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Supporting Information for

The requirements at the C-3 position of alkylquinolones for signalling in *Pseudomonas aeruginosa*

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General Considerations

Solvents and reagents were used as obtained from commercial sources and without purification. ^{1}H NMR (600 MHz) spectra and ^{1}H NMR (300 MHz) spectra were recorded on Bruker Avance 600 and Bruker Avance 300 NMR spectrometers respectively in proton coupled mode. ^{13}C NMR (150 MHz) spectra and ^{13}C NMR (75.5 MHz) spectra were recorded on Bruker Avance 600 and Bruker Avance 300 NMR spectrometers respectively in proton decoupled mode at 20 °C in deuterated dimethyl sulphoxide using tetramethysilane as internal standard. Low-resolution mass spectra were recorded on a Waters Quattro Micro triple quadropole instrument in electrospray ionisation (ESI) mode using 50% acetonitrile-water containing 0.1% formic acid as eluent; samples were made up in acetonitrile. High resolution precise mass spectra (HRMS) were recorded on a Waters LCT Premier Tof LC-MS instrument in electrospray ionisation (ESI) mode using 50% acetonitrile-water containing 0.1% formic acid as eluent; samples were made up in acetonitrile. Infrared spectra were measured as pressed potassium bromide (KBr) for solids or thin films on sodium chloride plates for liquids on a Perkin-Elmer FT-IR spectrometer.

Synthetic Procedures and Spectral Data

Methyl-3-oxodecanoate

2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's acid) (40 g, 270 mmol) was dissolved in distilled dichloromethane (400 mL). The solution was cooled to 0°C. To the cooled solution were added pyridine (45 mL, 556 mmol) and octanoyl chloride (51.1 mL, 299.5 mmol), drop-wise. The solution was stirred at 0°C for 1.5 h and then at room temperature for 1.5 h. The mixture was washed with 5% HCl (3 x 150 mL) and with distilled water (150 mL). The solution was then dried with anhydrous $MgSO_4$ filtered and concentrated *in vacuo* to yield acyl Meldrum's acid as a brown oil which was used in the subsequent step without further purification.

Acyl Meldrum's acid was dissolved in MeOH (360 mL) and heated at reflux for 5 h with constant stirring. After allowing to cool, the reaction mixture was concentrated *in vacuo* yielding the crude product as an orange oil. Purification was achieved by fractional distillation affording the β-keto ester as a pale yellow oil (40 g, 72 % yield). The analytical data was consistent with that previously published.[1]

2-Heptylquinolin-4(1*H*)-one (HHQ, 1)

To a solution of methyl 3-oxodecanoate (20g, 100 mmol) in hexane (150 mL) were added aniline (9.21 mL, 100 mmol) and *p*-toluene sulfonic acid (400 mg, 2 mmol). The reaction mixture was heated at reflux (>70°C) overnight using a Dean-Stark system. Upon completion, the reaction mixture was concentrated *in vacuo* to afford the crude β-enamino ester, which was then added drop-wise to refluxing diphenyl ether (30 mL, >260°C). Reflux was maintained for approx. 1.5 h. After cooling to room temperature, ether (approx. 400 mL) was added to the reaction mixture and left overnight at 5°C, allowing the quinolone product to precipitate. The quinolone was collected by vacuum filtration as a white solid (10.016 g, 41%), recrystallized from hot methanol (if necessary) and dried *in vacuo*. The analytical data was consistent with that previously published.[2]

2-Heptyl-6-nitroquinolin-4(1*H*)-one (2)[3]

HHQ (486 mg, 2 mmol) was dissolved in conc. H_2SO_4 (2.4 mL) and the solution was cooled down on an ice bath to 0ºC. In a separate vial, conc. H_2SO_4 (0.24 mL) and conc. HNO_3 (0.24 mL) were combined and also cooled on ice. The nitric acid solution was added dropwise over aprox. 5 min to the HHQ solution whilst maintaining the temperature between 0-5ºC. The resulting yellow solution was allowed to warm to room temperature with stirring for a further 2 h. Iced water (5 mL) was added to the solution and a solid crashed out. It was filtered and recrystallised from hot ether and the minimum amount of MeOH. Then was left on the fridge overnight. The white solid obtained was filtered, washed with ether several times and dried under vacuum (388 mg, 67 %).

Large Scale synthesis: HHQ (4.740 g, 19.5 mmol) was dissolved in conc. H_2SO_4 (23.4 mL) and the solution was cooled down on an ice bath to 0ºC. In a separate vial, conc. H_2SO_4 (2.3 mL) and conc. HNO_3 (2.3 mL) were combined and also cooled on ice. The nitric acid solution was added dropwise over aprox. 10 min to the HHQ solution whilst maintaining the temperature between 0-5ºC. The resulting yellow solution was allowed to warm to room temperature with stirring for a further 2 h. Iced water (50 mL) was added to the black solution and a solid crashed out. It was filtered and recrystallised from hot ether and the minimum amount of MeOH. Then was left on the fridge overnight. The white solid obtained was filtered, washed with ether several times and dried under vacuum (3.737 g, 66 %).

White solid; yield 3.737 g (66 %); m.p. = 183-185°C (Et_2O); IR (KBr): ν 3102, 2952, 1659, 1616, 1524, 1494, 1371, 1356, 1194 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 0.86 (3H, t, *J* = 6.8 Hz), 1.20-1.40 (8H, m), 1.65-1.75 (2H, m), 2.68 (2H, t, *J* = 7.7 Hz), 6.22 (1H, s), 7.78 (1H, d, *J* = 9.2 Hz), 8.44 (1H, dd, *J* = 9.2, ^4J = 2.7 Hz), 8.82 (1H, d, ^4J = 2.6 Hz), 12.34 (1H, bs). ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 13.9, 22.0, 28.1, 28.3, 28.4, 31.1, 33.3, 108.8, 120.0, 121.3, 123.0, 126.0, 142.3, 143.7, 156.3, 175.6; HRMS calcd. (%) for $C_{16}H_{21}N_2O_3$: 289.1552; found: 289.1538.

Methyl 2-methyl-3-oxodecanoate[2]

To a round bottomed flask containing dry potassium carbonate (2.46 g, 17.8 mmol) was added a solution of methyl 3-oxodecanoate (2.74 g, 13.7 mmol) in acetone (35 mL). The resulting mixture was allowed to stir for 20 min before the addition of methyliodide (1.02 mL, 16.4 mmol). The reaction mixture was allowed to stir at reflux for 6 h. The mixture was removed from the heat, allowed to cool and the solvent removed *in vacuo* to yield crude product which was used in the next step without further purification.

2-Heptyl-3-methylquinolin-4(1*H*)-one[2]

To a solution of methyl 2-methyl-3-oxodecanoate in hexane (26.4 mL) were added aniline (1.2 mL, 13.2 mmol) and *p*-toluene sulfonic acid (53 mg, 0.26 mmol). The reaction mixture was heated at reflux (>70°C) overnight using a Dean-Stark system. Upon completion, the reaction mixture was concentrated *in vacuo* to afford the crude β-enamino ester, which was then added drop-wise to refluxing diphenyl ether (7 mL, >260°C). Reflux was maintained for approx. 1.5 h. After cooling to room temperature, ether (approx. 20 mL) was added to the reaction mixture and left overnight at 5°C, allowing the quinolone product to precipitate. The quinolone was collected by vacuum filtration as a white solid (10.016 g, 41%), recrystallized from hot methanol (if necessary) and dried *in vacuo* (261 mg, 8%).

2-Heptyl-3-methyl-6-nitroquinolin-4(1*H*)-one (3)

2-Heptyl-3-methylquinolin-4(1*H*)-one (166 mg, 0.64 mmol) was dissolved in conc. H_2SO_4 (1.2 mL) and the solution was cooled down on an ice bath to 0ºC. In a separate vial, conc. H_2SO_4 (0.1 mL) and conc. HNO_3 (0.1 mL) were combined and also cooled on ice. The nitric acid solution was added

dropwise over aprox. 5 min to the quinolone solution whilst maintaining the temperature between 0-5ºC. The resulting yellow solution was allowed to warm to room temperature with stirring for a further 3 h. Iced water (5 mL) was added to the solution and a solid crashed out. It was filtered and recrystallised from hot ether and the minimum amount of MeOH. Then was left on the fridge overnight. The white solid obtained was filtered, washed with ether several times and dried under vacuum.

Yellow solid; yield 30 mg (16 %); m.p. = > 250°C (Et_2O); IR (KBr): ν 3252, 3103, 2929, 1640, 1606, 1559, 1497, 1345, 1222 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 0.86 (3H, t, *J* = 6.5 Hz), 1.20-1.45 (8H, m), 1.55-1.75 (2H, m), 2.02 (3H, s), 2.71 (2H, t, *J* = 7.8 Hz), 7.68 (1H, d, *J* = 9.2 Hz), 8.37 (1H, dd, *J* = 9.2, ^4J = 2.7 Hz), 8.84 (1H, d, ^4J = 2.6 Hz), 11.91 (1H, bs). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 10.2, 13.9, 22.0, 28.1, 28.4, 28.8, 31.2, 31.6, 115.9, 119.4, 121.6, 121.9, 125.2, 142.1, 142.8, 151.0, 175.9; HRMS calcd. (%) for $C_{17}H_{23}N_2O_3$: 303.1709; found: 303.1704.

3-Chloro-2-heptyl-6-nitroquinolin-4(1*H*)-one (4)

To a stirred solution of 2-heptyl-6-nitroquinolin-4(1*H*)-one (576 mg, 2 mmol) in MeOH (33 mL), sodium dichloroisocyanurate (242 mg, 1.1 mmol) and water (7 mL) were added and the reaction mixture allowed to stir at 20 ºC overnight. The mixture was then filtered and the solid washed several times with MeOH. The solid was recrystallised (if necessary) from hot MeOH.

White solid; yield 159 mg (25 %); m.p. = > 250°C (Et_2O); IR (KBr): ν 3239, 3108, 2925, 1641, 1601, 1569, 1500, 1363, 1343, 1155 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 0.86 (3H, t, *J* = 6.8 Hz), 1.20-1.45 (8H, m), 1.65-1.80 (2H, m), 2.87 (2H, t, *J* = 7.9 Hz), 7.77 (1H, d, *J* = 9.2 Hz), 8.44 (1H, dd, *J* = 9.2, ^4J = 2.7 Hz), 8.86 (1H, d, ^4J = 2.6 Hz), 12.53 (1H, bs). ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 13.9, 22.0, 27.4, 28.3, 28.6, 31.0, 32.6, 115.1, 120.0, 121.8, 122.4, 125.9, 142.1, 142.8, 152.2, 170.6; HRMS calcd. (%) for $C_{16}H_{20}ClN_2O_3$: 323.1162; found: 323.1153.

