Lysosome specific theranostic NO donor inhibits cancer cells by stimuli responsive molecular self-decompostion with an on-demand fluorescence pattern

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Synthesis

Synthesis of 4-(methylamino)benzaldehyde (1): 4-bromobenzaldehyde (1.85 g, 10.0 mmol), 40% aqueous of methylamine (4 ml) was added into a 35 ml screw tube. The mixture was stirred at 100 ℃ for 16 h. Then the reaction mixture was extracted with ethyl acetate (10 ml×3). The organic phase was dried by anhydrous Na_2SO_4 and purified by silica gel column chromatography using the petroleum ether and ethyl acetate (5:1, v/v) as an eluent.[1] The product was pale yellow waxy solid, yield 78 %. ^{1}H NMR (400 MHz, DMSO) δ 9.60 (s, 1H), 7.61 (d, J = 8.7 Hz, 2H), 6.88 (d, J = 4.5 Hz, 1H), 6.62 (d, J = 8.7 Hz, 2H), 2.76 (d, J = 5.0 Hz, 3H).

Synthesis of (4-(methylamino)phenyl)methanol (2): 1 (1.09 g, 8.1 mmol) was dissolved in methanol and stirred at 0 ℃. Then the $NaBH_4$ (0.40 g, 10.0 mmol) was added. The mixture was stirred at 0 ℃ for 4 h. Then mixture of ice and water was added slowly to the reaction mixture. After the bubbles were disappeared, methanol was evaporated under vacuum, the water phase was extracted by ethyl acetate (10 ml×3). The organic phase was dried by anhydrous Na_2SO_4 and purified by a flash silica gel column

chromatography using the petroleum ether and ethyl acetate (5:1, v/v) as an eluent, yield 93 %. The pale yellow waxy solid was taken to the next procedure without further purification.

Synthesis of N-(4-(hydroxymethyl)phenyl)-N-methylnitrous amide (3):

2 (0.46 g, 3.4 mmol) was dissolved in a mixed solvent of HCl, acetic acid, dichloromethane (1:10:2, v/v) and cooled to 0 ℃ with an ice-water bath. $NaNO_2$ (0.28 g, 4.1 mmol) was added slowly while the mixture was being constantly stirred. The reaction mixture was allowed to warm to room temperature. After stirred for 2 h, concentrated aqueous solution of $NaHCO_3$ was added to adjust the pH to neutral. Then the mixture was extracted with dichloromethane (10 ml×3). The organic layer was dried by anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to afford the solid. The yellow solid was dissolved in methanol and the pH was adjusted to ~10 with the 1 M NaOH aqueous solution. The solution was stirred for 2 h. Then the solution was diluted with water and extracted with ethyl acetate (10 ml×3). The organic layer was dried by anhydrous Na_2SO_4, filtered and evaporated under reduced pressure to afford the brown solid. The solid was purified with a silica column chromatography using the petroleum ether and ethyl acetate (5:1, v/v) as an eluent, yield 70 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 – 7.52 (m, 1H), 7.48 (d, J = 8.7 Hz, 1H), 4.76 (s, 1H), 3.46 (s, 2H), 1.89 (s, 1H).

Synthesis of intermediates 4 and 4’

6-Bromo-2-butyl-1H-benzo[de]isoquinoline-1,3(2H)-dione (4): 6-bromo-1H,3H-benzo[de]isochromene-1,3-dione (1.39 g, 5.0 mmol), n-butylamine (4.38 ml, 45.0 mmol) and ethanol (100 ml) were added into a 250 ml round bottom flask. The mixture was refluxed over night during which the mixture became clear. Then the reaction mixture was cooled at 4 ℃. The brown solid was filtered off and dried in vacuum,[2, 3] yield 68 %. ^{1}H NMR (600 MHz, DMSO) δ 8.54 (ddd, *J* = 9.5, 7.9, 1.0 Hz, 1H), 8.32 (d, *J* = 7.8 Hz, 1H), 8.21 (d, *J* = 7.8 Hz, 1H), 7.99 (dd, *J* = 8.4, 7.3 Hz, 1H), 4.09 – 3.95 (m, 1H), 1.67 – 1.56 (m, 1H), 1.45 – 1.27 (m, 1H), 0.93 (t, *J* = 7.4 Hz, 2H).

6-Bromo-2-(2-morpholinoethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (4'): The preparation was the same as **4** except that n-butylamine was replaced by 2-morpholinoethan-1-amine. The yield was 79 %. ^{1}H NMR (600 MHz, DMSO) δ 8.50 (dd, *J* = 26.2, 7.7 Hz, 1H), 8.28 (d, *J* = 7.7 Hz, 1H), 8.17 (d, *J* = 7.7 Hz, 1H), 7.96 (t, *J* = 7.7 Hz, 1H), 4.15 (t, *J* = 6.7 Hz, 1H), 3.53 (s, 2H), 2.56 (t, *J* = 6.7 Hz, 1H), 2.46 (s, 2H).

Synthesis of intermediates 5 and 5':

6-azido-2-butyl-1H-benzo[de]isoquinoline-1,3(2H)-dione (5): 4 (0.33 g, 1.0 mmol) and NaN_3 (0.26 g, 4.0 mmol) (HYPERTOXIC!)was added into a 100 ml round bottom flask containing 50 ml N,N-dimethylformamide (DMF). The mixture was stirred at 100 ℃ overnight. Then the DMF was evaporated slowly. The residue was wash with water, dried under vacuum

and purified by silica gel chromatography using dichloromethane and methanol (5:1, v/v) as an eluent, yield 65 %. ^{1}H NMR (600 MHz, DMSO) δ 8.51 (dd, *J* = 7.2, 1.1 Hz, 1H), 8.45 (d, *J* = 8.0 Hz, 1H), 8.39 (dd, *J* = 8.4, 1.1 Hz, 1H), 7.85 (dd, *J* = 8.4, 7.3 Hz, 1H), 7.73 (d, *J* = 8.0 Hz, 1H), 4.12 – 3.94 (m, 2H), 1.61 (dd, *J* = 10.5, 4.5 Hz, 2H), 1.35 (dd, *J* = 15.0, 7.5 Hz, 2H), 0.93 (t, *J* = 7.4 Hz, 3H).

6-Azido-2-(2-morpholinoethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (5'): The synthesis was the same as **5** except that **4** was replaced by **4'**. The yield was 74 %. ^{1}H NMR (600 MHz, DMSO) δ 8.49 (dd, *J* = 7.2, 0.8 Hz, 1H), 8.42 (d, *J* = 8.0 Hz, 1H), 8.36 (d, *J* = 8.3 Hz, 1H), 7.86 – 7.80 (m, 1H), 7.70 (d, *J* = 8.0 Hz, 1H), 4.17 (s, 2H), 3.55 (s, 5H), 2.58 (s, 2H), 2.48 (d, *J* = 1.7 Hz, 1H).

Synthesis of intermediates 6 and 6':

6-Amino-2-butyl-1H-benzo[de]isoquinoline-1,3(2H)-dione (6): 5 (0.12 g, 0.4 mmol) and $Na_2S{\cdot}xH_2O$ (0.32 g, 0.4 mmol) were added into a 50 ml round bottom flask containing 25 ml DMF. The mixture was stirred at r.t. for 4 h. Then the solvent was evaporated under vacuum. The residue was resolved in methanol and the undissolved substance was filtered off. The solvent was evaporated under vacuum and the orange solid was dried under vacuum overnight, yield 59 %. ^{1}H NMR (600 MHz, DMSO) δ 8.60 (dd, *J* = 8.4, 1.0 Hz, 1H), 8.41 (dd, *J* = 7.3, 1.0 Hz, 1H), 8.18 (d, *J* = 8.4 Hz, 1H), 7.64 (dd, *J* = 8.3, 7.3 Hz, 1H), 7.42 (s, 2H), 6.84 (d, *J* = 8.4 Hz, 1H), 4.03

– 3.97 (m, 2H), 1.62 – 1.52 (m, 2H), 1.32 (dd, *J* = 15.0, 7.4 Hz, 2H), 0.91 (t, *J* = 7.4 Hz, 3H).

6-amino-2-(2-morpholinoethyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (6'): The compound was prepared by the same way as **6** except that **5** was replaced by **5'**. The yield was 62 %. ^{1}H NMR (600 MHz, DMSO) δ 8.61 (d, *J* = 8.2 Hz, 1H), 8.42 (d, *J* = 7.1 Hz, 1H), 8.18 (d, *J* = 8.4 Hz, 1H), 7.75 – 7.59 (m, 1H), 7.46 (s, 1H), 6.84 (d, *J* = 8.4 Hz, 1H), 4.14 (t, *J* = 7.0 Hz, 2H), 3.60 – 3.49 (m, 4H), 2.54 (s, 1H), 2.53 (s, 1H), 2.45 (s, 2H).

Synthesis of Mo-Nap-NO: 6' (0.13 g, 0.5 mmol), N,N-diisopropyl-ethylamine (DIPEA) (0.16 g, 1.2 mmol) and catalytic amount of 4-dimethylaminopyridine (DMAP) (3 mg) was added into a 50 ml round bottom flask containing 25 ml anhydrous toluene which equipped with a rubber plug. The mixture was stirred at 0 ℃ for 15 min. Then the triphosgene (0.07 g, 0.25 mmol) was injected. The mixture was stirred at 0 ℃ for 2 h. **3** (0.10g, 0.6 mmol) dissolved in 10 ml anhydrous toluene was added slowly into the reaction mixture. The mixture was stirred at 0 ℃ overnight. Then the row product was filtered after the mixture was stored at -20 ℃ for 2 h. The pale yellow solid was washed with cold methanol and dried under vacuum, yield 49 %. ^{1}H NMR (600 MHz, DMSO) δ 10.43 (s, 1H), 8.72 (d, *J* = 8.5 Hz, 1H), 8.49 (dd, *J* = 14.3, 7.5 Hz, 2H), 8.21 (d, *J* = 8.2 Hz, 1H), 7.83 (dd, *J* = 8.4, 7.4 Hz, 1H), 7.69 (dd, *J* = 22.5, 8.6 Hz, 4H), 5.35 (s, 2H), 4.17 (t, *J* = 7.0 Hz, 2H), 3.53 (s, 4H), 3.45 (s, 3H), 2.56 (t, *J*

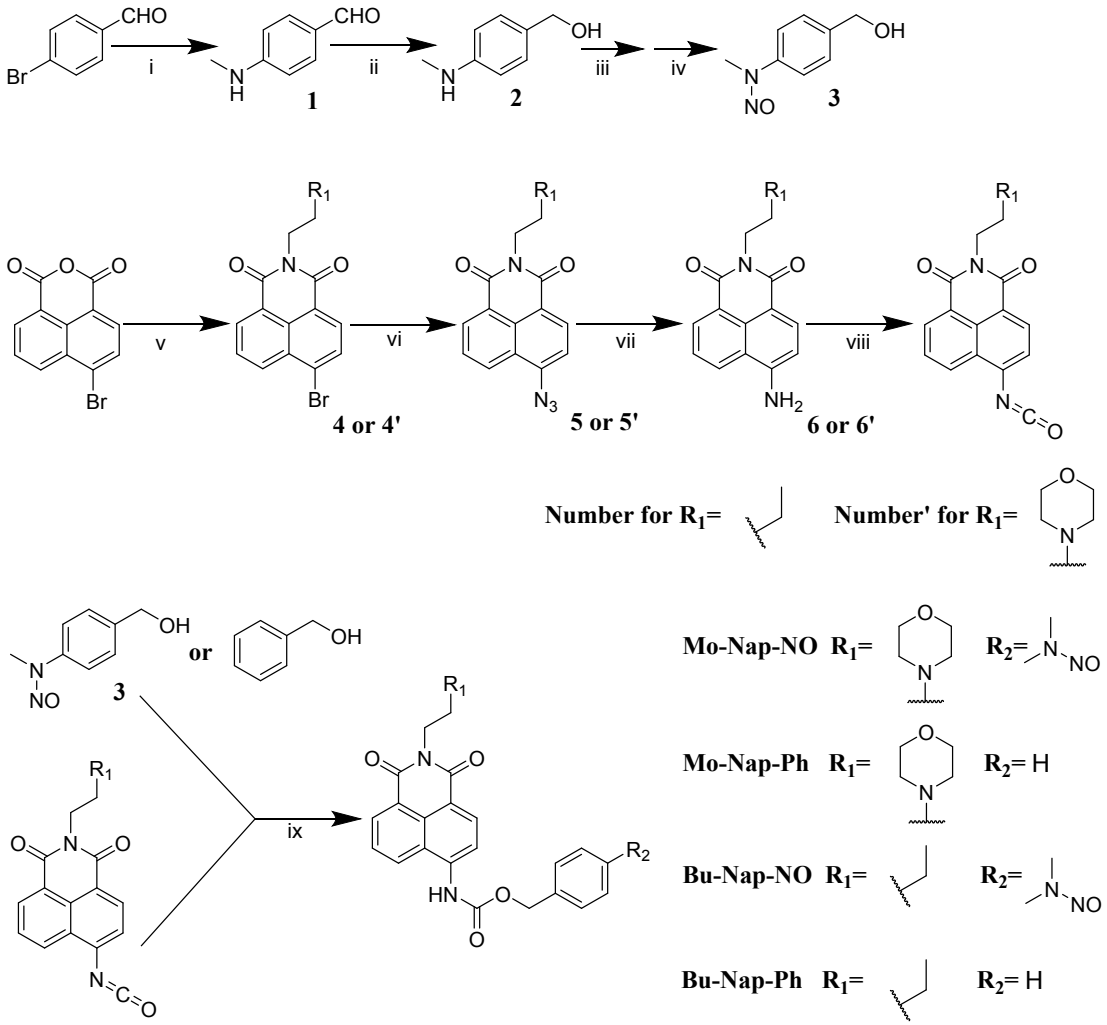
= 6.9 Hz, 2H), 2.46 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 163.89, 163.32, 154.36, 142.20, 141.14, 135.82, 132.18, 131.39, 129.84, 129.76, 128.76, 126.83, 124.22, 122.59, 119.88, 118.55, 117.44, 66.68, 66.42, 56.03, 53.87, 37.16, 32.01.

