Biotin-decorated NIR-absorbing Nanosheets for Targeted Photodynamic Cancer Therapy

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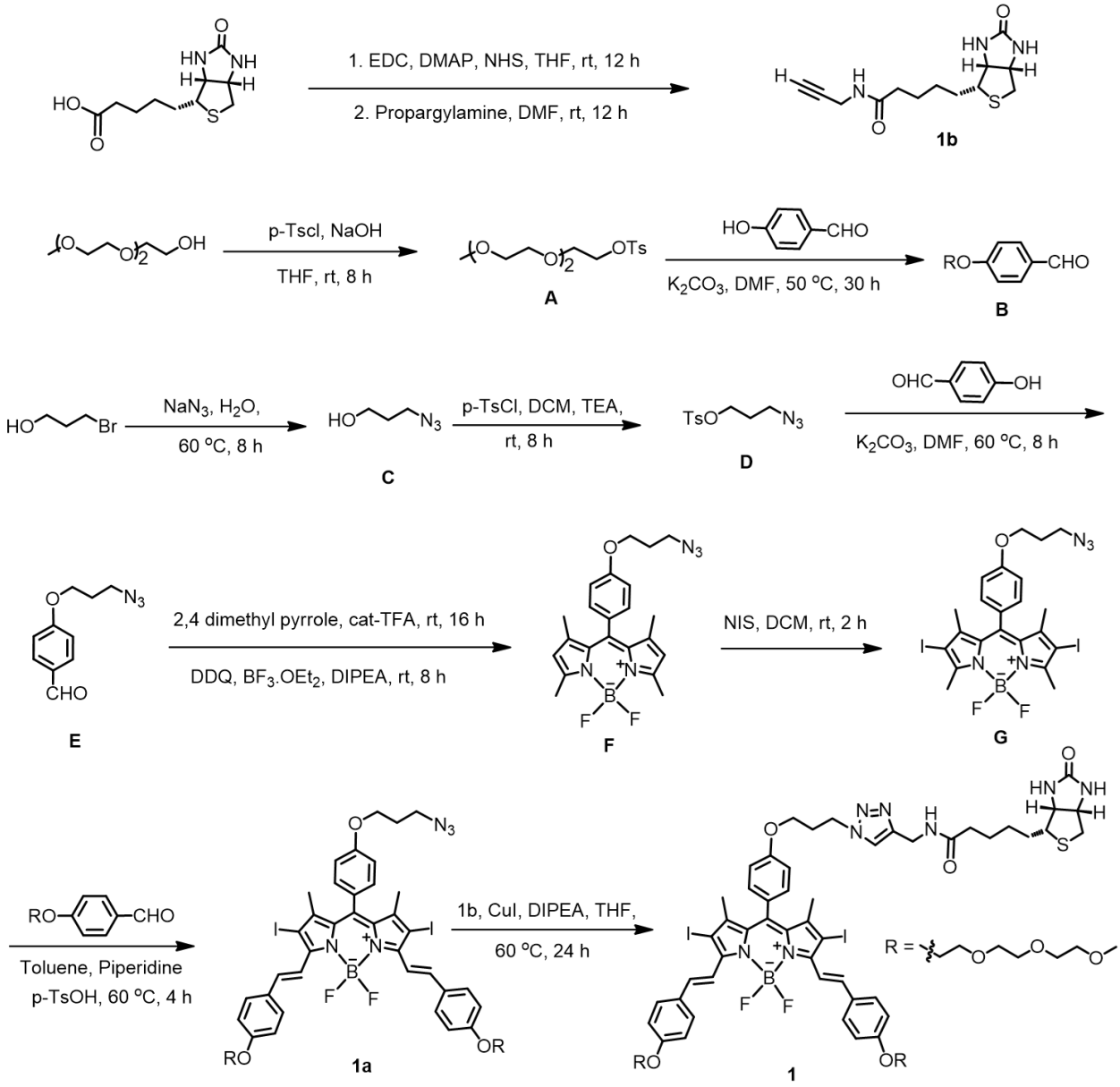
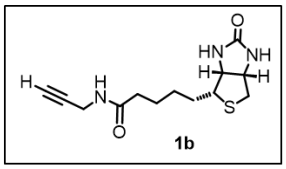
Synthesis of amphiphile 1



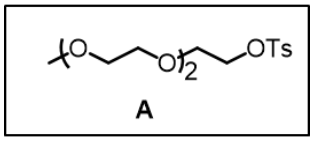
Scheme S1: Synthesis scheme for **1**.

Synthesis of 1b: To a solution of biotin (0.5 g, 2.06 mmol) in dry DMF (20mL) was added N-hydroxysuccinimide (NHS) (0.258 g, 2.455 mmol) and 1- ethyl-3-(3 dimethylaminopropyl)carbodiimide (EDC.HCl) (0.470 g, 2.455 mmol). The reaction mixture was stirred at room temperature for 24 hrs. The solvent was removed under reduced pressure and the crude product was washed with methanol and dried under vacuum. To a solution of the crude product in 15 ml of dry DMF was added propargylamine (0.112 mL, 1.759 mmol) and triethylamine

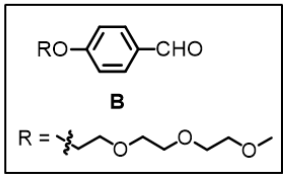


(0.366 mL, 2.198 mmol) and stirred the reaction mixture at room temperature for 24 h. After reaction completion, solvent was removed under reduced pressure and crude product was purified by washing with methanol to get desired product as color less solid. (92%) M.P. 210-212 ˚C, TLC (DCM: MeOH, 95:5), R_f = 0.35; ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm) = 1.20-1.25 (m, 2H), 1.38-1.43, (m, 3H), 1.51-1.53 (m, 1H), 1.99 (t, J = 6 Hz, 2H), 2.43 (s, 1H), 2.49 (s, 1H), 3.00 (t, J = 6 Hz, 2H),), 3.00 (t, J = 6 Hz, 2H), 3.41 (s, 1H), 3.75 (t, J = 6 Hz, 2H), 4.04 (t, J = 6Hz, 1H), 4.22 (t, J = 6Hz, 1H), 6.28 (s, 1H), 8.16 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm) = 25.58, 28.18, 28.48, 28.64, 35.32, 55.85, 59.65, 61.50, 73.25, 81.82, 163.17, 172.26; HR-MS (m/z): $[M+Na]^+$ $C_{13}H_{19}N_3O_2SNa$: 304.77 (cal.), 305.09 (expt.).

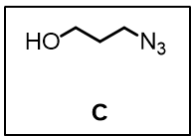
Synthesis of A: To a solution of triethylene glycol monomethyl ether (10 g, 60.9 mmol) in 50 mL of THF, a solution containing aq. NaOH (9.744 mmol) was added. To this, a 20 ml THF solution of p-TsCl (1.16 g, 6.09 mmol) was added at 0 ºC slowly and stirred at room temperature for 3 h. The reaction mixture was diluted with 100 mL water and extracted with DCM. The solvent was removed under reduced pressure and crude product was purified through column chromatography by using DCM: MeOH as an eluent to get desired product **A** as colourless liquid. (88%), TLC (DCM: MeOH, 99:1), R_f = 0.35; ^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm) = 2.44 (s, 3H), 3.37 (s, 3H), 3.52- 3.69 (m, 10H), 4.15 (t, J = 8 Hz, 2H), 7.33-7.34 (d, J = 6 Hz, 2H), 7.79-7.80 (d, J = 6 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$), δ (ppm) = 20.64, 62.00, 58.00, 67.65, 68.22, 69.52, 69.72, 70.88, 126.90, 128.80, 132.00, 143.78; HR-MS (m/z): $[M+Na]^+$ of $C_{14}H_{22}O_6SNa$: 341.38 (cal.), 341.10 (expt.).



Synthesis of B: To a solution of 4-hydroxy benzaldehyde (2.8 g, 22.8 mmol) and K_2CO_3 (6.3 g, 45.6 mmol) in 50 mL of dry DMF was added a solution of compound **A** (8 g, 25.12 mmol) in dry DMF and the reaction was stirred at 50 ºC for 30 h. The compound was extracted with DCM and washed with water and solvent was removed under reduced pressure, purified by using column chromatography using DCM: MeOH as an eluent to get desired product as colourless liquid(76%). TLC (DCM: MeOH, 98:2), R_f = 0.32; ^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm) = 3.30 (s, 2H), 3.47 (t, J = 6 Hz, 2H), 3.52-3.68 (m, 6H), 4.14 (t, J = 8 Hz, 2H), 6.95 (d, J = 8 Hz, 2H), 7.75 (d, J = 6 Hz, 2H), 9.81(s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$), δ (ppm) = 58.02, 66.854, 68.54, 69.57, 69.65, 69.85, 70.92, 106.99, 107.30, 137.29, 159.39, 190; GC-MS (EI)- m/z of $C_{14}H_{20}O_5$: 268.30 (cal.), 268.00 (expt.).

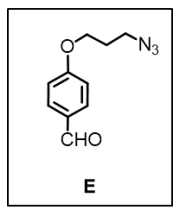


Synthesis of C: To a solution of 3-bromo-1-propanol (5 g, 40.94 mmol) in water, sodium azide (5.32 g, 81.88 mmol) in 30mL water was added and the reaction was stirred at 60 °C for 8 h. The product was extracted with dichloromethane and solvent was removed under reduced pressure to get the desired product as colourless liquid (96%). TLC (petroleum ether: DCM, 60:40), R_f = 0.3; ^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm) = 1.77 (t, J = 8 Hz, 2H), 3.38 (t, J = 6 Hz, 2H), 3.69 (t, J = 5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$), δ (ppm) = 30.45, 47.51, 58.94; GC-MS (EI)- m/z of $C_3H_7N_3O$: 101.09 (cal.), 101.00 (expt.).



C

Synthesis of D: The compound **C** (5.3 g, 55.13 mmol) and triethylamine (27.84 g, 275.65 mmol) were dissolved in dry DCM under nitrogen atmosphere. To this, a solution of p-TsCl (14.71 g, 77.19 mmol) in DCM was added slowly and the reaction was stirred at room temperature for 12 h. After reaction completion monitored by TC, solvent was removed under reduced pressure and the crude product was purified by column chromatography using dichloromethane and petroleum ether (3:7) as eluent to afford the desired product as colourless liquid (78.8%) TLC (petroleum ether: DCM, 70:30); R_f = 0.36; ^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm) = 1.82 (t, J = 8 Hz, 2H), 2.38 (s, 3H), 3.31 (t, J = 8 Hz, 2H), 4.04 (t, J = 8 Hz, 2H), 7.30 (d, J = 8 Hz, 2H), 7.73 (d, J = 6 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$), δ (ppm) =20.64, 27.45, 46.27, 65.96, 120.90, 128.92, 131.77, 144.02; HR-MS (m/z): $[M+Na]^+$ of $C_{10}H_{13}N_3O_3SNa$: 278.29 (cal.), 278.05 (expt.).



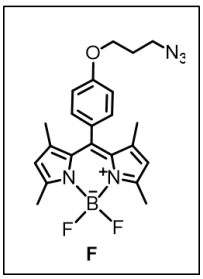
D

Synthesis of E: The 250 ml two neck round bottomed flask was charged with p-hydroxybenzaldehyde (2.0 g, 16.45 mmol) and potassium carbonate (4.54 g, 32.90 mmol) in dry DMF (40 ml). To this, a solution of compound **D (**4.2 g, 16.45 mmol) in dry DMF was added and stirred the reaction at 50 ºC for 30 h. Solvent was removed under reduced pressure, the crude mixture was extracted with dichloromethane and then purified by column chromatography using 1:1 mixture of petroleum ether and DCM to afford desired product as colorless oil (92%). TLC (DCM: petroleum ether, 70:30), R_f = 0.4; ^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm) = 2.10 (t, J = 10 Hz, 2H), 3.89 (t, J = 6 Hz, 2H), 4.22 (t, J = 6 Hz, 2H), 7.03 (d, J =8 Hz, 2H), 7.85 (d, J = 8 Hz, 2H), 9.89 (s, 1H); ^{13}C NMR (125MHz, $CDCl_3$), δ (ppm) = 31.85, 59.76, 65.62, 114.77, 130.00, 132.03, 163.93, 190.85; HR-MS (m/z): $[M+H]^+$ of $C_{10}H_{11}N_3O_2$: 206.08 (cal.), 206.08 (expt.).

