Materials and methods

1. Materials and cells

2, 3, 3-Trimethylindolenine, anhydrous sodium acetate, di-tert-butyl dicarbonate, L-lysine methyl ester dihydrochloride and O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) and O-(Benzotriazol-1-yl)-N, N, N', N'-tetramethyluronium tetrafluoroborate (TBTU) were purchased from Aladdin (Shanghai, China). Cystamine dihydrochloride, anhydrous N, N-dimethylformamide (DMF) and anhydrous dimethyl sulfoxide (DMSO) were obtained from J&K Scientific Ltd. (Beijing, China). Salicylaldehyde, formaldehyde, hydrochloric acid, hexamethylenetetramine, acetic acid, N-bromosuccinimide, methyl 5-methyl-2-nitrobenzoate, benzoyl peroxide (BPO), carbon tetrachloride (CCl_4), potassium carbonate, toluene, lithium hydroxide, triethylamine (TEA), acetic anhydride and 1-bromohexadecane were purchased from Adamas-beta (Shanghai, China). Trifluoroacetic acid (TFA) and N, N-diisopropylethylamine (DIEA) were purchased from Astabiochem Ltd. (Chengdu, China). Boc-Arg (Pbf)-OH was obtained from GL Biochem Ltd. (Shanghai, China). Cell counter kit-8 (CCK-8) was purchased from Dojindo Laboratories (Kumamoto, Japan). Nicotinamide adenine dinucleotide, reduced disodium salt, trihydrate (NADH) and L-Glutathione (GSH) was obtained from Beyotime (Nantong, China). Nitroreductase (NTR) from Escherichia coli was purchased from Sigma-Aldrich (USA). PEI (MW = 25000) and Lipofectamine 2000 were obtained from Thermofisher (USA). Other chemicals and reagents were of analytical grade or better. All buffers were prepared by MilliQ ultrapure water and filtered (0.22 μm) prior to use.

Human cervical carcinoma cells (HeLa) or human umbilical vein endothelial cells (HUVEC) were incubated in DMEM (Dulbecco's Modified Eagle Medium, Hyclone, USA), supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Hyclone, USA).

2. Synthesis of NTR and GSH-responsive amphiphilic molecule (RNNF molecule).

Compound 1: Salicylaldehyde (20 g, 164 mmol) and formaldehyde (9.84 g, 328 mmol) were dissolved in hydrochloric acid (200 mL) and stirred for 5 h at room temperature. White precipitates were separated and washed with brine salt solution twice. The filtrate was re-dissolved in ethyl acetate and the resulting solution was dried with magnesium sulfate. After filtration and concentration, the precipitates were recrystallized under petroleum ether. Then, a solution of crude product (18 g, 106 mmol) and hexamethylenetetramine (29.64 g, 212 mmol) in acetic acid was stirred for 1 h at 115 ^{o}C, before180 mL of hydrochloric acid was added into the mixture and stirred for another 1 h at 115 ^{o}C. After cooling to room temperature, the white solids were precipitated out gradually and washed twice by saturated brine solution. The precipitate was dissolved in dichloromethane again and dried by anhydrous magnesium sulfate. The crude product was obtained after filteration and rotary evaporation. Finally, Compound **1** was purified by column chromatography and obtained as white solids (5.54 g, 34.9% yield). ^{1}H NMR (400 MHz, DMSO-*d*6), δ (ppm): 10.43 (s, 1H), 9.90 (s, 1H), 8.21 (s, 1H), 8.01 (d, *J* = 8.0 Hz, 1H), 7.17 (d, *J* = 8.0 Hz, 1H). MS: calcd. For $C_8H_6O_3$ *m/z* 150.03 [M]; found *m/z* 149.05 $[M]^-$.

Compound 2: Methyl 5-methyl-2-nitrobenzoate (20 g, 50 mmol), BPO (24.4 g, 50 mmol), and N-

bromosuccinimide (35.6 g, 100 mmol) were dissolved in CCl_4 (400 mL) and stirred for 4 h at 85 °C. After filtered and concentrated in vacuum, the residue was obtained and then dissolved in small amount of ethyl acetate. The supernatant was collected and concentrated by rotary evaporation to obtain the crude product. The 3.46 g compound **2** was obtained as yellow solids through purified by column chromatography (24.6% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.91 (d, *J* = 8.0 Hz, 1H), 7.75 (s, 1H), 7.65 (d, *J* = 8.0 Hz, 1H), 4.50 (s, 2H), 3.94 (s, 3H). MS (MALDI-TOF-MS): calcd. For $C_9H_8BrNO_4$ *m/z* 272.96 [M]; found *m/z* 273.76 [M].

Compound 3: Compound **2** (1.96 g, 13 mmol) and potassium carbonate (3.02 g, 22 mmol) were dissolved in anhydrous DMF (6 mL) and stirred for 10 min at 0 °C, followed by the addition of compound **1** (3.0 g, 11 mmol) solution in 6 mL anhydrous DMF. The resulting solution was stirred overnight at room temperature under N_2. After concentrated in vacuum, the mixture was dissolved in dichloromethane (200 mL) and washed with saturated brine solution. The organic phase was collected and dried with anhydrous sodium sulfate. Then, the crude product was concentrated in vacuum and purified by silica column chromatography to obtain compound **3** (0.61 g) as tawny and viscous solids (16.2% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 10.55 (s, 1H), 9.98 (s, 1H), 8.40 (d, *J* = 4.0 Hz, 1H), 8.15 (d, *J* = 4.0 Hz, 1H), 8.02 (s, 1H), 7.84 (d, *J* = 4.0 Hz, 1H), 7.75 (s, 1H), 7.16 (d, *J* = 8.0 Hz, 1H), 5.40 (s, 2H), 3.95 (s, 3H). MS: calcd. For $C_9H_8BrNO_4$ *m/z* 343.07 [M]; found *m/z* 342.09 $[M]^-$.

Compound 4: 2, 3, 3-Trimethylindolenine (10 g, 60 mmol) and 1-Bromohexadecane (28.86 g, 94 mmol) were dissolved in 80 mL of toluene and reacted for 72 h at 130 °C. After cooling to room temperature, the crude product (compound 4) was obtained by removing redundant toluene and purifying by column chromatography (10.4 g, 35.8% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.62 (d, *J* = 12.2 Hz, 4H), 7.57 (d, *J* = 2.5 Hz, 2H), 4.75 (t, *J* = 6.2 Hz, 2H), 3.15 (s, 3H), 1.67 (s, 6H), 1.25 (s, 28H), 0.88 (t, *J* = 6.8 Hz, 3H). MS: calcd. For $C_{27}H_{46}N^+$ *m/z* 384.36 [M]; found *m/z* 384.34 [M].

Compound 5: Compound **4** (2.02 g, 4.4 mmol), anhydrous sodium acetate (0.43 g, 5.4 mmol) and Compound **3** (0.6 g, 1.8 mmol) were dissolved in 120 mL acetic anhydride and stirred for 2 h in dark at 80 °C under N_2. Then, the mixture was dissolved in 200 mL dichloromethane. The organic phase was washed with saturated solution of $NaHCO_3$, NaH_2PO_4 and NaCl, respectively. Afterwards, the organic phase was dried with anhydrous sodium sulfate and concentrated in vacuum. At last, compound **5** was obtained and purified by column chromatography (0.71 g, 32.9% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 10.31 (s, 2H), 9.31 (d, *J* = 12.0 Hz, 1H), 8.94 (s, 1H), 8.83 (m, 2H), 8.62 (d, *J* = 16.0 Hz, 1H), 8.04 (d, *J* = 8.0 Hz, 1H), 7.95 (d, *J* = 8.0 Hz, 1H), 7.87 (d, *J* = 8.0 Hz, 1H), 7.62 (d, *J* = 4.0 Hz, 2H), 7.57 (d, *J* = 4.0 Hz, 2H), 7.49 (d, *J* = 4.0 Hz, 1H), 7.09 (s, 1H), 6.75 (d, *J*=8.0 Hz, 1H), 6.52 (d, *J*=8.0 Hz, 1H), 5.55 (s, 2H), 5.26 (t, *J*=8.0 Hz, 4H), 3.94 (s, 3H), 2.01 (s, 12H), 1.23 (s, 56H), 0.86 (t, *J* = 6.0 Hz, 6H). MS (MALDI-TOF-MS): calcd. For $C_{71}H_{101}N_3O_5^{2+}$ *m/z* 1075.77 [M]; found *m/z* 1075.15 [M].

Compound 6: Compound **5** (1 g, 0.8 mmol) was dissolved in 8 mL mixed solvent of methanol and DMF (1/1, v/v). After the addition of 2 mL 1 M aqueous solution of lithium hydroxide, the obtained solution was stirred for 1 h at room temperature in dark and then concentrated in vacuum. After adjusted to pH = 2 with 1 M hydrochloric acid, the mixture was added 100 mL of dichloromethane and washed by saturated brine solution. Then, the organic phase was dried with anhydrous sodium

sulfate and concentrated in vacuum. Red brown powder of compound **6** was obtained and purified by column chromatography (1.61 g, 98.1% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 5.35 (s, 4H), 5.19 (t, *J* = 12.0 Hz, 3H), 1.94 (s, 12H), 1.22 (m, 56H), 0.85 (t, *J* = 6.0 Hz, 6H). MS (MALDI-TOF-MS): calcd. For $C_{70}H_{99}N_3O_5^{2+}$ *m/z* 1061.76 [M]; found *m/z* 1060.80 [M].

Compound 7: Cystamine dihydrochloride (8 g, 35 mmol), triethylamine (7.07 g, 70 mmol) and di-tert-butyl dicarbonate (6.7 g, 31 mmol) were dissolved in 500 mL methanol and stirred at 30 °C for overnight. After concentrated in vacuum by rotary evaporation, the residue was dissolved in 200 mL dichloromethane and washed by saturated brine solution. The organic phase was dried with anhydrous sodium sulfate and concentrated in vacuum. At last, compound **7** was obtained and purified by column chromatography (3.6 g, 47.4% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 4.98 (s, 1H), 3.45 (m, 2H), 3.01 (t, *J* = 6.2 Hz, 2H), 2.79 (t, *J* = 6.3 Hz, 4H), 1.45 (s, 9H). MS: calcd. For $C_9H_{20}N_2O_2S_2$ *m/z* 252.10 [M]; found *m/z* 253.08 $[M]^+$.

Compound 8: Compound **6** (1.6 g, 1.3 mmol), TBTU (0.84 g, 2.6 mmol) and DIEA (0.34 g, 2.6 mmol) were dissolved in 50 mL anhydrous DMF, followed by the addition of compound **7** (0.5 g, 1.9 mmol) in 10 mL DMF. The resulted solution was stirred for overnight at 30 °C under N_2 in dark and then concentrated in vacuum. After addition of 100 mL of dichloromethane, the reaction mixture was washed by saturated solution of $NaHCO_3$, NaH_2PO_4 and NaCl, sequentially. The organic solvent was dried with anhydrous sodium sulfate and removed under reduced pressure. The crude product was purified by column chromatography to obtain 0.27 g compound **8** (14.2% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 8.60 (m, 2H), 8.45 (d, *J* = 8.0 Hz, 1H), 8.10 (s, 1H), 7.91 (d, *J* = 8.0 Hz, 2H), 7.65 (d, *J* = 8.0 Hz, 1H), 7.62 (d, *J* = 8.0 Hz, 1H), 7.59 (br, 2H), 7.54 (d, *J* = 8.0 Hz, 2H), 7.07 (s, 2H), 6.76 (s, 1H), 6.52 (s, 1H), 5.53 (s, 2H), 5.20 (d, *J* = 4.0 Hz, 4H), 3.60 (m, 2H), 3.41 (m, 2H), 3.09 (m, 2H), 2.85 (t, *J* = 6.0 Hz, 2H), 1.91 (s, 12H), 1.35 (s, 9H), 1.24 (s, 56H), 0.86 (t, *J* = 6.0 Hz, 6H). MS (MALDI-TOF-MS): calcd. For $C_{79}H_{117}N_5O_6S_2^{2+}$ *m/z* 1295.84 [M]; found *m/z* 1295.02 [M].

Compound 9: Compound **8** was dissolved in 6 mL of dichloromethane and trifluoroacetic acid mixture (1: 1) under stirring at 0 °C. After stirring for 5 h at 30 °C in dark, the redundant solvent was removed under reduced pressure. Then, the residue was dissolved in 200 mL dichloromethane and washed by saturated brine solution. The organic phase was dried with anhydrous sodium sulfate and concentrated in vacuum. compound **9** was purified by column chromatography and obtained as red brown solids (0.20 g, 86.9% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 5.51 (s, 2H), 4.62 (br, 4H), 3.73 (br, 2H), 3.38 (br, 2H), 3.01 (br, 4H), 1.82 (s, 12H), 1.23 (s, 56H), 0.86 (t, *J* = 6.0 Hz, 6H). MS (MALDI-TOF-MS): calcd. For $C_{74}H_{109}N_5O_4S_2^{2+}$ *m/z* 1195.79 [M]; found *m/z* 1195.38 [M].

