Supporting Information

A novel chemiluminescent probe for hydrazine detection in water and HeLa cells.

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1. Experimental Section

General information

All of the reagents are analytically sourced from commercial reagent companies and do not require further purification for direct use. The water used in the laboratory comes from the Milli-Q reference system. Thin-layer chromatography (TLC) and silica gel for column chromatography comes from Qingdao Marine chemical plant (200-300 mesh). The spectra of chemiluminescent and fluorescence were analyzed using Molecular Devices SpectraMax M5. Spectrometer. NMR spectra were obtained on an Bruker Avance 400 MHz， Bruker Avance 600 MHz apparatus. 96-well black plate imaging was used with Bio-rad Biorad Gel Doc XR. Cell imaging was performed using Axio Observer D1.

Cell culture and fluorescence imaging

Adenocarcinoma of human alveolar basal epithelial cells (HeLa cells) were cultured in DMEM high glucose media supplemented with 10% fetal bovineserum, 1% Penstrep, 0.2% Amphotericin B. The cells were grown overnight at 37°C incubator with 5% CO_2. HeLa were seeded at a density of 0.3x106 cells in 35 mm dish and kept overnight prior to cell imaging studies.

Synthetic route

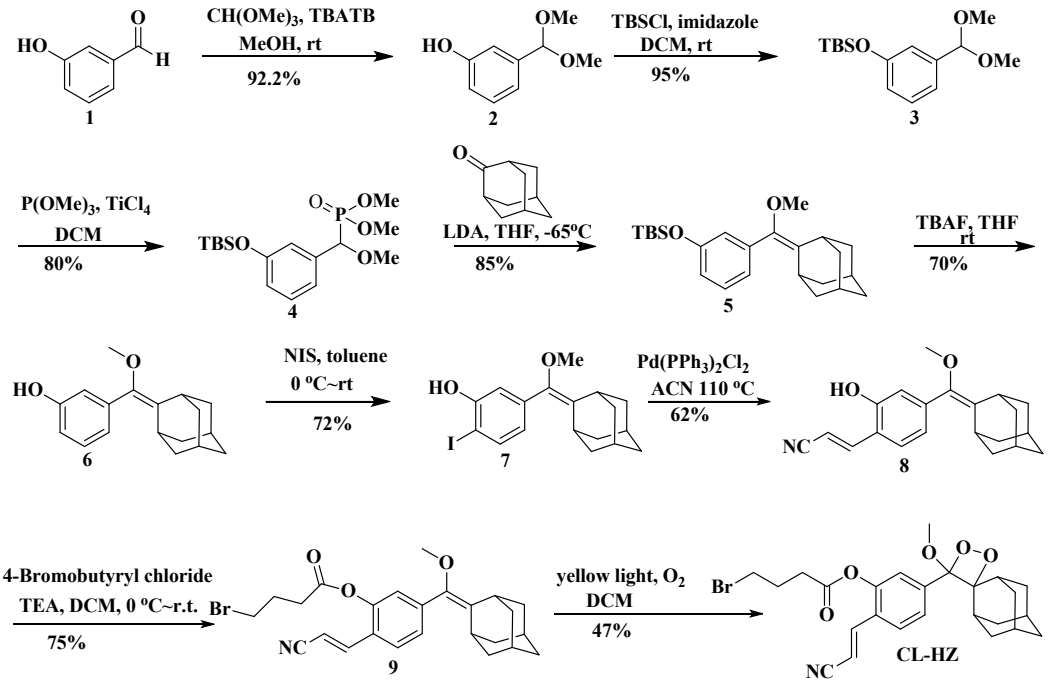
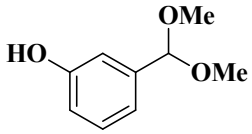


Figure S1. Synthetic route of **CL-HZ**

2. The synthesis process and Characterization Data of the Compounds

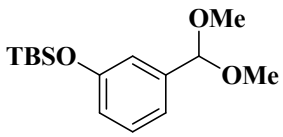
3-(dimethoxymethyl)phenol

3-(dimethoxymethyl)phenol was synthesized according to previous reported. 3-hydroxybenzaldehyde (2441 mg, 20 mmol) was dissolved in 30 ml of methanol. Trimethyl orthoformate (3180mg, 30 mmol) and tertrabutylammonium tribromide (482 mg, 1 mmol) were added and the solution was stirred overnight at room temperature. The reaction was monitored by TLC. After completion, the reaction mixture was diluted with EtOAc (100 ml) and washed with 0.01M $NaHCO_3$ (120 ml), aq NaCl (100ml). Organic phase was dried over with Na_2SO_4 and concentrated under reduced pressure. Purification by column chromatography (diluting with Pet:EtOAc= 80:20) afforded 3110mg (92.2% yield) of colorless oil.



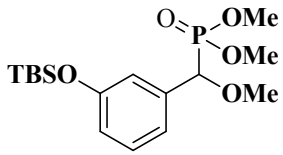
tert-butyl(3-(dimethoxymethyl)phenoxy)dimethylsilane

3-(dimethoxymethyl)phenol (3110mg, 18.45 mmol) and imidazole (1.88 g, 27.6 mmol) were dissolved in 20 ml of DCM. TBDMSCl (3336 mg, 22.14 mmol) was added and the solution was stirred at room temperature for 60 minutes and monitored by TLC. After completion, the white precipitate was filtered off and the solvent was evaporated under reduced pressure. Purification by column chromatography (Diluting with Pet:EtOAc =95:5) afforded compound 3 as a colorless oil (4945 mg, 95% yield). 1H NMR (500 MHz, MeOD) δ 7.25 (t, J = 7.9 Hz, 1H), 7.03 (d, J = 7.9 Hz, 1H), 6.95 – 6.90 (m, 1H), 6.82 (ddd, J = 7.9, 2.5, 0.9 Hz, 1H), 5.34 (s, 1H), 3.32 (s, 6H), 1.01 (s, 9H), 0.21 (s, 6H). MS (ESI^+): Calculated for C15H26O3Si: $[M+H]^+$ 282.17，found 283.11



