Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information

Design and synthesis of a NAD(P)H:quinone oxidoreductase 1-activatable photosensitiser for controlled photodynamic therapy

Evelyn Y. Xue,‡ Fangyuan Kang,‡ Yimin Zhou and Dennis K. P. Ng*

Department of Chemistry, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China

‡ These authors contributed equally to this work.

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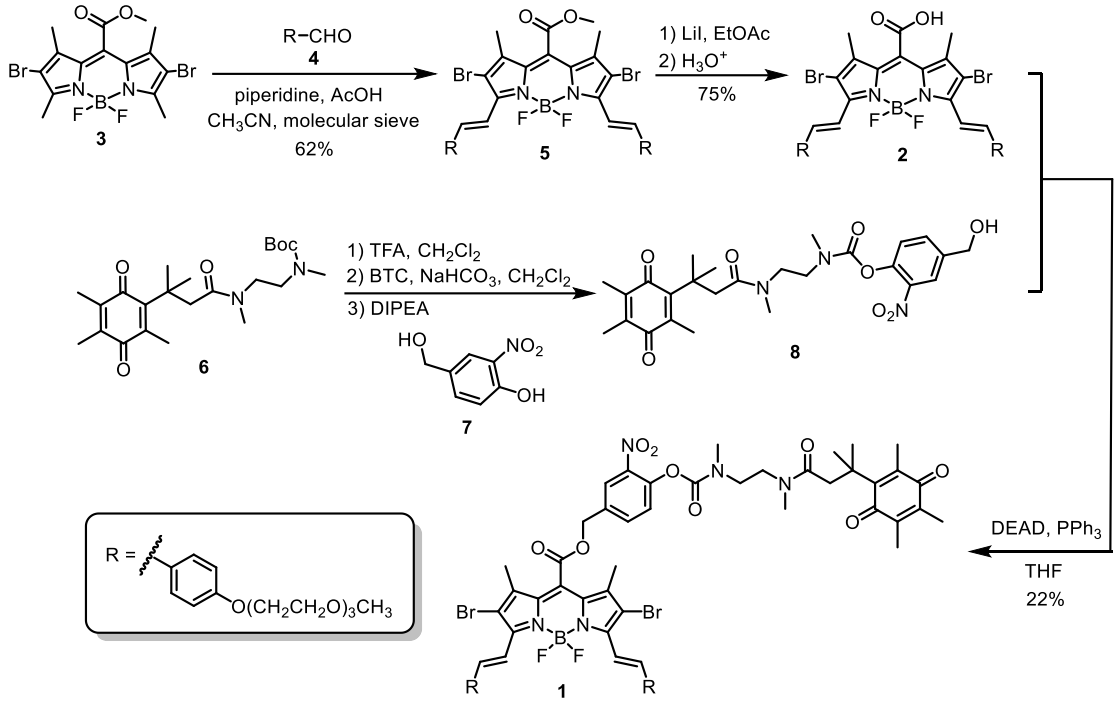

Scheme S1. Synthesis of NQO1-activatable distyryl BODIPY **1**.

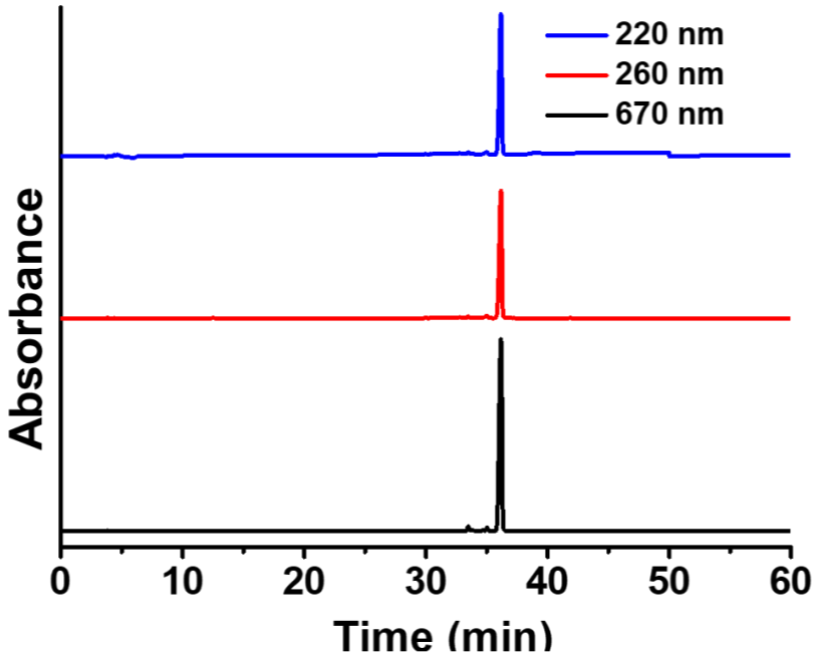

Fig. S1 HPLC chromatograms of **1** monitored at 220, 260 and 670 nm.

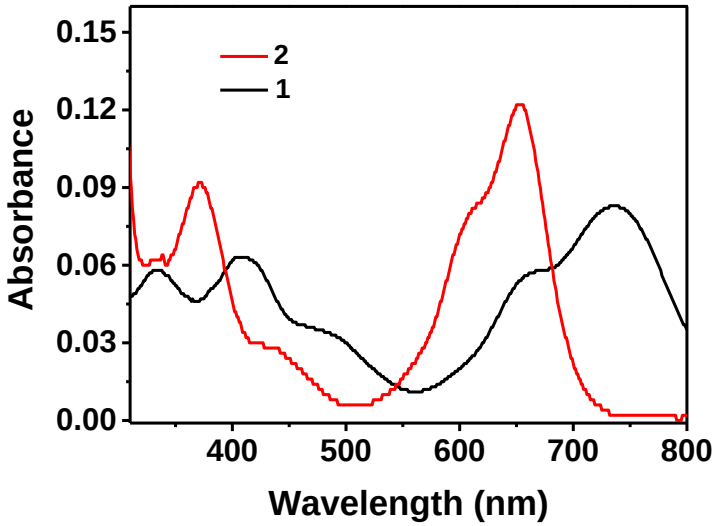

Fig. S2 Electronic absorption spectra of **1** and **2** (both at 2 μM) in PBS at pH 7.4 with 0.5% (v/v) DMF.

Table S1. Electronic absorption and fluorescence data for **1** and **2**.[a]

Compound	λ_{abs} (nm) (log ε)	λ_{em} (nm)[b]	Φ_f[c]	Φ_Δ[d]
1	330 (4.39), 408 (4.42), 655 (4.37), 732 (4.55)	745	0.026	0.03
2	371 (4.66), 612 (4.61), 652 (4.78)	688	0.59	0.15

[a] In PBS at pH 7.4 with 0.5% (v/v) DMF. [b] Excited at 610 nm. [c] Fluorescence quantum yield (Φ_f) with reference to ZnPc (Φ_f = 0.28 in DMF). [d] Relative to methylene blue (Φ_Δ = 0.52 in H_2O) using 9,10-anthrancenediyl-bis(methylene)dimalonic acid as the singlet oxygen scavenger.

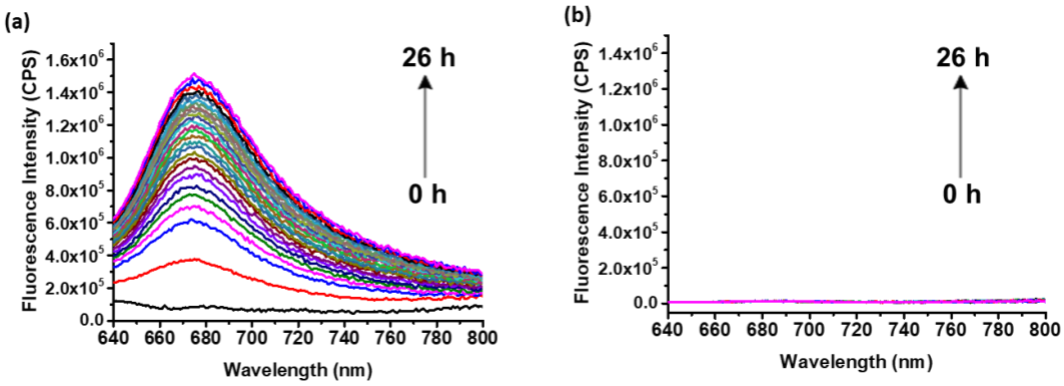

Fig. S3 Change in fluorescence spectrum of **1** (2 μM) in DMEM containing 1 mM of NADPH in the (a) presence and (b) absence of NQO1 (80 μg mL^{-1}).