Compound 10: Boc-Arg (Pbf)-OH (6 g, 11 mmol), HBTU (5.7 g, 15 mmol) and DIEA (1.9 g, 15mmol) were dissolved in 50 mL anhydrous DMF, followed by the addition of L-lysine methyl ester dihydrochloride (1.2 g, 5.0 mmol) in 50 mL anhydrous DMF. The mixture was stirred for 48 h at 30 °C under N_2. The organic phase was removed in vacuum. Afterwards, the 200 mL of dichloromethane was added and the mixture was washed by saturated solution of $NaHCO_3$, NaH_2PO_4 and NaCl, respectively. The organic phase was dried with anhydrous magnesium sulfate and concentrated in vacuum by rotary evaporation. 3.3 g pure product was obtained through purified by column chromatography (56.8% yield). This pure product was dissolved in 10 mL of

methanol, followed by the addition of 5 mL of 1 M sodium hydroxide aqueous solution and stirred for overnight at 30 °C. The pH of the solution was brought to 2 by adding 1 M hydrochloric acid. Then, the 200 mL of dichloromethane was added and the mixture was washed by saturated brine solution. The organic phase was dried with anhydrous magnesium sulfate and concentrated in vacuum. Compound **10** was obtained as white powder (2.7 g, 90% yield) after purified by column chromatography. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 4.36 (br, 3H), 3.38 (br, 6H), 2.96 (s, 4H), 2.53 (s, 6H), 2.47 (s, 6H), 2.08 (s, 6H), 1.84 (br, 6H), 1.68 (br, 6H), 1.46 (s, 12H), 1.39 (s, 18H), 1.25 (m, 2H). MS (MALDI-TOF-MS): calcd. For $C_{54}H_{86}N_{10}O_{14}S_2$ *m/z* 1162.58 [M]; found *m/z* 1163.63 [M].

Compound 11: Compound **10** (0.46 g, 0.39 mmol), TBTU (0.17 g, 0.52 mmol) and DIEA (0.07 g, 0.52 mmol) were dissolved in 50 mL anhydrous DMF. Then, compound **9** (0.36 g, 0.26 mmol) was also dissolved in 10 mL anhydrous DMF and dropwise added into the mixture and stirred for 48 h at 30 °C under N_2 in dark. After removing organic phase and adding 100 mL of dichloromethane, the mixture was washed by saturated solution of $NaHCO_3$, NaH_2PO_4 and NaCl, respectively. The organic phase was dried with anhydrous sodium sulfate and concentrated in vacuum. 0.54 g compound **11** was obtained after purified by silica column chromatography (82% yield). ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 7.95 (br, 2H), 7.57 (br, 6H), 7.20 (d, *J* = 8.0 Hz, 2H), 7.04 (d, *J* = 8.0 Hz, 1H), 6.86 (s, 1H), 5.76 (s 2H), 4.29 (s, 3H), 4.16 (m, 4H), 3.69 (m, 4H), 3.20 (br, 6H), 2.94 (br, 8H), 2.55 (s, 6H), 2.47 (s, 6H), 2.07 (s, 6H), 1.78 (m, 6H), 1.60 (m, 6H), 1.45 (s, 24H), 1.37 (s, 18H), 1.24 (s, 58H), 0.87 (t, *J* = 6.0 Hz, 6H). MS (MALDI-TOF-MS): calcd. For $C_{128}H_{193}N_{15}O_{17}S_4^{2+}$ *m/z* 2341.36 [M]; found *m/z* 2341.33 [M].

Compound 12: Compound **11** (0.2 g, 0.08 mmol) was dissolved in 2 mL of dichloromethane and the same volume of trifluoroacetic acid was added under stirring at 0 °C. The mixture was kept for 5 h at 30 °C in dark and then the redundant solvent was removed in vacuum by rotary evaporation. The product was precipitated by diethyl ether and concentrated in vacuum to obtain 0.14 g compound **12** (97% yield). ^{1}H NMR (400 MHz, CD_3OD), δ (ppm): 5.47 (s, 2H), 4.40 (m, 1H), 3.96 (m, 4H), 3.71 (m, 4H), 3.50 (m, 2H), 3.18 (m, 2H), 2.97 (br, 4H), 2.69 (m, 4H), 1.74 (m, 6H), 1.58 (m, 6H), 1.45 (s, 12H), 1.29 (s, 58H), 0.91 (t, *J* = 6.0 Hz, 6H). MS (MALDI-TOF-MS): calcd. For $C_{128}H_{193}N_{15}O_{17}S_4^{2+}$ *m/z* 1637.09 [M]; found *m/z* 1637.08 [M].

3. Self-assembly and characterization of assemblies

The RLS, RNNF, RNNS-1, RNNS-2, RNNS-3 and RNNS-4（Table S1）were prepared by the injection method. Briefly, 100 μL dimethylsulfoxide with 1 mg assemblies were dropped into 1 mL ultrapure water with rapid stirring. The assemblies were obtained after 15 min. Then, fluorescence spectra of NTR-responsive molecule (RNNF) in various solutions with maximal excitation wavelength (dotted line, Em 870 nm) and emission wavelength (solid line, Ex 690 nm) was detected by fluorescence spectrometer (HITACHI, F-7000, Japan). The size and zeta potential were detected by dynamic light scattering (DLS) with Zetasizer Nano ZS (Malvern, Worcestershire, UK) at 25 °C. The morphologies of assemblies were observed by transmission electron microscopy (JEM-2100 Plus, JEOL, Japan).

4. The selective disassembly response of assemblies

The sensitive responsiveness of assemblies to NTR and GSH was proved through the change of size and zeta potential at 25 °C. The changes of size and zeta potential for various assemblies (RNNF,

RLS and RNNS) were detected after incubation with 10 mM GSH, 5 μg/mL NTR and 1 mM NADH, or mixture of GSH and NTR for different time (0 h, 0.5 h, 1 h, 2 h and 3 h) in 37 ^{o}C, respectively.

5. Gene condensation and release

The complexes were formed by incubating assemblies with pDNA (200 ng) at various N/P ratios for 20 min at room temperature. Then, the complexes were incubated with 10 mM GSH, 5 μg/mL NTR and 1 mM NADH, or mixture of GSH and NTR for 1 ~ 2 h at 37 ^{o}C in culture media containing 10 % FBS. The gel electrophoresis assay was used to evaluate the gene condensation and release ability of assemblies. All samples were loaded into 1 % agarose gel for electrophoresis (85 V, 1 h). The gels were stained with GelRed and visualized by the Molecular Imager ChemiDoc XRS+ (Bio-Rad, USA).

6. In vitro cytotoxicity

The cytotoxicity of assemblies was tested by Cell Counting Kit-8 (CCK-8) assay. Briefly, Human cervical carcinoma cells (HeLa) were seeded into 96-well plates with a density of 1×10^4 per well and incubated for 24 h. Cell were exposed to various concentrations of different assemblies in serum-free medium. After 4 h incubation, the media were removed and fresh media containing 10 % FBS were added. After other 24 h, the cells were washed with PBS buffer (pH = 7.4 and incubated with fresh serum-free media containing 10 % CCK-8 for 2 h. Then the absorbance was detected by microplate reader (Bio-Rad 550, USA) under 450 nm.

7. Detection the content of NTR in cells

The content of NTR content in cells was detected by Human NTR ELISA Kit (Mlbio, Shanghai). Firstly, HeLa and human umbilical vein endothelial cells (HUVEC) were collected to the concentration of 1×10^7 cell/mL with PBS solution (pH 7.4). After 5 times rupture through repetitive freeze-thawing, the supernatant was collected by centrifugation at 3000 rpm/min for 20 min. Then, the detection of NTR content could refer to the protocol of kit. Briefly, the samples or standards were added into precoated plate. Then, horseradish peroxidase (HRP) labeled detection antibody were incorporated into each well for 60 min incubation at 37 ^{o}C. After the addition of 3,3',5,5'-tetramethylbenzidine (TMB), the samples turned to blue and were detected at a wavelength of 450 nm.

8. Intracellular fluorescence recovery

The fluorescence recovering assay in cells was detected by laser scanning confocal microscope (LSCM, Leica TCS SP5, Germany). HeLa or HUVEC were seeded in a 35 mm confocal dish (Φ = 15 mm) with a density of 1×10^4 cells per well and incubated overnight. And then, the media were replaced by fresh one containing RNNS-3/DNA complexes with N/P = 30 (300 ng of DNA). After co-incubation for different time (1 h, 2 h, 4 h and 6 h), the cells were washed by PBS (pH = 7.4) to remove the unused complexes which did not be internalized. The modified NIR dyes were excited by 663 nm laser and the channel (dichroic mirror 700 ~ 800 nm) was used to detect the emission signals.

9. In vitro gene transfection efficiency

In vitro gene transfection efficiency was detected on HeLa. The cells were seeded in 96-well plates with a density of 1×10^4 cells per well. After 24 h, the media were replaced with 100 μL of fresh media with or without 10 % FBS. Simultaneously, gene complexes containing 200 ng pEGFP

were added into per well with different N/P ratios (N/P = 20, 30, 40 and 60). Lipofectamine 2000 and PEI (MW = 25000) were acted as control groups. The dosage of Lipofectamine 2000 was used following the specification and the N/P ratio of PEI/ pEGFP was 10. Then, the media were replaced by the fresh one containing 10 % serum after 4 h incubation at 37 ^{o}C. The pictures of cells were taken by fluorescence microscope (Leica, Germany) after other 44 h. In addition, the transfection efficiency was quantitatively analyzed by flow cytometry. HeLa were seeded in 12-well culture plates at a density of 1×10^5 per well and grew overnight. Different complexes (RNNS-3/pEGFP, PEI/pEGFP, Lipo2000/pEGFP) containing 1 μg of pEGFP were added to each well. After transfection for 48 h, cells were washed by PBS, trypsinized and collected into tubes. The percentage of pEGFP positive expressing cells was determined with a flow cytometer (Millipore Guava) by counting at least 1×10^4 gated events per sample.

10. *In vivo* imaging and gene transfection

All the animal experiments were conducted with the approval of the Institutional Animal Care and Use Committee of Sichuan University (Chengdu, China) as well as the Regulations for the Administration Affairs Concerning Experimental Animals. Healthy BALB/c nude mice (18-20 g) were lived in a pathogen-free environment according to NIH guidance and fed with sterilized food and distilled water. HeLa (1×10^7) were injected into the right flank region of female BALB/c nude mice to form a xenograft tumor model. When the tumor reached 150-200 mm^3, the nude mice were randomly divided into two groups, which received intratumoral injection of RNNS-3/pEGFP (N/P = 30) or PEI/pEGFP (N/P = 10) complexes with dosage of 5 μg pEGFP, respectively. At the desired time (2, 4 and 6 h), mice were observed by CRI Maestro Imaging System (Cambridge Research and Instrumentation Inc., USA). In 6 h, some mice in RNNS-3/pEGFP group were sacrificed, and the tissues containing heart, liver, spleen, lung, kidney, and tumor were isolated for Near Infrared (NIR) imaging. In 48 h, mice were sacrificed, and the isolated tumors were immediately frozen and cut into 10 μm thick sections for fluorescence microscope observation (Leica, Germany).

11. Statistical analysis

Every experiment repeated at least five times. Data are presented as mean ± standard deviations (mean ± S.D.). Differences was evaluated by one-way ANOVA using Graghpad Prism 7.0 (GraphPad Software Inc, California, USA). A p value of <0.05 was considered statistically significant.

Table S1. The particle size and zeta potential of assemblies and their gene complexes.

