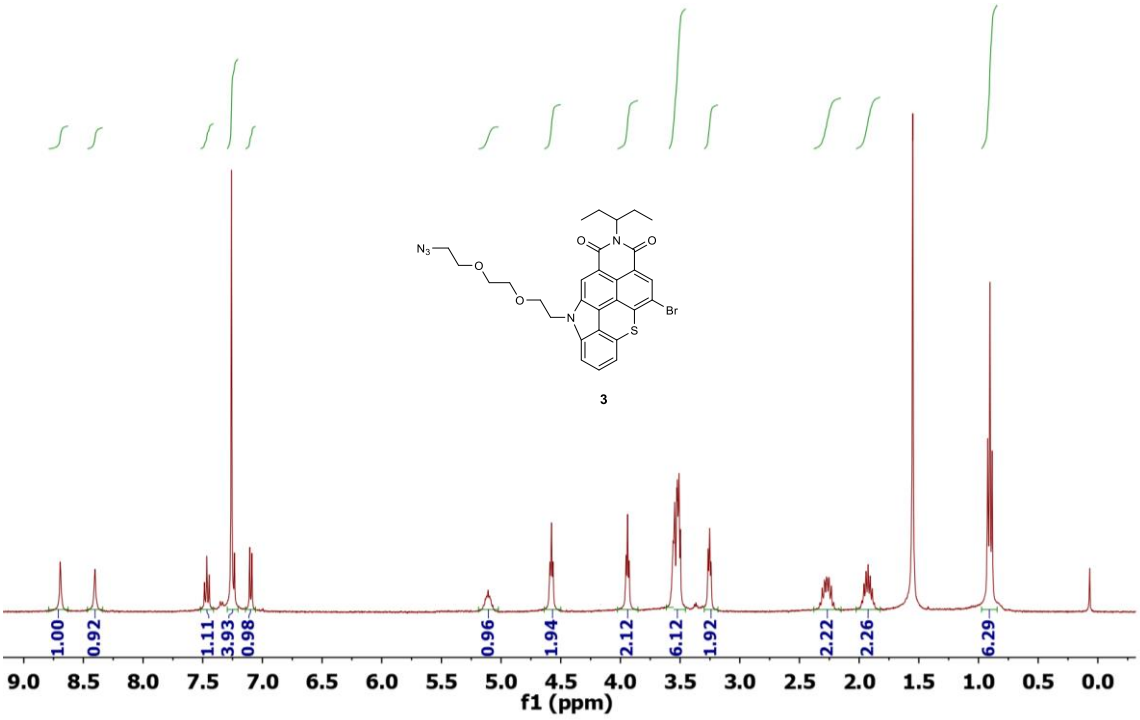


Figure S1. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298K) of compound **3.**

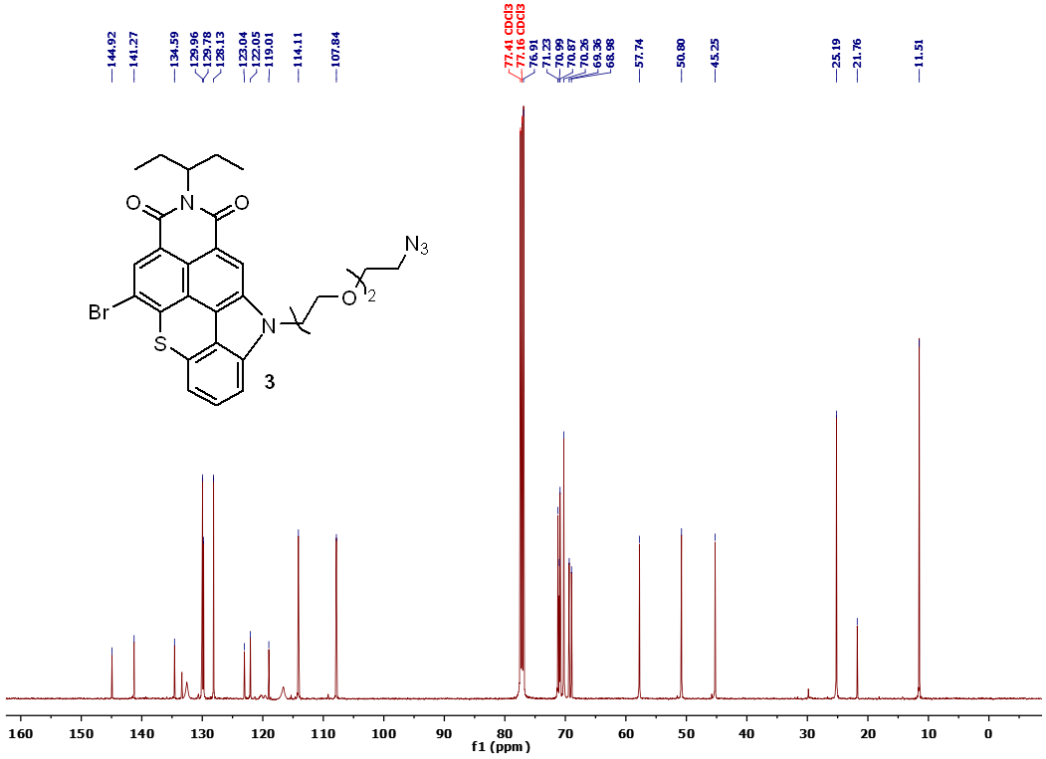


Figure S2. $^{13}C\{^{1}H\}$ NMR spectrum (126 MHz, $CDCl_3$, 298K) of compound **3**.

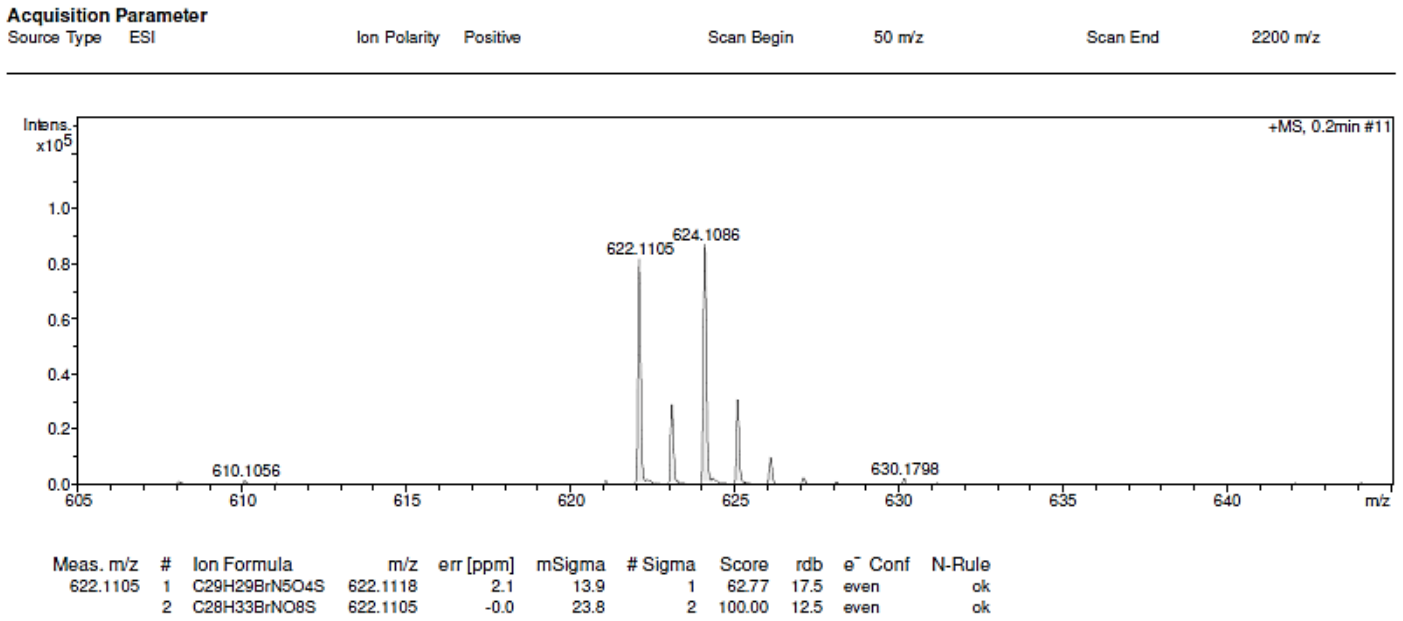


Figure S3. High resolution ESI-TOF (positive mode) mass spectrum of compound **3.**