dimethyl((3-((tert-butyldimethylsilyl)oxy)phenyl)(methoxy)methyl)phosphonate

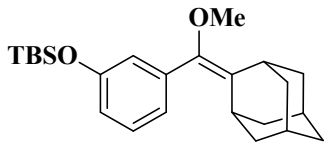
tert-butyl(3-(dimethoxymethyl)phenoxy)dimethylsilane (4945 mg, 17.52 mmol) and trimethyl phosphite (3261 mg, 26.29 mmol) were dissolved in 50 ml of DCM. The reaction mixture was cooled to 0℃ and titanium (IV) chloride (4986 mg, 26.29 mmol) was dropwised to the reaction mixture. The mixture was stirred for 30 minutes and monitored by TLC. After completion, the solution was poured into a aq$NaHCO_3$ (150 ml) at 0℃. Stirring for 10 minutes, 150 ml of DCM was added and the phases were separated. Organic phase was dried over with Na_2SO_4 and concentrated under reduced pressure. Purification by column chromatography (Hex:EtOAc 30:70) afforded compound 4 as a colorless oil (5385 mg, 80% yield). 1H NMR (500 MHz, MeOD) δ 7.29 (t, J = 7.8 Hz, 1H), 7.05 (d, J = 7.8 Hz, 1H), 6.98 (d, J = 1.4 Hz, 1H), 6.87 (d, J = 7.8 Hz, 1H), 4.68 (d, J = 15.6 Hz, 1H), 3.77 (d, J = 10.5 Hz, 3H), 3.67 (d, J = 10.5 Hz, 3H), 3.37 (s, 3H), 1.02 (s, 9H), 0.24 (s, 6H). MS (ESI^+): Calculated for C16H29O5PSi: $[M+H]^+$ 360.15，found 361.33



(3-((1r,3r,5R,7S)-adamantan-2-ylidene(methoxy)methyl)phenoxy)(tert-butyl)dimethylsilane

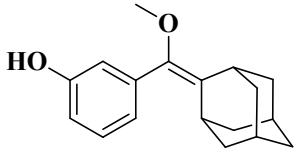
dimethyl((3-((tert-butyldimethylsilyl)oxy)phenyl)(methoxy)methyl)phosphonate (5385 mg, 14.01 mmol) was dissolved in 40 ml of anhydrous THF under N2 atmosphere at -65℃(with dry ice acetone bath). LDA (2.0 M in THF, 8.4 ml, 16.8 mmol) was added and the solution was stirred for 20 minutes. A solution of 2-adamantanone (2520 mg, 16.8 mmol) in 20 ml of THF was added, and after 15 minutes of stirring at -65℃, the reaction was removed to room temperature and stirred

for additional 30 minutes. Reaction was monitored by TLC. Ater completion, reaction mixture was diluted with EtOAc (160 ml) and washed with brine (160 ml). Organic phase was dried over with Na_2SO_4 and concentrated under reduced pressure. Purification by column chromatography (diluting with Pet/EtOAc95:5) afforded compound 5 as a colorless oil (4569 mg, 85% yield). 1H NMR (500 MHz, $CDCl_3$) δ 7.40 (d, J = 7.7 Hz, 1H), 7.31 – 7.25 (m, 2H), 6.99 (d, J = 7.70 Hz, 1H), 3.54 – 3.20 (m, 4H), 2.30 (s, 2H), 2.09 – 1.69 (m, 12H), 1.57 (d, J = 12.5 Hz, 1H), 0.99 (s, 9H), 0.21 (s, 6H). MS (ESI^+): Calculated for C24H36O2Si: $[M+H]^+$ 384.25， found 385.33



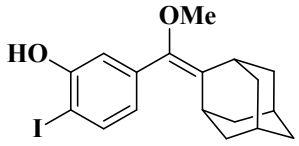
3-((1r,3r,5R,7S)-adamantan-2-ylidene(methoxy)methyl)phenol

(3-((1r,3r,5R,7S)-adamantan-2-ylidene(methoxy)methyl)phenoxy)(tert-butyl)dimethylsilane (4569 mg, 11.9 mmol) was dissolved in 40 ml of THF. TBAF (1.0 M in THF, 14.28 ml, 14.28 mmol) was added and the solution was stirred at room temperature for 30 minutes. The reaction was monitored by TLC. After completion, reaction mixture was diluted with EtOAc (150 ml) and washed with aq NaCl (100 ml). Organic phase was dried over with Na_2SO_4 and concentrated under reduced pressure. Purification by column chromatography (diluting with Pet:EtOAc 85:15) afforded compound 6 as white solid (2250 mg, 70% yield). 1H NMR (500 MHz, $CDCl_3$) δ 7.21 (t, J = 7.6 Hz, 1H), 6.88 (d, J = 7.6 Hz, 1H), 6.85 – 6.82 (m, 1H), 6.79 – 6.75 (m, 1H), 5.30 (s, 1H), 3.32 (s, 3H), 3.24 (s, 1H), 2.65 (s, 1H), 2.01 – 1.88 (m, 4H), 1.88 – 1.68 (m, 9H). MS (ESI^+): Calculated for C18H22O2: $[M+H]^+$ 270.16， found 271.18



5-((1r,3r,5R,7S)-adamantan-2-ylidene(methoxy)methyl)-2-iodophenol

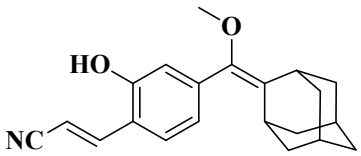
3-((1r,3r,5R,7S)-adamantan-2-ylidene(methoxy)methyl)phenol (2250 mg, 8.3 mmol) was dissolved in 150 ml of Toluene and cooled to 0℃. NIS (2249 mg, 10 mmol) was added in portions. After added, the reaction mixture was removed to room temperature for 3 hours and monitored by TLC. After completion, reaction was quenched with saturated $Na_2S_2O_3$, diluted with EtOAc (200 ml) and washed with aq NaCl (200 ml). The organic phase was dried over with Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (diluting with Pet:EtOAc 85:15) to afford compound 7 as a white solid (2366 mg , 72% yield). 1H NMR (500 MHz, $CDCl_3$) δ 7.63 (d, J = 8.1 Hz, 1H), 6.97 (d, J = 1.9 Hz, 1H), 6.67 (dd, J = 8.1, 1.9 Hz, 1H), 3.32 (s, 3H), 3.24 (s, 1H), 2.65 (s, 1H), 2.02 – 1.90 (m,4H), 1.87 – 1.68 (m, 8H). MS (ESI^+): Calculated for C18H21IO2: $[M+H]^+$ 396.08，found 397.18



(E)-3-(4-((1r,3r,5R,7S)-adamantan-2-ylidene(methoxy)methyl)-2-hydroxyphenyl)acrylonitrile