	Molar Ratio of RNNF/RLS	N/P	Z-Average (nm)	PDI	Zeta potential (mv)
RNNF	-	-	341.4±6.8	0.31±0.02	29.4±0.8
RLS	-	-	169.8±3.6	0.25±0.07	27.1±0.3
RNNS-1	1/1	-	184.8±1.3	0.19±0.008	20.5±0.9
RNNS-2	1/2	-	142.1±0.1	0.27±0.003	22.5±0.9
RNNS-3	1/3	-	136.6±1.5	0.25±0.1	21.9±1.2
RNNS-4	1/4	-	182.3±2.3	0.27±0.03	21.6±0.3
RNNS-1/DNA	1/1	30	188.7±0.3	0.21±0.01	18.6±1.1
RNNS-2/DNA	1/2	30	197.0±0.5	0.28±0.01	21.2±0.4
RNNS-3/DNA	1/3	30	177.9±1.1	0.16±0.01	19.2±1.4
RNNS-4/DNA	1/4	30	227.3±1.8	0.19±0.01	20.5±1.2

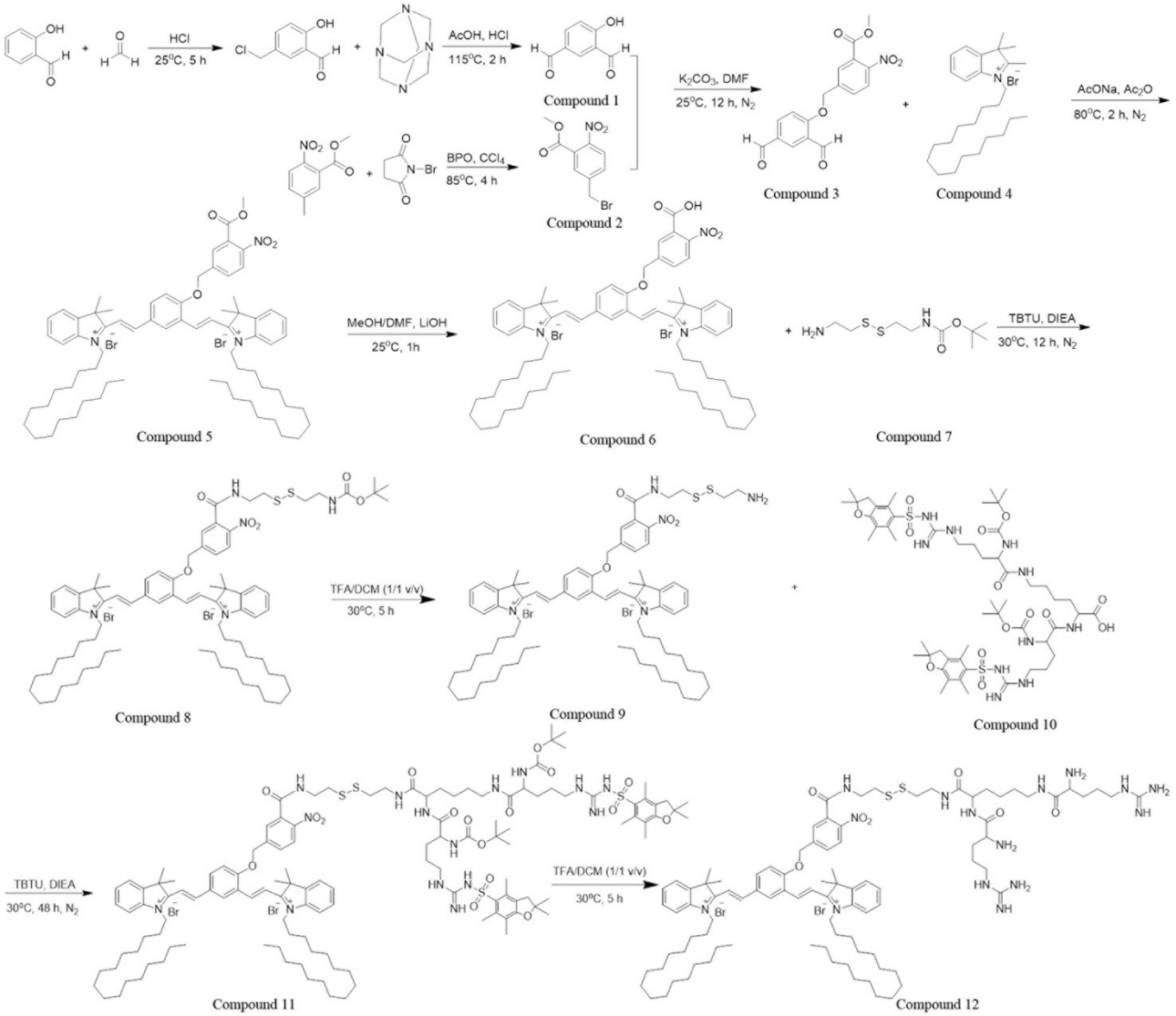

Figure S1. Synthesis of nitroreductase (NTR) and glutathione (GSH)-responsive amphiphilic molecule (RNNF molecule).

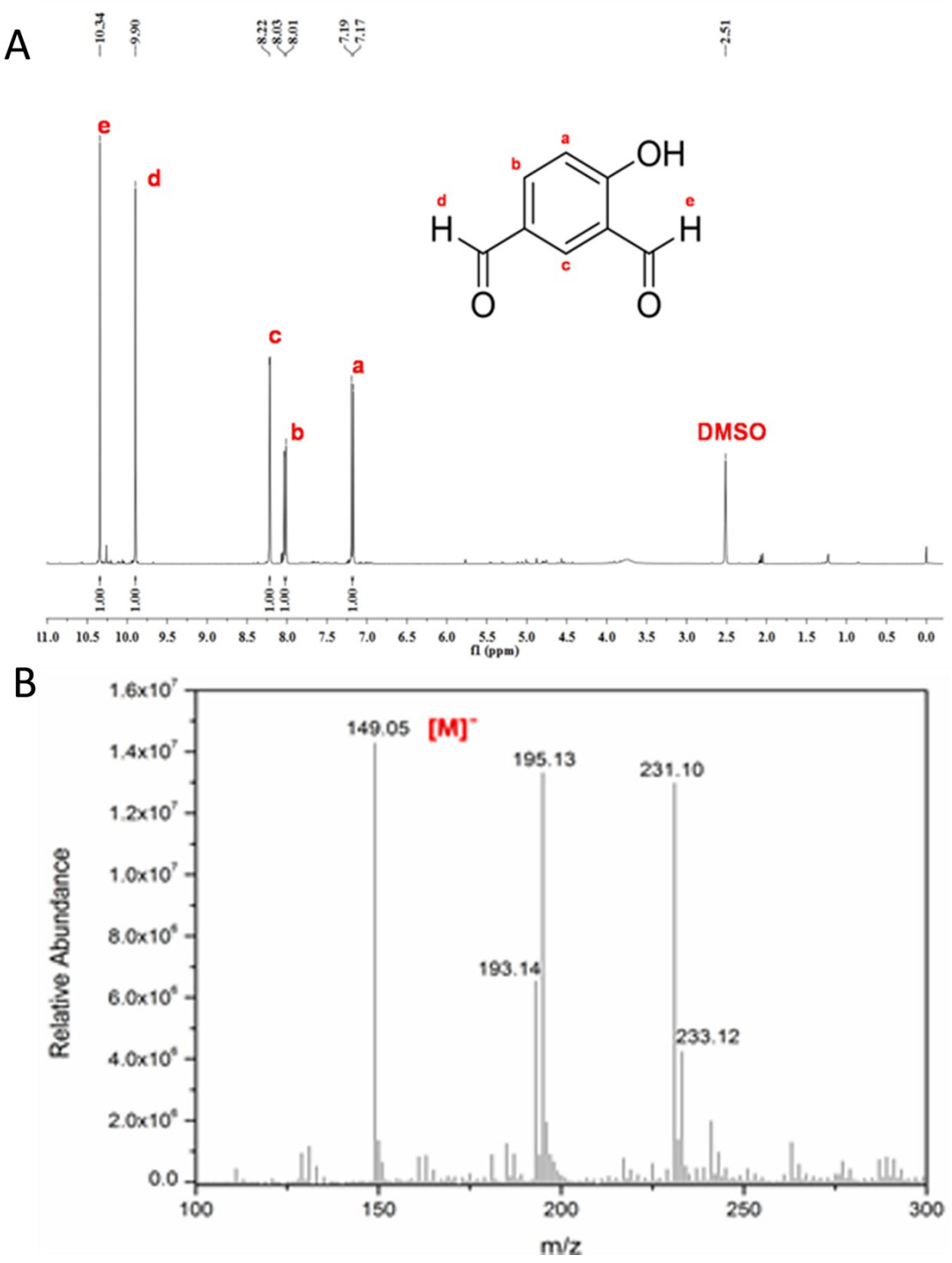

Figure S2. Chemical characterization (^{1}H NMR spectrum (A) and ESI MS (B)) of compound **1**.

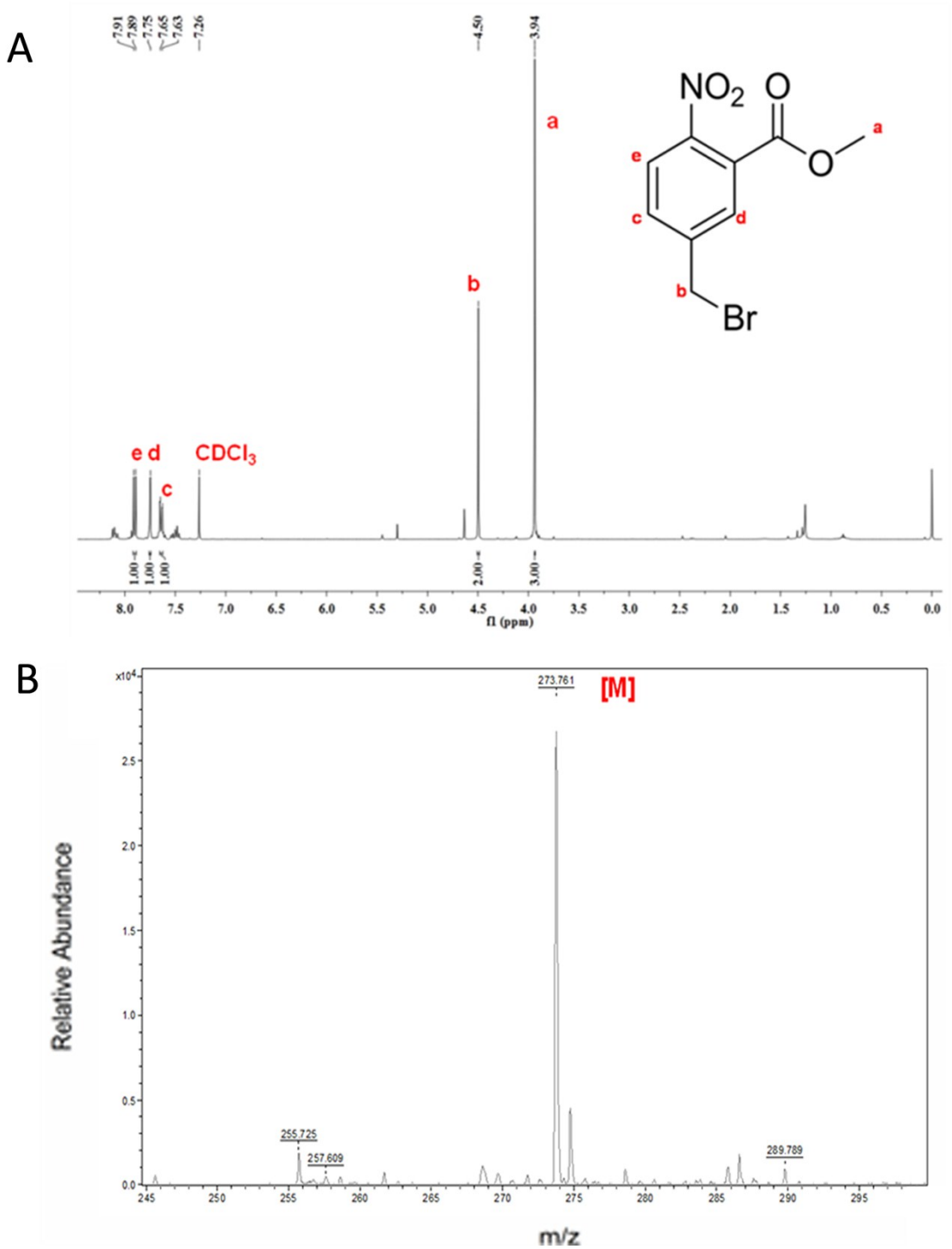

Figure S3. Chemical characterization (^{1}H NMR spectrum (A) and ESI MS (B)) of compound **2**.