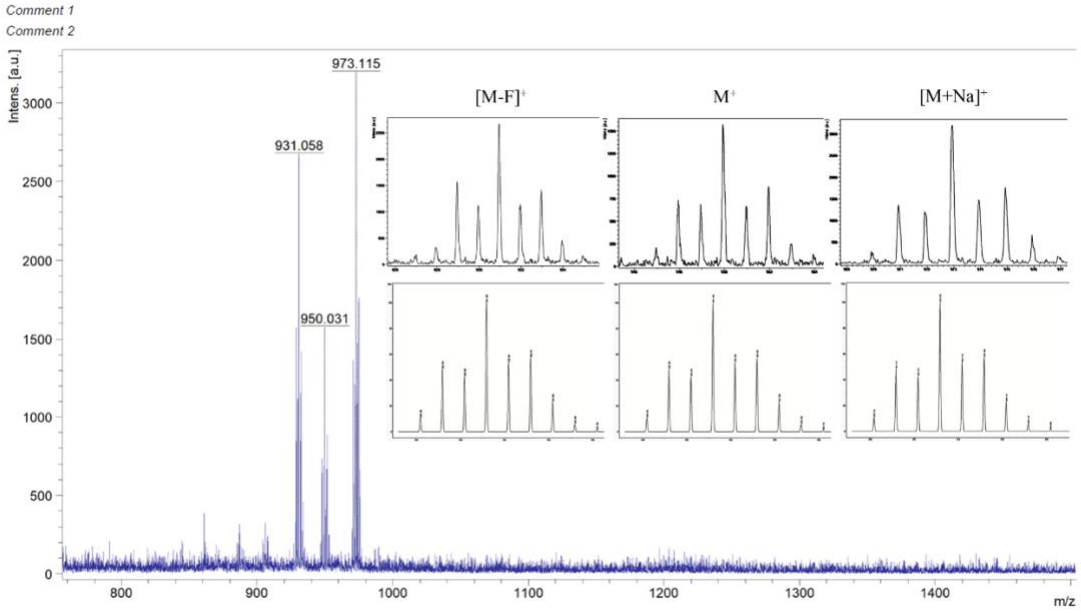

Fig. S4 MALDI-TOF mass spectrum of **1** (2 μM) after being treated with NQO1 (80 μg mL^{-1}) and NADPH (1 mM) in DMEM at 37 ℃ for 26 h. The insets show the experimental (upper row) and simulated (lower row) isotopic patterns of the $[M-F]^+$, M^+ and $[M+Na]^+$ ions of **2**.

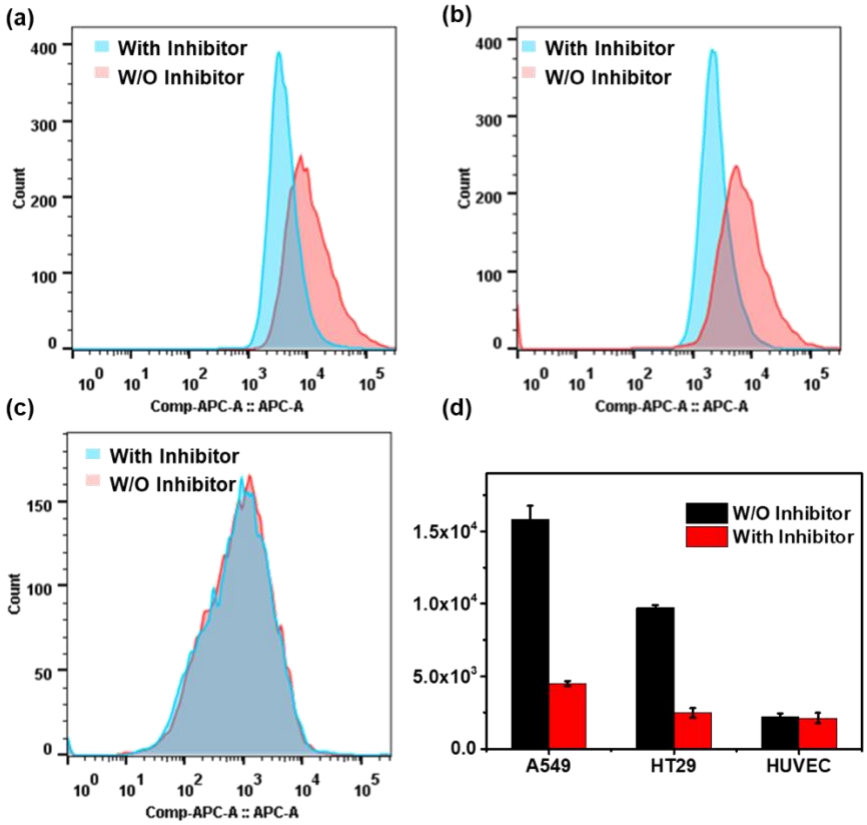

Fig. S5 Flow cytometric analysis for (a) A549 cells, (b) HT29 cells and (c) HUVECs after being incubated with **1** (4 μM) for 3 h with or without pre-incubation with dicoumarol (50 μM) for 1 h. Figure (d) shows the corresponding quantified fluorescence intensities. Data are reported as the mean ± standard deviation of three independent experiments.

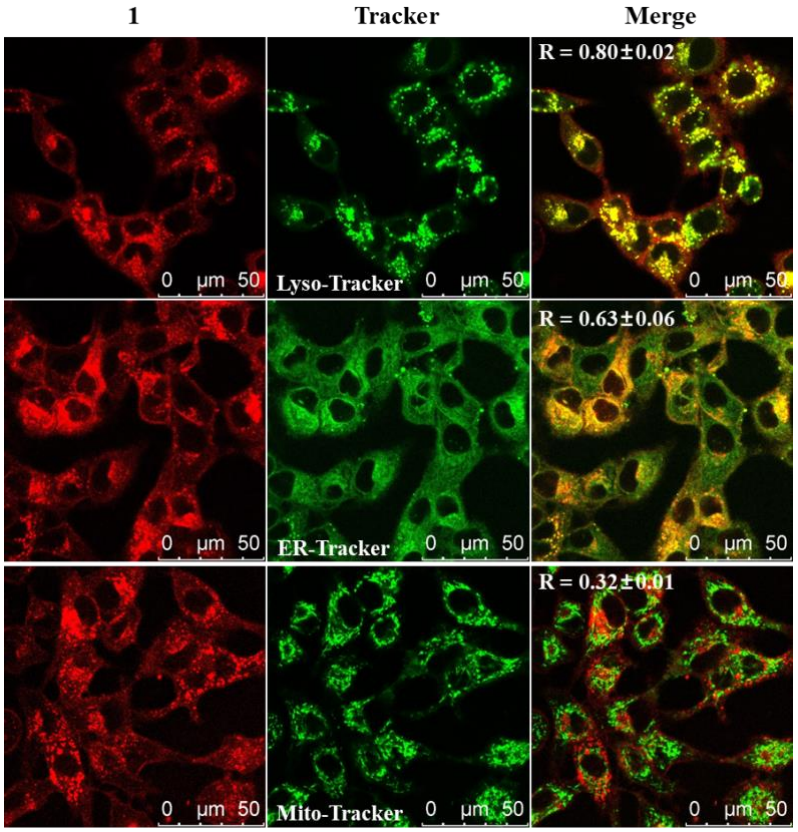

Fig. S6. Visualisation of the intracellular fluorescence of the activated form of compound **1** and various subcellular trackers, as well as their overlapped images. A549 cells were incubated with **1** (4 μM) for 3 h, and then with LysoTracker Green DND-26 (2 μM for 15 min), MitoTracker Green FM (0.2 μM for 15 min) or ER-Tracker green (0.25 μM for 30 min) at 37 °C. The Pearson's coefficients are given in the corresponding merged images.

