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Electronic Supplementary Information (ESI)

Antitumor Effects of New Glycoconjugated Pt^{II} Agents Dual-targeting GLUT1 and Pgp Proteins

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Table of Contents

Experimental Details

Materials and instrumentation

All chemicals and reagents were commercially available, such as ethyl acetate (AcOEt), petroleum ether (PE), methanol (MeOH), N, N-Dimethylformamide (DMF), and dichloromethane (DCM). Solvents were analytical or higher grade. K_2PtCl_4 and cisplatin (CDDP) were purchased from a local chemical company (purity 99%). Bovine serum albumin (BSA) was purchased from Sigma. 3-(4,5-dimathylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Annexin V-FITC/PI assay kit, Reactive oxygen species (ROS) kit, SDS-PAGE kit, 5′-dGMP, and Hoechst 33342 were from Solarbio. Fetal bovine serum (FBS) was from Biological Industries. RPMI 1640 and 0.25% trypsin/EDTA solutions were from Gibco. GLUT1/SLC2A1 Rabbit mAb (GLUT1), P Glycoprotein Rabbit mA (Pgp), and GAPDH was purchased from Affinity. All buffer solutions were prepared using deionized and sonicated triple-distilled water.

Nuclear Magnetic Resonance Spectroscopy (NMR) was recorded on a Bruker AVANCE III 400MHz spectrometer at room temperature. Electrospray ionization mass spectrometry (ESI-MS) data were measured by Agilent 6520 Q-TOF LC-MS. Infrared spectroscopy was performed on a Bruker Vector 22 FT-IR spectrophotometer. The electronic and fluorescence spectra were obtained on an Agilent Cary 100 spectrophotometer and an MPF-4 fluorescence spectrophotometer, respectively. Inductively coupled plasma mass spectrometry (ICP-MS) was measured by Thermo iCAP Q ICP-MS. Confocal laser scanning microscopy (CLSM) was measured in the Olympus FV 1000.

Synthetic procedures for the ligands

Preparation of 2,3,4,6-tetra-O-acetyl-β-D-glucose isothiocyanate (1a)

A mixture of potassium thiocyanate (2.3633 g, 24.32 mmol), tetra-butylammonium

iodide (4.4914 g, 12.16 mmol) and activated 4 Å molecular sieves in anhydrous acetonitrile (40 mL) were stirred at room temperature for 3 h under Ar_2. Then, acetobromo-α-D-glucose (5.00 g, 12.16 mmol) were added in dark and refluxed overnight until the reaction was completed as detected by TLC. Acetonitrile was removed under reduced pressure. The residue was purified by flash column chromatography to give **1a**. (silica gel, AcOEt/PE 2:3) (yield: 3.83 g, 80.89%). ^{1}H NMR (400 MHz, Chloroform-d): 5.15 (td, *J* = 9.4, 4.0 Hz, 1H), 5.05 (td, *J* = 9.9, 9.4, 4.3 Hz, 2H), 4.96 (dd, *J* = 8.5, 4.4 Hz, 1H), 4.17 (dd, *J* = 10.9, 5.9 Hz, 1H), 4.14-4.01 (m, 1H), 3.68 (d, *J* = 9.1 Hz, 1H), 2.10-1.86 (m, 12H). ^{13}C NMR (101 MHz, Chloroform-d): 169.54, 169.07, 168.20, 168.03, 143.20, 82.46, 73.03, 71.46, 70.85, 66.62, 60.49, 19.68, 19.51.

Preparation of 2,3,4,6-tetra-O-acetyl-β-D-glucose thiosemicarbazide (2a)

A solution of **1a** (2.00 g, 5.14 mmol) in CH_2Cl_2 (25 mL) was slowly added to hydrazine monohydrate (0.31 g, 6.17 mmol) in CH_2Cl_2 (10 mL) at 0 °C under Ar_2. During the addition, a white precipitate was formed. After stirring for 3 h, the precipitate was recrystallized with methanol, and filtered to get a white solid (yield: 1.97 g, 91%). ^{1}H NMR (400 MHz, Chloroform-d): 8.08 (d, *J* = 9.2 Hz, 1H), 7.66 (s, 1H), 5.69 (t, *J* = 8.5 Hz, 1H), 5.31 (td, *J* = 9.5, 2.6 Hz, 1H), 5.03 (tdd, *J* = 9.8, 7.2, 2.6 Hz, 2H), 4.32-4.19 (m, 1H), 4.09-4.01 (m, 1H), 3.80 (ddd, *J* = 9.8, 4.9, 2.5 Hz, 3H), 2.05-1.92 (m, 12H). ^{13}C NMR (101 MHz, Chloroform-d): 183.70, 170.86, 170.69, 169.88, 169.61, 82.07, 73.50, 72.75, 70.64, 68.26, 61.61, 20.77, 20.61.

Preparation of 2-Pyridinecarboxaldehyde-4-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl) thiosemicarbazone (HL^1)

2-Pyridinecarboxaldehyde (0.3052 g, 2.8496 mmol) was added to a solution of **2a** (1.00 g, 2.3746 mmol) in methanol (25 mL). Then, the mixture was refluxed at 65 ℃ until the reaction was completed as detected by TLC. The solution was collected and concentrated, and the residue was purified by flash column chromatography (silica gel, AcOEt/DCM 1:3). A white solid was gotten (*MW*= 510.51, yield: 0.67 g, 55.3076%). ^{1}H NMR (400 MHz, Chloroform-d): 9.42 (s, 1H), 8.55 (dt, *J* = 4.8, 1.4 Hz, 1H), 8.32 (d, *J* = 8.6 Hz, 1H), 8.03 (dt, *J* = 7.7, 1.1 Hz, 1H), 7.81 (s, 1H), 7.72 (td, *J* = 7.8, 1.8

Hz, 1H), 7.30- 7.22 (m, 1H), 5.64 (t, *J* = 9.0 Hz, 1H), 5.36 (t, *J* = 9.5 Hz, 1H), 5.09 (dt, *J* = 15.1, 9.6 Hz, 2H), 4.10-4.02 (m, 2H), 3.86 (ddd, *J* = 10.1, 4.6, 2.1 Hz, 1H), 2.03 (s, 3H), 1.98 (d, *J* = 3.7 Hz, 9H). ^{13}C NMR (101 MHz, Chloroform-d): 180.14, 170.75, 169.96, 169.67, 151.78, 148.60, 137.62, 134.31, 125.84, 124.36, 82.09, 73.64, 72.98, 70.67, 68.40, 61.78, 20.82, 20.72, 20.67. FT-IR (KBr, cm^{-1}): 599.76, 778.63, 831.18, 895.78, 922.78, 1038.49, 1233.27, 1368.75, 1436.73, 1468.55, 1531.22, 1586.19, 1752.04, 2957.83, 3331.96.

Preparation of 2-Pyridinecarboxaldehyde-4-(β-D-glucopyranosyl) thiosemicarbazone (HL^2)

Sodium methoxide (0.2647 g, 4.901 mmol) was added to a solution of **HL^1** (0.50 g, 0.9801 mmol) in anhydrous methanol (20 mL) in Ar_2. The mixture was stirred at 0 ℃ for 2 h. Then, the solution was neutralized with HCl, it was filtered to remove sodium ions, the solvent was evaporated using a rotary evaporator and the residue was purified by flash column chromatography (silica gel, MeOH/DCM 1:15). A white solid was gotten (*MW* = 342.37, yield: 0.24 g, 71.5780%). ^{1}H NMR (400 MHz, DMSO-d_6): 11.02 (s, 1H), 9.09 (s, 1H), 8.85 (d, *J* = 5.2 Hz, 1H), 8.39 (d, *J* = 3.8 Hz, 2H), 7.85 (s, 1H), 5.37 (t, *J* = 8.9 Hz, 1H), 3.86 (d, *J* = 2.7 Hz, 1H), 3.84 (d, *J* = 2.7 Hz, 1H), 3.81 (d, *J* = 2.7 Hz, 1H), 3.77 (s, 1H), 3.57 (dd, *J* = 12.1, 8.7 Hz, 2H), 3.49-3.41 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6): 179.46, 153.42, 149.75, 143.58, 137.23, 124.85, 121.32, 84.59, 79.19, 78.02, 72.34, 70.24, 61.23. FT-IR (KBr, cm^{-1}): 630.47, 782.83, 893.16, 940.45, 1037.05, 1076.58, 1082.85, 1217.36, 1238.57, 1275.70, 1434.80, 1474.81, 1552.92,1567.38, 3173.82, 3398.49.

Preparation of 2-Acetylpyridine-4-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl) thiosemicarbazone (HL^3)

A yellowish white solid **HL^3** was obtained according to the procedure described for **HL^1** (*MW* = 524.54, yield: 0.85 g, 6829%). ^{1}H NMR (400 MHz, Chloroform-d): 8.92 (s, 1H), 8.64 (d, *J* = 4.8 Hz, 1H), 8.53 (s, 1H), 8.24 (d, *J* = 8.0 Hz, 1H), 7.82 (t, *J* = 7.8 Hz, 1H), 7.43-7.32 (m, 1H), 5.77 (t, *J* = 9.0 Hz, 1H), 5.44 (q, *J* = 9.8 Hz, 1H), 5.19 (dt, *J* = 25.3, 9.7 Hz, 2H), 4.40 (dd, *J* = 12.5, 4.6 Hz, 1H), 4.16 (dd, *J* = 12.4, 2.2 Hz, 1H),

3.95 (ddd, *J* = 10.3, 4.7, 2.2 Hz, 1H), 2.44 (s, 3H), 2.15-1.88 (m, 12H). ^{13}C NMR (101 MHz, Chloroform-d): 178.64,170.14, 169.65, 168.82, 168.59, 147.55, 146.95, 135.70, 123.30, 122.89, 119.89, 81.37, 72.56, 71.64, 69.57, 67.37, 60.63, 19.76, 19.72, 19.60, 10.25. FT-IR (KBr, cm^{-1}): 509.63, 782.83, 861.64, 919.43, 1038.49, 1233.27, 1366.82,1438.17, 1465.65, 1522.54, 1749.14, 2942.20, 3351.73.

Preparation of 2-Acetylpyridine-4-(β-D- glucopyranosyl) thiosemicarbazone (HL^4)

A white solid **HL^4** was obtained according to the procedure described for **HL^3** (*MW* = 356.39, yield: 0.215g, 63.29%). ^{1}H NMR (400 MHz, DMSO-d_6): 10.70 (s, 1H), 8.61 (q, *J* = 6.1 Hz, 2H), 8.43-8.31 (m, 1H), 7.85 (p, *J* = 7.6 Hz, 1H), 7.43 (ddd, *J* = 15.3, 9.7, 5.5 Hz, 1H), 5.43 (p, *J* = 8.8 Hz, 1H), 5.07 (dt, *J* = 15.0, 7.4 Hz, 2H), 4.94 (q, *J* = 5.2 Hz, 1H), 4.50 (dt, *J* = 11.4, 5.6 Hz, 1H), 4.11 (dq, *J* = 10.5, 5.3 Hz, 1H), 3.66 (p, *J* = 6.4 Hz, 1H), 3.39-3.31 (m, 1H). ^{13}C NMR 101 MHz, DMSO-d_6): 180.22, 154.97, 149.77, 149.05, 136.96, 124.64, 121.51, 84.51, 79.14, 77.98, 72.53, 70.28, 61.25, 12.94. FT-IR (KBr, cm^{-1}): 627.72, 782.48, 859.14, 892.89, 1033.67, 1076.10, 1212.06, 1252.07, 1268.47,1311.86,1405.87, 1432.87, 1476.26, 1540.38, 1601.13, 2912.03, 3276.03.

Preparation of Acetobromo-α-D-galactose (1b)

To a stirred solution of AcO_2 (222 mmol) in glacial acetic acid, D-galactose (5.00 g, 27.7680 mmol) was added slowly for 40 min, then 3 drops of $HClO_4$ were added. After the solution became clear, 66.85mmol (11 mL) of HBr-acetic acid (33%) was added in dark and the mixture stirred at room temperature until the reaction was completed as detected by TLC. Then, the solution was neutralized with $NaHCO_3$ and extracted with CH_2Cl_2. The residue obtained was purified by flash column chromatography after removing the solvent (silica gel, AcOEt/PE 2:3). (yield: 8.12 g, 71.11%). ^{1}H NMR (400 MHz, Chloroform-d): 6.63 (d, *J* = 3.9 Hz, 1H), 5.45 (dd, *J* = 3.3, 1.4 Hz, 1H), 5.34 (dd, *J* = 10.7, 3.3 Hz, 1H), 4.98 (dd, *J* = 10.7, 4.0 Hz, 1H), 4.42 (td, *J* = 6.5, 1.3 Hz, 1H), 4.12 (dd, *J* = 11.4, 6.3 Hz, 1H), 4.07-4.01 (m, 1H), 2.09 (s, 3H), 2.05 (s, 3H), 2.00 (s, 3H), 1.95 (s, 3H). ^{13}C NMR (400 MHz, Chloroform-d): 170.33, 170.08, 169.91, 169.77,

88.12, 71.05, 67.98, 67.76, 66.96, 60.83, 20.77, 20.66, 20.61, 20.58.

Preparation of 2,3,4,6-tetra-O-acetyl-β-D- galactose isothiocyanate (2b)

The compound **2b** was synthesized from **1b** following a procedure same as that described for **1a**. (yield: 2.55 g, 53.90%). ^{1}H NMR (400 MHz, Chloroform-d): 5.44 (d, *J* = 3.3 Hz, 1H), 5.36 (dd, *J* = 10.3, 8.7 Hz, 1H), 5.11-4.99 (m, 2H), 4.17 (d, *J* = 6.5 Hz, 2H), 4.01 (t, *J* = 6.5 Hz, 1H), 2.21 (s, 3H), 2.16 (s, 3H), 2.09 (s, 3H), 2.03 (s, 3H). ^{13}C NMR (101 MHz, Chloroform-d): 170.37, 170.09, 169.96, 169.17, 143.75, 84.00, 72.96, 70.62, 69.29, 66.87, 61.27, 20.70, 20.65, 20.55.

Preparation of 2,3,4,6-tetra-O-acetyl-β-D- galactose thiosemicarbazide (3b)

The compound **3b** was synthesized from **2b** following a procedure same as that described for **2a** (yield: 1.80 g, 83.15%). ^{1}H NMR (400 MHz, Chloroform-d): 8.14 (d, *J* = 9.3 Hz, 1H), 7.76 (s, 1H), 5.67 (t, *J* = 9.1 Hz, 1H), 5.40 (d, *J* = 3.2 Hz, 1H), 5.30-5.06 (m, 2H), 4.09 (dt, *J* = 5.9, 3.4 Hz, 2H), 4.02 (q, *J* = 6.6, 5.2 Hz, 1H), 3.79 (s, 2H), 2.10 (s, 3H), 2.00 (d, *J* = 6.1 Hz, 6H), 1.94 (s, 3H). ^{13}C NMR (101 MHz, Chloroform-d): 183.77, 171.18, 169.82, 82.48, 72.44, 70.95, 68.48, 67.34, 61.23, 20.90, 20.79, 20.69, 20.61.

Preparation of 2-Pyridinecarboxaldehyde-4-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl) thiosemicarbazone (HL^{5})

The **HL^{5}** was synthesized from **3b** following a procedure same as that described for **HL^{1}.** A white solid was gotten (*MW* = 510.51, yield: 0.73 g, 60.26%). ^{1}H NMR (400 MHz, Chloroform-d): 9.95 (s, 1H), 8.67 (d, *J* = 4.7 Hz, 1H), 8.47 (d, *J* = 8.6 Hz, 1H), 8.17 (d, *J* = 8.0 Hz, 1H), 7.99 (s, 1H), 7.83 (td, *J* = 7.8, 1.8 Hz, 1H), 7.37 (dd, *J* = 7.3, 5.0 Hz, 1H), 5.74 (t, *J* = 8.9 Hz, 1H), 5.54 (d, *J* = 3.4 Hz, 1H), 5.40 (t, *J* = 9.7 Hz, 1H), 5.29 (dd, *J* = 10.3, 3.4 Hz, 1H), 4.22 (d, *J* = 5.8 Hz, 2H), 4.19-4.13 (m, 1H), 2.22 (s, 3H), 2.10 (d, *J* = 2.4 Hz, 6H), 2.06 (s, 3H). ^{13}C NMR (101 MHz, Chloroform-d): 179.32, 171.54, 170.54, 170.18, 169.79, 152.21, 149.48, 143.50, 136.90, 124.66, 120.96, 82.63, 72.39, 70.74, 68.36, 67.27, 61.09, 20.86, 20.78, 20.70, 20.59. FT-IR (KBr, cm^{-1}): 586.74, 779.11, 920.85, 954.60, 1050.5, 1081.88, 1220.25, 1369.71, 1436.73, 1468.55, 1529.29, 1751.07, 2966.99, 3339.19.

Preparation of 2-Pyridinecarboxaldehyde-4-(β-D-galactopyranosyl) thiosemicarbazone (HL^{6})

The **HL^{6}** was synthesized from **HL^{5}** following a procedure same as that described for

HL[2]. A white solid was gotten (*MW* = 342.37, yield: 0.24 g, 71.58%). ^{1}H NMR (400 MHz, DMSO-d_6): 11.98 (s, 1H), 8.68 (d, *J* = 9.1 Hz, 1H), 8.63-8.57 (m, 1H), 8.33 (d, *J* = 8.0 Hz, 1H), 8.18 (s, 1H), 7.87 (td, *J* = 7.7, 1.7 Hz, 1H), 7.42 (ddd, *J* = 7.5, 4.9, 1.2 Hz, 1H), 5.35 (t, *J* = 9.0 Hz, 1H), 4.94 (s, 2H), 4.66 (s, 1H), 4.47 (s, 1H), 3.88-3.67 (m, 2H), 3.61-3.43 (m, 4H). ^{13}C NMR (101 MHz, DMSO-d_6): 179.36, 149.94, 143.75, 137.08, 124.82, 121.05, 85.01, 77.38, 74.69, 69.91, 68.72, 60.72. FT-IR (KBr, cm^{-1}): 517.51, 706.65, 782.83, 832.74, 943.08, 1040.42, 1073.20, 1213.02, 1240.02, 1275.21, 1478.67, 1546.65, 1566.90, 3180.09, 3238.91.

Preparation of 2-Acetylpyridine-4-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl) thiosemicarbazone (HL[7])

The **HL[7]** was synthesized from **3b** following a procedure same as that described for **HL[3].** A white solid was gotten (*MW* = 524.54, yield: 0.85 g, 68.29%). ^{1}H NMR (400 MHz, Chloroform-d): 8.95 (s, 1H), 8.61 (dt, *J* = 4.8, 1.4 Hz, 1H), 8.47 (dd, *J* = 16.0, 9.0 Hz, 1H), 8.25 (d, *J* = 8.0 Hz, 1H), 7.78 (td, *J* = 7.8, 1.8 Hz, 1H), 7.32 (ddd, *J* = 7.5, 4.8, 1.2 Hz, 1H), 5.73 (t, *J* = 9.0 Hz, 1H), 5.55-5.46 (m, 1H), 5.36 (td, *J* = 9.8, 9.2, 1.8 Hz, 1H), 5.25 (td, *J* = 9.8, 3.4 Hz, 1H), 4.21-4.01 (s, 3H), 2.43 (s, *J* = 2.1 Hz, 3H), 2.18 (s, *J* = 2.9 Hz, 3H), 2.06 (s, *J* = 4.8 Hz, 6H), 2.02 (s, 3H). ^{13}C NMR (101 MHz, Chloroform-d): 179.63, 171.45, 170.46, 170.12, 169.70, 154.16, 149.04, 148.69, 136.47, 124.26, 120.96, 82.68, 72.39, 70.74, 68.34, 67.29, 61.08, 20.83, 20.72, 20.67, 20.54, 11.33. FT-IR (KBr, cm^{-1}): 591.06, 785.46, 856.39, 919.43, 956.21,1048.13, 1082.37,1222.66, 1369.71, 1438.66, 1467.10, 1523.51, 1750.11, 2965.84, 3338.87.

Preparation of 2-Acetylpyridine-4-(β-D-galactopyranosyl) thiosemicarbazone (HL[8])

The **HL[8]** was synthesized from **L[7]** following a procedure same as that described for **HL[4].** A white solid was gotten (*MW* = 356.39, yield: 0.22 g, 64.76%). ^{1}H NMR (400 MHz, DMSO-d_6): 10.71 (s, 1H), 8.61 (d, *J* = 4.8 Hz, 1H), 8.51 (d, *J* = 9.2 Hz, 1H), 8.29 (d, *J* = 8.1 Hz, 1H), 7.86 (td, *J* = 7.7, 1.8 Hz, 1H), 7.43 (dd, *J* = 7.3, 5.0 Hz, 1H), 5.35 (t, *J* = 9.0 Hz, 1H), 5.02 (d, *J* = 5.6 Hz, 1H), 4.83 (d, *J* = 5.3 Hz, 1H), 4.62 (t, *J* = 5.4 Hz, 1H), 4.39 (d, *J* = 5.4 Hz, 1H), 3.72 (dt, *J* = 10.3, 4.6 Hz, 2H), 3.54 (qd, *J* = 8.8, 5.5 Hz, 1H), 3.44 (dq, *J* = 9.3, 5.5 Hz, 3H), 2.43 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): 179.63, 154.48, 149.27, 148.62, 136.56, 124.17, 120.79, 84.37, 76.80, 74.01, 69.58,

68.19, 60.12, 12.52. FT-IR (KBr, cm^{-1}): 680.75, 744.40, 780.55, 848.51, 1046.69, 1078.99, 1173.00, 1214.95, 1275.70, 1316.20, 1432.39, 1478.19, 1540.87, 2908.65, 3258.19, 3330.99.

