Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Effects of side chains on DNA binding, cell permeability, nuclear localization and cytotoxicity of 4-Aminonaphthalimides

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Experimental section

Materials. 4-bromo-1,8-naphthalanhydride was purchased from Liaoning Dye Chemical Co. Itd (Liaoyang,china). 3-dimethylaminopropylamine, 1, 3-di-boc-2-methylisothioure, EB and DAPI were purchased from Alfa Aesar Inc. (Ward Hill, MA). Pyridine, pyridine-HCl, cyanamide, ethylenediamine and guanidinium hydrochloride were purchased from jk-chemical Company (Beijing). Ethanol, DMSO and Other reagents were purchased from Beijing Chemical Plant, China. Distilled-deionized water was used throughout this work. All chemical reagents were used without further purification. Deionized water was purified by a UPHW-III-90T Milli-Q water purification system (chengdu,china). All of the titration experiments were performed at room temperature.

Nucleic acid Samples. CT-DNA was purchased from Sigma-Aldrich Co. LLC. (USA). CT-DNA was quantitated by absorption at 260 nm. The concentration was expressed in base pairs. Ribonucleic acid (RNA), from torula yeast was obtained from Sigma-Aldrich. RNA was dissolved in the pH 7.4 Tris-HCl buffer containing diethypyrocarbonate (DEPC). 100-6000 bp DNA marker and 20-500 bp DNA marker were purchased from Takara Biotechnology Co., Ltd (Dalian, China). Single-stranded DNA (ss-DNA) A40 was obtained from Sangon Biotech Co., Ltd (Shanghai, China). Stock solution of DNAs was prepared in Tris-HCl buffer (25 mM, pH 7.6) and stored at -20ºC.

Instruments. 1H NMR spectra were recorded at 300 MHz, and 13C NMR spectra were recorded at 75 MHz on a Brucker AM 300 spectrometer with tetramethylsilane (TMS) as the internal standard. J values were given in hertz. Low-resolution mass spectra (MS) were recorded on a LC-MS 2010A (Shimadzu) instrument using standard conditions. High-resolution MS were obtained on a Bruker Daltonics flex-Analysis. UV-visible absorption spectra were recorded on a Hitachi U-2550 UV/vis spectrophotometer (Kyoto, Japan). Fluorescence emission spectra were recorded on a Hitachi F-4600 fluorescence spectrofluorometer (Kyoto, Japan). The absorption and fluorescence spectra were recorded in a 1.0-cm quartz cuvette.

Synthesis and characterization

Synthesis of compound N-dimethylaminopropyl-4-bromine-1,8-naphthalimide. 3.5 g (12.6 mmol) 4-bromine-1,8-naphthalic anhydride was dissolved in ethanol (300 mL) and heated to reflux. Then 1.7 mL of 3-dimethylaminopropylamine (13.3 mmol) was added to the mixture slowly. The resulted mixture was heated to reflux with stirring for 1 h. The reaction was stopped after the solution became clear. After cooling down to room temperature, water was added to the reaction mixture
in order to precipitate the crude product. The precipitate was collected by vacuum filtration, and then washed with water and ethanol for three times respectively, and finally dried under vacuum (yield 95%);

^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 8.59 (d, J = 6.0 Hz, 1H), 8.49 (d, J = 9.0 Hz, 1H), 8.34 (d, J = 6.0 Hz, 1H), 7.97 (d, J = 6.0 Hz, 1H), 7.89 (t, J = 7.5 Hz, 1H), 4.19 (t, J = 7.5 Hz, 2H), 2.23 (s, 6H), 1.94-1.84 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 163.58, 163.56, 133.20, 132.00, 131.18, 131.10, 130.60, 130.20, 128.97, 128.09, 123.15, 122.29, 57.33, 45.46, 39.20, 26.11; MS (ESI): m/z 361.2 $(M+H)^+$, Calcd for $C_{17}H_{18}BrN_2O_2$ $(M+H)^+$, 361.1.

Synthesis of compound N-dimethylaminopropyl-4-dimethylaminopropyl)amino-1,8-naphthalimide (DND). 1.16 g (3.22 mmol) N-dimethylaminopropyl-4-bromine-1,8-naphthalimide was added in 8 mL DMSO, and then added 800 μL 3-dimethylaminopropylamine (6.37 mmol) into the solution. The resulting mixture was heated to 80

°C for stirring 12 h. After cooled to room temperature, DMSO was evaporated under reduced pressure. The residue was washed with water and methanol for five times and precipitate was collected by vacuum filtration. The product was dried in a vacuum oven overnight at 60 °C (0.82 g, 66.5%).