Synthesis of Mo-Nap-Ph: The preparation was the same as Mo-Nap-NO except that the **3** was replaced by benzyl alcohol. The product was collected as pale yellow solid, yield 61 %. ^{1}H NMR (600 MHz, DMSO) δ 10.50 (s, 1H), 8.75 (d, *J* = 8.5 Hz, 1H), 8.50 (dd, *J* = 16.0, 7.7 Hz, 2H), 8.21 (d, *J* = 8.2 Hz, 1H), 7.85 (t, *J* = 7.9 Hz, 1H), 7.51 (d, *J* = 7.4 Hz, 2H), 7.43 (t, *J* = 7.4 Hz, 2H), 7.38 (d, *J* = 7.2 Hz, 1H), 5.28 (s, 2H), 4.18 (t, *J* = 6.7 Hz, 2H), 3.53 (s, 5H), 2.59 – 2.54 (m, 2H), 2.46 (s, 3H). ^{13}C NMR (151 MHz, DMSO) δ 163.94, 163.38, 154.47, 141.29, 136.71, 132.20, 131.44, 129.99, 128.98, 128.80, 128.68, 128.66, 128.62, 126.87, 124.36, 122.58, 118.75, 117.45, 67.01, 66.69, 56.04, 53.87, 37.17.

Synthesis of Bu-Nap-NO: The synthetic method was the same as Mo-Nap-NO except that the **6'** was replaced by **6**. The product was pale yellow solid, yield 55 %. ^{1}H NMR (600 MHz, DMSO) δ 10.42 (s, 1H), 8.72 (d, *J* = 8.5 Hz, 1H), 8.50 (dd, *J* = 14.3, 7.7 Hz, 2H), 8.22 (d, *J* = 8.2 Hz, 1H), 7.84 (t, *J* = 7.9 Hz, 1H), 7.69 (dd, *J* = 23.2, 8.4 Hz, 4H), 5.34 (s, 2H), 4.04 (t, *J* = 7.4 Hz, 2H), 3.45 (s, 3H), 1.61 (dt, *J* = 14.9, 7.5 Hz, 2H), 1.35 (dd, *J* = 14.8, 7.4 Hz, 2H), 0.93 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, DMSO) δ 163.93, 163.38, 154.40, 142.19, 141.13, 135.85, 132.14, 131.38,

129.82, 128.79, 126.86, 124.33, 122.68, 119.90, 118.69, 117.57, 66.40, 32.03, 30.14, 20.28, 14.20.

Synthesis of Bu-Nap-Ph: The preparation was the same as Bu-Nap-NO except that the **3** was replaced by benzyl alcohol. The pale yellow solid was collected with a yield of 68 %. ^{1}H NMR (600 MHz, DMSO) δ 10.36 (s, 1H), 8.69 (d, *J* = 7.9 Hz, 1H), 8.46 (dd, *J* = 10.3, 7.8 Hz, 2H), 8.22 – 8.15 (m, 1H), 7.80 (d, *J* = 7.1 Hz, 1H), 7.50 (s, 2H), 7.42 (d, *J* = 6.5 Hz, 2H), 7.37 (s, 1H), 5.27 (s, 2H), 4.01 (d, *J* = 5.5 Hz, 2H), 1.59 (d, *J* = 5.9 Hz, 2H), 1.33 (s, 2H), 0.99 – 0.81 (m, 3H). ^{13}C NMR (151 MHz, DMSO) δ 163.88, 163.32, 154.39, 141.13, 136.68, 132.11, 131.31, 129.68, 128.98, 128.75, 128.68, 126.77, 124.18, 122.63, 118.44, 117.44, 67.06, 30.13, 20.28, 14.19.



Scheme S1 Synthesis route of products. i. 40% aqueous of methylamine, 100 ℃, 16 h; ii. $NaBH_4$, methanol, ice-water bath to room temperature, 4 h; iii. $NaNO_2$, dichloromethane, HCl, acetic acid, ice-water bath to room temperature, 3 h; iv. 1 M aqueous of NaOH, methanol, 6 h; v. N-butylamine or 2-morpholinoethan-1-amine, ethanol, reflux overnight; vi. NaN_3, DMF, 100 ℃, overnight; vii. Na_2S, DMF, room temperature, 4 h; viii. Triphosgene, DIPEA, DMAP, toluene, ice-water bath, 4 h; ix. **3** or benzyl alcohol, ice-water bath to room temperature overnight.

Table S1 Photophysics parameters of the NO donors before and after 460 nm light irradiation for 10 min.

Compounds		ε/(L*mol^{-1}*cm^{-1})			λ_{Em}/nm	I_L/I_D[a]
Mo-Nap-NO	Dark	23900 (255 nm)	16300 (370 nm)	2600 (430 nm)	550	14.9
	Light	29600 (255 nm)	6300 (370 nm)	13100 (430 nm)		
Bu-Nap-NO	Dark	21400 (260 nm)	16400 (372 nm)	1200 (435 nm)	539	21.6
	Light	34400 (260 nm)	3800 (372 nm)	14200 (435 nm)		

[a] I_L/I_D=(Fluorescence intensity of irradiated samples)/(Fluorescence intensity of samples without irradiation).

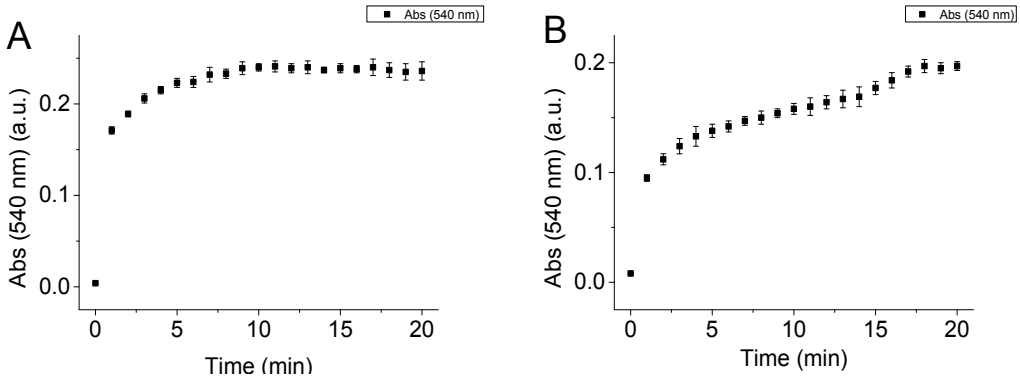


Figure S1 Griess assay to confirm the NO release of (A) Mo-Nap-NO and (B) Bu-Nap-NO. The data was presented as the mean ± SD for three independent experiments.

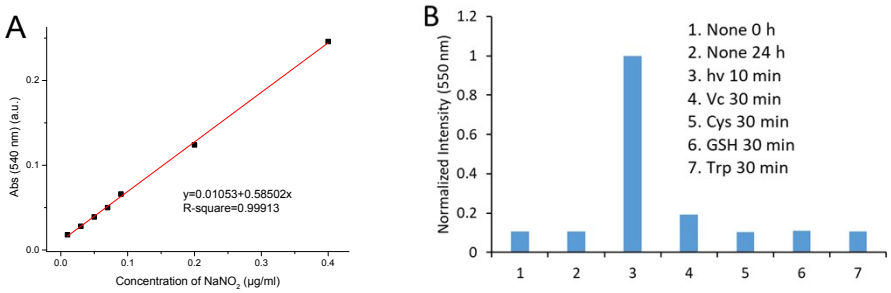


Figure. S2 (A) Calibration curve of griess assay. (B) Dark stability of Mo-Nap-NO (10 μM) without or with the presence of biological reductants [Ascorbic acid (10 mM), Cysteine (10 mM), Glutathione (10 mM) in phosphate buffer (0.01 mM, pH = 7.4, containing 1 % DMSO) and Tryptophan (10 mM) in DMSO]. The fluorescence intensity is collected at 550 nm.

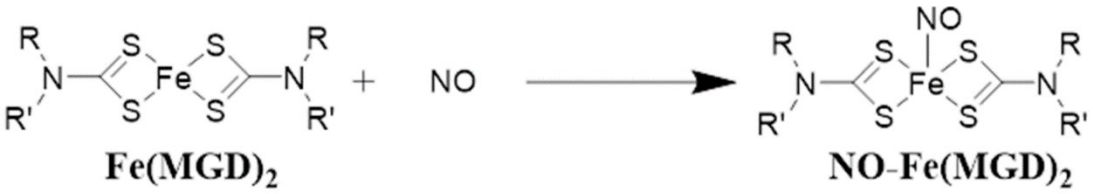


Figure. S3 Mechanism illustration of Ferro-di(N-methyl-D-glucamine-dithiocarbamate) as a NO trap for electron paramagnetic resonance experiment.

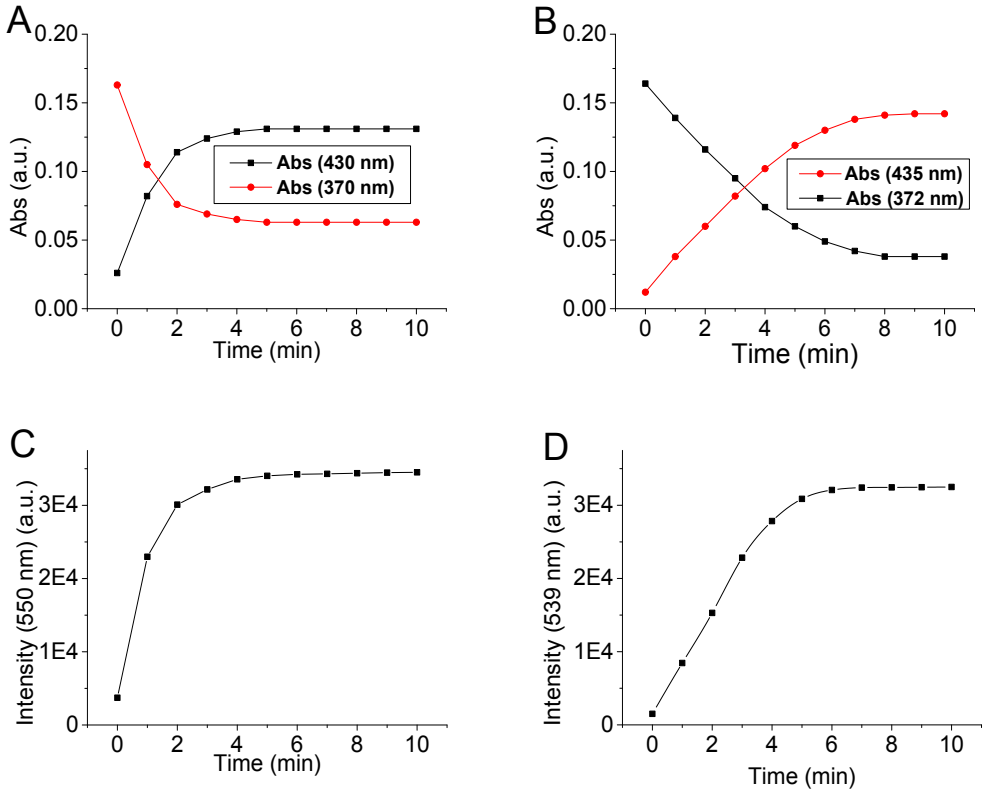


Figure S4 Kinetic curves of UV-Vis absorption and fluorescence emission titration experiments of NO donors. (A) UV-Vis absorption titration of Mo-Nap-NO. (B) UV-Vis absorption titration of Bu-Nap-NO. (C) Fluorescence emission titration of Mo-Nap-NO. (D) Fluorescence emission titration of Bu-Nap-NO. 10 μM of complexes were irradiated by series of dose of 460 nm light irradiation. For Mo-Nap-NO, the solvent was PBS (0.01 mM, pH=7.4, containing 1 % DMSO). For Bu-Nap-NO, the

solvent was PBS (0.01 mM, pH=7.4)/DMSO=1:1, v/v.

