Electronic Supplementary Material (ESI) for ChemComm.


# Electronic Supplementary Information

## *"Off-on" switching of Intracellular Singlet Oxygen Release under Biocompatible Conditions*

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**Contents**

## 1. General

**Materials:** All reagents and solvents were purchased from commercial suppliers and used without further purification. Reactions were monitored by thin layer chromatography using Merck TLC Silica gel 60 $F_{254}$. Column chromatography was performed by using Merck Silica Gel 60 (particle size: 0.040-0.063 mm, 230-400 mesh ASTM).

**Instruments:** The $^{1}H$ and $^{13}C$ NMR spectra were recorded using Bruker DPX-400 (operating at 400 MHz for $^{1}H$ NMR and 100 MHz for $^{13}C$ NMR) at 298 K using deuterated solvents with tetramethylsilane (TMS) as internal standard. Chemical shifts were reported in parts per million (ppm) and coupling constants (*J* values) are given in Hz. Splitting patterns are indicated as follow s, singlet; d, doublet; t, triplet; m, multiplet. The UV-Vis absorption spectra were performed by using Varian Cary-100 Bio UV-Vis spectrophotometer. Mass spectra were recorded with Agilent Technologies 6224 TOF LC/MS.

## 2. Synthetic Procedures and Characterization

(Please note that the numbering of the compounds is different in SI.)

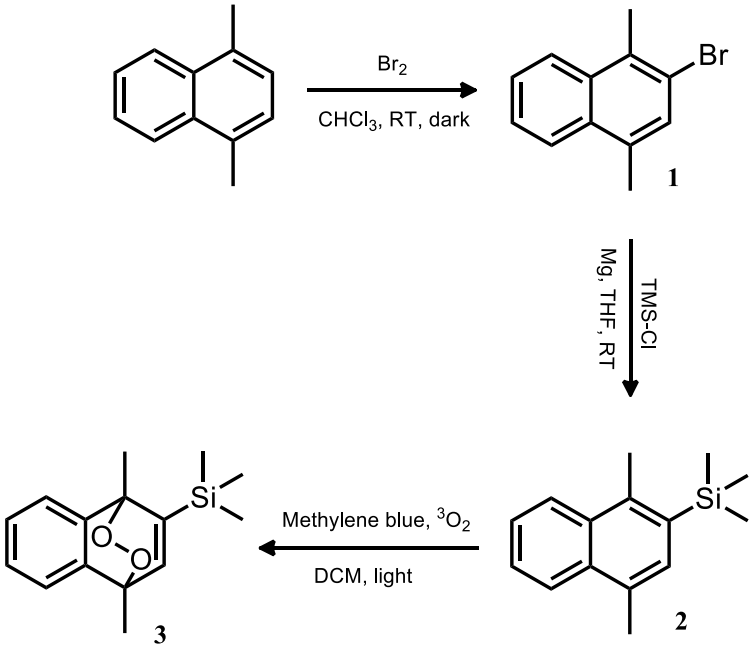
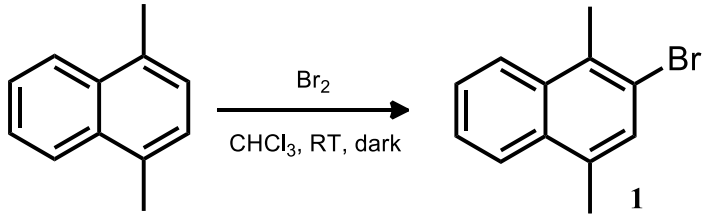


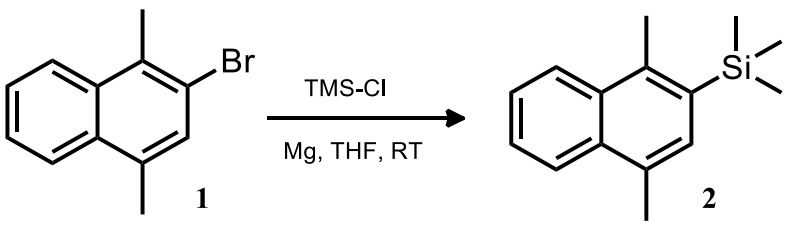
**Figure S1.** Synthetic pathway for 1,4-Dimethyl-2-TMS-naphthalene-endoperoxide.

