New Cy5 photosensitizers for cancer phototherapy: low singlet-triplet gap provides high quantum yield of singlet oxygen

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Content

1. Figure S1-S12 and Table S1-S2.

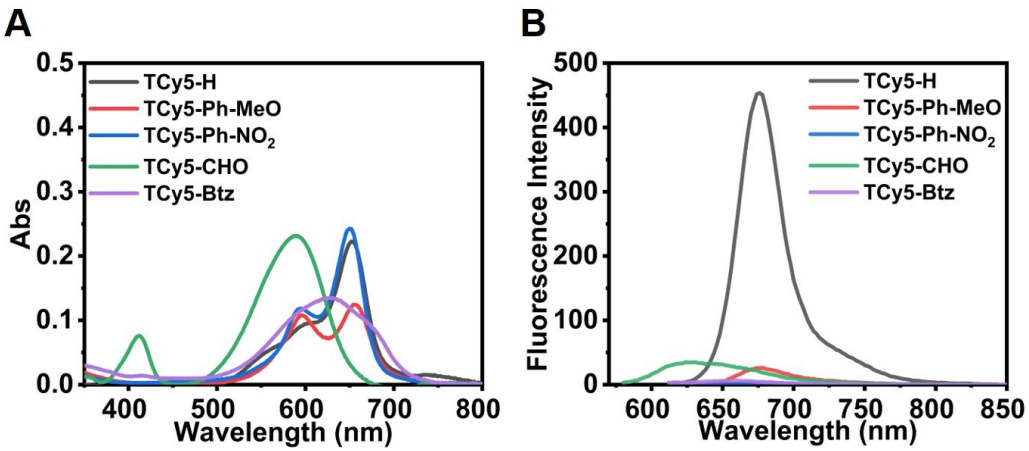


Figure S1. Absorption (A) and fluorescence (B) of **TCy5** compounds (4 μM) in ultrapure water. The slit of **TCy5** is 5/5.

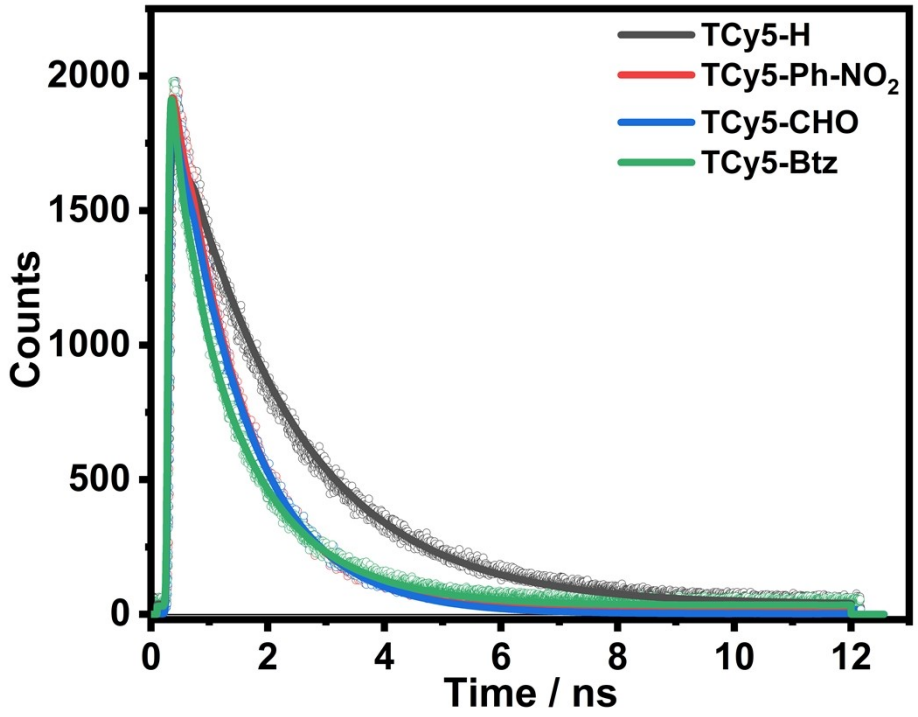


Figure S2. Time-correlated single-photon counting fluorescence intensity decay of TCy5 in air-saturated DCM. The solid lines are fitting curves. λ_{ex} = 400 nm, λ_{probe} = 690 nm (TCy5-H), 684 nm (TCy5-Ph-NO_2), 665 nm (TCy5-CHO) and 673 nm (TCy5-Btz).

Table S1. the fluorescence decay fitting curve parameters of dyes in DCM[a]

Compounds	τ_1 (A_1)	τ_2 (A_2)
TCy5-H	2.0 ns (100 %)	-
TCy5-Ph-NO_2	1.2 ns (100 %)	-
TCy5-CHO	1.2 ns (100 %)	-
TCy5-Btz[b]	1.3 ns (67 %)	0.28 ns (33 %)

[a]Fluorescence lifetimes measured by TCSPC technique for TCy5 compounds, the percentages of fractional amplitudes exhibit in brackets. [b]The fluorescence decay curves are fitted by the double exponential functions (τ_1, τ_2, A_1, A_2).

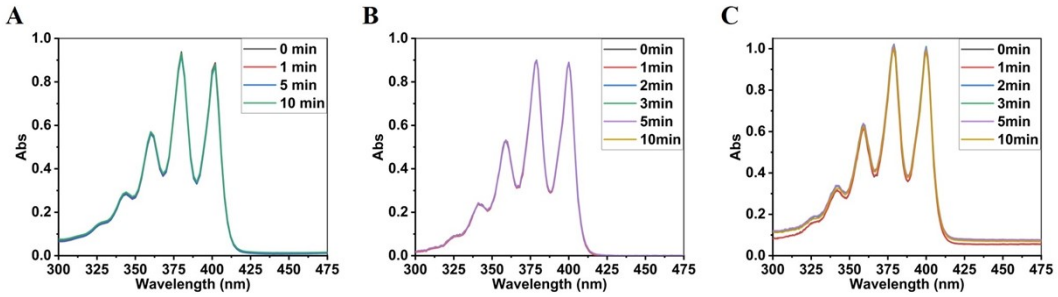


Figure S3. UV–vis absorption spectra of ABDA in **TCy5** solutions (10 μM) irradiated for different durations with NIR light irradiation (20 mW cm^{-2}). In aqueous solutions, the absorbance of ABDA at 380 nm was recorded upon different irradiation time to obtain the decay rate of the photosensitizing process. A (**TCy5-H**, 660 nm), B (**TCy5-Ph-OMe**, 660 nm), C (**TCy5-Ph-NO_2**, 660 nm).

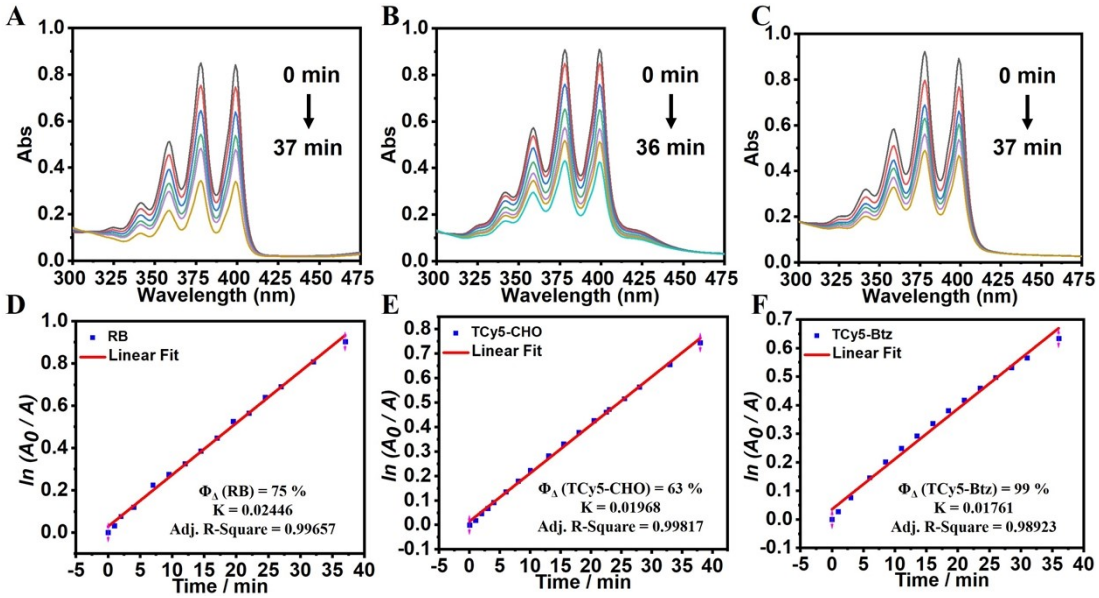


Figure S4. UV–vis absorption spectra of ABDA in (A) **Rose Bengal** (**RB**) (1×10^{-5} M) (B) **TCy5-CHO** (1×10^{-5} M), (C) **TCy5-Btz** (1×10^{-5} M), irradiated for different durations with light irradiation (660 nm / 550 nm, 1 mW cm^{-2}). The linear fit of (D) **RB**, (E) **TCy5-CHO**, (F) **TCy5-Btz** based on absorbance of ABDA at 380 nm in aqueous solutions for different durations with light irradiation.

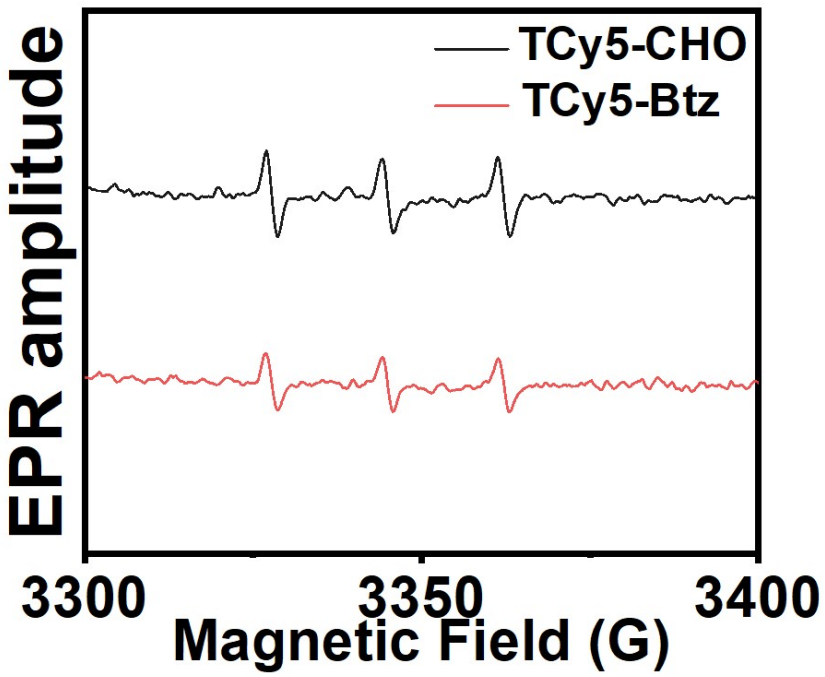


Figure S5. EPR signals of TEMP for $^1O_2$ characterization of **TCy5-CHO**, **TCy5-Btz**.

