Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information for

Synthesis and photochemical properties of caged peroxides for photocontrol of oxidative stress in cells

by

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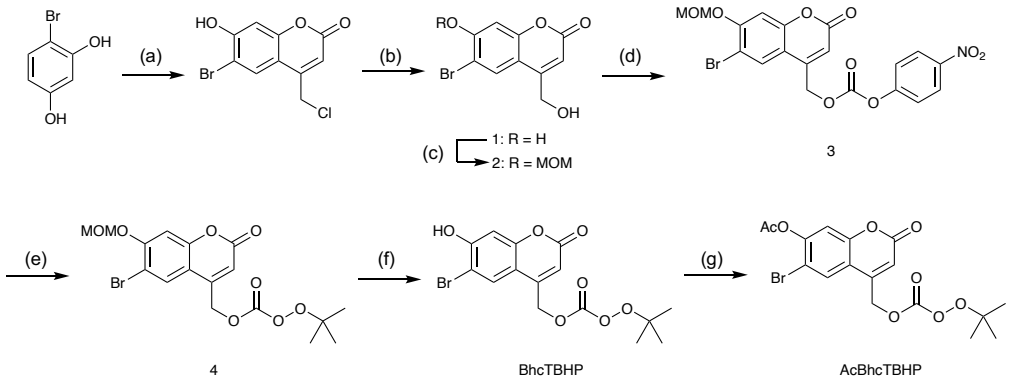
Contents:

1. General Experimental Section

All chemicals used in this study were commercial products. ^{1}H NMR spectra were obtained on a JEOL ECA-500 spectrometer at 500 MHz, JEOL ECZ-400 spectrometer at 400 MHz. ^{13}C NMR spectra were obtained on a JEOL ECA-500 spectrometer at 125 MHz, JEOL ECZ-400 spectrometer at 100 MHz. Spectra were obtained in $CDCl_3$, DMSO-d_6, or CD_3OD. Chemical shifts of ^{1}H NMR were referenced to tetramethylsilane (0.00 ppm). Chemical shifts of ^{13}C NMR were referenced to $CDCl_3$ (77.0 ppm), DMSO-d_6, (39.5 ppm) or CD_3OD (49.0 ppm). Column chromatography was conducted by hand using silica-gel (Taiko-shoji AP-300S) or on a Biotage Accelerated Chromatographic Isolation System with a silica-gel-packed column (FL60D). Reactions were monitored by silica gel TLC (Merck Silica gel 60 PF254) with visualization of components by UV light (254 nm and 365 nm) or with visual observation of the dye spots. High-resolution mass spectra (HRMS) were performed on a LCMS-IT-TOF (Shimadzu) to detect synthesized compounds. Light irradiation in TBHP-releasing evaluations were performed using LED light (M375L4 or M365L3, Thorlabs). Absorbance measurements were performed on UV-VIS spectrophotometer (Agilent 8453, Agilent or Duetta, HORIBA).

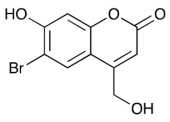
2. Synthesis and Characterization of Compounds

2-1. Synthesis of BhcTBHP and AcBhcTBHP



Scheme S1. Synthesis of BhcTBHP and AcBhcTBHP: (a) ethyl 4-chloroacetoacetate, rt, 2 h; (b) 3 M HCl aq./DMF (3:4), reflux, 15 h, 40% (2 steps); (c) DIPEA, MOMCl, CH_2Cl_2, 0 °C, 1 h, 71%; (d) *p*-nitrophenyl chloroformate, DIPEA, CH_2Cl_2, N_2, 0 °C to rt, 14 h, 66%; (e) TBHP in decane (5.5 M), K_2CO_3, THF/DMF (5:1), Ar or N_2, −40 °C then −10 °C, 18 h, 27%; (f) CH_2Cl_2/TFA (1:1), rt, 1 h, 90%; (g) AcCl, py, rt, 3 h, 54%

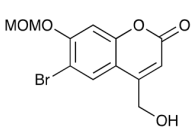
6-bromo-7-hydroxy-4-(hydroxymethyl)-2*H*-1-benzopyran-2-one (1) (CAS: 223420-42-5)



4-Bromoresorcinol (5.00 g, 26.5 mmol) was dissolved in methane sulfonic acid (40 mL). Ethyl 4-chloroacetoacetate (4.22 mL, 39.7 mmol) was added and the reaction mixture was stirred for 2 h at room temperature. The mixture was poured into ice water (100 mL) and then stirred for 30 min to give a light blue precipitate. After the precipitate was collected by filtration and washed with cold water to afford 6-bromo-4-(chloromethyl)-7-hydroxy-2*H*-1-benzopyrane-2-one[1] (10.4 g, crude) as a white solid. This material was used for the next step without further purification. ^{1}H NMR (400 MHz; DMSO-d_6) δ 5.00 (2H, s), 6.48 (1H, s), 6.92 (1H, s), 8.00 (1H, s), 11.59 (1H, s).

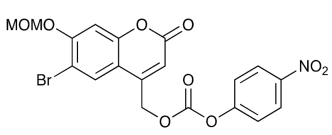
A solution of the crude (10.4 g) in 3 M HCl aq./DMF (3:4, 30 mL/40 mL) was refluxed for 15 h. After cooling to room temperature, the mixture was extracted with EtOAc (500 mL). The organic layer was washed with 1 M HCl aq. (100 mL × 3), brine (100 mL × 3), dried over anhydrous $MgSO_4$, and filtered. The solvent was removed under reduced pressure and the residue was washed with EtOAc (100 mL) to afford compound **1** (2.87 g, 40% in 2 steps) as a yellow solid. ^{1}H NMR (400 MHz; DMSO-d_6) δ 4.70 (2H, s), 5.63 (1H, s), 6.27 (1H, s), 6.90 (1H, s), 7.84 (1H, s), 11.41 (1H, s).

6-bromo-4-(hydroxymethyl)-7-(methoxymethoxy)-2*H*-benzopyran-2-one (2)[2] (CAS: 640721-02-4)



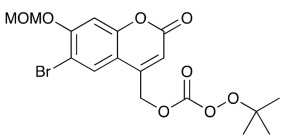
Compound **1** (1.20 g, 4.43 mmol) was dissolved in CH_2Cl_2 (40 mL). DIPEA (1.00 mL, 5.76 mmol) and MOMCl (473 µL, 5.76 mmol) were added at 0 °C under N_2 atmosphere and the mixture was stirred for 1 h at 0 °C. The reaction mixture was diluted with $CHCl_3$ (100 mL) and washed with saturated $NaHCO_3$ aq. (50 mL × 3), 5% citric acid aq. (50 mL × 3), dried over anhydrous $MgSO_4$, filtered. The solvent was removed under reduced pressure to afford compound **2** (988 mg, 71%) as a yellow solid. ^{1}H NMR (500 MHz; DMSO-d_6) δ 3.42 (3H, s), 4.72 (2H, dd, J = 1.5 Hz, 5.6 Hz), 5.42 (2H, s), 5.67 (1H, t, J = 5.6 Hz), 6.36 (1H, s), 7.26 (1H, s), 7.95 (1H, s).

[6-bromo-7-(methoxymethoxy)-2-oxo-2*H*-1-benzopyran-4-yl]methyl 4-nitrophenyl carbonate (3)[3] (CAS: 640721-04-6)



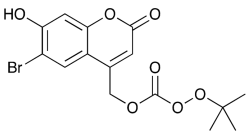
Compound **2** (356 mg, 1.14 mmol) was dissolved in anhydrous CH_2Cl_2 (5 mL). DIPEA (990 µL, 5.69 mmol) and *p*-nitrophenyl chloroformate (458 mg, 2.27 mmol) were added at 0 °C under N_2 atmosphere and the mixture was stirred for 14 h at room temperature. The reaction mixture was diluted with $CHCl_3$ (20 mL) and washed with saturated $NaHCO_3$ aq. (15 mL × 3), brine (20 mL), dried over anhydrous $MgSO_4$, and filtered. The solvent was removed under reduced pressure and the residue was washed with hexane/EtOAc (75:25→0:100) to afford compound **3** (990 mg, 66 %) as a white solid. ^{1}H NMR (400 MHz; $CDCl_3$) δ 3.53 (3H, s), 5.34 (2H, s), 5.42 (2H, s), 6.50 (1H, s), 7.20 (1H, s), 7.44 (2H, d, *J* = 9.2 Hz), 7.71 (1H, s), 8.32 (2H, d, *J* = 9.2 Hz).

[6-bromo-7-(methoxymethoxy)-2-oxo-2*H*-1-benzopyran-4-yl]methyl 1,1-dimethylethyl carbonoperoxoate (4)



Compound **3** (100 mg, 0.208 mmol) and K_2CO_3 (26 mg, 0.208 mmol) were suspended in anhydrous THF/DMF (5:1, 5 mL/1 mL). After cooling to −40 °C, *tert*-butyl hydroperoxide (TBHP) (5.5 M in decane, 75 µL, 0.416 mmol) was added under Ar atmosphere. After stirring for 18 h at −10 °C, the reaction mixture was purified by flash chromatography on silica gel (hexane: EtOAc = 90:10→20:80→50:50→5:95) to afford **4** (24 mg, 27%) as a yellow solid. ^{1}H NMR (400 MHz; $CDCl_3$) δ 1.37 (9H, s), 3.52 (3H, s), 5.33 (2H, s), 5.35 (2H, d, *J* = 0.8 Hz), 6.43 (1H, s), 7.18 (1H, s), 7.67 (1H, s); ^{13}C NMR (100 MHz; $CDCl_3$) δ 26.0, 56.9, 65.0, 85.1, 95.3, 104.2, 108.8, 112.0, 112.2, 127.5, 146.9, 154.1, 154.3, 156.7, 160.0; HRMS-ESI (m/z) $[M+H]^+$ calcd for $C_{17}H_{20}BrO_8^+$: 431.0336; found 431.0337

(6-bromo-7-hydroxy-2-oxo-2H-1-benzopyran-4-yl) methyl 1,1-dimethylethyl carbonoperoxoate (BhcTBHP)

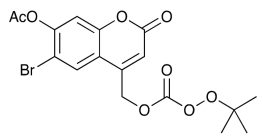


Compound **4** (63 mg, 0.146 mmol) was dissolved in anhydrous CH_2Cl_2 (1 mL) and TFA (1 mL). After stirring for 1 h at room temperature, the reaction mixture was concentrated. The residue was purified by flash chromatography on silica gel (hexane: EtOAc = 3: 1) to afford **BhcTBHP** (51 mg, 90%) as a white solid.

^{1}H NMR (400 MHz; DMSO-d_6) δ 1.28 (9H, s), 5.52 (2H, s), 6.24 (1H, s), 6.92 (1H, s), 7.92 (1H, s), 11.6 (1H, brs); ^{13}C NMR (125 MHz; DMSO-d_6) δ 25.5, 65.6, 84.5, 103.2,

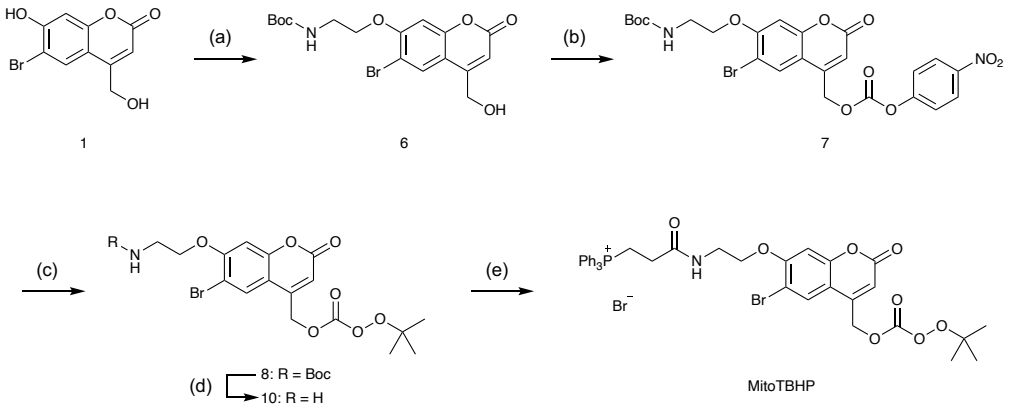
106.3, 109.9, 110.2, 128.8, 148.5, 153.5, 153.9, 157.7, 159.5; HRMS-ESI (m/z) $[M+H]^+$ calcd for $C_{15}H_{16}BrO_7^+$: 387.0074; found 387.0071.

[7-(acetyloxy)-6-bromo-2-oxo-2*H*-1-benzopyran-4-yl]methyl 1,1-dimethylethyl carbonoperoxoate (AcBhcTBHP)



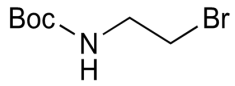
BhcTBHP (33.7 mg, 0.0870 mmol) was dissolved in pyridine (1 mL, 12.4 mmol). Acetyl chloride (30.8 μL, 0.435 mmol) was added and the mixture was stirred for 3 h at room temperature. The reaction mixture was diluted in EtOAc (10 mL) and washed with brine (5 mL), dried over anhydrous $MgSO_4$, filtered. The solvent was removed under reduced pressure and the residue was purified by flash chromatography on silica gel (hexane: EtOAc = 5: 1) to afford **AcBhcTBHP** (37.3 mg, 54%) as a white solid. ^{1}H NMR (400 MHz; $CDCl_3$) δ 1.37 (9H, s), 2.41 (3H, s), 5.36 (2H, s), 6.55 (1H, s), 7.22 (1H, s), 7.74 (1H, s); ^{13}C NMR (100 MHz; $CDCl_3$) δ 20.9, 26.0, 64.8, 85.2, 112.4, 113.2, 114.5, 116.3, 127.8, 146.4, 150.9, 153.4, 154.0, 159.2, 168.0; HRMS-ESI (m/z) $[M+H]^+$ calcd for $C_{17}H_{18}BrO_8^+$: 429.0180; found 429.0194.

2-2. Synthesis and Characterization of MitoTBHP



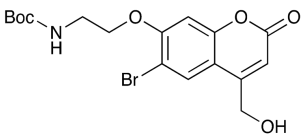
Scheme S2. Synthesis of MitoTBHP: (a) K_2CO_3, *N*-(*tert*-butoxycarbonyl)-2-bromomethylamine (**5**), DMF, rt, 48 h, 31%; (b) *p*-nitrophenyl chloroformate, DIPEA, CH_2Cl_2, N_2, 0 °C then rt, 2 h, 45%; (c) TBHP in decane (5.5 M), K_2CO_3, THF/DMF (5:1), Ar or N_2, −40 °C then −10 °C, 18 h, 58%; (d) CH_2Cl_2/TFA (1:1), rt, 1 h; (e) (2-carboxyehyyl)triphenyl phosphonium bromide (**9**), EDC•HCl, HOSu, N_2, rt, 1 h, 39% (2 steps)

***N*-(*tert*-butoxycarbonyl)-2-bromoethylamine (5)**[4] (CAS: 39684-80-5)



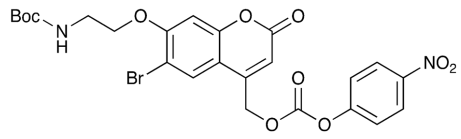
N-Boc-ethanolamine (3.00 g, 18.6 mmol) was dissolved in CH_2Cl_2 (120 mL). After cooling to 0 °C, carbon tetrabromide (6.80 g, 20.5 mmol) and triphenylphosphine (PPh_3) (5.38 g, 20.5 mmol) were added and the mixture was stirred for 4.5 h at 0 °C. The reaction mixture was concentrated, and the residue was purified by flash chromatography on silica gel (hexane: Et_2O = 4: 1) to afford **5** (2.61 g, 63%) as a colorless oil. ^{1}H NMR (400 MHz; $CDCl_3$) δ 1.46 (9H, s), 3.45–3.47 (2H, m), 3.52–3.55 (2H, m), 5.03 (1H, brs).