***Synthesis of Compound 1*:**[1]



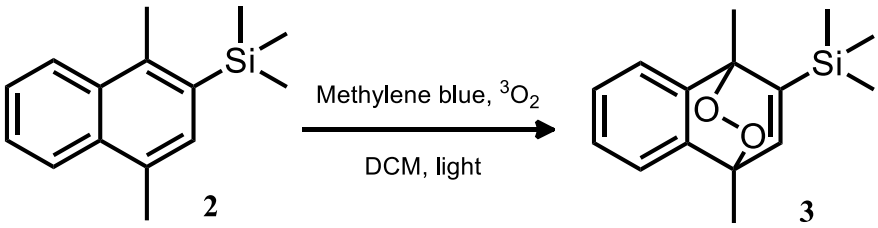

1,4-Dimethylnaphthalene (1.56 g, 10.0 mmol) was dissolved in 15 mL chloroform under Argon atmosphere and exclusion of light. Bromine solution (0.54 mL, 10.5 mmol) was added to the reaction mixture over 10 minutes period of time at 0 °C, in an ice bath. Reaction was allowed to warm at room temperature for 2 hours while it was stirring. Reaction was monitored by TLC. Then, the reaction mixture was diluted with 20 mL chloroform and washed with 25 mL saturated $Na_2S_2O_3$ solution, 25 mL water, and 25 mL saturated NaCl solution, accordingly. Organic layer was combined and dried over anhydrous $Na_2SO_4$. The solution was filtered through a thin pad of silica gel. After removal of the solvent by rotary evaporator, the crude product was purified by silica gel column chromatography with Hexane as the eluent. The product was obtained in colorless oil form with 93% yield (2.18 g, 9.3 mmol). $^1$H NMR (400 MHz, $CDCl_3$): δ 8.08-8.06 (m, 1H), 8.00-7.98 (m, 1H), 7.57 (t, $J$ = 4 Hz, 2H), 7.51 (s, 1H), 2.80 (s, 3H), 2.66 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 133.94, 133.53, 131.77, 131.27, 130.5, 126.4, 125.5, 124.97, 124.81, 122.4, 18.97, 18.65.

***Synthesis of Compound* 2:**



First of all, Mg (0.15 g, 6.2 mmol) was activated with catalytic amount of iodine in 5 mL of THF for 15 minutes with vigorous stirring. Including the first step, all reaction conditions were carried out under $CaCl_2$ tube. Compound **1** (0.60 g, 2.6 mmol) was added to the reaction mixture in ice-bath. Followingly, TMSCl (0.33 g, 3.1 mmol) was dissolved in 3 mL THF and was added to the reaction mixture dropwise. The reaction mixture was allowed to stir at room temperature for 24 hours. Then, the mixture was extracted with water and DCM for three times. After removal of the solvent by rotary evaporator, the crude product was purified by silica gel column chromatography with Hexane: EtOAc (95:5, v/v) as the eluent. The product was obtained in colorless oil form with 48% yield (0.29 g, 1.25 mmol). $^1$H NMR (400 MHz, DMSO): δ 8.11-8.08 (m, 1H), 8.01-7.98 (m, 1H), 7.57 (t, *J* = 4 Hz, 2H), 7.35 (s, 1H), 2.74 (s, 3H), 2.61 (s, 3H), 0.38 (s, 9H). $^{13}$C NMR (100 MHz, $CDCl_3$): δ 138.8, 133.0, 132.5, 131.4, 130.9, 126.47, 126.06, 124.91, 124.84, 19.65, 19.58, 0.9. MS (TOF-ESI) m/z calcd for $C_{15}H_{20}Si$: 227.1262 $[M-H]^-$, found: 227.1234 $[M-H]^-$, Δ= 12.02 ppm.

***Synthesis of Compound 3*:**




Compound **2** (0.15 g, 0.66 mmol) was dissolved in 20 mL DCM. The reaction mixture was cooled to – 78 °C. Methylene blue (0.02 g, 0.07 mmol) was added into the solution and mixture was stirred for 10 hours under oxygen atmosphere. During the reaction, water-cooled 400 W Hg arc lamp (white light irradiation) was used. After removal of the solvent by rotary evaporator, the crude product was purified by silica gel column chromatography with Hexane: EtOAc (95:5, v/v) as the eluent. The product was obtained in colorless oil form with 51%

yield (0.09 g, 0.34 mmol). $^1$H NMR (400 MHz, DMSO): δ 7.38-7.34 (m, 2H), 7.27-7.25 (m, 2H), 6.94 (s, 1H), 1.86 (s, 3H), 1.77 (s, 3H), 0.15 (s, 9H). MS (TOF-ESI) m/z calcd for $C_{15}H_{20}O_2Si$: 261.1305 $[M+H]^+$, found: 261.1296 $[M+H]^+$, Δ= 3.46 ppm.

### 3. Reaction rate experiments

With the intention of getting more quantitative results, the NMR investigations of two systems were performed as a function of time, the solvent being DMSO-$d_6$. It was observed that the TBAF introduced endoperoxide had a half-life of **5.0** hours, while the endoperoxide itself had a half-life of **800** hours according to appearance/disapearance of their normalized integral values of the selected peaks. The rate constant and half-life calculations were done in accordance to the first-order reaction rate equations. The equations are given below:

$$\ln[A] = -kt + \ln[A]_0 \quad , \quad t_{1/2} = 0.693/k$$

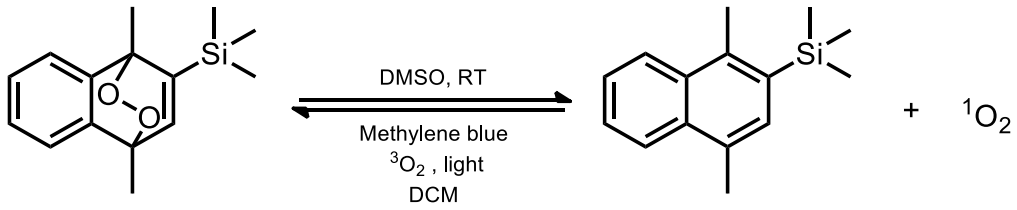



**Figure S2.** Reversible reaction of parent naphthalene-endoperoxide.

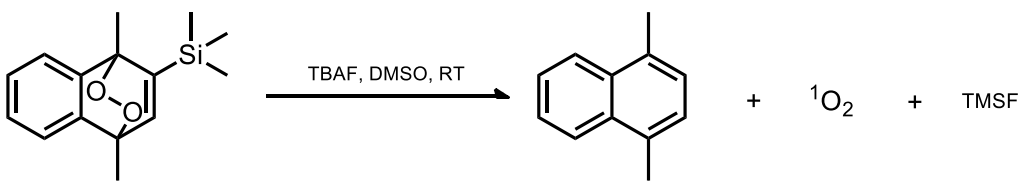



**Figure S3.** Irreversible reaction of TBAF and TMS-naphthalene-endoperoxide.

In the following NMR spectra, it is possible to observe the evolution of peaks (8.11-8.08, 8.01-7.98, 7.57, and 7.35 ppm) which belongs to Compound **2** due to endoperoxide cycloreversion. While the peaks of parent compound increases, the peaks of endoperoxide (Compound **3**) (7.38-7.34, 7.27-7.25, and 6.94 ppm) decreases.

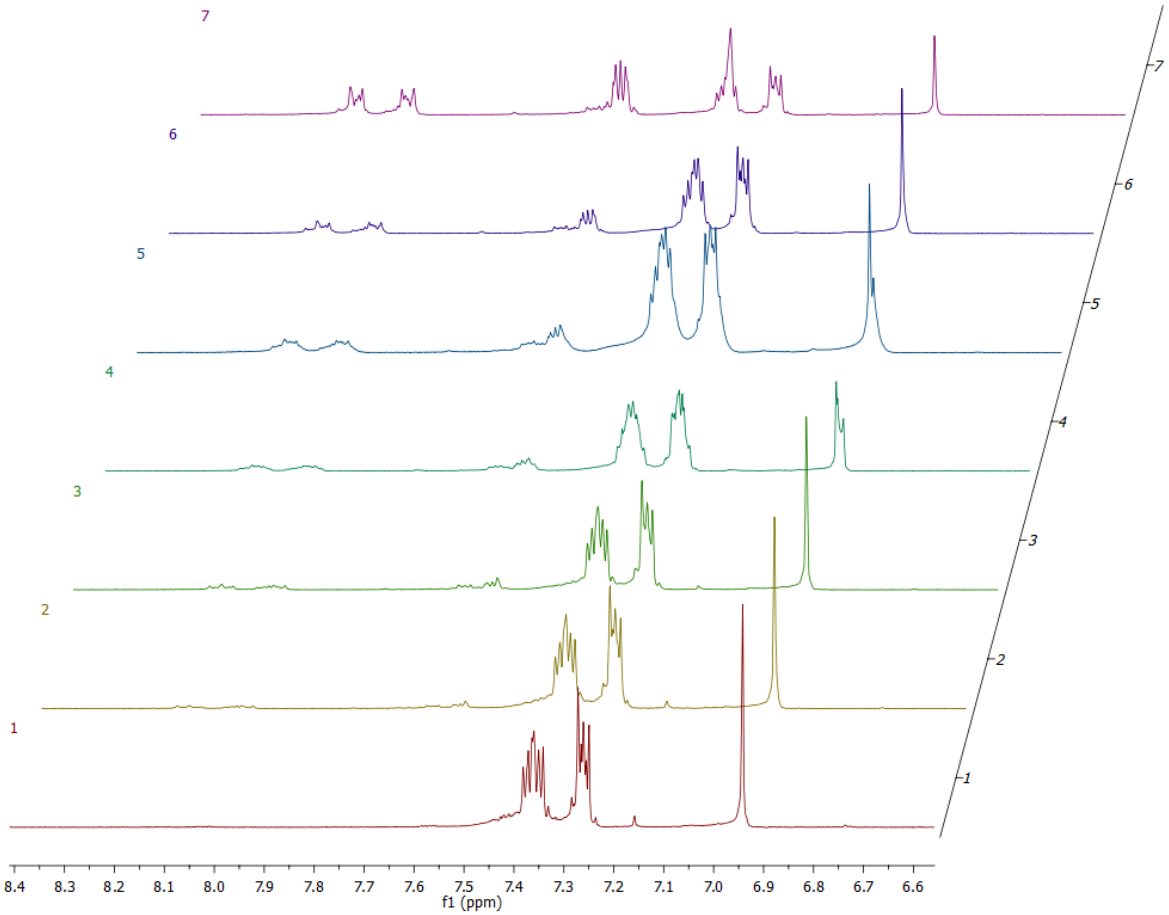



**Figure S4.** Evolution of the NMR spectra of parent-naphthalene- endoperoxide with time at room temperature in DMSO-$d_6$ as the solvent.

(Spectra 1- 0 hours, Spectra 2- 27.5 hours, Spectra 3- 67.5 hours, Spectra 4- 115.5 hours, Spectra 5- 153.5 hours, Spectra 6- 251 hours, Spectra 7- 990 hours).