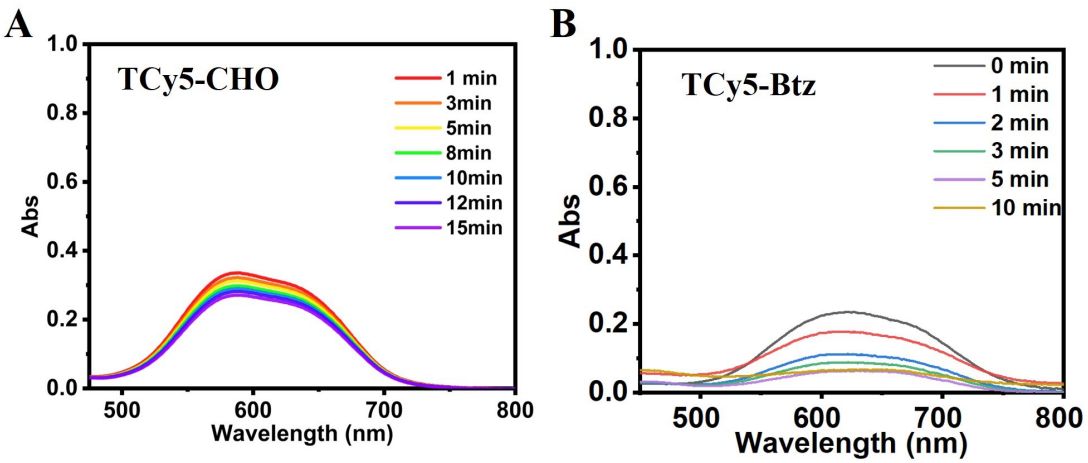


Figure S6. UV-vis spectra of (A) **TCy5-CHO** and (B)**TCy5-Btz** at absorption maximum against exposure time (10 mW cm^{-2}).

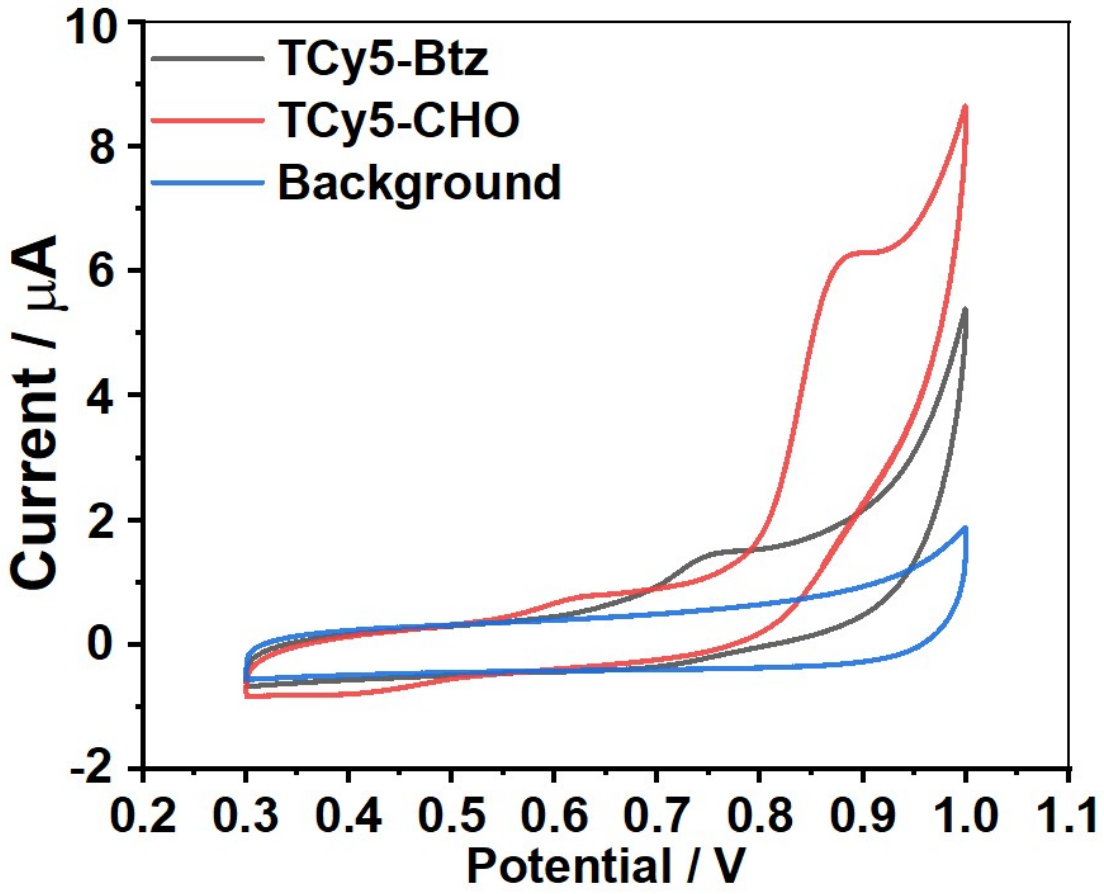


Figure S7. Cyclic voltammograms of **TCy5-CHO**, **TCy5-Btz**. Conditions: H_2O : EtOH = 3 : 4 containing 0.19 M KCl as the supporting electrolyte and Ag/AgCl as the reference electrode. Scan rates: 100 mV/s. c = 1.15 × 10^{-3} M, 20 °C.

Table S2. Electrochemical Redox Potentials of the Compounds

	$E_{1/2}$(OX) (V vs Ag/AgCl)	$E_{1/2}$(RED) (V vs Ag/AgCl)
TCy5-CHO	+0.63, +0.88	+0.44
TCy5-Btz	+0.75	-[a]

[a]Not observed.

The electrochemical properties of **TCy5-Btz**, **TCy5-CHO** are studied. For **TCy5-CHO**, a reversible reduction waves at +0.63 V and +0.44 V are observed (vs Ag/AgCl). The irreversible oxidation waves, for **TCy5-CHO** and **TCy5-Btz,** are observed at +0.88 and +0.75 V, respectively. Compared to the oxidation potential of **TCy5-CHO** and **TCy5-Btz**, the **TCy5-Btz** is more easily oxidized by $^1O_2$ matching with the sharp photobleaching *in vitro* experiment.

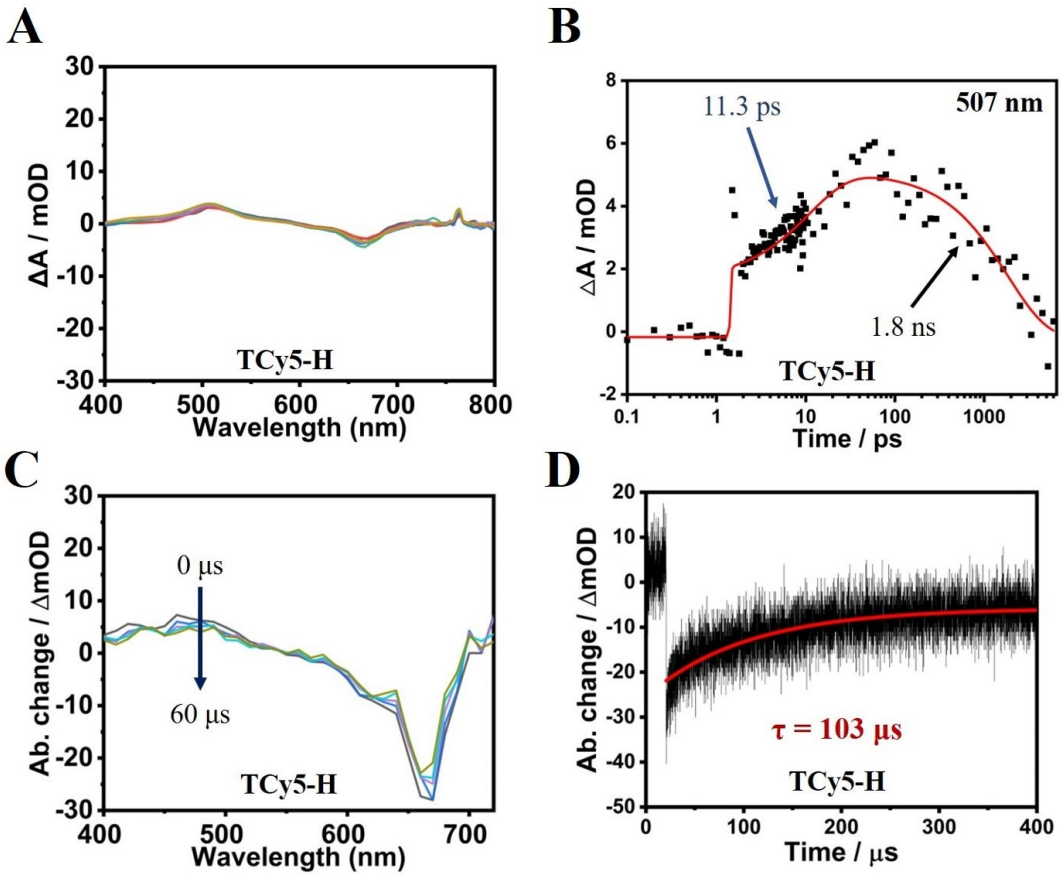


Figure S8. (A) Femtosecond transient absorption spectra of TCy5-H. (B) the kinetics at 507 nm of TCy5-H, the lifetimes associated with the components of monoexponential fitting are shown in the insets. λ_{ex} = 380 nm, in air-saturated DCM, c (TCy5-H) = 50 μM. (C) Nanosecond transient absorption spectra, and (D) decay trace at 667 nm of TCy5-H. Excited with nanosecond laser at 355 nm, c = 3.0 x 10^{-5} M in deaerated DCM; at room temperature. Lifetime of the monoexponential fitting functions is given in the graph.

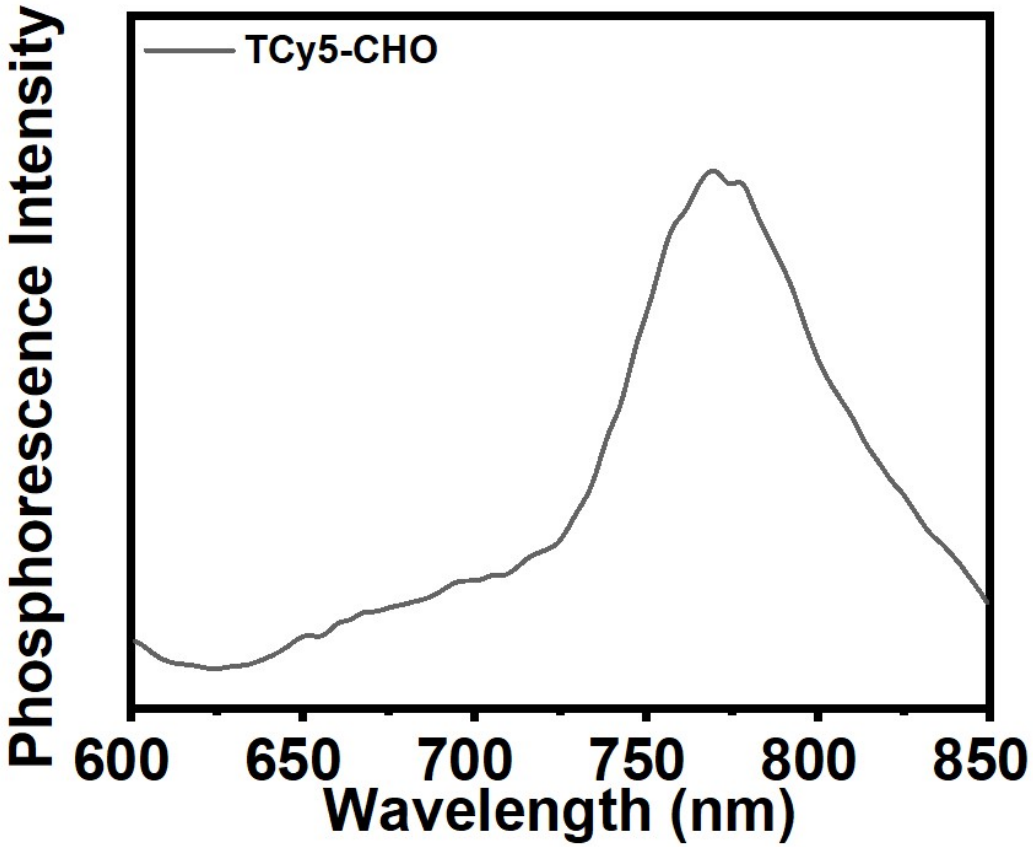


Figure S9. Phosphorescence spectrum of TCy5-CHO (10 μM) in 2-methyltetrahydrofuran. λ_{ex} = 580 nm, in liquid nitrogen.