1,1-dimethylethyl *N*-(2-{[6-bromo-4-(hydroxymethyl)-2-oxo-2*H*-1-benzopyran-7-yl]oxy}ethyl)carbamate (6)[5] **(**CAS: 1096159-07-7)



Compound **1** (629 mg, 2.32 mmol) and K_2CO_3 (1.60 g, 11.6 mmol) were suspended in anhydrous DMF (10 mL). A solution of **5** (2.61 g, 11.6 mmol) in anhydrous DMF (7 mL) was added at room temperature under N_2 atmosphere. After stirring for 48 h at room temperature, the reaction mixture was concentrated. The residue was dissolved in $CHCl_3$ (200 mL) and washed with water (200 mL ×2), brine (200 mL), dried over anhydrous $MgSO_4$ filtered. The solvent was removed under reduced pressure and the residue was purified by flash chromatography on silica gel (hexane: EtOAc = 1: 1) to afford compound **6** (294 mg, 31%) as a yellow solid. ^{1}H NMR (400 MHz; DMSO-d_6) δ 1.38 (9H, s), 3.32–3.37 (2H, m, overlapped with solvent peak), 4.16 (2H, t , *J* = 5.5 Hz), 4.72 (2H, dd, *J* = 1.4 Hz, 5.5 Hz), 5.67 (1H, t, *J* = 5.5 Hz), 6.34 (1H, s), 7.02 (1H, t, *J* = 5.5 Hz), 7.24 (1H, s), 7.91 (1H, s).

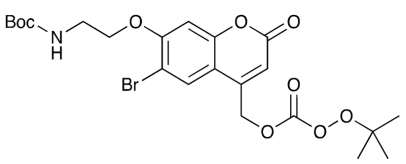
(6-bromo-7-{2-[*N*-(*tert*-butoxycarbonyl)-amino]ethoxy}-2-oxo-2*H*-1-benzopyran-4-yl)methyl 4-nitrophenyl carbonate (7)



Compound **6** (131 mg, 0.316 mmol) and DIPEA (275 μL, 1.58 mmol) were dissolved in anhydrous CH_2Cl_2 (5 mL). After cooling to 0 °C, *p*-nitrophenyl chloroformate (127 mg, 0.632 mmol) was added under N_2 atmosphere. After stirring for 2 h at room temperature, the reaction mixture was diluted in $CHCl_3$ (50 mL). The organic layer was washed with saturated $NaHCO_3$ aq. (30 mL ×3), brine (50 mL), dried over anhydrous $MgSO_4$, filtered. The solvent was removed under reduced pressure and the

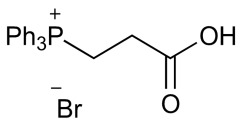
residue was purified by flash chromatography on silica gel (hexane: EtOAc = 3: 1 to 1: 1) to afford **7** (83.3mg, 45%) as a white solid. ^{1}H NMR (400 MHz; $CDCl_3$) δ 1.46 (9H, s), 3.65–3.67 (2H, m), 4.15 (2H, t, *J* = 5.2 Hz), 5.03 (1H, brs), 5.42 (2H, s), 6.49 (1H, s), 6.88 (1H, s), 7.44 (2H, d, *J* = 9.2 Hz), 7.70 (1H, s), 8.32 (2H, d, *J* = 9.2 Hz); ^{13}C NMR (125 MHz; DMSO-*d*$_6$) δ 28.3, 65.8, 68.3, 77.9, 101.7, 107.2, 111.2, 111.3, 122.7, 125.5, 128.6, 145.3, 148.2, 151.5, 154.2, 155.2, 155.7, 157.5, 159.5. one carbon is overlapped with solvent.; HRMS-ESI (m/z) $[M+Na]^+$ calcd for $C_{24}H_{23}BrN_2NaO_{10}^+$: 601.0428; found 601.0431.

(6-bromo-7-{2-[*N*-(*tert*-butoxycarbonyl)-amino]ethoxy}-2-oxo-2*H*-1-benzopyran-4-yl)methyl dimethylethyl carbonoperoxoate (8)



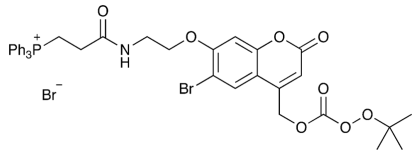
Compound **7** (122.1 mg, 0.211 mmol) and K_2CO_3 (58 mg, 0.422 mmol) was suspended in anhydrous THF/DMF (3.0 mL/0.6 mL). After cooling to −40 °C, TBHP (5.5 M in decane, 77 μL, 0.422 mmol) was added under N_2 atmosphere and the mixture was stirred for 18 h at −10 °C. The reaction mixture was diluted with CH_2Cl_2 (60 mL) washed with brine (80 mL), dried over anhydrous $MgSO_4$, filtered. The solvent was removed under reduced pressure and the residue was purified by flash chromatography on silica gel (hexane: EtOAc = 3: 1) to afford **8** (65.4 mg, 58%) as a yellow solid. ^{1}H NMR (400 MHz; $CDCl_3$) δ 1.37 (9H, s), 1.46 (9H, s), 3.65–3.66 (2H, m), 4.13–4.15 (2H, m), 5.03 (1H, brs), 5.35 (2H, d, *J* = 0.4 Hz), 6.42 (1H, s), 6.85 (1H, s), 7.67 (1H, s); ^{13}C NMR (100 MHz; $CDCl_3$) δ 26.0, 28.5, 39.8, 65.0, 69.2, 80.0, 85.1, 101.6, 108.4, 111.5, 111.7, 127.5, 147.0, 154.1, 154.6, 155.9, 157.9, 160.0; HRMS-ESI (m/z) $[M+Na]^+$ calcd for $C_{22}H_{28}BrNNaO_9^+$: 552.0840; found 552.0870.

(2-carboxyethyl)triphenylphosphonium bromide (9) [6] (CAS: 51114-94-4)



A solution of 3-bromopropionic acid (200 mg, 1.31 mmol) and PPh_3 (334 mg, 1.31 mmol) in anhydrous MeCN was refluxed under Ar for 28 h. After the solvent was removed under reduced pressure, the residue was washed with Et_2O to afford **9** (459 mg, 84%) as a white solid. ^{1}H NMR (500 MHz; DMSO-*d*$_6$) δ 2.55–2.60 (2H, m), 3.78–3.84 (2H, m), 7.77–7.91 (15H, m), 8.34 (1H, s).

[6-bromo-7-(2-{[1-oxo-3-(triphenylphosphonio)propyl]amino}ethoxy)-2-oxo-2*H*-1-benzopyran-4-yl]methyl dimethylethyl carbonoperoxoate bromide (MitoTBHP)



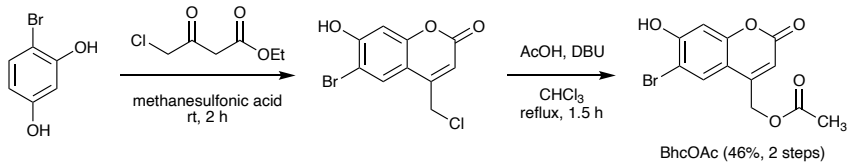
A solution of **8** (77 mg, 0.146 mmol) in anhydrous CH_2Cl_2 (2 mL) and TFA (2 mL) was stirred for 1 h at room temperature, the reaction mixture was concentrated to obtain **10** (86.1 mg, crude) as an off-white solid. This material was used for the next step without further purification. ^{1}H NMR (500 MHz; CD_3OD) δ 1.33 (9H, s), 3.49 (2H, t, *J* = 4.9 Hz), 4.41–4.43 (2H, m), 5.48 (2H, d, *J* = 1.0 Hz), 6.36 (1H, s), 7.16 (1H, s), 7.93 (1H, s).

A solution of **9** (121 mg, 0.292 mmol), EDC•HCl (84.0 mg, 0.438 mmol) and HOSu (50 mg, 0.438 mmol) in anhydrous CH_2Cl_2 (3 mL) was stirred at room temperature under N_2 for 1 h. After removal of the solvent under reduced pressure, the activated ester of **9** as a light-yellow solid that was used directly in next step.

Crude **10** and activated ester of **9** were dissolved in dry DMF (2.0 mL). DIPEA was added and the mixture was stirred at room temperature under Ar for 1.5 h. After the solvent was removed under reduced pressure, the residue was purified by preparative HPLC (PU-4086-Bynary pump and UV-970, JASCO) using reverse phase column (TSKgel ODS-80Ts (TOSHO), 5 μm, 20×250 mm) eluted with H_2O/MeCN containing 0.05% formic acid to afford **MitoTBHP** (47.2 mg, 39% in 2 steps) as a white solid. ^{1}H NMR (500 MHz; $CDCl_3$) δ 1.36 (9H, s), 2.95–2.96 (2H, m), 3.66–3.67 (2H, m), 3.78–3.81 (2H, m), 4.18 (2H, t, *J* = 6.5 Hz), 5.33 (2H, s), 6.37 (1H, s), 6.92 (1H, s), 7.61 (1H, s), 7.73–7.85 (15H, m), 9.63 (1H, brs); ^{13}C NMR (100 MHz; DMSO-d_6) δ 16.9 (d, ^{1} *J*(C,P) = 53.7 Hz), 25.5, 27.5, 38.0, 65.6, 67.9, 84.5, 101.8, 107.2, 111.0, 111.3, 118.3 (d, ^{1} *J*(C,P) = 85.3 Hz), 128.6, 130.2 (d, ^{3} *J*(C,P) = 12.5 Hz), 133.7 (d, ^{2} *J*(C,P) = 9.5 Hz), 134.9, 148.3, 153.5, 154.2, 157.3, 159.4, 169.2 (d, ^{3} *J*(C,P) = 14.4 Hz); HRMS-ESI (m/z) $[M]^+$ calcd for $C_{38}H_{38}BrNO_8P^+$: 746.1513, found 746.1533.

2-3. Synthesis and Characterization of BhcOAc and BhcO^{t}Bu

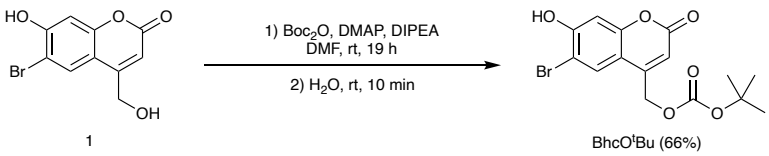
4-[(acetyloxy)methyl]-6-bromo-7-hydroxy-2*H*-1-benzopyran-2-one (BhcOAc) (CAS: 223420-18-6)



Scheme S3. Synthesis of BhcOAc

4-Bromoresorcinol (500 mg, 26.5 mmol) was dissolved in methanesulfonic acid (4 mL). Ethyl 4-chloroacetoacetate (422 μL, 3.98 mmol) was added and the reaction mixture was stirred for 2 h at room temperature. The mixture was poured into ice water (10 mL) and then stirred for 30 min to give a light blue precipitate. After the precipitate was collected by filtration and washed with cold water to afford 6-bromo-4-(chloromethyl)-7-hydroxy-2*H*-1-benzopyrane-2-one[1] (604 mg, crude) as a white solid. A mixture of the crude (100 mg), DBU (206 μL, 1.38 mmol) and AcOH (59 μL, 1.04 μL) in benzene was refluxed for 1.5 h. After cooling, the mixture was diluted with $CHCl_3$ (20 mL) and quenched with 1 M HCl (2.0 mL). The organic layer was separated and dried over anhydrous $MgSO_4$. After filtration, the solvent was removed under reduced pressure and the residue was purified by flash chromatography on silica gel (MeOH: $CHCl_3$ = 0: 100→5: 95) to afford compound **BhcOAc** (50 mg, 46%, 2 steps) as a white solid. ^{1}H NMR (500 MHz; DMSO-d_6) δ 2.18 (3H, s), 5.33 (2H, d, *J* =1.5 Hz), 6.23 (1H, s), 6.90 (1H, s), 7.90 (1H, s), 11.53 (1H, brs).

(6-bromo-7-hydroxy-2-oxo-2*H*-1-benzopyran-4-yl)methyl 1,1-dimethylethyl carbonate (BhcO^{t}Bu)



Scheme S4. Synthesis of BhcO^{t}Bu

Compound **1** (20 mg, 0.074 mmol), Boc_2O (35 mg, 0.16 mmol) and DMAP (3.6 mg, 0.030 mmol) were dissolved in dry DMF (3 mL). DIPEA (154 μL, 0.16 mmol) was added and the mixture was stirred at room temperature under N_2 for 19 h. H_2O (20 mL) was added, the resulting solution was stirred for 10 min and extracted with $CHCl_3$ (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and filtered. The solvent was removed under reduced pressure and the residue was purified by flash chromatography on silica gel (EtOAc: hexane = 1: 3→1: 2) to afford compound **BhcO^{t}Bu** (20.3 mg, 66%) as a white solid. ^{1}H NMR (500 MHz; DMSO-d_6) δ 1.46 (9H, s), 5.34 (2H, s), 6.16 (1H, s), 6.92 (1H, s), 7.88 (1H, s), 11.58 (1H, brs); ^{13}C NMR (125 MHz; DMSO-d_6) δ 27.3, 63.6, 82.7, 103.2, 106.3, 109.2, 110.4, 128.6, 149.7, 152.3, 153.9, 157.6, 159.6; HRMS-ESI (m/z) $[M+H]^+$ calcd for $C_{15}H_{16}BrO_6^+$: 371.0125, found 371.0128.

3. TBHP-releasing evaluation by fluorescence spectroscopy

A solution of caged compound (50 μM) in 100 mM phosphate buffer (pH 7.4, 0.5% DMF) was irradiated at 375 nm for 0–5 min. To an irradiated solution was added a solution of NBzF (final 5 μM). The mixture was kept for 1 h at 37 °C and diluted to three times. The reacted sample was analyzed with a fluorescence spectroscopy (Duetta, HORIBA) with 5 nm and 5 nm for excitation and emission. The path length was 1 cm with a cell volume of 1.0 mL.