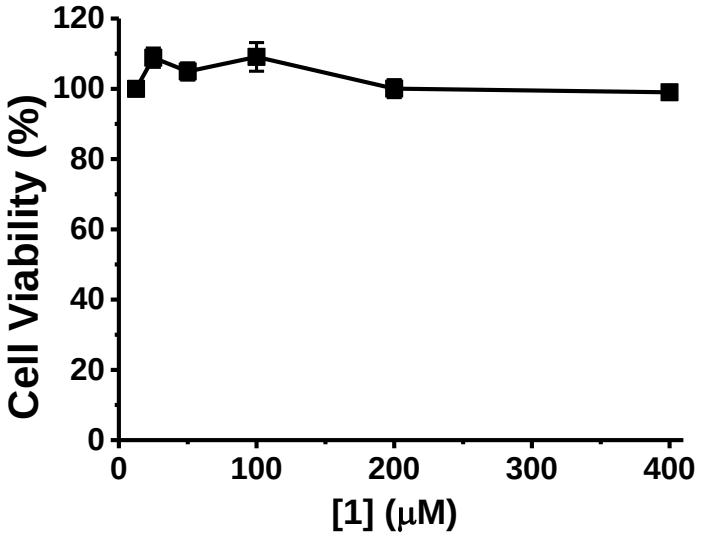

Fig. S7 Cytotoxicity of **1** against A549 cells. The cells were incubated with **1** for 3 h, followed by the dark treatment for 20 min.

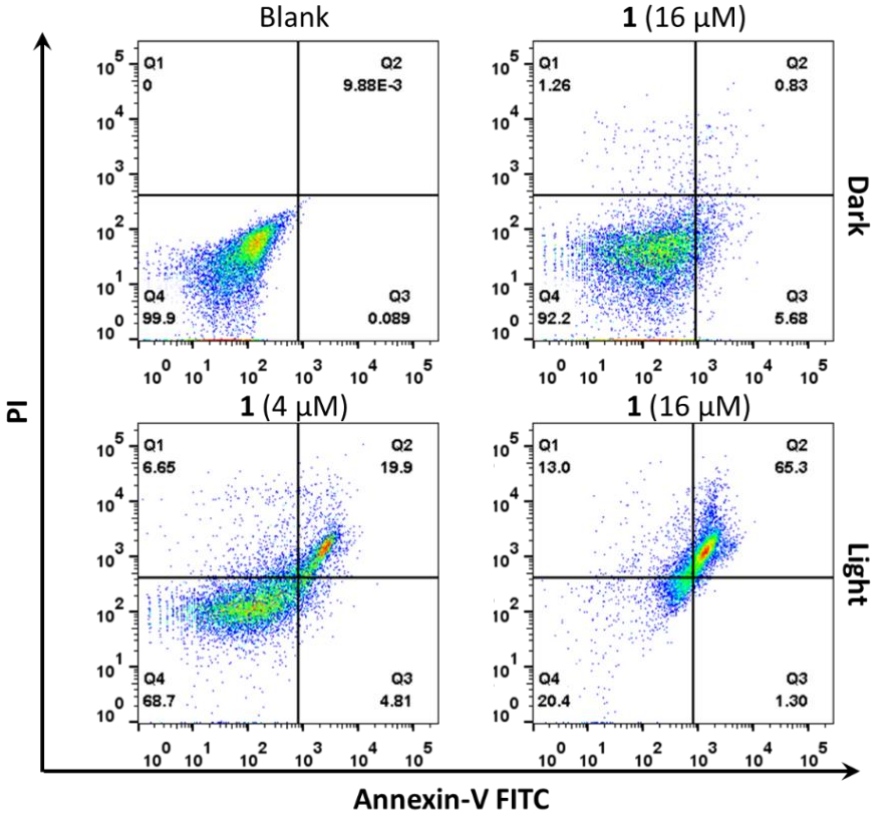

Fig. S8 Cell apoptotic analysis by Annexin V-FITC/propidium iodide (PI) assay for A549 cells after being incubated with **1** (4 or 16 μM) for 3 h. In the absence of light irradiation, there was a large population of viable cells located in the lower left quadrant, showing that **1** was essentially non-toxic in the dark. Upon light irradiation (λ > 610 nm, 23 mW cm^{-2}) for 20 min, a small population of cells undergoing apoptosis (19.9% and 4.8% in the upper and lower right quadrants, reflecting the late and early apoptotic stages respectively) when 4 μM of **1** was used. When 16 μM of **1** was used, the total apoptotic cells population was increased to 66.6%. Under all these conditions, the population of necrotic cells shown in the upper left quadrant was relatively small, suggesting that apoptosis was the major cell death mechanism for this compound.

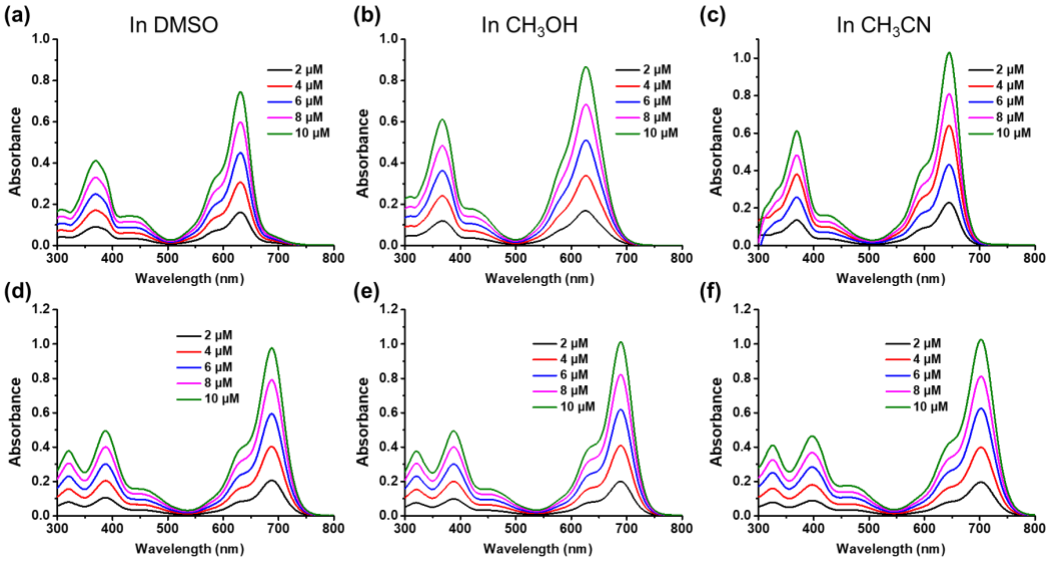

Fig. S9 Electronic absorption spectra of (a-c) **1** and (d-e) **2** in DMSO, CH_3OH and CH_3CN respectively at different concentrations.

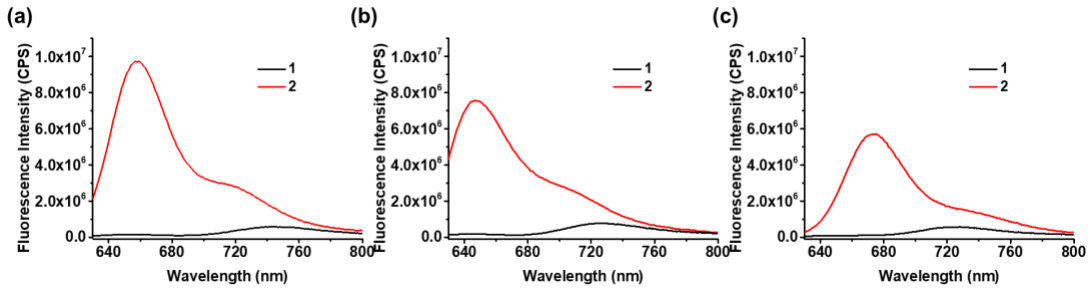

Fig. S10 Fluorescence spectra of **1** and **2** (both at 2 μM) in (a) DMSO, (b) CH_3OH and (c) CH_3CN.

