

Supporting Information

***Meso* pyridinium BODIPY-based long wavelength infrared mitochondria-targeting fluorescent probe with high photostability**

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Apparatus and reagents

All reactions were carried out under an atmosphere of nitrogen or argon in air-dried glassware with magnetic stirring. Air- and/or moisture-sensitive liquids were transferred with syringe. Organic solutions were concentrated by rotary evaporation at 25-60 °C at 15-30 Torr. All solvents and common materials were purchased from commercial suppliers and used as received without further purification. Solvents used were purified by standard methods prior to use. The progress of reaction was monitored using thin layer chromatography (TLC) (silica gel 60, F254 0.25 mm), and components were visualized by observation under UV light (254 and 365 nm) or by treating the TLC plates with phosphomolybdic acid (PMA), KMnO_4 , or ninhydrin followed by heating. Column chromatography was carried out as “Flash Chromatography” using SepaBean machine. ^1H NMR spectra were recorded on Varian Model Mercury 400 or Bruker Advance IV 600 MHz spectrometer with tetramethylsilane (TMS) as internal standard. ^{13}C NMR spectra were recorded on a Varian Model Mercury 151 MHz spectrometer with tetramethylsilane (TMS) as internal standard. Recorded shifts are reported in parts per million (δ): Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant (J , Hz) and integration.

Mito-Tracker Green, Lyso-Tracker Green DND-26, and ER-Tracker Green were purchased from Beyotime Biotechnology, China. Mito-Tracker Deep Red FM were purchased from Yeasen Biotechnology, China. Electrospray ionization (ESI) mass spectra were acquired with Agilent 1100 Series LC/MSD and AB SCIEX Triple TOFTM 5600 + mass spectrometer. HRMS (high resolution mass spectrometry, DART positive) spectra were obtained on Thermo Fisher Scientific LTQ FT Ultra. Spectrometer UV-visible spectra were acquired on a 759S double beam UV-visible spectrophotometer (Lengguang Tech). Fluorescence spectra were measured by a F98 fluorescence spectrophotometer (Lengguang Tech). All spectra experiments were performed in a $1 \times 1 \text{ cm}^2$ quartz cuvette.

Synthetic procedure

1,3,5,7-Tetramethyl-8-(3-pyridinyl)-4,4'-difluoroboradiazaindacene (1a)

2,4-Dimethyl-pyrrole (620 μL , 6 mmol) and 3-pyridinylcarbaldehyde (285 μL , 3 mmol) were dissolved in 250 mL of anhydrous dichloromethane under N_2 atmosphere. TFA (300 μL) was added, and the solution was stirred at room temperature for 2 h. A solution of p-chloranil (885 mg, 3.6 mmol) in 100 mL of dichloromethane was dropped in the reaction flask, and the stirring was continued for 2 h followed by the addition of DIPEA (3.87 g, 30 mmol) and $\text{BF}_3 \cdot \text{OEt}_2$ (4.5 mL, 36 mmol) at 0 $^\circ\text{C}$. After stirring for 3 h, the reaction mixture was washed three times with water (300 mL), dried over anhydrous sodium sulfate, and the solvent was removed under vacuum. The raw material was purified by column chromatography, affording the title compound as orange solid (370 mg, 38%). ^1H NMR (600 MHz, CDCl_3) δ 8.77 (d, $J = 4.8$ Hz, 1H), 8.59 (s, 1H), 7.67 (d, $J = 7.7$ Hz, 1H), 7.48 (dd, $J = 7.6, 5.0$ Hz, 1H), 6.03 (s, 2H), 2.58 (s, 6H), 1.40 (s, 6H). ^{13}C NMR (151 MHz, CDCl_3) δ 155.75, 149.58, 147.89, 142.16, 136.64, 135.39, 130.87, 130.67, 123.11, 121.15, 14.37, 14.02. ESI-MS [m/z] 326.2 ($\text{M} + \text{H}^+$) (325.2 calculated for $\text{C}_{18}\text{H}_{18}\text{BF}_2\text{N}_3$).

1,3,5,7-Tetramethyl-8-(2-pyridinyl)-4,4'-difluoroboradiazaindacene (1b)

2,4-Dimethyl-pyrrole (620 μL , 6 mmol) and 2-pyridinylcarbaldehyde (285 μL , 3 mmol) reacted according to the procedure for **1a**, and the title compound was obtained as orange solid (399 mg, 42%). ^1H NMR (600 MHz, CDCl_3) δ 8.80 (d, $J = 4.3$ Hz, 1H), 7.86 (t, $J = 7.6$ Hz, 1H), 7.45 (m, 2H), 6.00 (s, 2H), 2.57 (s, 6H), 1.33 (s, 6H). ^{13}C NMR (151 MHz, CDCl_3) δ 155.73, 153.26, 149.55, 141.95, 137.85, 136.44, 130.76, 123.79, 123.30, 120.64, 14.06, 13.14. ESI-MS [m/z] 326.2 ($\text{M} + \text{H}^+$) (325.2 calculated for $\text{C}_{18}\text{H}_{18}\text{BF}_2\text{N}_3$).

1,7-Dimethyl-3,5-bis(4-methoxystyryl)-8-(3-pyridinyl)-4,4'-difluoroboradiazaindacene (2a)

The compound **1a** (100 mg, 0.3 mmol), 4-methoxybenzaldehyde (418 mg, 3.0 mmol) was dissolved in toluene (10 mL), then added glacial acetic acid (0.1 mL), piperidine (0.1 mL), reflux in a double-neck flask for 5 h, remove the oil bath after the reaction, extract with dichloromethane and water (25 mL \times 3), dry with anhydrous sodium sulfate, filter and evaporate the solvent, and purify by column chromatography to obtain the title compound as dark green solid (58 mg, 34%). ^1H NMR (600 MHz, Chloroform-*d*) δ 8.78 – 8.75 (m, 1H), 8.64 – 8.61 (m, 1H), 7.69 (dt, $J = 7.7, 2.0$ Hz, 1H), 7.65 – 7.57 (m, 6H), 7.47 (dd, $J = 7.7, 4.9$ Hz, 1H), 7.26 – 7.21 (m, 1H), 6.96 – 6.91 (m, 4H), 3.86 (s, 6H), 1.44 (s, 6H). ^{13}C NMR (151 MHz, CDCl_3) δ 160.58, 153.32, 150.12, 149.12, 141.24, 136.56, 136.46, 133.41, 133.24, 131.66, 129.45, 129.18, 123.63, 118.02, 117.08, 117.06, 117.04, 114.33, 55.41, 15.28. ESI-HRMS [m/z] 562.2469 ($\text{M} + \text{H}^+$) (562.2472 calculated for $\text{C}_{34}\text{H}_{30}\text{BF}_2\text{N}_3\text{O}_2$).

1,7-Dimethyl-3,5-bis(4-(1,4,7-trioxaoctyl)styryl)-8-(3-pyridinyl)-4,4'-difluoroboradiazaindacene (2b)

The compound **1a** (100 mg, 0.3 mmol), 4-(2-(2-methoxyethoxy)ethoxy)benzaldehyde (336 mg, 1.5 mmol) reacted according to the procedure for **2a**, and the title compound was obtained as dark green solid (88 mg, 39%). ^1H NMR (600 MHz, Chloroform-*d*) δ 8.78 – 8.75 (m, 1H), 8.62 (d, $J = 2.2$ Hz, 1H), 7.70 (dt, $J = 7.7, 2.0$ Hz, 1H), 7.63 – 7.55 (m, 6H), 7.47 (dd, $J = 7.7, 5.0$ Hz, 1H), 7.23 (d, $J = 16.2$ Hz, 2H), 6.97 – 6.93 (m, 4H), 6.64 (s, 2H), 4.19 (t, $J = 4.9$ Hz, 4H), 3.89 (t, $J = 4.8$ Hz, 4H), 3.76 – 3.72 (m, 4H), 3.62 – 3.58 (m, 4H), 3.41 (s, 6H), 1.43 (s, 6H). ^{13}C NMR (151 MHz, CDCl_3) δ 159.78, 153.32, 150.03, 149.02, 141.21, 136.66, 136.47, 133.33, 133.24, 131.69, 129.57, 129.14, 123.66, 118.04, 117.07, 117.05, 114.98, 71.98, 70.82, 69.71, 67.52, 59.11, 15.28. ESI-HRMS [m/z] 738.3534 ($\text{M} + \text{H}^+$) (738.3520 calculated for $\text{C}_{42}\text{H}_{46}\text{BF}_2\text{N}_3\text{O}_6$).

1,7-Dimethyl-3,5-bis(4-methoxystyryl)-8-(2-pyridinyl)-4,4'-difluoroboradiazaindacene (2c)

The compound **1b** (100 mg, 0.3 mmol), 4-methoxybenzaldehyde (418 mg, 3.0 mmol) reacted according to the procedure for **2a**, and the title compound was obtained as dark green solid (48 mg, 27%). ^1H NMR (600 MHz, Chloroform-*d*) δ 8.79 (d, $J = 4.9$ Hz, 1H), 7.83 (td, $J = 7.7, 1.8$ Hz, 1H), 7.65 – 7.54 (m, 6H), 7.47 (d, $J = 7.7$ Hz, 1H), 7.42 (dd, $J = 7.7, 5.0$ Hz, 1H), 7.21 (d, $J = 16.2$ Hz, 2H), 6.93 (d, $J = 8.5$ Hz, 4H), 6.61 (s, 2H), 3.85 (s, 6H), 1.37 (s, 6H). ^{13}C NMR (151 MHz, CDCl_3) δ 160.45, 154.31, 153.37, 150.12, 141.11, 136.87, 136.02, 135.07, 133.21, 129.55, 129.10, 124.96, 123.80, 117.55, 117.32, 117.30, 117.28, 114.28, 55.38, 13.99. ESI-HRMS [m/z] 562.2456 ($\text{M} + \text{H}^+$) (562.2472 calculated for $\text{C}_{34}\text{H}_{30}\text{BF}_2\text{N}_3\text{O}_2$).

1,7-Dimethyl-3,5-bis(4-(1,4,7-trioxaoctyl)styryl)-8-(2-pyridinyl)-4,4'-difluoroboradiazaindacene (2d)

The compound **1b** (100 mg, 0.3 mmol), 4-(2-(2-methoxyethoxy)ethoxy)benzaldehyde (336 mg, 1.5 mmol) reacted according to the procedure for **2a**, and the title compound was obtained as dark green solid (93 mg, 41%). ^1H NMR (600 MHz, Chloroform-*d*) δ 8.81 – 8.77 (m, 1H), 7.84 (td, $J = 7.7, 1.7$ Hz, 1H), 7.63 – 7.54

(m, 6H), 7.49 – 7.40 (m, 2H), 7.20 (d, $J = 16.3$ Hz, 2H), 6.96 – 6.92 (m, 4H), 6.61 (s, 2H), 4.19 (t, $J = 4.9$ Hz, 4H), 3.89 (dd, $J = 5.6, 4.1$ Hz, 4H), 3.76 – 3.72 (m, 4H), 3.62 – 3.56 (m, 4H), 3.41 (s, 6H), 1.37 (s, 6H). ^{13}C NMR (151 MHz, CDCl_3) δ 159.66, 154.23, 153.38, 150.06, 141.10, 136.96, 136.05, 134.94, 133.21, 129.68, 129.08, 125.00, 123.86, 117.58, 117.31, 114.94, 71.97, 70.80, 69.72, 67.50, 59.11, 13.99. ESI-HRMS [m/z] 738.3527 ($\text{M}+\text{H}^+$) (738.3520 calculated for $\text{C}_{42}\text{H}_{46}\text{BF}_2\text{N}_3\text{O}_6$).

1,7-Dimethyl-3,5-bis(4-methoxystyryl)-8-(N-methyl-3-pyridinyl)-4,4'-difluoroboradiazaindacene Iodide (3a)

2a (56 mg, 0.1 mmol) and methyl iodide (647 μL , 2 mmol) were dissolved in 5 mL of MeCN, and the solution was kept under reflux in sealed tube at 60 °C. After stirring for 12 h, the solvent was removed under vacuum and the crude product was purified by silica gel column chromatography to give dark blue solid (68 mg, 97%). ^1H NMR (600 MHz, $\text{DMSO}-d_6$) δ 9.38 (s, 1H), 9.22 (d, $J = 6.3$ Hz, 1H), 8.89 (d, $J = 8.0$ Hz, 1H), 8.35 (dd, $J = 8.0, 6.2$ Hz, 1H), 7.65 – 7.59 (m, 6H), 7.43 (d, $J = 16.5$ Hz, 2H), 7.11 – 7.04 (m, 6H), 4.46 (s, 3H), 3.83 (s, 6H), 1.48 (s, 6H). ^{13}C NMR (151 MHz, DMSO) δ 161.17, 153.88, 147.11, 146.56, 145.69, 141.62, 138.55, 134.13, 132.79, 130.12, 130.04, 129.60, 129.12, 128.72, 128.61, 128.56, 126.56, 124.34, 119.54, 116.04, 115.25, 55.86, 49.06, 48.93, 16.17, 11.45. ESI-HRMS [m/z] 576.2615 (M^+) (576.2628 calculated for $\text{C}_{35}\text{H}_{33}\text{BF}_2\text{N}_3\text{O}_2$).