3-Bromo-2-heptyl-6-nitroquinolin-4(1*H*)-one (5)

To a stirred solution of 2-heptyl-6-nitroquinolin-4(1*H*)-one (576 mg, 2 mmol) in acetic acid (4 mL) was added a solution of bromine (0.12 mL, 2.3 mmol) in acetic acid (1.2 mL) dropwise over 10 min. The reaction vessel was covered in aluminium foil and the reaction allowed to stir at 20ºC for 5h. On completion, the mixture was then poured into 1% aqueous sodium sulfite (30 mL). The precipitate was filtered and washed with ether. The solid was recrystallised from EtOH.

White solid; yield 436 mg (59 %); m.p. = > 250°C (Et_2O); IR (KBr): ν 3234, 3107, 2922, 1640, 1597, 1557, 1495, 1343, 1148 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 0.85 (3H, t, *J* = 6.7 Hz), 1.20-1.45 (8H, m), 1.60-1.75 (2H, m), 2.86 (2H, t, *J* = 7.9 Hz), 7.72 (1H, d, *J* = 9.2 Hz), 8.40 (1H, dd, *J* = 9.2, ^4J = 2.7 Hz), 8.80 (1H, d, ^4J = 2.6 Hz), 12.46 (1H, bs). ^{13}C-NMR (75 MHz, DMSO-d_6) δ:13.9, 22.0, 27.5,

28.3, 28.6, 31.1, 34.6, 107.2, 119.9, 121.6, 122.0, 126.0, 142.3, 142.9, 153.6, 170.9; HRMS calcd. (%) for $C_{16}H_{20}BrN_2O_3$: 367.0657; found: 367.0654.

2-Heptyl-3-iodo-6-nitroquinolin-4(1*H*)-one (6)

To a stirred solution of 2-heptyl-6-nitroquinolin-4(1*H*)-one (1.973 g, 6.85 mmol) in glacial acetic acid (50 mL) was added *N*-iodosuccinimide (1.575 g, 7 mmol) portionwise and the reaction mixture allowed to stir at 20ºC for 2 h. The precipitate was filtered and washed with ether. The solid was recrystallised from Ether:MeOH (if needed).

Pale yellow solid; yield 2.426 g (86 %); m.p. = 236-240°C (Et_2O); IR (KBr): ν 3229, 3100, 2926, 1637, 1607, 1568, 1488, 1338, 1141, 836 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 0.85 (3H, t, *J* = 6.7 Hz), 1.20-1.45 (8H, m), 1.60-1.75 (2H, m), 2.90 (2H, t, *J* = 7.8 Hz), 7.70 (1H, d, *J* = 9.2 Hz), 8.38 (1H, dd, *J* = 9.1, ^4J = 2.5 Hz), 8.77 (1H, d, ^4J = 2.4 Hz), 12.48 (1H, bs). ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 13.9, 22.0, 27.8, 28.3, 28.7, 31.1, 38.8, 87.5, 119.4, 119.7, 122.2, 126.0, 142.6, 143.0, 156.1, 172.9; HRMS calcd. (%) for $C_{16}H_{20}IN_2O_3$: 415.0519; found: 415.0510.

3-Fluoro-2-heptyl-6-nitroquinolin-4(1*H*)-one (7)

To a stirred solution of Selectfluor® (2.140 g, 6.8 mmol) in EtOH (28 mL) was added 2-heptyl-6-nitroquinolin-4(1*H*)-one (1.152 g, 4 mmol). The reaction was allowed to stir at 20ºC for 7 days. The reaction mixture was filtered and solvent removed *in vacuo* to yield the crude product. Purification was achieved using silica column chromatography eluting with 98:2 DCM:MeOH.

Yellow solid; yield 55 mg (4 %); m.p. = 236-239°C (Et_2O); IR (KBr): ν 3246, 3081, 2957, 1647, 1587, 1503, 1378, 1344 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 0.85 (3H, t, *J* = 6.3 Hz), 1.20-1.40 (8H, m), 1.65-1.75 (2H, m), 2.77 (2H, t, *J* = 6.4 Hz), 7.76 (1H, d, *J* = 9.2 Hz), 8.41 (1H, dd, *J* = 9.0, ^4J = 1.9 Hz), 8.87 (1H, d, ^4J = 1.9 Hz), 12.30 (1H, bs). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 13.9, 22.0, 27.3, 27.4, 28.3, 28.5, 31.1, 120.0, 121.5 (d, $^4J_{(C,F)}$ = 4.0 Hz), 124.6 (d, $^3J_{(C,F)}$ = 10.3 Hz), 125.5, 141.7, 141.9 (d, $^2J_{(C,F)}$ = 26.5 Hz), 142.2, 145.2 (d, $^1J_{(C,F)}$ = 233.4 Hz), 166.9 (d, $^2J_{(C,F)}$ = 15.0 Hz); ^{19}F NMR (282 MHz, DMSO-d_6): δ -160.4. HRMS calcd. (%) for $C_{16}H_{20}FN_2O_3$: 307.1458; found: 307.1452.

2-Heptyl-3-iodo-quinolin-4(1*H*)-one[3]

To a stirred solution of HHQ (729 g, 3 mmol) in glacial acetic acid (22 mL) was added *N*-iodosuccinimide (690 mg, 3.07 mmol) portionwise and the reaction mixture allowed to stir at 20ºC for 3 h. The precipitate was filtered and washed with ether (950 mg, 86%).

3-Deutero-2-heptylquinolin-4(1*H*)-one

Zinc dust (834 mg, 13.3 mmol) and 2-heptyl-3-iodo-quinolin-4(1*H*)-one (950 mg, 2.57 mmol) were evacuated and refilled with nitrogen three times in a dry Schlenk tube. Mono*deutero*acetic acid (15 ml)

(previously degassed by three freeze-pump-thaw cycles and stored under nitrogen) was added and the mixture was stirred at 20ºC under nitrogen overnight. Potassium carbonate (9 g) in deuterium oxide (5 mL) was added to basify the mixture and was allowed to stir under nitrogen for 10 min. DCM (*ca*. 50 mL) was used to dissolve the solid that formed. The mixture was filtered. The aqueous layer was washed again with DCM (2 x 75 ml). The organic layers were combined, dried over $MgSO_4$, evaporated *in vacuo* giving the title compound as a white solid (535 mg, 85%,). 94% deuteration was indicated by integration of the H-3 singlet in ^{1}H NMR.

3-Deutero-2-heptyl-6-nitroquinolin-4(1*H*)-one (8)

3-Deutero-2-heptylquinolin-4(1*H*)-one (535 mg, 2.19 mmol) was dissolved in conc. H_2SO_4 (2.4 mL) and the solution was cooled down on an ice bath to 0ºC. In a separate vial, conc. H_2SO_4 (0.24 mL) and conc. HNO_3 (0.24 mL) were combined and also cooled on ice. The nitric acid solution was added dropwise over aprox. 5 min to the HHQ solution whilst maintaining the temperature between 0-5ºC. The resulting yellow solution was allowed to warm to room temperature with stirring for a further 2 h. Iced water (5 mL) was added to the solution and a solid crashed out. It was filtered and recrystallised from hot ether and the minimum amount of MeOH. Then was left on the fridge overnight. The pale yellow solid obtained was filtered, washed with ether several times and dried under vacuum. 92% deuteration was indicated by integration of the H-3 singlet in ^{1}H NMR.

Pale yellow solid; yield 85 mg (13 %); m.p. = 174-176°C (Et_2O); IR (KBr): ν 3228, 3108, 2921, 1655, 1611, 1460, 1412, 1350, 1193cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 0.86 (3H, t, *J* = 6.8 Hz), 1.20-1.40 (8H, m), 1.60-1.75 (2H, m), 2.65 (2H, t, *J* = 7.5 Hz), 6.13 (0.08H, s), 7.73 (1H, d, *J* = 9.0 Hz), 8.42 (1H, dd, *J* = 9.1, ^4J = 2.7 Hz), 8.81 (1H, d, ^4J = 2.7 Hz), 12.10 (1H, bs). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 14.0, 22.1, 28.36, 28.44, 28.5, 31.2, 33.4, 108.2 (d, $J_{(C,D)}$ = 22.9 Hz), 120.5, 121.1, 122.3, 126.4, 143.2, 143.4, 157.9, 174.7; HRMS calcd. (%) for $C_{16}H_{20}DN_2O_3$: 290.1615; found: 290.1611.

Methyl 2-heptyl-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate

To a suspension of 55-65% NaH (3.84 g, 8 mmol) in dry DMF (24 mL) was added a solution of methyl 3-oxononanoate (1.602 g, 8 mmol) in dry DMF (16 mL) via a dropping funnel under a nitrogen atmosphere, and the reaction mixture stirred at room temperature. Once the evolution of H_2 had ceased, a solution of 5-nitroisotoic anhydride (1.498 g, 7.2 mmol) in dry DMF (15 mL) was added dropwise via a dropping funnel. The reaction mixture (orange) was then stirred at room temperature overnight. The reaction mixture was concentrated *in vacuo* (Kugelrohr) to approximately 25% original volume. 1M HCl was then added to precipitate out the crude product, which was collected by vacuum filtration and dried *in vacuo* (yellow solid). The crude product was recrystallized from hot MeOH, allowed to crash out on ice, collected and dried *in vacuo* to give a pale yellow solid (0.859 g, 2.48 mmol, 31%).

Pale yellow solid; yield 0.859 g (31%); m.p. = 235-239°C (MeOH); IR (KBr): ν 2950, 2928, 1727, 1661, 1615, 1504, 1343 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ: 0.86 (3H, t, *J* = 6.8 Hz), 1.18-1.65 (8H, m), 1.66 (2H, m), 2.65 (2H, t, *J* = 7.8 Hz), 3.80 (3H, s), 7.77 (1H, d, *J* = 9.1 Hz), 8.45 (1H, dd, *J* = 9.1, ^{4}J = 2.6 Hz), 8.81 (1H, d, *J* = 2.6 Hz), 12.30 (1H, bs); ^{13}C-NMR (75 MHz, DMSO-d_6): δ: 14.4, 22.5, 28.7, 29.1, 29.2 31.5, 32.5, 52.5, 116.2, 120.6, 122.0, 124.1, 127.0, 143.5, 143.6, 154.7, 167.1, 173.6; HRMS calcd. (%) for $C_{18}H_{23}N_2O_5$: 347.1607; found: 347.1594.

