Supporting Information

A novel hydrazino-substituted naphthalimide-based fluorogenic probe for *tert*-butoxy radicals

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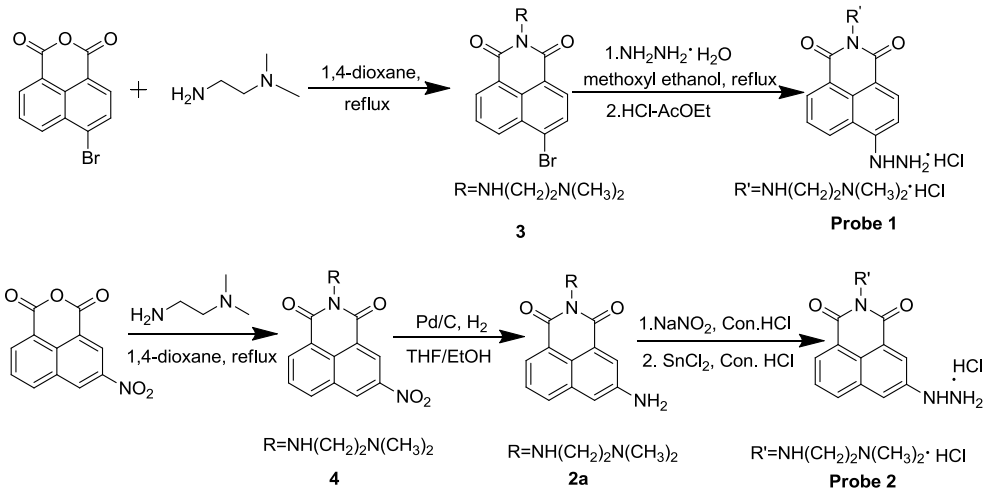
1. Materials and instruments

All reagents and solvents available from commercial sources were used as received unless otherwise noted. Twice-distilled water was used throughout all experiments. Water used for the fluorescence studies was doubly distilled and further purified with a Mill-Q filtration system. Melting points were determined on an electrothermal melting point apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR were recorded on a Bruker 300 MHz NMR and 600 MHz NMR spectrometer. Mass spectra were performed by the analytical and the mass spectrometry facilities at Shandong University. Absorption spectra were recorded using Shimadzu UV-1700 UV-visible spectrometer. Fluorescence spectra were obtained with Varioskan microplate reader (Thermo Electron Corporation). Fluorescence imaging was performed using an OLMPUS CK30-F200 fluorescence microscope. HPLC analysis was carried out on Agilent Technologies S1260 infinity. .

2. Synthesis

Scheme S1. Synthesis of probes 1 and 2



6-bromo-2-(2-(dimethylamino)ethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (3)

4-Bromo-1, 8-naphthalic anhydride (2.77 g, 10 mmol) was dissolved in 80 mL 1,4-dioxane, and N^1,N^1-dimethylethane-1,2-diamine (1.31 mL, 12 mmol) was added. The solution was stirred and refluxed at 103 °C for 4 h. The mixture was then cooled and poured into water to precipitate out a yellowish solid. The sediment were collected by filtration, washed with water, dried, and recrystallized from AcOEt-*n*-methanol to yield compound **2** as a pale-yellow solid.[1] Yield: 2.87 g, 83.2%. M.p.: 146.0~147.0 °C.[2]

2-(2-(dimethylamino)ethyl)-6-hydrazinyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione dihydrochloride (probe 1)

Compound 3 (347.8 mg, 1 mmol) and 183.4 μL 80% hydrazine hydrate (3 mmol) were added into 3mL of methoxyl ethanol under a nitrogen atmosphere and refluxed at 123°C for 3h. After the mixture cooled to room temperature, the precipitated solids were filtered, washed with ethanol and chloroform, dried in vacuum to give a pink powdery solid. The product is dissolved in methanol and a small amount of DMF is used for solubilization. Then excess EtOAc solution saturated with HCl gas was added and stirred to precipitate. Filtered and washed with AcOEt to afford a dark yellow power, **probe 1**.[3] Yield: 276.8 mg, 74.6%. Decomposition point: 209.0~211.0 °C. ^{1}HNMR (DMSO-d_6, 600 MHz): δ10.70 (br s., 2H), 10.33 (s, 1H), 10.20 (s, 1H) 8.68 (dd, 1H, J_1=8.4 Hz, J_2=1.2 Hz), 8.50 (dd, 1H, J_1=7.8 Hz, J_2=1.2 Hz), 8.41 (d, 1H, J=8.4 Hz), 7.83 (dd, 1H, J_1=8.4, J_2=7.8 Hz), 7.23 (d, 1H, J=8.4 Hz), 4.38 (t, 2H, J=6.0 Hz), 3.43 (dt, 2H, J=5.4 Hz, J=4.8 Hz), 2.87 (d, 6H, J=3.6 Hz); ^{13}C-NMR(DMSO-d_6,150 MHz): δ164.269, 163.514, 147.684, 132.942, 131.191, 129.242, 128.826, 125.946, 122.326, 120.080, 113.408, 106.926, 54.755, 42.693, 35.053. HRMS (ESI) m/z calcd. for $C_{16}H_{18}N_4O_2$ ([M + H]$^+$) 299.1503; found 299.1498.

2-(2-(dimethylamino) ethyl)-5-nitro-1*H*-benzo [*de*]isoquinoline-1,3(2*H*)-dione (4)

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The solution of 3-nitro-1, 8-naphthalic anhydride (1.01 g, 4.15 mmol) and N^1,N^1-dimethylethane -1,2-diamine (1.078 mL, 9.80 mmol) in 30 mL 1,4-dioxane was refluxed for 2.5 h. The postprocessing is similar to **3**. Recrystallized from AcOEt, to yield a off-white solid product.[1] Yield: 1.02 g, 78%. M.p.: 139.0~140.0°C.[4]

5-amino-2-(2-(dimethylamino)ethyl)-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (2a)

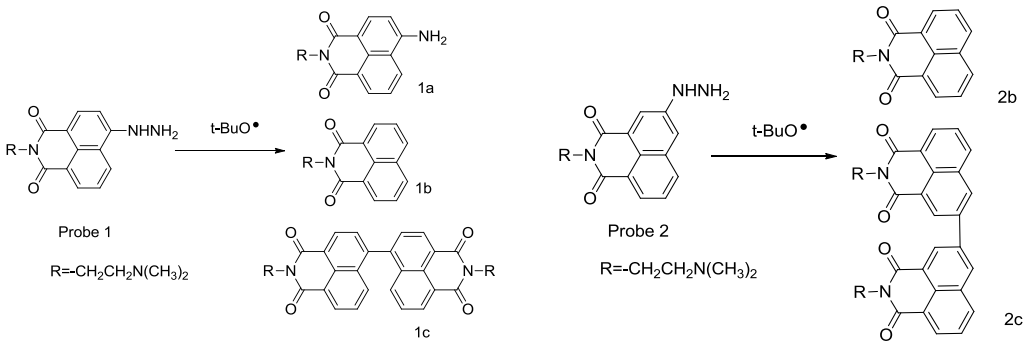
To the solution of compound **5** (0.90 g, 2.88 mmol) in 40 mL of THF/EtOH (1:1), 10% Pd/C (268.8 mg) was added. The mixture was stirred overnight under a hydrogen atmosphere (H_2 balloon). The reaction mixture was filtered over celite, concentrated in vacuo, and recrystallized from methanol to afford a yellow solid.[5] Yield: 594.3 mg, 73.0%. ^{1}HNMR (DMSO-d_6, 300 MHz): δ8.07 (dd, 1H, J_1=7.2 Hz, J_2=0.9 Hz), 8.03 (d, 1H, J=8.4 Hz), 7.96 (d, 1H, J=2.4 Hz), 7.61 (dd, 1H, J_1=8.1 Hz, J_2=7.2 Hz), 7.27 (d, 1H, J=2.4 Hz), 5.984 (s, 2H), 4.13 (t,2H, J=6.9 Hz), 2.49 (t, 2H, J=6.9 Hz), 2.20 (s, 6H). M.p.: 169.0~170.0 °C.[4]

2-(2-(dimethylamino)ethyl)-5-hydrazinyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione dihydrochloride (probe 2)

A stirred solution of **4** (284.1 mg, 1 mmol) in concentrated hydrochloric acid (3 mL) was treated dropwise at -5 °C with sodium nitrite (71.01 mg, 1 mmol) in cold water (1 mL), care being taken not to allow the temperature to rise above 0 °C. After the addition, the diazotization reaction was continued for 1 h. The solution was poured in a thin stream into a solution of stannous chloride dihydrate (1.12 g, 5 mmol) in cold hydrochloric acid (6 mL). After 1 h, the reaction mixture was filtered, washed with concentrated hydrochloric (5 mL), followed with ethyl acetate (10 mL) and finally with ether (10 mL), and dried in vacuum dryer to give a light bright-yellow product.[6] Yield: 340.2 mg, 91.6%. ^{1}HNMR (DMSO-d_6, 300 MHz): δ9.98 (br s, 2H), 9.65 (br s, 1H), 8.91 (s, 1H), 8.31 (dd, 1H, J_1=7.2 Hz, J_2=0.9 Hz), 8.27 (d, 1H, J=8.4 Hz), 8.20 (d, 1H, J=2.4 Hz), 7.81 (dd, 1H, J_1=8.1Hz, J_2=7.2 HZ), 7.77 (d, 1H, J=2.4 Hz), 4.39 (t, 2H, J=5.7 Hz), 3.45 (t, 2H, J=5.7 Hz), 2.89 (s, 6H); ^{13}CNMR (DMSO, 150 MHz): δ164.553, 164.256, 146.446, 133.119, 132.963, 128.206, 127.970, 123.411, 123.160, 122.553, 120.821, 112,929, 55.191, 43.130, 35.616. HRMS (ESI) m/z calcd. for $C_{16}H_{18}N_4O_2$ ([M + H]$^+$) 299.1503; found 299.1502.

3. Reaction of probes with tert-butoxy radical.

Scheme S2. Reaction of probe 1 and probe 2 with *tert*-butoxy radical



3.1 Reaction of probe 1 with *tert*-butoxy radical

The tert-butoxyl radical was generated from the Fenton reagent (Fe^{2+}: TBHP=10:1). To fresh prepared solution of $FeSO_4{\cdot}7H_2O$ (561.6 mg, 20.3 mmol) and tert-Butyl hydroperoxide (TBHP, 20.2 μL, 0.203 mmol) in 6 mL water, the solution of probe 1 (50.1 mg, 0.149 mmol) in 2 mL H_2O was added. Gas was observed immediately, and the reaction mixture was stirred for 20 min at room temperature until TLC indicated that the starting material was consumed**.** The reaction was washed with saturated aq. Na_2CO_3 to alkalinity and then extracted with EtOAc (100 mL × 3). The combined organic layers were dried over anhydrous $MgSO_4$, filtered and concentrated. According to TLC and HRMS (**Figure S20)** of the mixture, three major compounds, m/z of whose [M + H]$^+$ are respectively 269.1284 (**1b**), 284.1390 (**1a**) and 535.2328 (**1c**), were generated from the reaction. Presumably, the structures is shown in Figure S14,[7, 8] and **1a** was included (**Scheme S2**). The compounds were isolated by HPLC at the condition of 70% CH_3OH (1% triethylamine) in H_2O. Meantime, comparing to the standard substance by HPLC (t_R≈11.8min, CH_3OH: H_2O = 6.5: 3.5, 0.5% triethylaminein in CH_3OH), we confirmed the structure of 1a. Compound 1a: ^{1}HNMR (DMSO-d_6, 600 MHz): δ8.64 (d, 1H, J=8.4Hz), 8.43 (d, 1H, J=7.2Hz), 8.19 (d, 1H, J=8.4Hz), 7.66 (t, 1H, J=7.2Hz), 7.50 (s, 2H), 6.85 (d, 1H, J=8.4Hz), 4.17 (t, 2H, 6.6Hz), 2.69 (br s., 2H), 2.33 (s, 6H). HRMS (ESI) m/z calcd. for $C_{16}H_{18}N_3O_2$ ([M + H]$^+$) 284.1394; found 284.1393. Compound 1b: HNMR (DMSO--d_6) δ8.52 (dd, 2H, J_1=7.5Hz,

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J_2=1.2Hz), 8.49 (d, 2H, J_1=8.4Hz, J_2=1.2Hz), 7.91 (t, 2H, J=7.8Hz), 4.16 (t, 1H, J=6.9Hz), 2.54 (2H), 2.21 (s, 6H, J=8.4Hz). MS (ESI) m/z calcd. for $C_{16}H_{16}N_2O_2$ ($[M + H]^+$) 269.13; found 269.4. Compound 1c: MS (ESI) m/z calcd. for $C_{32}H_{30}N_4O_4$ ($[M + H]^+$) 535.23; found 535.4.