1,7-Dimethyl-3,5-bis(4-methoxystyryl)-8-(N-benzyl-3-pyridinyl)-4,4'-difluoroboradiazaindacene Bromide (3b)

2a (56 mg, 0.1 mmol) and benzyl bromide (493 μL , 2 mmol) reacted according to the procedure for **3a**, and the title compound was obtained as dark blue solid (53 mg, 72%). ^1H NMR (600 MHz, $\text{Methanol}-d_4$) δ 9.41 (d, $J = 16.8$ Hz, 2H), 8.76 (s, 1H), 8.35 (s, 1H), 7.60 (d, $J = 8.1$ Hz, 4H), 7.52 – 7.43 (m, 5H), 7.35 (d, $J = 7.8$ Hz, 4H), 6.99 (d, $J = 8.2$ Hz, 4H), 6.84 (s, 2H), 5.94 (s, 2H), 3.85 (s, 6H), 1.30 (d, $J = 9.0$ Hz, 6H). ^{13}C NMR (151 MHz, MeOD) δ 162.74, 145.22, 139.34, 134.91, 131.48, 131.19, 130.97, 130.90, 130.85, 130.76, 130.60, 130.36, 130.30, 129.88, 129.51, 129.39, 128.31, 128.04, 124.36, 124.27, 120.13, 117.41, 116.64, 115.82, 115.67, 115.60, 65.28, 55.97, 55.78, 15.95, 13.56. ESI-HRMS [m/z] 652.2933 (M^+) (652.2941 calculated for $\text{C}_{41}\text{H}_{37}\text{BF}_2\text{N}_3\text{O}_2$).

1,7-Dimethyl-3,5-bis(4-(1,4,7-trioxaoctyl)styryl)-8-(N-methyl-3-pyridinyl)-4,4'-difluoroboradiazaindacene Iodide (3c)

According to the procedure described for **3a**, **2b** (74 mg, 0.1 mmol) was treated with methyl iodide to give **3c** as dark blue solid (86 mg, 98%). ^1H NMR (600 MHz, $\text{Methanol}-d_4$) δ 9.24 (d, $J = 1.7$ Hz, 1H), 9.14 (dt, $J = 6.3, 1.4$ Hz, 1H), 8.77 (d, $J = 8.0$ Hz, 1H), 8.31 (dd, $J = 8.0, 6.2$ Hz, 1H), 7.60 (d, $J = 8.8$ Hz, 4H), 7.58 – 7.42 (m, 4H), 7.02 (d, $J = 8.7$ Hz, 4H), 6.90 (s, 2H), 4.54 (s, 3H), 4.24 – 4.16 (m, 4H), 3.90 – 3.82 (m, 4H), 3.74 – 3.68 (m, 4H), 3.61 – 3.55 (m, 4H), 3.37 (s, 6H), 1.53 (s, 6H). ^{13}C NMR (151 MHz, MeOD) δ 161.82, 155.85, 148.11, 147.80, 146.78, 142.30, 139.21, 137.32, 134.05, 130.83, 130.37, 129.83, 128.52, 120.18, 117.56, 116.27, 73.04, 71.63, 70.80, 68.84, 59.18, 49.62, 16.24. ESI-HRMS [m/z] 752.3662 (M^+) (752.3677 calculated for $\text{C}_{43}\text{H}_{49}\text{BF}_2\text{N}_3\text{O}_6$).

1,7-Dimethyl-3,5-bis(4-(1,4,7-trioxaoctyl)styryl)-8-(N-benzyl-3-pyridinyl)-4,4'-difluoroboradiazaindacene Bromide (3d)

According to the procedure described for **3a**, **2b** (74 mg, 0.1 mmol) was treated with benzyl bromide to give **3d** as dark blue solid (74 mg, 82%). ^1H NMR (600 MHz, $\text{Methanol}-d_4$) δ 9.42 – 9.38 (m, 2H), 8.74 (dt, $J = 7.9, 1.5$ Hz, 1H), 8.34 (t, $J = 7.2$ Hz, 1H), 7.63 – 7.53 (m, 7H), 7.53 – 7.40 (m, 6H), 7.02 – 6.96 (m, 4H), 6.83 (s, 2H), 4.19 – 4.15 (m, 4H), 3.87 – 3.83 (m, 4H), 3.72 – 3.66 (m, 4H), 3.59 – 3.53 (m, 4H), 3.36 (s, 6H), 1.30 (s, 6H). ^{13}C NMR (151 MHz, MeOD) δ 161.81, 155.81, 148.73, 146.83, 145.95, 141.98, 139.29, 137.86, 134.90, 134.00, 131.47, 130.96, 130.78, 130.58, 130.38, 130.31, 128.25, 120.21, 117.50, 116.25, 73.03, 71.62, 70.79, 68.83, 66.55, 59.17, 16.01. ESI-HRMS [m/z] 828.3984 (M^+) (828.3990 calculated for $\text{C}_{49}\text{H}_{53}\text{BF}_2\text{N}_3\text{O}_6$).

1,7-Dimethyl-3,5-bis(4-methoxystyryl)-8-(N-methyl-2-pyridinyl)-4,4'-difluoroboradiazaindacene Iodide (3e)

According to the procedure described for **3a**, **2c** (56 mg, 0.1 mmol) was treated with methyl iodide to give **3e** as dark blue solid (69 mg, 98%). ^1H NMR (600 MHz, $\text{Methanol}-d_4$) δ 9.23 (d, $J = 6.3$ Hz, 1H), 8.78 (s, 1H), 8.40 (d, $J = 7.8$ Hz, 1H), 8.35 (s, 1H), 7.62 (d, $J = 8.2$ Hz, 4H), 7.51 (t, $J = 16.5$ Hz, 4H), 6.99 (d, $J =$

8.2 Hz, 4H), 6.93 (s, 2H), 4.32 (s, 3H), 3.85 (s, 6H), 1.41 (s, 6H). ¹³C NMR (151 MHz, MeOD) δ 163.03, 157.35, 150.96, 149.64, 148.46, 141.21, 140.52, 132.99, 131.23, 130.63, 130.58, 130.47, 120.62, 117.27, 115.75, 115.58, 56.01, 46.96, 13.47. ESI-HRMS [*m/z*] 576.2612 (M⁺) (576.2628 calculated for C₃₅H₃₃BF₂N₃O₂).

1,7-Dimethyl-3,5-bis(4-methoxystyryl)-8-(N-benzyl-2-pyridinyl)-4,4'-difluoroboradiazaindacene Bromide (3f)

According to the procedure described for **3a**, **2c** (56 mg, 0.1 mmol) was treated with benzyl bromide to give **3f** as dark blue solid (59 mg, 80 %). ¹H NMR (600 MHz, Methanol-*d*₄) δ 9.66 (d, *J* = 6.3 Hz, 1H), 8.79 (s, 1H), 8.43 (s, 2H), 7.64 (d, *J* = 8.2 Hz, 4H), 7.51 – 7.45 (m, 4H), 7.34 (d, *J* = 7.6 Hz, 2H), 7.27 (d, *J* = 7.5 Hz, 3H), 7.20 (t, *J* = 7.6 Hz, 2H), 7.00 (d, *J* = 8.2 Hz, 4H), 6.77 (s, 2H), 5.80 (s, 2H), 3.86 (s, 6H), 1.15 (s, 4H). ¹³C NMR (151 MHz, MeOD) δ 163.02, 157.14, 150.76, 149.34, 148.57, 141.25, 140.44, 133.83, 133.26, 132.13, 131.19, 131.14, 130.64, 130.62, 130.51, 130.20, 121.85, 120.68, 117.31, 115.76, 64.70, 56.02, 13.91. ESI-HRMS [*m/z*] 652.2937 (M⁺) (652.2941 calculated for C₄₁H₃₇BF₂N₃O₂).

1,7-Dimethyl-3,5-bis(4-(1,4,7-trioxaoctyl)styryl)-8-(N-methyl-2-pyridinyl)-4,4'-difluoroboradiazaindacene Iodide (3g)

According to the procedure described for **3a**, **2d** (74 mg, 0.1 mmol) was treated with methyl iodide to give **3g** as dark blue solid (88 mg, 99%). ¹H NMR (600 MHz, Methanol-*d*₄) δ 9.30 – 9.23 (m, 1H), 8.81 (td, *J* = 7.9, 1.4 Hz, 1H), 8.45 (dd, *J* = 7.9, 1.5 Hz, 1H), 8.37 (ddd, *J* = 7.9, 6.2, 1.5 Hz, 1H), 7.64 – 7.61 (m, 4H), 7.60 – 7.50 (m, 4H), 7.04 – 7.01 (m, 4H), 6.98 (s, 2H), 4.35 (s, 3H), 4.23 – 4.17 (m, 4H), 3.88 – 3.84 (m, 4H), 3.73 – 3.67 (m, 4H), 3.60 – 3.55 (m, 4H), 3.37 (s, 6H), 1.45 (s, 6H). ¹³C NMR (151 MHz, MeOD) δ 162.14, 157.29, 150.90, 149.66, 148.47, 141.22, 140.45, 132.95, 132.89, 130.66, 130.64, 130.59, 122.29, 120.68, 117.39, 116.34, 73.03, 71.63, 70.78, 68.87, 59.18, 46.98, 13.52. ESI-HRMS [*m/z*] 752.3662 (M⁺) (752.3677 calculated for C₄₃H₄₉BF₂N₃O₆).

1,7-Dimethyl-3,5-bis(4-(1,4,7-trioxaoctyl)styryl)-8-(N-benzyl-2-pyridinyl)-4,4'-difluoroboradiazaindacene Bromide (3h)

According to the procedure described for **3a**, **2d** (74 mg, 0.1 mmol) was treated with benzyl bromide to give **3h** as reddish-brown solid (78 mg, 86%). ¹H NMR (600 MHz, Methanol-*d*₄) δ 9.68 (d, *J* = 6.2 Hz, 1H), 8.81 (td, *J* = 7.9, 1.4 Hz, 1H), 8.47 – 8.42 (m, 2H), 7.67 – 7.46 (m, 8H), 7.31 – 7.27 (m, 3H), 7.24 – 7.19 (m, 2H), 7.06 – 7.01 (m, 4H), 6.81 (s, 2H), 5.83 (s, 2H), 4.22 – 4.18 (m, 4H), 3.89 – 3.84 (m, 4H), 3.73 – 3.67 (m, 4H), 3.60 – 3.56 (m, 4H), 3.38 (s, 6H), 1.19 (s, 6H). ¹³C NMR (151 MHz, MeOD) δ 162.14, 157.12, 150.74, 149.34, 148.60, 141.27, 140.39, 133.82, 133.27, 132.13, 131.19, 131.15, 130.70, 130.65, 130.61, 130.20, 121.87, 120.71, 117.44, 116.36, 73.04, 71.64, 70.79, 68.87, 64.70, 59.18, 13.92. ESI-HRMS [*m/z*] 828.3985 (M⁺) (828.3990 calculated for C₄₉H₅₃BF₂N₃O₆).

1,7-Dimethyl-3,5-bis(4-(1,4,7-trioxaoctyl)styryl)-8-(N-(4-(chloromethyl)benzyl)-2-pyridinyl)-4,4'-difluoroboradiazaindacene Chloride(3i)

According to the procedure described for **3a**, **2d** (74 mg, 0.1 mmol) was treated with α,α',α4 mg, 0.1 mmol) wto give **3i** as reddish-brown solid (18 mg, 24%). ¹H NMR (600 MHz, Methanol-*d*₄) δ 9.67 (d, *J* = 6.1 Hz, 1H), 8.81 (d, *J* = 1.4 Hz, 1H), 8.45 (s, 1H), 8.39 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.62 (d, *J* = 8.6 Hz, 4H), 7.48 (d, *J* = 12.3 Hz, 2H), 7.38 – 7.32 (m, 2H), 7.23 (q, *J* = 8.1 Hz, 4H), 7.02 (d, *J* = 8.6 Hz, 4H), 6.76 (s, 2H), 5.79 (s, 2H), 4.50 (s, 2H), 4.21 – 4.17 (m, 4H), 3.88 – 3.84 (m, 4H), 3.73 – 3.69 (m, 4H), 3.60 – 3.56 (m, 4H), 3.37 (s, 6H), 1.17 (s, 6H). ¹³C NMR (151 MHz, MeOD) δ 160.64, 155.57, 149.25, 148.23, 147.19, 139.83, 139.59, 139.00, 135.01, 132.38, 131.74, 130.56, 129.77, 129.68, 129.33, 129.26, 129.23, 128.86, 127.25, 124.88, 120.29, 119.49, 115.98, 114.91, 71.57, 70.16, 69.33, 67.42, 63.20, 57.74, 44.59, 42.09, 12.60, 10.13. ESI-HRMS [*m/z*] 876.3758 (M⁺) (876.3765calculated for C₅₀H₅₄BClF₂N₃O₆).