2-Heptyl-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid[4]

A suspension of methyl 2-heptyl-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (0.375 g, 1.08 mmol) in 10% aqueous NaOH (77 mL) was refluxed at 97^{0}C with stirring for 4 hours (yellow suspension to orange solution). The reaction mixture was then cooled and extracted with two 5 mL portions of ethyl acetate. The aqueous layer was then acidified to pH 4.5 with concentrated HCl (orange solution to yellow solution) on ice, causing a white precipitate to crash out. This was then collected via vacuum filtration, and recrystallized in hot methanol to yield a white solid (0.209 g, 0.63 mmol, 58%).

White solid; yield 0.209 g (58%); m.p. = 194-197°C (MeOH); IR (KBr) ν 3440, 3041, 2932, 2852, 1681, 1418 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ: 0.87 (3H, t, *J* = 6.7 Hz), 1.21-1.48 (8H, m), 1.62-1.75 (2H, quin, *J* = 7.8 Hz), 3.21-3.32 (2H, m overlapping with H_2O of DMSO), 7.93 (1H, d, *J* = 9.2 Hz), 8.60 (dd, 1H, *J* = 9.2, ^{4}J = 2.7 Hz), 8.94 (1H, d, *J* = 2.7 Hz), 13.23 (1H, bs), 15.67 (1H, bs); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 14.4, 22.5, 28.8, 29.6 (2 x C), 31.6, 33.9, 108.2, 121.4, 122.0, 123.2, 128.1, 142.3, 144.7, 164.5, 166.0, 179.0; HRMS calcd (%) for $C_{17}H_{21}N_2O_5$: 333.1450; found: 333.1451.

2-Heptyl-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxamide (9)[4]

N,*N*'-Carbonyldiimidazole (0.195 g, 1.2 mmol) was added to a solution of 2-heptyl-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (0.2 g, 0.6 mmol) in dry DMF (3 mL), and the reaction mixture stirred for 4 hours at 65^{0}C (yellow to orange solution). The cooled reaction mixture was then added to iced concentrated $NH_3.H_2O$ (15 mL) and the resulting solution stirred at room temperature overnight. The reaction mixture was then concentrated by approximately 50% *in vacuo* (Kugelrohr) and iced water (10 mL) was added to precipitate the solid product (pale yellow) which was collected by vacuum filtration and dried in *in vacuo* (pale yellow solid, 0.129 g, 0.39 mmol, 65%).

Pale yellow solid; yield 0.129 g (65%); m.p. = 238-241 °C; IR (KBr) ν 3392, 2976, 1651, 1502, 1409, 1342 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ: 0.86 (3H, t, *J* = 6.8 Hz), 1.20-1.40 (8H, m) 1.70 (2H, quin, *J* = 7.3 Hz), 3.01 (2H, t, *J* = 7.3 Hz), 7.35 (1H, bs), 7.76 (1H, d, *J* = 9.0 Hz), 8.45 (1H, dd, *J* = 9.0, ^{4}J = 2.6 Hz), 8.55 (1H, bs), 8.87 (1H, d, *J* = 2.6 Hz), 12.30 (1H, bs); ^{13}C-NMR (75 MHz, DMSO-d_6): δ: 14.4, 22.5, 28.8, 29.5, 29.7, 31.6, 33.2, 115.8, 120.4, 122.3, 124.4, 126.8, 142.9, 143.6, 158.2, 167.2, 175.5; HRMS calcd (%) for $C_{17}H_{21}N_3O_4$: 332.1610; found: 332.1597.

2-Heptyl-*N*-methyl-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxamide (10)

N,*N*'-Carbonyldiimidazole (0.0195 g, 0.12 mmol) was added to a solution of 2-heptyl-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (0.02 g, 0.06 mmol) in dry DMF (1 mL), and the reaction mixture stirred for 4 hours at 65^0C (yellow to orange solution). The cooled reaction mixture was then added to iced $NH_2CH_3.H_2O$ (1.5 mL, 40%) and the resulting solution stirred at room temperature overnight. The reaction mixture was then added to iced water (5 mL) to precipitate the solid product (pale yellow) which was collected by vacuum filtration and dried in *in vacuo*. (0.0110 g, 0.0318 mmol, 53%).

Pale yellow solid; yield 11 mg (53%); m.p. = 243-245°C; IR (KBr) ν 3302, 3100, 2928, 1634, 1568, 1501, 1337, 1216 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ: 0.86 (3H, t, *J* = 6.6 Hz), 1.22-1.42 (8H, m) 1.67 (2H, m), 2.77 (3H, d, *J* = 4.0 Hz), 2.94 (2H, t, *J* = 8.0 Hz), 7.77 (1H, d, *J* = 9.1 Hz), 8.45 (1H, dd, *J* = 9.1, ^4J = 2.7 Hz), 8.86 (1H, d, *J* = 2.6 Hz), 8.98 (1H, bs), 12.32 (1H, bs); ^{13}C-NMR (75 MHz, DMSO-d_6): δ: 14.4, 22.5, 26.2, 28.8, 29.5, 29.7, 31.6, 33.1, 116.0, 120.6, 122.3, 124.3, 126.7, 143.1, 143.4, 157.8, 165.8, 175.3; HRMS calcd. (%) for $C_{18}H_{23}N_3O_4$: 346.1767; found: 346.1773.

2-Heptyl-*N*,*N*-dimethyl-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxamide (11)

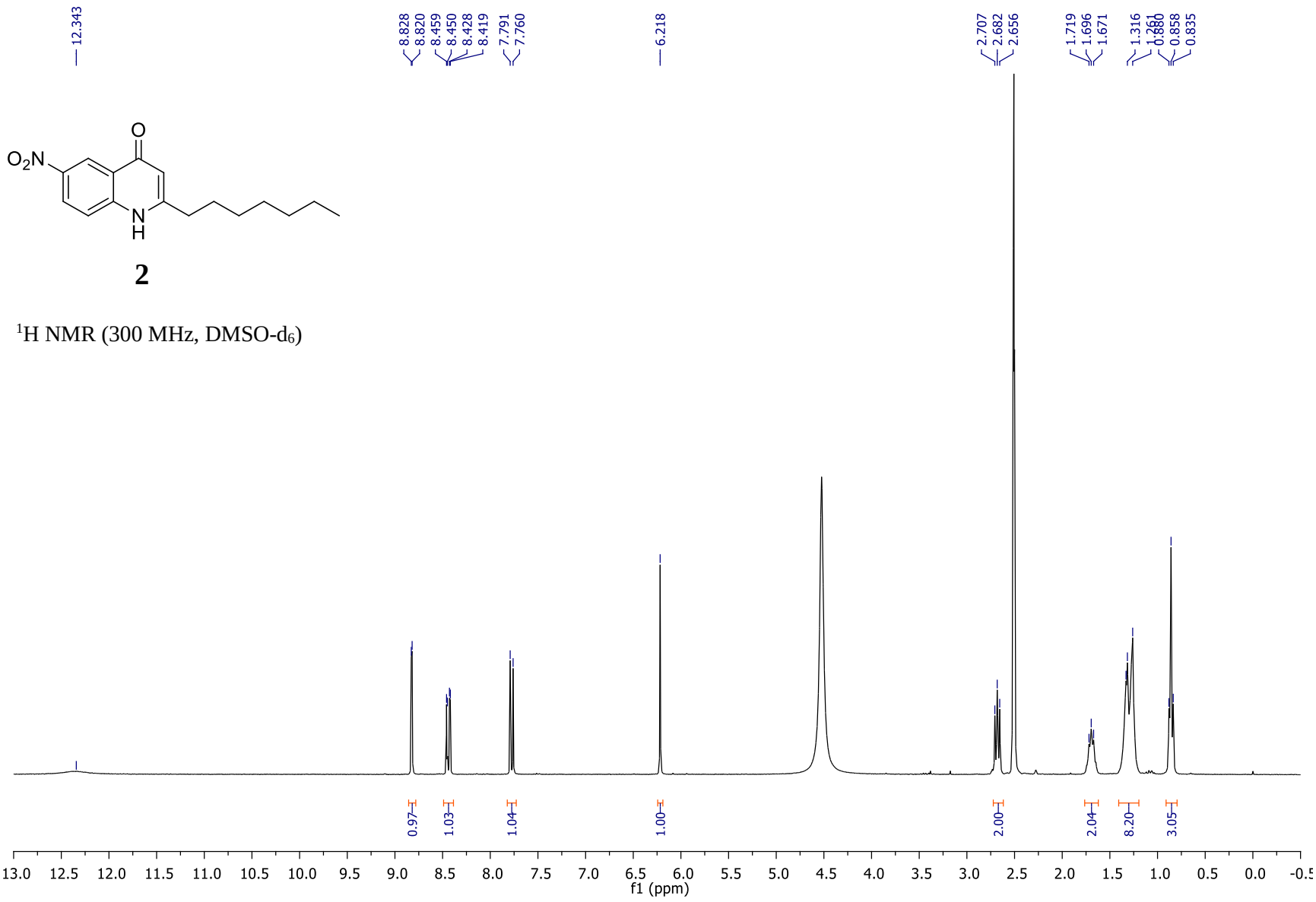
N,*N*'-Carbonyldiimidazole (0.097 g, 0.6 mmol) was added to a solution of 2-heptyl-6-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (0.1 g, 0.3 mmol) in dry DMF (1.5 mL), and the reaction mixture stirred for 4 hours 30 mins at 65^0C (yellow to orange solution). The cooled reaction mixture was then added to iced $NH(CH_3)_2.Et_2O$ (8 mL, 33%) and the resulting (orange) solution stirred at room temperature overnight. The reaction mixture was concentrated *in vacuo* and then added to iced water (4 mL) to precipitate the solid product (pale yellow) which was collected by vacuum filtration and dried in *in vacuo.* (0.059 g, 0.177 mmol, 59%).

Pale yellow solid; yield 59 mg (59%); m.p. = 236-238°C; IR (KBr) ν 3229, 3096, 2922, 1621, 1556, 1504, 1334, 1130 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ: 0.86 (3H, t, *J* = 6.6 Hz), 1.18-1.42 (8H, m) 1.65 (2H, m), 2.4-2.6 (2H, m overlapping with quin. of DMSO), 2.84 (3H, s), 2.99 (3H, s), 7.75 (1H, d, *J* = 9.2 Hz), 8.44 (1H, dd, *J* = 9.2, ^4J = 2.6 Hz), 8.82 (1H, d, *J* = 2.6 Hz), 12.25 (1H, bs); ^{13}C-NMR (75 MHz, DMSO-d_6): δ: 14.4, 22.5, 28.7 (2 x C), 29.2, 31.5, 32.1, 34.5, 37.7, 119.5, 120.4, 122.1, 123.6, 126.6, 143.2, 143.7, 152.9, 166.6, 173.3; HRMS calcd. (%) for $C_{19}H_{25}N_3O_4$: 360.1923; found: 360.1919.