4. Analysis by LC-MS or HPLC

The result samples for product analysis were analyzed by a LC-MS (Shimadzu) using reverse phase column (TSKgel ODS -80Ts (TOSHO), 5 μm, 4.6×75 mm or Symmetry C18 (Waters), 5 μm, 4.6×75 mm) eluted with H_2O/MeCN (90/10 for 5 min, then 90/10 to 5/95 over 15 min) containing 0.05% formic acid. The absorbance at 330 nm or 254 nm was monitored. Flow rate was 0.5 mL/min. The result samples for time courses analysis were analyzed by a HPLC (1260 infinity series, Agilent) using reverse phase column (TSKgel ODS -80Ts (TOSHO), 5 μm, 4.6×75 mm) eluted with H_2O/MeCN (90/10 for 5 min, then 90/10 to 5/95 over 15 min) containing 0.05% formic acid. The absorbance at 330 nm was monitored. Flow rate was 0.5 mL/min.

5. Determination of the Quantum yield of disappearance (Φ_{dis})

A solution (3 mL) of caged compounds (10 μM) in 100 mM KCl and 10 mM KMops buffer (pH 7.2, 1% DMF) was irradiated with a 365 nm LED in a quartz cuvette. The duration of each irradiation period ranged from 0 to 300 s. After each irradiation period, 50 μL of the irradiated solution was analysed by HPLC. The time-dependent curves were plotted to calculate irradiation times (s) at which 90% of the starting materials reacted ($t_{90\%}$). The quantum yield of disappearance (Φ_{dis}) was calculated according to a previously published method[7,8].

$\Phi_{dis}=1/(t_{90\%} \times I \times \sigma_{365})$

I [einstein $cm^{-2}s^{-1}$] is the number of photons, σ_{365} [cm^2mol^{-1}] is the decadic extinction coefficient at 365 nm (σ_{365} [cm^2mol^{-1}] = $10^3 \times \varepsilon_{365}$ [$M^{-1}cm^{-1}$]). I was measured using potassium ferrioxalate actinometry in the same cuvette used for the above photoreactions.

6. Computational Procedure

Geometry optimization of BhcTBHP and BhcOtBu in the S_0 ground state and S_1 excited state was performed using B3LYP/6-31G(d) and TD-B3LYP/6-31G(d) methods, respectively, with Gaussian 09 or 16 program package. The water solvent effects were included by PCM method[9]. Normal mode analysis was performed to confirm the absence of imaginary frequencies for the optimized structures.

7. Cell culture

MCF-7 cells were maintained in MEM supplemented with 10% FBS and 50 U mL^{-1}, penicillin and 0.05% streptomycin and kanamycin under the conditions of 5% CO_2 at 37 °C.

8. MTT assay of AcBhcTBHP and MitoTBHP

MCF-7 cells (8.0×10^3 cells/per well) were planted on 96-well-plate (TTP Techno Plastic Product AG) and incubated for 24 h. The cells were treated with 0–100 μM caged compounds for 15 min with HBSS. After irradiation (375 nm, 6.1 mW/cm^2) or not irradiation for 15 min, the irradiated cells were incubated for 1 h and washed with HBSS. A solution of MTT reagent (5 mg/mL, 10 μL/well) was added. The cells were incubated

for 4 h and dissolved with DMSO (100 μL/well). The absorbance at 570 nm was measured by a micro plate leader (Multiskan JX plate, Thermo Fisher Scientific). Control cells treated with 1% DMF media (as a vehicle) and not irradiated were considered 100% viable.

9. LDH assay to evaluate phototoxicity.

MCF-7 cells (0.5×10^4 cells/per well) were planted on 96-well-plate (TTP Techno Plastic Product AG) and incubated for 24 h. The cells were irradiated at 375 nm (12 mW/cm^2 or 30 mW/cm^2) for 15 min in HBSS (100 μL) and incubated for 1 or 24 h in DMEM (Phenol Red (−), 1% FBS). After incubation, the plate was centrifuged for 3 min at 1,000 rpm. The supernatant (50 μL) of each well was reacted with 50 μL of LDH color reagents (Wako) for 45 min at 37 °C. The reaction was stopped with 0.5 M HCl and the absorbance at 560 nm was measured by a micro plate leader (SpectraMax^{TM} iD3, MOLECULAR DEVICES). Positive control (PC) was treated with 1% Triton X-100 for 15 min.

10. Confocal fluorescence imaging

Confocal fluorescence images were acquired by using an Olympus IX83 microscope equipped with a laser diode illuminator (LDI with 7 laser lines, 89 North), an EMCCD camera (Hamamatsu Photonics, ImagEM), and a disk scan confocal unit (DSU). Fluorescence images were acquired with a 130 W Hg lump or a LED illuminator (89 North, LDI with 7 laser lines). Experiments were performed with a 10× objective lens or a 20× objective lens.

Filter set of a 130 Hg lump: DSU-FITC (Ex= 465–500 nm, Em = 516–556 nm, DM = 495 nm). Filter set of a LED illuminator (89 North, LDI with 7 laser lines): FITC (Ex = 470 nm, Em = 516–556 nm, DM = 495 nm) or Rhodamine (Ex = 555 nm, Em = 572–642 nm, DM = 562 nm)

Cells were planted on Advanced TC glass-bottomed dishes (CELLviewTM Cell Culture Dish, Greiner) and were allowed to grow 60–80% confluence. For all imaging experiments, HBSS containing calcium and magnesium without phenol red (Gibco) was used.

Imaging using NBzFDA: The cells were incubated with 50 μM AcBhcTBHP or MitoTBHP (from 5 mM stock solution in DMF) for 15min and washed with HBSS. After

irradiation at 375 nm for 5 or 15 min, the irradiated cells were incubated with 5 μM NBzFDA (from 0.5 mM stock solution in DMF) for 60 min.

Imaging using JC-1: After preincubation of 2 μM JC-1 (from 0.2 mM stock solution in DMF) for 60 min, the cells were treated with 50 μM MitoTBHP or AcBhcTBHP (from 5 mM stock solution in DMF) for 15 min and washed with HBSS. The cells were irradiation at 375 nm for 15 min and then incubated for 60 min.

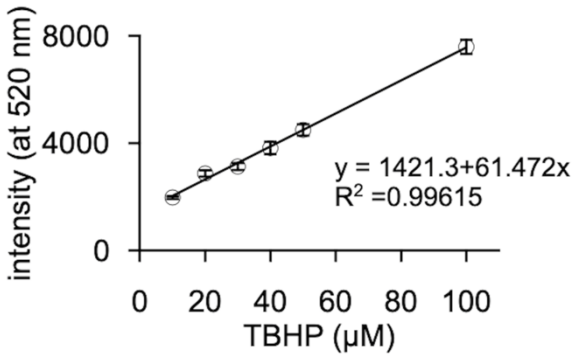


Figure S1. Calibration curve of TBHP determined NBzF: TBHP (10–100 μM) reacted with NBzF (5 μM) in 100 mM phosphate buffer (pH7.4) at 37 °C for 60 min.

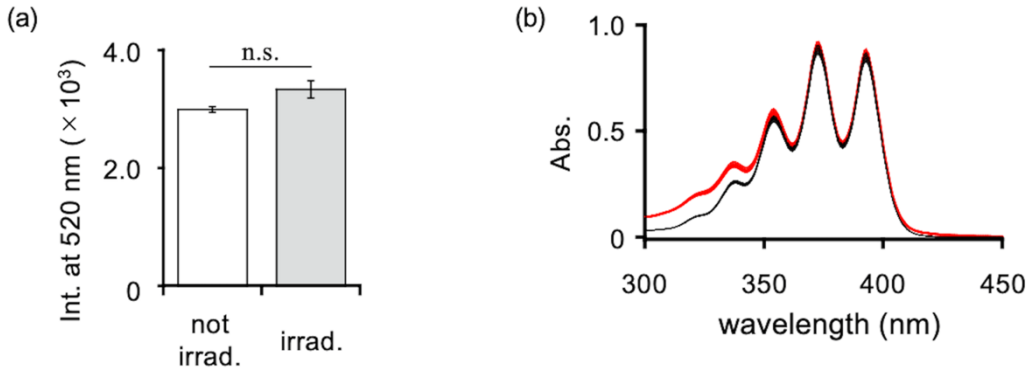


Figure S2. Evaluation of effects as a photosensitizer: (a) Fluorescence intensity of NBzF in BhcOtBu solutions with or without irradiation. A solution of 50 μM BhcOtBu in 100 mM phosphate buffer (pH 7.4, 0.5% DMF) was irradiated at 375 nm (2.5 mW/cm^2) or not irradiated for 5 min. To an irradiated solution was added a solution of NBzF (final 5 μM). The mixture was kept for 1 h at 37 °C and analysed. Error bars denote ± SE (n=3). n.s.: not significant (Student's t-test). (b) Absorption spectra of 9,10-diphenylantharcene after irradiation in the presence of BhcTBHP. A solution of 60 μM 9,10-diphenylanthracene in MeCN (1%DMF) including BhcTBHP was irradiated for 600 s. These absorption spectra were measured every 30 s. Red: 10 μM BhcTBHP irradiated at 375 nm (2.5 mW/cm^2), Black: control

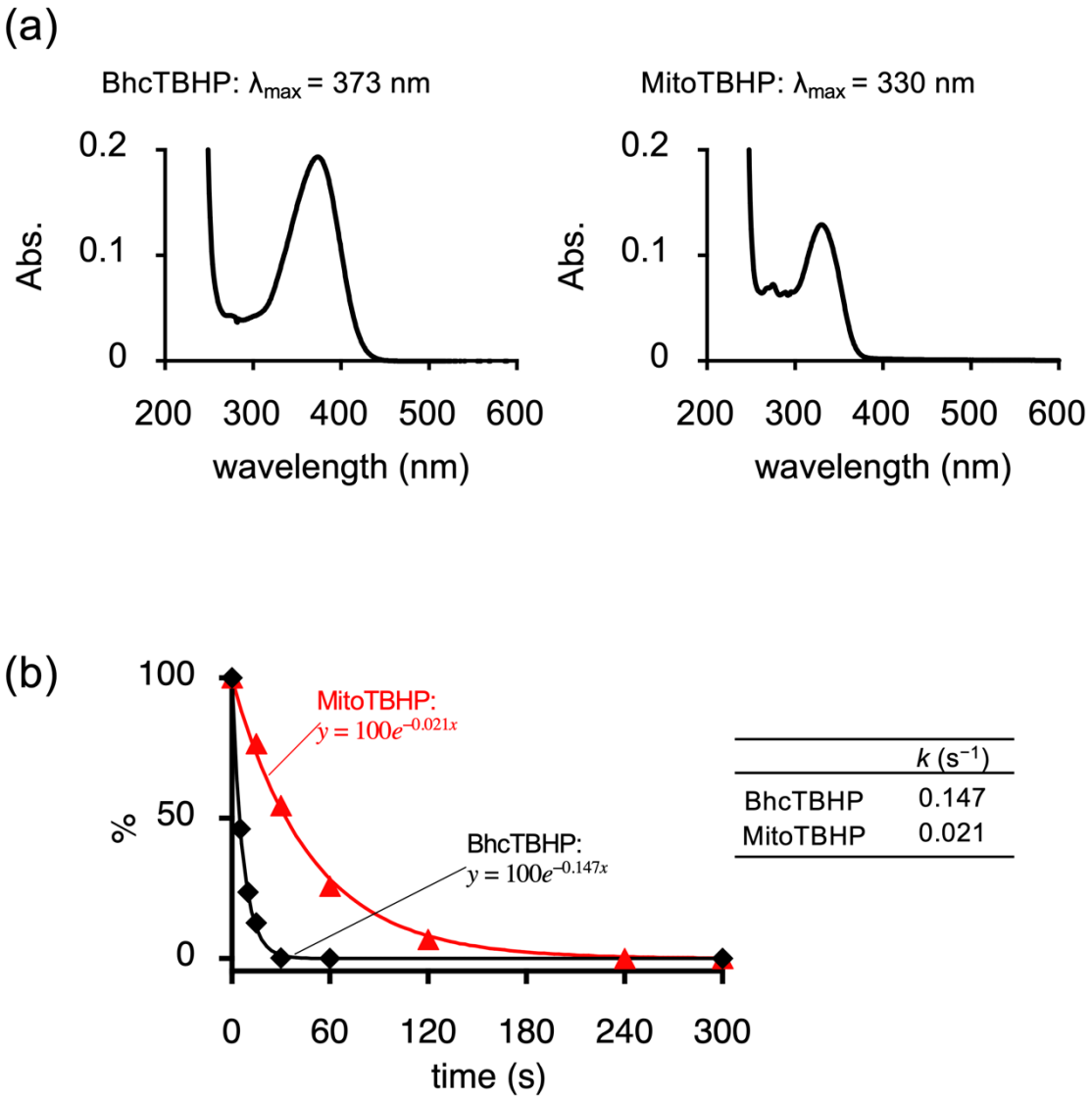


Figure S3. UV measurement and the reaction rate of BhcTBHP or MitoTBHP. (a)The solution of 10 μM BhcTBHP (from 0.1 mM stock DMF solution) or MitoTBHP (from 10 mM stock DMF solution) was measured in 100 mM KCl and 10 mM KMops buffer (pH 7.2). left: BhcTBHP: λ_{max}= 373 nm (ε = 19,300), right; MitoTBHP: λ_{max}= 330 nm (ε = 12,900). (b)10 μM solution of BhcTBHP or MitoTBHP in 100 mM KCl–10 mM KMops buffer (pH 7.2) was irradiated at 365 nm (5 mW/cm^2). The reaction was monitored by HPLC over time and changes in the peak areas of BhcTBHP and MitoTBHP were examined.

Table S1. TDDFT B3LYP/6-31G(d) calculated electronic transitions.