^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 8.67 (d, *J* = 9.0 Hz, 1H), 8.33 (d, *J* = 6.0 Hz, 1H), 8.16 (d, *J* = 6.0 Hz, 1H), 7.89 (s, 1H), 7.59 (t, *J* = 7.5 Hz, 1H), 6.71 (d, *J* = 9.0 Hz, 1H), 3.39 (t, *J* = 6.0 Hz, 2H), 2.87-2.80 (m, 4H), 2.51 (s, 12H), 1.96-1.84 (m, 4H); MS (ESI): m/z 383.2 $(M+H)^+$; Calcd for $C_{22}H_{31}N_4O_2$ $(M+H)^+$, 383.2.

Synthesis of compound N-dimethylaminopropyl-4-aminoethylamino-1,8-naphthalimide (DNE). 1.2 g (3.3 mmol) N-dimethylaminopropyl-4-bromine-1,8-naphthalimide was added in 10 mL ethylenediamine. The resulting mixture was heated to 60 °C and stirred 12 h. After cooled to room temperature, the excess ethylenediamine was evaporated under reduced pressure. The residue was washed with methanol for three times and precipitated with dichloromethane. Then the product was dried in a vacuum oven overnight at 60 °C (0.95 g, 84.3%).

^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 8.58 (d, *J* = 6.0 Hz, 1H), 8.46 (d, *J* = 6.0 Hz, 1H), 8.17 (d, *J* = 9.0 Hz, 1H), 7.63 (t, *J* = 7.5 Hz, 1H), 7.21 (t, *J* = 7.5 Hz, 2H), 3.45-3.39 (m, 2H), 3.18 (t, *J* = 6.0 Hz, 2H), 2.45 (t, *J* = 6.0 Hz, 1H), 2.27 (s, 6H), 2.04-1.85 (m, 2H). MS (ESI): m/z 341.3 $(M+H)^+$; Calcd for $C_{19}H_{25}N_4O_2$ $(M+H)^+$, 341.2.

Synthesis of compound N-dimethylaminopropyl-4- guanidinoethylamino-1,8-naphthalimide (DNG). 360 mg (1.3 mmol) 1,3-di-boc-2-methylisothioure and 180 mg (0.6 mmol) N-dimethylaminopropyl-4-aminoethylamino-1,8-naphthalimide were added in 7 mL DMF at the room temperature. Then 0.6 mL (4.3 mmol) triethylamine and mercury-(II) chloride (0.5 g, 1.4 mmol) were added to this solution simultaneously. The resulting mixture was stirred for 24 h at the room temperature. The reaction suspension was added and diluted with CH_2Cl_2. The organic layer was separated and dried over Na_2SO_4. After evaporated under reduced pressure and the residue was purified by column chromatography (CH_2Cl_2/MeOH, 10:3, V/V). Depotection of Di-Bocgunidine: the Di-Bocgunidine was dissolved in HCl-MeOH (10 M, 10 mL) and stirred for 3 days at 15 °C. Then the solvent was removed under vacuum. The resulting precipitate was further purified by HPLC and finally an orange solid was obtained. (123 mg, 24.7%)

^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 9.59 (s, 1H), 8.71 (d, *J* = 9.0 Hz, 1H), 8.46 (d, *J* = 6.0 Hz, 1H), 8.29 (d, *J* = 9.0 Hz, 1H), 7.98 (s, 1H), 7.83 (s, 1H), 7.71 (t, *J* = 7.5 Hz, 1H), 7.40-7.33(m, 3H), 6.87 (d, *J* = 9.0 Hz, 1H), 4.08 (t, *J* = 6.0 Hz, 2H), 3.54-3.39(m, 4H), 2.77 (s, 6H), 2.01 (t, *J* = 7.5 Hz, 2H); ^{13}C NMR (DMSO, 75 MHz) δ (ppm): 163.49, 163.05, 156.90, 137.71,131.60,131.29, 129.27, 128.57, 127.59, 126.76, 123.45, 122.41, 119.94, 55.93, 54.16, 41.72, 37.09, 22.80, 18.52. MS (ESI): m/z 383.2, $(M-H)^-$; HRMS (ESI): m/z Calcd for $C_{20}H_{27}N_6O_2$ $(M+H)^+$, 383.2190, found, 383.2184.

Synthesis of compound N-aminoethyl-4-bromine-1,8-naphthalimide. 4.3 g (15.5 mmol) 4-bromine-1,8-naphthalic anhydride was dissolved in ethanol (330 mL) and heated to reflux. Then 0.95 mL of ethylenediamine (17.6 mmol) was added to the mixture slowly. The resulted mixture was heated to reflux with stirring for 3 h. The reaction was stopped after the solution became clear and then turbid. After the solution was cooled to room temperature, precipitate was obtained. The precipitate was collected by vacuum filtration, and then washed with water and ethanol for three times respectively, and finally dried under vacuum (3.33g, 72.3%). MS (ESI): m/z 319.1 $(M+H)^+$; Calcd for $C_{14}H_{12}$ BrN_2O_2 $(M+H)^+$, 319.0.