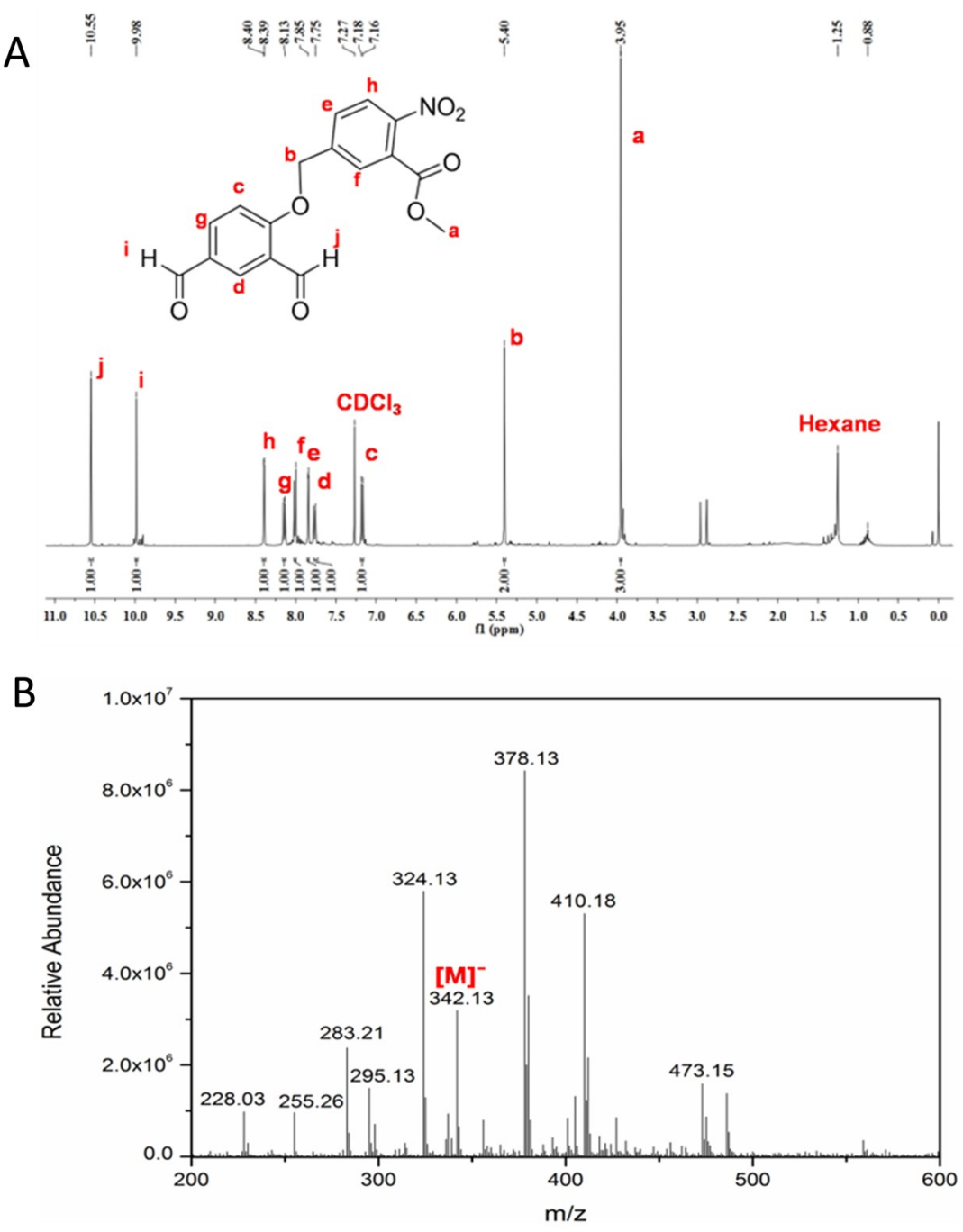

Figure S4. Chemical characterization (^{1}H NMR spectrum (A) and ESI MS (B)) of compound **3**.

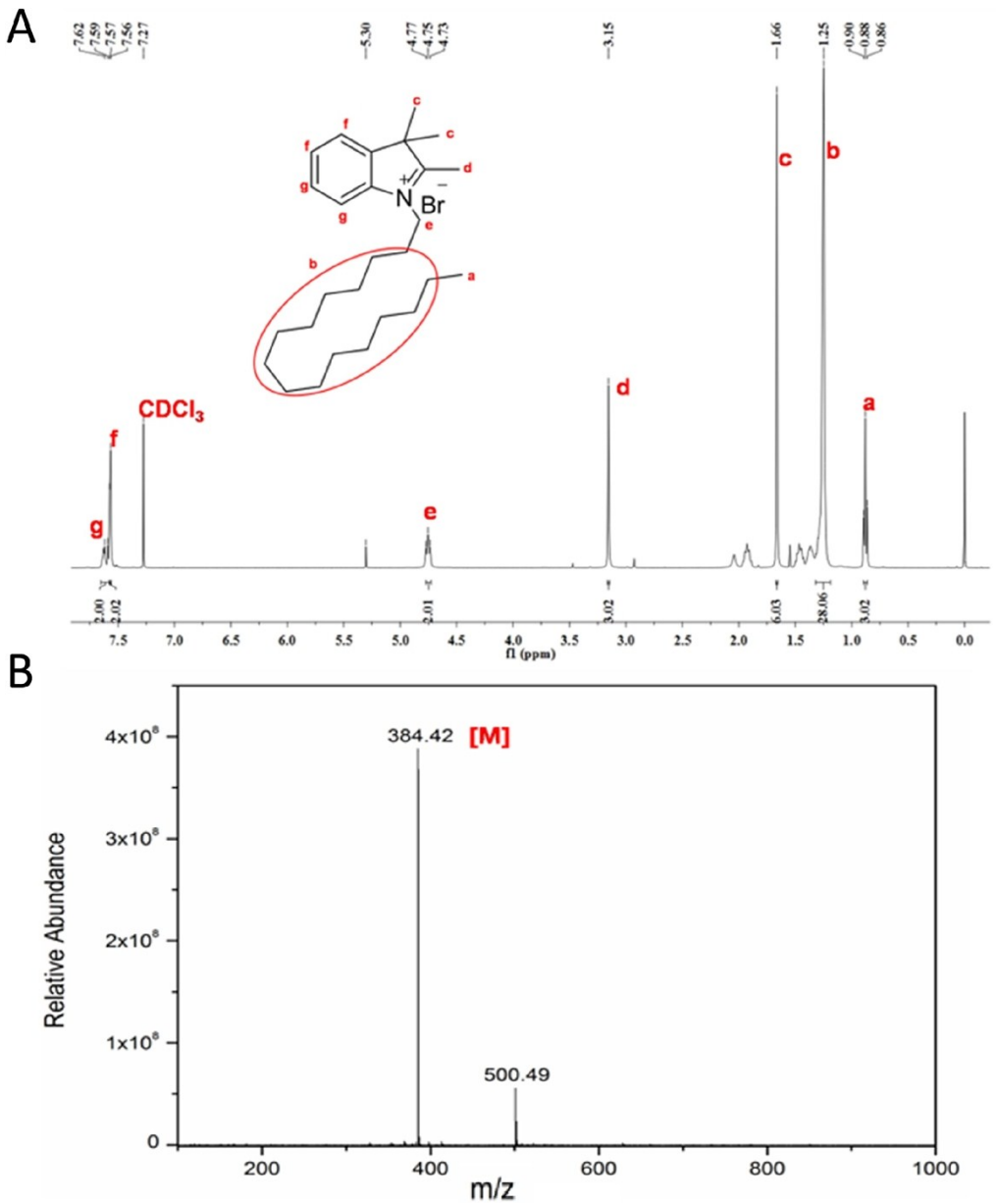

Figure S5. Chemical characterization (^{1}H NMR spectrum (A) and ESI MS (B)) of compound **4**.

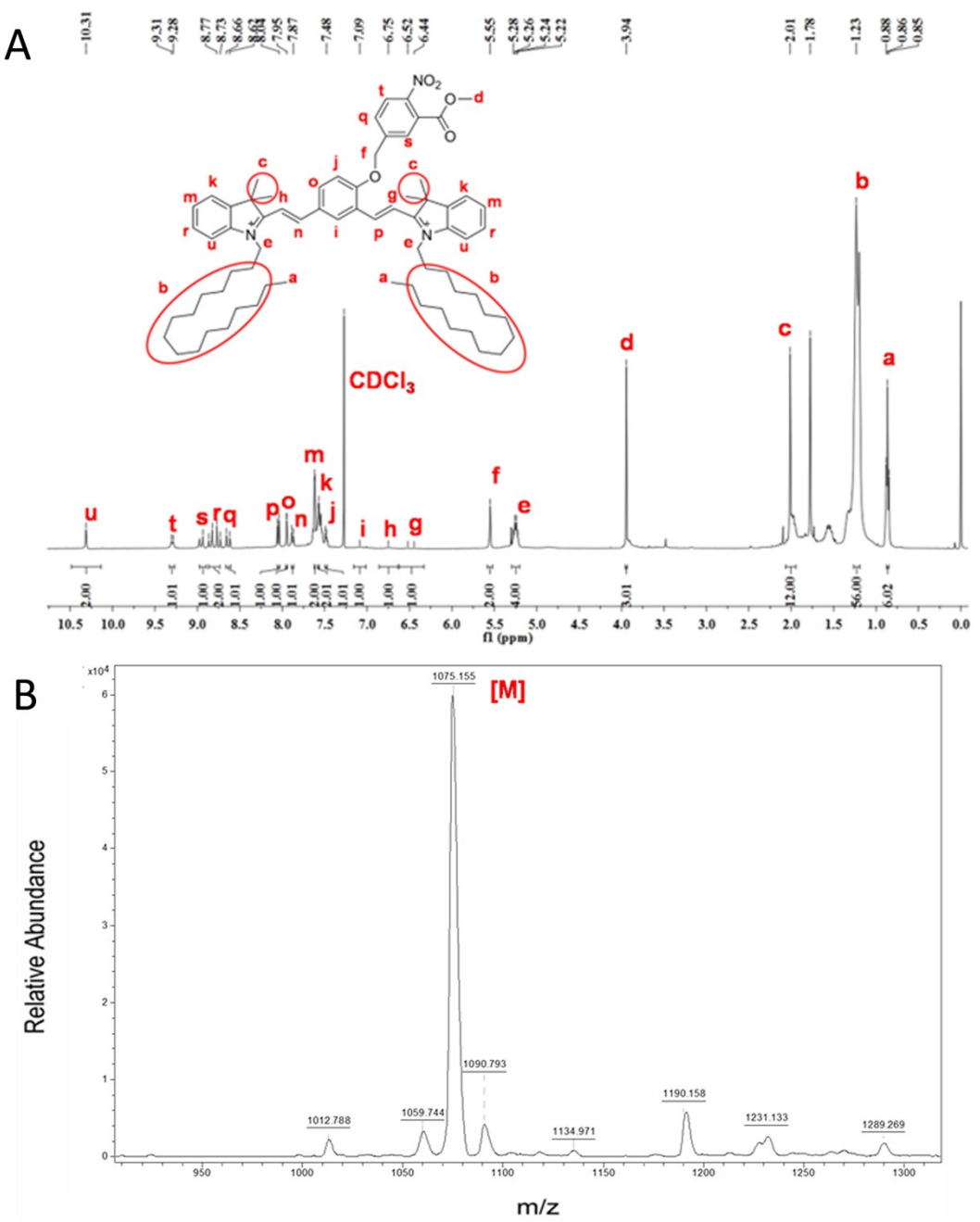

Figure S6. Chemical characterization (^{1}H NMR spectrum (A) and ESI MS (B)) of compound **5**.

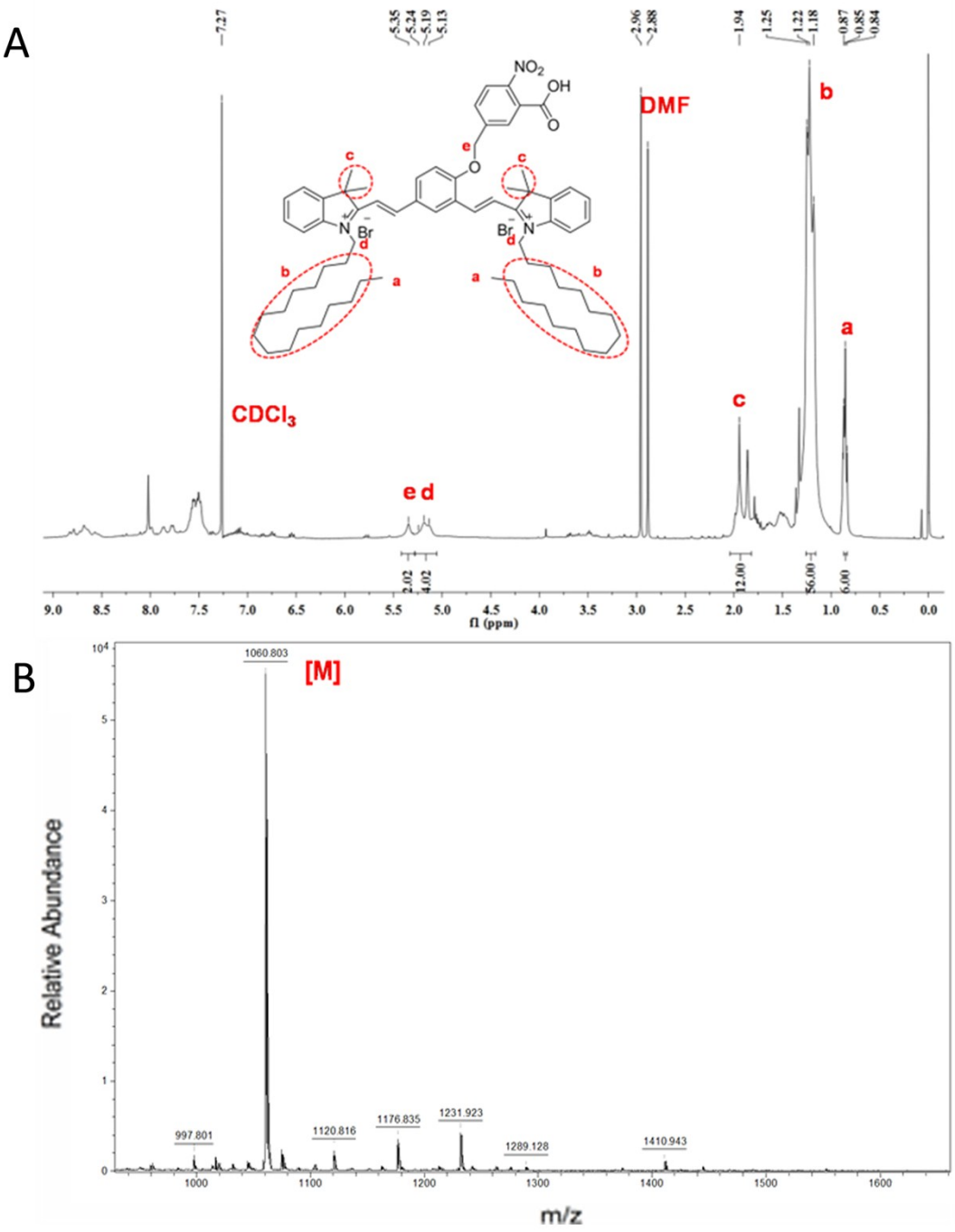

Figure S7. Chemical characterization (^{1}H NMR spectrum (A) and ESI MS (B)) of compound **6**.