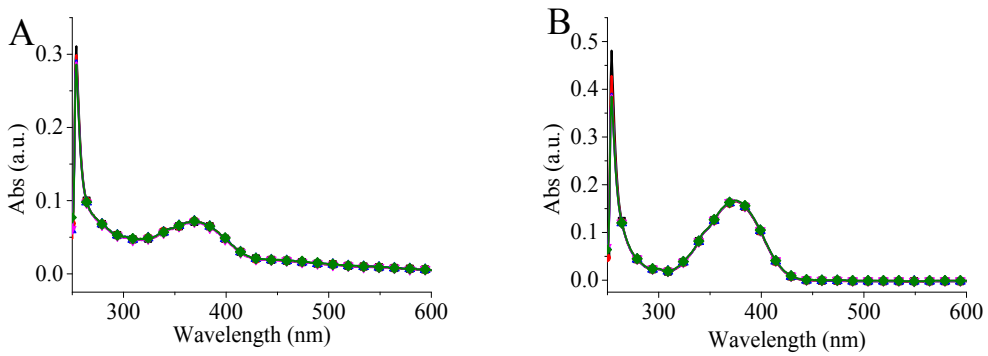


Figure. S5 UV-Vis absorption titration of (A) Mo-Nap-Ph and (B) Bu-Nap-Ph under the 460 nm light irradiation. 10 μM of compounds were irradiated by 460 nm light for 5, 10, 15, 20, 25 min. For Mo-Nap-Ph, the solvent was PBS (0.01 mM, pH=7.4, containing 1 % DMSO). For Bu-Nap-Ph, the solvent was PBS (0.01 mM, pH=7.4)/DMSO=1:1, v/v.

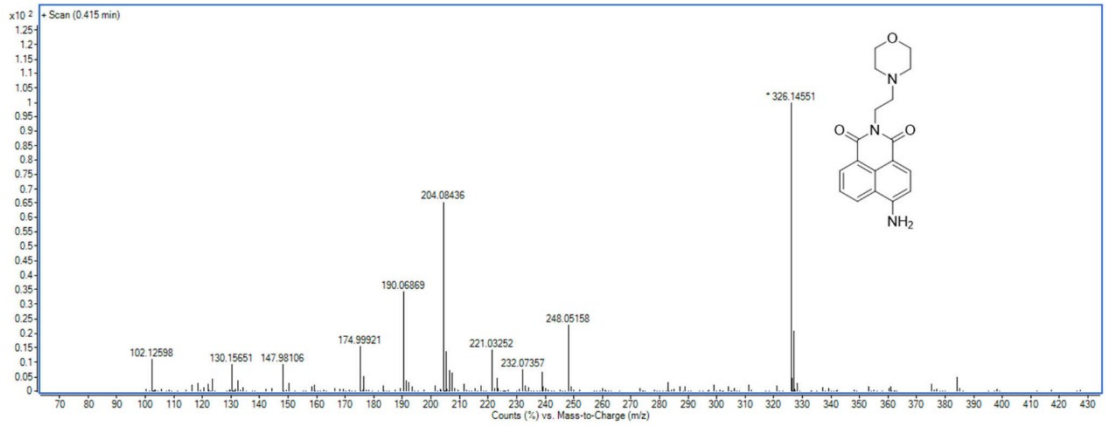
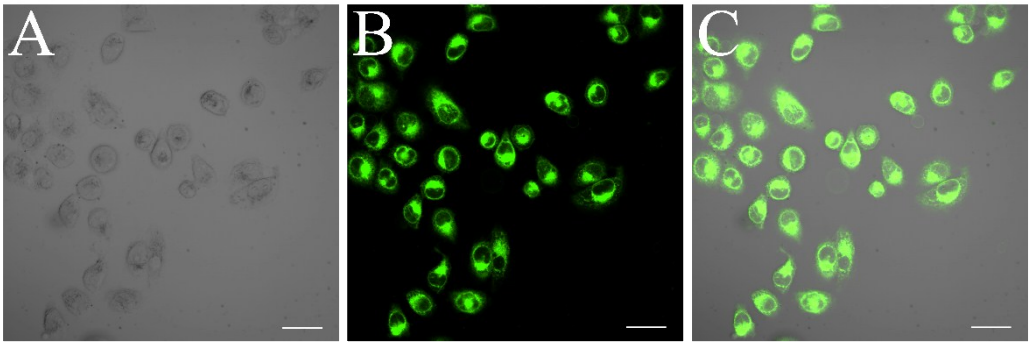


Figure. S6 Mass spectra of Mo-Nap-NO irradiated by 460 nm light.



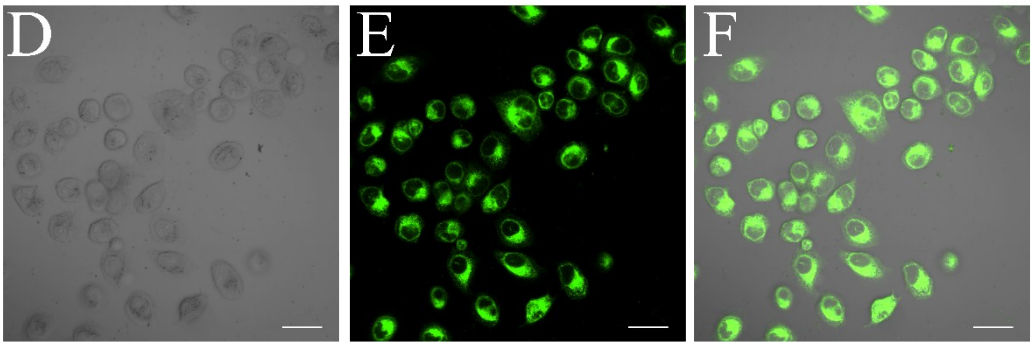


Figure. S7 The cells in Figure S6A (bright field), B (green Channel) and C (merged image) were loaded with 20 μM of Mo-Nap-Ph and irradiated by 460 nm light for 10 min. The cells in Figure S6D (bright field), E (green Channel) and F (merged image) were loaded with 20 μM of Bu-Nap-Ph and irradiated by 460 nm light for 10 min. Green channel: λ_{EX}=405 nm, λ_{EM} =500-580 nm. Scale bar, 30 μm.

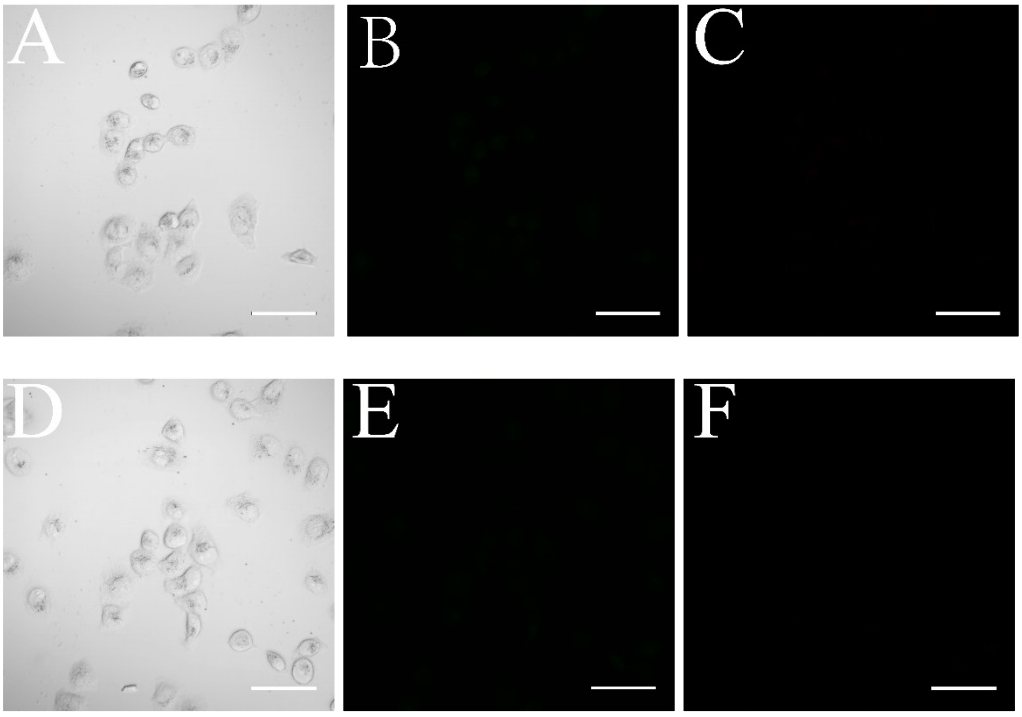


Figure. S8 Cell images of A549 cells treated with compounds and NO-tracker. The cells in Figure 3A (bright field), B (green Channel) and C (red Channel) were co-loaded with 20 μM of Mo-Nap-NO and 5 μM of NO-

tracker without 460 nm irradiation. The cells in Figure 3D (bright field), E (green Channel) and F (red Channel) were co-loaded with 20 μM of Bu-Nap-NO and 5 μM of NO-tracker without 460 nm irradiation. Green channel: λ_{EX}=405 nm, λ_{EM} =500-580 nm; Red channel: λ_{EX}=561 nm, λ_{EM} =600-640 nm. Scale bar, 60 μm.