Table S2. Electronic absorption and fluorescence data for **1** and **2** in different organic solvents.

		In DMSO	In CH_3OH	In CH_3CN
1	λ_{abs} (nm) (log ε)	372 (4.61), 594 (4.54), 632 (4.87)	367 (4.79), 586 (4.63), 627 (4.94)	370 (4.77), 598 (4.61), 645 (4.99)
	λ_{em}[a] (nm)	745	726	728
2	λ_{abs} (nm) (log ε)	327 (4.62), 400 (4.67), 657 (4.51), 703 (5.02)	321 (4.57), 390 (4.68), 622 (4.53), 688 (4.98)	321 (4.58), 389 (4.69), 620 (4.51), 690 (5.01)
	λ_{em}[a] (nm)	657	646	673

[a] Excited at 610 nm.

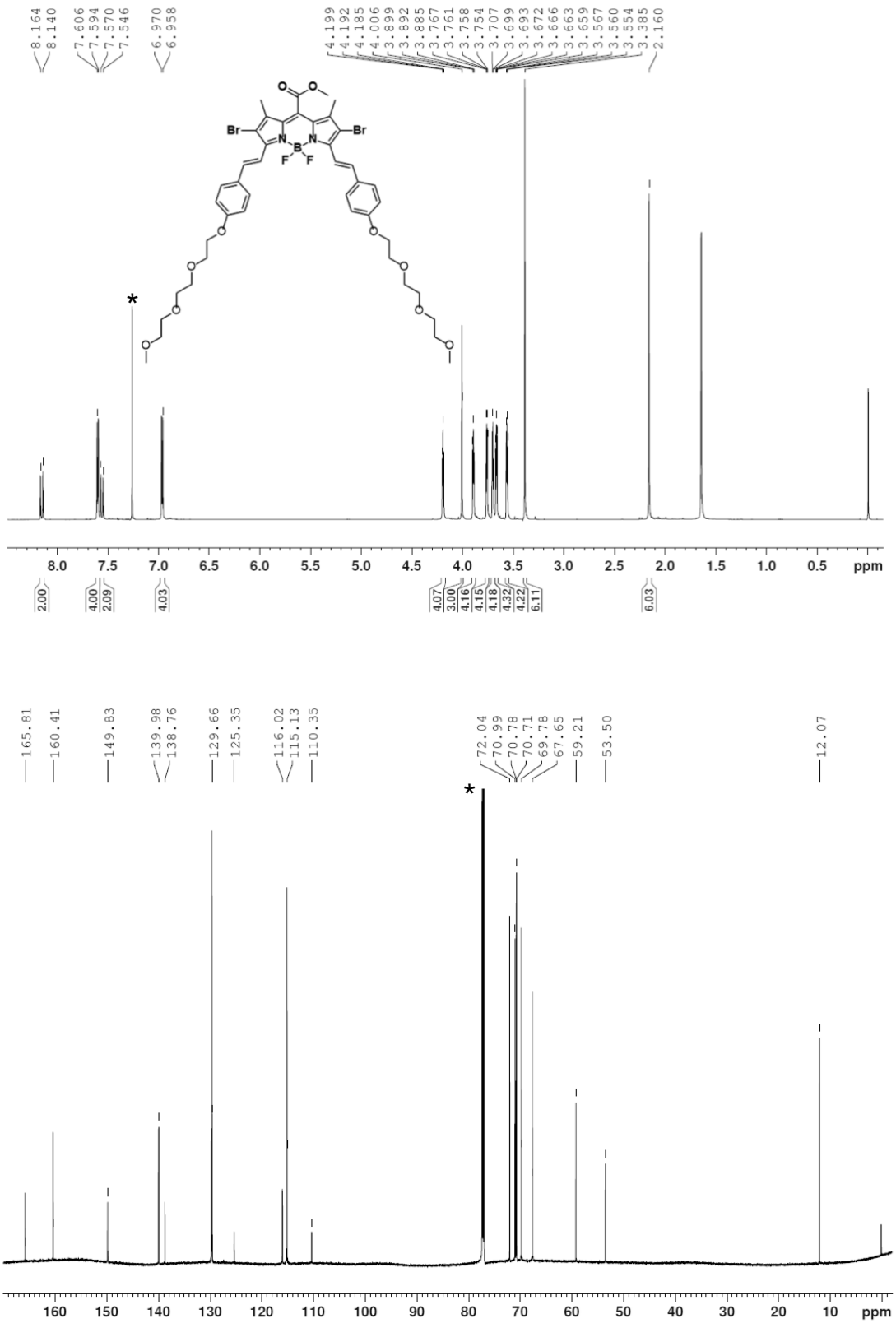

Fig. S11 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **5** in $CDCl_3$.

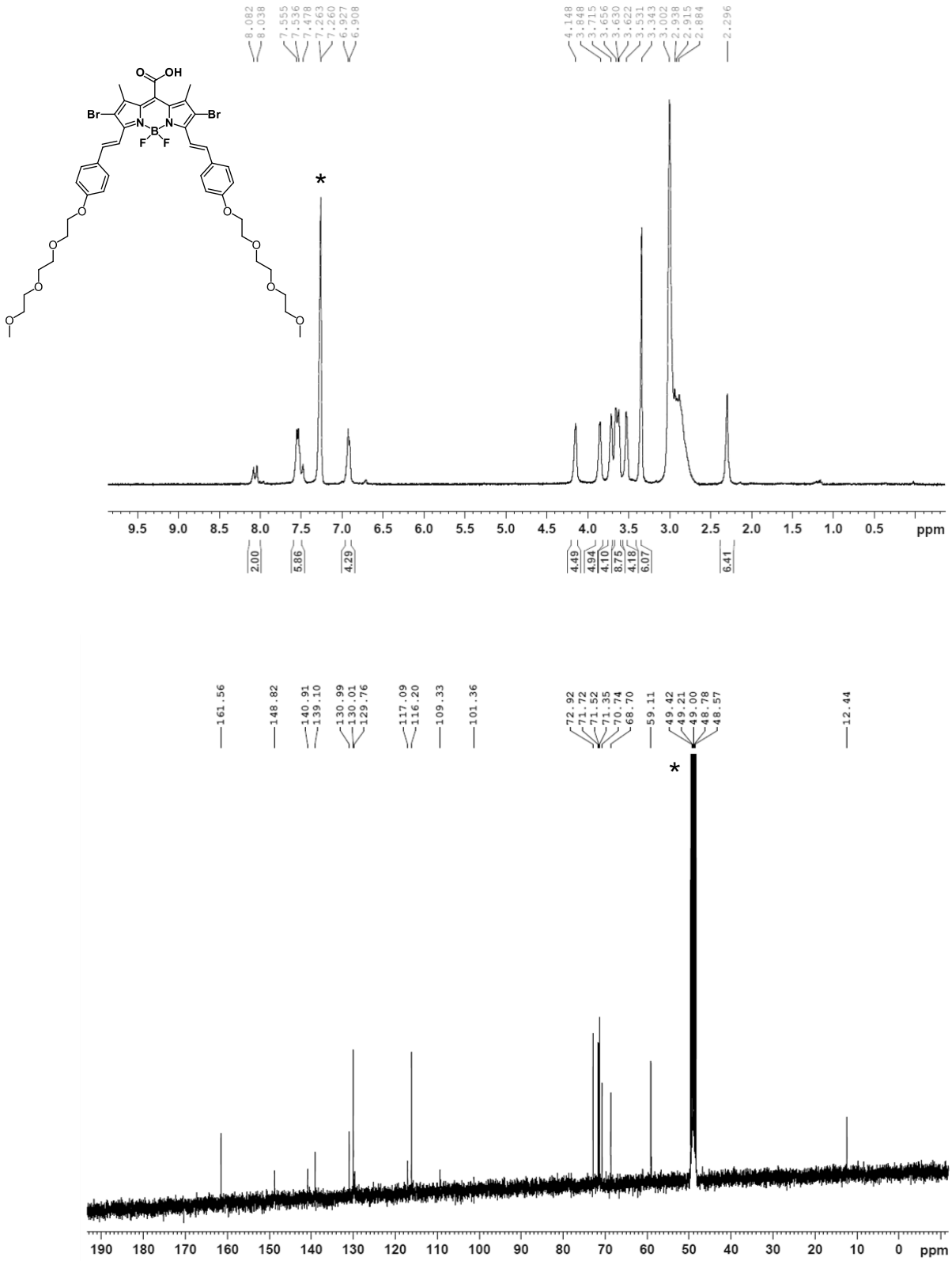

Fig. S12 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **2** in $CDCl_3$ and CD_3OD respectively.

