Supplementary information

Enzyme-induced liquid-to-solid phase transition of a mitochondria-targeted AIEgen in cancer theranostics

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1. Materials and methods

The chemicals and solvents were purchased from Sigma-Aldrich, TCI or Spectrochem and used without further purification if not mentioned. TLC plates (Silica gel 60 F254) from Sigma were used for monitoring the reaction. For column chromatography 100-200 mesh silica gel was used as stationary phase. Reverse phase HPLC purification was performed in Agilent and Waters HPLC instrument with C18 semipreparative column. ^{1}H and ^{13}C NMR spectra were recorded in Bruker AV−400 and JEOL-600 MHz spectrometers using tetramethylsilane (TMS) as internal standard. MALDI-TOF and HRMS were acquired from Bruker Autoflex Speed MALDI-TOF spectrometer and Agilent 6538 UHD HRMS/Q-TOF high-resolution spectrometer, respectively. A Bruker IFS 66/V spectrometer was employed to perform Fourier Transform Infrared (FTIR) spectroscopy. Cell media DMEM/DMEM-F12 (Dulbecco's Modified Eagle Media), Glasgow's Minimum Essential Medium (GMEM), RPMI, fetal bovine serum (FBS), penicillin-streptomycin (PS), PBS (phosphate buffered saline) were purchased from Gibco. LysoTracker Blue DND-22 and MitoTracker Green FM were purchased from Invitrogen. MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and NADH, dicoumarol were purchased from Spectrochem. The enzyme Nitroreductase (NTR) from *E. Coli* (#N9284) was obtained from Sigma Aldrich. Details of antibodies used in the study are listed in Table S1. The absorbance spectra were recorded in PerkinElmer LAMBDA 750 and Agilent Cary series UV-Vis/NIR spectrophotometer. The fluorescence spectra were recorded in PerkinElmer FL6500 and Agilent Cary fluorescence spectrophotometer. The absorbance and fluorescence on 96 well plates were measured by Spectramax i3 (Molecular devices) and Tecan Spark Control Magellan plate reader instrument. The live cell imaging was performed in Leica DMi8 fluorescence microscope. Confocal imaging was conducted using an Olympus Fluoview3000 confocal laser scanning microscope, and the processing was carried out with the integrated software of the same system. Atomic force microscopy was executed in Bioscope Resolve (Bruker) and the processing was done by NanoScope Analysis 1.8 software. Transmission electron microscopy (TEM) studies were performed in JEOL, JEM 2100 plus operated at 200 kV and dynamic light scattering (DLS) experiments were executed in Zetasizer instrument and the data was analyzed by Zetasizer software. Data processing and analysis was done using Graphpad Prism 8, Origin 8.5 and ImageJ software.

Table S1. List of antibodies used in the study.

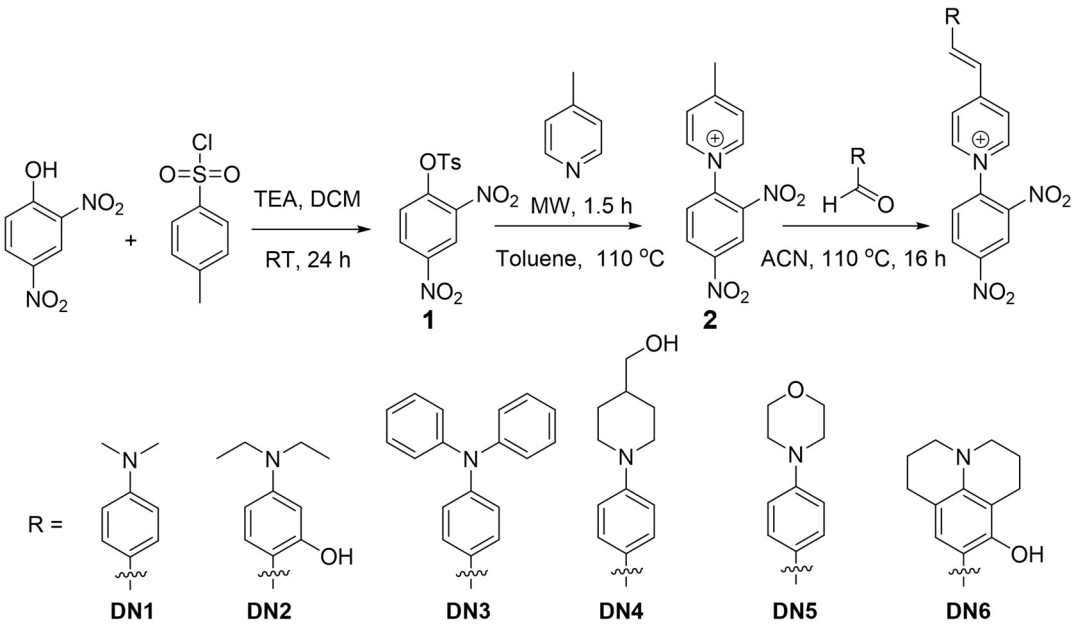
Sl No	Antibody	Application	Dilution	Origin	Vendor	Catalogue
1	Drp1	Immunofluorescence	1:250	Rabbit	Abclonal	#A17069
2	Fis1	Immunofluorescence	1:200	Rabbit	Abclonal	#A5821
3	Nrf2	Immunofluorescence	1:500	Rabbit	Invitrogen	PA5-88084
4	FITC-Annexin V	Confocal imaging	5 µL	-	BD Biosciences	560931
5	Alexa488-conjugated secondary	Immunofluorescence	1:500	Goat	Invitrogen	#A-11034

2. Synthetic procedures

Synthesis of 2,4-dinitrophenyl tosylate (1). To a solution of 2,4-dinitrophenol (1 mmol) in 10 mL dichloromethane, p-toluene sulfonyl chloride (1.1 mmol) and triethylamine (2.5 mmol) was added dropwise and stirred for 12 h at room temperature. The completion of reaction was monitored by thin layer chromatography (TLC). The reaction mixture was extracted from water/DCM mixture and the organic layer was dried over anhydrous sodium sulphate followed by evaporation in vacuo. The crude product was washed with methanol and then filtered. White solid, Yield: 55%, ^{1}H NMR (400 MHz, $CDCl_3$): δ_{ppm} 8.76 (d, J = 2.7 Hz, 1H), 8.49 (dd, J = 9.0, 2.7 Hz, 1H), 7.81 (d, J = 6.7 Hz, 2H), 7.73 (d, J = 9.0 Hz, 1H), 7.40 (d, J = 8.1 Hz, 2H), 2.50 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ_{ppm} 147.3, 146.1, 145.4, 142.6, 131.0, 130.4, 128.8, 128.7, 126.4, 121.7, 22.0. HRMS (ESI, m/z) calcd. for $C_{13}H_{20}N_2O_7S$ $[M+Na]^+$: 361.0101, Found: 361.0090.

Synthesis of N-(2,4-dinitrophenyl)-4-methyl pyridinium tosylate (2). A mixture of 2,4-dinitrophenyl tosylate (1.1 mmol) and 4-methyl pyridine (1mmol) was dissolved in 5 mL toluene. The reaction mixture was refluxed for 1.5 h in microwave reactor at 110 ºC and left to cool to room temperature. The completion of reaction was monitored by thin layer chromatography (TLC). Toluene was evaporated under vacuo and the crude product was washed with diethyl ether. Black tar, Yield: 80%, ^{1}H NMR (400 MHz, DMSO-d_6): δ_{ppm} 9.21 (d, J = 6.8 Hz, 2H), 9.08 (d, J = 2.6 Hz, 1H), 8.92 (dd, J = 8.7, 2.6 Hz, 1H), 8.37 (d, J = 8.7 Hz, 1H), 8.25 (d, J = 6.7 Hz, 2H), 7.43 (d, J = 8.0 Hz, 2H), 7.09 (d, J = 7.9 Hz, 2H), 2.77 (s,

3H), 2.28 (s, 3H). ^{13}C NMR (150 MHz, DMSO-d_6): δ_{ppm} 163.5, 149.5, 146.2, 145.4, 143.7, 139.1, 138.2, 132.5, 130.7, 128.8, 128.6, 126.0, 122.0, 40.6, 22.6, 21.3. HRMS (ESI, m/z) calcd. for $C_{12}H_{10}N_3O_4$ $[M]^+$: 260.0666, Found: 260.0669.



Scheme S1. Synthesis of **DN1** to **DN6**.

General procedure for synthesis of the aldehydes

K_2CO_3 (2 g, 15 mmol,) was weighed in a100 mL round bottom flask and 10 mL of DMF was added. 4-Fluorobenzaldehyde (1.05 mL, 10 mmol) and the corresponding amine (20 mmol) was added sequentially. The flask was transferred to an oil bath at 65 ºC and later the temperature was increased to 80 ºC and was stirred for 16 h. The completion of reaction was monitored by thin layer chromatography (TLC). DMF was evaporated in vacuo and the reaction mixture was extracted from ethyl acetate: water mixture. The organic layer was collected and dried under vaccuo. The crude product (**3** and **4**) was purified using column chromatography (ethyl acetate: hexane).

Synthesis of 4-morpholinobenzaldehyde (3). Off white solid, Yield: 70%, ^{1}H NMR (400 MHz, $CDCl_3$): δ_{ppm} 9.81 (s, 1H), 7.78 (d, J = 9.0 Hz, 2H), 6.92 (d, J = 8.9 Hz, 2H), 3.89 – 3.77 (m, 4H), 3.38 – 3.28 (m, 4H). ^{13}C NMR (150 MHz, $CDCl_3$): δ_{ppm} 190.7, 155.2, 131.9, 127.8, 113.6, 66.6, 47.3. HRMS (ESI, m/z) calcd. for $C_{11}H_{13}NO_2$ $[M]^+$: 191.0946, Found: 191.0946.

Synthesis of 4-(4-(hydroxymethyl)piperidin-1-yl)benzaldehyde (4). Pale yellow solid, Yield: 60%, ^{1}H NMR (400 MHz, $CDCl_3$) δ_{ppm} 9.75 (s, 1H), 7.77 – 7.70 (m, 2H), 6.94 – 6.85

(m, 2H), 4.03-3.92 (m,2H), 3.54 (d, J = 6.3 Hz, 2H), 2.99-2.88 (m, 2H), 1.92 – 1.81 (m, 2H), 1.42 – 1.28 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_{ppm} 190.4, 155.0, 132.0, 126.4, 113.5, 67.4, 47.4, 4.58, 28.3. HRMS (ESI, m/z) calcd. for $C_{13}H_{17}NO_2$ $[M]^+$: 219.1259, Found: 219.1257.

General procedure for synthesis of the DN compounds (DN1 to DN6)

To a suspension of N-(2,4-dinitrophenyl)-4-methyl pyridinium tosylate salt (1 mmol) in acetonitrile, the corresponding aromatic aldehyde (4 mmol) was added. The reaction mixture was stirred at 110 ºC under reflux and completion of reaction was monitored by TLC. Following completion of reaction, the solvent was evaporated in vacuo. The crude product was purified by reverse phase chromatography (C18 column, grad. 60-85% acetonitrile: water).

DN1: Violet solid, Yield: 30%, ^{1}H NMR (400 MHz, DMSO-d_6): δ_{ppm} 9.11 (d, J = 2.5 Hz, 1H), 8.96 – 8.91 (m, 3H), 8.37 (d, J = 8.7 Hz, 1H), 8.23 (d, J = 1.7 Hz, 1H), 8.25 – 8.15 (m, 2H), 7.71 – 7.65 (m, 2H), 7.31 (d, J = 15.9 Hz, 1H), 6.87 – 6.81 (m, 2H), 3.07 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ_{ppm} 156.8, 153.1, 149.2, 146.1, 144.1, 143.8, 139.3, 132.5, 131.7, 130.6, 122.9, 122.1, 122.0, 117.2, 112.6, 40.7. HRMS (ESI-TOF) m/z: $[M]^+$ Calcd. for $C_{21}H_{19}N_4O_4^+$: 391.1401, Found: 391.1401.

DN2: Blue solid, Yield: 30%, ^{1}H NMR (400 MHz, DMSO-d_6) δ_{ppm} 10.54 (s, 1H), 9.04 (d, J = 2.5 Hz, 1H), 8.86 (dd, J = 8.7, 2.6 Hz, 1H), 8.71 (d, J = 7.1 Hz, 2H), 8.30 (d, J = 8.7 Hz, 1H), 8.17 (d, J = 15.7 Hz, 1H), 8.03 (d, J = 7.2 Hz, 2H), 7.51 (d, J = 9.2 Hz, 1H), 7.22 (d, J = 15.6 Hz, 1H), 6.35 (dd, J = 9.1, 2.6 Hz, 1H), 6.20 (d, J = 2.6 Hz, 1H), 3.37 (q, J = 7.0 Hz, 4H), 1.11 (t, J = 7.0 Hz, 6H). ^{13}C NMR (150 MHz, MeOD) δ_{ppm} 157.7, 153.3, 149.1, 143.9, 142.8, 142.0, 139.2, 131.9, 131.3, 129.5, 128.5, 125.6, 121.9, 120.3, 111.8, 105.8, 96.7, 44.5, 11.7. HRMS (ESI-TOF) m/z: $[M]^+$ Calcd. for $C_{23}H_{23}N_4O_5^+$: 435.1663, Found: 435.1650.

DN3: Deep pink solid, Yield: 50%, ^{1}H NMR (400 MHz, DMSO-d_6) δ_{ppm} 9.12 (d, J = 2.5 Hz, 1H), 9.10 – 9.06 (m, 2H), 8.95 (dd, J = 8.7, 2.6 Hz, 1H), 8.41 – 8.33 (m, 3H), 8.20 (d, J = 15.5 Hz, 1H), 7.73 – 7.67 (m, 2H), 7.47 – 7.38 (m, 5H), 7.24 – 7.14 (m, 6H), 6.99 – 6.94 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ_{ppm} 156.0, 150.3, 148.8, 145.9, 144.5, 143.9, 143.3, 138.7, 132.0, 130.4, 130.1, 129.9, 127.5, 125.8, 124.9, 122.6, 121.5, 120.1, 120.0. HRMS (ESI-TOF) m/z: $[M]^+$ Calcd. for $C_{31}H_{23}N_4O_4^+$: 515.1714, Found: 515.1708.

DN4: Violet solid, Yield: 25%, ^{1}H NMR (400 MHz, DMSO-d_6) δ_{ppm} 9.11 (d, J = 2.6 Hz, 1H), 9.00 – 8.91 (m, 2H), 8.37 (d, J = 8.7 Hz, 1H), 8.25 (d, J = 7.2 Hz, 2H), 8.16 (d, J = 15.9 Hz, 1H), 7.67 (d, J = 9.0 Hz, 2H), 7.47 (d, J = 8.0 Hz, 1H), 7.34 (d, J = 15.9 Hz, 1H), 7.08 (m, 2H),

4.01 (d, J = 13.2 Hz, 2H), 3.29 (d, J = 6.1 Hz, 2H), 2.89 (t, J = 12.7 Hz, 2H), 2.29 (s, 1H), 1.83 – 1.71 (m, 2H), 1.64 (d, J = 10.9 Hz, 1H), 1.20 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ_{ppm} 156.3, 152.8, 148.7, 145.04, 143.9, 138.8, 132.0, 131.2, 130.1, 128.0, 125.5, 123.5, 121.8, 117.5, 114.1, 65.6, 46.9, 38.3, 28.0. HRMS (ESI-TOF) m/z: [M]+ Calcd. for $C_{25}H_{25}N_4O_5^+$: 461.1819, Found: 461.1812.

DN5: Red solid, Yield: 25%, ^{1}H NMR (400 MHz, DMSO-d_6) δ_{ppm} 9.11 (d, J = 2.5 Hz, 1H), 9.06 – 9.01 (m, 2H), 8.95 (dd, J = 8.7, 2.6 Hz, 1H), 8.39 (d, J = 8.8 Hz, 1H), 8.33 – 8.28 (m, 2H), 8.19 (d, J = 16.1 Hz, 1H), 7.75 – 7.68 (m, 2H), 7.42 (d, J = 16.1 Hz, 1H), 7.12 – 7.05 (m, 2H), 3.75 (t, J = 5.0 Hz, 4H), 3.38 – 3.29 (m, 4H). ^{13}C NMR (150 MHz, DMSO-d_6) δ_{ppm} 156.9, 153.6, 149.4, 145.3, 144.8, 143.9, 139.3, 132.6, 131.3, 130.6, 125.4, 122.7, 122.1, 119.0, 114.7, 66.40, 47.4. HRMS (ESI-TOF) m/z: $[M]^+$ Calcd. for $C_{23}H_{21}N_4O_5^+$: 433.1506, Found: 433.1491.

DN6: Blue solid, Yield: 30%, ^{1}H NMR (400 MHz, MeOD) δ_{ppm} 9.06 (d, J = 2.6 Hz, 1H), 8.70 (dd, J = 8.7, 2.6 Hz, 1H), 8.32 – 8.18 (m, 3H), 8.04 (d, J = 8.7 Hz, 1H), 7.68 (d, J = 7.3 Hz, 2H), 7.18 (s, 1H), 6.92 (d, J = 15.4 Hz, 1H), 3.26 (m, 4H), 2.62 (t, J = 6.3 Hz, 2H), 2.55 (t, J = 6.5 Hz, 2H), 1.87 (q, J = 5.9 Hz, 4H). ^{13}C NMR (151 MHz, MeOD) δ_{ppm} 156.7, 156.0, 149.3, 143.9, 142.3, 141.3, 139.4, 131.2, 129.5, 121.9, 119.6, 116.5, 113.2, 112.6, 50.2, 49.0, 31.9, 27.01, 21.5, 20.7, 20.6. HRMS (ESI-TOF) m/z: $[M]^+$ Calcd. for $C_{25}H_{23}N_4O_5^+$: 459.1663, Found: 459.1651.

DN6R: Dark pink solid, ^{1}H NMR (400 MHz, DMSO-d_6) δ_{ppm} 9.04 (s, 1H), 8.49 (d, J = 7.1 Hz, 2H), 8.20 (dd, J = 15.8, 6.5 Hz, 1H), 7.89 (d, J = 7.2 Hz, 2H), 7.25 (s, 1H), 7.09 (d, J = 15.5 Hz, 1H), 6.98 (d, J = 8.4 Hz, 1H), 6.19 (s, 1H), 6.14 – 5.99 (m, 1H), 3.24 (m, 4H), 2.66 (t, J = 6.4 Hz, 2H), 2.61 (t, J = 6.4 Hz, 2H), 1.86 (q, J = 5.6 Hz, 4H). ^{13}C NMR (150 MHz, DMSO-d_6) δ_{ppm} 154.6, 147.4, 145.2, 143.5, 138.4, 127.8, 125.5, 122.0, 115.2, 115.1, 111.3, 107.6, 50.0, 49.4, 49.1, 27.4, 22.0, 21.5, 21.1. HRMS (ESI-TOF) m/z: $[M]^+$ Calcd. for $C_{25}H_{27}N_4O^+$: 399.2179, Found: 399.2186.

3. UV-Vis absorption spectroscopy

The compound stock solution of 10 mM was prepared in DMSO and diluted in 2 mL PBS (10 mM, pH = 7.4) for recording the spectra. All UV-Vis absorption spectra were taken in 10 mm path length quartz cuvette and baseline corrected with respect to the corresponding solvent. The absorption spectra of all the compounds in fixed concentration (10 μM) in PBS (10 mM, pH = 7.4) were recorded.

4. Fluorescence spectroscopy

All fluorescence spectra were taken in quartz photoluminescence cuvette with excitation and emissions slit width 10 nm and scan rate 200 nm/min. The fluorescence spectra of the probes at fixed concentration (10 μM) in 2 mL PBS (10 mM, pH = 7.4) were obtained with excitation wavelength at their corresponding absorbance maxima (λ_{ex} for **DN1**, **2**, **3**, **4**, **5** and **6** are 525, 558, 511, 461, 448 and 515 nm respectively).

5. Chemical reduction of dinitro compounds

The reduction of the synthesized dinitro-compounds by sodium dithionite ($Na_2S_2O_4$) was studied by UV-Vis absorption and fluorescence spectroscopy. In a quartz photoluminescence cuvette 2 mL MQ water was taken and baseline was corrected. After that 1 μL of compound solution from 10 mM stock was added to make the final concentration 5 μM and absorbance and fluorescence spectra were taken. Sodium dithionite was added to make the final concentration 0.5 mM, incubated for 2 min and absorption and fluorescence spectra were measured (λ_{ex} for reduced **DN1**, **2**, **3**, **4**, **5** and **6** are 478, 507, 475, 425, 422 and 537 nm respectively).

6. Viscosity dependent fluorescence studies

The fluorescence spectra of the compounds were taken at 10 μM concentration in mixture of glycerol and PBS (10 mM, pH = 7.4) in different glycerol percentages (0%, 10%, 20%, 50%, and 70%). $Na_2S_2O_4$ was added to make its final concentration 1 mM and fluorescence spectra (λ_{ex} at corresponding absorbance maxima) were taken after 2 min incubation.

7. pH dependent absorbance and fluorescence studies

"The absorbance and fluorescence spectra of the compounds at a fixed concentration of 10 μM were recorded at various pH conditions (3, 4, 5, 6, 7, 8, and 9) in PBS (10 mM). $Na_2S_2O_4$ was

then added to compound solution to achieve a final concentration of 1 mM. Subsequently, the absorbance and fluorescence spectra (λ_{ex} at corresponding absorbance maxima) of the reduced compounds were recorded after a 2 min incubation period.

8. Nitroreductase enzyme assay

To check the effect of NTR on the synthesized probes, fluorescence assay in PBS (10 mM, pH=7.4) was performed in 96 black well plate (50 μL each well) and the end point data was taken in plate reader according to the excitation and emission maxima of each probe and their reduced form (chemical reduction). The concentration of the enzyme, NADH and compound used was 2 μM, 0.5 mM and 10 μM respectively and the reaction mixture was incubated for 2 h. The concentration dependent effect of the enzyme on the probes **DN1**, **DN2**, **DN5** and **DN6** was also checked with enzyme concentrations 10 nM to 2 μM. Limit of detection (LOD) value was calculated by the formula

$$\text{LOD} = 3.3 \times \text{standard deviation } (F\text{-}F_0)/\text{Slope}$$

where, F is fluorescence intensity with a particular enzyme concentration and F_0 is intensity without adding enzyme and slope corresponds to $(F\text{-}F_0)$ vs. concentration plot.

To perform the enzyme kinetic studies, time dependent emission of the probes (**DN1**, **2**, **5**, **6** in 10 μM concentration) on addition of NTR in different concentrations (500 nM and 2 μM) and NADH (0.5 mM) was recorded until saturation in plate reader at their respective emission maxima in reduced form (λ_{em} for reduced **DN1**, **2**, **3**, **4**, **5** and **6** are 617, 608, 593, 610, 610 and 628 nm respectively).

9. Analytical HPLC study

DN6 (100 μM) was reduced by NTR (5 μM) and NADH (2.5 mM) in PBS (10 mM, pH = 7.4) for 2 h. The reaction mixture (100 μL) was extracted with ethyl acetate (300 μL) for 1 min and the organic layer was subjected to analytical HPLC study to get the extracted ion chromatogram (XIC) for the unreduced ($[M]^+$ = 459.1649) and reduced **DN6** ($[M]^+$ =399.2170).

10. Dicoumarol inhibition

DN6 (10 μM) was reduced by NTR (2 μM) and NADH 90.5 mM), preincubated (for 15 min) with dicoumarol (50, 100 and 200 μM) for 2 h in PBS (10 mM, pH = 7.4) and the endpoint

emission was recorded using plate reader in 96 black well plate (50 µL each well) according to the excitation and emission maxima of their reduced form.

11. Selectivity experiments

DN molecules (10 µM) were incubated with NTR (2 µM) and NADH (0.5 mM), KCl, NaCl, $CaCl_2$, $FeSO_4$, $CuCl_2$ (100 mM) glucose (10 mM), ascorbate (10 mM), tyrosine, arginine, glycine, glutamic acid, cysteine, glutathione (10 mM), H_2O_2 (10 mM), DNA (3 µg/µL), bovine serum albumin (BSA) (10 mM) for 2 h in PBS (10 mM, pH = 7.4) and the endpoint emission was recorded using plate reader in 96 black well plate (50 µL each well) according to the excitation and emission maxima of their reduced form.

