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A pH sensitive ratiometric fluorophore and its application for monitoring the intracellular and extracellular pHs simultaneously

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Contents:

1. Synthesis and characterization

Synthesis of compound 1a N-hydroxyethyl-4-bromine-1,8-naphthalimide. 2.0 g (7.22 mmol) 4-bromine-1,8-naphthalic anhydride was dissolved in ethanol (160 mL) and heated to reflux. Then 440 μL of ethanolamine (7.3 mmol) was added to the mixture slowly after the temperature cooling down to 50 ^{o}C. The resulted mixture was heated to reflux with stirring for 1 h. The reaction was over when the solution became clear. When the solution was cooled to room temperature, precipitate was emerged. The precipitate was collected by vacuum filtration, and then washed with water and ethanol for three times respectively, and finally dried by vacuum (yield 90%);

^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 8.65 (d, J = 9.0 Hz, 1H), 8.57 (d, J = 9.0 Hz, 1H), 8.40 (d, J = 9.0 Hz, 1H), 8.03 (d, J = 9.0 Hz, 1H), 7.84 (t, J = 7.5 Hz, 1H) 4.44 (t, J = 6.0 Hz, 2H), 3.98 (t, J = 6.0 Hz, 2H), 2.09 (s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 162.9, 162.9, 132.5, 131.5, 131.3, 130.8, 129.7, 128.9,128.7, 128.2, 122.8, 122.0, 57.7, 41.9; MS (ESI): m/z 320.1 $(M+H)^{+}$.

Synthesis of compound 2a (ENNA). 430 mg (1.3 mmol) compound 1a N-hydroxyethyl-4-bromime-1,8-naphthalimide, 200 mg (2.4 mmol) 3-amino-1,2,4-triazole and 120 mg (3 mmol) NaOH were dissolved in DMSO (8 mL). The resulting mixture was heated to 150 ^{o}C for 12 h with stirring under an atmosphere of nitrogen. After cooled to room temperature, the solution was evaporated under reduced pressure. The residue was purified by column chromatography using silica-gel (100-200 mesh) and 8% methanol in dichloromethane as eluent to give a salmon pink solid compound (128 mg, 30.7%).

^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 13.28 (s, 1H), 8.54 (d, J = 9 Hz, 1H), 8.43 (d, J = 9 Hz, 1H), 8.31 (s, 1H), 7.62 (d, J = 6 Hz, 1H), 7.17 (d, J = 9 Hz, 1H), 4.15 (t, J = 6 Hz, 2H), 3.59 (t, J = 7.5 Hz, 2H); ^{1}H NMR (DMSO-d_6+NaOH solid, 300 MHz) δ (ppm): 8.37 (d, J = 9 Hz, 1H), 8.22 (s, 1H), 8.11 (d, J = 9 Hz, 1H), 7.33 (d, J = 6 Hz, 1H), 6.89 (d, J = 9 Hz, 1H), 4.18 (t, J = 7.5 Hz, 2H), 3.55 (t, J = 7.5 Hz, 2H); ^{13}C NMR (DMSO-d_6+NaOH solid, 75 MHz,) δ (ppm): 163.2, 162.7, 155.6, 154.62 154.3, 139.7, 133.7, 132.7, 132.2, 113.7, 113.3, 111.3, 103.3, 101.5, 58.2, 41.4; MS (ESI): m/z 320.1 $(M-H)^{-}$; HRMS (ESI): m/z Calcd for $C_{16}H_{10}N_5O_3$ $(M-H)^{-}$ 320.078903, found, 320.078494.

Synthesis of compound 1b N-hexanoic acid-4-bromine-1,8-naphthalimide. 4.0 g (14.44 mmol) 4-bromine-1,8-naphthalic anhydride was dissolved in ethanol (300 mL), and heated to reflux. Then water-alcohol mix solution of 6-amino-hexanoic acid (2.1 g, 15.9 mmol) was added to the mixture slowly after the temperature cooling down to 50 ^{o}C. The resulted mixture was heated to reflux with stirring for 3 h. The reaction was over when the solution became clear. After the solution was cooled to room temperature, added much water to the mixture solution and precipitate was emerged. Primrose solid was obtained by vacuum filtration, and then washed with water and ethanol for three times respectively, and finally dried by vacuum (yield 95%);

^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 8.39 (d, J = 7.2 Hz, 1H), 8.33 (d, J = 8.4 Hz, 1H), 8.14 (d, J = 7.8 Hz, 1H), 8.04 (d, J = 7.8 Hz, 1H), 7.84 (t, J = 8.0 Hz, 1H), 3.94 (t, J = 7.4 Hz, 2H), 2.21 (t, J = 7.4 Hz, 2H), 1.58 (m, 1.65~1.50, 4H), 1.32 (m, 1.38~1.29, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 174.4, 162.6, 162.5, 132.3, 131.4, 130.7, 129.5, 129.0, 128.5, 127.9, 122.4, 121.7, 33.5, 27.1, 26.0, 24.2; MS (ESI): m/z 390.2 $(M+H)^{+}$.

Synthesis of compound 2b ANNA. 663 mg (1.7 mmol) N- hexanoic acid -1,8-naphthalimide, 260mg (3.1 mmol) 3-amino-1,2,4-triazole and 140 mg (3.5 mmol) NaOH were dissolved in DMSO (10 mL). The resulting mixture was heated to 135 ^{o}C for 12 h with stirring under an atmosphere of nitrogen. After cooled to room temperature, the solution was evaporated under reduced pressure. The residue was purified by column chromatography using silica-gel (100-200 mesh) and 15% methanol in dichloromethane (contained 0.1% TEA) as eluent to give a salmon pink solid compound (254 mg, 38.3%).

^{1}H NMR (DMSO-d_6+NaOH solid, 300 MHz) δ (ppm): 8.35 (d, J = 8.4 Hz, 1H), 8.22 (s, 1H), 8.10 (d, J = 9.0 Hz, 1H), 7.32 (d, J = 8.4 Hz, 1H), 6.88 (d, J = 9.0 Hz, 1H), 4.03 (t, J = 7.4 Hz, 2H), 2.06 (t, J = 7.2 Hz, 2H), 1.55 (m, 1.63~1.47, 4H), 1.28 (m, 1.38~1.17, 2H); ^{13}C NMR (DMSO-d_6 +NaOH solid, 75 MHz) δ (ppm): 175.8, 163.0, 164.5, 155.6, 154.6, 154.2, 139.7, 133.6, 132.6, 132.1, 113.6, 113.3, 111.3, 103.4, 101.4, 35.7, 27.7, 26.6, 25.5, 25.3; MS (ESI): m/z 390.1 $(M-H)^{-}$; HRMS (ESI): m/z Calcd for $C_{20}H_{18}N_5O_4$ $(M+H)^{+}$ 392.13533, found, 392.13583.

Synthesis of compound 1c N-hexadecyl-4-bromine-1,8-naphthalimide. 3.1 g (11.2 mmol) 4-bromine-1,8-naphthalic anhydride was dissolved in ethanol (300 mL), and heated to reflux. Then alcohol solution of 1-Hexadecylamine (3.2 g, 13.2 mmol) was added to the mixture slowly after the temperature cooling down to 50 ^{o}C. The mixture solution turned to be clear after the temperature rised to 85 ^{o}C and then it turned back to turbid solution. The resulted mixture was heated to reflux with stirring for 5 h. Then the solution was cooled to room temperature, more precipitate was emerged. Gray solid was obtained by vacuum filtration, and then washed with water and ethanol for three times respectively, and finally dried by vacuum (yield 92%);

^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 8.65 (d, J = 7.2 Hz, 1H), 8.55 (d, J = 8.4 Hz, 1H), 8.40 (d, J = 7.8 Hz, 1H), 8.03 (d, J = 7.8 Hz, 1H), 7.84 (t, J = 7.8 Hz, 1H), 4.15 (t, J = 7.7 Hz, 2H), 1.71 (dd, J = 10.8 Hz, 6.6 Hz, 2H), 1.3 (m, 1.36~1.24, 26H), 0.87 (t, J = 6.3 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ (ppm): 163.7, 133.3, 132.1, 131.3, 130.3, 129.1, 128.2, 132.3, 123.3, 122.4, 40.7, 32.0, 29.8, 29.8, 29.7, 29.7, 29.5, 28.2, 27.2, 22.8, 14.2; MS (ESI): m/z 500.2 $(M+H)^{+}$.

Synthesis of compound 2c HNNA. 0.95 g (1.9 mmol) N- hexadecyl -1,8-naphthalimide, 227mg (2.7 mmol) 3-amino-1,2,4-triazole and 124 mg (3.1 mmol) NaOH were dissolved in DMSO (15 mL). The resulting mixture was heated to 135 ^{o}C for 12 h with stirring under an atmosphere of nitrogen. After cooled to room temperature, the solution was evaporated under reduced pressure. The residue was purified by column chromatography using silica-gel (100-200 mesh) and 3% methanol in dichloromethane (contained 0.1% TEA) as eluent to give a salmon pink solid compound (401 mg, 42.1%).

^{1}H NMR (DMSO-d_6+NaOH solid, 300 MHz) δ (ppm): 8.35 (d, J = 8.1 Hz, 1H), 8.21 (s, 1H), 8.09 (d, J = 8.7 Hz, 1H), 7.32 (d, J = 8.4 Hz, 1H), 6.86 (d, J = 8.7 Hz, 1H), 4.04 (t, J = 7.4 Hz, 2H), 1.58 (t, J = 7.4 Hz, 2H), 1.25 (m, 1.30~1.21, 26H), 0.83 (t, J = 6.9 Hz, 3H); ^{13}C NMR (DMSO-d_6 +NaOH solid, 75 MHz) δ (ppm): 163.0, 162.5, 155.7, 154.6, 154.2, 139.7, 133.6, 132.6, 132.1, 113.6, 113.3, 111.3, 103.3, 101.4, 31.3, 29.0, 28.9, 28.9, 18.8, 28.7, 27.7, 26.7, 22.1, 14.0; MS (ESI): m/z 500.4 $(M-H)^{-}$; HRMS (ESI): m/z Calcd for $C_{30}H_{40}N_5O_2$ $(M+H)^{+}$ 502.31765, found, 502.31797.

Synthesis of N-butyl-4-butylamine-1,8-naphthalimide (the standard for Φ_f measurement). 1.5 g (5.42 mmol) 4-bromine-1,8-naphthalic anhydride was dissolved in 120 mL ethanol, and heated to reflux.

Then 400 μL of butylamine (5.6 mmol) was added to the mixture slowly after the temperature cooling down to 50 ^{o}C. The resulted mixture was heated to reflux with stirring for 1 h. The reaction was over when the solution became clear. After the solution was cooled to room temperature, added water to the solution and then precipitate was emerged. White solid was obtained by vacuum filtration, and then washed with water and ethanol for three times respectively and finally dried by vacuum. N-butyl-4-bromine-1,8-napthalimide was obtained (yield 81.1%). Then 100 mg (0.302 mmol) N-butyl-4-bromine-1,8-napthalimide was dissolved to 5 ml of ethylene glycol monomethyl ether, adding 130 μL (1.8 mmol) of butylamine slowly with magnetic force stirring. The resulted mixture was heated to reflux with stirring for 1 h. After the solution stay overnight at room temperature, precipitate was emerged and solid was obtained by vacuum filtration. The residue was purified by column chromatography using silica-gel (100-200 mesh) and 3% methanol in dichloromethane as eluent to give yellow solid compound (rate of purity 98%, yield 128 mg, 30.7%), further purified by HPLC, purity reached to 99.9%.

^{1}H NMR (DMSO-d_6, 300 MHz) δ (ppm): 8.71 (d, J = 9.0 Hz, 1H), 8.43 (d, J = 6.0 Hz, 1H), 8.26 (d, J = 9.0 Hz, 1H), 7.74 (t, J = 6.0 Hz, -NH-, 1H), 7.67 (t, J = 9.0 Hz, 1H), 6.78 (d, J = 9.0 Hz, 1H), 4.01 (t, J = 7.5 Hz, 2H), 3.39 (t, J = 6.0 Hz, 2H), 1.74-1.67 (m, 2H), 1.65-1.54 (m, 2H), 1.50-1.42 (m, 2H), 1.40-1.30(m, 2H), 0.98-0.89 (m, 6H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ (ppm): 150.7, 150.7, 134.3, 130.6, 129.4, 128.6, 124.2, 121.9, 120.1, 107.5, 103.7, 42.6, 29.9, 29.8, 19.8, 13.8, 13.7; MS (ESI): m/z 325.0 $(M+H)^{+}$

2. Optical spectral behavior of ANNA at different pH

The absorbance and fluorescence spectra of ANNA at different pH were collected at the same condition as that of ENNA. These results show that ANNA has the same optical spectral behavior as ENNA, indicating that changing the substitutent at 9-position (N-9) of naphthalimides do not affect their optical spectral behavior.