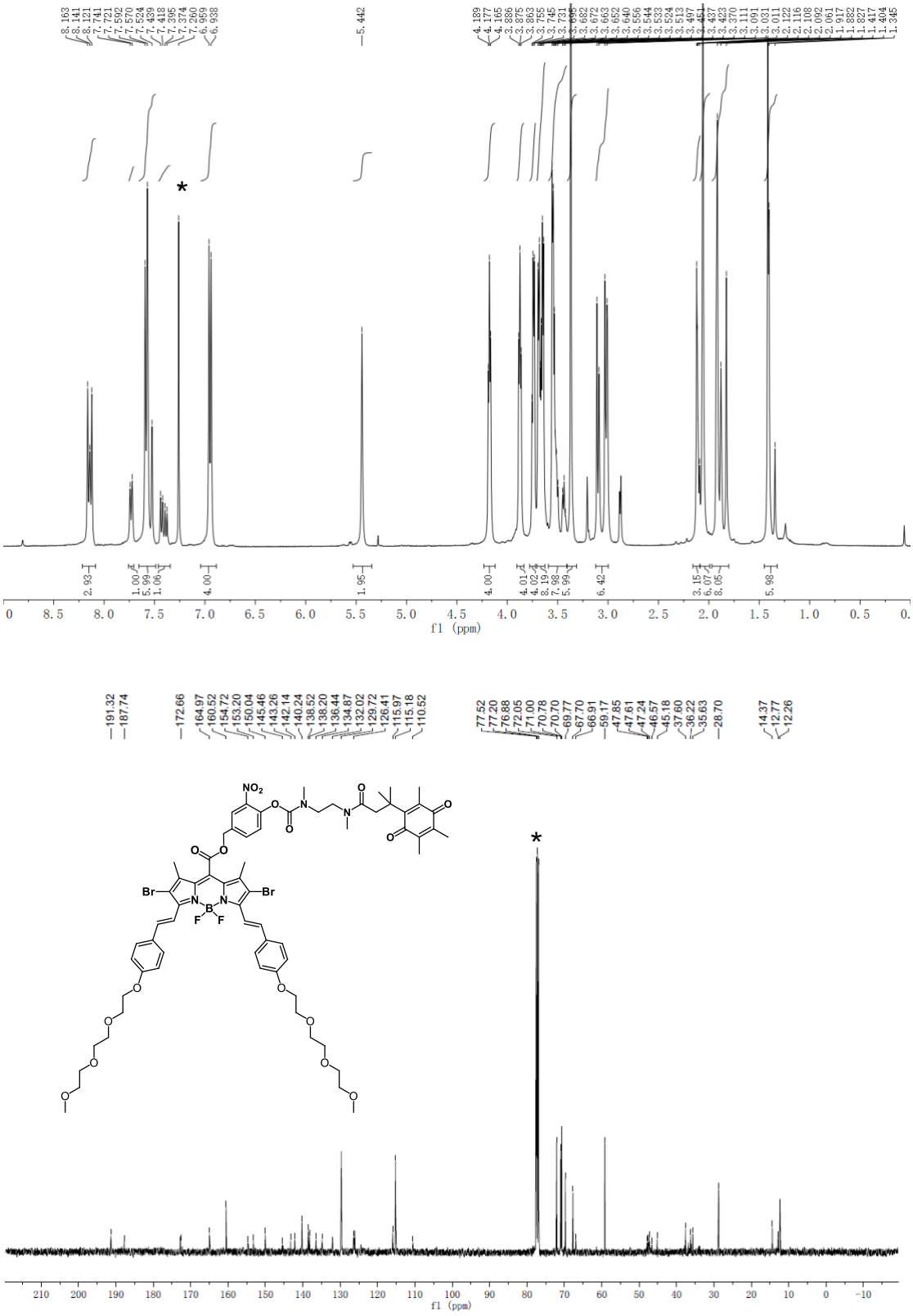

Fig. S13 ^{1}H (top) and $^{13}C\{^{1}H\}$ (bottom) NMR spectra of **1** in $CDCl_3$.

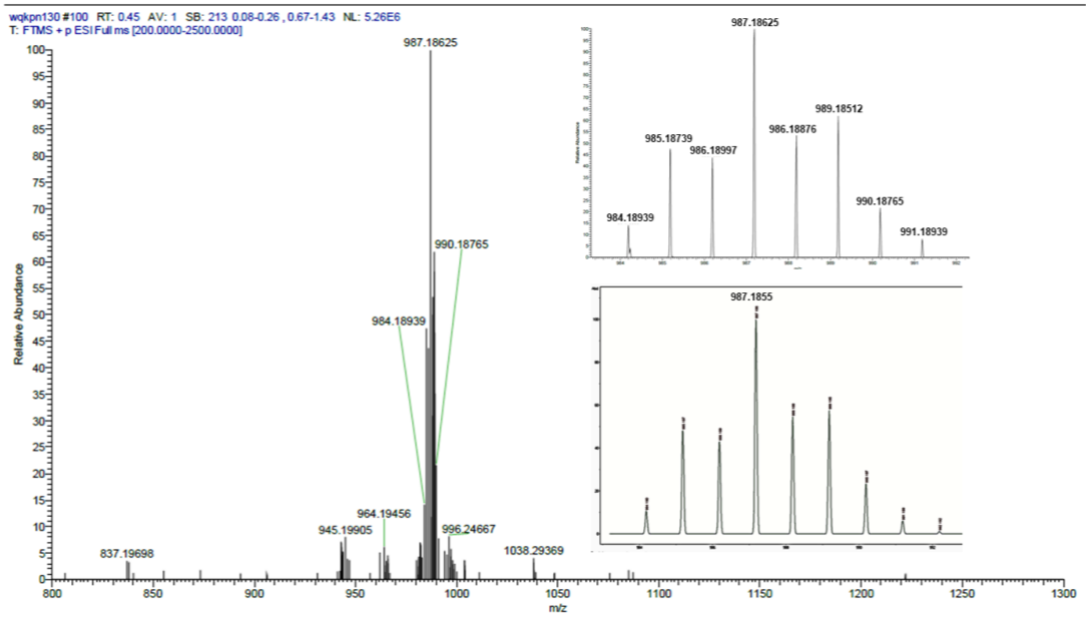

Fig. S14 ESI mass spectrum of **5**. The insets show the experimental (upper) and simulated (lower) isotopic patterns of the sodiated molecular ion $[M+Na]^+$.