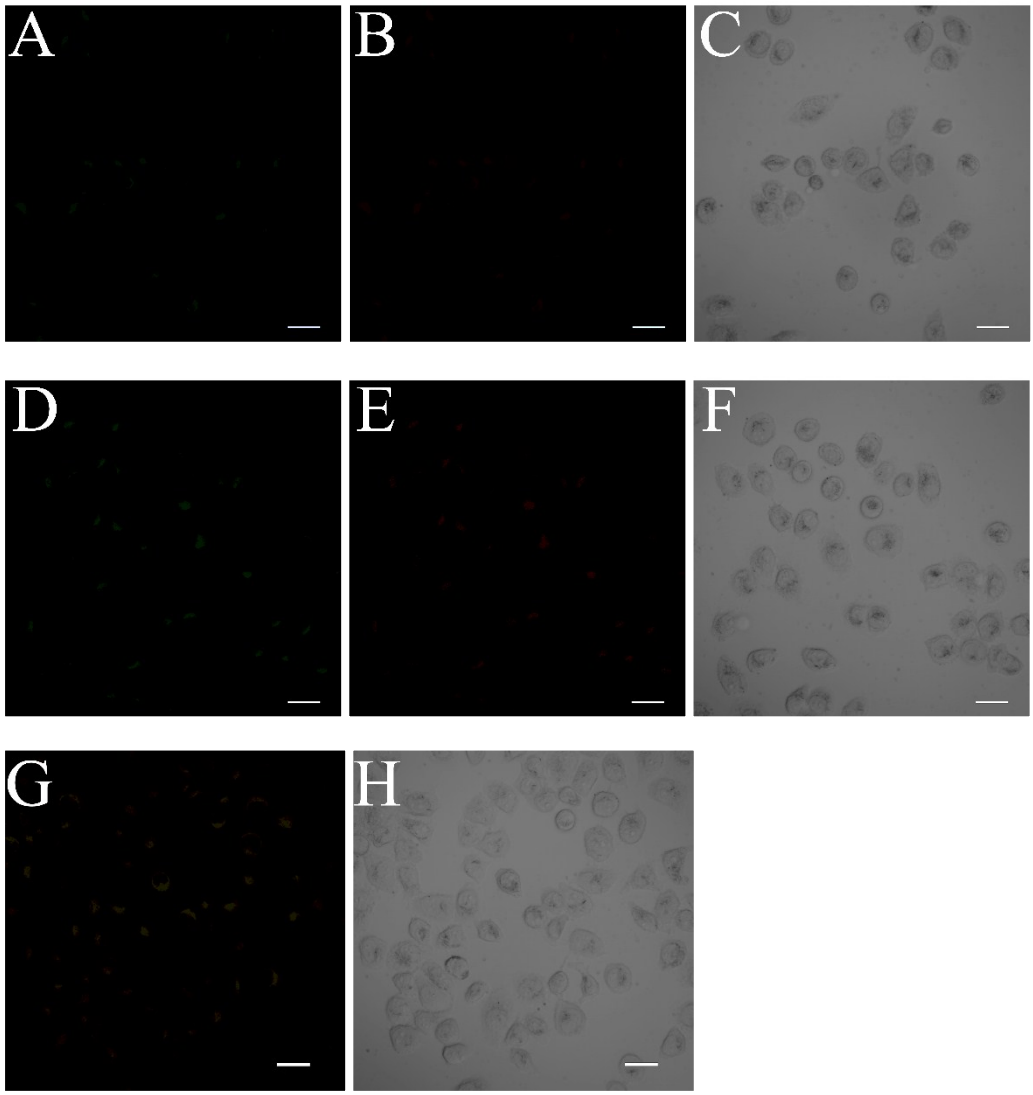


Figure. S9 The cells in Figure S7A (green channel), B (red Channel) and C (merged image) were co-loaded with 20 μM of Mo-Nap-Ph and 5 μM of NO-tracker and irradiated by 460 nm light for 10 min. The cells in Figure S7D (green channel), E (red Channel) and F (merged image) were co-

loaded with 20 μM of Bu-Nap-Ph and 5 μM of NO-tracker and irradiated by 460 nm light for 10 min. The cells in Figure S7G (red channel), 7H (merged image) were loaded with 5 μM of NO-tracker as a negative control. Green channel: λ_{EX}=405 nm, λ_{EM} =500-580 nm; Red channel: λ_{EX}=561 nm, λ_{EM} =600-640 nm. Scale bar, 30 μm.

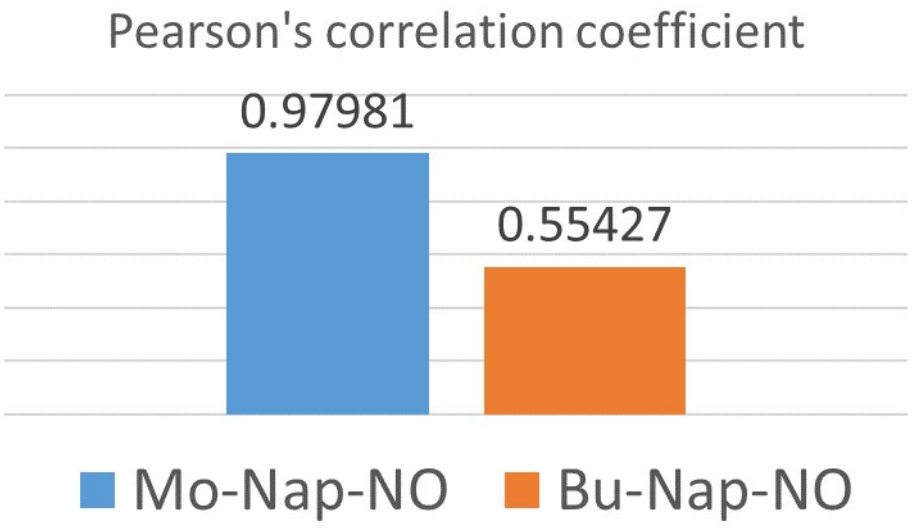
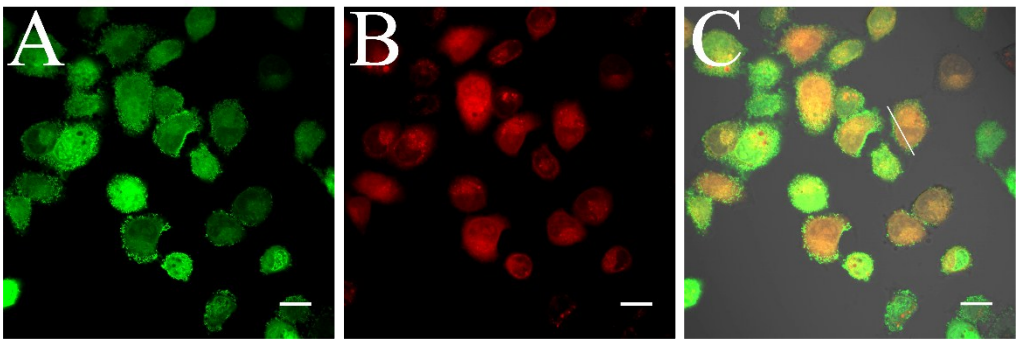


Figure. S10 Pearson's correlation coefficient of Mo-Nap-NO and Bu-Nap-NO towards Lyso-tracker.



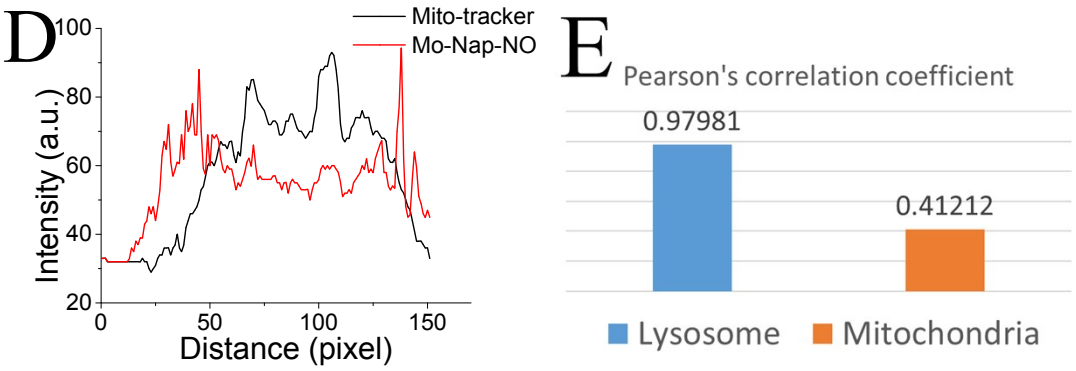


Figure. S11 Cell images of A549 cells treated with the compounds and Mito-tracker. The cells in Figure 3A (green channel), B (red Channel) and C (merged image) were co-loaded with 20 μM of Mo-Nap-NO and 5 μM of Mito-tracker and irradiated by 460 nm light for 10 min. (D) Intensity profile of the linear ROI across the cell (white line in images C). (E) Pearson's correlation coefficient of Mo-Nap-NO towards Lyso-tracker and Mito-tracker. Green channel: λ_{EX}=405 nm, λ_{EM} =500-580 nm; Red channel: λ_{EX}=561 nm, λ_{EM} =580-640 nm. Scale bar, 20 μm.

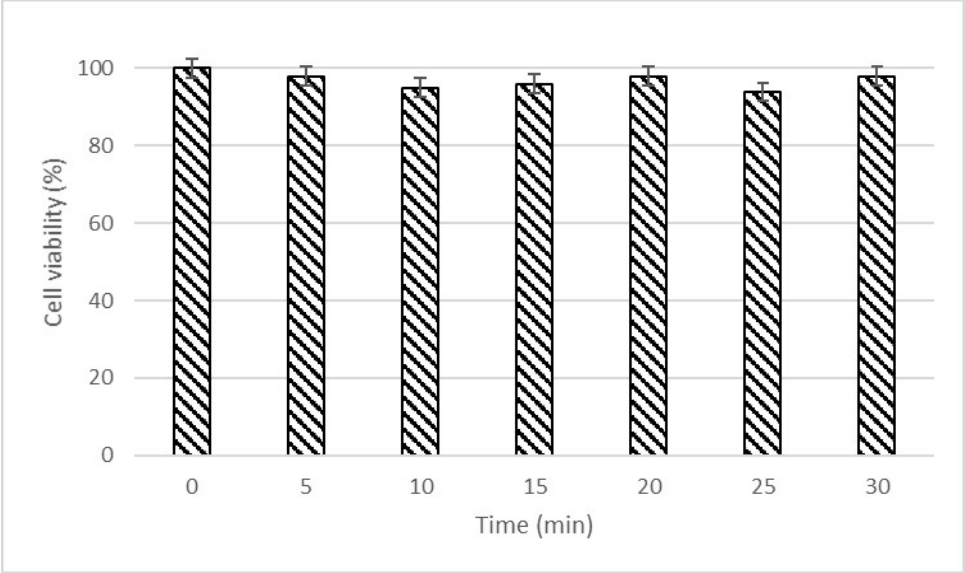


Figure. S12 Cell viability of A549 cells after irradiated by 460 nm light for indicated period.

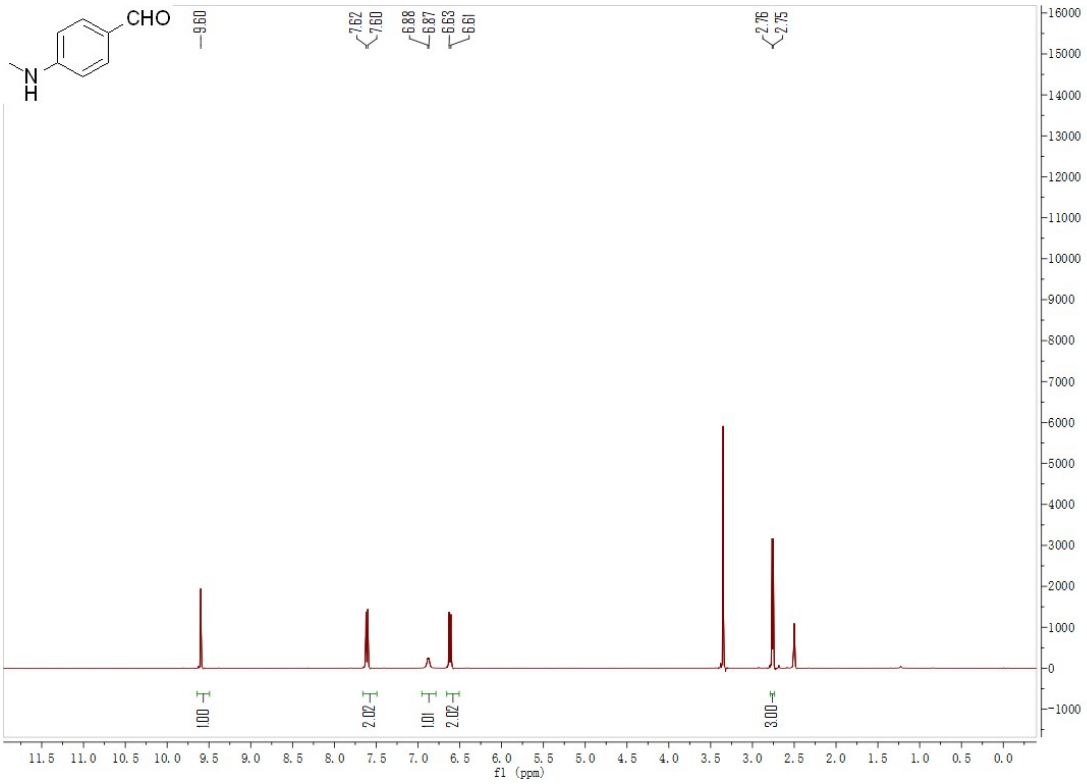


Figure. S13 ^{1}H NMR spectrum of **1**. (DMSO-d_6).