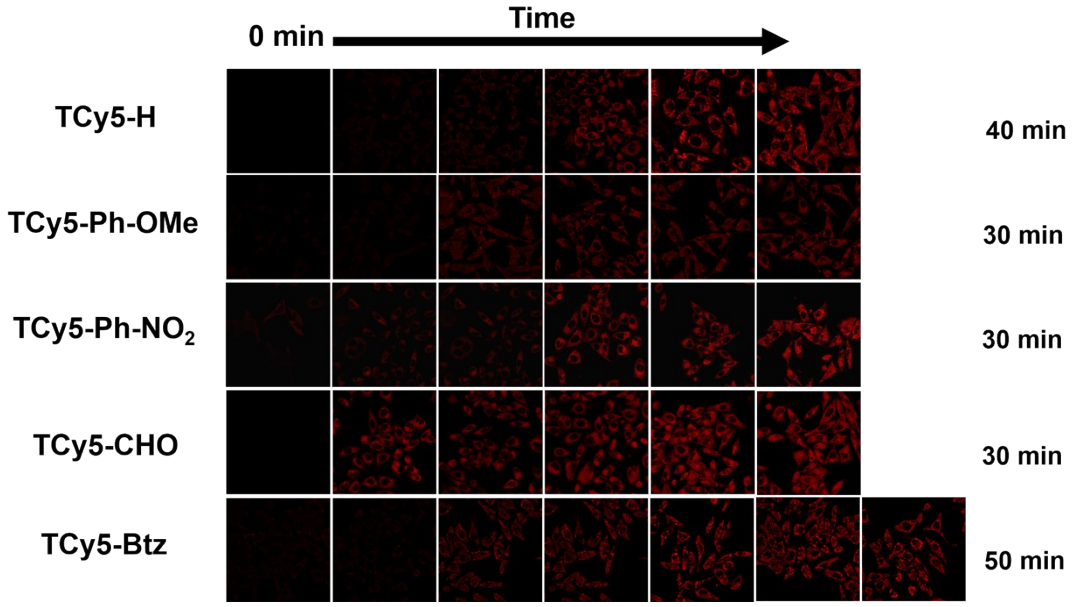


Figure S10. Cellular uptake of **TCy5** in MCF7 cells. Emissions are collected at 650-730 nm (λ_{ex} = 640 nm).

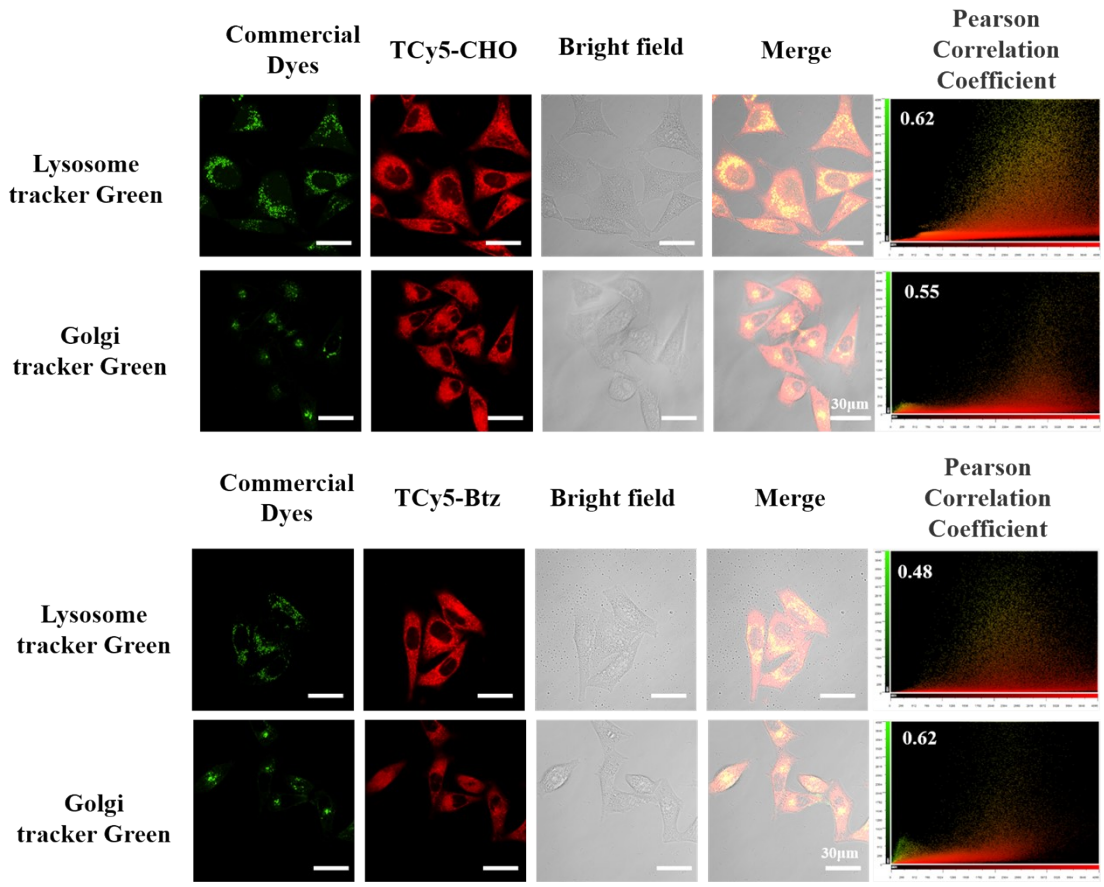


Figure S11. Fluorescence imaging of MCF7 cells labeled with **TCy5-CHO/TCy5-Btz** and their colocalization with Lyso- and Golgi-tracker Green, respectively. The correlation coefficient is shown in the graph at the end of each line. λ_{ex} = 488 nm, λ_{em} = 500-580nm, λ_{ex} = 640 nm, λ_{em} = 650-730 nm. Scale bar = 30 μm.

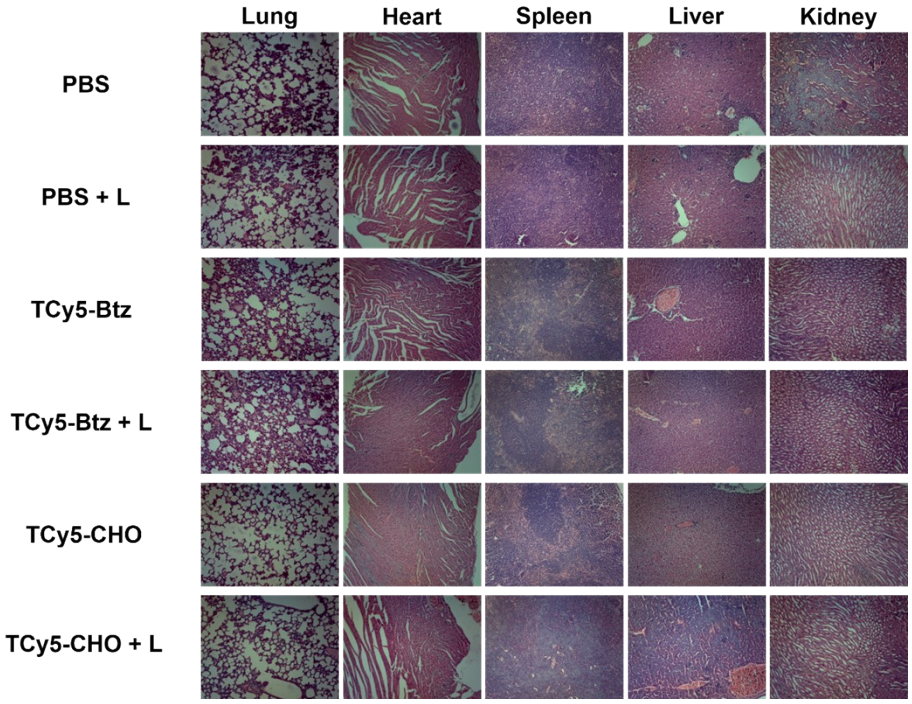


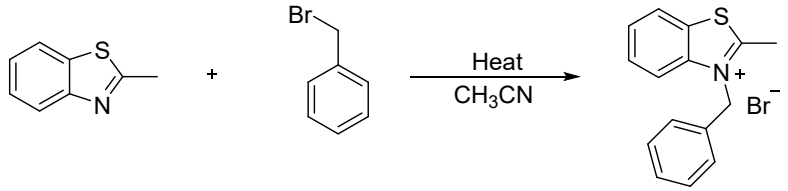
Figure S12. Typical images of H&E-stained lung, heart, spleen, liver, and kidney from mice 3 days post different treatments of Balb/C mice: A) PBS, B) PBS + light (660 nm, 50 mW cm^{-2}), C) **TCy5-Btz**, D) **TCy5-Btz** + light (660 nm, 50 mW cm^{-2}), E) **TCy5-CHO,** F) **TCy5-CHO** + light (660 nm, 50mW cm^{-2}).

2. General Information

In this report, the general chemicals including 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Energy Chemical Co., and J&K Scientific Ltd., and all of the solvents were of analytic grade. Flash column chromatography was performed with silica gel (200–300 mesh) and dichloromethane/methanol was used as eluent. Singlet Oxygen Sensor Green (SOSG) was obtained from life technologies. Mitochondrial Membrane Potential Assay Kit with JC-1 and Calcein-AM/PI was obtained from Beijing Solarbio Life Sciences & Technology Co., Ltd. Lyso-, Golgi- and Mito-Tracker was purchased from Life Technologies Co. (USA). All the other solvents and reagents used in this study were of analytical grade. NMR spectra were detected by Bruker Avance III 500 spectrometer. Chemical shift (δ) was reported as ppm in DMSO-d_6 with TMS as the internal standard. The mass spectrum was recorded on Agilent 6224 TOF LC/MS instruments. Absorption and emission spectra for six **TCy5** were performed with a Lambda 35 UV-visible spectrophotometer (PerkinElmer) and a VAEIAN CARY Eclipse fluorescence spectrophotometer (Serial No. FL0812-M018), respectively. Fluorescence quantum yield was obtained with the HAMAMATSU absolute fluorescence quantum yield spectrometer (Serial No. C11347). Nanosecond time-resolved transient absorption spectra were recorded on a LP980 laser flash photolysis spectrometer (Edinburgh Instruments Ltd.) in combination with a Nd:YAG laser (Surelite I-10, Continuum Electro-Optics, Inc.). Confocal laser scanning microscope (CLSM) images were performed on Olympus FV3000 confocal laser scanning microscope. For solution and in vitro tests, stock solution of **TCy5** in DMSO (10 mM) was prepared. For in vivo test, **TCy5** was dissolved in saline solution.