3-Fluoro-2-heptylquinolin-4(1H)-one (12)

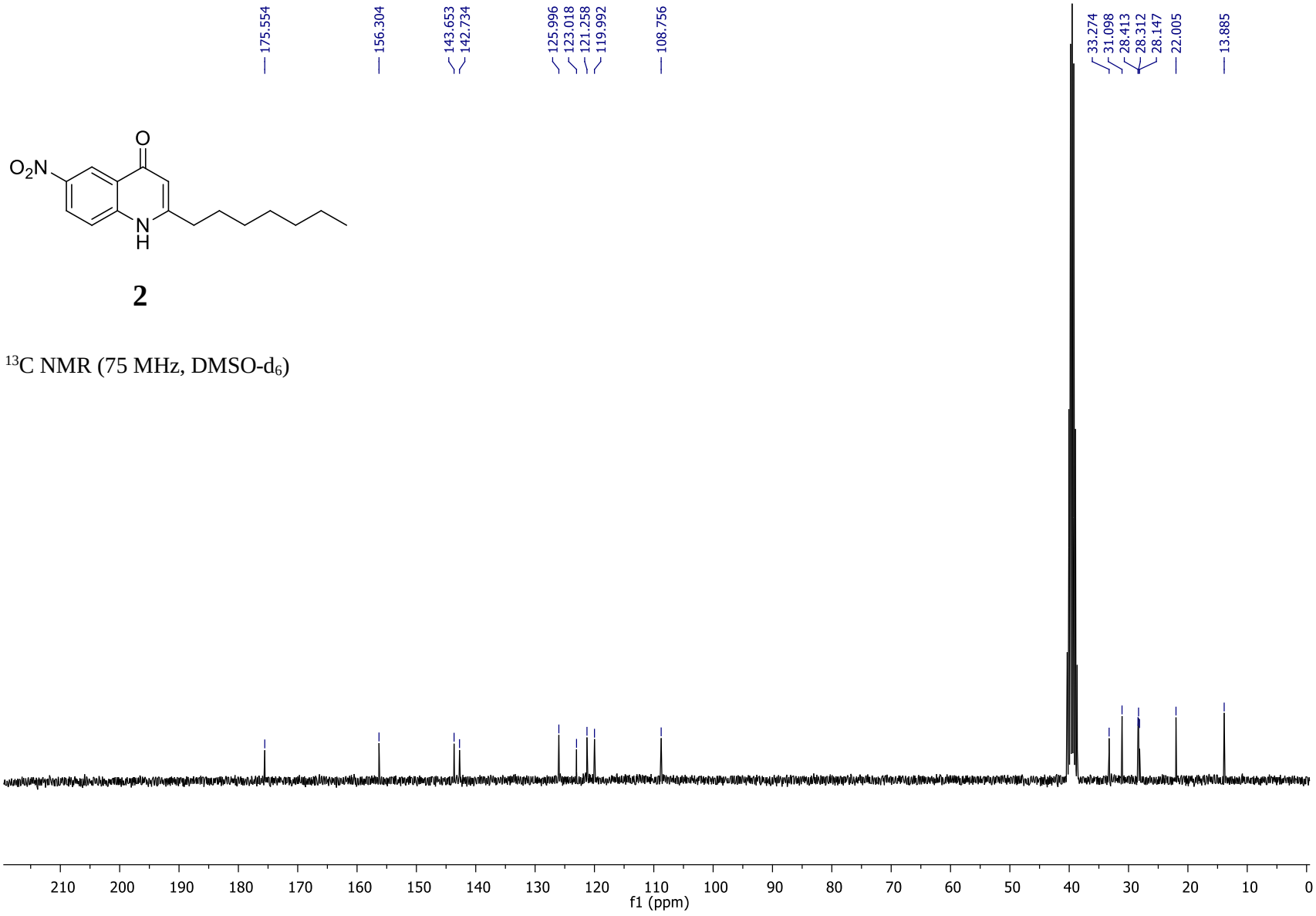
To a stirred solution of Selectfluor® 16 (0.509 g, 1.44 mmol) in EtOH (6 mL) was added HHQ (0.206 g, 0.85 mmol). The reaction was allowed to stir at room temperature for 7 days. The reaction mixture was filtered and solvent removed in vacuo to yield the crude product as a bright yellow solid.

Purification was achieved using silica column chromatography eluting with 90:10 ethyl acetate:hexane to yield 17 as a white solid (0.018 g, 8%). m.p. 152–155 °C. IR (KBr) ν: 3369, 2957, 2928, 1694, 1615, 1508, 1483, 1108 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.80 (3H t, *J* = 6.7 Hz), 1.10-1.38 (8H, m), 1.70-1.82 (2H, m), 2.83-2.92 (2H, m), 7.35 (1H, t, *J* = 7.6 Hz), 7.61 (1H, ddd, *J* = 8.4, 7.0, ^{4}J = 1.4 Hz), 7.75 (1H, d, *J* = 8.4 Hz), 8.44 (1H, dd, *J* = 8.2, ^{4}J = 1.0 Hz), 11.41 (1H, bs) ppm; ^{13}C-NMR (100 MHz, CDCl3): δ 14.0, 22.5, 28.4, 28.7, 28.9, 29.3, 31.6, 119.1, 123.4, 124.9 (d, $^{4}J_{C\text{-}F}$ = 4.6 Hz), 125.8 (d, $^{3}J_{C\text{-}F}$ = 8.3 Hz), 131.5, 138.9, 142.9 (d, $^{2}J_{C\text{-}F}$ = 26.5 Hz), 145.6 (d, $^{1}J_{C\text{-}F}$ = 229.4 Hz) 168.0 (d, $^{2}J_{C\text{-}F}$ = 12.9 Hz) ppm; ^{19}F-NMR (282 MHz, CDCl3): δ -161.1 (CF) ppm; HRMS (ESI) m/z calcd for $C_{16}H_{21}FNO$ [(M + H)+]: 262.1607, found 262.1601.



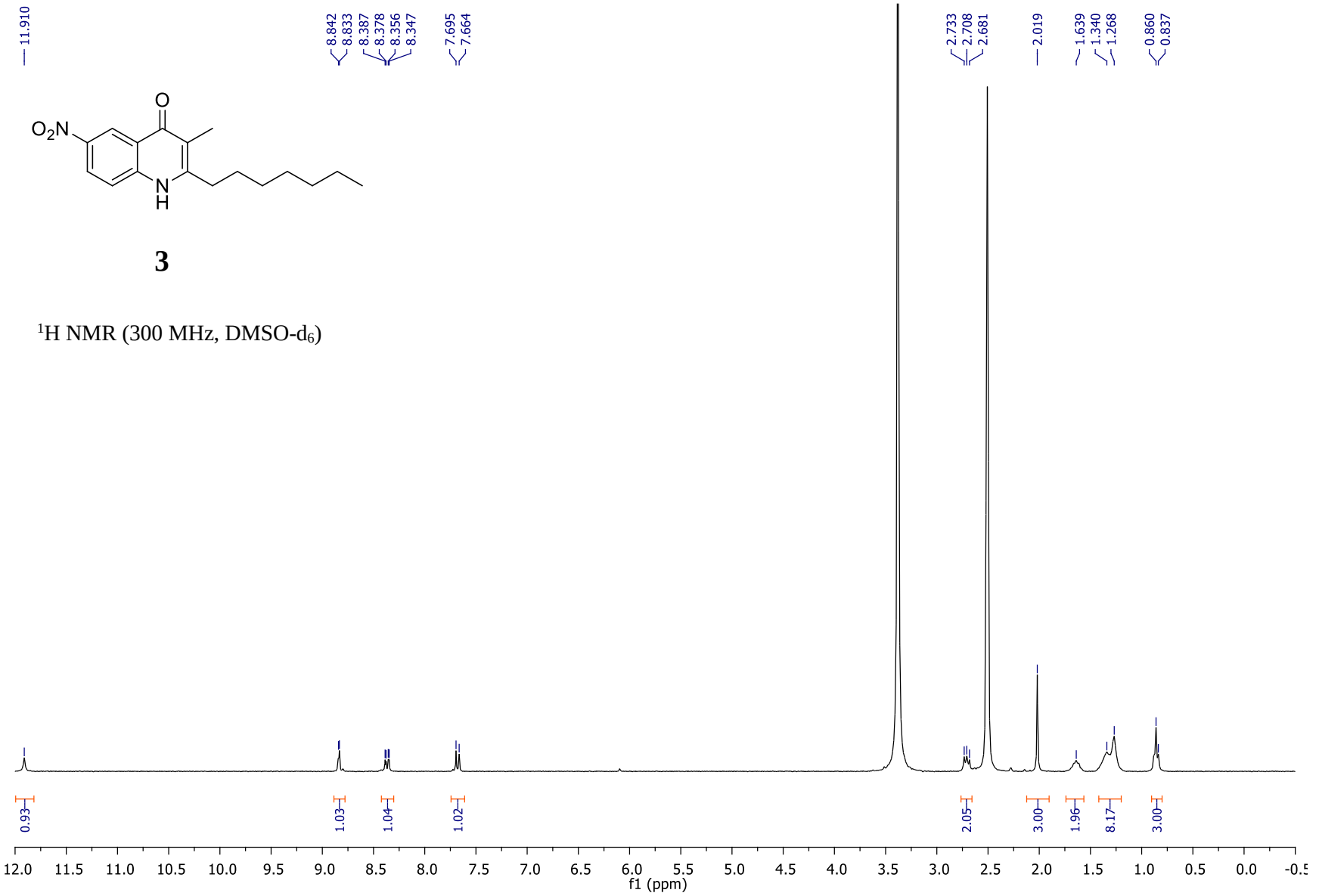
2

^{1}H NMR (300 MHz, DMSO-d_6)