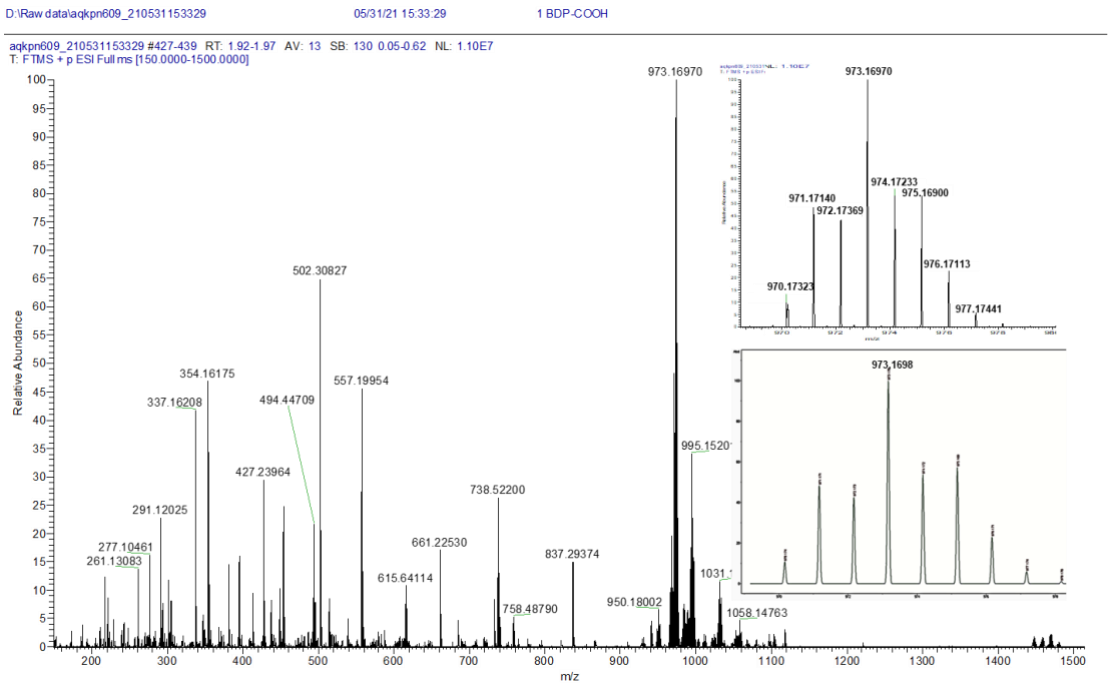

Fig. S15 ESI mass spectrum of **2**. The insets show the experimental (upper) and simulated (lower) isotopic patterns of the sodiated molecular ion $[M+Na]^+$.

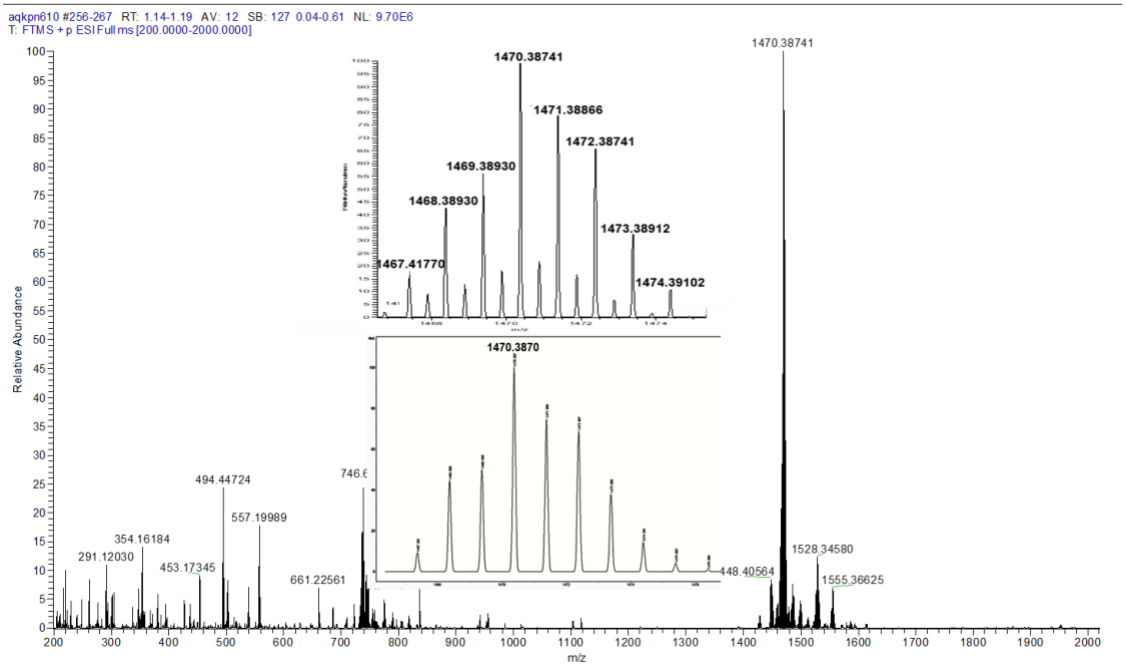

Fig. S16 ESI mass spectrum of **1**. The insets show the experimental (upper) and simulated (lower) isotopic patterns of the sodiated molecular ion $[M+Na]^+$.

Experimental section

General

All reactions were performed under an atmosphere of nitrogen. CH_2Cl_2, DMF and tetrahydrofuran (THF) were dried using an INERT solvent purification system. All other solvents and reagents were of reagent grade and used as received. Chromatographic purification was performed on silica gel (Macherey-Nagel 230-400 mesh) column with the indicated eluent. Size-exclusion chromatography was carried out on Bio-Beads S-X1 beads (200-400 mesh) with THF as the eluent. Compounds **3**,[R1] **4**[R2] and **6**[R3] were prepared as described.

^1H and $^{13}C\{^1H\}$ NMR spectra were recorded on a Bruker AVANCE III 700 spectrometer (^1H, 700 MHz; ^{13}C, 176.0 MHz) or on a Bruker AVANCE III 400 spectrometer (^1H, 400 MHz; ^{13}C, 100.6 MHz) in deuterated solvents. Spectra were referenced internally by using the residual solvent [^1H: $\delta = 7.26$ (for $CDCl_3$)] or solvent [^{13}C: $\delta = 77.2$ (for $CDCl_3$), $\delta = 49.0$ (for CD_3OD)] resonances relative to $SiMe_4$. Electrospray ionisation (ESI) mass spectra were recorded on a Thermo Finnigan MAT 95 XL mass spectrometer. MALDI-TOF mass spectra were recorded on a Bruker Daltonics Autoflex III spectrometer. Electronic absorption and steady-state fluorescence spectra were taken on a Shimadzu UV-1800 UV-Vis spectrophotometer and a Horiba FluoroMax spectrofluorometer respectively.

HPLC analysis was performed on an Apollo-C18 column (5 μm, 4.6 mm × 150 mm) at a flow rate of 1 mL min^{-1} at room temperature, using a Waters system equipped with a Waters 1525 binary pump with a Waters 2998 photodiode array detector. The

solvents used for HPLC analysis were of HPLC grade. The conditions were set as follows: solvent A = 0.1% trifluoroacetic acid (TFA) in acetonitrile and solvent B = 0.1% TFA in deionised water. The gradient was 30% A + 70% B in the first 5 min, and then changed to 100% A in 30 min and maintained under this condition for 10 min, and then changed back to 100% B in 5 min and kept at this condition for 10 min.

Preparation of 5

To a solution of benzaldehyde **4** (3.43 g, 12.8 mmol) in CH_3CN (100 mL), piperidine (1.2 mL) and acetic acid (1.2 mL) were added. The mixture was stirred at room temperature for 15 min before the addition of BODIPY **3** (0.70 g, 1.51 mmol) and dry molecular sieves. The resulting mixture was heated at 65 ^oC for 20-30 min. After filtration and removing the solvent under reduced pressure, the residue was chromatographed twice on a short silica gel column with EtOAc as the eluent. The target compound **5** was obtained as a purple solid (0.90 g, 62%). ^1H NMR (700 MHz, $CDCl_3$): δ 8.15 (d, *J* = 16.8 Hz, 2 H, C=CH), 7.60 (d, *J* = 8.4 Hz, 4 H, ArH), 7.56 (d, *J* = 16.8 Hz, 2 H, C=CH), 6.96 (d, *J* = 8.4 Hz, 4 H, ArH), 4.19 (t, *J* = 4.9 Hz, 4 H, OCH_2), 4.01 (s, 3 H, OCH_3), 3.89 (t, *J* = 4.9 Hz, 4 H, OCH_2), 3.75-3.77 (m, 4 H, OCH_2), 3.70 (t, *J* = 4.9 Hz, 4 H, OCH_2), 3.66-3.67 (m, 4 H, OCH_2), 3.56 (t, *J* = 4.9 Hz, 4 H, OCH_2), 3.39 (s, 6 H, OCH_3), 2.16 (s, 6 H, CH_3). $^{13}C\{^1H\}$ NMR (176.0 MHz, $CDCl_3$): δ 165.8, 160.4, 149.8, 140.0, 138.8, 129.7, 125.4, 116.0, 115.1, 110.4, 72.0, 71.0, 70.8, 70.7, 69.8, 67.6, 59.2, 53.5, 12.1. HRMS (ESI): *m/z* calcd for $C_{43}H_{51}BBr_2F_2N_2NaO_{10}$ $[M+Na]^+$, 987.1855, found 987.1862.