Comp.	State	E (eV)	λ (nm)	Contribution
BhcTBHP	1	2.5543	485.39	HOMO→LUMO (99%)
	2	3.2465	381.91	H−1→LUMO (98%)
	3	3.5359	350.65	H−2→LUMO(99%)
	4	4.2402	292.4	HOMO→L+1 (74%), HOMO→L+2 (24%)
	5	4.3603	284.35	HOMO→L+1 (25%), HOMO→L+2 (72%)
BhcOtBu	1	2.915	425.34	HOMO→LUMO (99%)
	2	3.5634	347.94	H−1→LUMO (97%)
	3	3.9194	316.34	H−2 →LUMO (97%), HOMO→L+1 (2.1%)
	4	4.3904	282.4	H−2→LUMO (2.2%), HOMO→L+1 (95%)
	5	4.4763	276.98	HOMO→L+2 (99%), H−1→L+5 (2.0%)

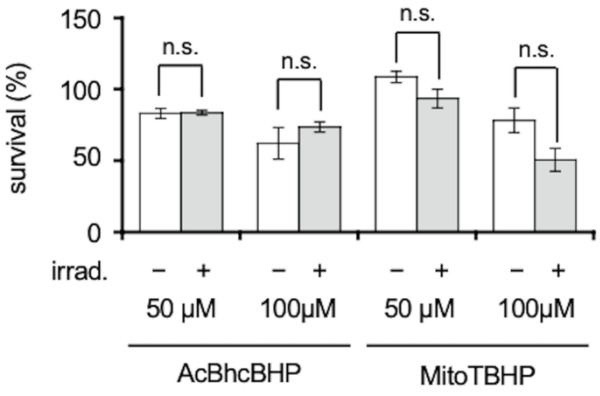


***Figure S4. MTT assay of AcBhcTBHP and MitoTBHP*:** Effects of caged compounds and irradiation on survival of MCF-7 cells. Cell viabilities were calculated as a 100 % of untreated control. Error bars denote standard error (n≧3). (Student's t-test)

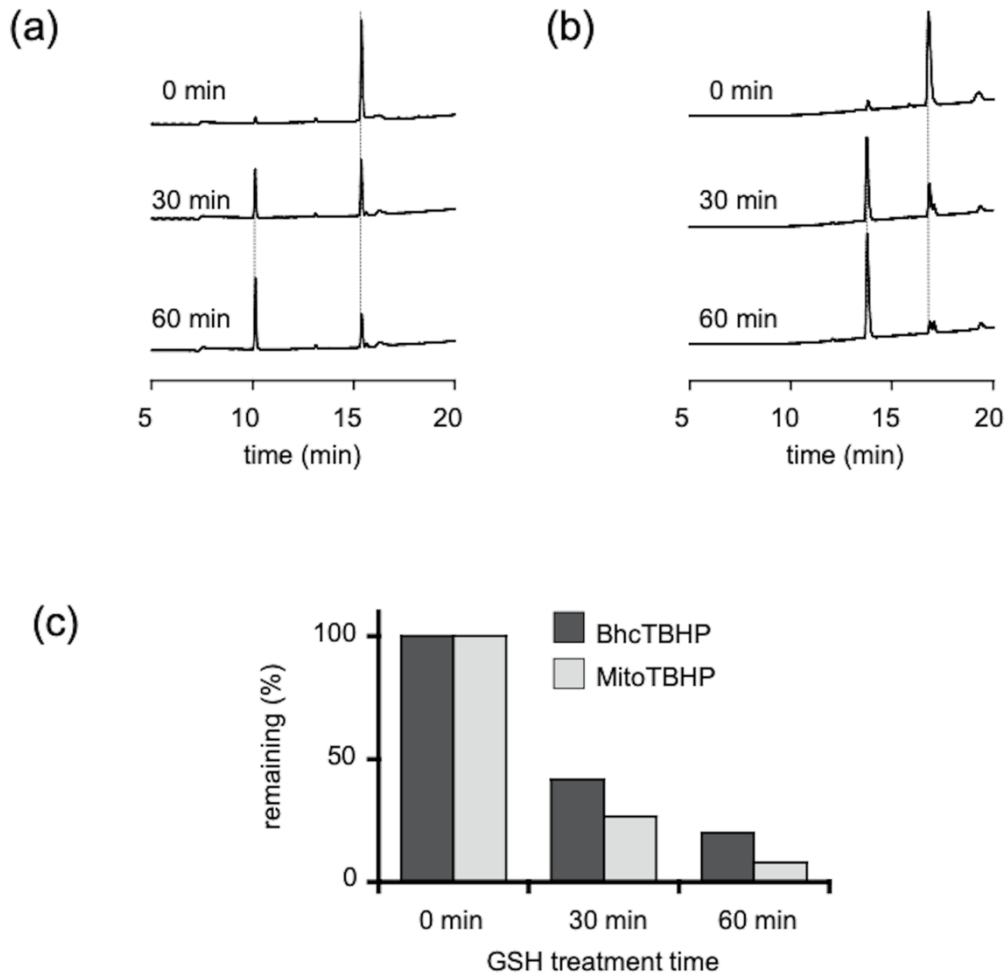


Figure S5. Stability of BhcTBHP and MitoTBHP to GSH: HPLC chromatograms of (a) BhcTBHP (100 μM) or (b) MitoTBHP (100 μM) before and after treatment with 1 mM GSH at 37 °C in 0.1 M phosphate buffer (pH 7.4, 1% DMF). (c) Remaining (%) of BhcTBHP and MitoTBHP treated with GSH (1 mM). These remaining (%) were calculated by area of HPLC.

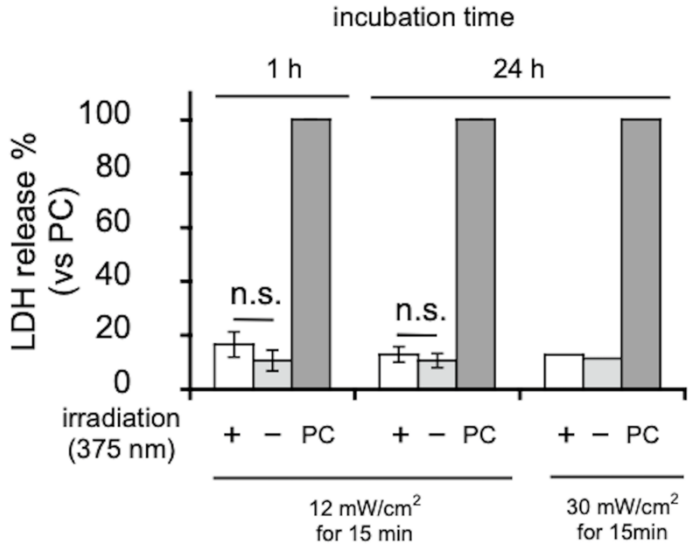


Figure S6. LDH assay to calculate phototoxicity: Effects of irradiation on injury of MCF-7 cells. LDH release (%) were calculated as a 100 % of positive control (PC). Error bars denote ± SE (12 mW/cm^2: n≧3). (Student's t-test)

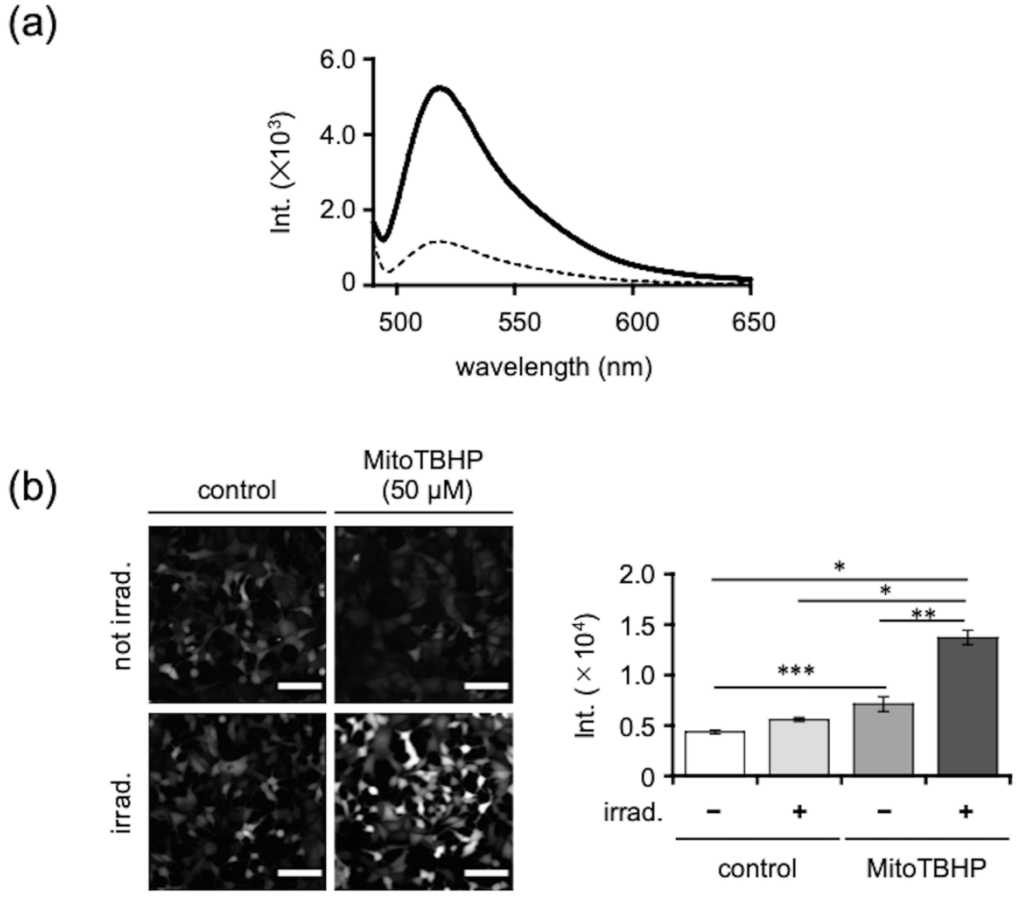


Figure S7. Evaluation of TBHP releasing from MitoTBHP: (a) A solution of MitoTBHP (50 μM) in 100 mM phosphate buffer (0.5% DMF) was irradiated at 375 nm (2.5 mW/cm^2) for 5 min. To an irradiated solution was added a solution of NBzF (final 5 μM). The mixture was kept for 1 h at 37 °C and analysed. solid: irrad. for 5 min, dot: in dark for 5 min. (b) Confocal fluorescence images of irradiated MCF-7 cells treated with MitoTBHP using NBzFDA. The cells were incubated with 50 μM MitoTBHP for 15 min and washed with HBSS. After irradiation at 375 nm (6.2 mW/cm^2) for 15 min, the irradiated cells were incubated with 5 μM NBzFDA for 60 min. Images were taken at Ex 465–500 nm and Em range 516–556 nm. Scale bar = 100 μm. Average cellular fluorescence intensity of the MCF-7 cells as determined using Image J. Error bars denote ± SE (n=3). *p<0.0001, **p<0.0005 and ***p<0.05 (Tukey's test)

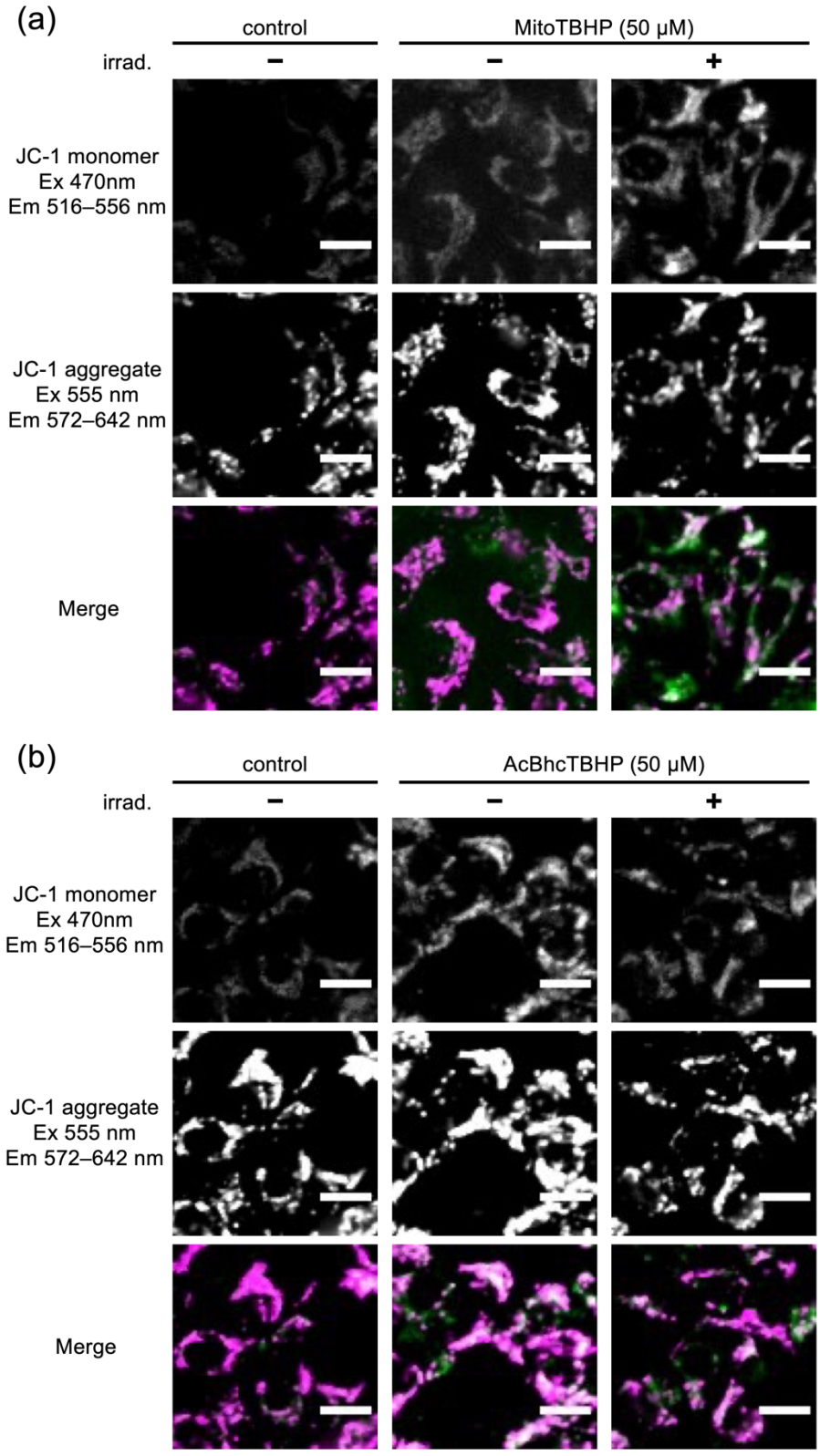


Figure S8. The enlarged images of Fig 5. (a) MitoTBHP, (b) AcBhcTBHP. Scale bar =20 μm