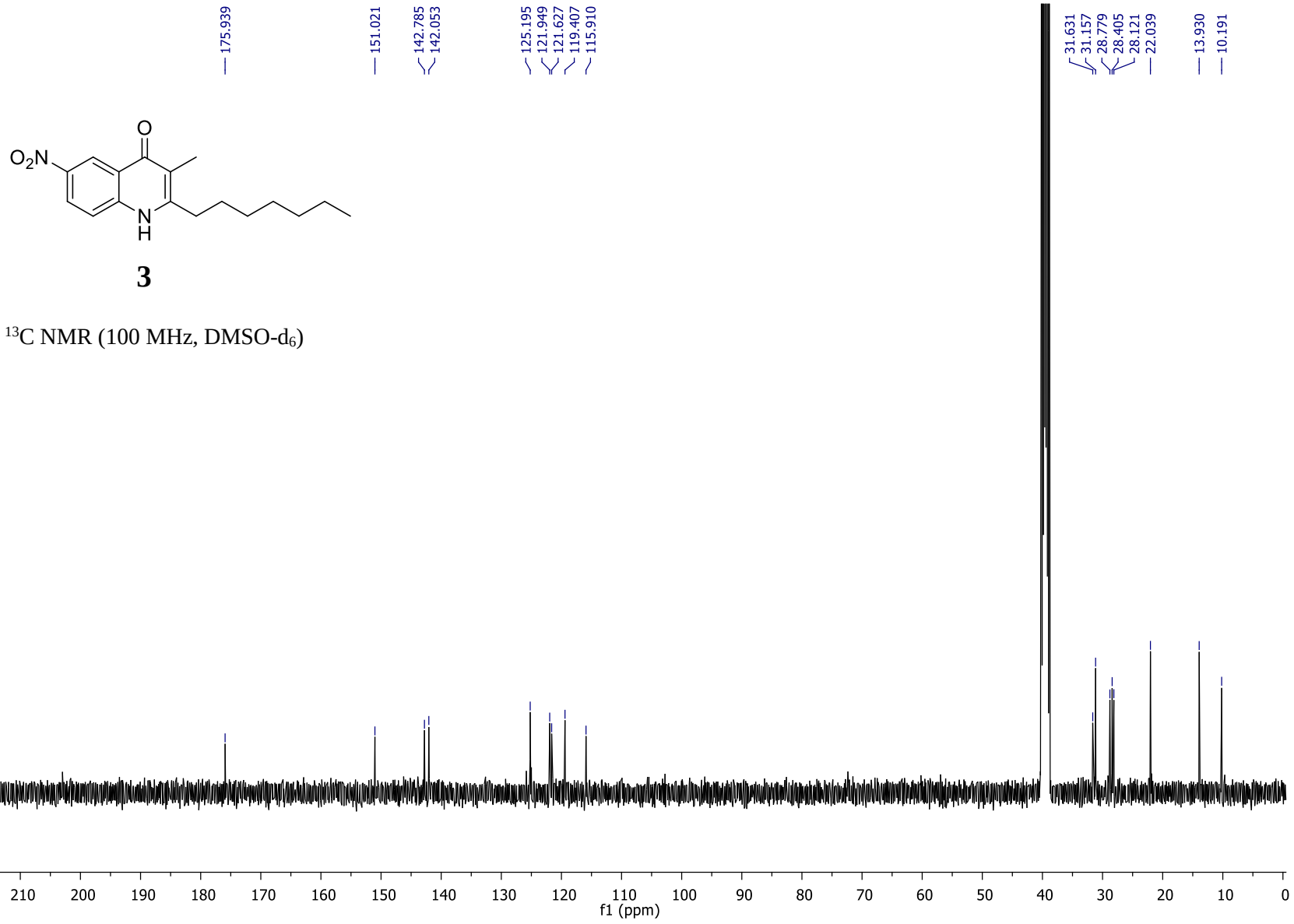
2

^{13}C NMR (75 MHz, DMSO-d_6)



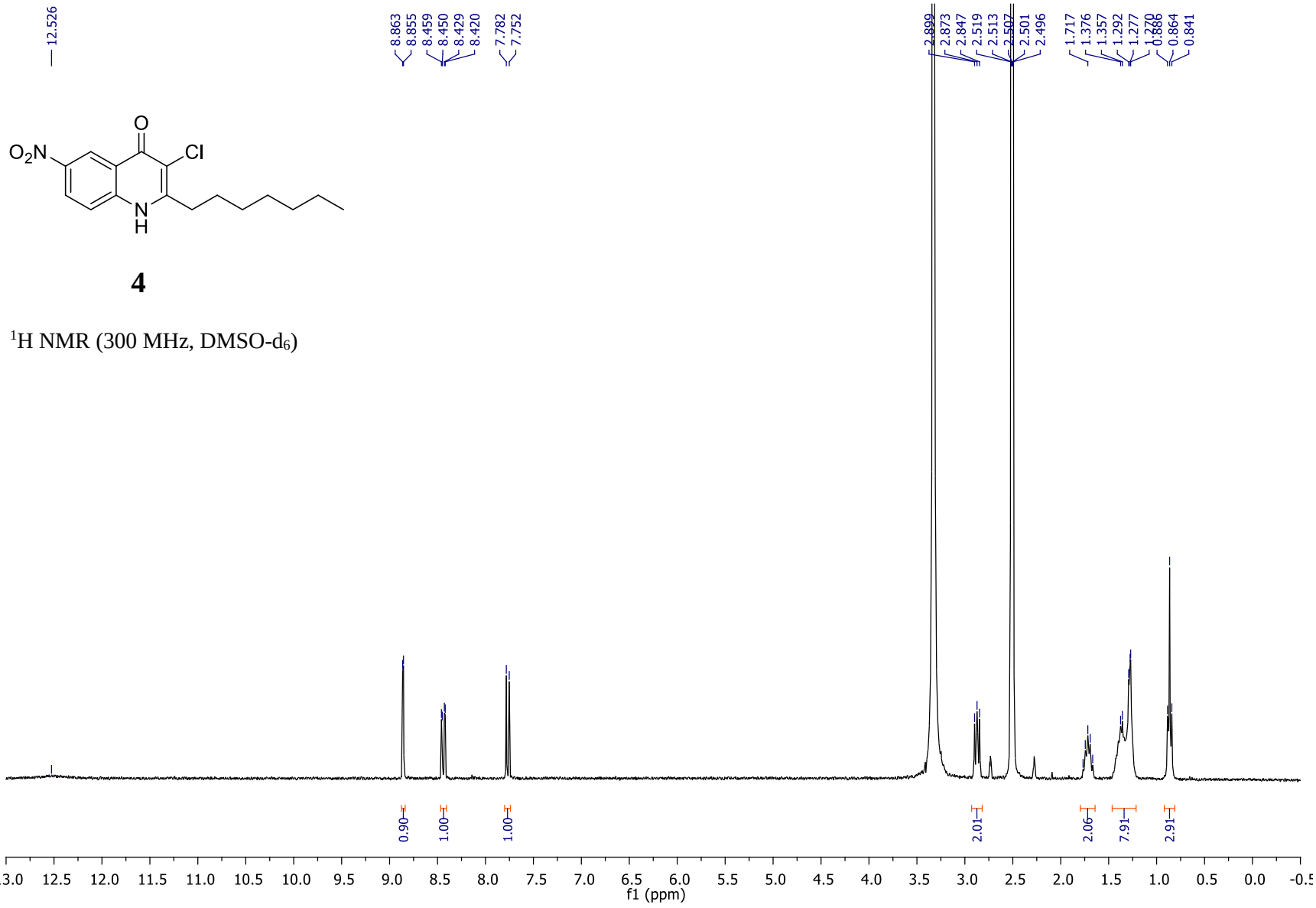
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^{1}H NMR (300 MHz, DMSO-d_6)



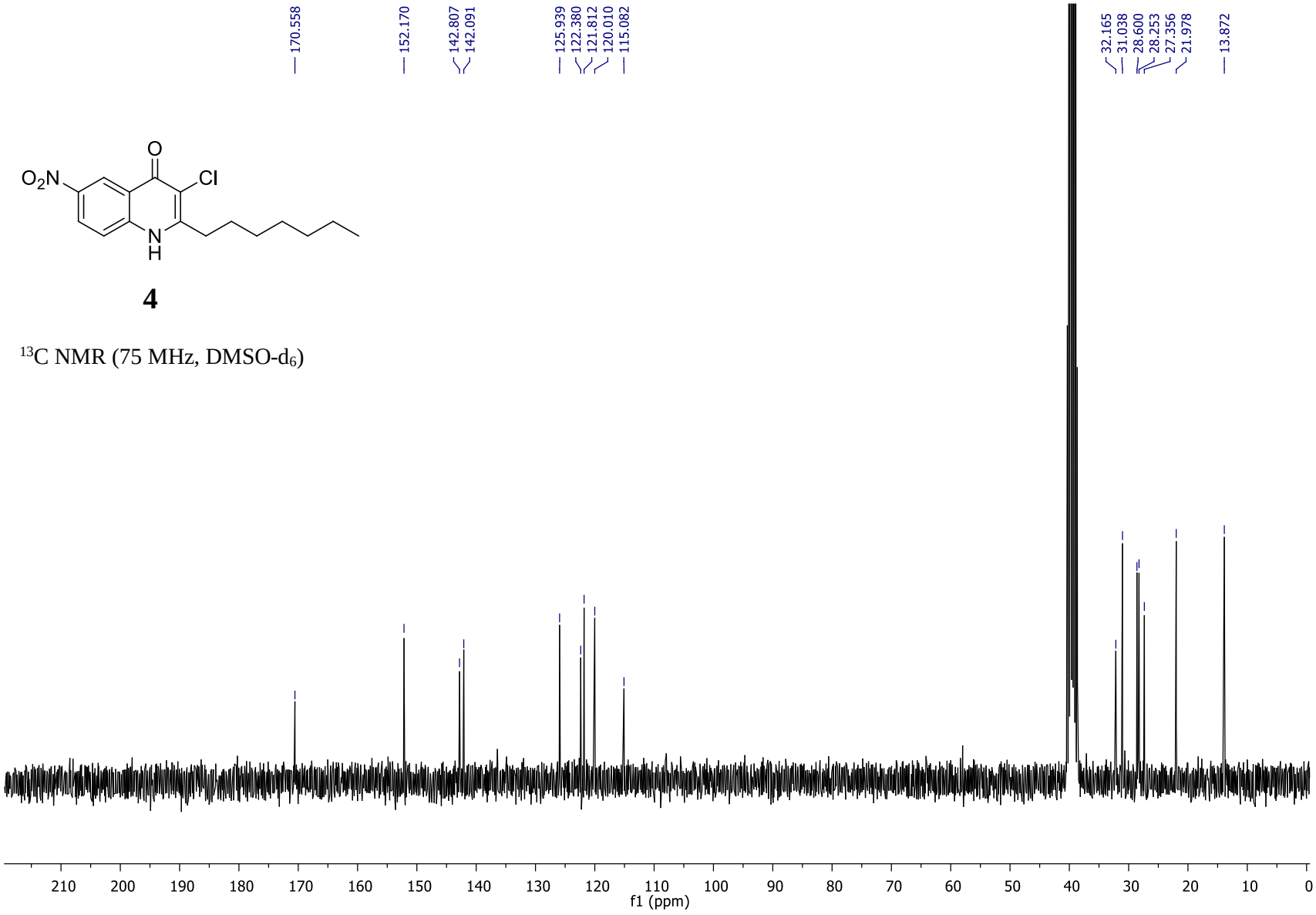
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^{13}C NMR (100 MHz, DMSO-d_6)