E

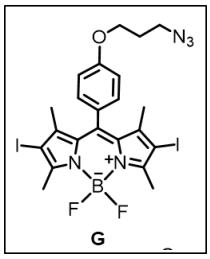
Synthesis of F: In a 100 mL two neck round bottomed flask, 2,4 dimethylpyrrole (1.85 g, 19.49 mmol) and compound **6 (**2.0 g, 9.74 mmol) were dissolved in dry DCM. To this solution, a catalytic amount of trifluoroacetic acid was added and the reaction mixture was stirred for 16

h at room temperature. A solution of 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ) (2 g, 8.85 mmol) in dry DCM was then added dropwise and the reaction mixture was stirred for another 15 min. Then $BF_3.OEt_2$ (29.8 mL) and triethyl amine (29.8 ml) were added and the reaction was further stirred for 3 h at room temperature. After reaction completion, the crude product was extracted with DCM and dried over Na_2SO_4. Solvent was removed under reduced pressure and then purified by column chromatography (33%). TLC (petroleum ether: DCM, 50:50); R_f = 0.42; ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm)=1.36 (s, 6H), 2.00-2.05 (m, 2H), 2.47 (s, 6H), 3.49 (t, J=6.45 Hz, 2H), 4.03 (t, J=5.8 Hz, 2H), 5.90 (s, 2H), 6.94 (d, J = 8.45 Hz, 2H), 7.11 (d, J = 8.45 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm) =14.60, 32.02, 60.27, 65.65, 115.05, 121.11, 127.23, 129.25, 131.84, 141.79, 143.15, 155.29, 159.38; HR-MS (m/z): $[M+Na]^+$ of $C_{12}H_{24}BF_2N_5ONa$: 446.20 (cal.), 446.19 (expt.).



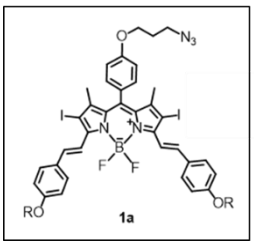
F

Synthesis of G: To a solution of compound **F** (0.258 g, 0.609 mmol) in 25 ml of dry DCM, N-iodosuccinimide (1.33 g, 5.91 mmol) was added and reaction mixture was stirred at room temperature for 2 h. Solvent was removed under reduced pressure and the crude product was purified by column chromatography. TLC (petroleum ether: DCM, 95:5); R_f = 0.5; ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm)= 1.37 (s, 6H), 2.04 (t, J = 6 Hz, 2H), 2.6 (s, 6H), 3.50 (t, J = 6.5 Hz, 2H), 4.05 (t, J = 6 Hz, 2H), 6.96 (d, J = 8.5 Hz, 2H), 7.07 (d, J = 8.5 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ (ppm)= 16.02, 17.21, 28.77, 29.71, 48.18, 64.72, 85.56, 115.37, 126.99, 129.18, 131.72, 141.46, 145.36, 156.62, 159.66; HR-MS (m/z): $[M+Na]^+$ of $C_{12}H_{22}BF_2I_2N_5ONa$: 697.99 (cal.), 697.98 (expt.).



G

Synthesis of 1a: The compound **G** (0.422 g, 0.625 mmol) and compound **B** (0.766 g, 2.5 mmol) were dissolved in 50 mL of dry toluene under nitrogen atmosphere. To this, p-toluene sulfonic acid (0.498 g, 6.25 mmol) and 0.5 ml of piperidine were added and reaction mixture was heated to 90 °C. The mixture of toluene and piperidine were repeatedly added to the reaction mixture until the complete consumption of starting material. Dean stark condenser was used with continues N_2 purging to remove the water and the crude reaction mixture was extracted with DCM, purified by column chromatography (63.4%). TLC (DCM: MeOH, 98:2); R_f = 0.36; ^{1}H NMR ($CDCl_3$, 500 MHz) δ (ppm) = 1.43 (s, 6H), 2.04 (t, J = 6.0 Hz, 2H), 3.31 (s, 6H), 3.48-3.82 (m, 22H), 4.05 (t, J = 6.0 Hz, 2H), 4.11 (t, J = 5.0 Hz, 4H), 6.88 (d, J = 8.0 Hz, 4H), 6.96 (d, J = 10Hz, 2H), 7.09 (d, J = 8.0 Hz, 2H), 7.49-7.52 (m, 6H), 8.05 (d, J = 10 Hz, 2H); ^{13}C NMR($CDCl_3$, 125 MHz) δ (ppm)= 13.84, 31.02, 48.43, 53.09,



1a

57.39, 59.11, 60.71, 64.59, 66.49, 68.04, 68.10, 68.67, 69.26, 69.32, 69.44, 69.58, 69.63, 69.83, 71.52, 73.58, 73.65, 78.64, 113.29, 113.92, 116.29, 116.40, 126.42, 127.80, 127.97, 128.70, 128.73, 132.54, 134.59, 137.18, 140.80, 151.54, 158.55; HR-MS (m/z): $[M+Na]^+$ of $C_{50}H_{58}BF_2I_2N_5O_9Na$: 1198.23 (cal.), 1198.22 (expt.).

Synthesis of 1: In a two neck round bottomed flask, compound **1a** (0.025 g, 0.02 mmol) and compound **1b** (0.0126 g, 0.024 mmol) were dissolved in 5 mL of freshly distilled THF and degassed for 15 min. To this, CuI (0.0076 g, 0.04 mmol) and DIPEA (0.01026 g, 0.08 mmol) were added and reaction mixture was stirred for 24 h at 60 °C. After completion of the reaction monitored by TLC, the crude product was purified by using column chromatography to afford desired product **1** as greenish solid (80%). TLC (DCM: MeOH, 90:10), R_f = 0.3; ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm) = 1.24 (s, 2H), 1.32 (s, 9H), 1.76 (s, 1H), 2.12 (s, 2H), 2.34 (s, 3H), 2.82 (s, 1H), 3.10 (s, 1H), 3.25 (s, 6H), 3.45 (s, 4H), 3.54 (s, 1H) 3.56 (s, 4H), 3.61(s, 1H), 3.78 (s, 4H), 4.11 (s, 3H), 4.18 (s, 2H), 4.32 (s, 4H), 4.57 (s, 3H), 4.62 (s, 2H), 6.35 (s, 1H), 6.41 (s, 1H), 7.08 (s, 8H), 7.09 (s, 2H), 7.36 (s, 2H), 7.47 (d, J = 15Hz, 2H), 7.58 (d, J = 6Hz, 4H), 8.01 (s, 1H), 8.06 (s, 2H), 8.28 (s, 1H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ (ppm) = 17.73, 28.57, 48.21, 60.67, 60.70, 67.92, 69.32, 70.23, 70.27, 70.44, 72.80, 115.83, 123.38, 139.10, 146.03, 160.41; HR-MS (m/z): $[M+Na]^+$ of $C_{63}H_{77}BF_2I_2N_8O_{11}SNa$: 1479.23 (cal.), 1479.35 (expt.).

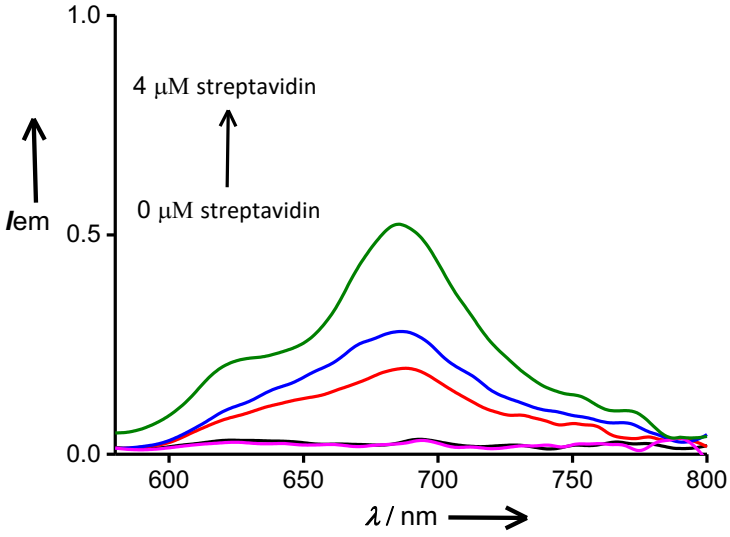


Figure S1: Fluorescence changes of the nanosheet at 693 nm when treated with different equivalences of streptavidin (0→4 μM) [nanosheet] = 4 μM.

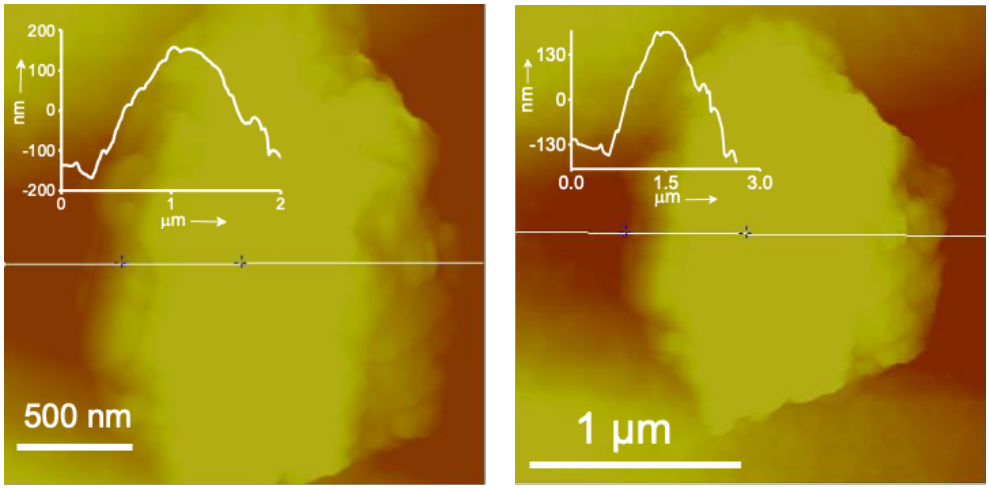


Figure S2. Additional AFM images for the nanosheets. The insets show the corresponding section analyses.

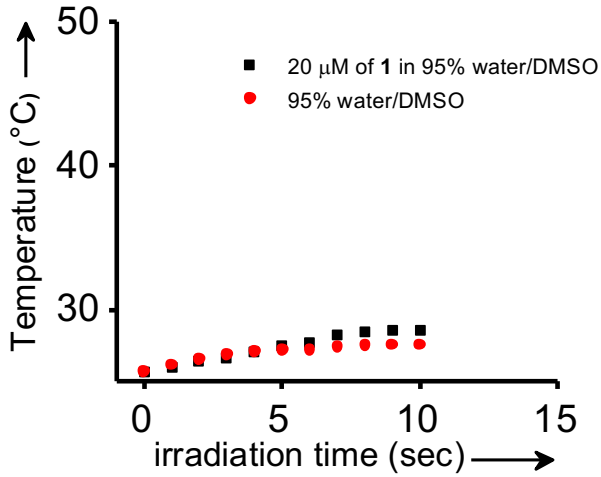


Figure S3. Graph showing the photothermal effect of the nanosheet when irradiated with a laser (635 nm, 1 W/cm^2).