Stability analysis

All Pt^{II} complexes were stable in the solid-state, and dissolved in DMF and diluted to the corresponding concentration using phosphate buffered saline (PBS). The solution stability of **1**-**8** was evaluated by UV−visible absorption spectroscopy. The absorption spectra of complexes were recorded at different times (0 h and 24 h).

Cell lines and culture conditions

Human lung adenocarcinoma cells (A549), human breast cancer cell line (MCF-7), human large-cell lung carcinoma (NCI-H460), Human epithelial cervical cancer cell line (HeLa), and human normal cells (BEAS-2B) were obtained from China Center for Type Culture Collection (CCTCC). A549, MCF-7, NCI-H460, HeLa, and BEAS-2B were grown in RPMI-1640 supplemented with 100 units mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, and 10% FBS. Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2.

Cell clonogenic assay

Briefly, A549 cells at a density of 300 cells per well were seeded in 6-well plates and incubated for 72 h in a 5% CO_2 atmosphere in 2 mL of complete medium at 37 ℃. Then cells were treated with **2** (0, 3.125, 6.25, 12.5, 25 and 50 μM) for 48 h. Then a fresh culture medium of 2 mL was added. One week later, cells were fixed with 4% paraformaldehyde solution for 30 min, and then stained with 1% crystal violet solution for 30 min.

Hoechst 33342 staining assay

A549 cells were seeded into a 12-well plate at a density of 2×10^5 cells per well and incubated with **2** at 0, 12.5, 25 and 50 μM for 24 h. Then, the medium was removed and washed with PBS, Hoechst 33342 at a concentration of 1 μg/mL was added in the dark at 37 °C for 20 min. After that, the cells were washed with PBS, an inverted fluorescence microscope was used for observation.

Annexin V-FITC/PI staining assay

The Annexin V-FITC/PI assay was performed by Annexin V-FITC Apoptosis Detection Kit. A549 cells at a density of 5×10^5 cells per well were treated with different concentrations (0, 12.5, 25 and 50 μM) of **2** for 48 h, then collected and washed with cold PBS. After that, the cells were digested with 0.25% trypsin and washed with cold PBS twice. Cells were resuspended in the 300 μL 1 × binding buffer, and 5 μL Annexin V-FITC was added to the solutions and incubated in the dark at room temperature for 15 min. They were incubated for another 5 min by propidium iodide (PI, 5 μL), then the same binding buffer (200 μL) was added. The samples were examined with flow cytometry.

ROS determination assay

The ROS generation in A549 cells exposed to **2** was evaluated by fluorescent probe DCFH-DA. Briefly, A549 cells at a density of 1.0×10^6 per well were seeded in a six-well plate at 37 °C with 5% CO_2 overnight. The cells were treated with positive control and **2** at concentrations of 3.125, 6.25, and 12.5 μM for 6 h. Then, the cells were washed twice with PBS and incubated with 10 μM DCFH-DA at 37 °C for 20 min. The cells were washed with PBS and observed under a fluorescent microscope equipped with $\lambda_{ex}/\lambda_{em}$ = 488/525 nm. The fluorescence intensity was analyzed by flow cytometry.

Western blots assay

Total protein was boiled with sample buffer, and separated by SDS-PAGE Kit according to the supplier's instructions, and then transferred to PVDF membranes and incubated with appropriate antibodies overnight. The membranes were washed with TBST buffer and incubated with the secondary antibody. After washing with TBST, the bands were observed.

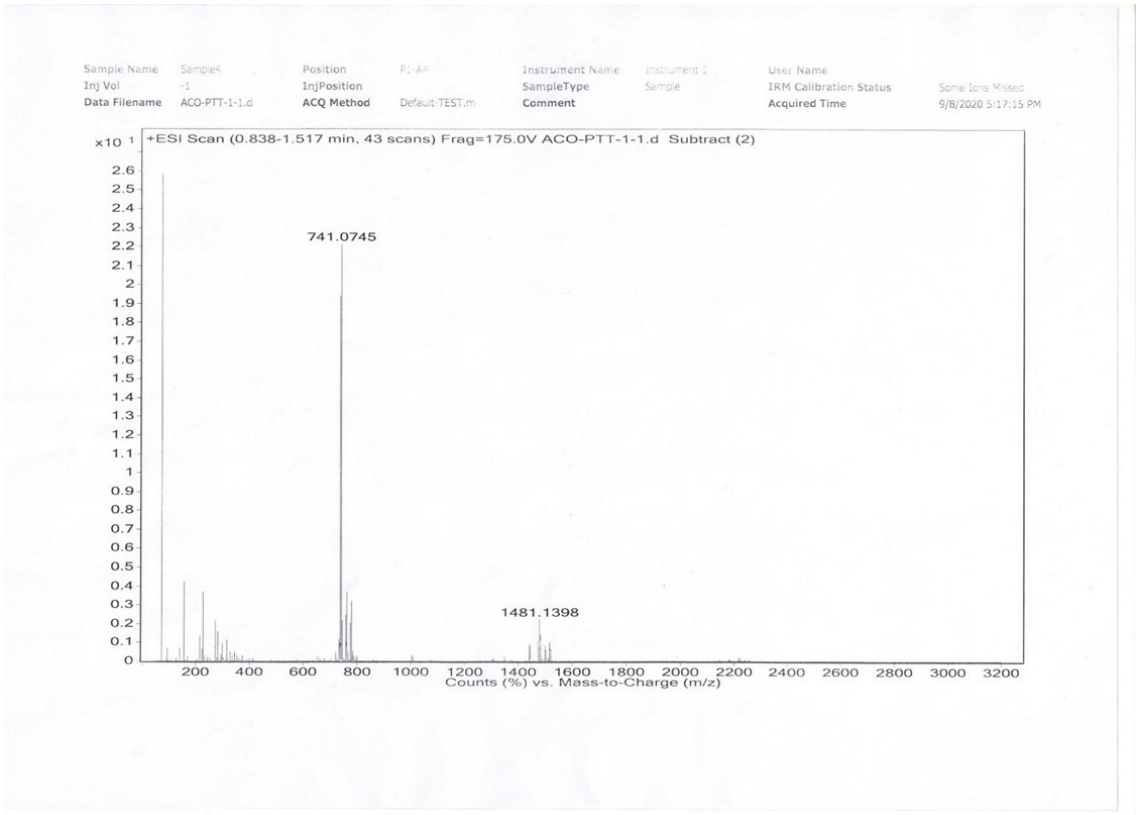
DNA binding assay

To study the binding ability of DNA with Pt^{II} complexes, 5'-dGMP was used as a model of DNA. Complex **2** (1 mM) and CDDP (1 mM) were incubated with 5'-dGMP (2mM) in standard culture conditions for 48 h, respectively. Then, the Pt^{II} complexes combined with 5'-dGMP to form Pt^{II}-dGMP, which were confirmed by ESI-MS.

BSA-binding assay

For BSA binding assay, fluorescence and electronic absorption spectroscopy were

carried out using BSA stock solution (BSA, 0.3 mM) at 4 °C overnight in 10 mM phosphate buffer (pH = 7.2). With increasing the concentration of complex by a fixed amount, the fluorescence spectra excitation at 292nm were recorded at 300-500 nm at different temperatures (298 K and 310 K). Electronic absorption spectroscopy of complex was recorded under the same conditions and carried out the mechanism using an Agilent Cary 100 UV-vis spectrophotometer. The data were recorded from 200 to 350 nm. Absorption titration experiments were done by keeping the concentration of BSA constant (0.3 μM) while adding the same concentration of complexes.



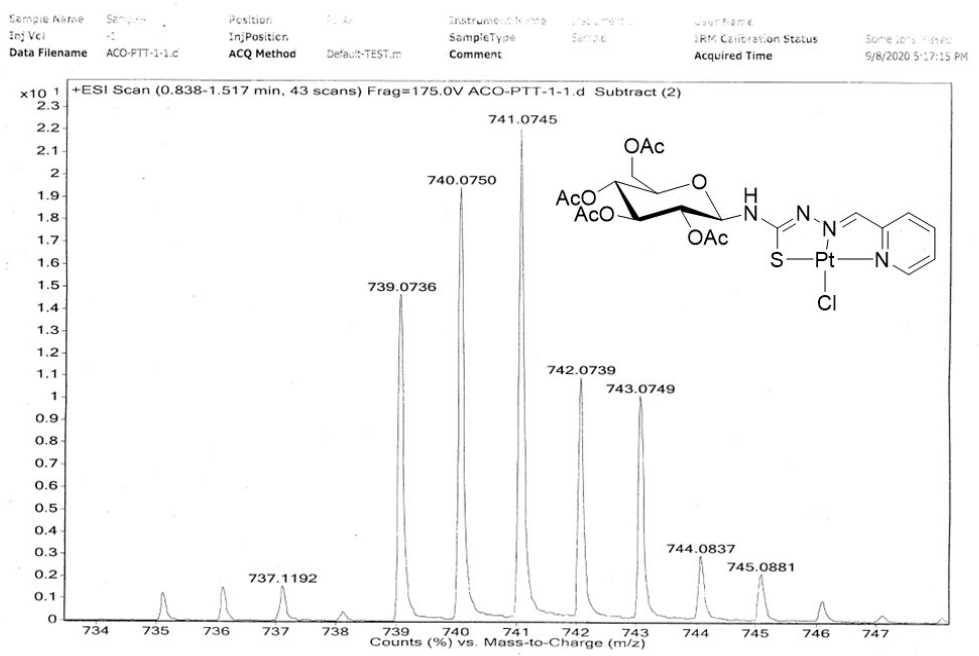
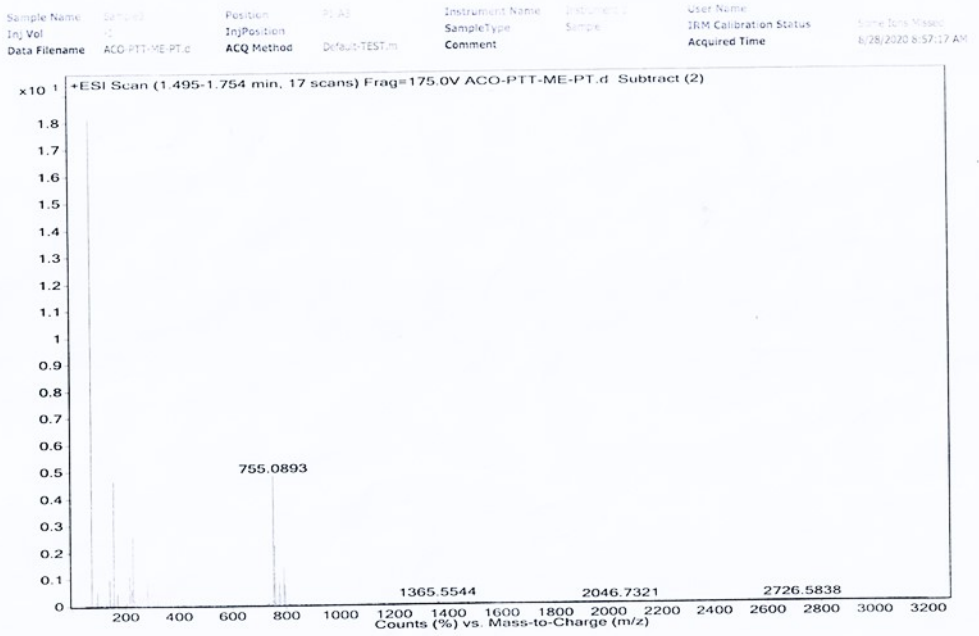


Fig. S1. ESI-MS spectrum for complex **1.**



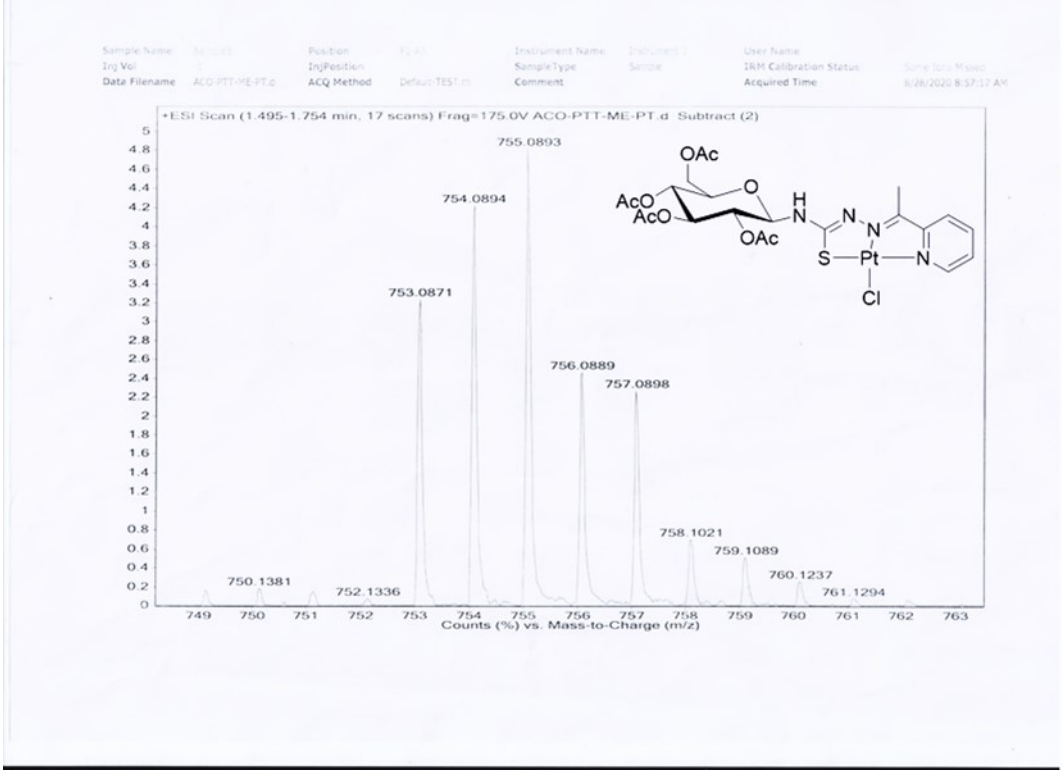
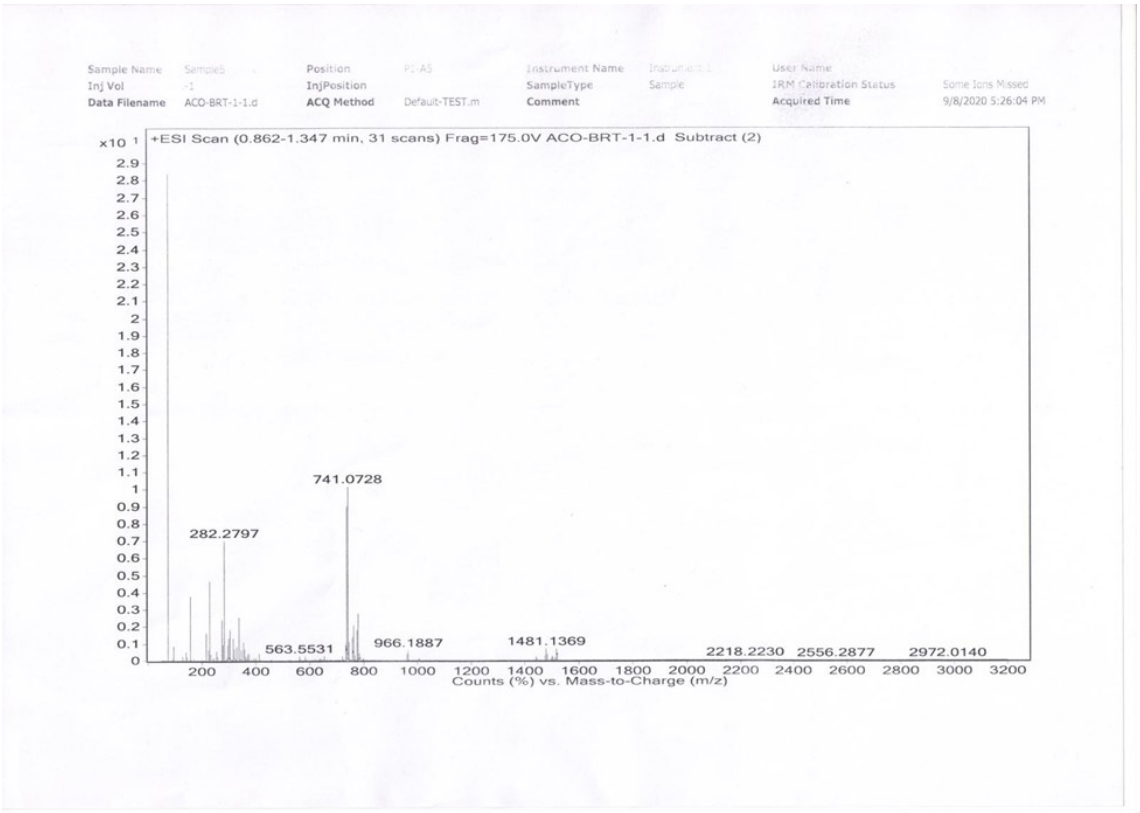


Fig. S2. ESI-MS spectrum for complex **2**.



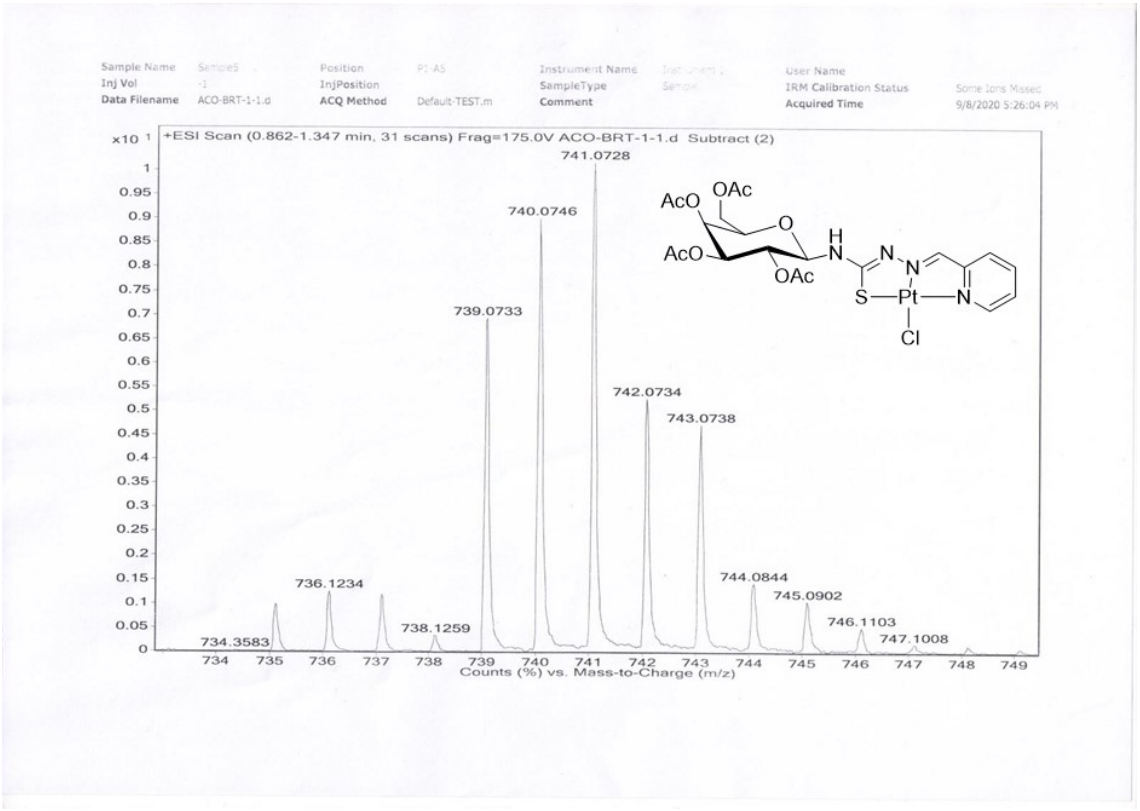
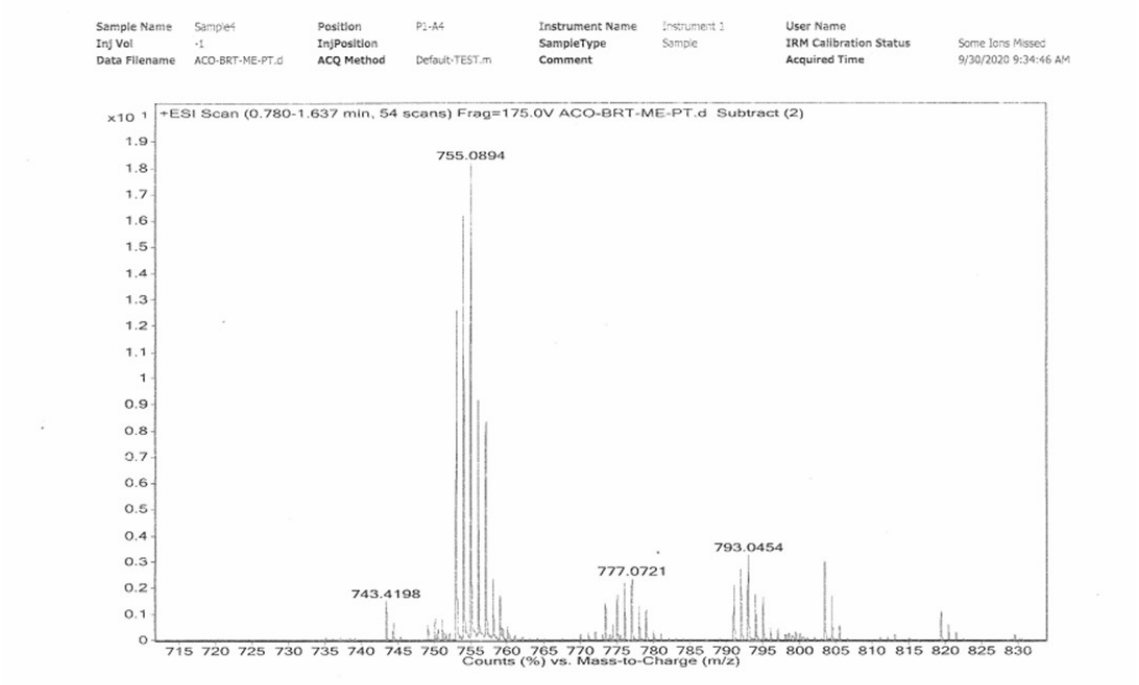


Fig. S3. ESI-MS spectrum for complex **3**.



