Supporting Information

for

Supramolecular crafting of stimuli-responsive, carrier-free, self-deliverable nanoparticles of Camptothecin and antisense DNA for combination cancer therapy

Devanathan Perumal, Jemshiya Kalathil, Jithu Krishna, Gowtham Raj, Kaloor S. Harikrishnan, Uthpala M. L., Ria Gupta and Reji Varghese*

Indian Institute of Science Education and Research Thiruvananthapuram

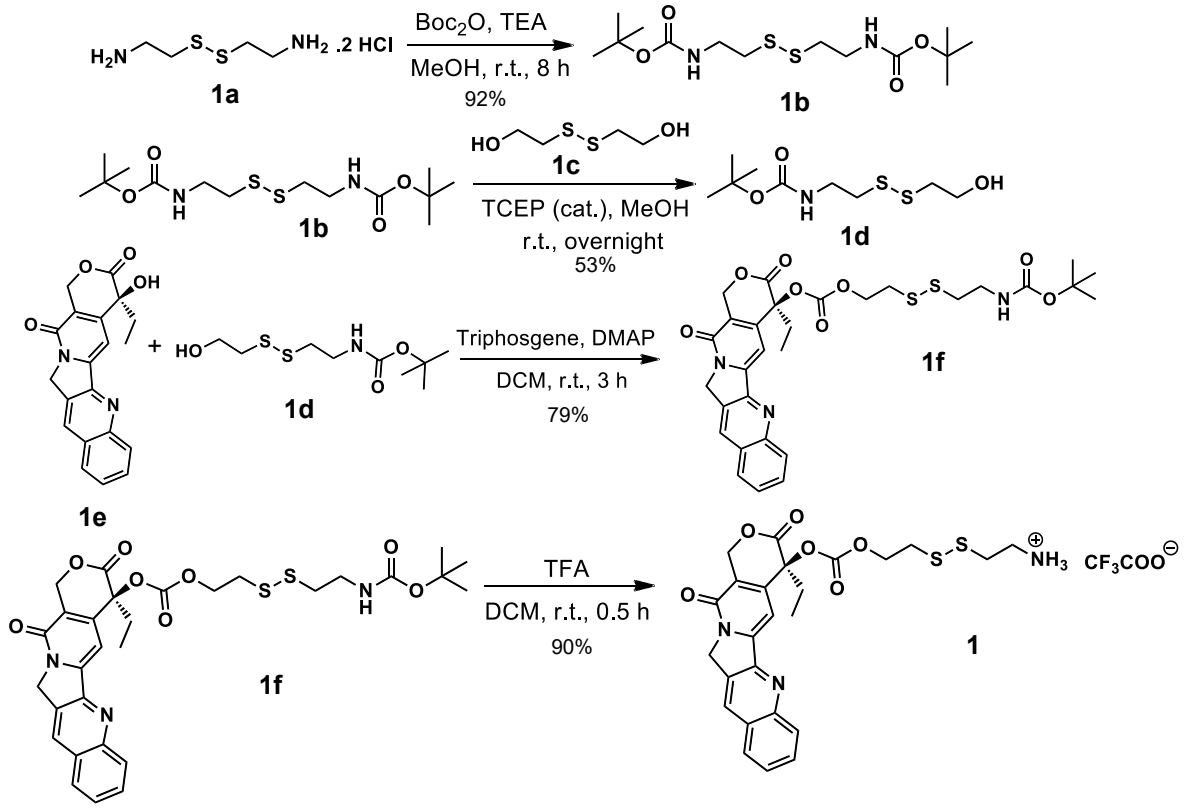
(IISER-TVM) Trivandrum-695551

Email: reji@iisertvm.ac.in

TABLE OF CONTENTS

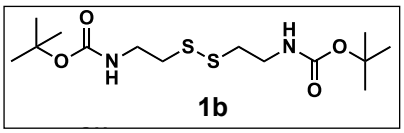
2. Experimental section

All chemicals used for the organic syntheses were purchased from Sigma Aldrich (http://www.sigmaaldrich.com/india.html) and were used as received. Solvents were dried following the standard procedures. TLC analyses were done on aluminium plates coated with silica gel 60 F254. Column chromatography was performed on 200-400 mesh silica gel. Melting points were measured on Stuart SMP30 melting point apparatus and are uncorrected. ^{1}H and ^{13}C-NMR spectra were recorded on 500 MHz Bruker Avans spectrometer using 1,1,1,1-tetramethylsilane (TMS) as internal standard. Water used for all studies was Milli Q deionised water (18.2 MΩ.cm). GC-MS analyses were performed on a Shimadzu GC-MS, QP-2010. Measurements were done in EI mode. ESI-HRMS measurements were performed on Thermal orbitrap mass spectrometer (Thermo exactive). Measurements were done in positive ion mode. AFM analyses were carried out on Multimode SPM (Veeco Nanoscope V). Samples were prepared by drop casting 2 μL solution of the sample on a freshly cleaved mica surface and dried under air. Imaging was done under ambient conditions in tapping mode. The probe used for imaging was antimony doped silicon cantilever with a resonant frequency of 300 kHz and spring constant of 40 Nm^{-1}. TEM analyses were carried out on FEI Tecnai TF30 HR-TEM. Samples were prepared by depositing 2 μL of the sample on a 400-mesh carbon coated copper grid (Ted Pella, Inc.). Samples were allowed to adsorb on the grid for 2 min and then excess sample was wicked with a piece of filter paper. Then the grid was dried under air. Absorption spectra were recorded using quartz cuvette of 10 mm path length on a Shimadzu UV-3600 Vis-NIR Spectrophotometer. Steady state fluorescence spectra were recorded on Horiba Jobin Yvon Fluorimeter equipped with thermostat peltier cell holder, in a quartz cuvette of 10 mm path length. Dynamic Light Scattering (DLS) was performed on Malvern Zetasizer Nano ZS equipped with 655 nm laser. Experiments were done at 20 °C at a back-scattering angle of 173º. The Hela Kyoto and MCF-7 cells were obtained from NCCS Pune, cultured in DMEM media supplied with 10% Fetal bovine serum with 1% penicillin-streptomycin and cultured at 37 °C in 5 % CO2 incubator. Both the mediums, FBS and antibiotics were purchased from Gibco-Thermo Fisher Scientific and the media. Flow cytometry analyses were carried out using the BD FACSAria™ III. Confocal microscopic images have been carried out using Nikon Eclipse Ti with a 100 X oil immersion objective. MTT assay has been carried on Tecan Infinite 200 PRO microplate reader.

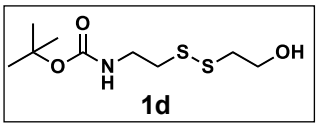


Scheme S1*: Synthesis scheme for the multi-step synthesis of* ***1****.*

Synthesis of 1b: To a solution of cystamine dihydrochloride (3.0 g, 13.76 mmol) in MeOH



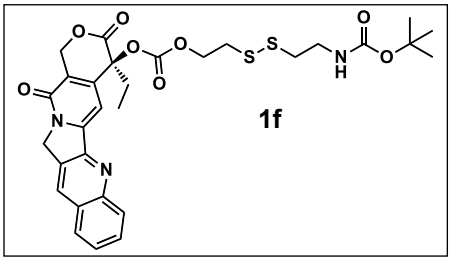
was added a solution of di-tert-butyl dicarbonate (12.3 g, 55 mmol) in MeOH and TEA (11.62 mL, 11.5 mmol) in a dropwise manner. The reaction mixture was stirred at room temperature for 8 h. The solvent was removed under vacuum and the crude reaction mixture was extracted with DCM/H_2O and then washed with 10% citric acid, followed by 0.5N NaOH solution. The organic layer was collected, dried over anhydrous Na_2SO_4 and then concentrated under vacuum to get desired product as off white solid (92% yield). TLC (PE: EA, 50:50), R_f = 0.51; ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm) = 6.97 (t, J= 5 Hz, 2H), 3.22 (t, J= 5 Hz, 4H), 2.76 (t, J= 5 Hz, 4H), 1.39 (s, 18H); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm) = 156.0, 78.28, 38.14, 28.69; HR-MS (m/z): $[M+Na]^+$ $C_{14}H_{22}N_2O_4S_2Na$: 375.14 (cal.), 375.04 (expt.).