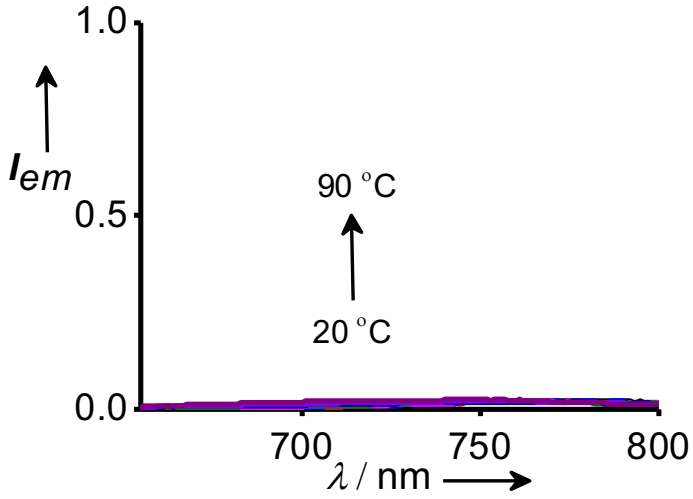


Figure S4. Temperature dependent emission studies of the nanosheet (λ_{exc} = 635 nm).

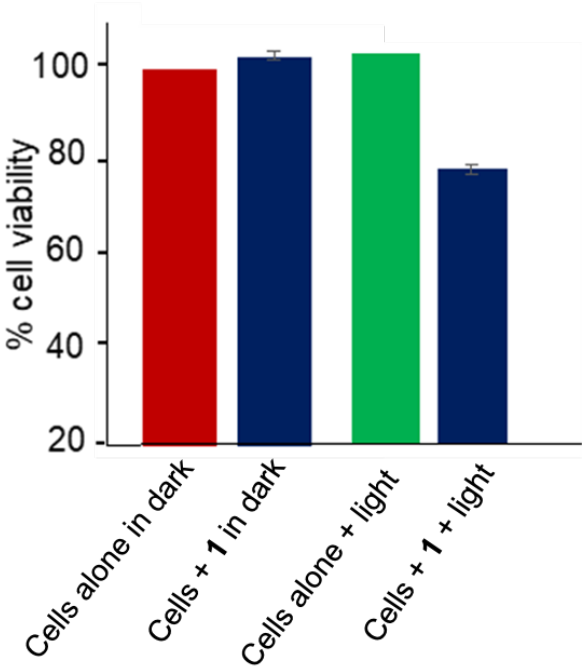


Figure S5. MTT assay of HeLa cells treated with **1** nanosheet in dark and light conditions.

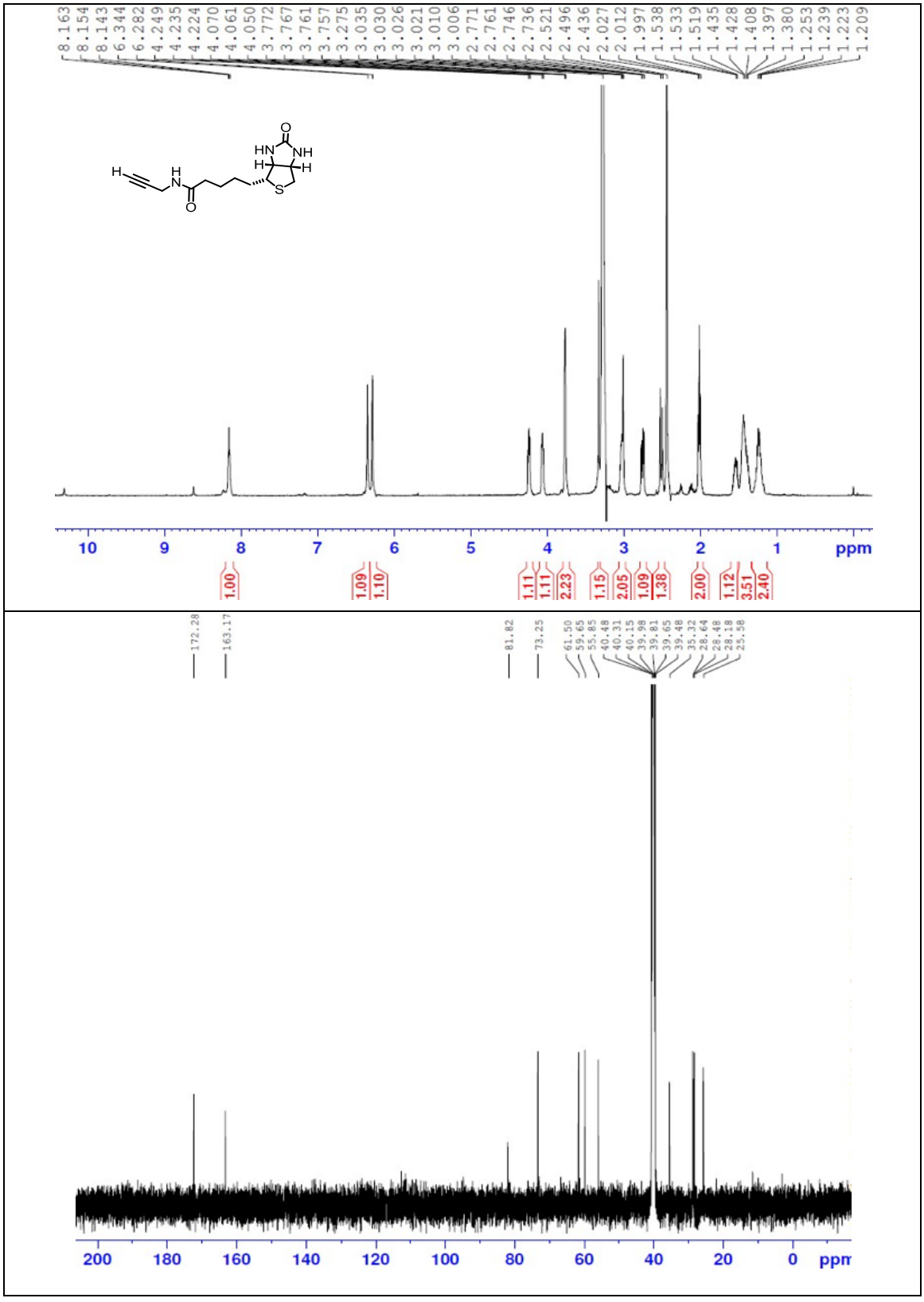


Figure S6. ^{1}H (above) and ^{13}C (below) NMR spectra of **1b**.

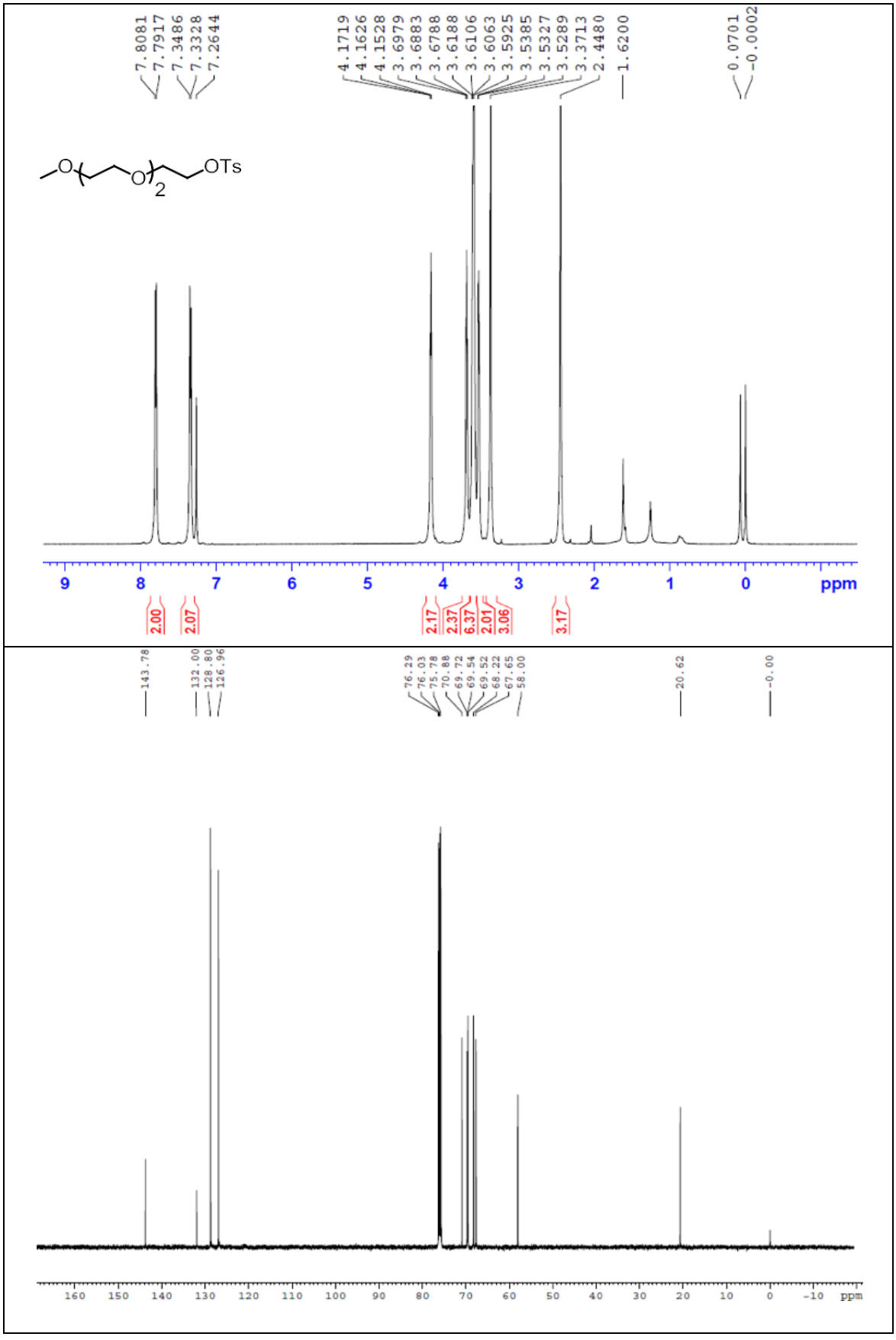


Figure S7. ^{1}H (above) and ^{13}C (below) NMR spectra of **A**.