3.2 Reaction of probe 2 with *tert*-butoxy radical

This reaction and postprocessing are analogous to the reaction of probe 1. But **2a** (**Scheme S2**) was not observed in the reaction according to the TLC and HRMS (**Figure S21**) of the crude products. Certainly, the main compounds, m/z of whose $[M + H]^+$ are 269.1285 (**2b**) and 535.2327 (**2c**), are similar to the corresponding products (**1b** and **1c**) in the reaction of probe 1. The two structures are shown in Figure S15.[7, 8] We also determine that the peak of 283.1439 is not the molecular ion peak of **2a**, because it is too far from 283.1321, which is m/z of $[M]^+$ of **2a**.

4. Fluorometeric Analysis

Note: All solutions were fresh and prepared in 50 mM phosphate buffer solution (PBS) at pH 6.0. The various solutions with needed concentrations were diluted from the prepared or purchased stock solutions. Before the stock solutions of Fe^{2+} and Fe^{3+} were used, the generated precipitation should be removed by centrifugation. Quartz cuvettes were used in UV studies, and 96-well plates were used in fluorescence studies. The fluorescence excitation wavelengths used were the UV λ_{max}.

4.1 Preparation of various analytes

Various analytes including ROS, RNS, RSS and inorganic ions were prepared according to the following methods.

Preparation of H_2O_2

The 30% stock H_2O_2 solution was diluted using PBS to afford the need solution.

Preparation of TBHP

The TBHP solution was obtained from *tert*-butyl hydroperoxide aqueous solutions.

Preparation of NOHP

The *n*-octyl hydroperoxides was synthesized as the literature[9]. The liquid product was identified by H^1 NMR and was diluted to prepare the NOHP solution.

Preparation of IPHP

The *iso*-propyl hydroperoxides was synthesized as the literature[10]. The liquid product was identified by H^1 NMR as well and was diluted to prepare the IPHP solution.

Preparation of ·OH, tBuO·, nOcO·, iPrO·

Hydroxyl radical, tert-butoxy radical, n-octoxy radical and iso-prooxy radical were generated in situ by Fenton reactions. 10 equiv of ferrous sulfate was added to H_2O_2, TBHP, NOHP, IPHP to prepare the radical solution, respectively.

Preparation of $\cdot CH_3$

The 0.1% (v/v) DMSO was added to the fresh fenton reagent (Fe^{2+} :H_2O_2, mol/mol = 10:1) to offer methyl radical.

Preparation of ClO^-

hypochlorite (ClO^-) were delivered from 6% NaClO aqueous solution.

Preparation of $O_2^{\cdot -}$

Superoxide radical was generated from xanthine/xanthine oxidase system. Xanthine oxidase was added first. After xanthine oxidase was dissolved, xanthine was added and the mixture was then stirred at 25 °C for 30 min. In addition, KO_2 was used as another source of suroeroxide radical.

Preparation of AcO_3H

The 20% stock solution of peroxyacetic acid was diluted to offer the need solution.

Preparation of ROO·

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The peroxyl radical was generated from AAPH (2,2'-azobis(2-amidinopropane) dihydrochloride). AAPH was added to PBS and the mixture was stirred at 25 °C for 30 min.

Preparation of $ONOO^-$

Peroxynitrite solution was synthesized as reported[11]. The peroxynitrite concentration was estimated by using an extinction coefficient of 1670 ± 50 cm^{-1} $(mol/L)^{-1}$ at 302 nm. The peroxynitrite solution prepared was usually very basic (pH 12). When larger volumes of peroxynitrite were added, part of the excess base was neutralized on the day of the experiment.

Preparation of Cys

The source of **Cys** was D-Cysteine hydrochloride monohydrate.

Preparation of PhSH

Thiophenol was diluted to prepared the need PhSH solution.

Preparation of NO_2^-

The source of $NO_2^-$was sodium nitrite.

Preparation of NO_3^-

The source of NO_3^- was sodium nitrate.

Preparation of NO

Nitric oxide was generated from Potassium Nitroprusside Dihydrate[12]. The experiments were performed under anaerobic conditions. Nitroprusside was added into degassed PBS under N_2 atmosphere then stirred for 30 min at 25 ^oC.

Preparation of Fe^{2+}

The source of Fe^{2+} was ferrous sulfate heptahydrate.

Preparation of Fe^{3+}

The source of Fe^{3+} was iron chloride hexahydrate.

4.2 Spectral Properties of Probes

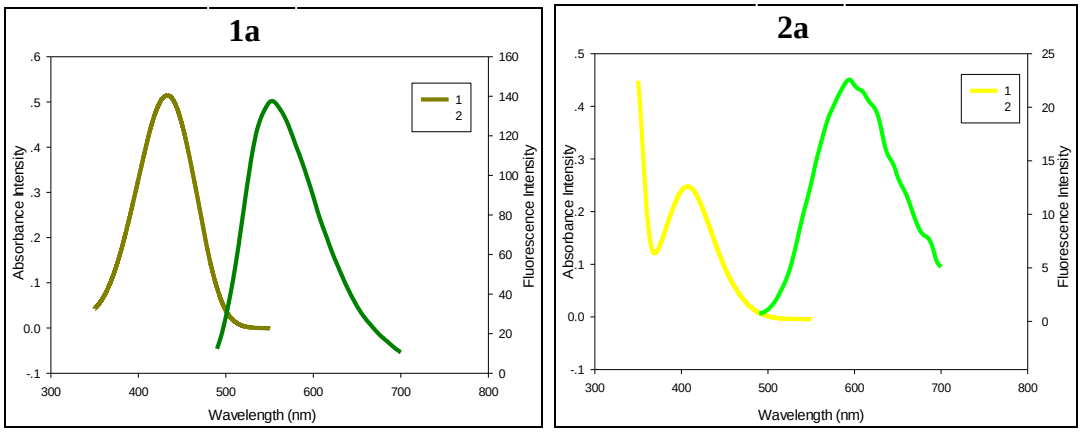

Figure S1. Absorbance spectra (line 1) and emission spectra (line 2) of 1a and 2a (50 μM)

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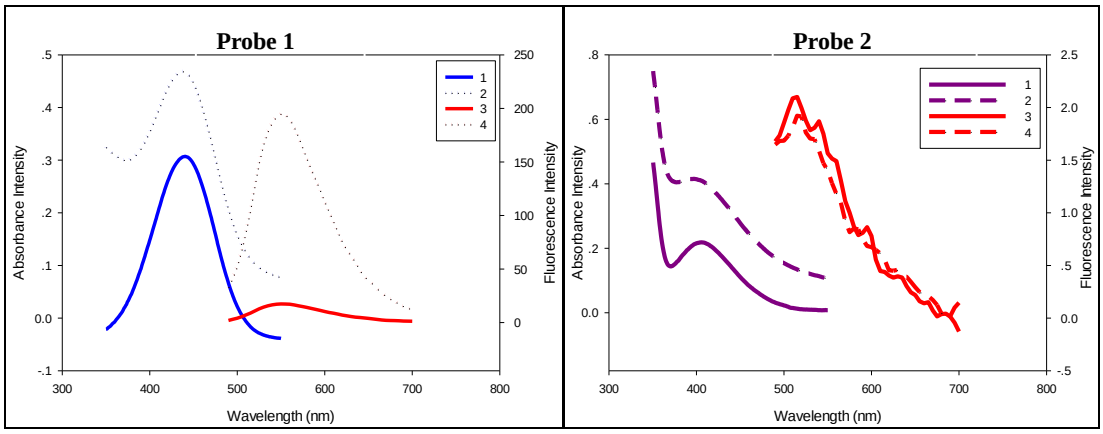

Figure S2. Absorbance spectra (line 1, 2) and emission spectra (line 3, 4) of probes **1** and **2** (50 μM) either with (line 2, 4) or without (line 1, 3) Fenton reagent (Fe^{2+}: TBHP, mol/mol = 10:1).

4.3 Evaluation of Selectivity of Probes

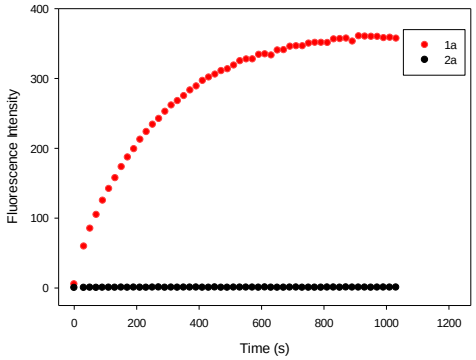

Figure S3. Time course of the interaction of two probes (50 μM) with *tert*-butoxy radicals (100.0 μM) in PBS (pH 6.0). Fluorescence intensity of 1a and 2a was recorded at 550 nm with λex = 440 nm, and 590 nm with λex = 405 nm, respectively.