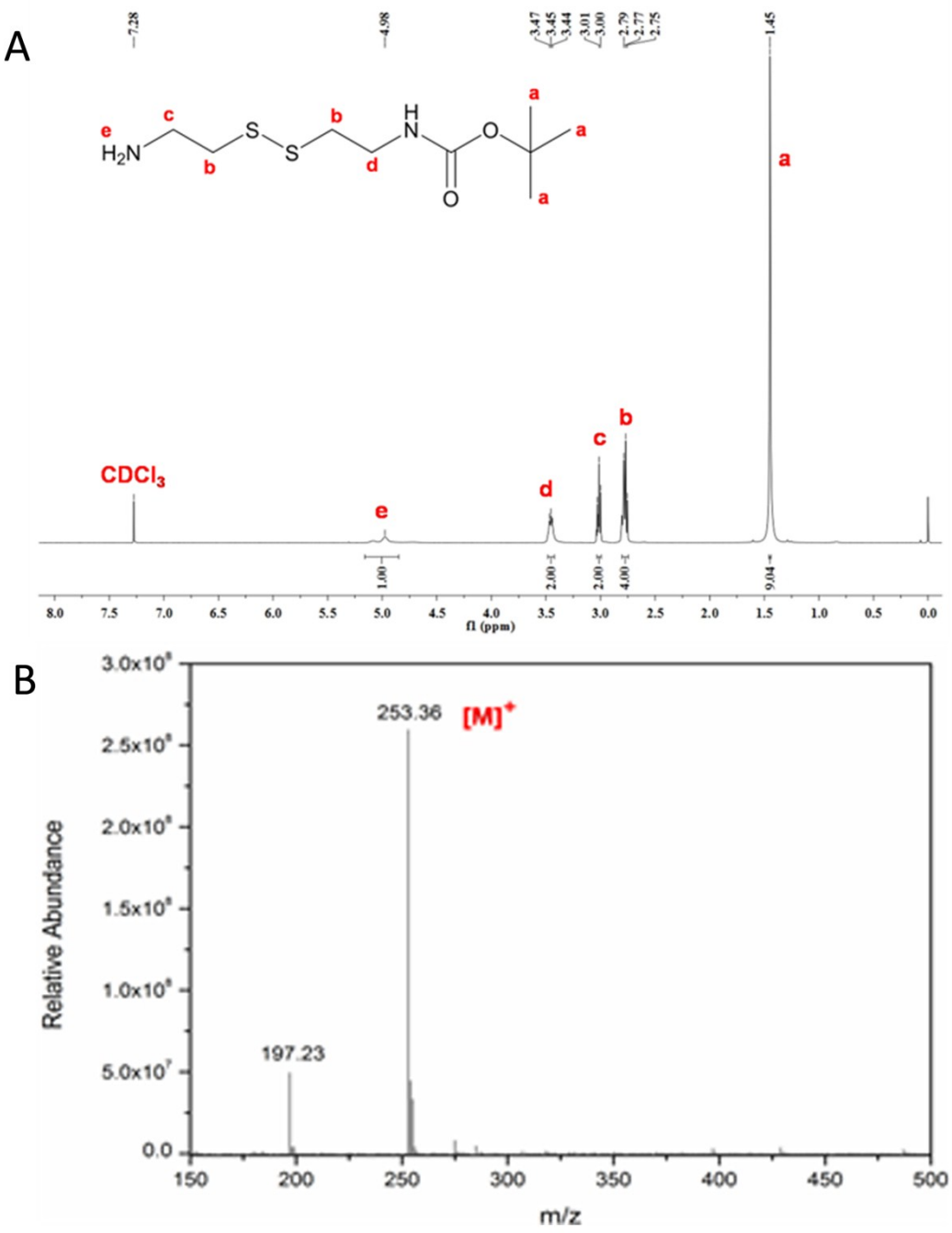

Figure S8. Chemical characterization (^{1}H NMR spectrum (A) and ESI MS (B)) of compound **7**.

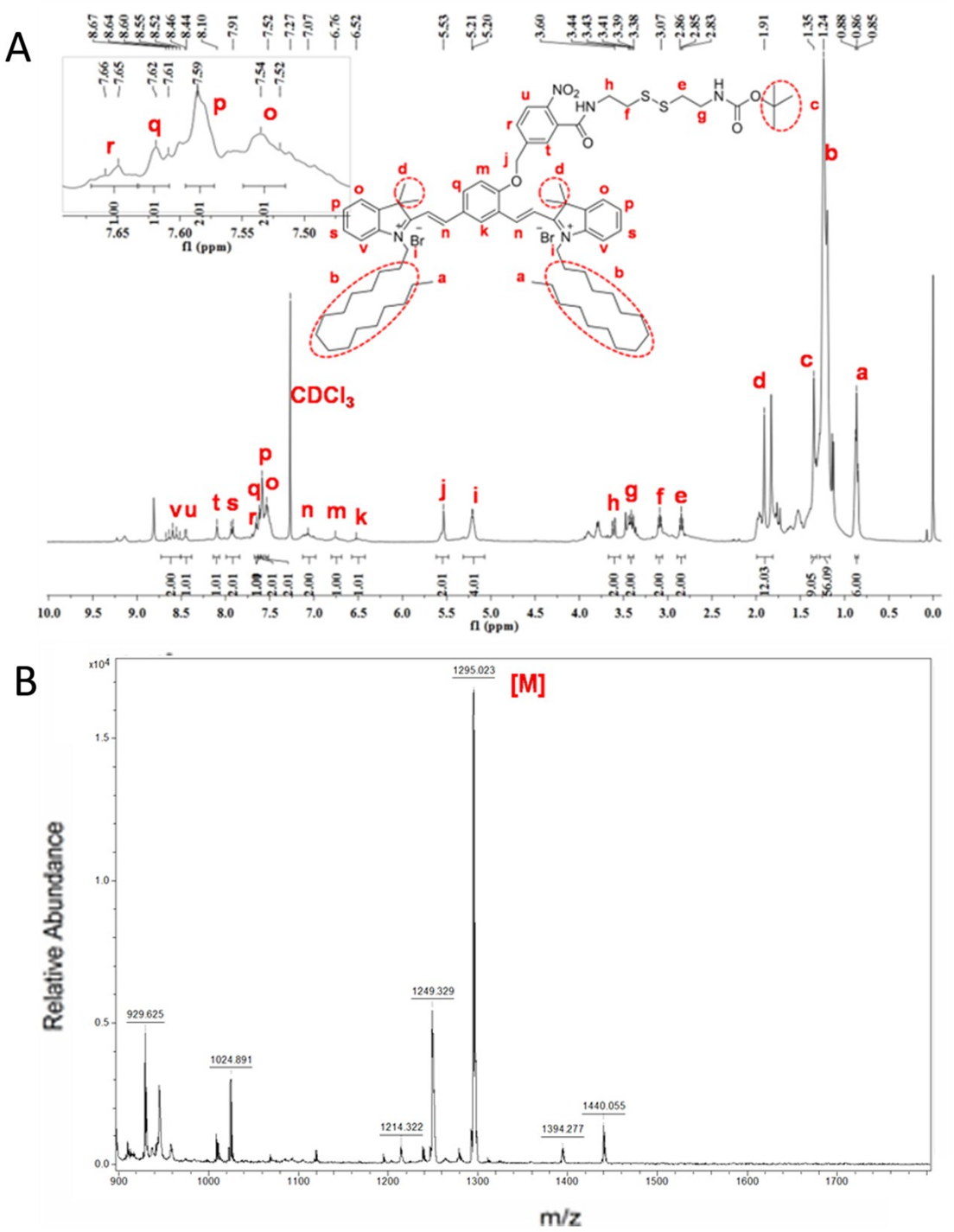

Figure S9. Chemical characterization (^{1}H NMR spectrum (A) and ESI MS (B)) of compound **8**.

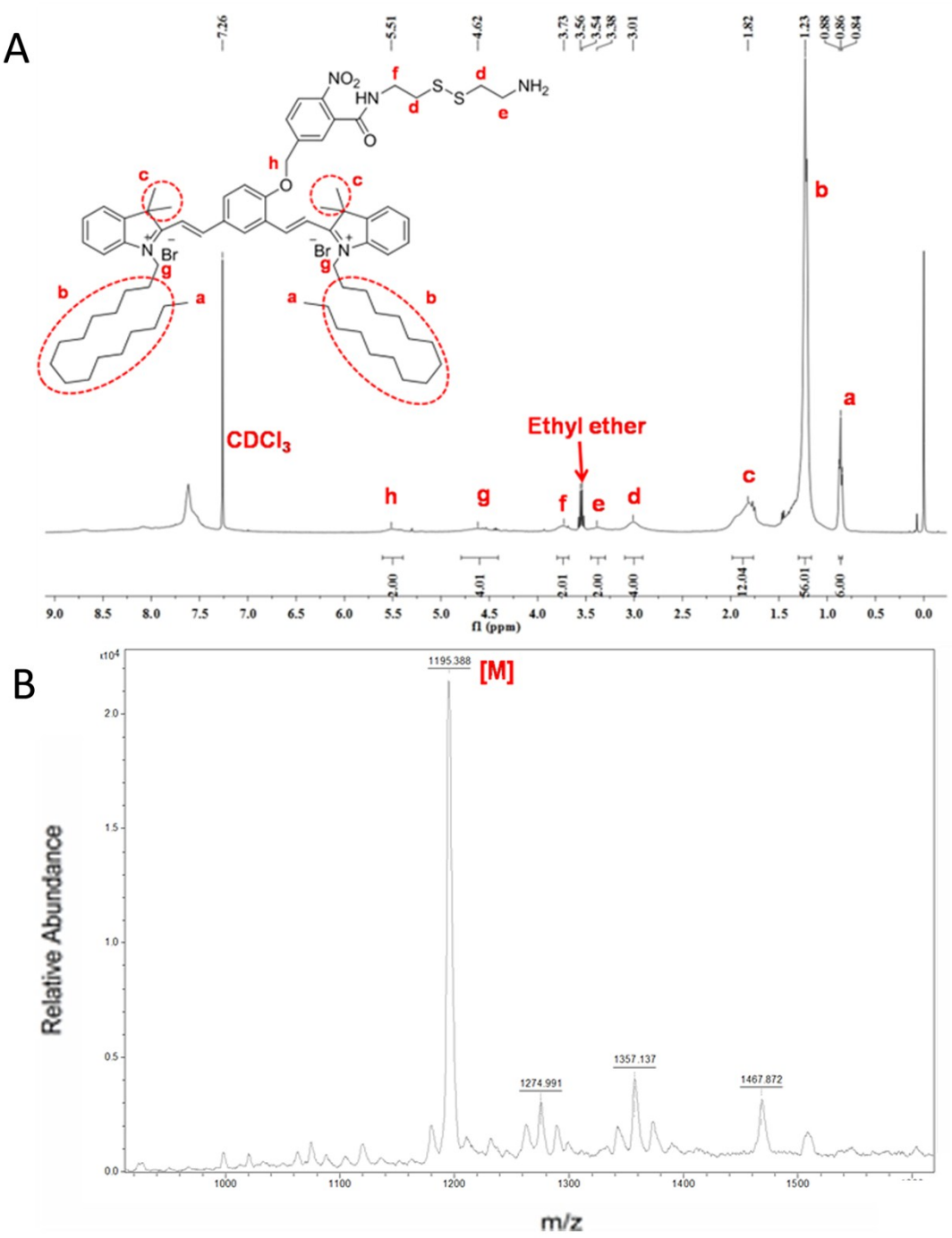

Figure S10. Chemical characterization (^{1}H NMR spectrum (A) and ESI MS (B)) of compound **9**.