Preparation of 2

To a solution of **5** (0.15 g, 0.15 mmol) in EtOAc (10 mL), lithium iodide (0.35 g, 2.6 mmol) was added. The mixture was stirred under reflux overnight. After cooling to room temperature, EtOAc (50 mL) was added, and the resulting mixture was washed with 0.1 N HCl (10 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was chromatographed on silica gel with EtOAc as the eluent, affording **2** as a brown powder (0.11 g, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (d, *J* = 17.6 Hz, 2 H, C=CH), 7.48-7.56 (m, 6 H, ArH and C=CH), 6.92 (d, *J* = 7.6 Hz, 4 H, ArH), 4.15 (virtual s, 4 H, OCH_2), 3.85 (virtual s, 4 H, OCH_2), 3,72 (virtual s, 4 H, OCH_2), 3.62-3.66 (m, 8 H, OCH_2), 3.53 (virtual s, 4 H, OCH_2), 3.34 (s, 6 H, OCH_3), 2.30 (s, 6 H, CH_3). ^{13}C{^{1}H} NMR (100.6 MHz, CD_3OD): δ 161.6, 148.8, 140.9, 139.1, 131.0, 130.0, 129.8, 117.1, 116.2, 109.3, 101.4, 72.9, 71.7, 71.5, 71.4, 70.7, 68.7, 59.1, 12.4. HRMS (ESI): *m/z* calcd for $C_{42}H_{49}BBr_2F_2N_2NaO_{10}$ $[M+Na]^+$, 973.1698, found 973.1697.

Preparation of 8

Compound **6** (126 mg, 0.3 mmol) was dissolved in TFA (5 mL) and CH_2Cl_2 (5 mL). The mixture was stirred at room temperature for 2 h. After that, the solvent was evaporated under reduced pressure. The crude product was re-dissolved in CH_2Cl_2 (20 mL), and this solution was then added in dropwise to a solution of bis(trichloromethyl) carbonate (BTC) (61 mg, 0.21 mmol) and $NaHCO_3$ (52 mg, 0.62 mmol) in CH_2Cl_2 (10

mL) at 0 °C. The resulting mixture was stirred at room temperature for 2 h. 4-Hydroxy-3-nitrobenzyl alcohol (**7**) (32 mg, 0.19 mmol) and *N,N*-diisopropylethylamine (DIPEA) (0.5 mL) were added into this mixture, and stirring was continued at room temperature overnight. The mixture was then mixed with water (20 mL), and the aqueous phase was extracted with CH_2Cl_2 (20 mL × 2). The combined organic portions were dried over anhydrous $MgSO_4$ and then evaporated *in vacuo*. The crude product was dissolved in EtOAc. The solution was then filtered through a short pad of silica gel to yield a yellow product after evaporation. The crude product was used directly in the next step without further purification.

Preparation of 1

To a mixture of **2** (86 mg, 0.09 mmol) and **8** (185 mg, 0.36 mmol) in THF (5 mL) at 0 °C was added another mixture of diethyl azodicarboxylate (DEAD) (62 mg, 0.36 mmol) and triphenylphosphine (PPh_3) (95 mg, 0.36 mmol) in THF (2 mL). The resulting mixture was stirred at room temperature for 24 h and then neutralised with 5% $NaHCO_3$ (aq). The aqueous layer was extracted with EtOAc (30 mL x 3). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel column using CH_2Cl_2 as the eluent, followed by size-exclusion chromatography using THF as the eluent, giving **1** as a blue solid (29 mg, 22%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 (d, J = 16.8 Hz, 2 H, C=CH), 8.14 (s, 1 H, ArH), 7.73 (d, J = 8.0 Hz, 1 H, ArH), 7.52-7.59 (m, 6 H, C=CH and ArH), 7.43 (d, J = 8.4 Hz) and 7.38 (d, J = 8.4 Hz) with

a total integration of 1 H for ArH, 6.95 (d, J = 8.4 Hz, 4 H, ArH), 5.44 (s, 2 H, $ArCH_2$), 4.18 (t, J = 4.8 Hz, 4 H, OCH_2), 3.88 (t, J = 4.8 Hz, 4 H, OCH_2), 3.73-3.76 (m, 4 H, OCH_2), 3.64-3.70 (m, 8 H, OCH_2), 3.42-3.56 (m, 8 H, OCH_2 and NCH_2), 3.37 (s, 6 H, OCH_3), 3.01-3.11 (m, 6 H, NCH_3), 2.11-2.12 (m, 3 H, CH_3), 2.06 (s, 6 H, pyrrole-CH_3), 1.83-1.92 (m, 8 H, CH_2 and CH_3), 1.35-1.42 (m, 6 H, CH_3). Some of the signals are split due to the presence of invertomers arising from the restricted nitrogen inversion. $^{13}C\{^1H\}$ NMR (100.6 MHz, $CDCl_3$): δ 191.3, 187.7, 172.7, 165.0, 160.5, 154.7, 153.2, 150.0, 145.5, 143.3, 142.1, 140.2, 138.5, 138.2, 136.4, 134.9, 132.0, 129.7, 126.4, 116.0, 115.2, 110.5, 72.1, 71.0, 70.8, 70.7, 69.8, 67.7, 66.9, 59.2, 47.9, 47.6, 47.2, 46.6, 45.2, 37.6, 36.2, 35.6, 28.7, 14.4, 12.8, 12.3. HRMS (ESI): *m/z* calcd. for $C_{68}H_{80}BBr_2F_2N_5NaO_{17}$ $[M+Na]^+$, 1470.3870, found 1470.3874. The purity of **1** was found to be >95% by HPLC analysis.

Determination of fluorescence quantum yields

The fluorescence quantum yields (Φ_f) were determined by the equation:[R4]

$$\Phi_{f(s)} = (F_s/F_{ref})(A_{ref}/A_s)(n_s^2/n_{ref}^2)\Phi_{f(ref)}$$

where the subscript s stands for the sample solution and ref refers to the reference. F, A and n are the measured fluorescence (area under the emission peak), the absorbance at the excitation position and the refractive index of the solvent respectively. ZnPc in DMF [$\Phi_{f(ref)}$ = 0.28][R5] was used as the reference. To minimise reabsorption of radiation by the ground-state species, the fluorescence spectra were obtained in very dilute solutions of which the absorbance at the excitation wavelength was less than 0.05.

Study of the singlet oxygen generation efficiency

The singlet oxygen generation efficiency of **1** and **2** was measured in PBS at pH 7.4 with 0.5% (v/v) DMF using DPBF as the singlet oxygen probe.[R5] A mixture of the sample (2 μM) and DPBF (30 μM) was irradiated with red light from a 300 W halogen lamp after passing through a water tank for cooling and a colour filter with a cut-on wavelength at 610 nm (Newport). The absorption maximum of DPBF at 417 nm was monitored along with the irradiation time.

Activation of 1 by NQO1

The activation of **1** by HQO1 was performed in a 1 cm × 1 cm quartz cuvette. A stock solution of **1** was prepared in DMF (2 mM), which was diluted to 2 μM with DMEM containing 1 mM of NADPH. Another stock solution of NQO1 in H_2O (1 mg mL^{-1}) was also prepared, which was then added to the above DMEM solution of **1** to make the final concentration of NQO1 to 80 μg mL^{-1}. The fluorescence spectra were recorded in the range of 640−800 nm at different time points upon excitation at 610 nm. The restoration in singlet oxygen generation was also studied spectroscopically as described above.