Synthesis of compound N-aminoethyl-4-aminoethylamino-1,8-naphthalimide (ENE). 0.8 g (2.6 mmol) N-aminoethyl-4-bromine-1,8-naphthalimide (5) was added in 8 mL ethylenediamine. The resulting mixture was

heated to 60 °C for stirring 12 h. After cooled to room temperature, the excess ethylenediamine was evaporated under reduced pressure. The residue was washed with methanol for three times and precipitated with dichloromethane. The product was dried in a vacuum oven overnight at 60 °C (0.56 g, 72.3%).

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm): 8.65 (dd, 1H), 8.56 (dd, 1H), 8.41 (d, J = 7.9 Hz, 1H), 8.02 (d, J = 7.8 Hz, 1H), 7.82 (dd, 2H), 4.27 (t, J = 6.6 Hz, 2H), 3.07 (t, J = 6.6 Hz, 2H). MS (ESI): m/z 299.1 $(M+H)^+$; Calcd for $C_{16}H_{18}N_4O_2$ $(M+H)^+$, 299.2.

Synthesis of compound N-guanidinoethyl-4-guanidinoethylamino-1,8-naphthalimide (GNG) (125 mg, 0.42 mmoles), pyridine (6 mL), pyridine-HCl (3 g) and DMAP (60 mg, 0.45 mmol) were mixed and stirred under N_2 at 120 °C. Cyanamide (527 mg, 12.6 mmoles, 30 equiv) was added in three equal portions over 48 hours. The reaction was removed from the heat, and 10 mL water was added. Acetic acid was added into the solution to adjust pH to neutral. The precipitate was removed and the solution was purified by HPLC and an orange solid was obtained (68 mg, 42.2%).

^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 9.92 (s, 1H,-COOH), 8.82 (d, *J* = 9.0 Hz, 1H), 8.75 (s, 1H), 8.43 (d, *J* = 6.0 Hz, H), 8.27 (d, *J* = 9.0 Hz, H), 8.09 (s, 2H, N-H), 7.94 (s, 5H, N-H), 7.68 (t, *J* = 7.5 Hz, H), 6.83 (d, *J* = 6.0 Hz, H), 4.14 (t, *J* = 12 Hz, 2H), 3.56 (s, 2H), 3.44 (s, 2H), 3.37 (s, 2H); ^{13}C NMR (DMSO, 75 MHz) δ (ppm): 175.16, 164.50, 163.61, 158.23, 158.18, 134.73, 131.26, 130.03, 129.37, 124.80, 122.28, 120.80, 108.43, 104.32, 41.41, 39.57. MS (ESI): m/z 383.3, $(M+H)^+$; Calcd for $C_{18}H_{23}N_8O_2$ $(M+H)^+$, 383.2.

Table S1. Fluorescence quantum yields of DNA, DND, DNE, ENA and GNG in different solvents at the room temperature. (λ_{ex} = 440 nm).

solvent	(DNE) Φ_f	(DND) Φ_f	(DNG) Φ_f	(ENE) Φ_f	(GNG) Φ_f
Acetic ether	0.52	0.69	0.20	0.59	0.04
Acetonitrile	0.55	0.72	0.38	0.72	0.66
DCM	0.55	0.82	0.30	0.17	0.15
Ethanol	0.55	0.21	0.47	0.70	0.61
DMF	0.80	0.11	0.51	0.66	0.93
H_2O	0.50	0.28	0.22	0.35	0.35
pbs 7.4	0.35	0.30	0.29	0.33	0.35
SDS (8mM)	0.83	0.57	0.84	0.80	0.80
CTAB(1mM)	0.35	0.40	0.31	0.60	0.40
Triton(1mM)	0.35	0.33	0.24	0.28	0.36

These molecules exhibited different Φ_fs in different solvents. This may be attributed to the different interactions of these molecules with solvents that might influence the extent of non-radiative decay. For example, the Φ_fs of these molecules in anion surfactant (SDS) solution were much higher than those in cationic surfactant (CTAB) and nonionic surfactant (Triton X-100) solution, which was attributed to the electrostatic interaction between these molecules and anionic micelle.