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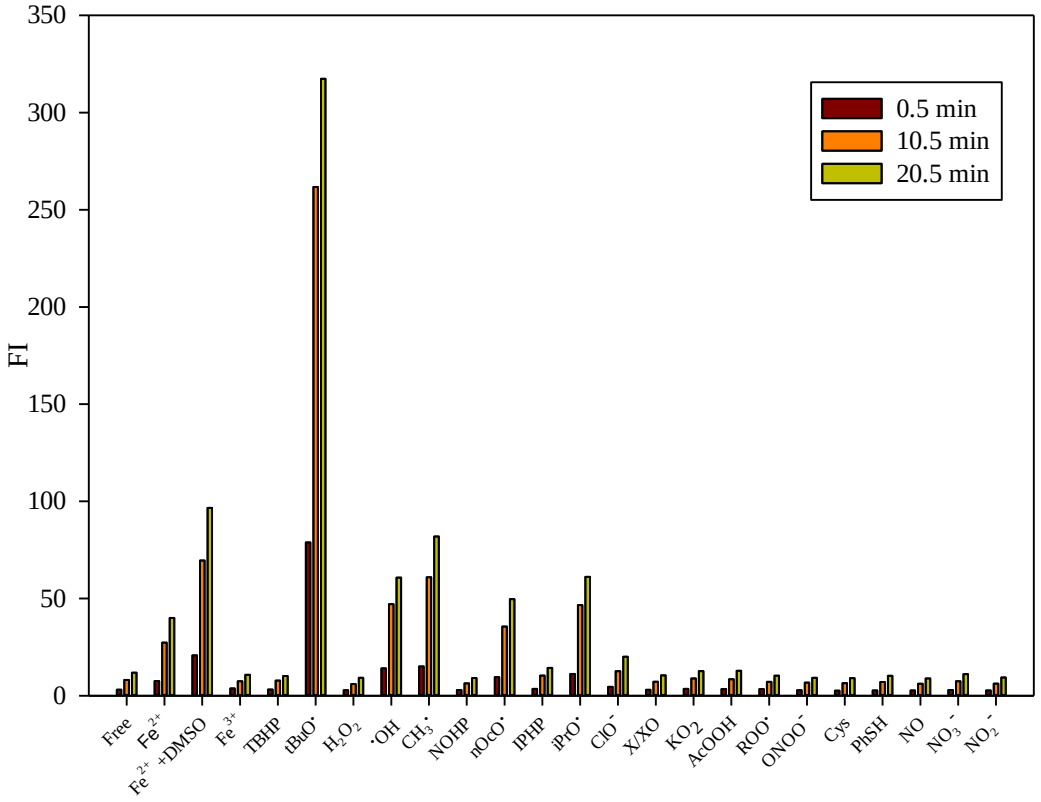

Figure S4. Fluorescence response of probe 1 (50 μM) in 50 mM PBS (pH=6.0) to various analytes at 550 nm (λ_{ex} = 440 nm). Data shown are for 50 μM of ROS, RNS and RSS, 0.5 mM of Fe^{2+}, Fe^{2+} (0.1%DMSO) and Fe^{3+}. Data were obtained after incubation with the appropriate analytes at room temperature for 0.5 min, 10.5 min and 20.5 min.

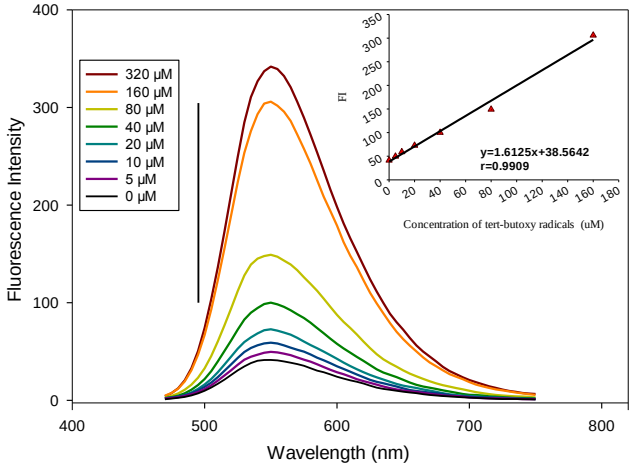

Figure S5. Concentration-dependent emission intensity changes of **probe 1** at room temperature in 50 mM PBS, pH=6.0. The emission spectra were obtained at 15 min after the addition of *tert*-butoxy radicals (0–320 μM) to 50 μM probe 1 with λex = 440 nm. **Inset:** Fluorescence intensity at 550nm as a function of concentration of *tert*-butoxy radicals.

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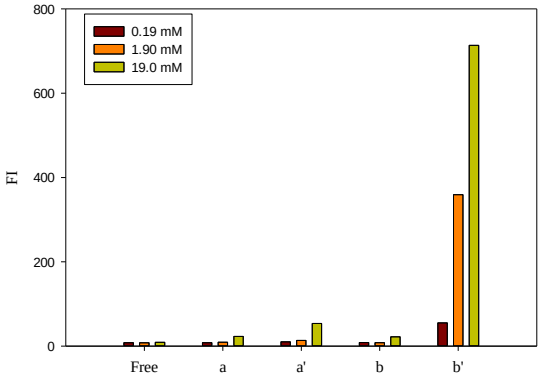

Figure S6. Fluorescence response of probe 1(50μM) to *tert*-butoxy radicals generated from the new systems[13]. (a) tBuOOtBu was diluted at room temperature. (a') tBuOOtBu was heated at 87 °C for 10min, and then diluted to the need solutions**.** (b) tBuONO was diluted at room temperature. (b') tBuONO was heated at 115 °C for 10min, and then diluted to the need solutions**..**

4.4 Quenching Test of TEMPO

The reactivity of TEMPO with **probe 1, 1a** and the mixture of **probe 1** and **tBuO·** was recorded via the fluorescence intensity at 550 nm, respectively.

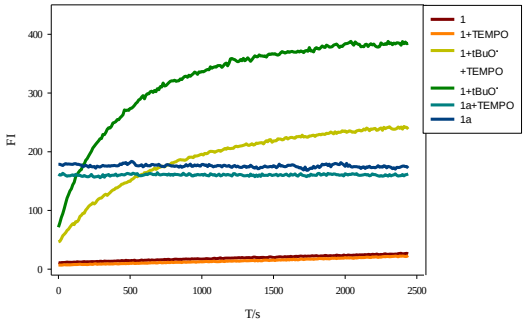

Figure S7. The reactions of TEMPO (2.5mg/ml) with probe 1 (50 μM), 1a (25 μM) and the mixture of probe (50μM) and tBuO· (50 μM) recorded by following the fluorescent intensity at 550 nm with λ_{ex} = 440 nm.

4.5 Kinetic Studies[14]

The reaction of probe 1 (5 μM) with *tert*-butoxy radicals (187.5μM, 250μM, 312.5μM) in PBS was monitored using the fluorescence intensity at 550 nm (λex = 440 nm), respectively. The apparent rate constant for the reaction was determined by fitting the fluorescence intensities to the pseudo first-order equation (S1):

$$\ln (F_t / F_{max}) = - k' t \quad (S1)$$

Where F_t is the fluorescence intensities at 550 nm at time t, and F_{max} is the maximum value obtained after the reaction was complete. k' is the observed rate constant. Figure S8 (a~c) are the pseudo first-order plots for the reaction of 1 with *tert*-butoxy radicals, and the slope of the line provides the pseudo-first-order rate constant for three reactions: $4.0 \times 10^{-3} s^{-1}$, 5.0×10^{-3} s^{-1}, $7.3 \times 10^{-3} s^{-1}$, respectively.

The pseudo-first-order rate constant, k', is related to the second-order rate constant, k ($M^{-1}s^{-1}$), by equation S2:

$$k' = k [M] \quad (S2)$$

Where M is the concentration of tert-butoxy radicals. The second-order rate constant for this reaction is the average of the three values. k = 21.56 $M^{-1}s^{-1}$.

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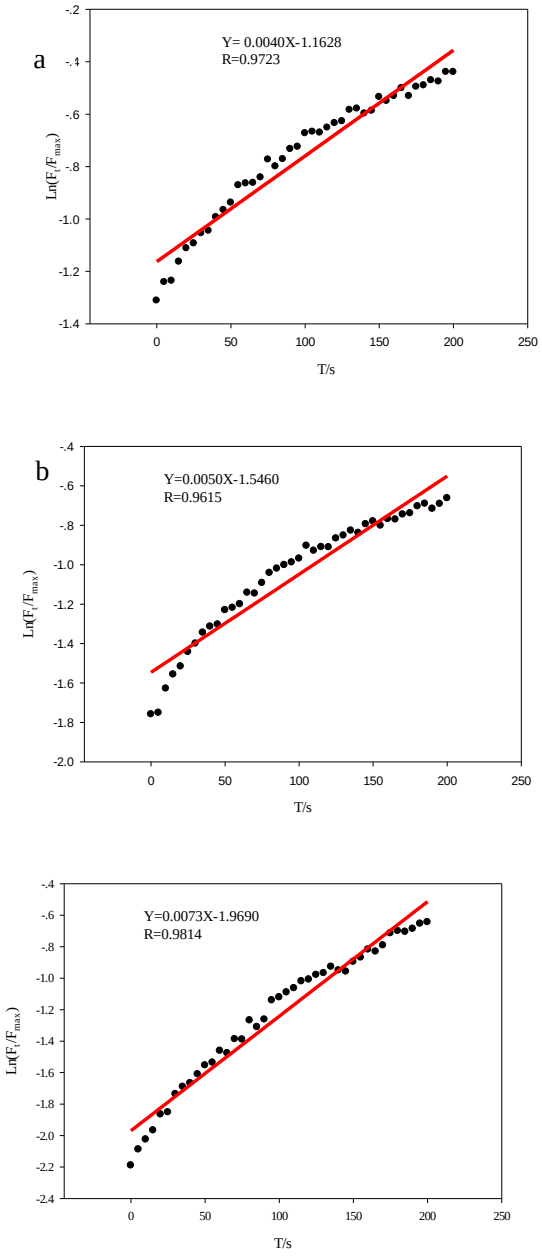

Figure S8. Pseudo first-order kinetic plots of the reaction of probe 1 (5 μM) with *tert*-butoxy radical in pH 6.0 PBS: (a) 37.5 equiv. of tBuO·, Slope = $4.0 \times 10^{-3}s^{-1}$. (b) 50.0 equiv. of tBuO·, Slope = 5.0×10^{-3} s^{-1}, (c) 62.5 equiv. of tBuO·, Slope = $7.3 \times 10^{-3}s^{-1}$..

5. Cell culture and fluorescent image assay

Human prostate cancer cell line (PC-3) was grown in RPMI-1640 medium supplemented with 10% (v/v) fetal bovine serum in an atmosphere of 5% CO_2, 95% air at 37 °C. Cells were plated on 24-well plate and allowed to adhere for 12 h~24h. After the medium was removed, the cells were carefully washed with RPMI-1640 medium, and then incubated at r.t. in the presence of probe **1** (10 μM, PBS, pH 6.0) for 4 min. Fluorescence imaging was performed after washing the cells three times with RPMI-1640 medium. Fluorescence imaging was performed using an OLMPUS CK30-F200 fluorescence microscope. The *tert*-butoxy radicals were prepared from the fenton reagent (10 μM) and the heated tBuONO (3.8 mM).

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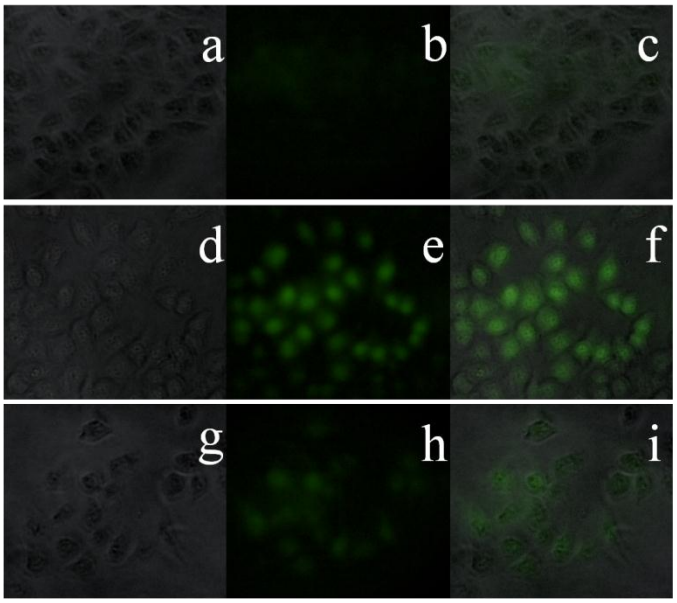

Figure S9. Fluorescence imaging of Fenton reagent (Fe^{2+}:TBHP, 10:1) induced tBuO• production in live PC-3 cells. **(a)** Bright-field image of live PC-3 cells incubated with only probe 1 (10 μM) for 3 min; **(b)** fluorescence image of (a) with green light excitation; **(c)** overlap of (a) and (b); **(d)** Bright-field image of live PC-3 cells preprocessed by Fenton reagent (10 μM) for 5 min and then incubated with probe 1 (10 μM) for 3 min; **(e)** fluorescence image of (d) with green light excitation; **(f)** overlap of (d) and **(e)**; **(g)** Bright-field image of live PC-3 cells incubated with Fenton reagent (10 μM) for 5 min firstly, then with catalase (1mg/mL) for 5 min, and finally with probe 1 (10 μM) for 3 min; **(h)** fluorescence image of **(g)** with green light excitation; **(i)** overlap of **(g)** and **(h)**.