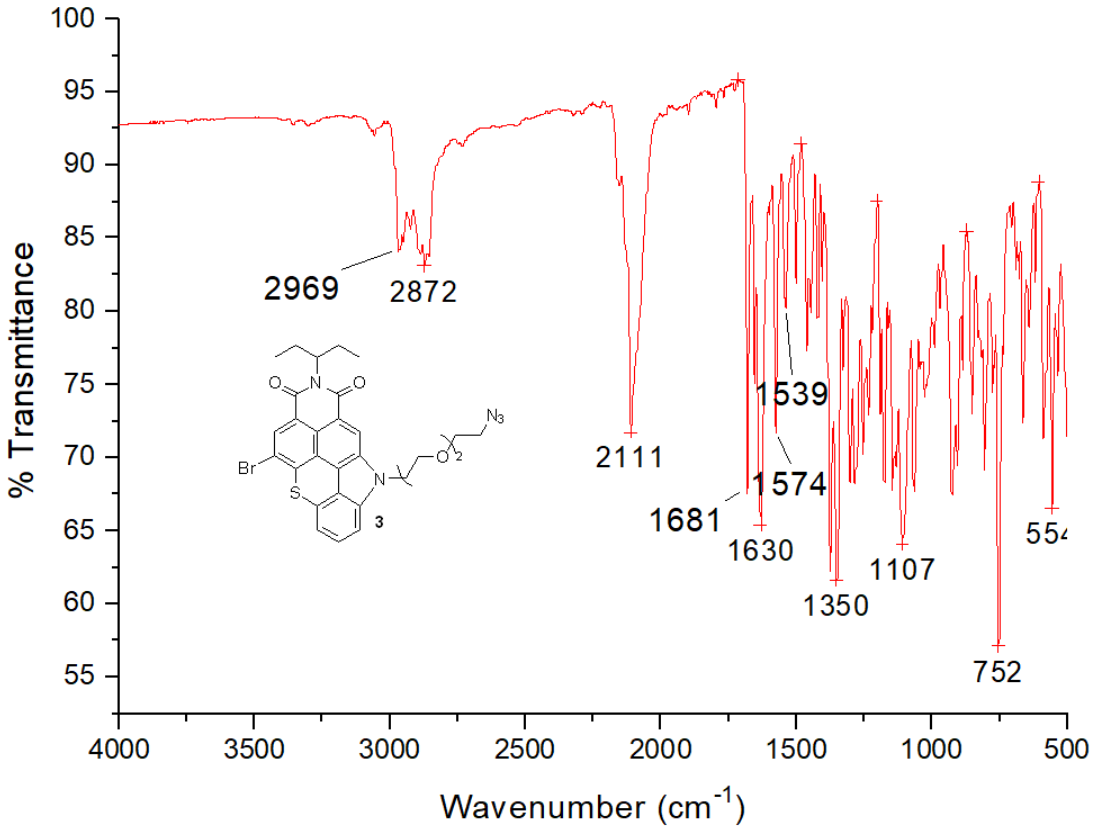


Figure S4. ATR-FTIR spectrum of compound **3.**

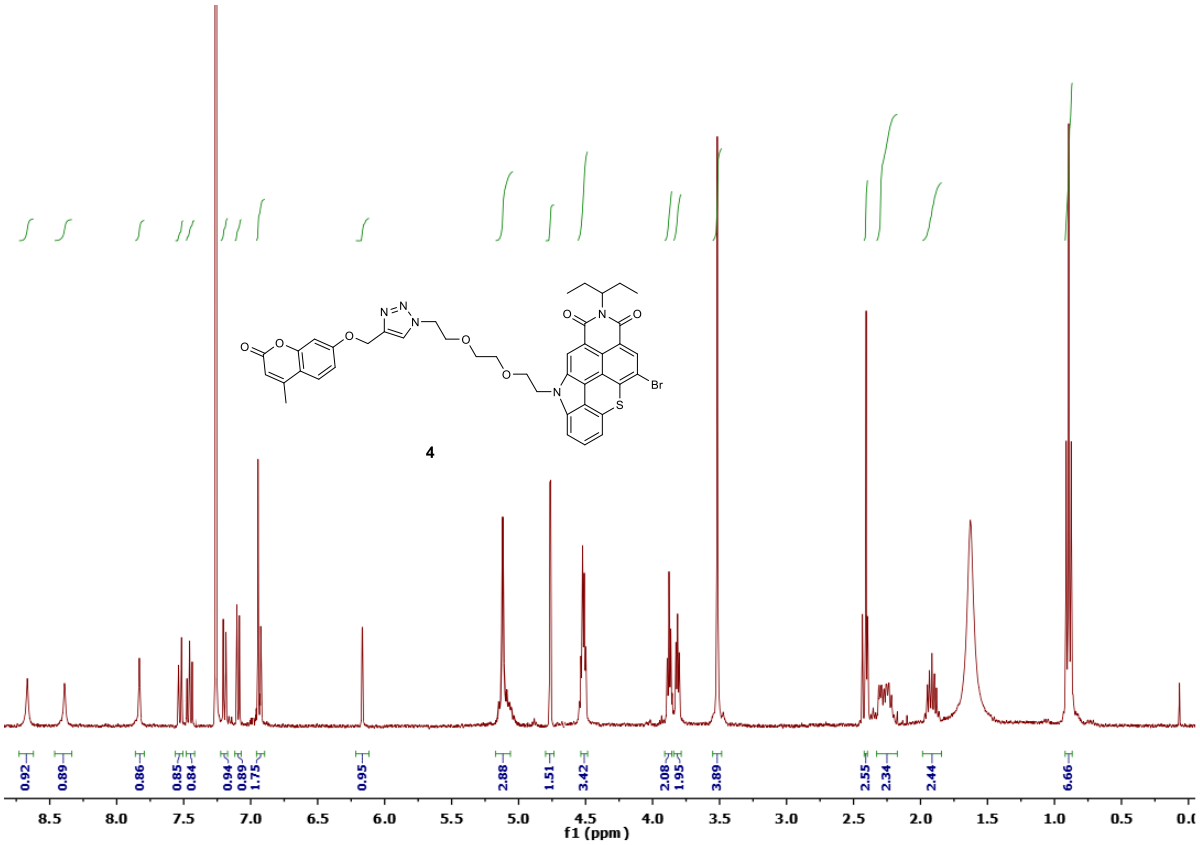


Figure S5. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298K) of compound **4.**

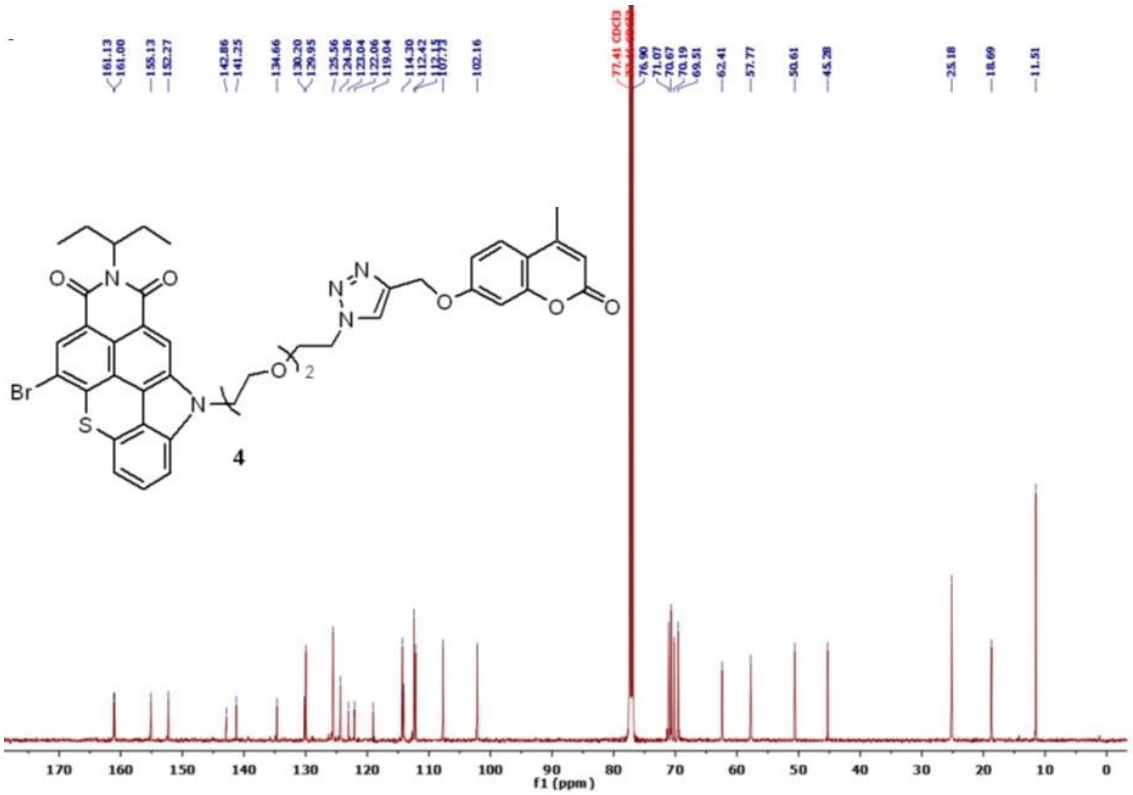
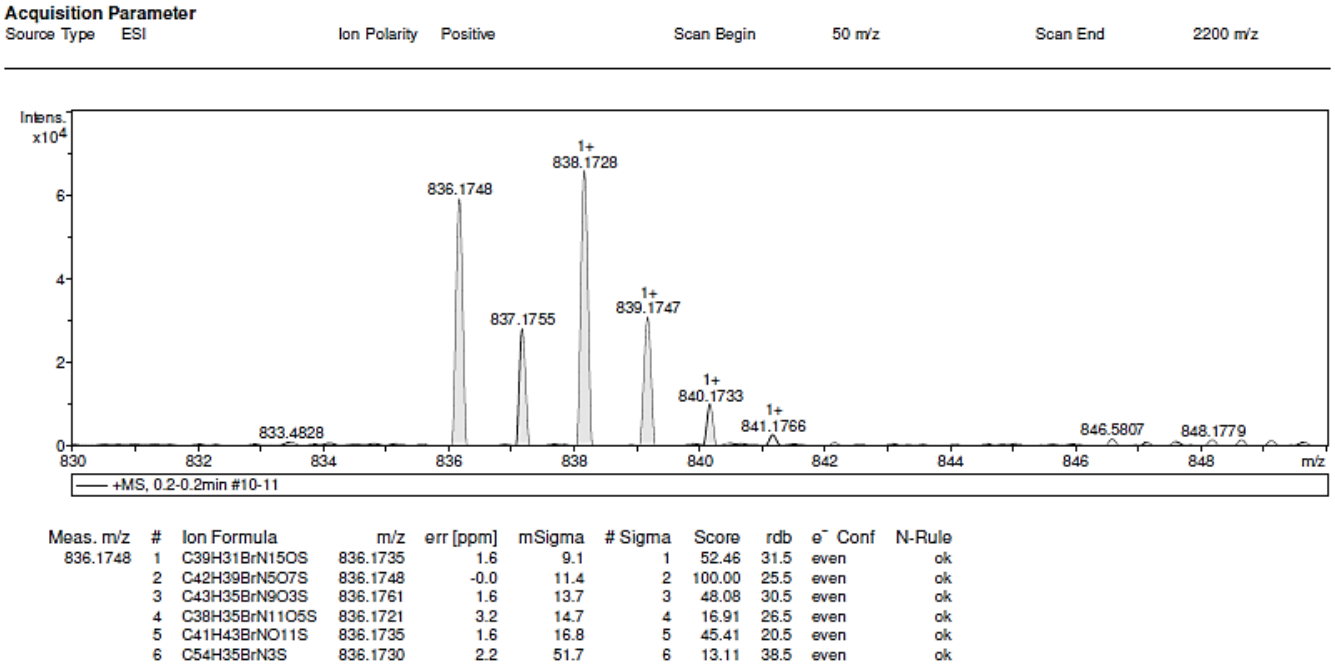


Figure S6. $^{13}C\{^{1}H\}$ NMR spectrum (126 MHz, $CDCl_3$, 298K) of compound **4**.



Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# Sigma	Score	rdb	e⁻ Conf	N-Rule
836.1748	1	C39H31BrN15OS	836.1735	1.6	9.1	1	52.46	31.5	even	ok
	2	C42H39BrN5O7S	836.1748	-0.0	11.4	2	100.00	25.5	even	ok
	3	C43H35BrN9O3S	836.1761	1.6	13.7	3	48.08	30.5	even	ok
	4	C38H35BrN11O5S	836.1721	3.2	14.7	4	16.91	26.5	even	ok
	5	C41H43BrNO11S	836.1735	1.6	16.8	5	45.41	20.5	even	ok
	6	C54H35BrN3S	836.1730	2.2	51.7	6	13.11	38.5	even	ok

Figure S7. High resolution ESI-TOF (positive mode) mass spectrum of compound **4.**

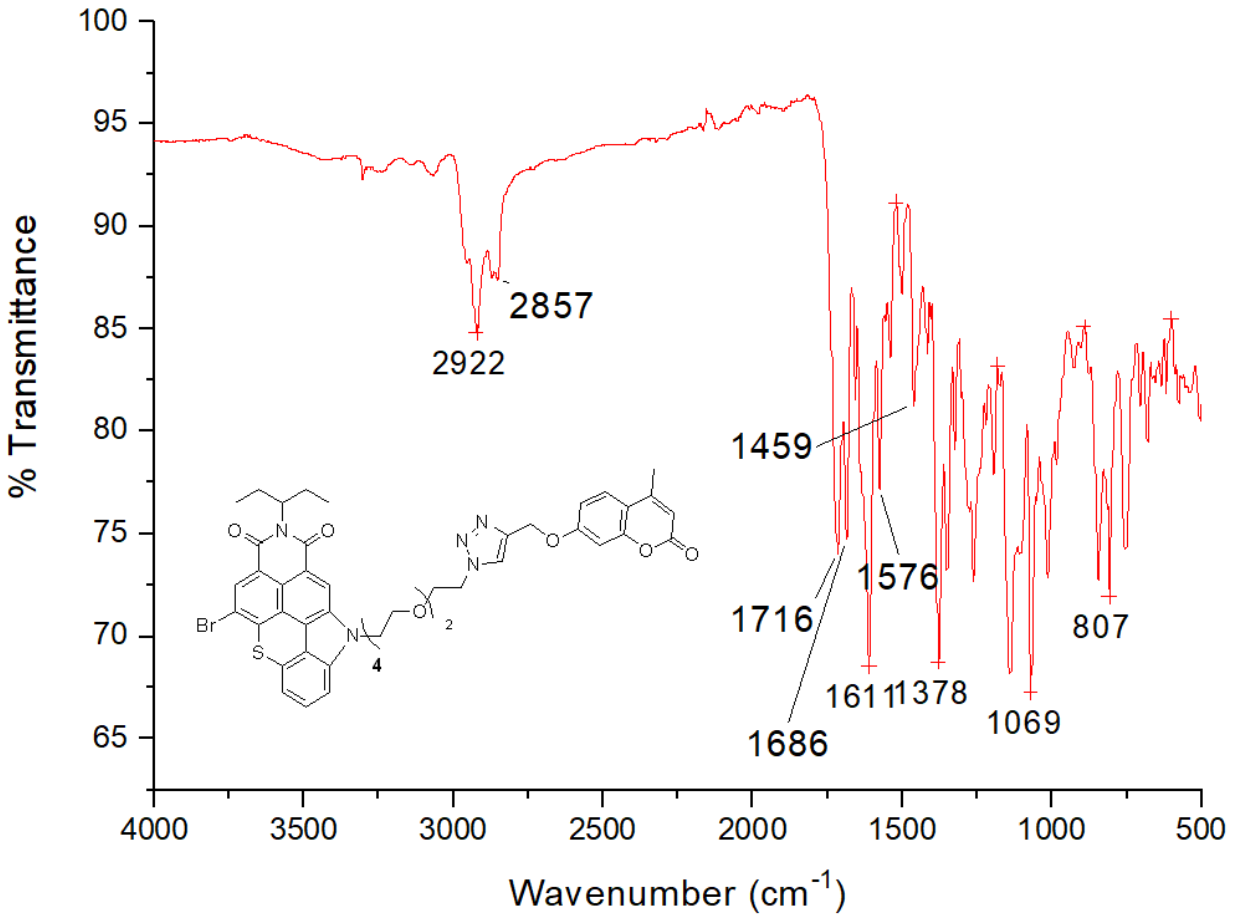


Figure S8. ATR-FTIR spectrum of compound **4.**

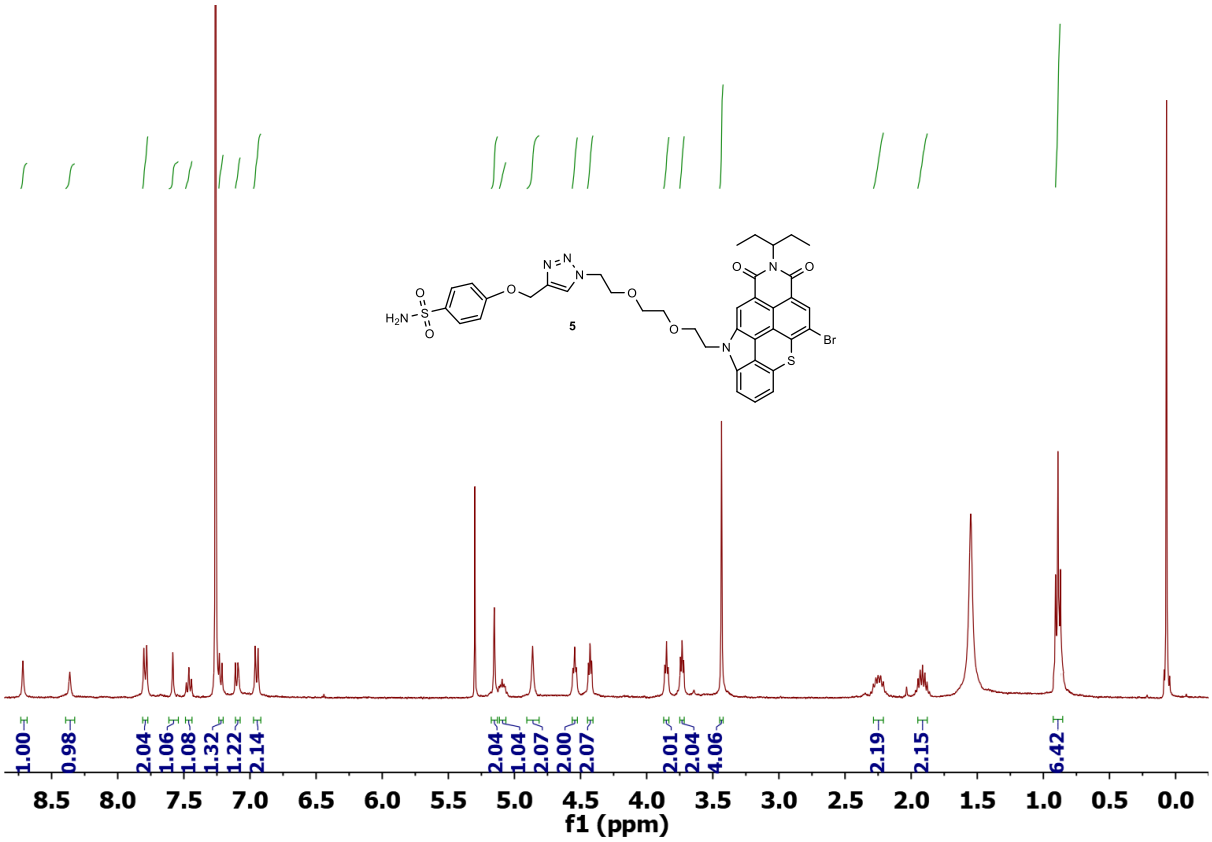


Figure S9. ^{1}H NMR spectrum (400 MHz,$CDCl_3$, 298K) of compound **5.**

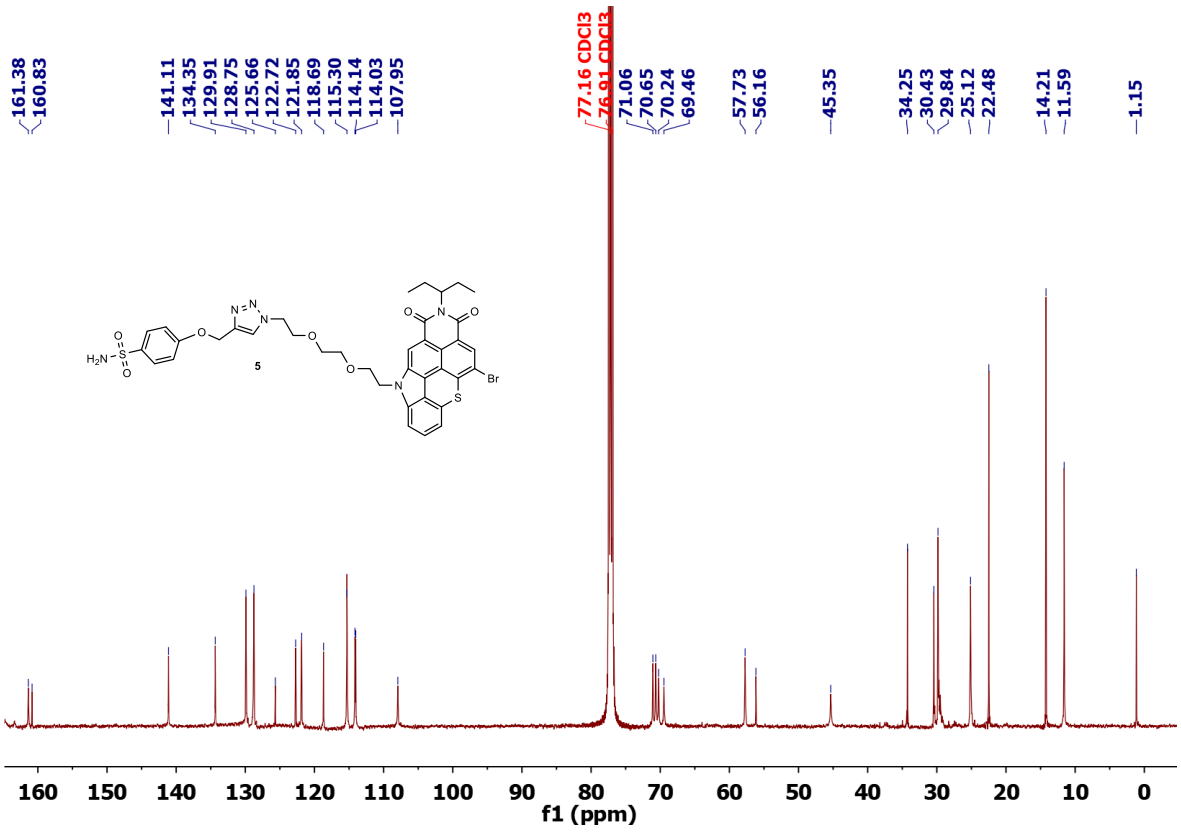


Figure S10. $^{13}C\{^{1}H\}$ NMR spectrum (126 MHz, $CDCl_3$, 298K) of compound **5.**