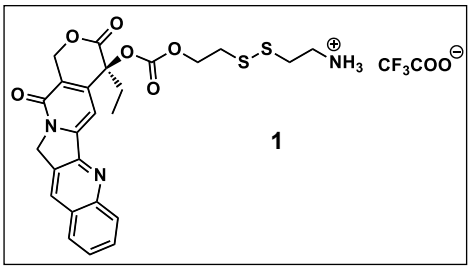
Synthesis of 1d: To a solution of compound **1b** (3.0 g, 8.5 mmol) and 2-hydroxyethyl disulfide (1.3 g, 8.4 mmol) in MeOH was added a catalytic amount of tris(2-carboxyethyl) phosphine hydrochloride and the reaction mixture was stirred at room temperature for 8 h. After completion of the reaction monitored by TLC, the solvent was removed under vacuum and the

crude reaction mixture was extracted with DCM/H_2O. The organic layer was collected, dried over anhydrous Na_2SO_4 and then concentrated under vacuum. The crude reaction mixture was purified using silica coloumn chromatography to get desired product as pale yellow oily liquid (53% yield). TLC (PE: EA, 70:30), R_f = 0.35; ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm) = 6.96 (s, 1H), 4.85 (t, J= 5 Hz, 1H), 3.63 (t, J= 10 Hz, 2H), 3.20 (t, J= 10 Hz, 2H), 2.80 (t, J= 10 Hz, 2H), 2.77 (t, J= 10 Hz, 2H) 1.37 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm)= 155.98, 78.24, 60.03, 41.56, 39.67, 38.16, 28.70; HR-MS (m/z): $[M+Na]^+$ $C_9H_{19}NO_3S_2Na$: 276.08 (cal.), 276.0 0 (expt.).

Synthesis of 1f: To a solution of camptothecin (0.1 g, 0.45 mmol), triphosgene (0.5 g, 1.68 mmol) and DMAP (0.56 g, 4.59 mmol) in dry DCM was added a solution of compound **1d** (0.17 g, 0.67 mmol) in dry DCM and the reaction mixture was stirred at room temperature for 3 h. After completion of the reaction monitored by TLC, the crude reaction mixture was extracted with DCM/H_2O. The organic layer was collected, dried over anhydrous Na_2SO_4 and then purified using silica coloumn chromatography to get desired product as pale yellow solid (79% yield). TLC (PE: EA, 10:90), R_f = 0.3; ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm) = 8.77 (s, 1H), 8.25 (d, J= 10 Hz, 2H), 8.19 (s, 1H), 7.95 (t, J= 10 Hz, 1H), 7.15 (s, 1H), 6.97 (s, 1H) 5.58 (s, 2H), 5.38 (s, 2H), 4.38 (d, J= 5 Hz, 2H), 3.21 (t, J= 5 Hz, 2H), 3.05 (t, J= 5 Hz, 2H), 2.80 (t, J= 5 Hz, 2H), 2.25 (t, J= 5 Hz, 2H), 1.40 (s, 9H), 0.92 (t, J= 5 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm)= 167.54, 156.99, 155.95, 153.28, 152.73, 148.40, 146.76, 145.20, 132.12, 130.93, 130.32, 129.51, 129.04, 128.53, 128.26, 119.63, 94.86, 78.36, 78.23, 66.94, 66.83, 50.84, 48.96, 31.18, 28.66, 17.71, 8.03; HR-MS (m/z): $[M+H]^+$ $C_{30}H_{34}N_3O_8S_2$: 627.17 (cal.), 627.80 (expt.).



Synthesis of 1: To a solution of compound **1f** (0.1 g, 0.15 mmol) in dry DCM was added TFA (0.5 mL, 2.94 mmol) and the reaction mixture was stirred at room temperature for 0.5 h. After completion of the reaction monitored by TLC, the crude reaction mixture was extracted with DCM/H_2O. The organic layer was collected, dried over anhydrous Na_2SO_4 and then purified using silica coloumn chromatography to get desired product as off white solid (90% yield). TLC ($CHCl_3$: MeOH, 90:10), R_f = 0.41; ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm) = 8.74 (s, 1H), 8.20 (d, J= 5 Hz, 2H), 7.91 (t, J= 5 Hz, 1H), 7.88 (t, J= 20 Hz, 1H), 7.77 (t, J= 20 Hz, 1H), 7.09 (s, 1H) 5.58 (s, 2H), 5.55 (d, J= 5 Hz, 2H), 5.35 (s, 2H), 3.09 (t, J= 5 Hz, 2H),



3.06 (t, J= 5 Hz, 2H), 3.04 (t, J= 5 Hz, 2H), 2.21 (t, J= 5 Hz, 2H), 0.95 (t, J= 10 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm)= 172.95, 167.55, 167.47, 158.74, 158.49, 158.24, 156.95, 156.89, 153.30, 153.20, 152.96, 150.47, 148.33, 148.26, 146.70, 146.65, 145.91, 145.20, 132.07, 131.95, 130.90, 130.78, 130.17, 130.05, 129.43, 128.97, 128.46, 128.37, 128.21, 128.12, 119.67, 119.57, 119.52, 118.89, 116.51, 97.19, 94.85, 79.66, 78.40, 78.33, 78.06, 72.86, 66.94, 66.80, 66.63, 65.73, 50.76, 50.66, 46.12, 38.34, 37.56, 37.26, 36.61, 31.15, 30.80, 29.48, 25.94, 18.91, 8.25; HR-MS (m/z): $[M+H]^+$ $C_{25}H_{26}N_3O_6S_2$: 527.18 (cal.), 528.19 (expt.).