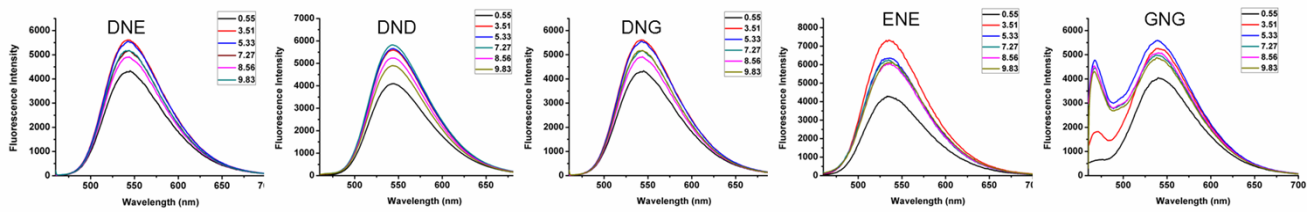


Figure S1. Fluorescence spectra of 10 μM DNE, DND, DNG, ENE and GNG measured in 50 mM HEPES buffer at pH 0.55, 3.51, 5.33, 7.27, 8.56, 9.83. λ_{ex} = 445 nm.

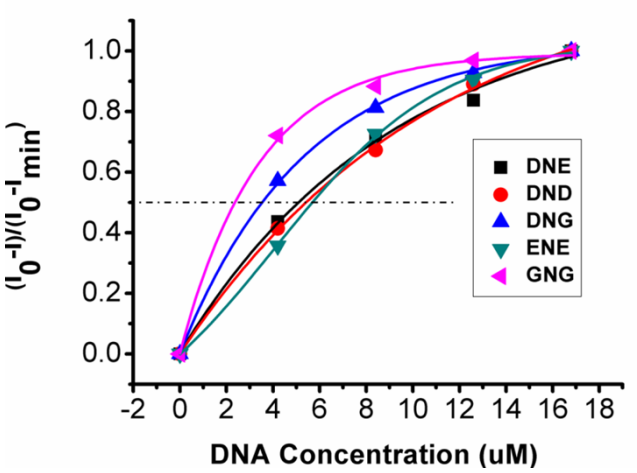


Figure S2. The curve of decreasing rate of fluorescence intensity of naphthalimides versus the concentration of CT-DNA (in base pairs).

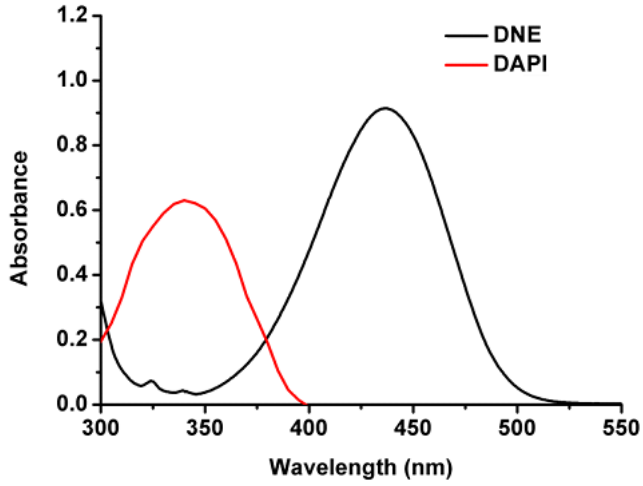


Figure S3. The absorption spectra of DNE(30 μM) and DAPI (30 μM) in PBS buffer (pH 7.4).

