

Supplementary Material

Antibacterial and antibiofilm activity of 8-hydroxyquinoline derivatives against *Mycobacterium* and *Staphylococcus* species

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1. General Experimental Procedure

The NMR spectra were recorded on the Bruker Avance, 400MHz NMR spectrometer using tetramethylsilane (TMS) as an internal standard CDCl₃ as solvent. The HR-ESIMS data were obtained using a Waters UPLC-MS system with PDA detector and Waters Synapt G2 QTOF mass spectrometer. The 8-hydroxyquinolines (HQ1-4) were purchased from Sigma Aldrich, South Africa.

2. Chemical Synthesis

The quinolines (1 mM) were taken into 10 mL of dried dimethyl formamide (DMF) in a round bottom flask. The corresponding alkyl bromide and potassium carbonate (3 mM each) were added to the quinoline solution and stirred for 6 h at room temperature. The progress of the reaction was monitored by TLC. After disappearance of the quinoline spot, the reaction was quenched by the addition of distilled water. The reaction mixture was then neutralized with 4% HCl solution and the product was extracted with ethyl acetate. The ethyl acetate layer was washed 4 times with water to remove the DMF. Finally, it was washed with brine, dried over anhydrous sodium sulfate (Na₂SO₄) and concentrated under vacuum to yield the crude product. The desired product was purified by using column chromatography. Yield of the reactions were between 60-95%. Compound structures were confirmed based on NMR and mass spectroscopic data.

2.1. 8-(Pentyloxy)quinoline (QD-1):

Yield: 91%; **¹H NMR (CDCl₃, 400 MHz):** δ_H, 0.91 (3H, t, *J* = 7.2 Hz), 1.40 (2H, m), 1.48 (2H, m), 2.00 (2H, m), 4.18 (2H, t, *J* = 7.0 Hz), 7.00 (1H, d, *J* = 7.7 Hz), 7.30-7.42 (3H, dd, *J* = 2.0, 2.2, 3.9, 4.1, 7.9, 8.2 Hz), 8.00 (1H, dd, *J* = 1.7, 8.3 Hz), 8.92 (1H, dd, *J* = 1.7, 4.2 Hz). **¹³C NMR (CDCl₃, 100 MHz):** δ_C, 14.03 (CH₃), 22.53 (CH₂), 28.20 (CH₂), 28.68 (CH₂), 68.95 (CH₂), 108.57 (CH), 119.27 (CH), 121.52 (CH), 126.74 (CH), 129.48 (C), 136.04 (CH), 140.16 (C), 149.12 (CH), 154.79 (C). HRESI-MS: *m/z*, 216.1403 [M+H]⁺ (Calcd; 216.1388 for C₁₄H₁₈NO).

2.2. 8-(Isopentyloxy)quinoline (QD-2):

Yield: 93%; **¹H NMR (CDCl₃, 400 MHz):** δ_H, 0.90 (6H, d, *J* = 5.8 Hz), 1.17 (1H, br s), 1.84 (2H, m), 4.17 (2H, t, *J* = 6.1 Hz), 6.95 (1H, br d, *J* = 7.6 Hz), 7.25-7.36 (3H, dd, *J* = 3.8, 4.1, 8.2 Hz), 8.00 (1H, dd, *J* = 1.7, 8.3), 8.85 (1H, dd, *J* = 1.7, 4.2). **¹³C NMR (CDCl₃, 100 MHz):** δ_C, 22.79 (2 x CH₃), 25.35 (CH), 37.73 (CH₂), 67.52 (CH₂), 108.65 (CH), 119.42 (CH), 121.59 (CH), 126.78 (CH), 129.58 (C), 135.99 (CH), 140.47 (C), 149.32 (CH), 154.97 (C). HRESI-MS: *m/z*, 216.1395 [M+H]⁺ (Calcd; 216.1388 for C₁₄H₁₈NO).

2.3. 8-((3-Methylbut-2-en-1-yl)oxy)quinoline (QD-3):

Yield: 84%; **¹H NMR (CDCl₃, 400 MHz):** δ_H, 1.703-1.707 (6H, s), 4.73 (2H, d, *J* = 6.6 Hz), 5.59 (1H, dt, *J* = 1.1, 6.6 Hz), 6.96 (1H, br d, *J* = 7.6 Hz), 7.27-7.37 (3H, dd, *J* = 1.5, 4.1, 7.7, 8.2 Hz), 8.01 (1H, br d, *J* = 8.3 Hz), 8.86 (1H, dd, *J* = 1.9, 2.2 Hz). **¹³C NMR (CDCl₃, 100 MHz):** δ_C, 18.44 (CH₃), 25.97 (CH₃), 65.85 (CH₂), 108.89 (CH), 119.48 (CH), 120.06 (CH), 121.66 (CH), 126.78 (CH), 129.58 (CH), 136.00 (CH), 137.59 (C), 140.54 (C), 149.38 (CH), 154.73 (C). HRESI-MS: *m/z*, 214.1236 [M+H]⁺ (Calcd; 214.1232 for C₁₄H₁₆NO).

2.4. 5,7-Dichloro-2-methyl-8-(pentyloxy)quinoline (QD-4):

Yield: 95%; **¹H NMR (CDCl₃, 400 MHz):** δ_H, 0.94 (3H, t, *J* = 7.3 Hz), 1.41 (2H, m), 1.55 (2H, m), 1.92 (2H, m), 2.76 (3H, s), 4.34 (2H, t, *J* = 6.7 Hz), 7.33 (1H, br d, *J* = 8.7 Hz), 7.53 (1H, br s), 8.33 (1H, br d, *J* = 8.6 Hz). **¹³C NMR (CDCl₃, 100 MHz):** δ_C, 14.27 (CH₃), 22.68 (CH₂), 25.70 (CH₃), 28.29 (CH₂), 30.14 (CH₂), 75.57 (CH₂), 122.92 (CH), 124.67 (C), 125.82 (C), 126.76 (C), 126.98 (CH), 133.38 (CH), 143.59 (C), 150.70 (C), 159.97 (C). HRESI-MS: *m/z*, 298.0766 [M+H]⁺ (Calcd; 298.0765 for C₁₅H₁₈Cl₂NO).

2.5. 5,7-Dichloro-8-(isopentyloxy)-2-methylquinoline (QD-5):

Yield: 89%; **¹H NMR (CDCl₃, 400 MHz):** δ_H, 0.96 (6H, d, *J* = 6.6 Hz), 1.78 (2H, m), 2.00 (1H, m), 2.73 (3H, s), 4.34 (2H, t, *J* = 6.7 Hz), 7.31 (1H, d, *J* = 8.7 Hz), 7.51 (1H, s), 8.30 (1H, d, *J* = 6.6 Hz). **¹³C NMR (CDCl₃, 100 MHz):** δ_C, 22.85 (2 x CH₃), 24.98 (CH₃), 25.70 (CH), 39.26 (CH₂), 73.95 (CH₂), 122.97 (CH), 124.71 (C), 125.89 (C), 126.84 (C), 127.00 (CH), 133.44 (CH), 143.61 (C), 150.71 (C), 160.03 (C). HRESI-MS: *m/z*, 298.0767 [M+H]⁺ (Calcd; 298.0765 for C₁₅H₁₈Cl₂NO).

2.6. 5,7-Dichloro-2-methyl-8-((3-methylbut-2-en-1-yl)oxy)quinoline (QD-6):

Yield: 82%; **¹H NMR (CDCl₃, 400 MHz):** δ_H, 1.70 (3H, s), 1.75 (3H, s), 2.76 (3H, s), 4.90 (2H, d, *J* = 7.4 Hz), 5.68 (1H, t, *J* = 7.4 Hz), 7.34 (1H, br d, *J* = 8.7 Hz), 7.51 (1H, br s), 8.32 (1H, br d, *J* = 8.6 Hz). **¹³C NMR (CDCl₃, 100 MHz):** δ_C, 18.20 (CH₃), 25.64 (CH₃), 25.99 (CH₃), 71.47 (CH₂), 120.33 (CH), 122.89 (CH), 124.58 (C), 125.92 (C), 126.89 (CH), 127.18 (C), 133.38 (CH), 139.40 (C), 143.69 (C), 150.11 (C), 159.91 (C). HRESI-MS: Molecular ion peak could not be found probably due to instability. The base peak was observed at *m/z* 227.9985 (Calcd; 227.9983 for C₁₀H₈Cl₂NO).

2.7. 2-Methyl-8-(pentyloxy)quinoline (QD-7):

Yield: 78%; **¹H NMR (CDCl₃, 400 MHz):** δ_H, 0.87 (3H, t, *J* = 7.2 Hz), 1.31-1.44 (4H, m), 1.94 (2H, m), 2.69 (3H, s), 4.12 (2H, t, *J* = 7.1 Hz), 6.94 (1H, d, *J* = 7.6 Hz), 7.19-7.31 (3H, d, *J* = 8.0, 10.2, 11.0 Hz), 7.91 (1H, d, *J* = 8.6 Hz). **¹³C NMR (CDCl₃, 100 MHz):** δ_C, 14.22 (CH₃), 22.72 (CH₂), 25.36 (CH₃), 28.31 (CH₂), 28.71 (CH₂), 69.21 (CH₂), 108.13 (CH), 119.35 (CH), 122.79 (CH), 126.01 (CH), 127.91 (C), 136.61 (CH), 139.62 (C), 154.33 (C), 158.20 (C). HRESI-MS: *m/z*, 230.1546 [M+H]⁺ (Calcd; 230.1545 for C₁₅H₂₀NO).

2.8. 8-(Isopentyloxy)-2-methylquinoline (QD-8):

Yield: 74%; **¹H NMR (CDCl₃, 400 MHz):** δ_H, 0.93 (6H, d, *J* = 6.4 Hz), 1.78-1.89 (3H, m), 2.71 (3H, s), 4.19 (2H, t, *J* = 7.1 Hz), 6.97 (1H, dd, *J* = 1.3, 7.5 Hz), 7.19-7.33 (3H, dd, *J* = 1.3, 8.0, 8.2, 8.4 Hz), 7.93 (1H, d, *J* = 8.4 Hz). **¹³C NMR (CDCl₃, 100 MHz):** δ_C, 22.97 (2 x CH₃), 25.59 (CH₃), 25.77 (CH), 37.69 (CH₂), 67.86 (CH₂), 109.21 (CH), 119.44 (CH), 122.73 (CH), 126.00 (CH), 127.92 (C), 136.51 (CH), 139.86 (C), 154.46 (C), 158.26 (C). HRESI-MS: *m/z*, 230.1551 [M+H]⁺ (Calcd; 230.1545 for C₁₅H₂₀NO).

2.9. 2-Methyl-8-((3-methylbut-2-en-1-yl)oxy)quinoline (QD-9):

Yield: 60%; **¹H NMR (CDCl₃, 400 MHz):** δ_H, 1.70 (3H, s), 1.75 (3H, s), 2.76 (3H, s), 4.91 (2H, t, *J* = 7.1 Hz), 5.68 (1H, t, *J* = 7.4 Hz), 6.97 (1H, dd, *J* = 1.3, 7.5 Hz), 7.19-7.33 (3H, dd, *J* = 1.4, 7.2, 8.1, 8.4 Hz), 7.93 (1H, d, *J* = 8.4 Hz). **¹³C NMR (CDCl₃, 100 MHz):** δ_C, 18.44 (CH₃), 25.64 (CH₃), 25.99 (CH₃), 71.47 (CH₂), 109.21 (CH), 119.44 (CH), 120.33 (CH), 122.73 (CH), 126.00 (CH), 127.92 (C), 136.51 (CH), 139.86 (C), 154.46 (C), 158.26 (C). HRESI-MS: Molecular ion peak was absent.

2.10. Pentyl 8-(pentyloxy)quinoline-2-carboxylate (QD-10):

Yield: 95%; **¹H NMR (CDCl₃, 400 MHz):** δ_H, 0.90 (3H, t, *J* = 7.1 Hz), 0.92 (3H, t, *J* = 7.3 Hz), 1.34-1.56 (8H, m), 1.84 (2H, m), 2.03 (2H, m), 4.17 (2H, t, *J* = 7.0 Hz), 4.39 (2H, t, *J* = 6.9 Hz), 7.03 (1H, d, *J* = 7.8 Hz), 7.36 (1H, br d, *J* = 7.6 Hz), 7.49 (1H, dt, *J* = 1.3, 7.8 Hz), 8.14 (1H, dd, *J* = 1.0, 8.5 Hz), 8.19 (1H, dd, *J* = 1.1, 8.6 Hz). **¹³C NMR (CDCl₃, 100 MHz):** δ_C, 14.16 (CH₃), 14.23 (CH₃), 22.57 (CH₂), 22.72 (CH₂), 28.30 (CH₂), 28.35 (CH₂), 28.47 (CH₂), 28.81 (CH₂), 66.32 (CH₂), 69.35 (CH₂), 109.34 (CH), 119.10 (CH), 121.58 (CH), 129.26 (CH), 130.71 (C), 137.18 (CH), 139.93 (C), 147.07 (C), 155.88 (C), 165.85 (C). HRESI-MS: *m/z*, 330.2076 [M+H]⁺ (Calcd; 330.2069 for C₂₀H₂₈NO₃).

2.11. Isopentyl 8-(isopentyloxy)quinoline-2-carboxylate (QD-11):

Yield: 95%; **¹H NMR (CDCl₃, 400 MHz):** δ_H, 0.95 (6H, dd, *J* = 0.7, 6.5 Hz), 0.98 (6H, d, *J* = 5.6 Hz), 1.71-1.93 (6H, m), 4.20 (2H, t, *J* = 6.0 Hz), 4.43 (2H, t, *J* = 6.9 Hz), 7.03 (1H, d, *J* = 6.1 Hz), 7.35 (1H, d, *J* = 8.3 Hz), 7.49 (1H, dt, *J* = 1.1, 8.0 Hz), 8.12 (1H, dd, *J* = 0.8, 8.5 Hz), 8.18 (1H, dd, *J* = 1.0, 8.5 Hz). **¹³C NMR (CDCl₃, 100 MHz):** δ_C, 22.72 (CH₃), 22.89 (CH₃), 25.33 (CH), 25.46 (CH), 37.40 (CH₂), 37.73 (CH₂), 64.78 (CH₂), 67.78 (CH₂), 109.28 (CH), 119.06 (CH), 121.53 (CH), 129.22 (CH), 130.66 (C), 137.11 (CH), 139.93 (C), 147.00 (C), 155.90 (C), 165.83 (C). HRESI-MS: *m/z*, 330.2079 [M+H]⁺ (Calcd; 330.2069 for C₂₀H₂₈NO₃).

2.12. 8-((3-Methylbut-2-en-1-yl)oxy)quinoline-2-carbaldehyde (QD-12):

Yield: 90%; **¹H NMR (CDCl₃, 400 MHz):** δ_H, 1.77 (6H, s), 4.84 (2H, d, *J* = 6.5 Hz), 5.63 (1H, dt, *J* = 1.3, 6.5 Hz), 7.10 (1H, d, *J* = 7.8 Hz), 7.40 (1H, td, *J* = 0.9, 8.2 Hz), 7.54 (1H, dt, *J* = 1.6, 8.0 Hz), 8.0 (1H, dd, *J* = 1.8, 8.4 Hz), 8.22 (1H, dd, *J* = 2.5, 8.4 Hz), 10.26 (1H, t, *J* = 0.8 Hz). **¹³C NMR (CDCl₃, 100 MHz):** δ_C, 18.59 (CH₃), 26.03 (CH₃), 66.44 (CH₂), 110.10 (CH), 117.96 (CH), 119.58 (CH), 119.64 (CH), 129.93 (CH), 131.56 (C), 137.43 (CH), 138.12 (C), 140.33 (C), 151.54 (C), 155.50 (C), 194.06 (CH). HRESI-MS: *m/z*, 242.1186 [M+H]⁺ (Calcd; 242.1181 for C₁₅H₁₆NO₂).

2.13. Pentyl 8-((3-methylbut-2-en-1-yl)oxy)quinoline-2-carboxylate (QD-12a):

Yield: 92%; **¹H NMR (CDCl₃, 400 MHz):** δ_H, 0.91 (3H, t, *J* = 7.12 Hz), 1.35-1.46 (4H, m), 1.76 (3H, s), 1.78 (3H, s), 1.85 (2H, m), 4.41 (2H, t, *J* = 7.00 Hz), 4.80 (2H, d, *J* = 6.4 Hz), 5.63 (1H, tt, *J* = 1.3, 6.5 Hz), 7.05 (1H, d, *J* = 7.2 Hz), 7.37 (1H, dd, *J* = 0.72, 8.2 Hz), 7.51 (1H, t, *J* = 8.0 Hz), 8.13 (1H, d, *J* = 8.48 Hz), 8.20 (1H, d, *J* = 8.5 Hz). **¹³C NMR (CDCl₃, 100 MHz):** δ_C, 14.17 (CH₃), 18.56 (CH₃), 22.58 (CH₂), 25.99 (CH₃), 66.35(CH₂), 66.40(CH₂), 109.77 (CH), 119.19 (CH), 120.00 (CH), 121.62 (CH), 129.20 (CH), 130.73 (C), 137.20 (CH), 137.44 (C), 140.08 (C), 147.15 (C), 155.62 (C), 165.82 (C). HRESI-MS: *m/z*, 328.1909 [M+H]⁺ (Calcd; 328.1913 for C₂₀H₂₆NO₃).

2.14. Isopentyl 8-((3-methylbut-2-en-1-yl)oxy)quinoline-2-carboxylate (QD-12b):

Yield: 89%; **¹H NMR (CDCl₃, 400 MHz):** δ_H, 0.96 (6H, d, *J* = 6.4 Hz), 1.76 (6H, s), 1.75-1.78 (3H, m), 4.44 (2H, t, *J* = 7.00 Hz), 4.79 (2H, d, *J* = 6.4 Hz), 5.66 (1H, tt, *J* = 1.4, 6.5 Hz), 7.05 (1H, d, *J* = 7.8 Hz), 7.37 (1H, d, *J* = 8.4 Hz), 7.50 (1H, t, *J* = 8.2 Hz), 8.14 (1H, d, *J* = 8.5 Hz), 8.20 (1H, d, *J* = 8.5 Hz). **¹³C NMR (CDCl₃, 100 MHz):** δ_C, 18.55 (CH₃), 22.75 (2 x CH₃), 25.43 (CH), 25.99 (CH₃), 37.40 (CH₂), 64.97 (CH₂), 66.31(CH₂), 109.76 (CH), 119.17 (CH), 119.98

(CH), 121.58 (CH), 129.20 (CH), 130.73 (C), 137.19 (CH), 137.46 (C), 140.07 (C), 147.10 (C), 155.61 (C), 165.80 (C).
HRESI-MS: m/z, 328.1913 [M+H]⁺ (Calcd; 328.1913 for C₂₀H₂₆NO₃).

3. Antistaphylococcal activity

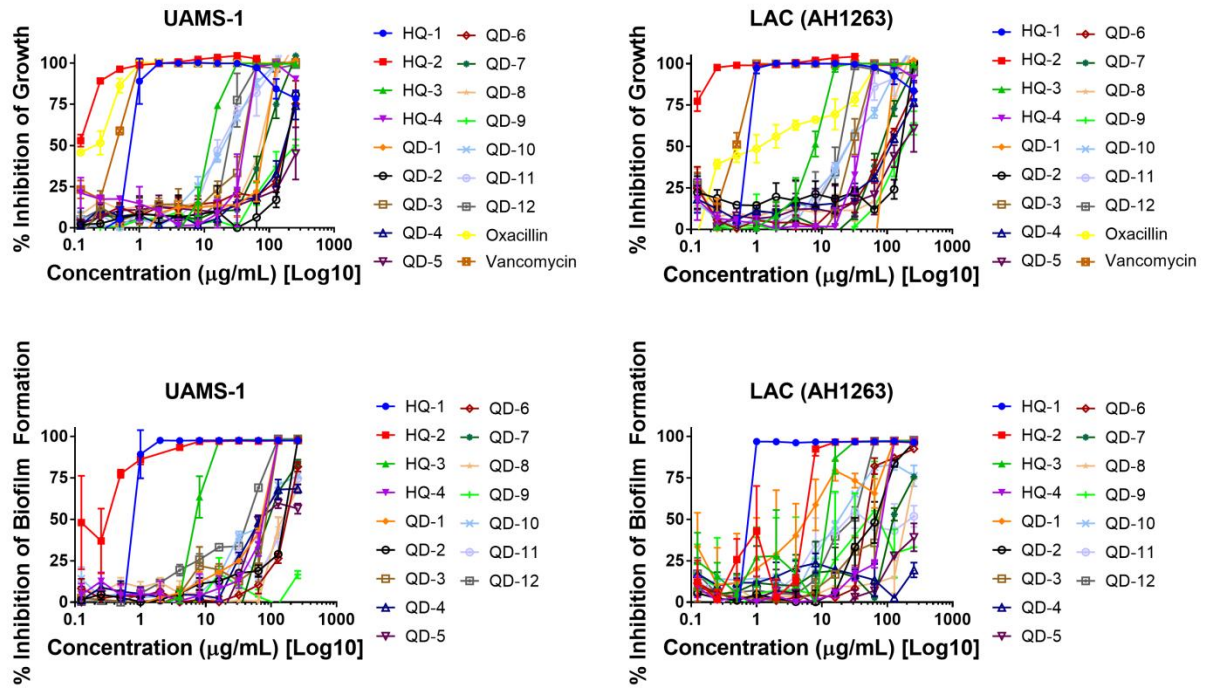


Figure S1: Antibacterial and antibiofilm activity of quinolines (HQ) and derivatives (QD) on *S. aureus* strains. Data points represented as % mean inhibition \pm SD.

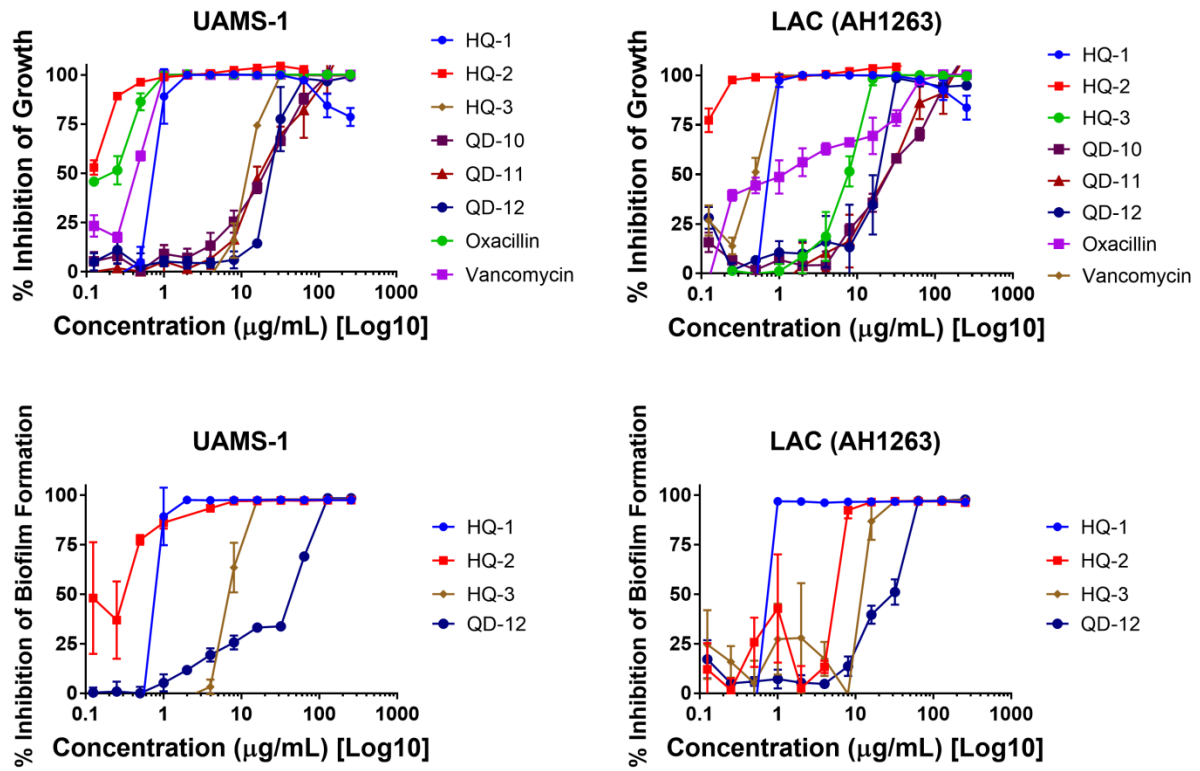


Figure S2: Top performers of the compounds tested against *S. aureus*. Data points represented as % mean inhibition \pm SD.