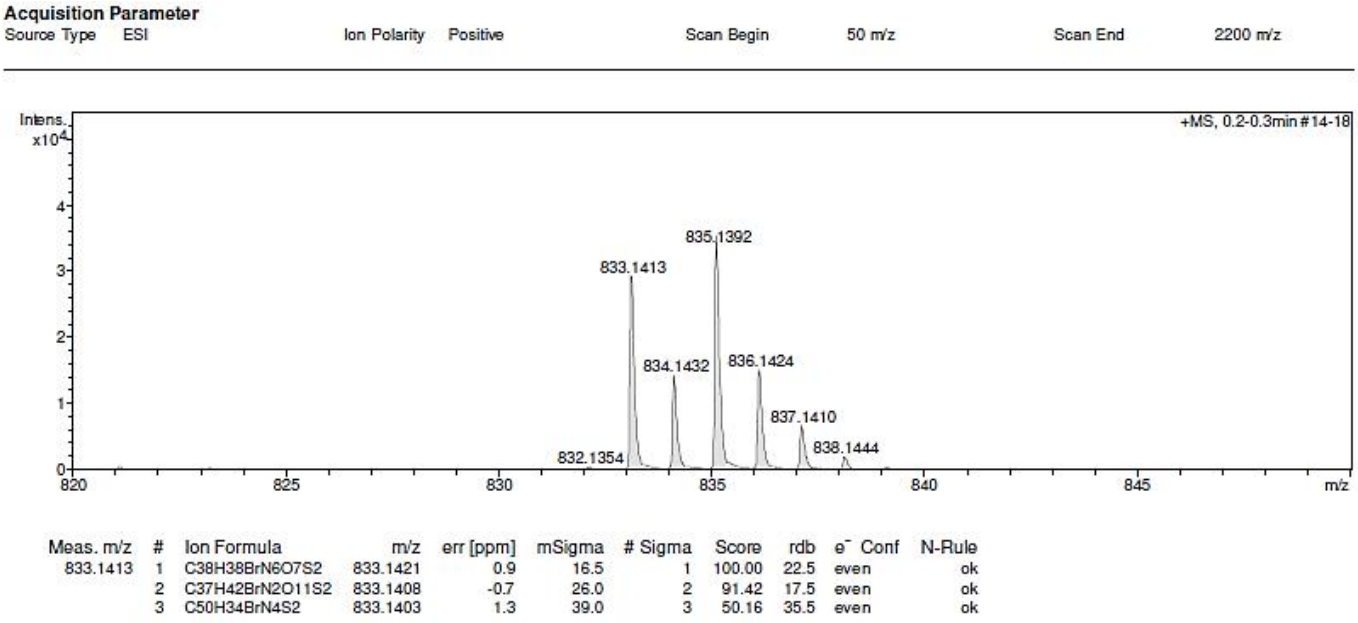


Figure S11. High resolution ESI-TOF (positive mode) mass spectrum of compound **5.**

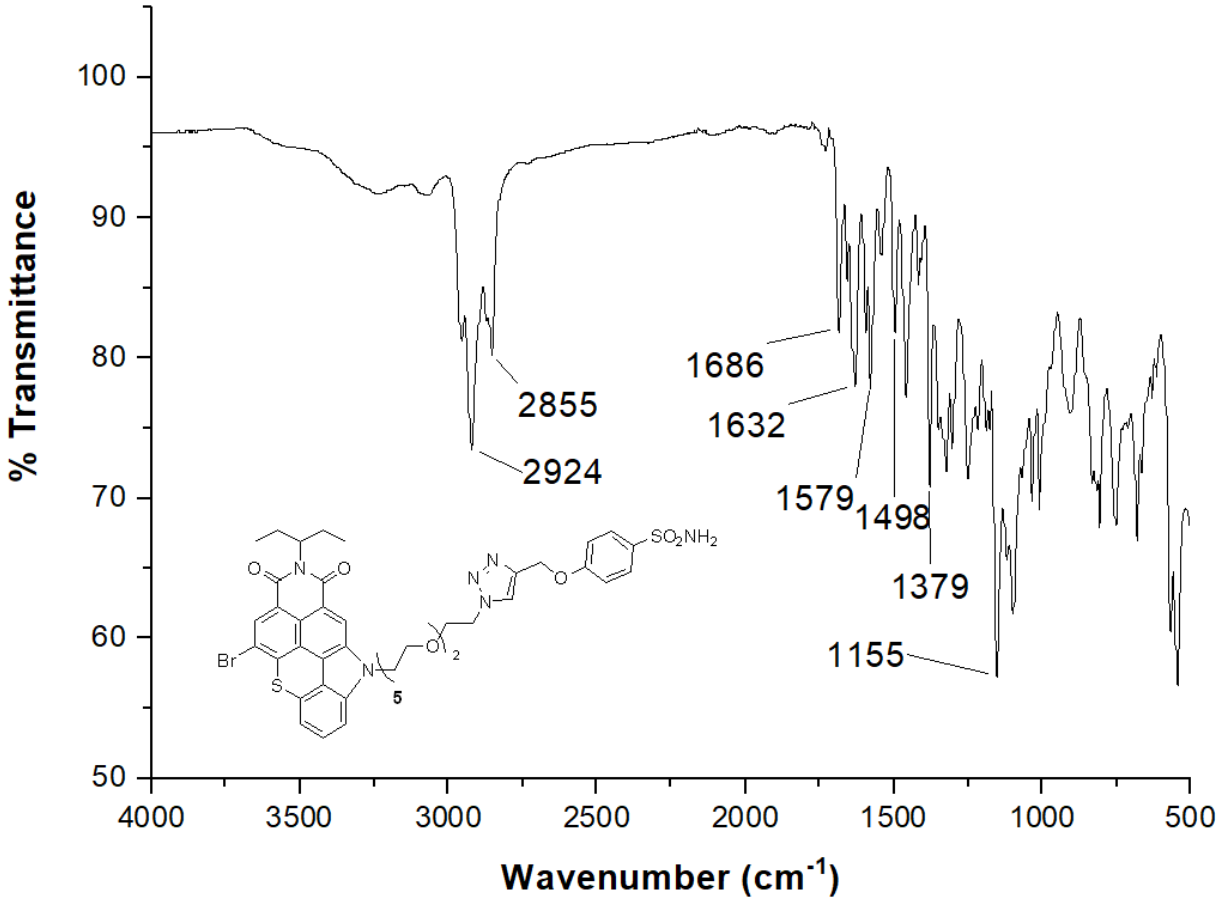


Figure S12. ATR-FTIR spectrum of compound **5.**

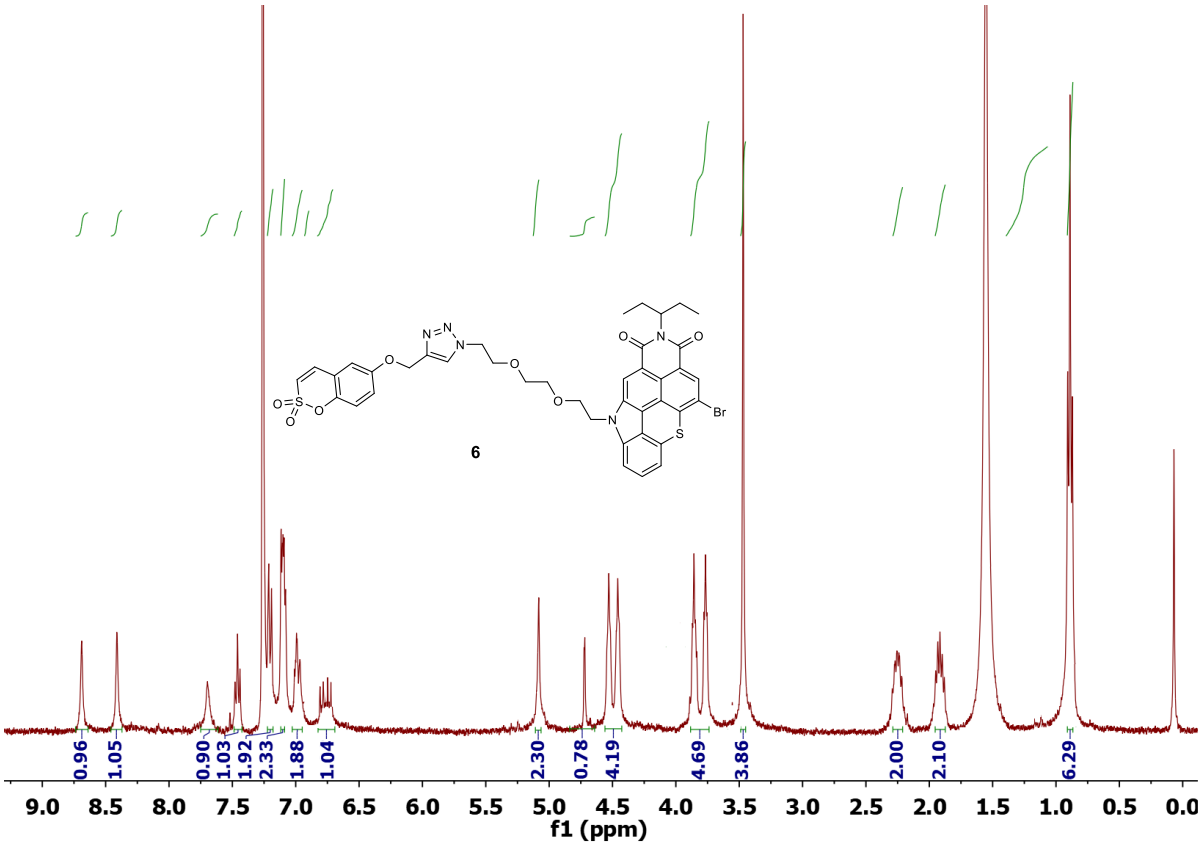


Figure S13. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298K) of compound **6.**