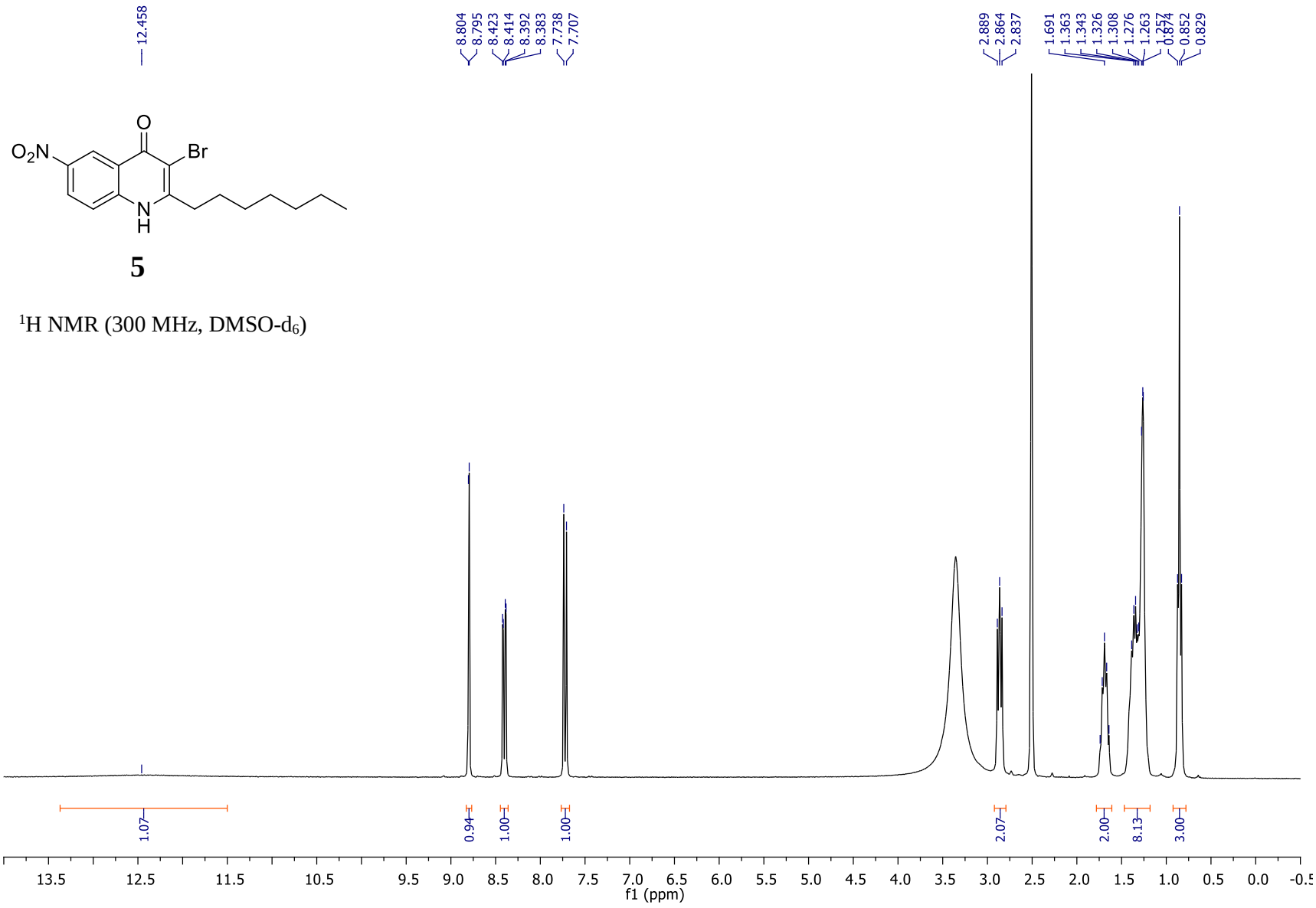
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^{1}H NMR (300 MHz, DMSO-d_6)



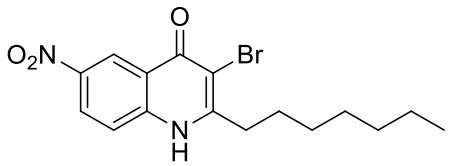
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^{13}C NMR (75 MHz, DMSO-d_6)



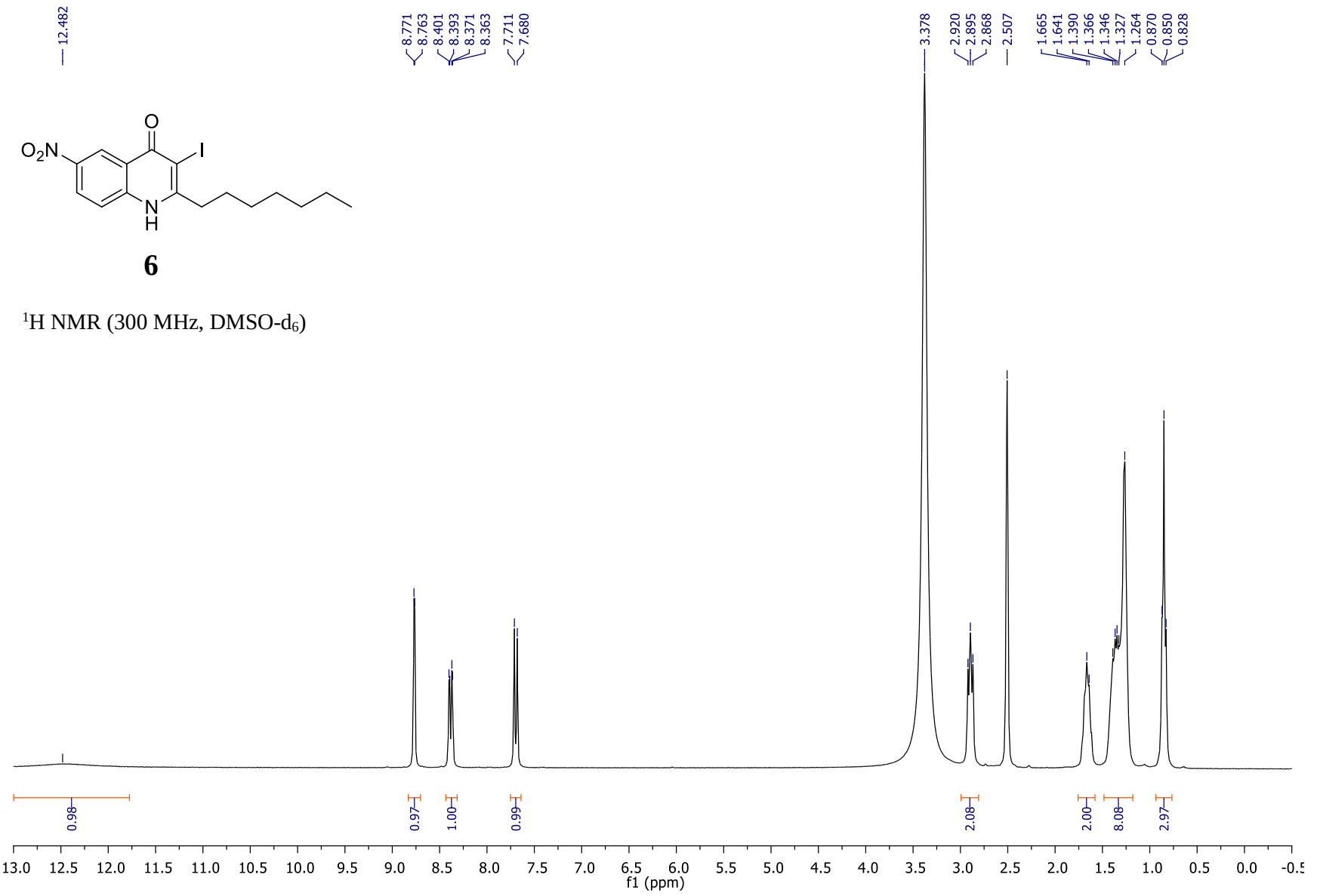
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^{1}H NMR (300 MHz, DMSO-d_6)