4. Spectroscopic data

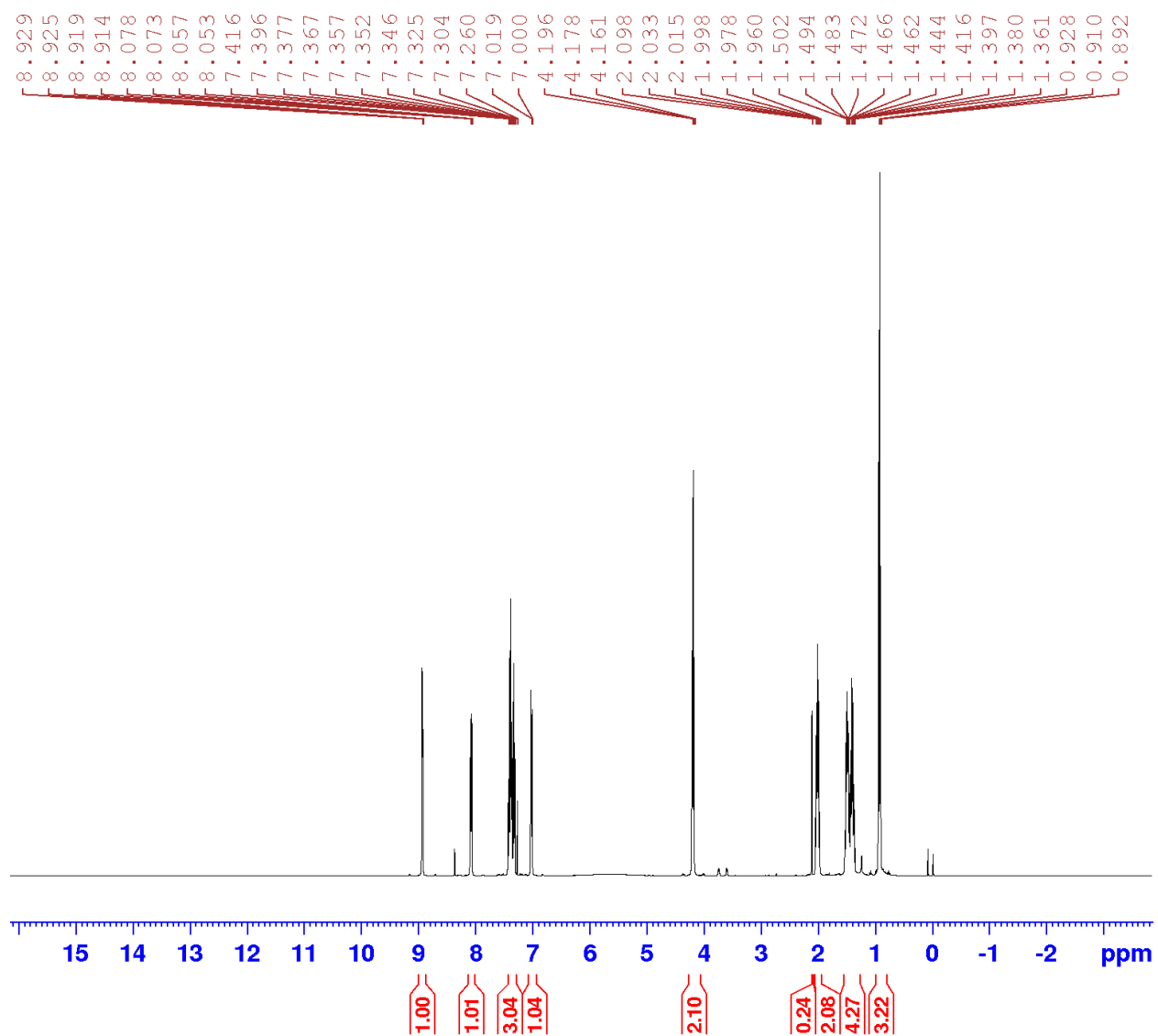


Figure S3: ¹H NMR of QD-1

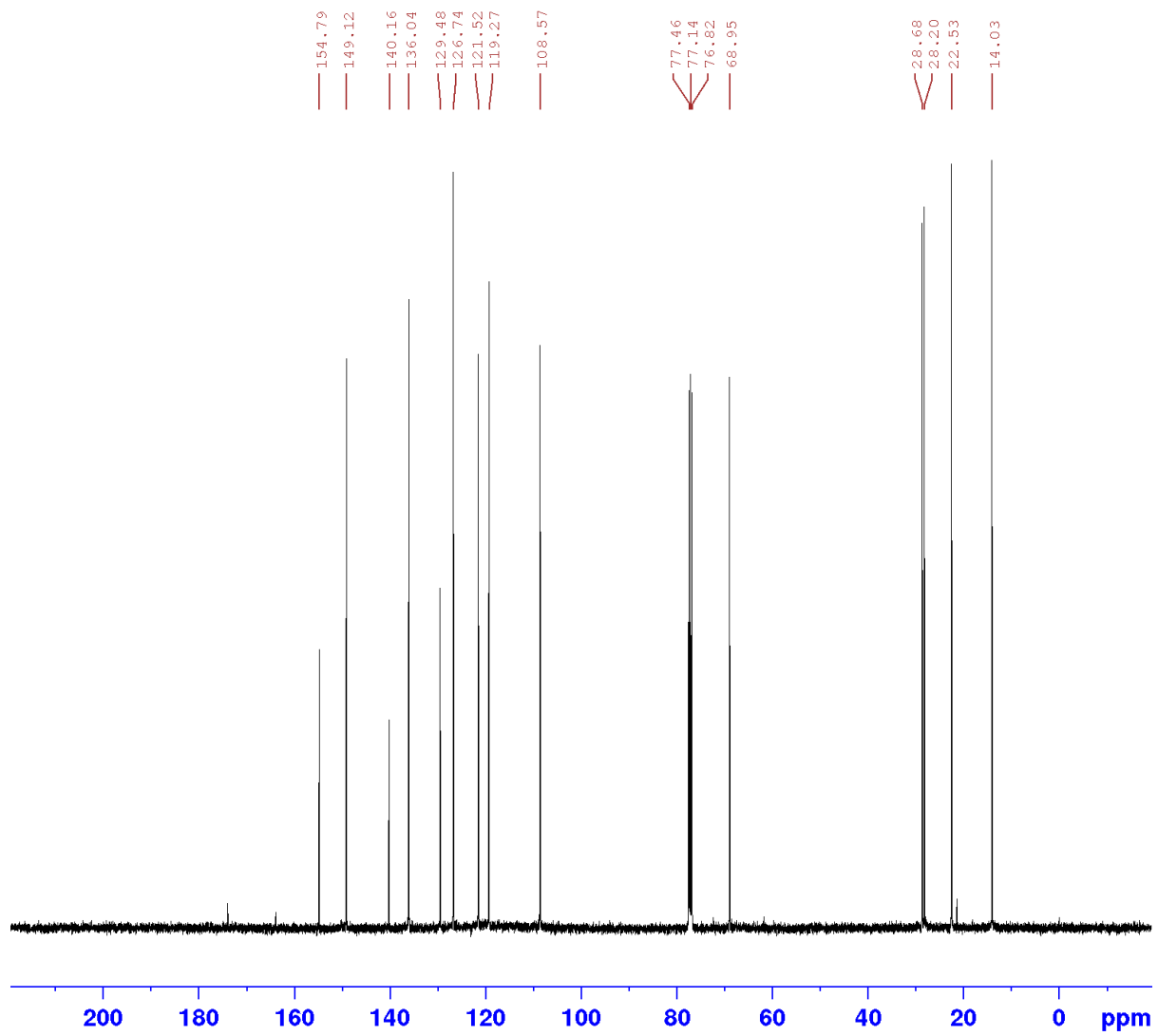


Figure S4: ^{13}C NMR of QD-1

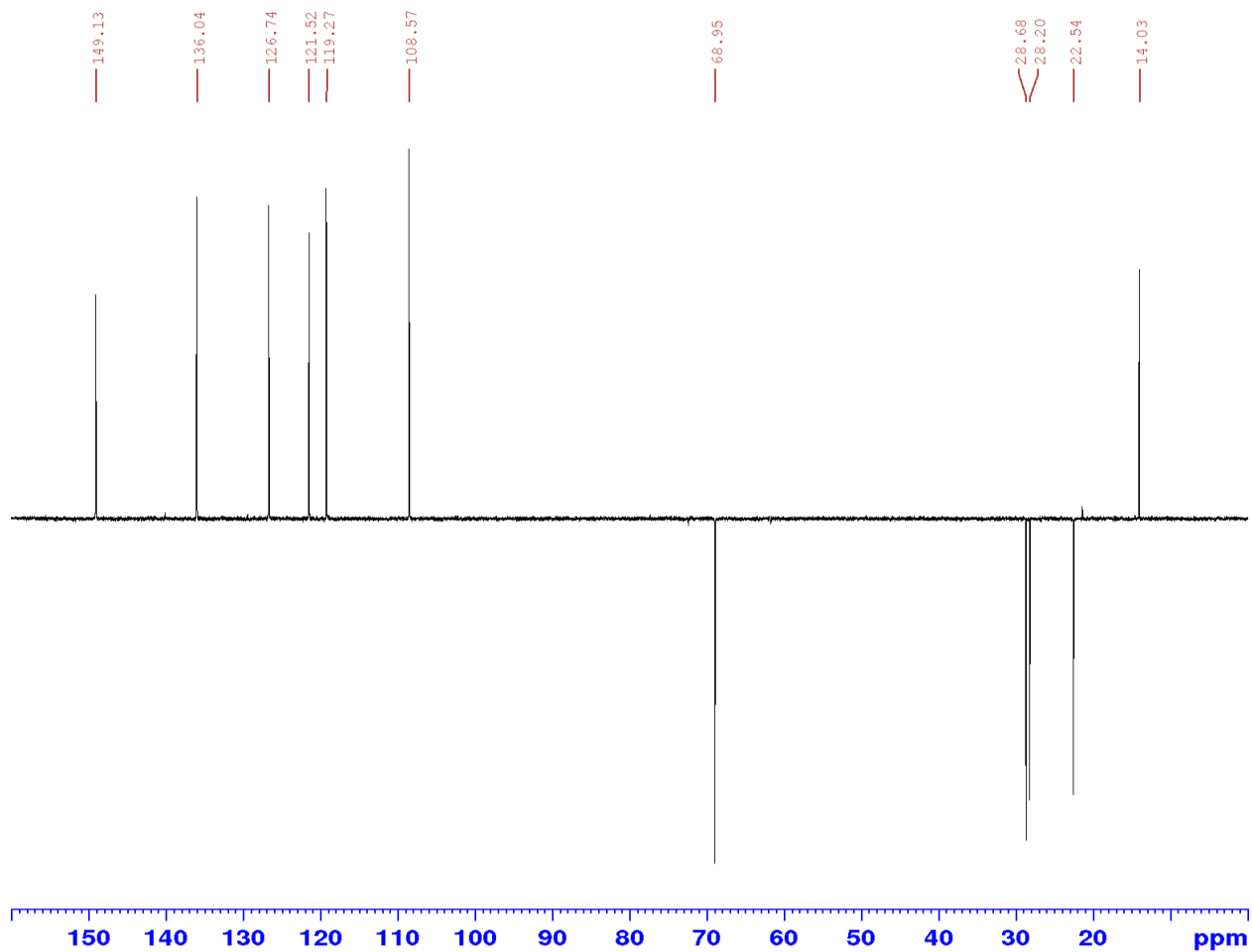


Figure S5: DEPT-135 NMR of QD-1

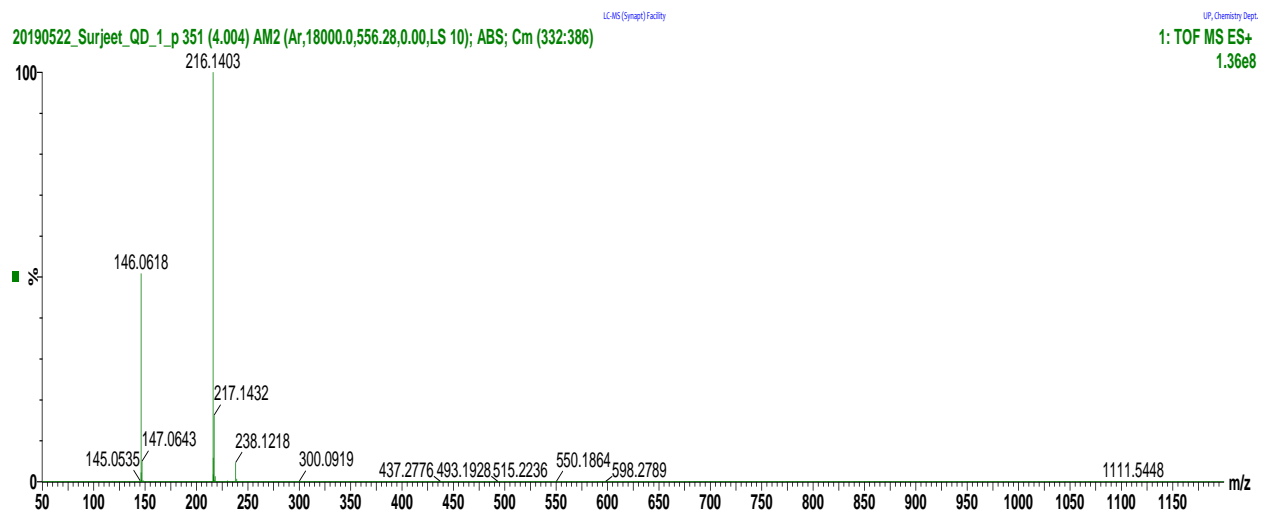


Figure S6: HRESI-MS of QD-1

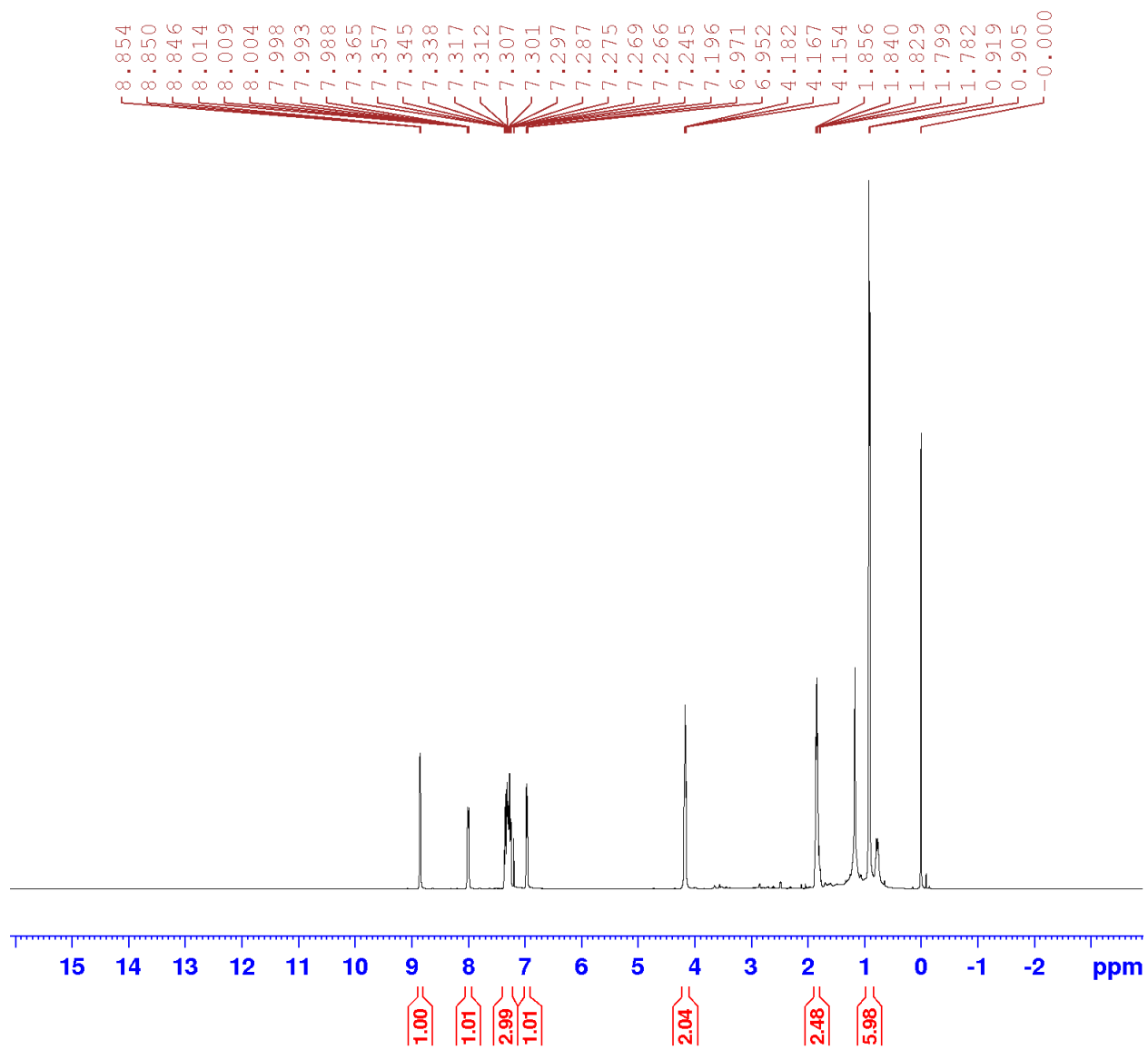


Figure S7: ¹H NMR of QD-2

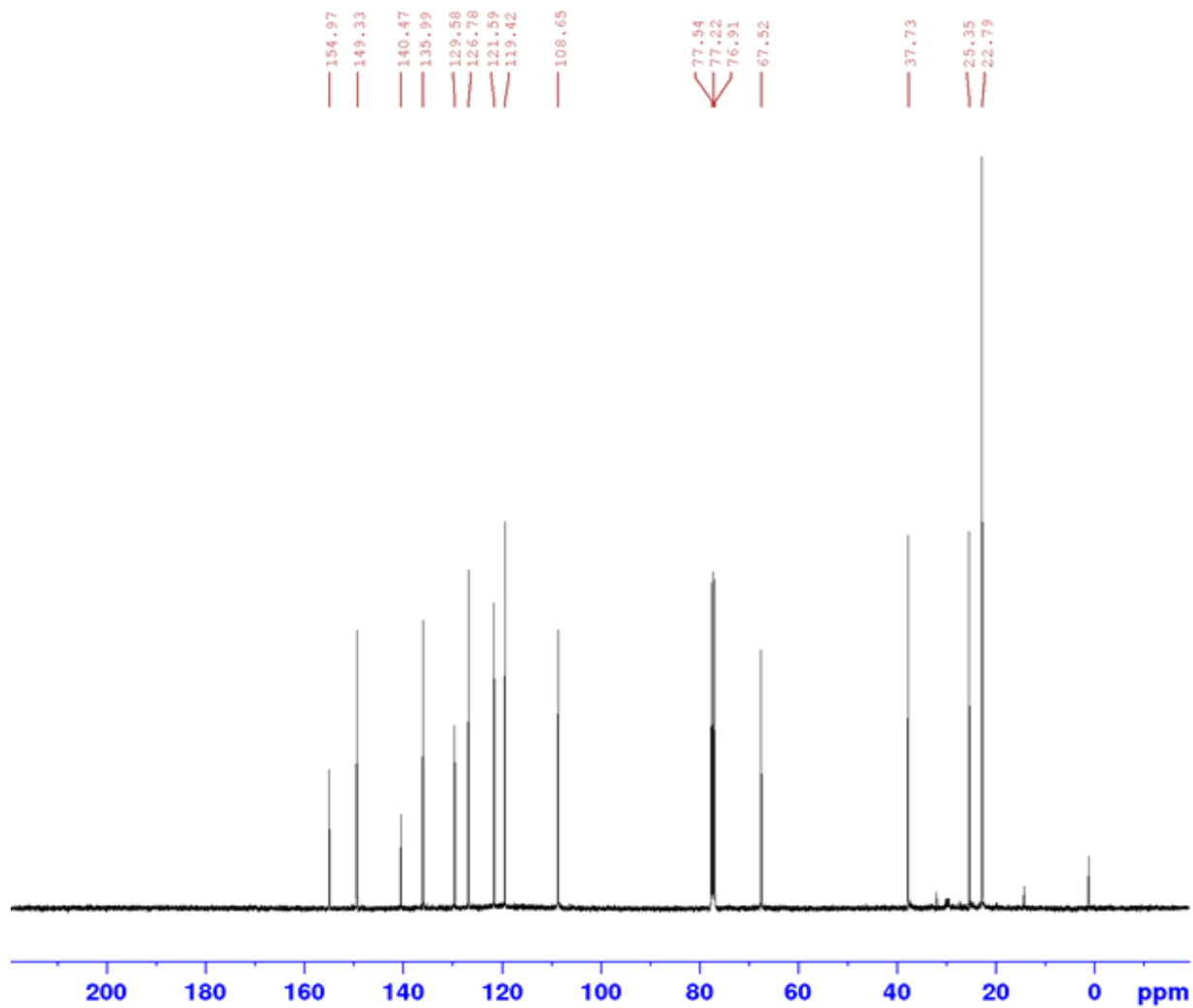


Figure S8: ^{13}C NMR of QD-2