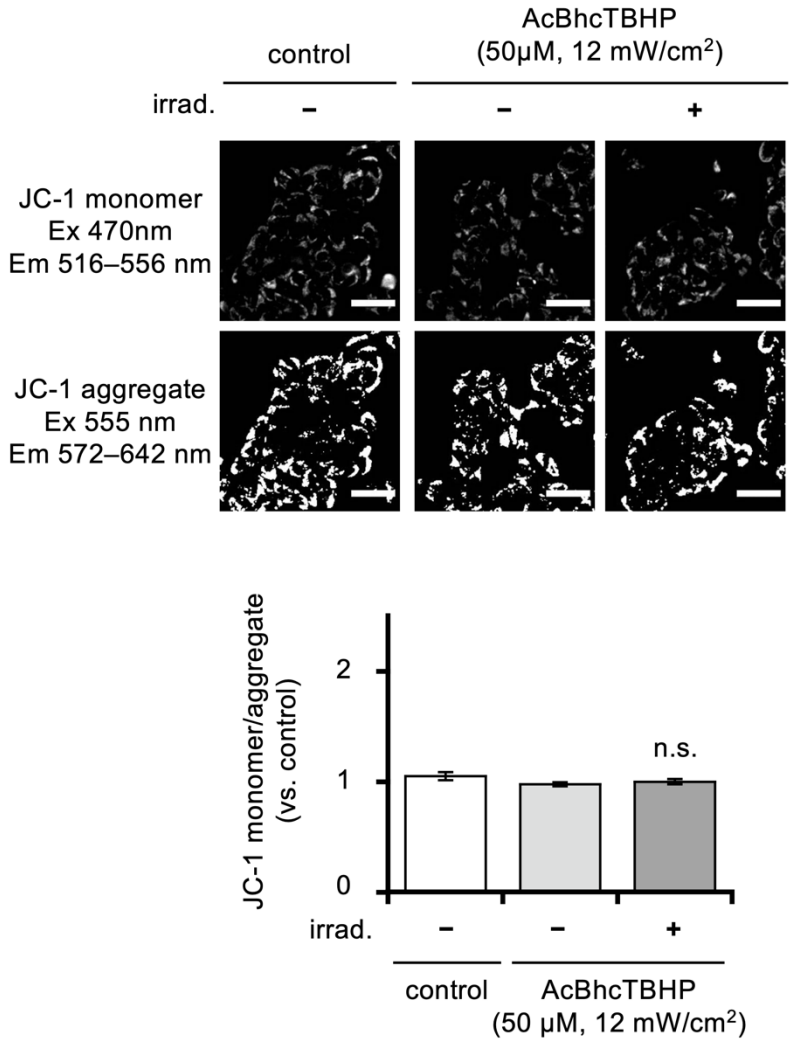


Figure S9. Confocal images using JC-1 treated with AcBhcTBHP and irradiated at 375 nm (12 mW/cm^2): MCF-7 cells were treated with AcBhcTBHP and irradiated at 375 nm (12 mW/cm^2) for 15 min using JC-1. After preincubation of 2 μM JC-1 for 60 min, the cells were treated with 50 μM AcBhcTBHP for 15 min and washed with HBSS. The cells were irradiated at 375 nm (12 mW/cm^2) for 15 min and then incubated for 60 min. Scale bar = 50 μm. The change of MMP of MCF-7 cells detected by the JC-1 ratio (JC-1 monomer fluorescence/JC-1 aggregate fluorescence) corrected with the control sample as 1.0. Average cellular fluorescence intensity of the MCF-7 cells was determined using Image J. Error bars denote ± SE (n=3). (Tukey's test)

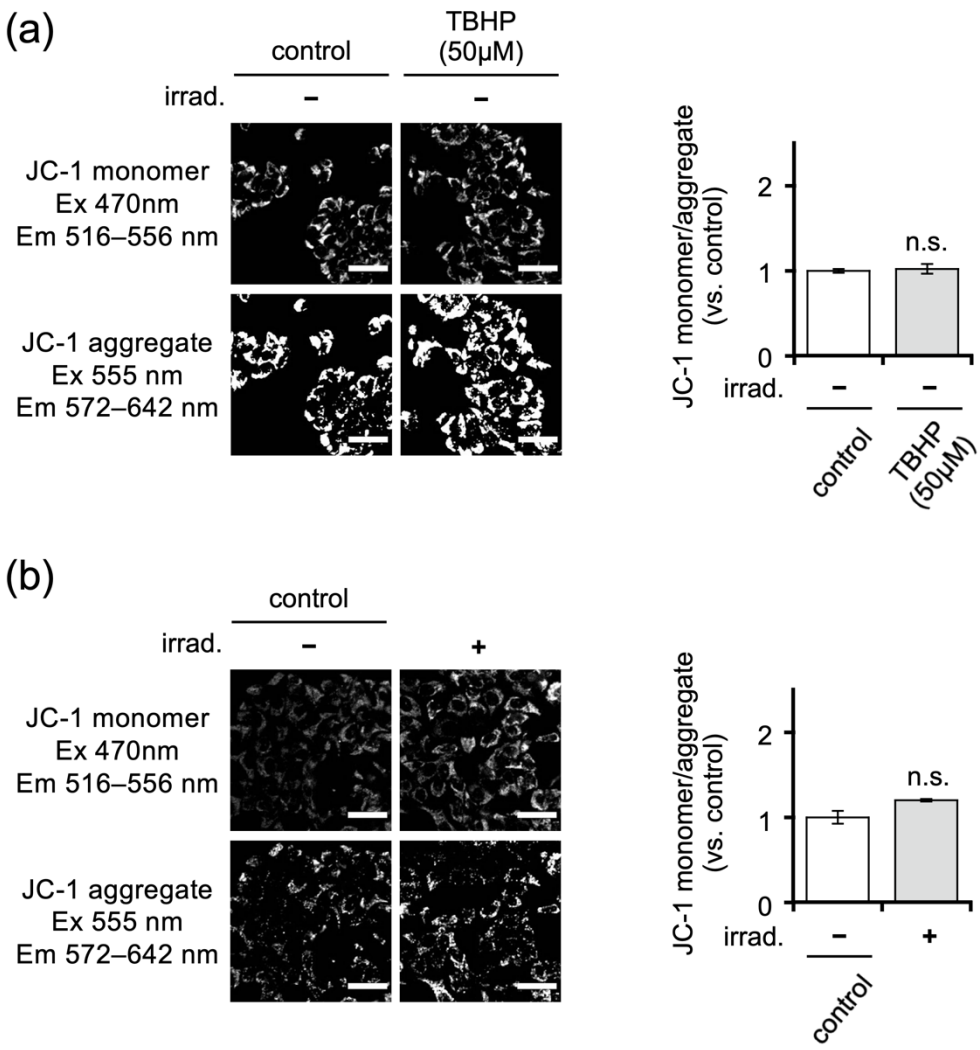


Figure S10. Confocal images using JC-1 treated with TBHP only or irradiation only: MCF-7 cells were treated with (a) TBHP or (b) irradiation only. After preincubation of 2 μM JC-1 for 60 min, the cells were washed with HBSS. (a) The cells were incubated for 30 min and then treated with 50 μM TBHP for 60 min. After incubation for 15 min, the cells were irradiated at 375 nm (2.5 mW/cm^2) for 15 min, and then incubated for 60 min. Scale bar = 50 μm. The change of MMP of MCF-7 cells detected by the JC-1 ratio (JC-1 monomer fluorescence/JC-1 aggregate fluorescence). These values were corrected with the control sample as 1.0. Average cellular fluorescence intensity of the MCF-7 cells was determined using Image J. Error bars denote ± SE (n=3). (Student's t-tests)

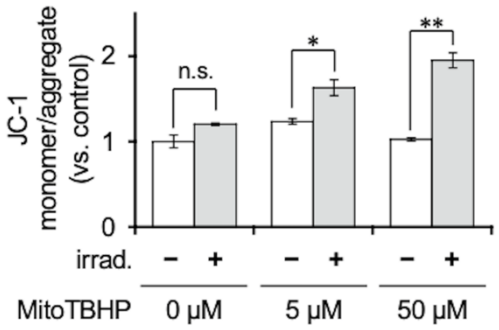


Figure S11. The change of MMP treated with MitoTBHP (0–50 μM):
MCF-7 cells were treated with MitoTBHP (0, 5, 50 μM). JC-1 ratio (JC-1 monomer fluorescence/JC-1 aggregate fluorescence) corrected with the control sample as 1.0. Average cellular fluorescence intensity of the MCF-7 cells was determined using Image J. Error bars denote ± SE (n=3). *p<0.05, ** p<0.001 (Student's t-test)

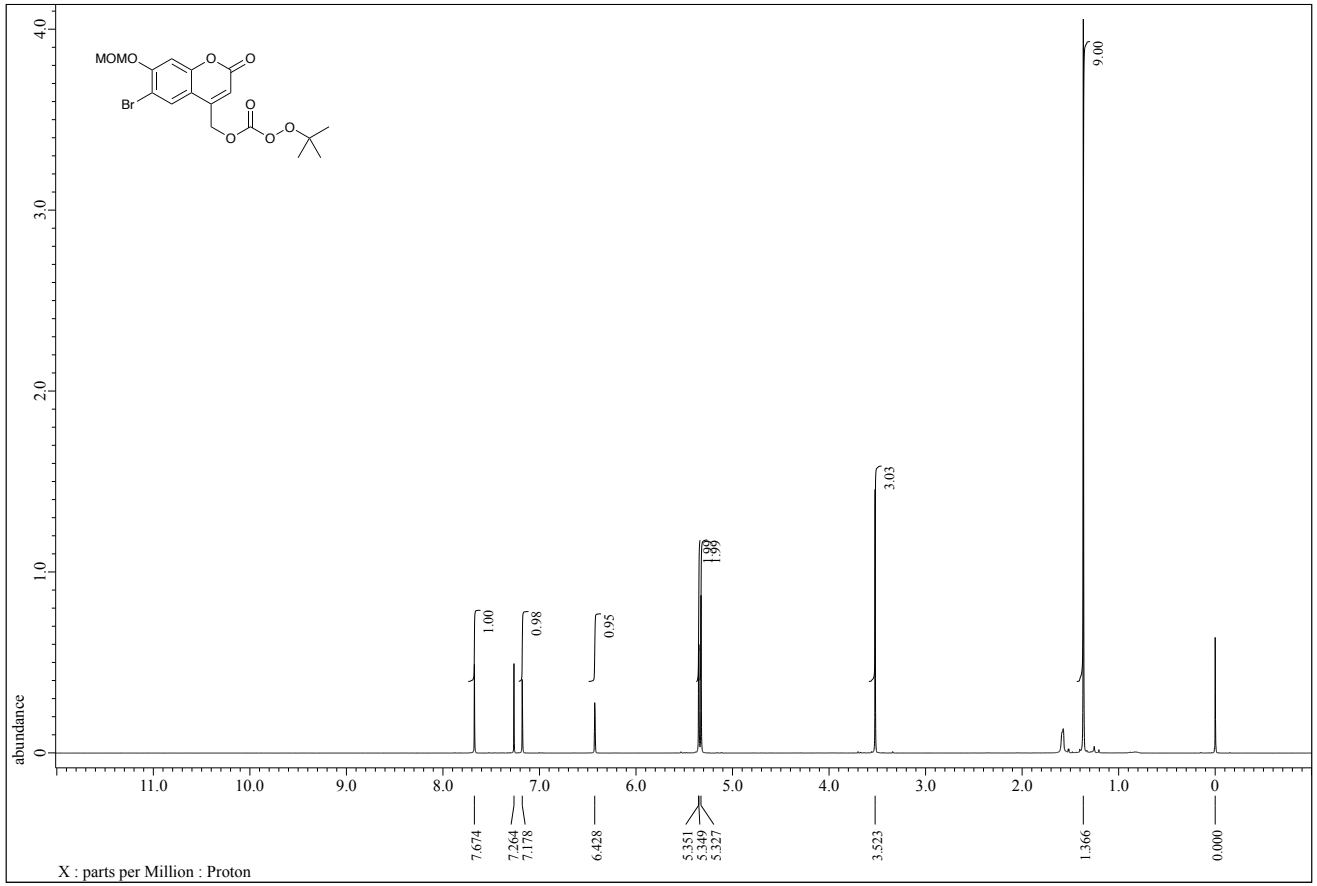


Figure S12. ^{1}H NMR spectrum of compound 4 in $CDCl_3$ (400 MHz)

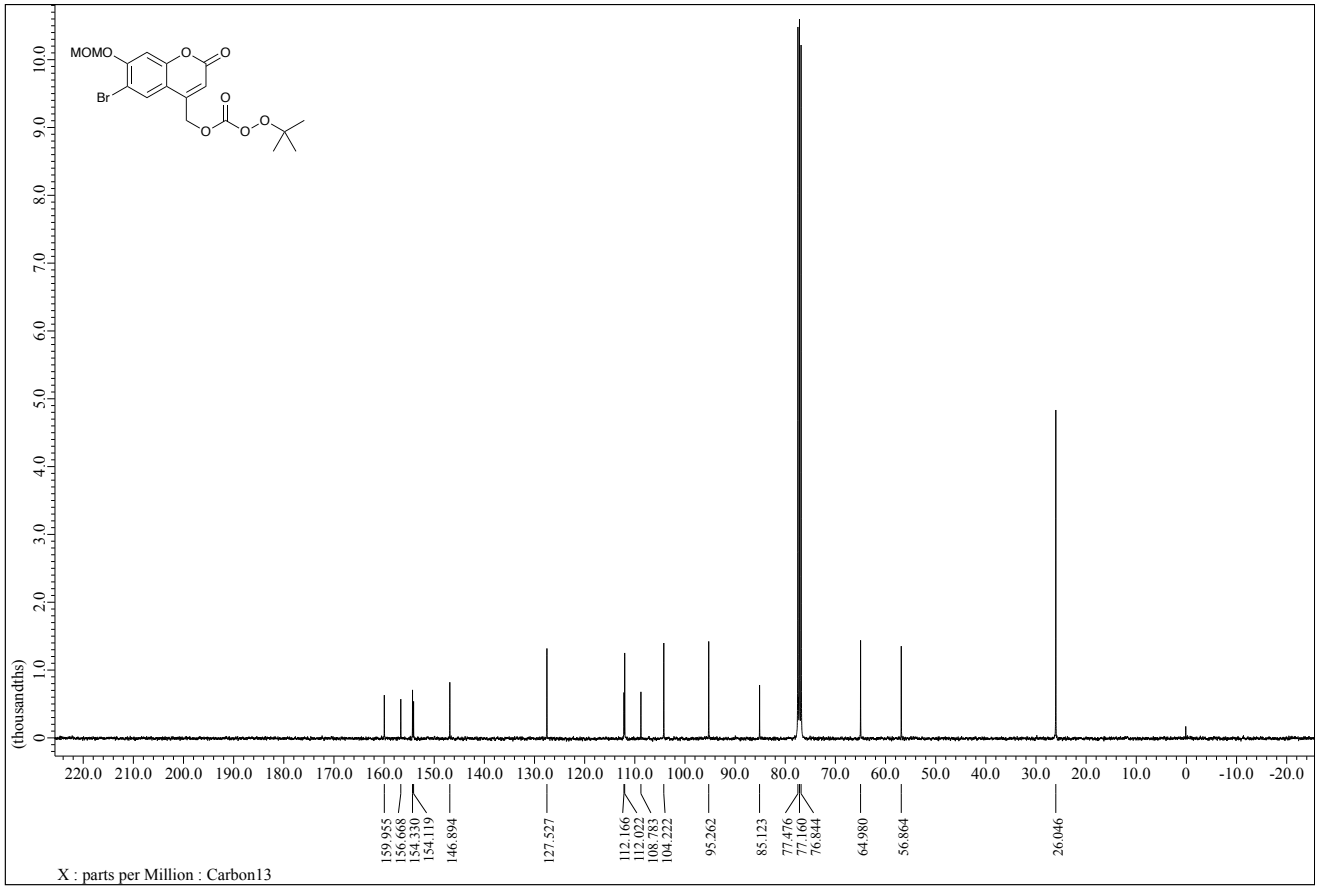


Figure S13. ^{13}C NMR spectrum of compound 4 in $CDCl_3$ (100 MHz)