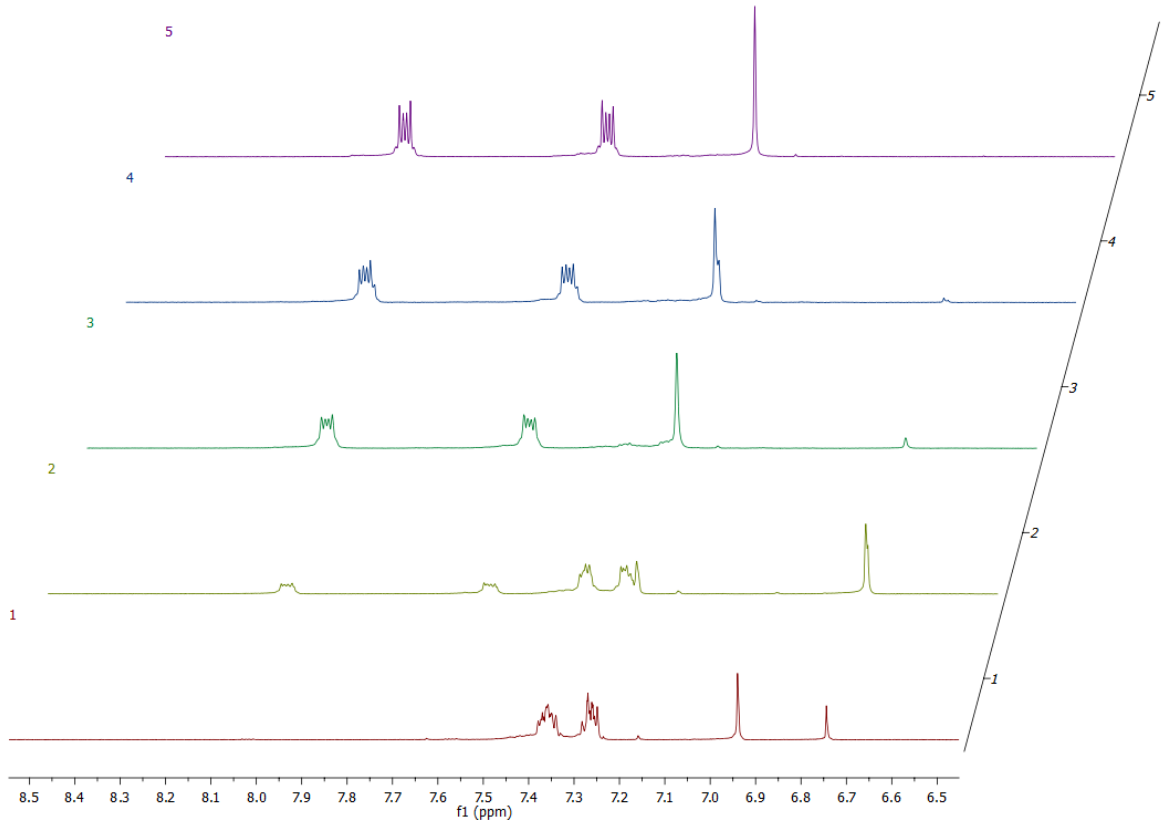



**Figure S5.** Evolution of the NMR spectra of TBAF added naphthalene- endoperoxide with time at room temperature in DMSO-$d_6$ as the solvent.

(Spectra 1- 0 hours, Spectra 2- 3.5 hours, Spectra 3- 20 hours, Spectra 4- 27.5 hours, Spectra 5- 45.5 hours).

NMR spectra which is given above represents the evolution of peaks (8.03-8.0, 7.58-7.55, and 7.24 ppm) which belongs to 1,4-dimethylnaphthalene due to endoperoxide cycloreversion. After the addition of TBAF, when the intensity of the parent naphthalene peaks increase, the peak intensities corresponding to endoperoxide/Compound **3** (7.38-7.34, 7.27-7.25, and 6.94 ppm) decreases.

## 4. Singlet Oxygen Trap Experiments

In singlet oxygen generation experiments, 1,3-Diphenylisobenzofuran (DPBF) was used as chemical singlet oxygen trap molecule in DMSO. This procedure includes approximately 130 µM Compound **3** mixed with trap compound (approximately 55 µM) in DMSO. TBAF (approximately 300 µM) was added. Measurements were taken at 30 minutes intervals at room temperature in dark conditions. Absorbance decrease of trap molecules at 414 nm was monitored, revealing singlet oxygen generation as a result of the silyl group removal.

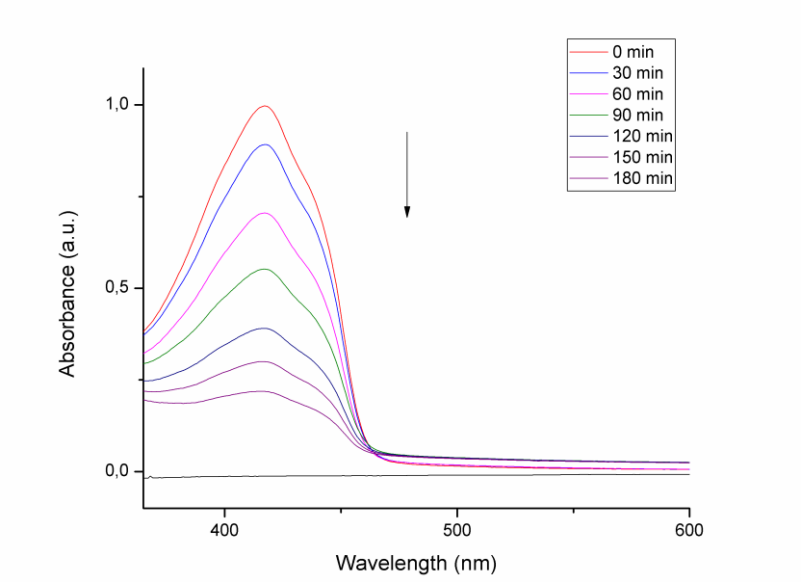



**Figure S6.** Decreasing absorbance peak for the singlet oxygen trap DPBF at 414 nm with time in DMSO in response to the addition of fluoride (TBAF) to compound **3**.