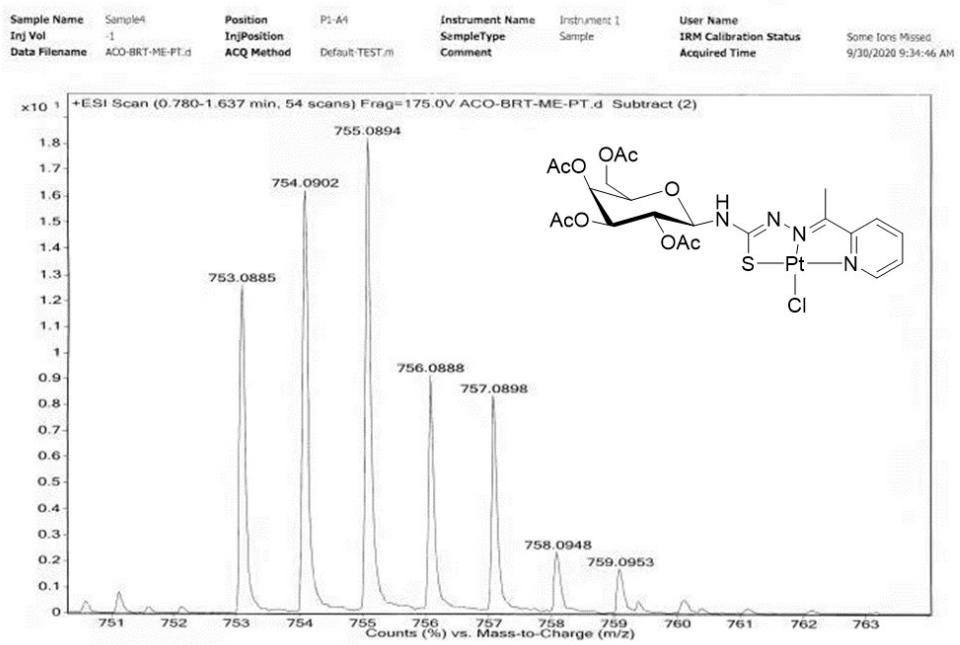
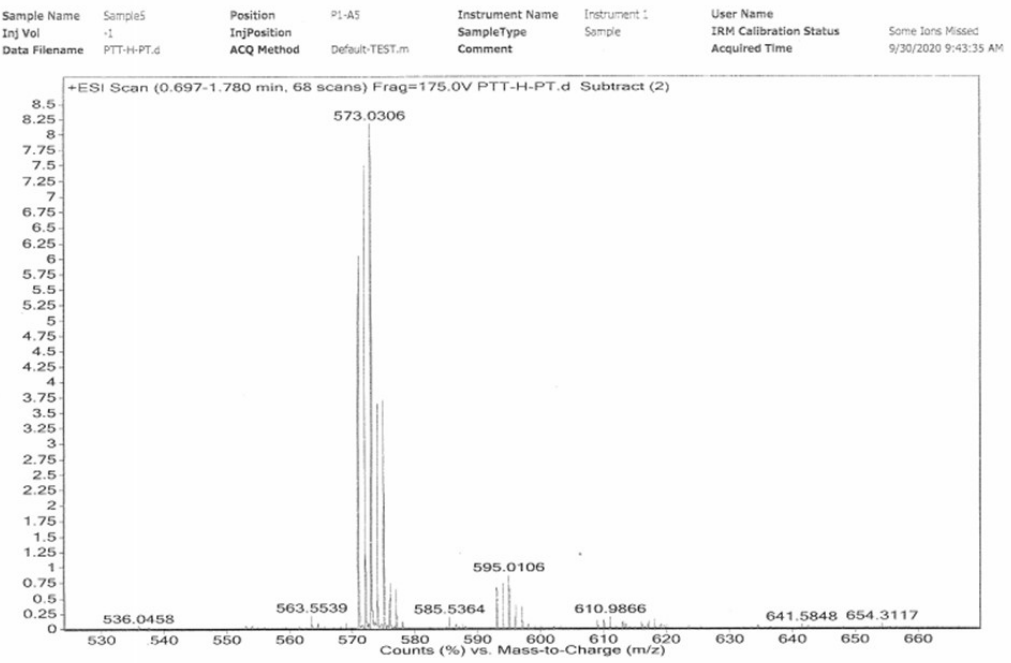


Fig. S4. ESI-MS spectrum for complex **4**.



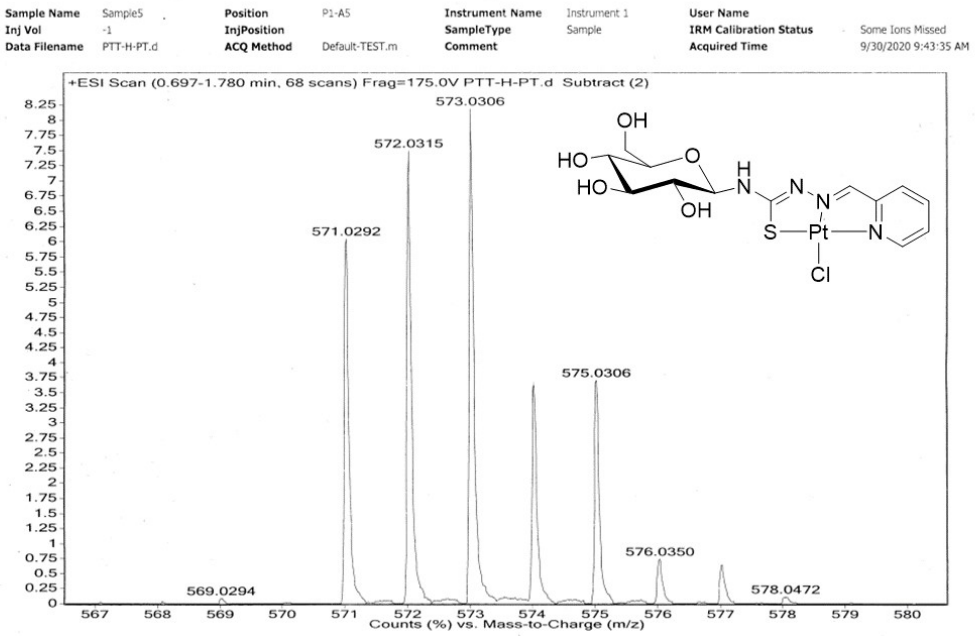
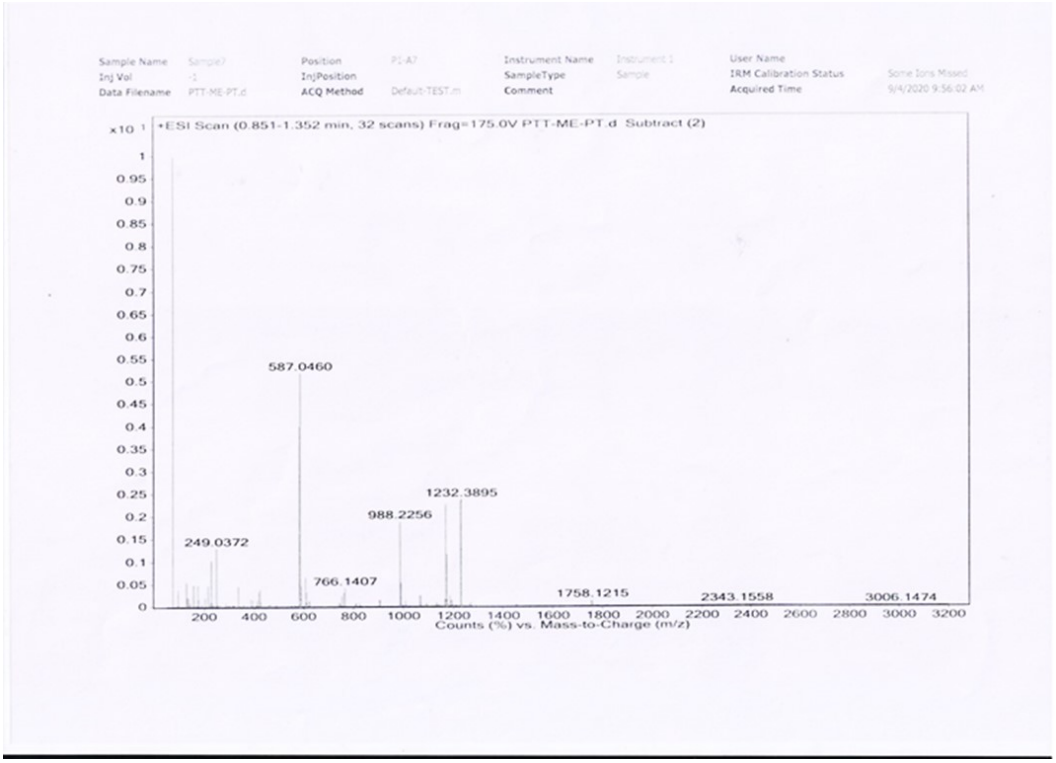


Fig. S5. ESI-MS spectrum for complex **5**.



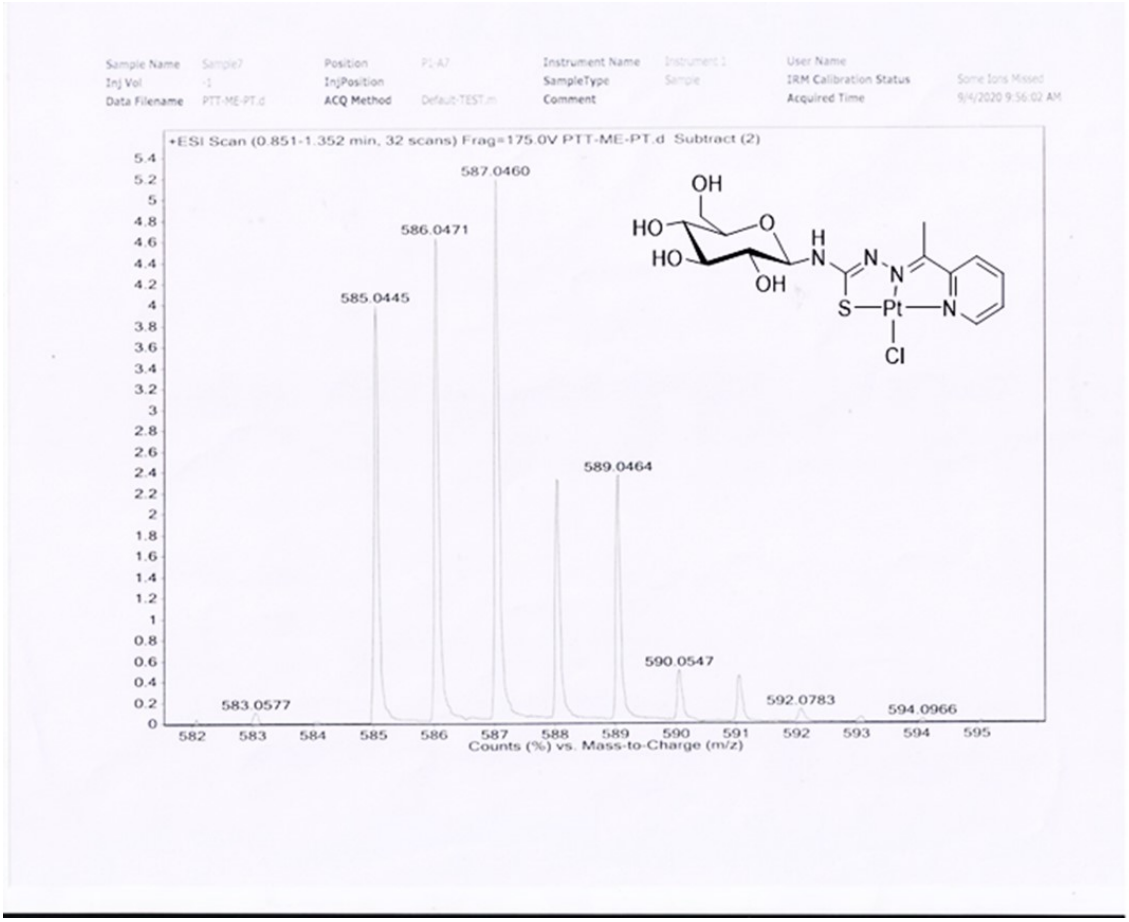
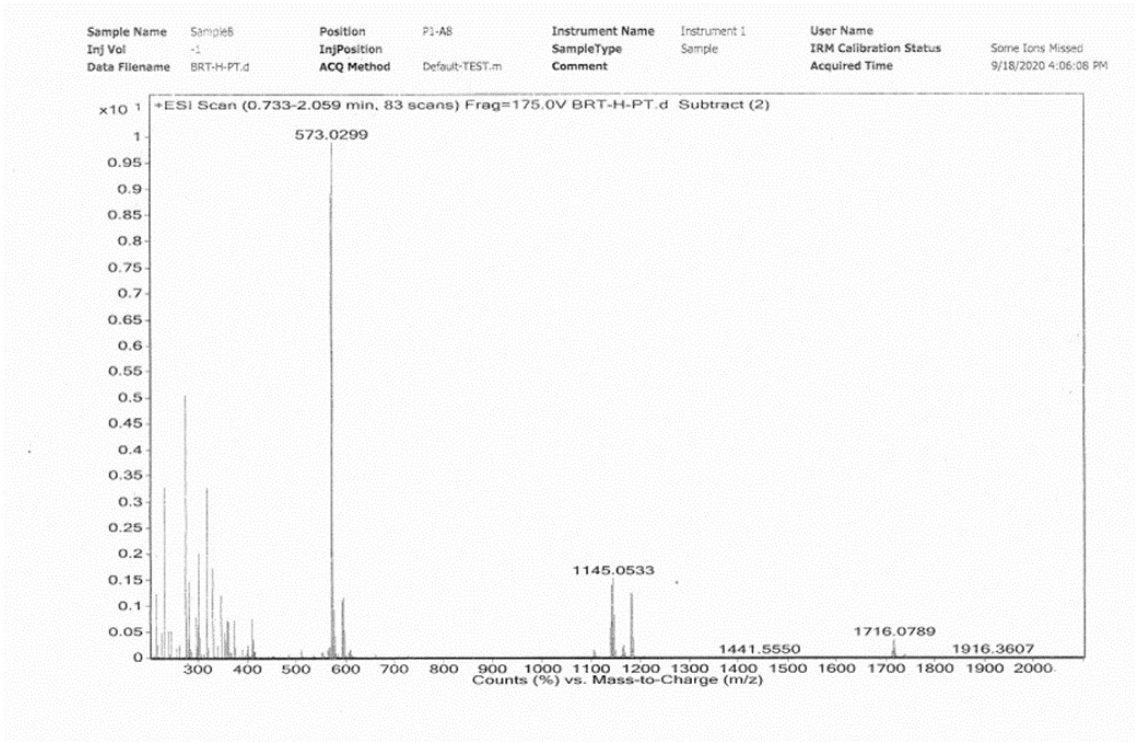


Fig. S6. ESI-MS spectrum for complex **6**.



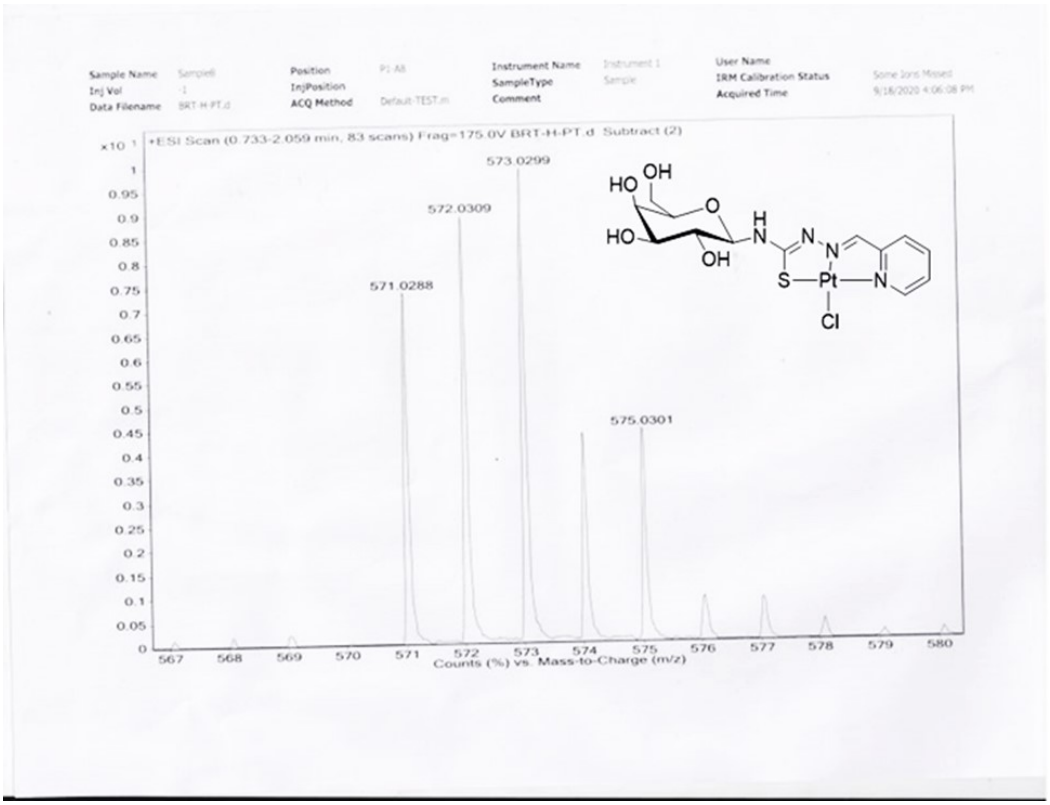
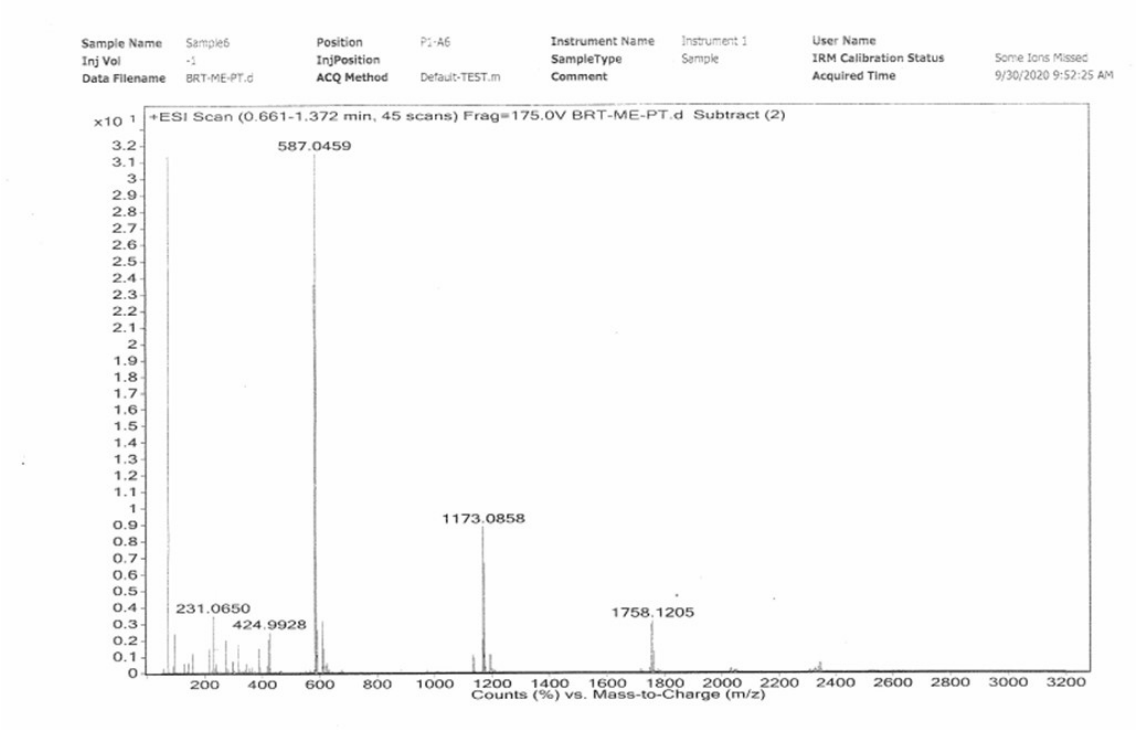


Fig. S7. ESI-MS spectrum for complex **7**.