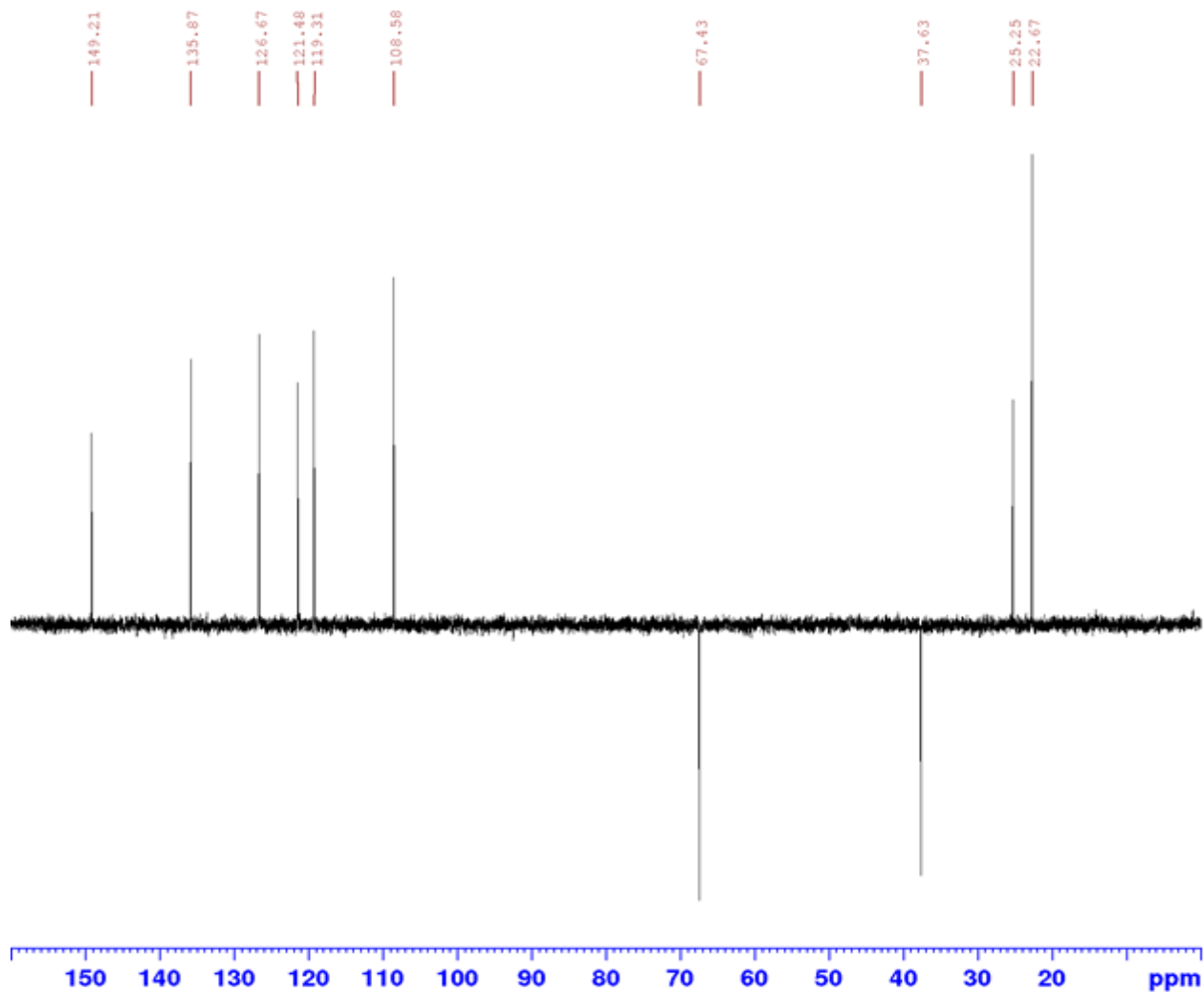


Figure S9: DEPT-135 NMR of QD-2

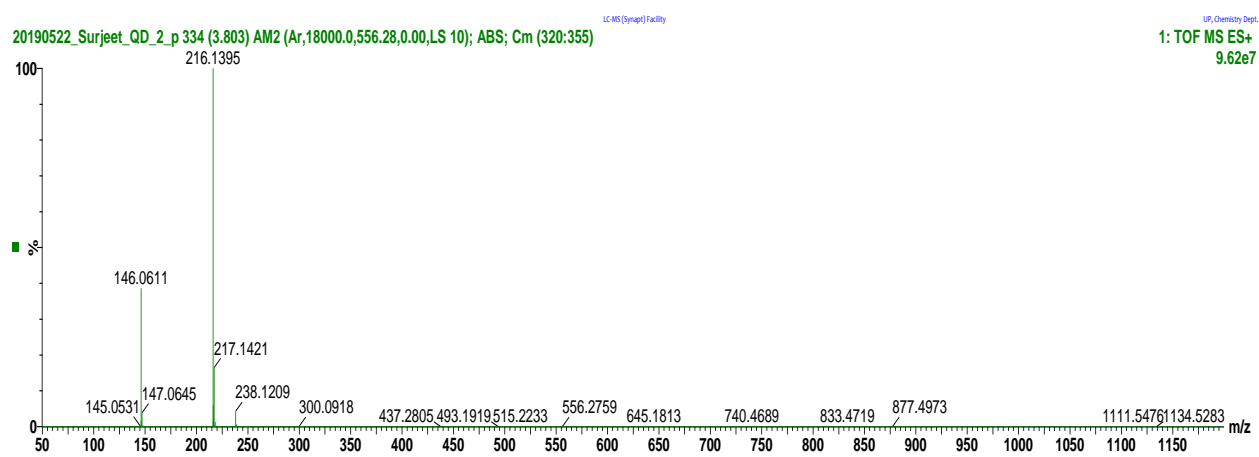


Figure S10: HRESI-MS of QD-2

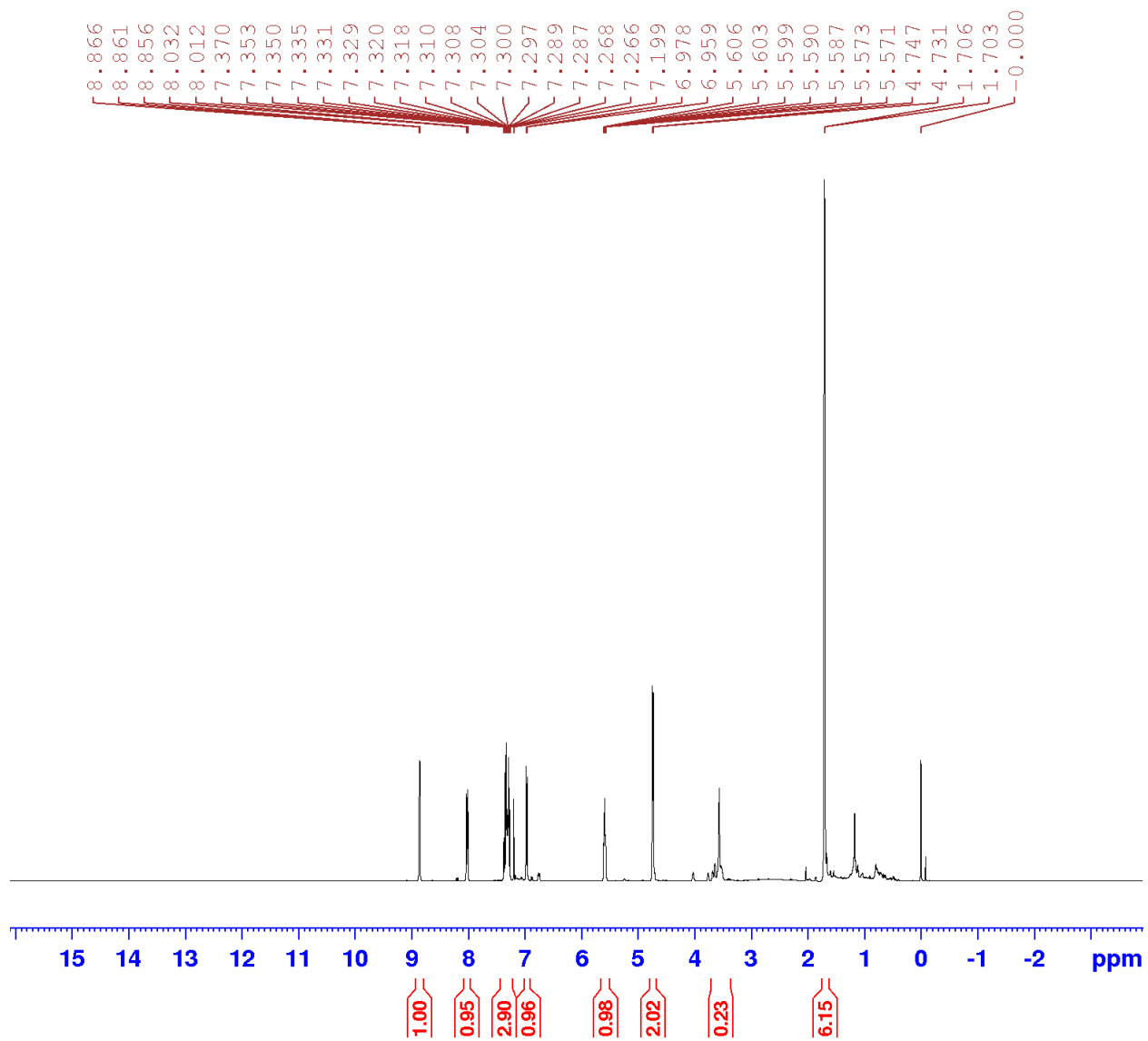


Figure S11: ¹H NMR of QD-3

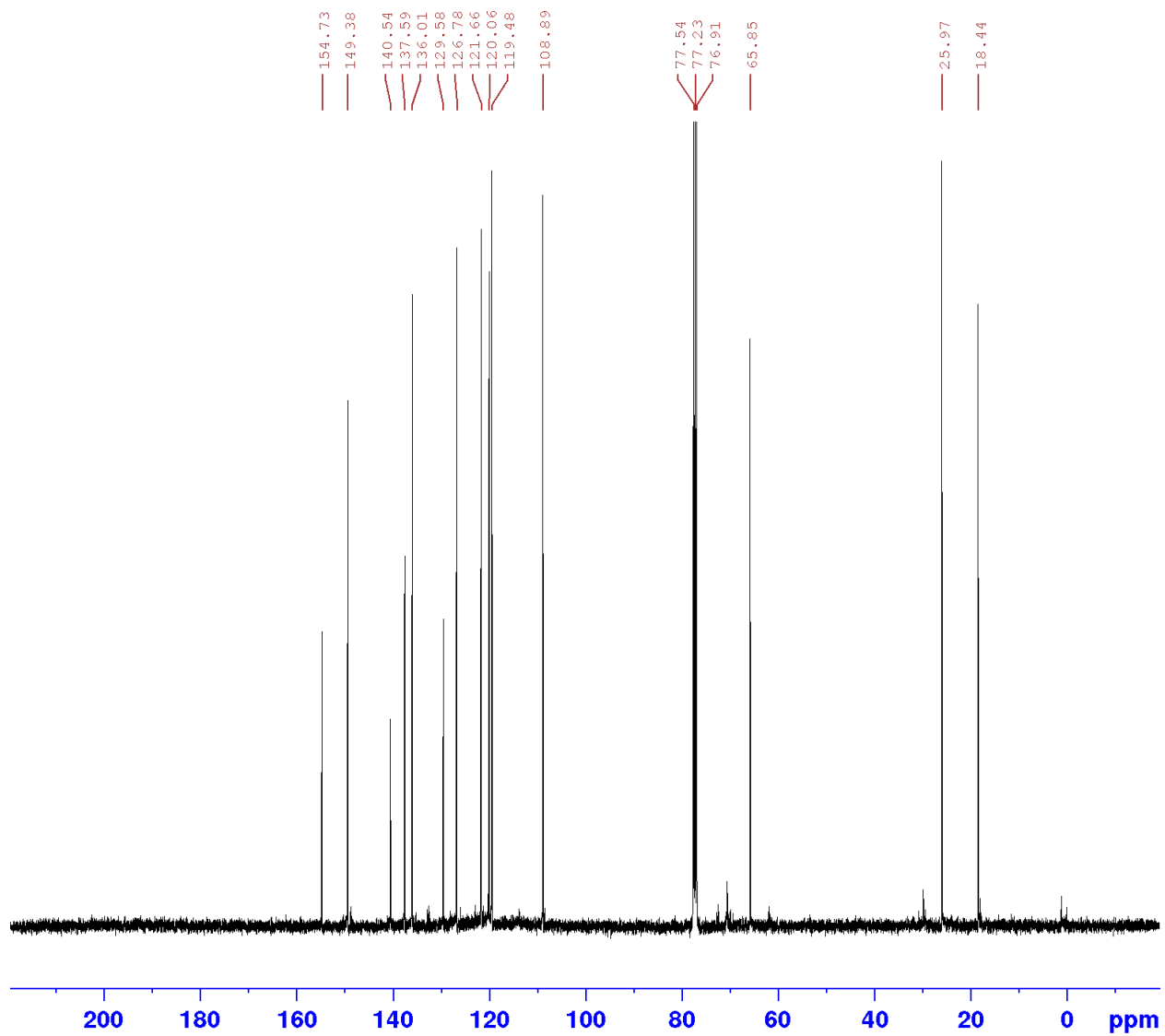


Figure S12: ¹³C NMR of QD-3

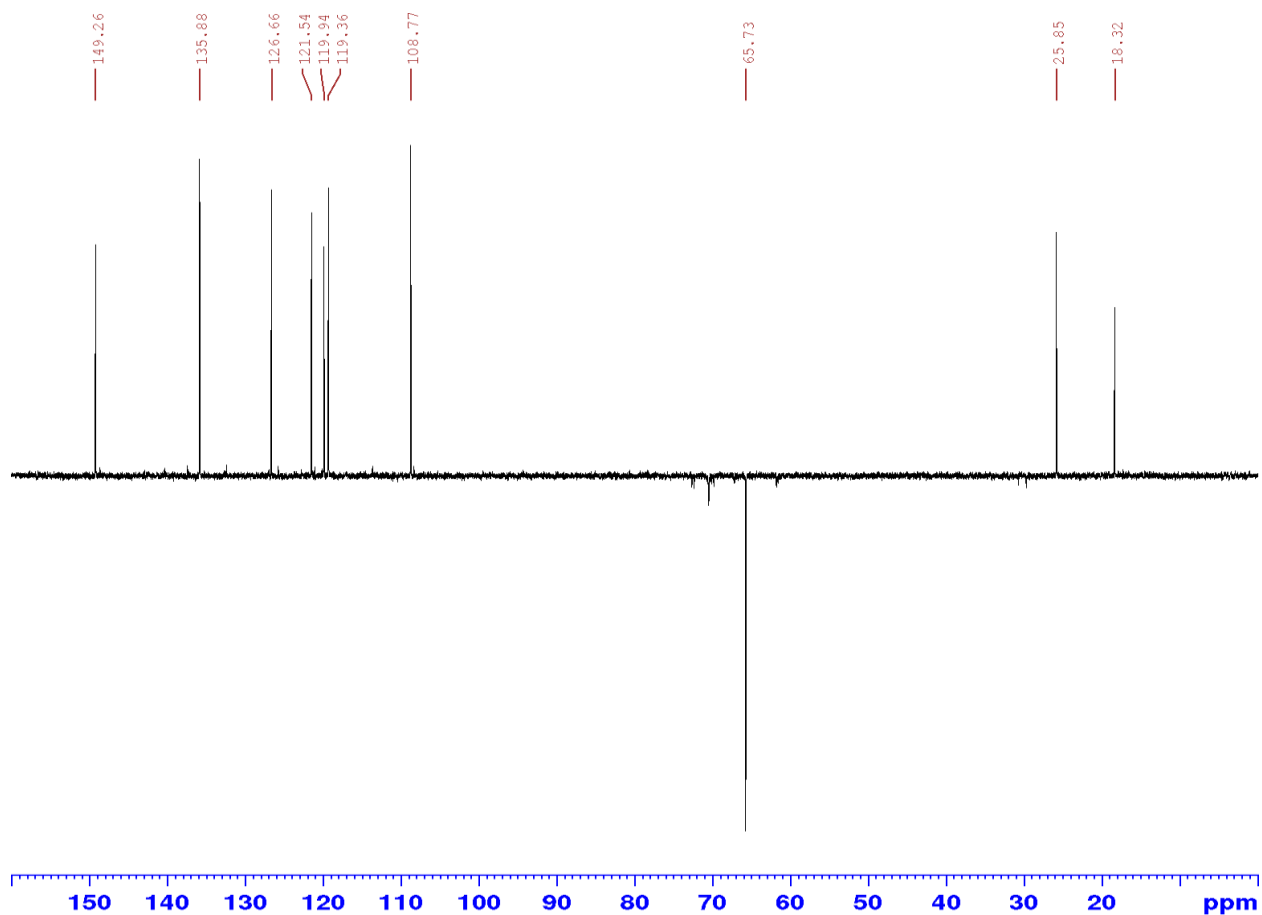


Figure S13: DEPT-135 NMR of QD-3

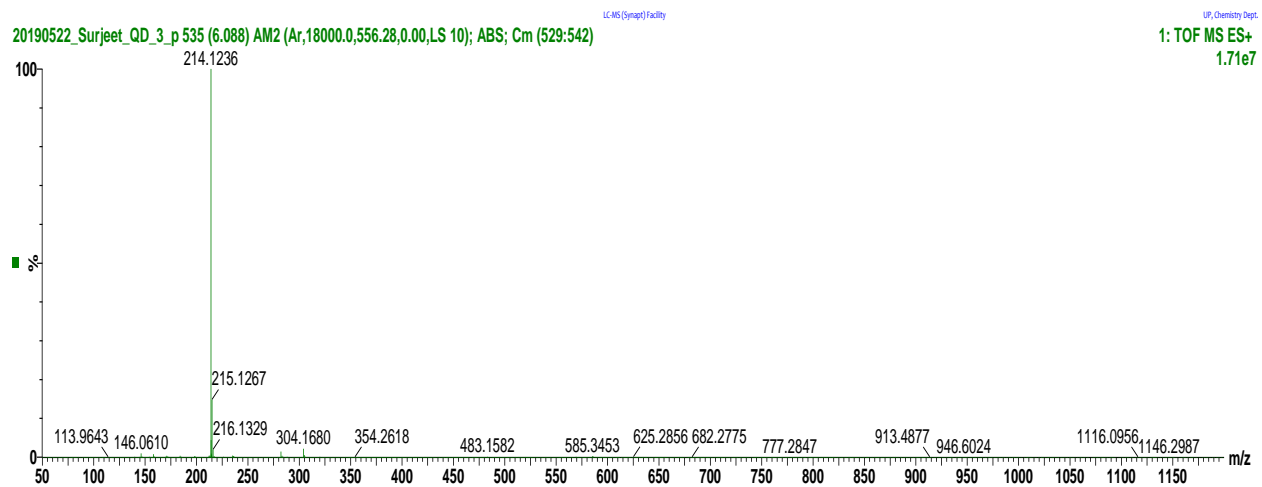


Figure S14: HRESI-MS of QD-3