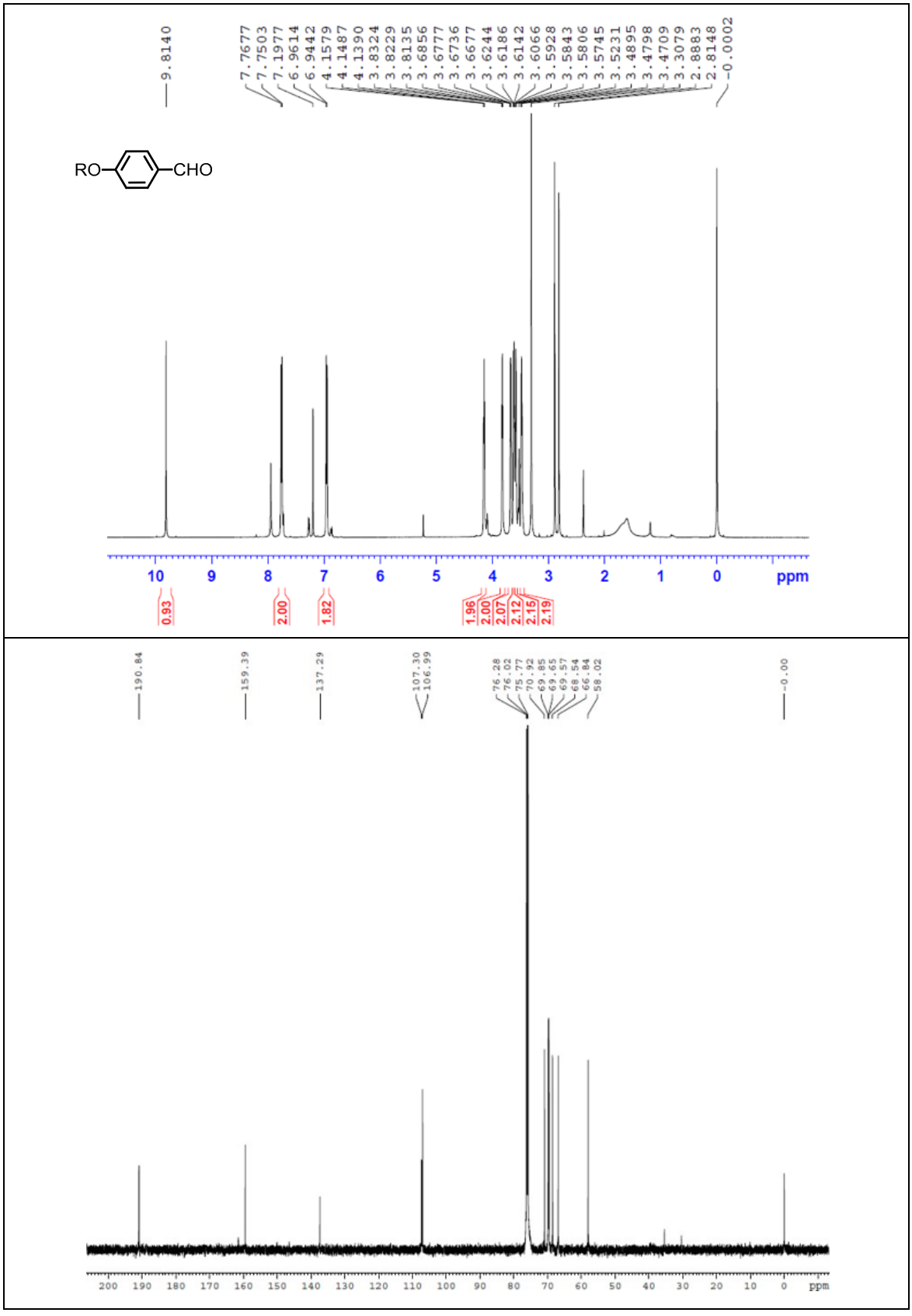


Figure S8. ^{1}H (above) and ^{13}C (below) - NMR spectra of **B**.

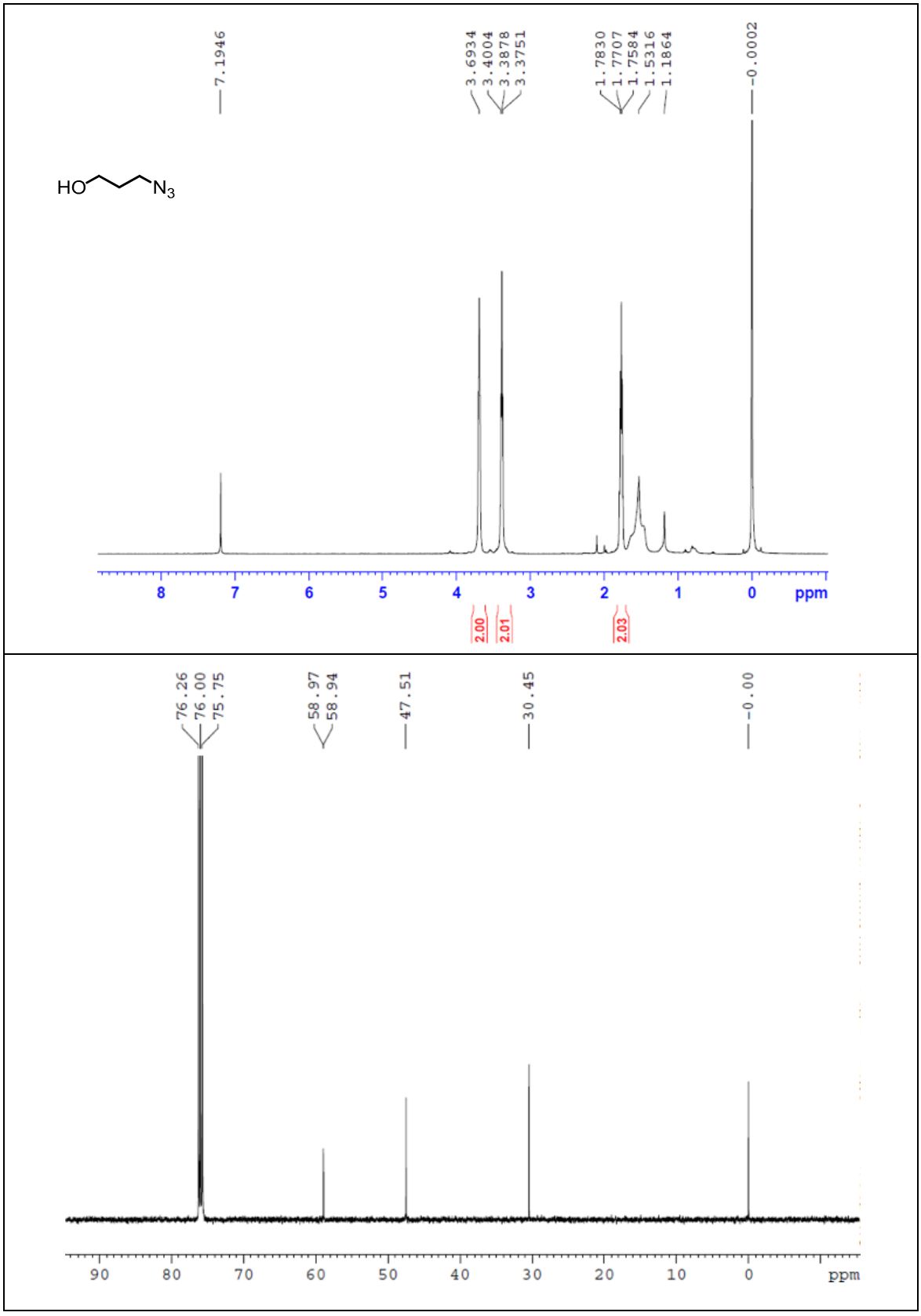


Figure S9. ^{1}H (above) and ^{13}C (below) - NMR spectra of **C**.

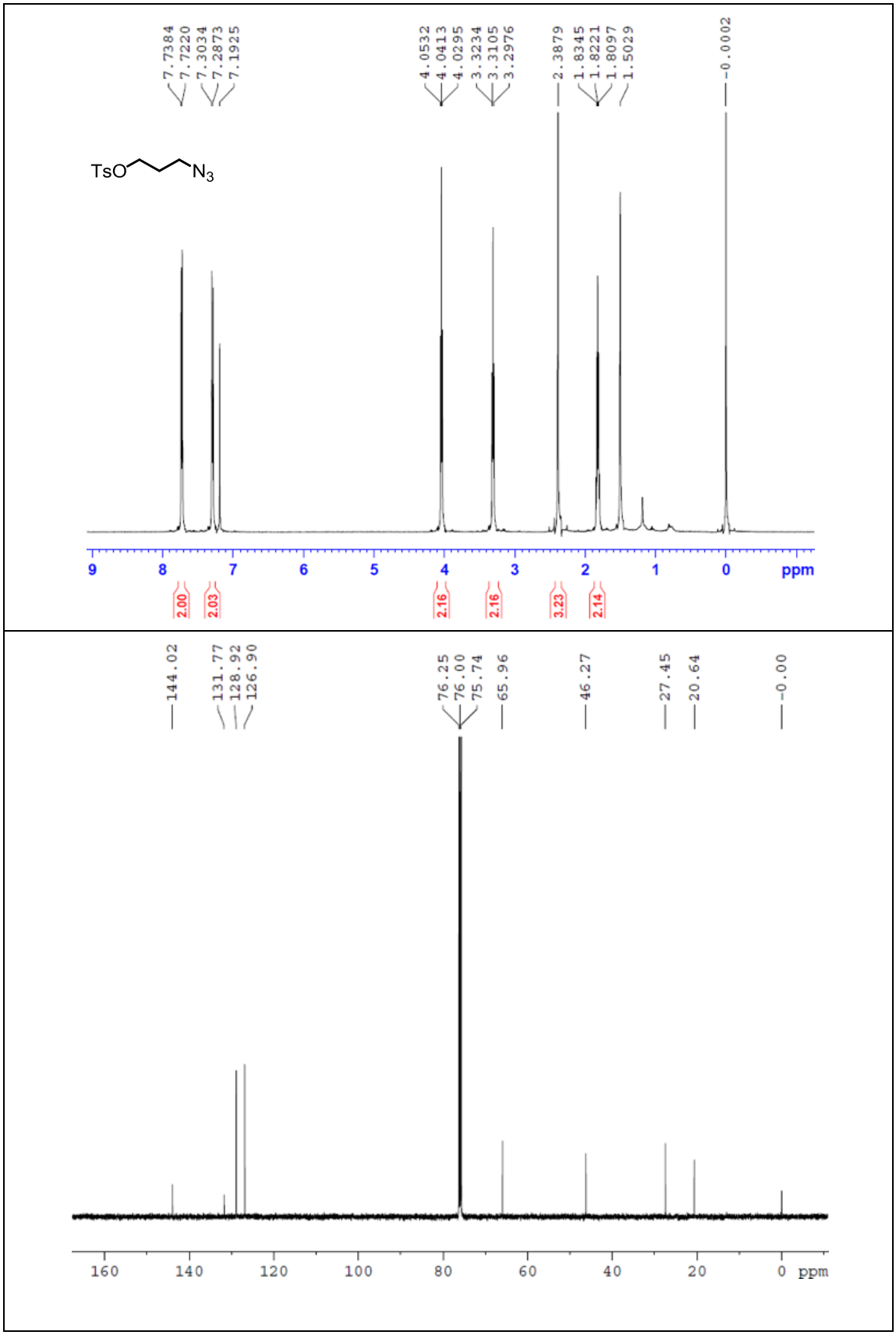


Figure S10. ^{1}H (above) and ^{13}C (below) NMR spectra of **D**.

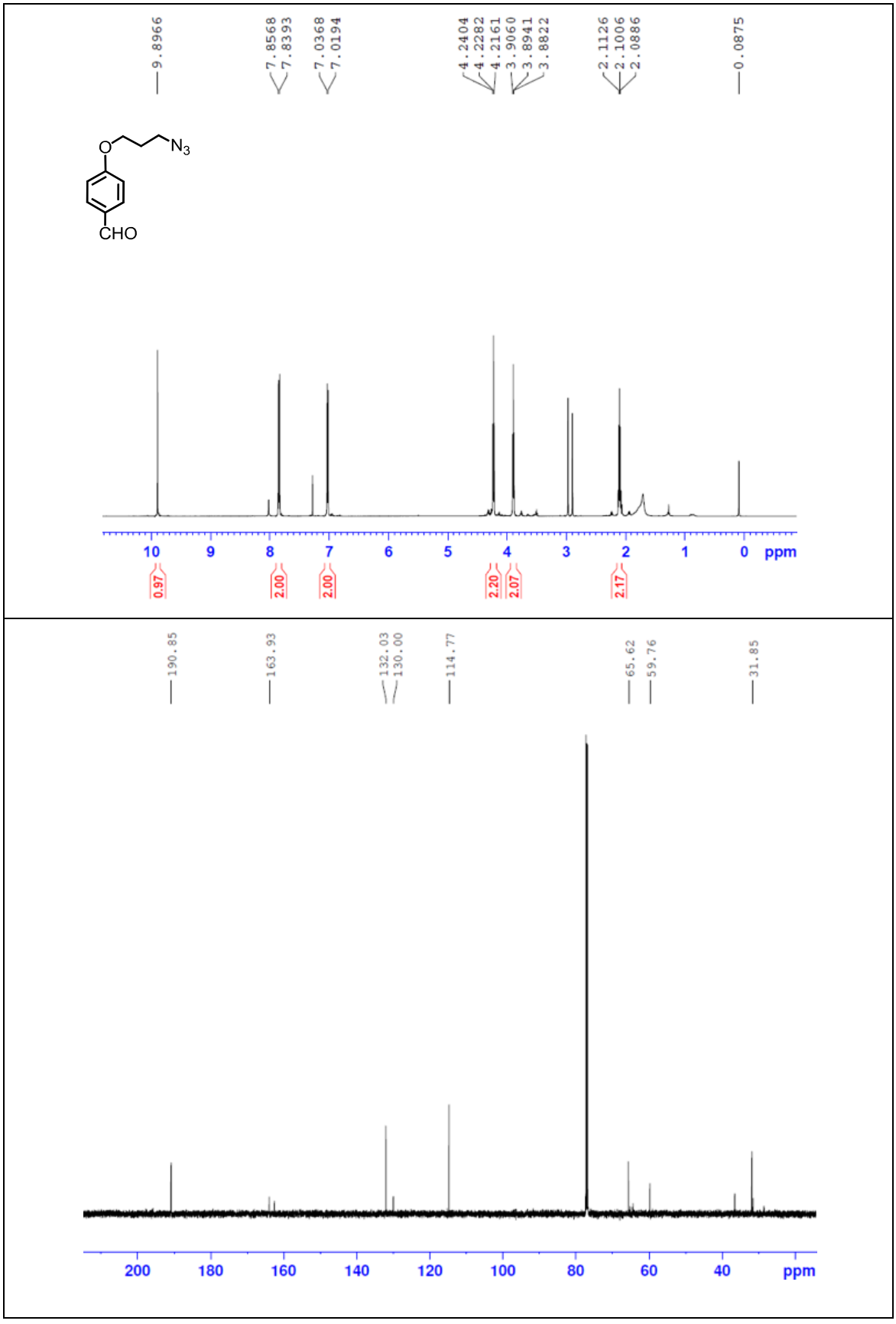


Figure S11. ^{1}H (above) and ^{13}C (below) NMR spectra of **E**.