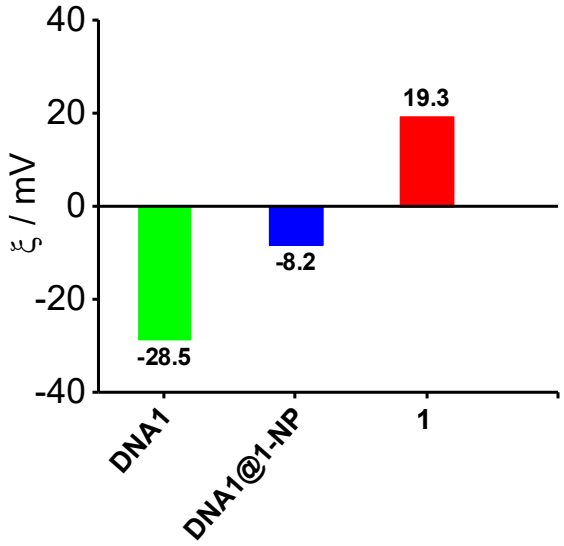


***Figure S1**: Zeta potential measurements of **DNA1** (green), **DNA1@1** ((blue) and **1** (red).*

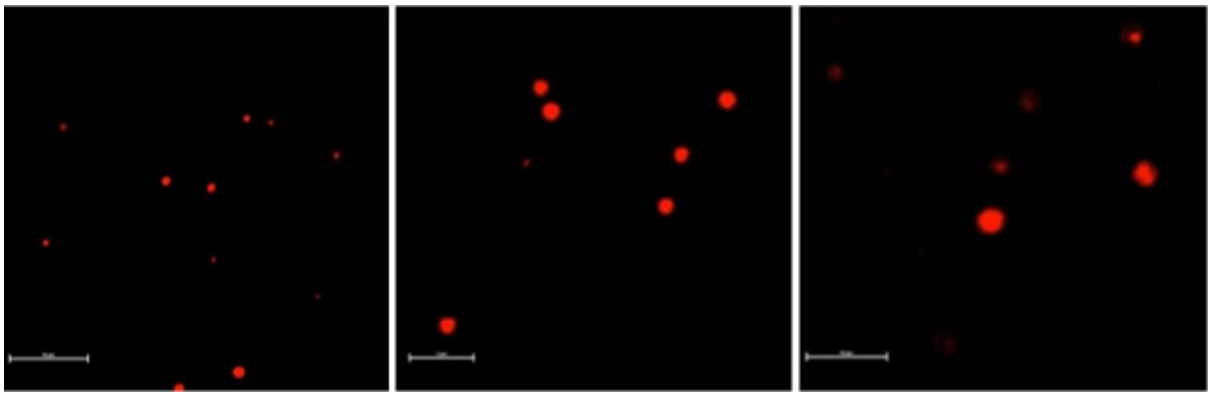


***Figure S2**. Additional CLSM images of **DNA3@1-NPs**.*

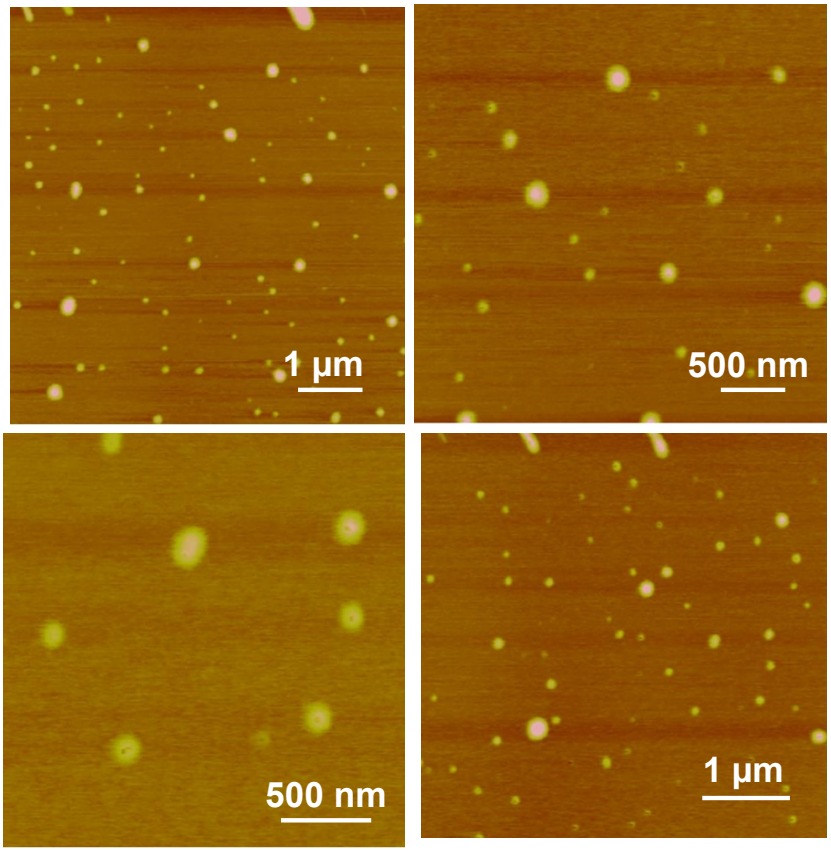


Figure S3. *Additional AFM images of* ***DNA1@1-NPs***.

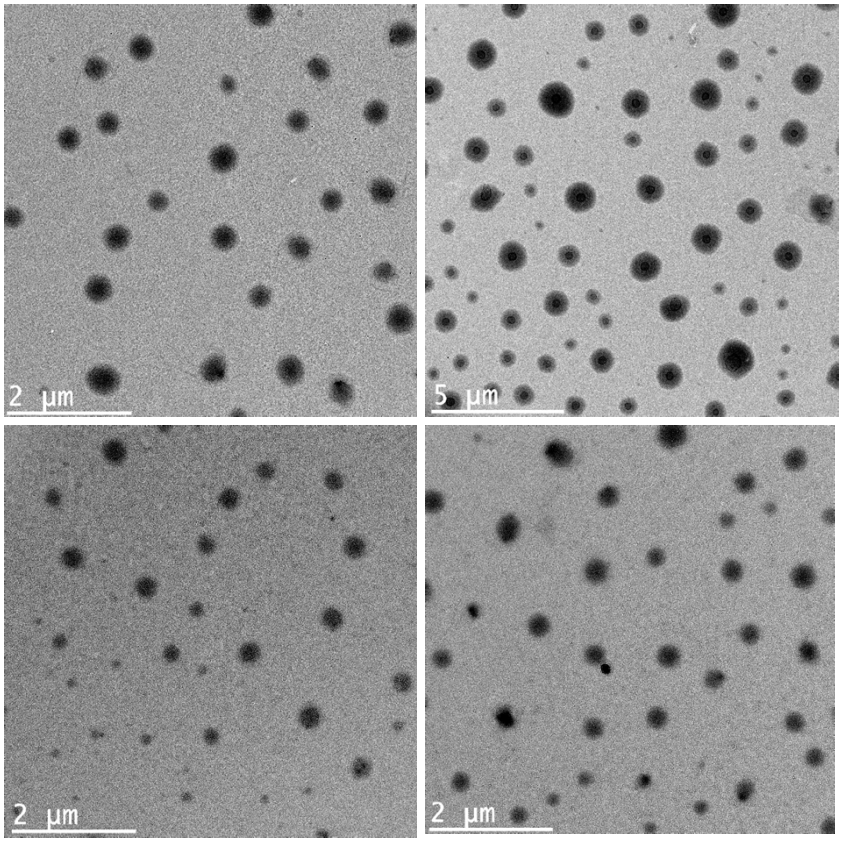


Figure S4. *Additional TEM images of* ***DNA1@1-NPs***.

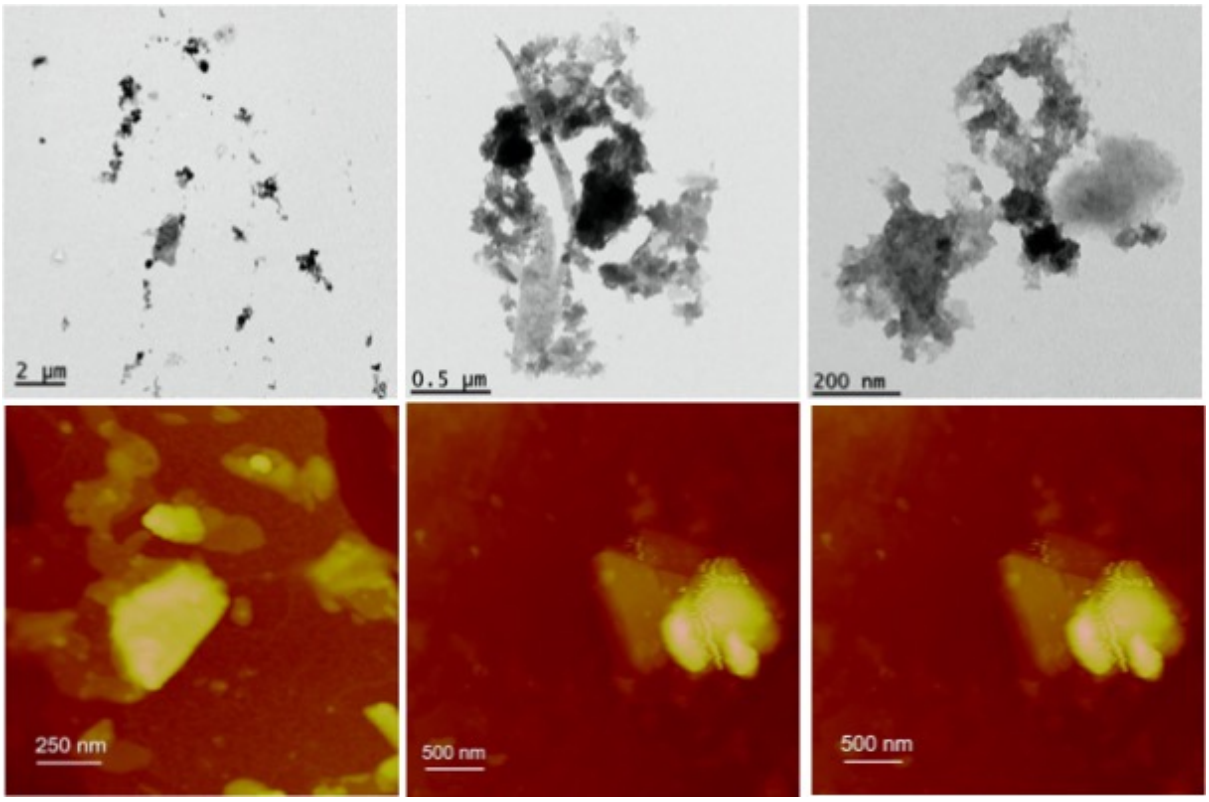


Figure S5*. TEM (top row) and AFM (bottom row) images of aggregates of* ***1*** *at various magnifications.*