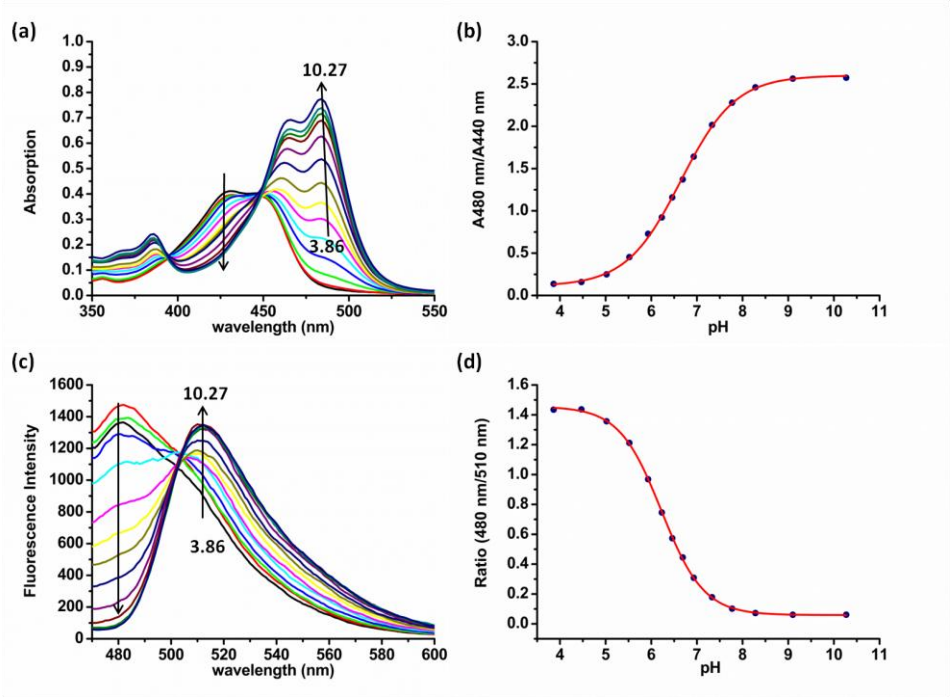


Figure S1. (a). Absorption spectra of 40 μM ANNA. All samples were measured in HEPES buffer at pH 3.86, 4.47, 5.02, 5.52, 5.93, 6.23, 6.46, 6.69, 6.93, 7.33, 7.77, 8.28, 9.10, 10.27. (b). Response curve of ratiometric absorbance

($A_{480\ nm}/A_{440\ nm}$) to pH. (c). Fluorescence spectra of 1.0 μM ANNA. All samples were measured in 20 mM HEPES buffer at pH 3.86, 4.47, 5.02, 5.52, 5.93, 6.23, 6.46, 6.69, 6.93, 7.33, 7.77, 8.28, 9.10, 10.27. (d) Response curve of ratiometic fluorescence intensity ($I_{480\ nm}/I_{510\ nm}$, λ_{ex} = 455 nm) to pH.

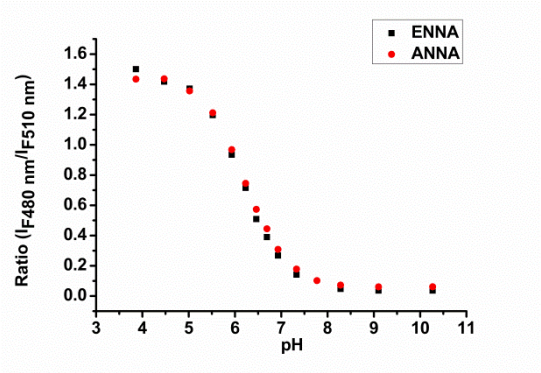


Figure S2. The comparison of fluorescence ratio curves of ANNA and ENNA against pH.

3. Reversibility of the fluorescence response to pH of ENNA and ANNA

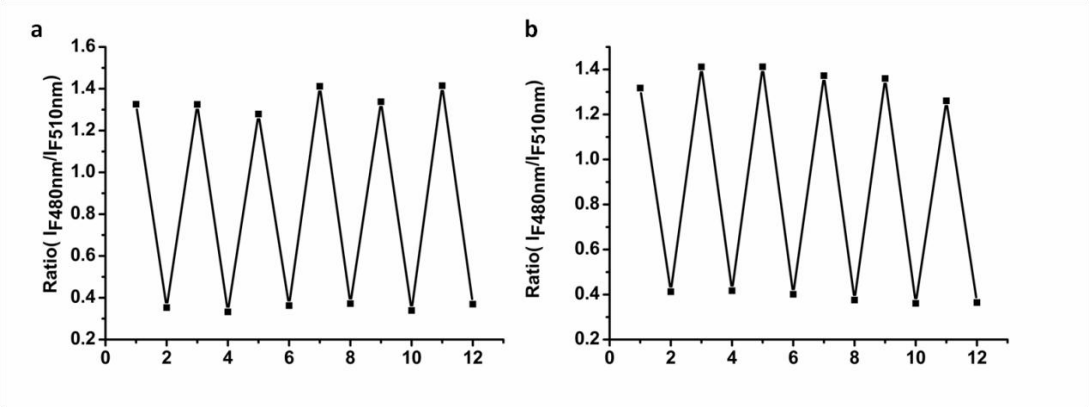


Figure S3. Fluorescence responses of ENNA and ANNA to acid/base cycles in HEPES (pH 5.52) solutions. The fluorescence intensities ratio at 480 nm to 510 nm was monitored by the alternate addition of NaOH (6 mM) and HCl (6 mM) under the excitation at 455 nm.

4. pH titrations in buffer solutions with different ionic strength

More pH dependence experiments in HEPES buffer solution at different ionic strengths were performed (NaCl: 0, 100, 150, 200 mM). The results indicated that there was no evident change in fluorescence emission intensity and wavelength, as well as the ratio of fluorescence intensity.

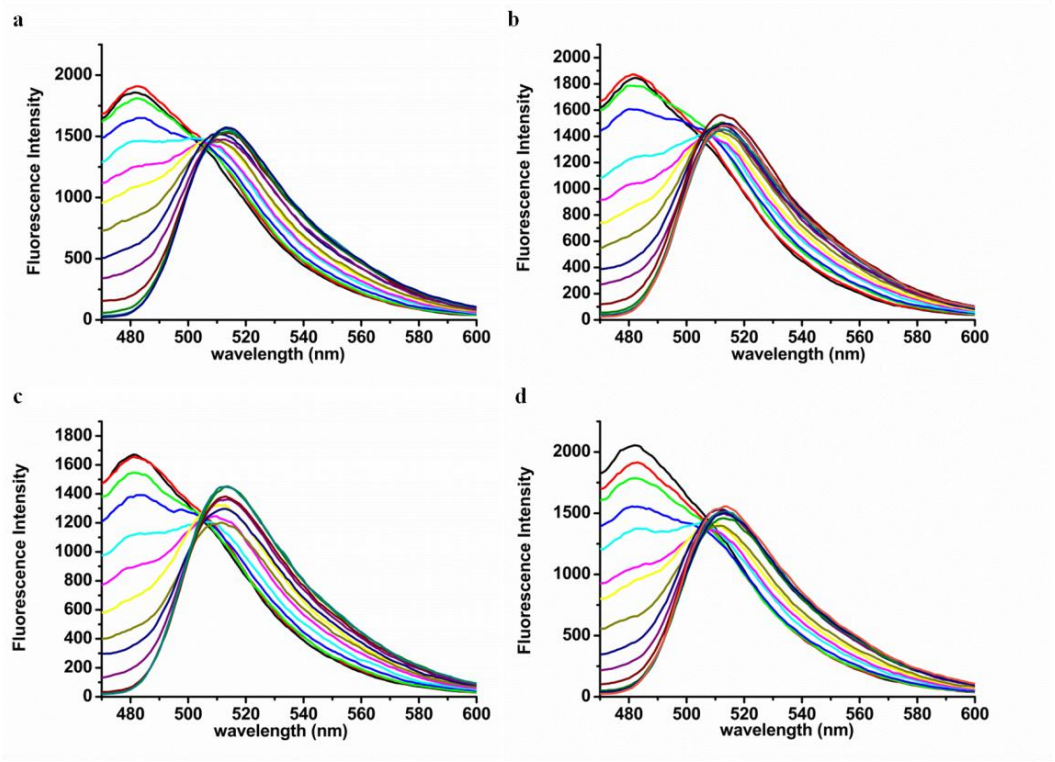


Figure S4. Fluorescence responce dependence on different pH value of 1.0 μM ENNA in HEPES buffer with different ionic strength. a: 0 mM NaCl, pH 4, 4.46, 5.04, 5.52, 5.85, 6.07, 6.28, 6.49, 6.75, 6.98, 7.47, 8.02, 8.87, 9.92; b: 100 mM NaCl, pH 3.94, 4.51, 5.19, 5.55, 5.99, 6.19, 6.37, 6.57, 6.05, 7.48, 8.02, 8.49, 9.33, 10.1; c: 150 mM NaCl, pH 3.86, 4.47, 5.02, 5.52, 5.93, 6.23, 6.46, 6.69, 6.93, 7.33, 7.77, 8.28, 9.10, 10.27; d: 200 mM NaCl, pH 3.87, 4.57, 4.98, 5.49, 5.83, 6.13, 6.3, 6.56, 6.89, 7.15, 7.45, 8.02, 8.93, 9.96. Spectra were obtained with excitation at 455 nm in 20 mM HEPES buffered aqueous solution.

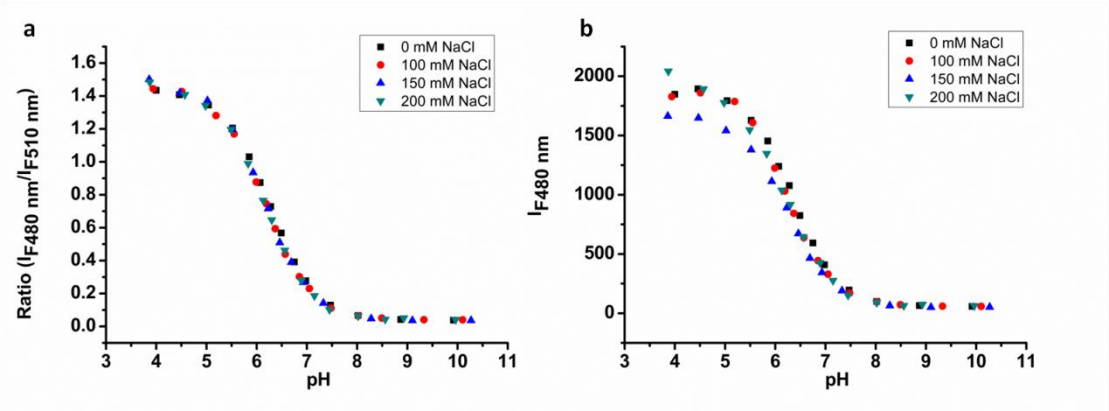


Figure S5. a. Response curve of fluorescence ratio ($I_{480\ nm}$ /$I_{510\ nm}$) against different pH value in HEPES buffer with different ionic strengths (NaCl: 0, 100, 150, 200 mM) with excitation at 455 nm. b. Response curve of fluorescence intensity at 480 nm against different pH value at different ionic strengths (NaCl: 0, 100, 150, 200 mM).