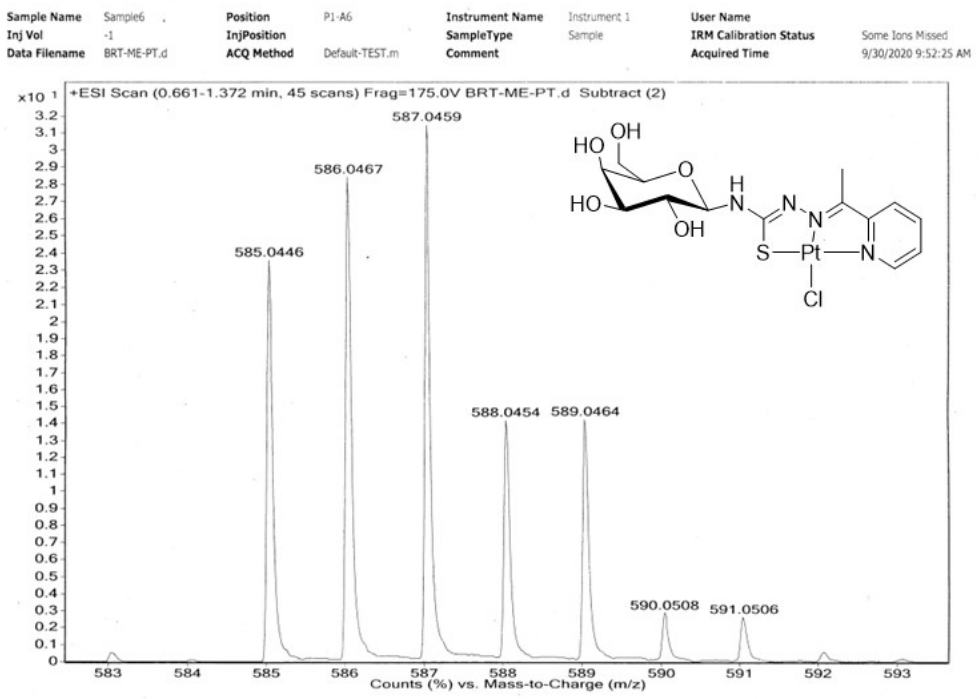


Fig. S8. ESI-MS spectrum for complex **8**.

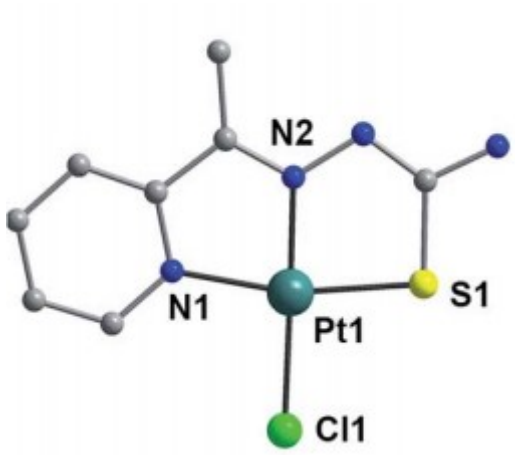


Fig. S9. DIAMOND views of [Pt(MH-TSC)Cl]**. H** atoms omitted for clarity.

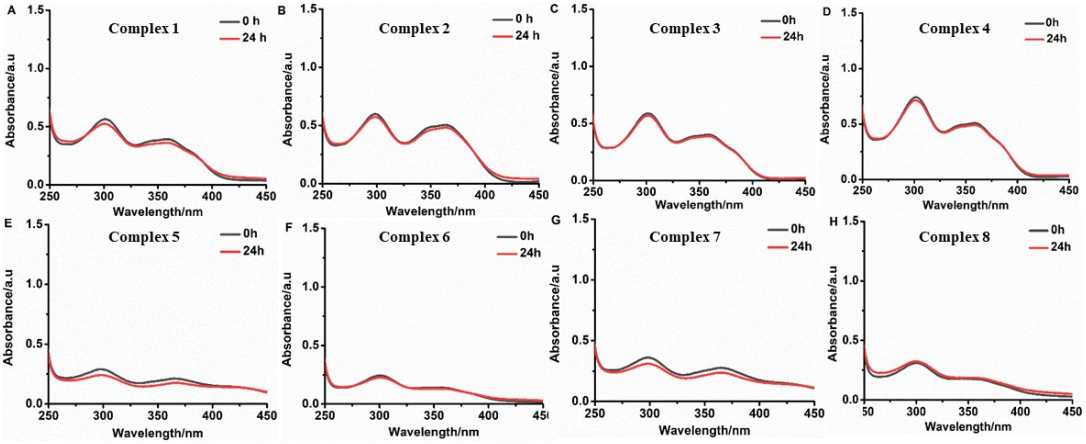


Fig. S10. Absorption spectra of complexes **1-8** in PBS at 0 h and 24 h, respectively.

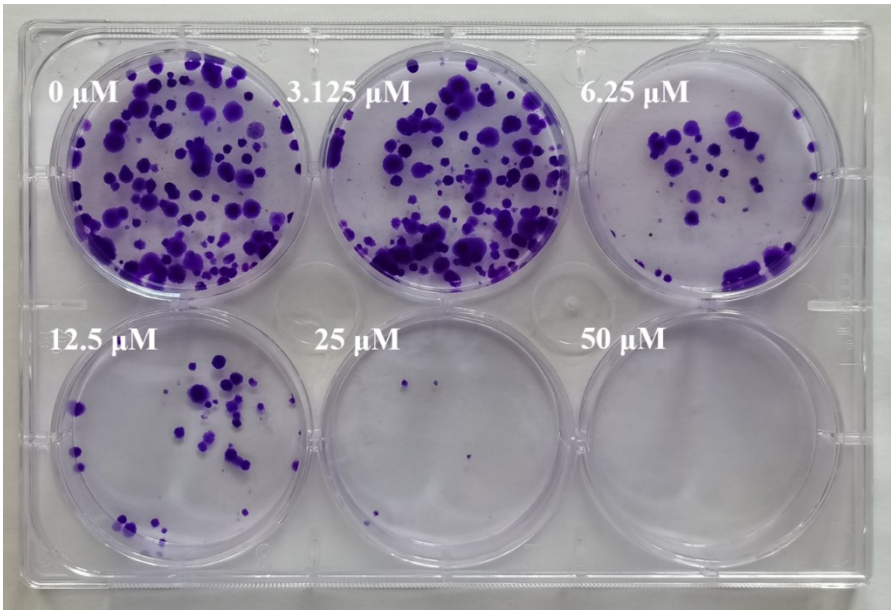


Fig. S11. Clonogenic capacity in A549 cells tested by colony-forming assay. The cells were exposed to 0 μM-50 μM of **2.**

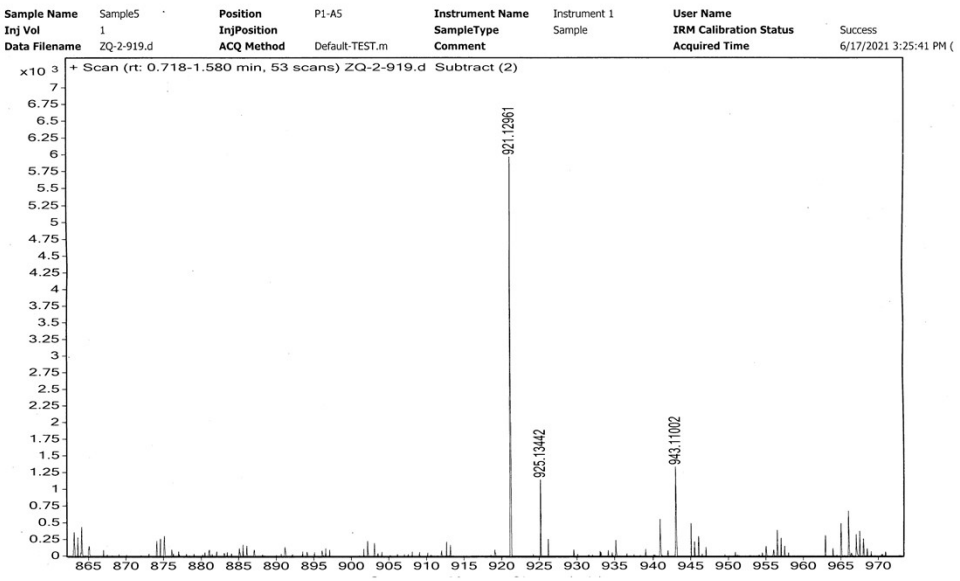


Fig. S12. ESI-MS of the reaction product of CDDP with 5'-dGMP.

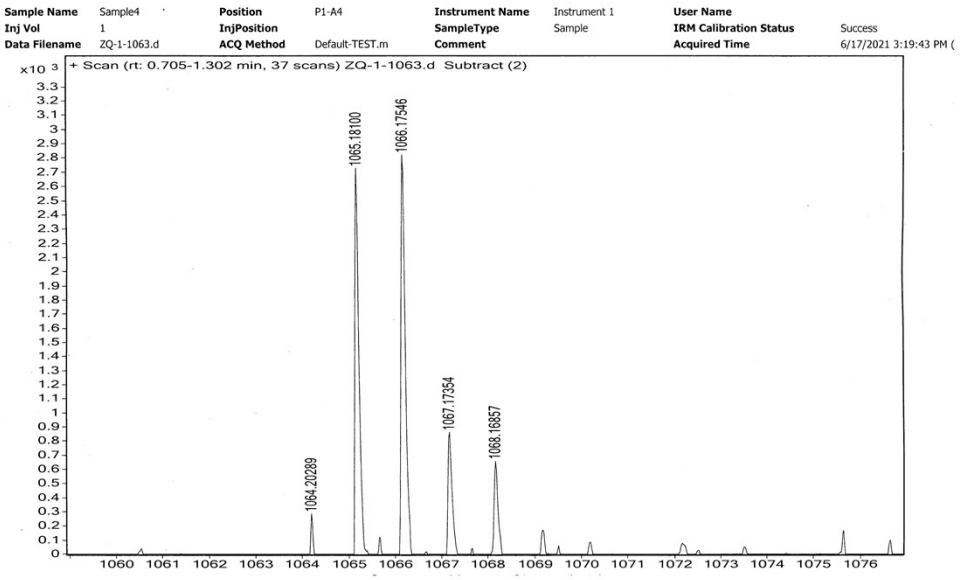


Fig. S13. ESI-MS of the reaction product of **2** with 5'-dGMP.

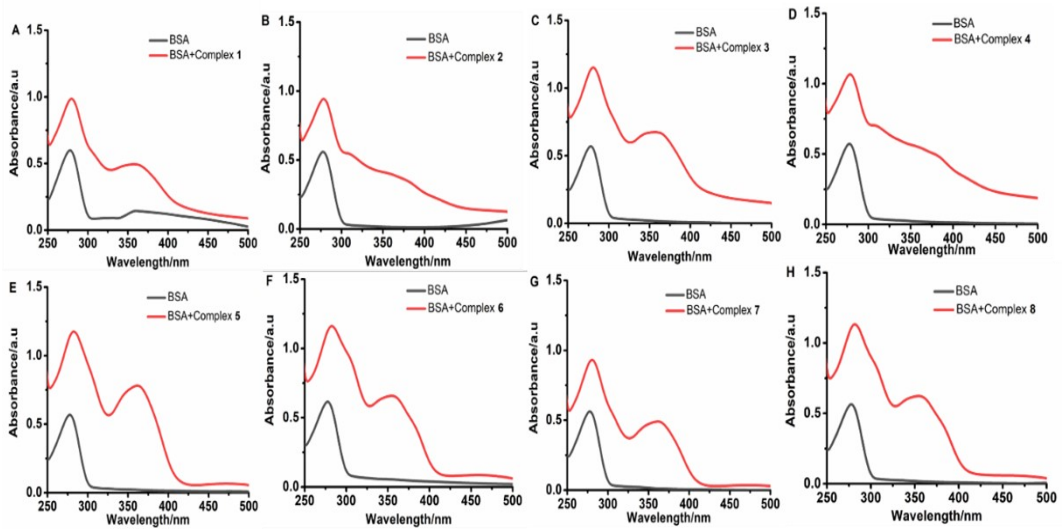


Fig. S14. UV-vis absorption spectra of BSA in the absence and presence of **1-8**.

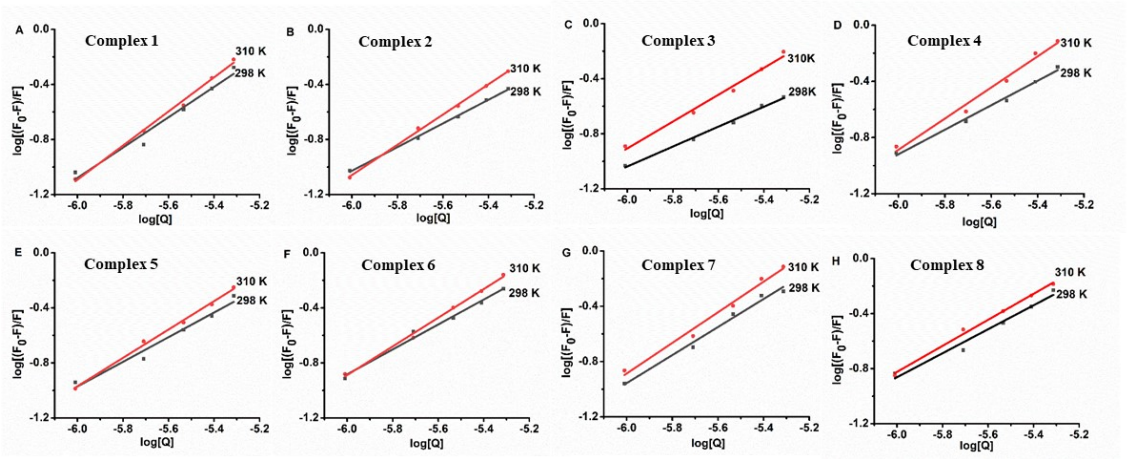


Fig. S15. Plot of Log $[(F_0 - F)/F]$ versus Log$[Q]$.

Table S1 The interaction quenching parameters and thermodynamic parameters of Pt^{II} complexes with BSA at different temperatures.

Complex	T (K)	$K_{SV}\times10^5$(M^{-1})	$K_q\times10^{13}$($M^{-1}S^{-1}$)	$K_b\times10^6$(M^{-1})	n	ΔH (kJ mol^{-1})	ΔG (kJ mol^{-1})	ΔS (J mol^{-1})
1	298	1.06	1.71	0.34	1.11	119.18	-31.56	505.83
	310	1.24	1.99	2.19	1.24		-37.64	
2	298	0.75	1.22	0.02	0.86	183.07	-24.54	696.67
	310	1.02	1.65	0.35	1.11		-32.91	
3	298	0.06	1.08	0.002	0.72	243.66	-18.84	880.83
	310	1.25	2.02	0.09	0.97		-29.41	
4	298	1.01	1.63	0.20	0.87	68.11	-30.24	330.01
	310	1.61	2.59	0.58	1.10		-34.21	
5	298	0.95	1.54	0.03	0.89	107.07	-25.54	445.01
	310	1.13	1.83	0.16	1.03		-30.88	
6	298	1.11	1.78	0.04	0.91	112.07	-26.25	464.17
	310	1.41	2.28	0.23	1.04		-31.82	

7	298	1.11	1.79	0.14	1.02	91.09	-29.35	404.17
	310	1.61	2.59	0.58	1.10		-34.21	
8	298	1.17	1.89	0.03	0.87	54.18	-25.54	265.5
	310	1.34	2.16	0.07	0.95		-28.75	

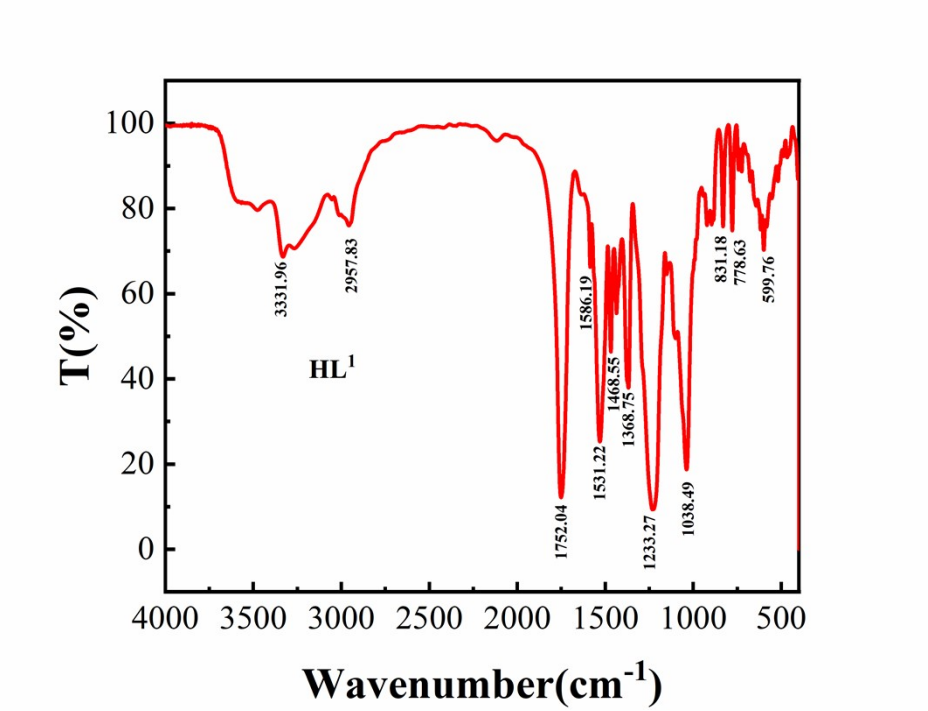


Fig. S16. IR spectra of **HL**[1].

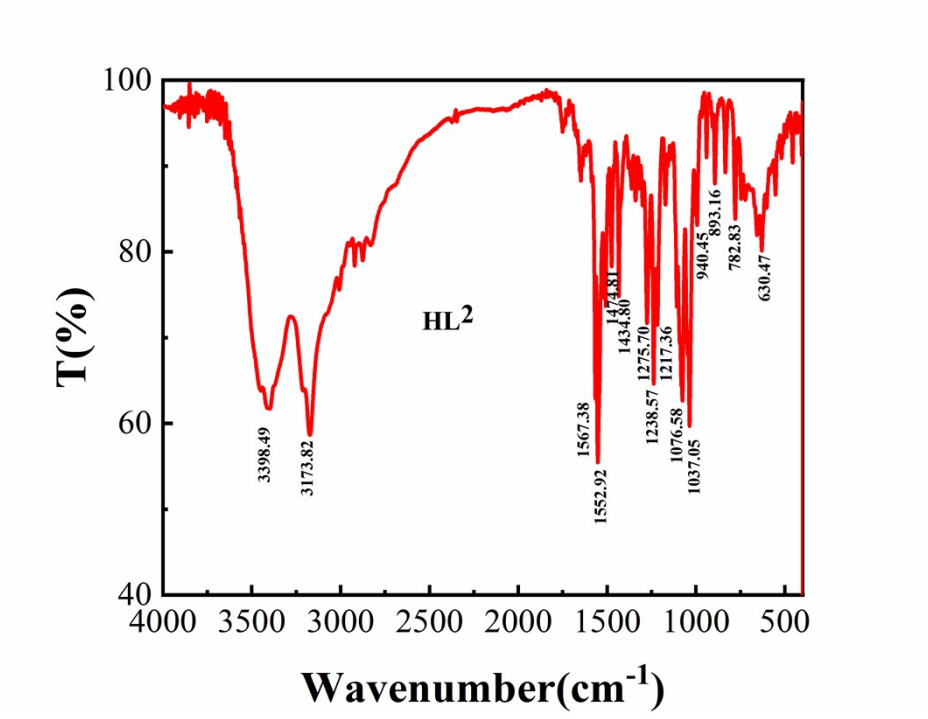


Fig. S17. IR spectra of **HL**[2].

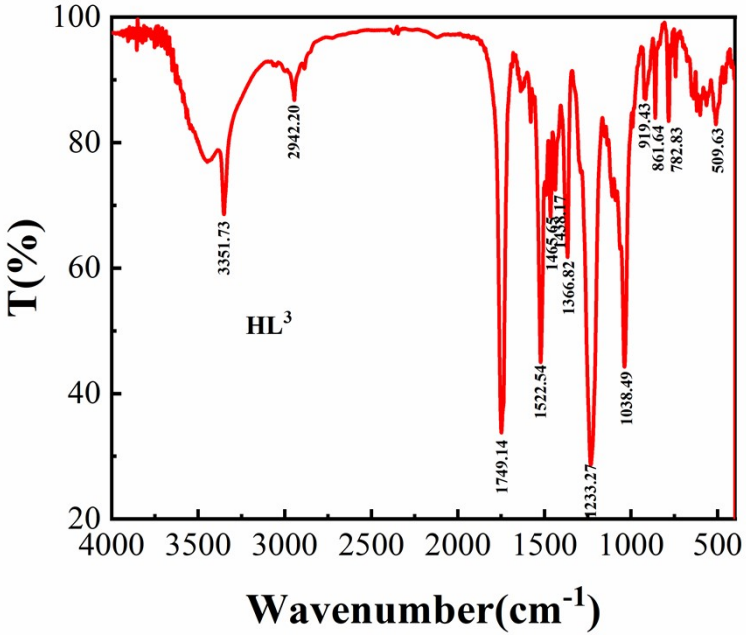


Fig. S18. IR spectra of **HL³**.