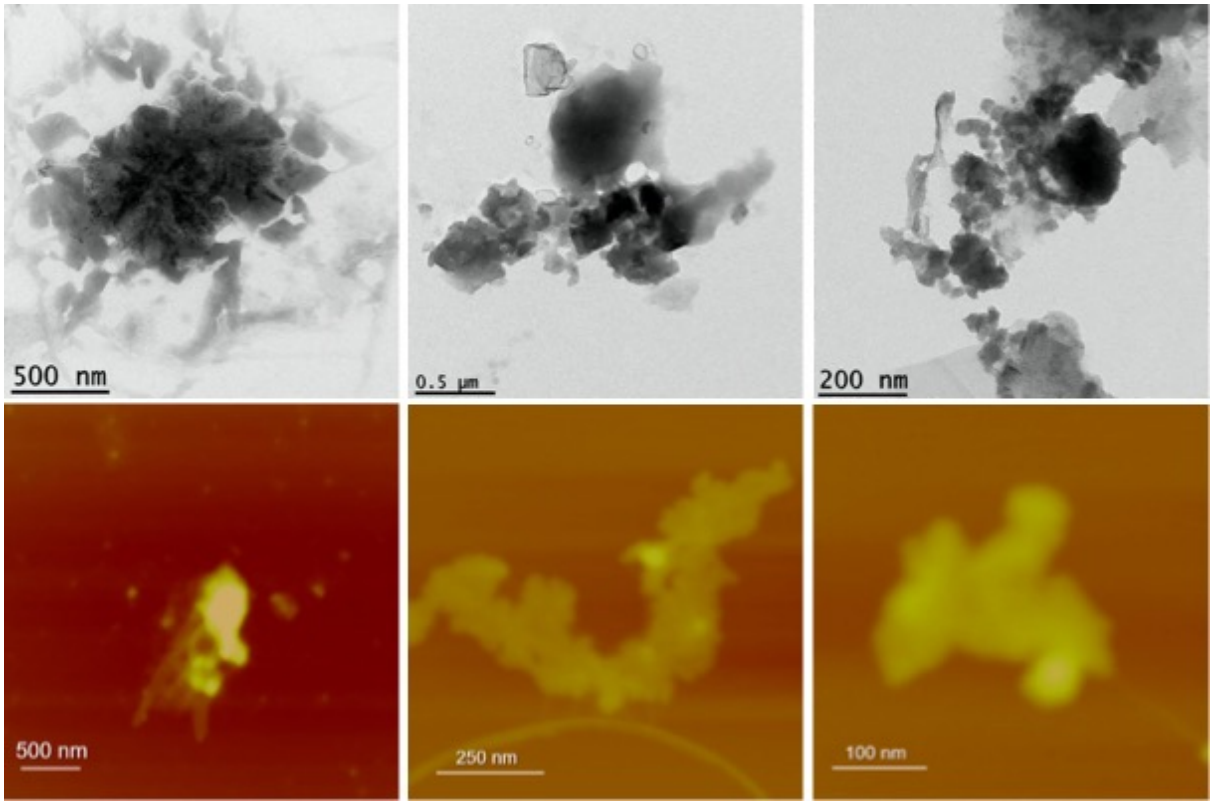


Figure S6. *TEM (top row) and AFM (bottom row) images of* ***1*** *aggregate after the addition of DNAs at various magnifications.*

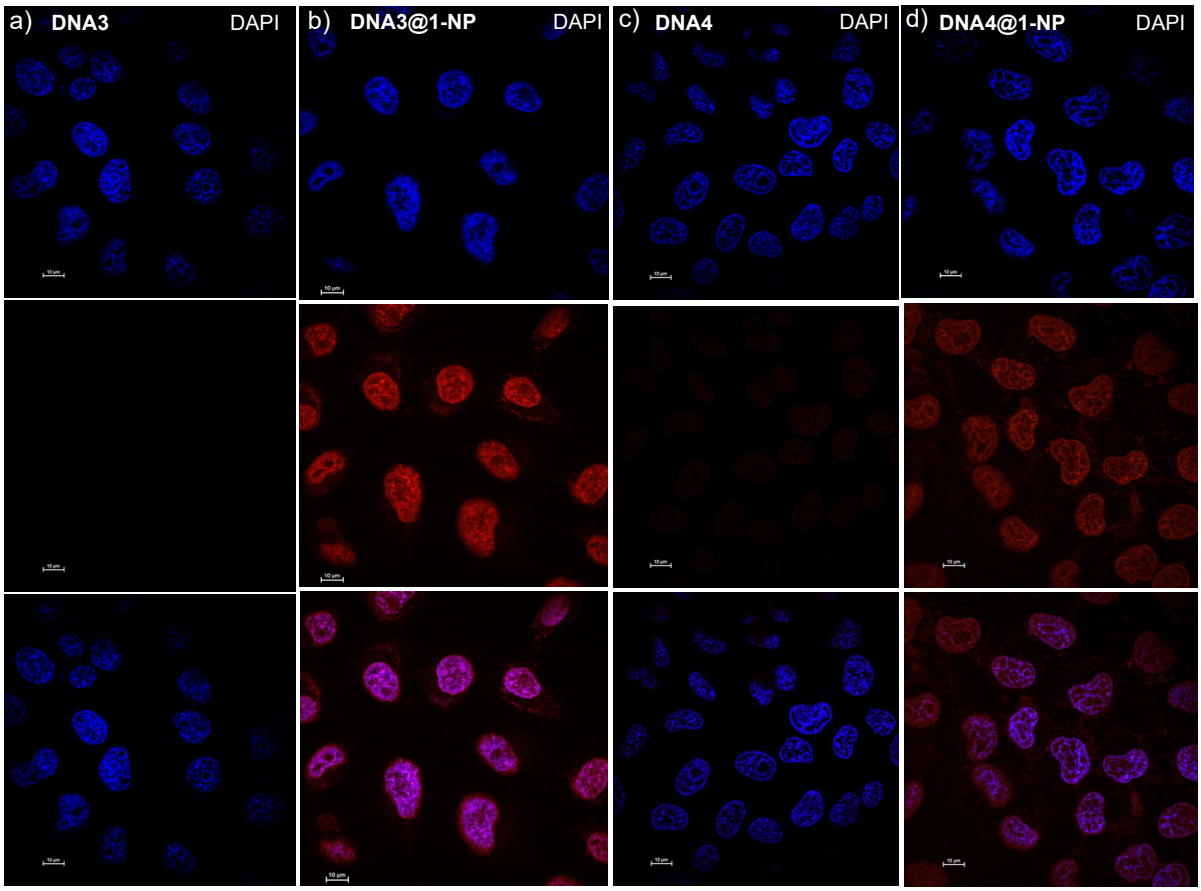


Figure S7*. CLSM images of HeLa Kyoto cells after incubation with (a)* ***DNA3****, (b)* ***DNA3@1-NP****, (c)* ***DNA4*** *and (d)* ***DNA4@1-NP*** *for 9 h (scale bar = 10 μm).*