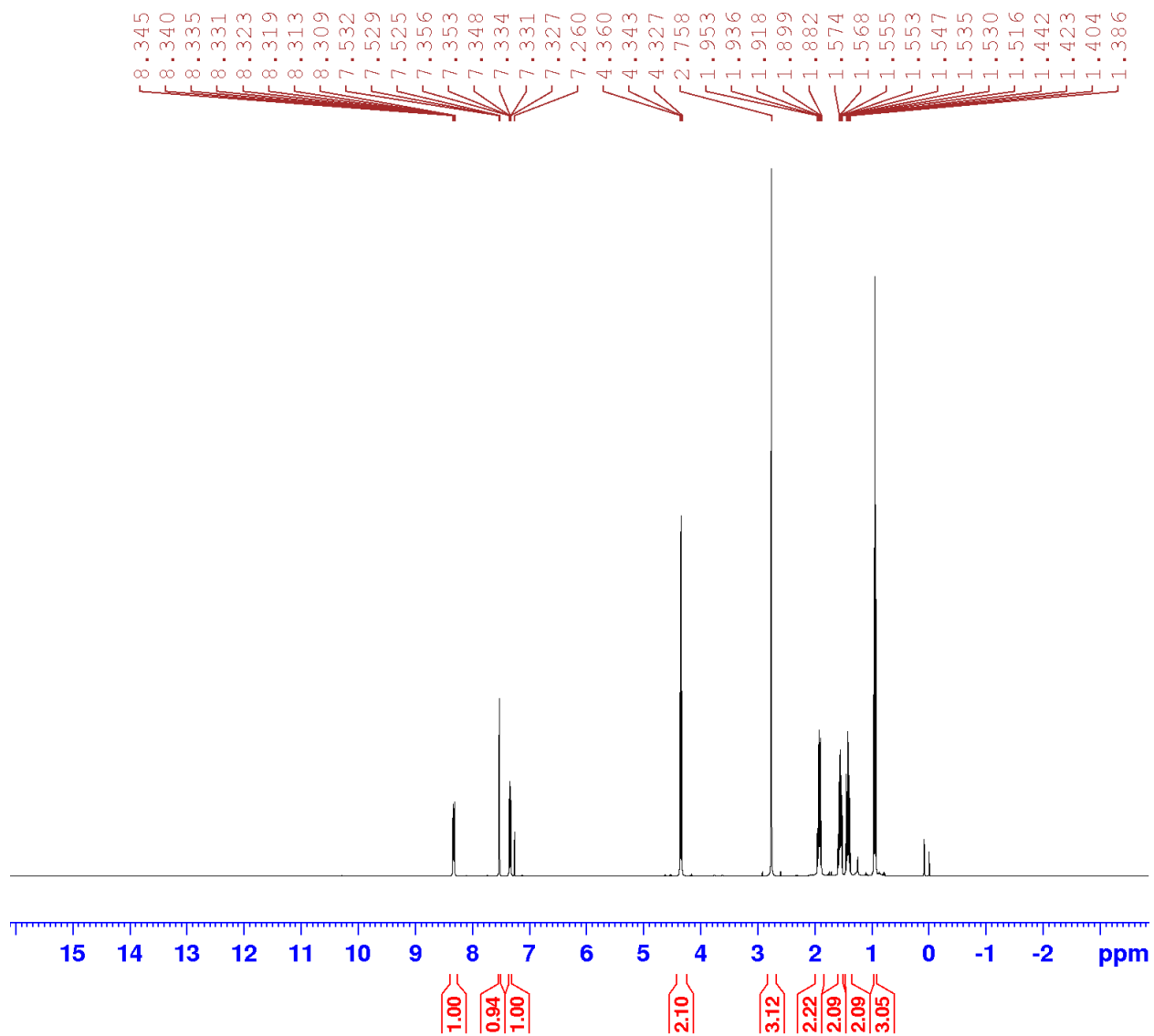


Figure S15: ¹H NMR of QD-4

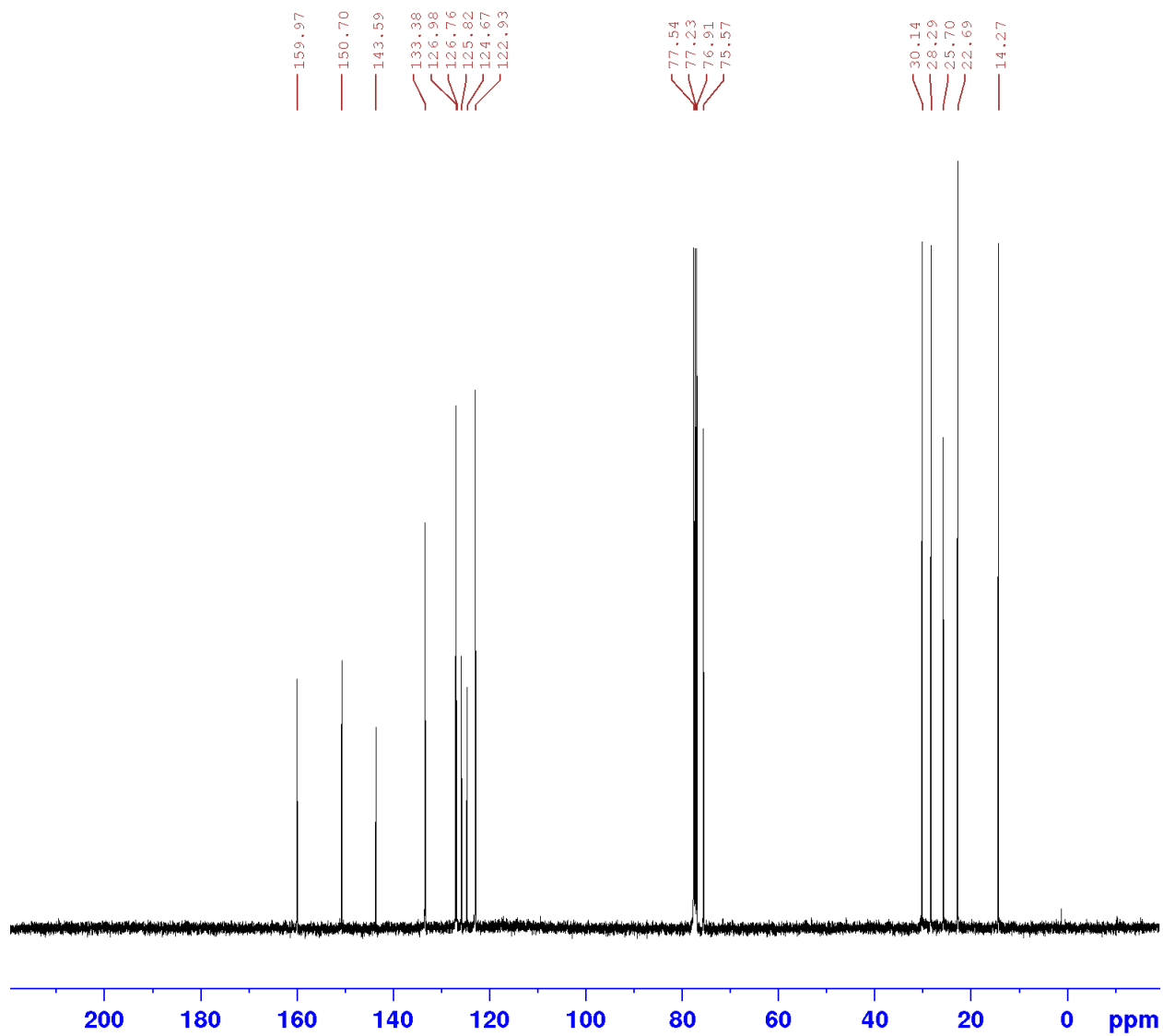


Figure S16: ¹³C NMR of QD-4

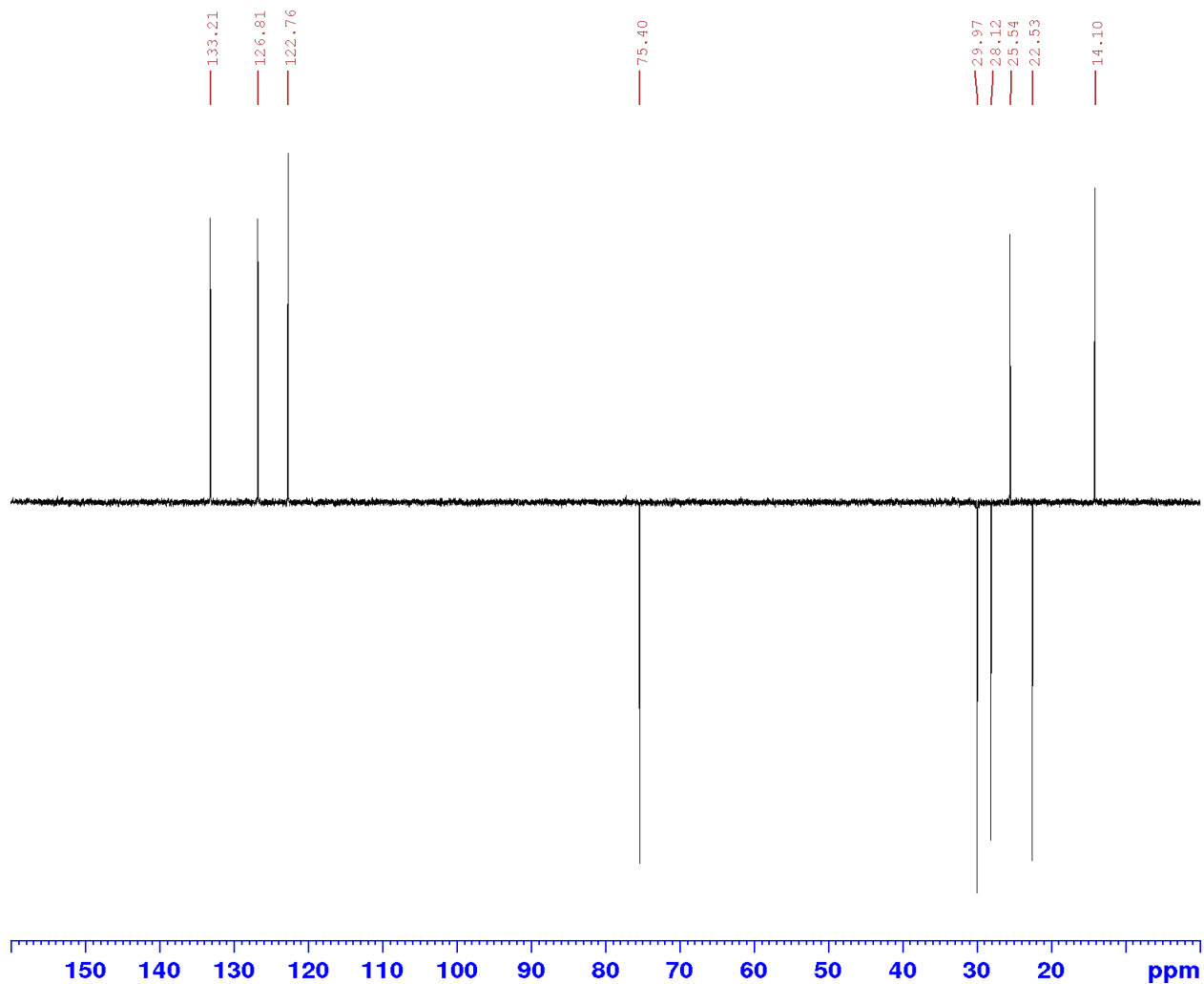


Figure S17: DEPT-135 NMR of QD-4

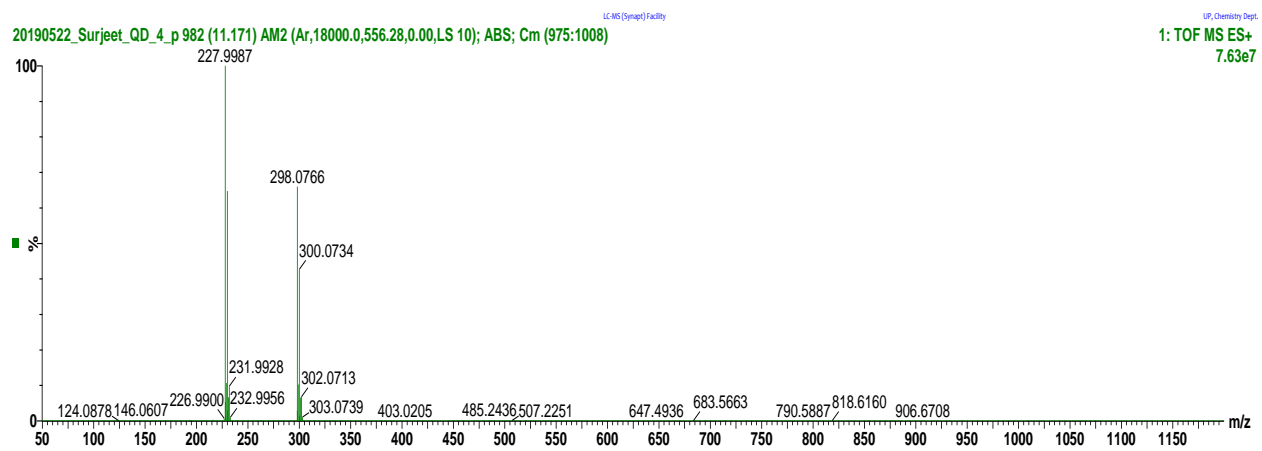


Figure S18: HRESI-MS of QD-4

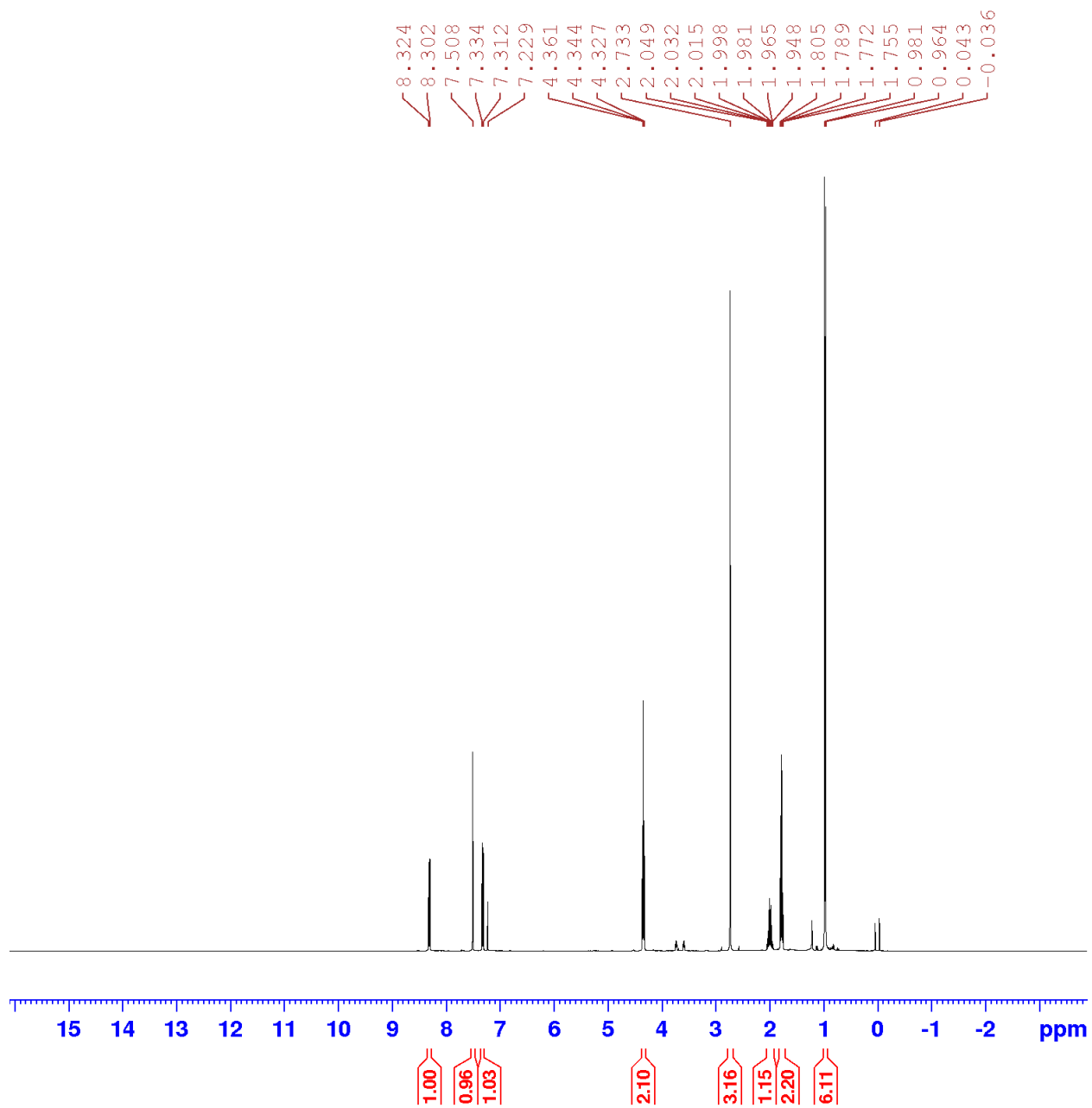


Figure S19: ¹H NMR of QD-5

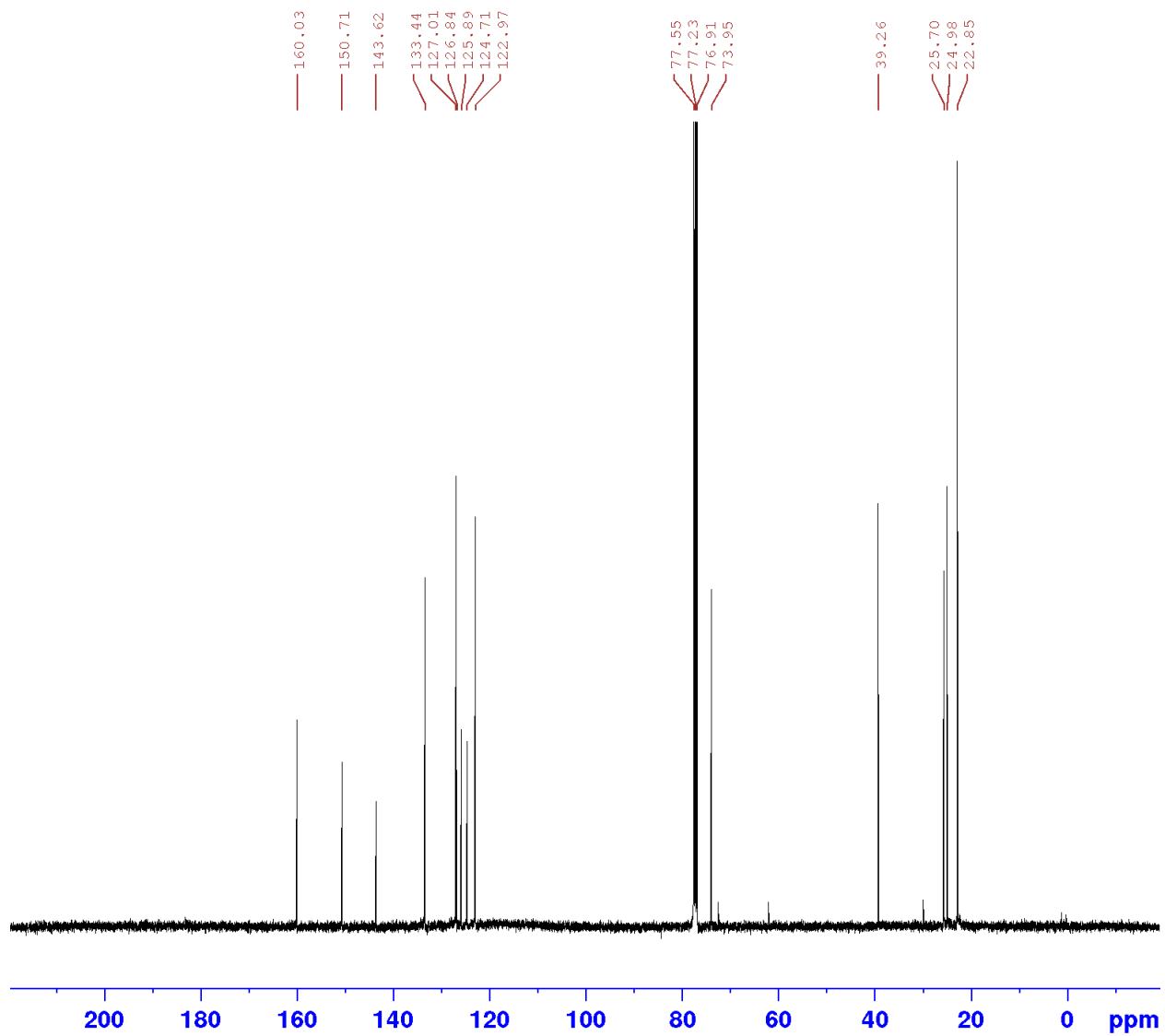


Figure S20: ^{13}C NMR of QD-5