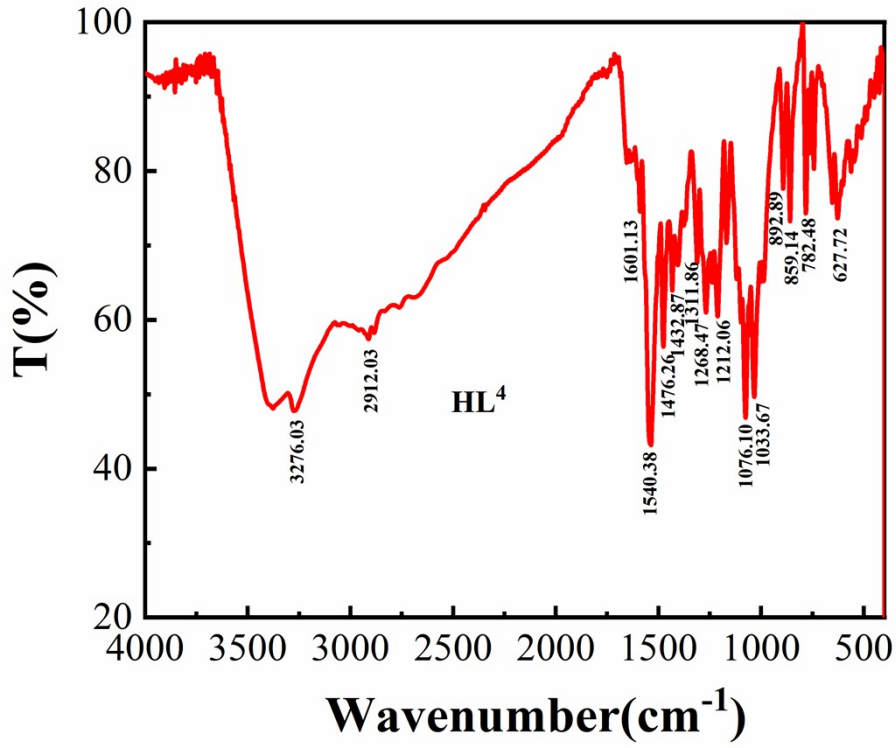


Fig. S19. IR spectra of **HL⁴**.

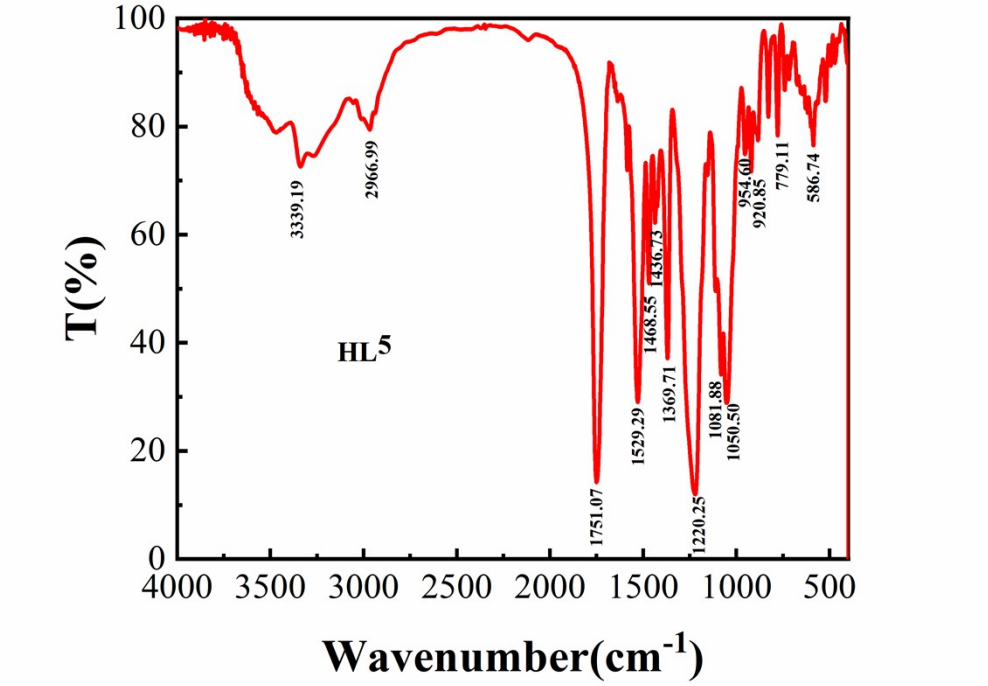


Fig. S20. IR spectra of **HL^5**.

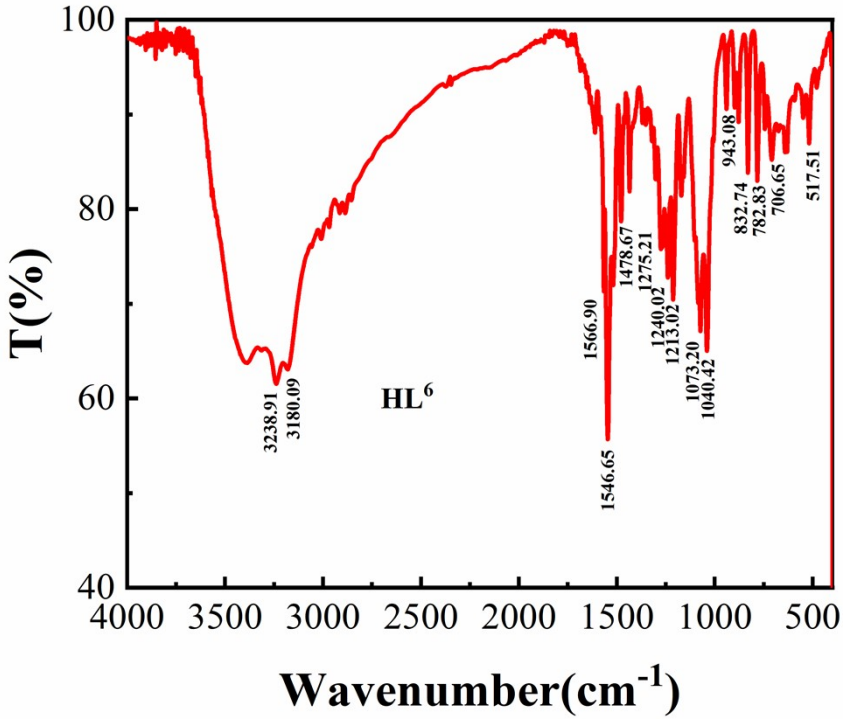


Fig. S21. IR spectra of **HL^6**.

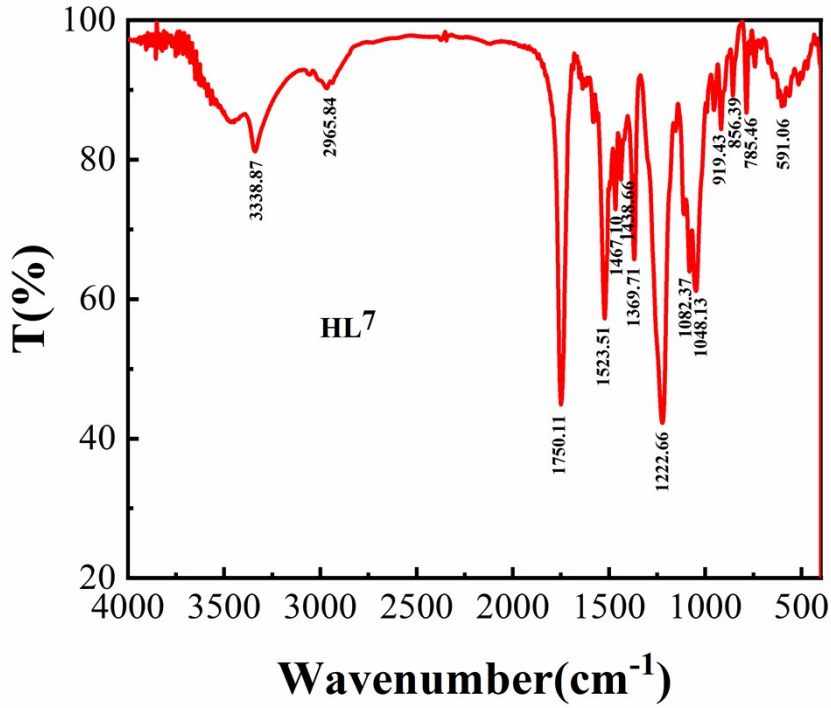


Fig. S22. IR spectra of **HL**[7].

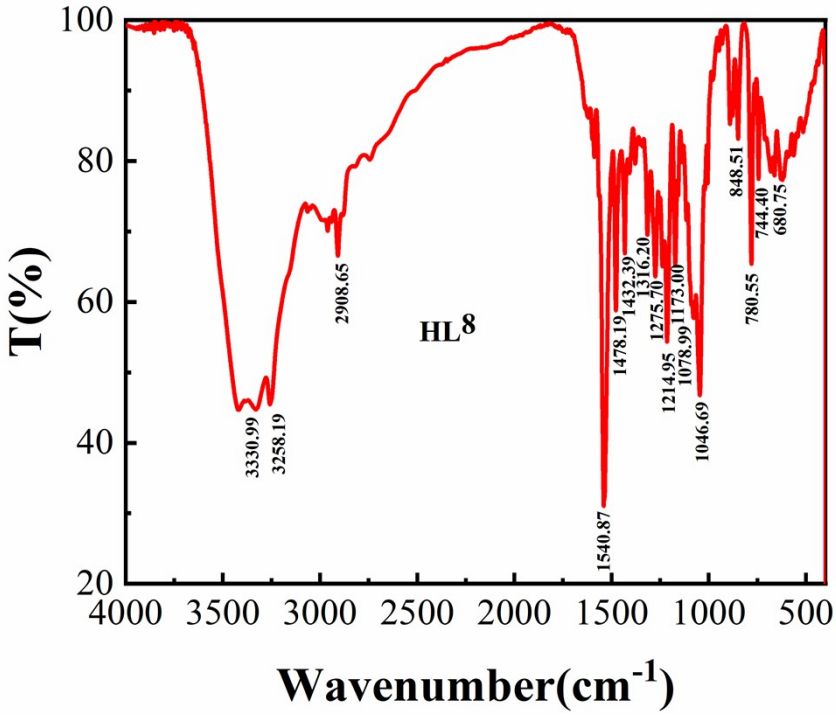


Fig. S13. IR spectra of **HL**[8].

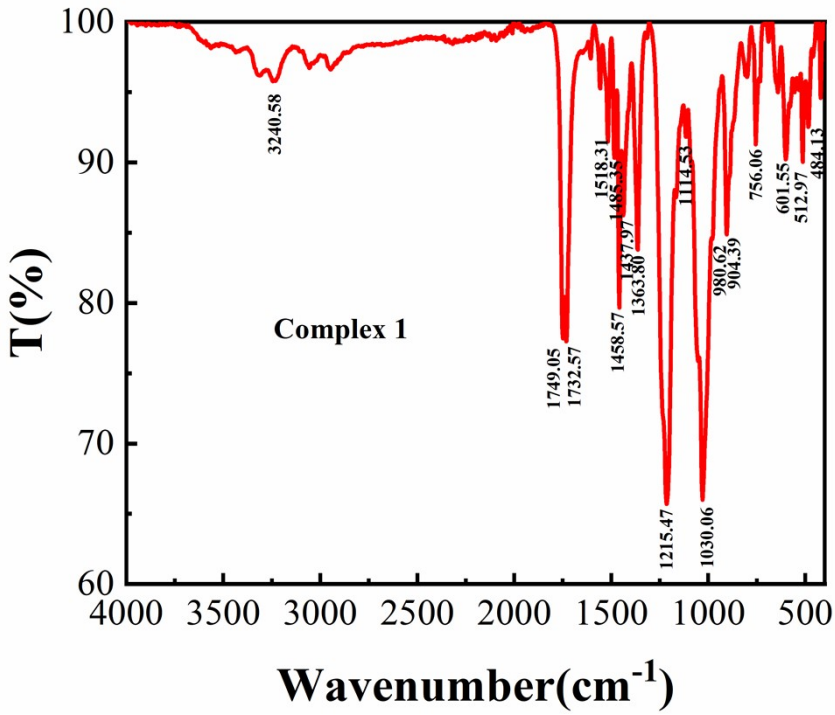


Fig. S24. IR spectra of **1**.

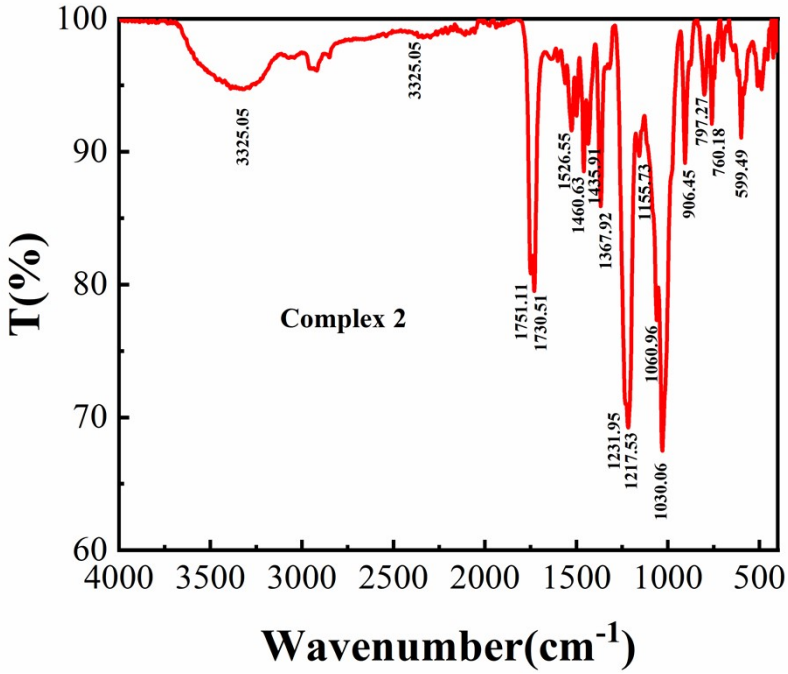


Fig. S25. IR spectra of **2**.

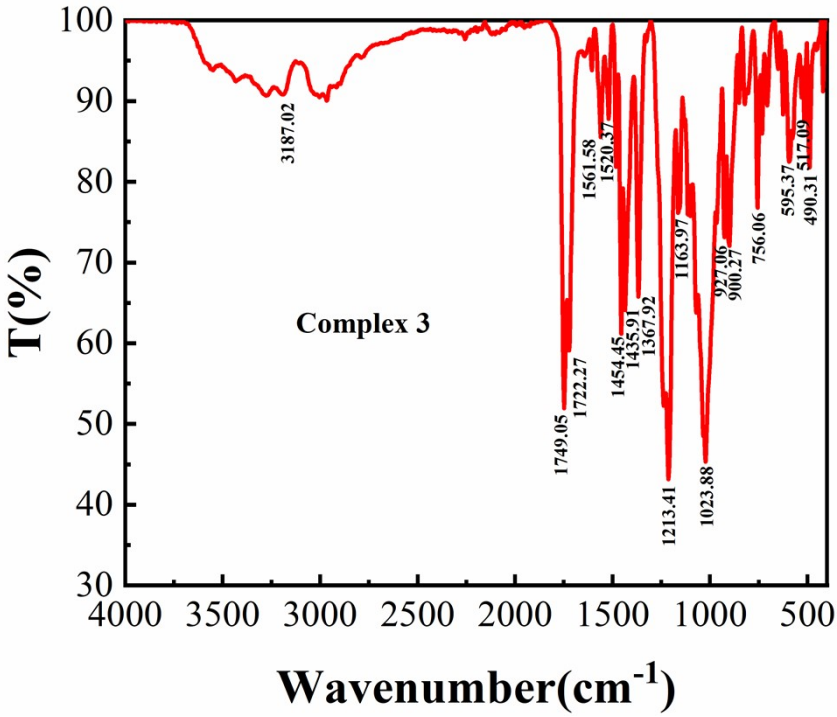


Fig. S26. IR spectra of **3**.

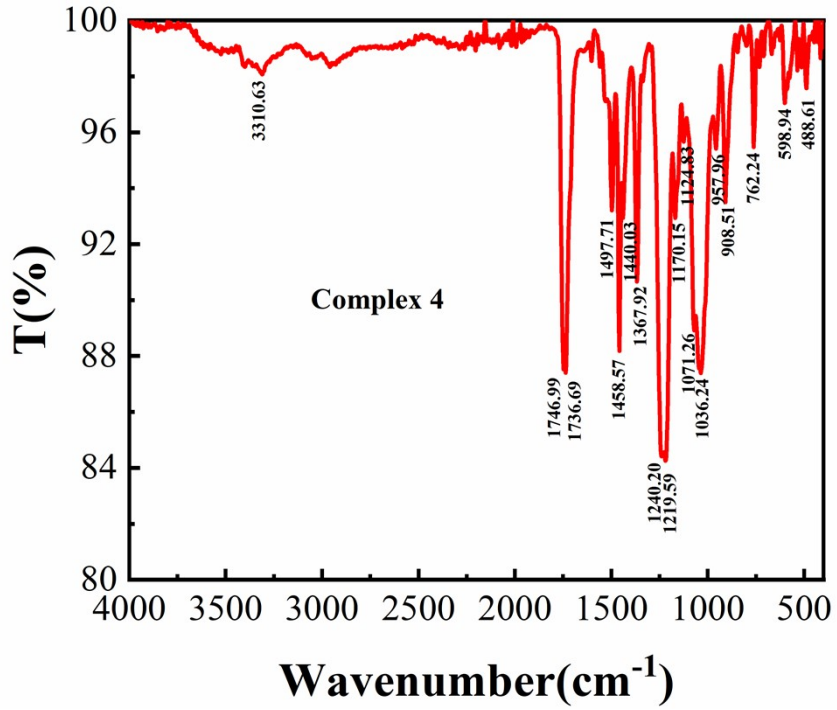


Fig. S27. IR spectra of **4**.

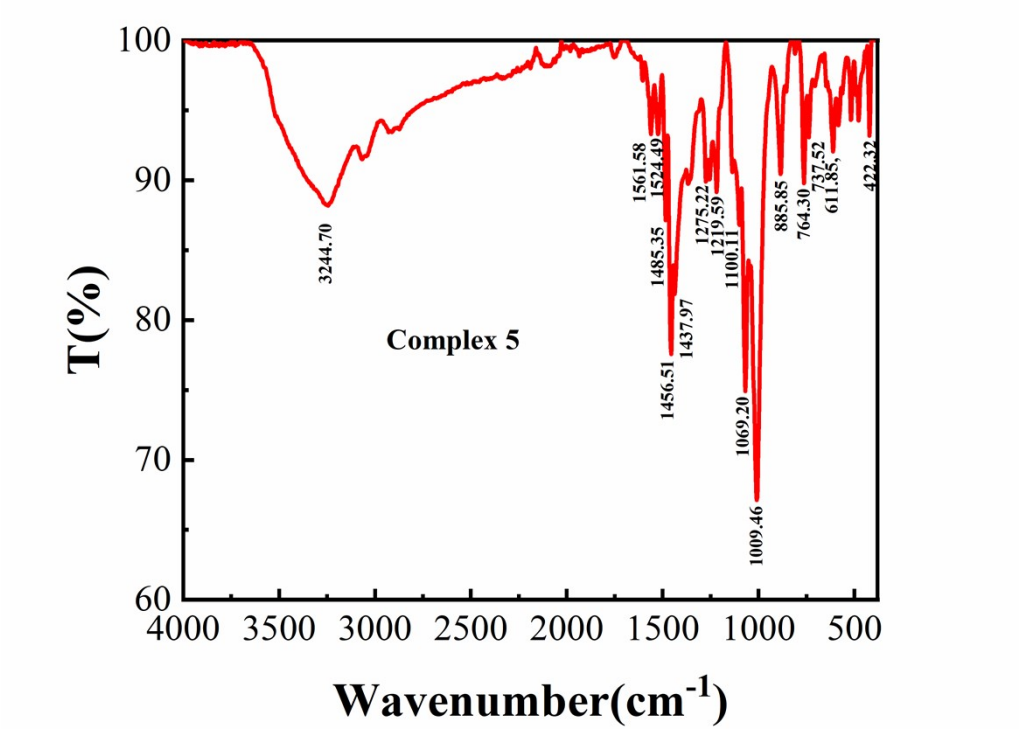


Fig. S28. IR spectra of **5**.

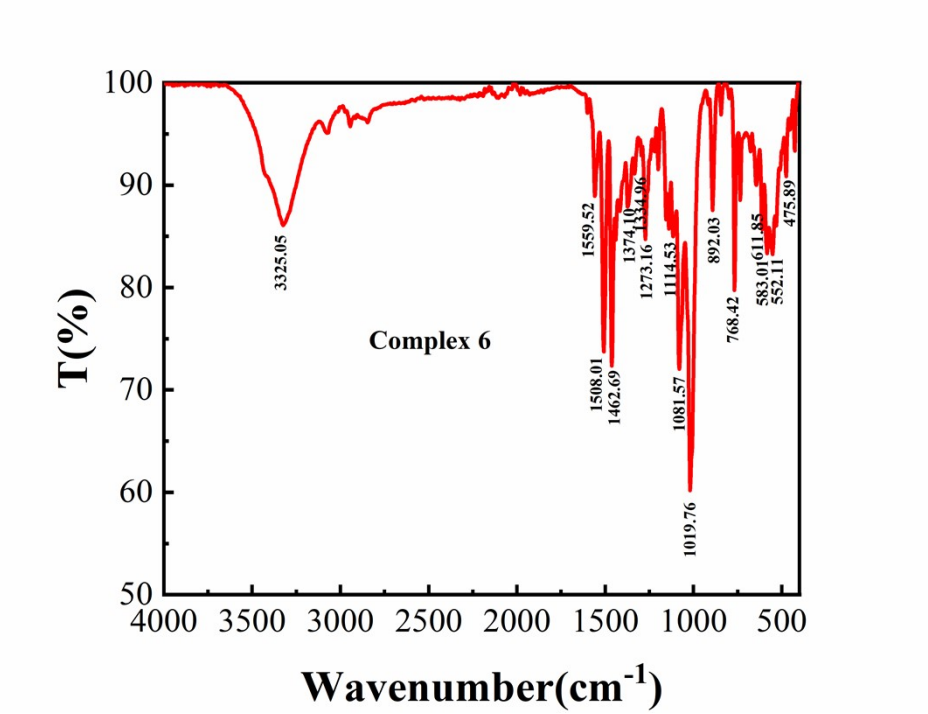


Fig. S29. IR spectra of **6**.