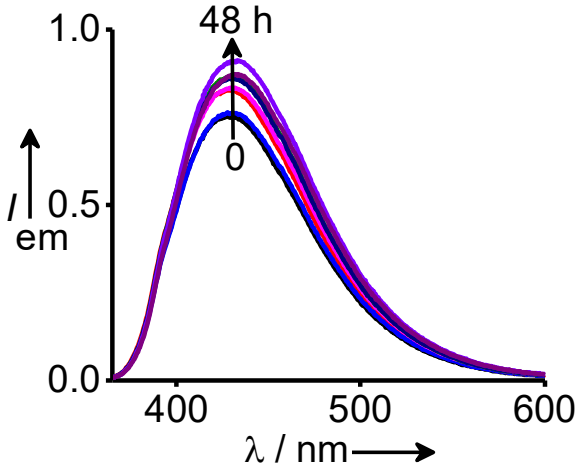


Figure S8*. Time-dependent (0 → 48h) fluorescence changes of* ***DNA1@1-NP*** *after the addition of 10 μM GSH.*

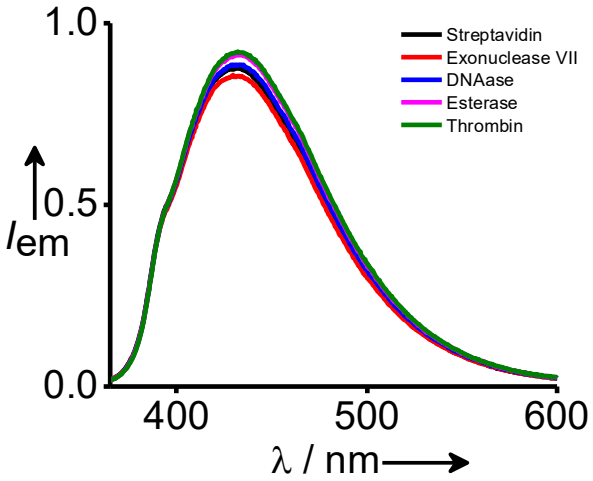


Figure S9. *Fluorescence changes of* ***DNA1@1-NP*** *with the addition of various analytes.*

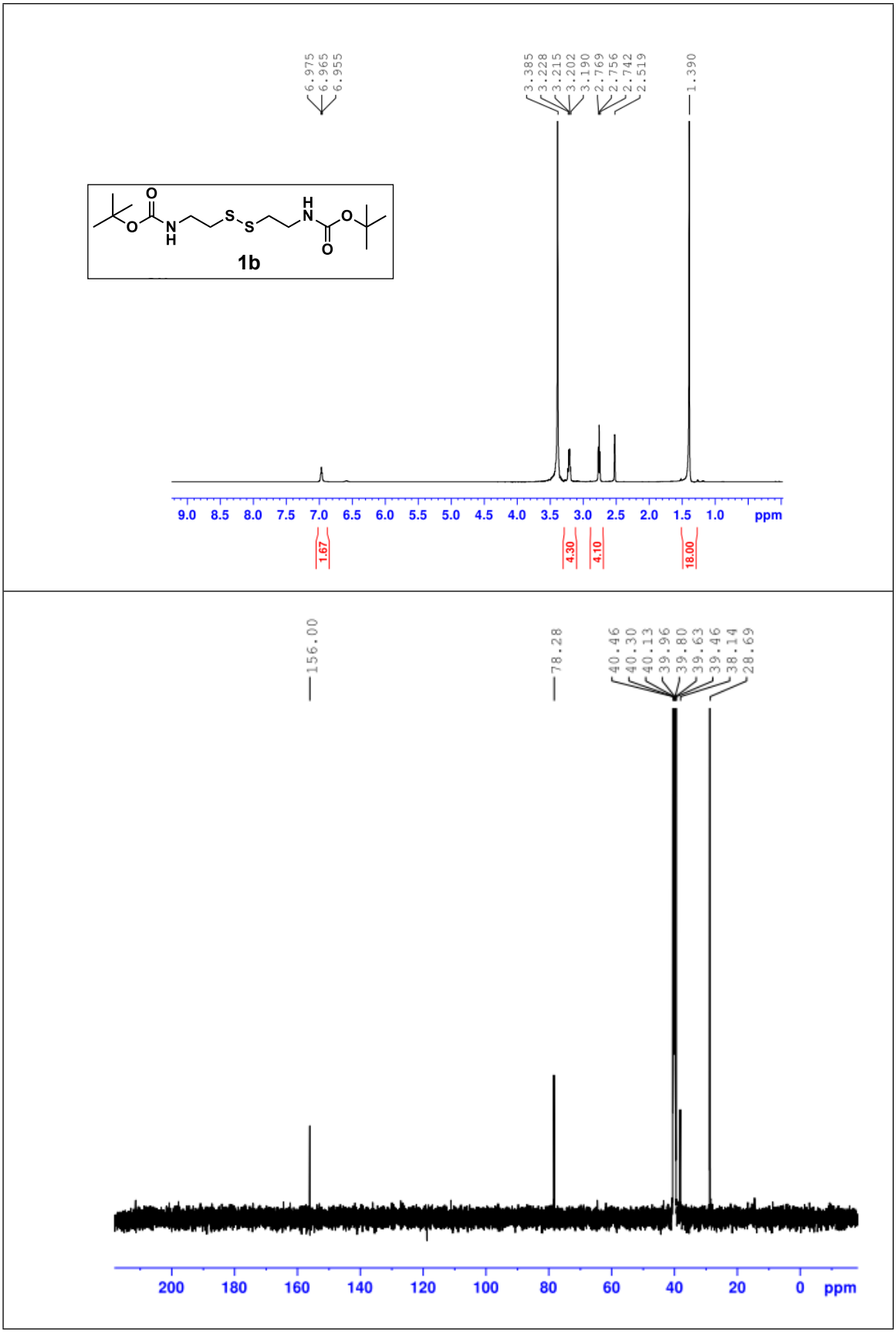


Figure S10. *^{1}H (above) and ^{13}C (below) NMR spectra of* ***1b***.