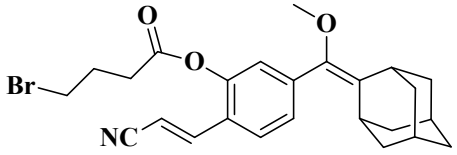
5-((1r,3r,5R,7S)-adamantan-2-ylidene(methoxy)methyl)-2-iodophenol (600 mg, 1.51mmol) was disslovd in 10 ml of Acetonitrile. Triethylamine (458 mg, 4.53 mmol), acrylonitrile (240 mg, 4.53mmol) and $Pd(PPh_3)_2Cl_2$ (52.5 mg, 0.075mmol) were added. The solution was thoroughly degassed by bubbling of N2. The reaction mixture was stirred at 110℃ for 4 hours under N2 atomsphere. Reaction was monitored by TLC. After completion, the solvent was evaporated under reduced pressure. Purification by column chromatography (diluting with Pet:EtOAc 40:60) afforded 300 mg (62% yield) of pale yellow solid. 1H NMR (500 MHz, $CDCl_3$) δ 7.59 (d, J = 16.7 Hz, 1H), 7.33 (d, J = 8.0 Hz, 1H), 7.28 (s, 1H), 6.91 (dd, J = 8, 1.4 Hz, 1H), 6.88 (d, J =

1.4 Hz, 1H), 6.27 (s, 1H), 6.17 (d, J = 16.7 Hz, 1H), 3.35 (s, 3H), 3.22 (s, 1H), 2.70 (s, 1H), 2.02 – 1.91 (m, 4H), 1.89 – 1.73 (m, 8H). MS (ESI^+): Calculated for C21H23NO2: $[M+H]^+$ 321.17， found 322.18



5-((1r,3r,5R,7S)-adamantan-2-ylidene(methoxy)methyl)-2-((E)-2-cyanovinyl)phenyl 4-bromobutanoate

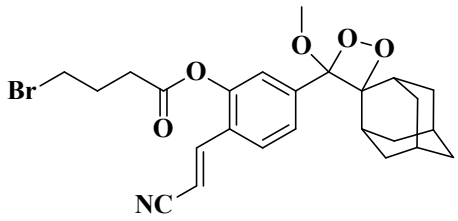
(E)-3-(4-((1r,3r,5R,7S)-adamantan-2-ylidene(methoxy)methyl)-2-hydroxyphenyl)acrylonitrile (100 mg, 0.31mmol) was disslovd in 4 ml of dichloromethane. Triethylamine (45 mg, 0.45 mmol) ws added and cooled to 0℃. 4-Bromobutyryl chloride (83 mg, 0.45mmol) was dropwised to the reaction mixture. The solution was removed to room temperature for 3 hours and monitored by TLC. After completion, the solvent was evaporated under reduced pressure. Purification by column chromatography (diluting with Pet:EtOAc 20:60) afforded 110 mg (75% yield) of pale yellow solid. 1H NMR (500 MHz, $CDCl_3$) δ 7.53 (d, J = 8.1 Hz, 1H), 7.47 (d, J = 16.7 Hz, 1H), 7.26 (dd, J = 8.1, 1.3 Hz, 1H), 7.13 (d, J = 1.3 Hz, 1H), 5.92 (d, J = 16.7 Hz, 1H), 3.58 (t, J = 6.3 Hz, 2H), 3.33 (s, 3H), 3.26 (s, 1H), 2.89 (t, J = 7.1 Hz, 2H), 2.71 (s, 1H), 2.39 – 2.29 (m, 2H), 2.03 – 1.92 (m, 4H), 1.90 – 1.75 (m, 8H). MS (ESI^+): Calculated for C25H28BrNO3: $[M+H]^+$ 469.13， found 470.18



2-((E)-2-cyanovinyl)-5-((1r,3r,5r,7r)-4'-methoxyspiro[adamantane-2,3'-[1,2]dioxetan]-4'-yl)phenyl 4-bromobutanoate

5-((1r,3r,5R,7S)-adamantan-2-ylidene(methoxy)methyl)-2-((E)-2-cyanovinyl)phenyl 4-bromobutanoate (30 mg, 0.064 mmol) and few milligrams of methylene blue were

dissolved in 6 ml of DCM. Oxygen was bubbled through the solution while irradiating with yellow light for 20 minutes. The reaction was monitored by TLC. After completion, the reaction mixture was concentrated by evaporation under reduced pressure. The crude product was purified by PTLC (with Pet:EtOAc 20:60) afforded 15 mg (47% yield) of white solid. 1H NMR (600 MHz, MeOD) δ 7.87 (d, J = 8.1 Hz, 1H), 7.62 (d, J = 16.7 Hz, 1H), 7.45 – 7.30 (m, 2H), 6.37 (d, J = 16.7 Hz, 1H), 3.62 (t, J = 6.5 Hz, 2H), 3.22 (s, 3H), 2.99 (s, 1H), 2.95 (t, J = 7.1 Hz, 2H), 2.34 – 2.30 (m, 2H), 2.12 (s, 1H), 1.96 – 1.47 (m, 12H). 13C NMR (151 MHz, DMSO) δ 171.13, 166.44, 143.59, 138.38, 127.79, 118.87, 111.31, 100.89, 95.19, 50.34, 36.07, 34.53, 33.03, 32.62, 32.22, 31.54, 27.69, 25.81, 25.66. .HRMS (ESI^+): Calculated for C25H28BrNO5: $[M+H]^+$ 502.1151，found 502.1150.



3. Supporting Figures

The detection limit was calculated based on the fluorescence titration. To determine the S/N ratio, the emission intensity of **CL-HZ** without hydrazine was measured by 11 times and the standard deviation of blank measurements was determined. The detection limit (DL) of **CL-HZ** for hydrazine was determined from the following equation: DL = 3σ / k, σ is the standard deviation of the blank solution; k is the slope of the calibration curve. From the graph we get slope = 3.634× 10^8, and σ value is 113.2 Thus using the formula we get the Detection Limit = 9.345× 10^{-7} M

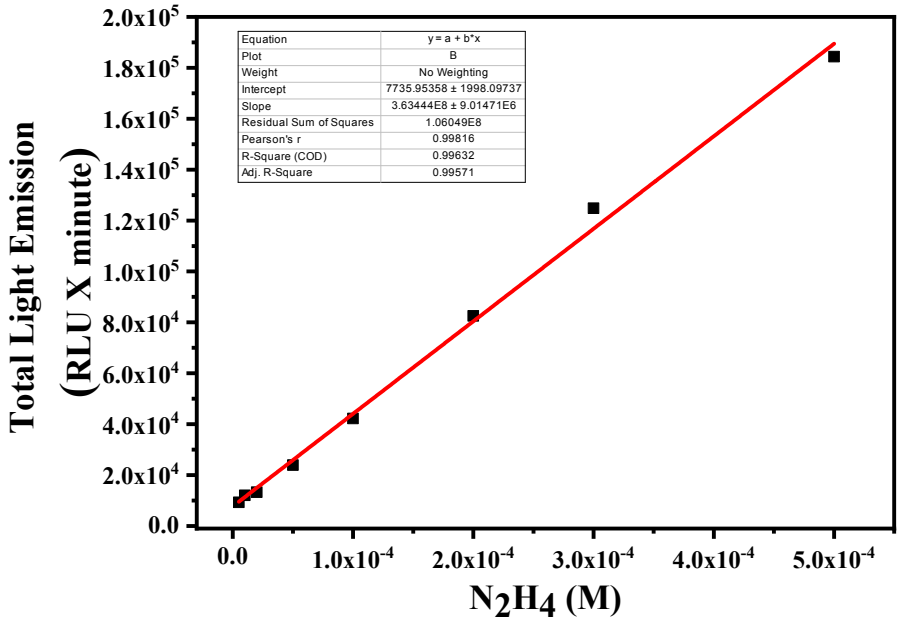


Figure S2. Linear response curve of **CL-HZ** of total light emission intensity depending on the N_2H_4 concentration (**Figure 2b** inset).