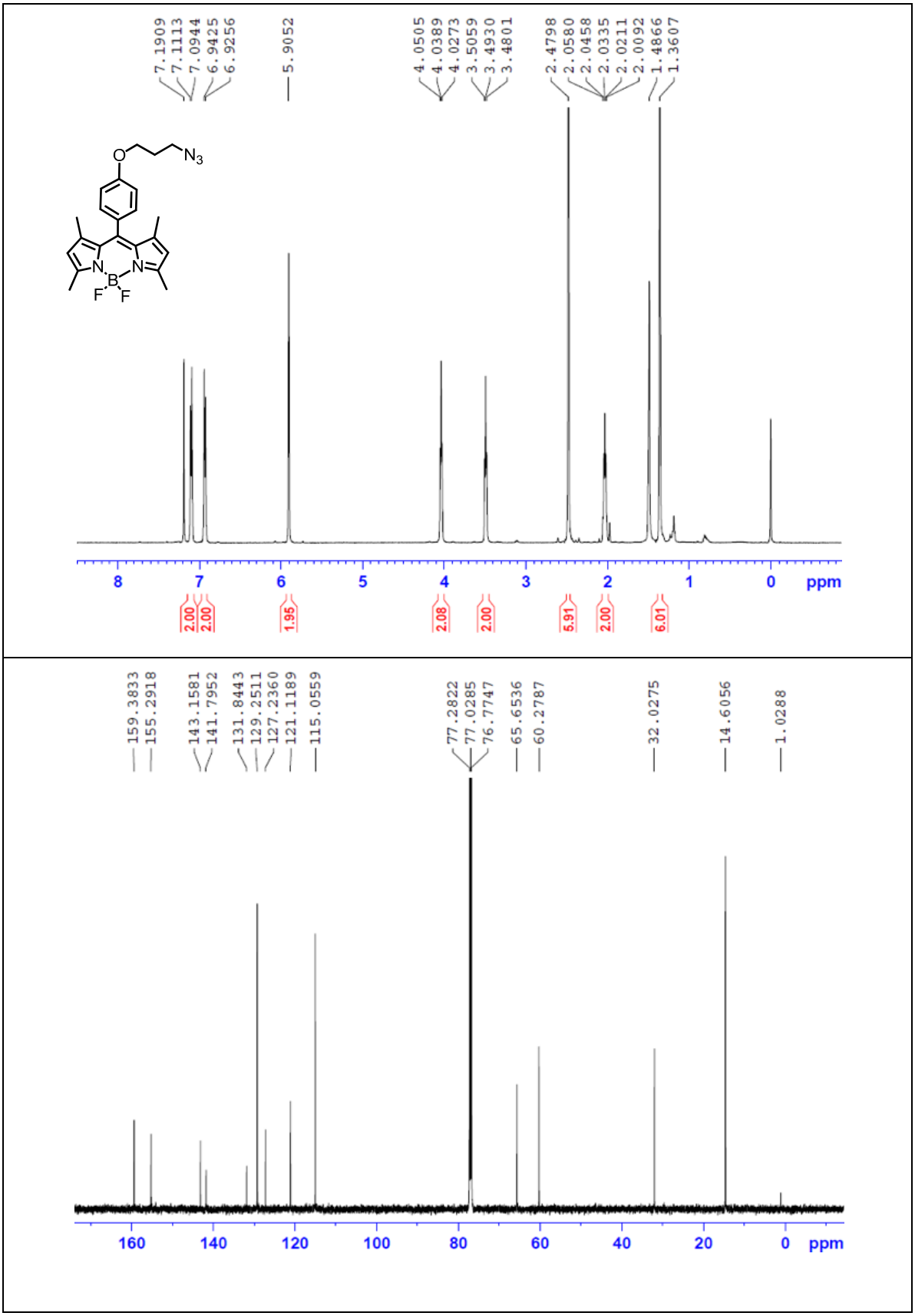


Figure S12. ^{1}H (above) and ^{13}C (below) - NMR spectra of **F**.

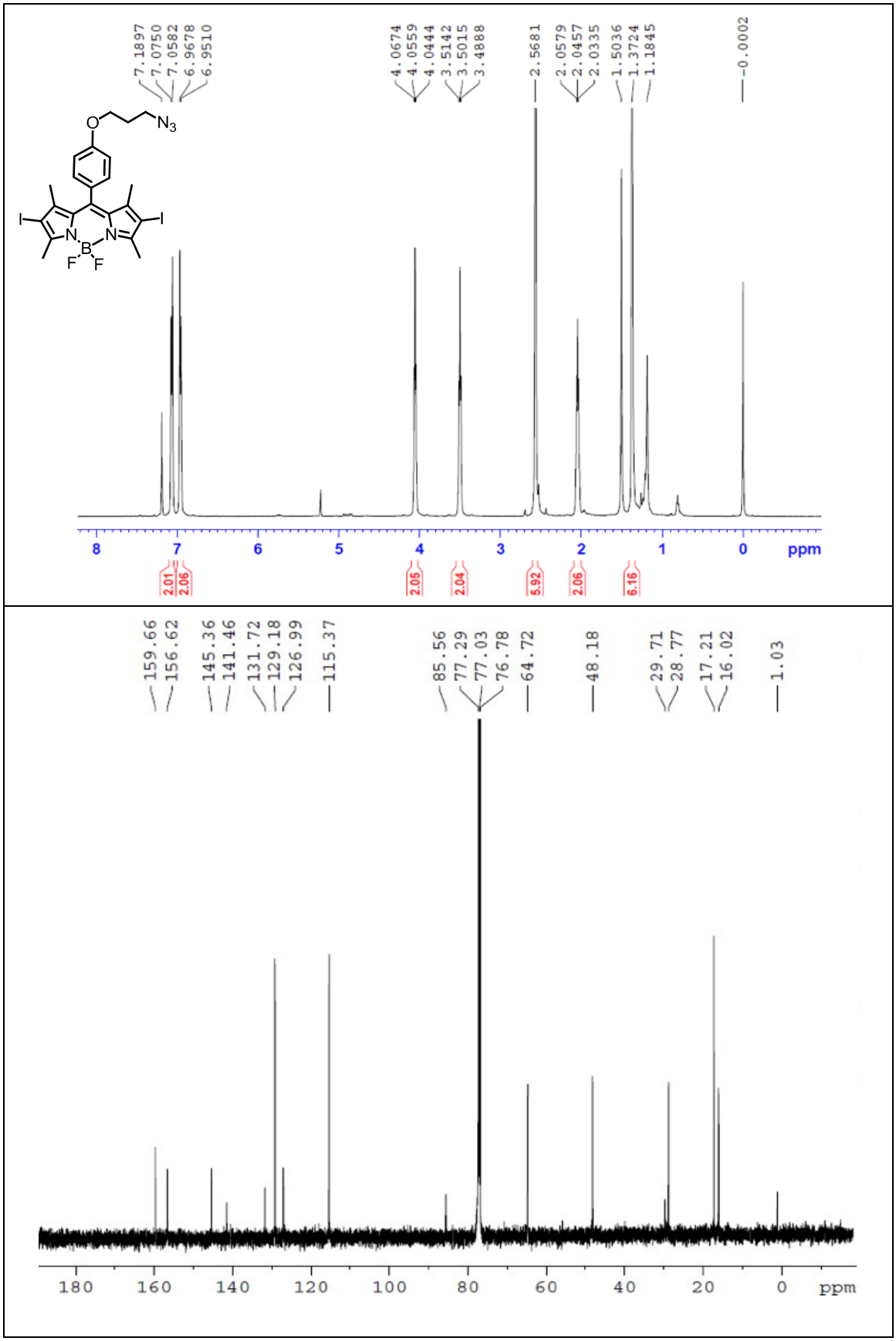


Figure S13. ^{1}H (above) and ^{13}C (below) - NMR spectra of **G**.

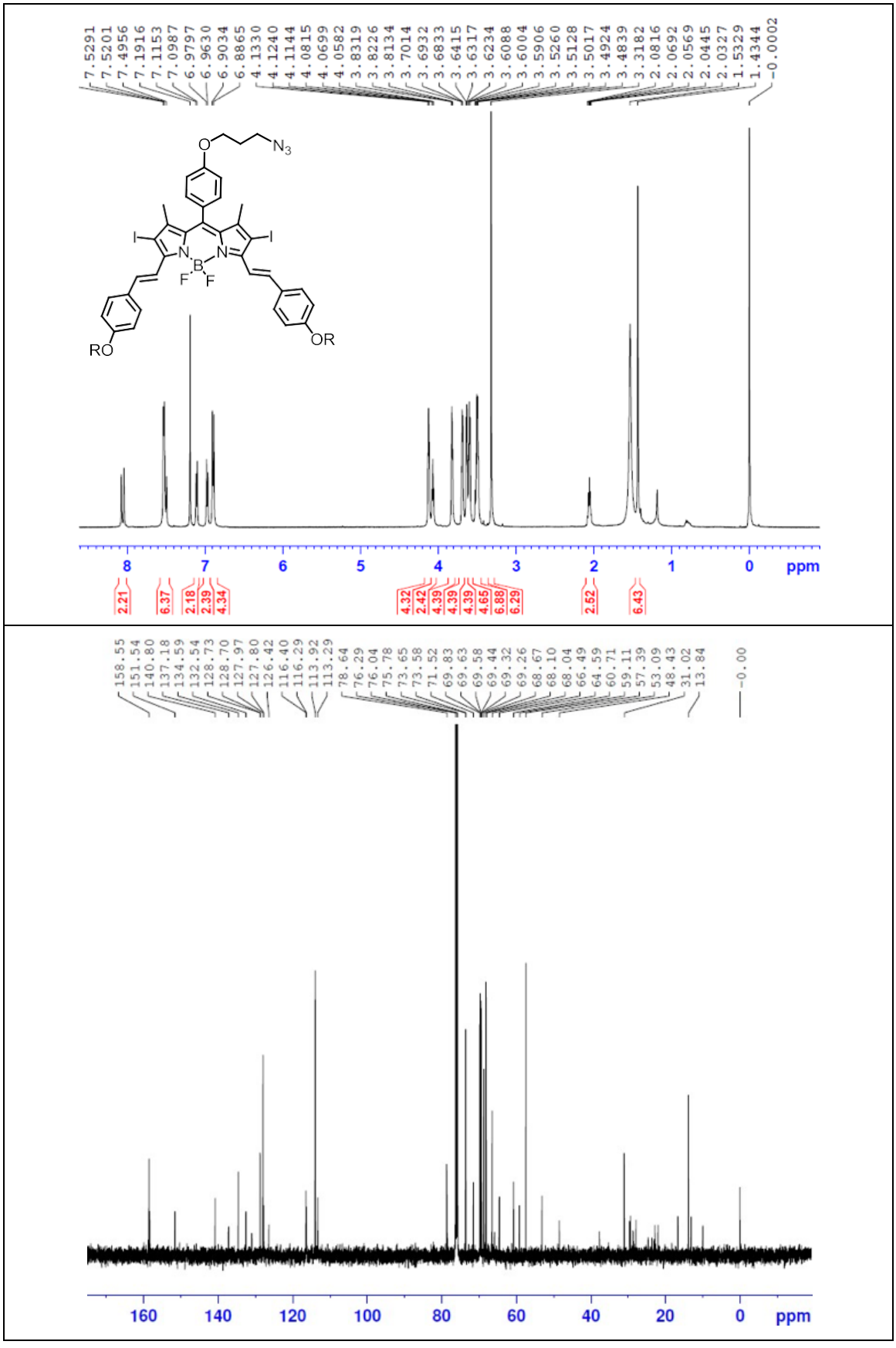


Figure S14. ^{1}H (above) and ^{13}C (below) NMR spectra of **1a**.