12. Enzyme responsive aggregation of DN6 (DN6R) to nanoaggregates

Time dependent absorbance study

To study the aggregation profile of **DN6** in PBS (10 mM, pH = 7.4), the UV-Vis absorbance of **DN6** (20 µM) at 575 nm was monitored with time in PBS (bad solvent) and at 615 nm in 80% MeOH in PBS (good solvent) in a 10 mm quartz absorbance cuvette in UV-Vis spectrophotometer. Further time dependent spectra of **DN6** (20 µM) in PBS and 80% MeOH in PBS were recorded for 3h. UV-Vis absorbance of **DN6** (20 µM) treated with $Na_2S_2O_4$ (2 mM) (chemical reduction) in PBS (at 537 nm) and 80% MeOH in PBS (at 551 nm) was monitored with time until saturation. Corresponding time dependent spectra were also recorded.

Temperature dependent absorbance after reduction

To confirm the effect of aggregation on absorbance properties of **DN6** (20 µM) after reduction by $Na_2S_2O_4$ (2 mM) in PBS (10 mM, pH = 7.4) for 3 h, absorbance spectra of the reduced species with increasing temperature (30, 35, 40, 45, 50, 55, 60, 65, 70, 72, 77, 80, 82.5, 85, 90, 92.5 ºC) was recorded in UV-Vis spectrophotometer with water-Peltier system.

Time dependent fluorescence spectroscopy

DN6 (20 µM) was treated with 2 mM of $Na_2S_2O_4$ and emission intensity at 610 nm (λ_{ex} = 537 nm) in 2 mL PBS (10 mM, pH = 7.4) and at 590 nm (λ_{ex} = 537 nm) in 2 mL 80% MeOH in PBS was recorded with time in fluorescence spectrophotometer.

13. Imaging experiments

Confocal imaging for studying enzyme responsive aggregation of DN6 (DN6R) to nanoaggregates

DN6 (20 μM) was reduced with $Na_2S_2O_4$ (2 mM) in filtered PBS (10 mM, pH =7.4). Enzymatic reduction was performed by incubating **DN6** (20 μM) with NTR (20 μM) and NADH (0.5 mM) in PBS at 37 ºC. The unreduced and reduced samples at different time points were drop-casted (6 μL) on clean glass slides, mounted with a round coverslip (18 mm), and immediately imaged in Olympus FLUOVIEW 3000 microscope with λ_{ex} = 561 nm for unreduced sample and λ_{ex} = 514 nm for the reduced samples with emission at 600-700 nm.

Fluorescence recovery after photobleaching (FRAP)

Enzymatic reduction was performed by incubating **DN6** (20 μM) with NTR (20 μM) and NADH (0.5 mM) in PBS (10 mM, pH =7.4) at 37 ºC for 15 min, 2 h and 4 h. The reduced samples (20 μL) were drop-casted on a confocal dish. For FRAP, the bleaching was carried out with 100% laser for 186 ms, with the fluorescent recovery monitored over 20 s. The recovery time was derived from a single exponential fit of the emission intensities plot using cell Sens Dimension software. The experiments were performed in triplicates.

Transmission electron microscopy for studying enzyme responsive aggregation of DN6 (DN6R) to nanoaggregates

DN6 (20 μM) was reduced with NTR (20 μM) and NADH (0.5 mM) in PBS (10 mM, pH =7.4) at 37 ºC with different incubation times. The reduced samples (5 μL) were placed on carbon-coated copper grids (200 mesh size), kept for 15 min, washed with filtered MiliQ water for two times, stained with uranyl acetate (0.5%), finally washed twice with filtered MiliQ water and dried in a desiccator under nitrogen purging. Samples were imaged in TEM to visualize the aggregates.

Atomic force microscopy for studying enzyme responsive aggregation of DN6 (DN6R) to nanoaggregates

Enzymatic reduction was performed by incubating **DN6** (20 μM) with NTR (20 μM) and NADH (0.5 mM) in PBS (10 mM, pH =7.4) at 37 ºC with different incubation times. 30 μL of each sample was drop-casted on freshly cleaved mica sheets (Agar Scientific). The samples were allowed to settle down onto surface for 20 min and the buffer was removed with blotting paper. The mica sheet was washed with 300 μL of filtered (0.22 μm) MiliQ water and dried at

37 ºC for 4 h. The samples were imaged in PeakForce Quantitative Nanomechanical Mapping (PFQNM) mode using Scanasyst-AIR probe with 5 nm tip radius, spring constant 0.4 N/m in Bioscope Resolve AFM (Bruker). The images were processed and analyzed using NanoScope analysis 1.9 software to calculate the mechanical stiffness (DMT modulus) and adhesion following the Derjaguin, Muller, and Toporov (DMT) adhesion theory. Multiple micrographs have been considered with ~20 datapoints from different region of the image for the analysis.

Effect of NaCl, hexanediol, DMF and THF on DN6R aggregation

DN6 (20 μM) was reduced by NTR (2 μM) and NADH (0.5 mM) for 2 h followed by addition of NaCl (100 mM), hexanediol (20%), DMF (20%), THF (20%) and incubated for 30 min and end point fluorescence reading was taken in plate reader. To see the concentration dependent effect of DMF, reduced **DN6** was treated with 5, 10, 20, 30 and 40% DMF in PBS (10 mM, pH =7.4) and fluorescence intensity was recorded after 30 min. Reduced **DN6** with and without the presence of 20% DMF and 20% THF in PBS (10 mM, pH = 7.4) was subjected to confocal imaging to visualize the formation of LLPS.

14. Enzyme encapsulated liposome formation

The enzyme mediated reduction and self-aggregation of **DN6** was studied in a minimalistic enzyme encapsulated liposome model. The liposomes were prepared using DPPC and cholesterol in 70:30 ratio.[1] The lipids were dissolved in chloroform (2 mM stock). The lipid solution was taken in a 5 mL RB and evaporated under reduced pressure to make a dehydrated lipid film. The RB was kept in desiccator overnight to remove the trace of $CHCl_3$. The lipid layer was rehydrated with 500 μL of NTR (5 μM) and NADH (0.5 mM) solution and vortexed for 15 min. The solution was frozen in liq. N_2 and thawed at 37 ºC. The unbound enzyme and NADH was removed by centrifugation (3000 rpm for 30 min at 25 ºC). Similarly, NADH (0.5 mM) and only PBS (10 mM, pH =7.4) encapsulated liposomes were prepared.

Confocal imaging

The liposomes were characterized by confocal imaging (DIC and fluorescence mode). **DN6** (20 μM) was incubated with the liposome solutions containing NTR-NADH and only NADH for 6 h. The sample solutions were drop-casted (6 μL) on clean glass slides, mounted with a coverslip, and immediately imaged in Olympus FLUOVIEW 3000 microscope with excitation at 561 nm for unreduced sample (only NADH containing liposomes) and 514 nm for the

reduced samples (NTR and NADH encapsulated vesicles with **DN6**) with emission in the range 600-700 nm.

Dynamic light scattering

The size of the liposomes containing NTR-NADH was measured by dynamic light scattering experiment (3 readings per sample) in disposable plastic cuvette (1 mL solution) using Zetasizer instrument. **DN6** (20 μM) was incubated with NTR-NADH encapsulated liposome solution at 37 ºC for 6 h. The solution was further subjected to DLS measurement.

TEM imaging

DN6 (20 μM) was incubated with NTR-NADH encapsulated liposome solution at 37 ºC for 6 h. The incubated solution and the only NADH encapsulated liposome solution (5 μL) were dropcasted on carbon-coated copper grids (200 mesh size), kept for 15 min, washed with filtered MiliQ water for two times, stained with uranyl acetate (0.5 %), finally washed twice with filtered MiliQ water and dried in a desiccator under nitrogen purging. Samples were imaged in TEM to visualize the liposomes and aggregates inside liposomes.

UV-Visible absorbance spectroscopy

UV-Visible absorbance of NADH at 340 nm was monitored for 6 h for NTR-NADH encapsulated liposomes treated with **DN6** (20 μM) in PBS (10 mM, pH =7.4) in three replicates using platereader. NTR-NADH encapsulated liposomes, NADH, NADH-NTR in PBS were taken as the control.

15. General cell culture

MCF-7 cells were cultured in DMEM media with 10% FBS and 1% PS and were incubated in humidified incubator with 5% CO_2 at 37 °C temperature. The cells were cultured in T25 flask and trypsinized at 80-85% confluency and seeded in 35 mm dishes or 96 well plates for further experiment. RAW, MDA-MB, HEK 293T AND L929 cells were cultured in DMEM media, SHSY-5Y cells in DMEM-F12 media and HCT-116 cells were cultured in RPMI media following the same procedure. BHK-21 cells were cultured in Glasgow's Minimum Essential Medium (GMEM) with peptone supplement**.**

16. Live cell imaging

Colocalization experiments

MCF-7 cells were seeded in 35 mm glass bottom confocal dishes and cultured at 37 °C with 5% CO_2. After 24 h, cells were washed with dPBS (10 mM, pH =7.4) and LysoTracker Blue (500 nM) in DMEM media with 2.5% FBS and 1% PS was added. Cells were incubated for 1 h at 37 °C followed by washing with dPBS. Then cells treated with MitoTracker Green FM (250 nM) and 2.5 μM of **DN6** in the above-mentioned media and incubated for 1 h. The cells were again washed with dPBS twice and fresh media was added to image the cells in live cell set up (Leica DMi8 system). The images were acquired in DAPI channel (λ_{ex}: 359 nm and λ_{em}: 461 nm) for Lysotracker Blue, FITC channel (λ_{ex}: 495 nm and λ_{em}: 519 nm) for MitoTracker Green FM and Rho channel (λ_{ex}: 553 nm and λ_{em}: 627 nm) for the probes and Y5 channel (λ_{ex}: 620 nm and λ_{em}: 660 nm) for **DN6** colocalization experiments. The images were deconvoluted by using Huygens essential software and quantified using Image J software.

In cellulo fluorescence spectra by confocal microscopy

MCF-7 cells were seeded onto 35 mm glass bottom confocal dishes and cultured for 24 h at 37 °C with 5% CO_2 in humidified condition. Cells were treated with 5 μM concentration of **DN6** in DMEM, 2.5% FBS and 1% PS media. After 1 h and 24 h of incubation, the cells were washed with Dulbecco's phosphate buffered saline (dPBS) to remove probe outside the cells and immediately imaged in Olympus FLUOVIEW 3000 microscope with excitation at 514 nm and emission in the range 590-750 nm.

Hypoxia responsive imaging

HEK293T cells were seeded onto 35 mm glass bottom confocal dishes and cultured for 6 h at 37 °C with 5% CO_2 in humidified condition. The cells were then supplied with different volume of DMEM, 2.5% FBS and 1% PS media (0.5, 1, 2, 3, 4 mL) to create hypoxic environment by changing the media height.[2] 24h the cells were treated with **DN6** (5 μM) for 1 h, washed with dPBS and imaged with confocal microscope (λ_{ex} = 514 nm and λ_{em} = 600-700 nm). The fluorescence intensity of the images was quantified by Image J to show the hypoxia responsiveness of **DN6**.

Cellular FRAP assay

MCF-7 cells were seeded onto 35 mm glass bottom confocal dishes and cultured for 24 h at 37 °C with 5% CO_2 in humidified condition. Cells were treated with 5 μM concentration of **DN6**

in DMEM, 2.5% FBS and 1% PS media. After 6 h and 24 h incubation, the cells were subjected to confocal imaging (λ_{ex} = 514 nm and λ_{em} = 600-700 nm). For FRAP, the bleaching was carried out with 100% laser for 186 ms, with the fluorescent recovery monitored over 10 s. The recovery time was derived from a single exponential fit of the emission intensities plot using cell Sens Dimension software. The experiments were performed in triplicates.

Inhibition of NTR in cells

MCF-7 cells were seeded onto 35 mm glass bottom confocal dishes and cultured for 24 h at 37 °C with 5% CO_2 in humidified condition. Cells were incubated with different concentrations of dicoumarol (50, 100, 200 μM) for 4 h followed by treatment with **DN6** (5 μM) in DMEM, 2.5% FBS and 1% PS media for 6 h. Cells were washed with PBS and subjected to confocal imaging. The **DN6** fluorescence in cells and number of droplets was quantified using ImageJ software.

Mitochondrial morphology change

MCF-7 cells were seeded onto 35 mm glass bottom confocal dishes and cultured for 24 h at 37 °C with 5% CO_2 in humidified condition. Cells were treated with 5 and 10 μM concentration of **DN6** in DMEM, 2.5% FBS and 1% PS media. After 18 h incubation, the cells were washed with Dulbecco's phosphate buffered saline (dPBS), incubated with MitoTracker Green FM (250 nM) in the above-mentioned media and incubated for 1 h. The cells were washed with dPBS and immediately imaged in Olympus FLUOVIEW 3000 microscope with λ_{ex} = 488 nm for MitoTracker Green and λ_{ex} = 561 nm for **DN6**.

17. Cell viability assay

To evaluate cell viability of **DN** compounds, MTT assay was performed in different cancer (MCF-7, HCT-116, RAW 264.7 and MDA-MB-231) and normal cell lines (HEK293T, L929, and BHK-21). Cells were cultured in T25 flask and then seeded in a 96 well plate. After 24 h the cells were treated with different concentrations of compounds and kept in incubator for 24 h. For rescue study, cells were treated with 5 μM of **DN6** and dicoumarol (5, 10, 20, 30 μM) and kept in incubator for 12 h. After that, the cells were treated with MTT solution (5 mg/mL). After 3 h the media containing MTT was removed without disturbing the formazan crystals and mixture of DMSO/methanol (1:1) was given to solubilize the crystals. The absorbance at 570 nm was recorded in plate reader instrument and percentage viability was calculated taking the control cells as 100%.

18. Characterization of intracellular reduction by mass spectrometry

MCF-7 cells were seeded onto 35 mm petri dishes and cultured for 24 h at 37 °C with 5% CO_2 in humidified condition. Cells were treated with 5 μM concentration of **DN6** and with DMSO vehicle in control in DMEM, 2.5% FBS and 1% PS media. After 24 h incubation, the cells were washed with Dulbecco's phosphate buffered saline (dPBS), trypsinized and centrifuged at 2500g for 7 min to pellet down the cells. The cells were lysed with RIPA lysis buffer and the lysate was extracted with ethyl acetate. The organic layer was subjected to MALDI-TOF mass spectrometry.

19. Live-dead staining of cells

MCF-7 cells were seeded in confocal dishes, at 60% confluency treated with **DN6** (5, 10 and 20 μM) for 18 h. After washing once with PBS (10 mM, pH = 7.4), the cells were incubated with Calcein AM (500 nM) for 30 min, washed once with PBS (10 mM, pH = 7.4) and imaged using confocal microscopy in FITC channel.

20. Mitochondrial membrane potential (MMP) measurement

MCF-7 cells were cultured in T25 flask in DMEM, 10% FBS and 1% PS media and then seeded in a 12 well plate. After 24 h the cells were treated with different concentrations of **DN6** (5, 10 and 20 μM) in three replicates. After 18 h the cells were washed with dPBS and treated with 50 nM Rhodamine123 in clear DMEM media for 30 min. The cells were washed with dPBS, trypsinized and centrifuged at 2500 xg for 6 min to pellet down the cells. After washing with dPBS two times the cells were suspended in dPBS and were subjected to flow cytometric analysis in FITC channel.

21. Cellular ROS measurement by DCFDA assay

MCF-7 cells were cultured in T25 flask in DMEM, 10% FBS and 1% PS media and then seeded in a 12 well plate. After 24 h the cells were treated with different concentrations of compound (5, 10 and 20 μM) in three replicates. After 18 h the cells were washed with dPBS and treated with 2 μM DCFDA clear DMEM media for 30 min. The cells were washed with dPBS, trypsinized and centrifuged at 2500 xg for 6 min to pellet down the cells. After washing with dPBS two times the cells were suspended in dPBS and were subjected to flow cytometric analysis in FITC channel.

22. Immunofluorescence

MCF-7 cells were seeded in confocal dishes, at 60% confluency treated with **DN6** (5, 10 and 20 μM) for 18 h and subjected to immunofluorescence. Cells were washed thrice with PBS (10 mM, pH =7.4) and fixed with 4% paraformaldehyde for 15 min. Cells were washed thrice with PBS and subjected to permeabilization with permeabilization buffer (PBS with 0.1% Triton-X) for 10 min. After washing the cells twice with PBS, blocking solution (10% goat serum) was treated for 30 min. Then the cells were incubated overnight with 1º antibody (rabbit Nrf2/Drp1/Fis1) of appropriate dilution at 4 ºC with gentle shaking. After recovering the 1º antibody, cells were washed thrice with PBS and treated with Alexa-488 conjugated anti-rabbit 2º antibody for 1 h at room temperature in dark followed by washing with PBS twice and counterstain with DAPI (1 μM) for 5 min. Cells were imaged by confocal microscopy and the fluorescence intensity was quantified using ImageJ software.

23. Apoptosis assay

MCF-7 cells were seeded in confocal dishes, at 60% confluency treated with **DN6** (5, 10 and 20 μM) for 18 h. After washing once with PBS, the cells were incubated with FITC-Annexin-V in 1X binding buffer (according to the protocol given in the kit) for 30 min, washed once with PBS and imaged using confocal microscopy and the fluorescence in the FITC channel was quantified by ImageJ software.

24. Spheroid culture[3]

96-well plates were coated with hot agarose solution (0.15% in PBS), kept for cooling down at room temperature and stored at 4 ºC in dark for further use. MCF-7 cells were seeded in the agarose coated 96-well plates, centrifuged (1000 xg for 10 min) using a plate centrifuge, and kept in incubator at 37 ºC and 5% CO_2. After 3 days, spheroids were treated with **DN6** (5, 10, 20 μM) and control was treated with vehicle DMSO. The wells containing spheroids were imaged using LeicaMICA confocal microscope (in Alexa Fluor 561 channel) up to 16 days. The area of the spheroid was quantified with ImageJ to show the inhibition of growth.

25. Results

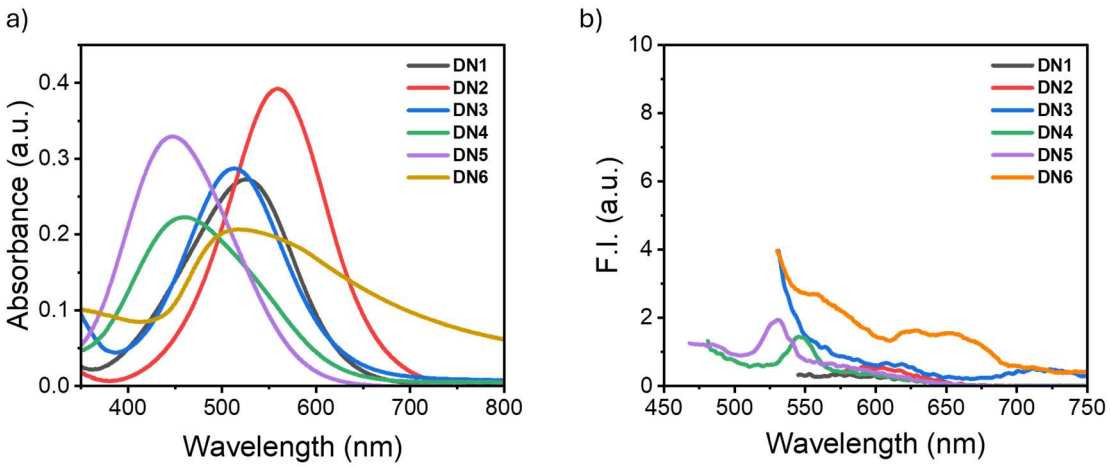


Fig. S1 a) Absorbance and b) emission spectra of **DN** compounds (10 μM) in PBS (10 mM, pH = 7.4) (F.I.: Fluorescence Intensity) (λ_{ex} for **DN1**, **2**, **3**, **4**, **5** and **6** are 525, 558, 511, 461, 448 and 515 nm respectively).

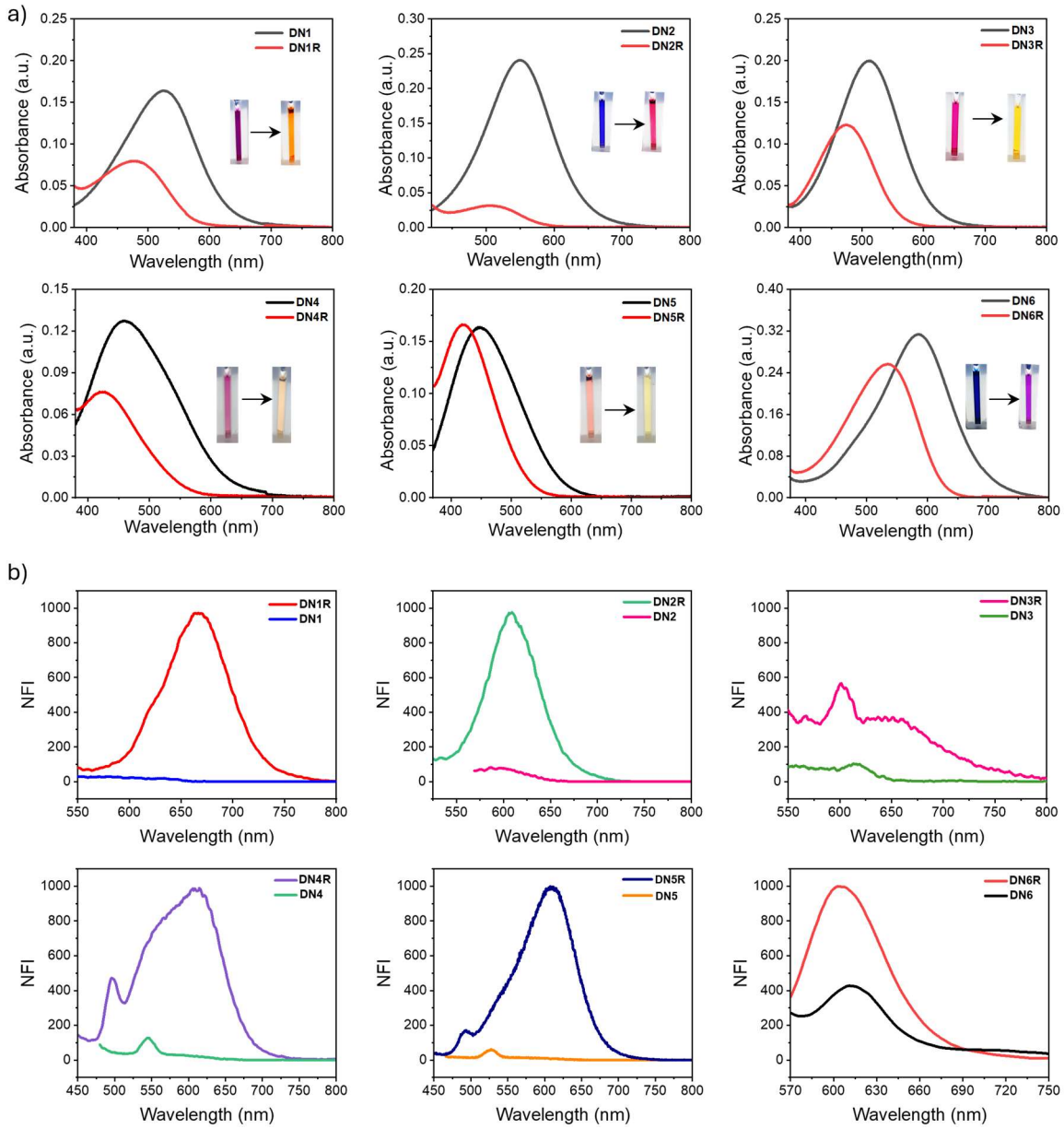


Fig. S2 a) Absorbance (inset – colorimetric change of **DN** compounds upon reduction) and b) fluorescence spectra of **DN** molecules (5 μM) before and after reduction by $Na_2S_2O_4$ (0.5 mM) in MQ water (λ_{ex} for reduced **DN1**, **2**, **3**, **4**, **5** and **6** are 478, 507, 475, 425, 422 and 537 nm respectively).