Supplementary optical spectra

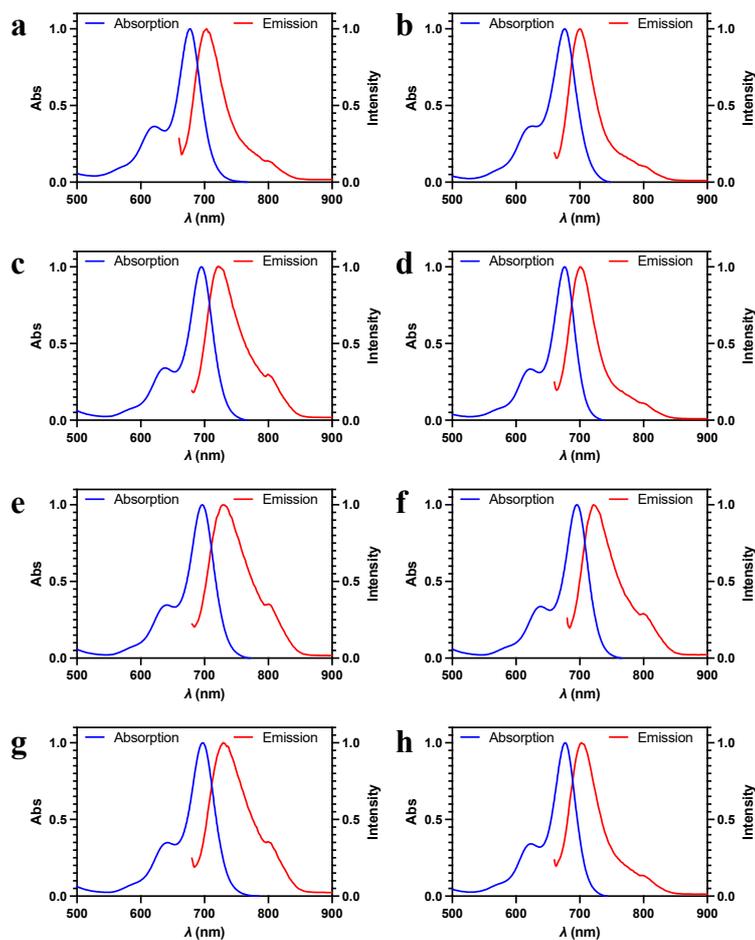


Figure S1. Normalized absorption and fluorescence emission spectra of probes in CHCl_3 . (a) **3a**; (b) **3b**; (c) **3c**; (d) **3d**; (e) **3e**; (f) **3f**; (g) **3g**; (h) **3h**.

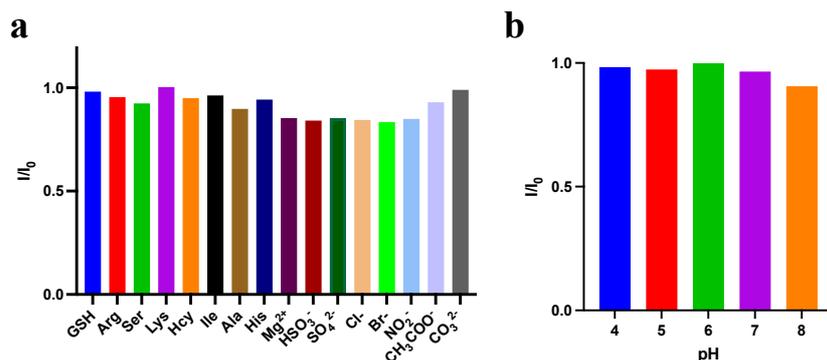


Figure S2 The normalized intensity of fluorescence of **3h** (10 μM) in PBS to (a) Common biological small molecules and ions interference; (b) pH from 4.0 to 8.0.

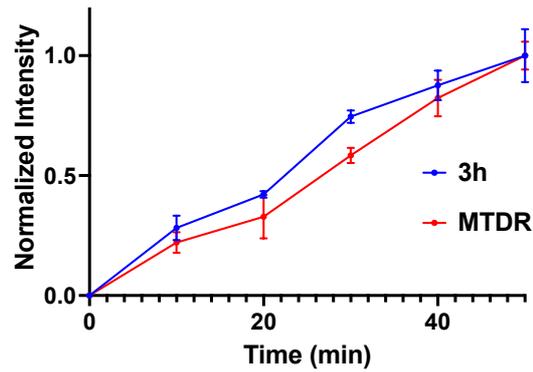


Figure S3. Cell uptake assay for HeLa cells were incubated with **3h** or **MTDR** (1 μ M) for different periods of time (0, 10, 20, 30, 40 and 50 min) at 37 $^{\circ}$ C.

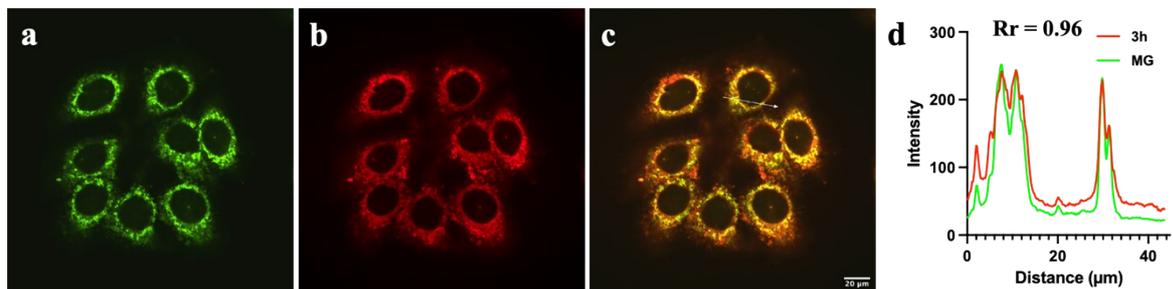


Figure S4. Confocal fluorescence images of HepG2 cells viewed in green channel ($\lambda_{\text{ex}} = 488$ nm) and red channel ($\lambda_{\text{ex}} = 633$ nm), respectively, after treatment with 500 nM of **MG** for 30 min followed by incubation with 5 μ M of **3h** for 30 min. (a) green channel; (b) red channel; (c) merged channel, (d) luminescence intensity profiles of ROI across HepG2 cells in (c). All images were obtained at 60 \times oil magnification.

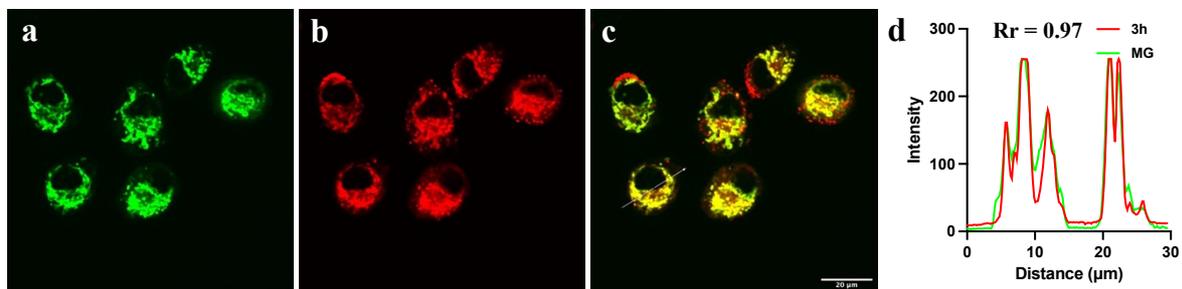


Figure S5. Confocal fluorescence images of A579 cells viewed in green channel ($\lambda_{\text{ex}} = 488$ nm) and red channel ($\lambda_{\text{ex}} = 633$ nm), respectively, after treatment with 500 nM of **MG** for 30 min followed by incubation with 5 μ M of **3h** for 30 min. (a) green channel; (b) red channel; (c) merged channel; (d) luminescence intensity profiles of ROI across HepG2 cells in (c). All images were obtained at 60 \times oil magnification.

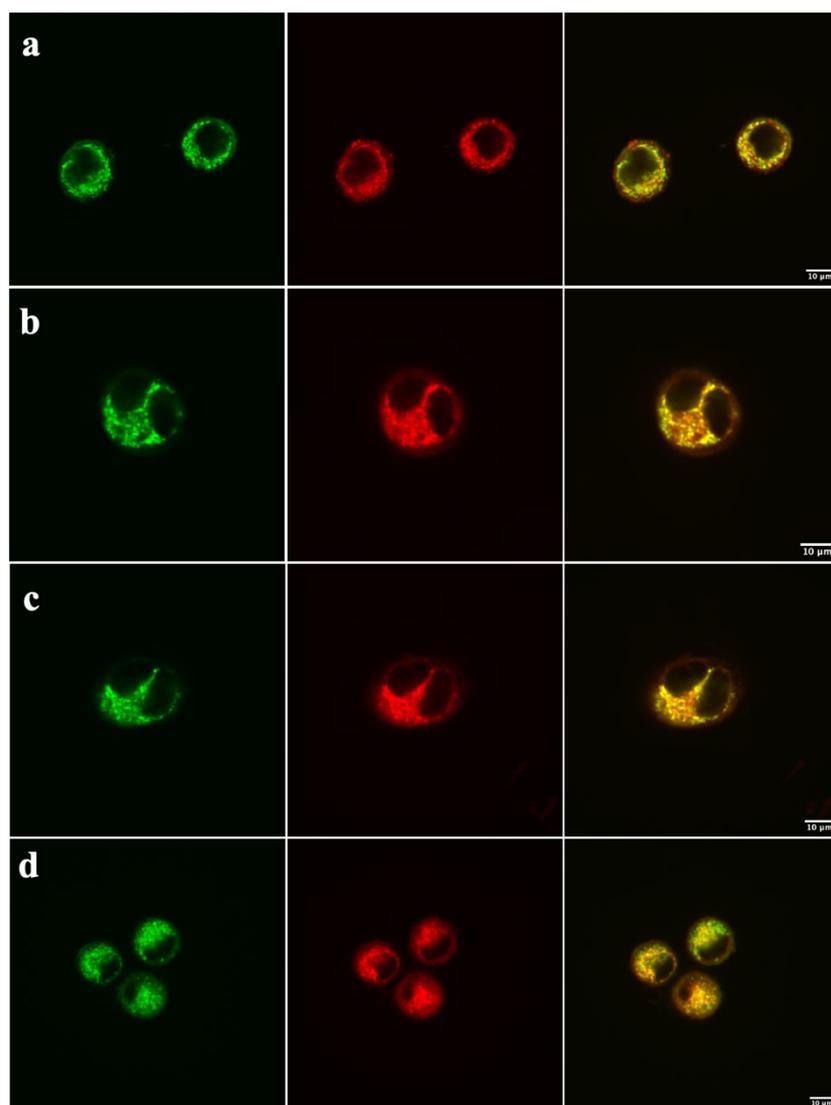


Figure S6. Subcellular location of **3h** in HeLa cells. Confocal fluorescence images of HeLa cells were viewed in green channel ($\lambda_{\text{ex}} = 488 \text{ nm}$) and red channel ($\lambda_{\text{ex}} = 633 \text{ nm}$), respectively, after treatment with 500 nM of **MG** for 30 min followed by incubation with **3h** in different concentrations for 30 min. (a) 100 nM; (b) 500 nM; (c) 1 μM ; (d) 2.5 μM . All images were obtained at 60 \times oil magnification.

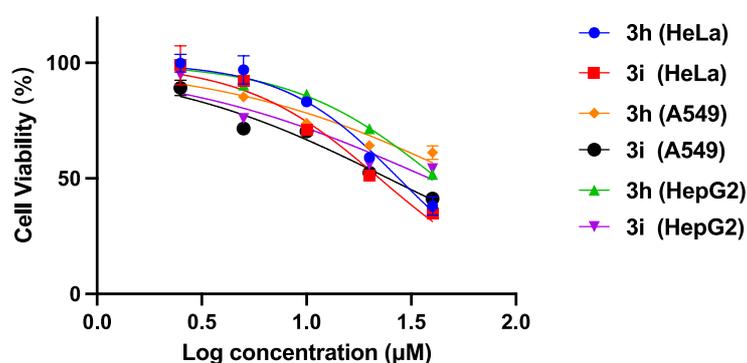


Figure S7. CCK-8 assessment of cytotoxicity in living HeLa, A549, and HepG2 cells. Cell viability assays were performed in HeLa cells following 12 h incubation of cells with increasing concentrations of probes. Data are presented as the mean \pm SEM of three independent experiments performed in triplicate.