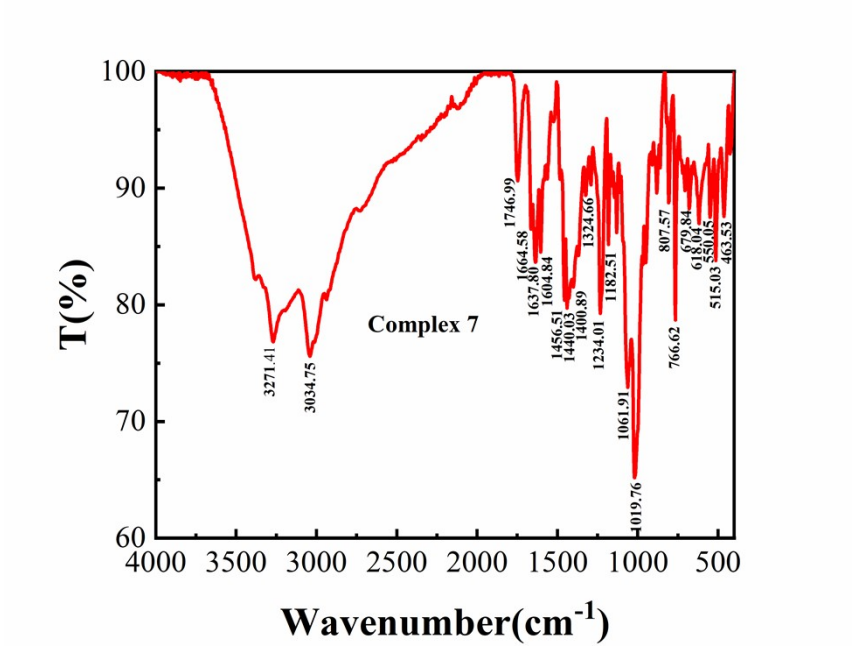


Fig. S30. IR spectra of **7**.

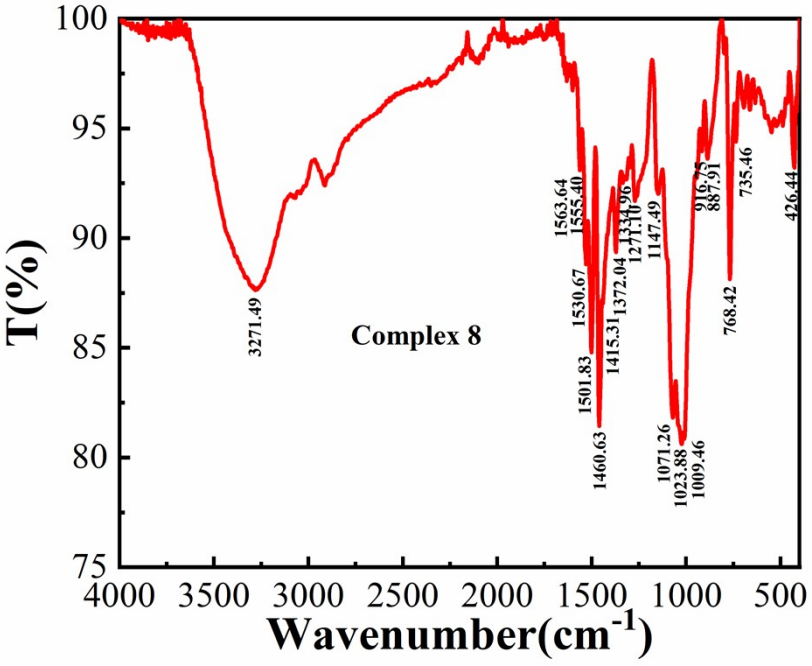


Fig. S31. IR spectra of **8**.

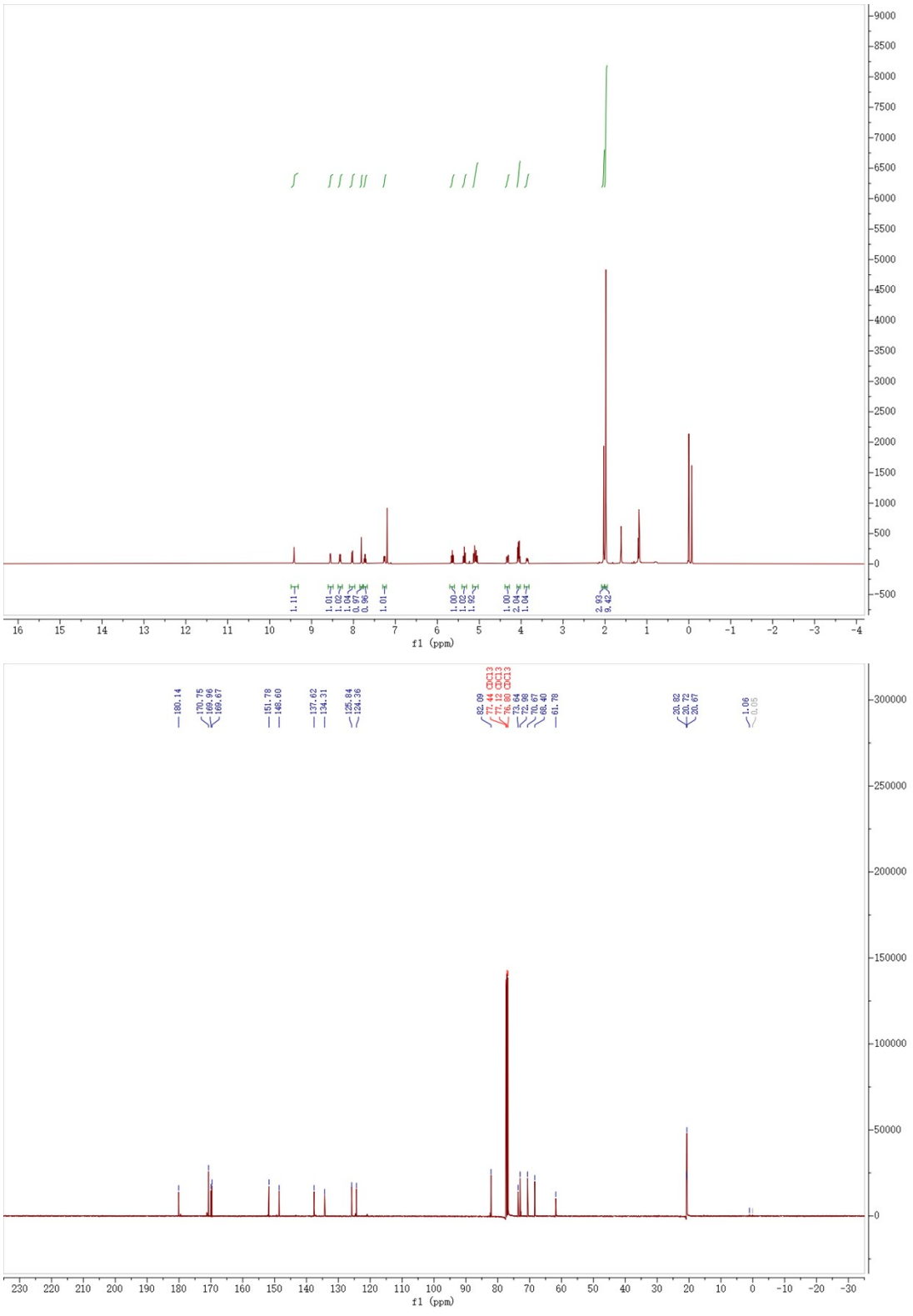


Fig. S32. NMR (^{1}H and ^{13}C) spectra of **HL^{1}**.

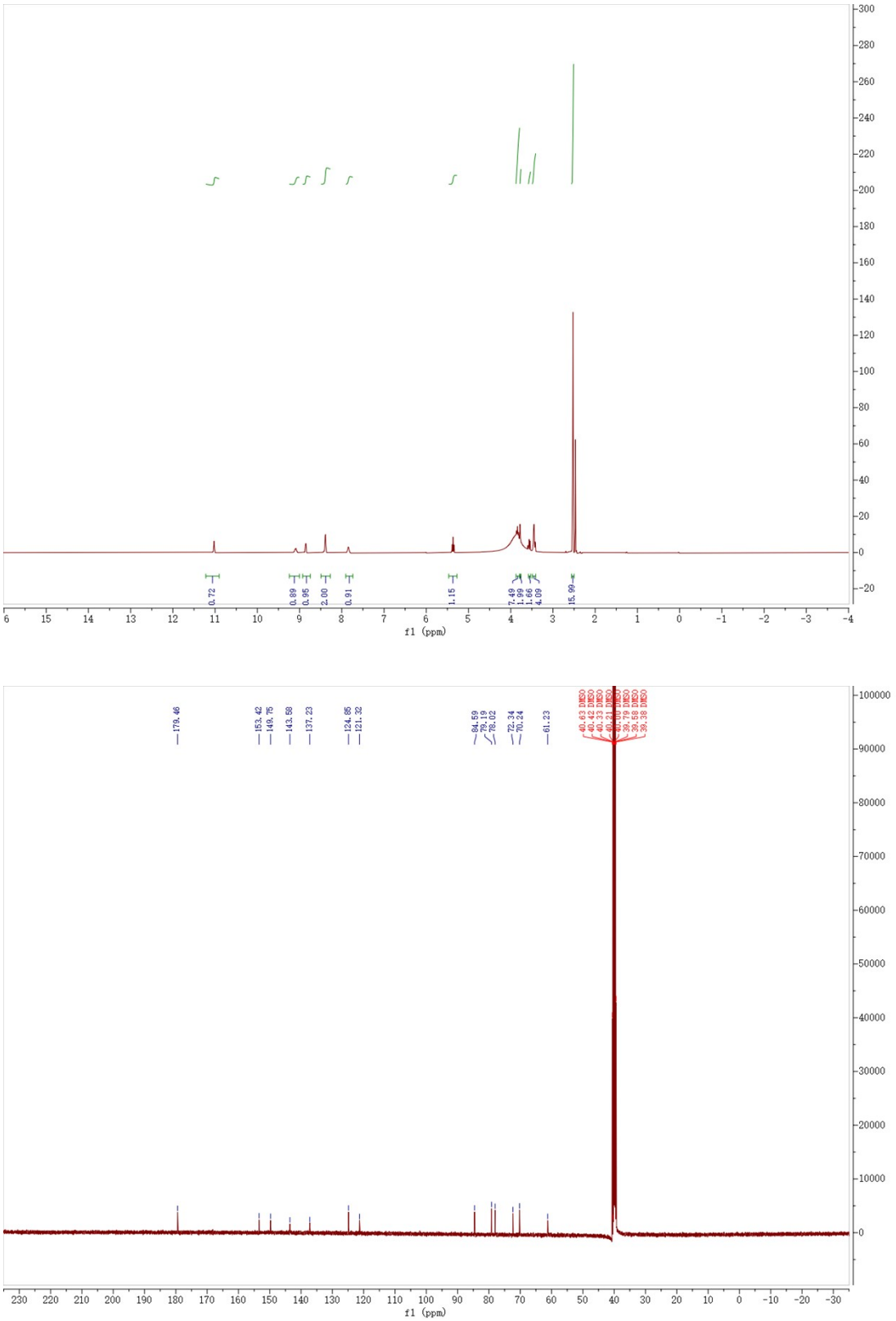


Fig. S33. NMR (^{1}H and ^{13}C) spectra of **HL²**.

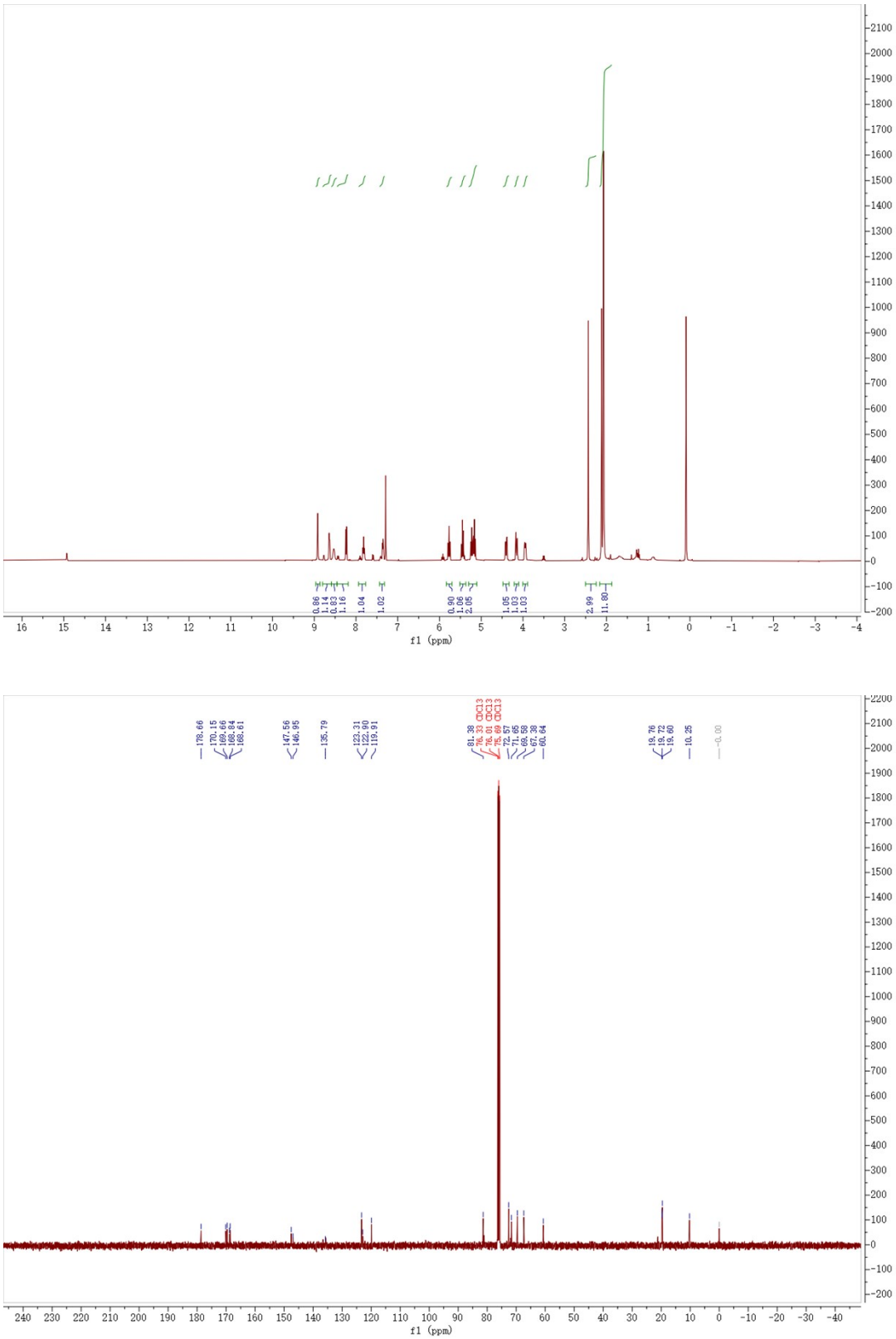


Fig. S34. NMR (^{1}H and ^{13}C) spectra of **HL^{3}**.

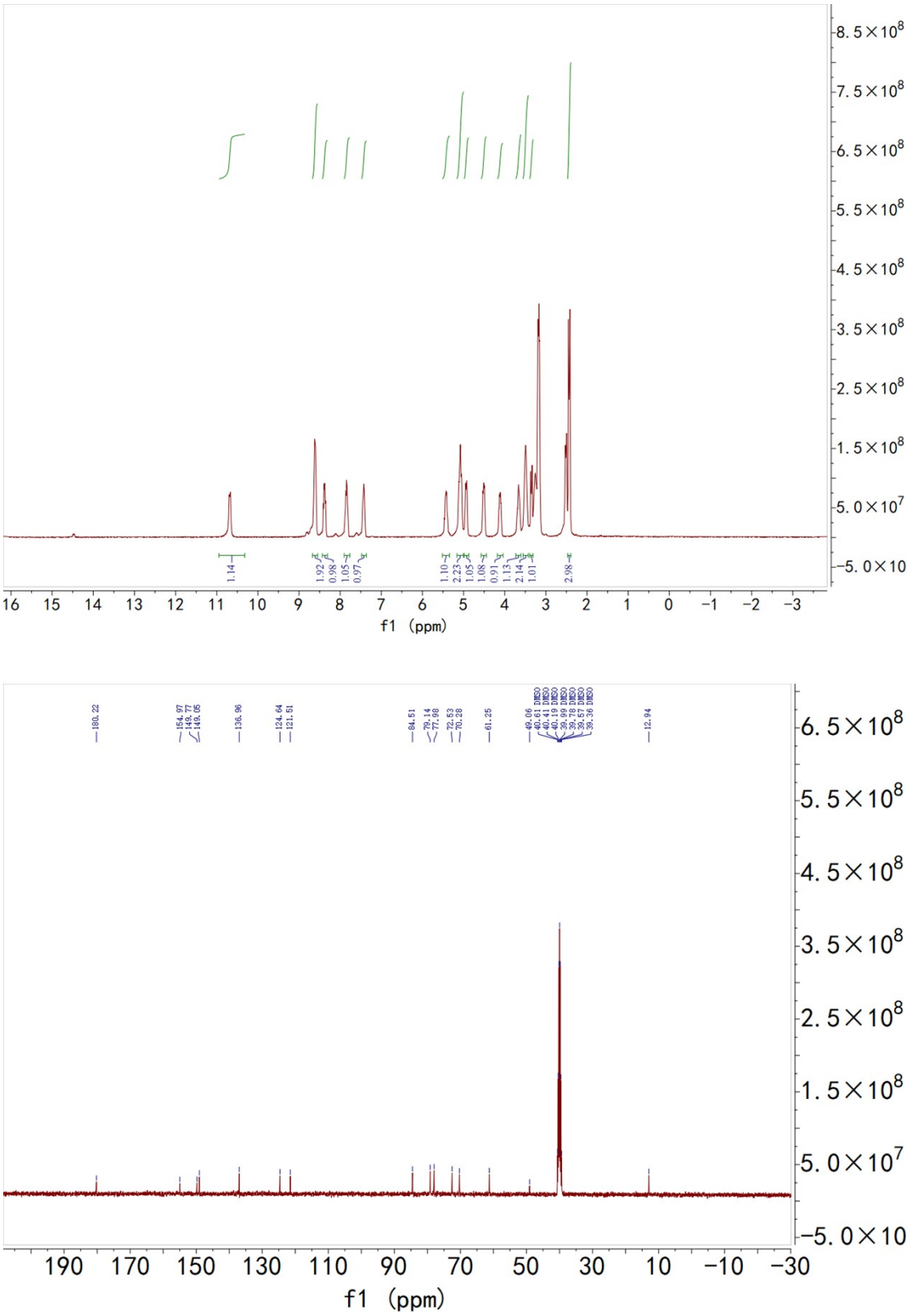


Fig. S35. NMR (^{1}H and ^{13}C) spectra of **HL^{4}**.

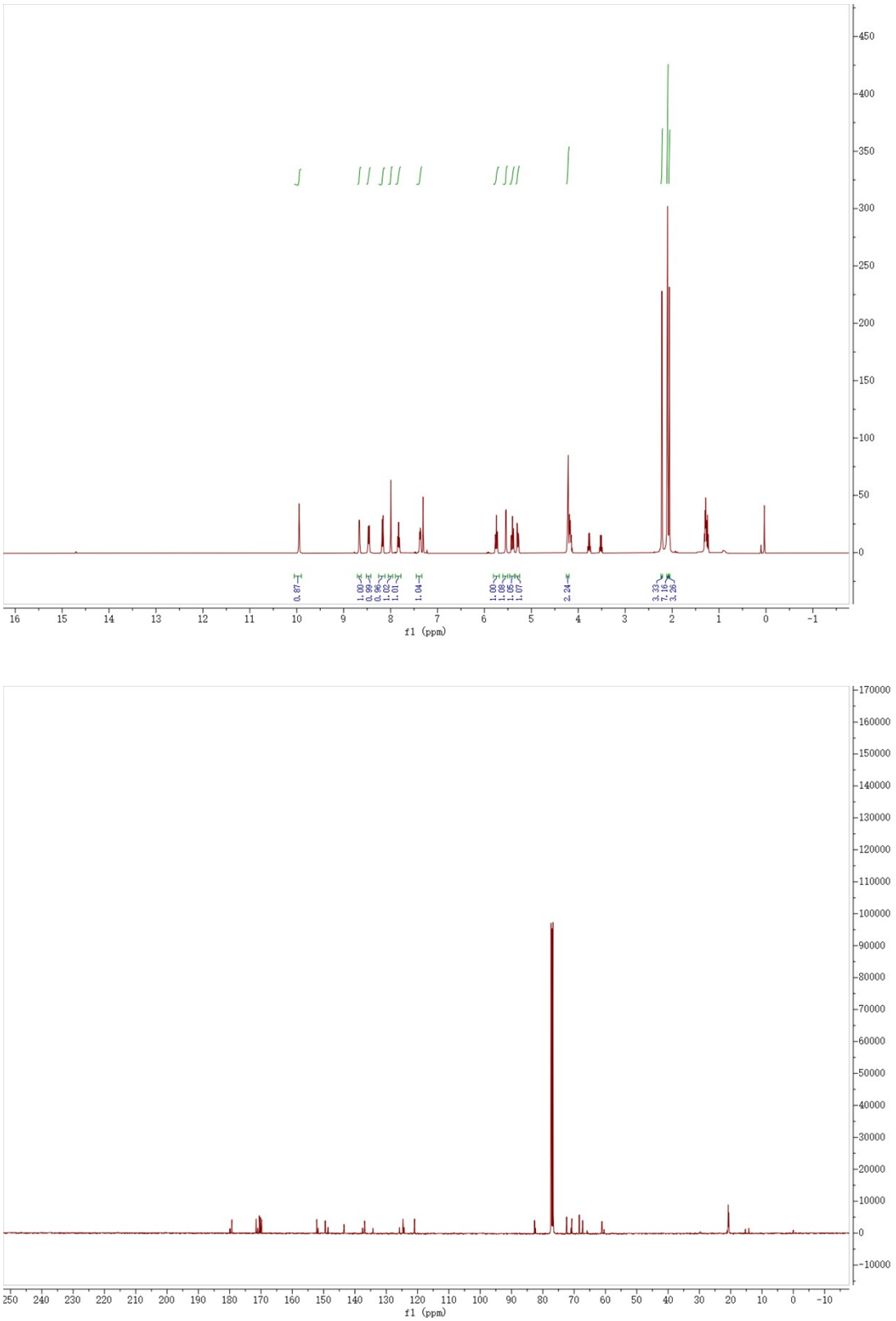


Fig. S36. NMR (^{1}H and ^{13}C) spectra of **HL^{5}**.