## 5. $^{1}$H-NMR and $^{13}$C-NMR Spectra of the Synthesized Compounds

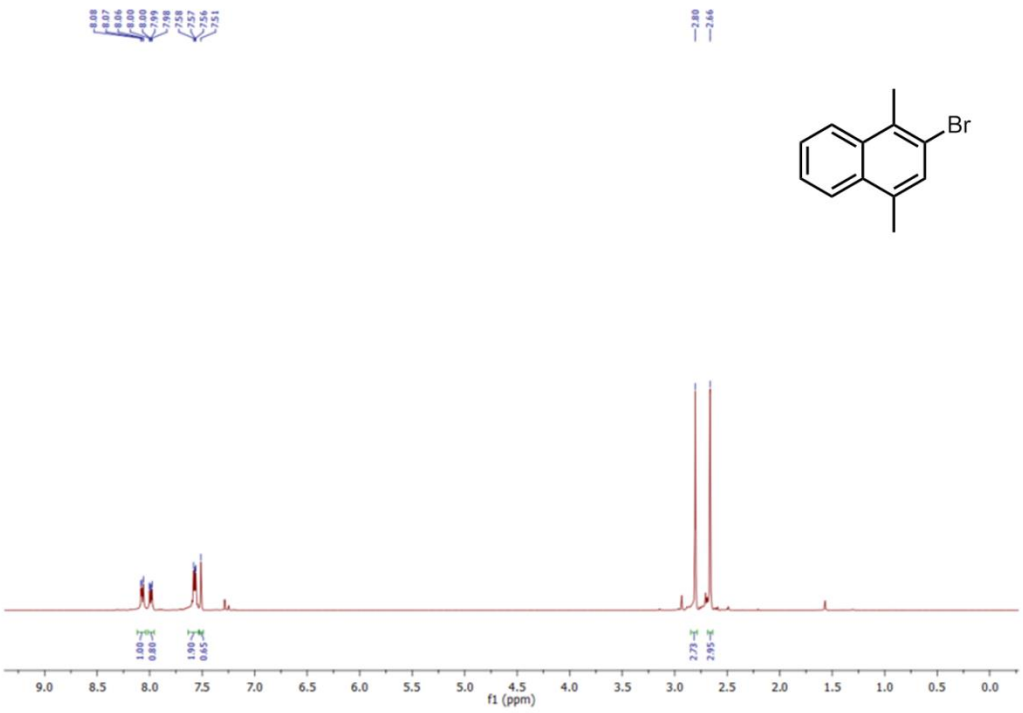



**Figure S7.** $^{1}$H-NMR Spectrum of Compound **1**

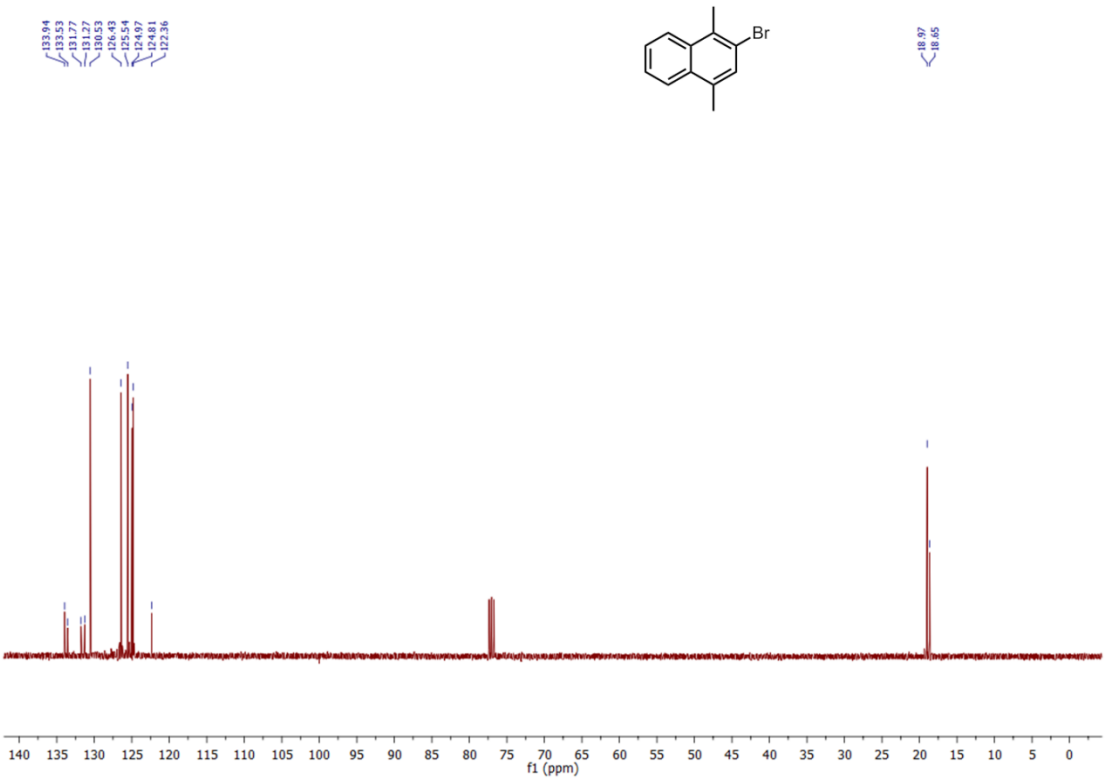



**Figure S8.** $^{13}$C-NMR Spectrum of Compound **1**

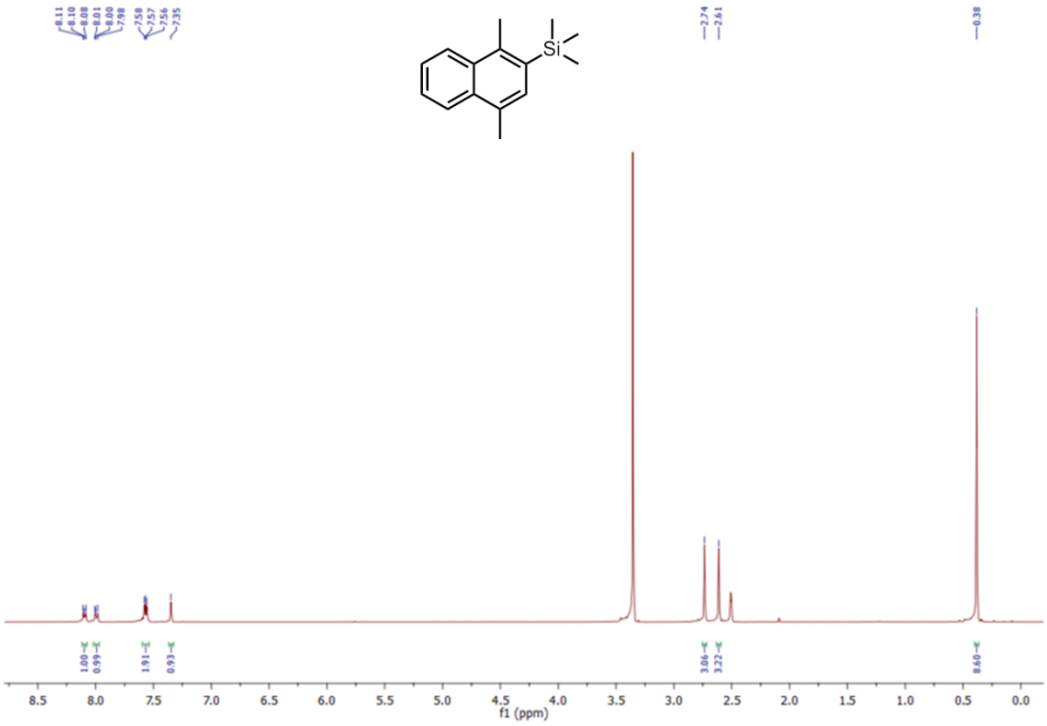



**Figure S9.** $^{1}$H-NMR Spectrum of Compound **2**

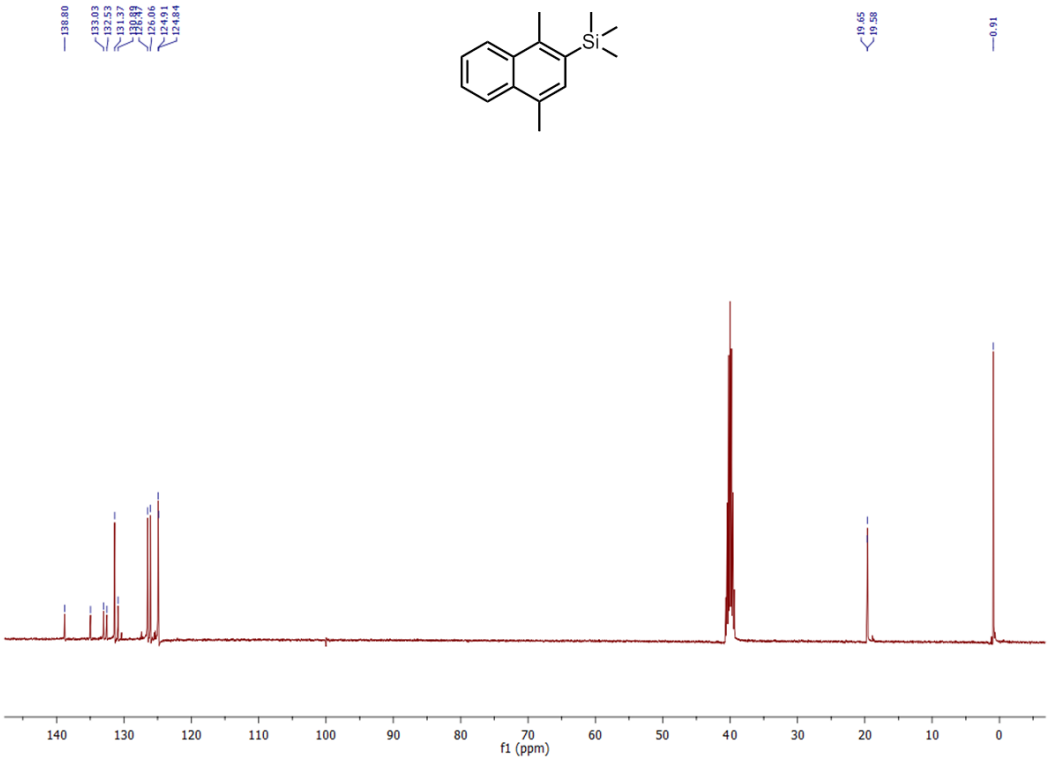



**Figure S10.** $^{13}$C-NMR Spectrum of Compound **2**

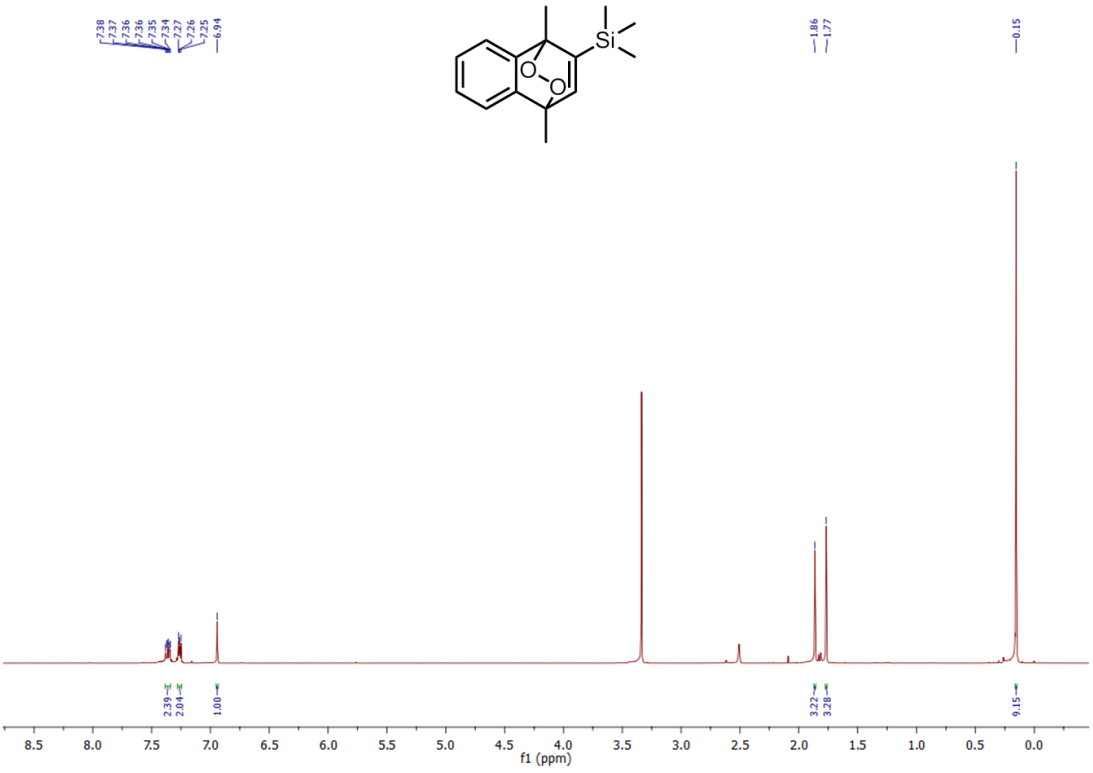



**Figure S11.** $^{1}$H-NMR Spectrum of Compound **3**

## 6. HRMS Spectra of the Synthesized Compounds

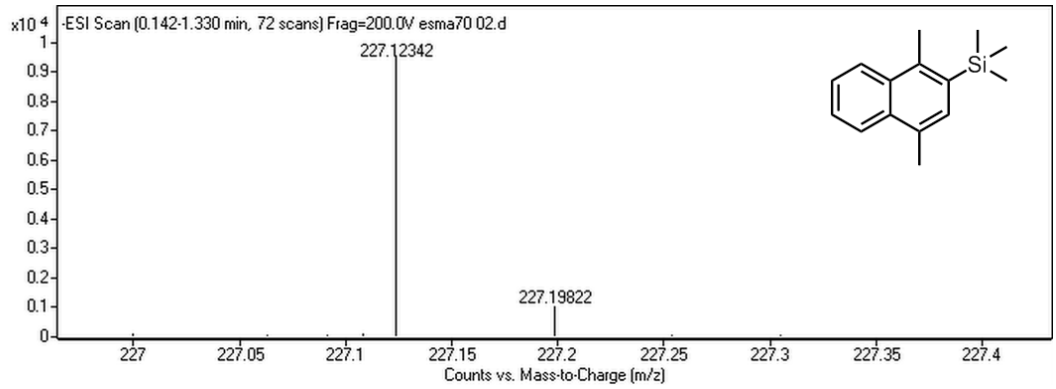



**Figure S12.** HRMs Spectrum of Compound **2**

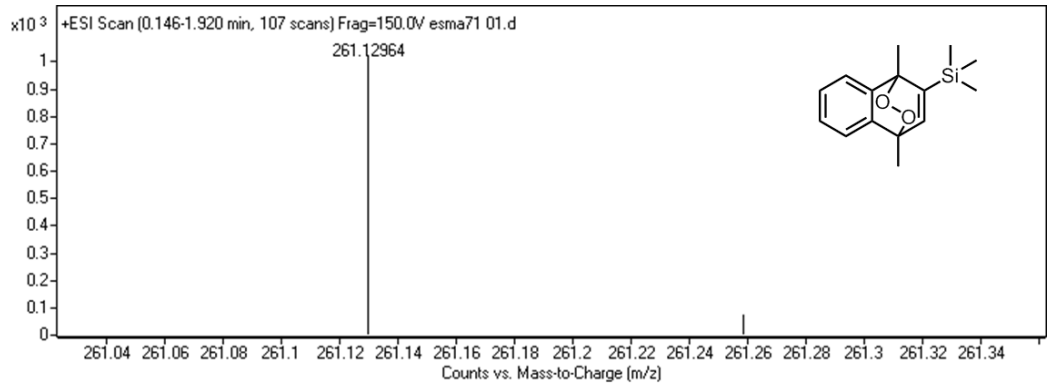



**Figure S13.** HRMs Spectrum of Compound **3**

## 7. Cell Culture Studies

### Cell Culture

MCF-7 human breast cancer cells were cultured in DMEM medium containing 10% fetal bovine serum (FBS), 1% penicillin and streptomycin at 37℃ in a humidified atmosphere