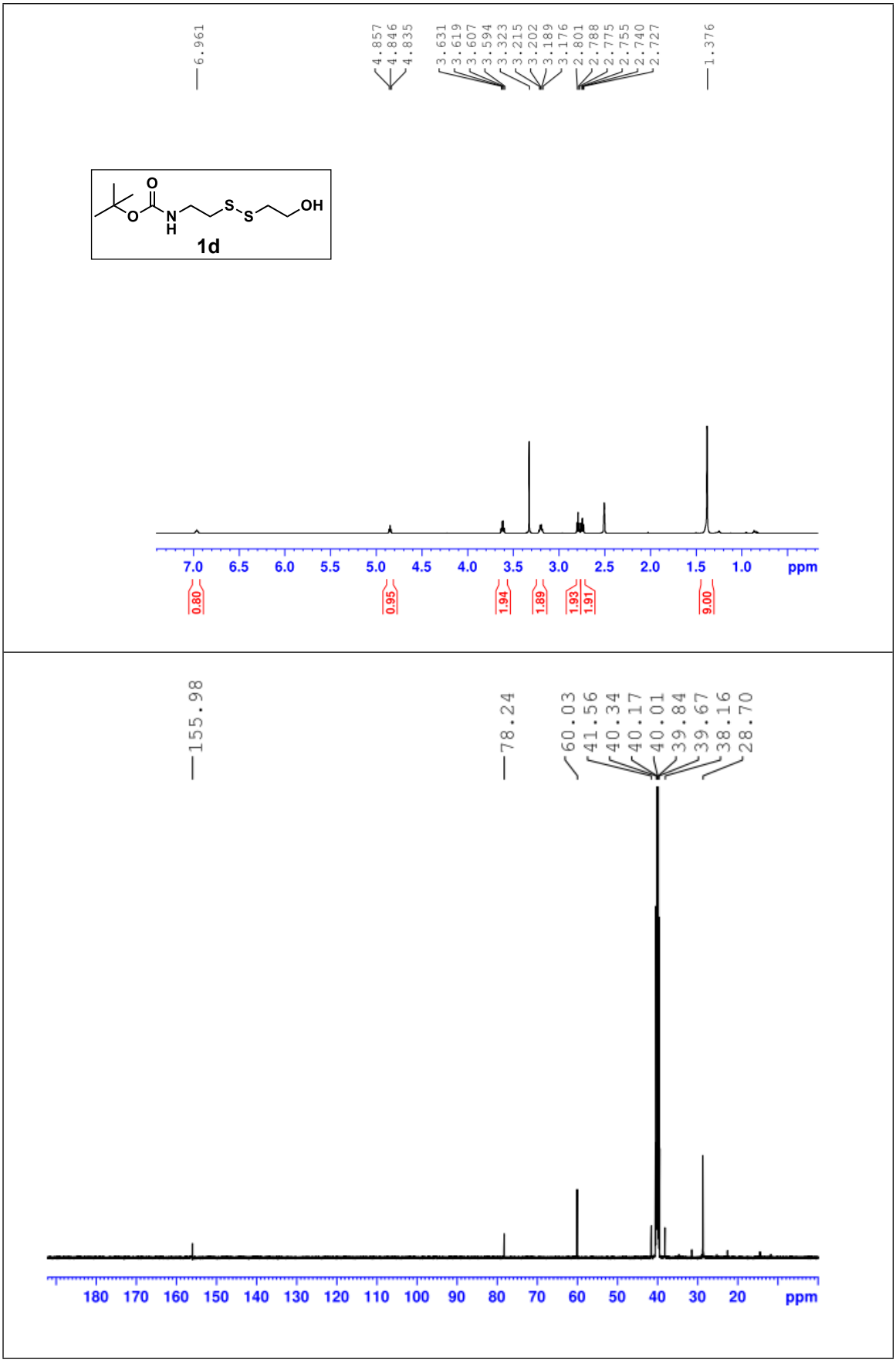


Figure S11. *^{1}H (above) and ^{13}C (below) NMR spectra of* ***1d***.

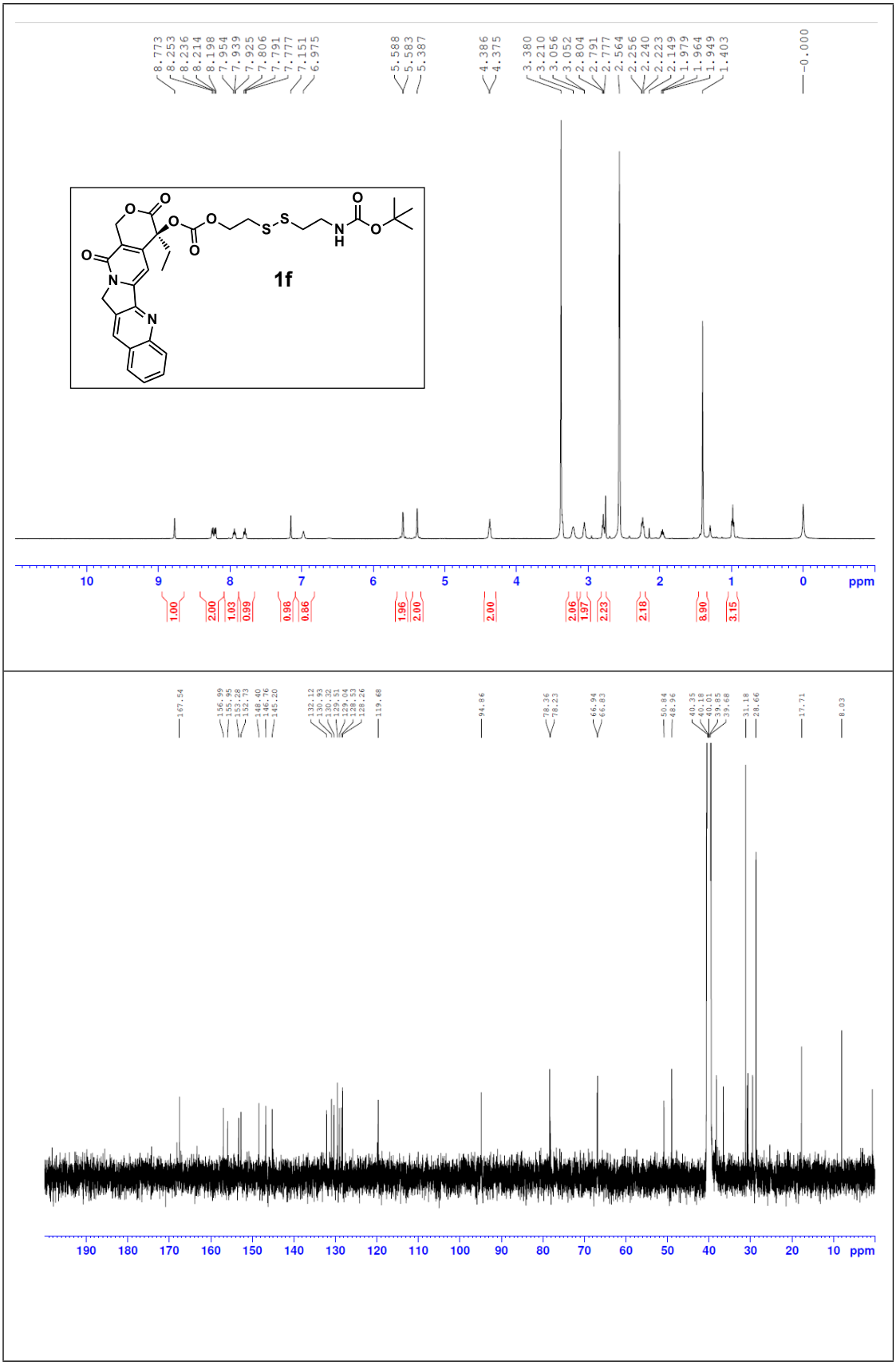


Figure S12. *^{1}H (above) and ^{13}C (below) NMR spectra of* ***1f***.