3. Preparation of TCy5 and the Spectral Data

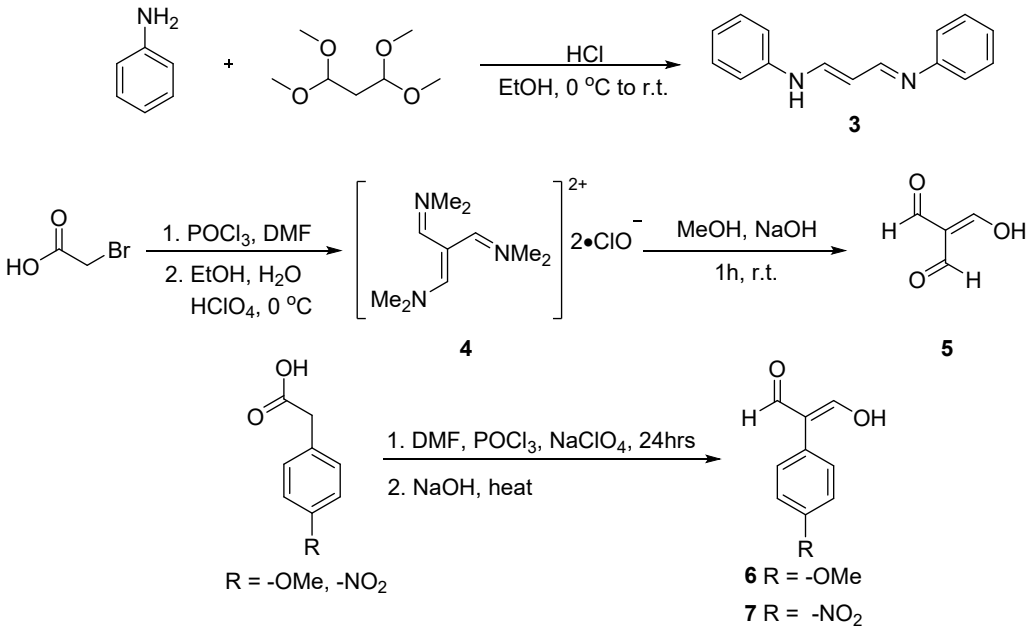
3.1 Preparation of quaternary ammonium salt and condensating agent



Scheme S1. Preparation of compounds **2**

Synthesis of compound 2: 3-benzyl-2-methylbenzo[d]thiazol-3-ium bromide

To a stirred solution of 2-Methylbenzothiazole (4 g, 26.8 mmol) in acetonitrile (10 mL) was added Benzyl bromide (3 g, 17.5 mmol) at room temperature. The reaction was heated to 60 ^{o}C for 24 hours. Subsequently, the reaction mixture was allowed to cool to room temperature. The precipitate was filtered and dried under vacuo to afford compound 2 as a pale green solid in 95 % yield (5.5 g).



Scheme S2. Preparation of compounds **3-7**

Synthesis of compound 3: N-((1E,3E)-3-(phenylimino)prop-1-en-1-yl)aniline

The title compound was prepared in accordance to the literature.[1] To a stirring mixture of 1,1,3,3-tetramethoxypropane (10 mL, 62 mol) and aniline (5.2 mL, 124 mol) in 100

mL ethanol was added to Conc. HCl (6 mL). The reaction mixture was stirred at ice-water bath for 1 hour followed by 3 hours at room temperature. The reaction mixture was then evaporated to remove EtOH, and then added water to the reaction residues, filtered, following washed with water and dry in vacuo to obtain 10 g (63 %) of product 3 as a yellow solid.

Synthesis of compound 4: Bis(perchlorate)aminalacetal

The title compound was prepared in accordance to the literature.[2] The round-bottom flask was charged with solid bromoacetic acid (9 g, 65 mmol, 1 equiv). The solid was dissolved in dimethylformamide (30 mL). The reaction solution was cooled in an ice bath, then POCl3 (29.8 g, 195 mmol, 3 equiv) was added to the stirring mixture dropwise. A colorless to yellowish slurry was obtained and the reaction mixture was stirred at 90 °C overnight. A suspension liquid was obtained following the reaction mixture was cooled to 0 °C. Ethanol (30 mL) was added dropwise within 30 minutes and the reaction mixture became clearer. Water (50 mL) was added dropwise within 20 minutes. Perchloric acid (70 % aq., 15 mL) was added dropwise and the reaction mixture was stirred for 3 hours at 0 °C. The yellow to orange solid was collected on a Büchner funnel, washed with ethanol, and dried overnight under reduced pressure. The desired product was obtained as a yellow solid (11.05 g, 29 mmol, 45 % yield). Which was used in the next step without further purification.

Synthesis of compound 5: Triformylmethane

The title compound was prepared in accordance to the literature.[2] A round bottom flask was charged with solid bis(perchlorate) aminalacetal (4.1 g, 11 mmol, 1 equiv), and dissolved in methanol (8 mL). A slurry was obtained. Colorless, solid NaOH were added under stirring until a clear solution was obtained. Addition of NaOH solid was continued until significant amounts of a colorless precipitate were formed which were collected over a Büchner funnel, washed with methanol (8 mL), and transferred to round-bottom flask. CH_2Cl_2 (8 mL) and 6 M aq. HCl (6 mL) were added to this flask. After stirring for 5 min, the mixture became more homogeneous. Stirring was continued for 70 min. The reaction mixture was transferred to a separatory funnel and the aq. phase was three times extracted with CH_2Cl_2. The pooled organic layers were dried

over $MgSO_4$, filtered, and dried under reduced pressure. The title compound was obtained as a colorless powder (0.96 g, 9.6 mmol, 89% yield).

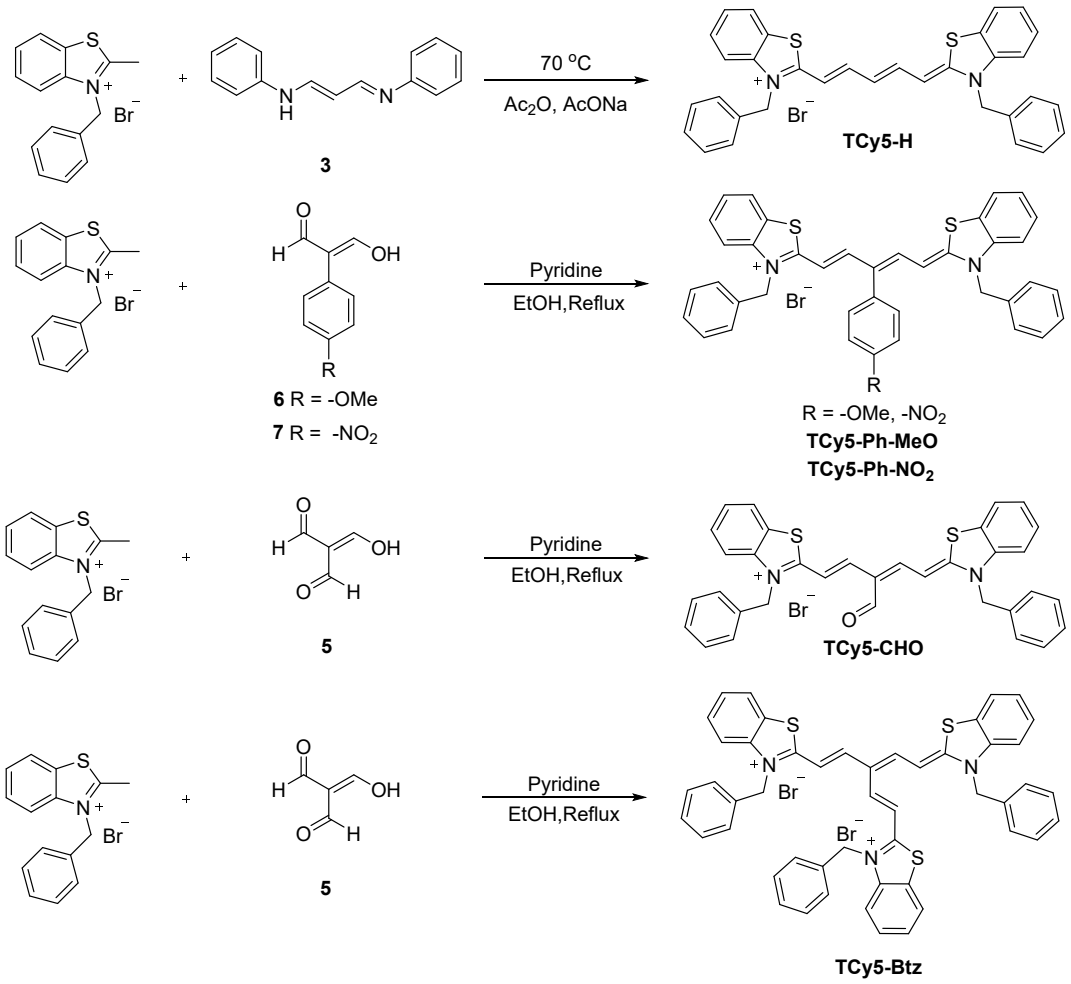
Synthesis of compound 6: (E)-3-hydroxy-2-(4-methoxyphenyl) acrylaldehyde

The title compound was prepared in accordance to the literature.[3] $POCl_3$ (2.1 mL, 22.5 mmol) was added dropwise to DMF (9 mL) at ice-water bath and stirred one hour. After that the mixture was stirred for three hours at room temperature. Subsequently, phenylacetic acid (7.5 mmol) was added to the mixture. The clear solution was heated to 90 °C keeping 4 hours, and then the reaction mixture cooled to room temperature. The crushed ice was added to the dark mixture followed by saturated $NaClO_4$ solution. The resulting white crystalline solid was filtered and carefully washed two times with saturated $NaClO_4$ solution to afford intermediate product, which was used in the next step without further purification. Intermediate product was added to 10 mL NaOH (0.4 g, 10 mmol) solution and the mixture was heated with stirring at 90 °C until the mixture was dissolved. The reaction mixture was cooled to room temperature and diluted with 10 mL water. The solution was acidified to pH 2 with 10% HCl solution resulting in the precipitation of the compound 6 (about 70 % yield).

Synthesis of compound 7: (E)-3-hydroxy-2-(4-methoxyphenyl) acrylaldehyde

Compound 7 was synthesized according to the synthetic procedure of compound 6 to give 60 % yield.

3.2 General procedure for the Synthesis of TCy5



Scheme S3. Preparation of **TCy5**

Method A:

A stirring mixture of condensating agent (0.5 mmol, 1 equiv), quaternary ammonium salt (1 mmol, 2 equiv) in ethyl alcohol (10 mL) was added a drop of pyridine. The resulting solution was heated to reflux and monitored by TLC analysis. After the yellow intermediate decreases obviously, the solvent was evaporated under reduced pressure to give a residue. The crude material was adsorbed onto silica gel and purified by column chromatography (dichloromethane/methyl alcohol = 20/1) to give the corresponding compound **TCy5**.