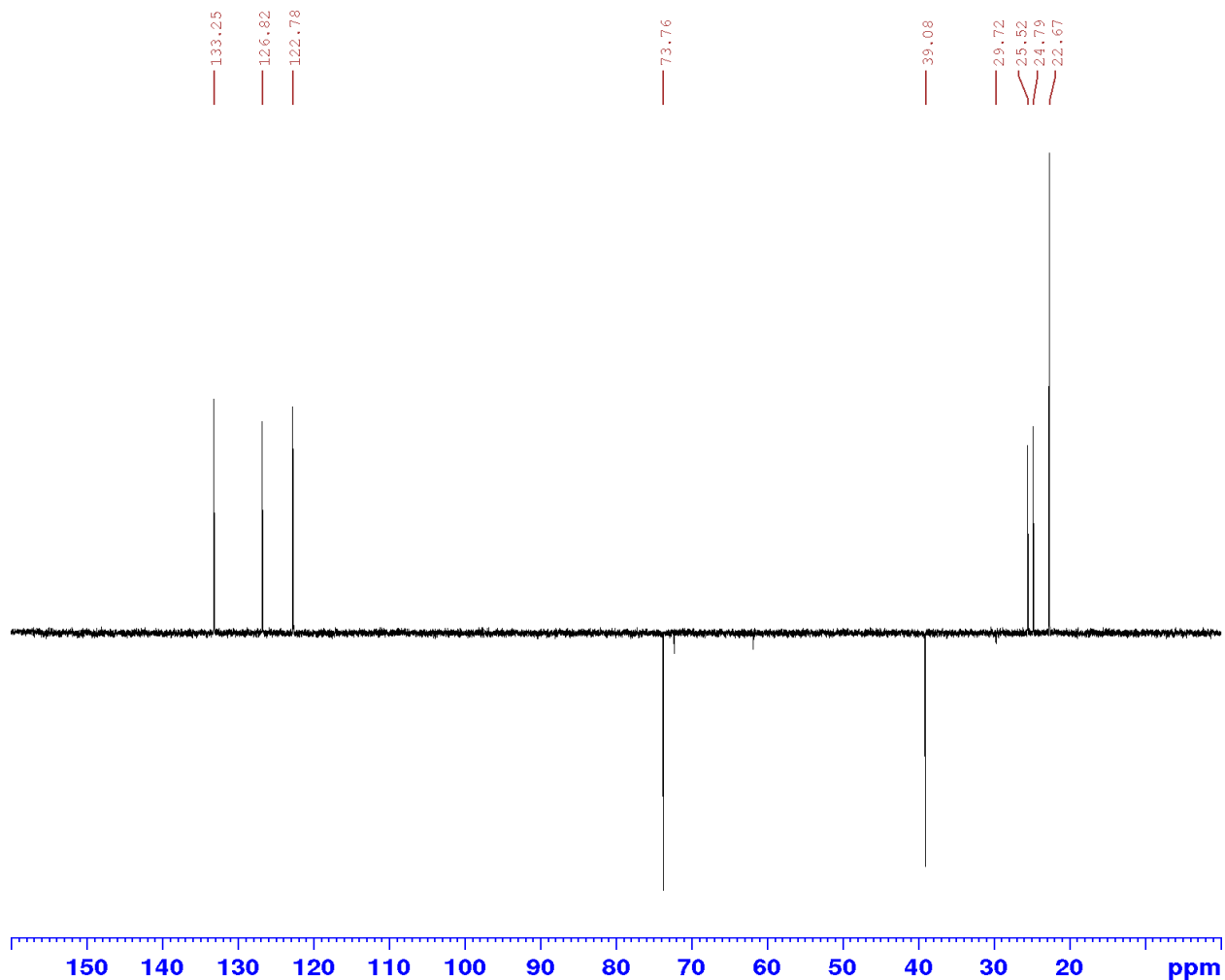


Figure S21: DEPT-135 NMR of QD-5

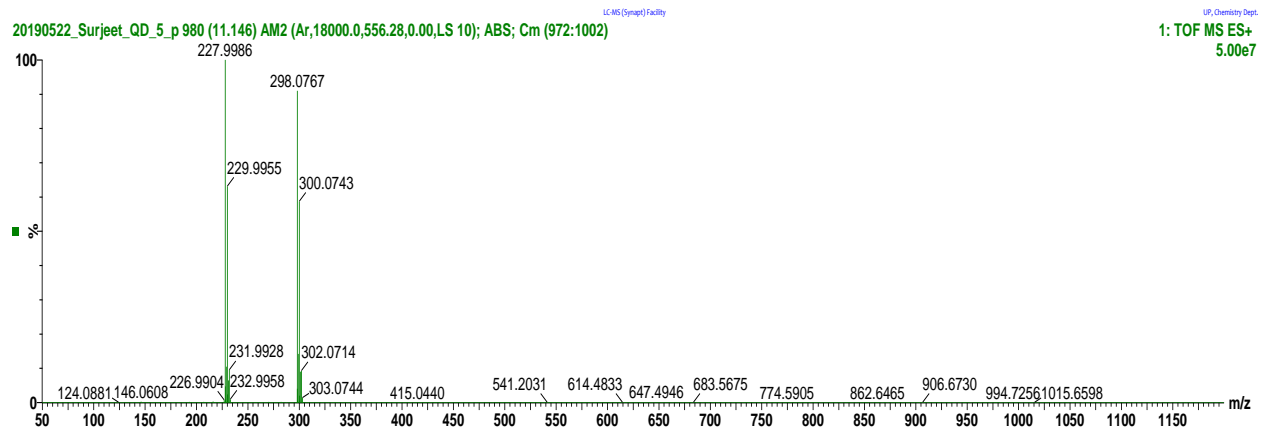


Figure S22: HRESI-MS of QD-5

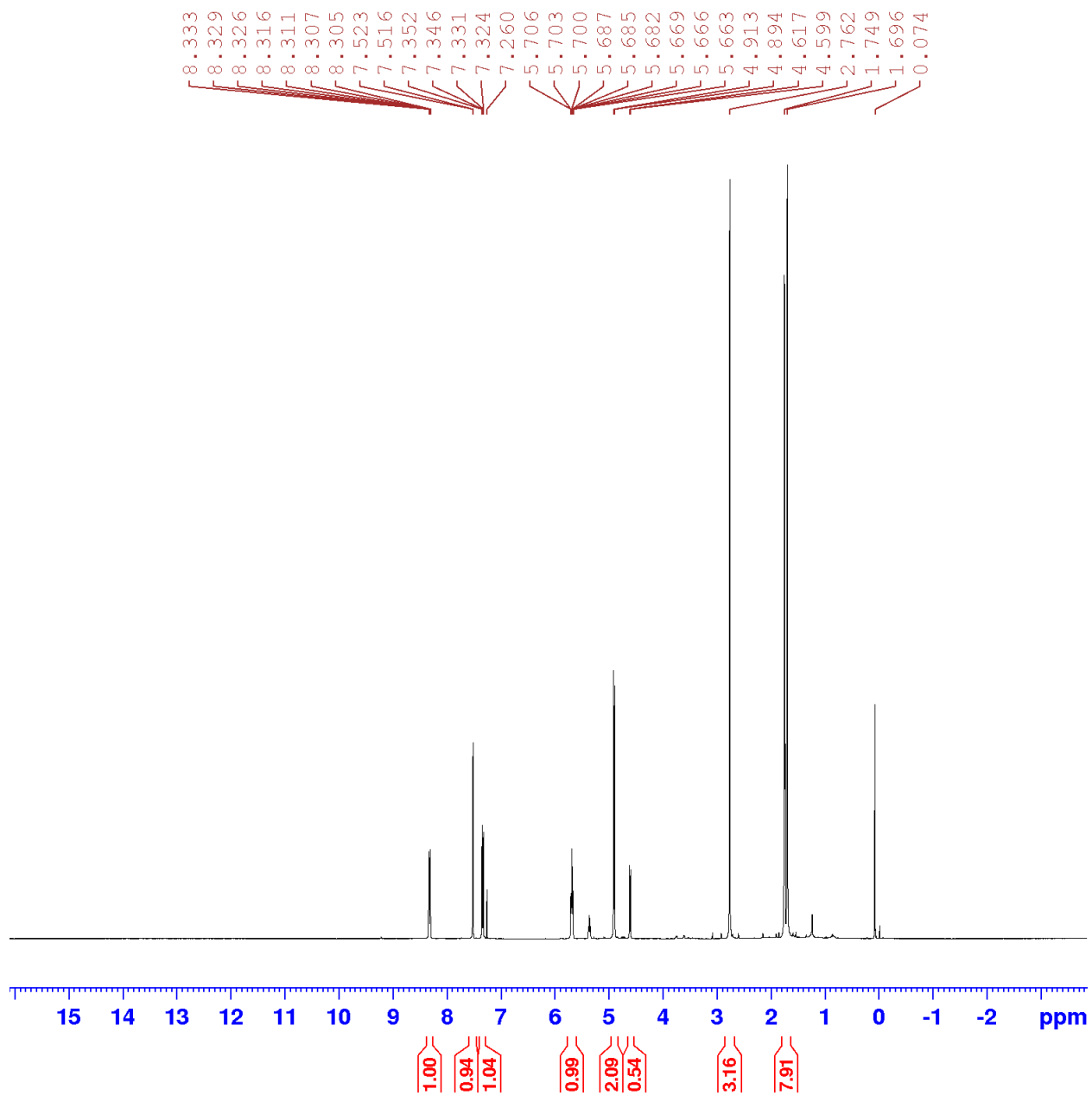


Figure S23: ¹H NMR of QD-6

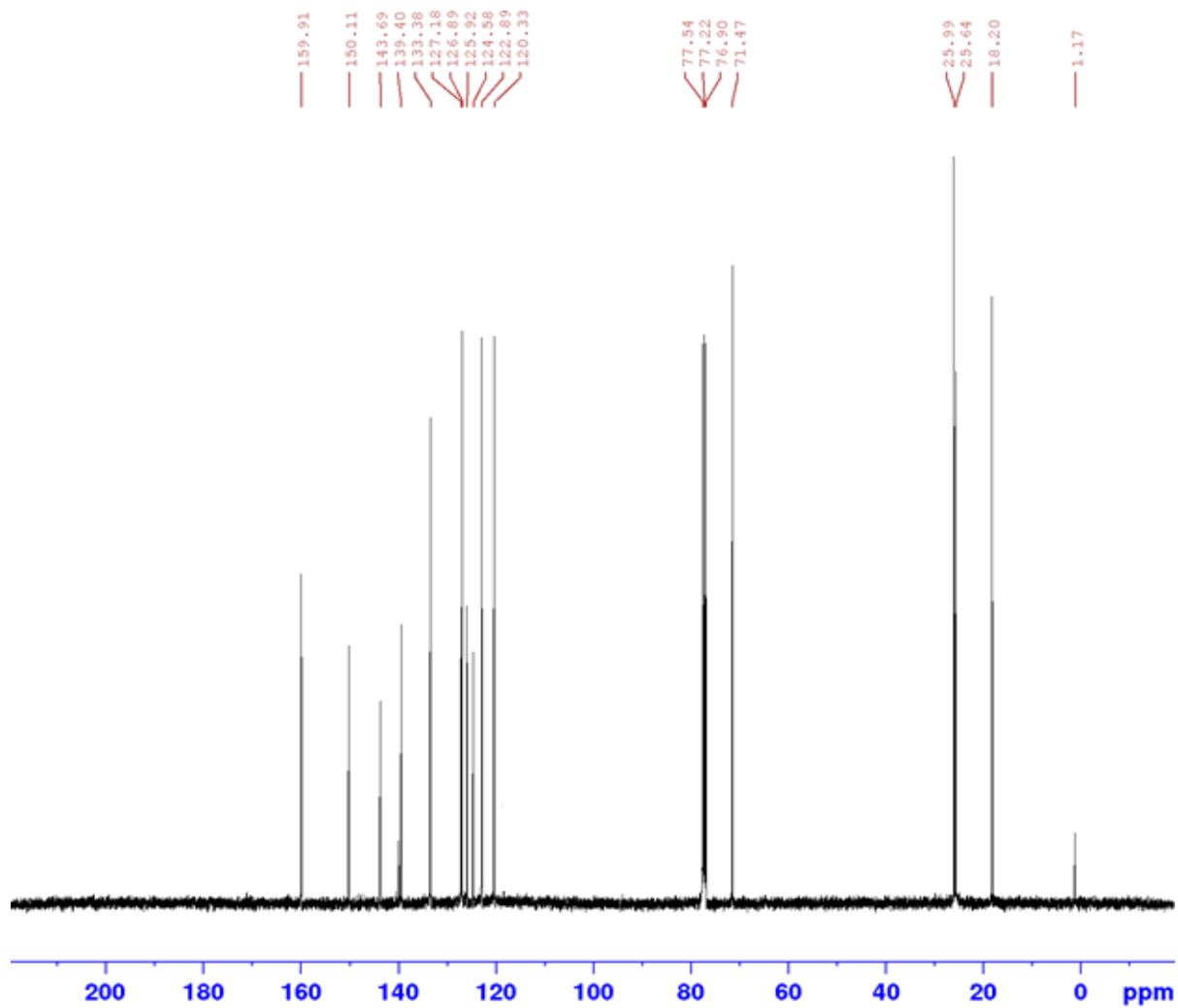


Figure S24: ¹³C NMR of QD-6

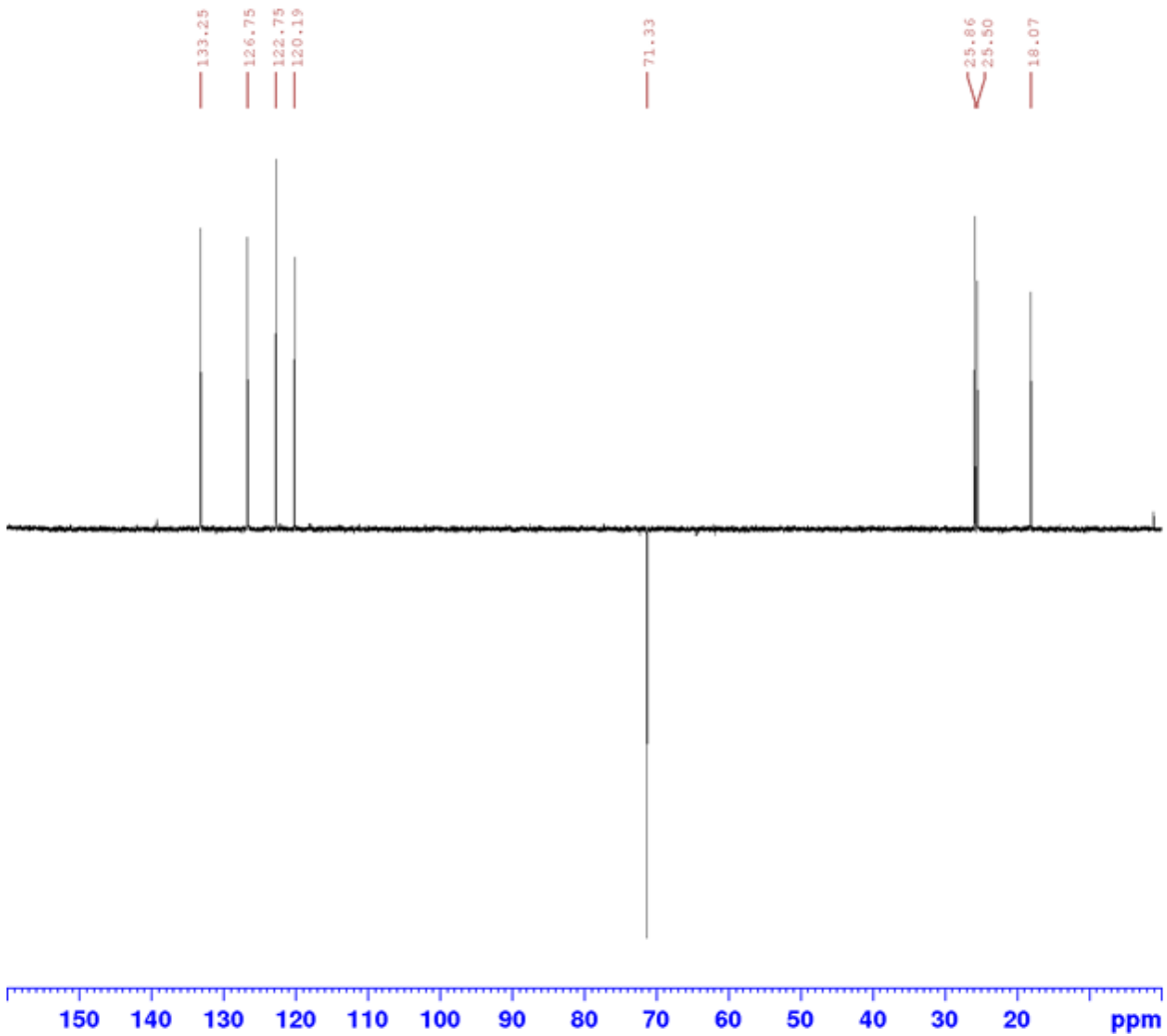


Figure S25: DEPT-135 NMR of QD-6

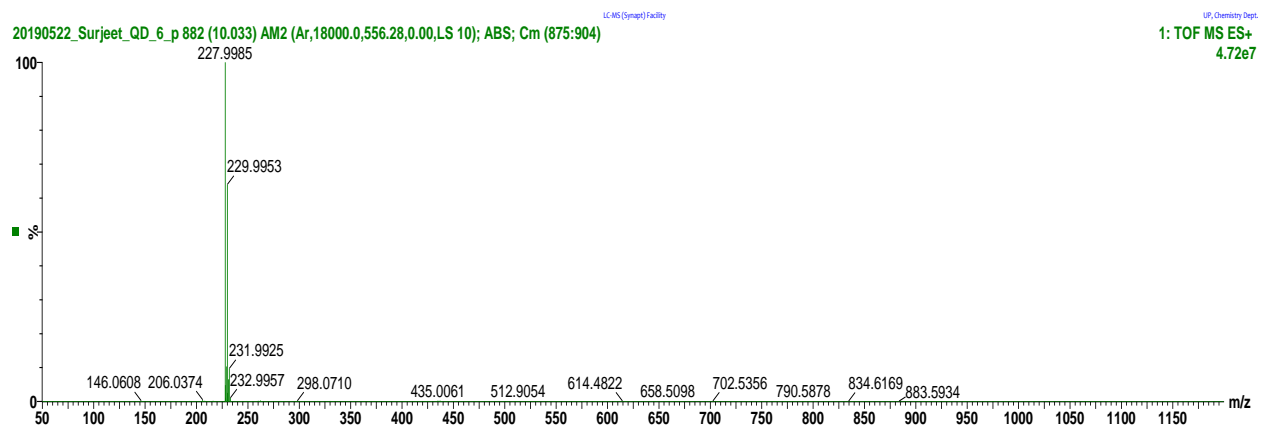
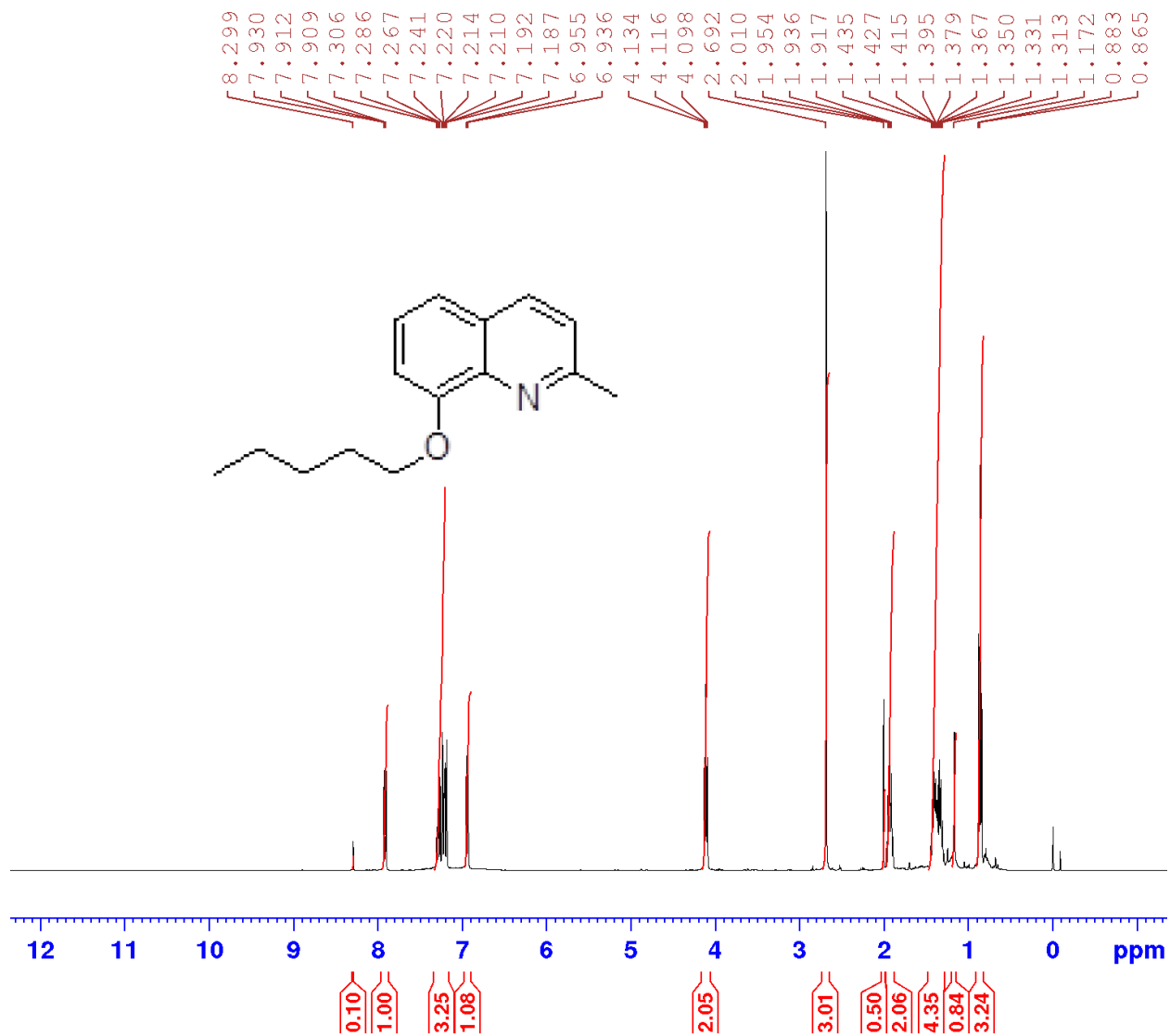