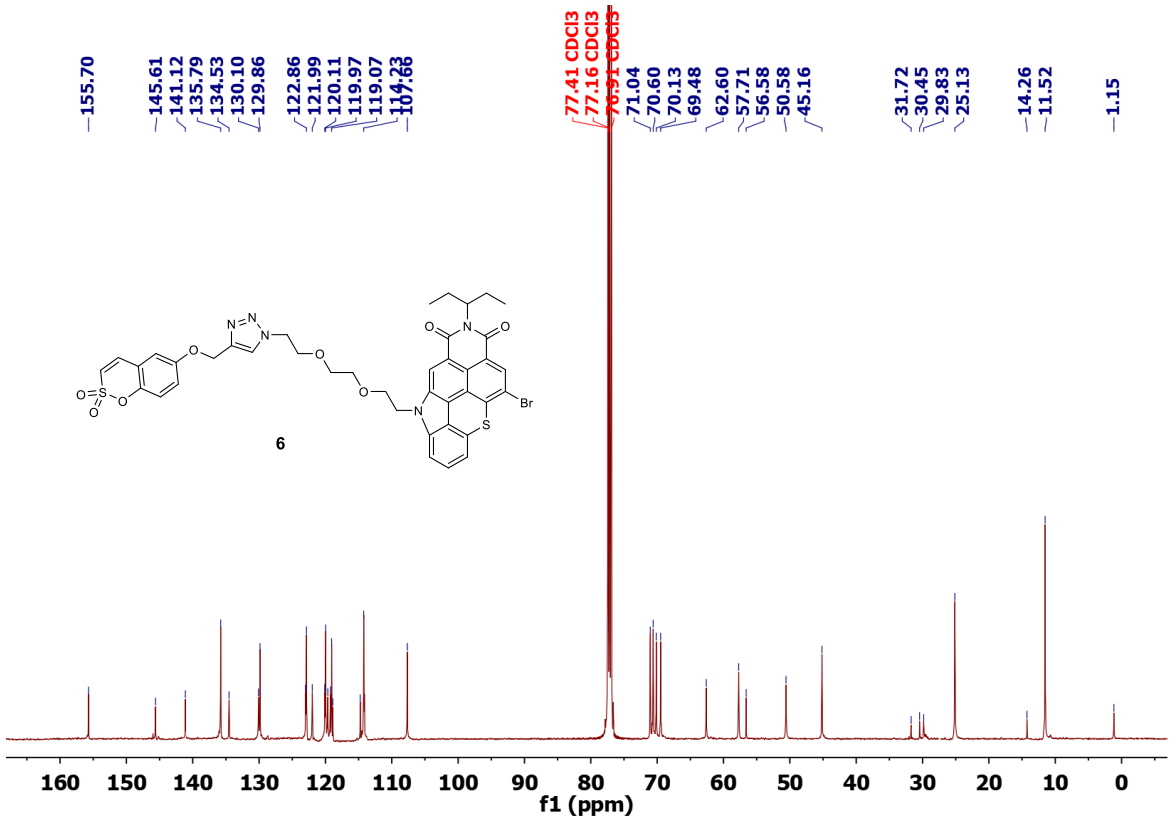
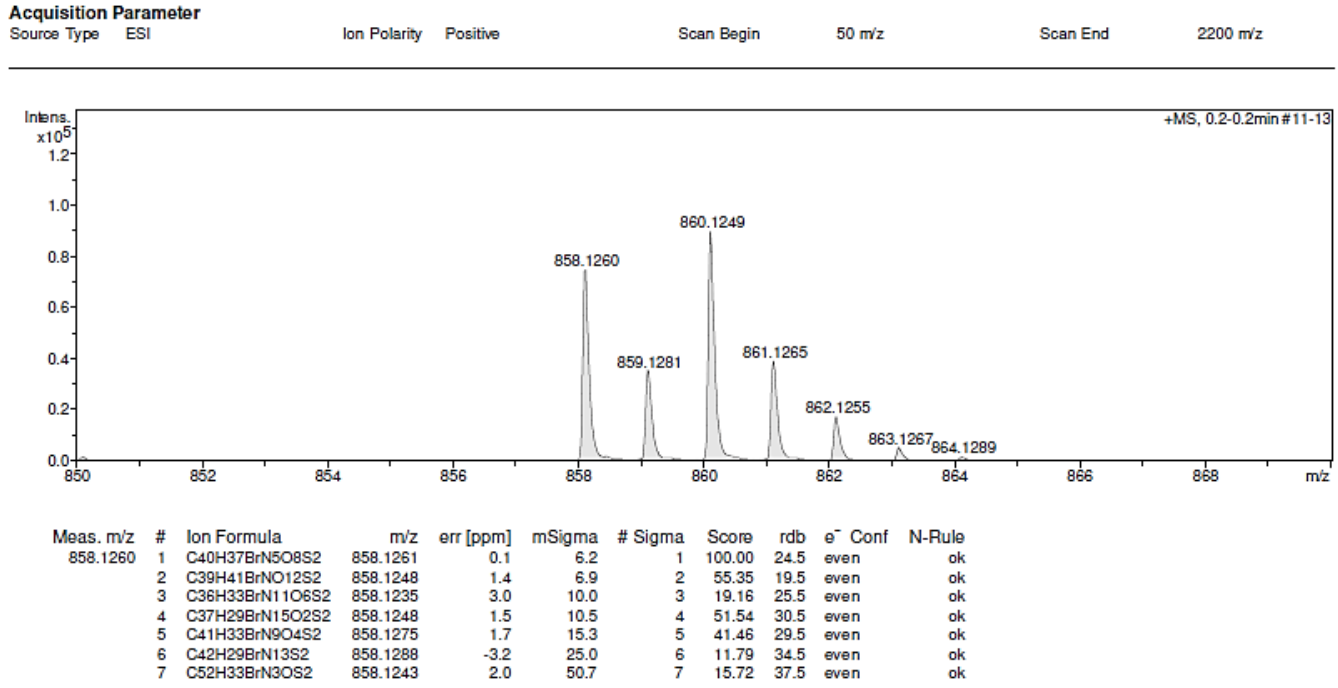


Figure S14. $^{13}C\{^{1}H\}$ NMR spectrum (126 MHz, $CDCl_3$, 298K) of compound **6.**



Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# Sigma	Score	rdb	e⁻ Conf	N-Rule
858.1260	1	C40H37BrN5O8S2	858.1261	0.1	6.2	1	100.00	24.5	even	ok
	2	C39H41BrNO12S2	858.1248	1.4	6.9	2	55.35	19.5	even	ok
	3	C36H33BrN11O6S2	858.1235	3.0	10.0	3	19.16	25.5	even	ok
	4	C37H29BrN15O2S2	858.1248	1.5	10.5	4	51.54	30.5	even	ok
	5	C41H33BrN9O4S2	858.1275	1.7	15.3	5	41.46	29.5	even	ok
	6	C42H29BrN13S2	858.1288	-3.2	25.0	6	11.79	34.5	even	ok
	7	C52H33BrN3OS2	858.1243	2.0	50.7	7	15.72	37.5	even	ok

Figure S15. High resolution ESI-TOF (positive mode) mass spectrum of compound **6.**