Cell lines and culture conditions

A549 human lung carcinoma cells (ATCC®CCL-185™) was maintained in DMEM (ThermoFisher Scientific, cat. no. 12100-046) supplemented with fetal bovine serum

(FBS) (ThermoFisher Scientific, cat. no. 10270-106) (10%) and a penicillin-streptomycin solution (100 units mL^{-1} and 100 μg mL^{-1} respectively). HT29 human colorectal adenocarcinoma cells (ATCC® HTB-38™) were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium (Invitrogen, no. 23400-021) supplemented with FBS (10%) and a penicillin-streptomycin solution (100 units mL^{-1} and 100 mg mL^{-1} respectively). HUVECs (ATCC®PCS-100-013) were maintained in Endothelial Cell Growth Medium (Sigma, cat. no. 211-500). All the cells were grown at 37 °C in a humidified 5% CO_2 atmosphere.

Confocal microscopic study

Approximately 2 × 10^5 A549 and HT29 cells and HUVECs in 2 mL of the culture medium were incubated on confocal dishes of 35 mm diameter overnight at 37 °C with 5% CO_2. The medium was then removed. The cells, after being rinsed with PBS, were incubated with **1** (4 μM) for 3 h. For the control group, the cells were first treated with the NQO1 inhibitor dicoumarol (50 μM) for 1 h, and then incubated with **1** (4 μM) for 3 h. After being washed with PBS twice, the cells were imaged using a Leica TCS SP8 high-speed confocal microscope equipped with a 638 nm argon laser. The BODIPY was excited at 638 nm and its fluorescence was monitored at 650−800 nm. The images were digitised and analysed using a Leica Application Suite X software.

Flow cytometric study

Approximately 2 × 10^5 A549 and HT29 cells and HUVECs in 2 mL of the culture

medium were seeded on dishes of 35 mm diameter and incubated overnight at 37 °C with 5% CO_2. After removal of the medium, the cells were treated as described above. After removing the medium and rinsing the cells with PBS for three times, the cells were harvested by 0.25% trypsin-ethylenediaminetetraacetic acid (0.4 mL). The activity of the trypsin was quenched with the cell culture medium (0.5 mL), and the mixture was centrifuged at 1500 rpm for 3 min. The pellet was washed with PBS (1 mL) and then centrifuged. The cells were suspended in Hank's Balanced Salt Solution (HBSS) (1 mL) and then subject to flow cytometric analysis using a BD FACSVerse flow cytometer (Becton Dickinson) with 10^4 cells counted in each sample. Cell fragments were excluded with the forward and side-scatter gating to ensure that all detected signals were originated from the relatively intact cells. Signals from **1** were recorded in Chanel APC-A. All experiments were performed in triplicate.

Study of subcellular localisation

Approximately 2×10^5 A549 cells on glass-bottom dishes of 35 mm diameter were incubated with **1** (4 μM) for 3 h. After being rinsed with PBS twice, the cells were stained with LysoTracker Green DND-26 (Thermo Fisher Scientific Inc., L7526) (2 μM for 15 min), MitoTracker Green FM (Thermo Fisher Scientific Inc., M7514) (0.2 μM for 15 min) or ER-Tracker Green (Thermo Fisher Scientific Inc., E34251) (0.25 μM for 30 min). The cells were then rinsed with PBS twice and refed with HBSS and examined using a confocal microscope (a Leica TCS SP8 high-speed imaging system with a CO_2 incubator) with a 488 nm argon laser and a solid-state 638 nm laser. All the

trackers were excited at 488 nm, and their fluorescence was monitored at 500–570 nm. The BODIPY was excited at 638 nm and its fluorescence was monitored at 650−800 nm.

Study of the intracellular ROS generation

Approximately 2×10^5 A549 and HT29 cells and HUVECs on glass-bottom dishes of 35 mm diameter were first treated with dicoumarol (50 μM) or the neat cell culture medium for 1 h, After being rinsed with PBS for three times, the cells were further incubated with **1** (4 μM) for 3 h. The medium was then removed, and the cells were rinsed with PBS twice. After that, the cells were further stained with H_2DCFDA (50 μM) for 30 min, followed by washing with PBS for three times. Finally, the cells were incubated in the dark or irradiated with the above light source ($\lambda > 610$ nm, 23 mW cm^{-2}) at ambient temperature for 20 min. The fluorescence of DCF in these cells was imaged immediately using confocal fluorescence microscopy. The DCF was excited at 488 nm, and the fluorescence was monitored at 515−580 nm.

Study of the photocytotoxicity

Approximately 1×10^4 A549 and HT29 cells and HUVECs per well in the culture medium were inoculated in 96-well plates and incubated overnight at 37 ^oC in a humidified 5% CO_2 atmosphere. The cells were first incubated with dicoumarol (50 μM) or the neat culture medium for 1 h. After being rinsed with PBS for three times, the cells were further incubated with **1** at different concentrations (0−16 μM) for 3 h.

After washing with PBS for three times, the cells were refed with 100 μL of the culture medium before being illuminated with the above light source (λ > 610 nm, 23 mW cm^{-2}) at ambient temperature for 20 min, giving a total fluence of 28 J cm^{-2}. Cell viability was determined by means of a colourimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[R6] After illumination, the cells were incubated at 37 °C under 5% CO_2 for 16 h. An MTT (Sigma) solution in PBS (3 mg mL^{-1}, 50 μL) was added to each well followed by incubation for 4 h under the same environment. After that, 70 μL of DMSO was added to each well. Solutions in all the wells were mixed until homogenous. The absorbance at 490 nm was measured using a plate reader (Tecan Spark 10M Microplate Reader). The average absorbance of the blank wells, which did not contain the cells, was subtracted from the readings of the other wells. The cell viability was then determined by the equation: %Viability = $[(\sum A_i/A_{control} \times 100)]/n$, where A_i is the absorbance of the i^{th} datum (i = 1, 2…n), $A_{control}$ is the average absorbance of the control wells, in which the photosensitiser was absent, and n (= 4) is the number of data points.

Annexin V-FITC/PI assay

A549 cells were seeded on 6-well plates and incubated in DMEM supplemented with 10% FBS for overnight. The medium was then removed and compound **1** (4 or 16 μM) in fresh medium was added. After incubation for 3 h, the medium was removed, followed by the dark or light (λ > 610 nm, 23 mW cm^{-2}) treatment for 20 min. After incubation for further 12 h, the cells were collected and washed with PBS. Finally, the

cells were stained with Annexin V-FITC and PI for 15 min before being subjected to flow cytometric analysis. Signals from Annexin V-FITC and PI were collected in the Chanel FITC and Chanel PerCP-Cy5.5 respectively.

Study of the *in vivo* activation

All experiments involving live animals were performed in strict accordance with the animal experimentation guidelines and were approved by the Animal Experimentation Ethics Committee of The Chinese University of Hong Kong (CUHK) (ref. no. 21-005-GRF). Licence to conduct animal experiments was obtained from the Department of Health, Government of the Hong Kong Special Administrative Region. Female Balb/c nude mice (20−25 g) were obtained from the Laboratory Animal Services Centre of The Chinese University of Hong Kong. The mice were kept under a pathogen-free condition with free access to food and water. HT29 cells (about 1×10^7 cells in 200 μL HBSS) were inoculated subcutaneously on the back of the mice. Once the tumour had grown to a size of *ca.* 100 mm^3, the mice were injected intratumourally with a solution of **1** (8 nmol) in water with 5% (v/v) DMSO (40 μL). For comparison, another group of mice were pre-treated with a solution of dicoumarol (80 nmol) in water with 5% (v/v) DMSO (40 μL) one hour before the injection of **1**. The fluorescence images of the mice were then captured at different time points up to 48 h using an Odyssey infrared imaging system (excitation wavelength = 680 nm, emission wavelength ≥ 700 nm). The images were digitised and analysed using the Odyssey imaging system software (9201-500). Three mice were used for each condition.

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