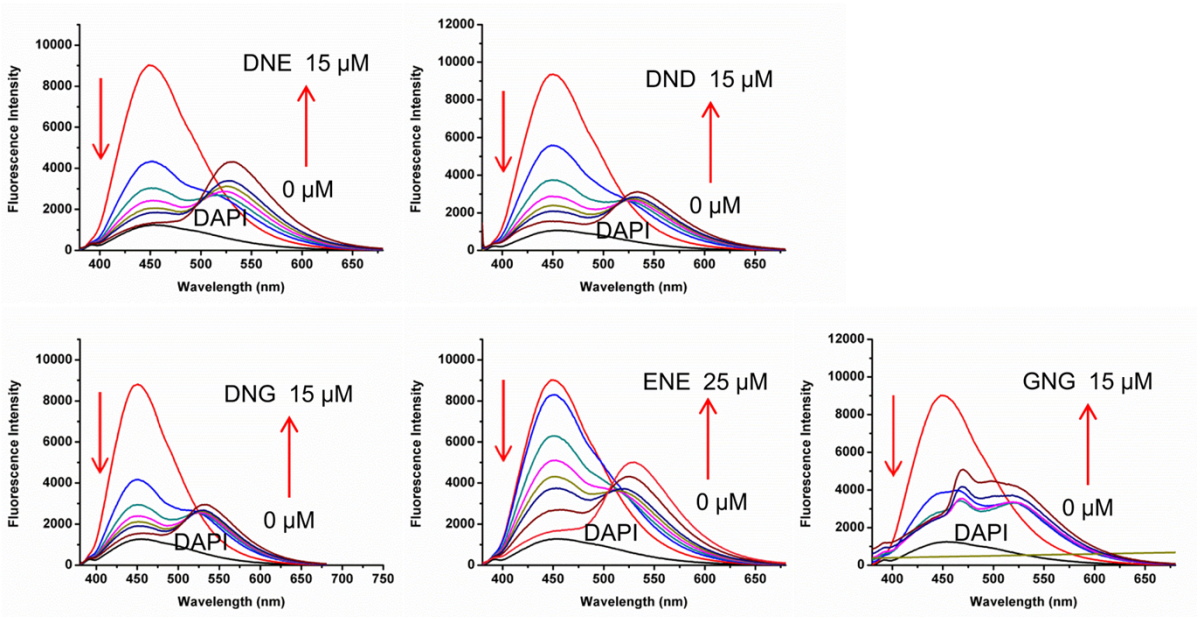


Figure S4. Fluorescence titration of DAPI-DNA complex with naphthalimides. 4.2 μM CT-DNA (in base pair) was added into the 1.0 μM DAPI in pH 7.4 PBS buffer. Then different concentrations of naphthalimides were added: 2.0, 4.0, 6.0, 8.0, 10 and 15 μM, excitation at 345 nm.

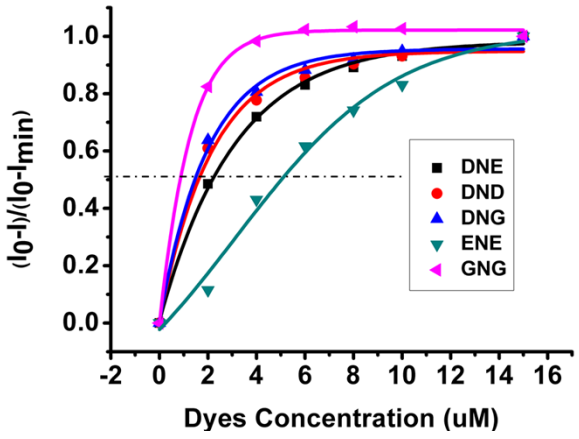


Figure S5. The curve of decreasing rate of fluorescence intensity of DAPI-CT-DNA complex versus the concentration of added naphthalimides.

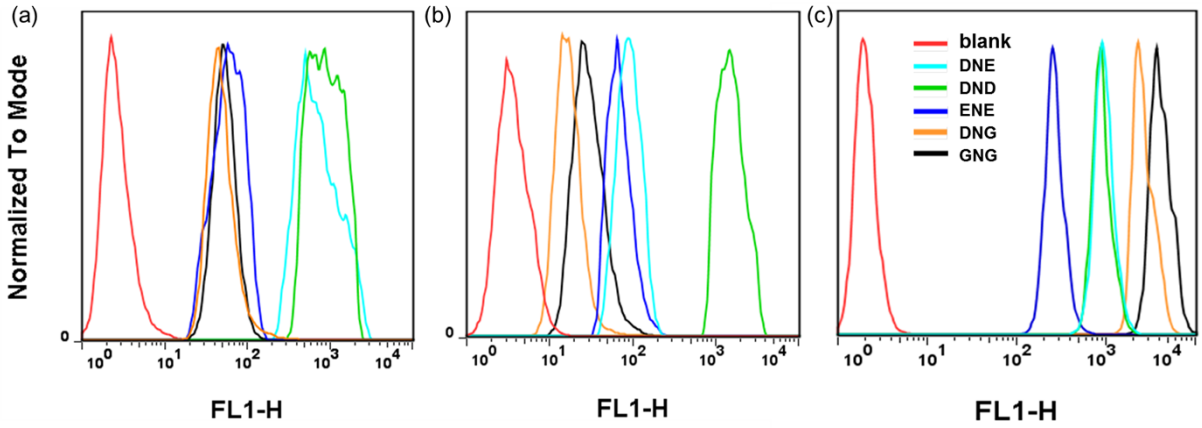
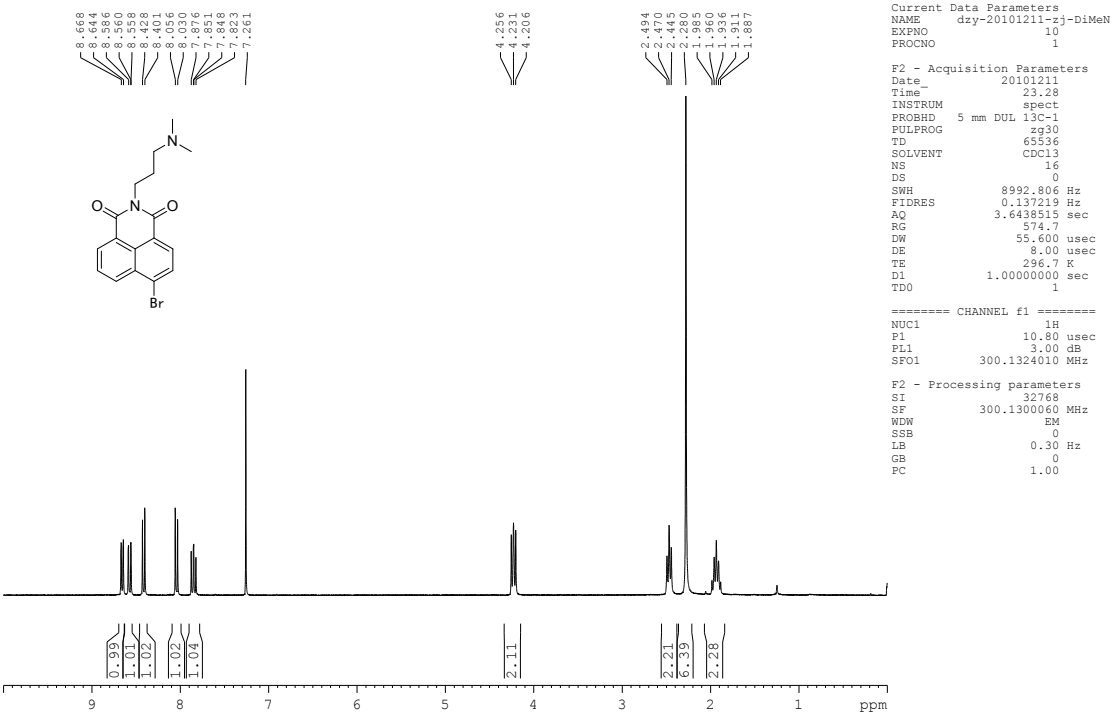