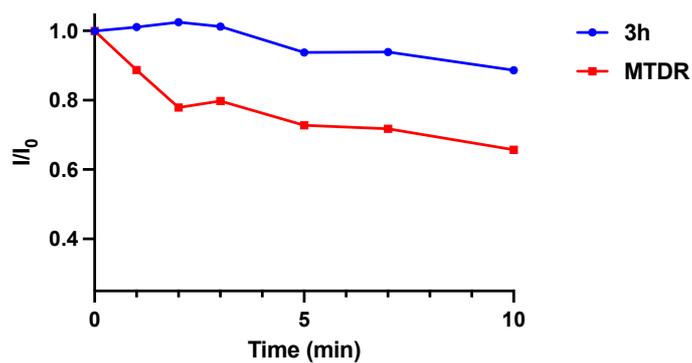


Figure S8. Normalized time-dependent intensity profiles of probes (1 μM) under light irradiation (Xe lamp, 150 mW cm^{-2}) in DMSO for 10 min.

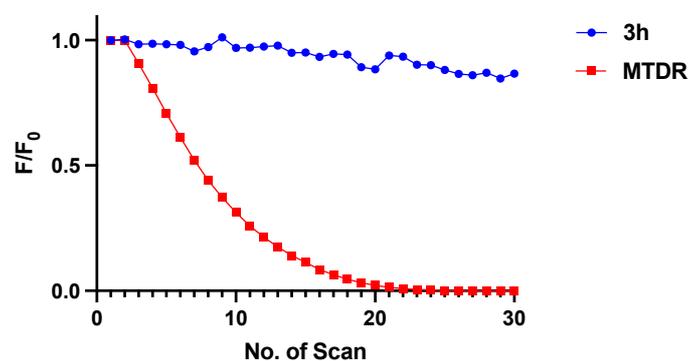


Figure S9. Normalized fluorescence intensity decay of **3h** compared with **MTDR**. F_0 is the max fluorescence intensity at initial scanning. F is the fluorescence intensity after several times of scanning. All images were obtained at 20x magnification.

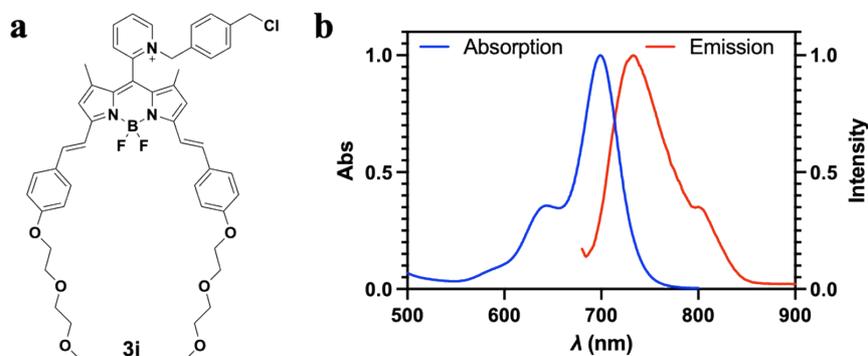


Figure S10. Chemical structure (a), absorption and fluorescence emission spectra (b) of probe **3i** in CHCl_3 .