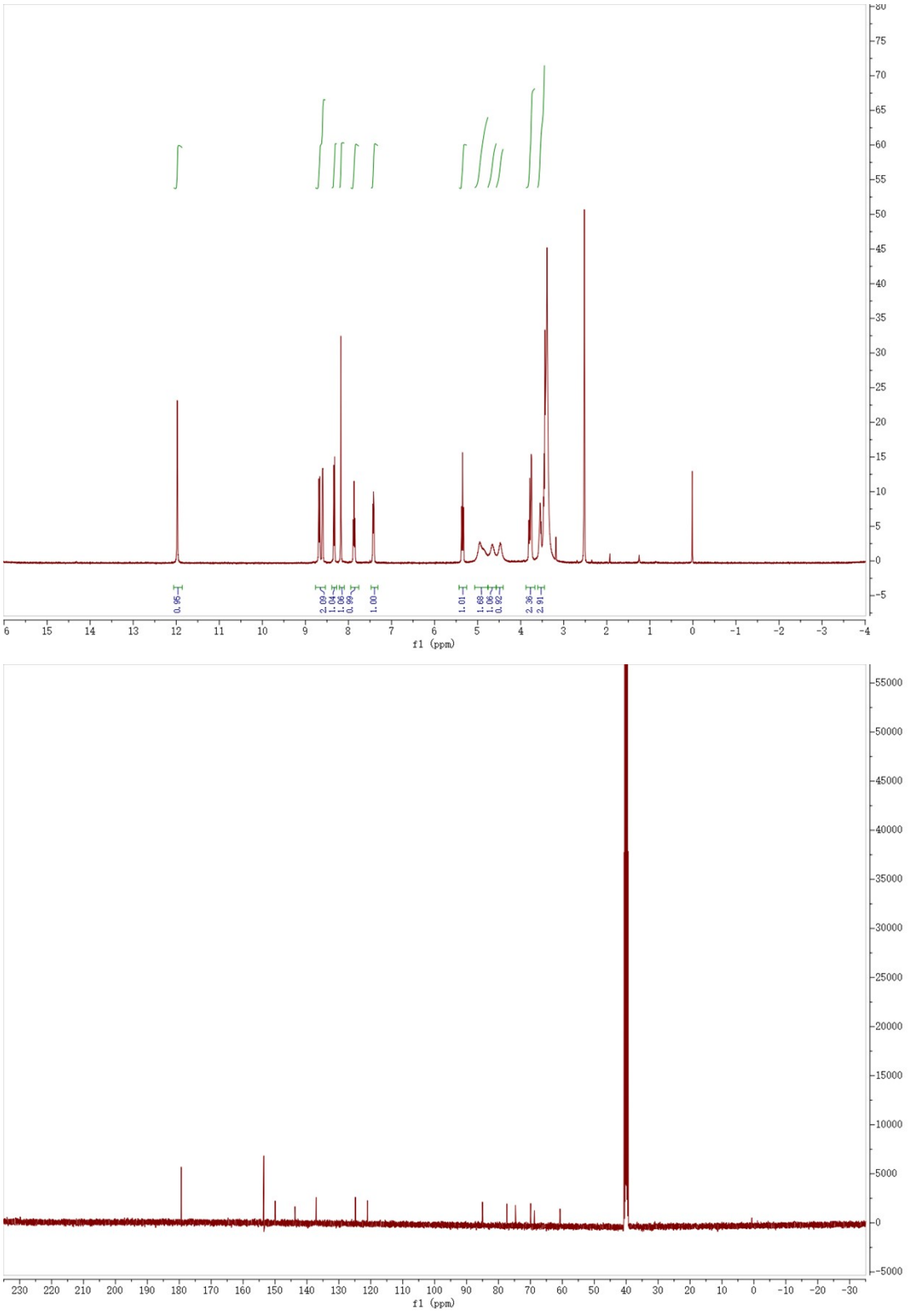


Fig. S37. NMR (^{1}H and ^{13}C) spectra of **HL^{6}**.

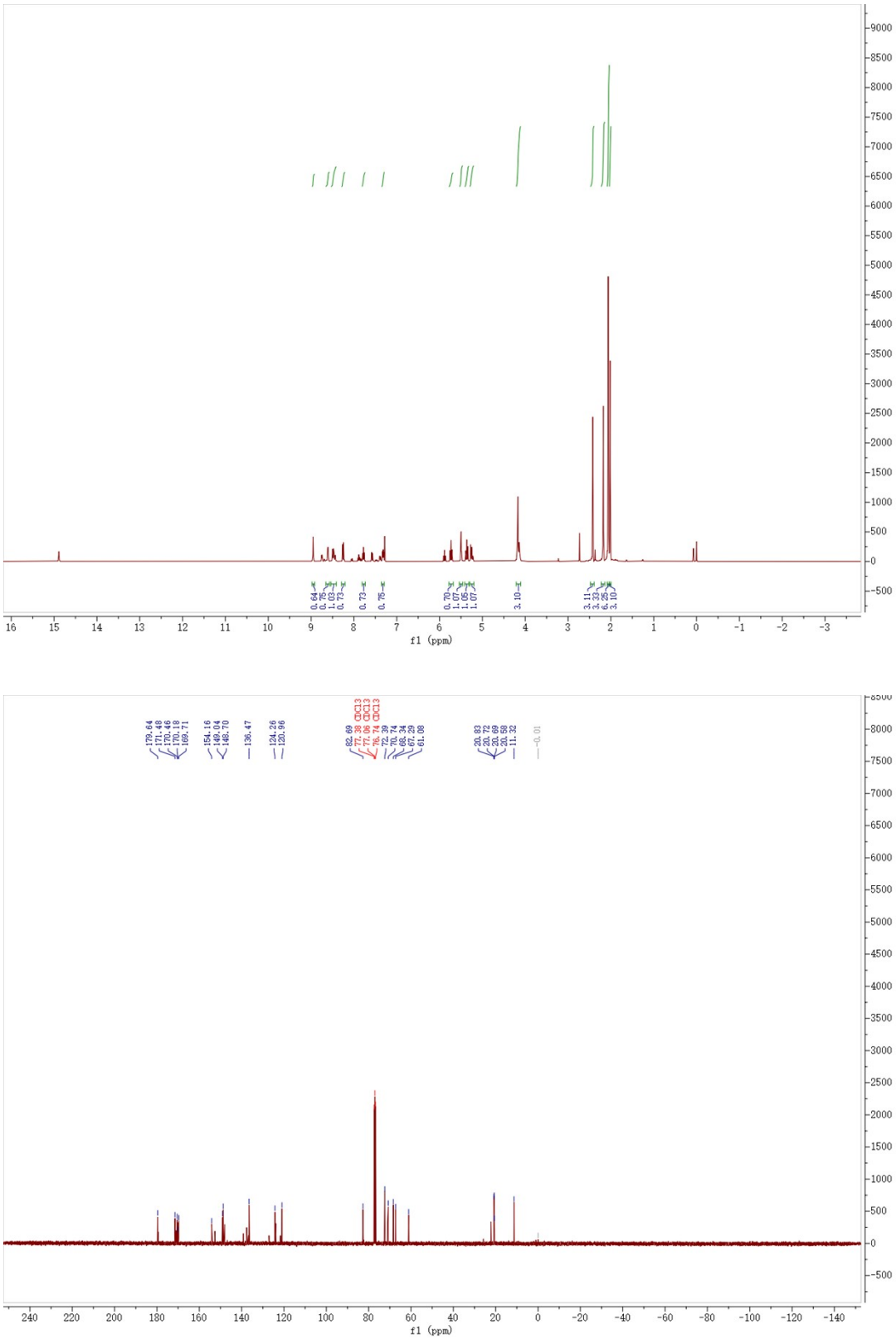


Fig. S38. NMR (^{1}H and ^{13}C) spectra of **HL**^{7}.

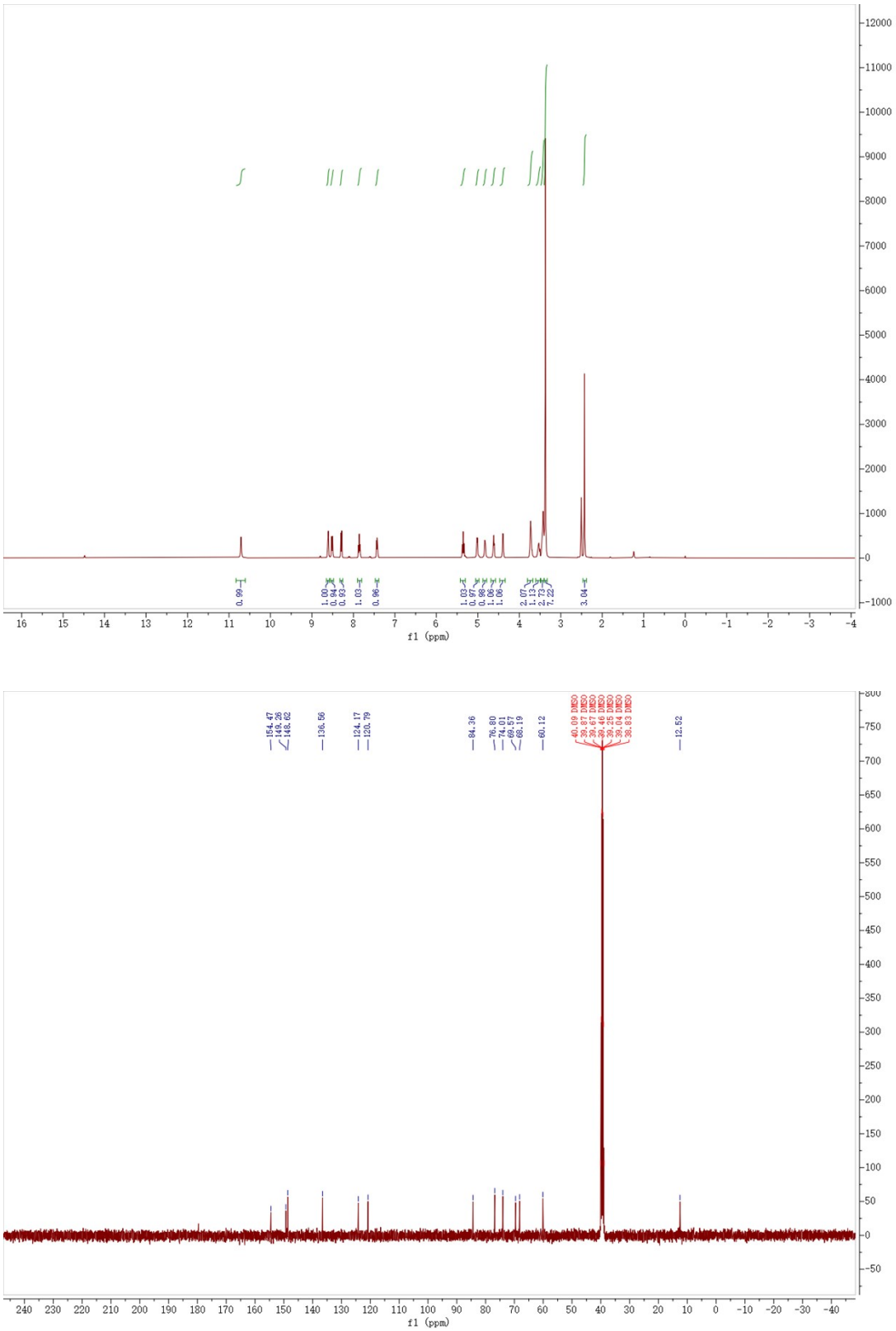


Fig. S39. NMR (^{1}H and ^{13}C) spectra of **HL^{8}**.

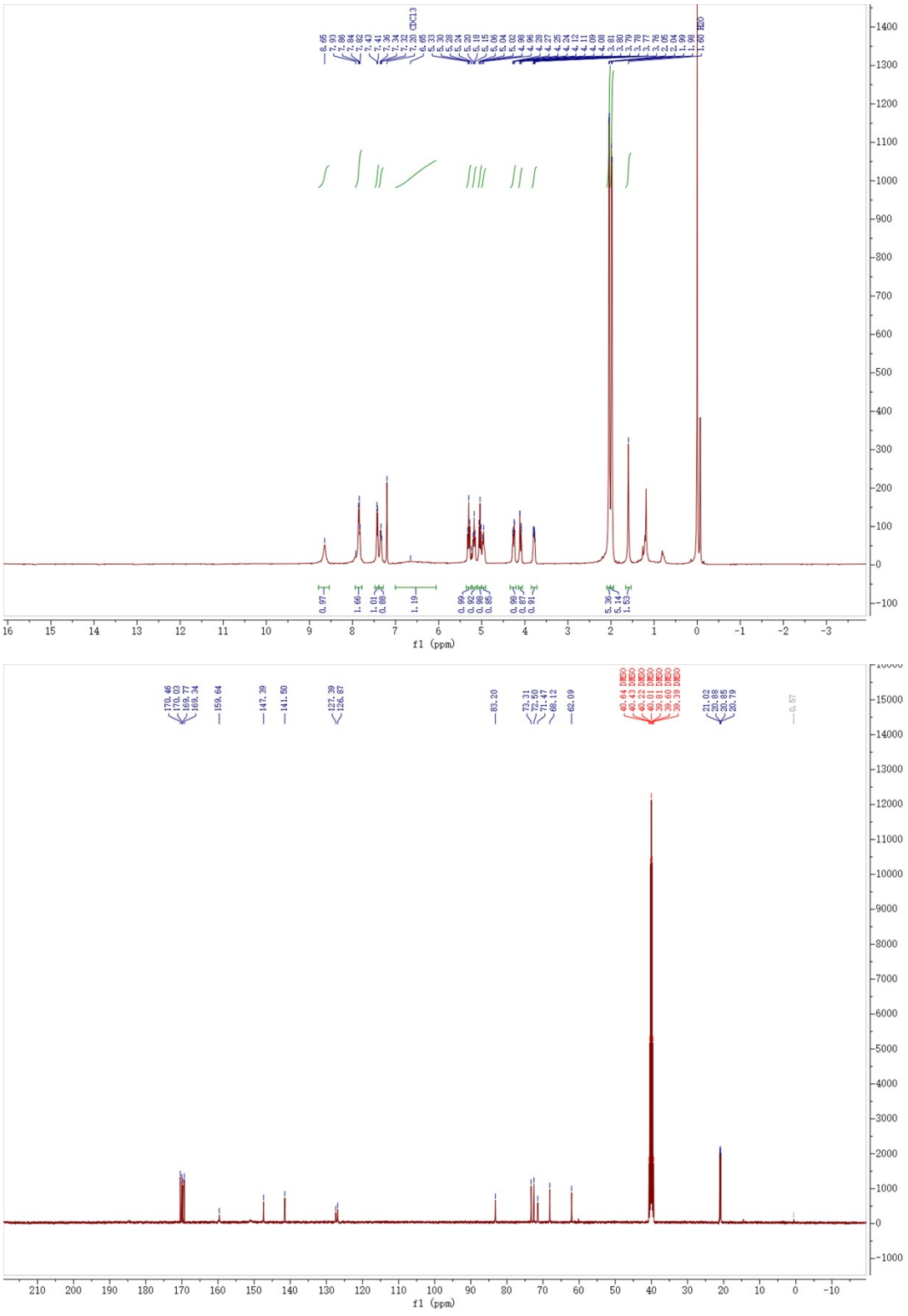


Fig. S40. NMR (^{1}H and ^{13}C) spectra of **1**.

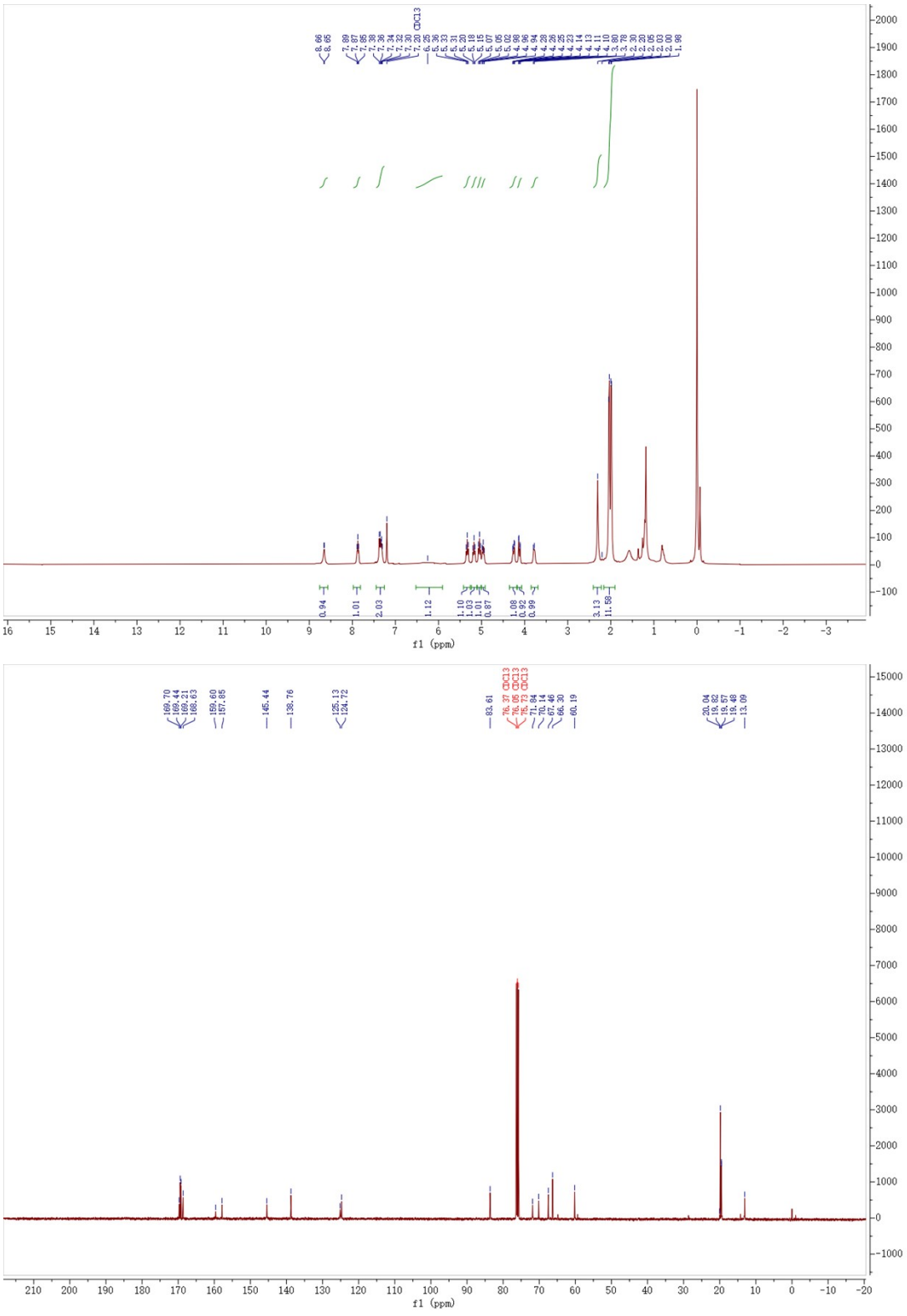


Fig. S41. NMR (^{1}H and ^{13}C) spectra of **2**.