Method B:

Both of condensating agent (0.5 mmol, 1 equiv) and quaternary ammonium salt (1 mmol, 2 equiv) were dissolved in acetic acid (20 mL). The resulting solution was stirred

in the room temperature and monitored by TLC analysis. After the yellow intermediate almost disappeared, the solvent was evaporated under reduced pressure to give a residue. Then, the residue was dissolved in 100 mL DCM, and organic layer was washed with saline and dried with anhydrous Na_2SO_4. Finally, the organic layer was evaporated to dryness and the crude material was adsorbed onto silica gel and purified by column chromatography (dichloromethane/methyl alcohol = 20/1) give the corresponding compound **TCy5**.

TCy5-H

The title compound was prepared according to Method B. The product was obtained as a purple powder with a metallic luster (146 mg, 49 % yield). **^{1}H NMR** (500 MHz, DMSO-d6): δ 8.03 (d, *J*= 8.0 Hz, 2H), 7.81 (t, *J*= 12.5 Hz, 2H), 7.68 (d, *J*= 8.0 Hz, 2H), 7.51 (t, *J*= 7.5 Hz, 2H), 7.44–7.34 (m, 6H), 7.64 (d, *J*= 7.0 Hz, 2H), 7.26 (d, *J*= 7.5 Hz, 4H), 6.63 (d, *J*= 12.5 Hz, 2H), 6.37 (t, *J*= 12.0 Hz, 1H), 5.66 (s, 4H) ppm; **^{13}C NMR** (125 MHz, DMSO-d_6): δ 164.25, 150.75, 141.47, 134.67, 128.96, 128.07, 127.96, 126.68, 125.27, 125.15, 123.17, 113.50, 101.00, 48.79, 40.00, 39.83, 39.6 6, 39.50, 39.33, 39.16, 39.00 ppm; HRMS(ESI): m/z calc. for $[C_{33}H_{27}N_2S_2]^+$ 515.1610, found 515.1605 $[M\text{-}Br]^+$.

TCy5-Ph-OMe

The title compound was prepared according to Method A. The product was obtained as a purple powder with a metallic luster (60 mg, 17 % yield). **^{1}H NMR** (500 MHz, DMSO-d6): δ 8.07 (d, *J*= 8.0 Hz, 2H), 7.92–7.80 (m, 4H), 7.55 (td, *J*= 7.5, 1.0 Hz, 2H), 7.43 (t, *J*= 7.5 Hz, 2H), 7.36–7.30 (m, 6H), 7.07–6.99 (m, 4H), 6.51 (d, *J*= 8.5 Hz, 2H), 6.80 (d, *J*= 8.5 Hz, 2H), 5.88 (d, *J*= 13.5 Hz, 2H), 5.41 (s, 4H) ppm; **^{13}C NMR** (125 MHz, DMSO-d_6): δ 164.11, 158.46, 141.78, 134.53, 130.52, 128.91, 128.26, 128.21, 128.03, 126.79, 125.25, 125.23, 123.29, 114.26, 113.43, 99.52, 55.18, 49.15 ppm; HRMS(ESI): m/z calc. for $[C_{40}H_{33}N_2OS_2]^+$ 621.2029, found 621.2033 $[M\text{-}Br]^+$.

TCy5-Ph-NO_2

The title compound was prepared according to Method A. The product was obtained as a purple powder with a metallic luster (251 mg, 70 % yield). **^{1}H NMR** (500 MHz,

DMSO-d_6): δ 8.22 (d, *J*= 8.5 Hz, 2H), 8.10 (d, *J*= 8.0 Hz, 2H), 7.95 (d, *J*= 14.0 Hz, 2H), 7.86 (d, *J*= 8.5 Hz, 2H), 7.57 (t, *J*= 8.0 Hz, 2H), 7.45 (t, *J*= 7.5 Hz, 2H), 7.35–7.24 (m, 6H), 6.20 (d, *J*= 8.5 Hz, 2H), 7.05–6.95 (m, 4H), 5.85 (d, *J*= 13.5 Hz, 2H), 5.48 (s, 4H) ppm; **^{13}C NMR** (125 MHz, DMSO-d_6): δ 165.51, 149.86, 146.96, 142.24, 135.13, 131.45, 129.39, 128.79, 128.53, 127.23, 125.98, 125.84, 124.60, 123.86, 114.21, 99.66, 74.36, 60.49, 58.49, 49.74 ppm; **HRMS**(ESI): m/z calc. for $[C_{39}H_{30}N_3O_2S_2]^+$ 636.1774, found 636.1779 $[M\text{-}Br]^+$.

TCy5-CHO

The title compound was prepared according to Method A. The product was obtained as a purple powder with a metallic luster (143 mg, 46 % yield). **^{1}H NMR** (500 MHz, DMSO-d_6): δ 9.75 (s, 1H), 8.20 (d, *J*= 7.5 Hz, 2H), 7.07 (d, *J*= 14.5 Hz, 2H), 7.94 (d, *J*= 8.5 Hz, 2H), 7.63 (t, *J*= 8.0 Hz, 4H), 7.54 (t, *J*= 8.0 Hz, 2H), 7.45–7.37 (m, 4H), 7.37–7.28 (m, 6H), 5.90 (s, 4H) ppm;**^{13}C NMR** (125 MHz, DMSO-d_6): δ 190.05, 169.72, 141.33, 134.31, 129.09, 128.56, 128.22, 126.88, 126.34, 125.98, 123.59, 117.68, 114.81, 49.84, 40.00, 39.83, 39.67, 39.50, 39.33, 39.17, 39.00 ppm; HRMS(ESI): m/z calc. for $[C_{34}H_{27}N_2OS_2]^+$ 543.1599, found 543.1564 $[M\text{-}Br]^+$.

TCy5-Btz

The title compound was prepared according to Method A, and the ratio of reagent was 3:1. The product was obtained as a purple powder with a metallic luster (139 mg, 30 % yield). **^{1}H NMR** (500 MHz, DMSO-d_6): δ 8.31 (d, *J*= 8.0 Hz, 3H), 8.11 (d, *J*= 14.5 Hz, 3H), 8.00 (d, *J*= 8.0 Hz, 3H), 7.70 (td, *J*= 7.5, 1.0 Hz, 3H), 7.62 (t, *J*= 7.5 Hz, 3H), 7.48 (d, *J*= 14.5 Hz, 3H), 7.44–7.40 (m, 6H), 7.38–7.32 (m, 9H), 6.09 (s, 6H) ppm; **^{13}C NMR** (125 MHz, DMSO-d_6): 169.36, 145.74, 141.32, 134.54, 129.19, 128.87, 128.33, 126.92, 126.61, 123.80, 117.26, 115.42, 104.27, 50.14, 39.99, 39.83, 39.66, 39.49, 39.33, 39.16, 38.99 ppm; HRMS(ESI): m/z calc. for $[C_{49}H_{39}N_3S_3]^{2+}$ 382.6148, found 382.6148 $[M\text{-}2Br]^{2+}$.

4. Analysis test procedure

Singlet oxygen ($^1O_2$) detection

The singlet oxygen generated by **TCy5** was measured using $^1O_2$ specificity capture agent, 9,10-anthracenedipropanoic acid (ABDA). Briefly, the absorbance of ABDA at 380 nm was adjusted to about 1.0 in UP water. Then, 10 μM **TCy5** was added to this cuvette. After blending well, the cuvette was irradiated with 660 nm monochromatic light for various time, and absorption spectra were measured immediately.

Procedure for $^1O_2$ Quantum Yield Measurement[4]

In 3 mL of the ultrapure water, 10 μM of targeted molecule was incubated for 1 min, and the absorbance of 9,10-anthracenediyl-bis(methylene) dimalonic acid (ABDA) at 380 nm was adjusted to about 1.0 in water. UV−vis absorption spectra were recorded after the probe was exposed to a corresponding monochromatic light with a power density of 1 mW/cm^2 for various time. The $^1O_2$ quantum yield was measured using Rose Bengal (RB) as the reference photosensitizer and calculated using the following equation:

$$\Phi_{probe} = \Phi_{RB}\frac{K_{probe}A_{RB}}{K_{RB}A_{probe}}$$

where K_{probe} and K_{RB} were the decomposition rate constants of ABDA in the presence of the probe and RB, respectively. Φ_{RB} was the $^1O_2$ quantum yield of RB (Φ_{RB} = 0.75 in water). $A_{RB}/A_{TCy5\text{-}CHO}$ and $A_{RB}/A_{TCy5\text{-}Btz}$ at corresponding monochromatic light were 1.048, 1.795, respectively (Figure S6). The natural logarithm of the absorbance ratio (A_0/A) of ABDA at 380 nm was plotted against irradiation time and the slope is regarded as the decomposition rate.

Cyclic Voltammetry

Cyclic voltammetry curves were recorded by a CHI660D electrochemical workstation (CHI instruments, Inc., Shanghai, China) at a scan rate of 100 mV/s. Electrochemical measurements were performed at room temperature (RT) using 0.19 M potassium chloride (the mixture of deionized water and ethyl alcohol ratio 4:3 was used as the

solvent) as the supporting electrolyte, a glassy carbon electrode as the working electrode, and a glassy carbon electrode as the counter electrode. The reference electrode was Ag/AgCl (0.22V vs NHE).

Laser flash photolysis

Nanosecond time-resolved transient absorption spectra were recorded on a LP980 laser flash photolysis spectrometer (Edinburgh Instruments Ltd.) in combination with a Nd:YAG laser (Surelite I-10, Continuum Electro-Optics, Inc.). Samples in nitrogen atmosphere were excited by a 555 nm laser pulse (1 Hz, 100 mJ per pulse, fwhm ≈ 7 ns) at room temperature. The absorbances of **TCy5-CHO** and **Cy5-CHO** were lower than 0.5 OD at 588 nm in 1 cm path length quartz cuvettes.

Time-correlated single photon counting (TCSPC)

Time-resolved photoluminescence (TRPL) measurements were carried out by using a time-correlated single photon counting (TCSPC) spectrometer (PicoHarp 300, PicoQuant).[5] The second harmonic of a Ti-sapphire laser (Mai Tai DeepSee, Spectra-Physics, 150 fs, 80 MHz) at 400 nm was used as the excitation source. The instrument response function (IRF) of the detection was 30 ps and all the fluorescence lifetimes were achieved by deconvolution fitting.

Computations details of B3LYP

Density Functional Theory (DFT) Calculations: The DFT and TD-DFT calculations were performed using the Gaussian09 program to optimize the molecular geometries of S_0 state, S_1 state and T_1 state (the atomic standard orientations after geometry optimization of ground state and excited state have been given in supporting information). Becke's three-parameter hybrid exchange functions with Lee–Yang–Parr gradient-corrected correlation functional (B3LYP functional) is the more reasonable choice to be in good accordance with the experimental results,[6] and used in both the DFT and TD-DFT methods in the sequential work. The 6-31G(d,p) basis set was chosen as the basis set throughout, which is an appropriate basis set for such organic compound.[7]

Femtosecond Transient Absorption Spectra

The femtosecond transient absorption spectra of compound TCy5-H and TCy5-CHO

were measured by a home-made ultrafast pump–probe setup. The pulse duration was 30 fs. The wavelength of the pump beam was chosen to be 380 nm. The used concentration of samples was 50 μM for both TCy5-H and TCy5-CHO in DCM at room temperature. Measurements were performed in 1 mm quartz cuvettes.