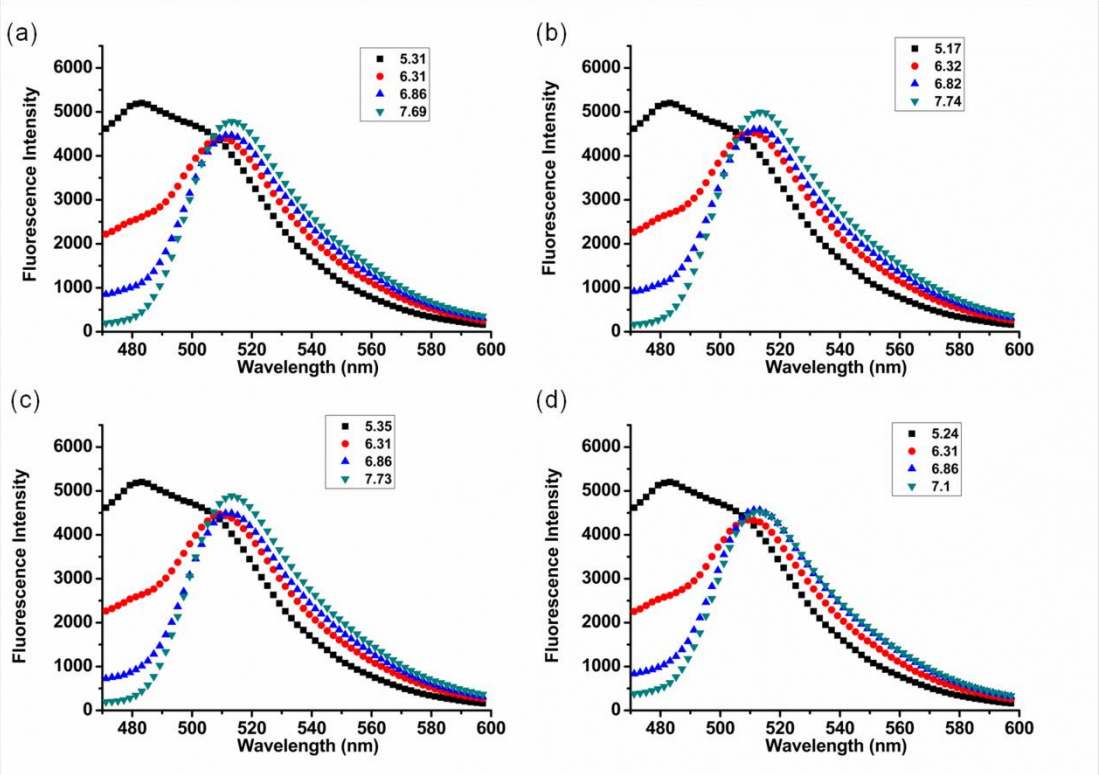


Figure S6. Fluorescence responce dependence on different pH value of 5.0 μM ENNA in PBS plus different concentration of NaCl. a: 0 mM NaCl, pH 5.31, 6.31, 6.86, 7.69; b: 100 mM NaCl, pH 5.17, 6.32, 6.86, 7.74; c: 150 mM NaCl, pH 5.35, 6.31, 6.86,7.73; d: 200 mM NaCl, pH 5.32, 6.31, 6.86, 7.1. Spectra were obtained with excitation at 455 nm in 20 mM PBS.

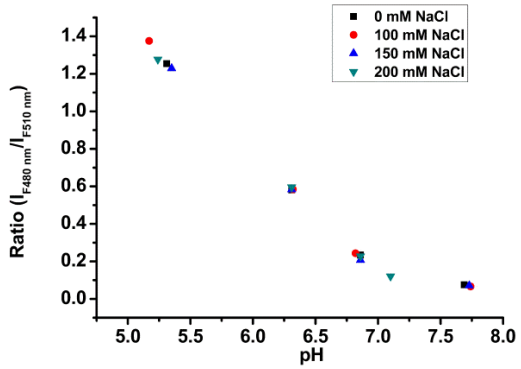


Figure S7. a. Response curve of fluorescence ratio ($I_{480\ nm}$ /$I_{510\ nm}$) against different pH value in PBS plus different concentration of NaCl. (NaCl: 0, 100, 150, 200 mM) with excitation at 455 nm in 20 mM PBS.

5. pKa determination of ENNA and ANNA

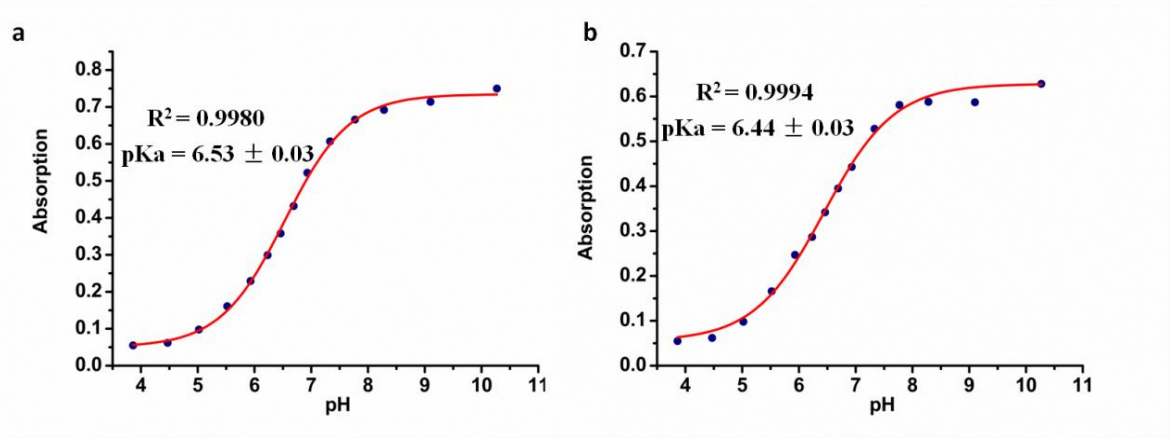


Figure S8. Response curve of absorbance of ENNA and ANNA to different pH. a: ENNA; b: ANNA. Absorption data (480 nm) were collected in HEPES buffer (containing 150 mM NaCl) at various pH.

6. ESI-MS spectrum of ENNA

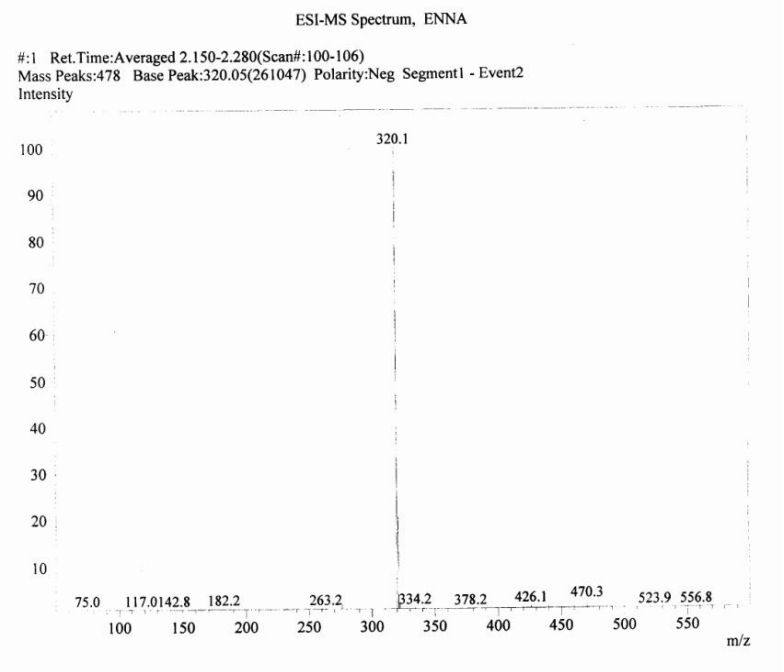


Figure S9. ESI-MS spectrum of ENNA, Exact Mass: 321.09

7. Stability of the ENNA

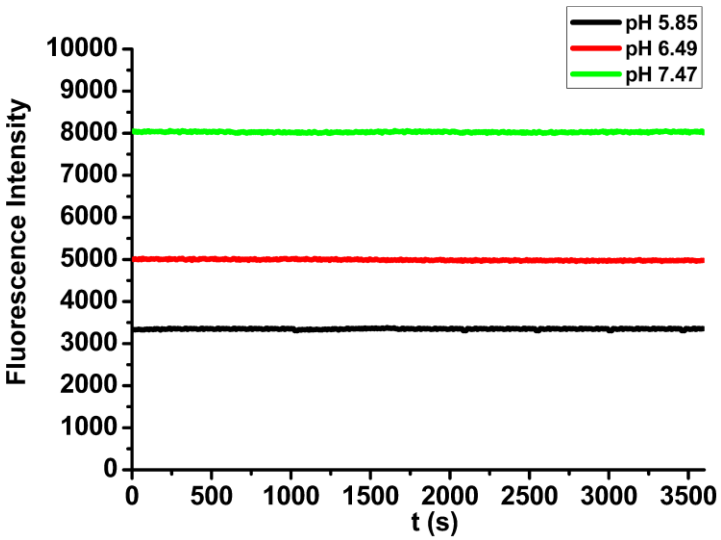


Figure S10. Time-dependent fluorescence intensities of the ENNA were measured by F-4600 (λ_{ex}: 480 nm and λ_{em}: 510 nm). The concentration was 5.0 μM in 20 mM HEPES.

8. Fluorescence spectra of HNNA

In Figure **S11**, we can see that HNNA do not exhibit fluorescence in pure water and buffer salt, but exhibit strong fluorescence in hydrophobic environment and organic solvent. SDS, CTAB and Triton X-100 (1mM for each) were dissolved in 50 mM HEPES, the pH of the surfactant solutions were adjusted by adding NaOH to the solution. 1μL of HNNA stock solution (5mM) was added to 1mL of surfactant solutions at different pH conditions, After standing for 0.5 h, the fluorescence spectra were collected.

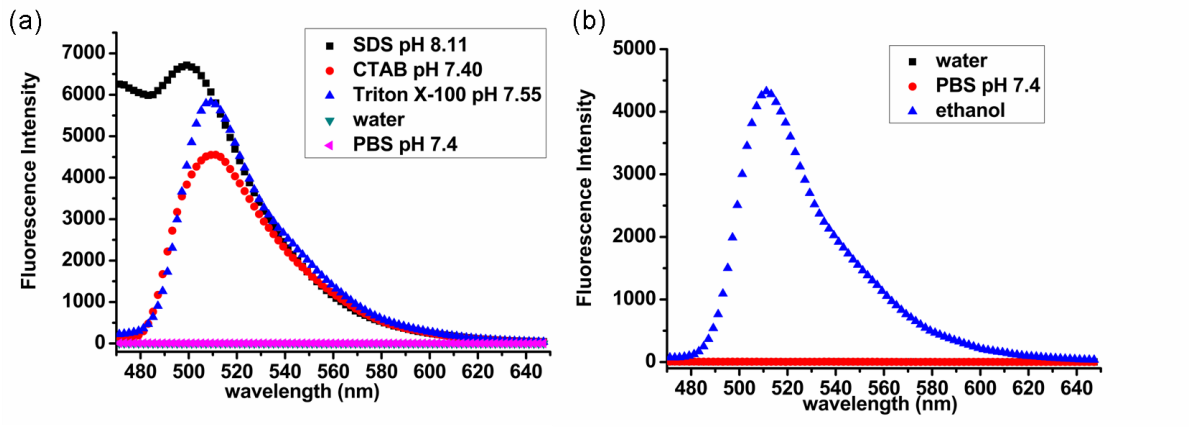


Figure S11. (a) Fluorescence spectra of HNNA in water (HNNA: 5 μM), pH 7.4 PBS (HNNA: 5 μM), SDS pH 7.40 (HNNA: 5 μM), CTAB pH 8.11 (HNNA: 3 μM), Triton X-100 pH 7.55 (HNNA: 3 μM); (b) Fluorescence spectra of HNNA in water (HNNA: 2 μM), PBS pH 7.4 (HNNA: 1 μM) and ethanol (HNNA: 2 μM), with excitation at 455 nm.

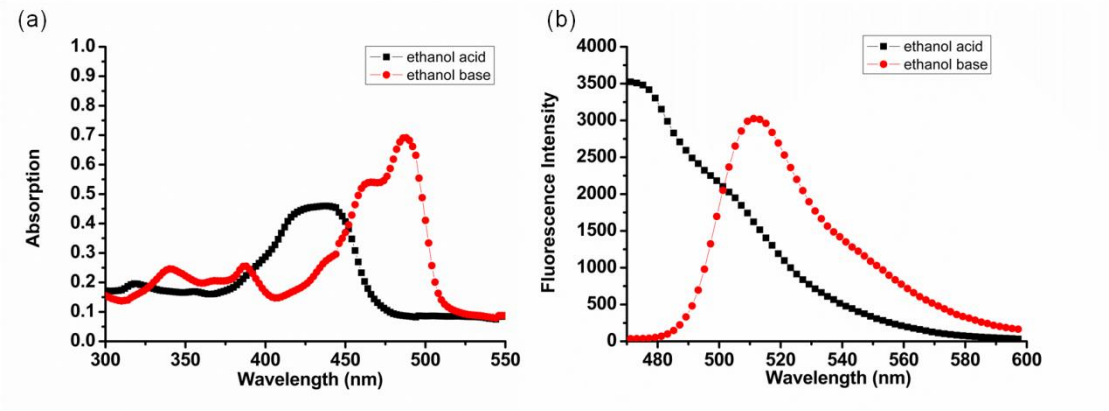


Figure 12. (a) Absorption spectra of 30 μM ENNA in acidic ethanol (Black line, plus HCl) and basic ethanol (red line, plus NaOH). (b) Fluorescence spectra of 1.0 μM HNNA in acidic ethanol (Black line, plus HCl) and basic ethanol (red line, plus NaOH). Spectra were collected with excitation at 455 nm.