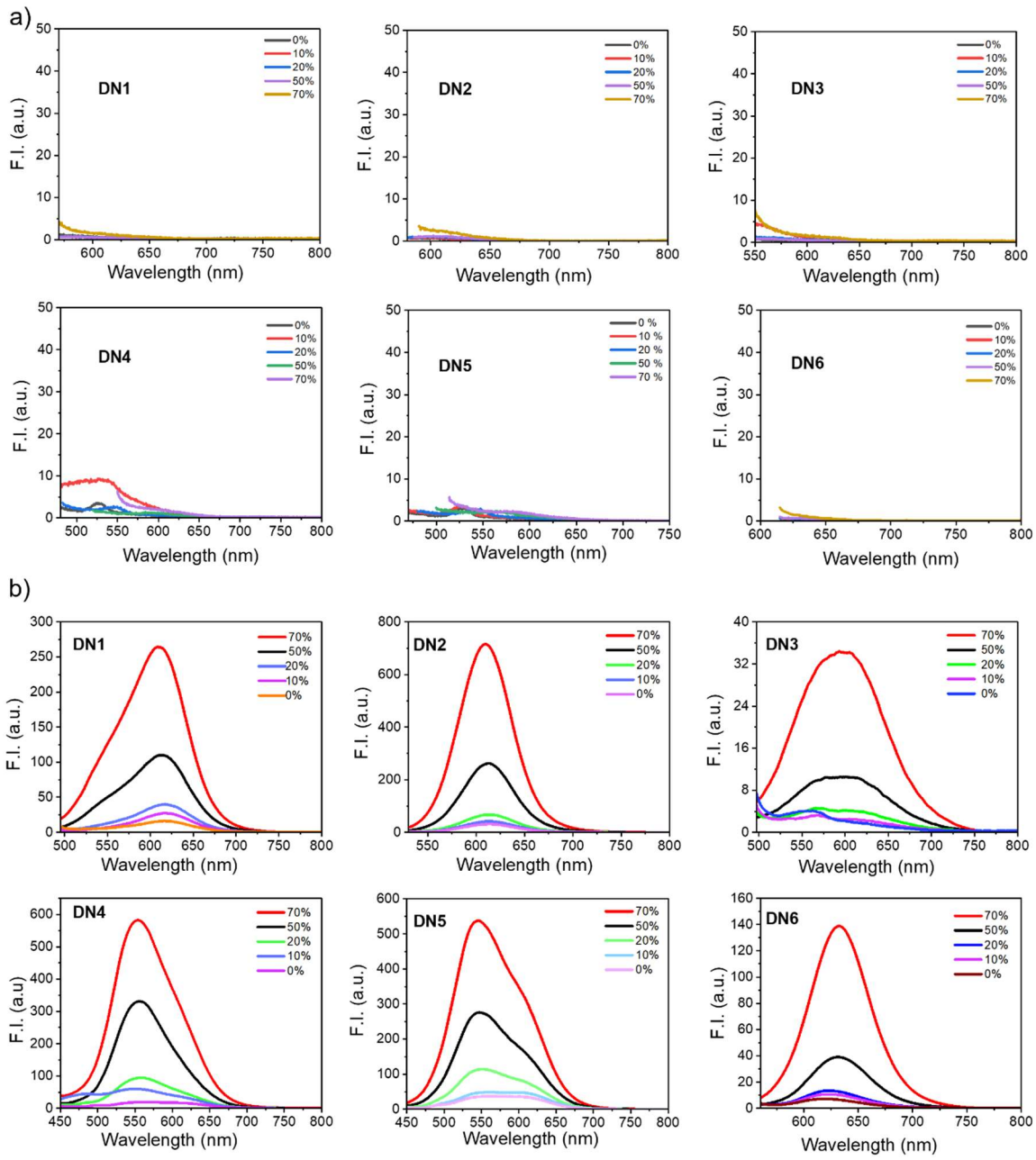


Fig. S3 Viscosity dependent fluorescence of the **DN** compounds (10 μM) in different percentages of glycerol (0, 10, 20, 50, 70%) in PBS (10 mM, pH =7.4) a) before and b) after reduction by $Na_2S_2O_4$ (1 mM) (λ_{ex} for reduced **DN1**, **2**, **3**, **4**, **5** and **6** are 477, 510, 477, 425, 420 and 538 nm respectively).

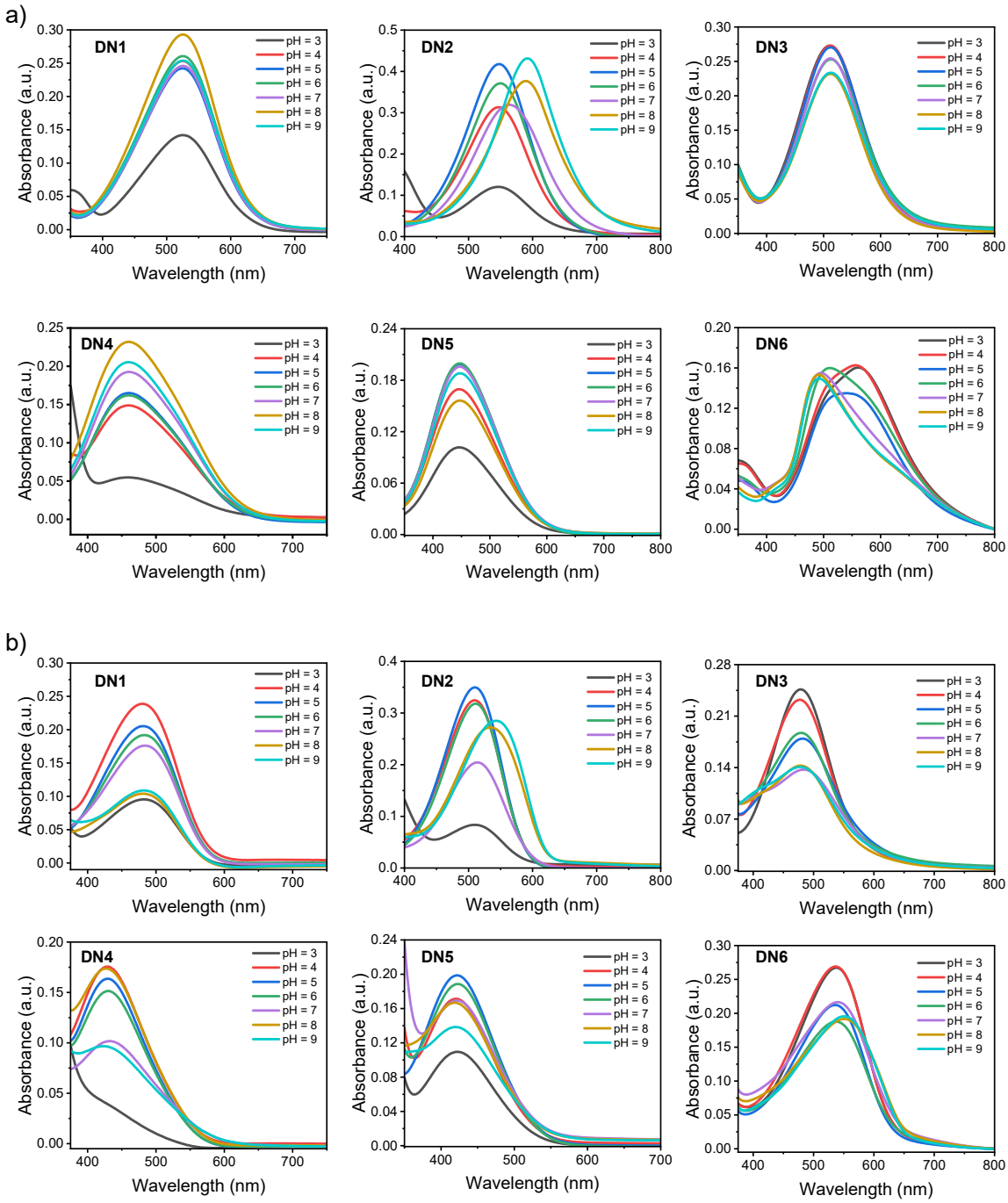


Fig. S4 pH dependent absorbance spectra of the **DN** compounds (10 μM) in different pH (3, 4, 5, 6, 7, 8 and 9) in PBS (10 mM) a) before and b) after reduction by $Na_2S_2O_4$ (1 mM).

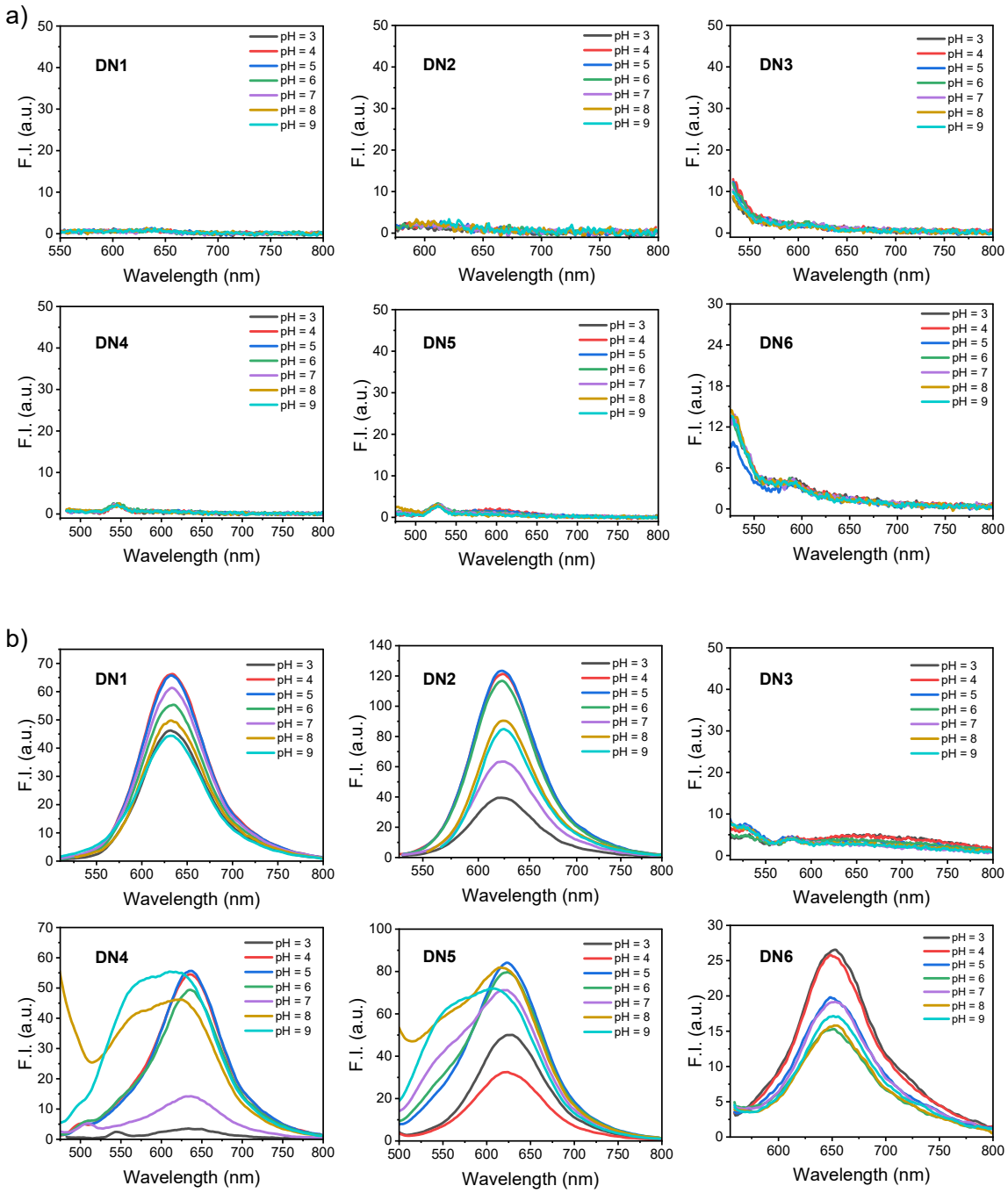


Fig. S5 pH dependent fluorescence of the **DN** compounds (10 μM) in different pH (3, 4, 5, 6, 7, 8 and 9) in PBS (10 mM) a) before and b) after reduction by $Na_2S_2O_4$ (1 mM).

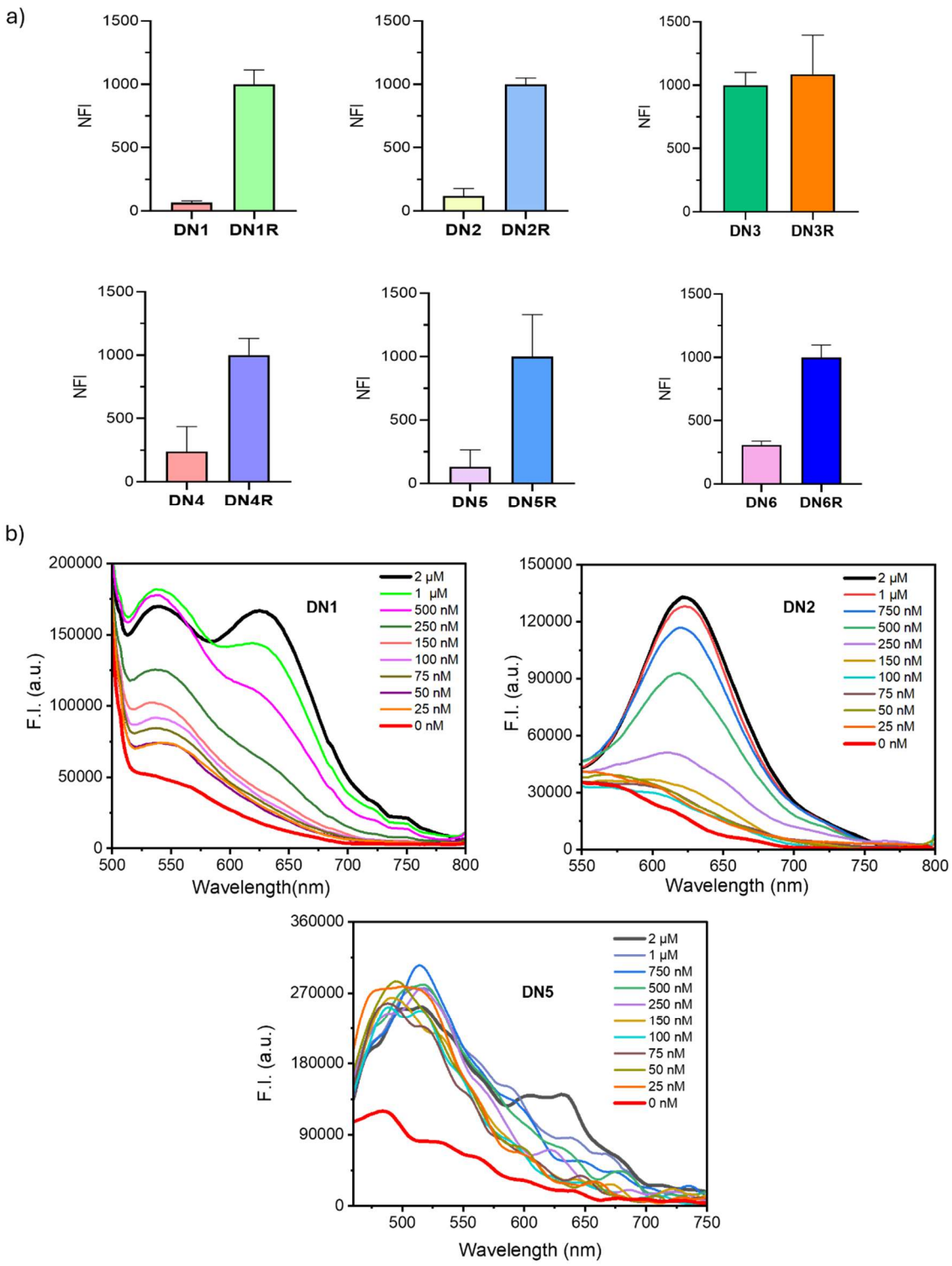


Fig. S6 a) Fluorescence response of **DN1**, **2**, **3**, **4**, **5** and **6** (10 μM) to NTR (2 μM) and NADH (0.5 mM) in PBS (λ_{ex} for reduced **DN1**, **2**, **3**, **4**, **5** and **6** are 478, 507, 475, 425, 422 and 538 nm respectively). b) Concentration dependent emission spectra of **DN1**, **2** and **5** (10 μM) with NTR (0-2 μM) and NADH (0.5 mM).

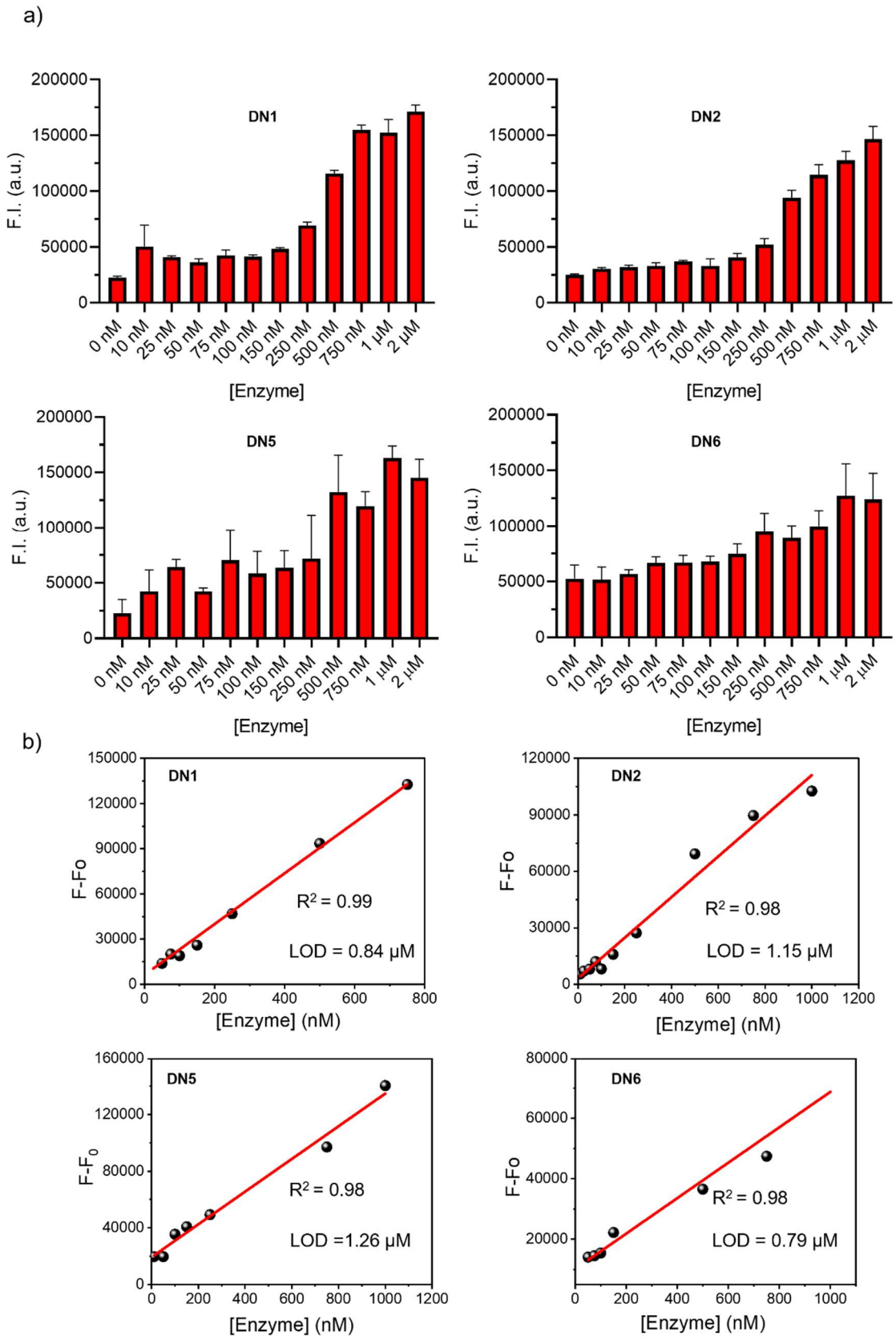


Fig. S7 a) Concentration dependent emission spectra of **DN1**, **2**, **5** and **6** (10 μM) with NTR (0-2 μM) and NADH (0.5 mM) in PBS (10 mM, pH = 7.4) (λ_{ex} for reduced **DN1**, **2**, **5** and **6** are 478, 507, 422

and 537 nm respectively). b) Determination of limit of detection (LOD) values of **DN1**, **2**, **5** and **6** for detection of NTR from the data in Fig. S5a.

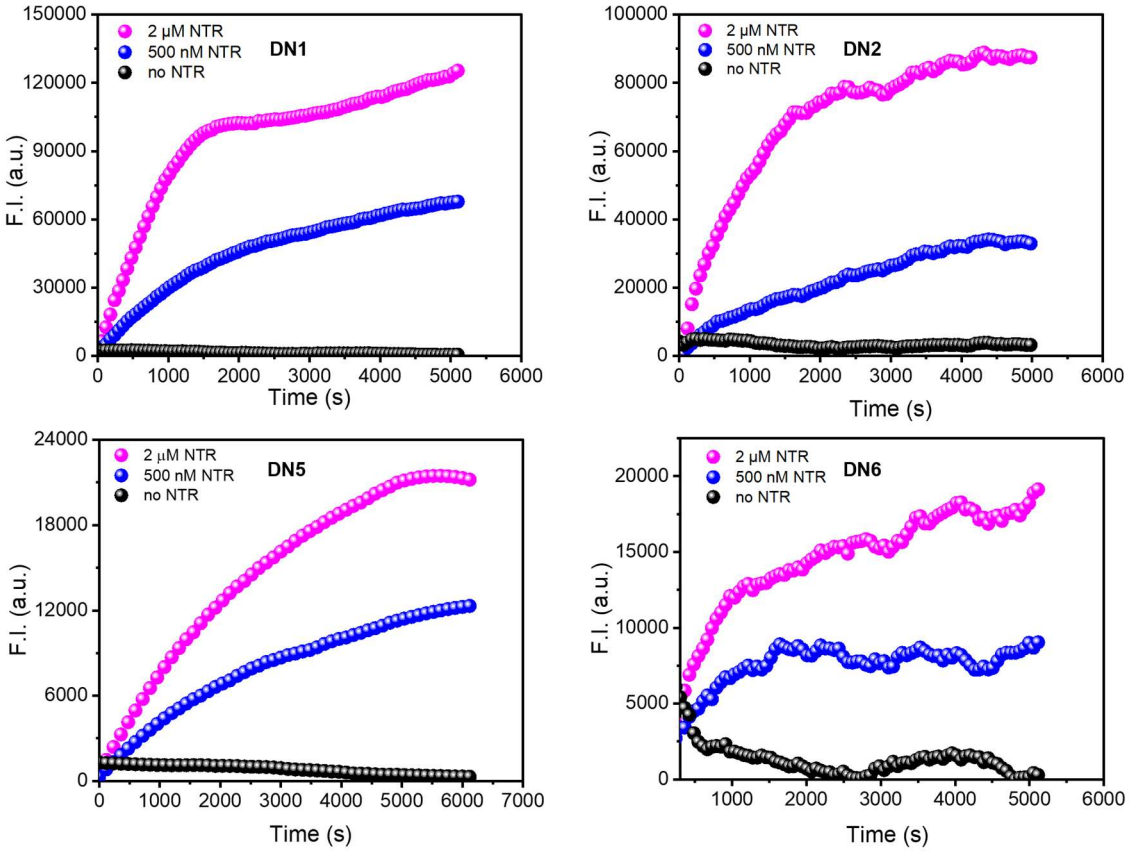


Fig. S8 Emission kinetics (λ_{ex} for reduced **DN1**, **2**, **5** and **6** are 477, 509, 422 and 537 nm respectively and λ_{em} are 617, 608, 610 and 632 nm respectively) of **DN1**, **2**, **5** and **6** (10 μM) by NTR (500 nM and 2 μM) and NADH (0.5 mM) in PBS (10 mM, pH =7.4).