Copies of NMR spectroscopic data

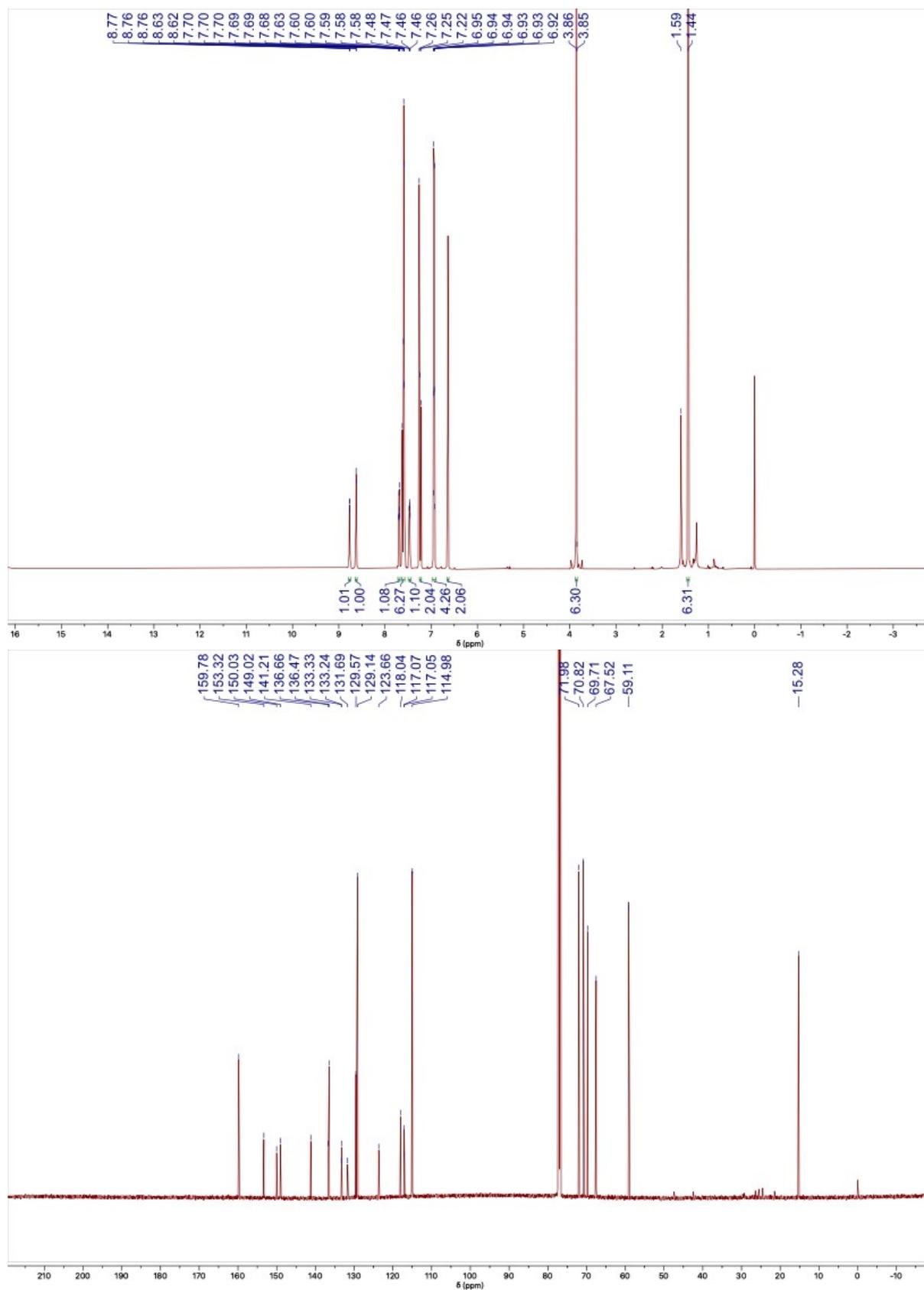


Figure S11. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectrum of **2a**

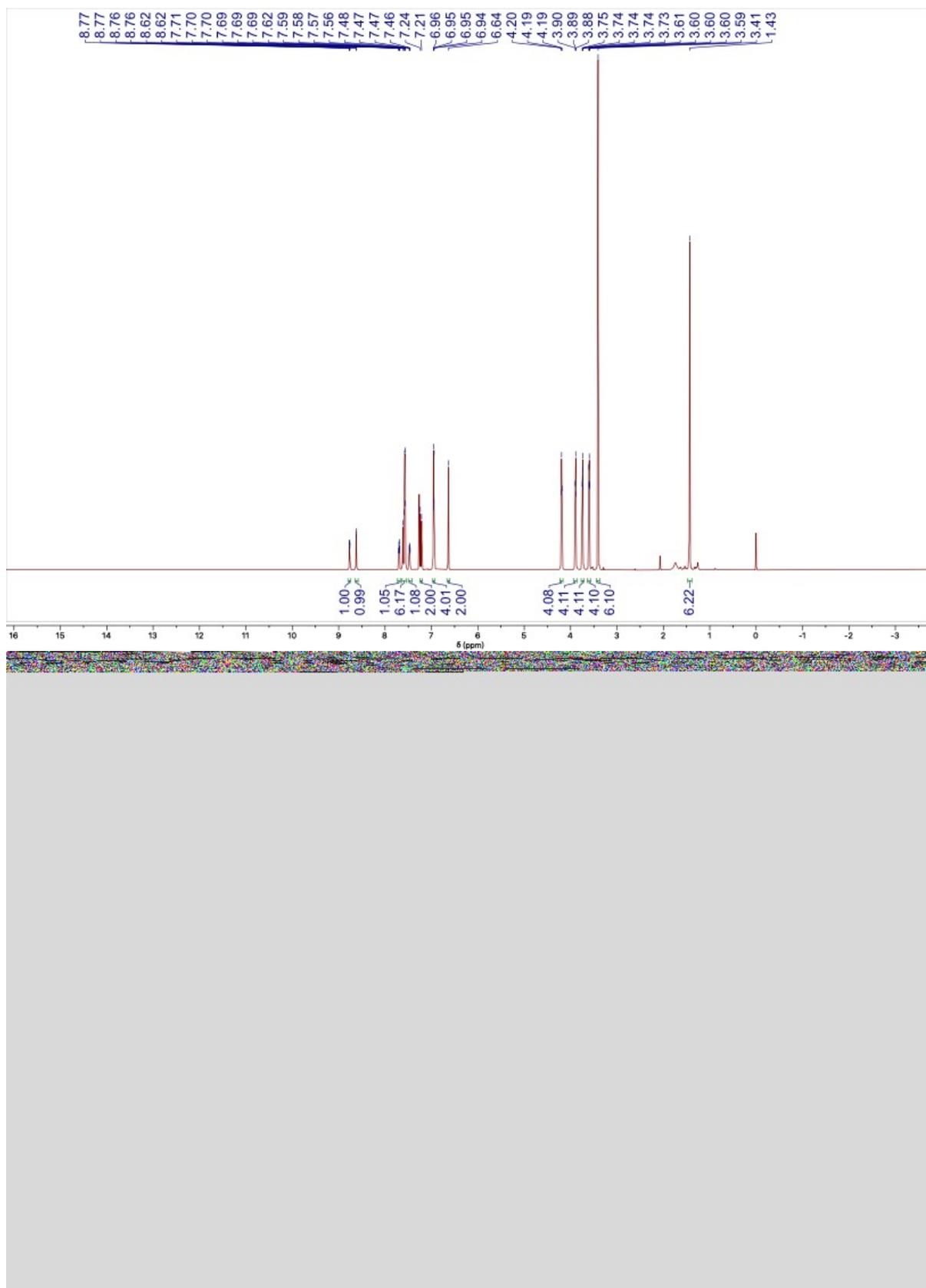


Figure S12. ^1H -NMR and ^{13}C -NMR spectrum of **2b**

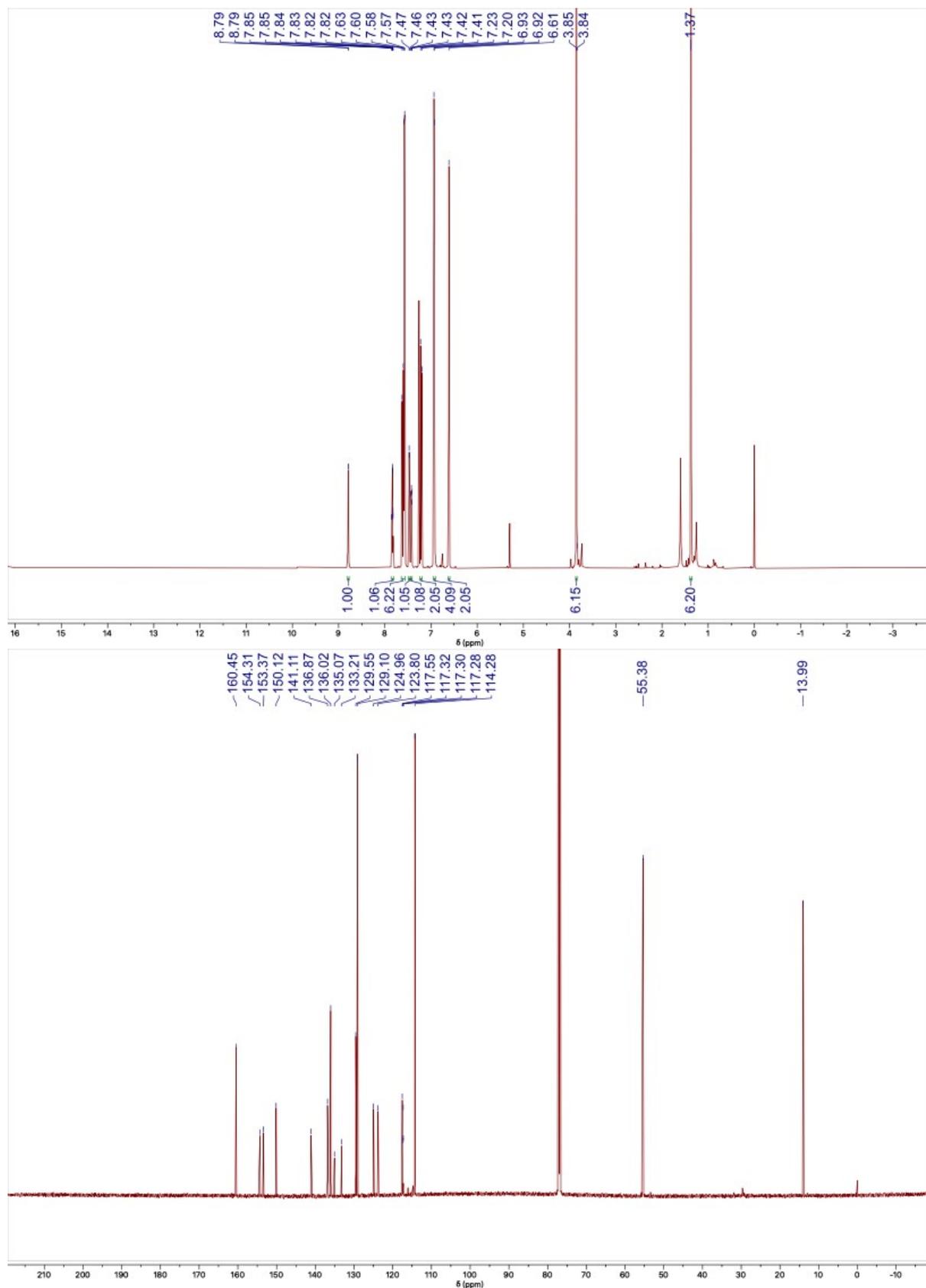


Figure S13. ^1H -NMR and ^{13}C -NMR spectrum of **2c**

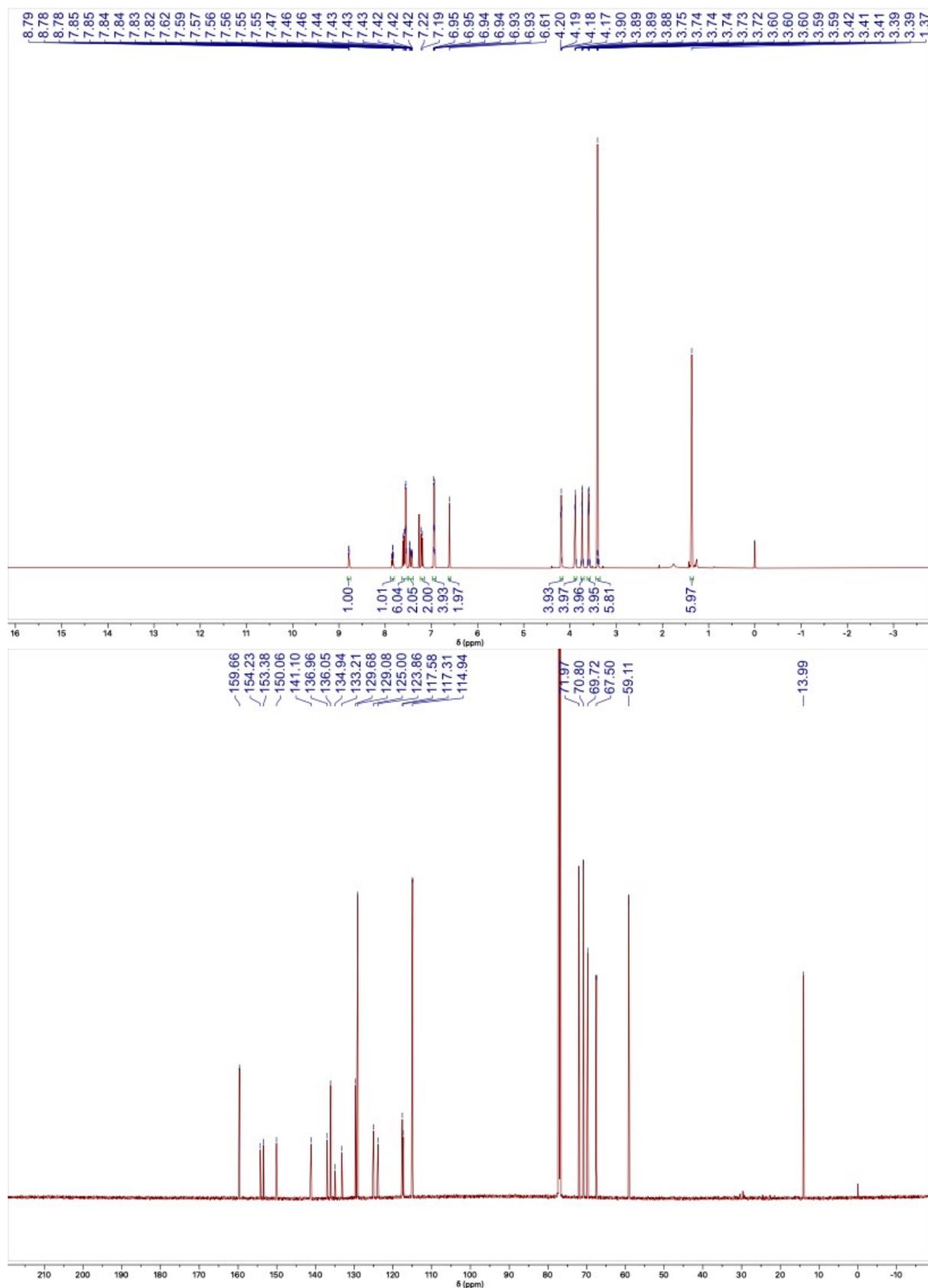


Figure S14. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectrum of **2d**

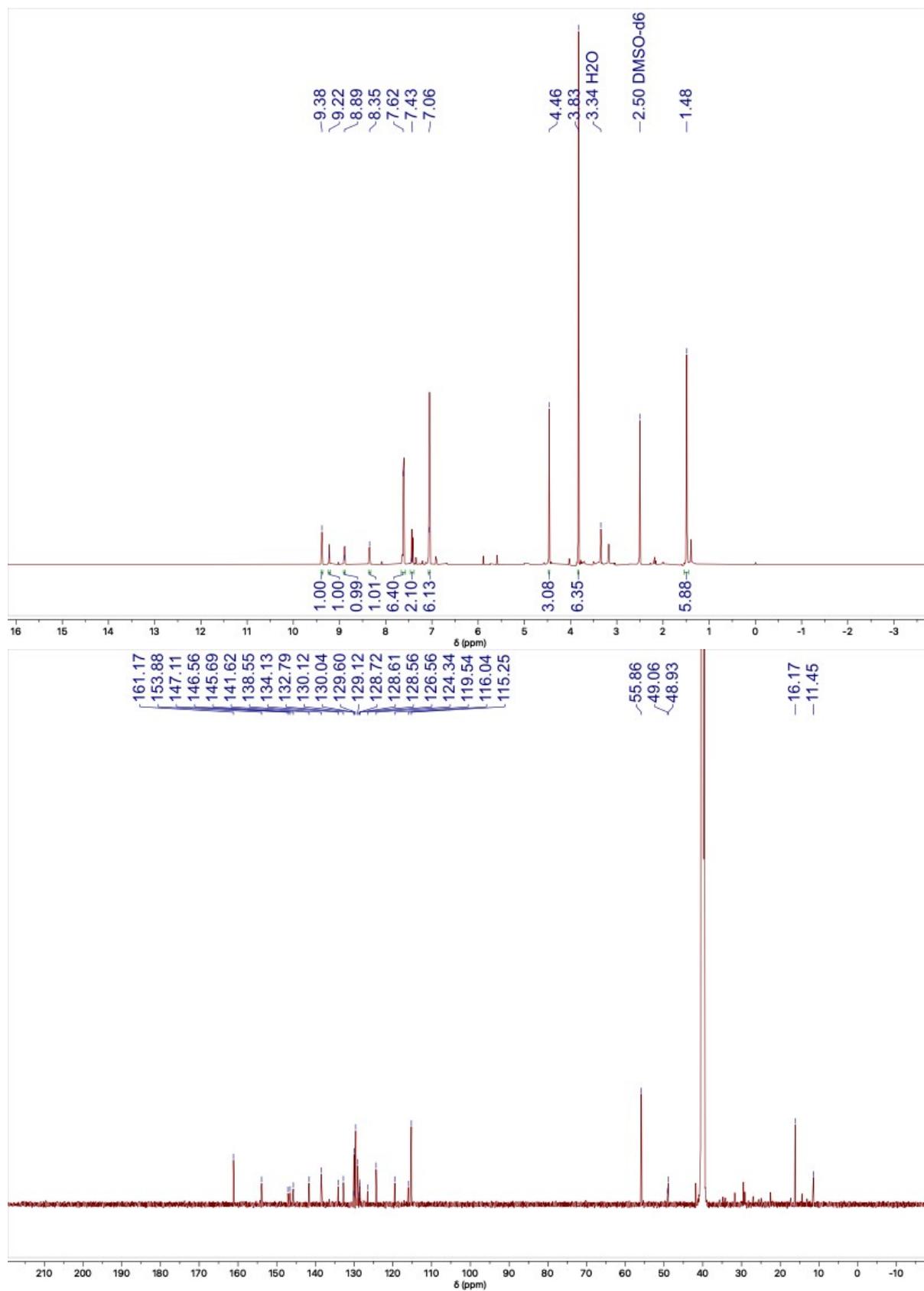


Figure S15. ^1H -NMR and ^{13}C -NMR spectrum of **3a**