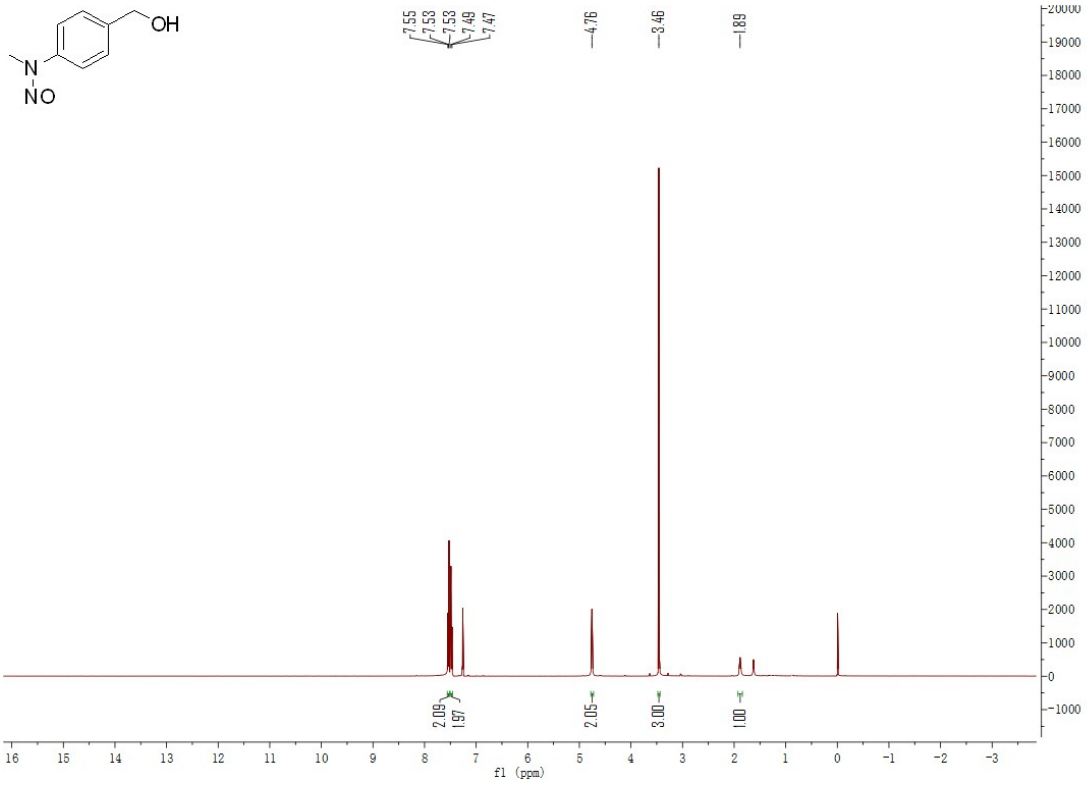


Figure. S14 ^{1}H NMR spectrum of **3**. ($CDCl_3$).

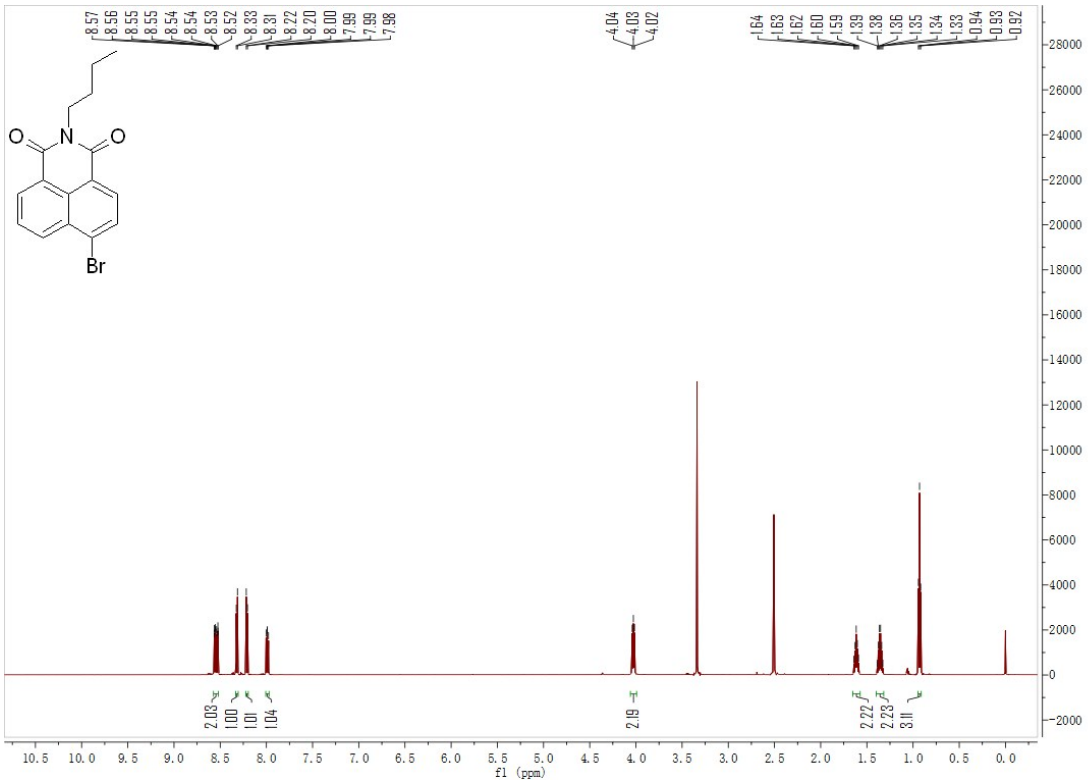


Figure. S15 ^{1}H NMR spectrum of **4**. (DMSO-d_6).

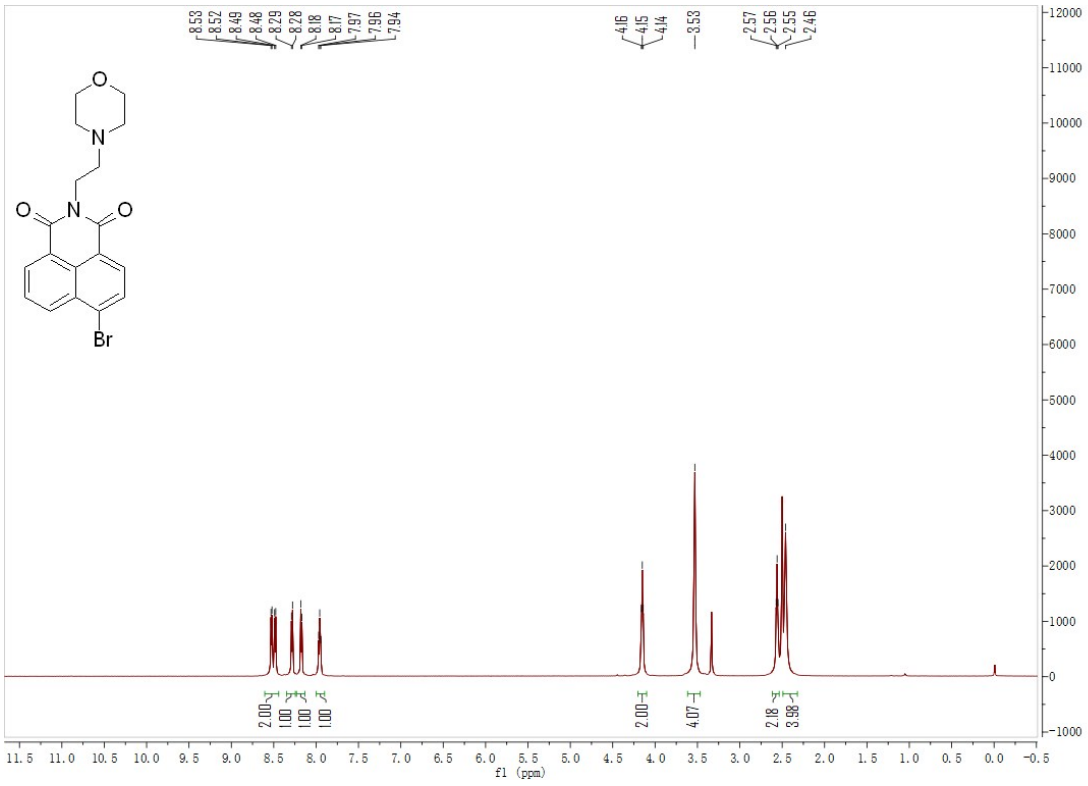


Figure. S16 ^{1}H NMR spectrum of **4'**. (DMSO-d_6).

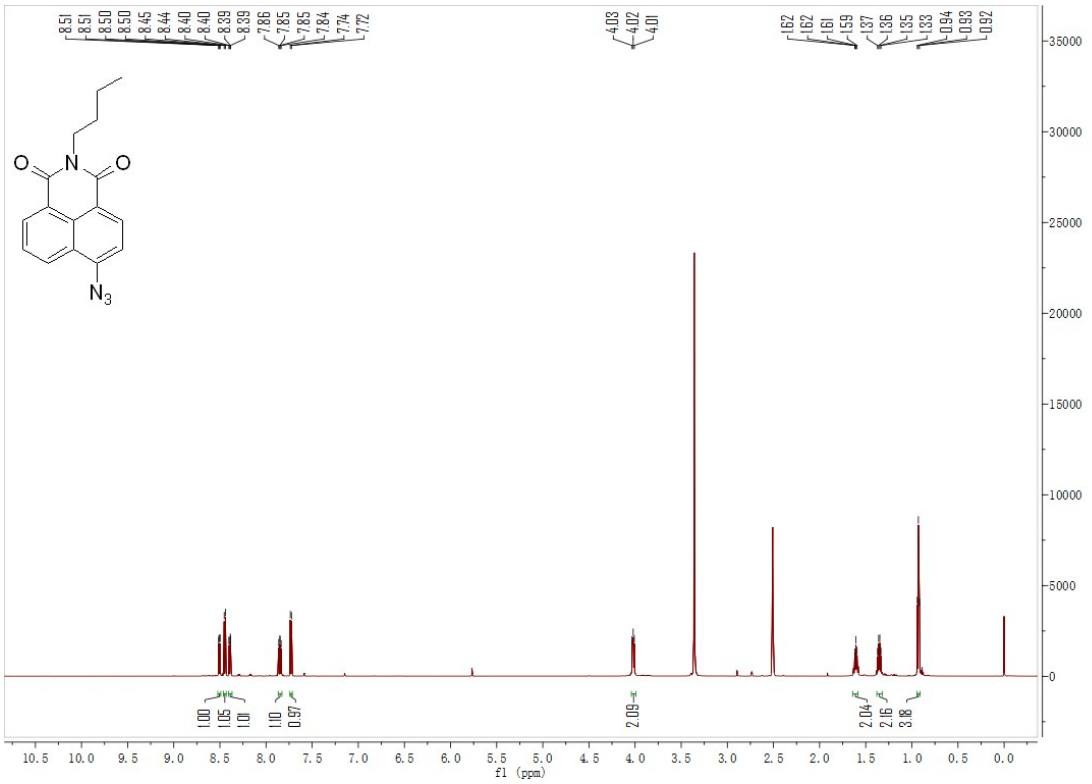


Figure. S17 ^{1}H NMR spectrum of **5**. (DMSO-d_6).