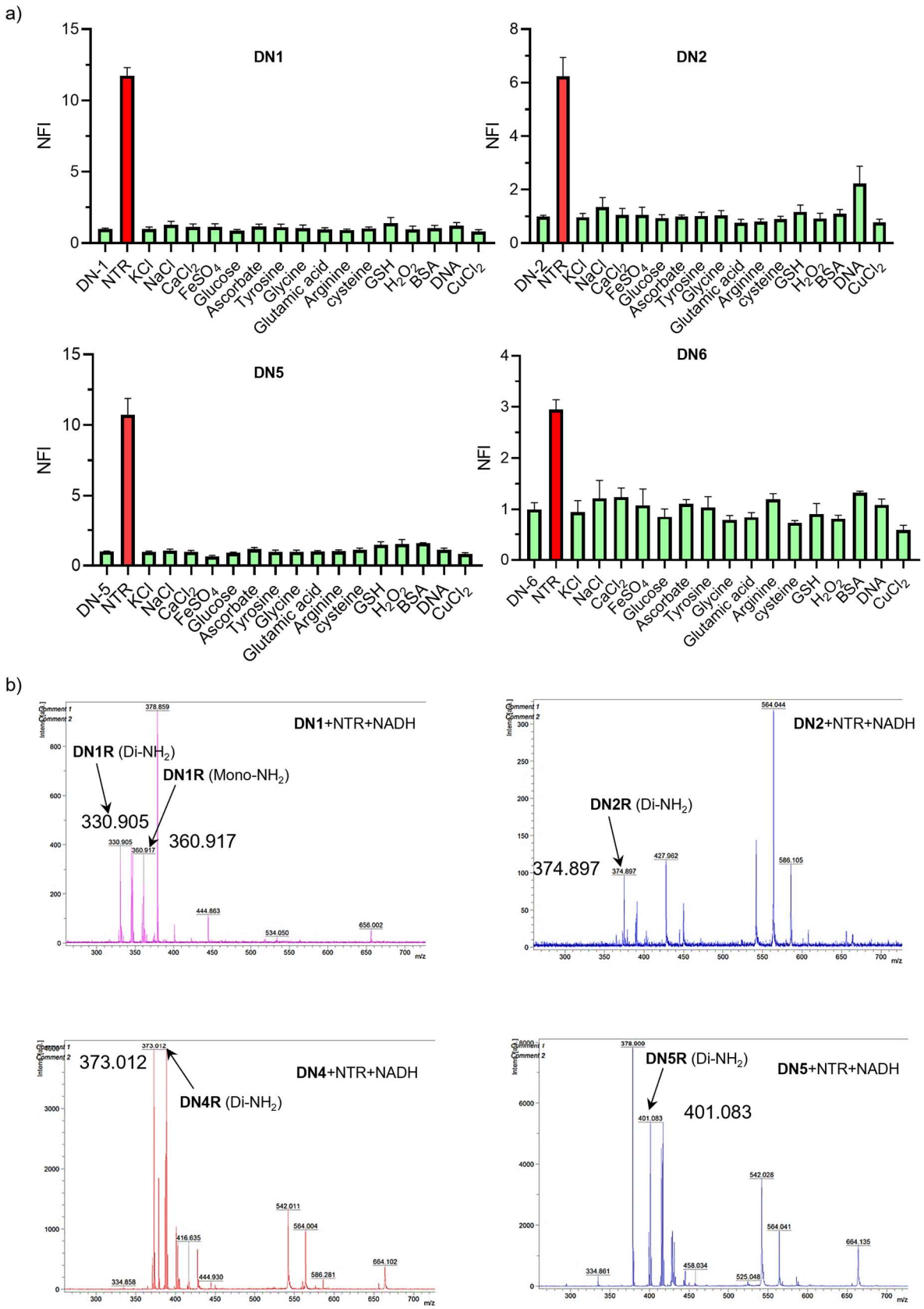


Fig. S9 a) Selectivity of **DN1**, **2**, **5** and **6** (10 μM) to NTR (2 μM)/NADH (0.5 mM). over other biologically active species KCl, NaCl, $CaCl_2$, $FeSO_4$, $CuCl_2$ (100 mM) glucose (10 mM), ascorbate (10

mM), tyrosine, arginine, glycine, glutamic acid, cysteine, glutathione (10 mM), H_2O_2 (10 mM), DNA (3 μg/μL), bovine serum albumin (BSA) (10 mM). b) MALDI-TOF mass spectrometry characterization of the reduced product of **DN1**, **2**, **4** and **5** (10 μM) after reduction by NTR (2 μM)/NADH (0.5 mM).

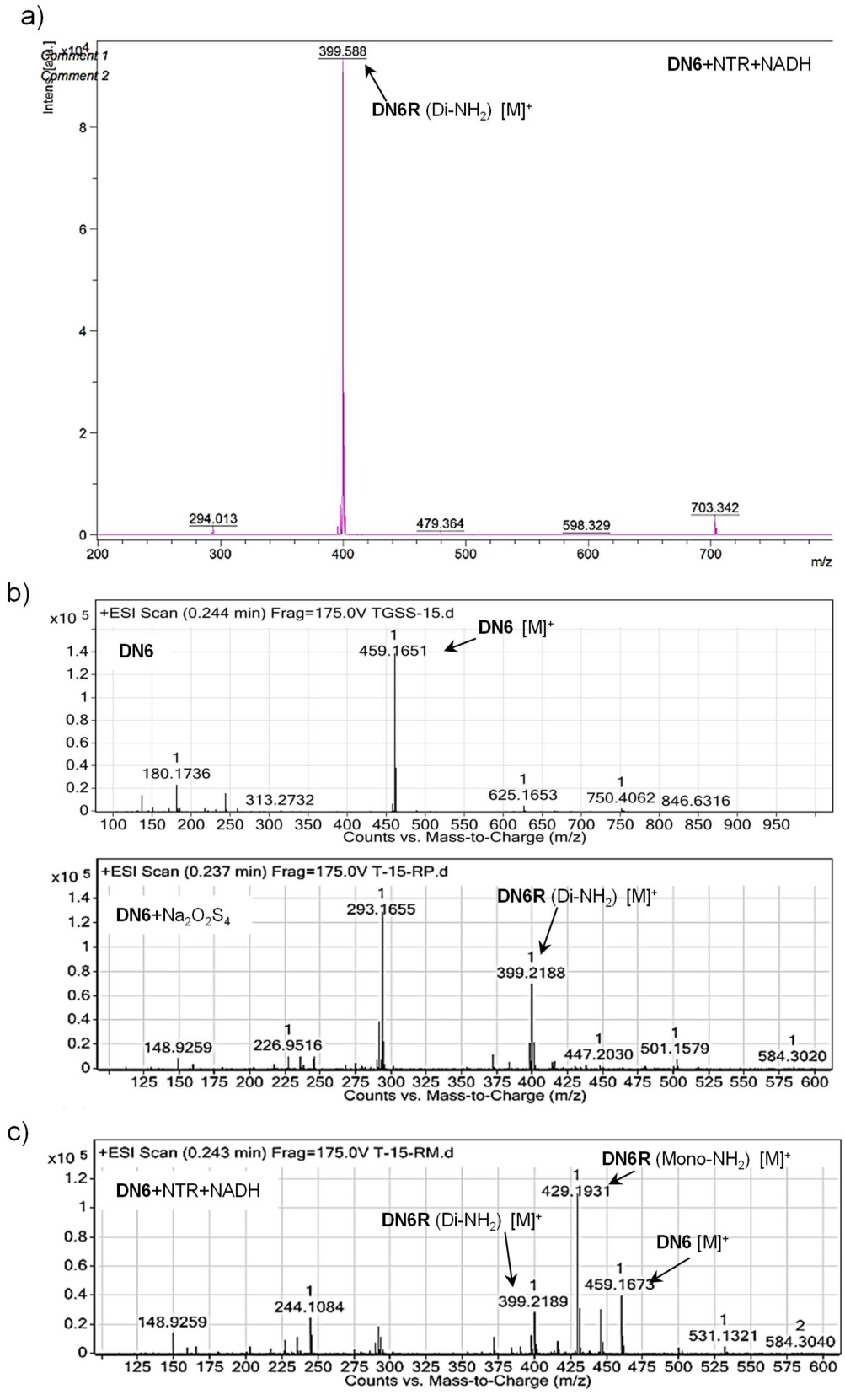


Fig. S10 a) MALDI-TOF mass spectrometry characterization of the di-reduced product of **DN6** (Mass = 399.588 for $[M]^+$) after reduction by NTR (2 μM)/NADH (0.5 mM). b) High resolution mass spectrometry characterization of **DN6** (Mass = 459.1651 for $[M]^+$) and the di-reduced product of **DN6** (10 μM) (Mass = 399.2188 for $[M]^+$) after reduction by $Na_2S_2O_4$ (0.5 mM). c) High resolution mass spectrometry characterization of monoreduced (Mass = 429.1931 for $[M]^+$) and the di-reduced (Mass = 399.2189 for $[M]^+$) product of **DN6** (10 μM) after reduction by NTR (2 μM)/NADH (0.5 mM).

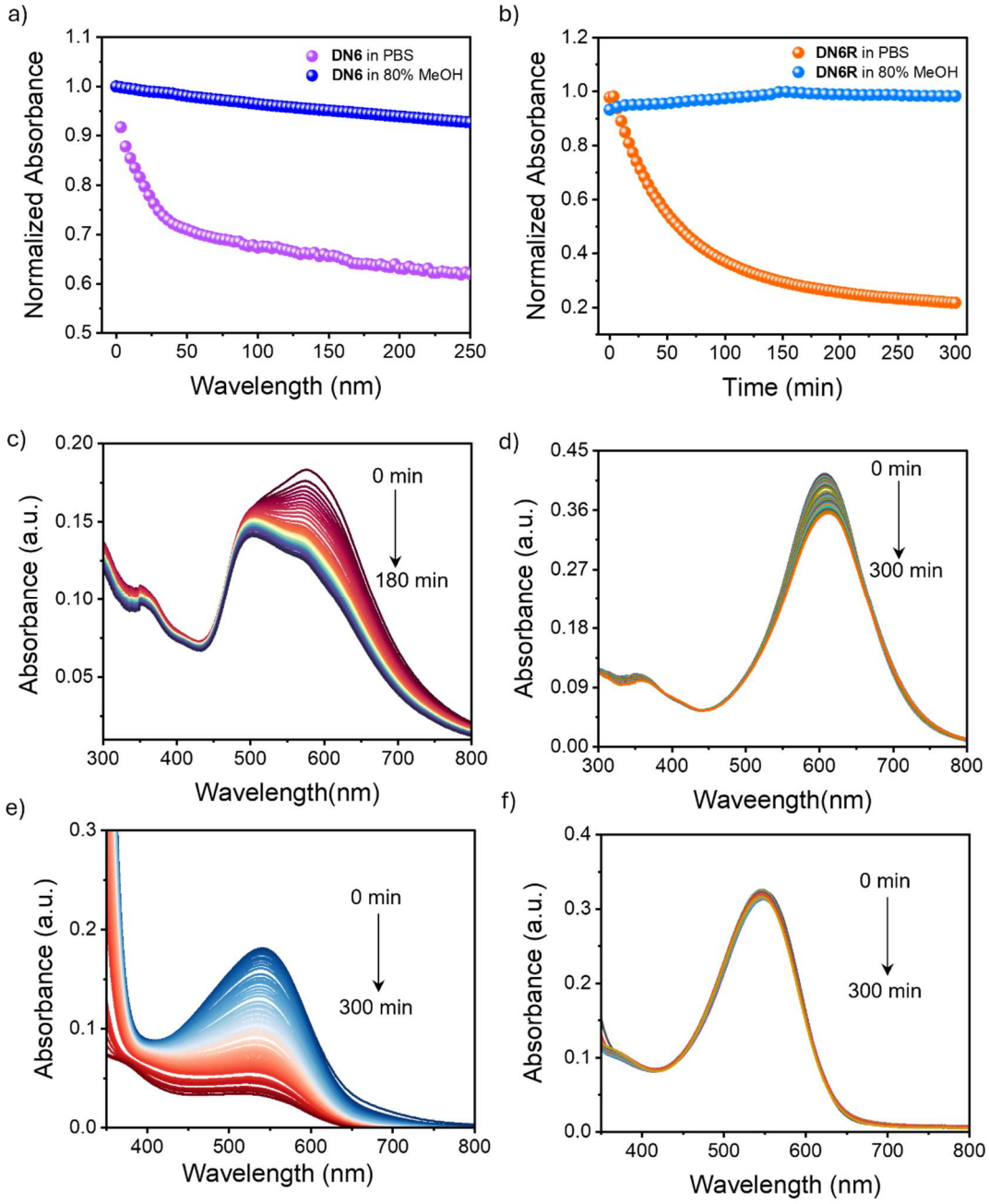


Fig. S11 a) Time dependent change in absorbance of **DN6** (20 μM) in PBS (10 mM, pH =7.4) and 80% methanol in PBS. b) Time dependent change in absorbance of **DN6** (20 μM) after reduction by $Na_2S_2O_4$

(0.5 mM) in PBS and 80% methanol in PBS. c) Time dependent absorbance spectra of **DN6** (20 μM) in PBS and d) in 80% methanol in PBS. e) Time dependent absorbance spectra of **DN6** (20 μM) after reduction by $Na_2S_2O_4$ (0.5 mM) in PBS and f) 80% methanol in PBS.

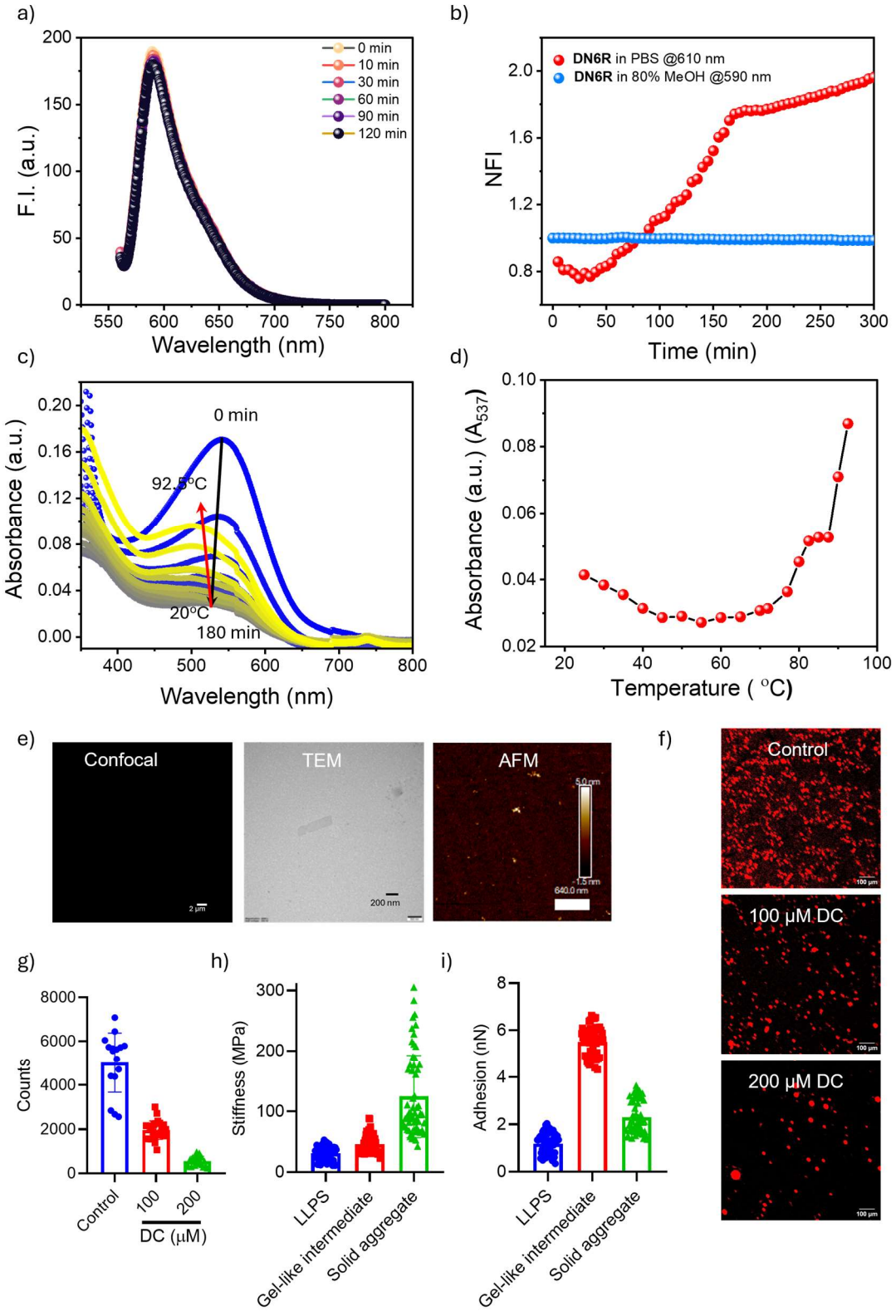


Fig. S12 a) Time dependent fluorescence spectra (λ_{ex} = 537 nm) of **DN6** (20 µM) after reduction by $Na_2S_2O_4$ (0.5 mM) in 80% methanol in PBS. b) Time dependent change in emission intensity of **DN6** (20 µM) after reduction by $Na_2S_2O_4$ (0.5 mM) in PBS (λ_{ex} = 537 nm, λ_{em} = 628 nm) and 80% methanol in PBS (λ_{ex} = 551 nm, λ_{em} = 590 nm). c) Temperature dependent absorbance spectra of reduced **DN6** (20 µM) after 3 h of aggregation in PBS. d) Temperature dependent increase in absorbance of reduced **DN6** (20 µM) aggregates. e) Confocal, TEM and AFM images for unreduced **DN6** (20 µM) in PBS. f) Confocal imaging showing concentration-dependent inhibition of NTR-driven LLPS formation of **DN6** (20 µM) by DC and g) its quantification. Time dependent h) mechanical stiffness (LLPS: 30.92, gel-like intermediate: 45.78, solid aggregate: 124.388 MPa and i) adhesion (LLPS: 1.17, gel-like intermediate: 5.50, solid aggregate: 2.30 nN) of **DN6** (20 µM) in PBS in the presence of NTR and NADH, determined from PeakForce QNM-AFM measurements.

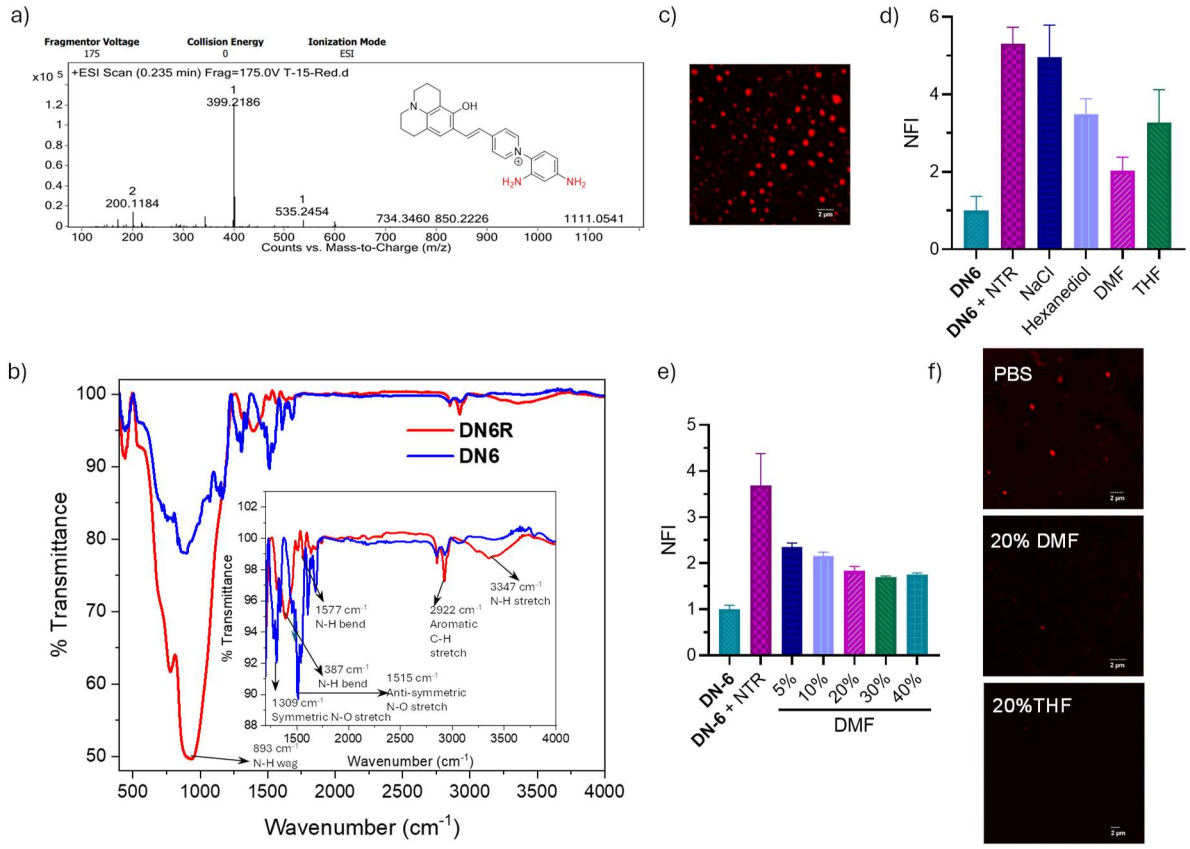


Fig. S13 a) HRMS spectra of reduced **DN6** purified by HPLC. b) Infrared spectroscopy for the characterization of **DN6** and **DN6R**. c) Confocal images of formation of LLPS by reduced **DN6**. d) Emission of **DN6** in presence of NaCl (100 µM), hexanediol (20%), DMF (20%) and THF (20%) post reduction by NTR. e) Decrement in emission of **DN6** by DMF in a concentration dependent manner after reduction by NTR. f) Inhibition in formation of LLPS by reduced **DN6** in presence of DMF and THF revealed by confocal imaging.

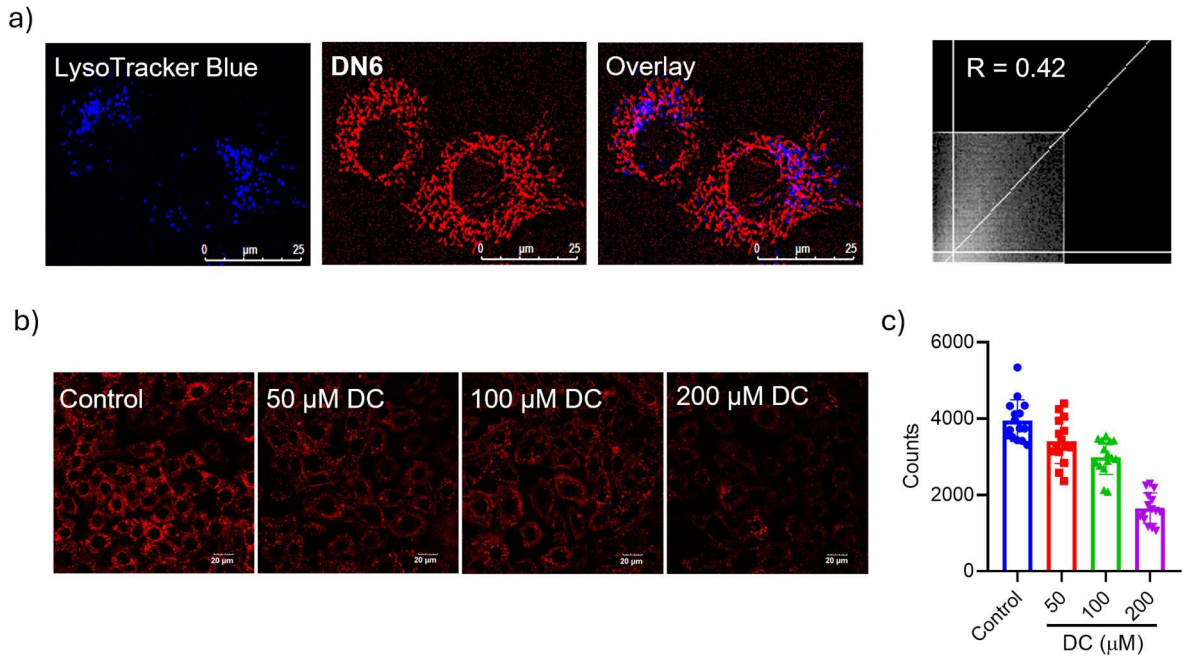


Fig. S14 a) Colocalization of **DN6** (2.5 μM) with LysoTracker Blue (500 nM). Pearson's colocalization coefficient, R = 0.42. b) Inhibition of cellular reduction of **DN6** (5 μM) by NTR inhibitor DC and c) quantification of number of droplets in cells.