9. Confocal imaging of Hela cells in low pH high K^+ buffer

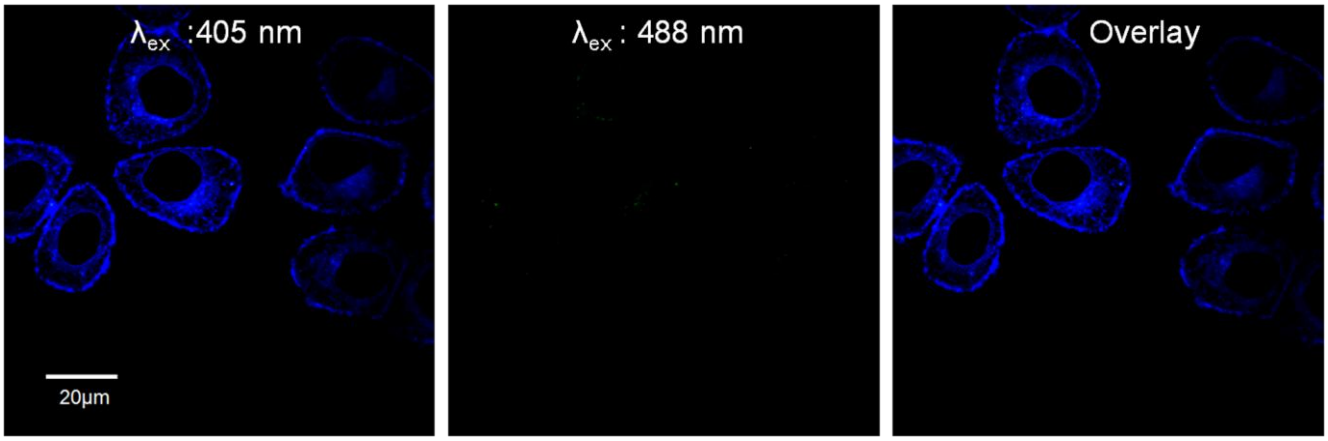


Figure S13 The confocal images of Hela cells. Images were collected at 5 min after changing buffer from PBS to high K^+ HEPES buffer (pH 5.5). Scale bar: 20 μm.

10. Confocal imaging of Hela cells treated with nigericin at different pH

The fluorescence imaging of living Hela cells treated with nigericin (5 μg/mL) in high K^+ HEPES-buffered solution at different pH values was also performed under the same condition as that of MCF-7. As shown in Figure S14a, HNNA localized inside the cytoplasm and cell membrane. As the pH increasing, the green channel become brighter and blue channel become darker. The ratiometric fluorescence images was obtained by green channel/blue channel. The curve of fluorescence ratio (F_{green}/F_{blue}) versus pH were obtained in the pH range of 6.4-8.1 (Figure S14b). The average pH of untreated Hela cells was determined to be 7.4 ± 0.02 base on the curve.

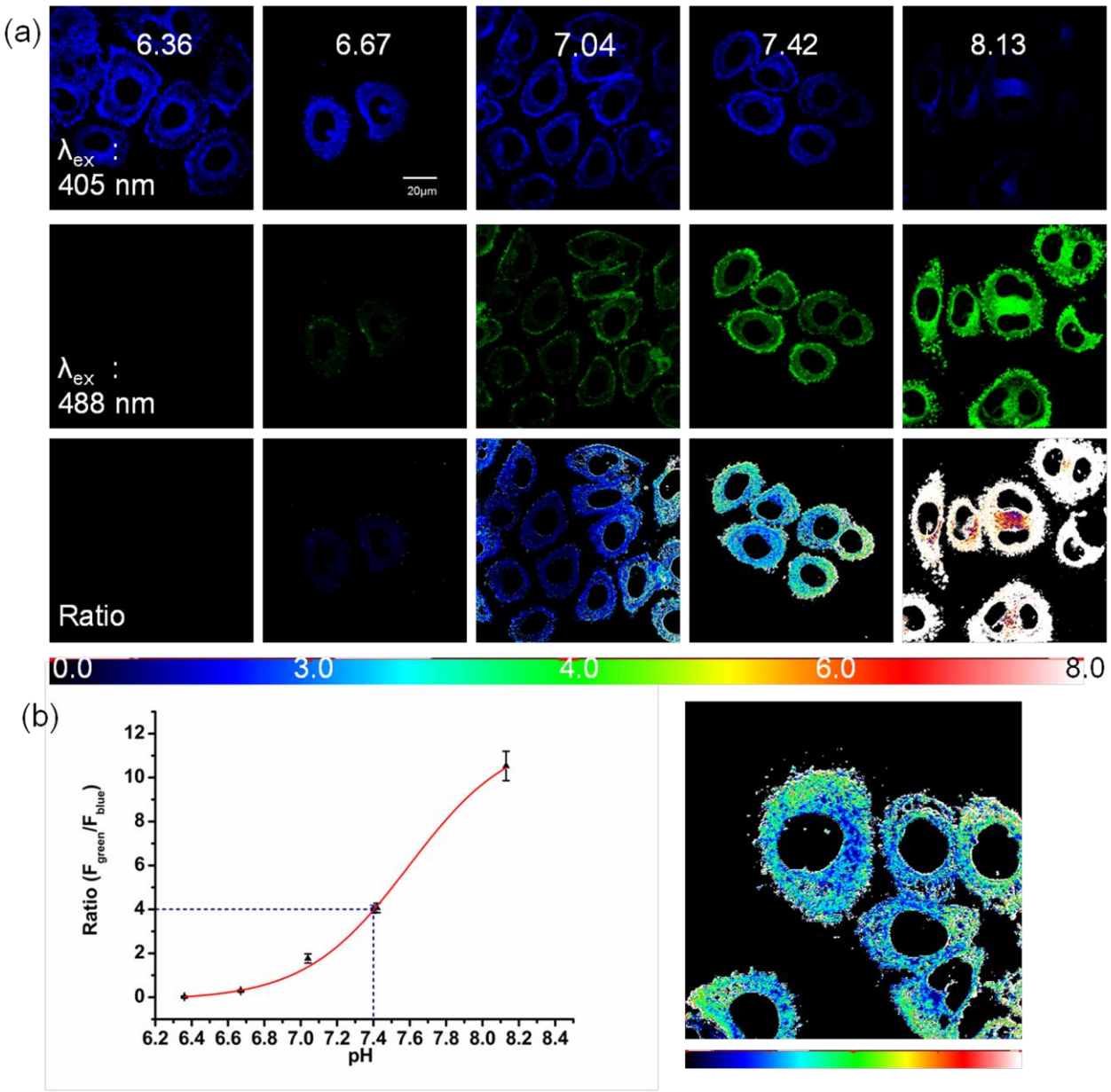
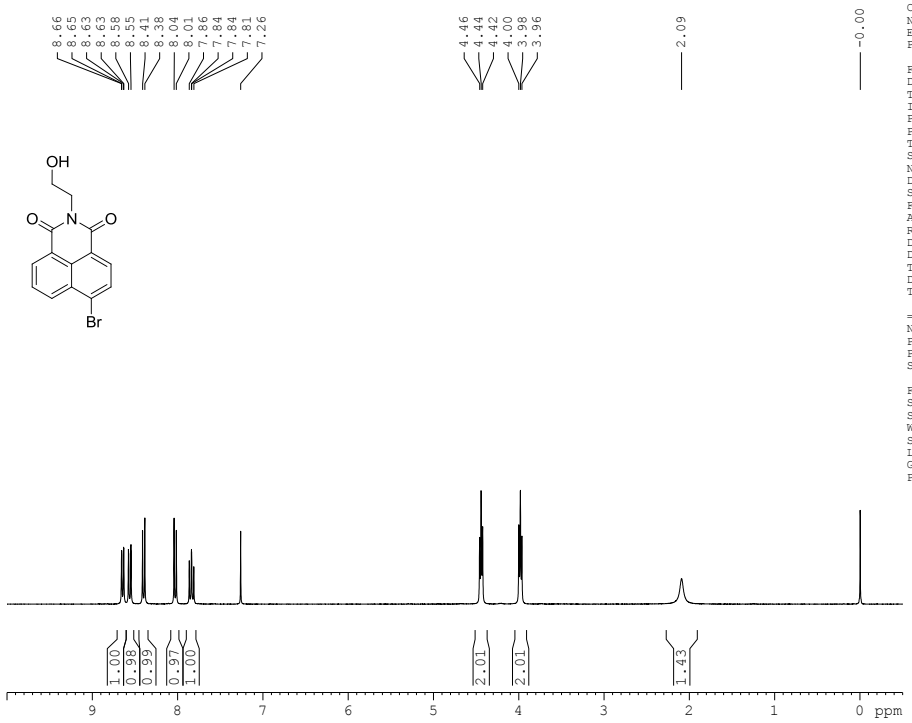


Figure S14. (a) Confocal fluorescence images of Hela cells incubated in high K^+ HEPES-buffered solution containing ionophores at different pH (b) pH map (right) calculation for untreated Hela cells based on the calibration curve (left) obtained using ionophores. Scale bar, 20 μm.

11. NMR spectra

Compound 1a N-hydroxyethyl-4-bromine-1, 8-naphthalimide

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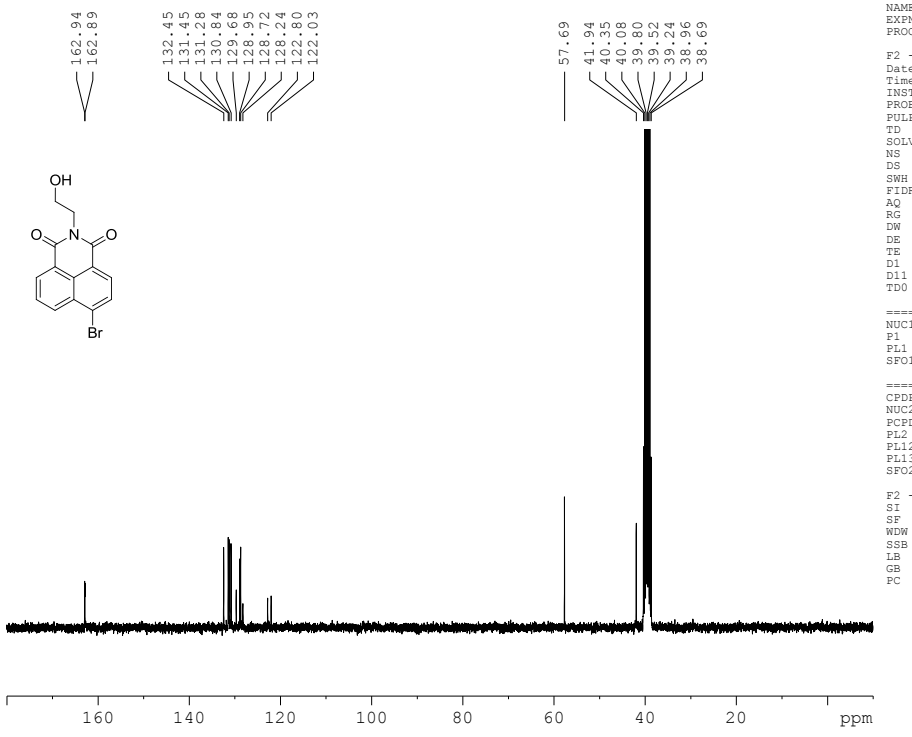