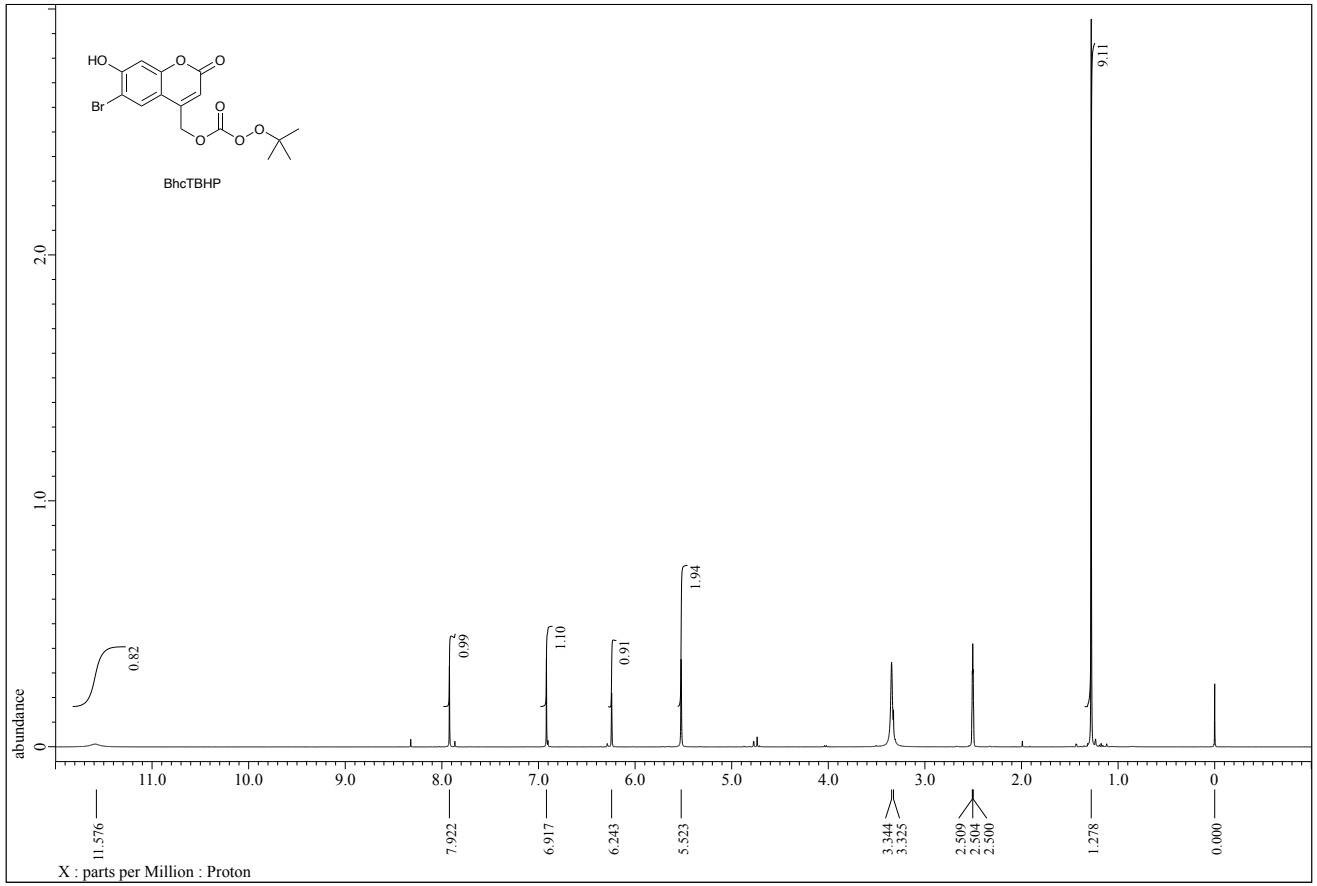


Figure S14. ^{1}H NMR spectrum of BhcTBHP in DMSO-d_6 (400 MHz)

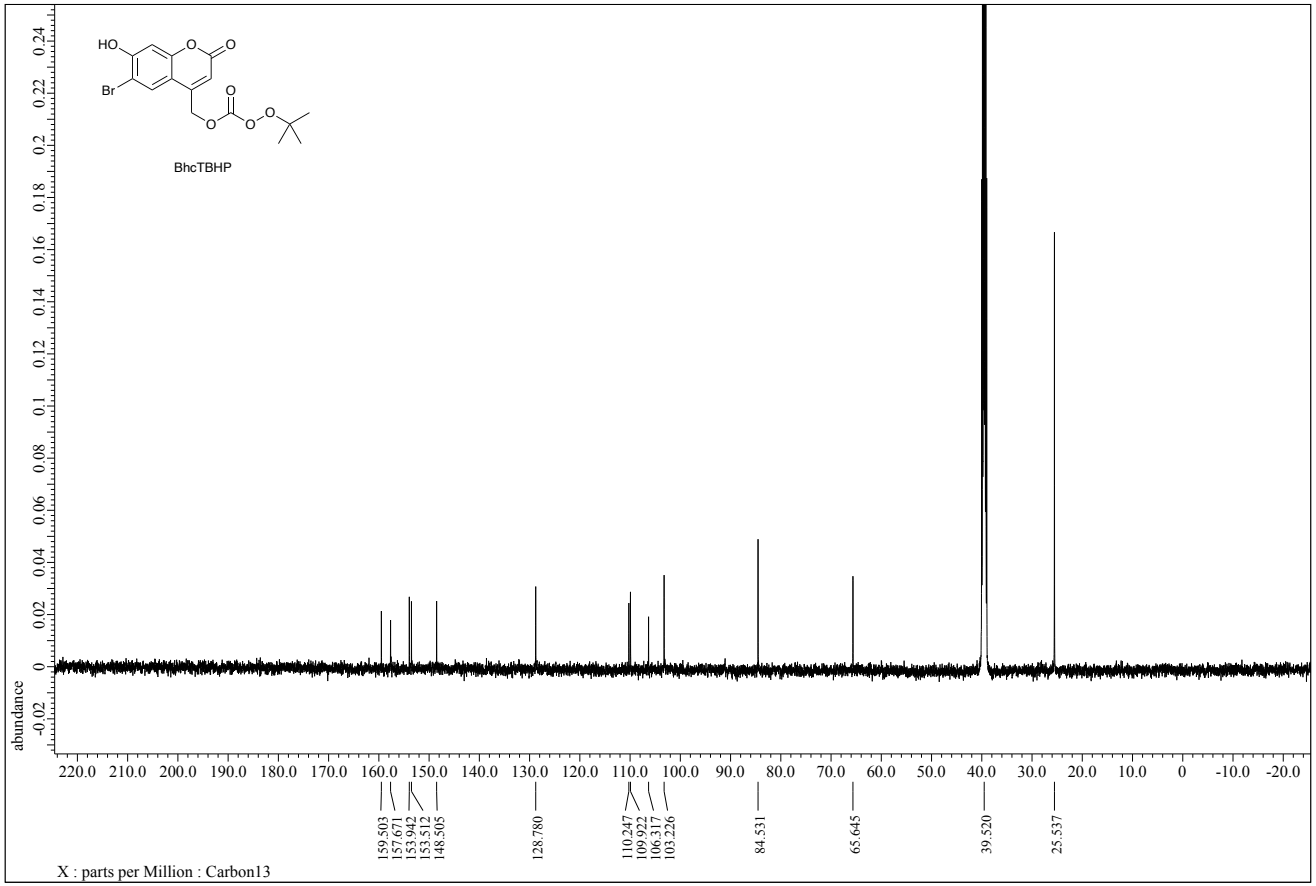


Figure S15. ^{13}C NMR spectrum of BhcTBHP in DMSO-d_6 (125 MHz)

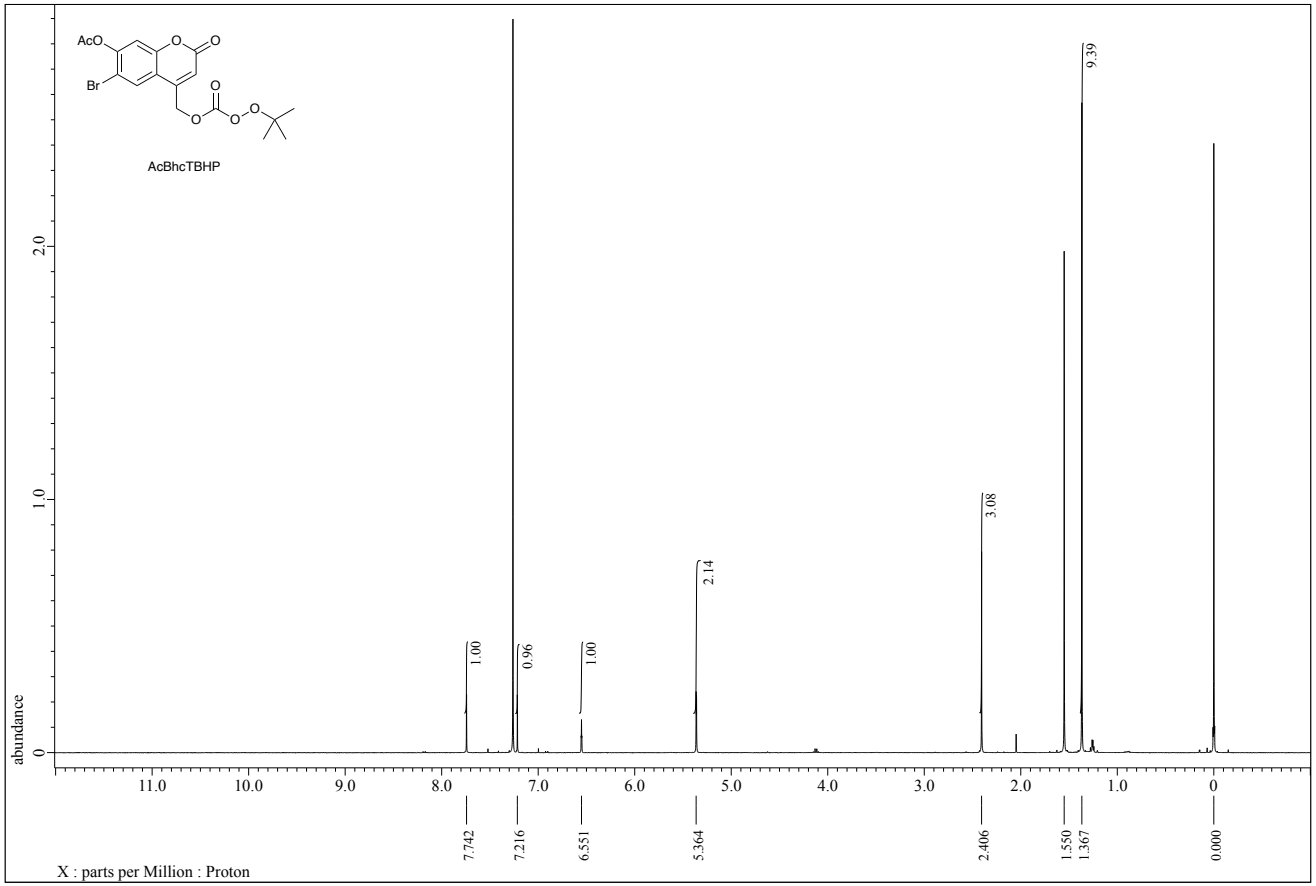


Figure S16. ^{1}H NMR spectrum of AcBhcTBHP in $CDCl_3$ (400 MHz)

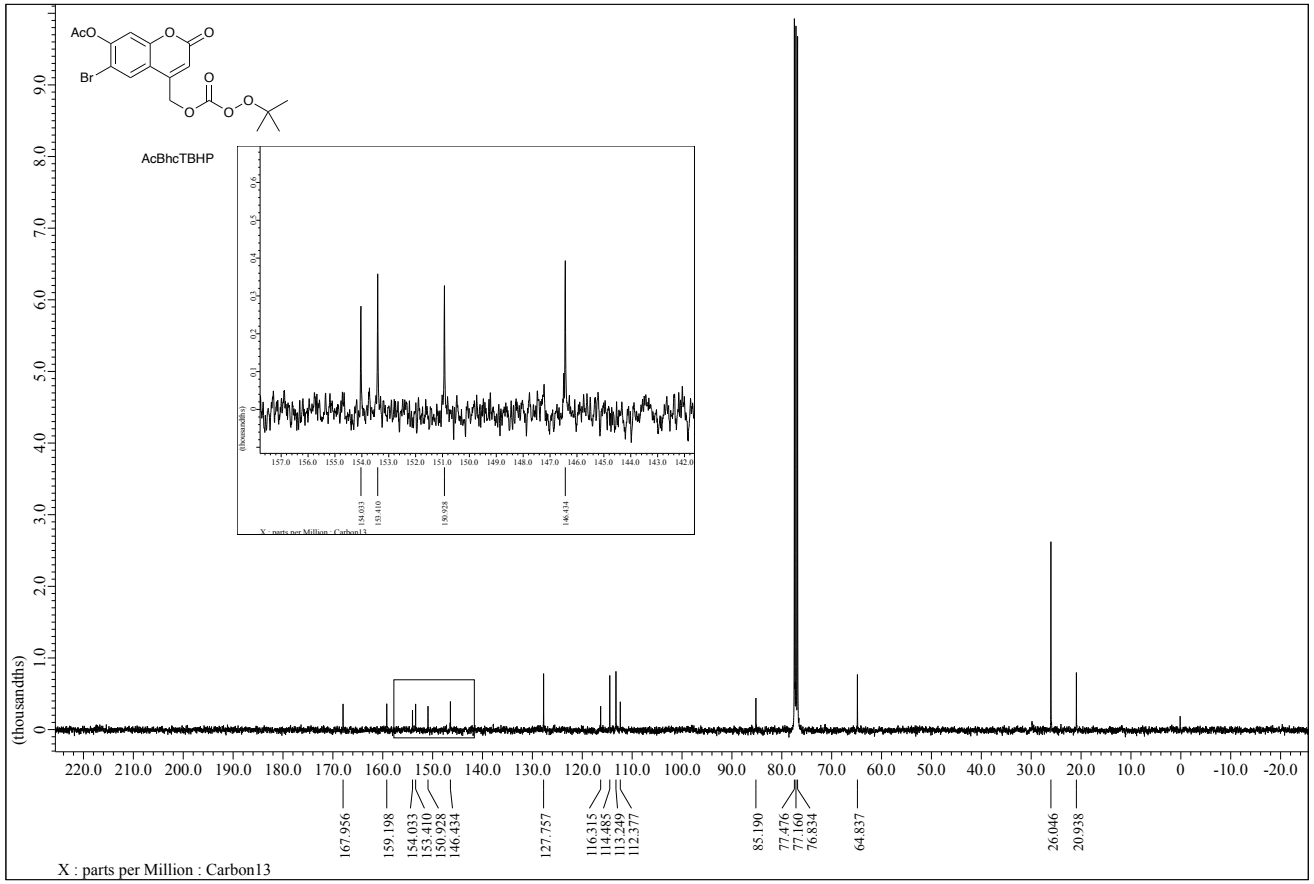


Figure S17. ^{13}C NMR spectrum of AcBhcTBHP in $CDCl_3$ (100 MHz)