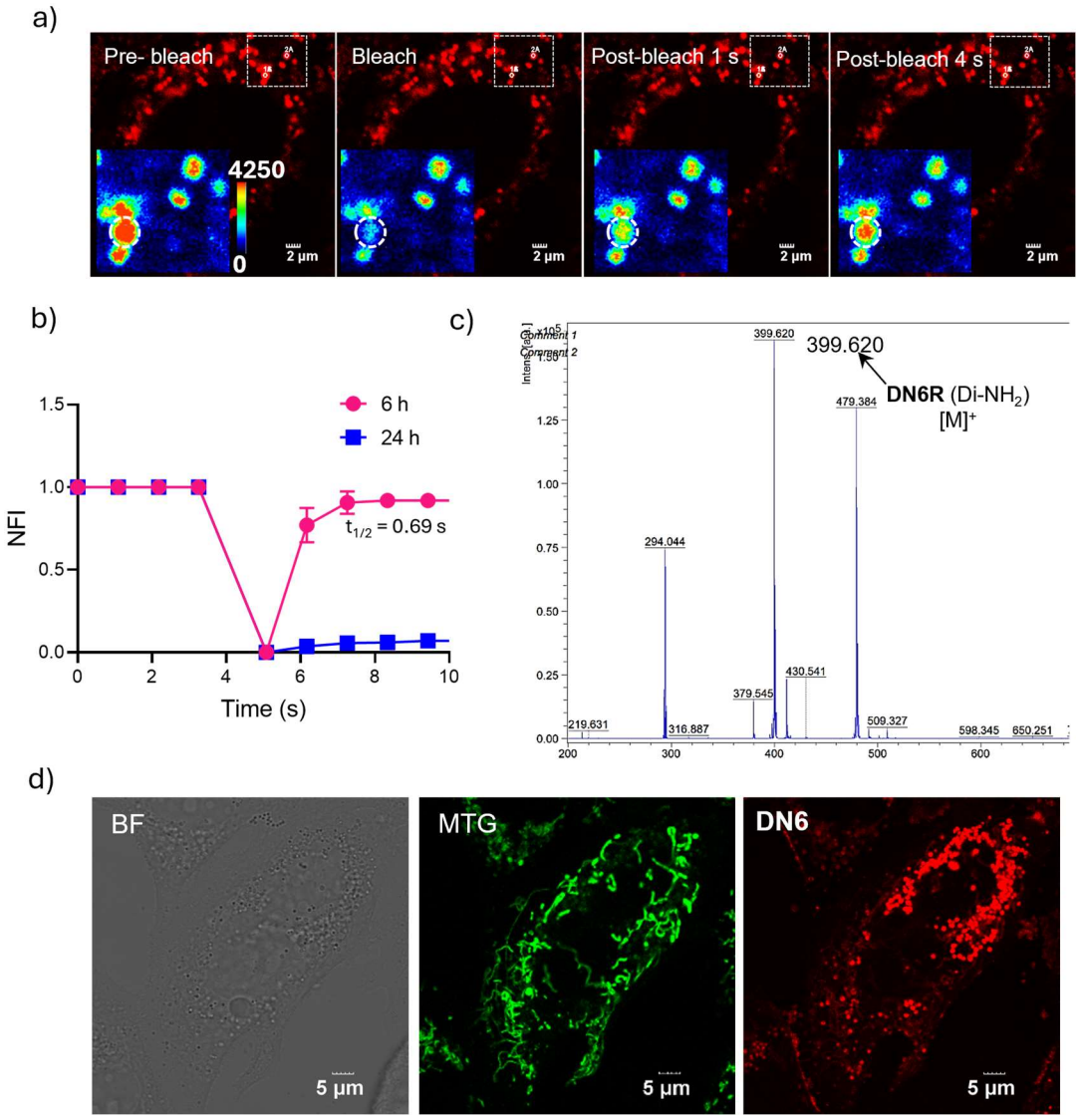
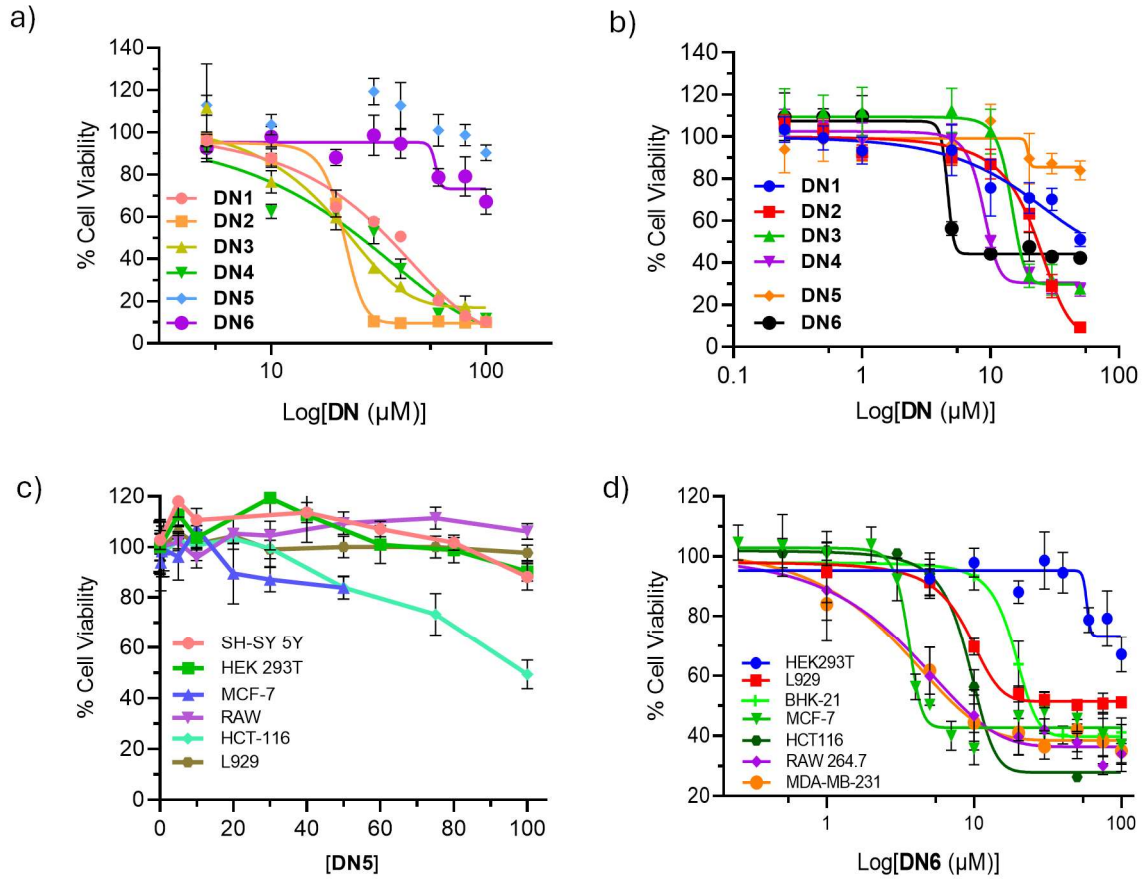


Fig. S15 a) FRAP study of droplets formed in MCF-7 cells after 6 h treatment of **DN6** and contour diagram (inset) (b) Frap recovery of the species formed by **DN6** in cells at 6 h and at 24 h. c) MALDI-TOF spectra of cell lysate after **DN6** treatment. d) Confocal images of intracellular fluorescent aggregates formed after 24h treatment with **DN6** (10 µM).



e)

Cell line	IC_{50}
HEK293T	>100μM
L929	100 μM
BHK-21	18.6 μM
MCF-7	3.7 μM
RAW	10 μM
MDA-MB	7.7 μM
HCT-116	10.4 μM

f)

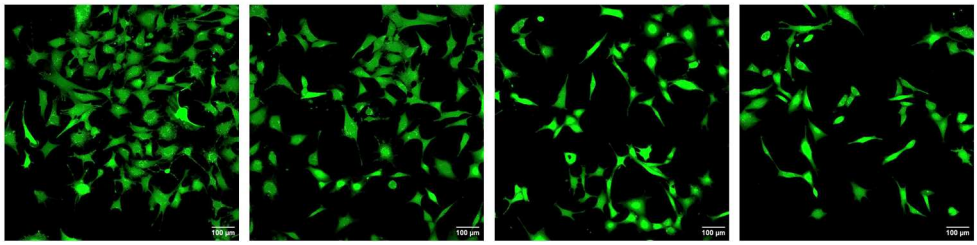


Fig. S16 a) Cell viability of HEK293T cells and b) MCF-7 breast cancer cells upon treatment with **DN** compounds. Cell viability of c) **DN5**, d) **DN6** and e) IC_{50} values of **DN6** in different cell lines. f) Live cell staining of MCF-7 cells with Calcein AM (500 nM) post treatment with **DN6** (5, 10, 20 μM).

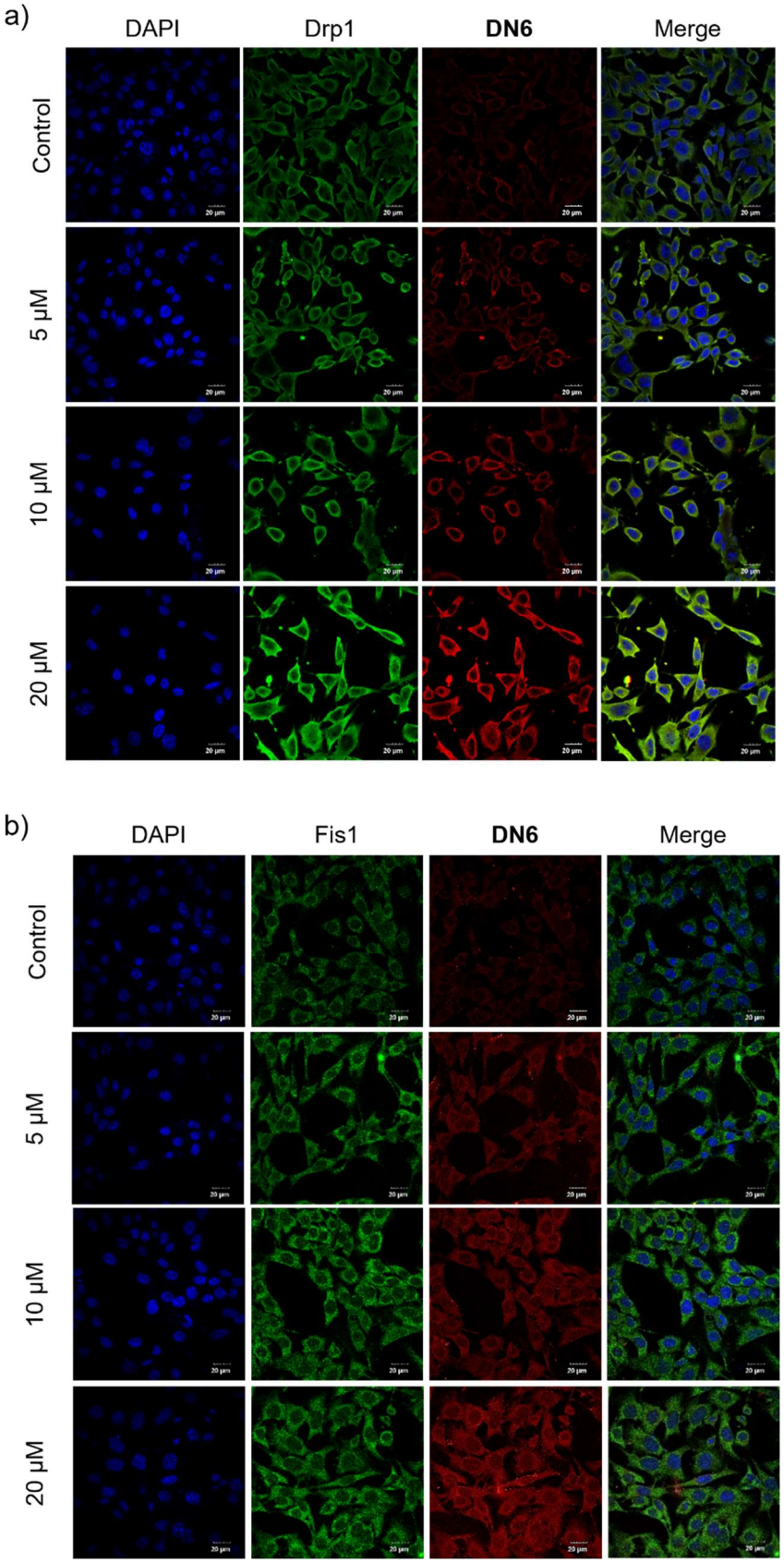


Fig. S17 Immunofluorescence study to show the increase in levels of a) Drp1 and b) Fis1 upon **DN6** treatment in MCF-7 cells.

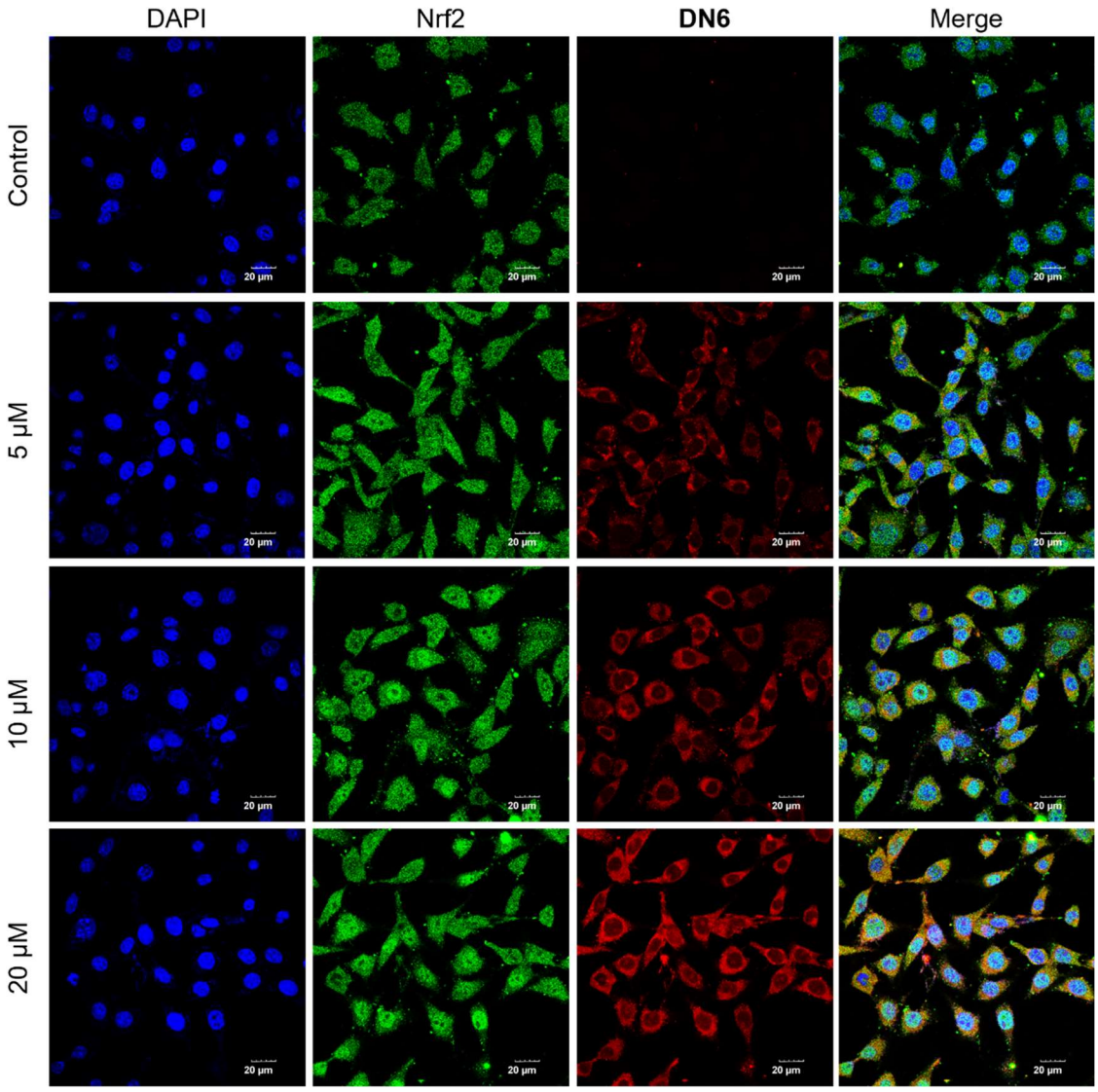
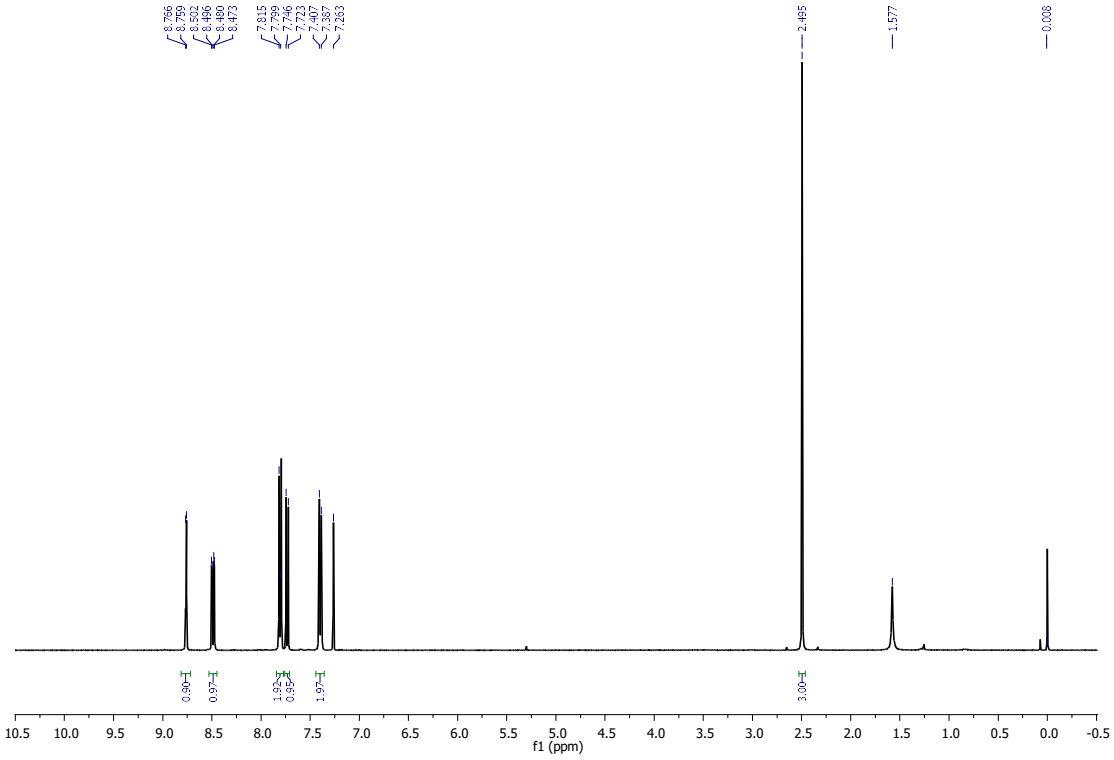
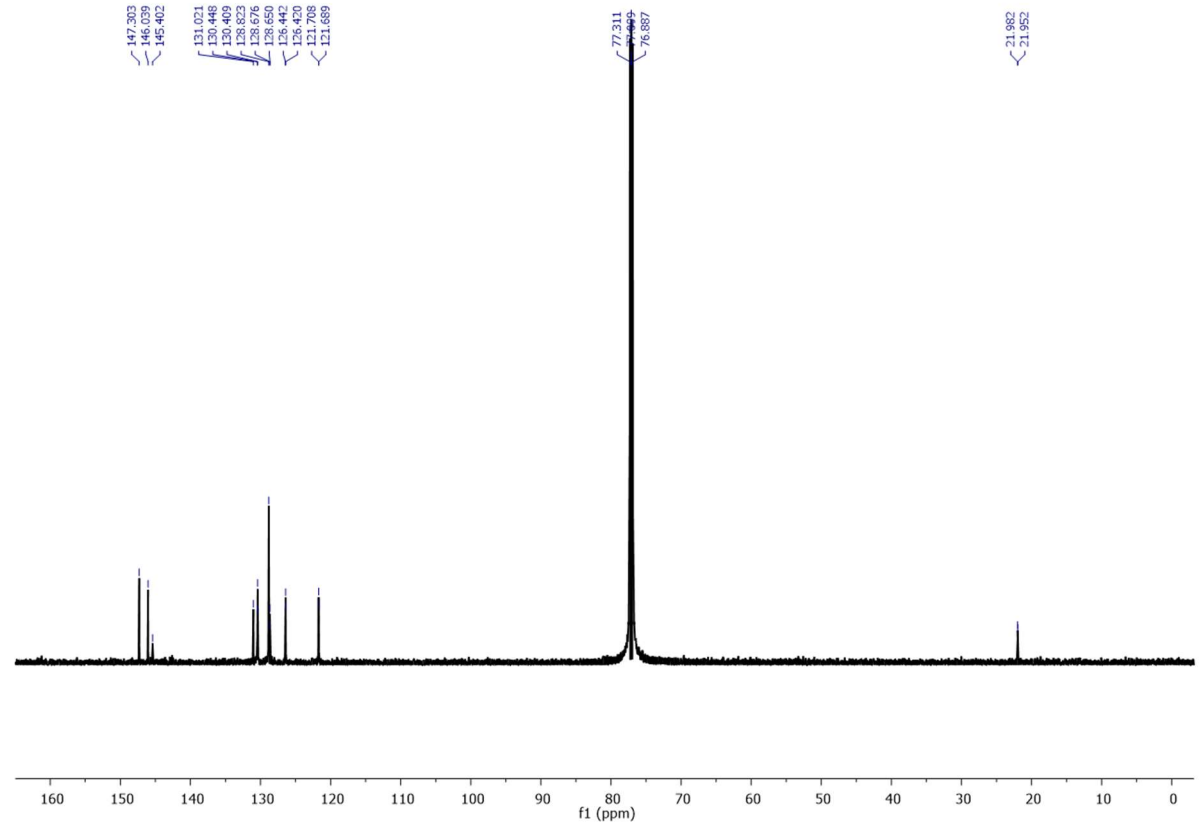


Fig. S18 Immunofluorescence study to show the nuclear translocation of Nrf2 upon **DN6** treatment in MCF-7 cells.