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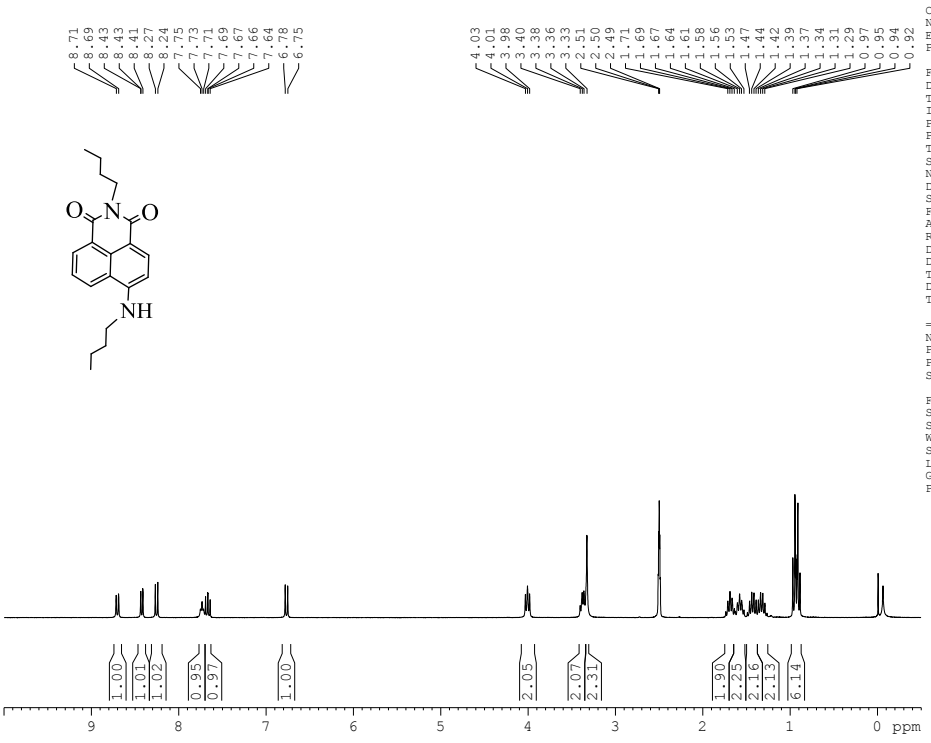
DW                27.800 usec
DE                 6.50 usec
TE                 298.9 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                  13C
P1                 12.50 usec
PL1                 2.00 dB
SFO1          75.4752953 MHz

======== CHANNEL f2 ========
CPDPRG2           waltz16
NUC2                   1H
PCPD2             100.00 usec
PL2                 3.00 dB
PL12               22.33 dB
PL13               23.00 dB
SFO2         300.1312005 MHz

F2 - Processing parameters
SI                  32768
SF           75.4677849 MHz
WDW                    EM
SSB                     0
LB                 1.00 Hz
GB                      0
PC                   1.40
```

N-butyl-4-butylamine-1, 8-naphthalimide

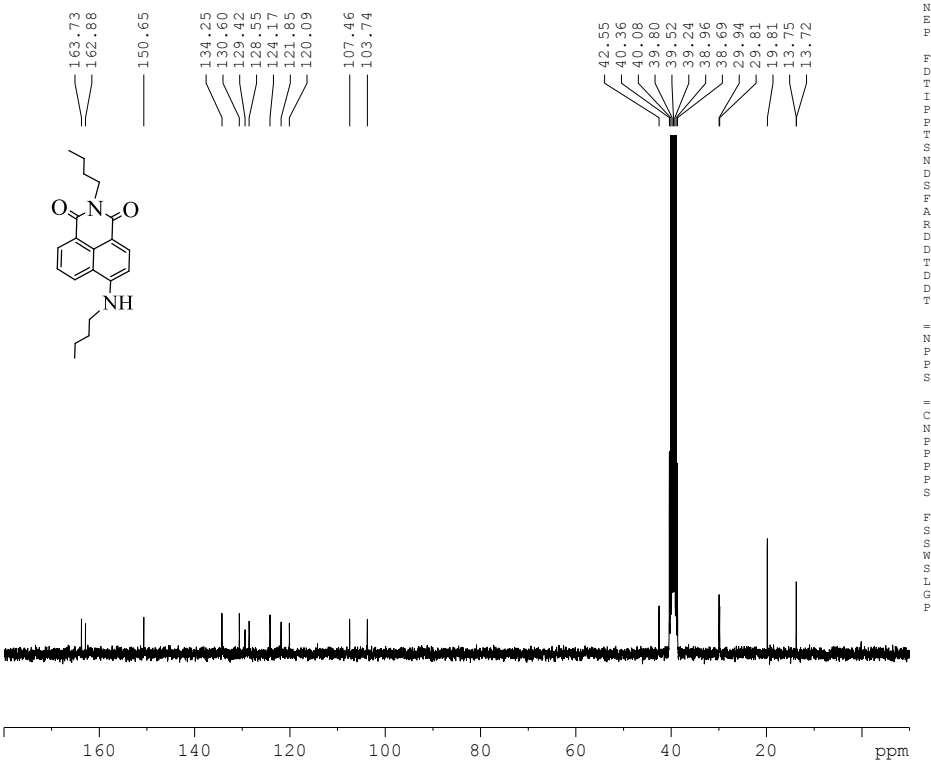



```
Current Data Parameters
NAME      dzy-20110709-DEND
EXPNO                    10
PROCNO                    1

F2 - Acquisition Parameters
Date_              20110709
Time                 20.08
INSTRUM              spect
PROBHD    5 mm DUL 13C-1
PULPROG              zg30
TD                  65536
SOLVENT              DMSO
NS                     16
DS                      0
SWH              8992.806 Hz
FIDRES           0.137219 Hz
AQ               3.6438515 sec
RG                    512
DW                 55.600 usec
DE                  8.00 usec
TE                 299.5 K
D1              1.00000000 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                   1H
P1                  10.80 usec
PL1                  3.00 dB
SFO1          300.1324010 MHz

F2 - Processing parameters
SI                  32768
SF            300.1300009 MHz
WDW                    EM
SSB                     0
LB                   0.30 Hz
GB                      0
PC                   1.00
```




```
Current Data Parameters
NAME      dzy-110710-zj-DNND
EXPNO                    10
PROCNO                    1

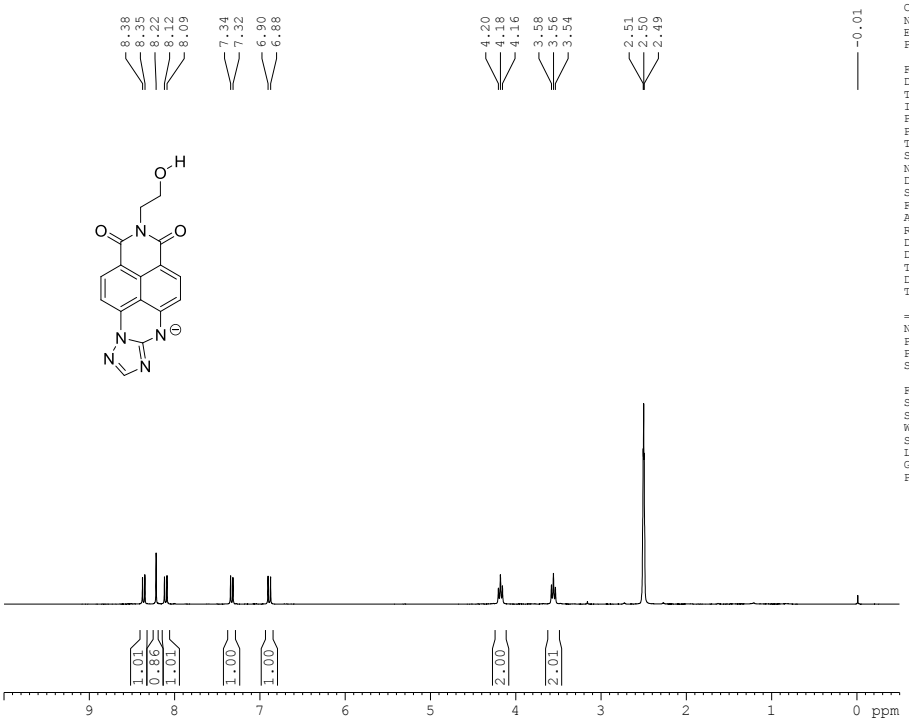
F2 - Acquisition Parameters
Date_              20110710
Time                 19.05
INSTRUM              spect
PROBHD    5 mm DUL 13C-1
PULPROG           zgpg30
TD                  65536
SOLVENT              DMSO
NS                    968
DS                      4
SWH             17985.611 Hz
FIDRES           0.274439 Hz
AQ               1.8219508 sec
RG                   4597.6
DW                 27.800 usec
DE                  6.50 usec
TE                 300.1 K
D1              2.00000000 sec
D11             0.03000000 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                  13C
P1                  12.50 usec
PL1                  2.00 dB
SFO1          75.4752953 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                   1H
PCPD2             100.00 usec
PL2                  3.00 dB
PL12                22.33 dB
PL13                23.00 dB
SFO2          300.1312005 MHz

F2 - Processing parameters
SI                  32768
SF            75.4677854 MHz
WDW                    EM
SSB                     0
LB                   1.00 Hz
GB                      0
PC                   1.40
```

compound 2a ENNA L^-



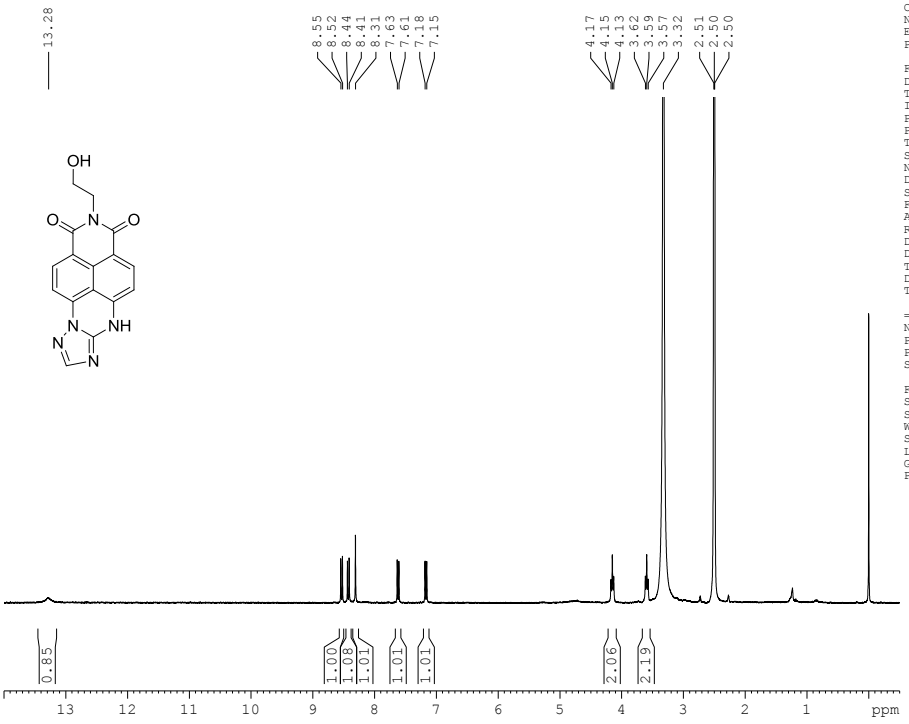

```
Current Data Parameters
NAME      dzy-20100910-1
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20100910
Time              15.31
INSTRUM           spect
PROBHD    5 mm DUL 13C-1
PULPROG           zg30
TD               65536
SOLVENT           DMSO
NS                   16
DS                    0
SWH           8992.806 Hz
FIDRES        0.137219 Hz
AQ            3.6438515 sec
RG                  362
DW               55.600 usec
DE                8.00 usec
TE                301.0 K
D1           1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 1H
P1                10.80 usec
PL1                3.00 dB
SFO1        300.1324010 MHz

F2 - Processing parameters
SI                32768
SF          300.1300011 MHz
WDW                  EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.00
```

ENNA LH



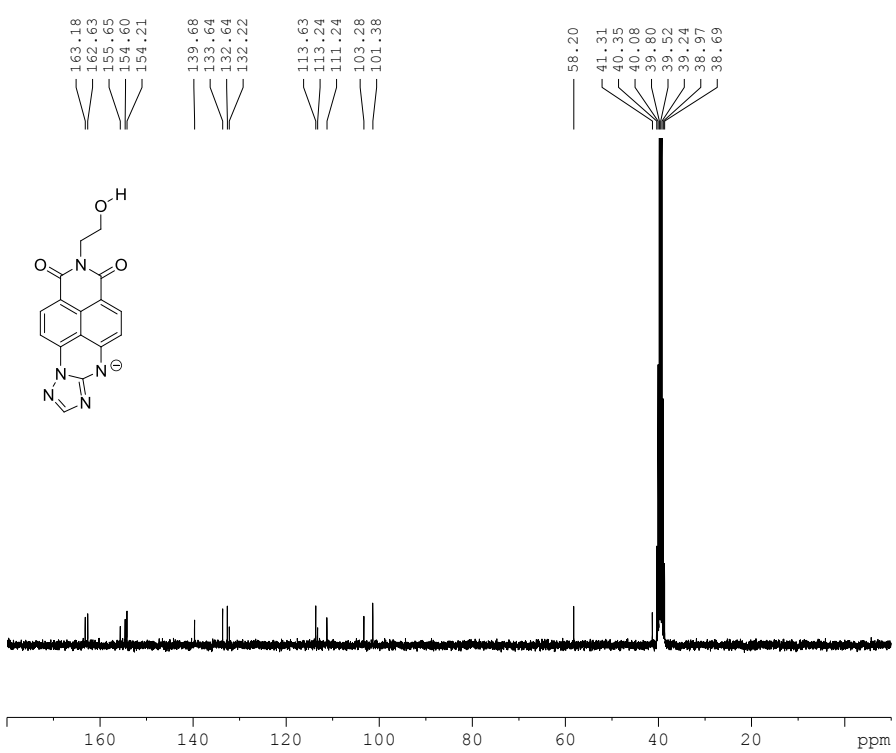