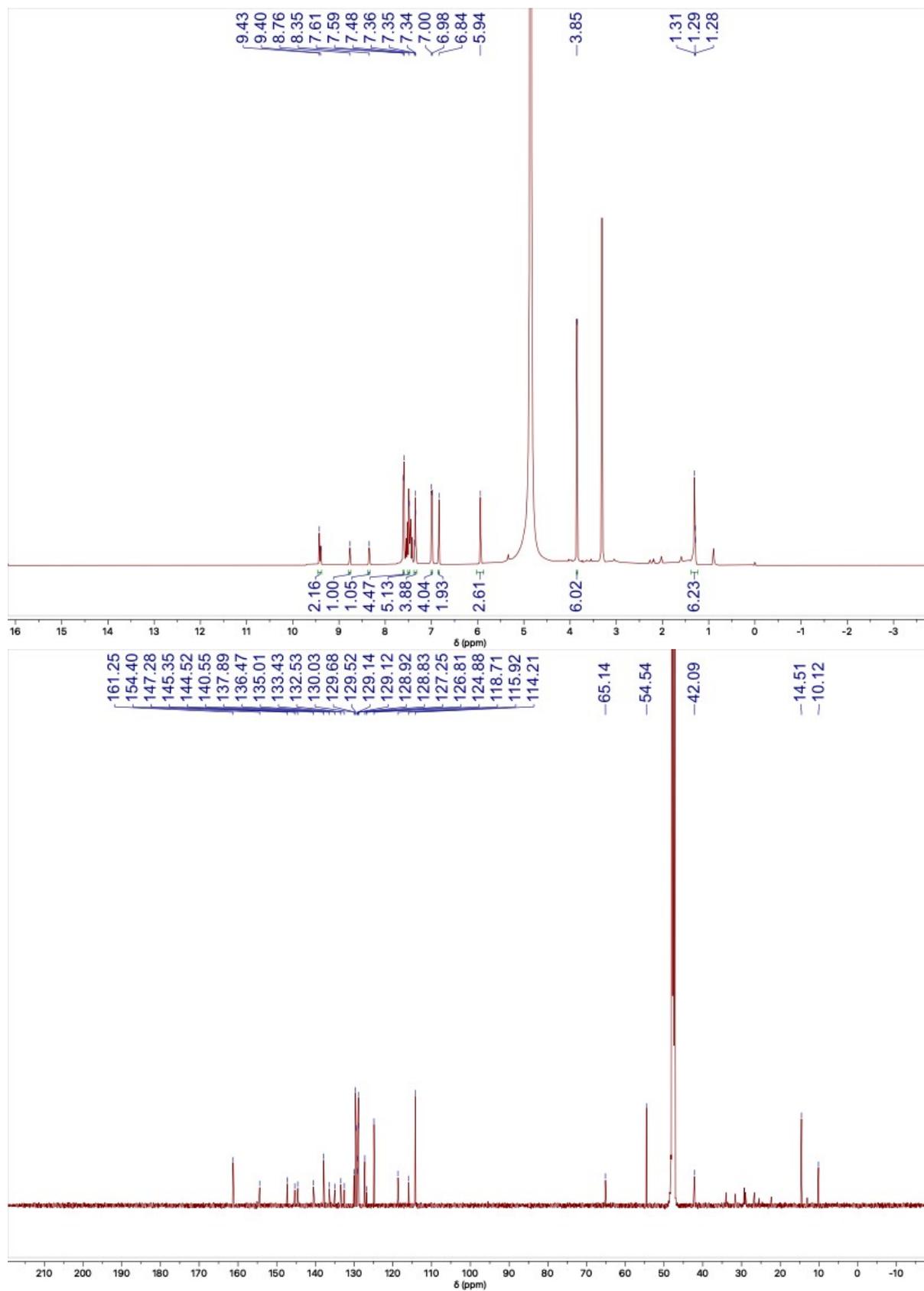


Figure S16. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectrum of **3b**

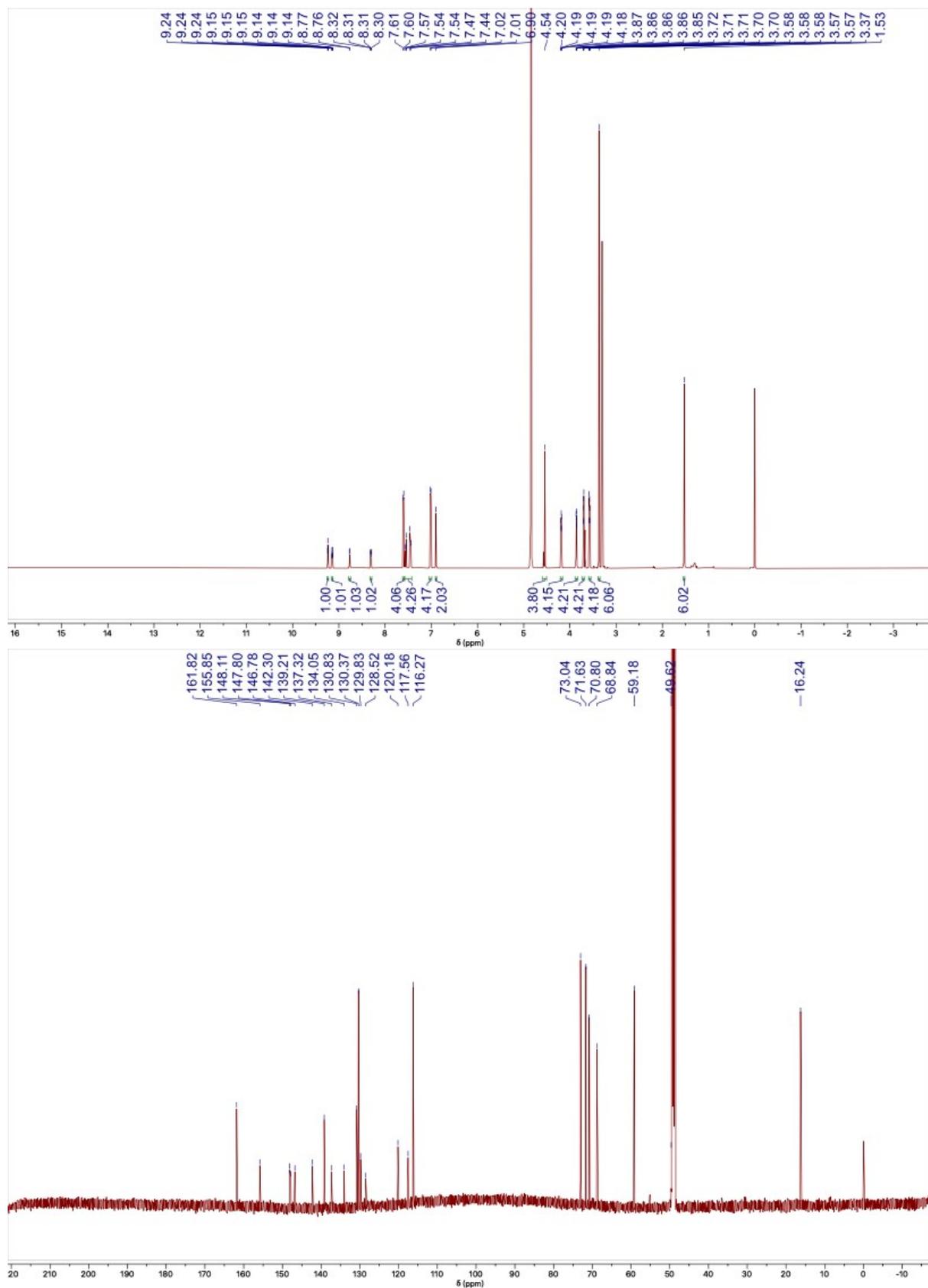


Figure S17. ¹H-NMR and ¹³C-NMR spectrum of 3c

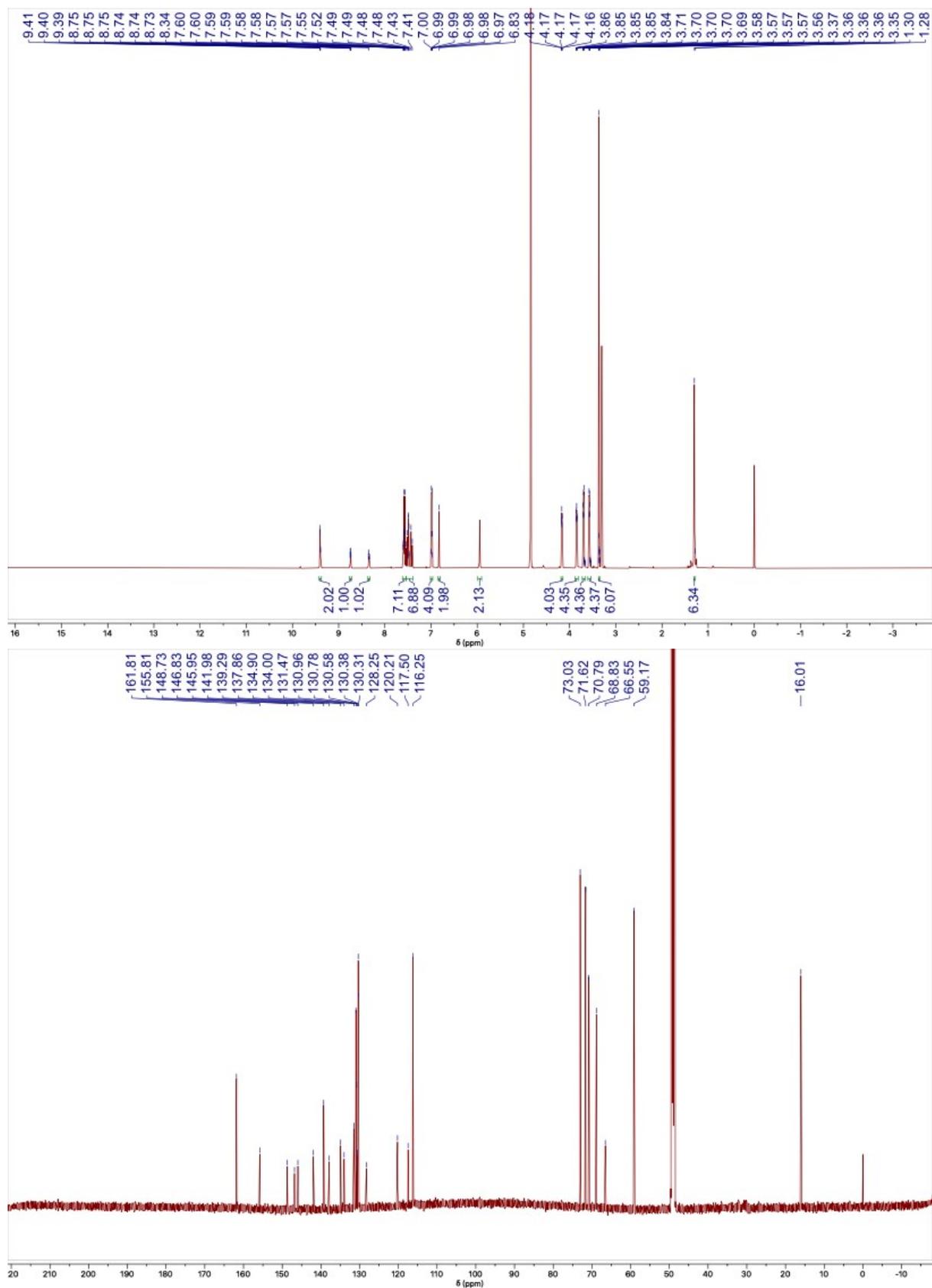


Figure S18. ¹H-NMR and ¹³C-NMR spectrum of **3d**

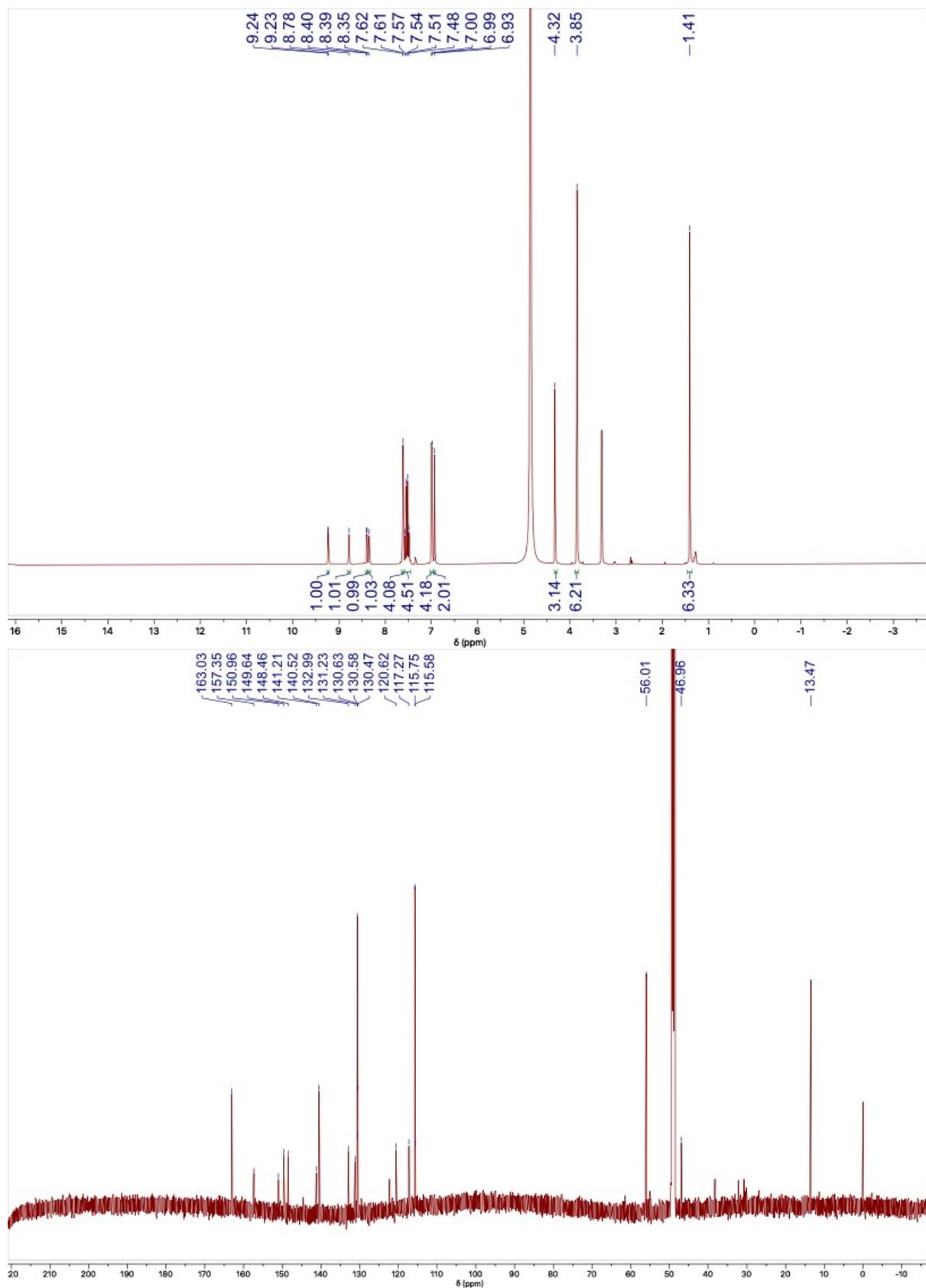


Figure S19. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectrum of **3e**

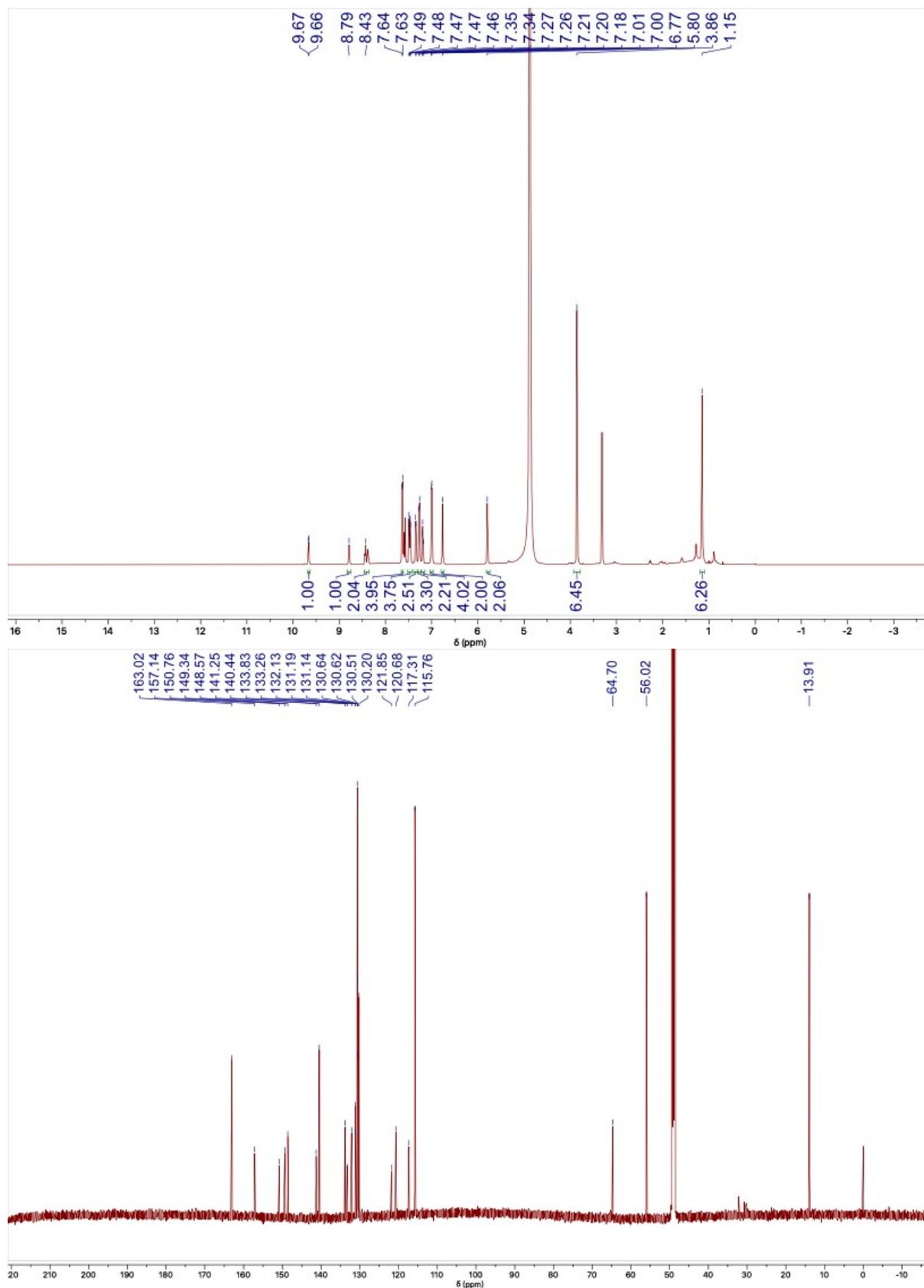


Figure S20. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectrum of 3f