Cell incubation

MCF7 cells, HepG2 cells and COS-7 cells were purchased from the Institute of Basic Medical Sciences (IBMS) of the Chinese Academy of Medical Sciences and cultured with Dulbecco's modified Eagle's medium (DMEM, Invitrogen) containing 10 % fetal bovine serum (Invitrogen) and 1 % antibiotics (penicillin/streptomycin, 100 U mL^{-1}) in an atmosphere of CO_2/ air = 5%/95% at 37 ºC.

Cytotoxicity assay

The cytotoxicity in the presence or absence of light was evaluated by reducing MTT (3-(4,5)-dimethylthiazol-2-yl)-3,5-diphenyltetrazolium bromide) to formazan crystals with mitochondrial dehydrogenases. MCF7 cells, HepG2 cells and COS-7 cells were seeded in a 96-well plate with a concentration of 1×10^5 cells per mL in 100 mL DMEM medium (containing 10 % FBS) incubated at 37 ºC for 24 h. For the photo-cytotoxicity evaluation, different concentrations of TCy5 from 0 to 2 μM in DMEM medium were added to the wells of cells, respectively. Then, the cells were further incubated for 1 h under normoxic conditions, respectively. Subsequently, the cells were subjected to 660 nm light (10 mW/cm^2, 10 min). Then the cells were further incubated for 24 h at 37 ºC. Next, MTT solution (5 mg/mL) was added in DMEM to each well following incubated the cells for 4 h, the solution in each well was removed out carefully, and added 100 μL DMSO to each well. The plate was then shaken for 10 min and the absorbance was determined on a microplate reader (Thermo Fisher Scientific) at 570 nm and 630 nm. The viability was expressed as a percent of the controlled one using the following equation:

$$\text{Cell viability (\%)} = \frac{OD_{dye570} - OD_{dye630}}{OD_{control570} - OD_{control630}} \times 100\%$$

For dark toxicity measurement of **TCy5**, the light irradiation was canceled and the other steps were same.

PDT in the 4T1 tumor bearing mice model

All procedures were in accordance with the Guide for the Care and Use of Laboratory Animal Resources and the National Research Council, and were approved by the Institutional Animal Care and Use Committee of the NIH. The 4–5 weeks old Balb/c mice were purchased from SPF experimental animal center of Dalian Medical University for transplanting 4T1 cells at a density of 5×10^6 under the subcutaneous of back. When the tumors volume reached 200 mm^3, the tumors mice were divided into 6 groups (PBS, PBS + Light, **TCy5-CHO**, **TCy5-CHO** + Light, **TCy5-Btz**, **TCy5-Btz** + Light, N = 5). The reagent (50 mM, 100 μL) was intratumorally injected into the tumor-bearing mice on day 0. After 2 h post-injection, tumor region was irradiated with 660 nm light at a power density of 50 mW cm^{-2} for 15 min. The volume of tumors and the weight of mice were measured by a vernier caliper diebus tertius after treatments. The tumors volume was measured using a vernier caliper and calculated using the following equation:

$$V = \frac{a \times b^2}{2}$$

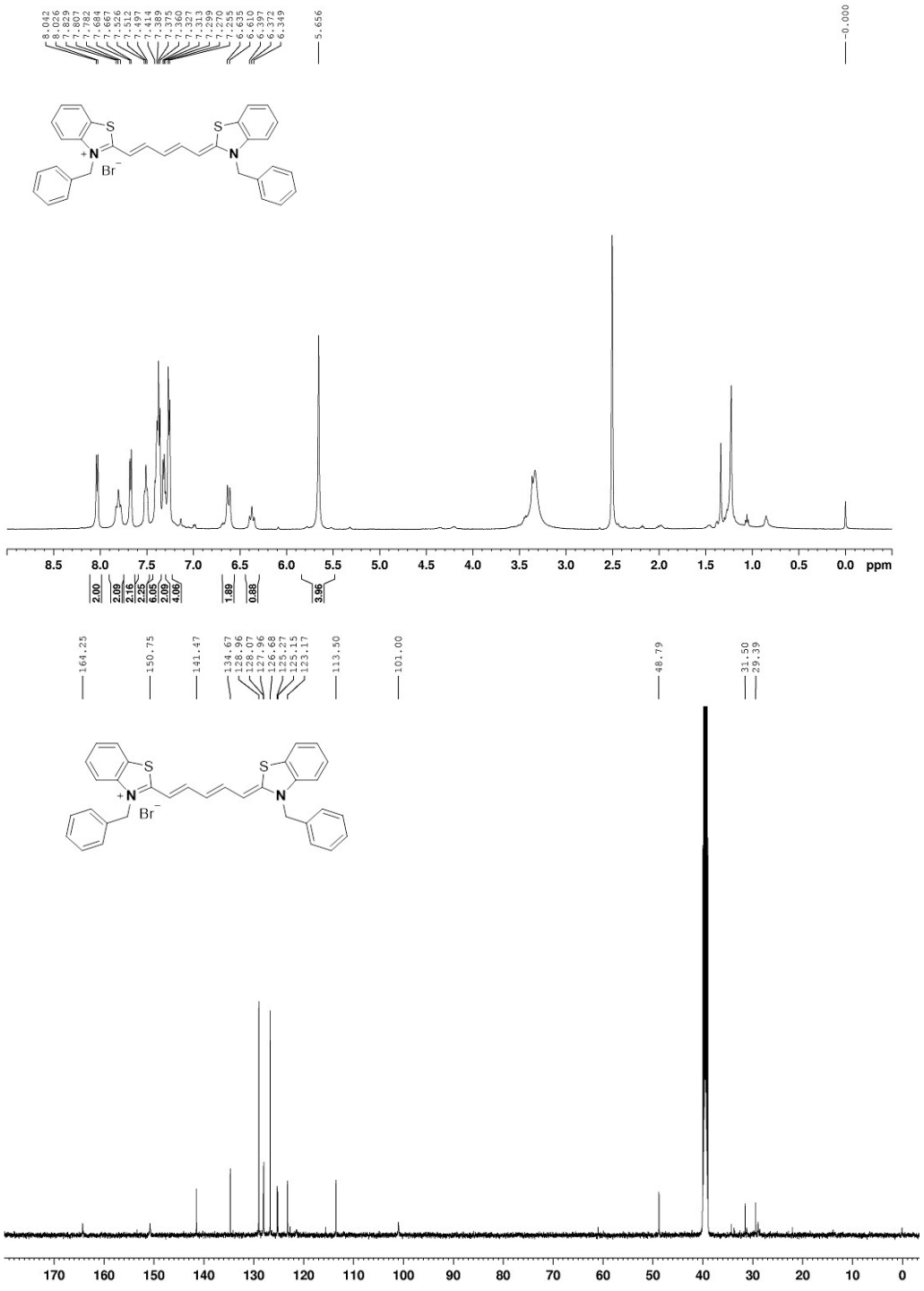
V represents the tumors volume, *a* and *b* represents the longer diameter and the shorter diameter, respectively

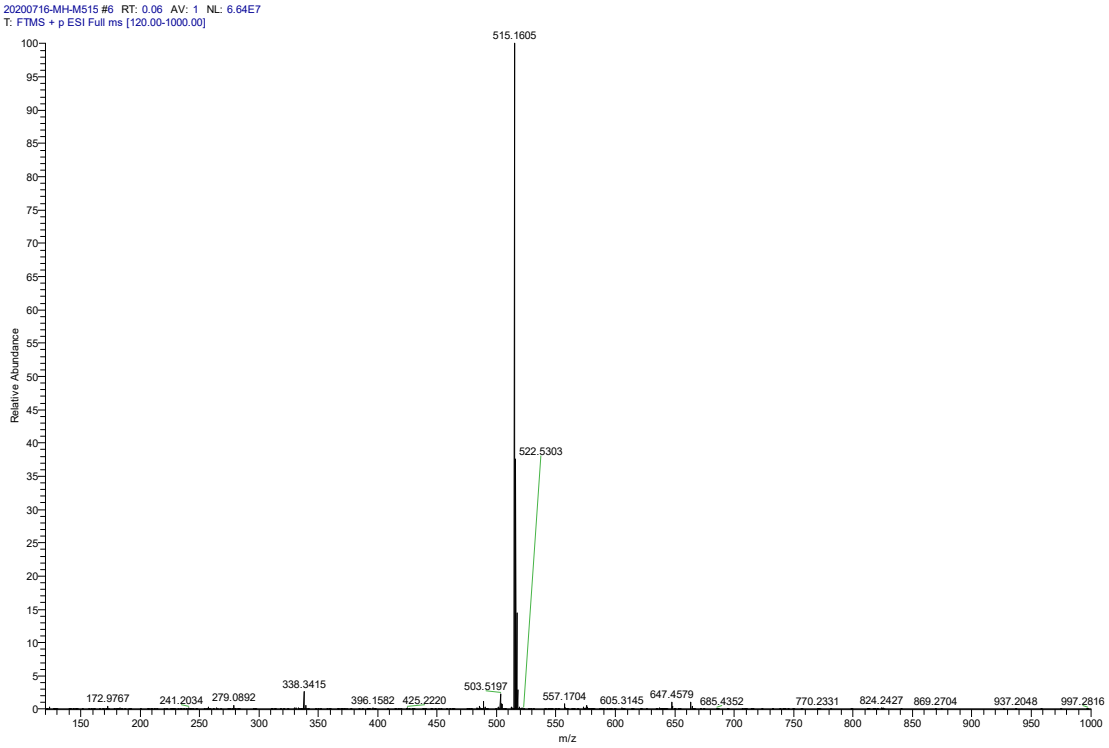
***In Vivo* Biosafety Assay**

The *in vivo* biosafety assay was performed by using measurement mice weight and H&E slice histological analysis. After the 21th day treatment, all mice were sacrificed and the tumor tissues were collected. The main organs including heart, liver, spleen, lung, kidneys were harvested for histological analysis by means of hematoxylin-eosin (H&E) staining.