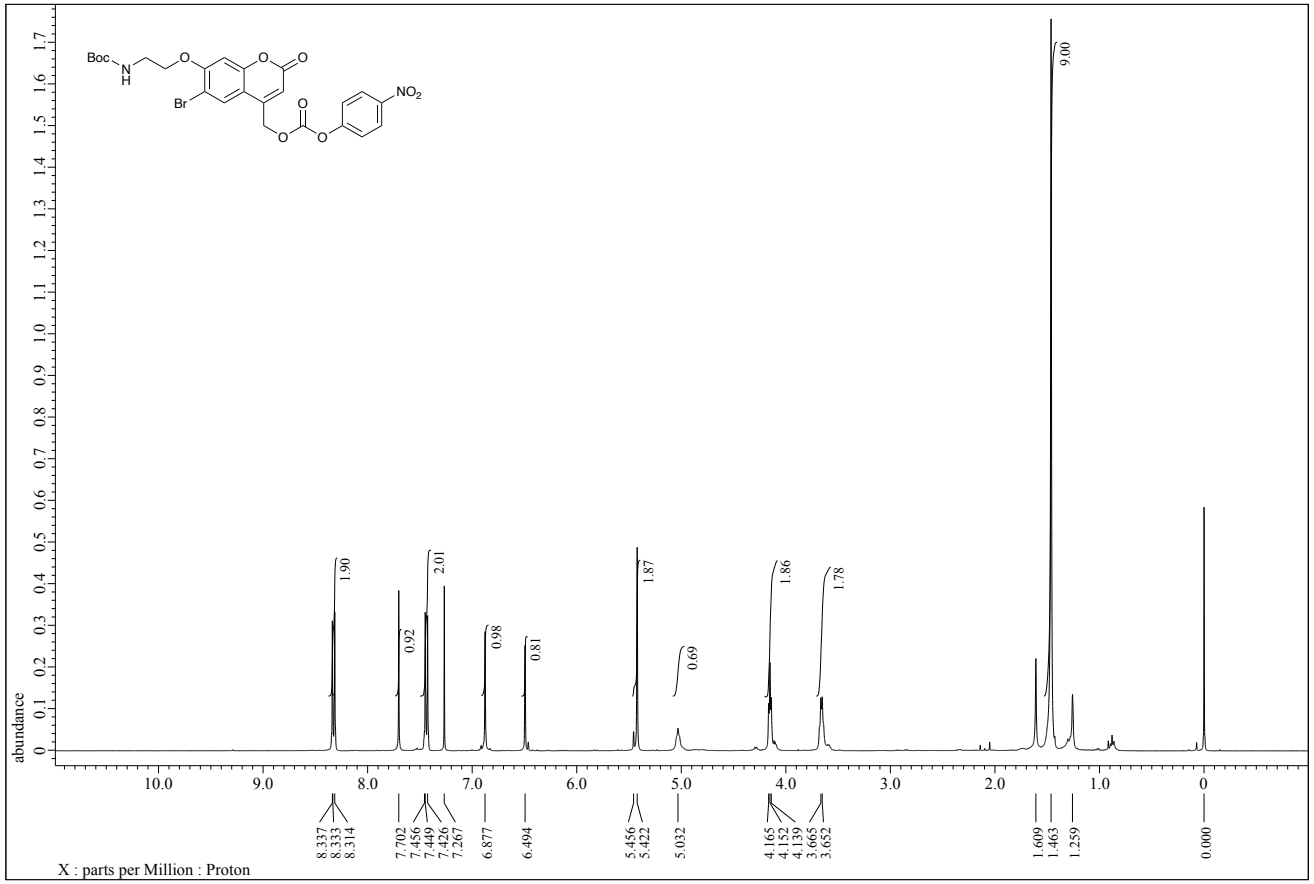


Figure S18. ^{1}H NMR spectrum of compound 7 in $CDCl_3$ (400 MHz)

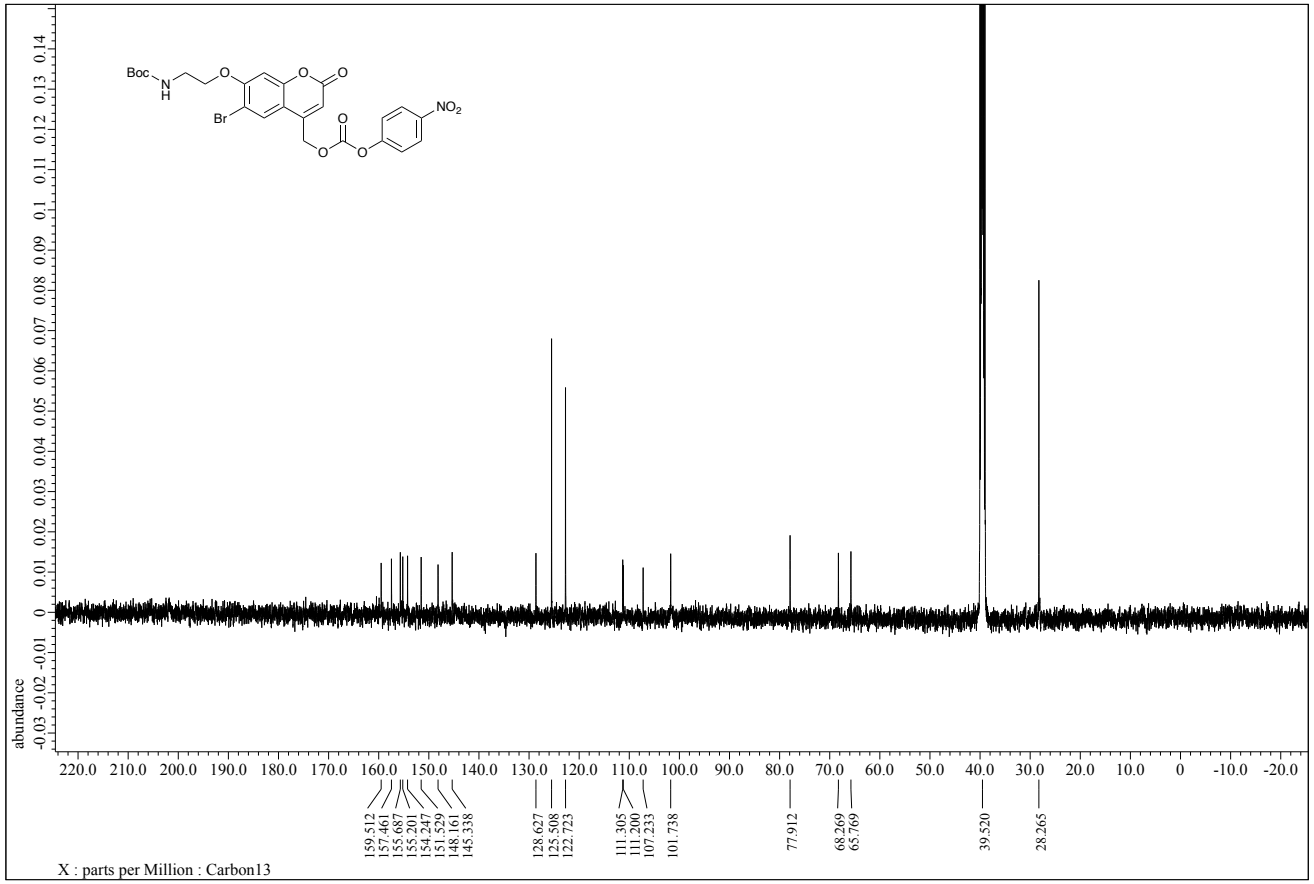


Figure S19. ^{13}C NMR spectrum of compound 7 in DMSO-d_6 (125 MHz)

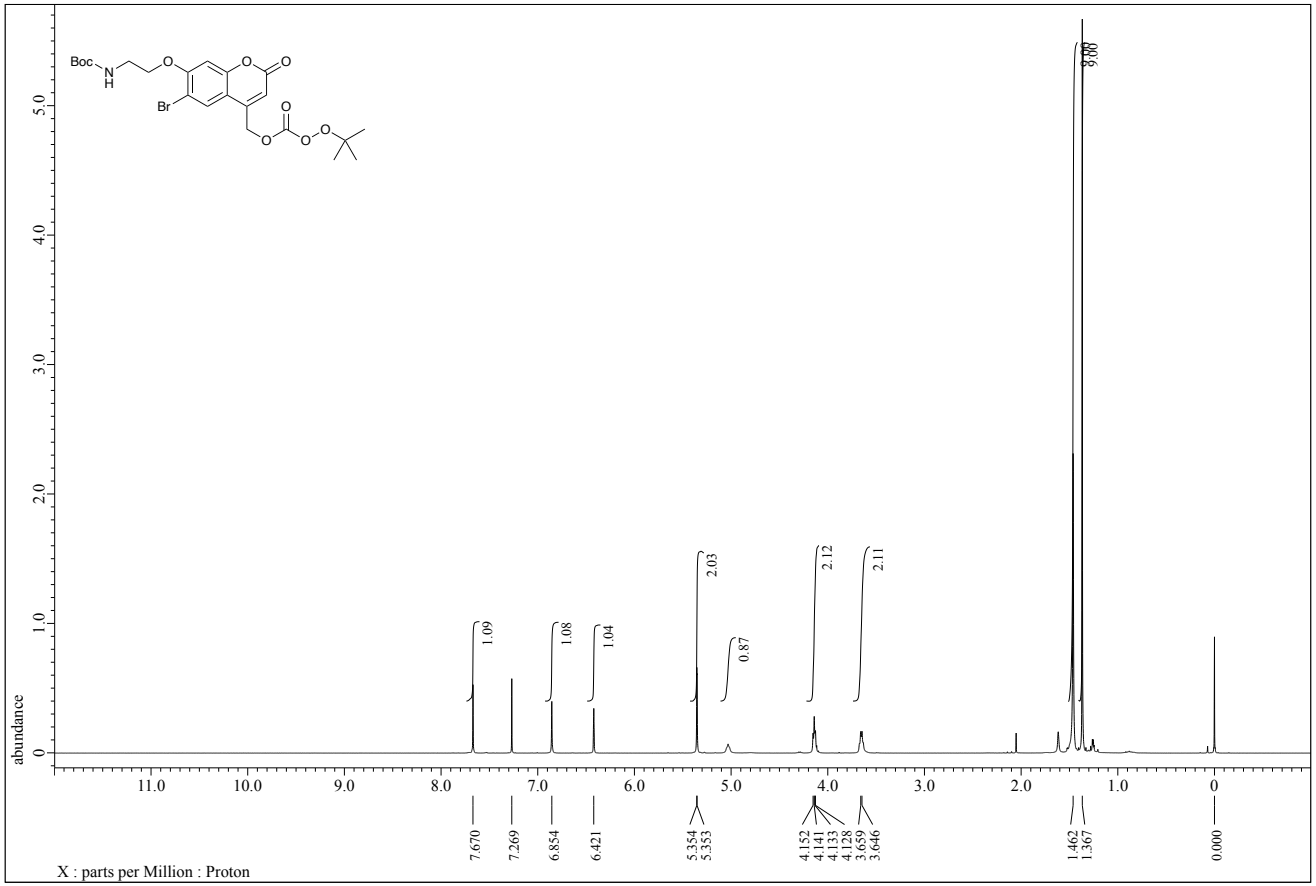


Figure S20. ^{1}H NMR spectrum of compound 8 in $CDCl_3$ (400 MHz)

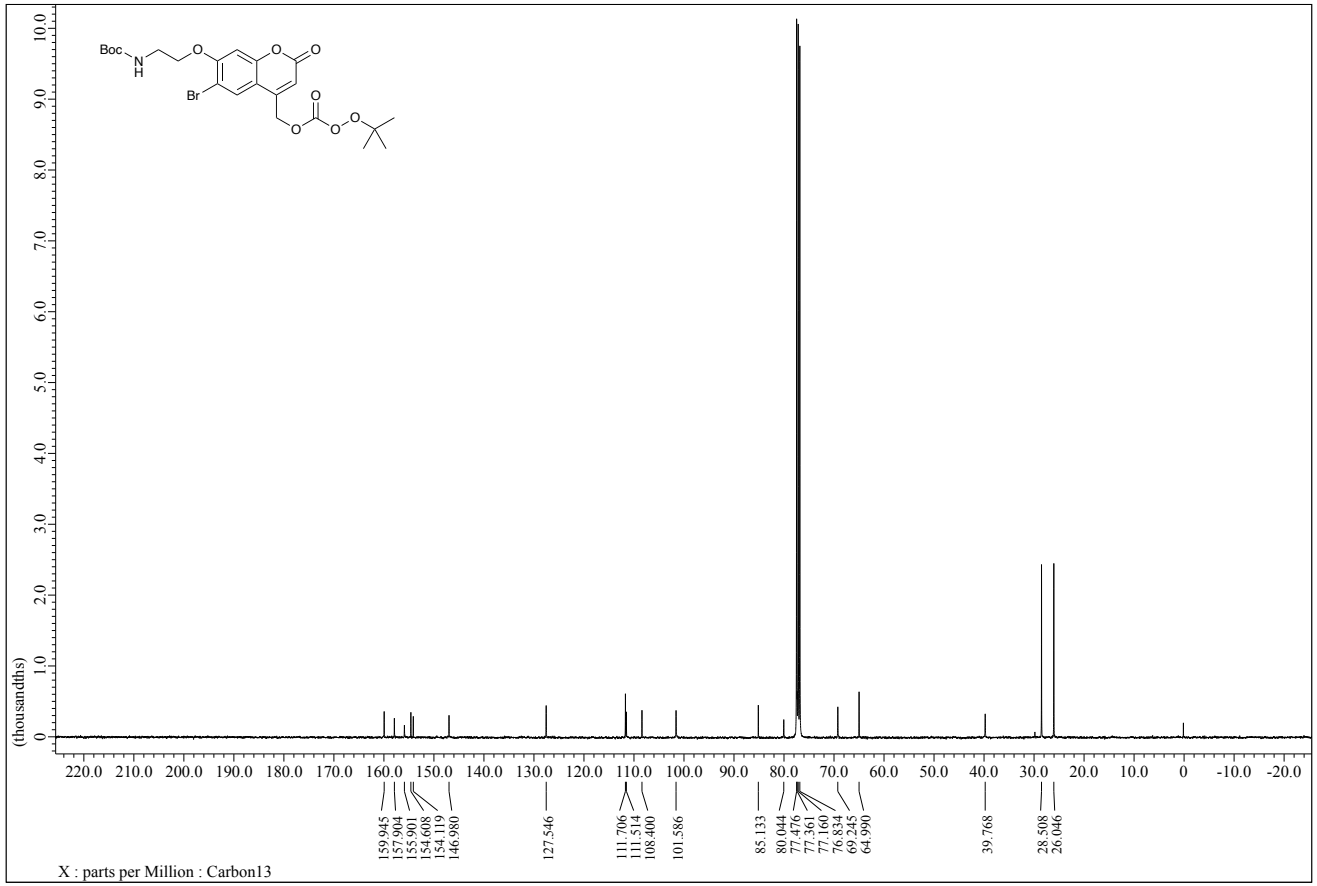


Figure S21. ^{13}C NMR spectrum of compound 8 in $CDCl_3$ (100 MHz)