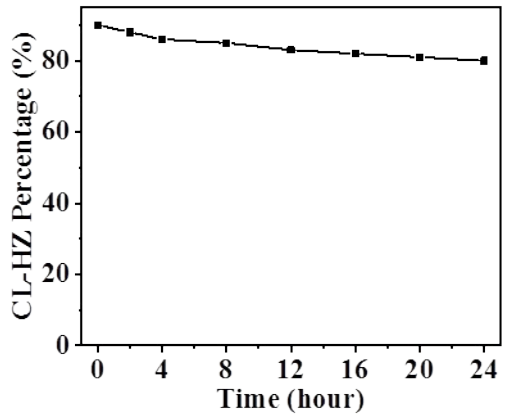


Figure S3. Stability of **CL-HZ** (100 μM) under physiological conditions. Probe was incubated in PBS (pH 7.4, 30% DMSO) at ambient temperature.

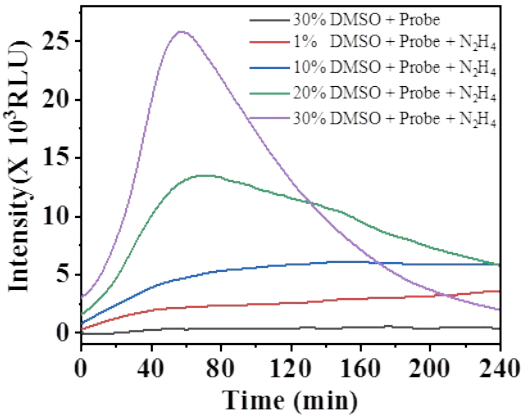


Figure S4. Chemiluminescence kinetic profile of probe CL-HZ (10 μM) in 1%-30% DMSO concentration reacted with 500 μM hydrazine.