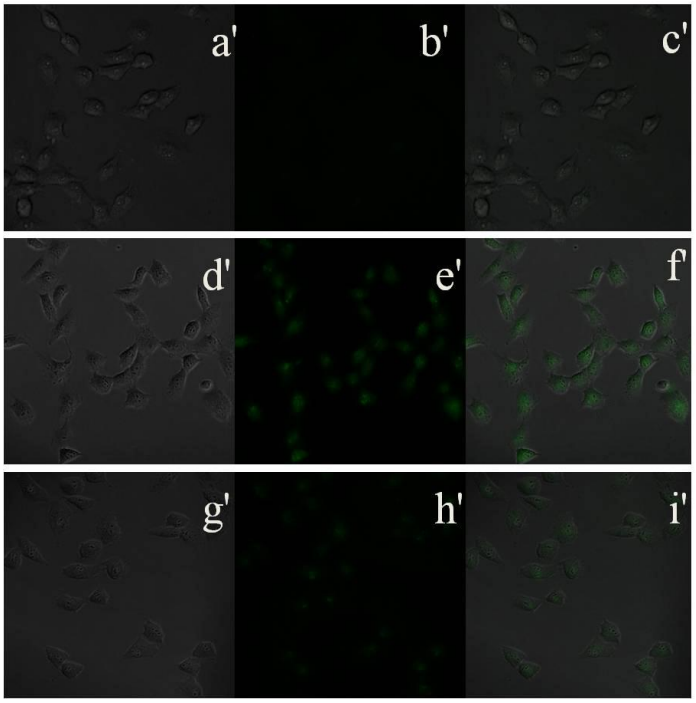

Figure S10. Fluorescence imaging of the heated tBuONO induced tBuO• production in live PC-3 cells. (a) Bright-field image of live PC-3 cells incubated with only probe 1 (10 μM) for 2 min; **(b)** fluorescence image of (a) with green light excitation; **(c)** overlap of (a) and (b); **(d)** Bright-field image of live PC-3 cells preprocess by tBuONO (3.8 mM) for 5 min and then incubated with probe 1 (10 μM) for 2 min; **(e)** fluorescence image of (d) with green light excitation; **(f)** overlap of (d) and (e); **(g)** Bright-field image of live PC-3 cells incubated with tBuONO (3.8 mM) for 5 min firstly, then with catalase (1mg/mL) for 5 min, and finally with probe 1 (10 μM) for 2 min; **(h)** fluorescence image of (g) with green light excitation; **(i)** overlap of (g) and (h).