```
Current Data Parameters
NAME      dzy-110702-zj-ENNA
EXPNO                 11
PROCNO                 1

F2 - Acquisition Parameters
Date_           20110702
Time              20.45
INSTRUM           spect
PROBHD    5 mm DUL 13C-1
PULPROG           zg30
TD               65536
SOLVENT           DMSO
NS                   96
DS                    0
SWH           6172.839 Hz
FIDRES        0.094190 Hz
AQ            5.3084660 sec
RG                  512
DW               81.000 usec
DE                6.50 usec
TE                299.5 K
D1           2.00000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                 1H
P1                10.80 usec
PL1                3.00 dB
SFO1        300.1318534 MHz

F2 - Processing parameters
SI                32768
SF          300.1300010 MHz
WDW                  EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.00
```

ENNA L$^-$




```
Current Data Parameters
NAME      dzy-20100910-1
EXPNO                 11
PROCNO                 1

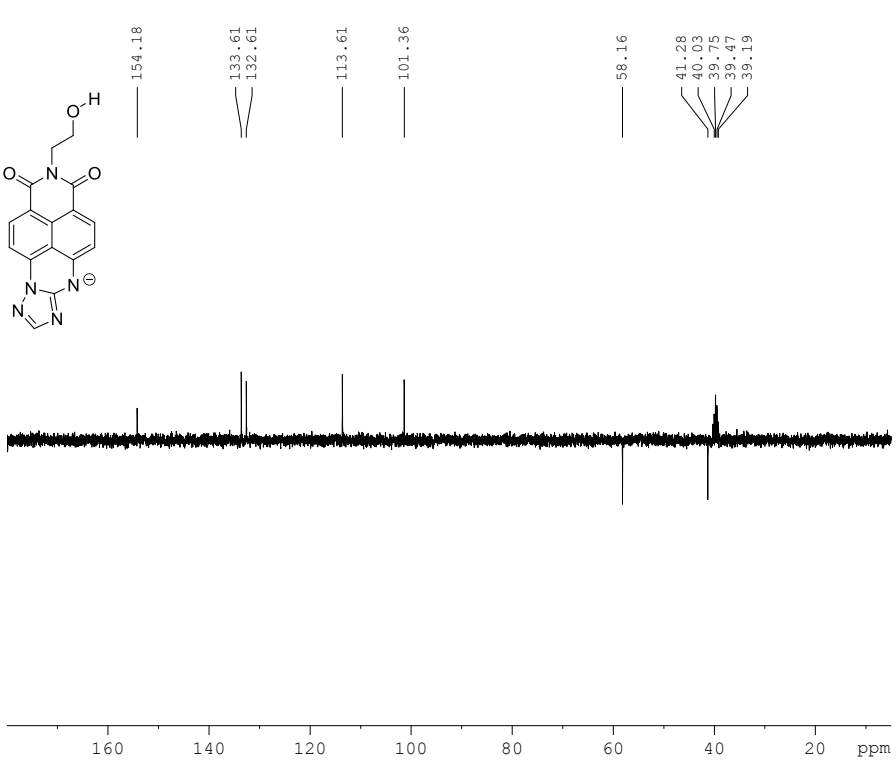
F2 - Acquisition Parameters
Date_           20100910
Time_              15.35
INSTRUM            spect
PROBHD   5 mm DUL 13C-1
PULPROG          zgpg30
TD                65536
SOLVENT             DMSO
NS                    274
DS                      4
SWH           17985.611 Hz
FIDRES         0.274439 Hz
AQ            1.8219508 sec
RG                16384
DW               27.800 usec
DE                8.00 usec
TE               301.6 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                 13C
P1                12.50 usec
PL1                2.00 dB
SFO1         75.4752953 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
PCPD2            100.00 usec
PL2                3.00 dB
PL12              22.33 dB
PL13              23.00 dB
SFO2        300.1312005 MHz

F2 - Processing parameters
SI                 32768
SF          75.4677849 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```

DEPT 135 (ENNA)




```
Current Data Parameters
NAME      dzy-20100910-1
EXPNO                 40
PROCNO                 1

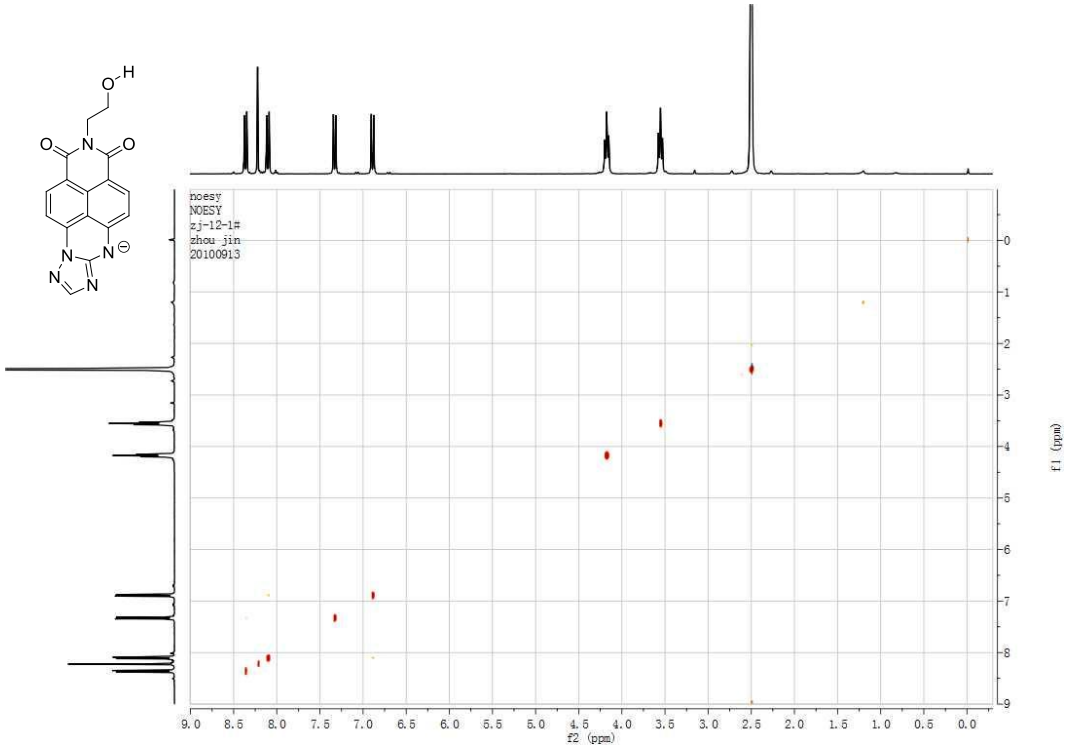
F2 - Acquisition Parameters
Date_           20100910
Time_              20.49
INSTRUM            spect
PROBHD   5 mm DUL 13C-1
PULPROG          dept135
TD                65536
SOLVENT             DMSO
NS                    144
DS                      4
SWH           17985.611 Hz
FIDRES         0.274439 Hz
AQ            1.8219508 sec
RG                16384
DW               27.800 usec
DE                8.00 usec
TE               301.5 K
CNST2        145.0000000
D1            2.00000000 sec
D2            0.00344828 sec
D12           0.00002000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                 13C
P1                12.50 usec
P2                25.00 usec
PL1                2.00 dB
SFO1         75.4752953 MHz

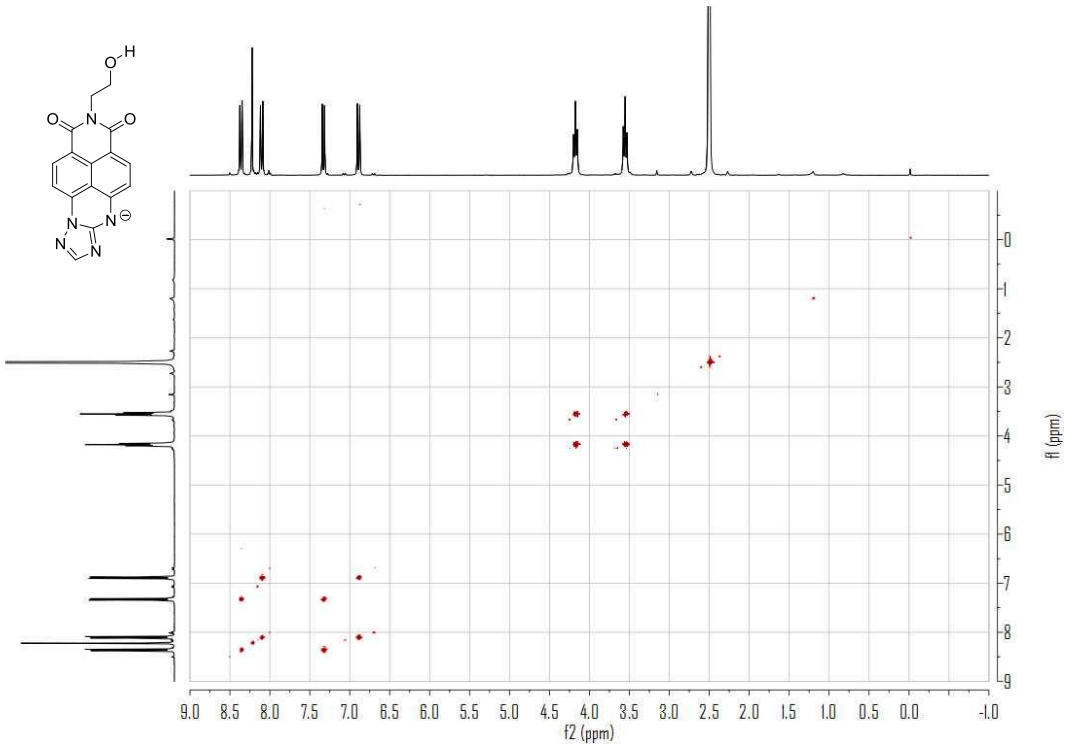
======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
P3                10.80 usec
P4                21.60 usec
PCPD2            100.00 usec
PL2                3.00 dB
PL12              22.33 dB
SFO2        300.1312005 MHz

F2 - Processing parameters
SI                 32768
SF          75.4677867 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```

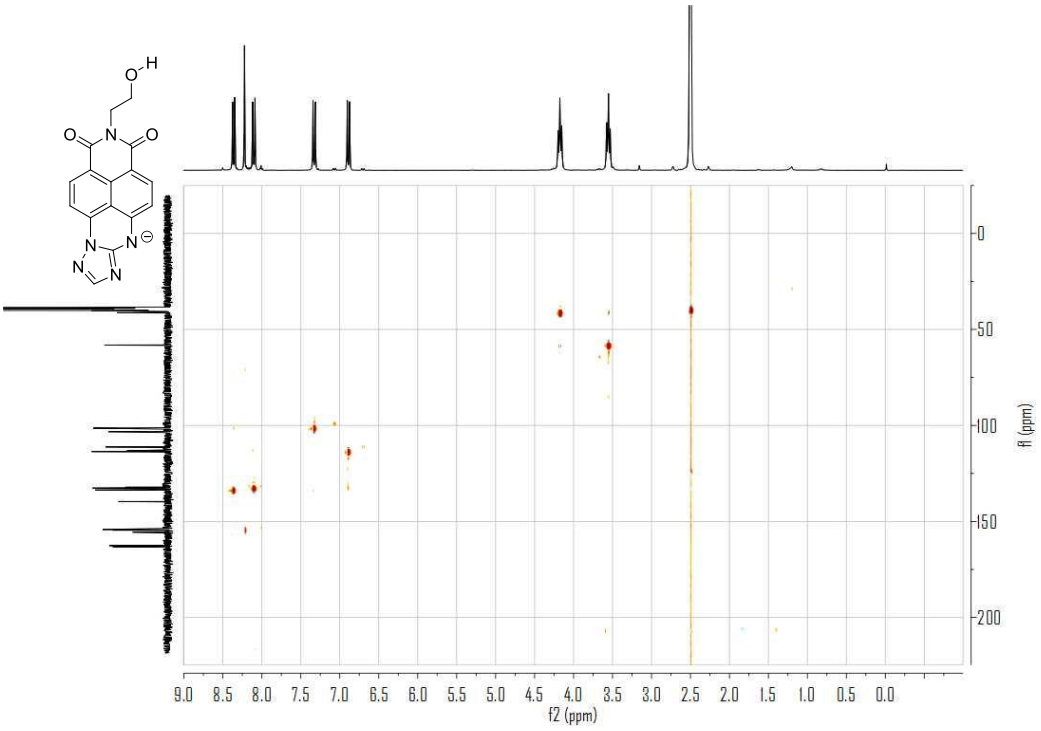
NOESY (ENNA)



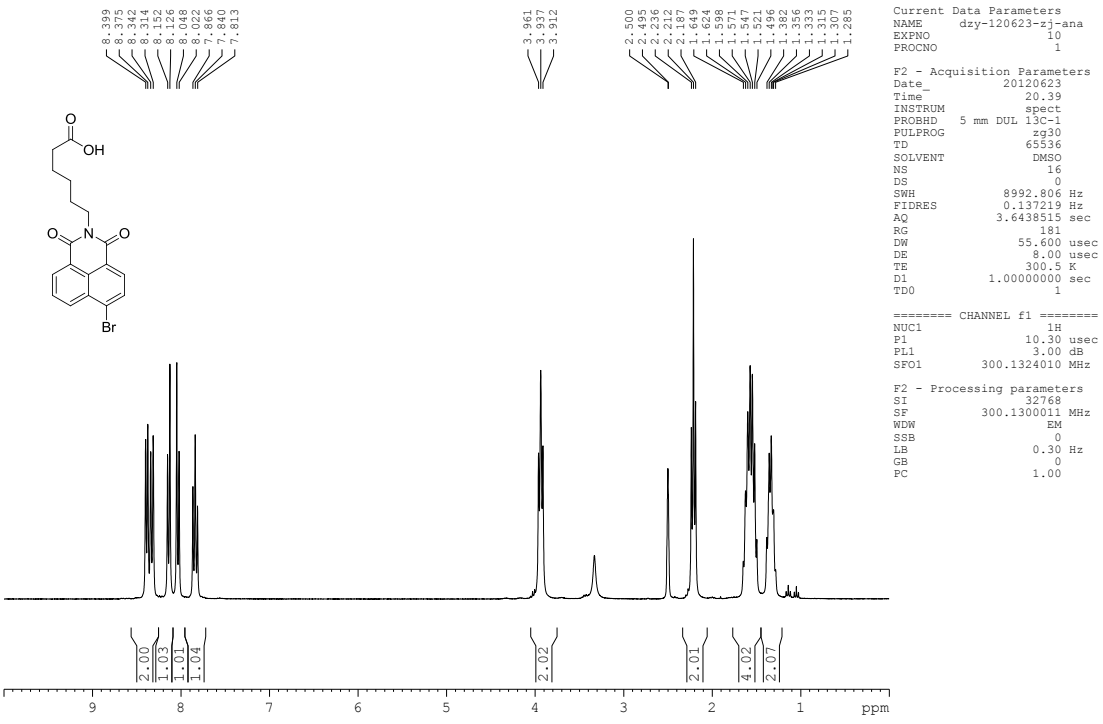
^{1}H, ^{1}H-COSY (ENNA)



HSQC (ENNA)



Compound 1b N-hexanoic acid-4-bromine-1, 8-naphthalimide

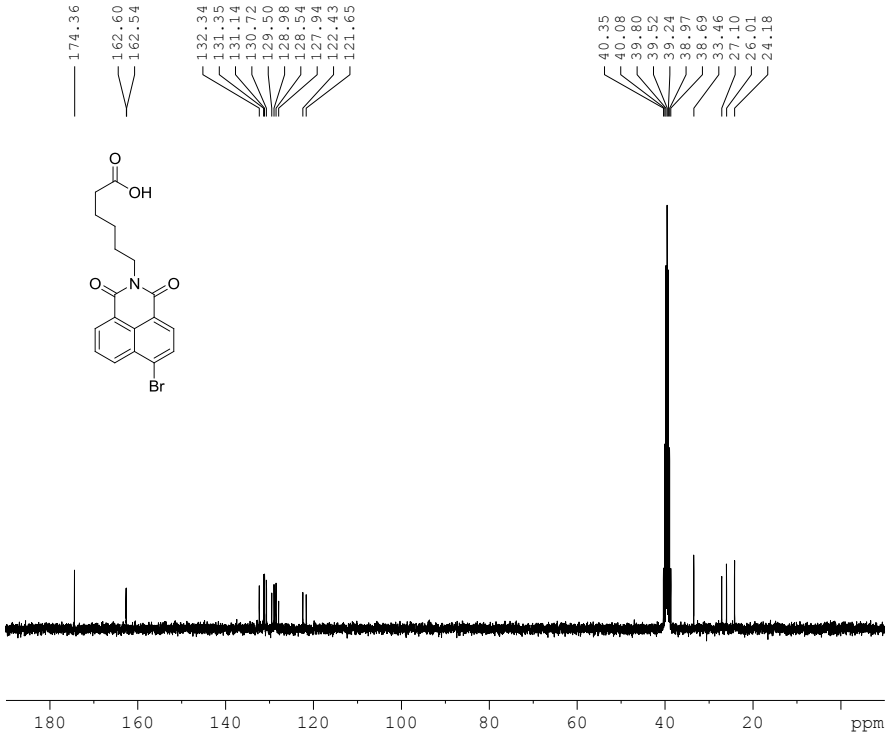