composed of 2% $O_2$ and 5% $CO_2$. The cells were maintained in an exponential growth phase by periodic subcultivation. The cell density was determined using a hemocytometer, and this was performed prior to any experiments.

**Cytotoxicity in vitro determined by MTT method:** MCF-7 cells were plated at 96-well plates ($1 \times 10^4$ cells per well and incubated in 100 μL). After 24 h of cell adhension, MCF-7 cells were treated with compound **3** at different concentrations (0, 1.25, 2.5, 5.0 and 10.0 μM) for 4 h, followed by treating with 5 mM NaF. After incubation for 24 h, MTT solution (100 μL of 0.5 mg/ml in DMEM) was added to each well, and the cells were further incubated at 37 ºC for 4 h. Subsequently, the medium was carefully removed, and 100 μL DMSO was added to dissolve the formazan crystals. The absorbance value was measured at 490 nm with a Bio-Rad microplate reader and the cell viability was calculated by the following formula:

$$\text{Cell viability (\%)} = \left(\text{OD}_{ps} - \text{OD}_{blank}/\text{OD}_{control} - \text{OD}_{blank}\right) \times 100\%$$

**Confocal imaging of cell Apoptosis.** Annexin V-FITC/PI Apoptosis Detection Kit was used for detection of Compound 3-induced cell Apoptosis. Briefly, MCF-7 cells were seeded onto 35 mm confocal dishes for 24 h, then cells were treated with following different treatments: group 1, without any treatments (control); group 2, incubated with 5 uM Compound 3 at 37

℃ for 4 h (Compound 3); group 3, incubated with 5 uM Compound 3 at 37 ℃ for 4 h and treated with 5 mM NaF (Compound 3 + NaF); group 4, incubated with 10 mM $NaN_3$ at 37 ℃ for 0.5 h and then with 5 uM Compound 3 for another 4 h, followed by treating with 5 mM NaF (Compound 3 + NaF + $NaN_3$). After incubation for 8 h, cells were stained with Annexin V-FITC/PI Apoptosis Detection Kit according to the manufacture instructions. Annexin V-FITC: Excitation wavelength: 488 nm, Emission was collected in the 500–540 nm range. PI: Excitation wavelength: 535 nm, Emission was collected in the 650–690 nm range.

**Intracellular singlet oxygen imaging:** 2′, 7′-dichlorofluorescin diacetate (DCFH-DA) was used as singlet oxygen probe. Group 1, incubated with 4 uM DCFH-DA at 37 ℃ for 0.5 h without any treatments (control); group 2, incubated with 5 uM Compound **3** at 37 ℃ for 4 h, and then incubated with 4 uM DCFH-DA for 0.5 h (Compound **3**); group 3, incubated with 5 uM Compound **3** at 37 ℃ for 3.5 h and then stained with 4 μM DCFH-DA for another 0.5 h, followed by treating with 5 mM NaF for 1.5 h (Compound **3** + NaF); group 4, incubated with 10 mM $NaN_3$ and 5 mM Compound **3** for 3.5 h, and then incubated with 4 uM DCFH-DA for 0.5 h, followed by treating with 5 mM NaF for 1.5 h (Compound **3**+NaF+$NaN_3$). Confocal fluorescence imaging was used to image intracellular $^1O_2$ level. Excitation wavelength: 488 nm. Emission was collected in the 505–555 nm range.

## 8. References

**1.** T. Koshiyama, K. Hirai and H. Tomioka, *J. Phys. Chem. A* 2002, **106**, 10261-10274.