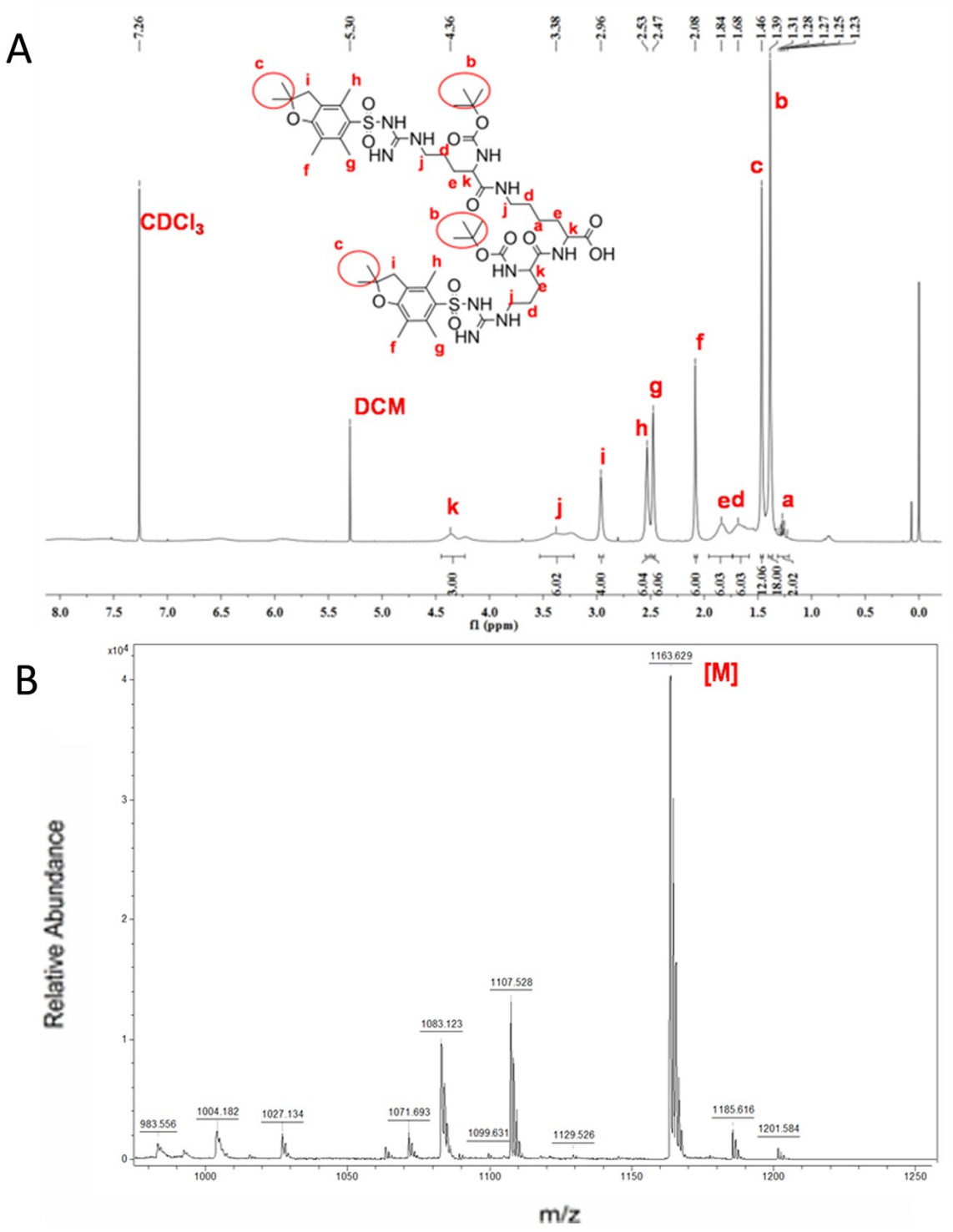

Figure S11. Chemical characterization (^{1}H NMR spectrum (A) and ESI MS (B)) of compound **10**.

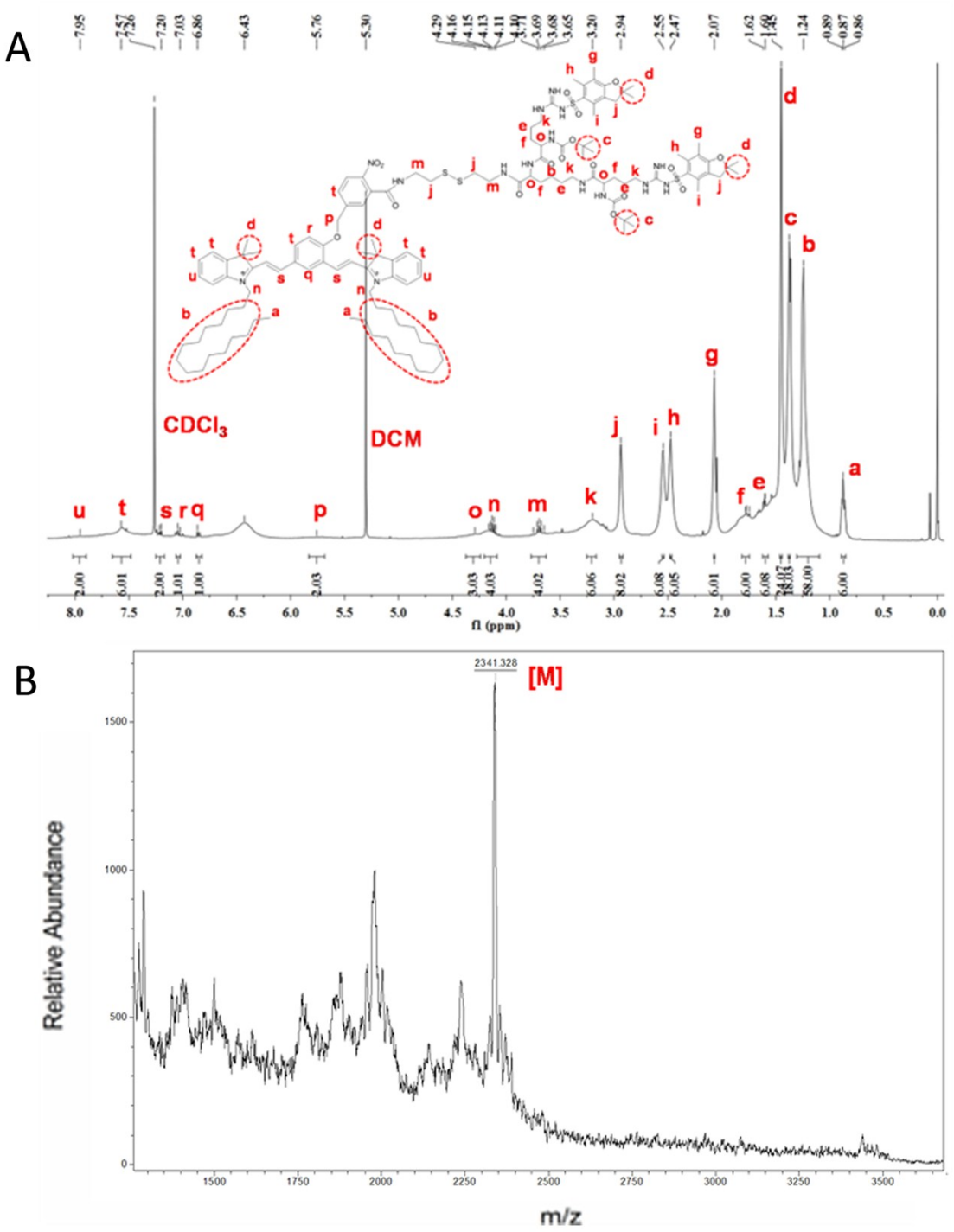

Figure S12. Chemical characterization (^{1}H NMR spectrum (A) and ESI MS (B)) of compound **11**.

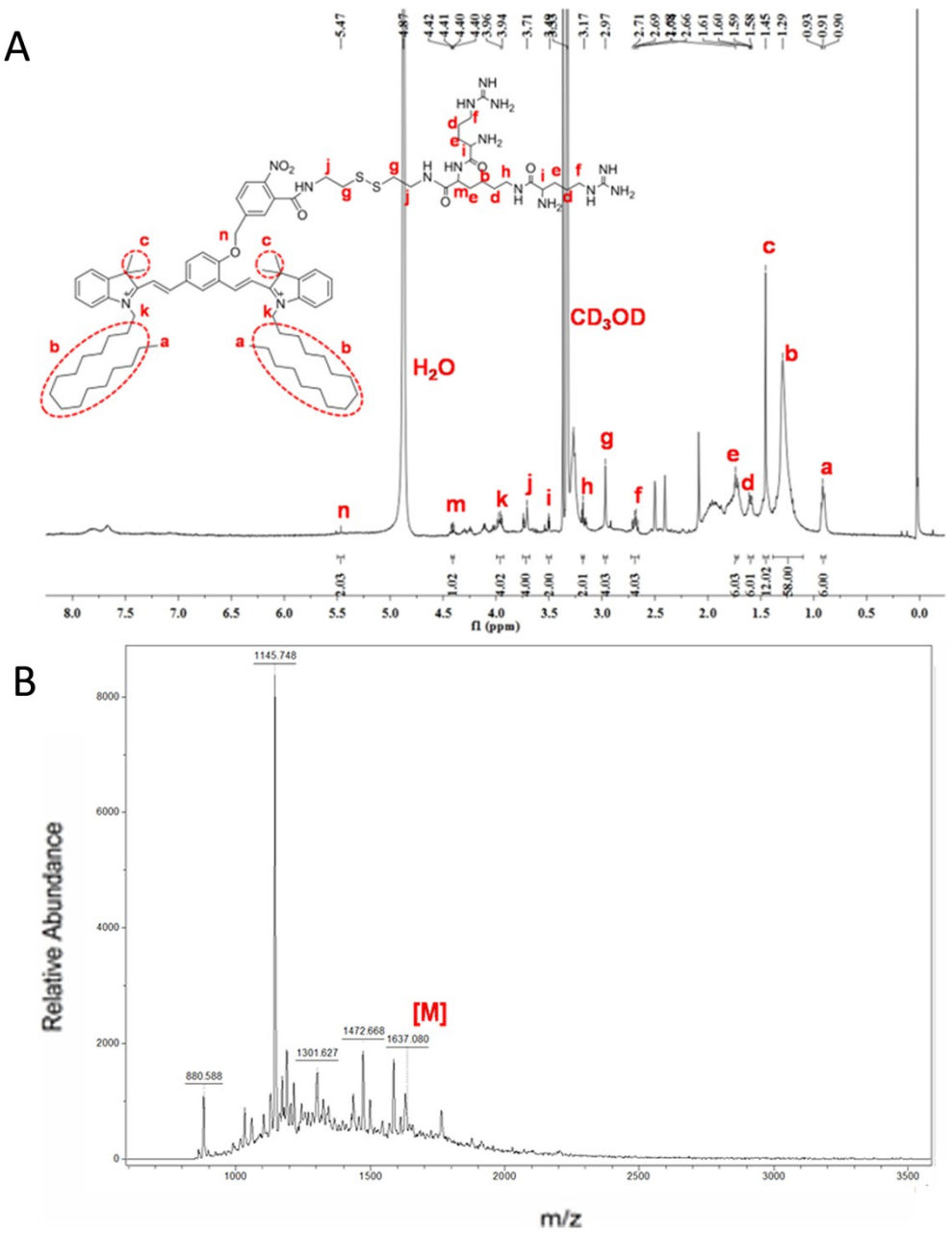

Figure S13. Chemical characterization (^{1}H NMR spectrum (A) and ESI MS (B)) of compound **12**.