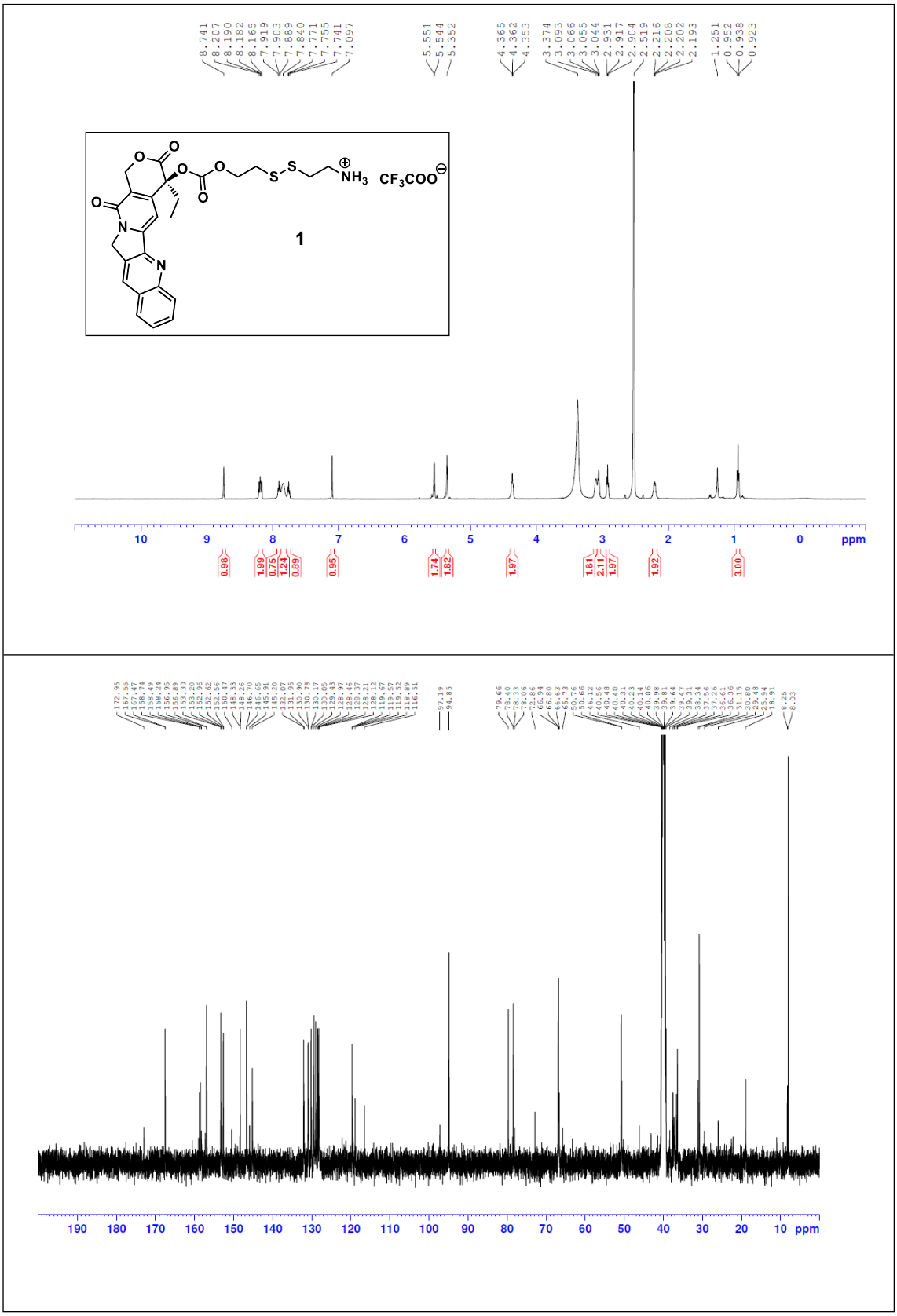


Figure S13. *^{1}H (above) and ^{13}C (below) NMR spectra of **1**.*

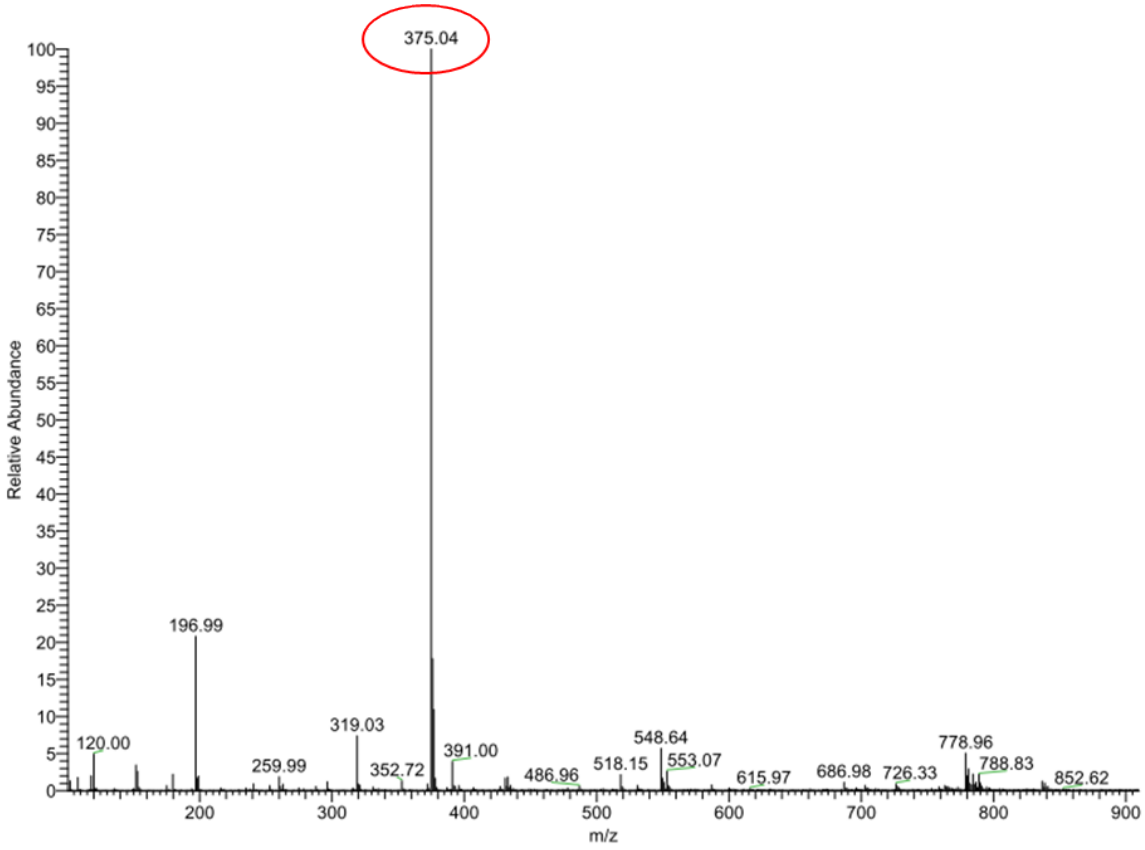


Figure S14. *LC-MS spectrum of compound* ***1b***.

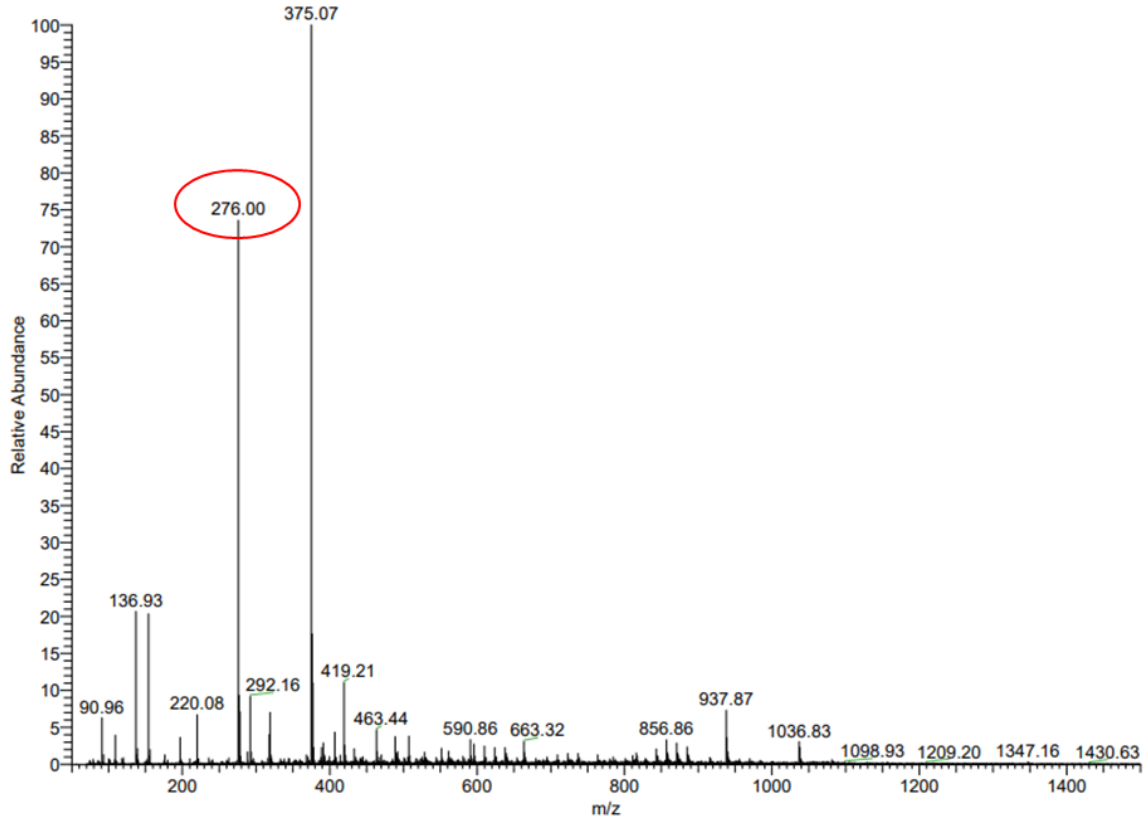


Figure S15. *LC-MS spectrum of compound* ***1d***.