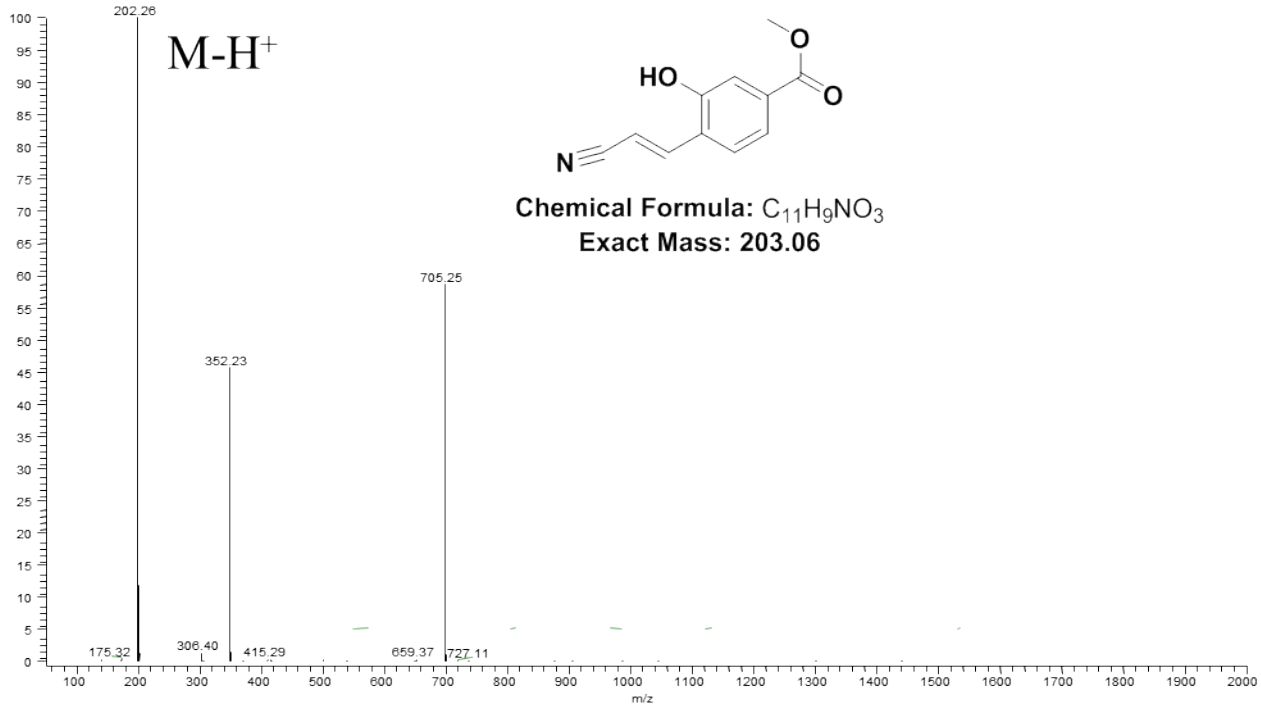


Figure S5. Mass spectrogram of compound D

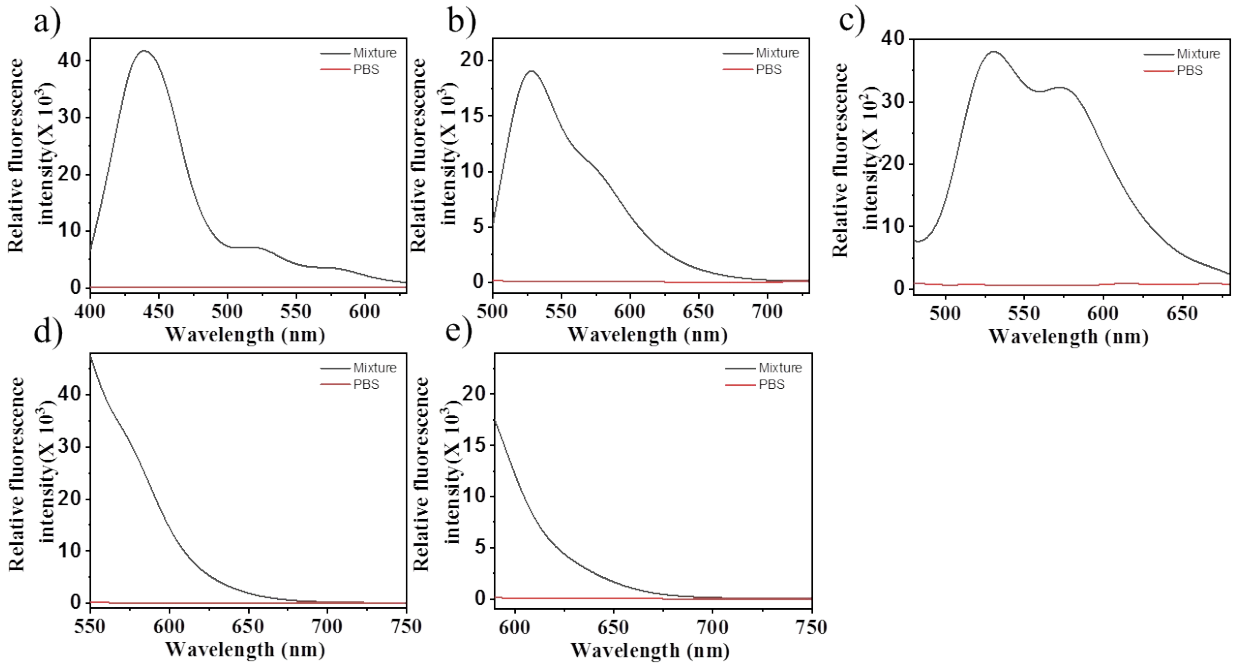


Figure S6. (a) Fluorescence spectral of the mixture in PBS (pH 7.4, 30% DMSO) (Ex= 350nm). (b) Ex= 405nm. (c) Ex= 500nm. (d) Ex= 550nm. (e) Ex= 600nm.The mixture (100 μM 4-Methyl-7-aminocoumarin, 100 μM fluorescein, 100 μM N-Butyl-4-Hydroxyl-1,8-Naphthalimide, 100 μM Rhodamine B, 100 μM Cyanine, 10 μM **CL-HZ**)