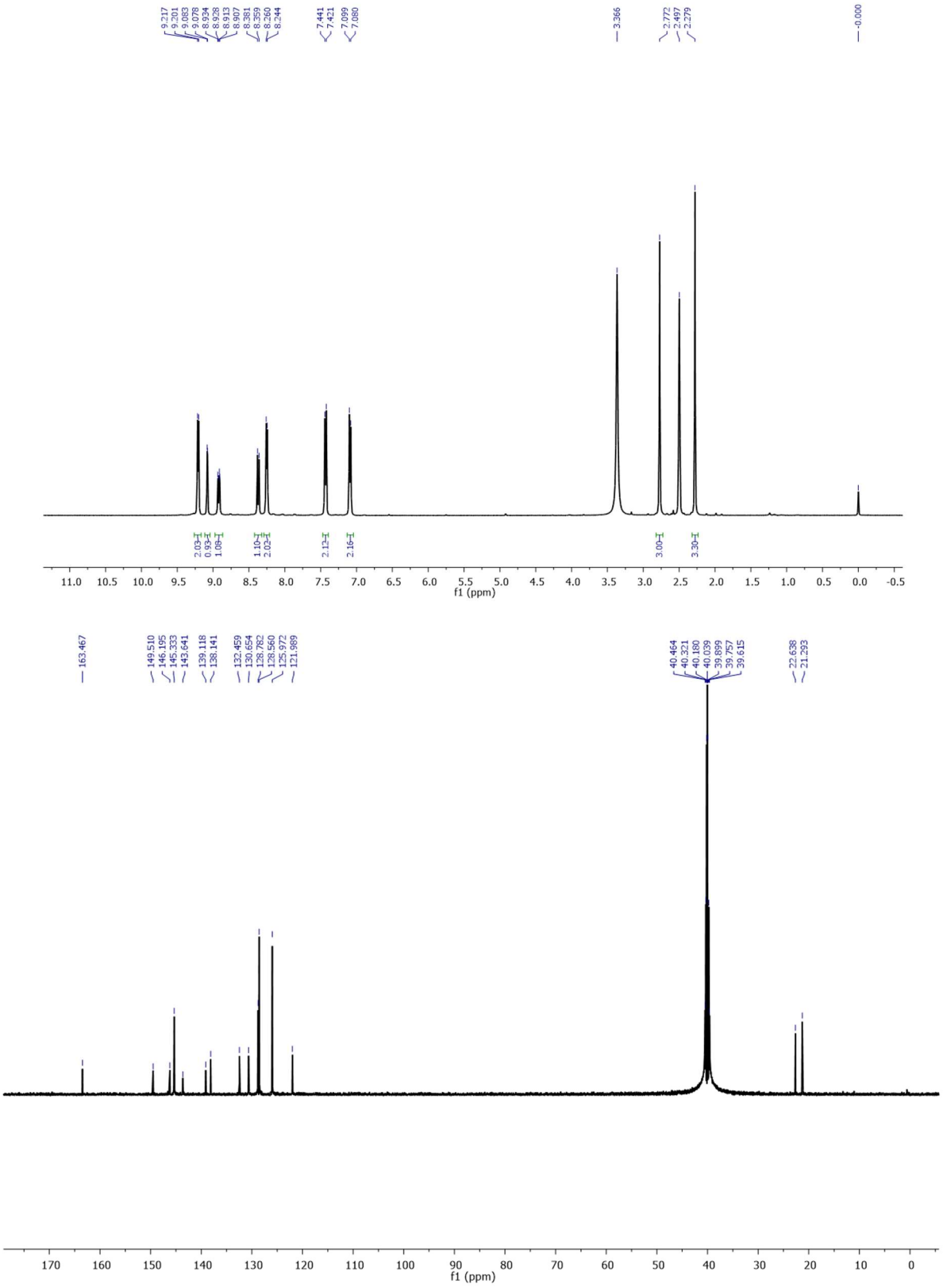
26. Appendix 1: Characterization data of compounds

1. ^{1}H and ^{13}C NMR spectra of **1**

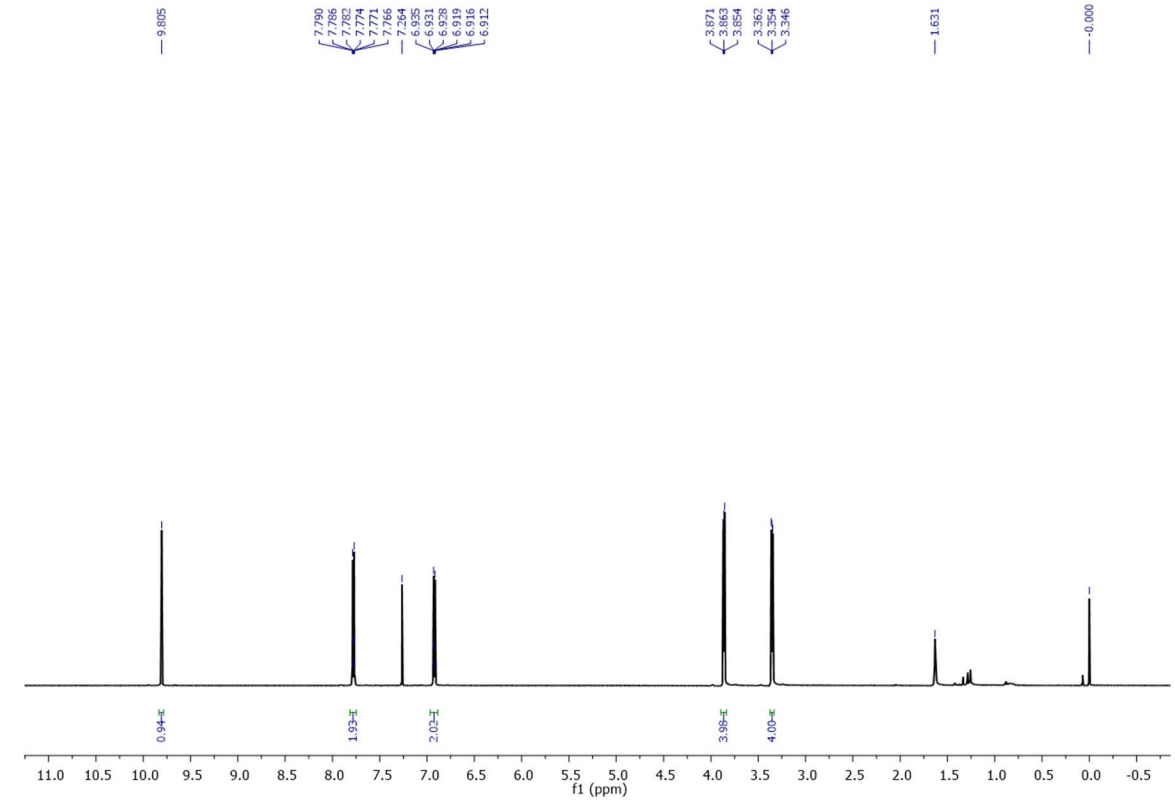


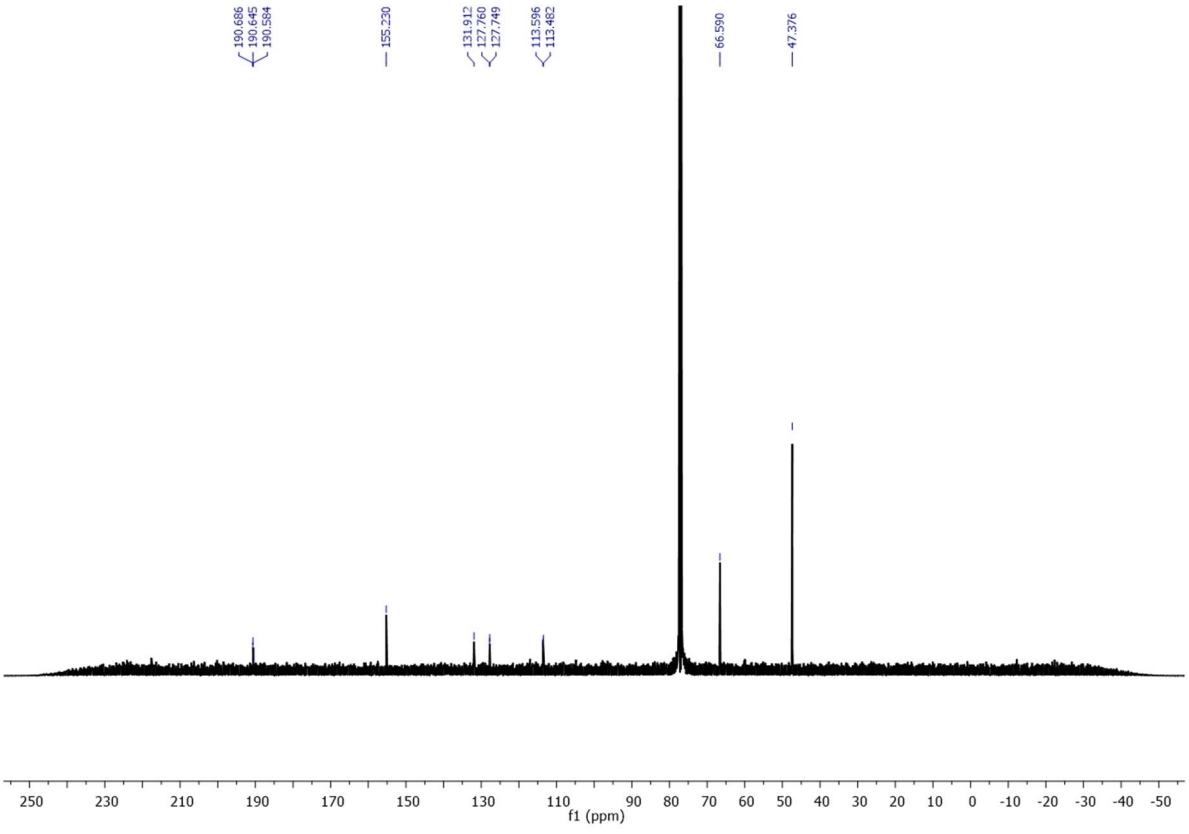


2. ^{1}H and ^{13}C NMR spectra of **2**

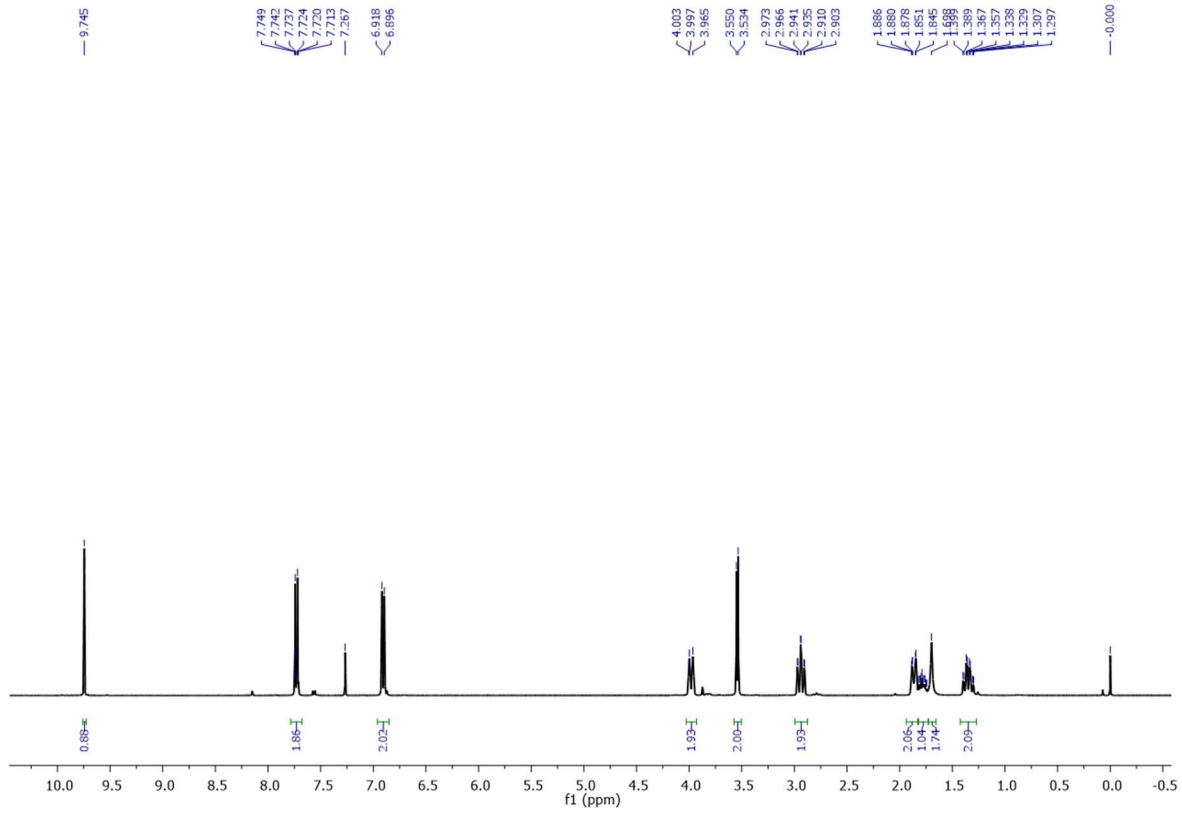


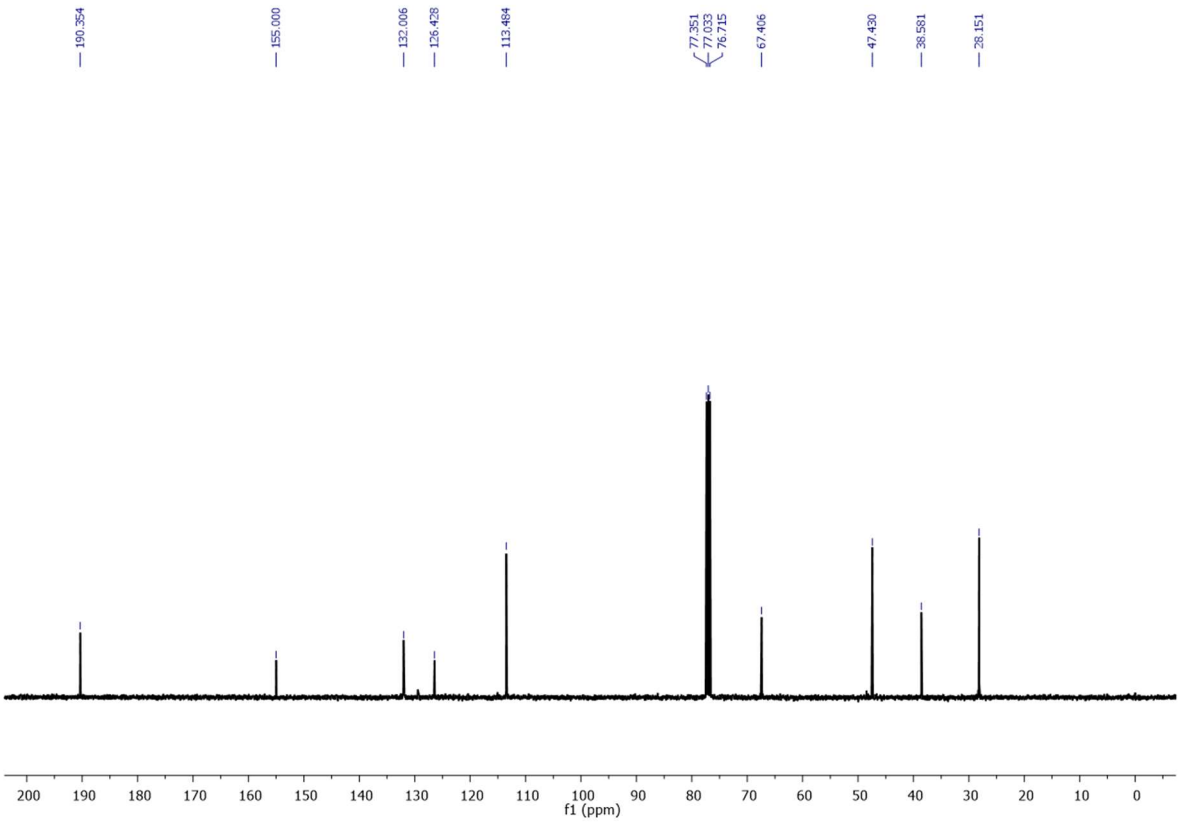
3. ^{1}H and ^{13}C NMR spectra of **3**



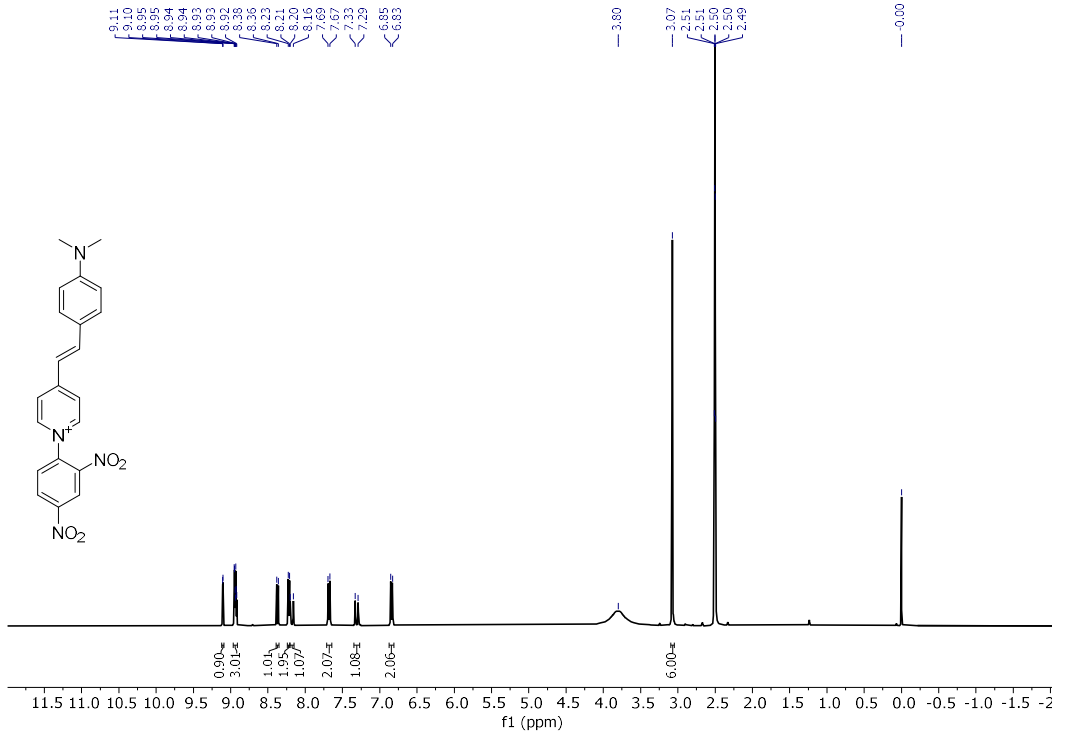


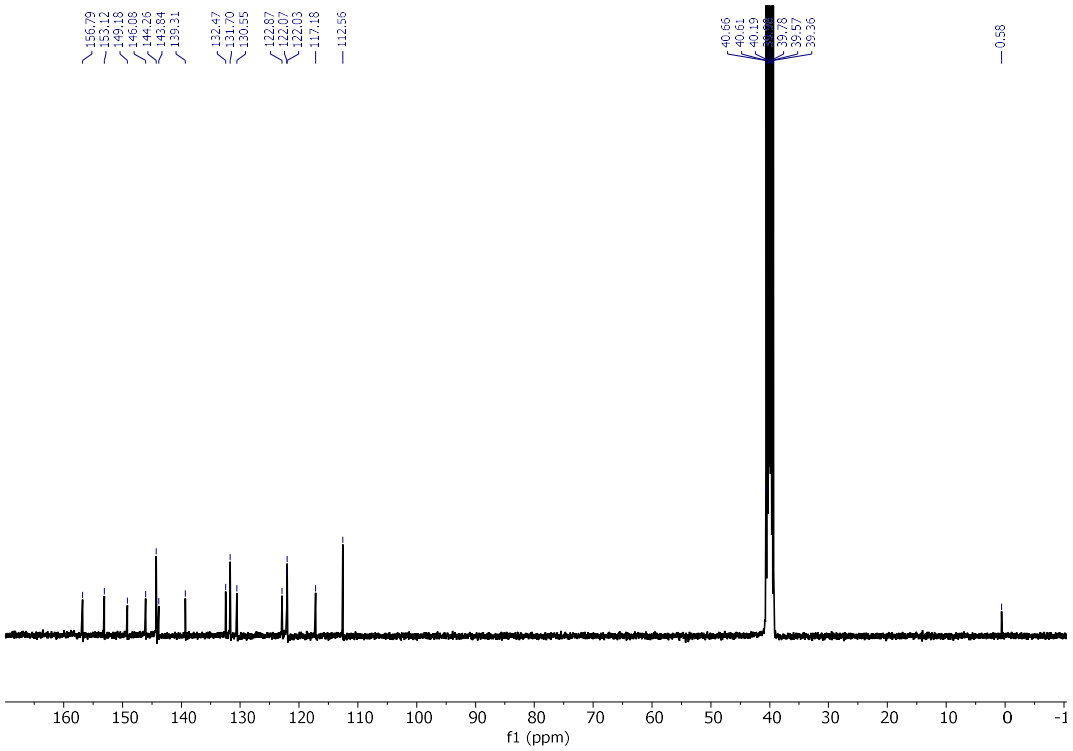
4. ^{1}H and ^{13}C NMR spectra of **4**



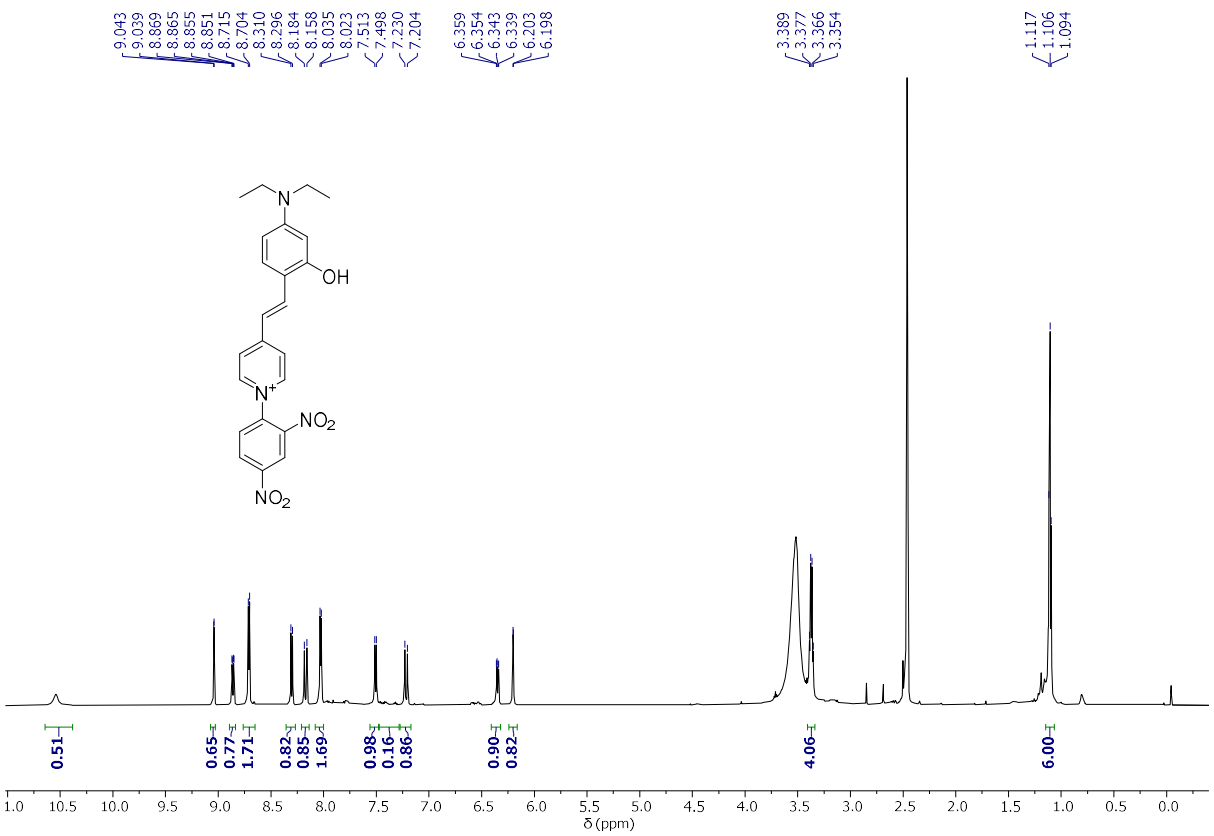


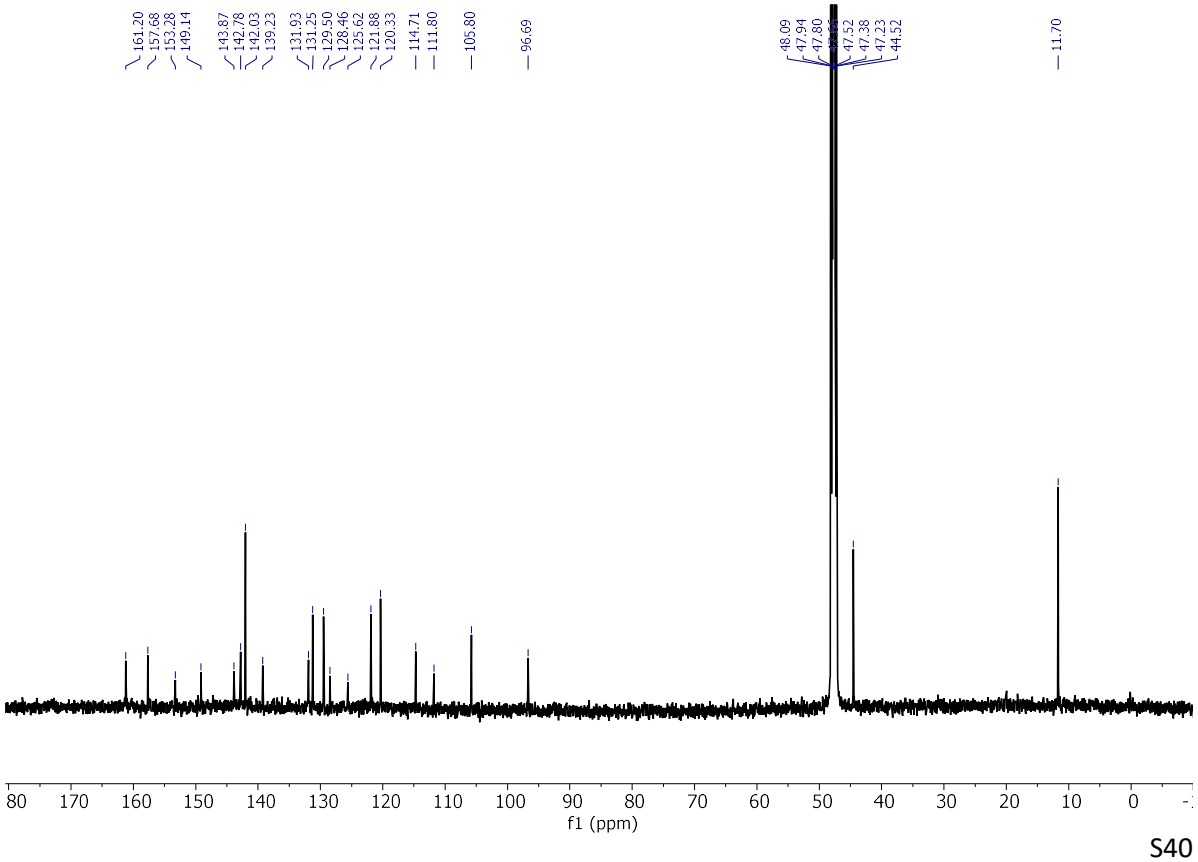
5. ^{1}H and ^{13}C NMR spectra of **DN1**