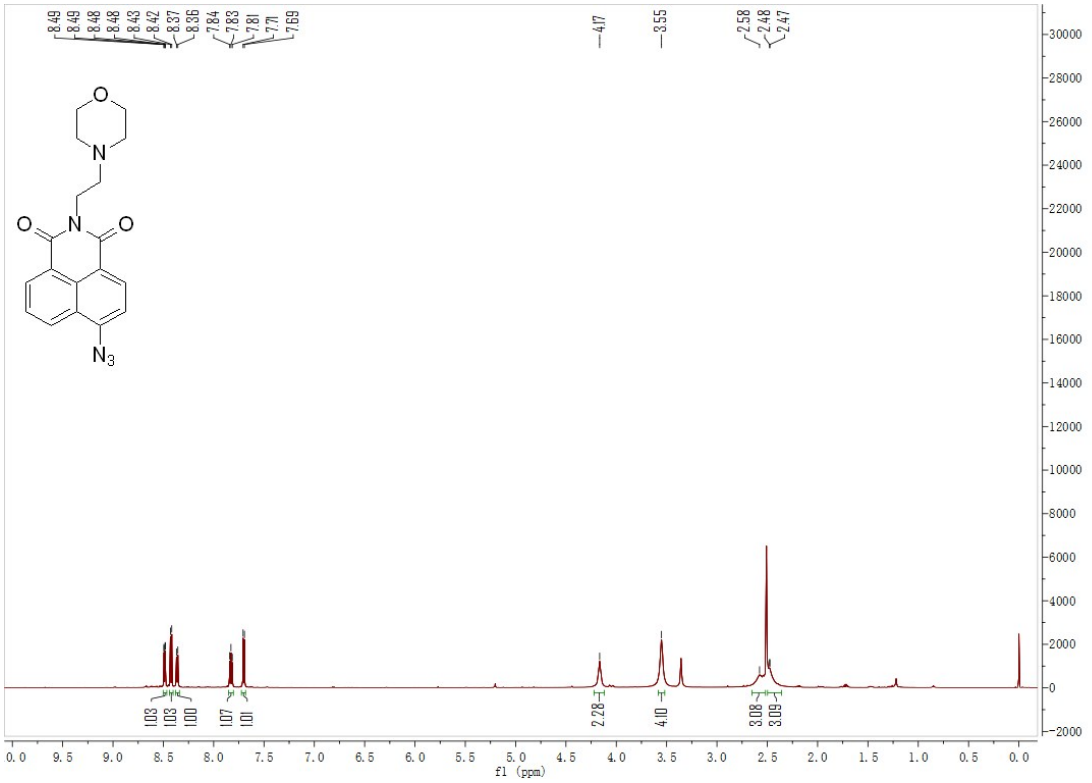


Figure. S18 ^{1}H NMR spectrum of **5'**. (DMSO-d_6).

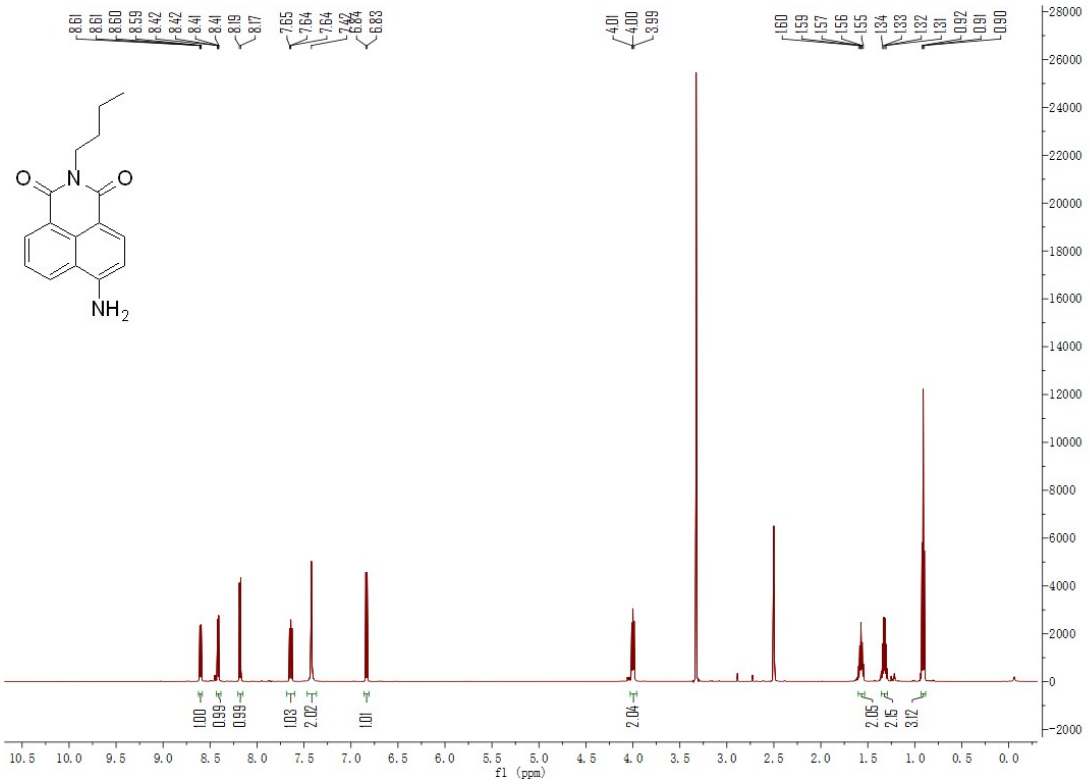


Figure. S19 ^{1}H NMR spectrum of **6**. (DMSO-d_6).

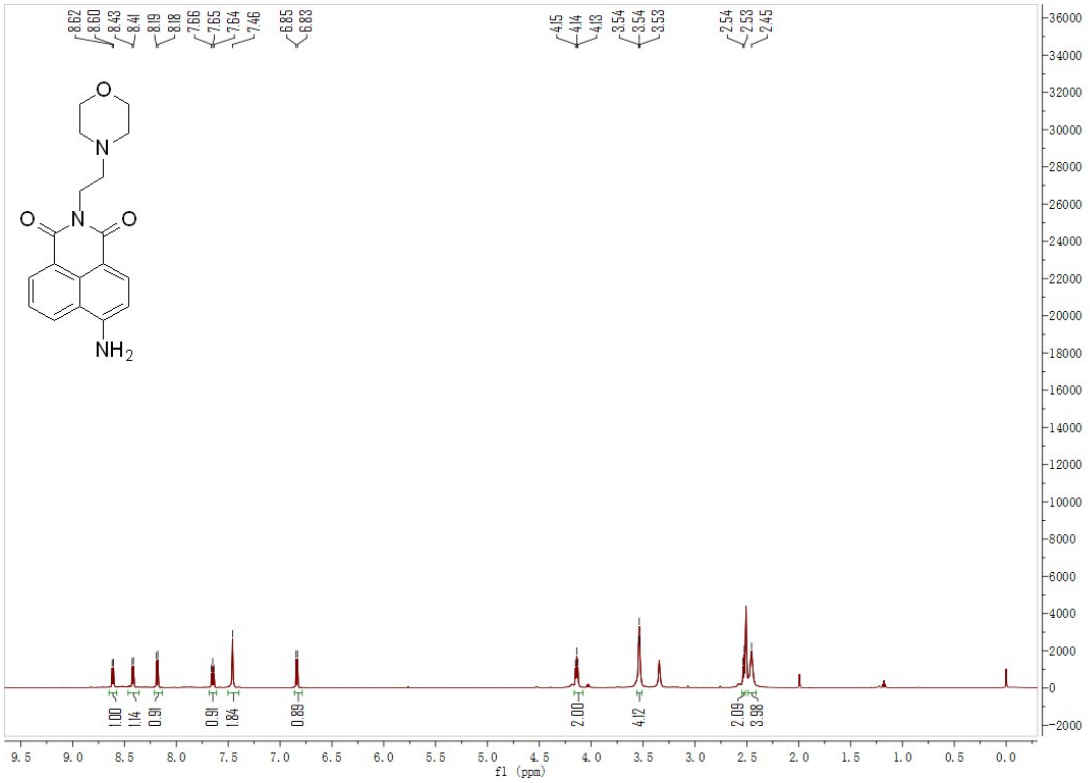


Figure. S20 ^{1}H NMR spectrum of **6'**. (DMSO-d_6).

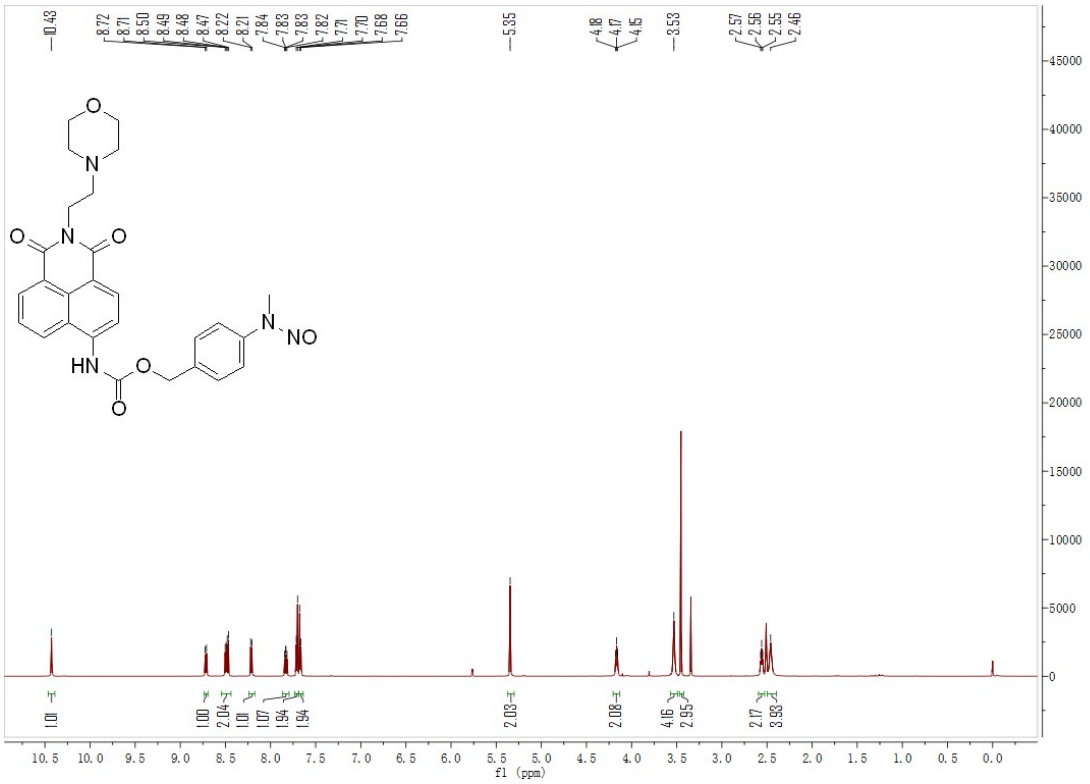


Figure. S21 ^{1}H NMR spectrum of Mo-Nap-NO. (DMSO-d_6).

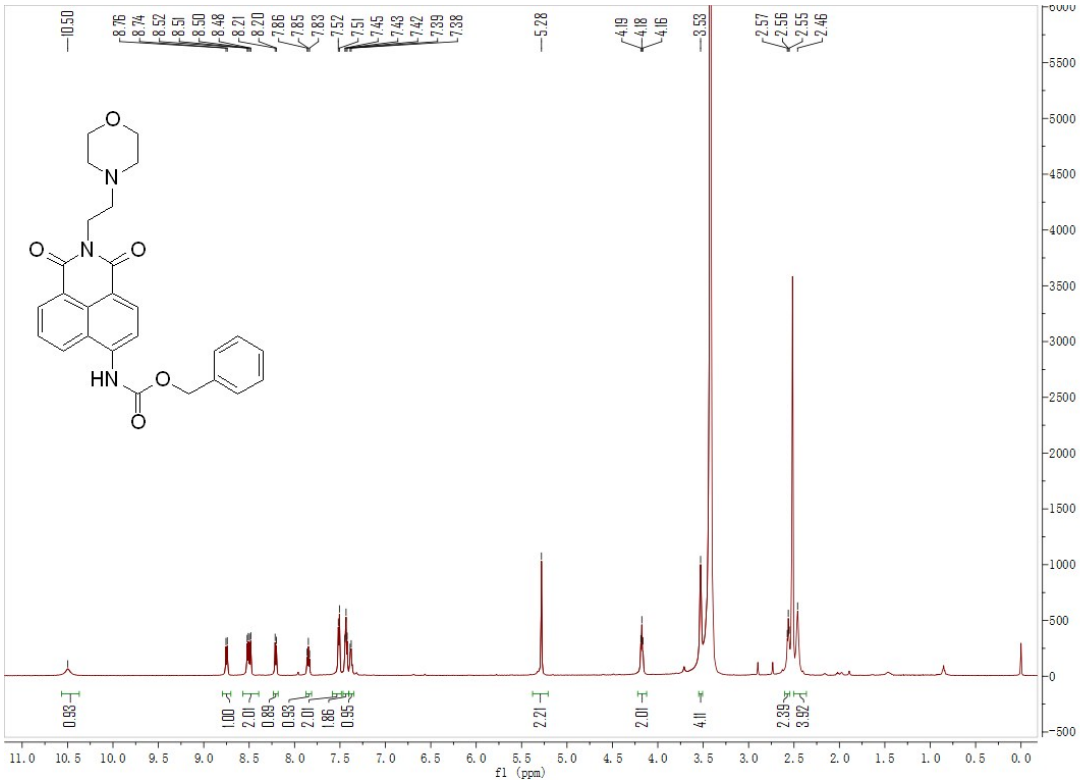


Figure. S22 ^{1}H NMR spectrum of Mo-Nap-Ph. (DMSO-d6).