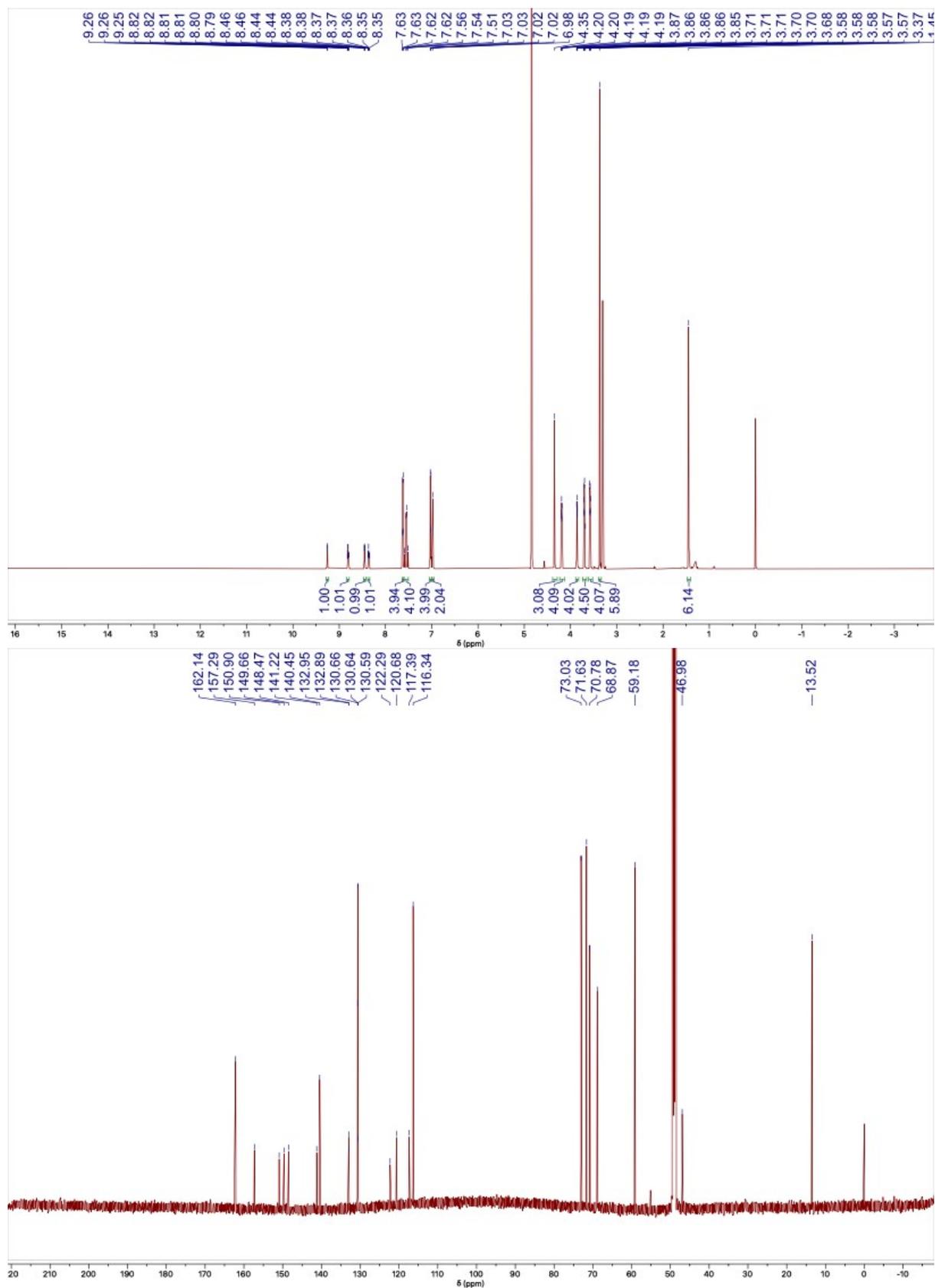


Figure S21. ¹H-NMR and ¹³C-NMR spectrum of **3g**

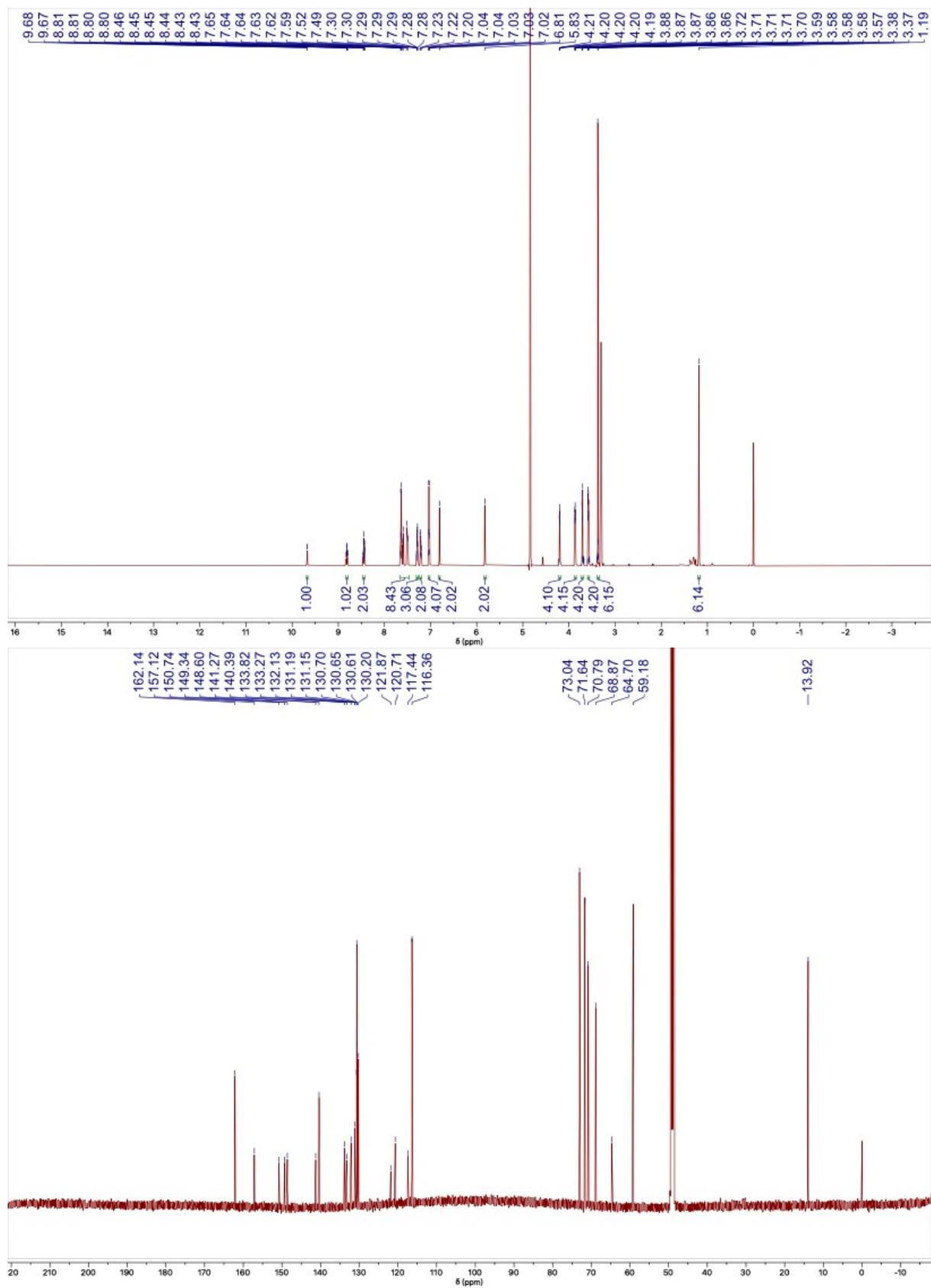


Figure S22. ^1H -NMR and ^{13}C -NMR spectrum of **3h**

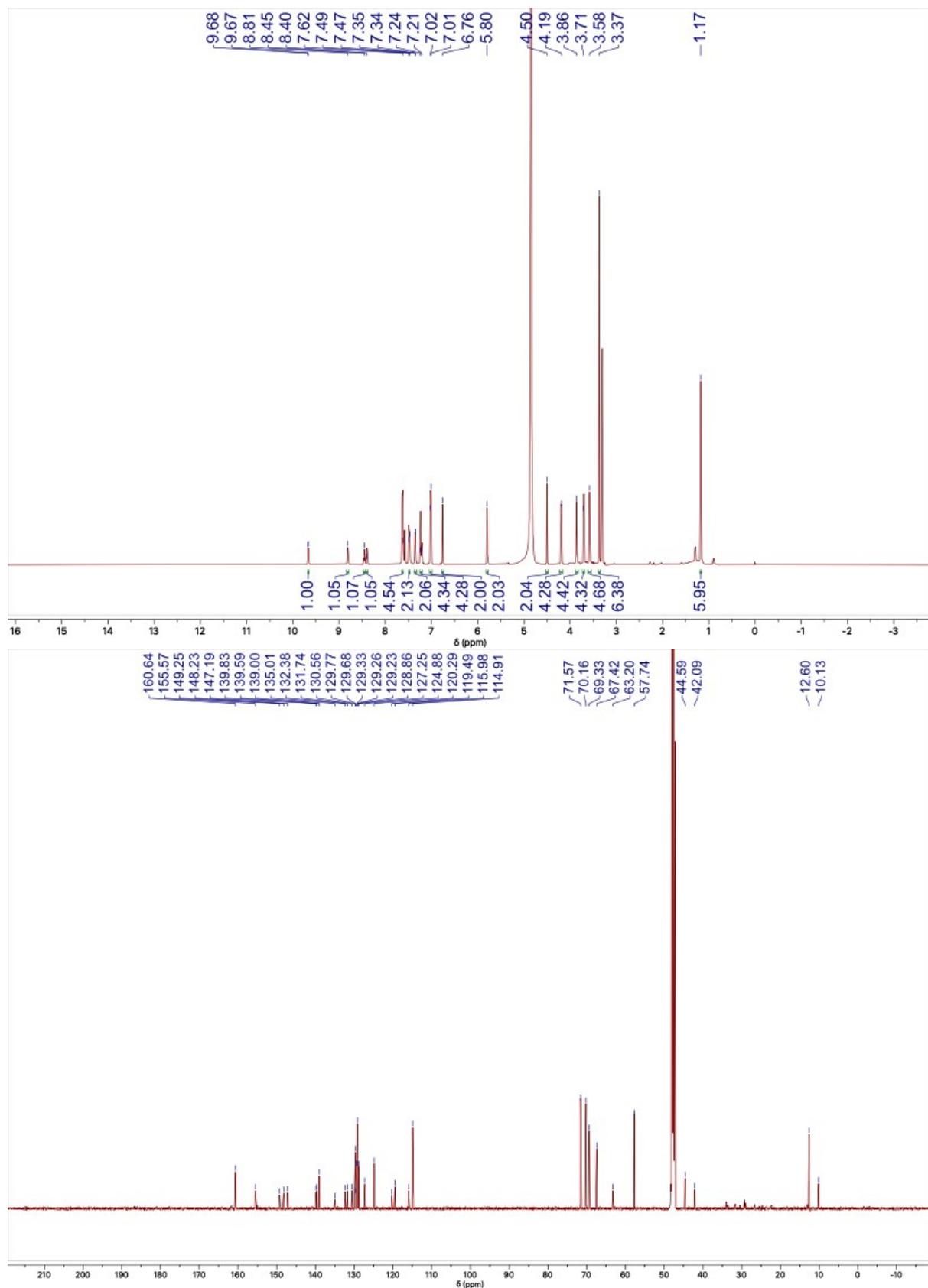


Figure S23. ^1H -NMR and ^{13}C -NMR spectrum of **3i**

Copies of mass spectrum characterization map

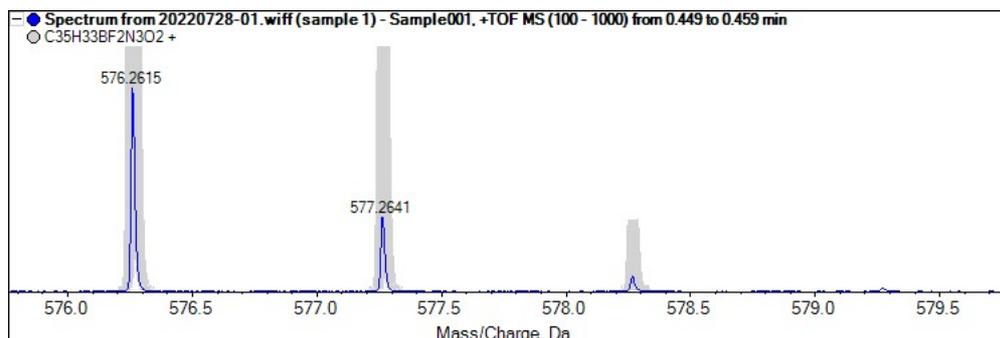


Figure S24 Mass spectrum characterization map of 3a

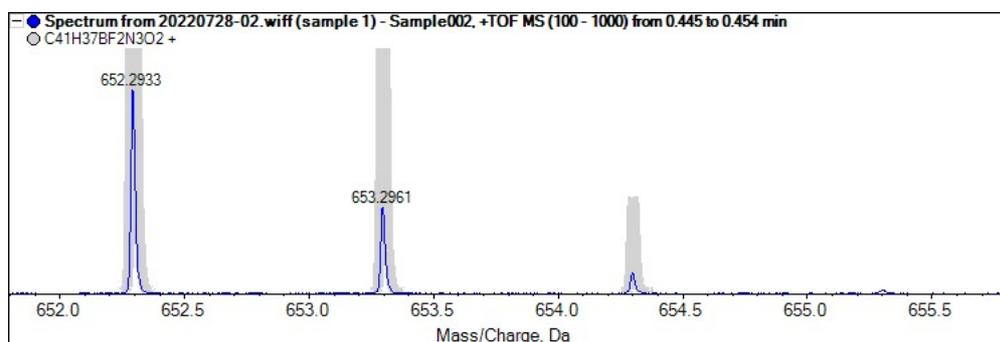


Figure S25 Mass spectrum characterization map of 3b

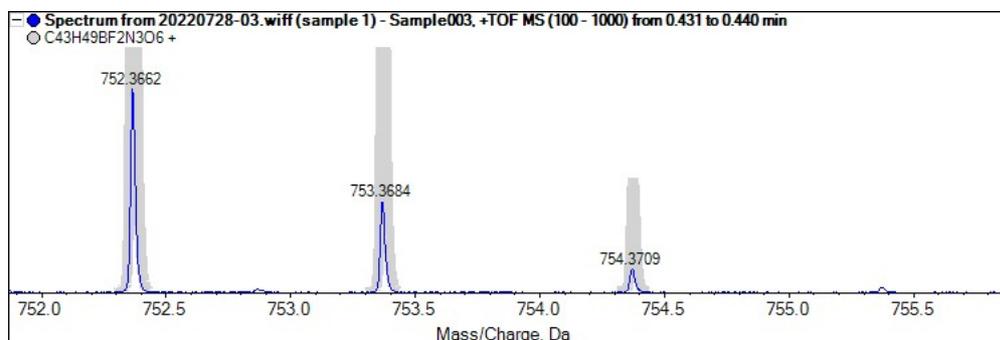