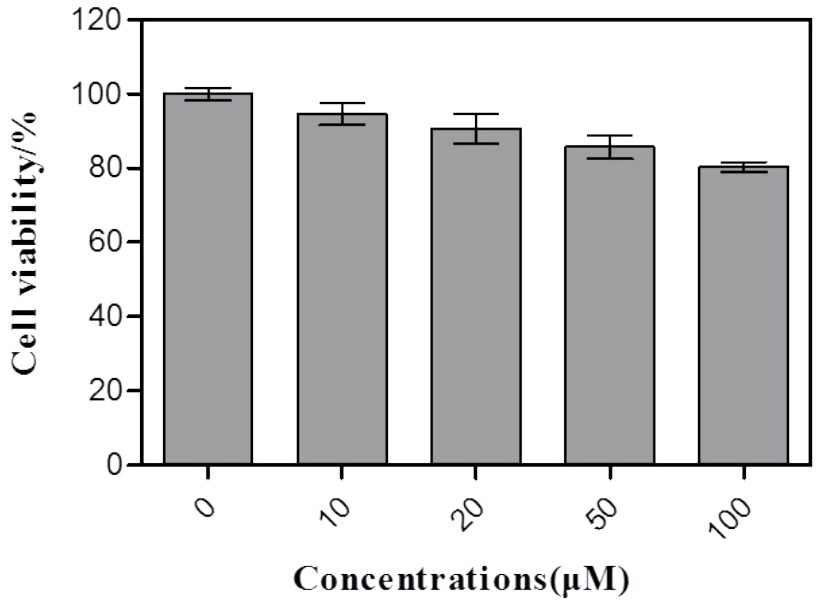
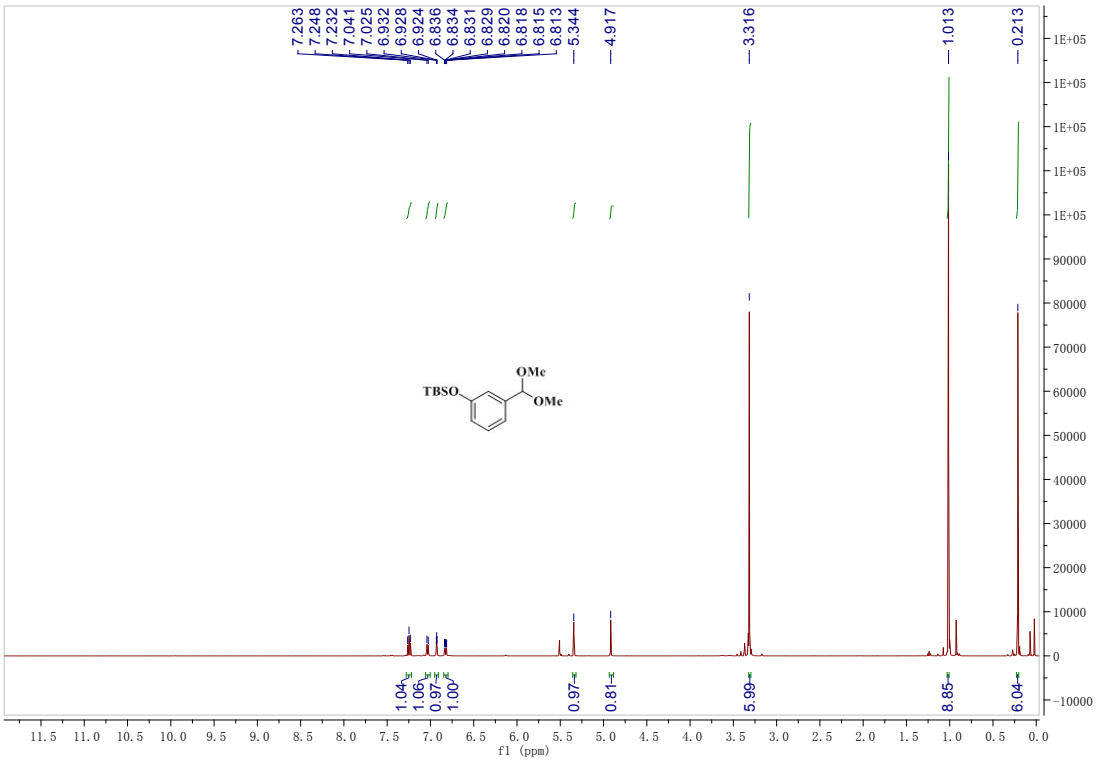
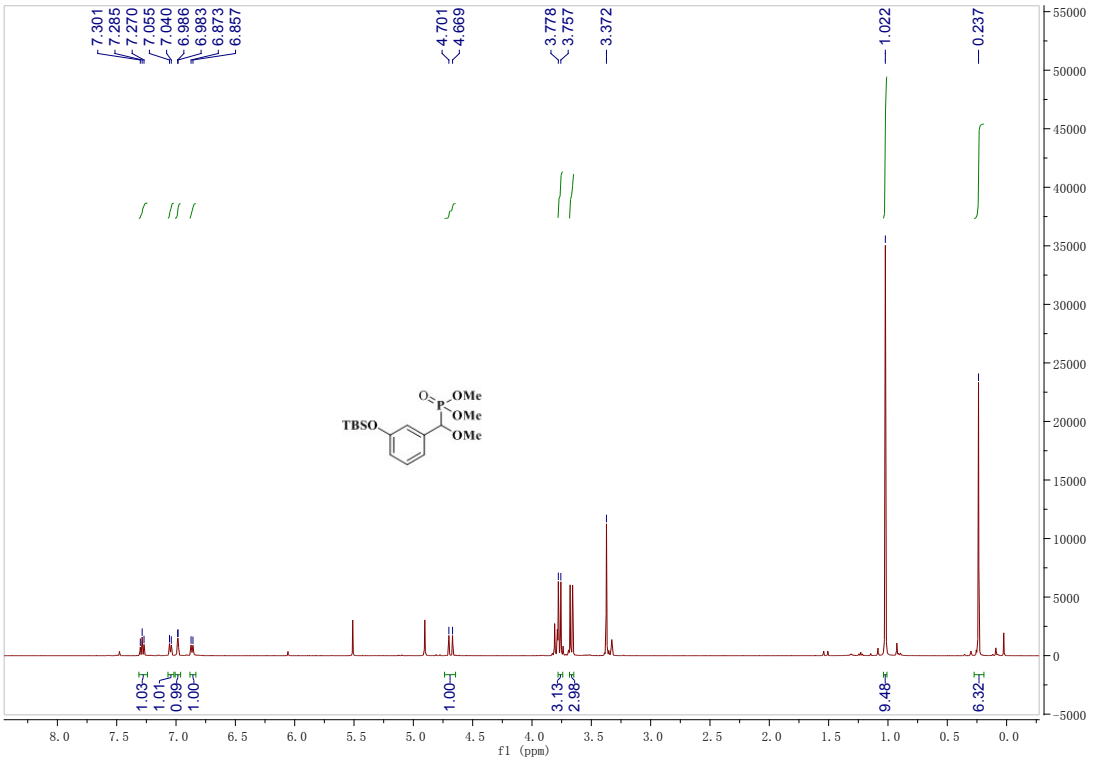
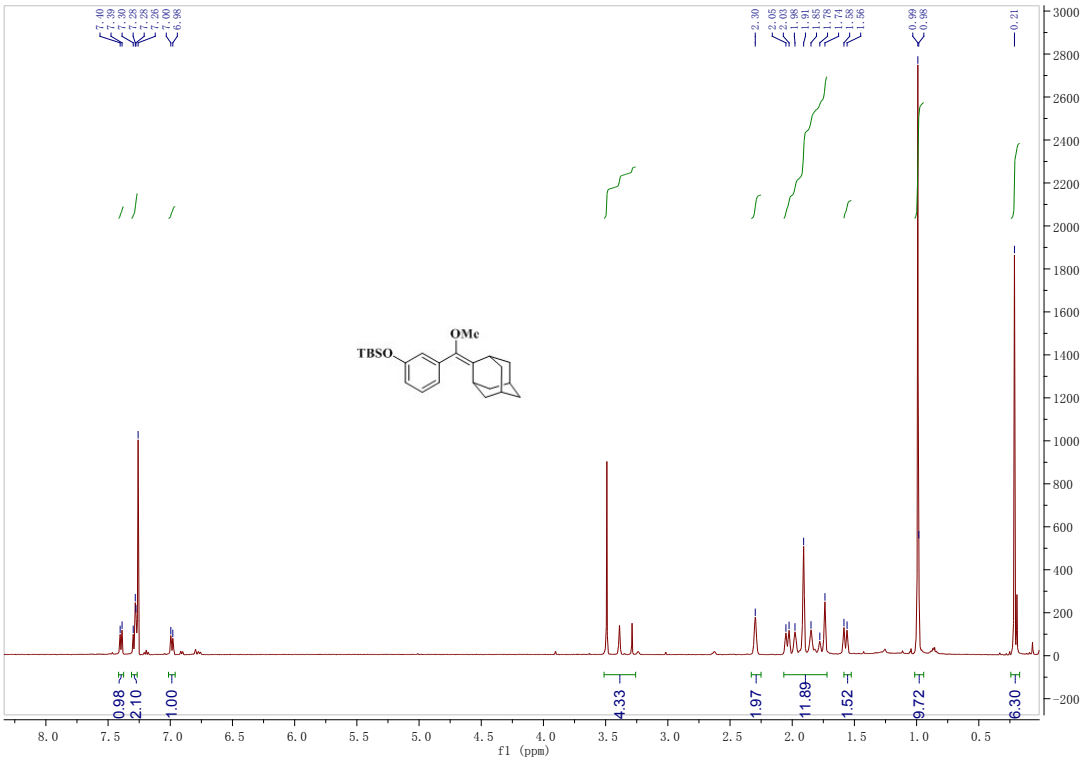


Figure S7. Cell viability of **CL-HZ** in 24 hours by a standard MTT assay (HeLa). The experiments were repeated five times and the data are shown as mean (±S.D.).