Figure S26: HRESI-MS of QD-6



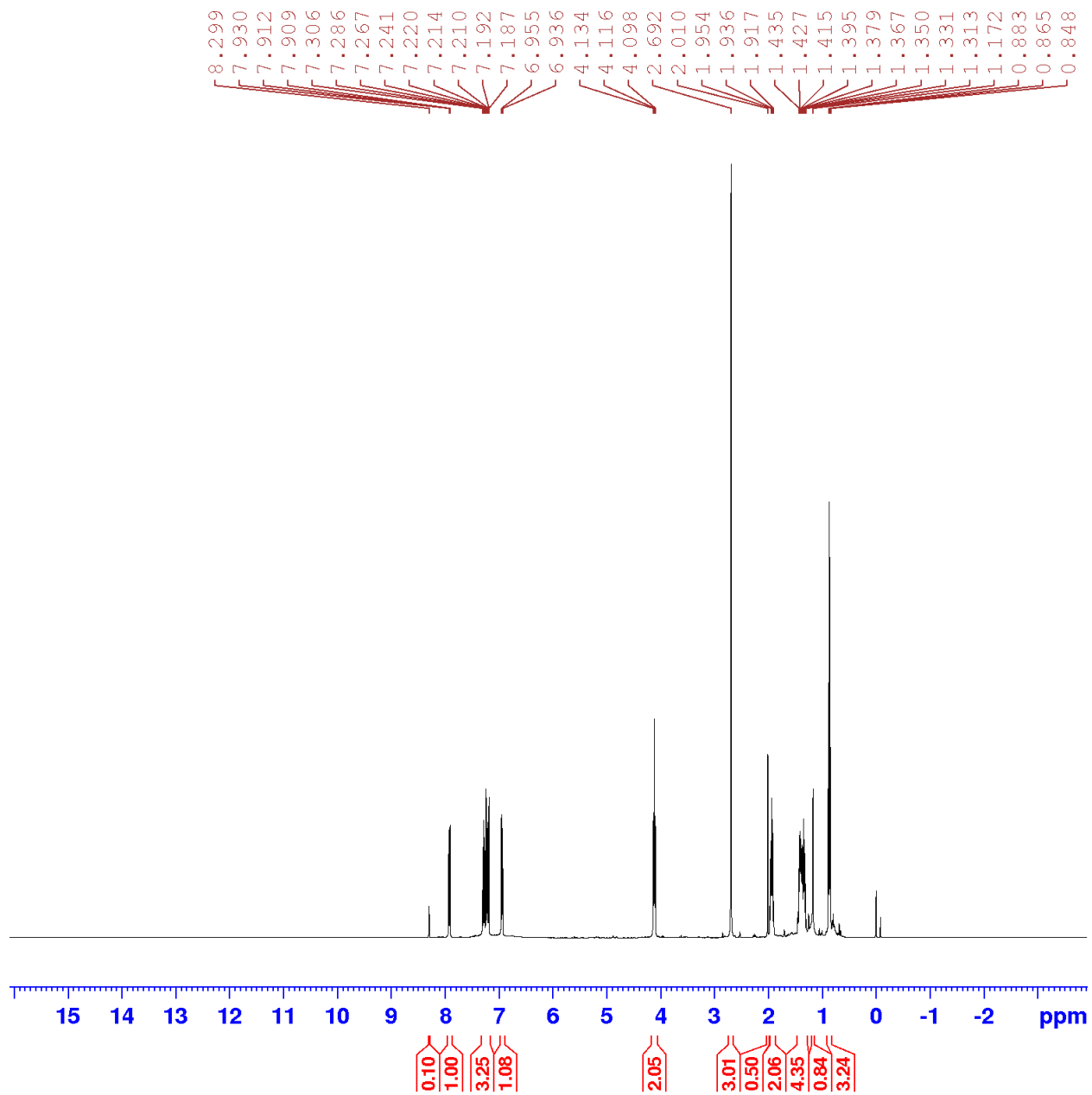


Figure S27: ¹H NMR of QD-7

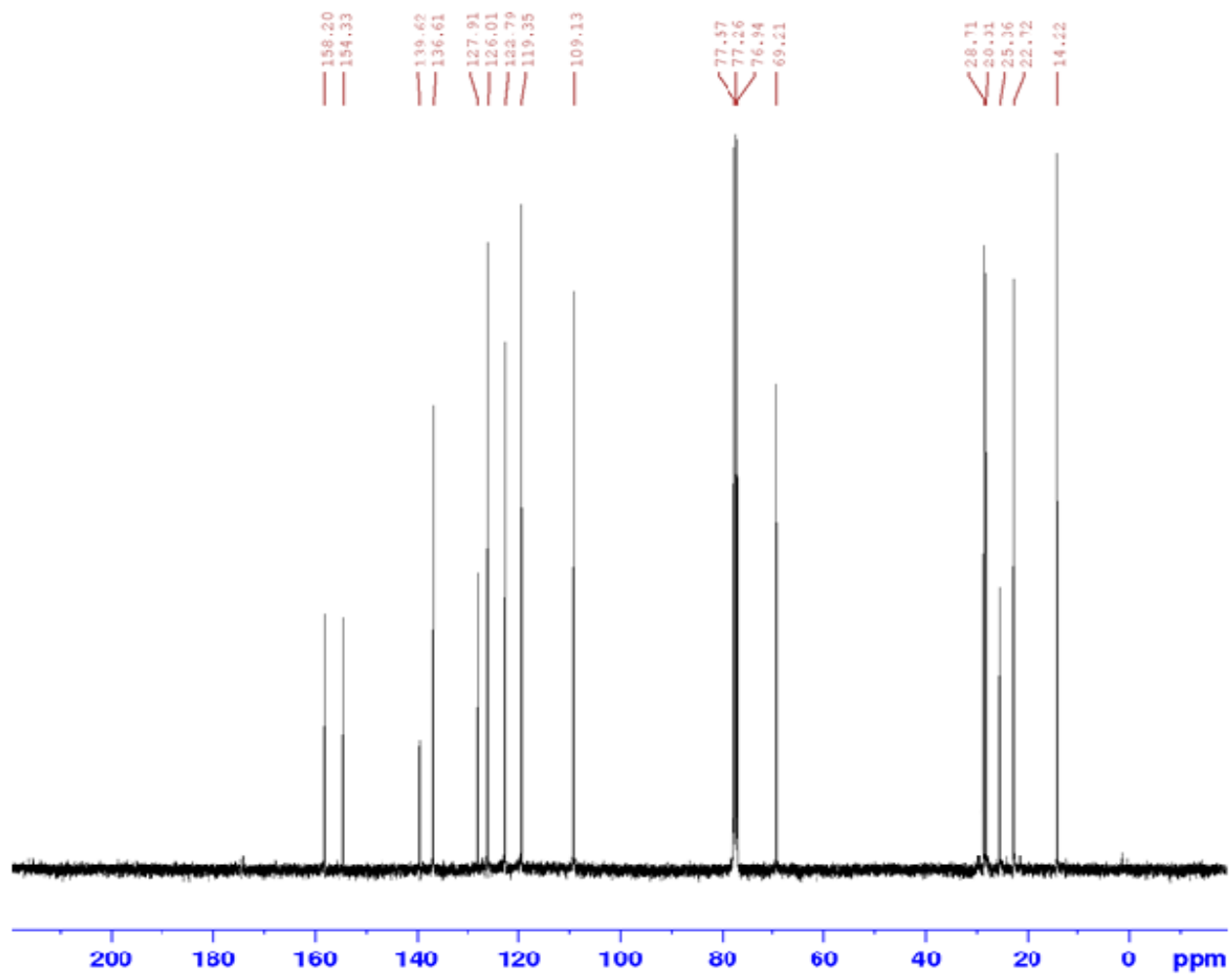


Figure S28: ^{13}C NMR of QD-7

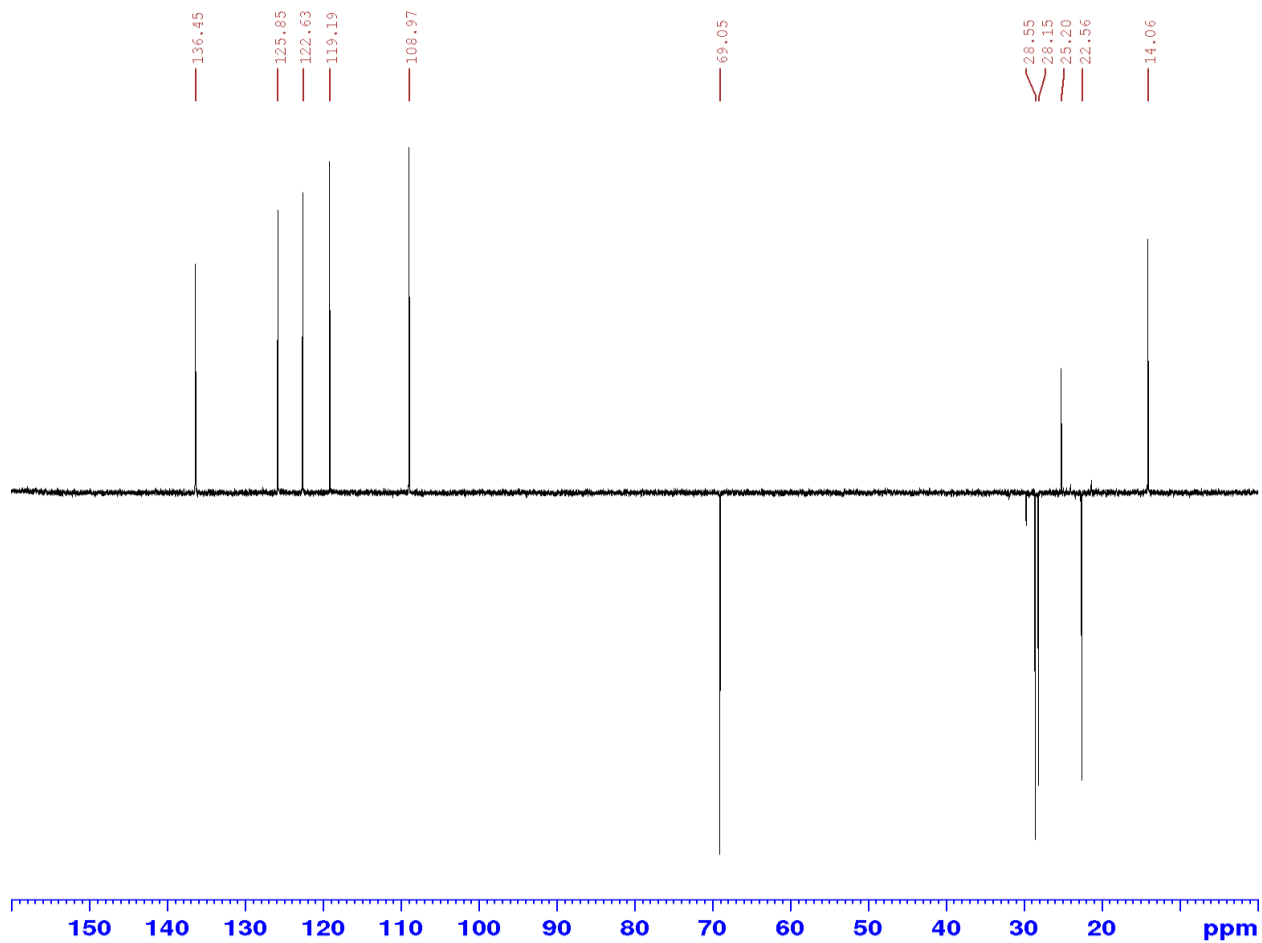


Figure S29: DEPT-135 NMR of QD-7

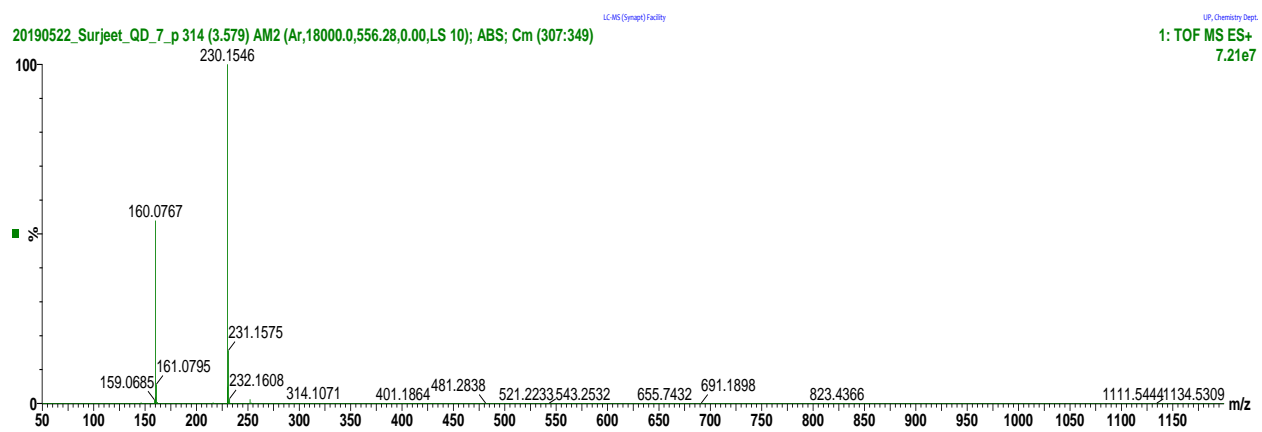


Figure S30: HRESI-MS of QD-7

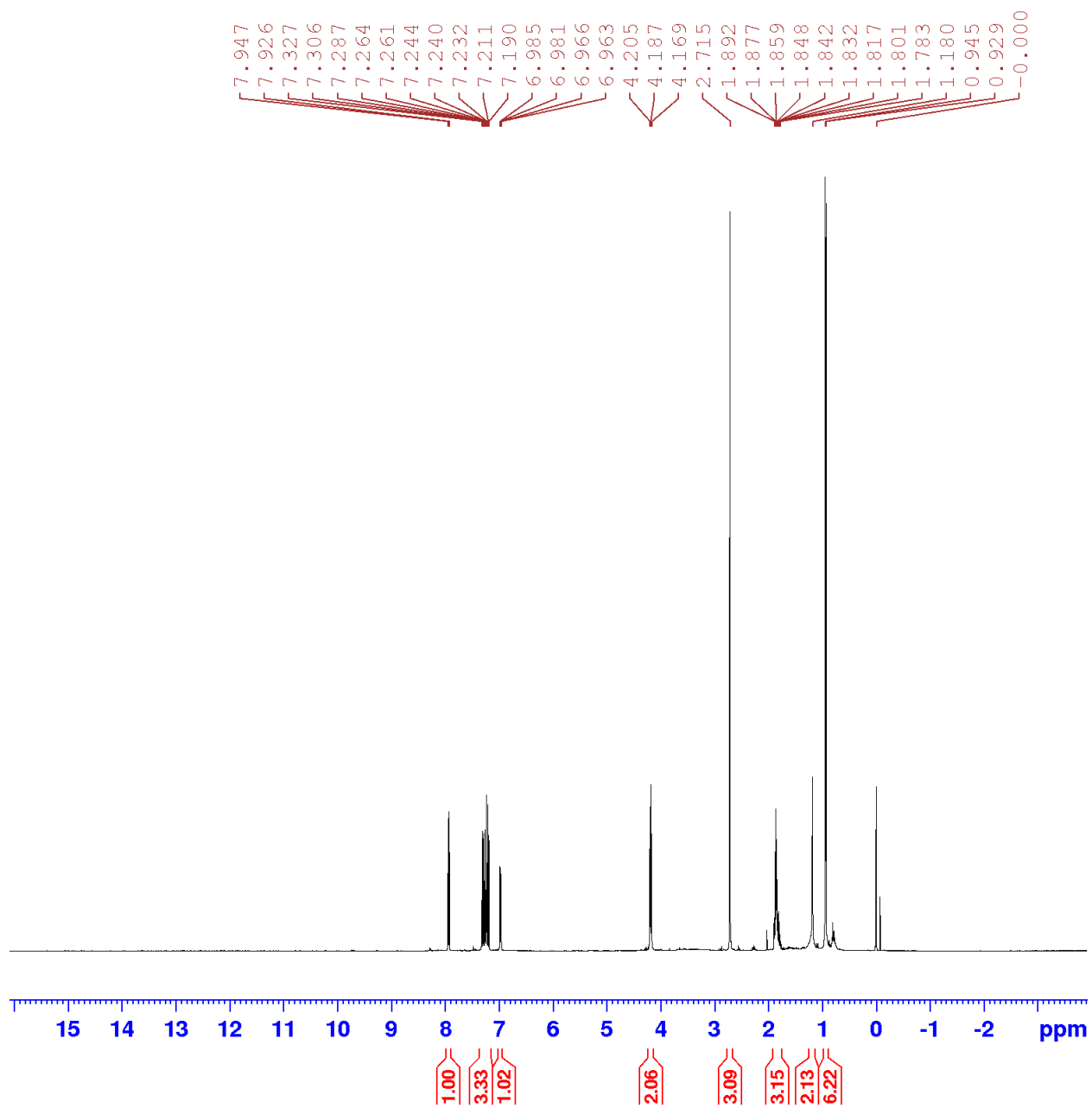


Figure S31: ¹H NMR of QD-8

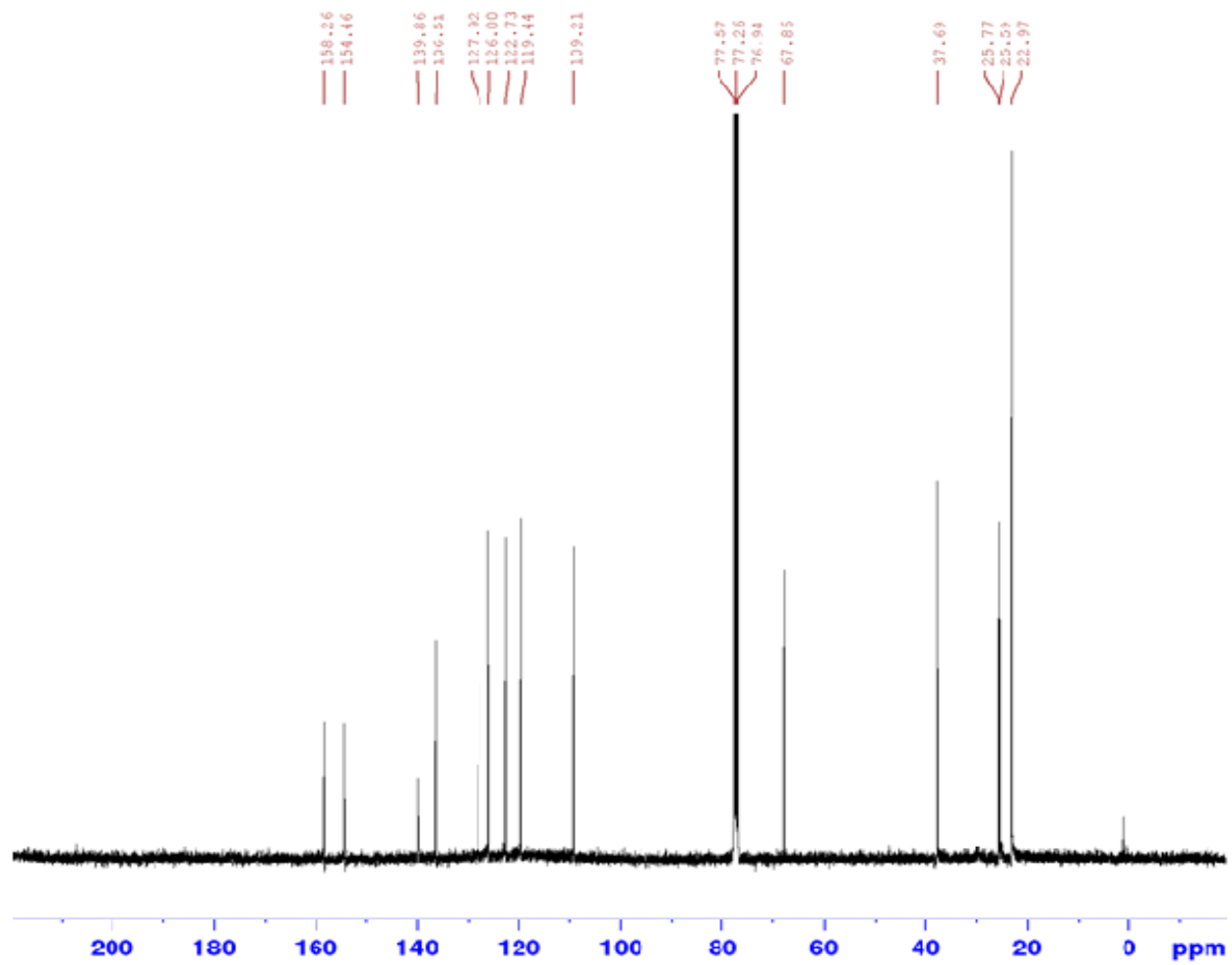


Figure S32: ^{13}C NMR of QD-8

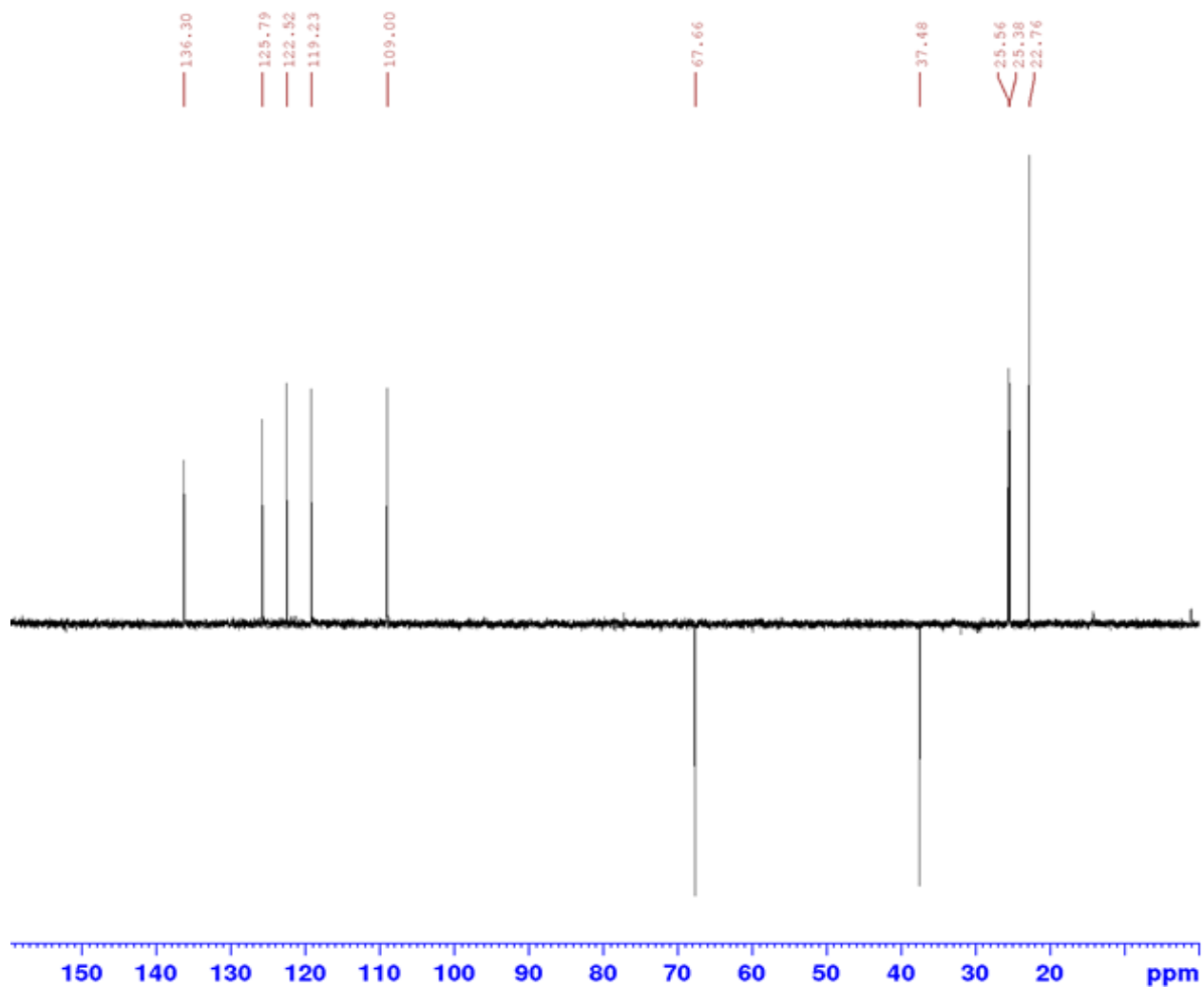


Figure S33: DEPT-135 NMR of QD-8

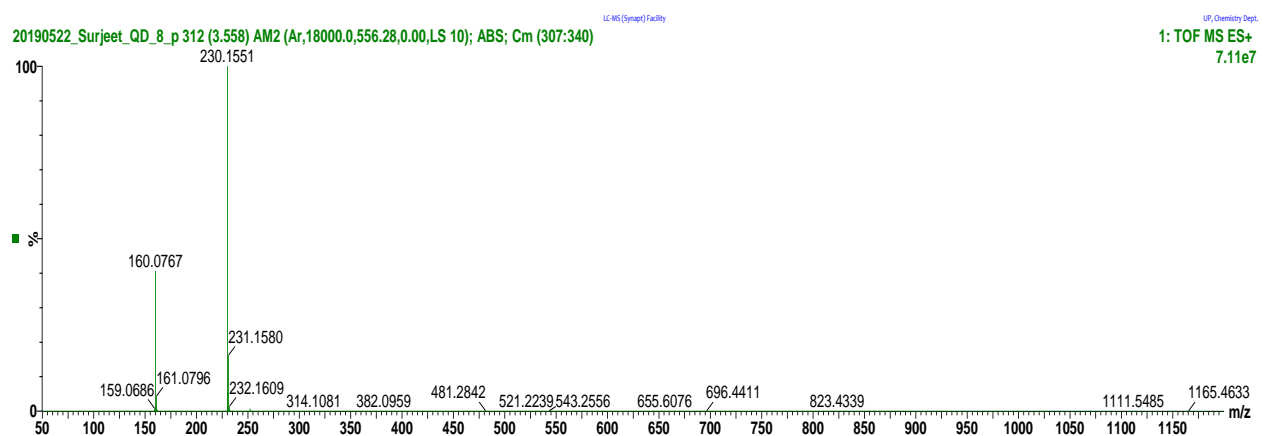


Figure S34: HRESI-MS of QD-8