Figure S6. Flow cytometry assay of MCF-7 cells stained by different naphthalimides (10 μM). (a) cells stained at 37 °C for 30 min; (b) cells stained at 4 °C for 30 min; (c) Fixed cells stained at room temperature for 20 min.

NMR spectra

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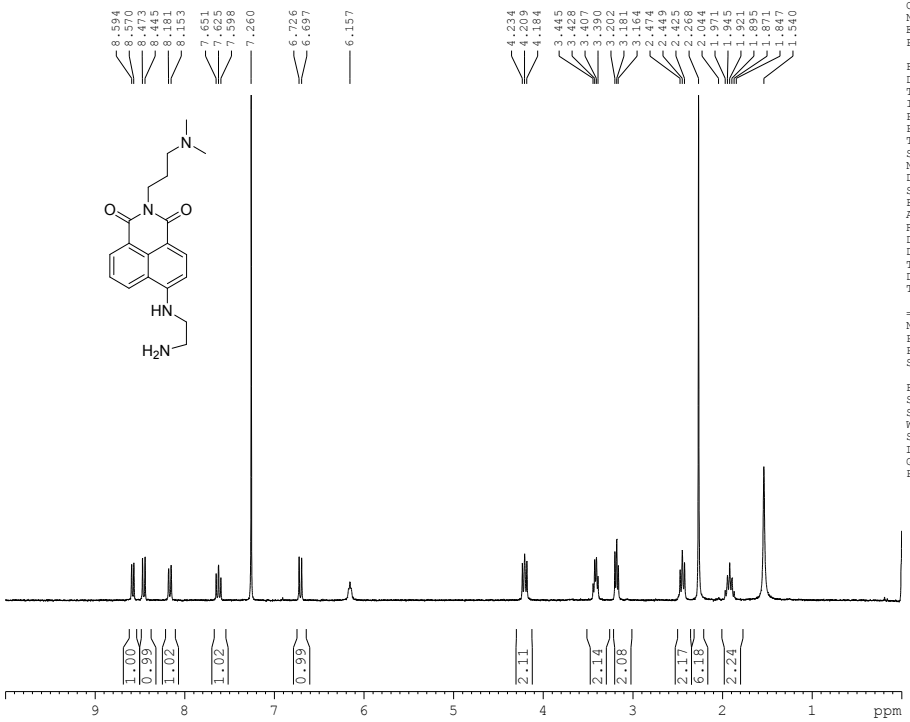