4. NMR Spectra



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OMe
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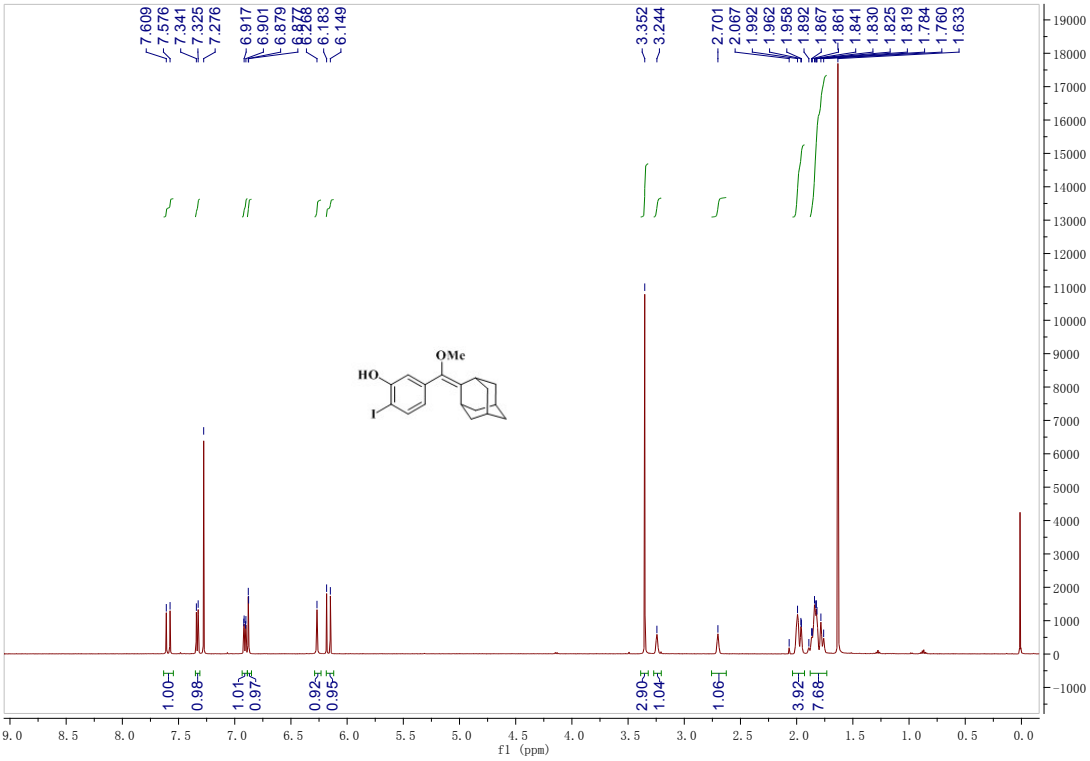
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OMe
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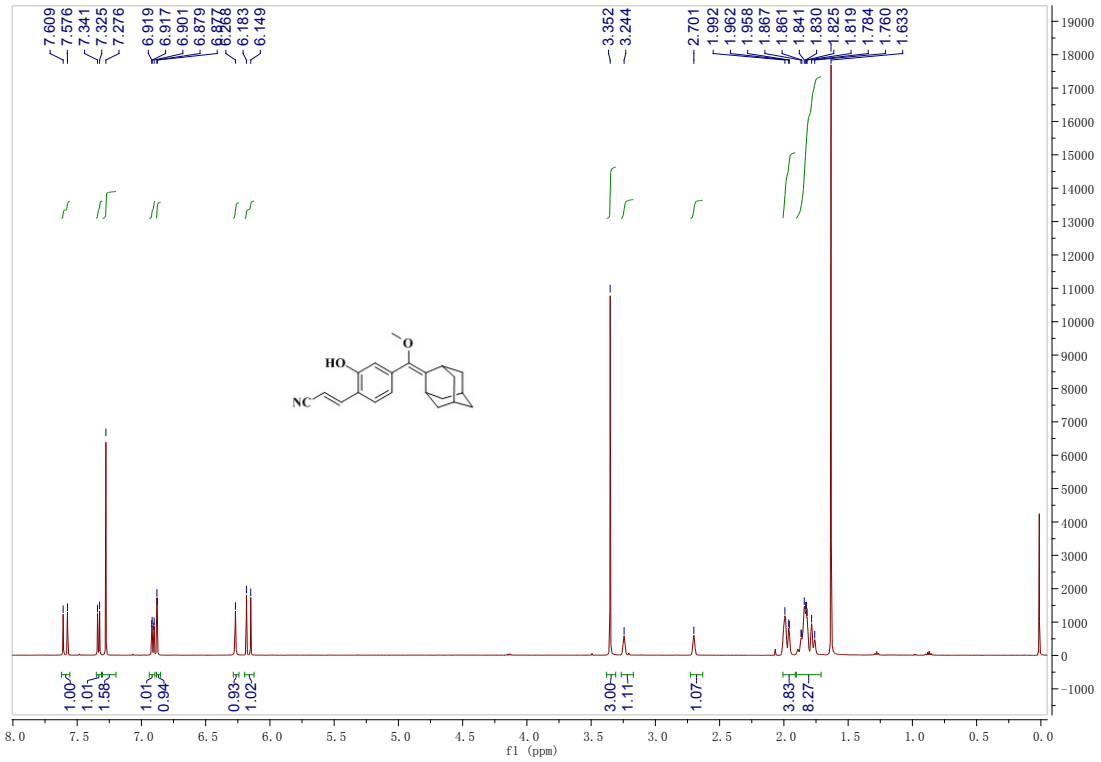
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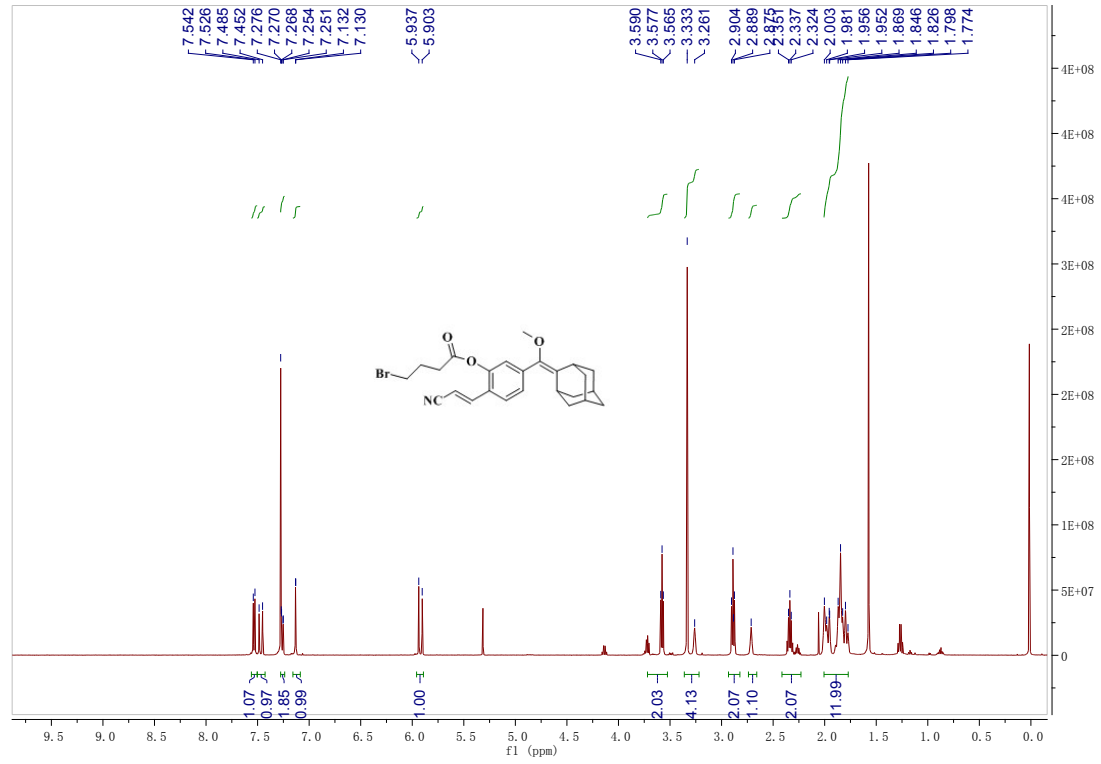