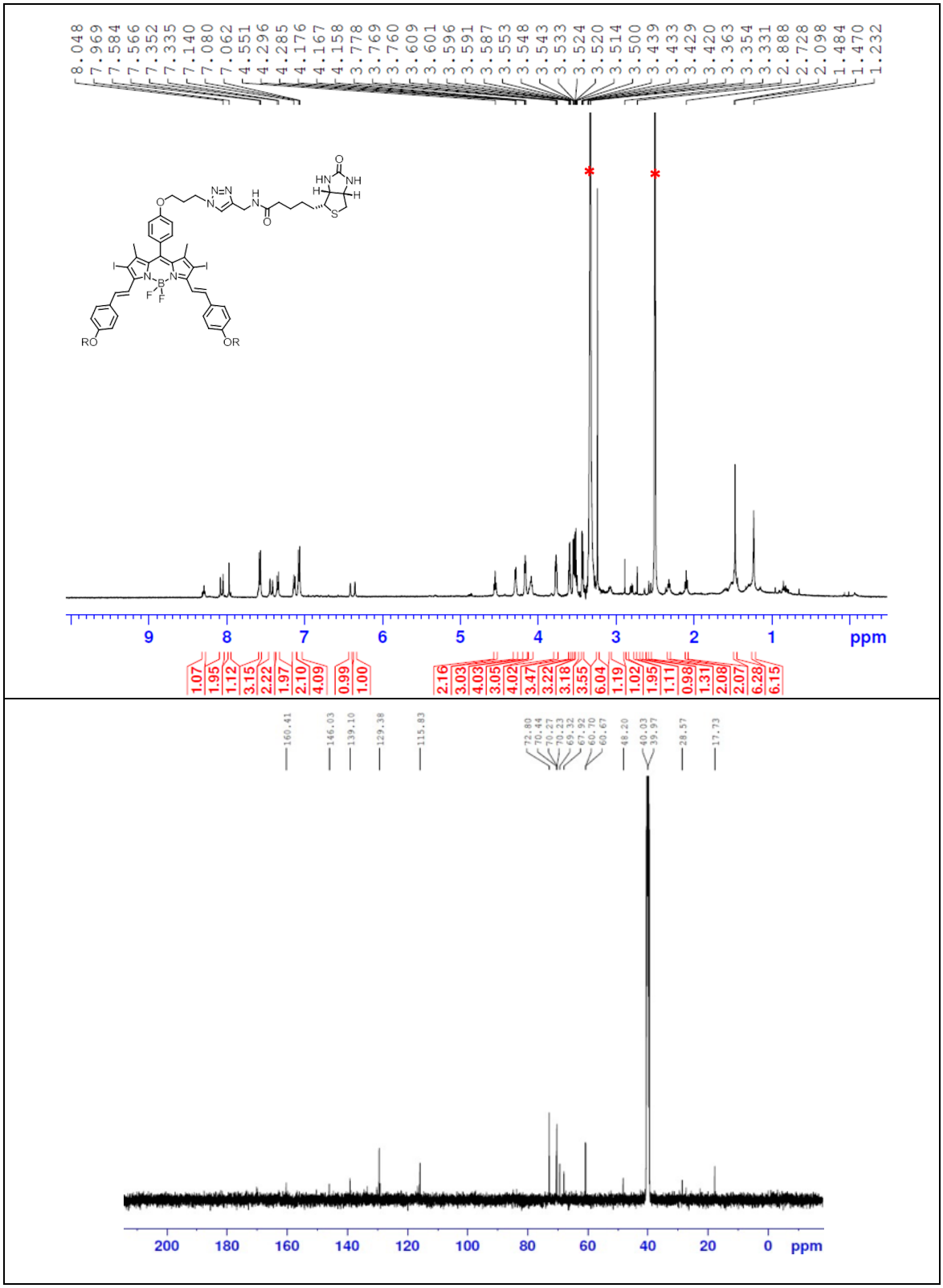


Figure S15. ^{1}H (above) and ^{13}C (below) - NMR spectra of **1**. Peaks marked with "*" in the ^{1}H-NMR spectrum are due to the solvents. Peaks correspond to **1** is not well resolved in ^{13}C NMR spectrum due to its poor solubility in DMSO and other common solvents used for NMR experiments.

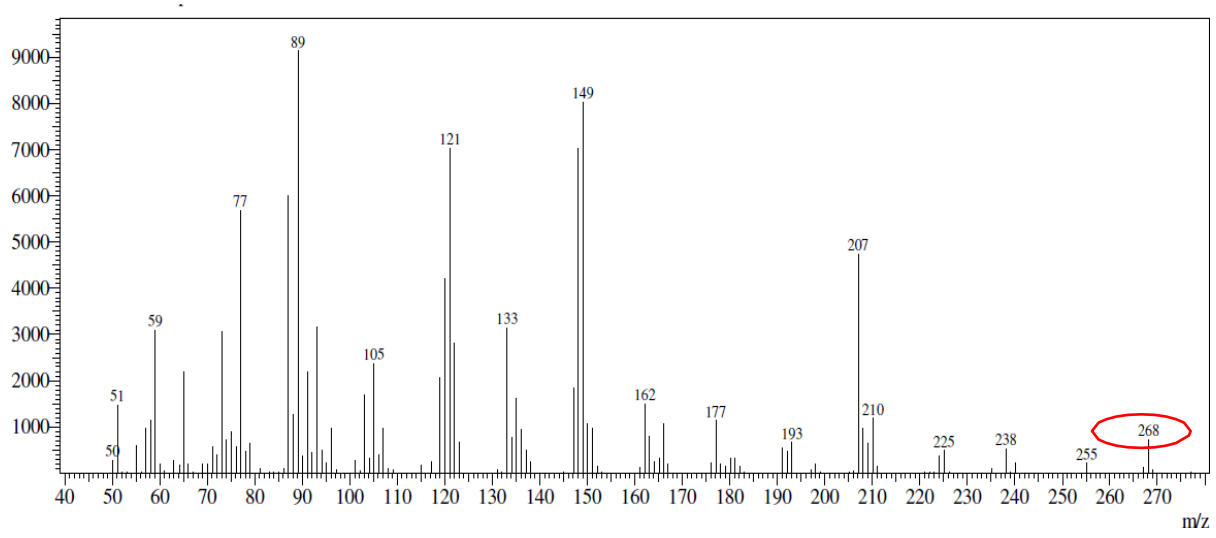


Figure S16. GC-MS spectrum of compound **B**.

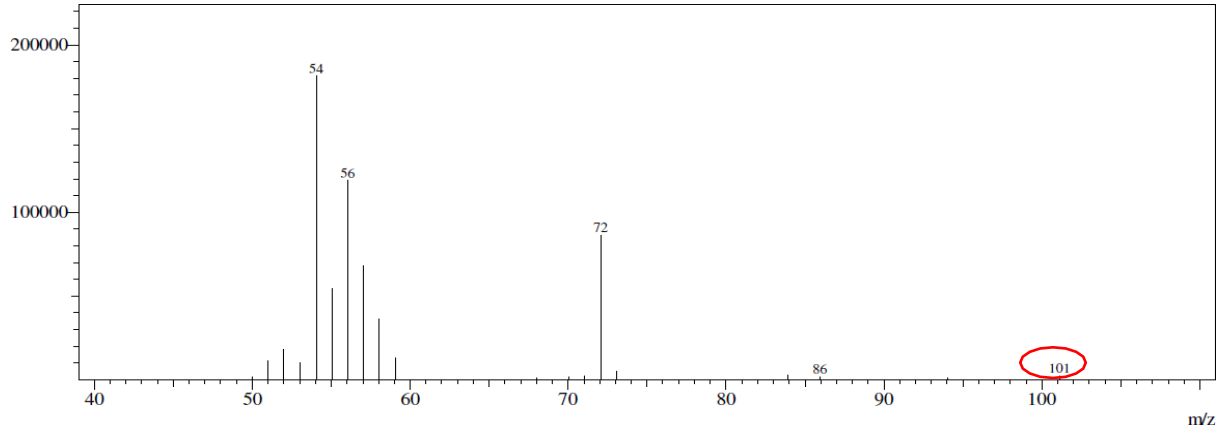


Figure S17. GC-MS spectrum of compound **C**.

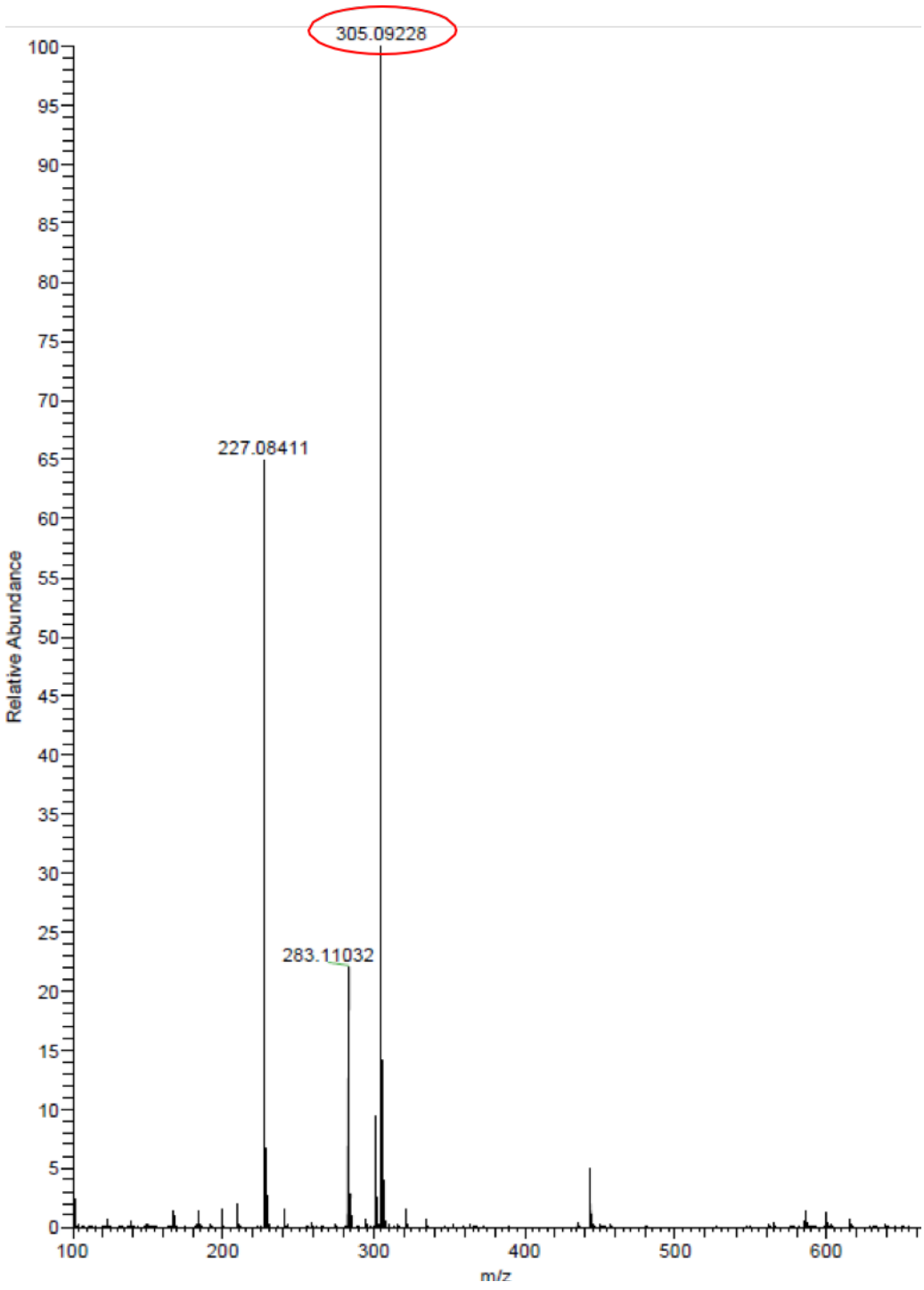


Figure S18. HR-MS spectrum of compound **1b**.