```
Current Data Parameters
NAME      dzy-120623-zj-ana
EXPNO                    10
PROCNO                    1

F2 - Acquisition Parameters
Date_              20120623
Time                  20.39
INSTRUM               spect
PROBHD   5 mm DUL 13C-1
PULPROG                zg30
TD                    65536
SOLVENT                DMSO
NS                       16
DS                        0
SWH                8992.806 Hz
FIDRES            0.137219 Hz
AQ                3.6438515 sec
RG                      181
DW                 55.600 usec
DE                  8.00 usec
TE                   300.5 K
D1              1.00000000 sec
TD0                       1

======== CHANNEL f1 ========
NUC1                     1H
P1                  10.30 usec
PL1                  3.00 dB
SFO1          300.1324010 MHz

F2 - Processing parameters
SI                    32768
SF            300.1300011 MHz
WDW                      EM
SSB                       0
LB                   0.30 Hz
GB                        0
PC                    1.00
```




```
Current Data Parameters
NAME      dzy-120623-zj-ana
EXPNO                   21
PROCNO                   1

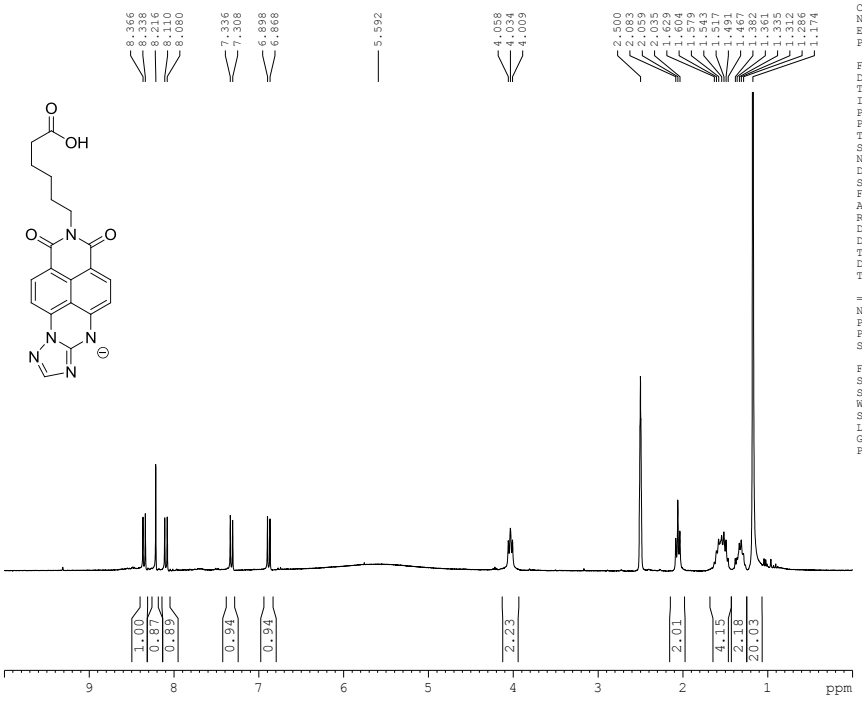
F2 - Acquisition Parameters
Date_           20120623
Time               20.48
INSTRUM            spect
PROBHD   5 mm DUL 13C-1
PULPROG          zgpg30
TD                65536
SOLVENT             DMSO
NS                    62
DS                     4
SWH            17985.611 Hz
FIDRES             0.274439 Hz
AQ              1.8219508 sec
RG                  3251
DW                27.800 usec
DE                 6.50 usec
TE                300.9 K
D1           2.00000000 sec
D11          0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  13C
P1                 12.50 usec
PL1                 2.00 dB
SFO1          75.4752953 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                  1H
PCPD2             100.00 usec
PL2                 3.00 dB
PL12               22.74 dB
PL13               23.00 dB
SFO2         300.1312005 MHz

F2 - Processing parameters
SI                 32768
SF            75.4677850 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```

Compound 2b ANNA

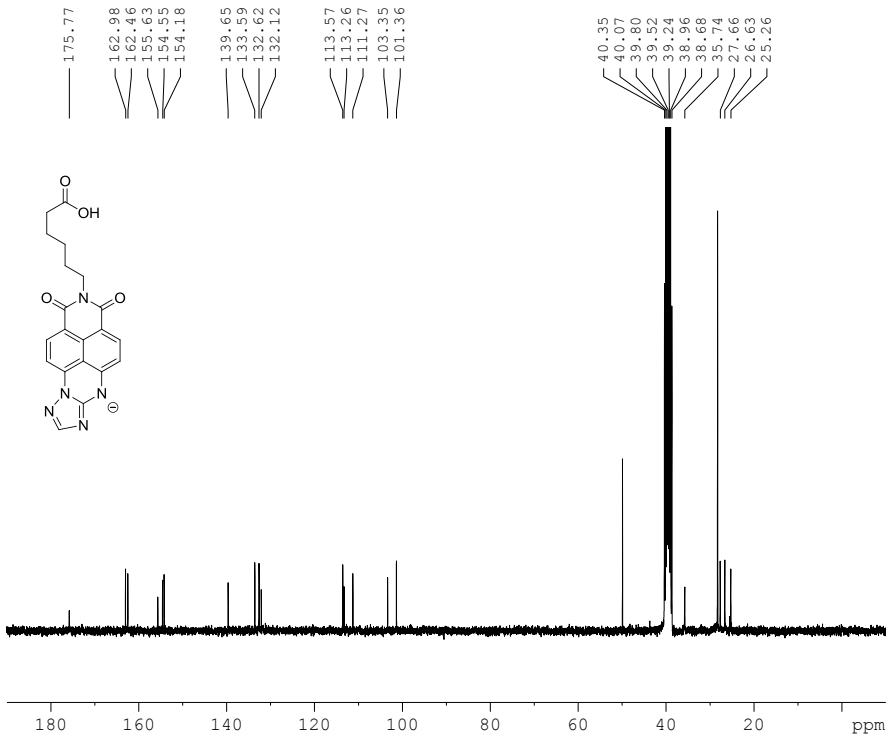



```
Current Data Parameters
NAME      dzy-111208-zj-annna
EXPNO                   10
PROCNO                   1

F2 - Acquisition Parameters
Date_           20111208
Time               11.24
INSTRUM            spect
PROBHD   5 mm DUL 13C-1
PULPROG             zg30
TD                65536
SOLVENT             DMSO
NS                    16
DS                     0
SWH             8992.806 Hz
FIDRES             0.137219 Hz
AQ              3.6438515 sec
RG                   256
DW                55.600 usec
DE                 8.00 usec
TE                296.2 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                   1H
P1                 10.80 usec
PL1                 3.00 dB
SFO1         300.1324010 MHz

F2 - Processing parameters
SI                 32768
SF            300.1300009 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```