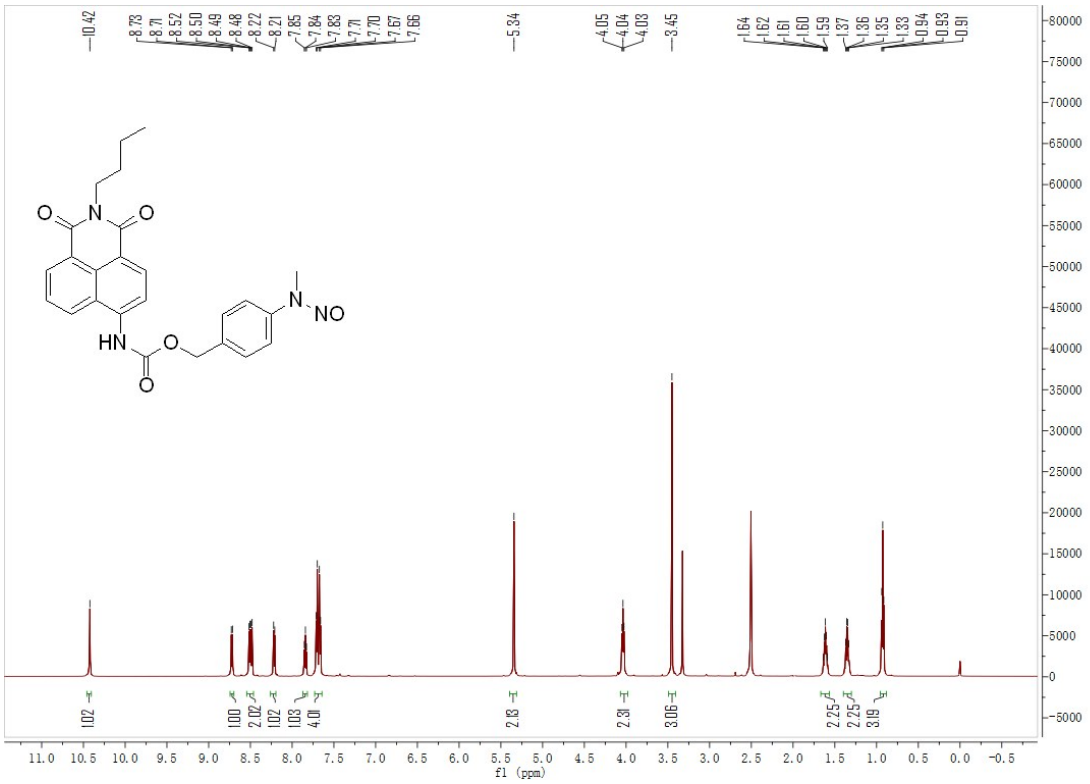


Figure. S23 ^{1}H NMR spectrum of Bu-Nap-NO. (DMSO-d6j).

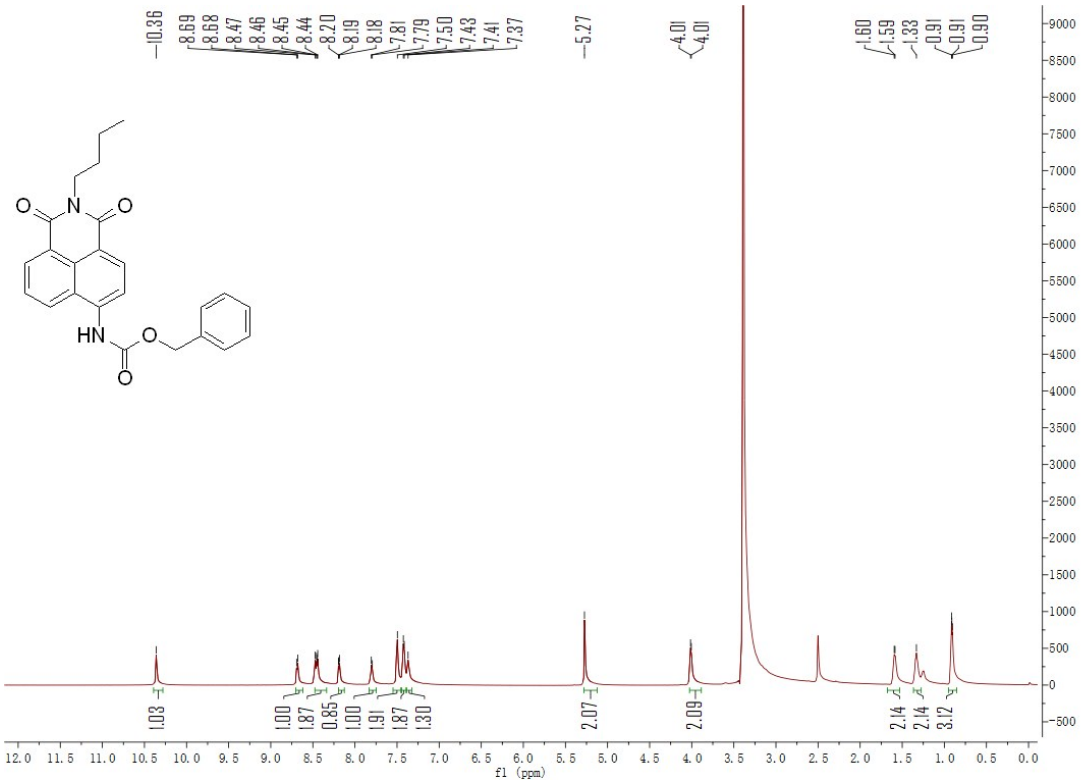


Figure. S24 ^{1}H NMR spectrum of Bu-Nap-Ph. (DMSO-d6).

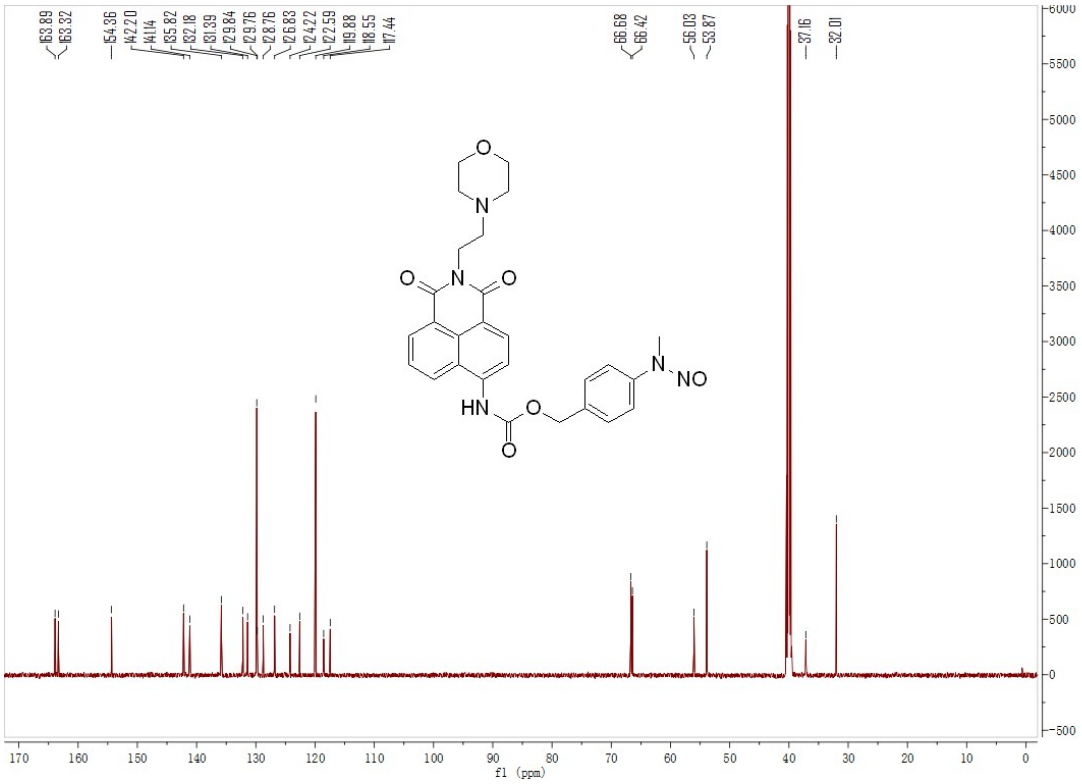


Figure. S25 ^{13}C NMR spectrum of Mo-Nap-NO. (DMSO-d6).

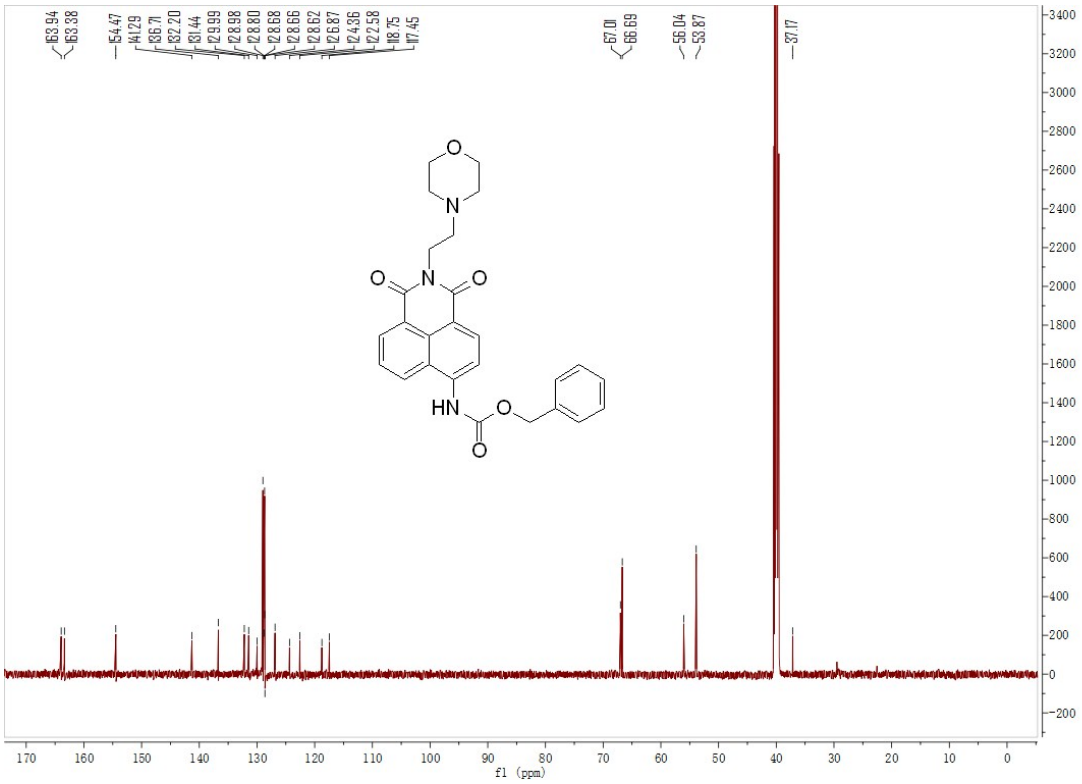


Figure. S26 ^{13}C NMR spectrum of Mo-Nap-Ph. (DMSO-d6).