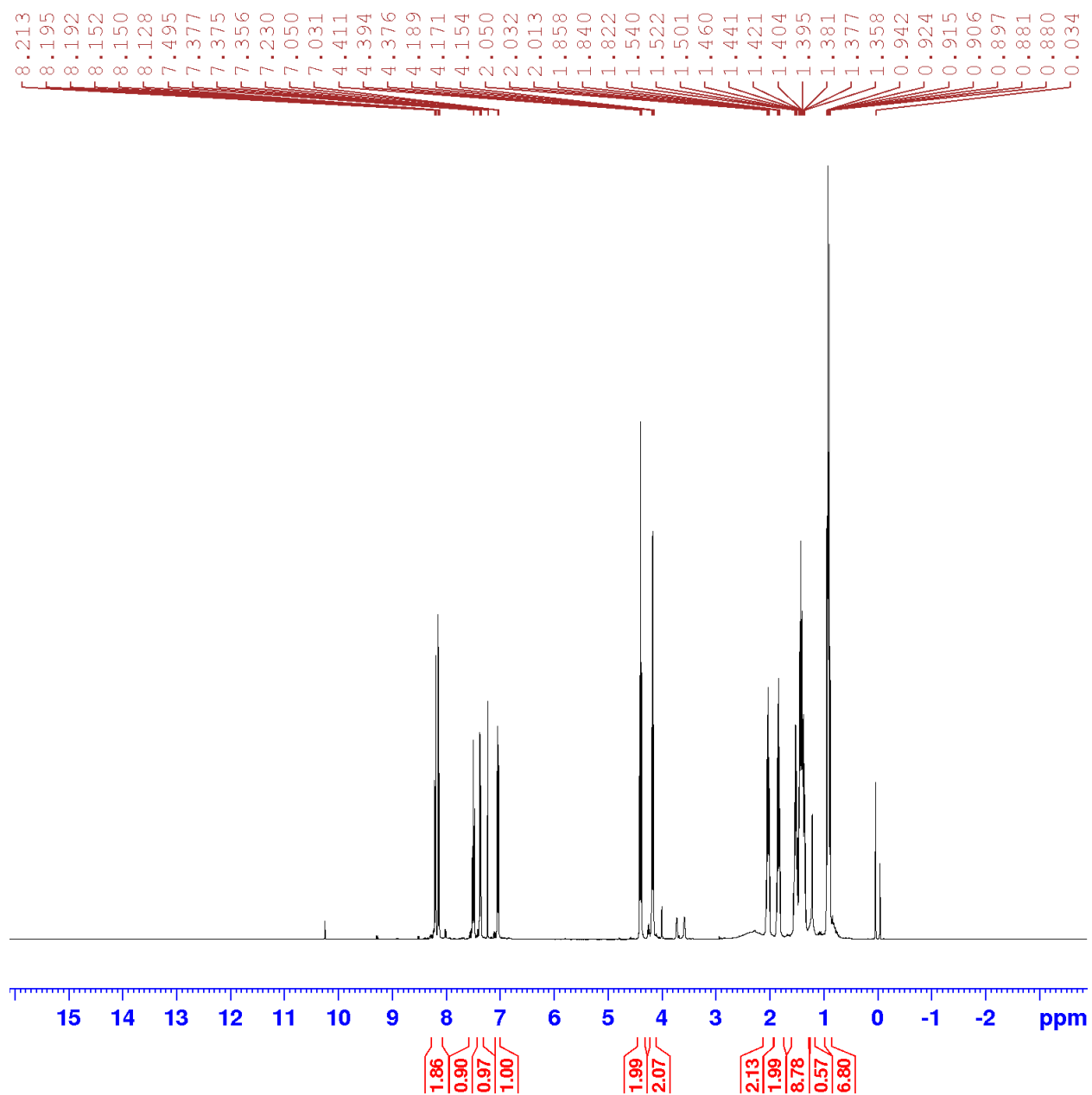


Figure S35: ¹H NMR of QD-10

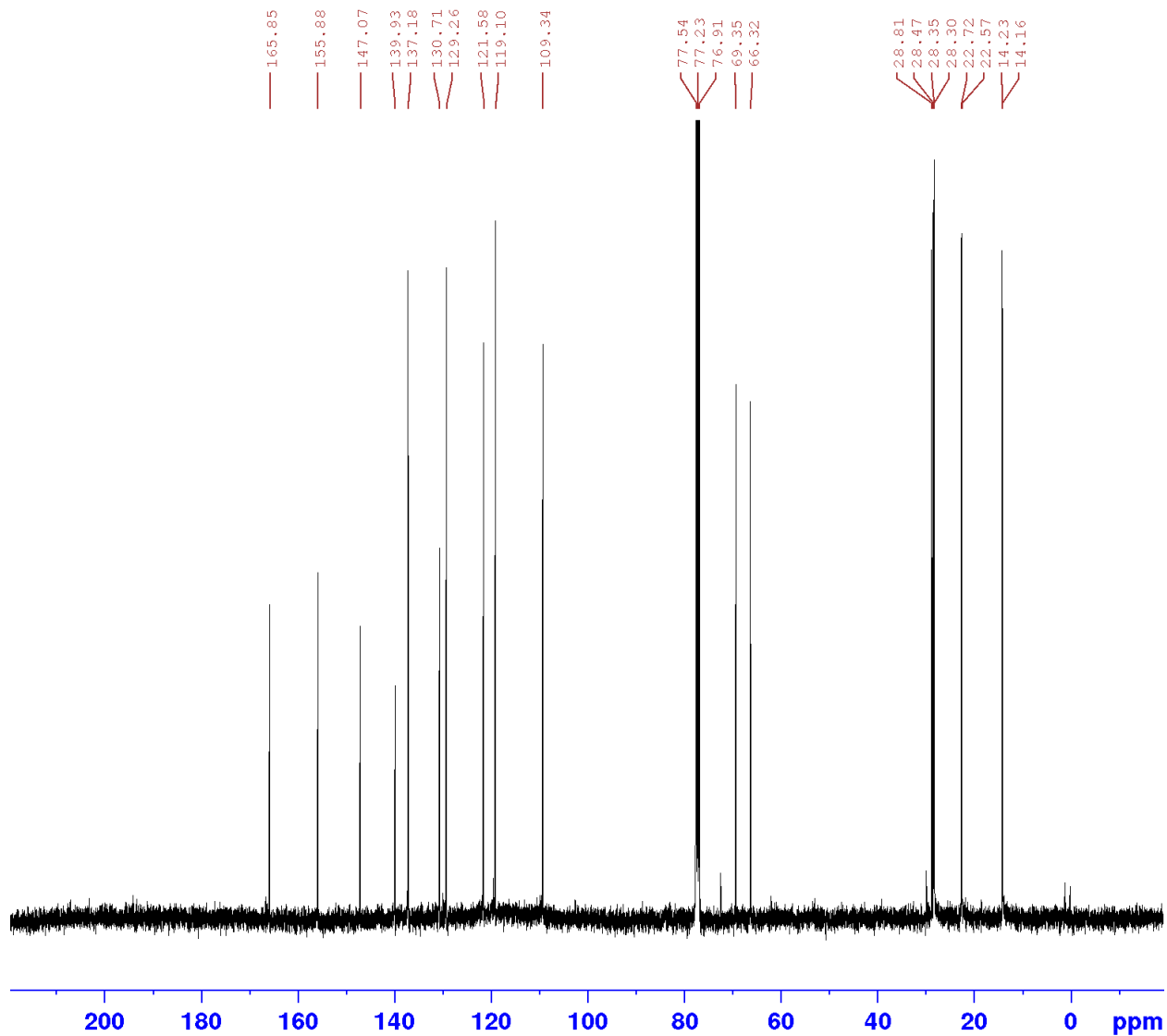


Figure S36: ^{13}C NMR of QD-10

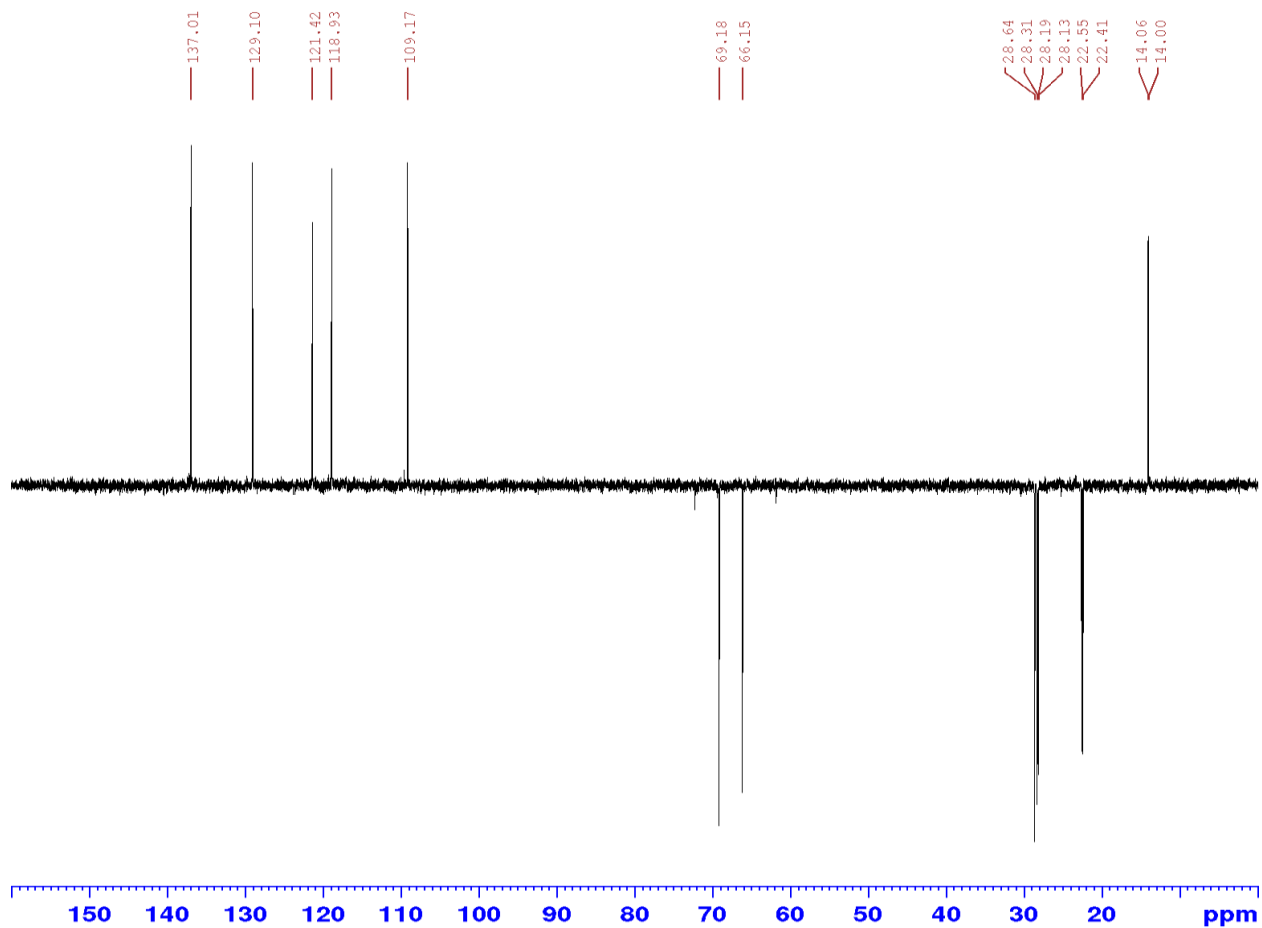


Figure S37: DEPT-135 NMR of QD-10

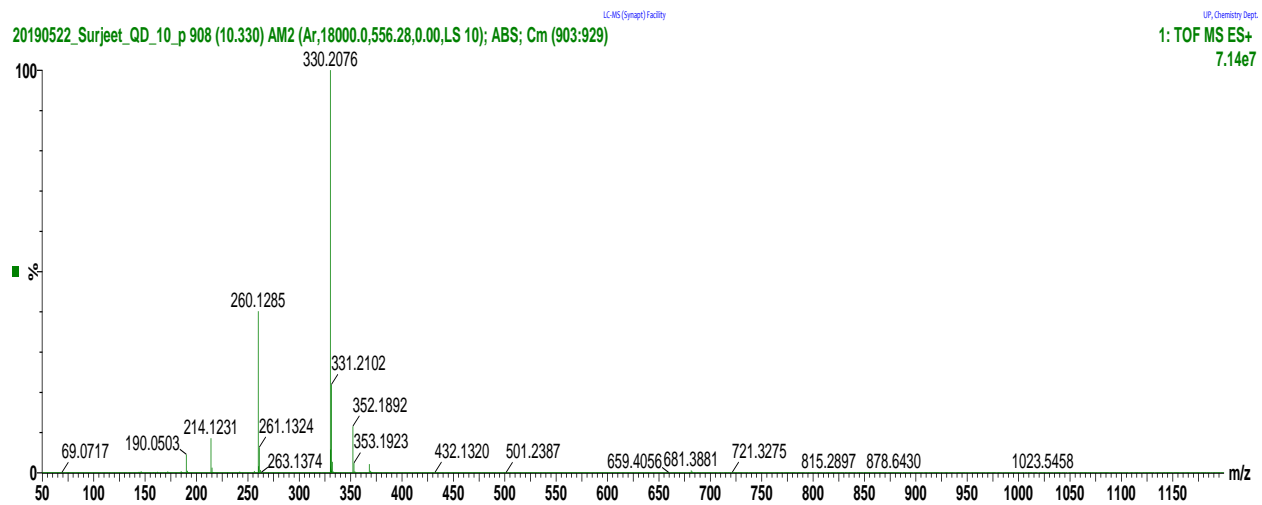


Figure S38: HRESI-MS of QD-10

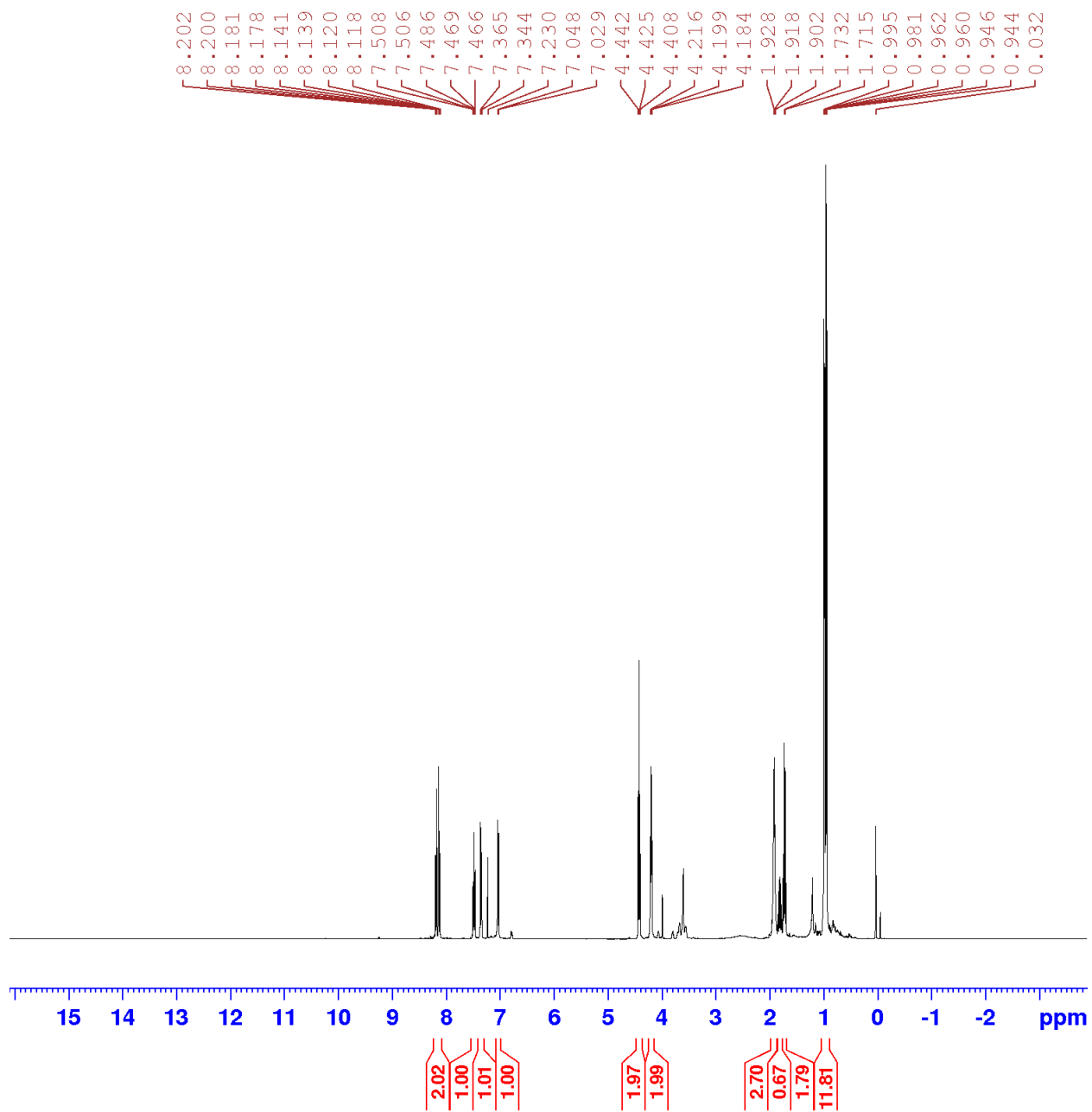


Figure S39: ¹H NMR of QD-11

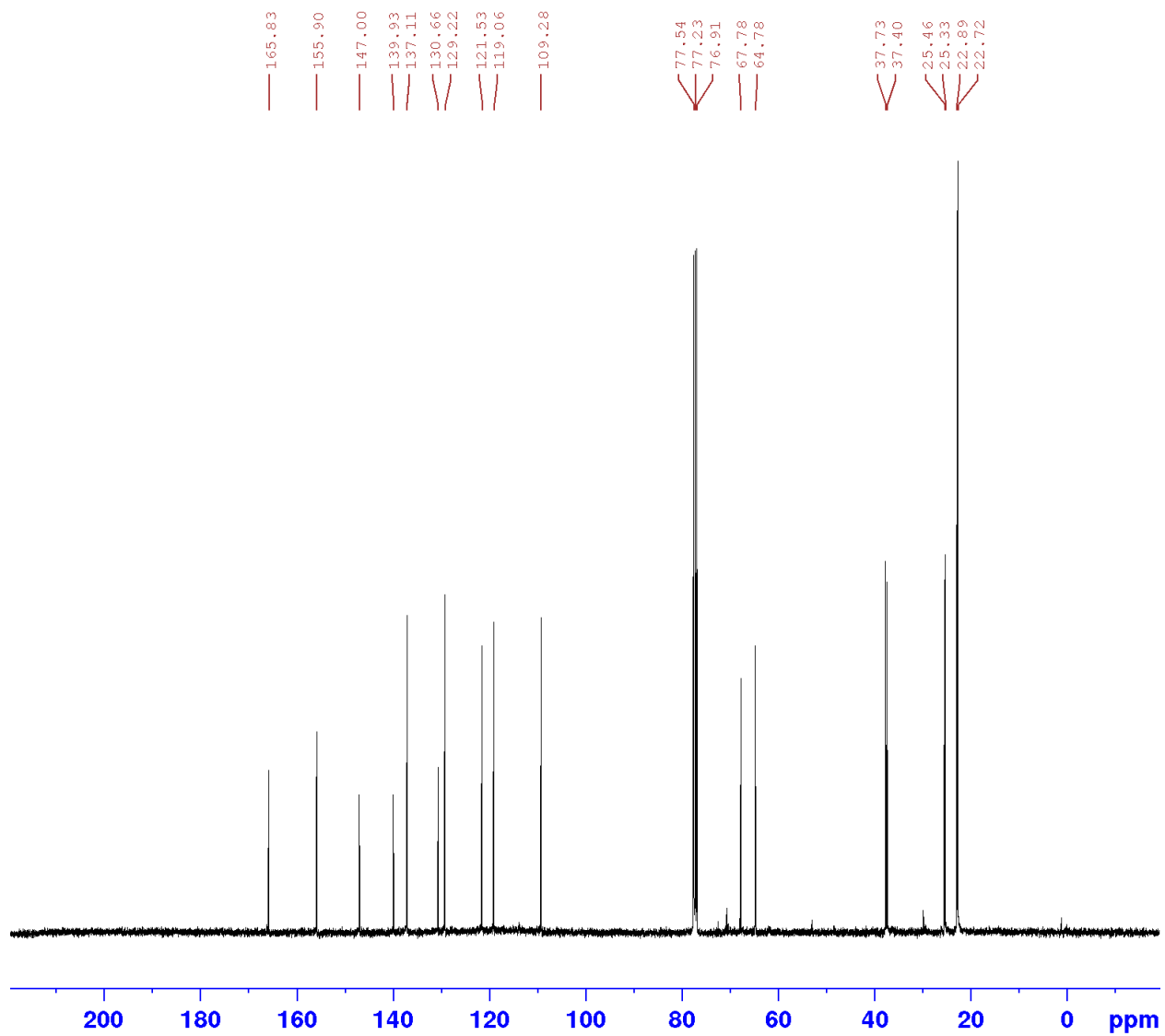


Figure S40: ^{13}C NMR of QD-11

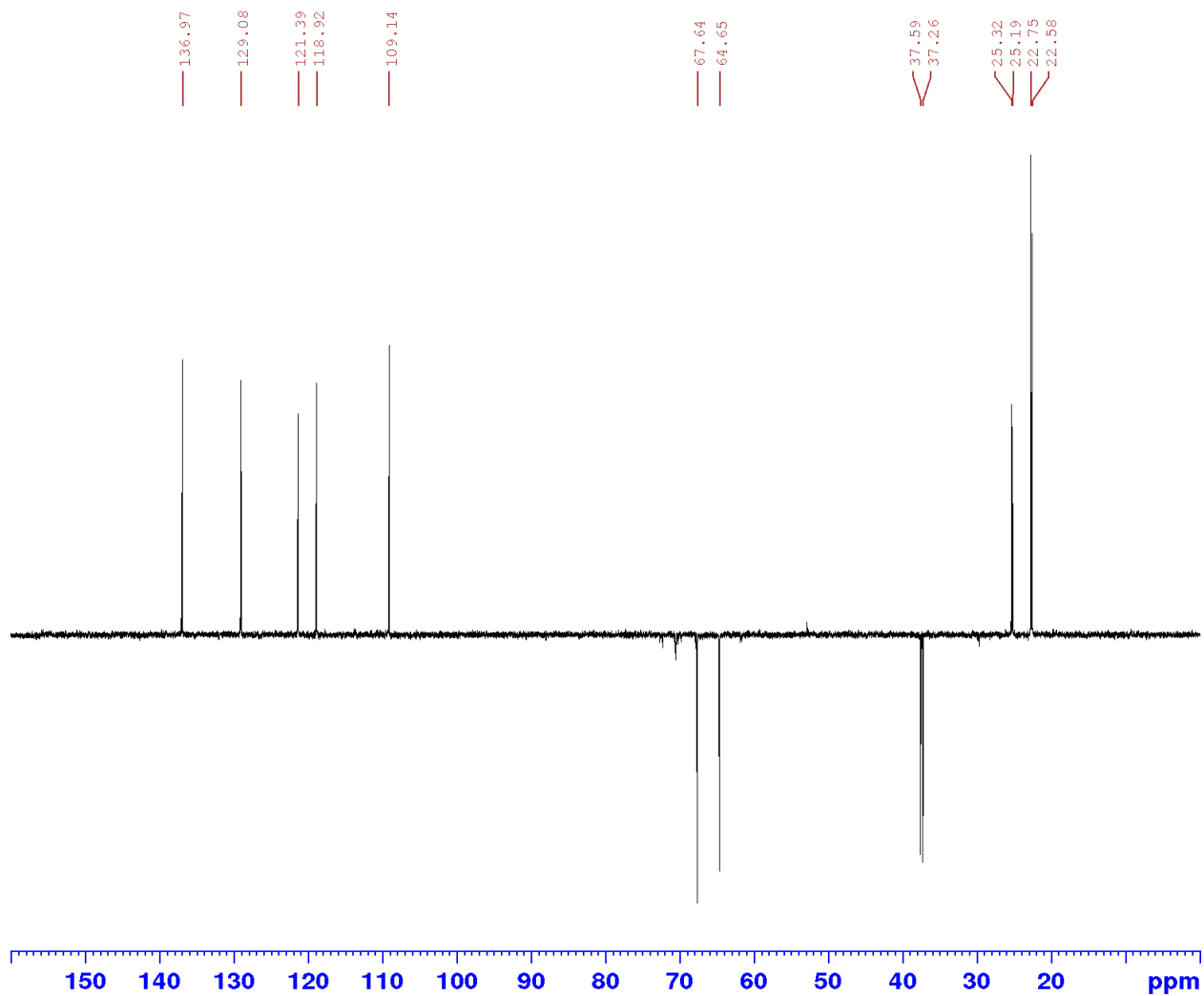


Figure S41: DEPT-135 NMR of QD-11

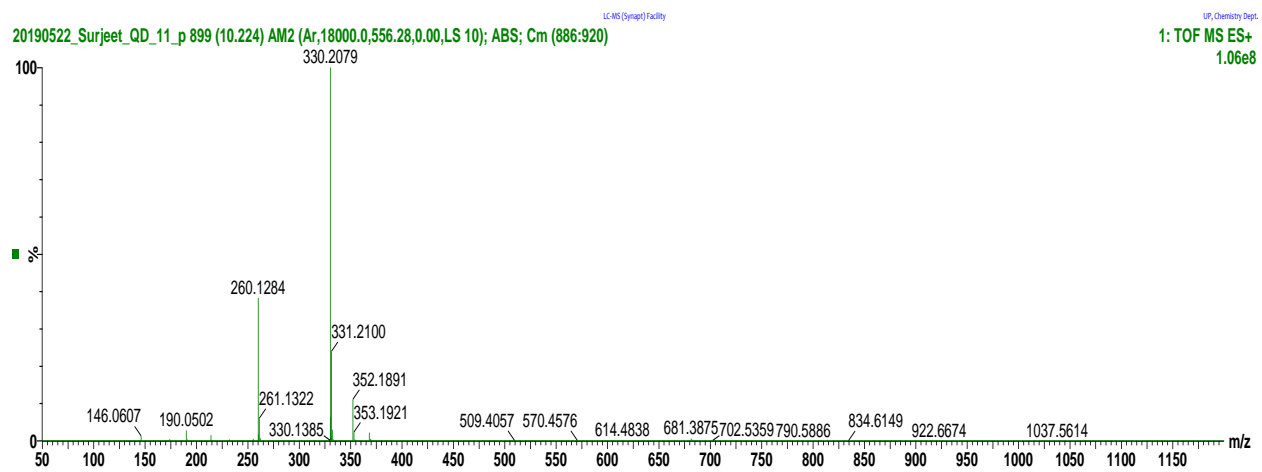


Figure S42: HRESI-MS of QD-11