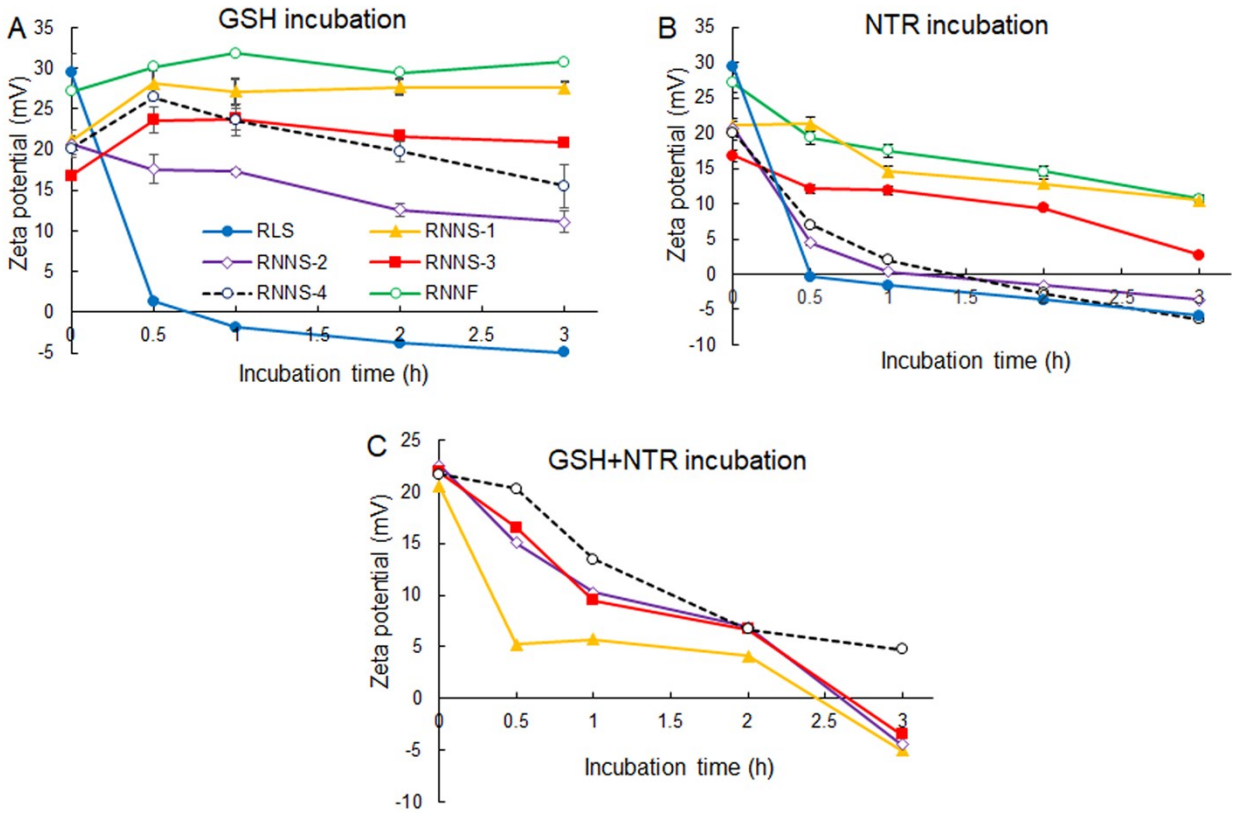

Figure S14. The changes of zeta potential with time for various assemblies (RNNF, RLS and RNNS) after incubation with 10 mM GSH (A), 5 μg/mL NTR and 1 mM NADH (B), or mixture of GSH and NTR (C), respectively. Date are presented as means ± SD (n = 5).

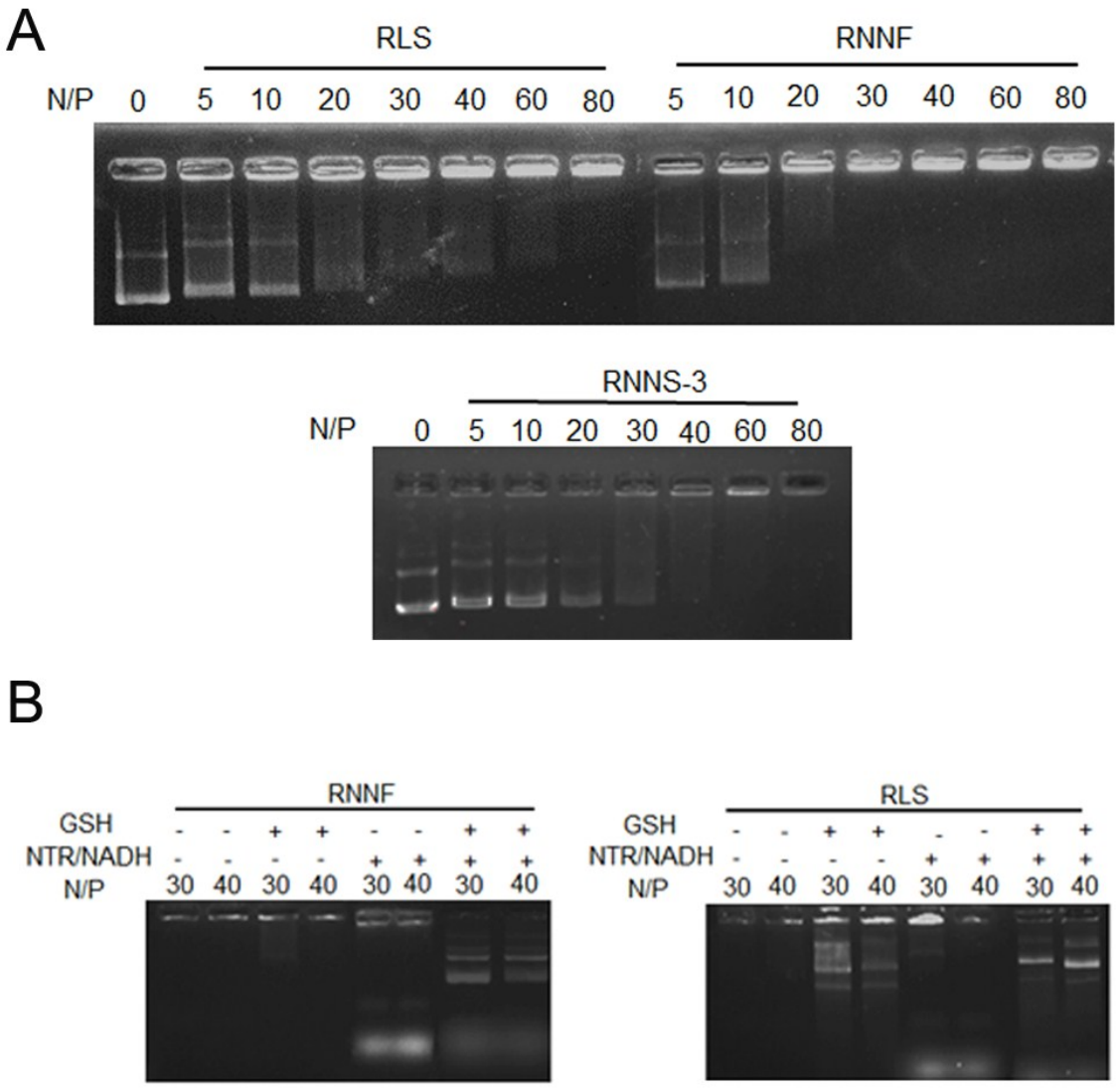

Figure S15. (A) Gel retardation assay of various assemblies/DNA complexes at different N/P ratios (0, 5, 10, 20, 40, 60). (B) The gene release ability of RNNF and RLS in the presence of 10 mM GSH, 5 μg/mL NTR and 1 mM NADH, or mixture of GSH and NTR, respectively.

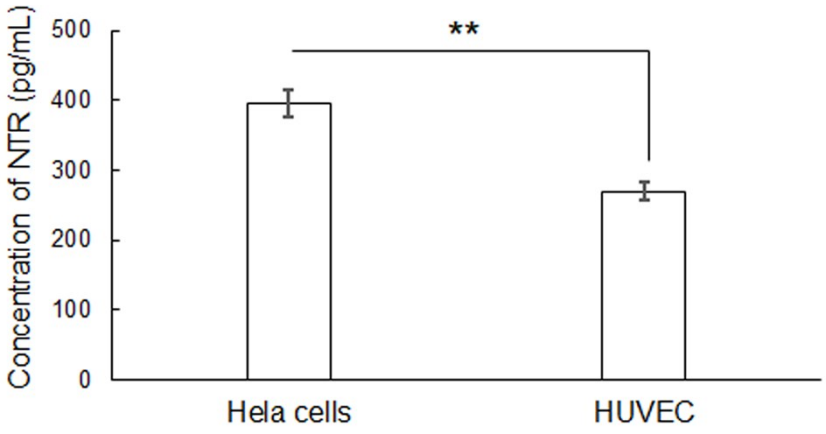

Figure S16. The content of NTR in Hela and HUVEC detected by Human NTR ELISA Kit. Date are presented as means ± SD (n = 5). ** $p < 0.01$.

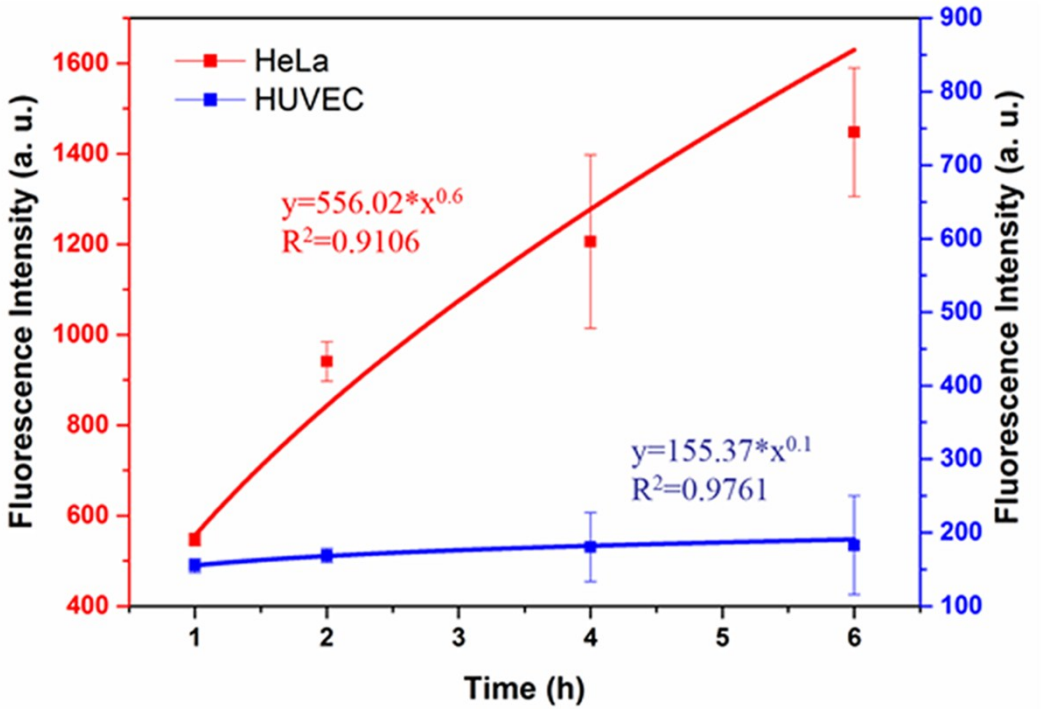

Figure S17. The semi-quantitative evaluation of fluorescence recovery in vitro on HeLa (red line) and HUVEC (blue line). Data are presented as means ± SD (n = 5).