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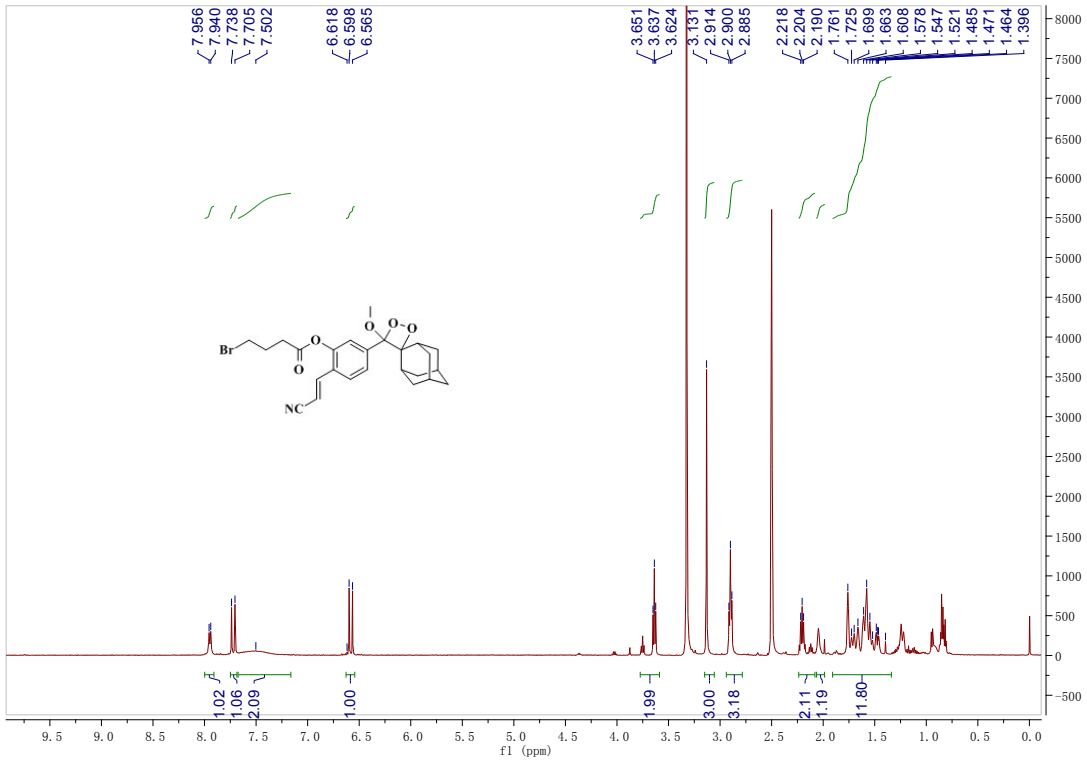
f1 (ppm)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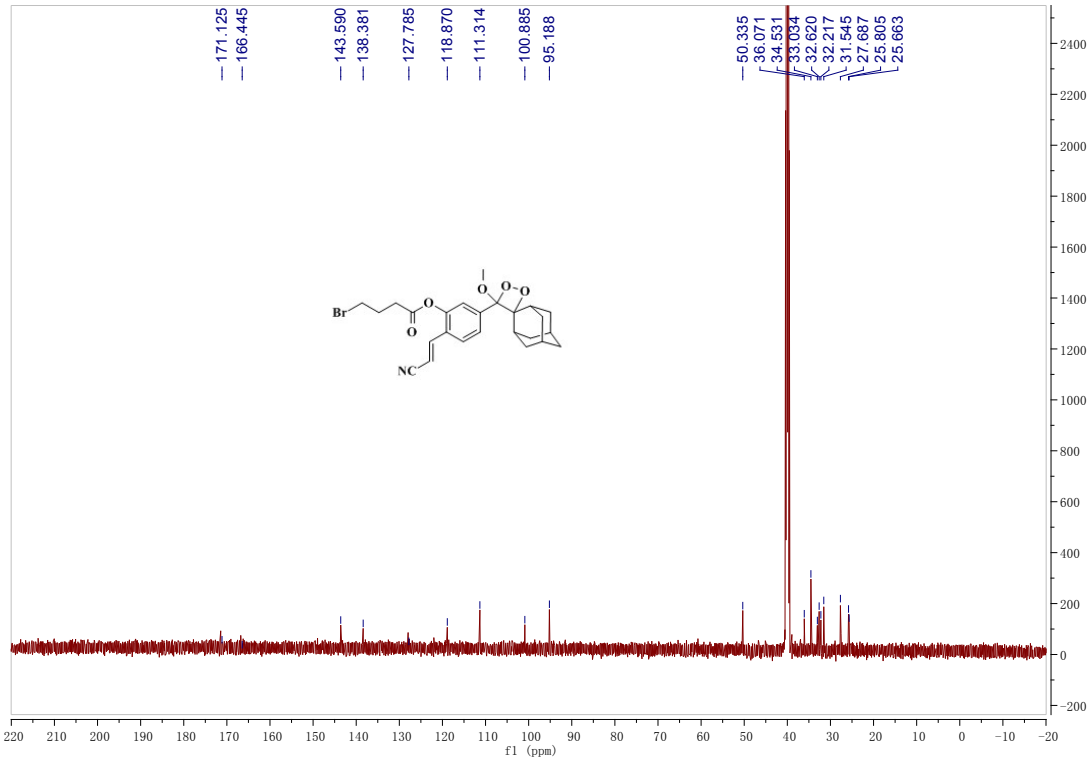
2.11
1.19
11.80
f1 (ppm)


171.125
166.445
143.590
138.381
127.785
118.870
111.314
100.885
95.188
50.335
36.071
34.531
33.034
32.620
32.217
31.545
27.687
25.805
25.663
Br
O
O
O
O
NC
f1 (ppm)