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6. NMR spectra

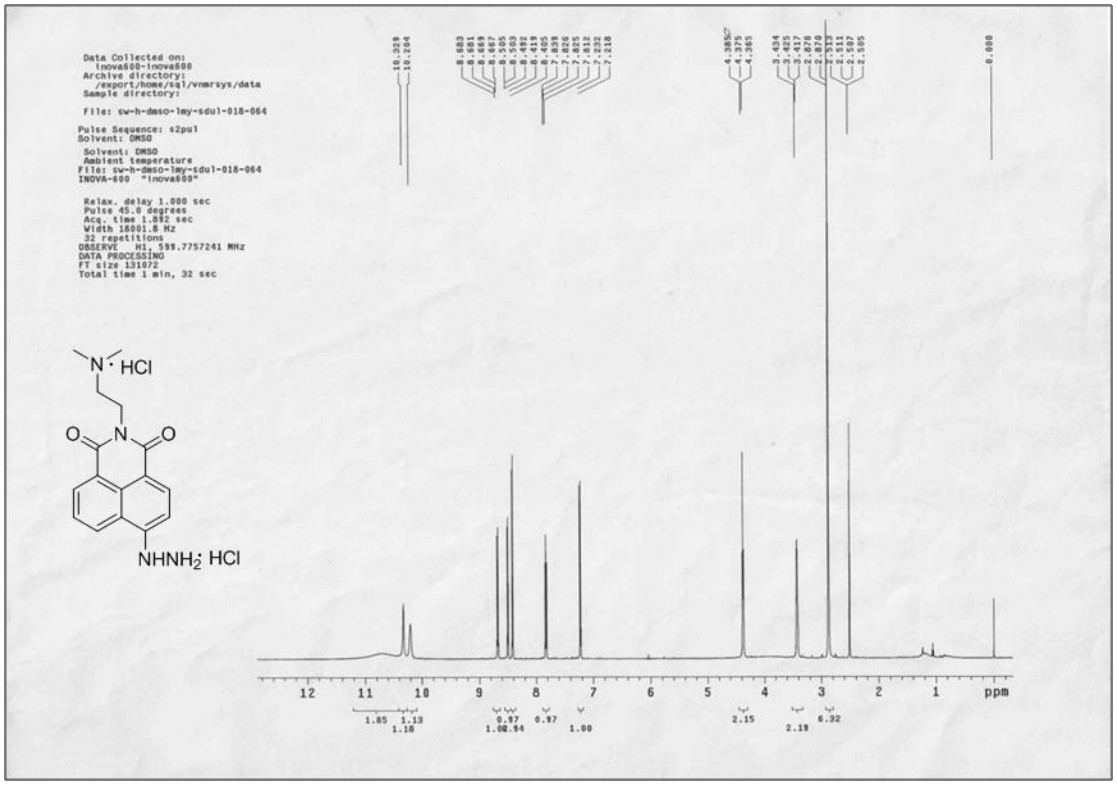


Figure S11. The 1HNMR (600 MHz) spectrum of **probe 1**

Supporting Information

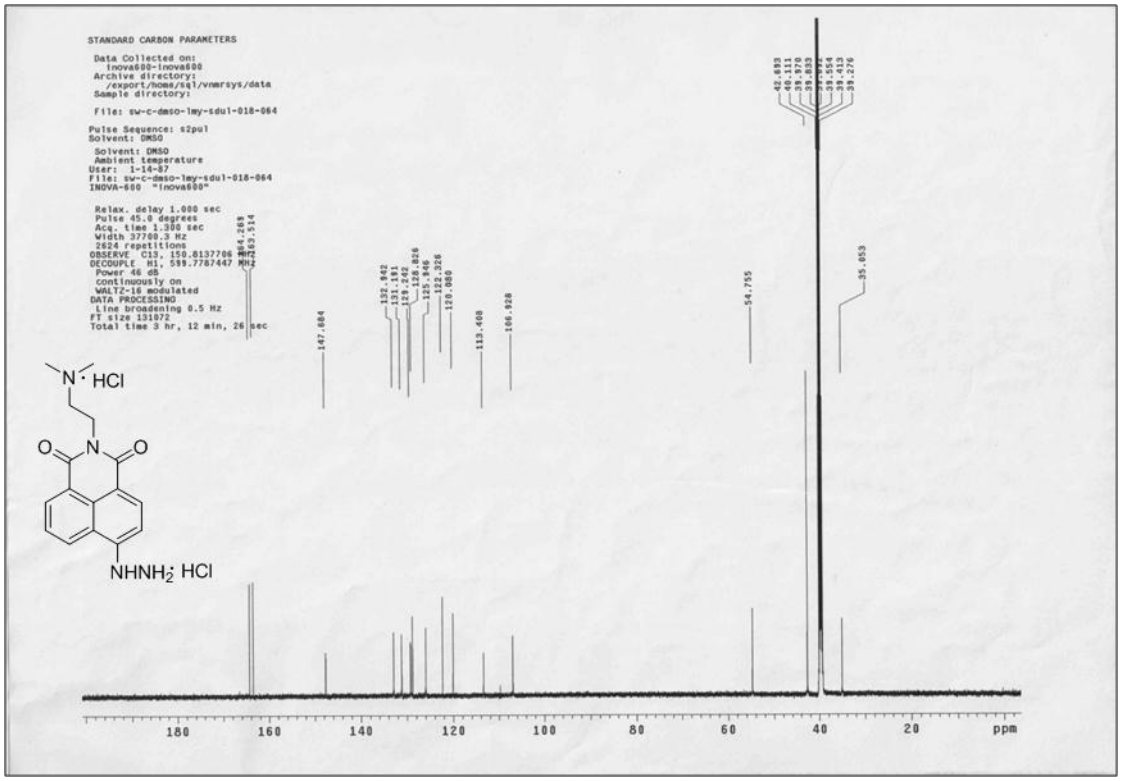


Figure S12. The 13CNMR (150 MHz) spectrum of **probe 1**

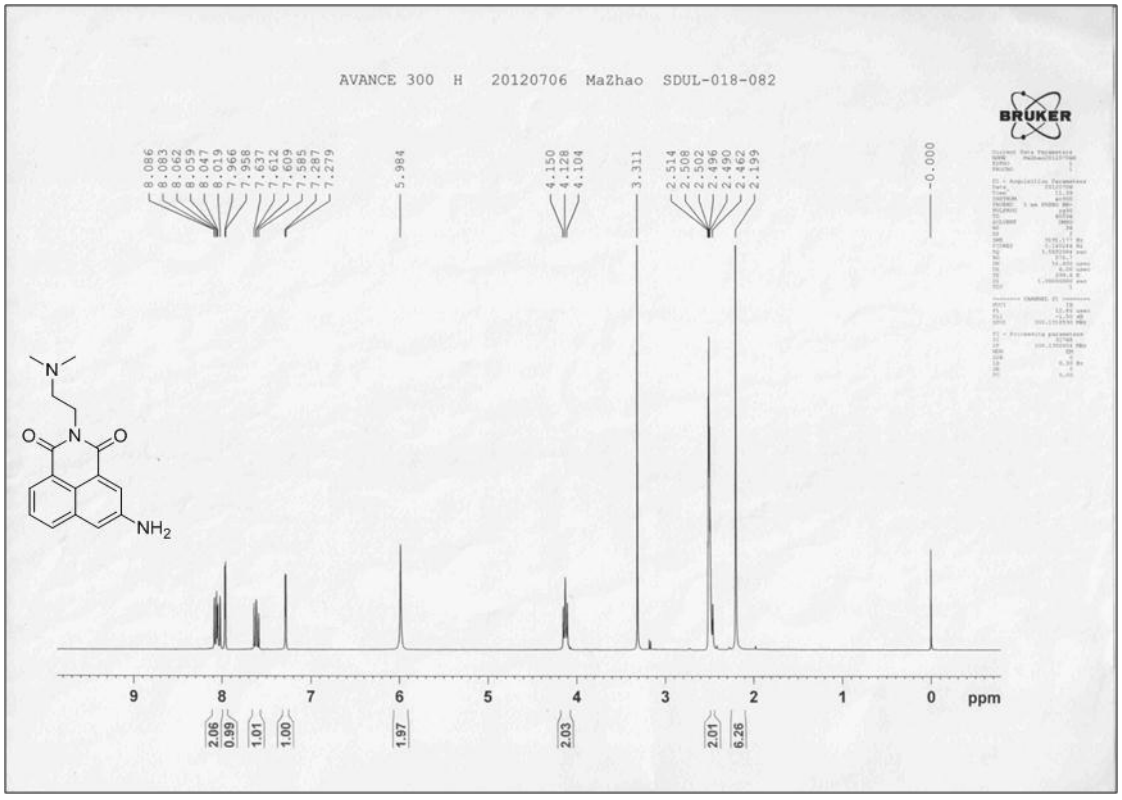


Figure S13. The ^{1}HNMR (300 MHz) spectrum of **3**

Supporting Information

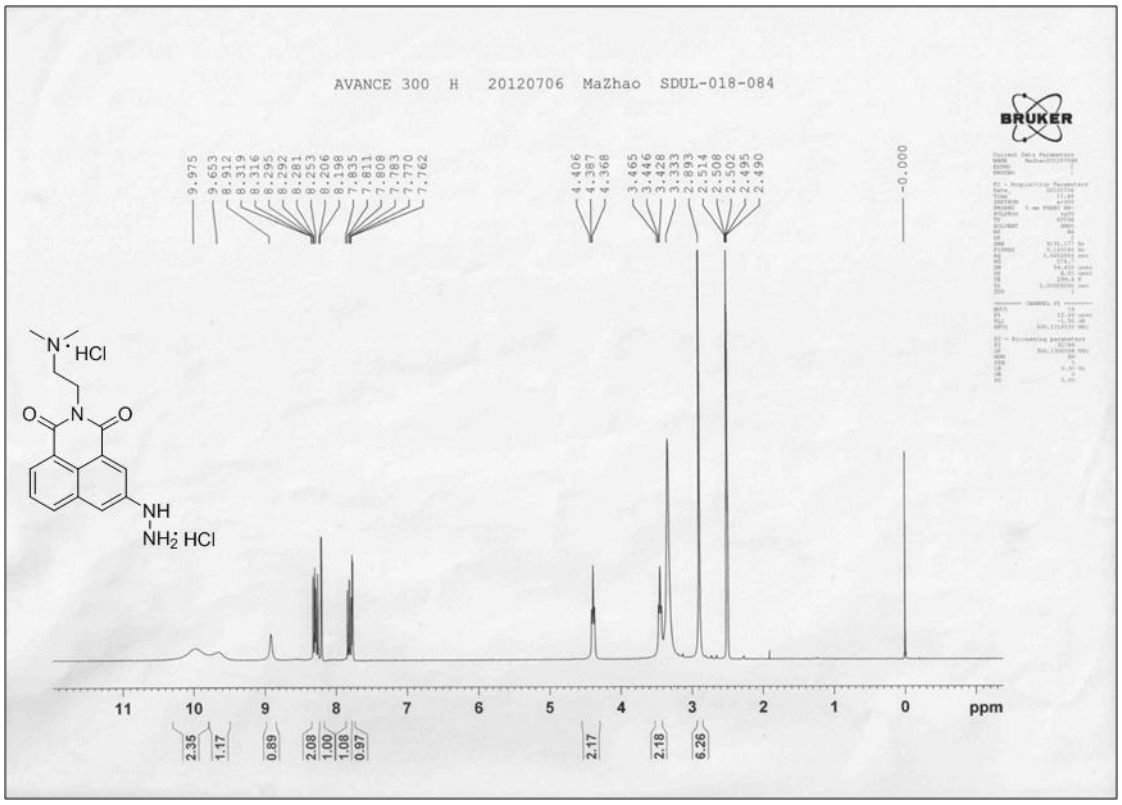


Figure S14. The ^{1}HNMR (300 MHz) spectrum of **probe 2**

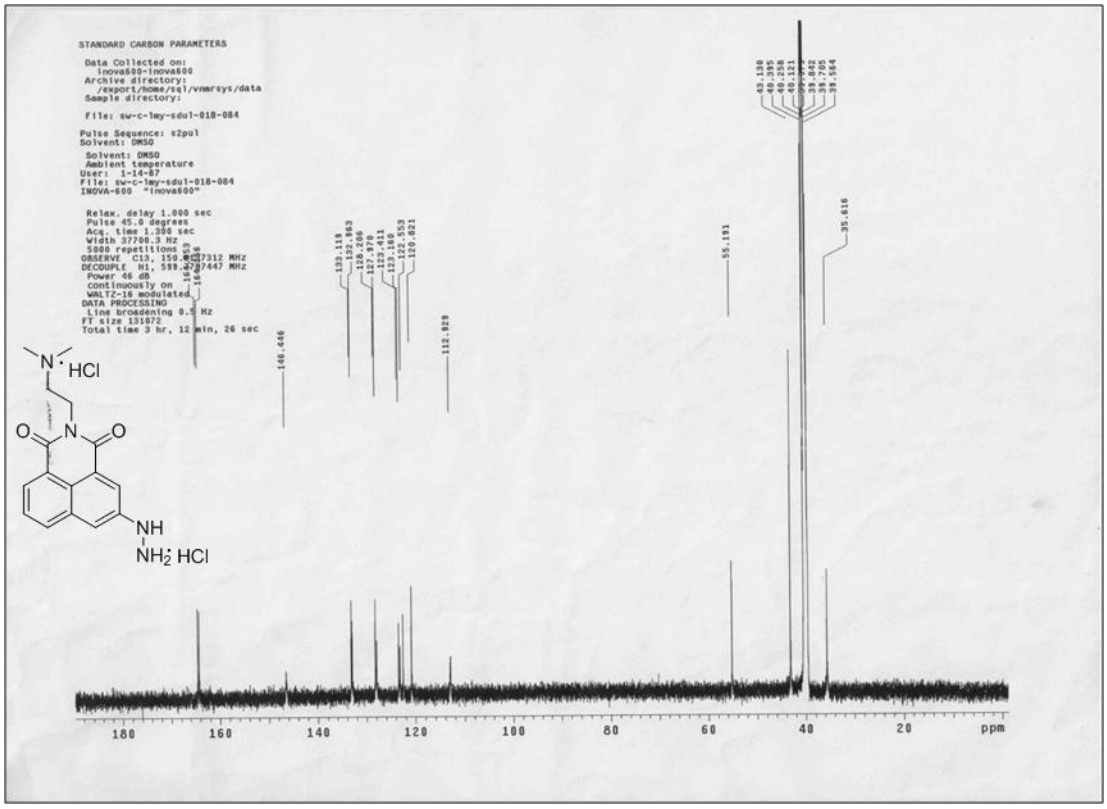


Figure S15. The ^{13}CNMR (150MHz) spectrum of **probe 2**

Supporting Information

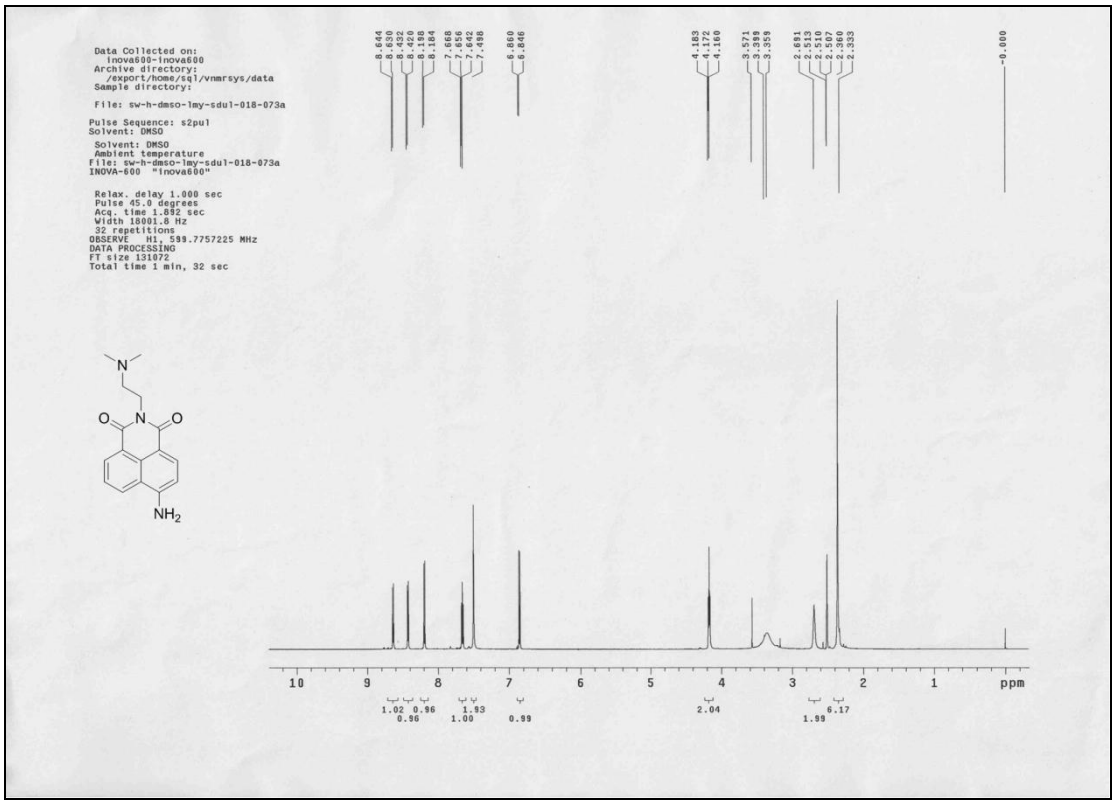


Figure S16. The ^{1}HNMR (600 MHz) spectrum of the standard substance of **1a**

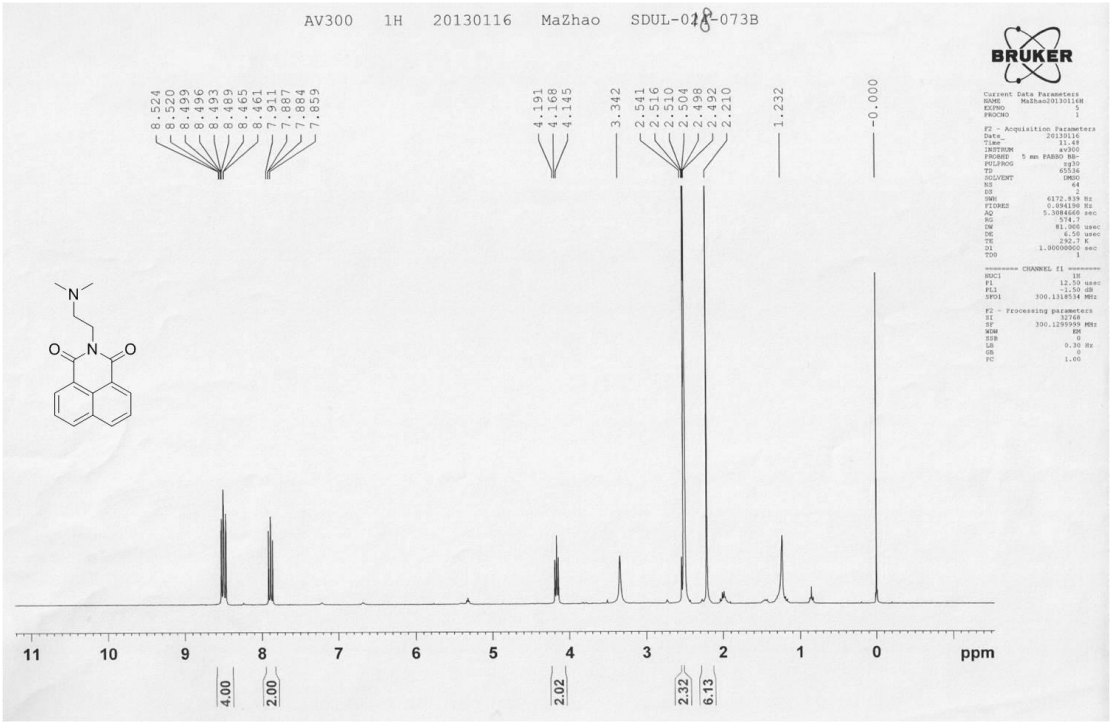
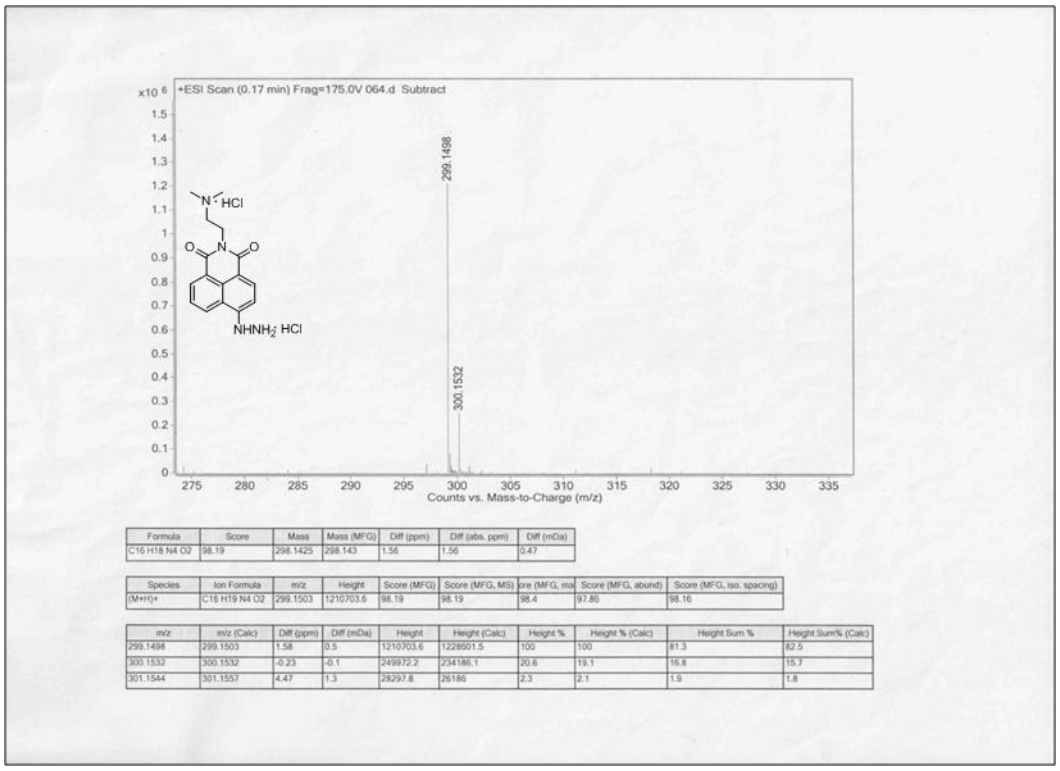


Figure S17. The ^{1}HNMR (300 MHz) spectrum of **1b**

7. HRMS and MS Data

Supporting Information

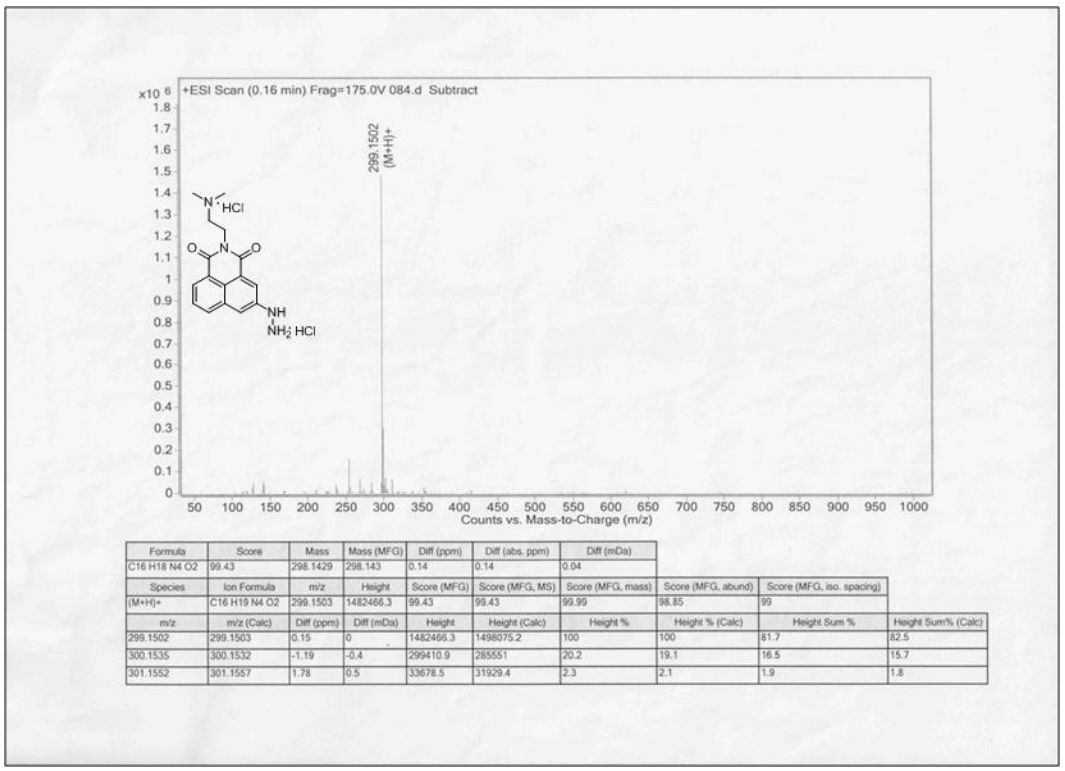


Formula	Score	Mass	Mass (MFG)	Diff (ppm)	Diff (abs. ppm)	Diff (mDa)
C16 H18 N4 O2	98.19	298.1425	298.143	1.56	1.56	0.47