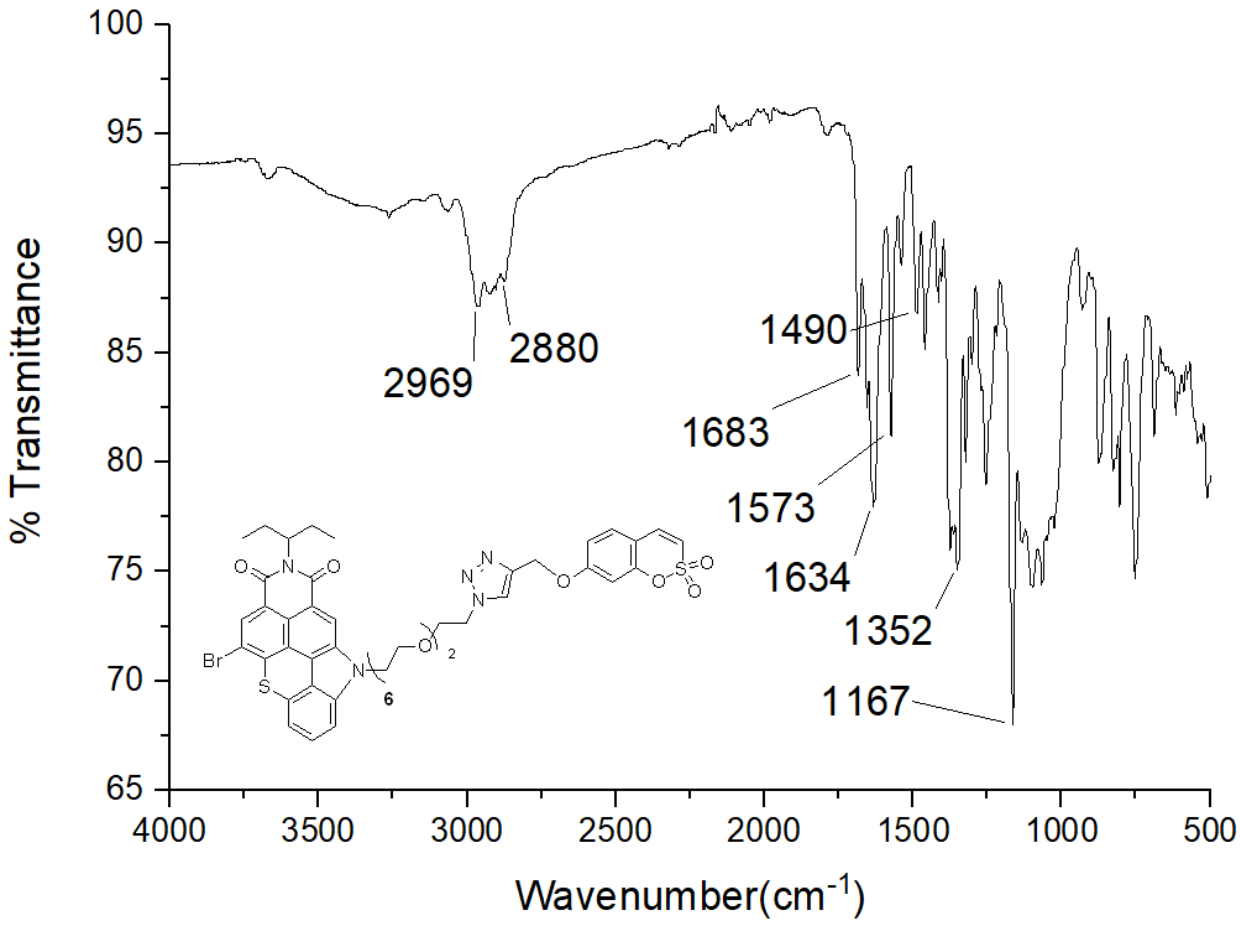


Figure S16. ATR-FTIR spectrum of compound **6**

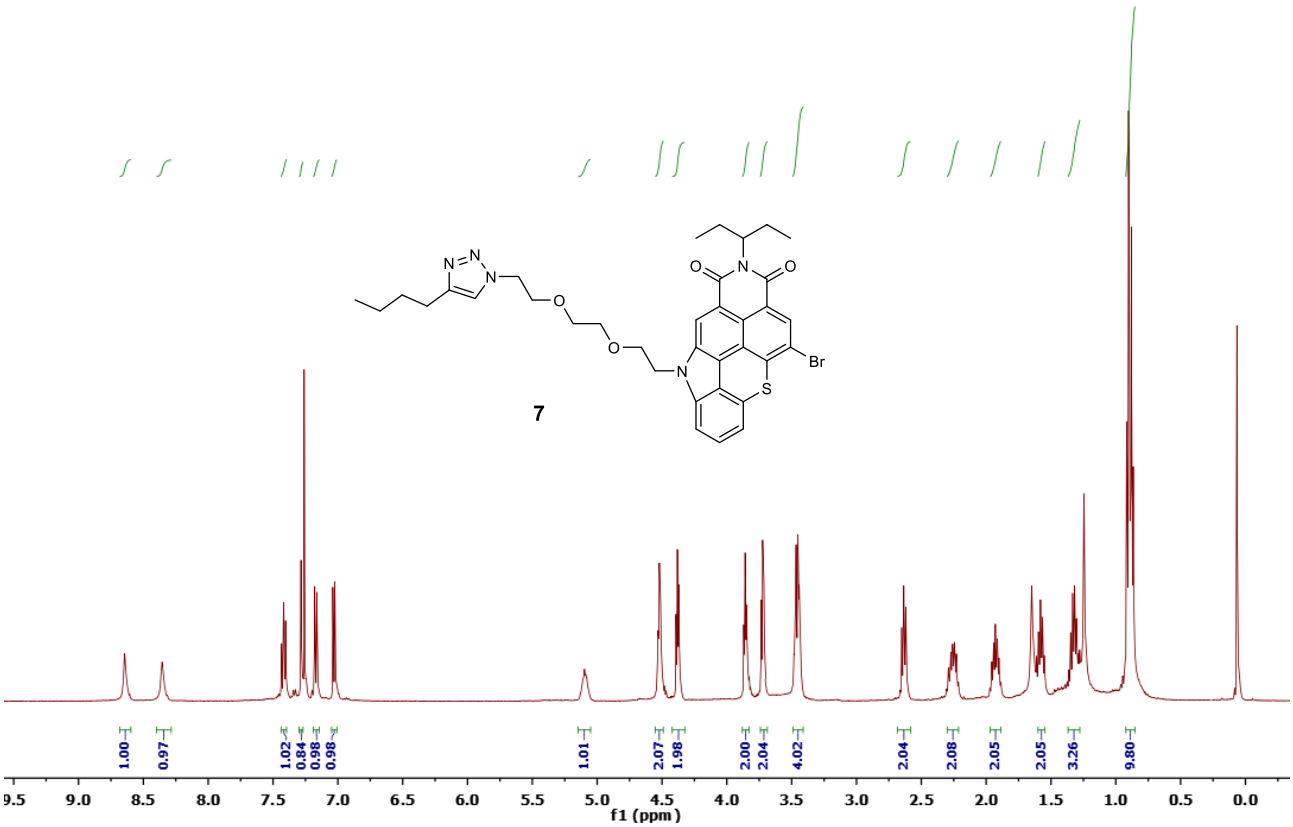


Figure S17. ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298K) of compound **7.**

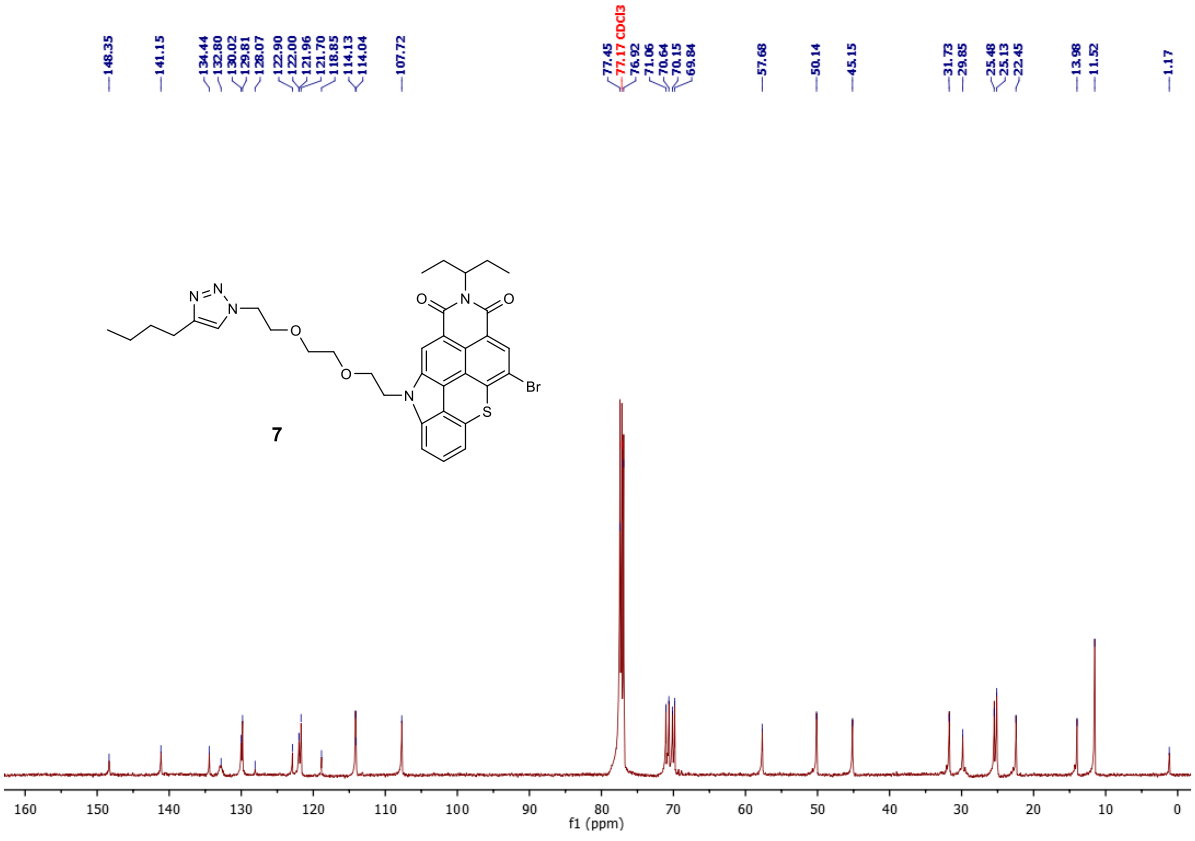
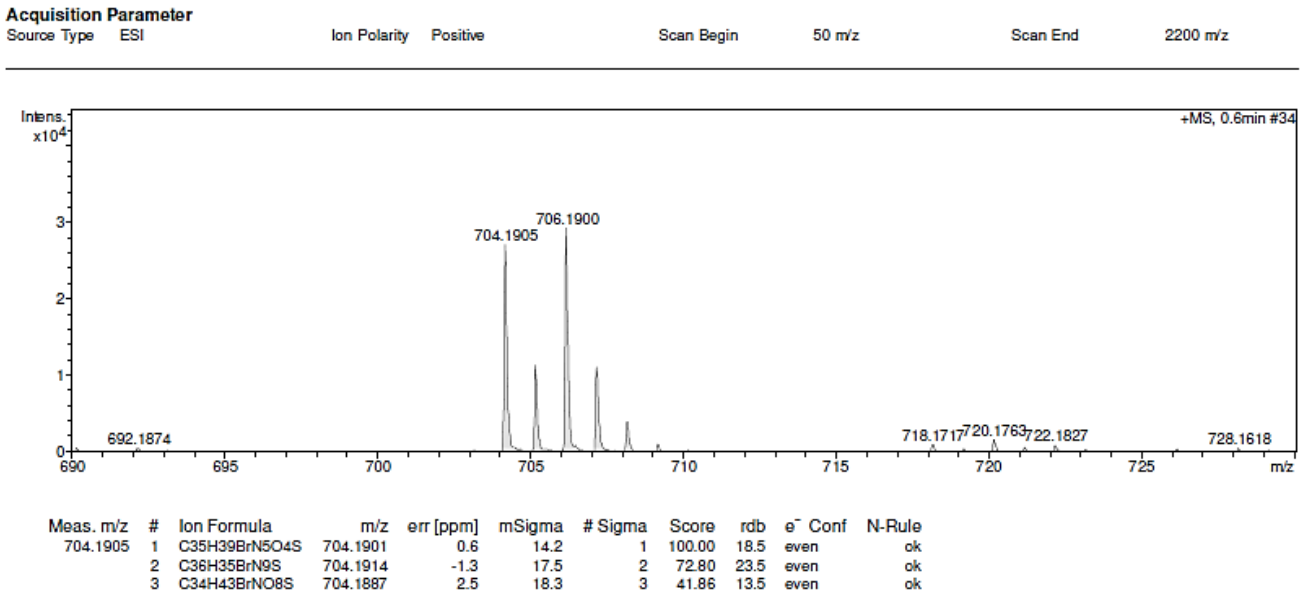


Figure S18. $^{13}C\{^{1}H\}$ NMR spectrum (126 MHz, $CDCl_3$, 298K) of compound **7**.



Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# Sigma	Score	rdb	e⁻ Conf	N-Rule
704.1905	1	C35H39BrN5O4S	704.1901	0.6	14.2	1	100.00	18.5	even	ok
	2	C36H35BrN9S	704.1914	-1.3	17.5	2	72.80	23.5	even	ok
	3	C34H43BrNO8S	704.1887	2.5	18.3	3	41.86	13.5	even	ok

Figure S19. High resolution ESI-TOF (positive mode) mass spectrum of compound **7.**

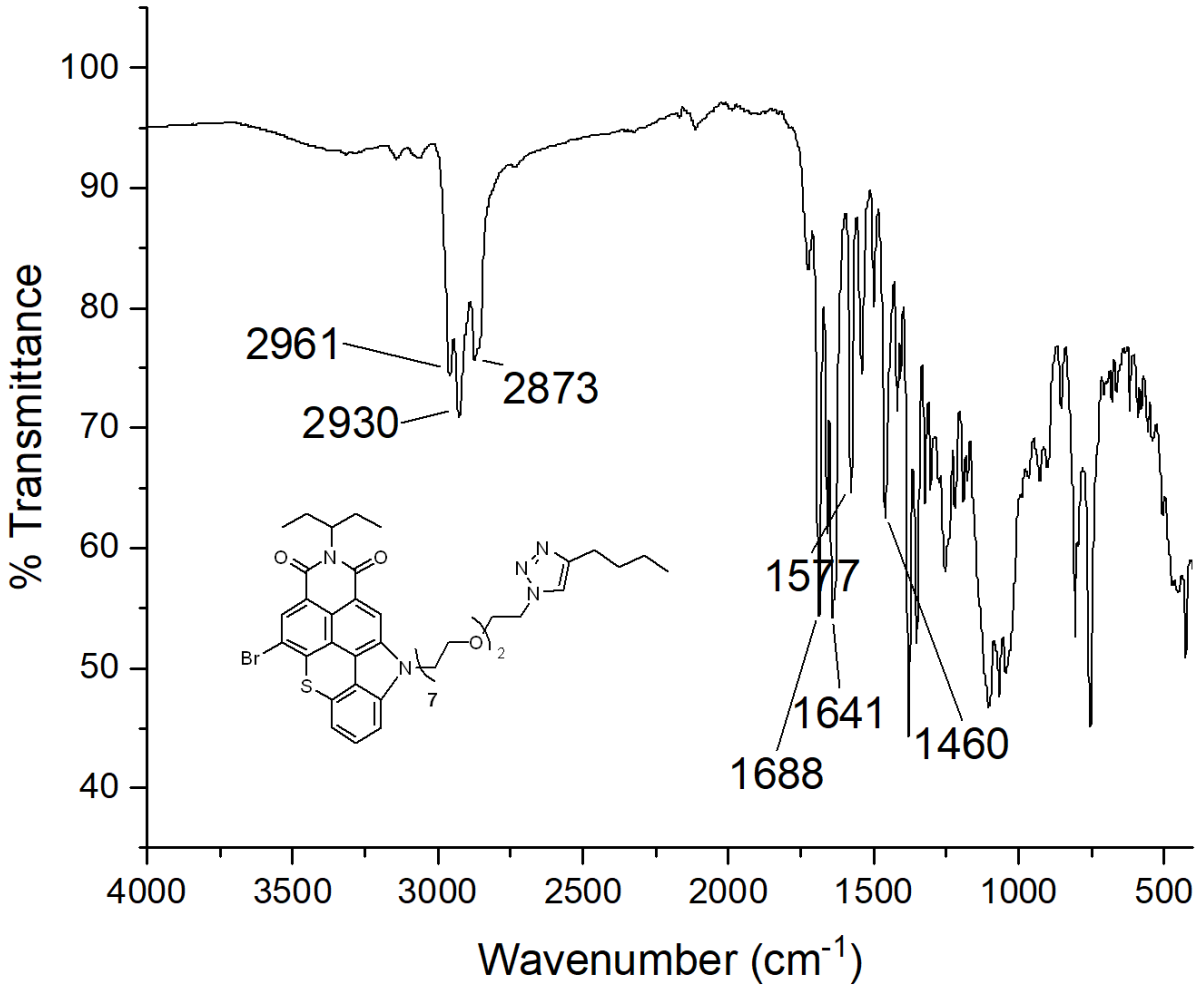


Figure S20. ATR-FTIR spectrum of compound **7.**

II. Optical properties

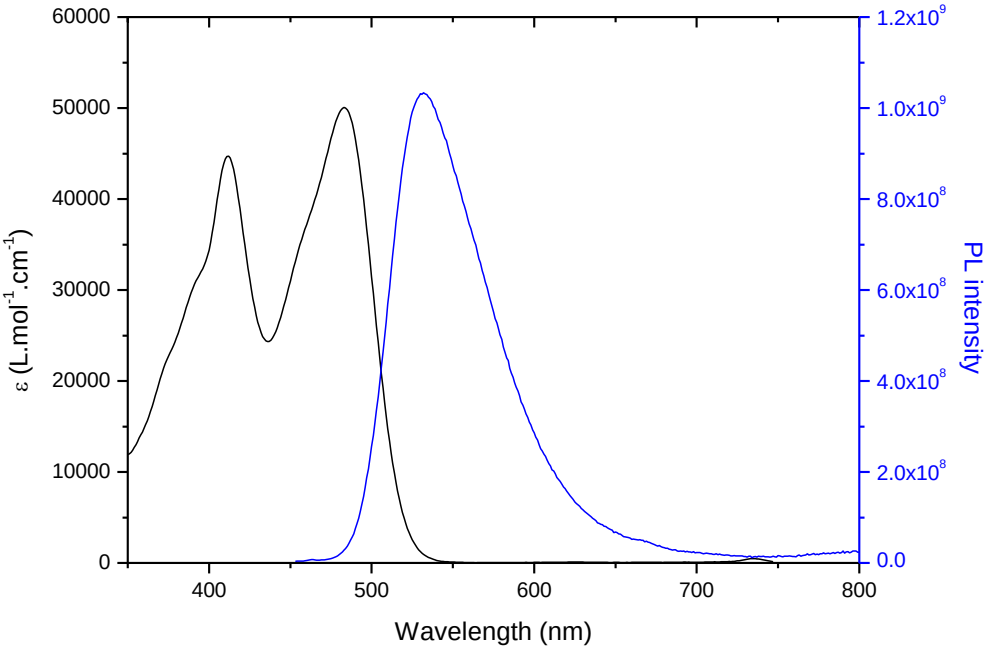


Figure S21. UV-visible absorption (black) and emission spectra (blue) of compound **5** in DMF (C= 1.2 x 10^{-5} mol.L^{-1}, λ_{exc} = 435 nm).

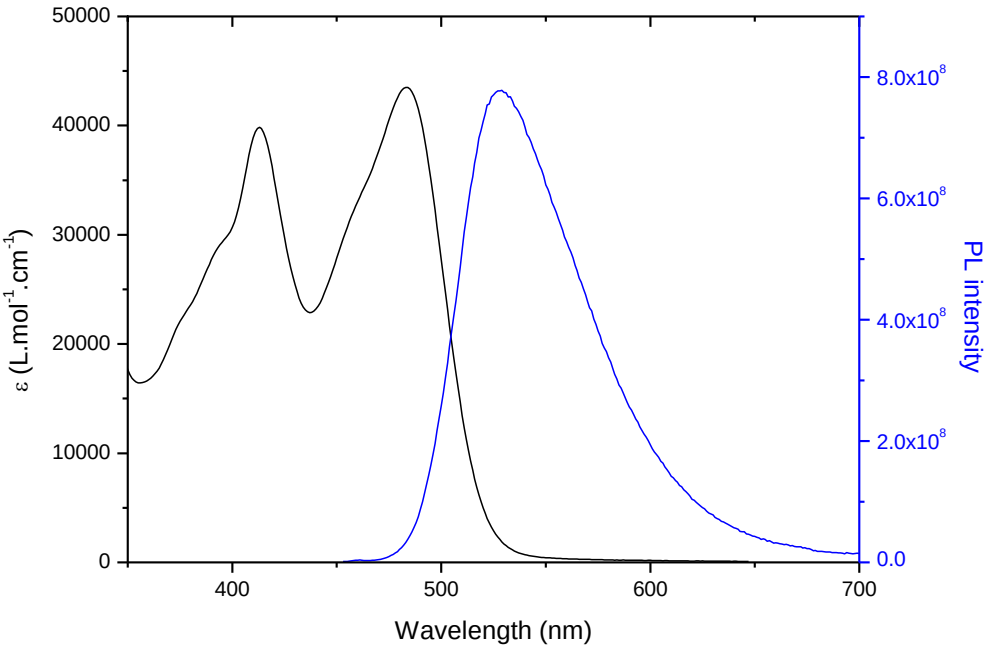


Figure S22. UV-visible absorption (black) and emission spectra (blue) of compound **6** in DMF (C= 1.1 x 10^{-5} mol.L^{-1}, λ_{exc} = 435 nm).

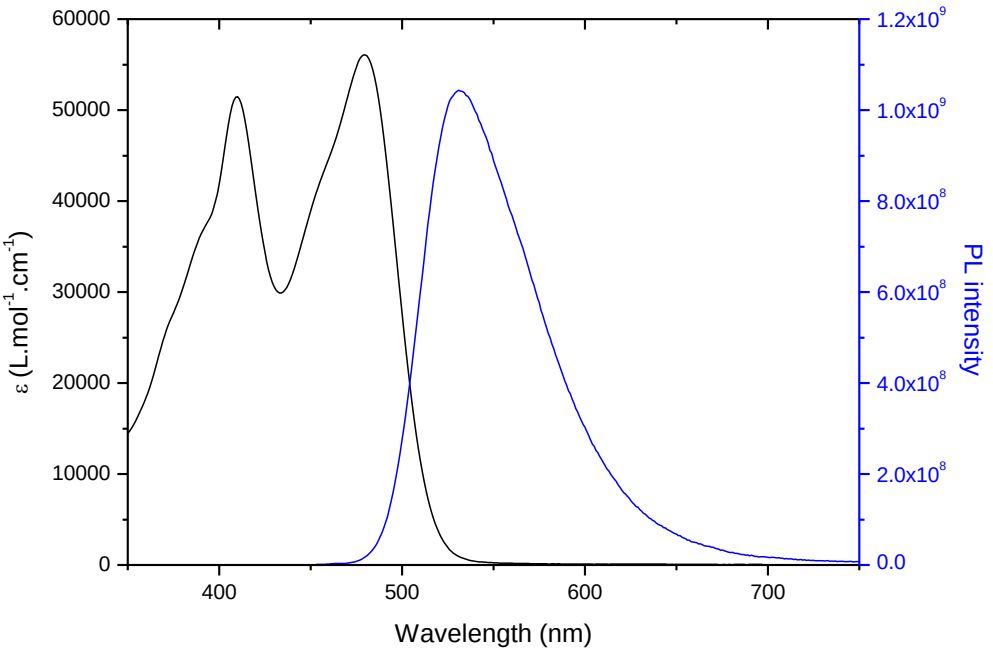


Figure S23. UV-visible absorption (black) and emission spectra (blue) of compound **7** in DMF (C= 1.1 x 10^{-5} mol.L^{-1}, λ_{exc} = 435 nm).