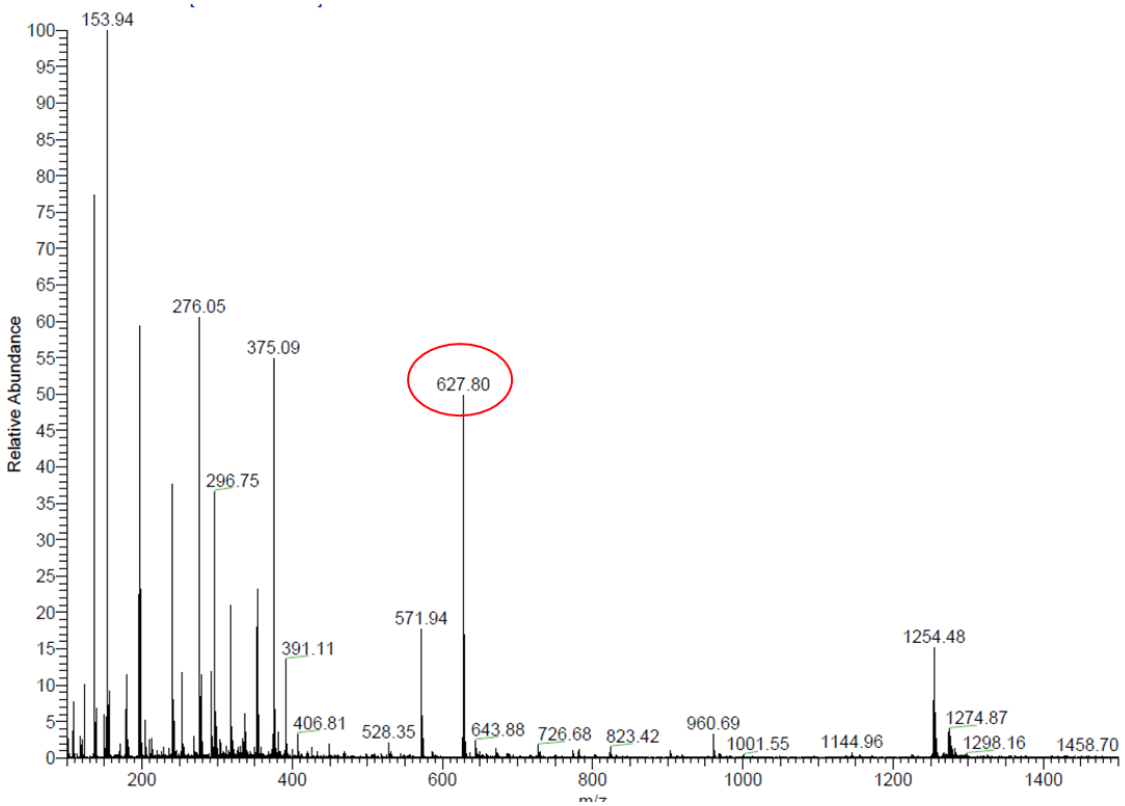


Figure S16**. LC-MS spectrum of compound **1f.

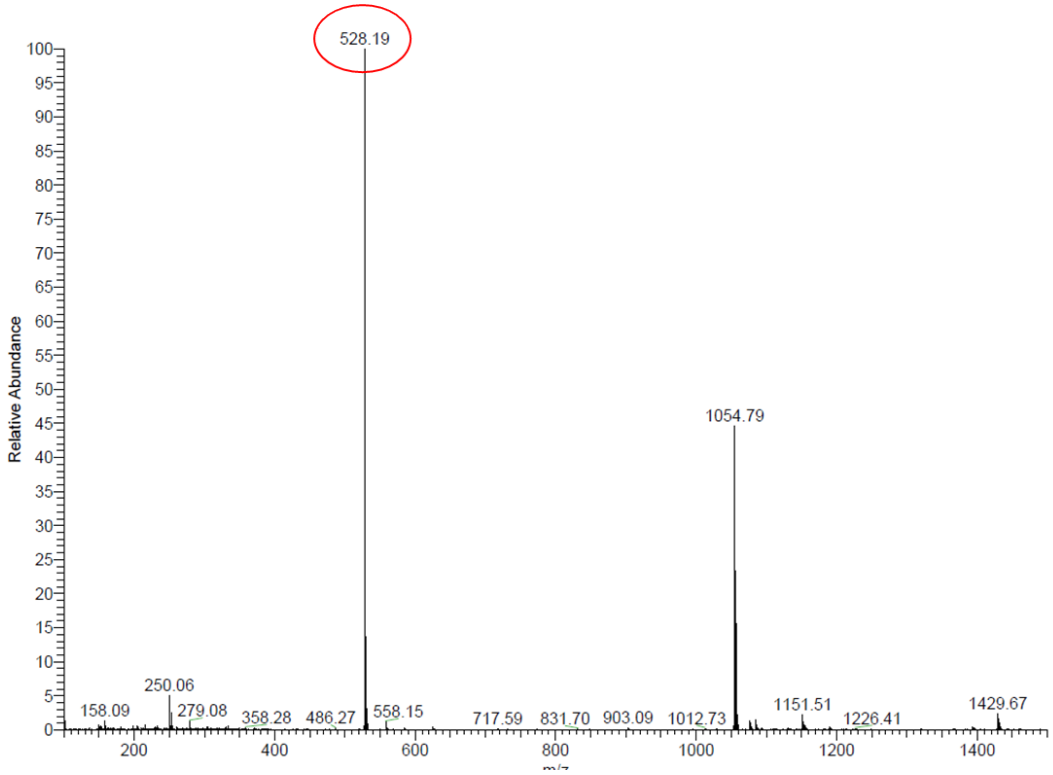


***Figure S17**. LC-MS spectrum of compound **1**.*

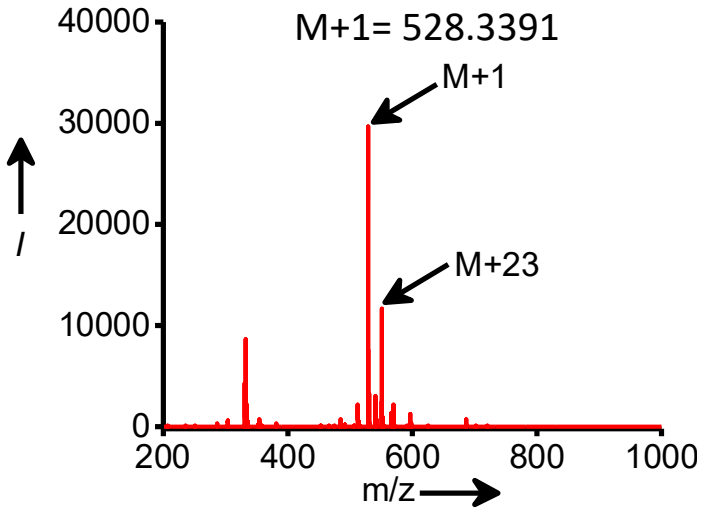


Figure S18. *MALDI-TOF spectrum of compound* ***1***.