Species	Ion Formula	m/z	Height	Score (MFG)	Score (MFG, MS)	ore (MFG, ma	Score (MFG, abund)	Score (MFG, iso. spacing)
(M+H)+	C16 H19 N4 O2	299.1503	1210703.6	98.19	98.19	98.4	97.86	98.16

m/z	m/z (Calc)	Diff (ppm)	Diff (mDa)	Height	Height (Calc)	Height %	Height % (Calc)	Height Sum %	Height Sum% (Calc)
299.1498	299.1503	1.58	0.5	1210703.6	1228601.5	100	100	81.3	82.5
300.1532	300.1532	-0.23	-0.1	249972.2	234186.1	20.6	19.1	16.8	15.7
301.1544	301.1557	4.47	1.3	28297.8	26186	2.3	2.1	1.9	1.8

Figure S18. High resolution mass spectrum of **Probe 1**



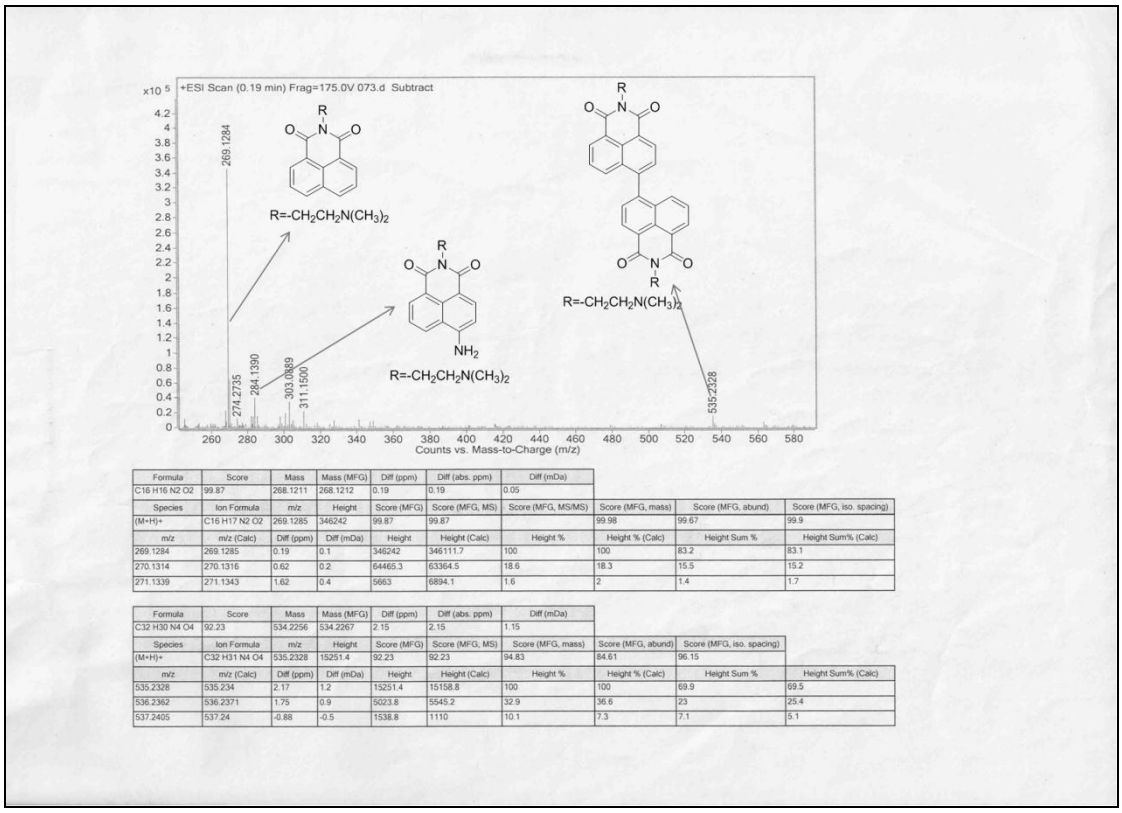
Formula	Score	Mass	Mass (MFG)	Diff (ppm)	Diff (abs. ppm)	Diff (mDa)
C16 H18 N4 O2	99.43	298.1429	298.143	0.14	0.14	0.04

Species	Ion Formula	m/z	Height	Score (MFG)	Score (MFG, MS)	Score (MFG, mass)	Score (MFG, abund)	Score (MFG, iso. spacing)
(M+H)+	C16 H19 N4 O2	299.1503	1482466.3	99.43	99.43	99.99	98.85	99

m/z	m/z (Calc)	Diff (ppm)	Diff (mDa)	Height	Height (Calc)	Height %	Height % (Calc)	Height Sum %	Height Sum% (Calc)
299.1502	299.1503	0.15	0	1482466.3	1498075.2	100	100	81.7	82.5
300.1535	300.1532	-1.19	-0.4	299410.9	285551	20.2	19.1	16.5	15.7
301.1552	301.1557	1.78	0.5	33678.5	31929.4	2.3	2.1	1.9	1.8