III CA inhibition assays

An SX.18V-R Applied Photophysics (Oxford, UK) stopped flow instrument was used to assay the catalytic/inhibition of various CA isozymes.[3] Phenol red (at the concentration of 0.2 mM has been used as an indicator, working at an absorbance maximum of 557 nm, with 10 mM HEPES (pH 7.4) as a buffer, 0.1 M Na_2SO_4 or $NaClO_4$ (for maintaining constant the ionic strength; these anions are not inhibitor in the used concentration), following the CA-catalyzed CO_2 hydration reaction for a period of 5–10 s. Saturated CO_2 solutions in water at 25 °C were used as substrate. Stock solutions of inhibitors were prepared at a concentration of 10 mM (in DMSO/water 1:1, v/v) and dilutions up to 0.01 nM done with the assay buffer mentioned above. At least 7 different inhibitor concentrations have been used for measuring the inhibition constant. Inhibitor and enzyme solutions were pre-incubated together for 6h at 4 °C prior to assay, in order to allow for the formation of the E-I complex. Triplicate experiments were done for each inhibitor concentration, and the values are reported throughout the paper is the mean of such results. The inhibition constants were obtained by nonlinear least squares methods using the Cheng-Prusoff equation, as reported earlier, and represent the mean from at least three different determinations. All CA isozymes used here were recombinant proteins obtained as reported earlier by our group and their concentration in the assay system was of 6–12 nM.[4-7]

IV. Biological assays

Cell Culture. Human breast cancer cells (MDA-MB-231) and MDA-MB-231-Luc-RFP were purchased from ATCC (American Type Culture Collection, Manassas, VA) and Amsbio, respectively. MDA-MB-231 cells were cultured in Dulbecco's Modified Eagle medium (DMEM) supplemented with 10% foetal bovine serum (FBS) and 50 µg mL^{-1} gentamycin. MDA-MB-231-Luc-RFP cells were maintained in the previously mentioned cell culture medium compositions in addition to 5 µg mL^{-1} blasticidin as a selection antibiotic. Cells were maintained in a humidified atmosphere, at 37 °C with 5% CO_2. DMEM, FBS and antibiotics were purchased from Gibco.

Dark Toxicity. MDA-MB-231 cells were seeded into a 96 multiwell at 5000 cells/ well in 200 µL of culture medium and allowed to grow for 24 h. The cells were then treated or not with **4**, **6, 7** or vehicle (DMSO) at increasing concentrations (from 0.1 to 100 $\mu g.mL^{-1}$) for 72 h and living cells were quantified. For that, cells were treated for 4 h with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Promega) in media. Then, MTT/ media solution was removed and the crystals thus formed were dissolved in EtOH/DMSO (1:1). As per manufacturer recommendations, absorbance was read at 540 nm.

PDT. MDA-MB-231 cells were seeded into a 384 multiwell at 1000 cells/ well in 50 µL of culture medium and allowed to grow for 24 h. The cells were then treated or not with **4**, **6** and **7** or vehicle (DMSO) at 0.5 $\mu g.mL^{-1}$ for 24 h. Then, cancer cells were irradiated or not at 470 ± 22 nm for 30 sec using GFP light cube of EVOS 5000 (ThermoFisher) (13.4 J cm^{-2}). Two days after irradiation, MTT assay was performed to evaluate the cell viability.

Confocal Microscopy Imaging. MDA-MB-231 cells were seeded into bottom glass dishes (World Precision Instrument, Stevenage, UK) at a density of 10^6 cells cm^{-2}. One day after seeding, cells were washed once and incubated with 1 mL of fresh medium containing **4**, **6** or **7** at a concentration of 10 $\mu g.mL^{-1}$ for 24 h. Fifteen minutes before the end of incubation, the cells were incubated with CellMask Orange for membrane cell staining (5 µg mL^{-1}, Thermo Fisher). Then, cells were washed several times with culture media to remove staining molecules excess. The cells were then visualized with an LSM 780 LIVE confocal microscope (Carl Zeiss, Le Pecq, France), at 488 nm and 561 nm for compounds and CellMask respectively, using high magnification (63×/1.4 OIL DIC Plan-Apo).

Experiments on Zebrafish Embryos. Casper zebrafish embryos were purchased from Zebrafish International Resource Center (ZIRC) as embryos and were raised to adulthood in circulating aquarium system at 28 °C, 80% humidity, 14 h light/10 h dark cycle, in the ZeNeuro platform (Inserm U1198), Montpellier University. Experiments with zebrafish embryos until 96 hpf are considered as in vitro studies according to the EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

Imaging on Zebrafish Embryos. Casper zebrafish embryos of 72 hours post fertilization (hpf) were anesthetized with tricaine solution (168 mg L^{-1}) for 10 min. Casper embryos (n=4/group) were injected or not with 10 nL of analogues at 1 $mg.mL^{-1}$ in 5% glucose, in the caudal vein. Then, zebrafishes were imaged 3 h after injection of derivatives **4** and **6**, and immediately after for **7**. Tile scans were performed at an excitation wavelength of 488 nm using confocal microscopy LSM880 and objective EC plan-Neofluar 10x/0.30 M27.

Study of PDT Efficiency in Zebrafish Embryos. MDA-MB-231-Luc-RFP cells were seeded in Nunc EasYFlask 75 cm^2. Twenty-four hours after seeding, the cells were treated or not with 10 $\mu g.mL^{-1}$ of analogue **4** for 24 h. After incubation, the cells were washed thrice with PBS and then were trypsinized and suspended in culture medium. The cells were counted and centrifuged and then suspended in the required volume of PBS containing 2% FBS to have a solution of 3×10^7 cells per 1 mL. The cell solution was kept on ice until injection. Casper embryos at 30 hpf were used. The embryos were anesthetized with tricaine solution 10 min prior to injection. Then, the embryos were placed on an agar mold for the microinjection of cells previously incubated (or not) with **4**. Each embryo received 2 pulses of 5 nL of cell suspension in the yolk. Injected Casper embryos with cells

without **4** were used as the control. After injection, the embryos were placed in a 100 mm × 20 mm Petri-dish containing 30 mL of water at 31°C. Twenty-four hours after injection, the embryos were anesthetized and imaged at 561 nm excitation using confocal microscopy LSM880 and objective EC plan-Neofluar 10x/0.30 M27 to visualize the xenograft in red. The embryos of control and compound **4** were divided into two groups: the no blue light irradiation group and the blue light irradiation group (n=10/group). Each embryo in the blue light irradiation group was exposed to light for 1 or 2 min using GFP light cube of EVOS 5000 (objective lens 4×, λex = 470 ± 22 nm, 9.41 J cm^{-2}). After irradiation, each embryo was placed in a well of 24-well plate with 2 mL of water and at 31°C. Twenty-four hours after irradiation, the embryos were imaged again. The fluorescence intensity of the xenograft before and after irradiation was quantified using imageJ program. The corrected total cell fluorescence (CTCF) was calculated as CTCF = Integrated Density – (Area of selected cell X Mean fluorescence of background readings) and the tumour regression (%) was calculated by considering the fluorescence intensity value of each embryo before irradiation is 100%.

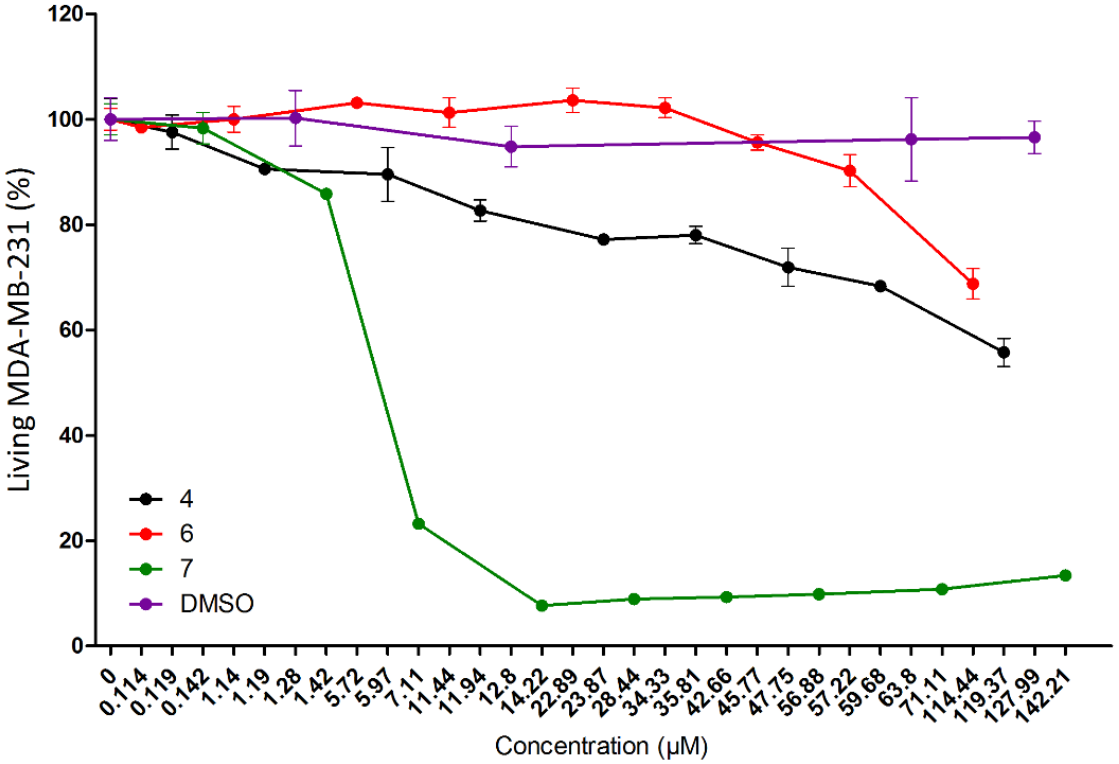


Figure S24; Toxicity study in human breast cancer cell line (MDA-MB 231) incubated 3 days with increasing concentrations of **4, 6, 7** and the corresponding volume of the vehicle (DMSO). Values are mean ± SEM of three experiments.

References:

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