Figure S26 Mass spectrum characterization map of 3c

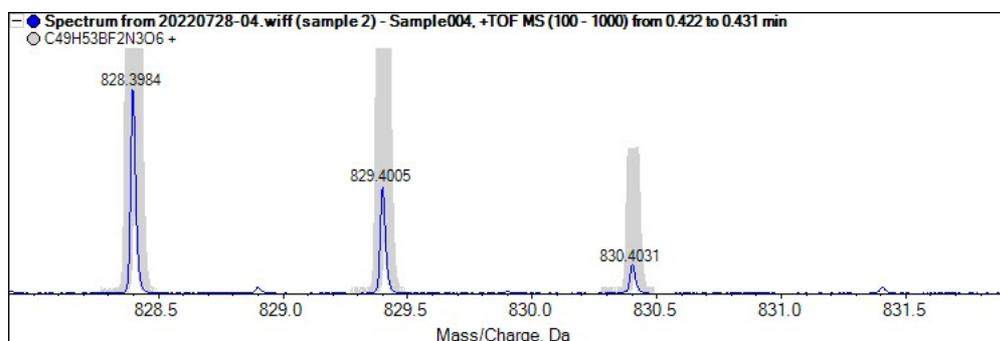


Figure S27 Mass spectrum characterization map of 3d

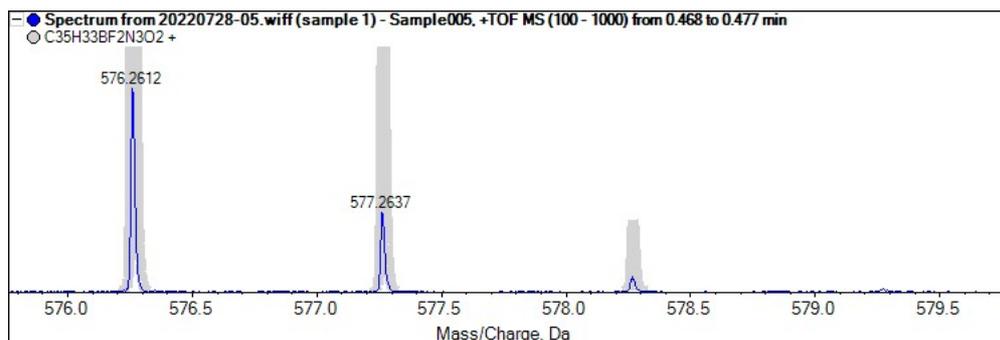


Figure S28 Mass spectrum characterization map of **3e**

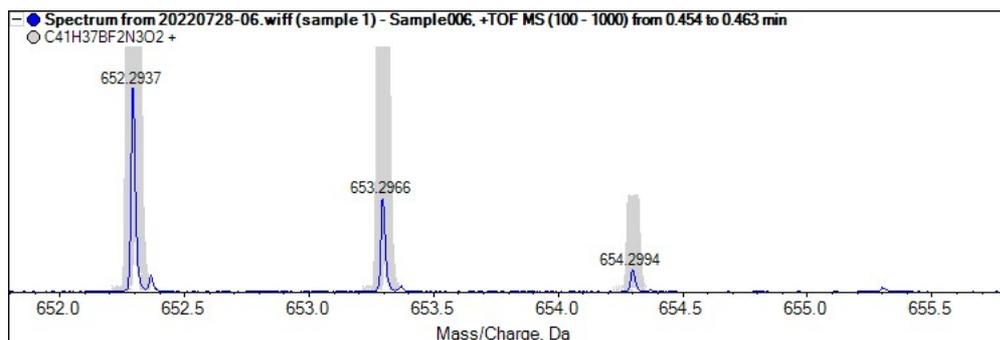


Figure S29 Mass spectrum characterization map of **3f**

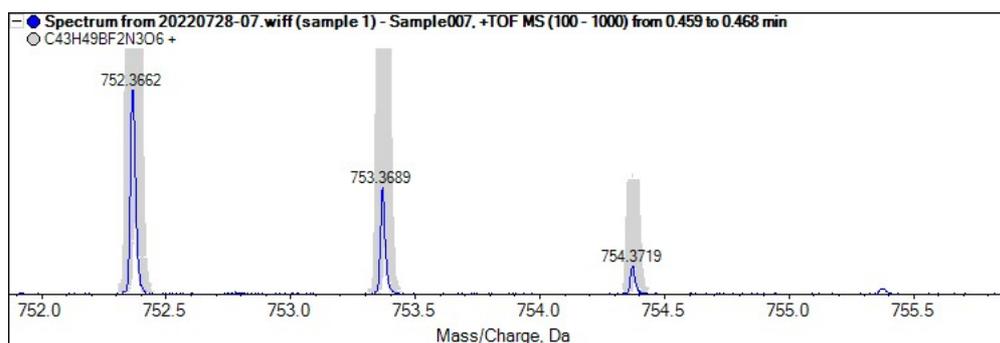


Figure S30 Mass spectrum characterization map of **3g**

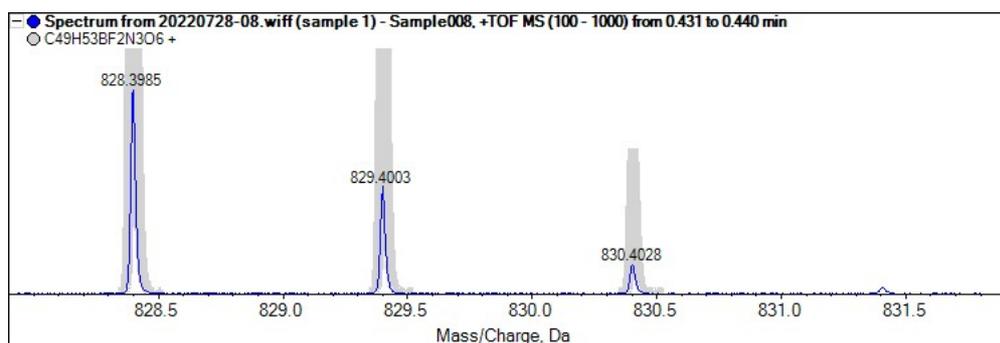


Figure S31 Mass spectrum characterization map of **3h**



Figure S32 Mass spectrum characterization map of **3i**