Figure S19. High resolution mass spectrum of **Probe 2**

Supporting Information



Formula	Score	Mass	Mass (MFG)	Diff (ppm)	Diff (abs. ppm)	Diff (mDa)
C16 H16 N2 O2	99.87	268.1211	268.1212	0.19	0.19	0.05

Species	Ion Formula	m/z	Height	Score (MFG)	Score (MFG, MS)	Score (MFG, MS/MS)	Score (MFG, mass)	Score (MFG, abund)	Score (MFG, iso. spacing)
(M+H)+	C16 H17 N2 O2	269.1285	346242	99.87	99.87		99.98	99.67	99.9

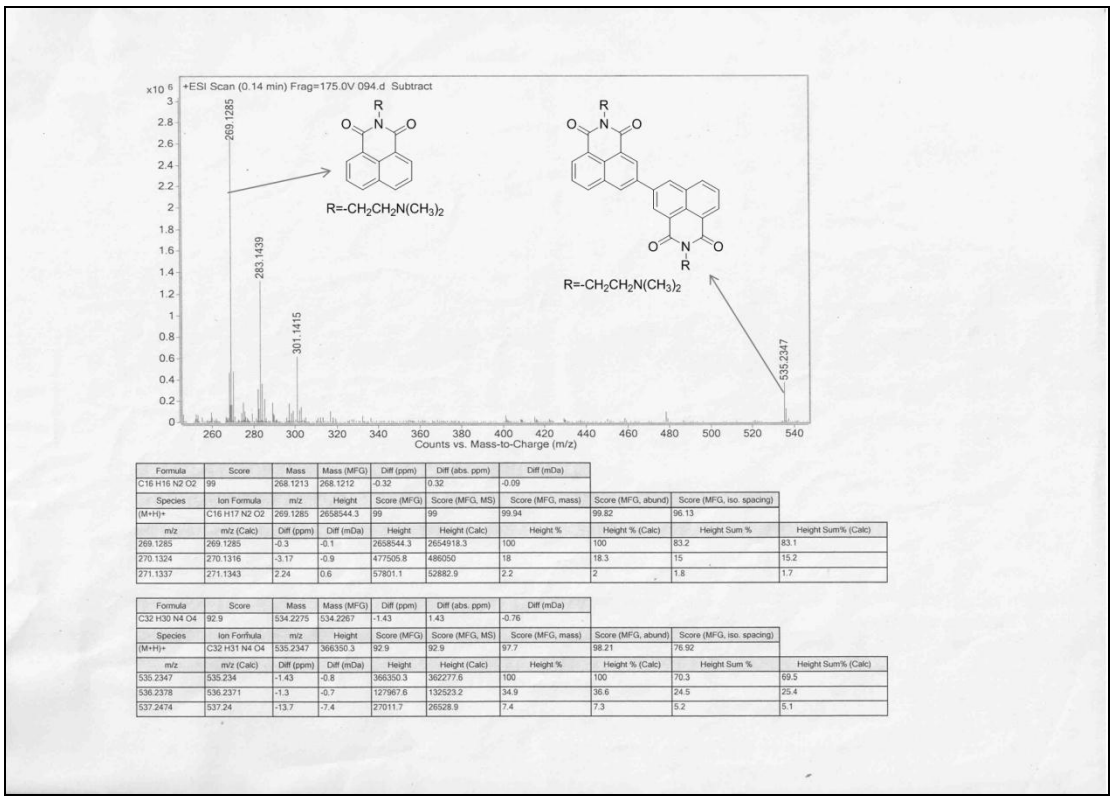
m/z	m/z (Calc)	Diff (ppm)	Diff (mDa)	Height	Height (Calc)	Height %	Height % (Calc)	Height Sum %	Height Sum% (Calc)
269.1284	269.1285	0.19	0.1	346242	346111.7	100	100	83.2	83.1
270.1314	270.1316	0.62	0.2	64465.3	63364.5	18.6	18.3	15.5	15.2
271.1339	271.1343	1.62	0.4	5663	6894.1	1.6	2	1.4	1.7

Formula	Score	Mass	Mass (MFG)	Diff (ppm)	Diff (abs. ppm)	Diff (mDa)
C32 H30 N4 O4	92.23	534.2256	534.2267	2.15	2.15	1.15

Species	Ion Formula	m/z	Height	Score (MFG)	Score (MFG, MS)	Score (MFG, mass)	Score (MFG, abund)	Score (MFG, iso. spacing)
(M+H)+	C32 H31 N4 O4	535.2328	15251.4	92.23	92.23	94.83	84.61	96.15

m/z	m/z (Calc)	Diff (ppm)	Diff (mDa)	Height	Height (Calc)	Height %	Height % (Calc)	Height Sum %	Height Sum% (Calc)
535.2328	535.234	2.17	1.2	15251.4	15158.8	100	100	69.9	69.5
536.2362	536.2371	1.75	0.9	5023.8	5545.2	32.9	36.6	23	25.4
537.2405	537.24	-0.88	-0.5	1538.8	1110	10.1	7.3	7.1	5.1

Figure S20. High resolution mass spectrum of crude products of **probe 1** with ***t*-BuO·**



Formula	Score	Mass	Mass (MFG)	Diff (ppm)	Diff (abs. ppm)	Diff (mDa)
C16 H16 N2 O2	99	268.1213	268.1212	-0.32	0.32	-0.09

Species	Ion Formula	m/z	Height	Score (MFG)	Score (MFG, MS)	Score (MFG, mass)	Score (MFG, abund)	Score (MFG, iso. spacing)
(M+H)+	C16 H17 N2 O2	269.1285	2658544.3	99	99	99.94	99.82	96.13

m/z	m/z (Calc)	Diff (ppm)	Diff (mDa)	Height	Height (Calc)	Height %	Height % (Calc)	Height Sum %	Height Sum% (Calc)
269.1285	269.1285	-0.3	-0.1	2658544.3	2654918.3	100	100	83.2	83.1
270.1324	270.1316	-3.17	-0.9	477505.8	486050	18	18.3	15	15.2
271.1337	271.1343	2.24	0.6	57801.1	52882.9	2.2	2	1.8	1.7

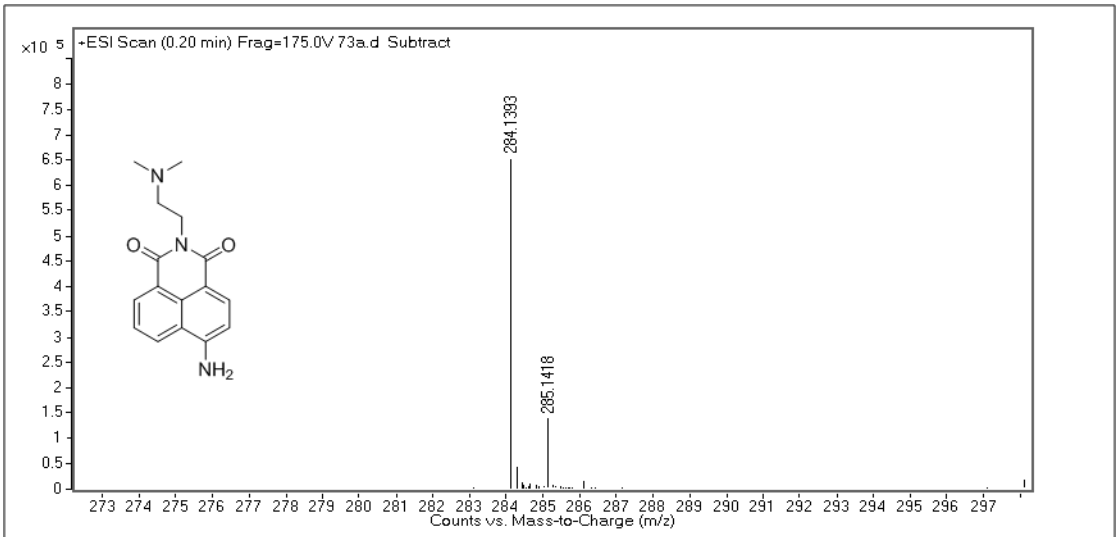
Formula	Score	Mass	Mass (MFG)	Diff (ppm)	Diff (abs. ppm)	Diff (mDa)
C32 H30 N4 O4	92.9	534.2275	534.2267	-1.43	1.43	-0.76

Species	Ion Formula	m/z	Height	Score (MFG)	Score (MFG, MS)	Score (MFG, mass)	Score (MFG, abund)	Score (MFG, iso. spacing)
(M+H)+	C32 H31 N4 O4	535.2347	366350.3	92.9	92.9	97.7	98.21	76.92

m/z	m/z (Calc)	Diff (ppm)	Diff (mDa)	Height	Height (Calc)	Height %	Height % (Calc)	Height Sum %	Height Sum% (Calc)
535.2347	535.234	-1.43	-0.8	366350.3	362277.6	100	100	70.3	69.5
536.2378	536.2371	-1.3	-0.7	127967.6	132523.2	34.9	36.6	24.5	25.4
537.2474	537.24	-13.7	-7.4	27011.7	26528.9	7.4	7.3	5.2	5.1

Figure S21. High resolution mass spectrum of crude products of **probe 2** with ***t*-BuO·**

Supporting Information



Formula	Score	Mass	Mass (MFG)	Diff (ppm)	Diff (abs. ppm)	Diff (mDa)
C16 H17 N3 O2	98	283.132	283.1321	0.25	0.25	0.07

Species	Ion Formula	m/z	Height	Score (MFG)	Score (MFG, MS)	Score (MFG, mass)	Score (MFG, abund)	Score (MFG, iso. spacing)
(M+H)+	C16 H18 N3 O2	284.1394	651859.4	98	98	99.96	95.15	97.51

m/z	m/z (Calc)	Diff (ppm)	Diff (mDa)	Height	Height (Calc)	Height %	Height % (Calc)	Height Sum %	Height Sum% (Calc)
284.1393	284.1394	0.23	0.1	651859.4	666154.3	100	100	81	82.8
285.1418	285.1424	2.16	0.6	137653.8	124466.7	21.1	18.7	17.1	15.5
286.1429	286.145	7.43	2.1	14836.6	13728.9	2.3	2.1	1.8	1.7