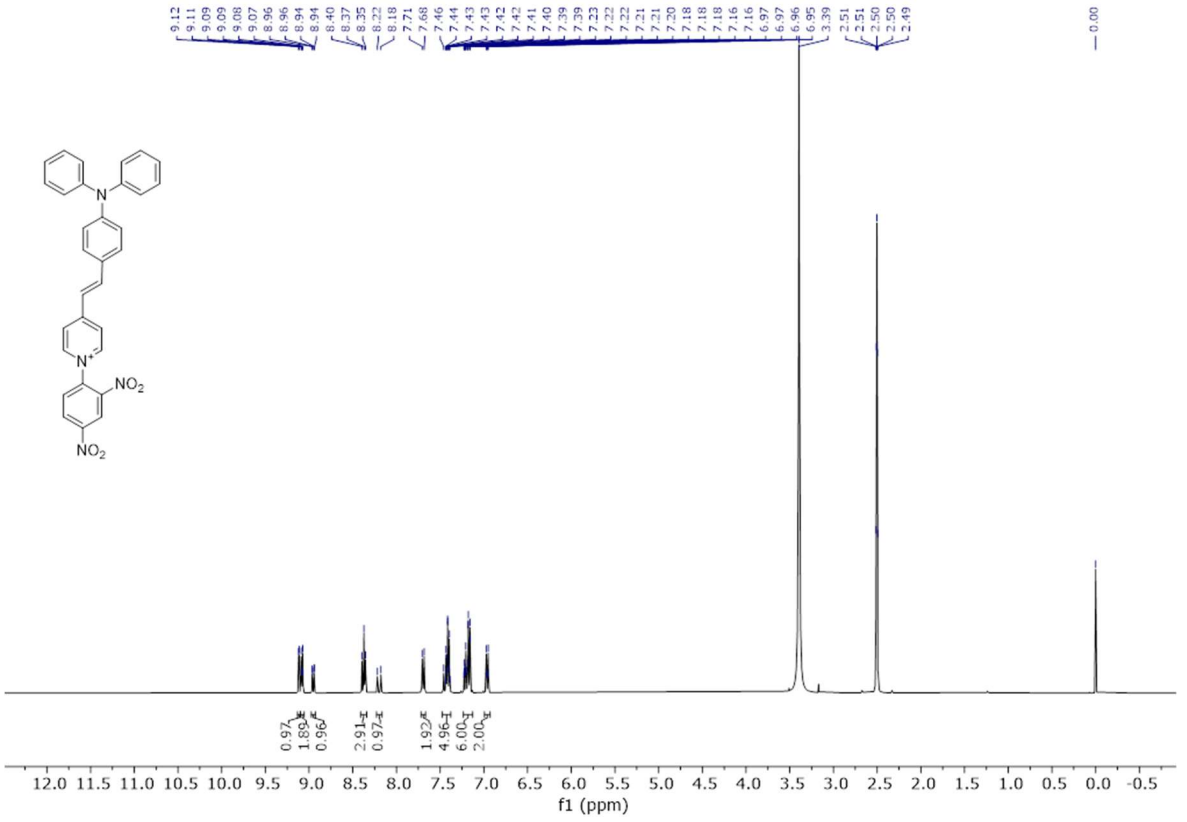


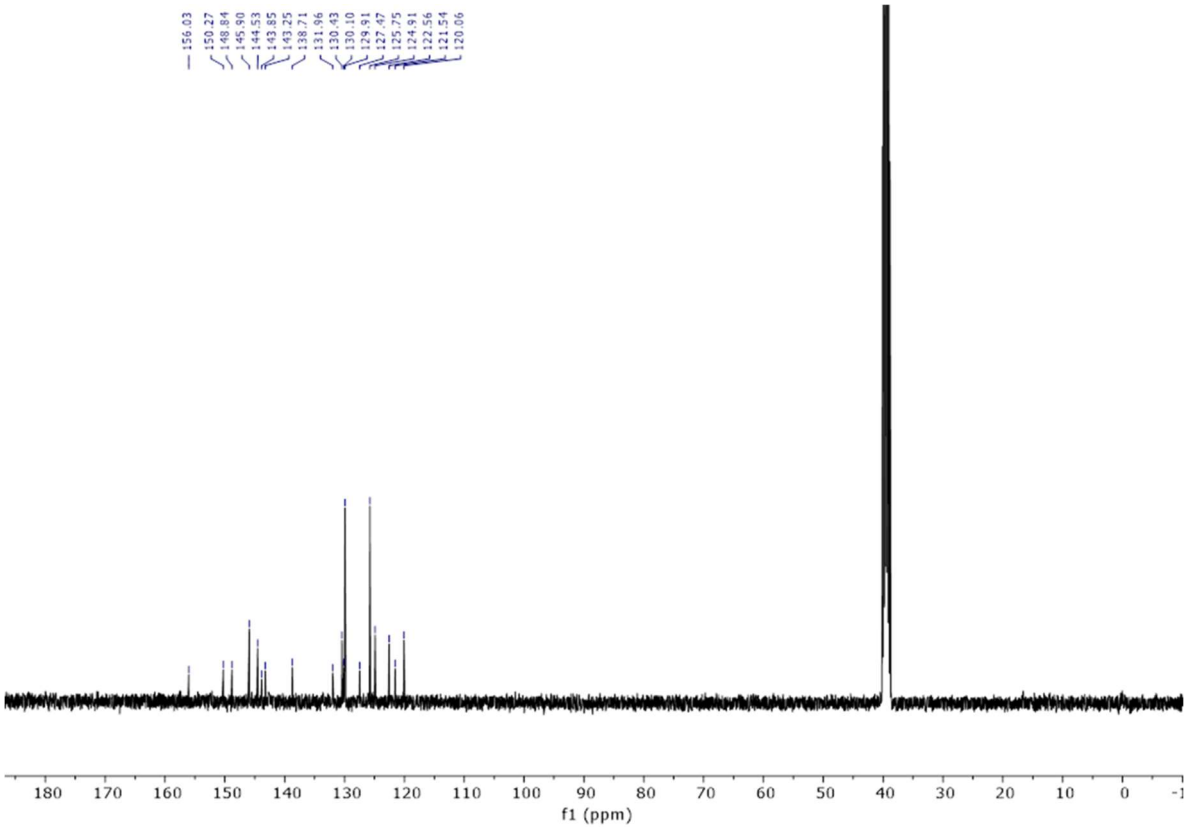
6. ^{1}H and ^{13}C NMR spectra of **DN2**



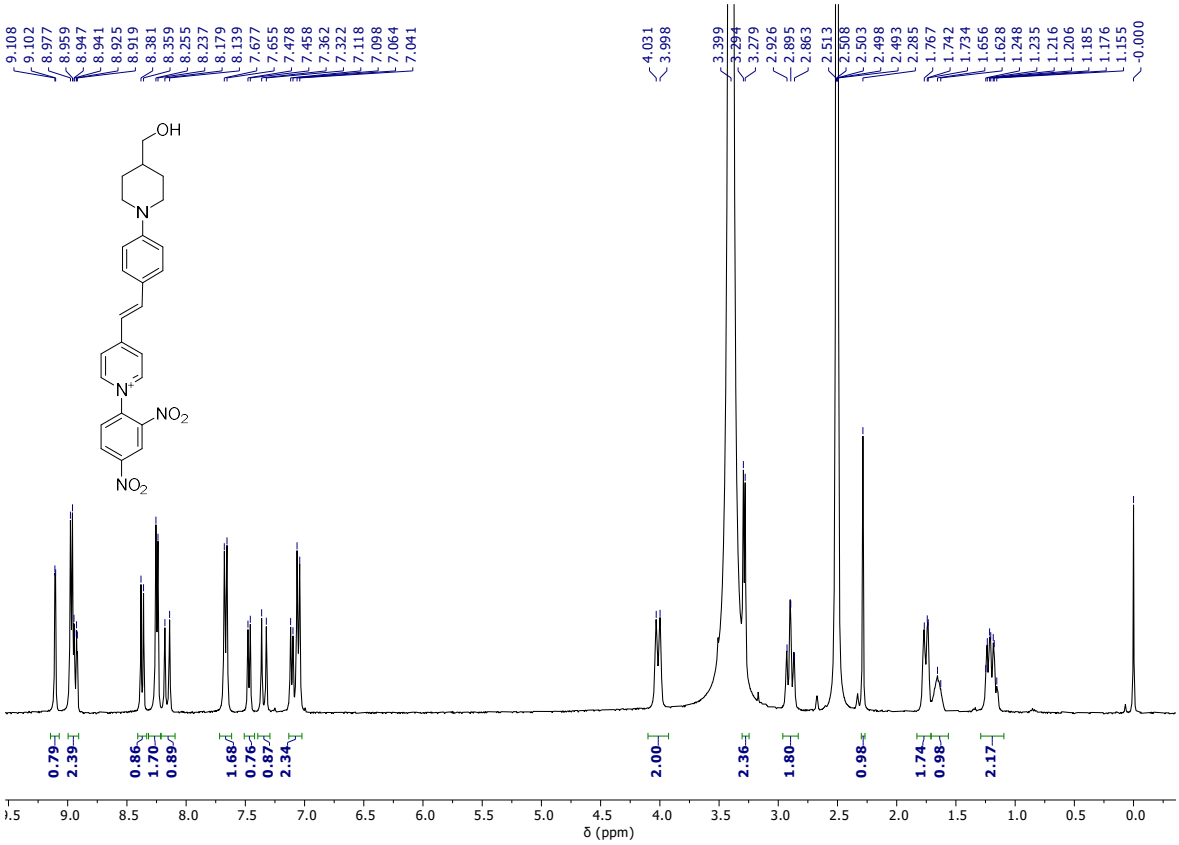


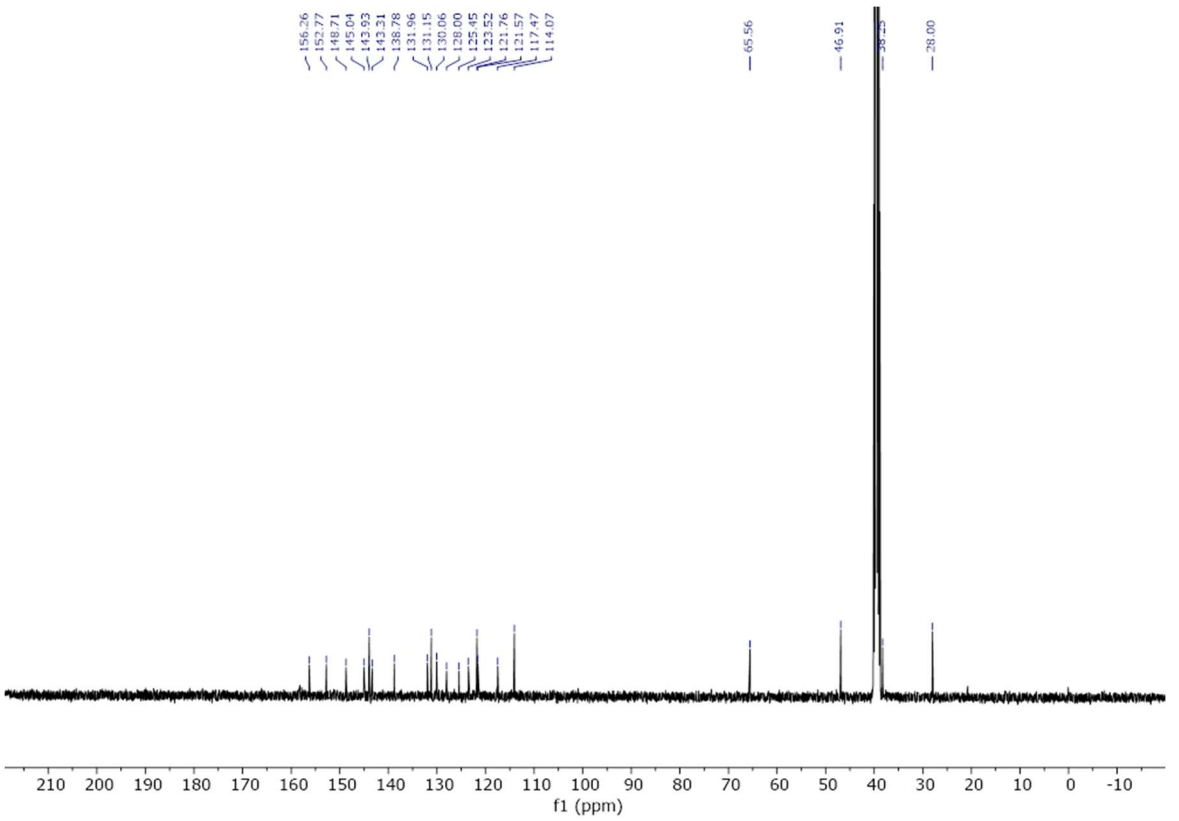
7. ^{1}H and ^{13}C NMR spectra of **DN3**



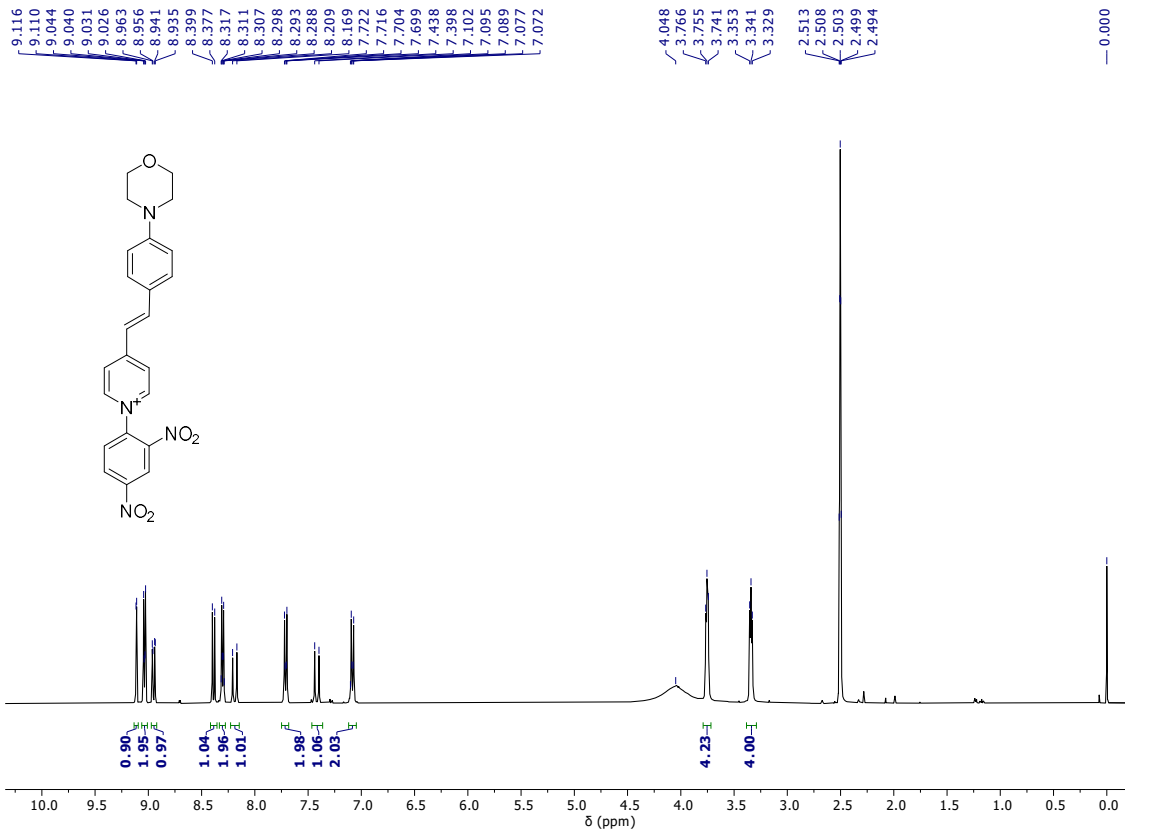


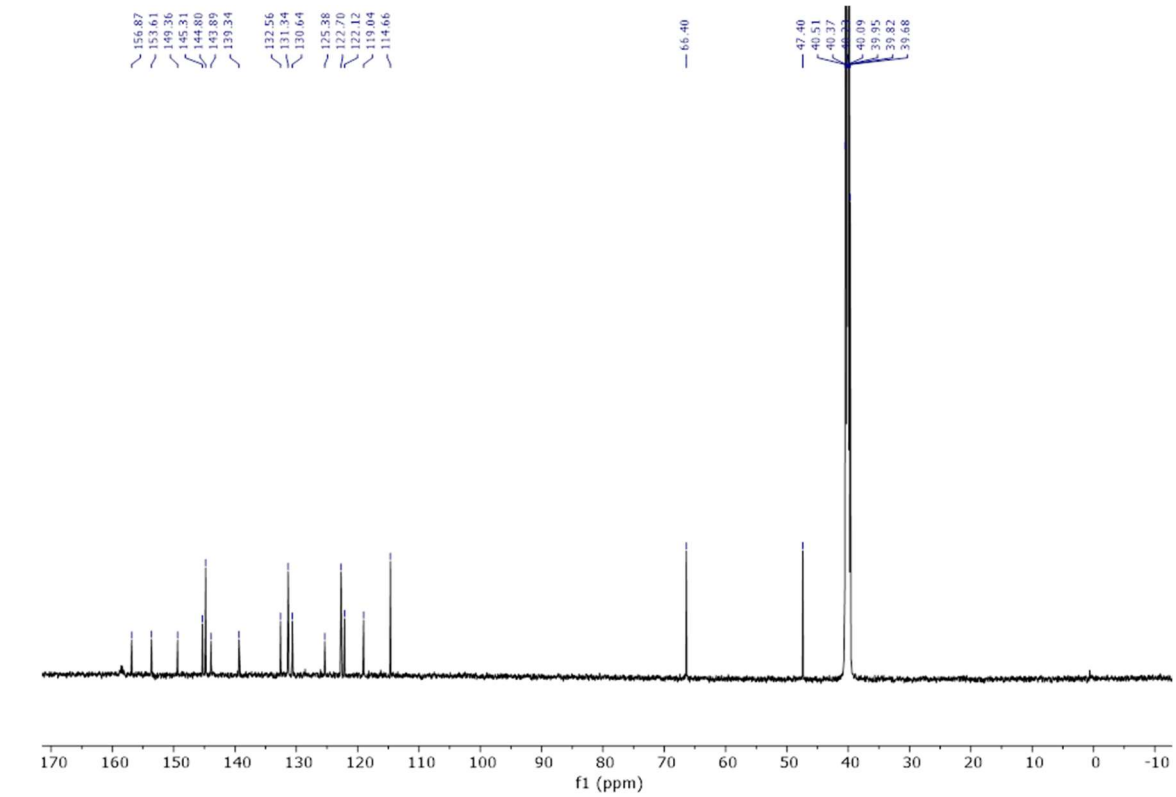
8. ^{1}H and ^{13}C NMR spectra of **DN4**



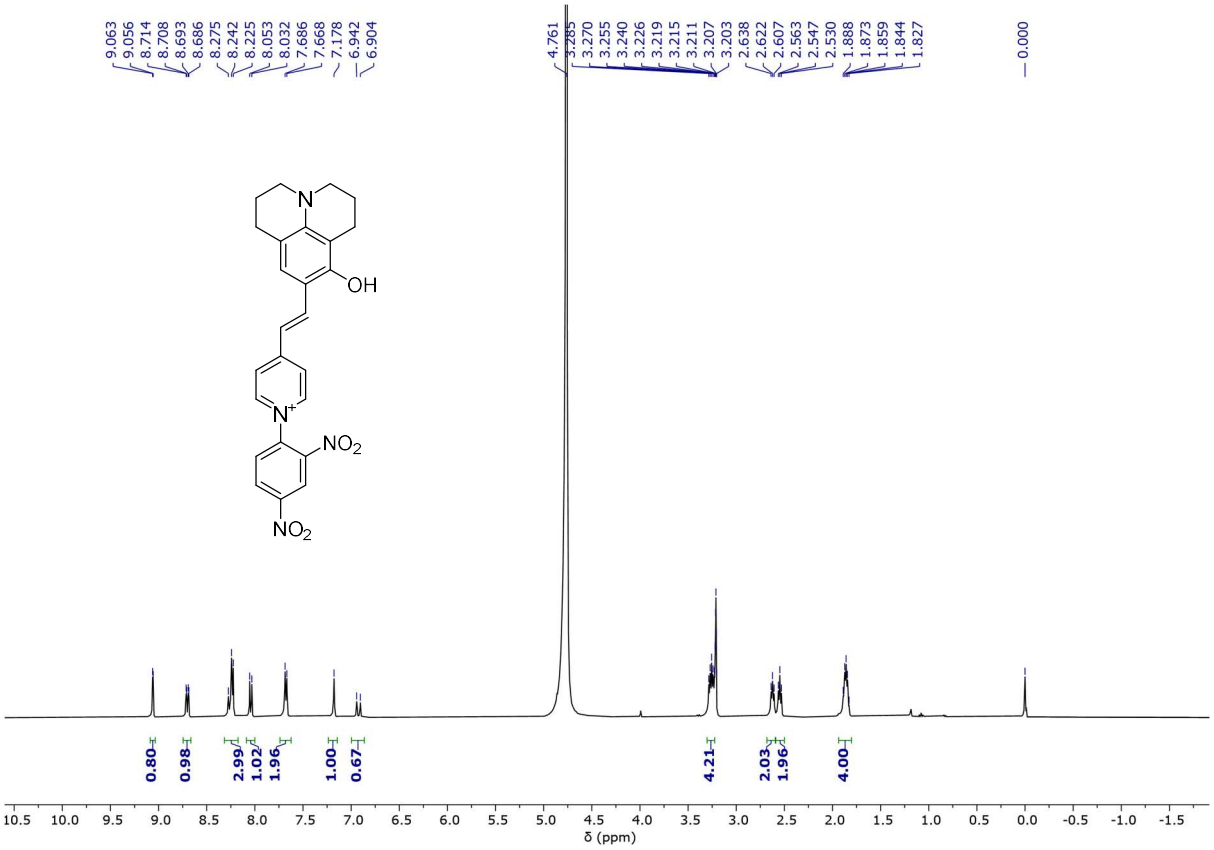


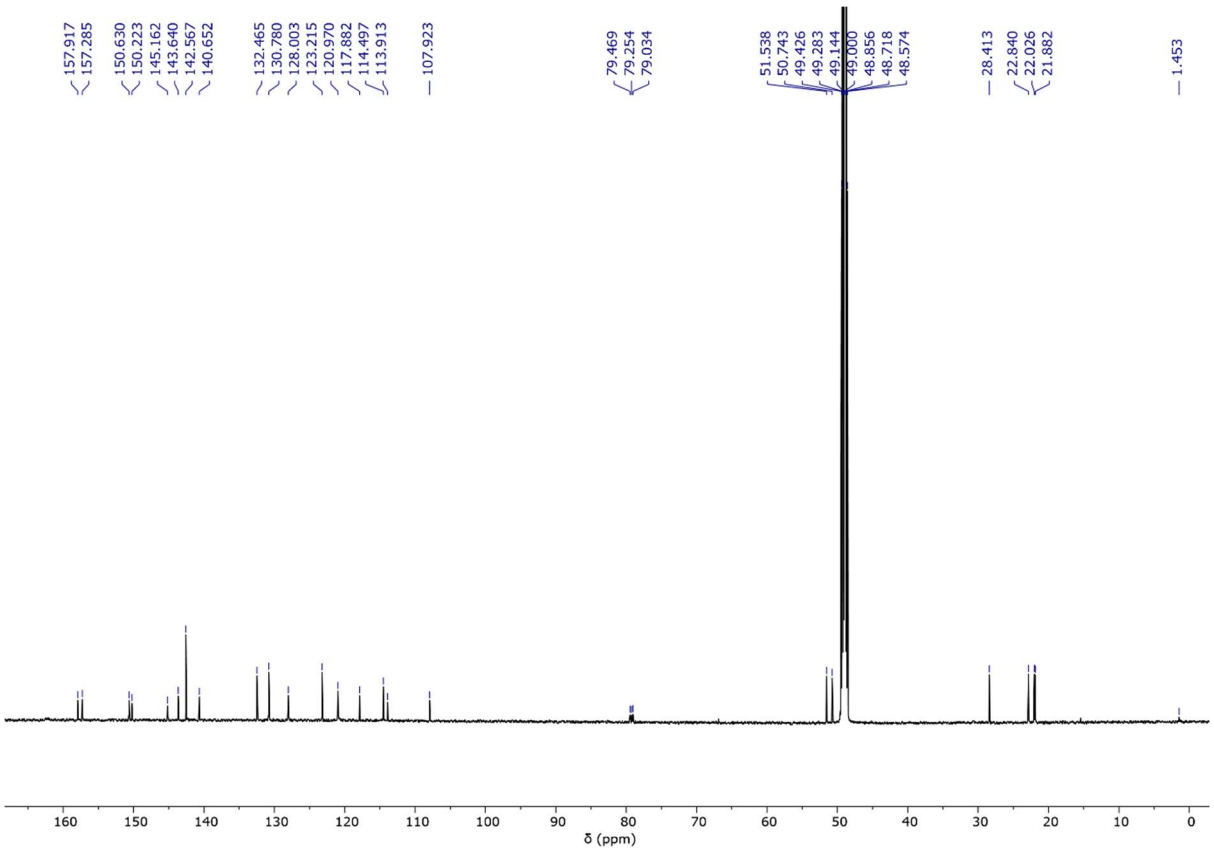
9. ^{1}H and ^{13}C NMR spectra of **DN5**