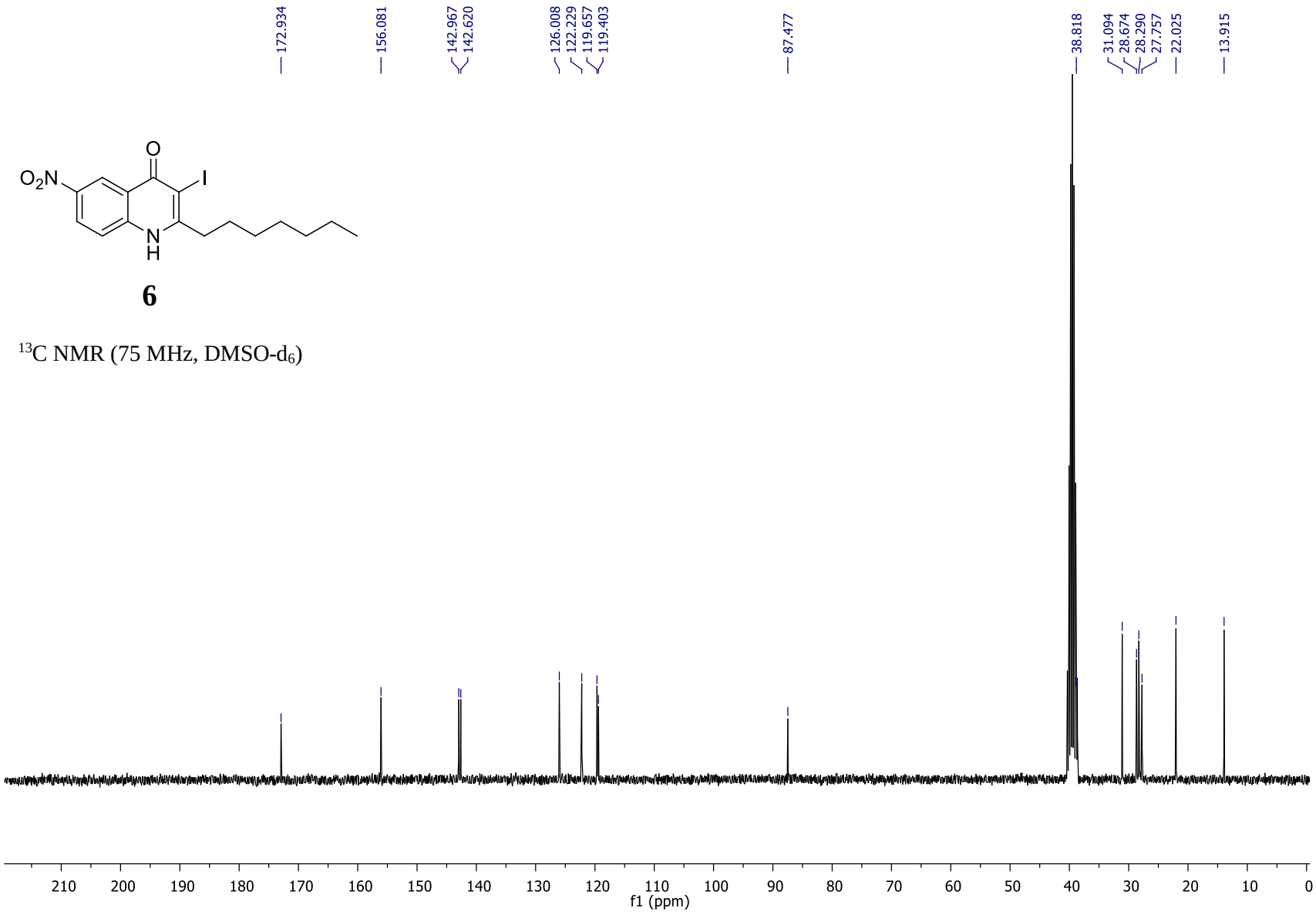
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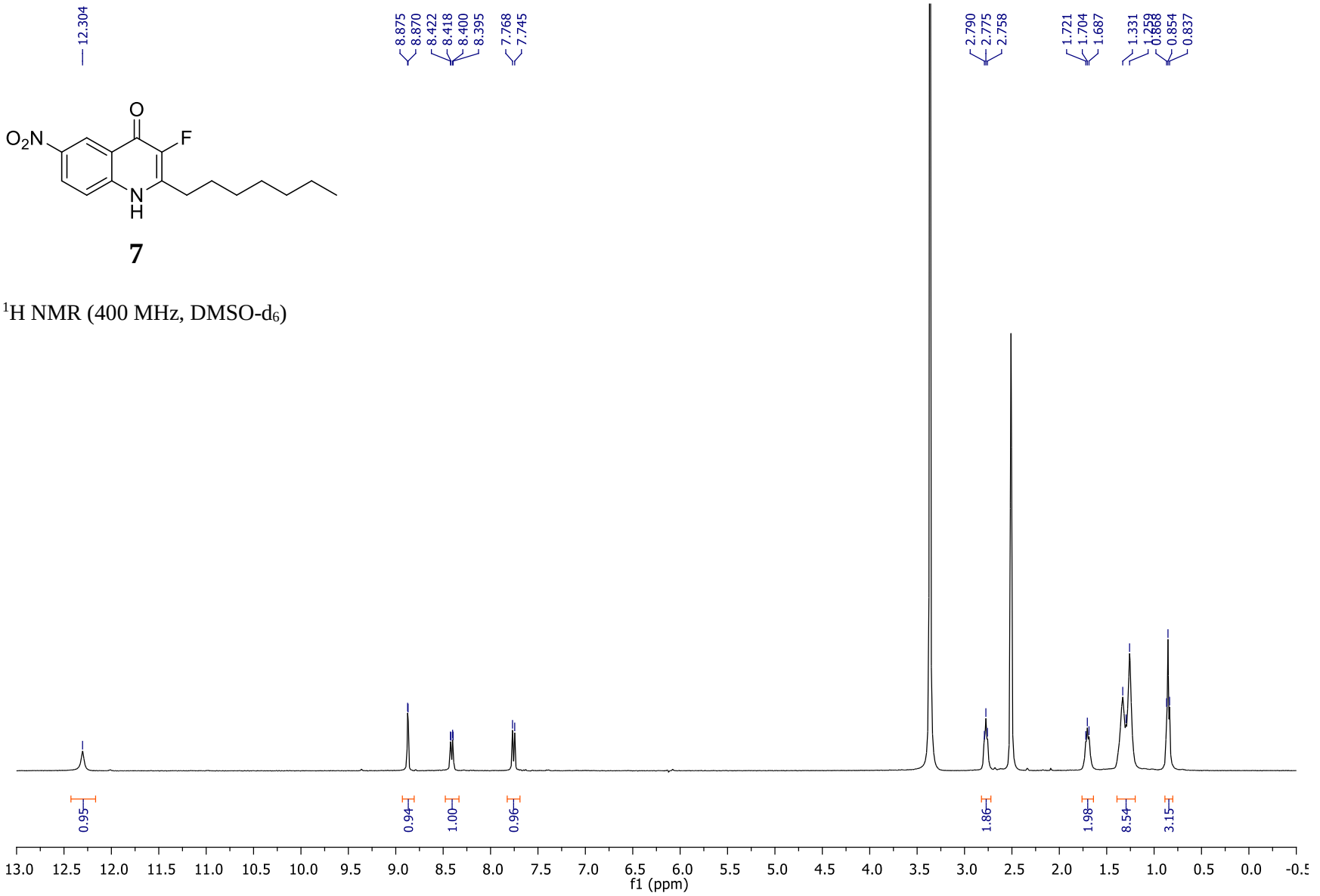
6

^{1}H NMR (300 MHz, DMSO-d_6)



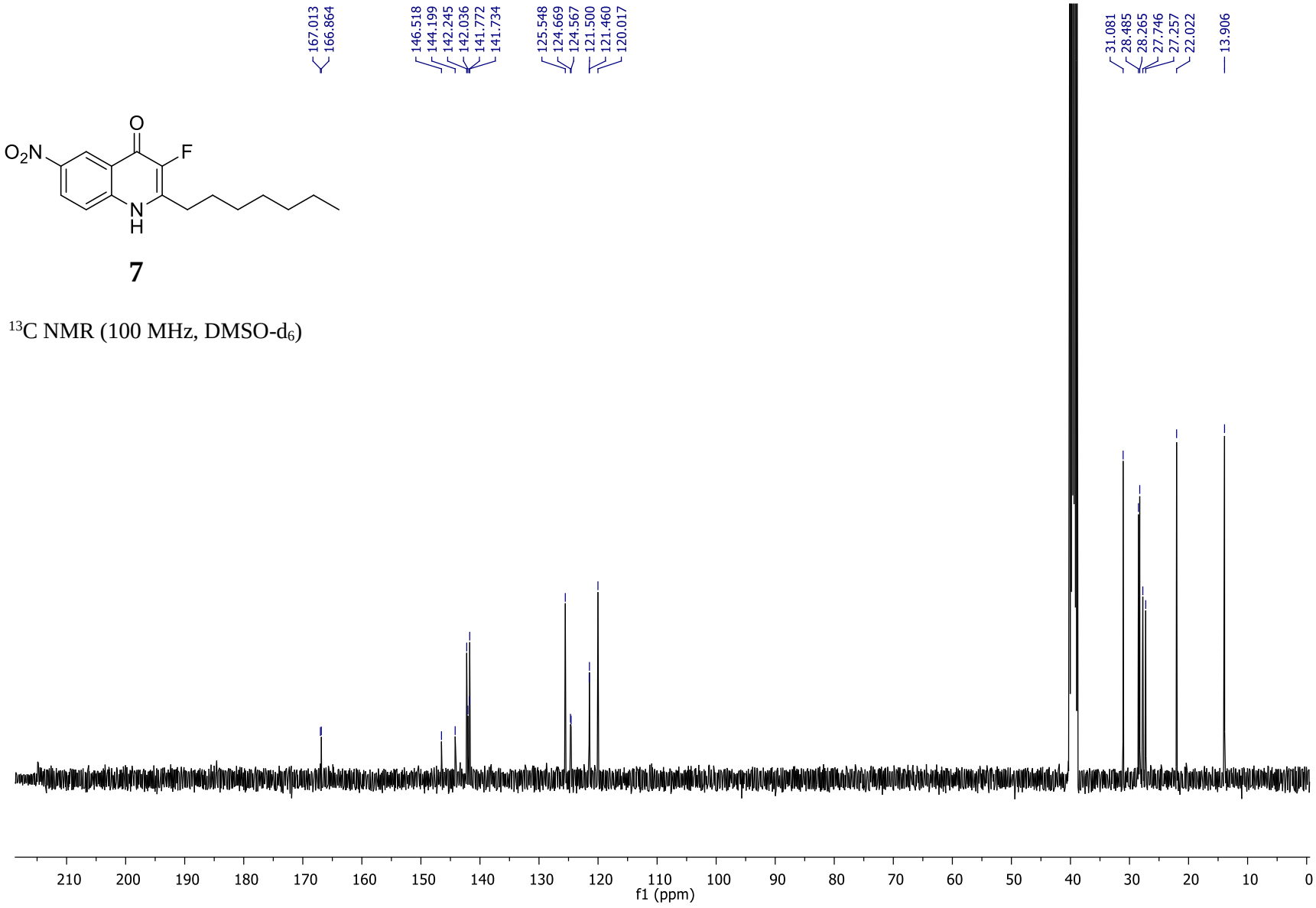
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^{13}C NMR (75 MHz, DMSO-d_6)



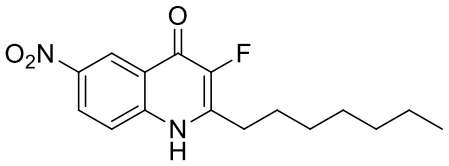
7

^{1}H NMR (400 MHz, DMSO-d_6)