Figure S22. High resolution mass spectrum of **1a**

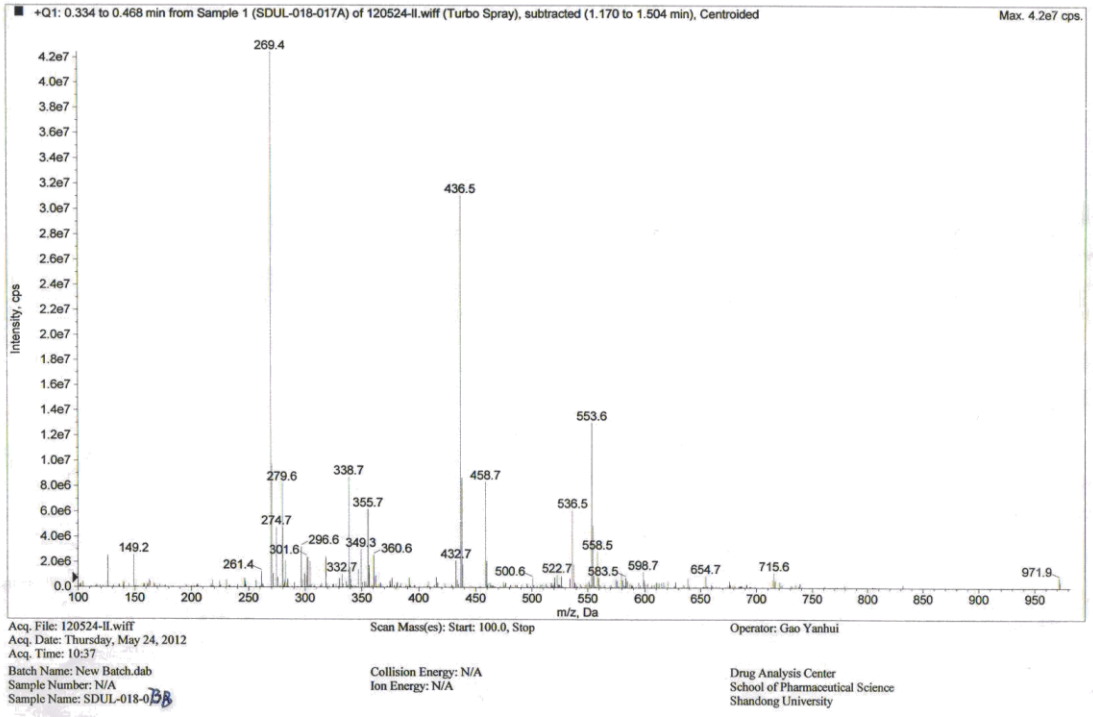


Figure S23. Mass spectrum of **1b**

Supporting Information

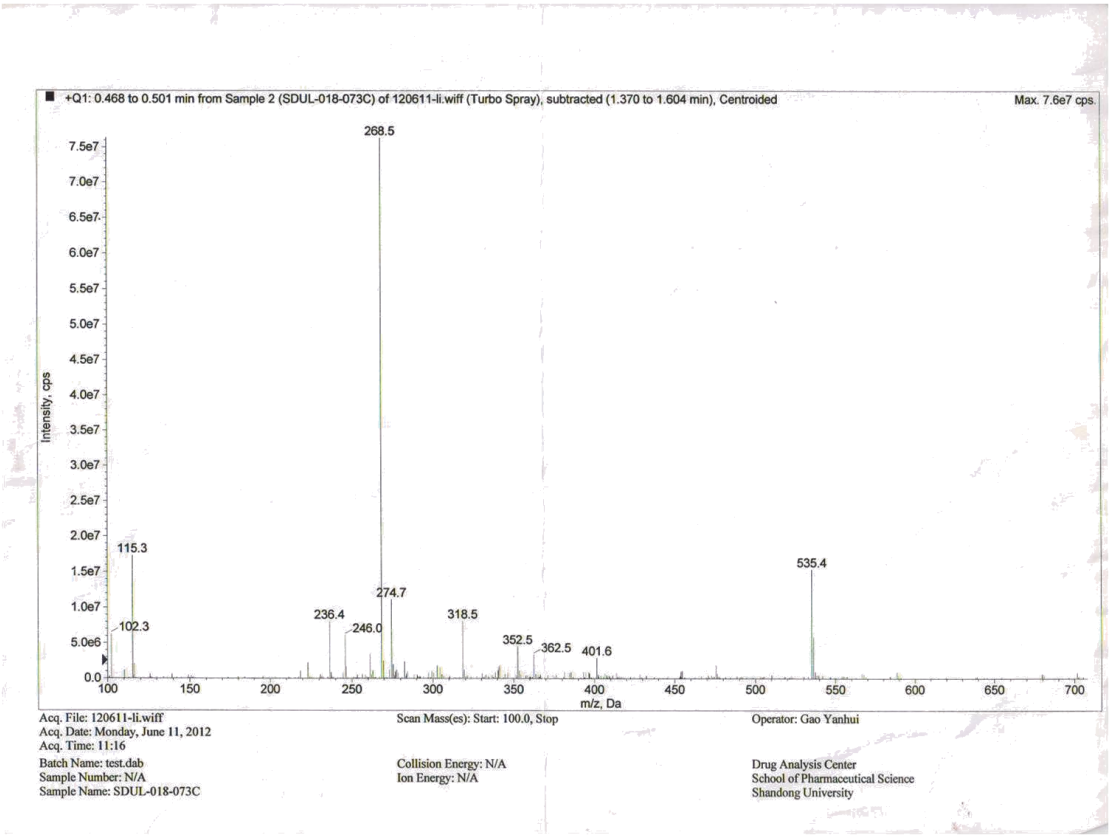


Figure S24. Mass spectrum of **1c**

8. HPLC data

Column: 250×10.00 mm

Stationary Phase: C_{18}

Mobile Phase: CH_3OH: H_2O = 6.5:3.5, 0.5 % triethylamine in CH_3OH.

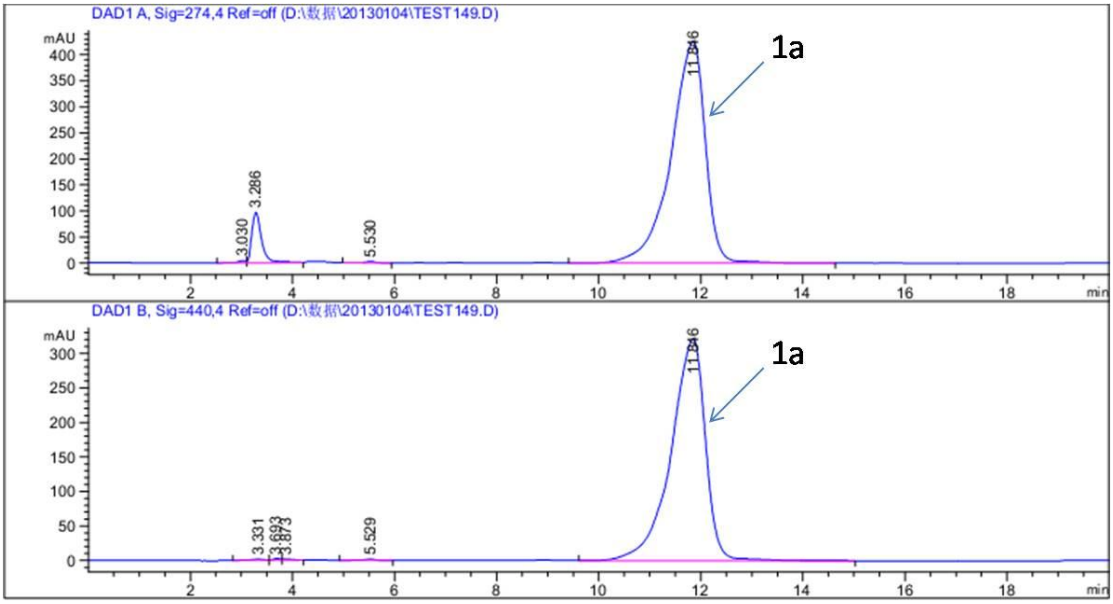


Figure S25. The HPLC chromatogram of **standard substance** of 6-amino-2-(2-(dimethylamino)ethyl)-1H- benzo[*de*]isoquinoline-1,3(2*H*)-dione, detected at 274 nm and 440 nm.

Supporting Information

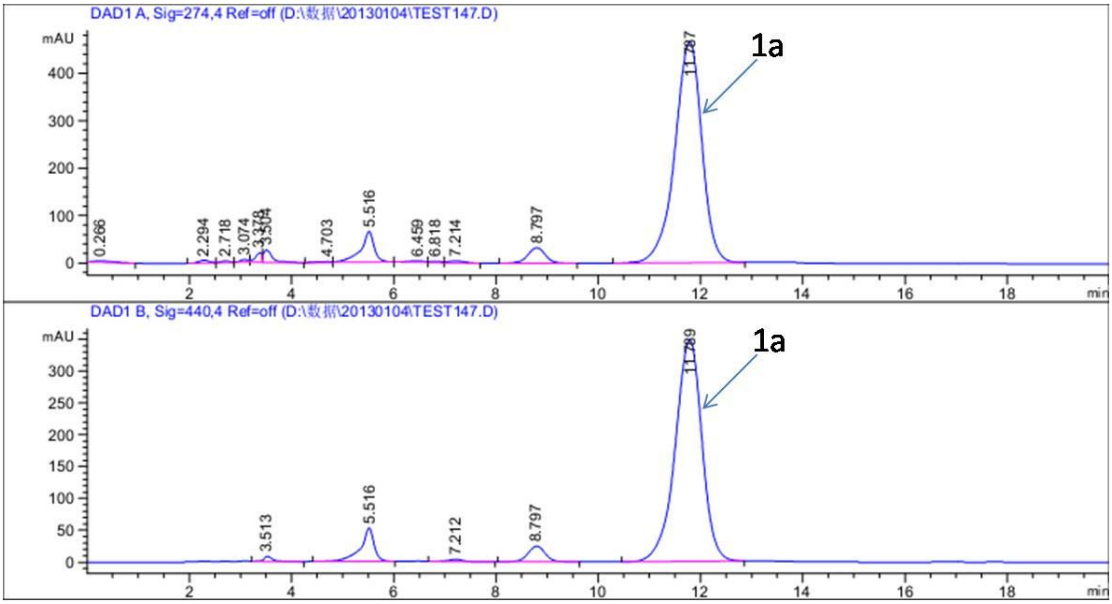


Figure S26. The HPLC chromatogram of **1a**, recorded at 274 nm and 440 nm.

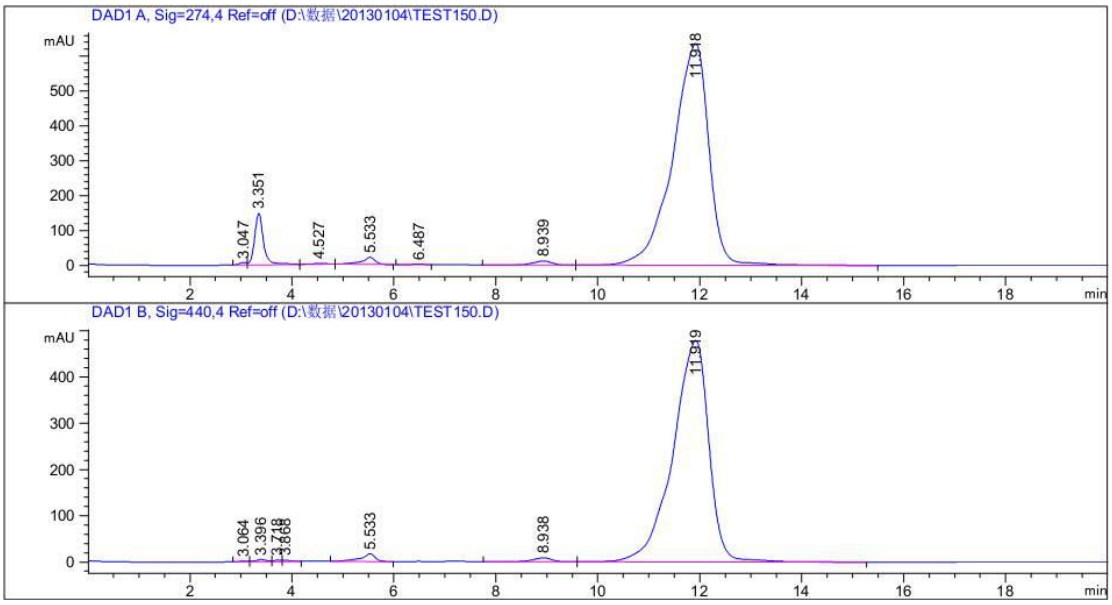


Figure S27. The HPLC chromatogram of the mixture of **the** standard substance and **1a**, recorded at 274 nm and 440 nm.