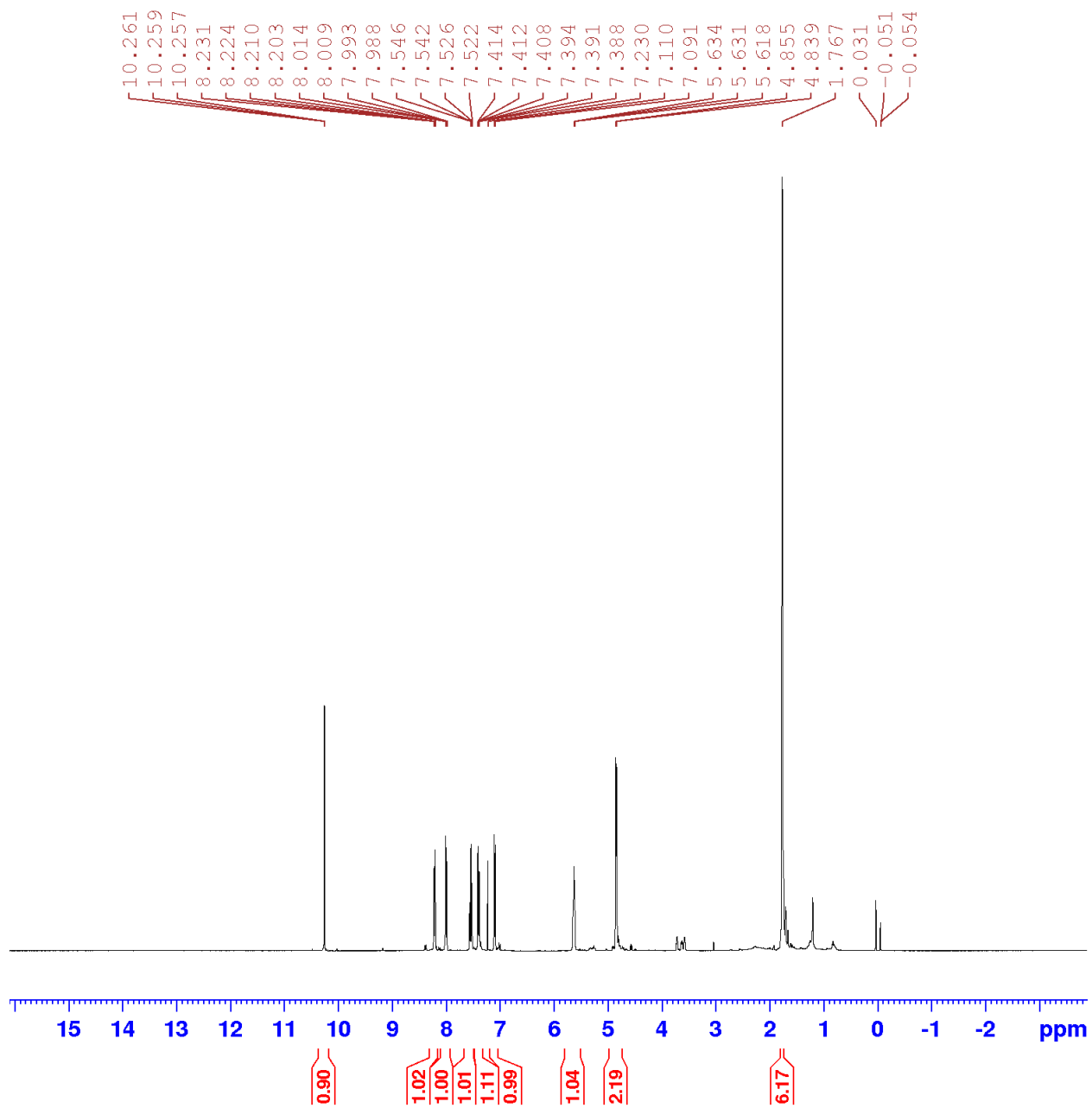


Figure S43: ¹H NMR of QD-12

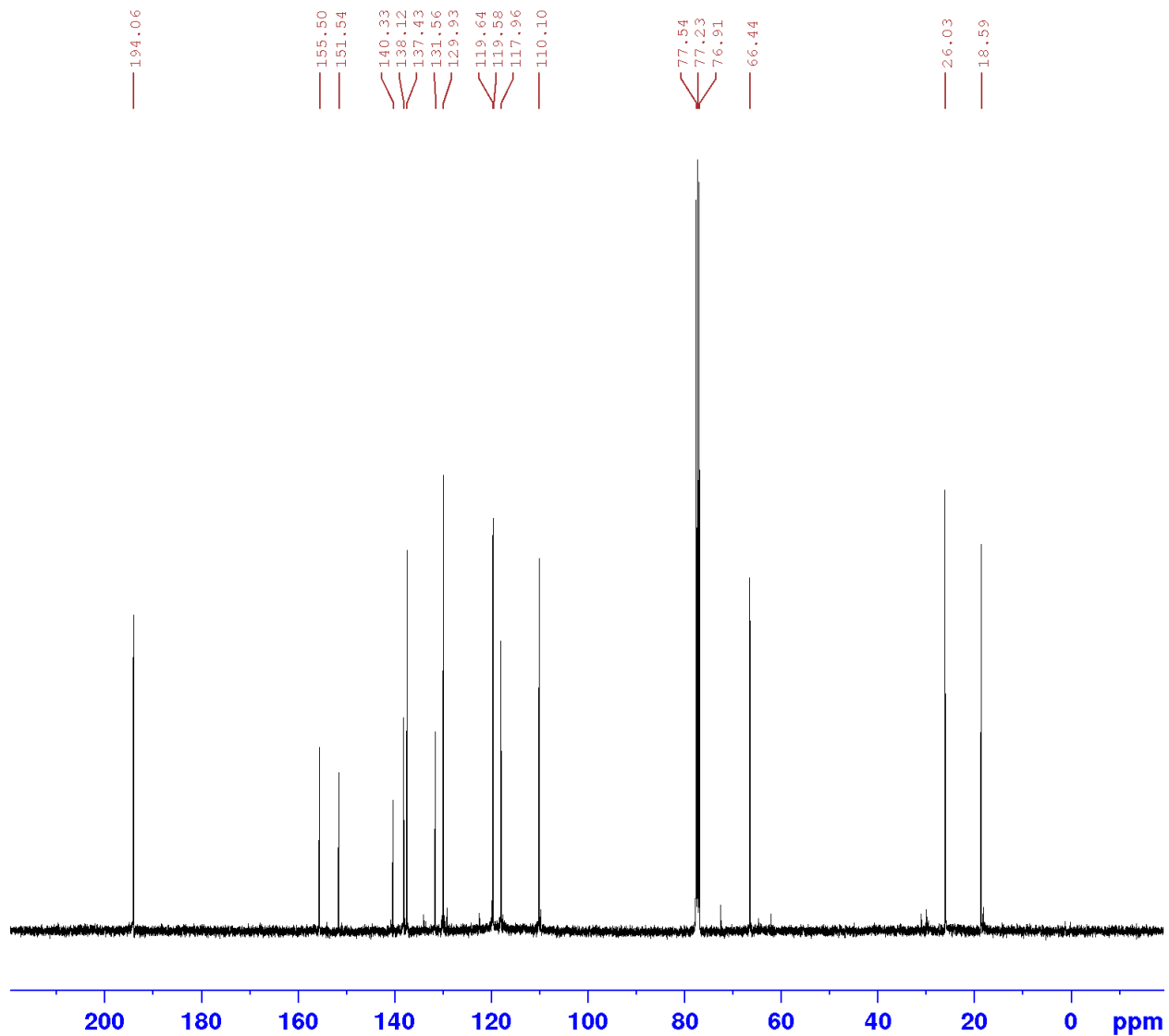


Figure S44: ^{13}C NMR of QD-12

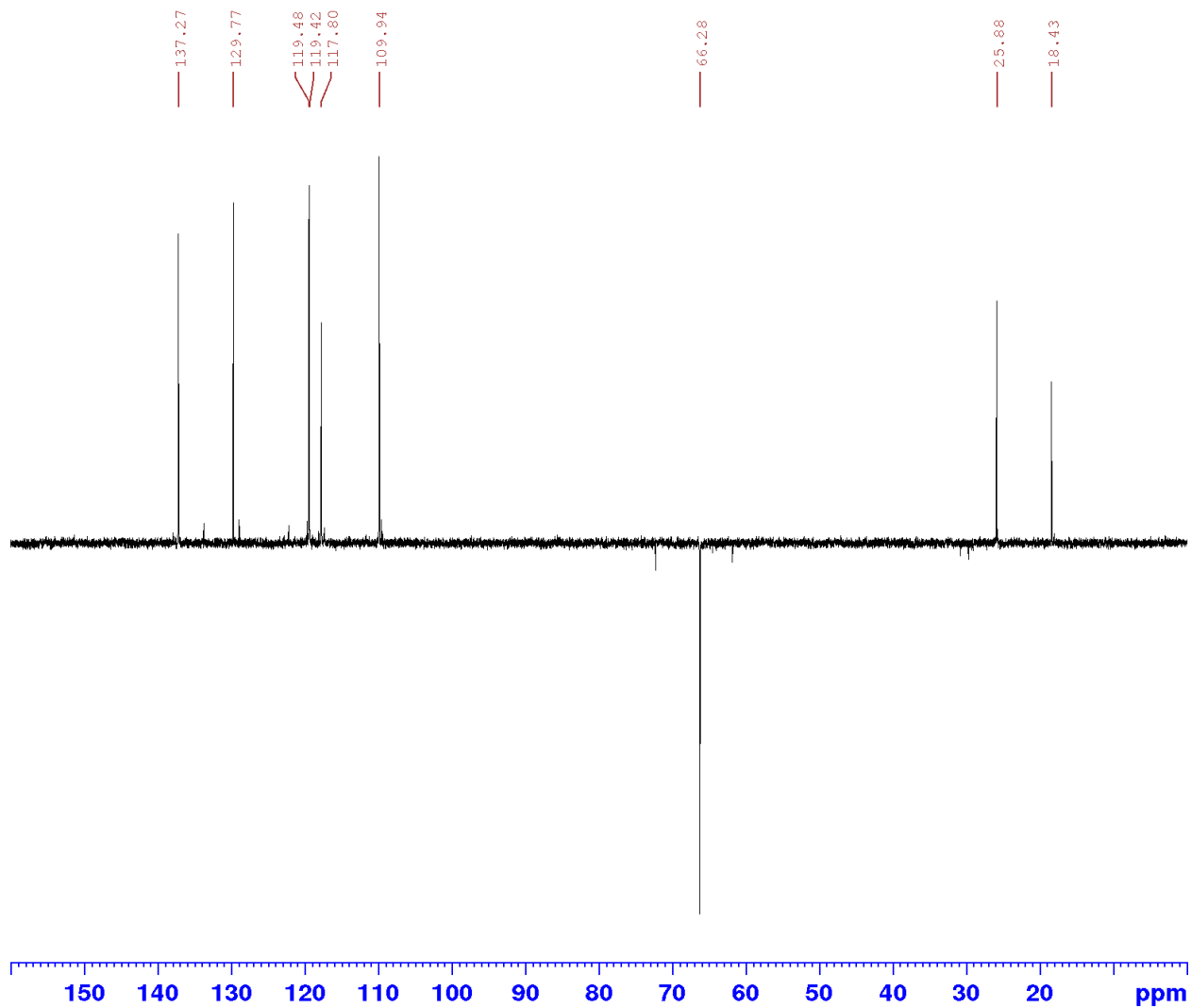


Figure S45: DEPT-135 NMR of QD-12

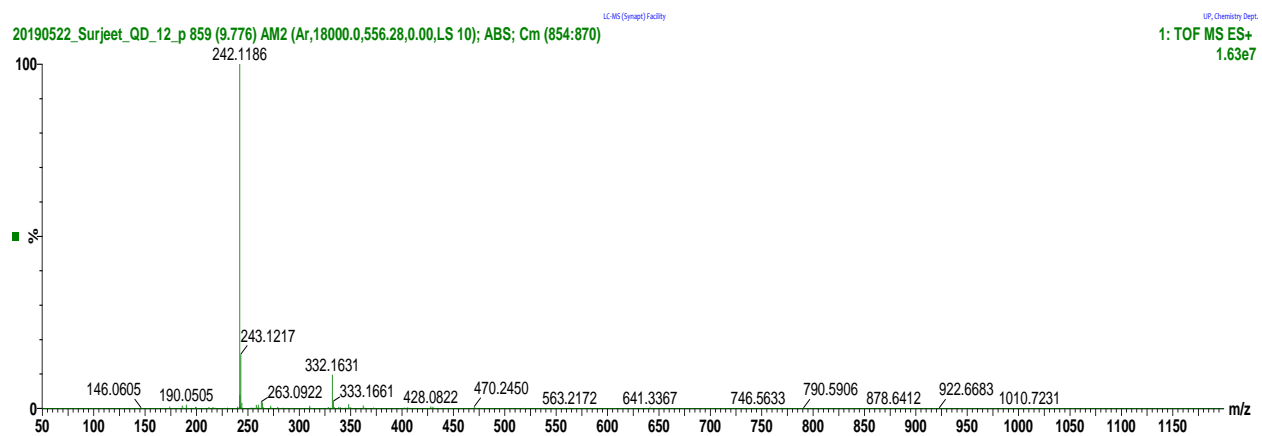


Figure S46: HRESI-MS NMR of QD-12

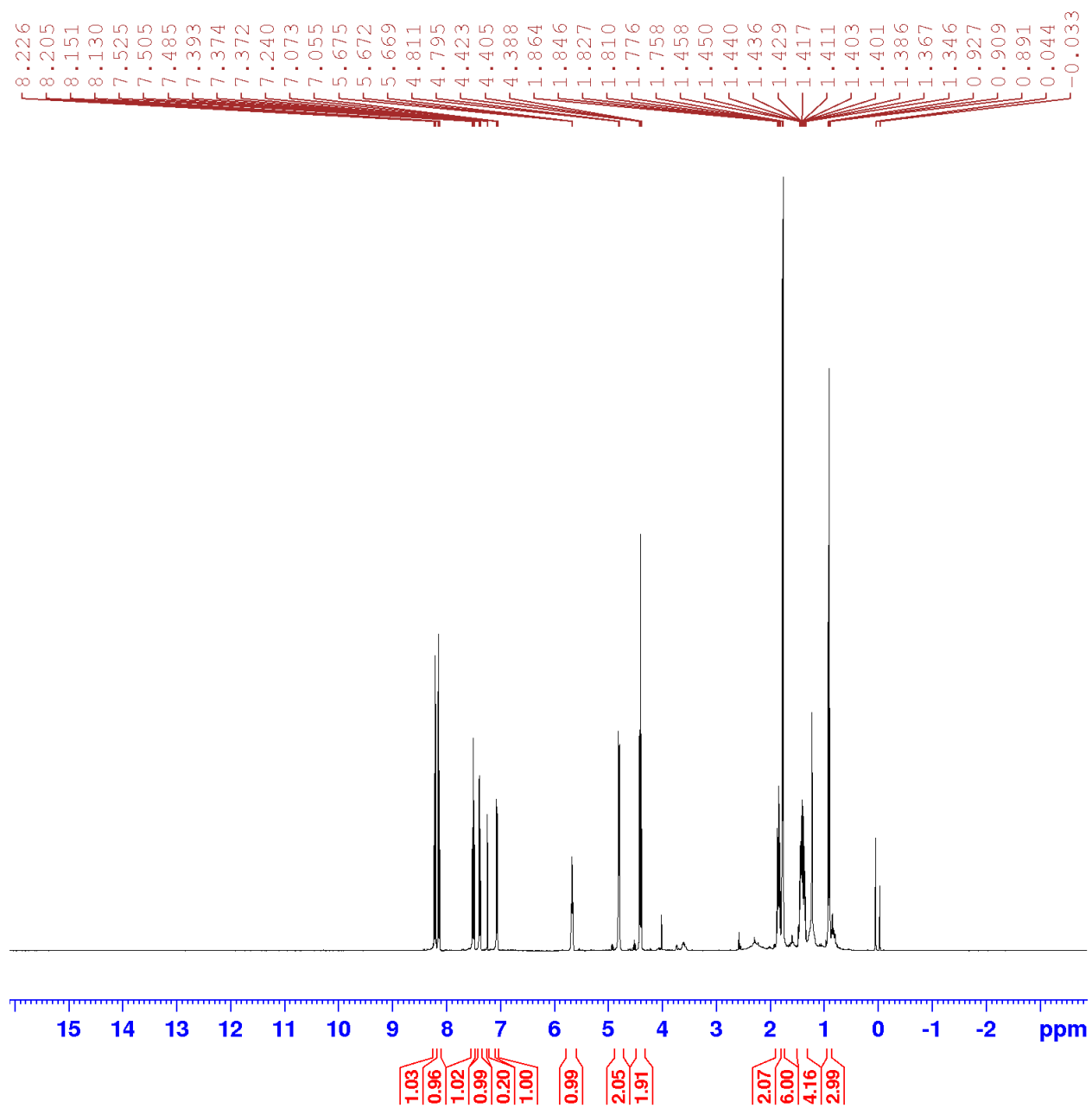


Figure S47: ¹H NMR of QD-12a

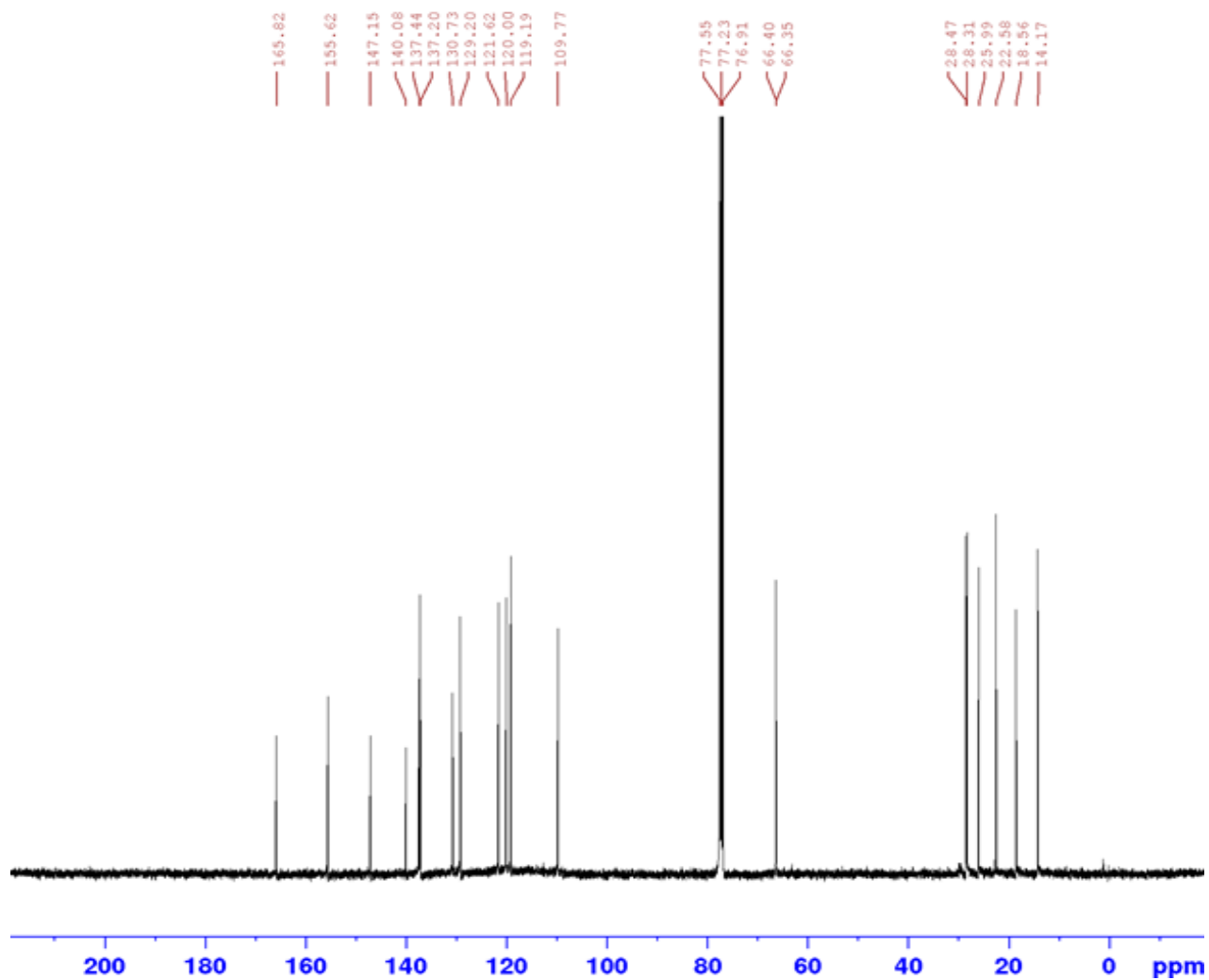


Figure S48: ^{13}C NMR of QD-12a

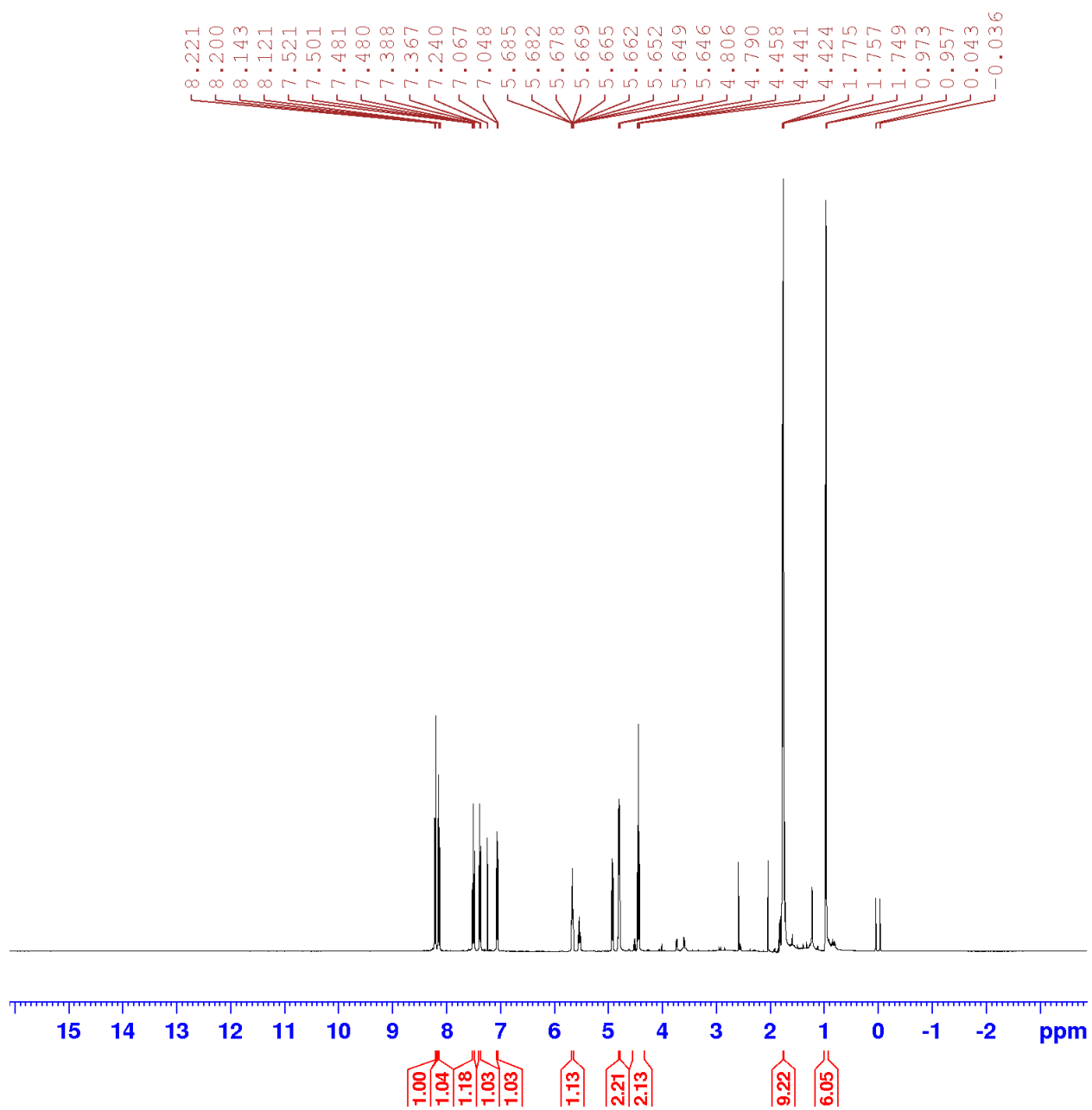


Figure S51: ¹H NMR of QD-12b

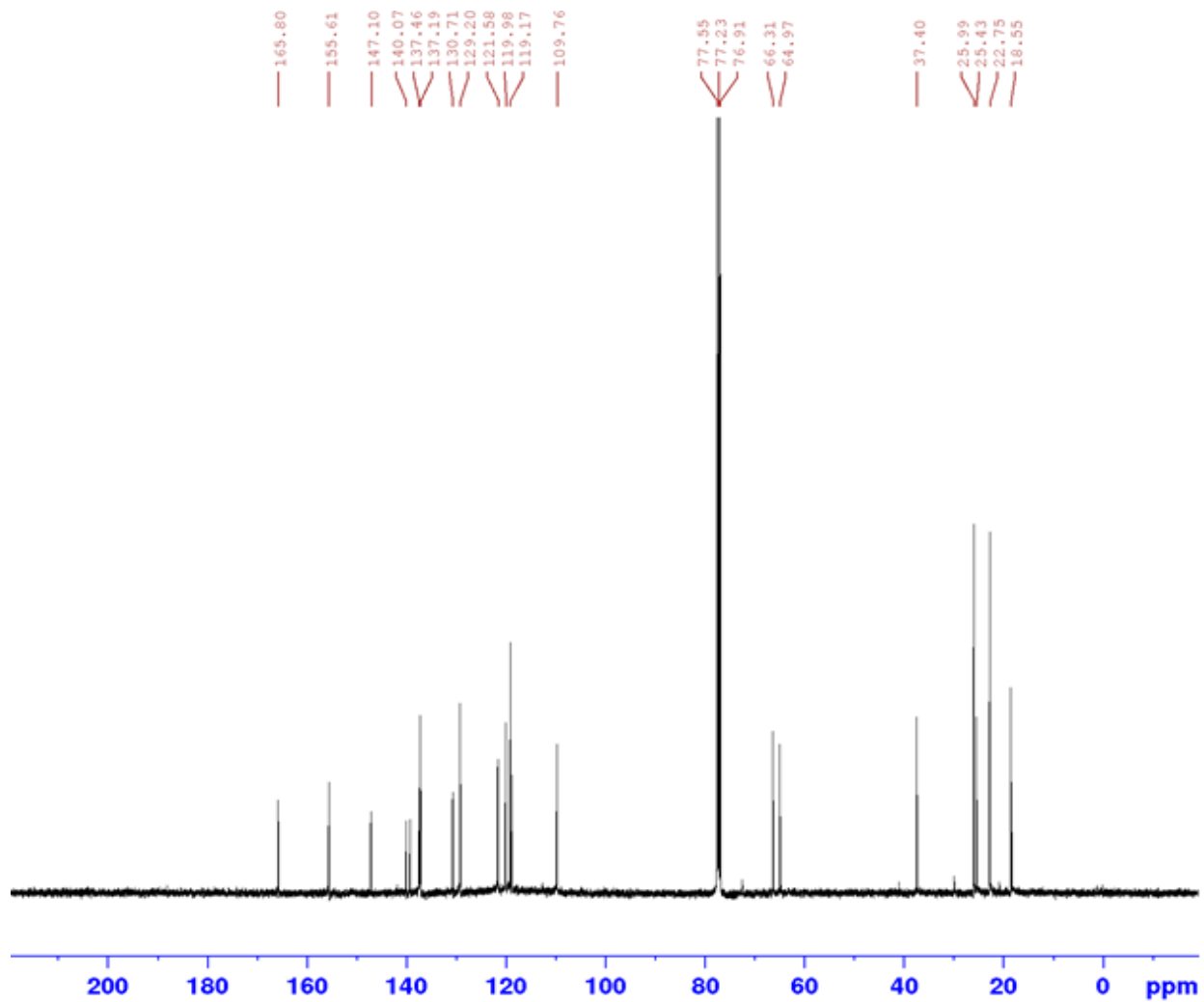


Figure S52: ^{13}C NMR of QD-12b