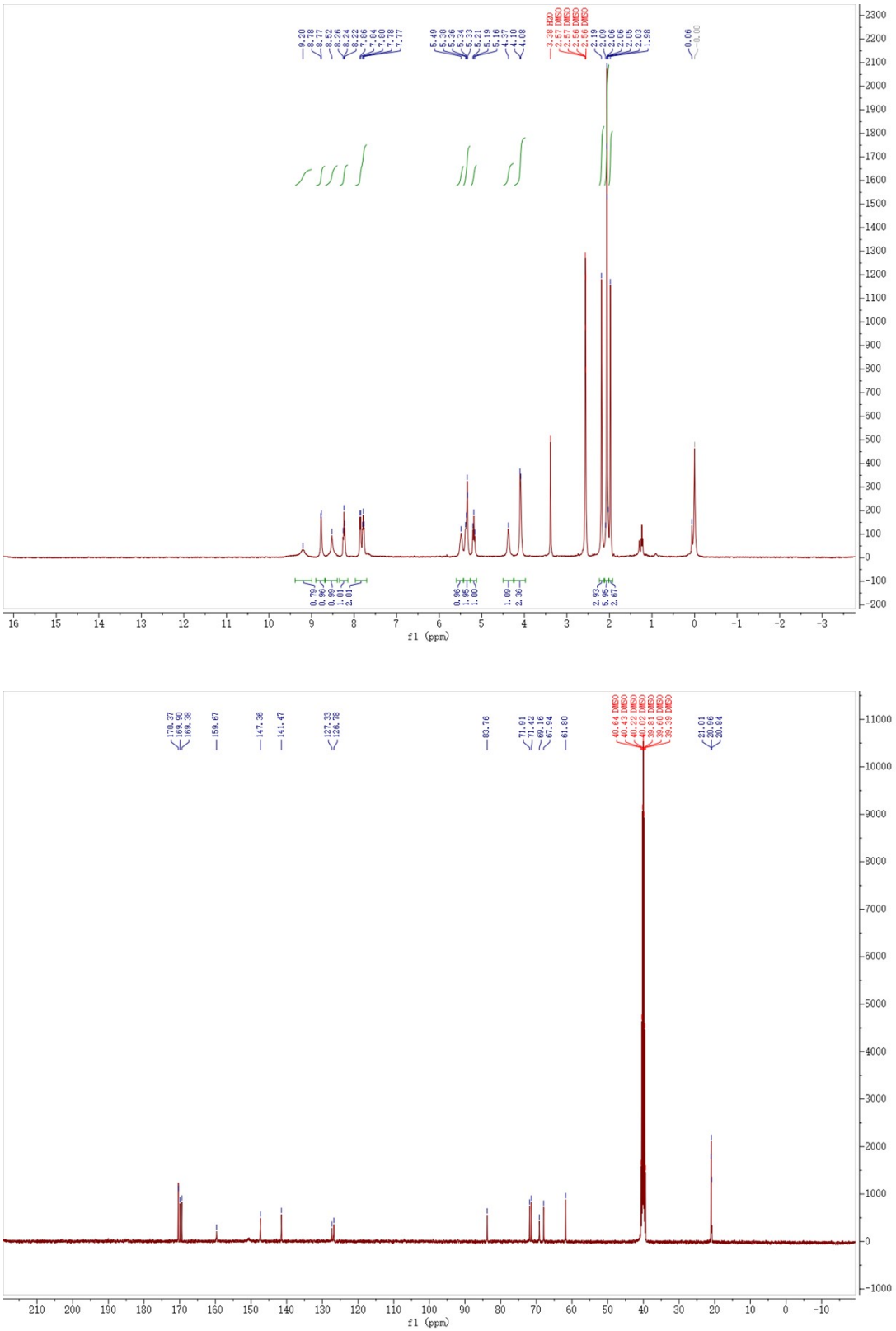


Fig. S42. NMR (^{1}H and ^{13}C) spectra of **3**.

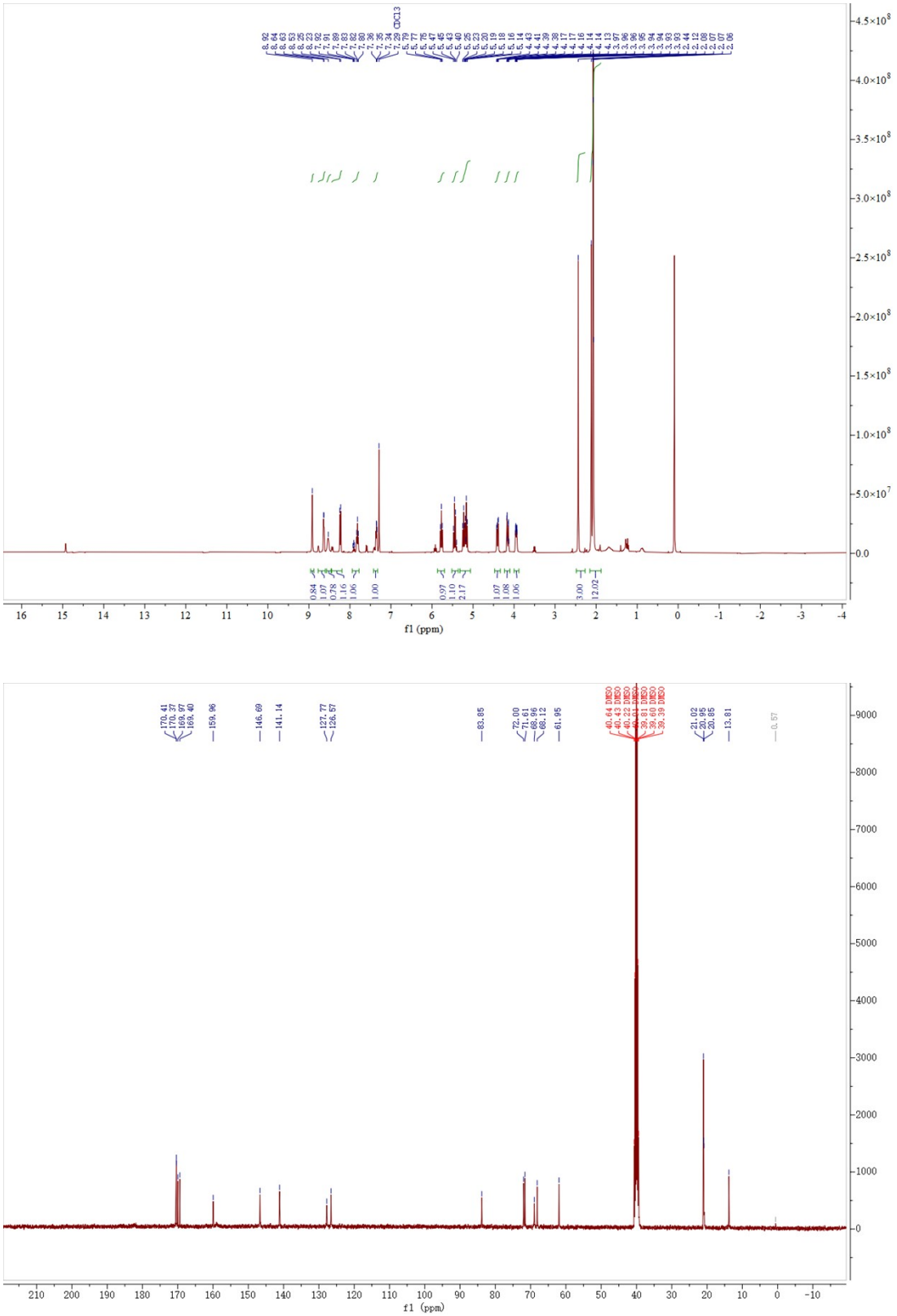


Fig. S43. NMR (^{1}H and ^{13}C) spectra of **4**.

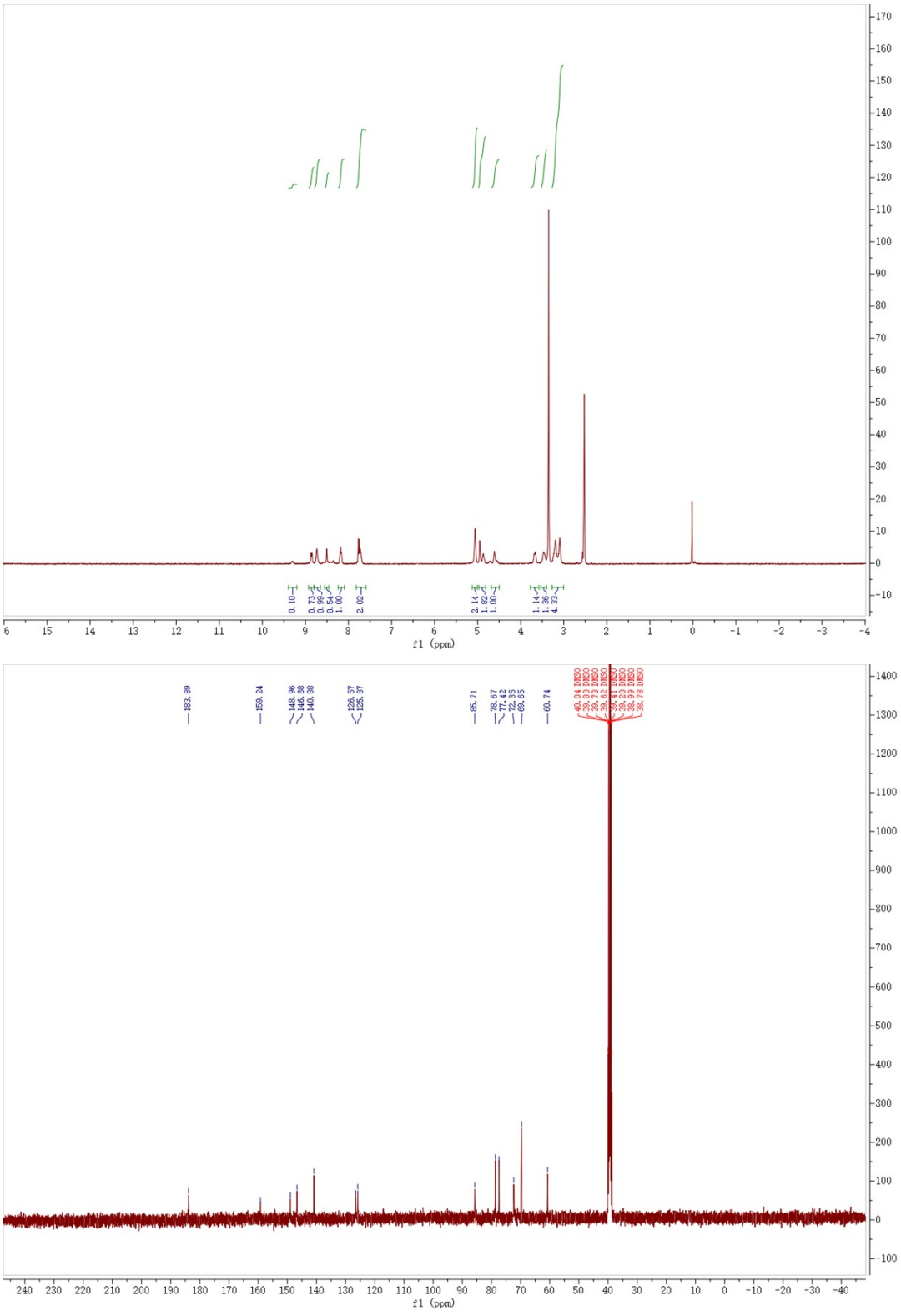


Fig. S44. NMR (^{1}H and ^{13}C) spectra of **5**.

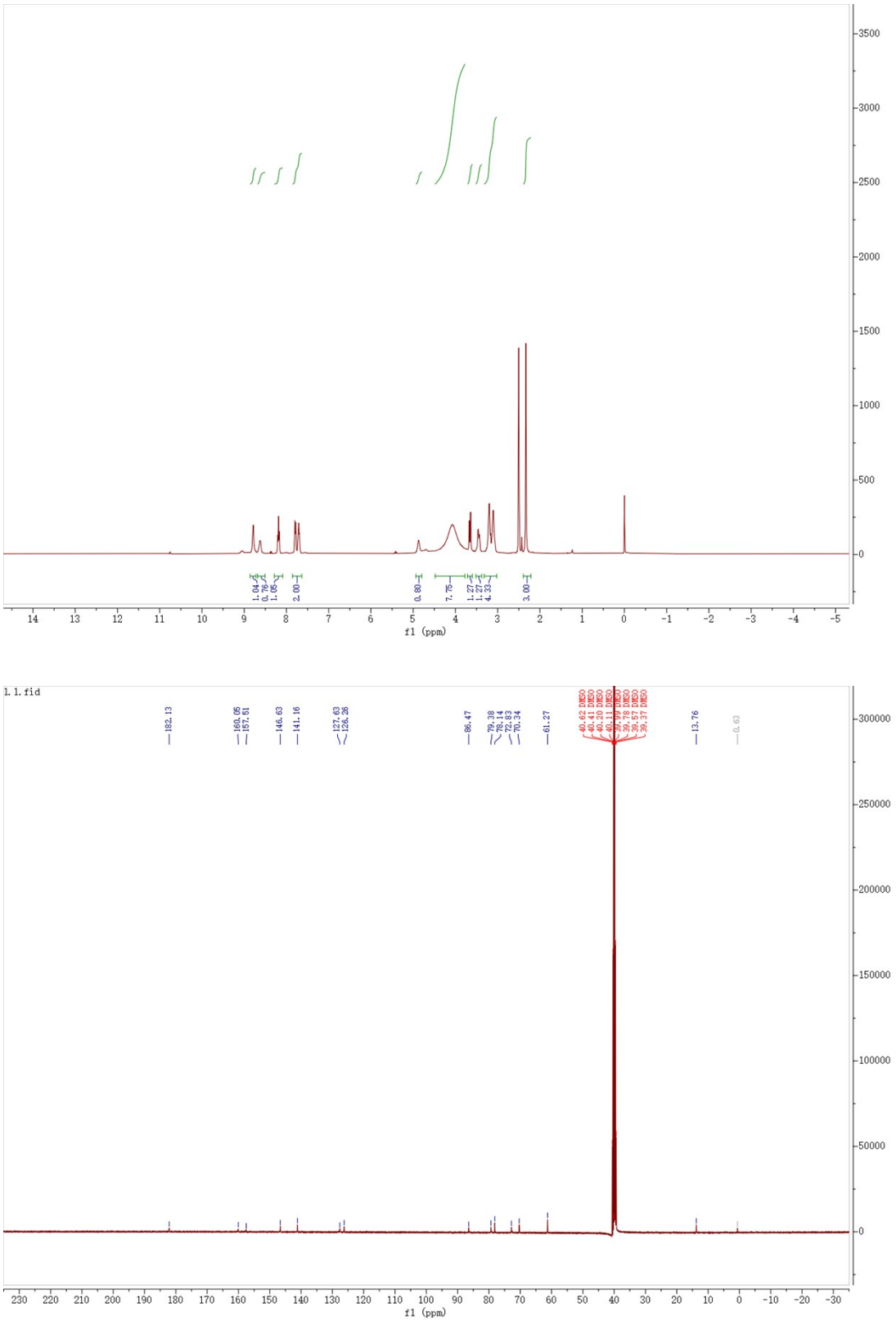


Fig. S45. NMR (^{1}H and ^{13}C) spectra of **6**.

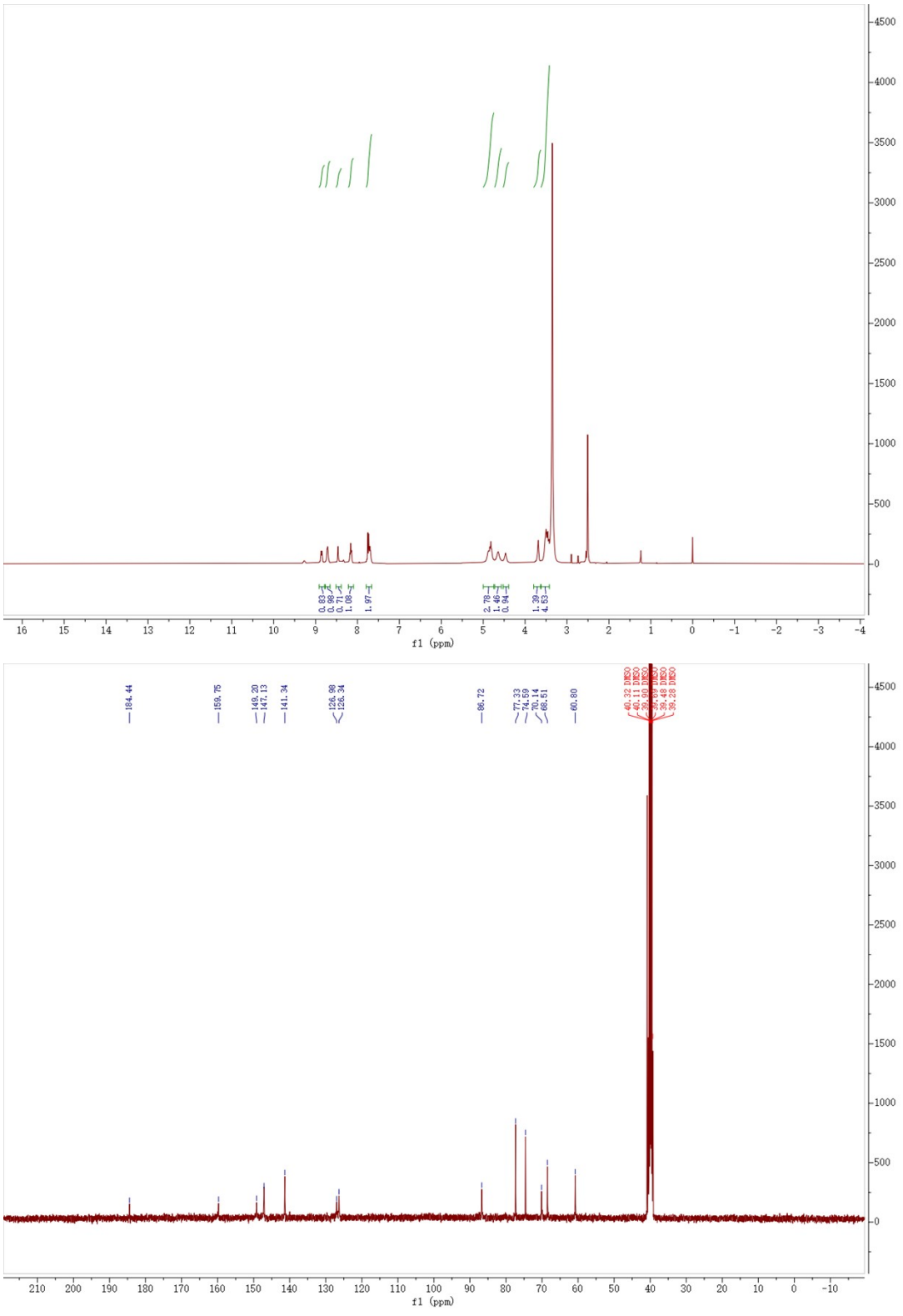


Fig. S46. NMR (^{1}H and ^{13}C) spectra of **7**.

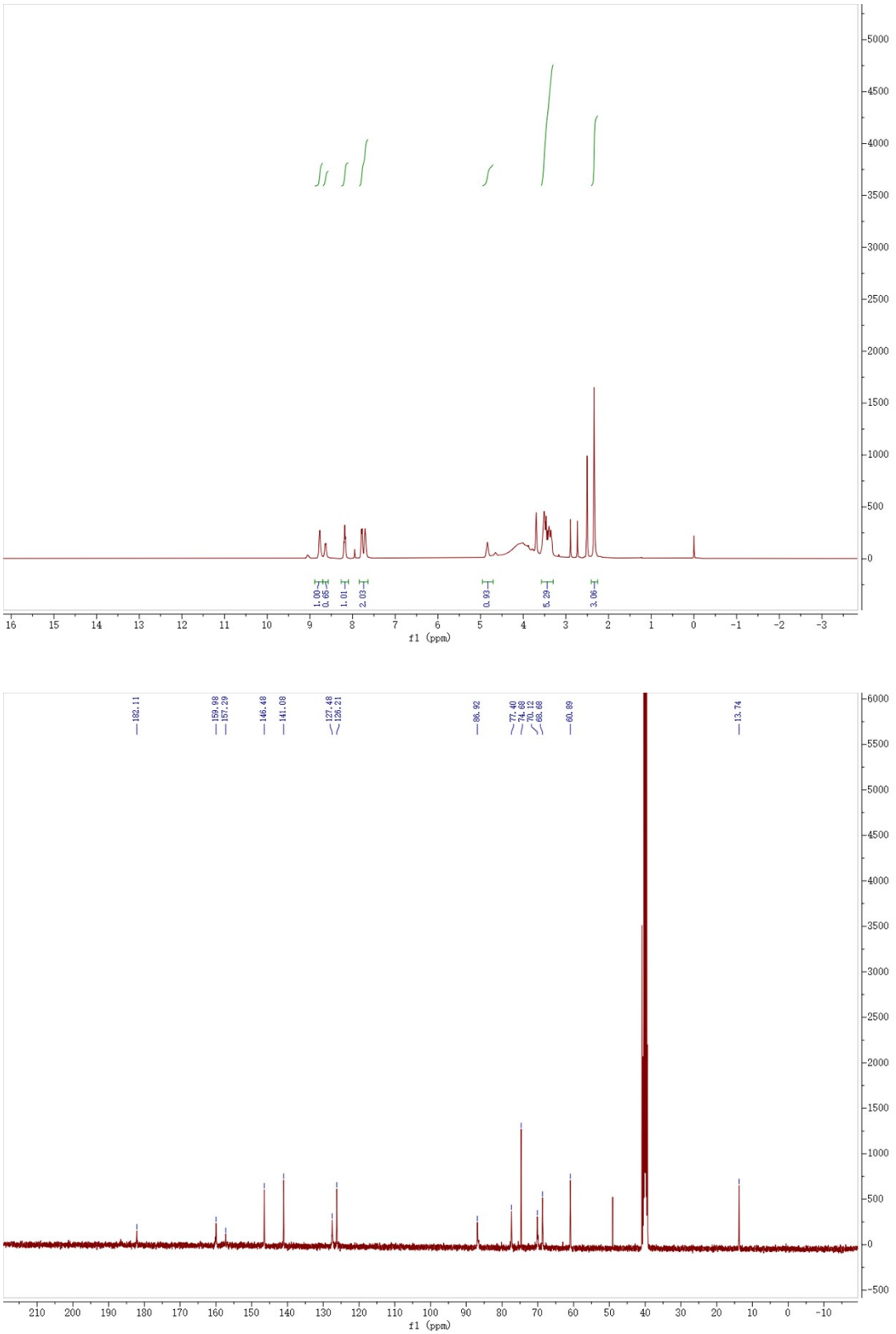


Fig. S47. NMR (^{1}H and ^{13}C) spectra of **8**.