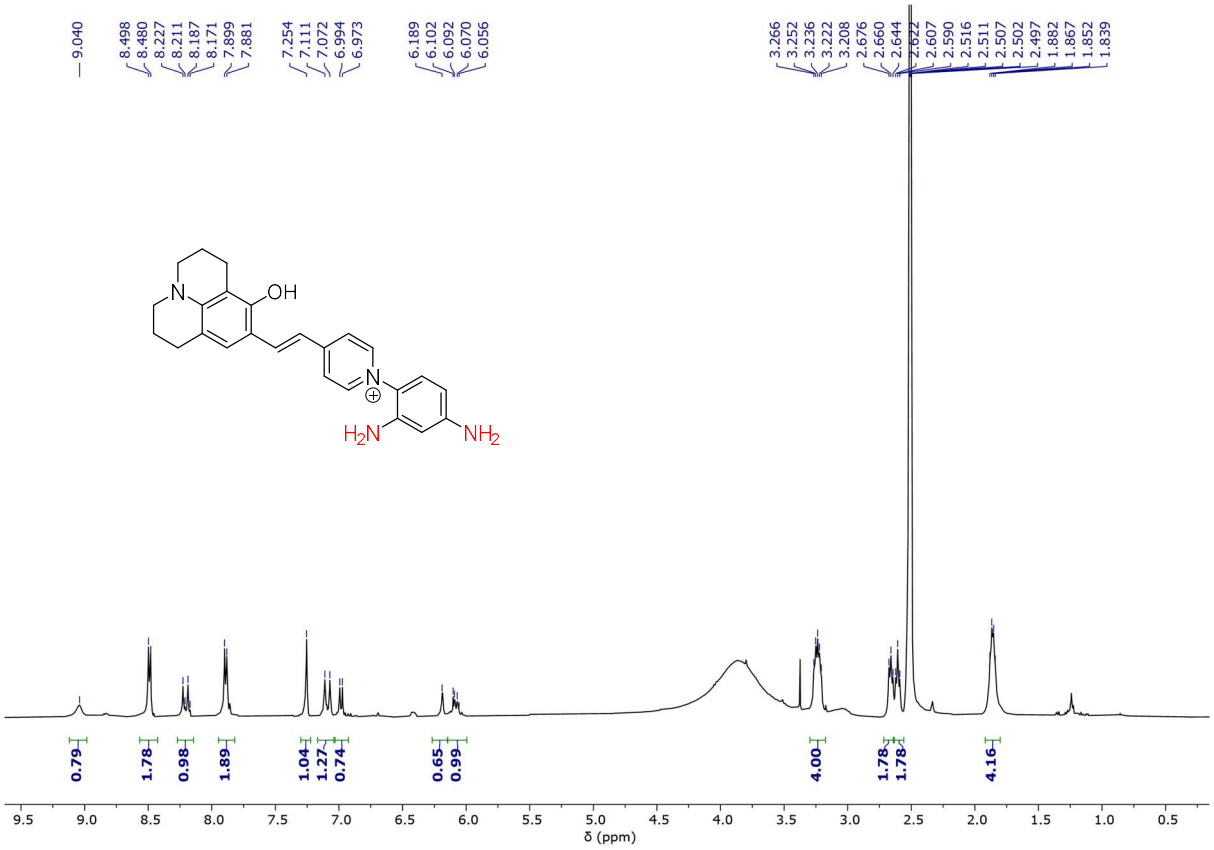


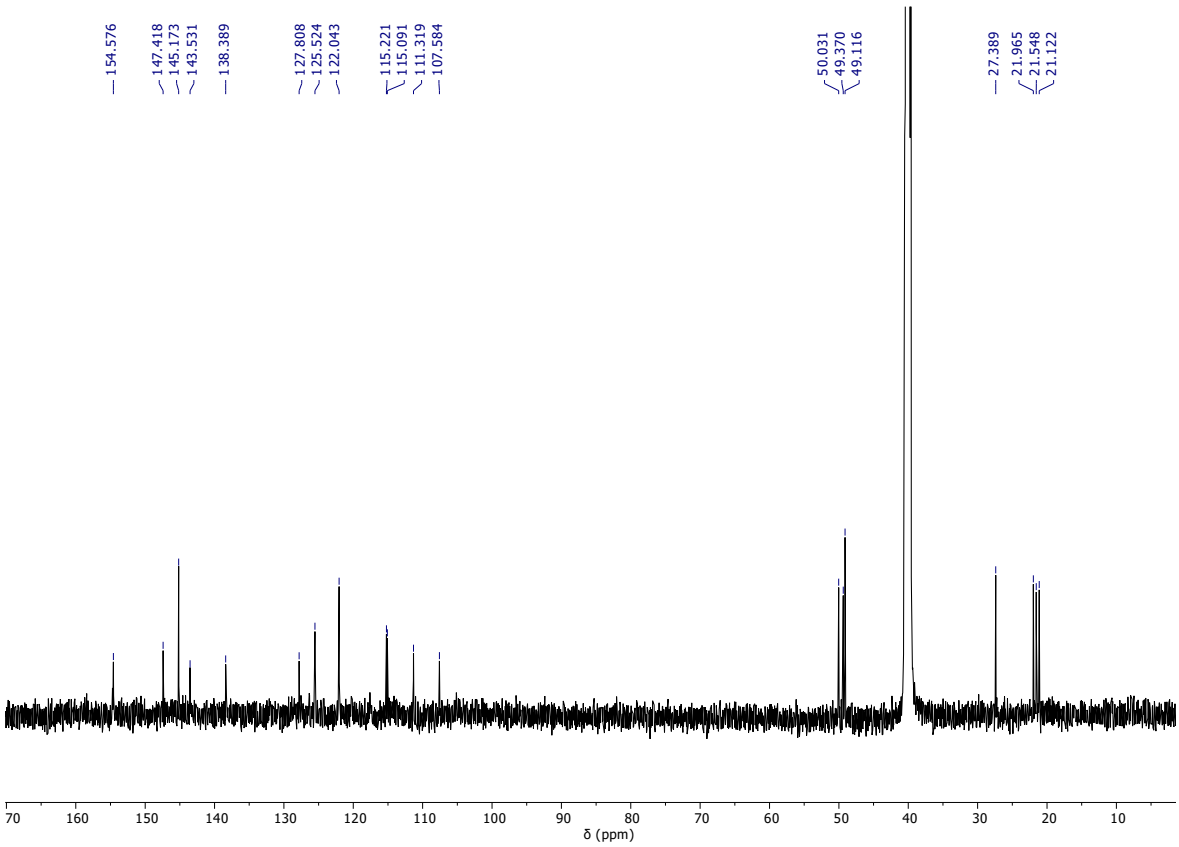
10. ^{1}H and ^{13}C NMR spectra of **DN6**





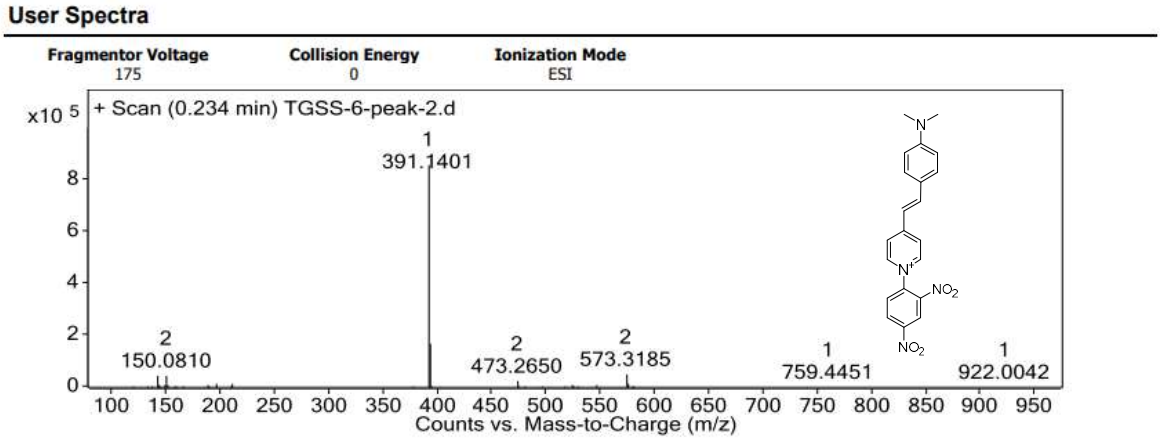
11. ^{1}H and ^{13}C NMR spectra of **DN6R**



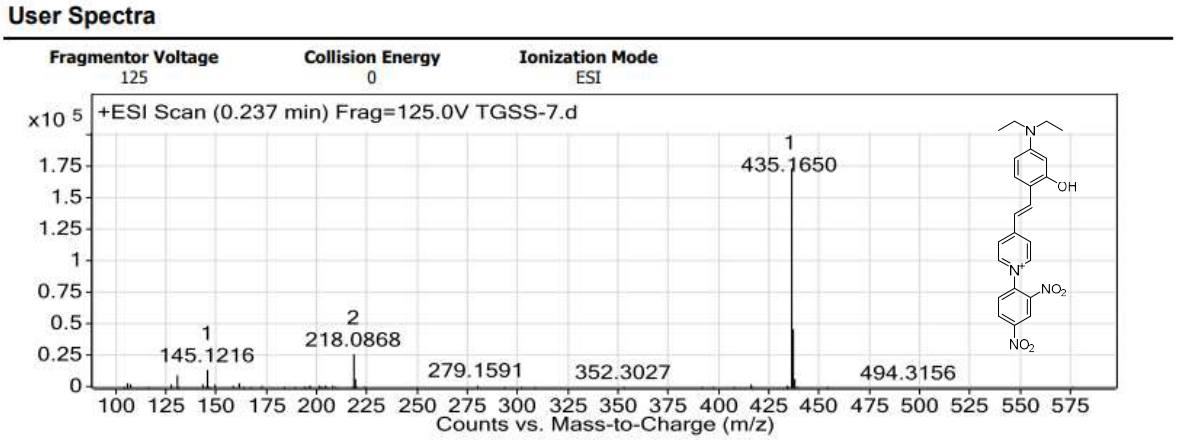


High-resolution mass spectrometry data (HRMS)

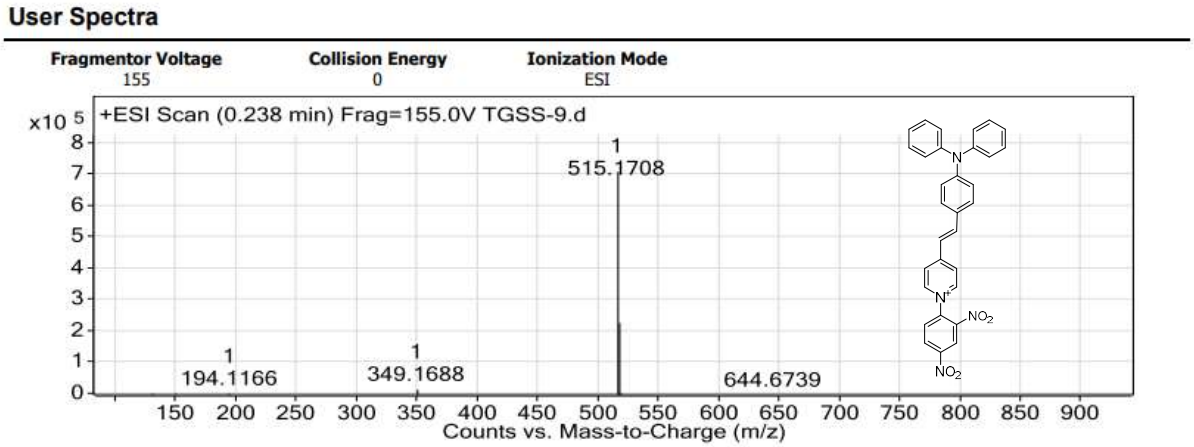
DN1



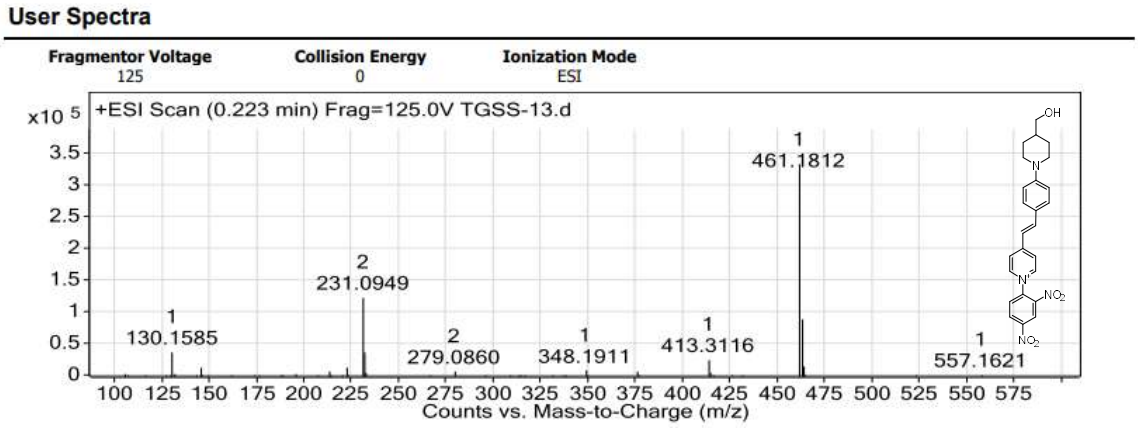
DN2



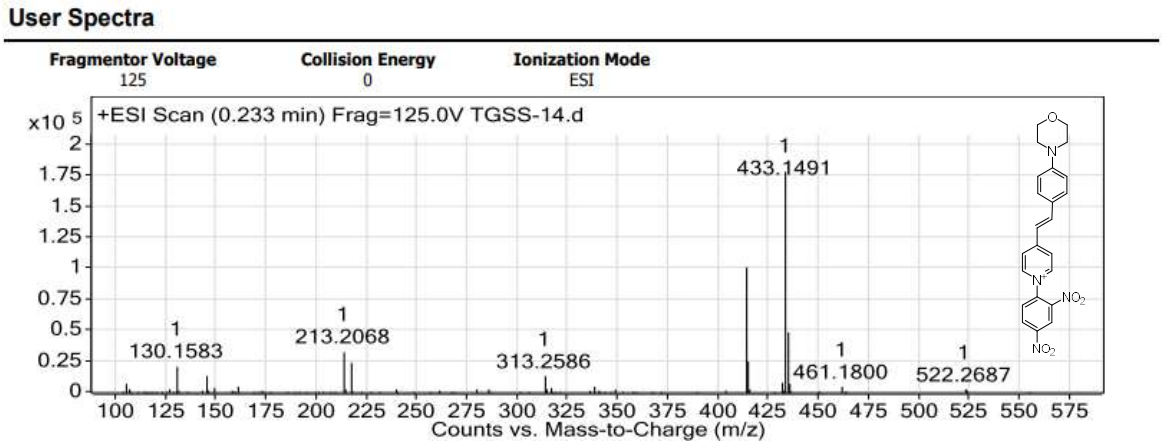
DN3



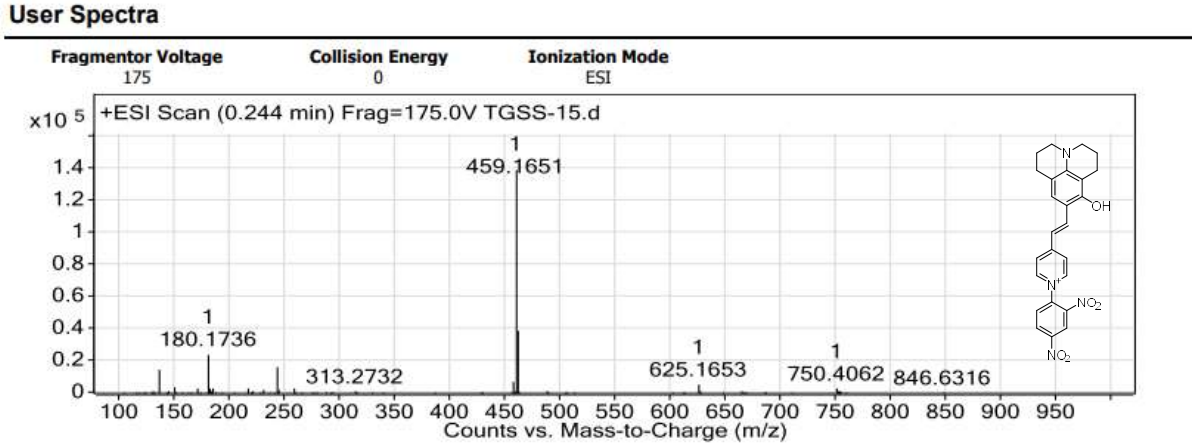
DN4


User Spectra
Fragmentor Voltage
125
Collision Energy
0
Ionization Mode
ESI
+ESI Scan (0.223 min) Frag=125.0V TGSS-13.d
x10 5
1
461.1812
2
231.0949
1
130.1585
2
279.0860
1
348.1911
1
413.3116
1
557.1621
Counts vs. Mass-to-Charge (m/z)

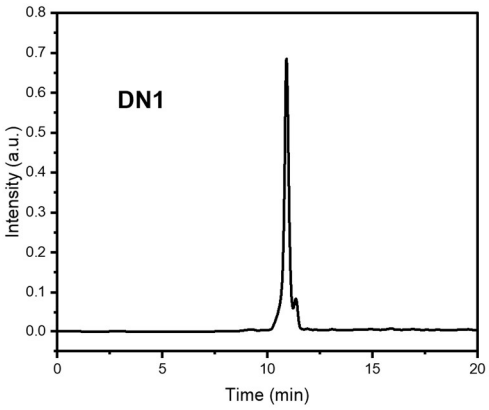
DN5

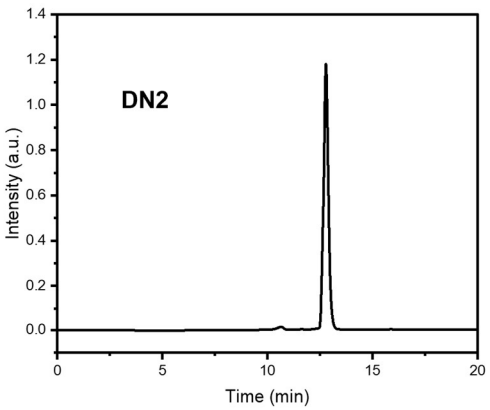

User Spectra
Fragmentor Voltage
125
Collision Energy
0
Ionization Mode
ESI
+ESI Scan (0.233 min) Frag=125.0V TGSS-14.d
x10 5
1
433.1491
1
213.2068
1
130.1583
1
313.2586
1
461.1800
1
522.2687
Counts vs. Mass-to-Charge (m/z)

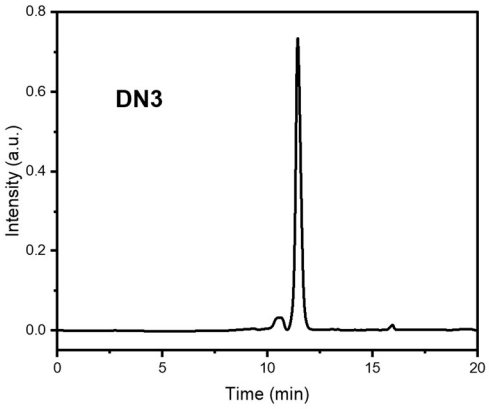
DN6

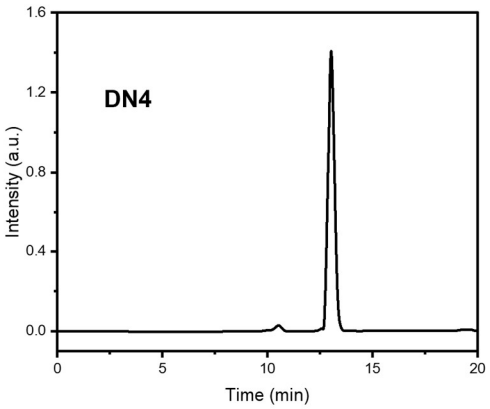

User Spectra
Fragmentor Voltage
175
Collision Energy
0
Ionization Mode
ESI
+ESI Scan (0.244 min) Frag=175.0V TGSS-15.d
x10 5
1
459.1651
1
180.1736
313.2732
1
625.1653
1
750.4062
846.6316
Counts vs. Mass-to-Charge (m/z)

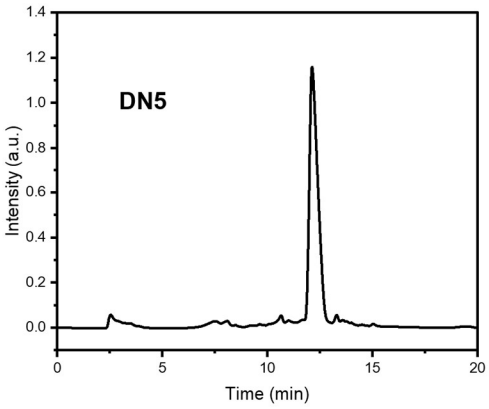
HPLC chromatograms

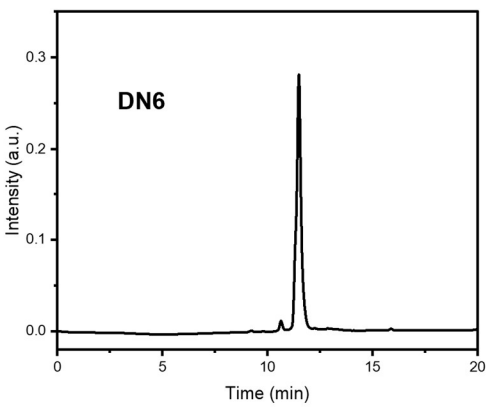


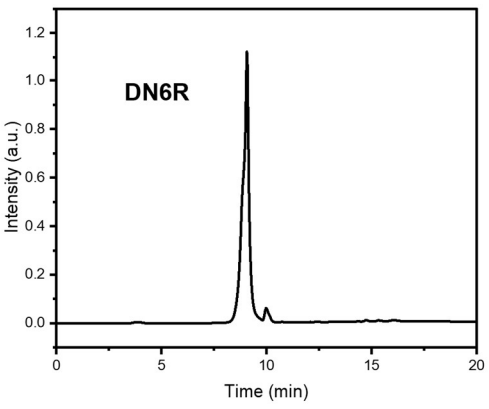












Sl No	Compound Name	Retention Time in HPLC (min)	Purity	Expected Mass	Observed Mass
1	**DN1**	10.91	96.27%	391.1401	391.1401
2	**DN2**	12.78	98.76%	435.1663	435.1650
3	**DN3**	11.44	95.02%	515.1714	515.1708
4	**DN4**	13.04	98.36%	461.1819	461.1812
5	**DN5**	12.13	95.53%	433.1506	433.1491
6	**DN6**	10.64	97.87%	459.1663	459.1651
7	**DN6R**	9.07	96.10%	399.2190	399.2186

27. References

1. K. Rajasekhar, C. Madhu and T. Govindaraju, *ACS Chem. Neurosci.*, 2016, **7**, 1300–1310.
2. H. Rinderknecht, S. Ehnert, B. Braun, T. Histing, A. K. Nussler and C. Linnemann, *Oxygen*, 2021, **1**, 46–61.
3. J. Friedrich, C. Seidel, R. Ebner and L. A. Kunz-Schughart, *Nat. Protoc.*, 2009, **4**, 309–324.