```
Current Data Parameters
NAME       dzy-1218-zj-1
EXPNO                 20
PROCNO                 1

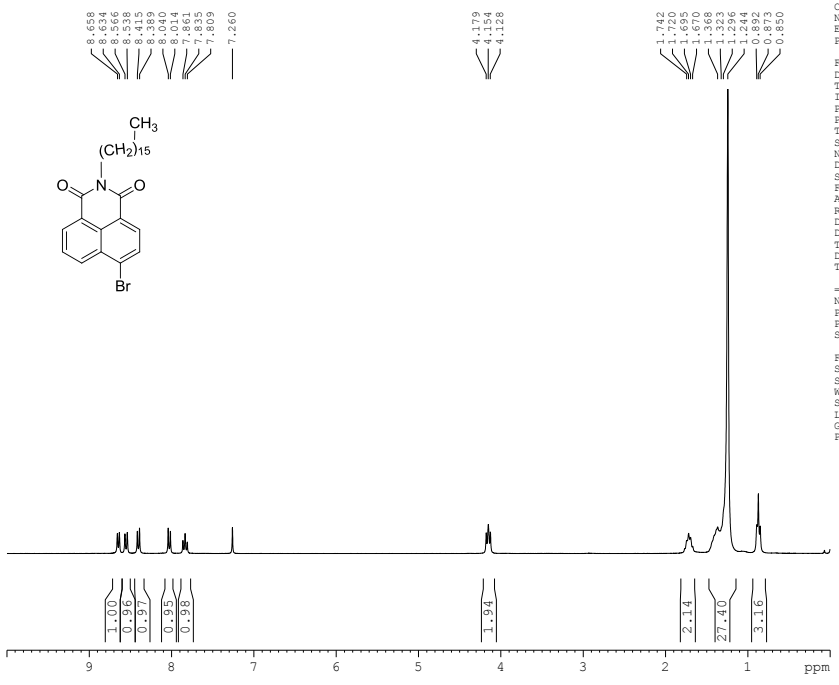
F2 - Acquisition Parameters
Date_           20111218
Time               16.00
INSTRUM            spect
PROBHD   5 mm DUL 13C-1
PULPROG          zgpg30
TD                65536
SOLVENT             DMSO
NS                  4024
DS                     4
SWH            17985.611 Hz
FIDRES             0.274439 Hz
AQ              1.8219508 sec
RG                  6502
DW                27.800 usec
DE                 6.50 usec
TE                298.1 K
D1            2.00000000 sec
D11           0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                 13C
P1                12.50 usec
PL1                2.00 dB
SFO1          75.4752953 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
PCPD2             100.00 usec
PL2                3.00 dB
PL12              22.33 dB
PL13              23.00 dB
SFO2         300.1312005 MHz

F2 - Processing parameters
SI                  32768
SF           75.4677839 MHz
WDW                    EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```

Compound 1c N- hexadecyl-4-bromine-1, 8-naphthalimide

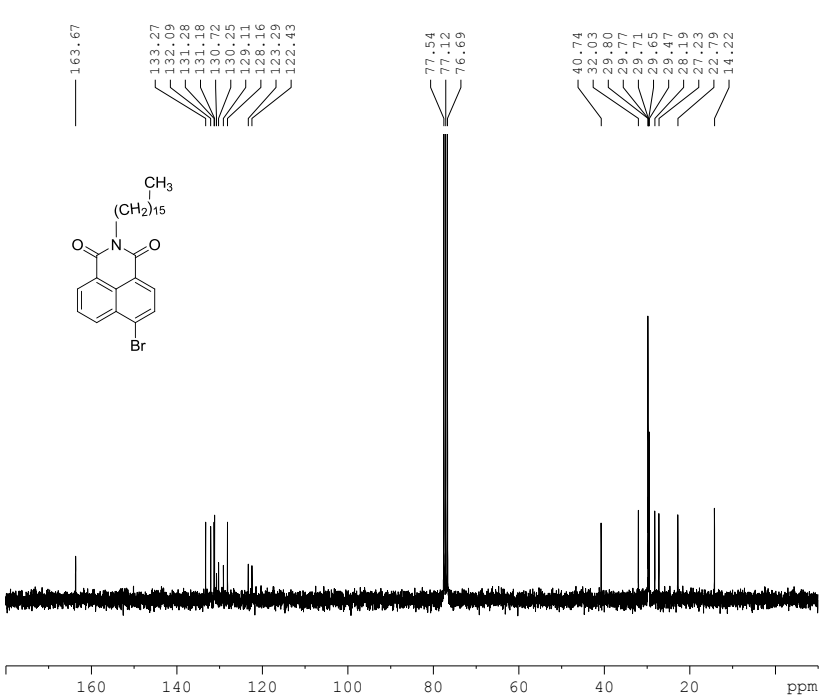



```
Current Data Parameters
NAME      dzy-zj-20120107-hna
EXPNO                  10
PROCNO                  1

F2 - Acquisition Parameters
Date_           20120107
Time               20.12
INSTRUM            spect
PROBHD   5 mm DUL 13C-1
PULPROG            zg30
TD                65536
SOLVENT           CDCl3
NS                   16
DS                    0
SWH             8992.806 Hz
FIDRES          0.137219 Hz
AQ              3.6438515 sec
RG                  256
DW                55.600 usec
DE                 8.00 usec
TE                297.7 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                 1H
P1                10.80 usec
PL1                3.00 dB
SFO1         300.1324010 MHz

F2 - Processing parameters
SI                  32768
SF           300.1300059 MHz
WDW                    EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```




```
Current Data Parameters
NAME      dzy-120108-zj-hna
EXPNO                  10
PROCNO                  1

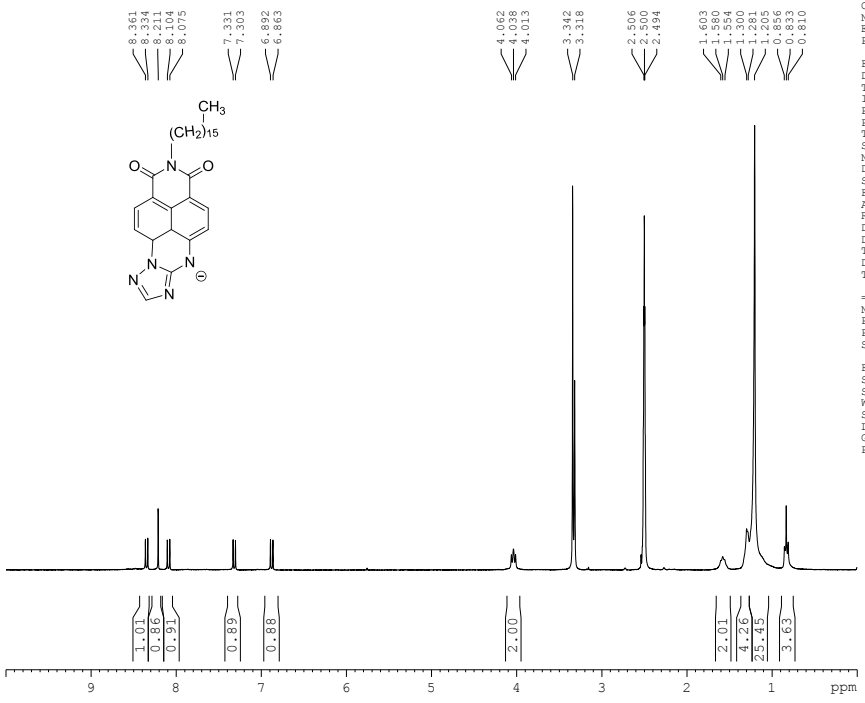
F2 - Acquisition Parameters
Date_            20120108
Time                10.47
INSTRUM             spect
PROBHD   5 mm DUL 13C-1
PULPROG           zgpg30
TD                 65536
SOLVENT             CDC13
NS                    166
DS                      4
SWH             17985.611 Hz
FIDRES          0.274439 Hz
AQ              1.8219508 sec
RG                  322.5
DW                27.800 usec
DE                 6.50 usec
TE                298.3 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                  13C
P1                  12.50 usec
PL1                  2.00 dB
SFO1          75.4752953 MHz

======== CHANNEL f2 ========
CPDPRG2           waltz16
NUC2                  1H
PCPD2              100.00 usec
PL2                  3.00 dB
PL12                22.33 dB
PL13                23.00 dB
SFO2         300.1312005 MHz

F2 - Processing parameters
SI                   32768
SF            75.4677410 MHz
WDW                     EM
SSB                      0
LB                    1.00 Hz
GB                       0
PC                    1.40
```

Compound 2c HNNA

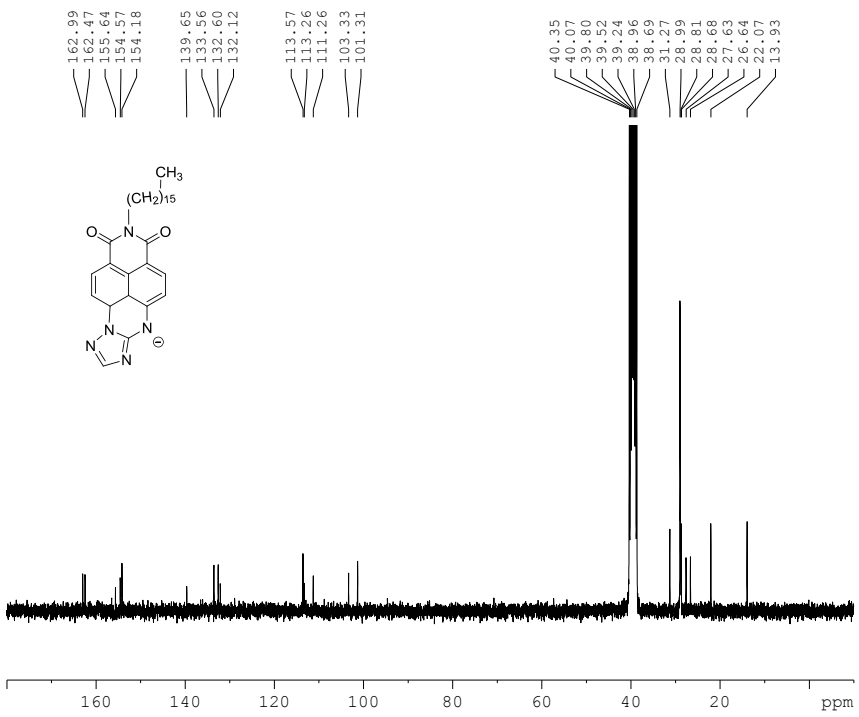



```
Current Data Parameters
NAME      dzy-120112-zj-HNNA-base
EXPNO                  10
PROCNO                  1

F2 - Acquisition Parameters
Date_            20120112
Time                13.03
INSTRUM             spect
PROBHD   5 mm DUL 13C-1
PULPROG              zg30
TD                 65536
SOLVENT              DMSO
NS                     16
DS                      0
SWH              8992.806 Hz
FIDRES          0.137219 Hz
AQ              3.6438515 sec
RG                    362
DW                55.600 usec
DE                 8.00 usec
TE                294.4 K
D1             1.00000000 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                   1H
P1                 10.80 usec
PL1                  3.00 dB
SFO1          300.1324010 MHz

F2 - Processing parameters
SI                   32768
SF            300.1300006 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00
```




```
Current Data Parameters
NAME      dzy-110610-zj-HNNA
EXPNO                     10
PROCNO                     1

F2 - Acquisition Parameters
Date_              20120610
Time                  12.01
INSTRUM               spect
PROBHD   5 mm DUL 13C-1
PULPROG             zgpg30
TD                   65536
SOLVENT                DMSO
NS                     5500
DS                        4
SWH              17985.611 Hz
FIDRES           0.274439 Hz
AQ               1.8219508 sec
RG                  3649.1
DW                  27.800 usec
DE                   6.50 usec
TE                  299.9 K
D1             2.00000000 sec
D11            0.03000000 sec
TD0                       1

======== CHANNEL f1 ========
NUC1                    13C
P1                    12.50 usec
PL1                    2.00 dB
SFO1           75.4752953 MHz

======== CHANNEL f2 ========
CPDPRG2            waltz16
NUC2                    1H
PCPD2               100.00 usec
PL2                    3.00 dB
PL12                  22.74 dB
PL13                  23.00 dB
SFO2          300.1312005 MHz

F2 - Processing parameters
SI                     32768
SF            75.4677843 MHz
WDW                       EM
SSB                        0
LB                    1.00 Hz
GB                         0
PC                    1.40
```