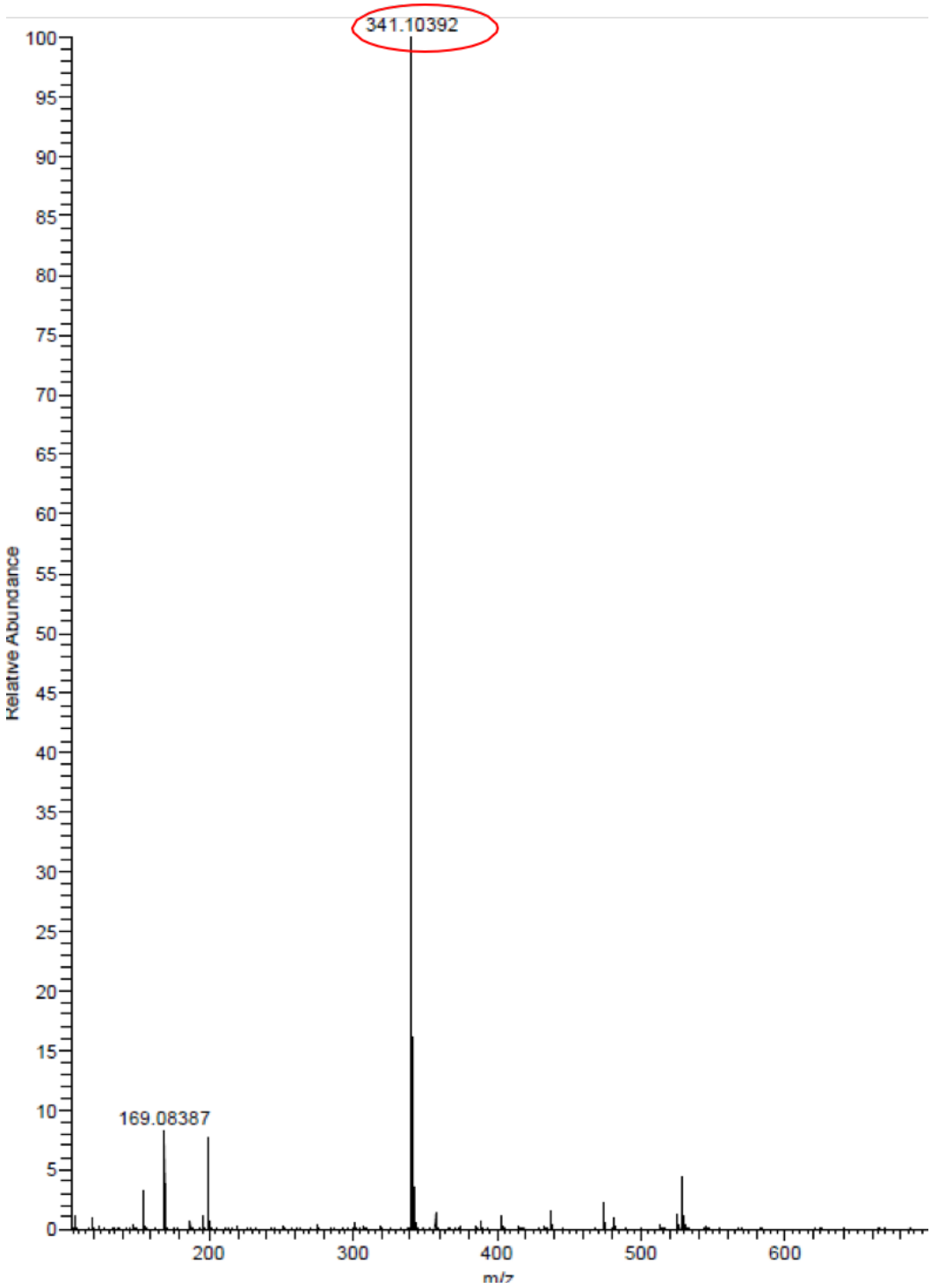


Figure S19. HR-MS spectrum of compound **A**.

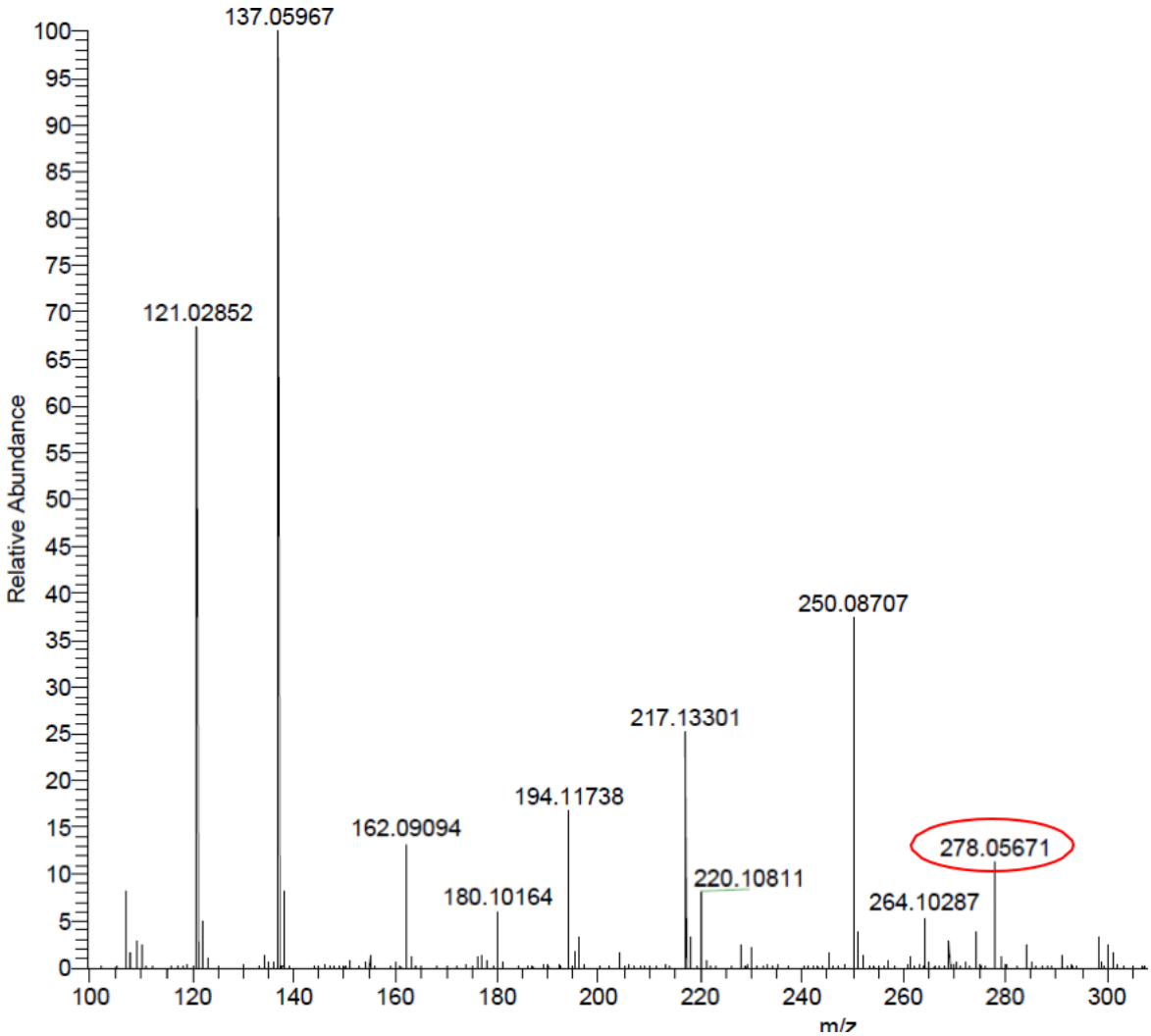


Figure S20. HR-MS spectrum of compound **D**.

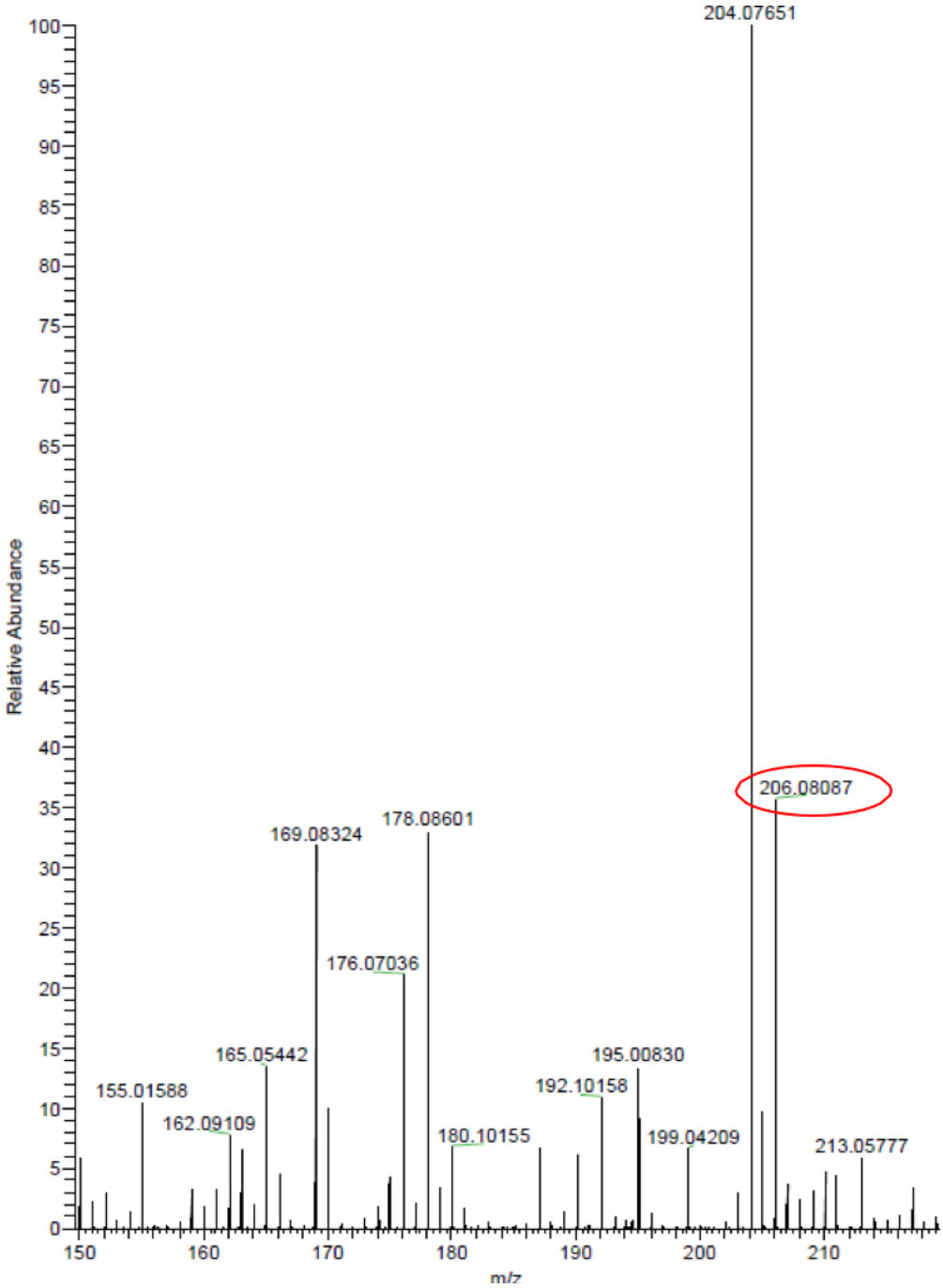


Figure S21. HR-MS spectrum of compound **E**.