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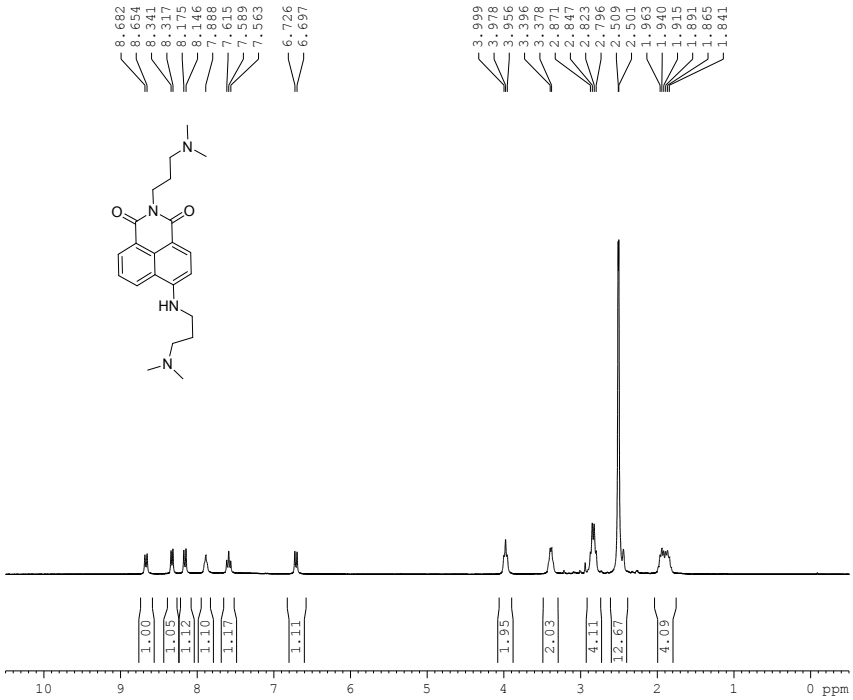


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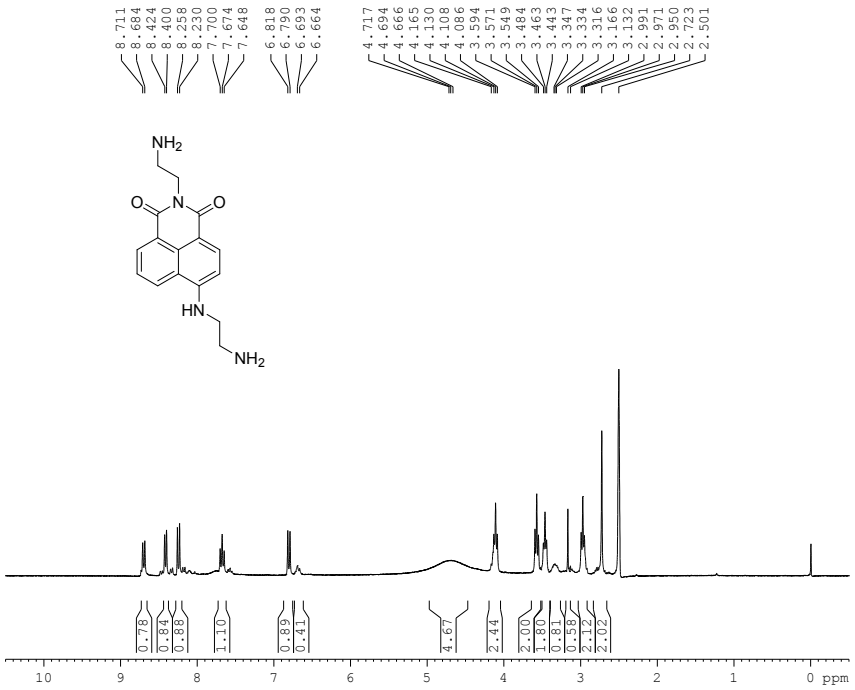