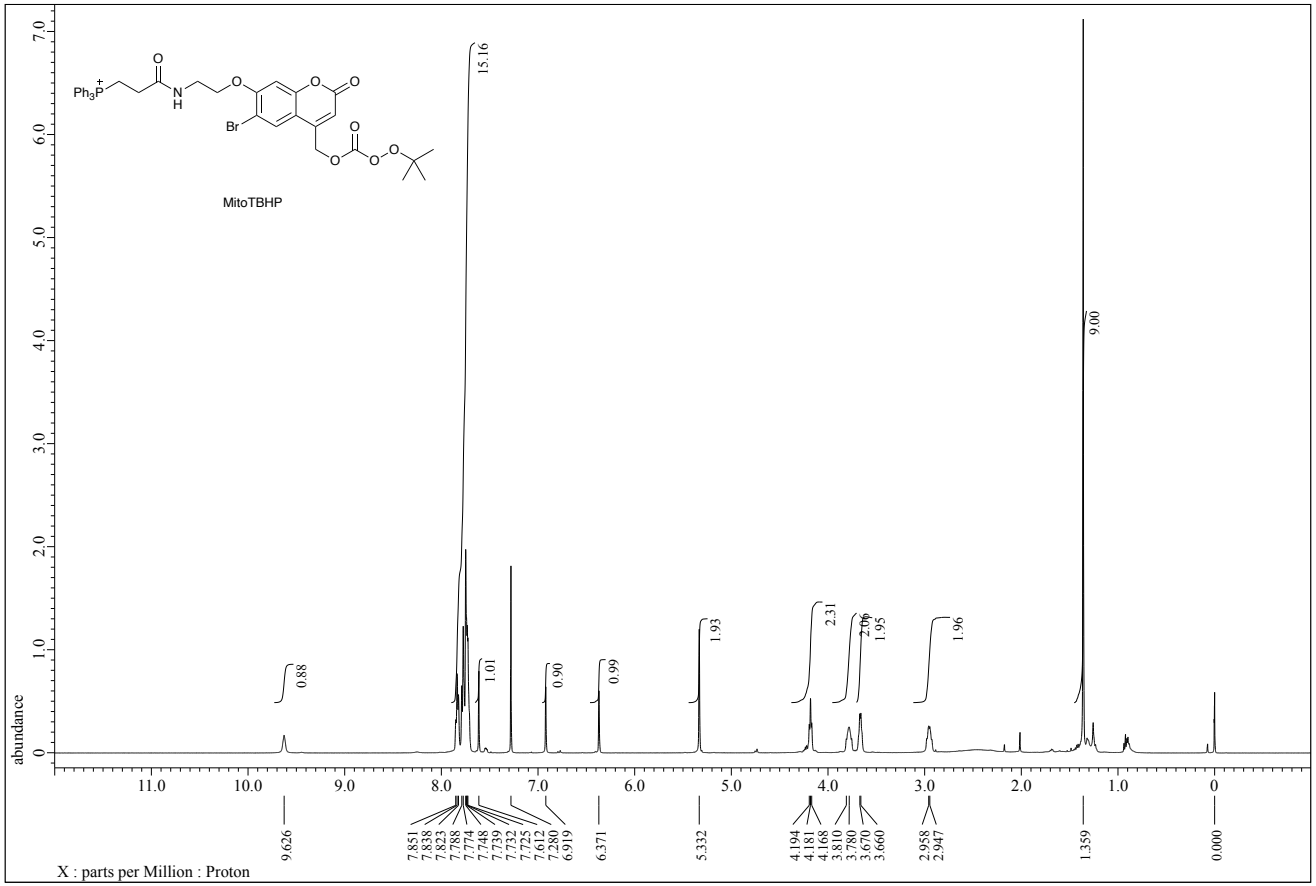


Figure S22. ^{1}H NMR spectrum of MitoTBHP in $CDCl_3$ (500 MHz)

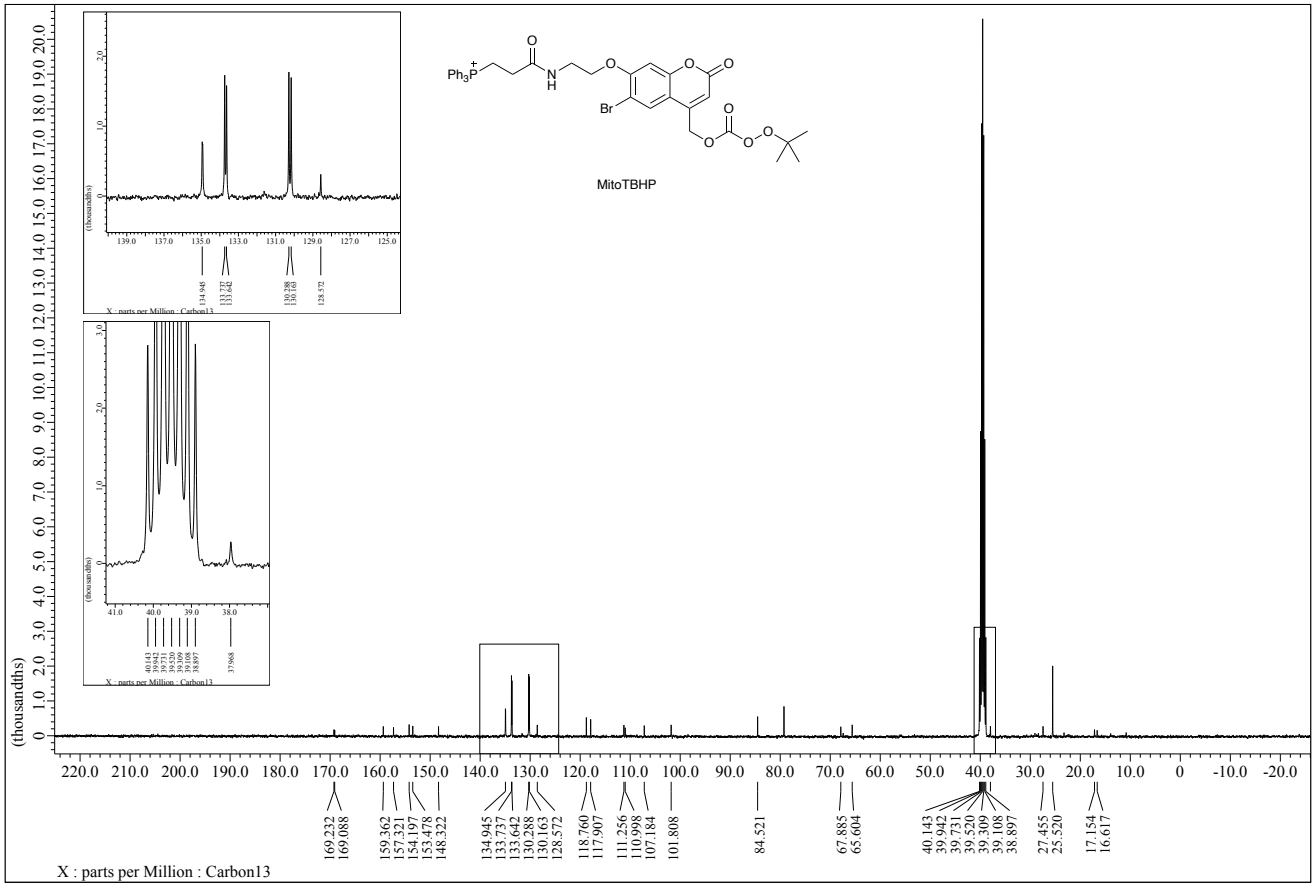


Figure S23. ^{13}C NMR spectrum of MitoTBHP in DMSO-d_6 (100 MHz)

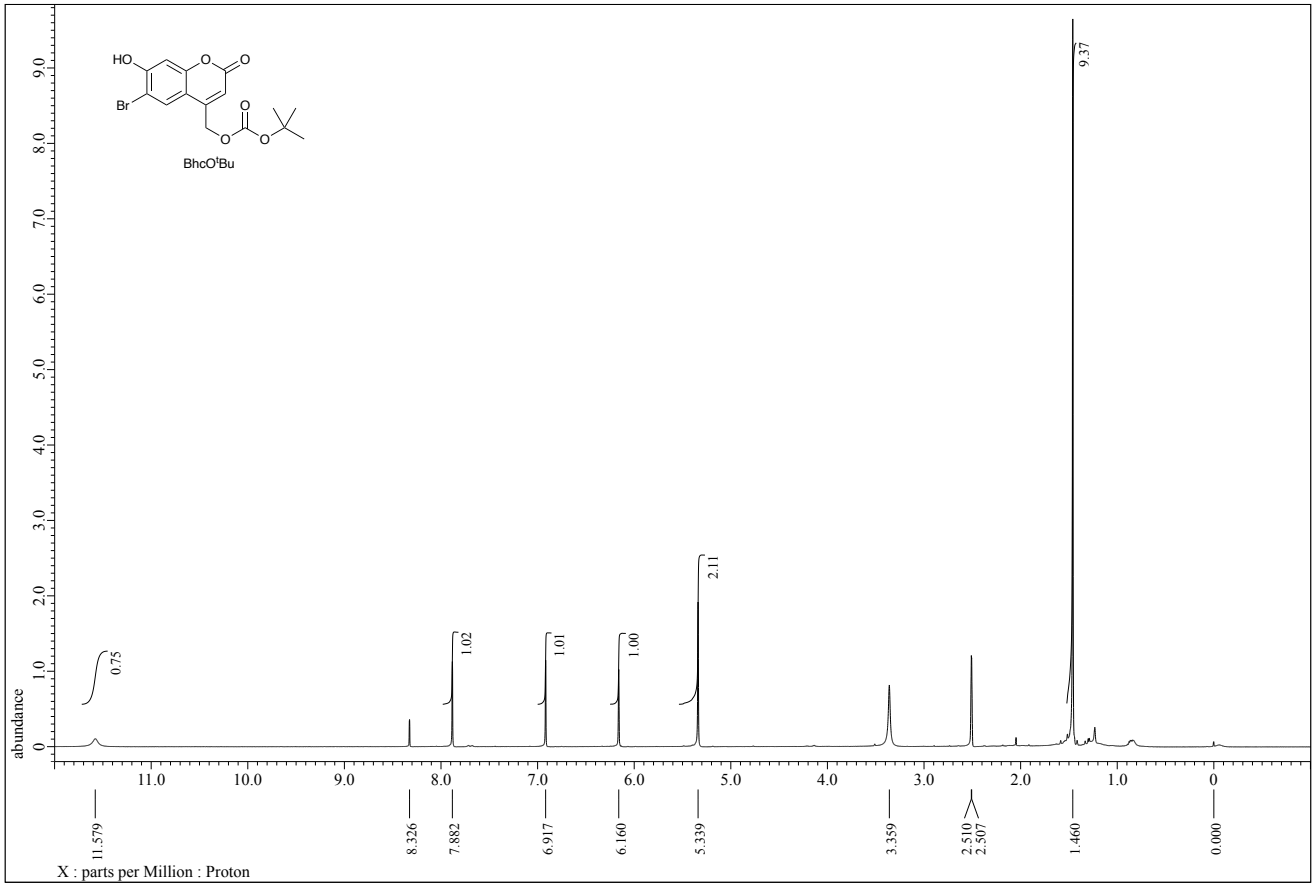


Figure S24. ^{1}H NMR spectrum of BhcO^{t}Bu in DMSO-d_6 (500 MHz)

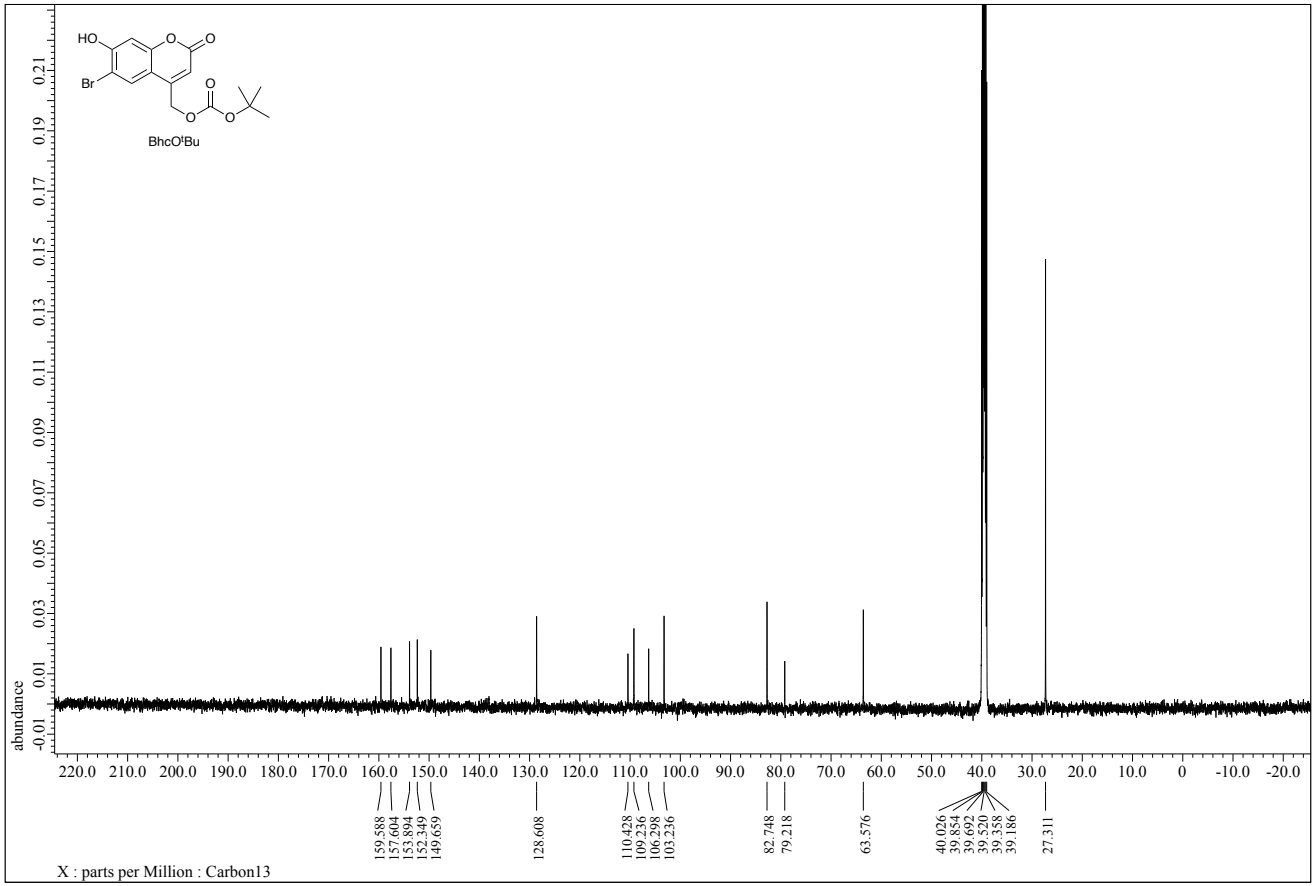


Figure S25. ^{13}C NMR spectrum of BhcO^{t}Bu in DMSO-d_6 (125 MHz)

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