5. ^{1}H NMR, ^{13}C NMR spectrogram and HRMS data

TCy5-H

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T: FTMS + p ESI Full ms [120.00-1000.00]
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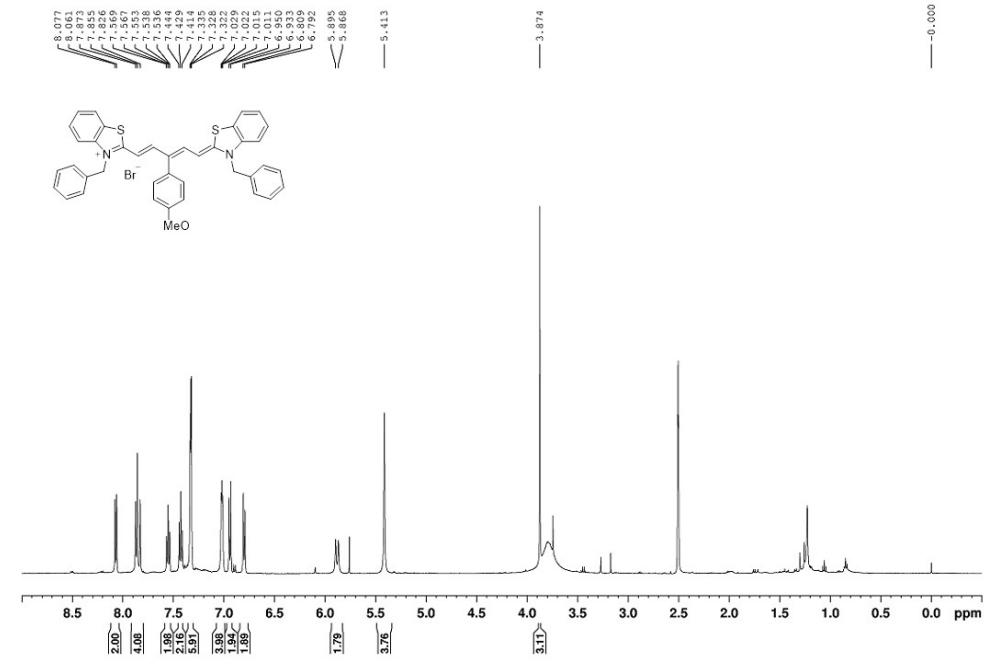
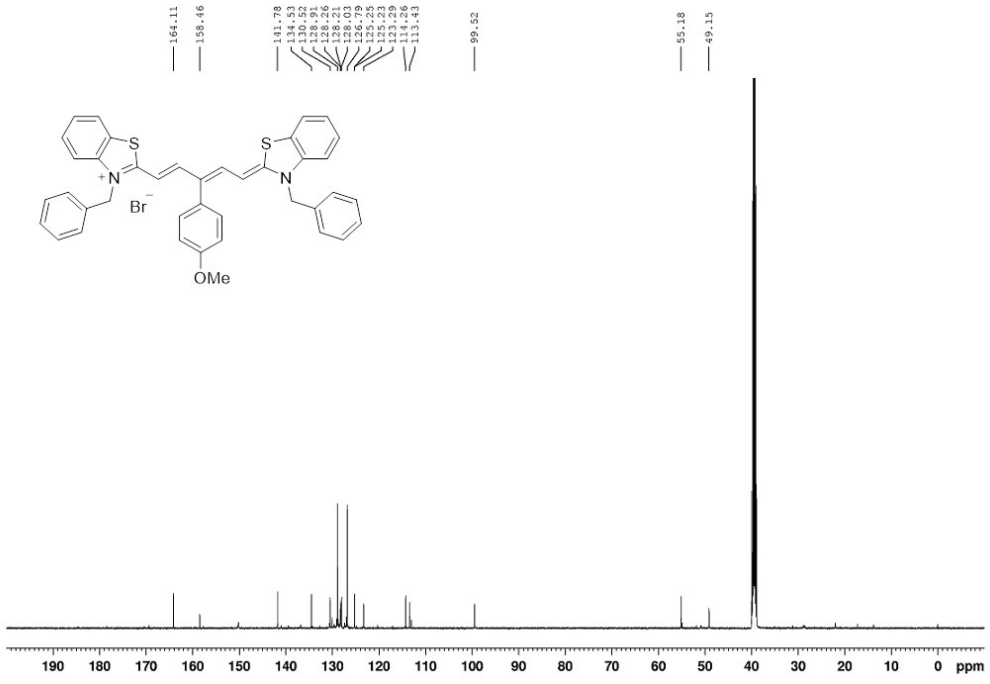
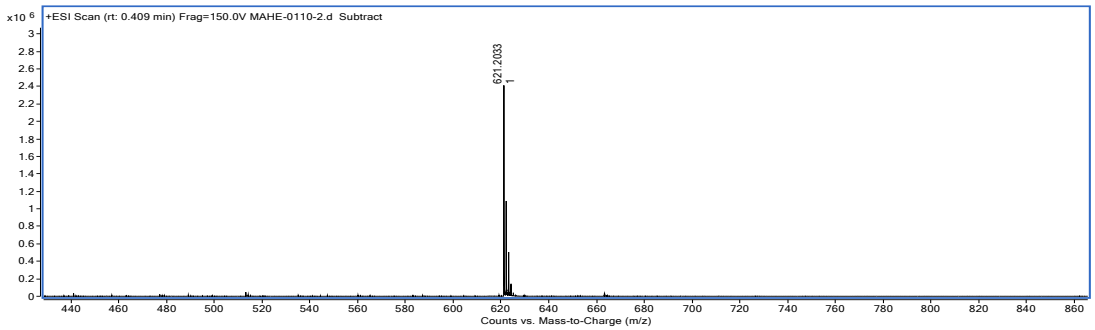
522.5303
338.3415
503.5197
172.9767
241.2034
279.0892
396.1582
425.2220
557.1704
605.3145
647.4579
685.4352
770.2331
824.2427
869.2704
937.2048
997.2816
Relative Abundance
m/z

TCy5-Ph-OMe


8.077
8.061
7.883
7.855
7.826
7.569
7.567
7.553
7.538
7.536
7.444
7.429
7.419
7.335
7.328
7.322
7.309
7.022
7.015
7.011
6.950
6.933
6.809
6.792
5.895
5.868
5.413
3.874
0.000
S
N
Br
MeO
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
2.00 4.08 1.98 2.16 5.97 3.98 1.94 1.89 1.79 3.76 3.11


164.11
158.46
141.78
134.53
130.52
128.91
128.26
128.21
126.03
126.79
125.25
125.23
123.29
114.26
113.43
99.52
55.18
49.15
S
N
Br
OMe
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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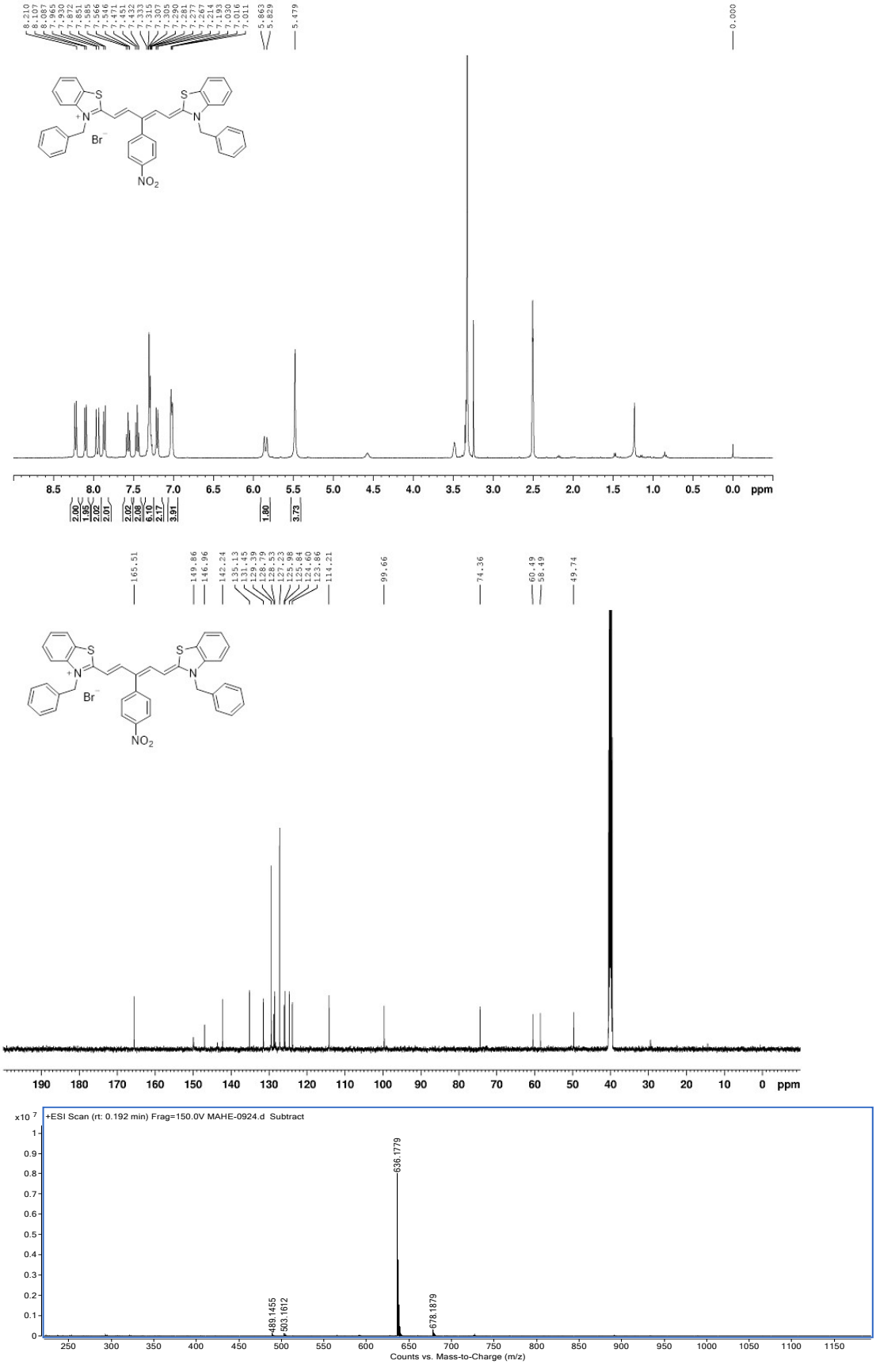
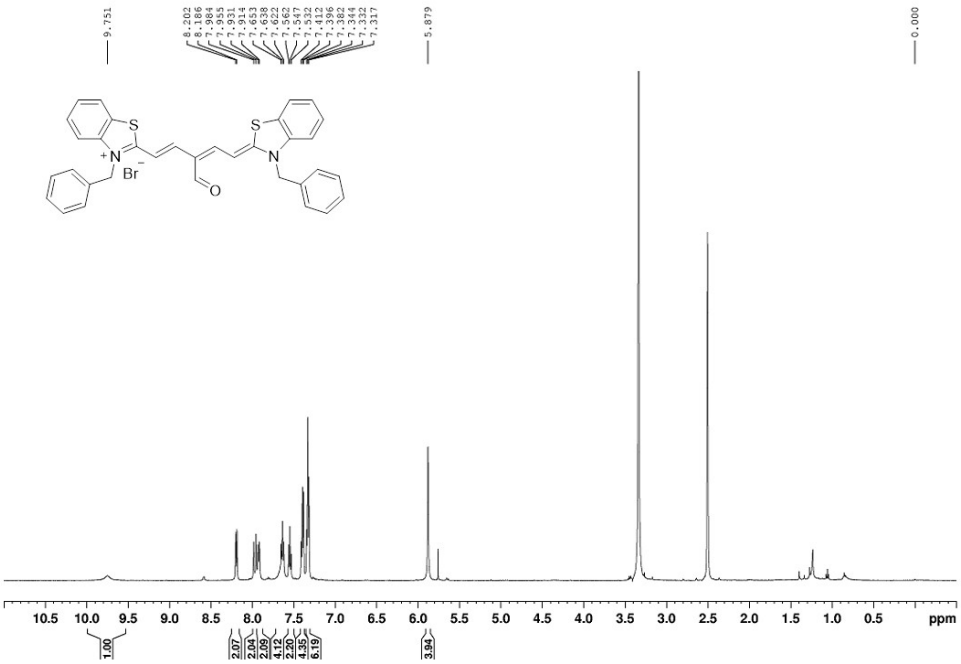
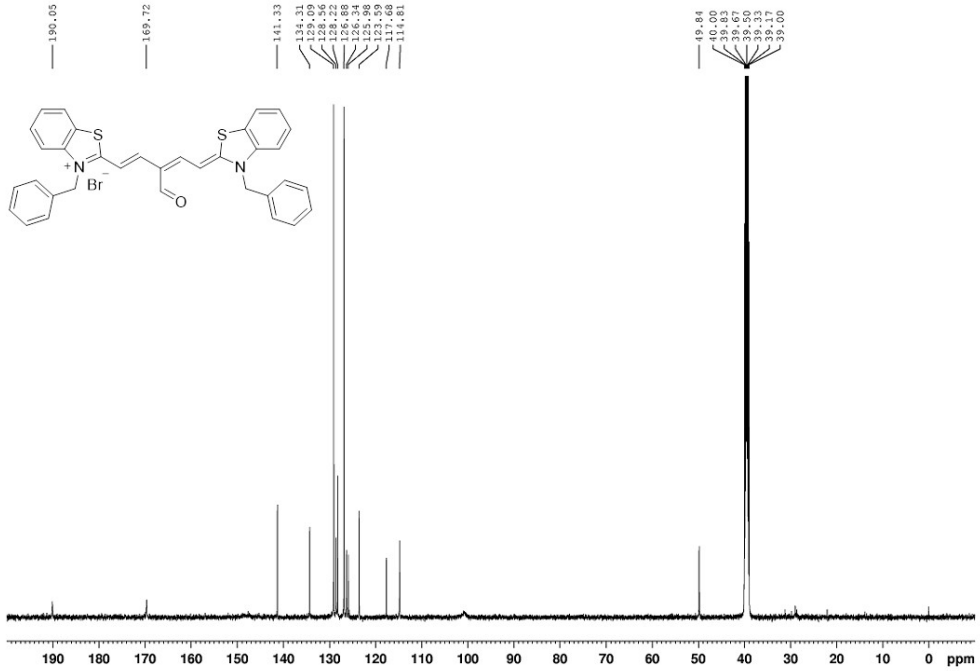
621.2033
1
Counts vs. Mass-to-Charge (m/z)