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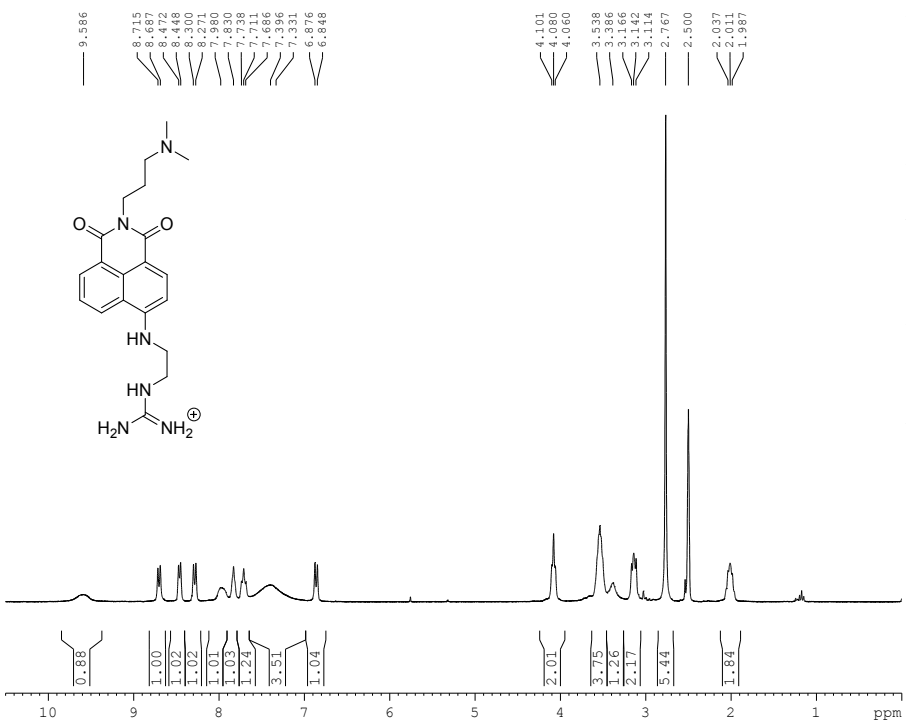


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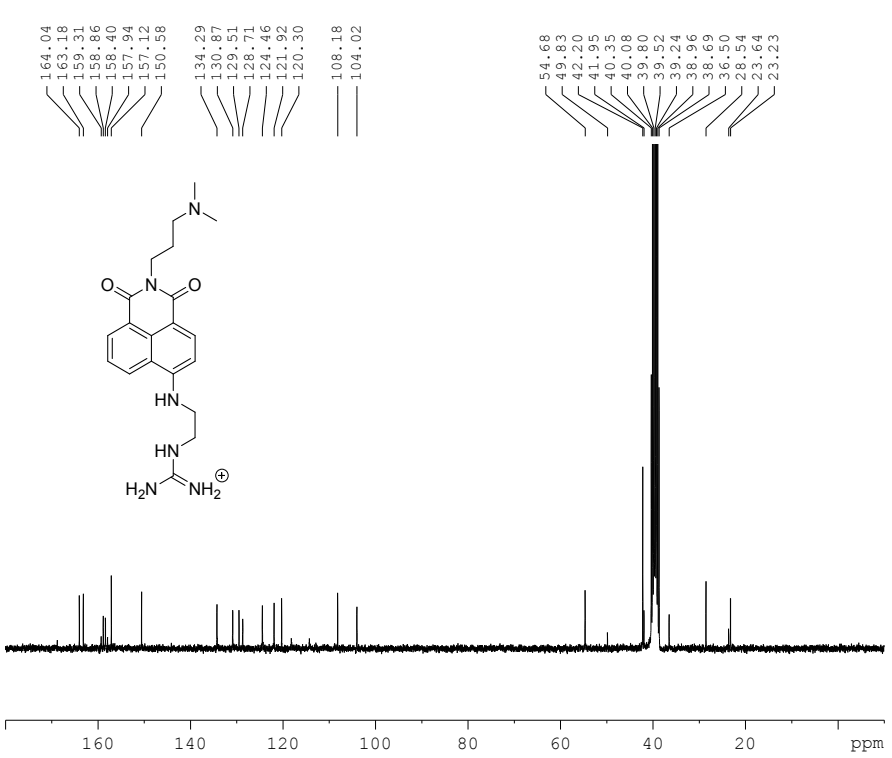


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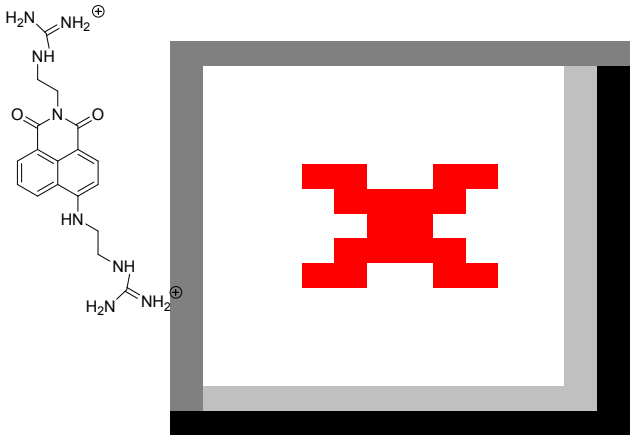

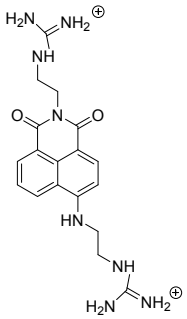
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