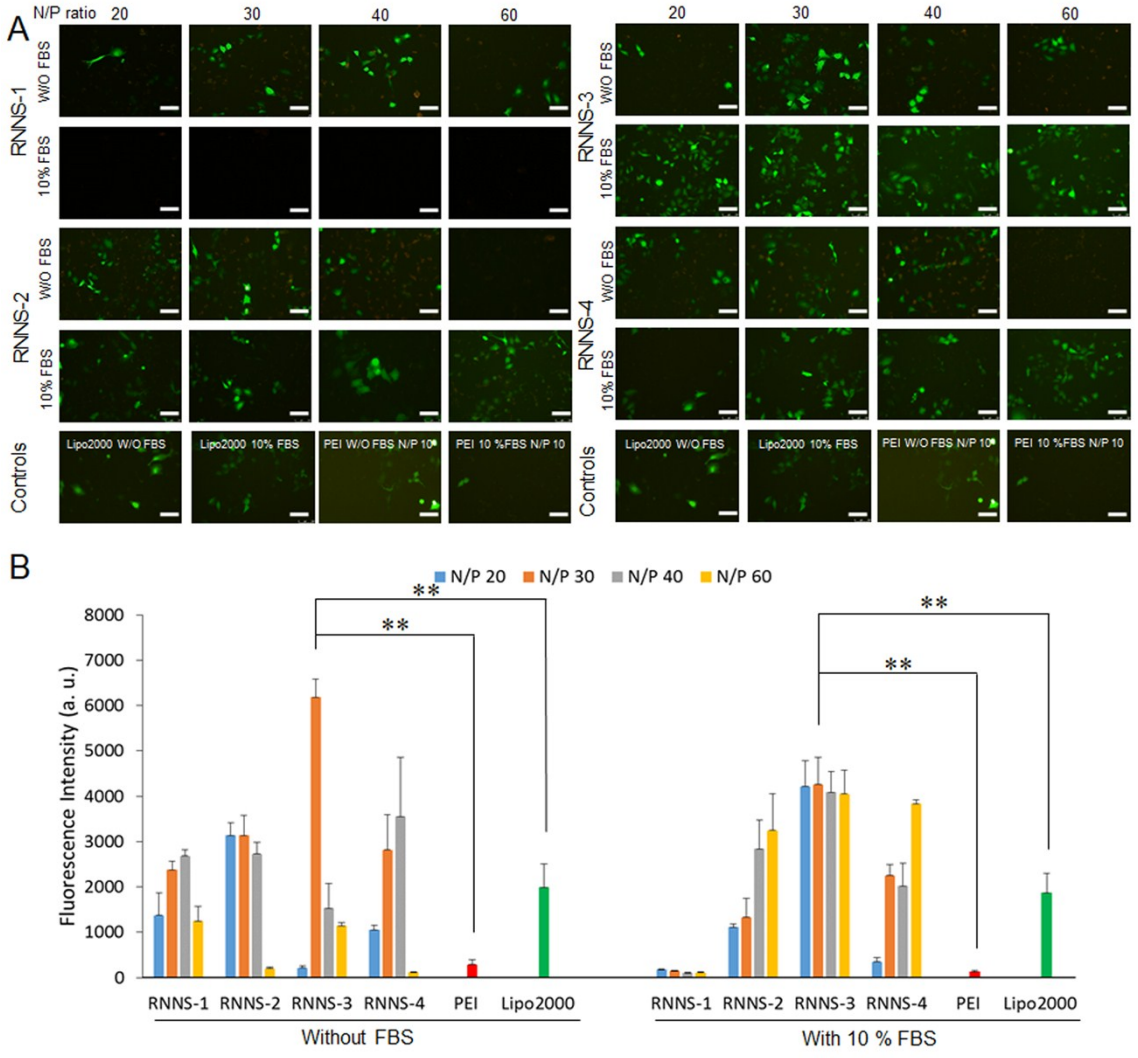

Figure S18. *In vitro* gene transfection efficiency on Hela. (A) Fluorescence microscopy images of Hela with EGFP transfection for 48 h in the culture medium without or with 10% FBS. pEGFP plasmid DNA were condensed by RNNS assemblies at different N/P ratios (20, 30, 40 and 60). Lipofectamine 2000 and PEI (MW = 25000) were acted as control groups. The N/P ratio of PEI/pEGFP complexes were 10. Scale bar= 100 μm. (B) Semi-quantification evaluation of EGFP positive cell percentage and mean fluorescent intensity (MFI) of Hela incubated with various complexes by Image-Pro Plus 6.0 software. Data are presented as means ± SD (n = 5), ** $p < 0.01$.

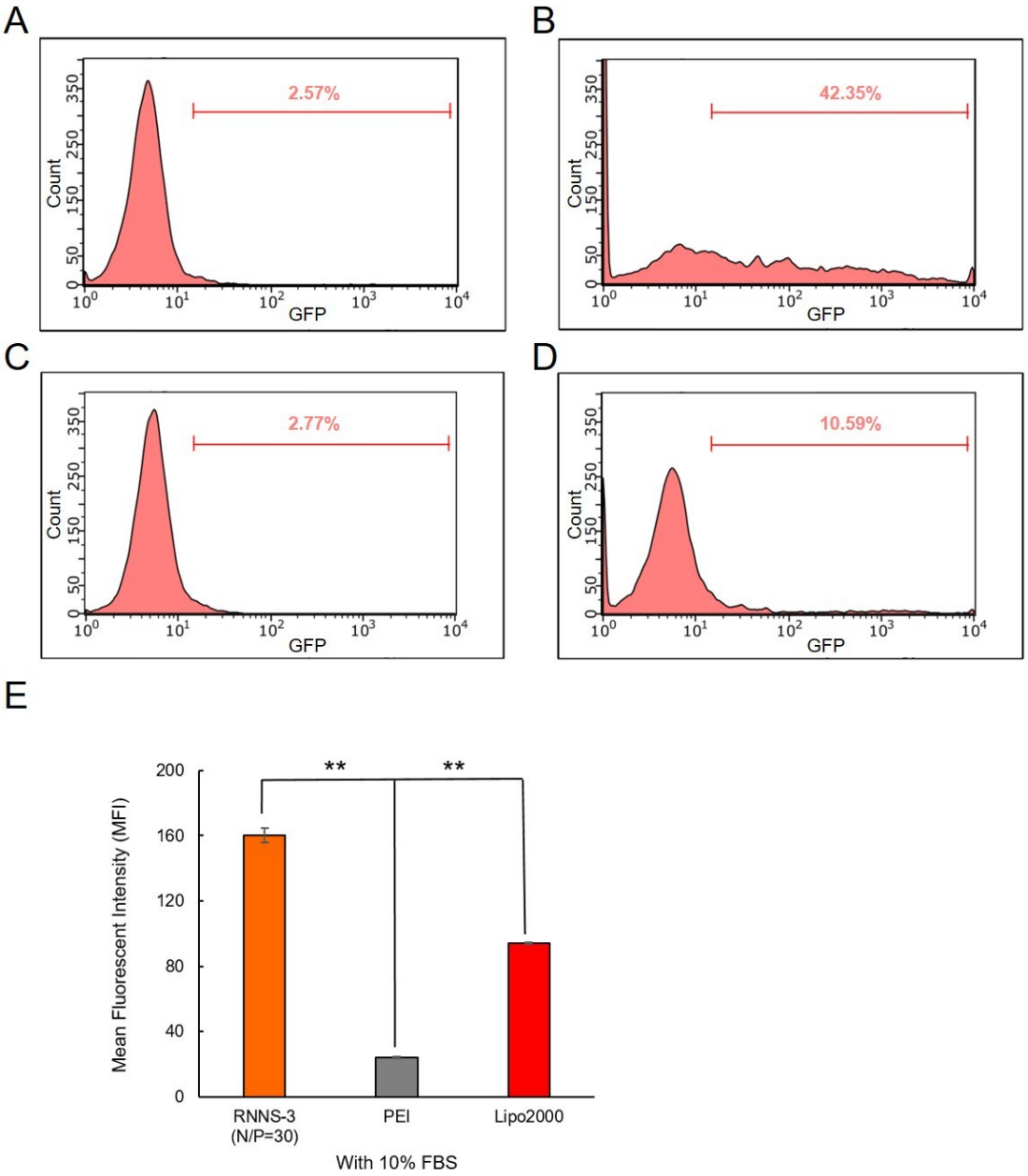

Figure S19. Representative flow cytometry data of various gene complexes (A) control, (B) RNNS-3/pEGFP, (C) PEI/pEGFP and (D) Lipo2000/pEGFP were detected on HeLa after 48 h incubation. (E) EGFP mean fluorescent intensity (MFI) of Hela incubated with various complexes by flow cytometry. Date were presented as means ± SD (n = 5). ** $p < 0.01$.

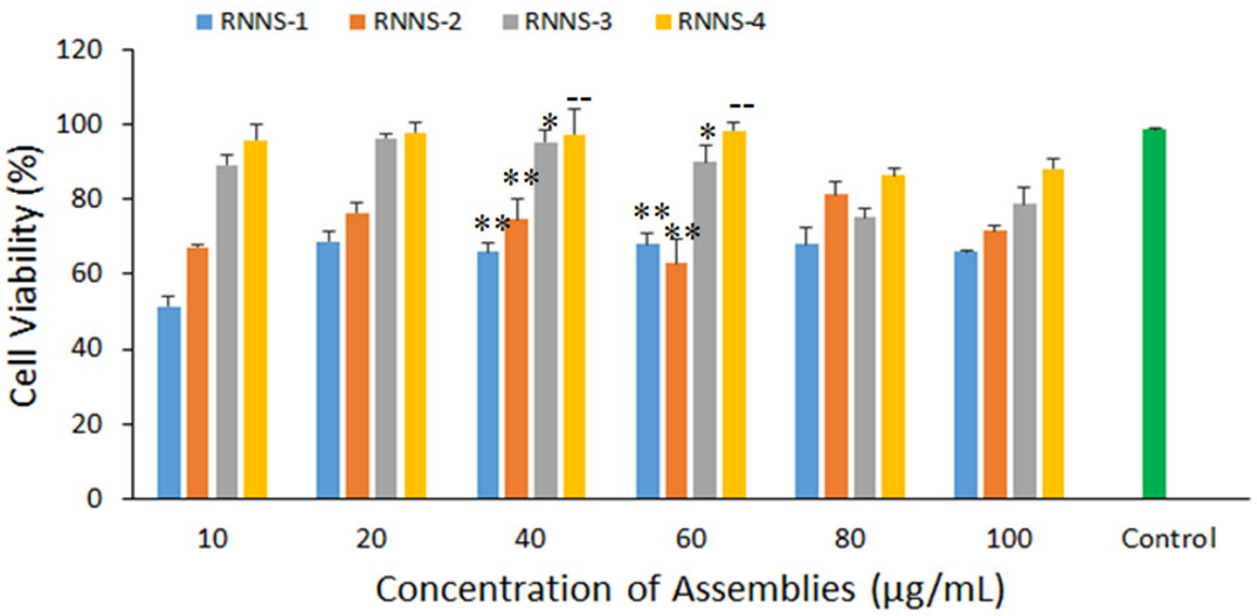

Figure S20. Cell viability of various formulated assemblies (RNNS-1, 2, 3 and 4) at different concentrations (10-100 μg/mL) by CCK-8 assay was detected on HeLa after 48 h incubation. Date were presented as means ± SD (n = 5). "*" $p < 0.05$, "**" $p < 0.01$, "--" no significant difference versus control.

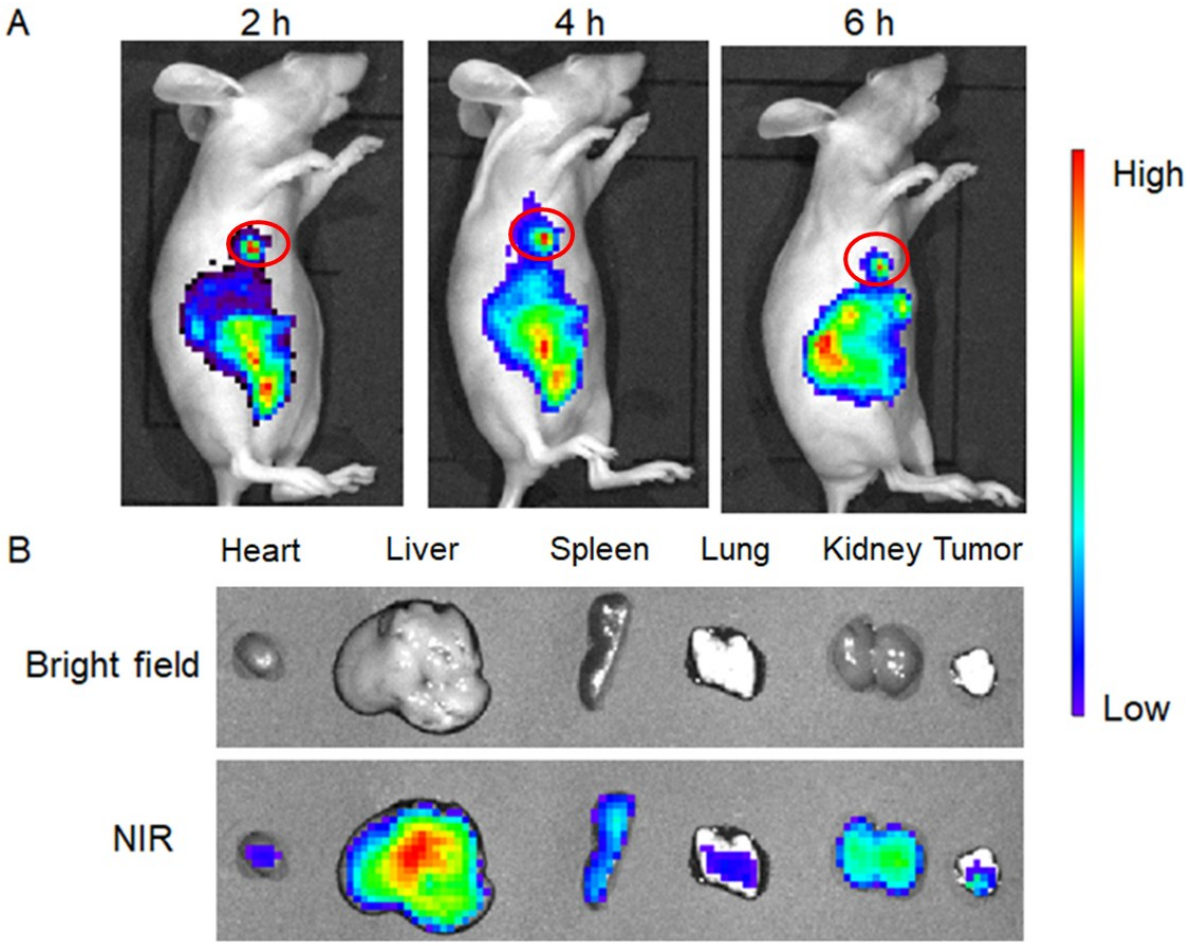

Figure S21. Fluorescence imaging for the RNNS-3/pEGFP complexes (N/P = 30) through intratumoral injection. (A) *In vivo* fluorescence imaging detected from NIR channel at different time (2, 4 and 6 h) after injection, respectively. The tumor site was marked by red circle. (B) The 6 h imaging of isolated tissues detected from NIR channel after injection (heart, liver, spleen, lung, kidney, and tumor), respectively.