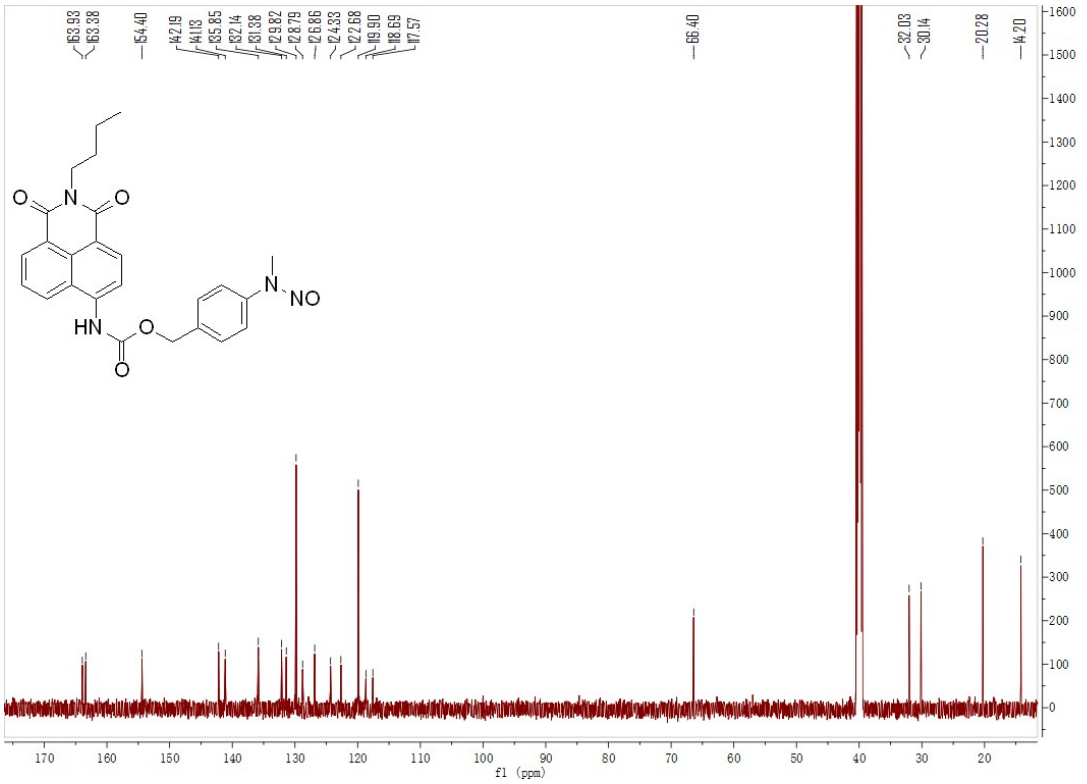


Figure. S27 ^{13}C NMR spectrum of Bu-Nap-NO. (DMSO-d6).

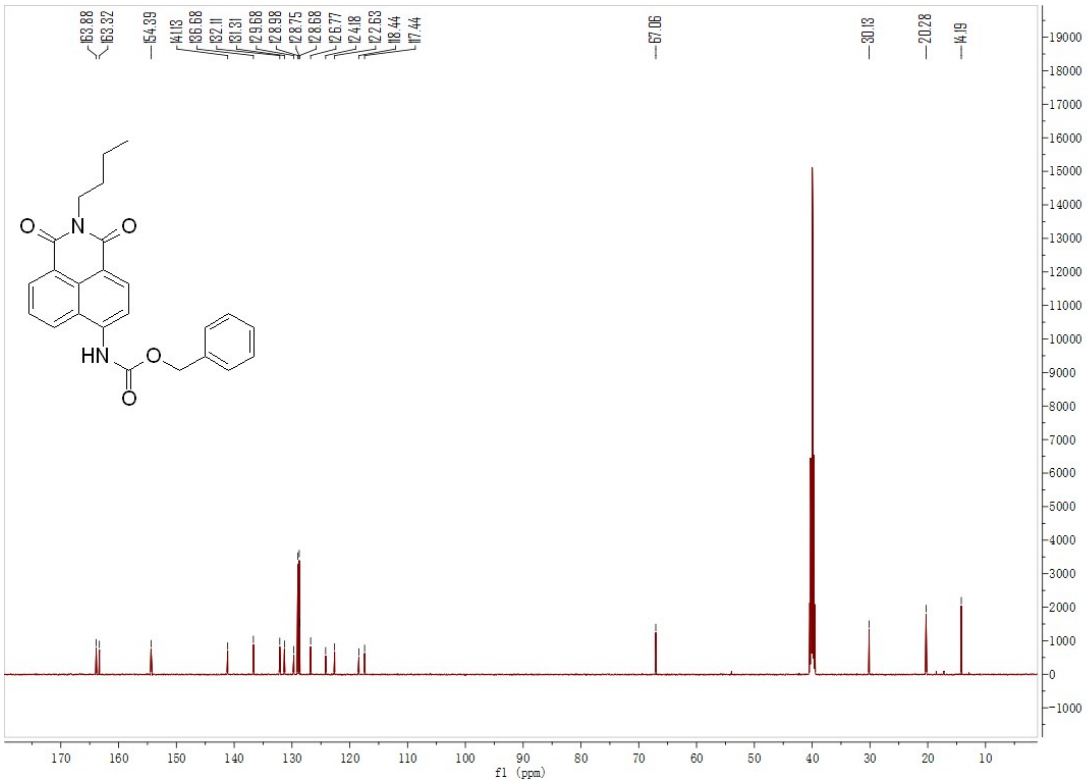


Figure. S28 ^{13}C NMR spectrum of Bu-Nap-Ph. (DMSO-d6).

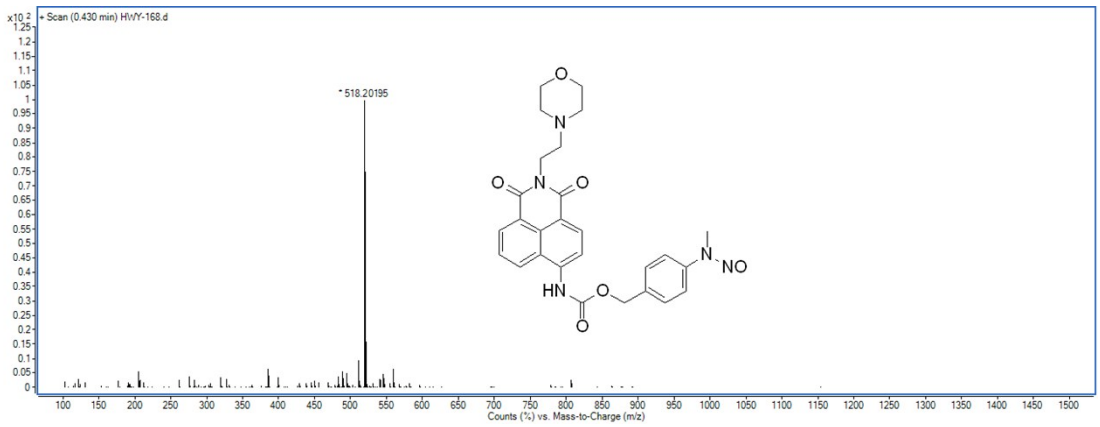


Figure. S29 Mass spectrum of Mo-Nap-NO. $[M+H]^+$.

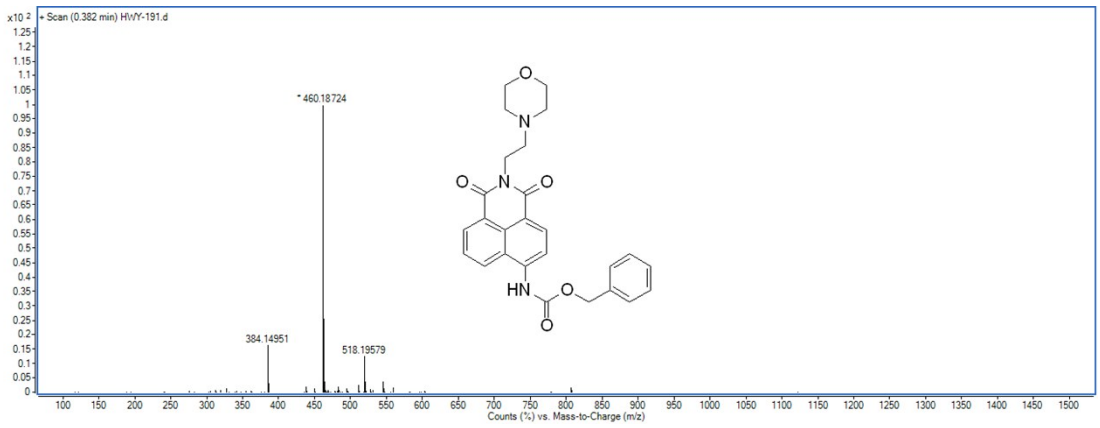


Figure. S30 Mass spectrum of Mo-Nap-Ph. $[M+H]^+$.

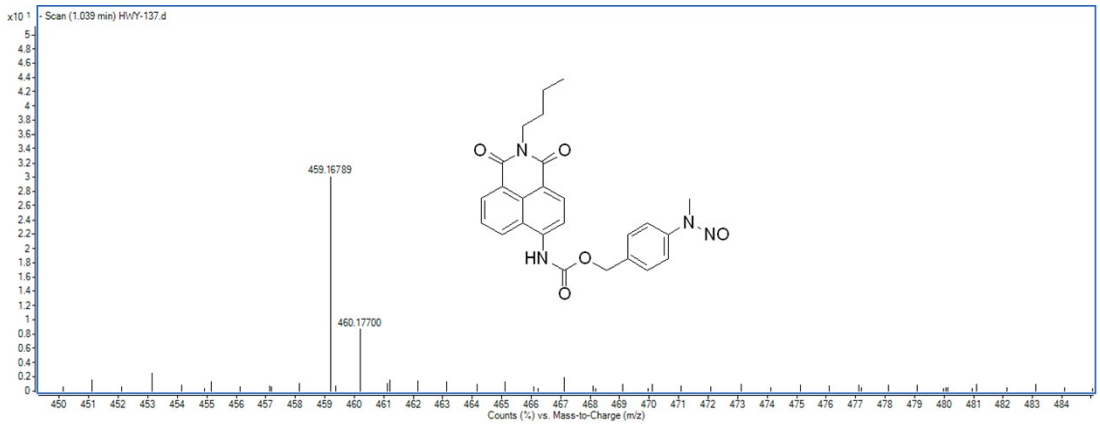


Figure. S31 Mass spectrum of Bu-Nap-NO. $[M-H]^-$.

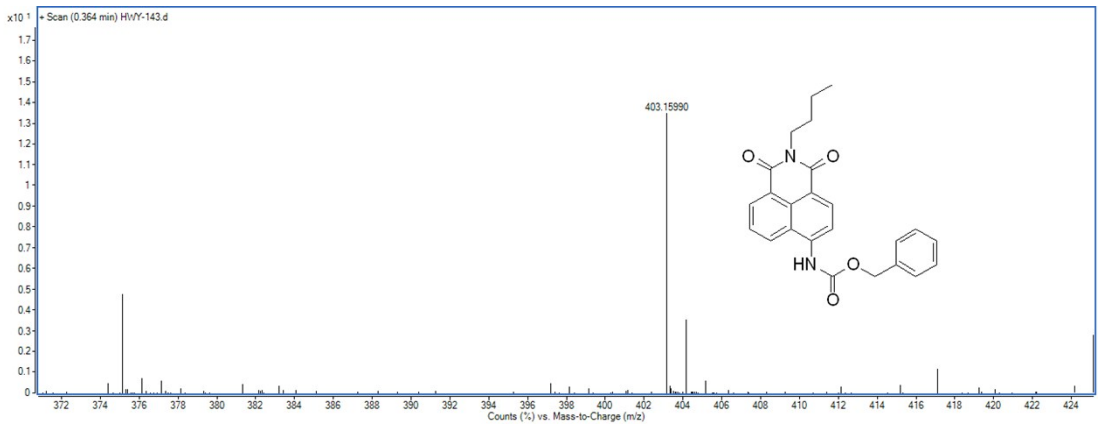


Figure. S32 Mass spectrum of Bu-Nap-Ph. $[M+H]^+$.

Reference:

[1] Jiao, J.; Zhang, X-R.; Chang, N-H.; Wang, J.; Wei, J-F.; Shi, X-Y. and Chen, Z-G. *J. Org. Chem.* **2011**, 76, 1180-1183.

[2] Zhang, Z.; Wu, J.; Shang, Z.; Wang, C.; Cheng, J.; Qian, X.; Xiao, Y.; Xu, Z. and Yang, Y. *Anal. Chem.* **2016**, 88, 7274−7280.

[3] Zhang, L.; Li, S.; Hong, M.; Xu, Y.; Wang, S.; Liu, Y.; Qian, Y. and Zhao, J. *Org. Biomol. Chem.* **2014**, 12, 5115-5125.