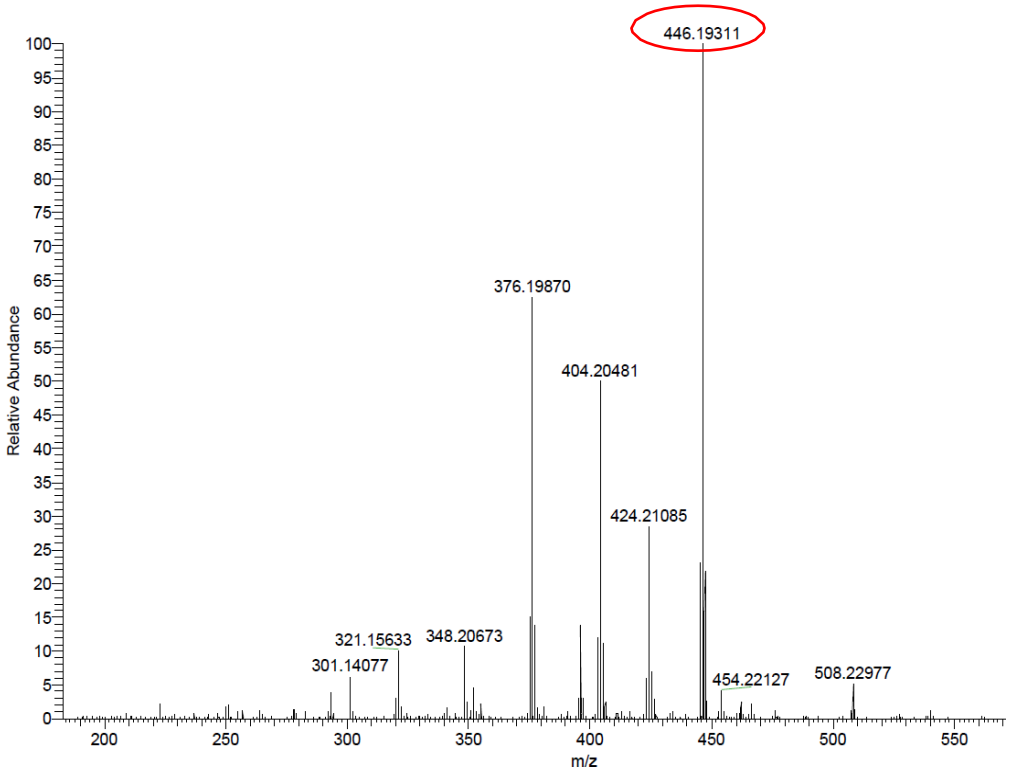


Figure S22. HR-MS spectrum of compound **F**.

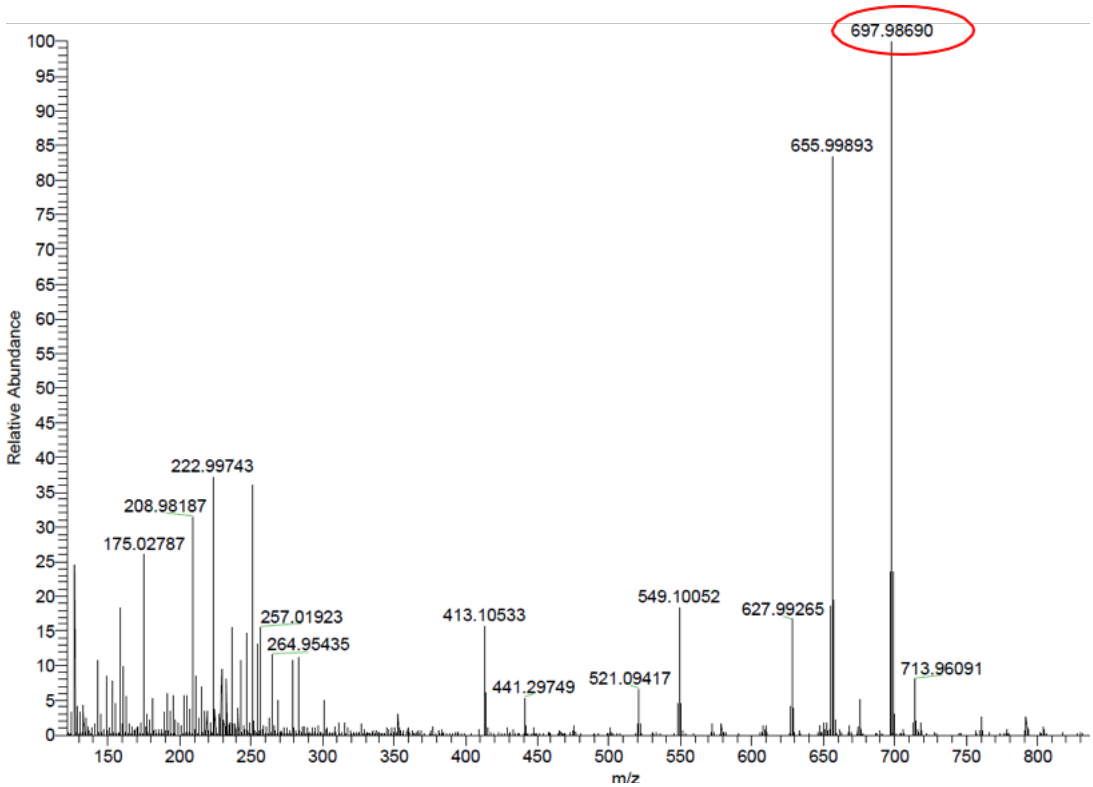


Figure S23. HR-MS spectrum of compound **G**.

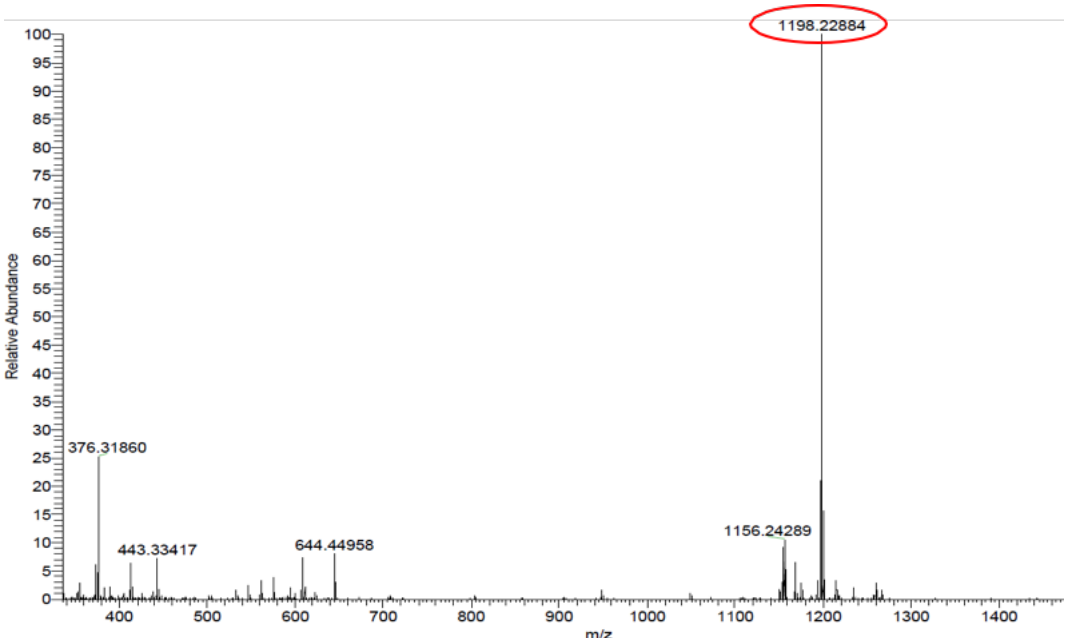


Figure S24. HR-MS spectrum of compound **1a**.

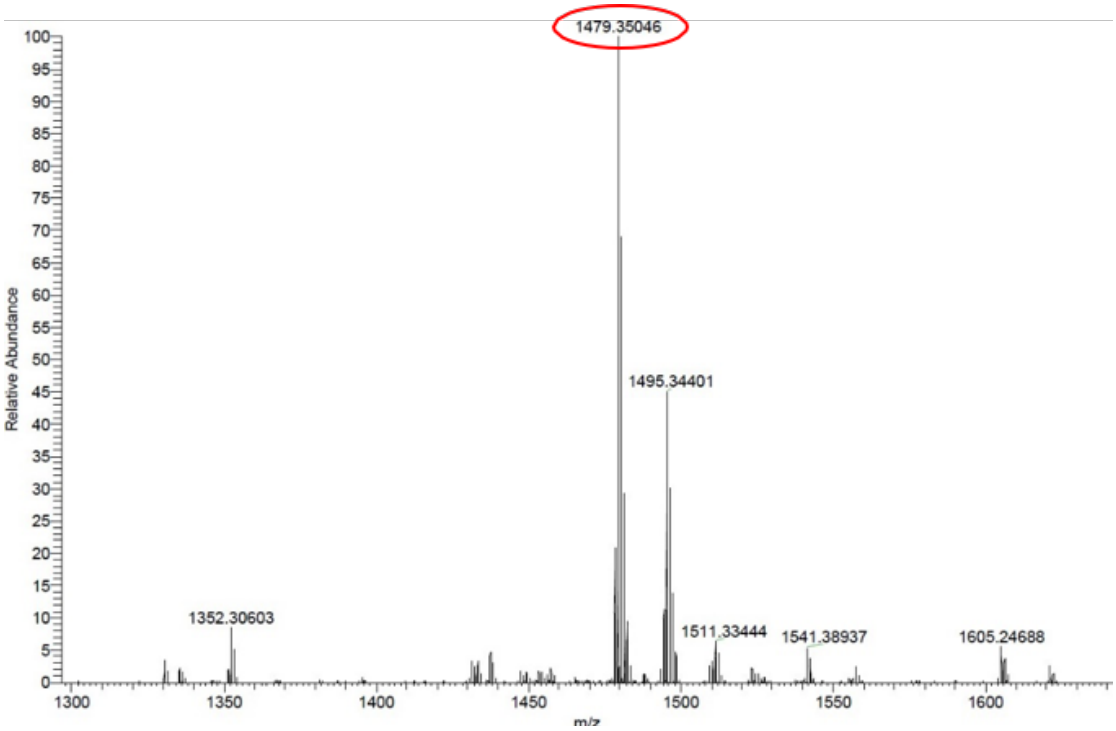


Figure S25. HR-MS spectrum of compound **1**.