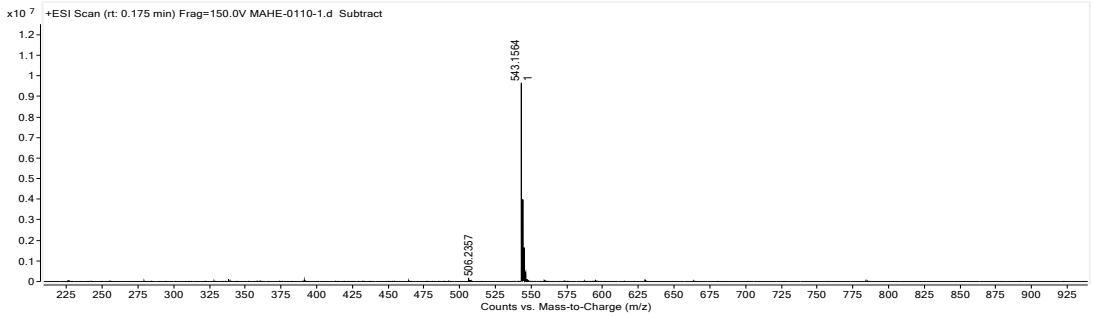
TCy5-Ph-NO_2


8.210
8.107
8.087
7.965
7.880
7.872
7.851
7.585
7.566
7.546
7.471
7.451
7.432
7.333
7.315
7.307
7.305
7.290
7.281
7.277
7.267
7.214
7.193
7.030
7.016
7.011
5.863
5.829
5.479
0.000
Br
NO2
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
2.00 1.95 2.02 2.01 2.02 2.06 6.10 2.17 3.91 1.80 3.73
165.51
149.86
146.96
142.24
135.13
131.45
129.39
128.53
127.23
125.98
125.84
124.60
123.66
114.21
99.66
74.36
60.49
58.49
49.74
Br
NO2
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm
x10 7
+ESI Scan (rt: 0.192 min) Frag=150.0V MAHE-0924.d Subtract
636.1779
489.1455
503.1612
678.1879
250 300 350 400 450 500 550 600 650 700 750 800 850 900 950 1000 1050 1100 1150
Counts vs. Mass-to-Charge (m/z)

TCy5-CHO


9.751
8.202
8.186
7.984
7.955
7.931
7.914
7.653
7.638
7.622
7.562
7.547
7.532
7.412
7.396
7.382
7.364
7.332
7.317
5.879
0.000
S
+N
Br
S
N
O
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
1.00
2.07
2.04
2.09
4.12
2.20
4.35
6.19
3.84


190.05
169.72
141.33
134.31
130.09
128.50
128.22
126.88
126.34
125.98
123.59
117.68
114.81
49.84
40.00
39.83
39.67
39.50
39.33
39.17
39.00
S
+N
Br
S
N
O
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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+ESI Scan (rt: 0.175 min) Frag=150.0V MAHE-0110-1.d Subtract
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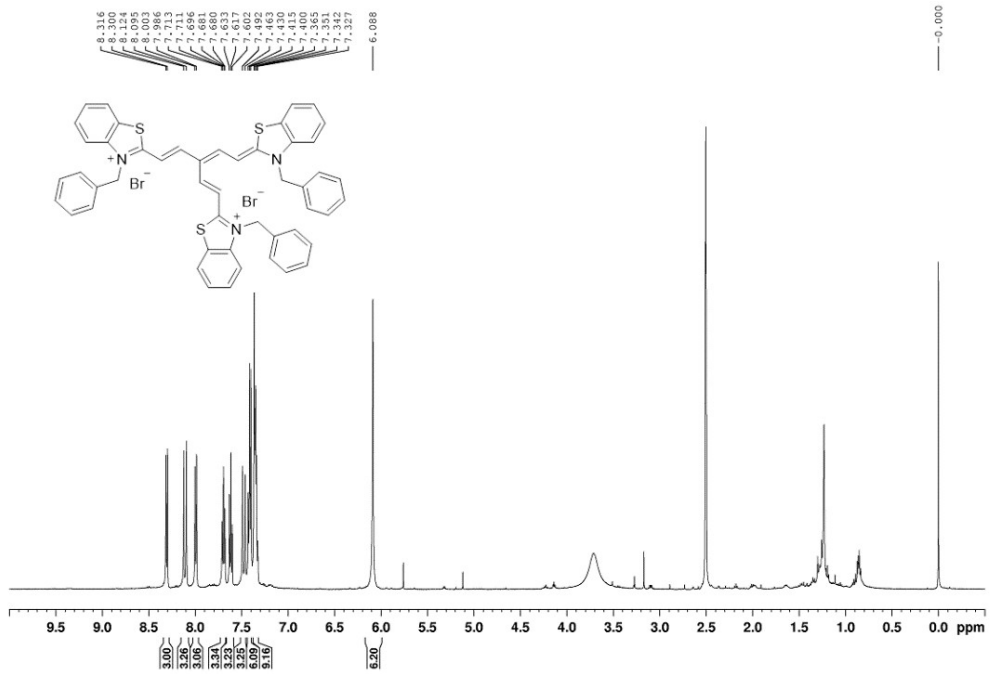
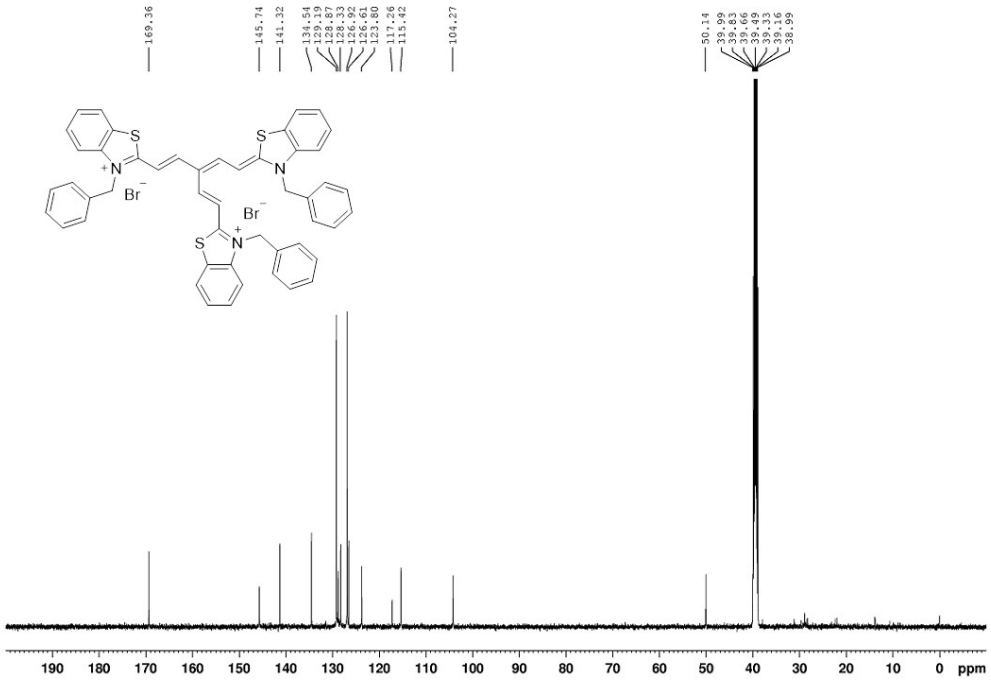
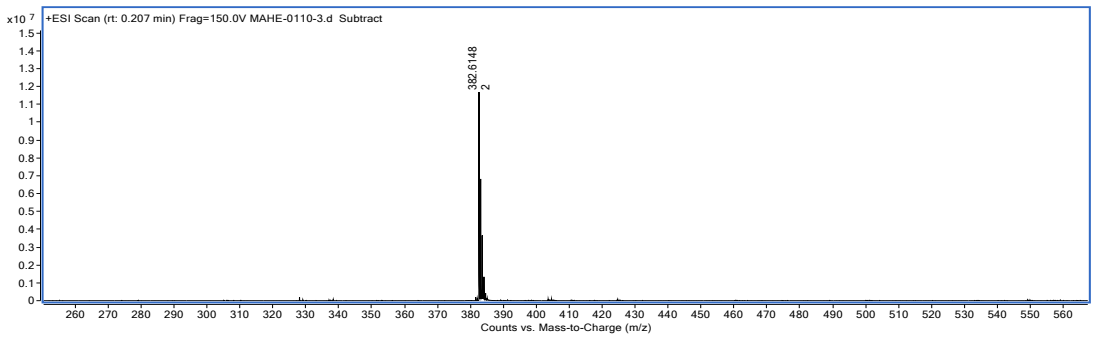
1
506.2357
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
225 250 275 300 325 350 375 400 425 450 475 500 525 550 575 600 625 650 675 700 725 750 775 800 825 850 875 900 925
Counts vs. Mass-to-Charge (m/z)

TCy5-Btz


8.316
8.300
8.214
8.195
8.003
7.986
7.713
7.711
7.696
7.681
7.680
7.633
7.617
7.602
7.492
7.463
7.430
7.415
7.400
7.365
7.351
7.342
7.327
6.088
0.000
Br⁻
Br⁻
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 ppm
3.00
3.26
3.06
2.34
3.23
3.25
6.09
9.16
6.20


169.36
145.74
141.32
134.54
129.19
128.87
128.33
126.92
126.61
123.80
117.26
115.42
104.27
50.14
39.99
39.83
39.66
39.49
39.33
39.16
38.99
Br⁻
Br⁻
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm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382.6148
2
260 270 280 290 300 310 320 330 340 350 360 370 380 390 400 410 420 430 440 450 460 470 480 490 500 510 520 530 540 550 560
Counts vs. Mass-to-Charge (m/z)

6. References

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[7] L. C. Zhou, G. Zhao, J. Liu, K. Han, Y. K. Wu, X. J. Peng, M. Sun, *Journal of Photochemistry and Photobiology A-chemistry* **2007**, *187*, 305-310.