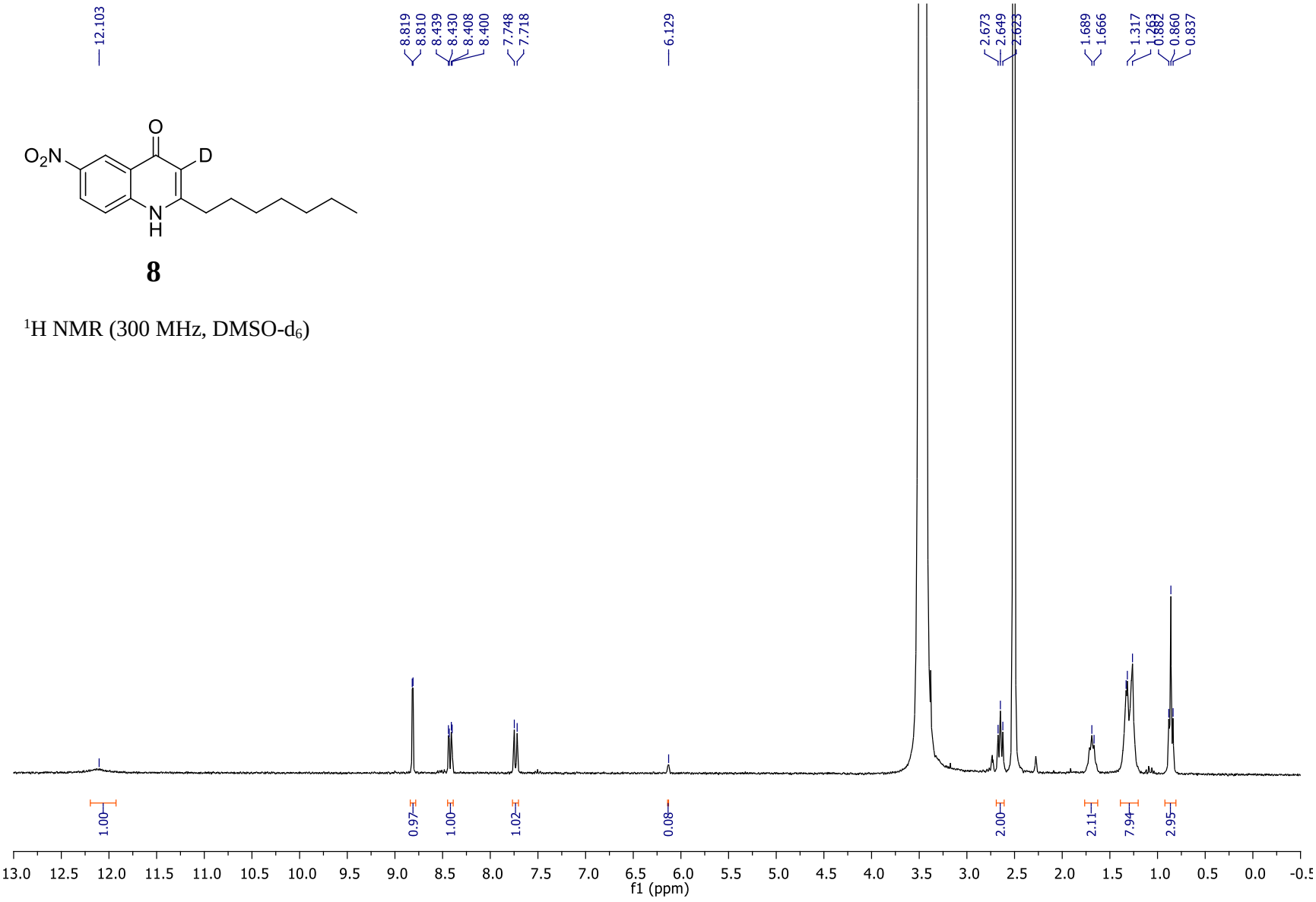
7

^{13}C NMR (100 MHz, DMSO-d_6)



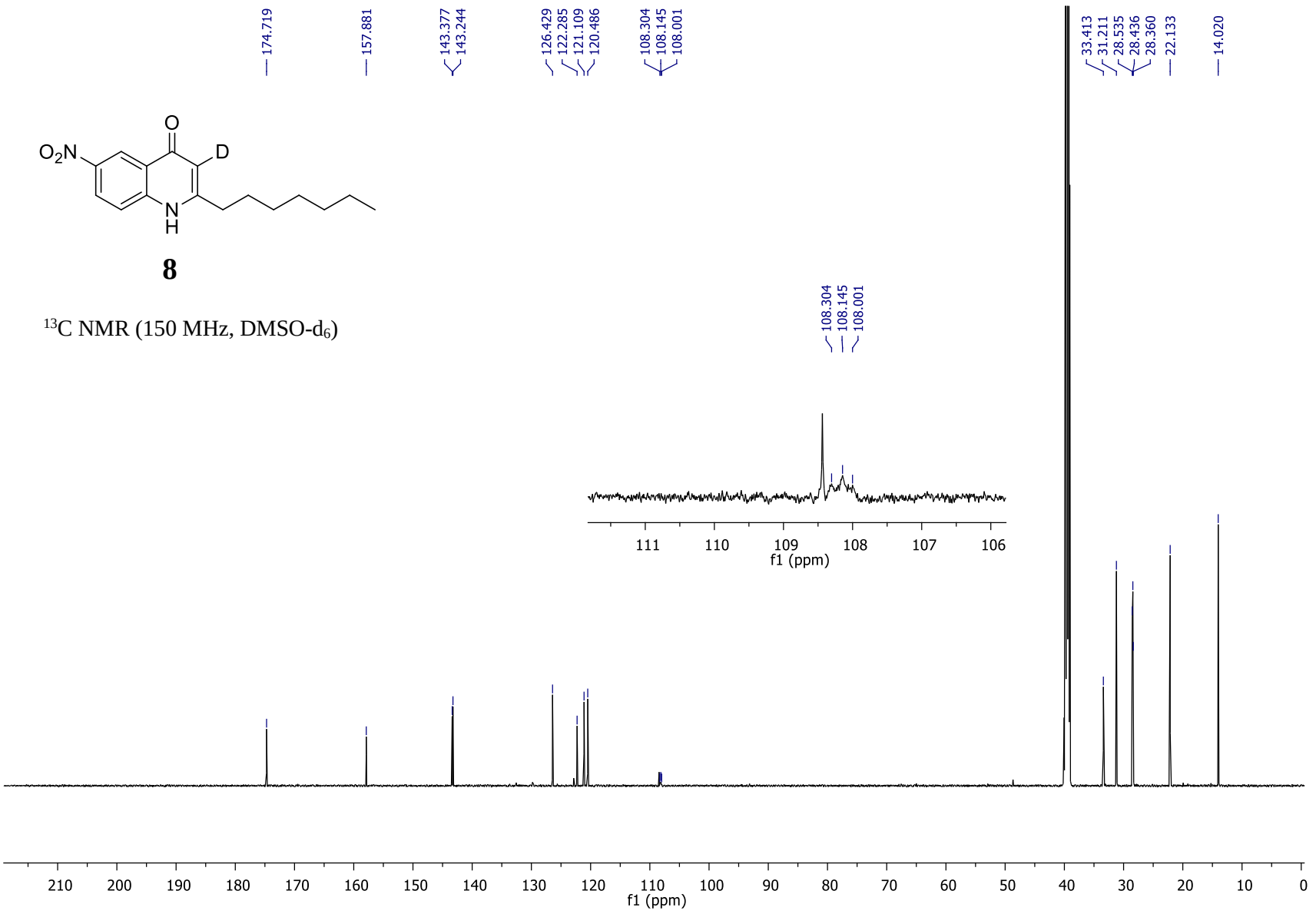
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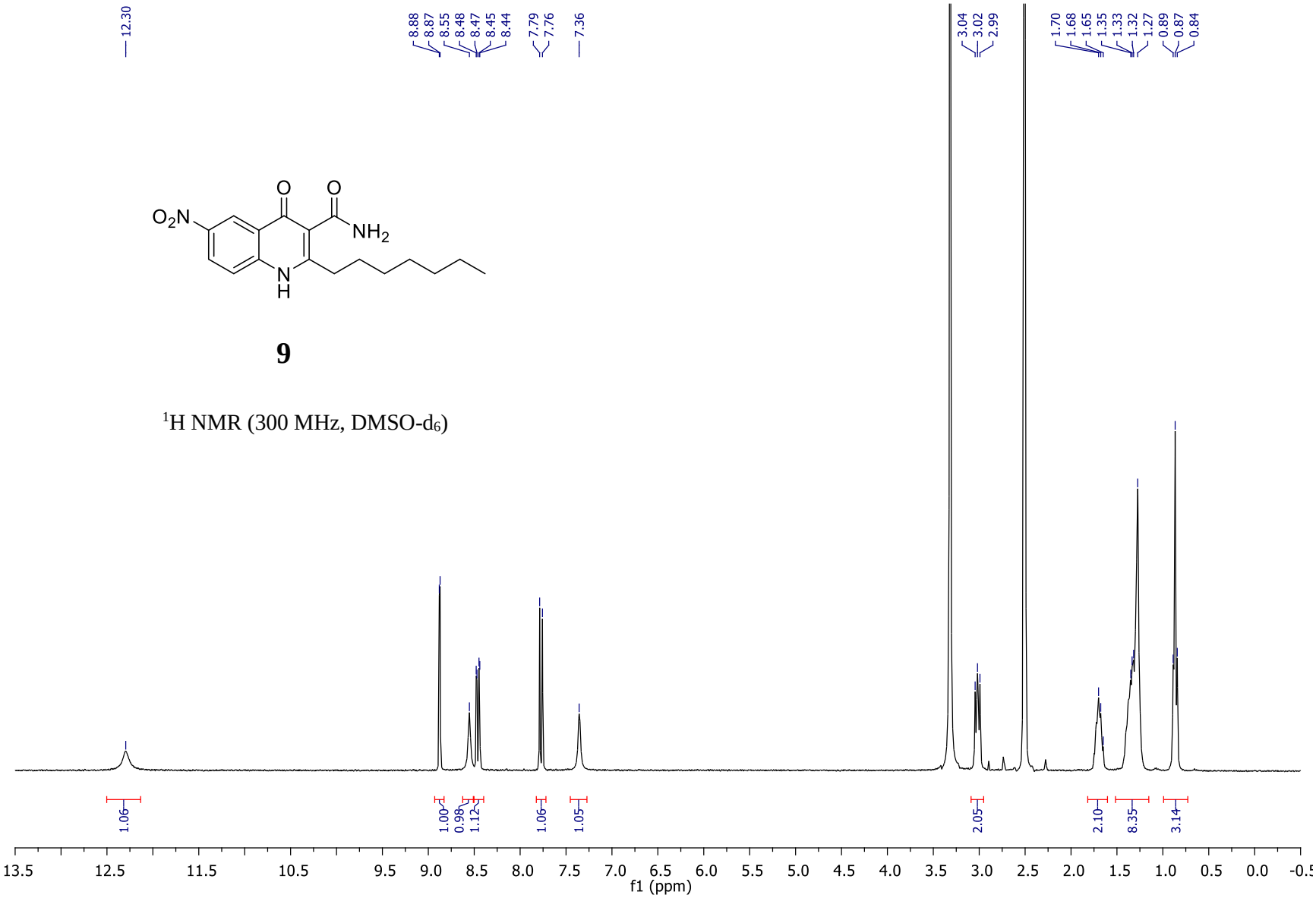
^{19}F NMR (282 MHz, DMSO-d_6)



8

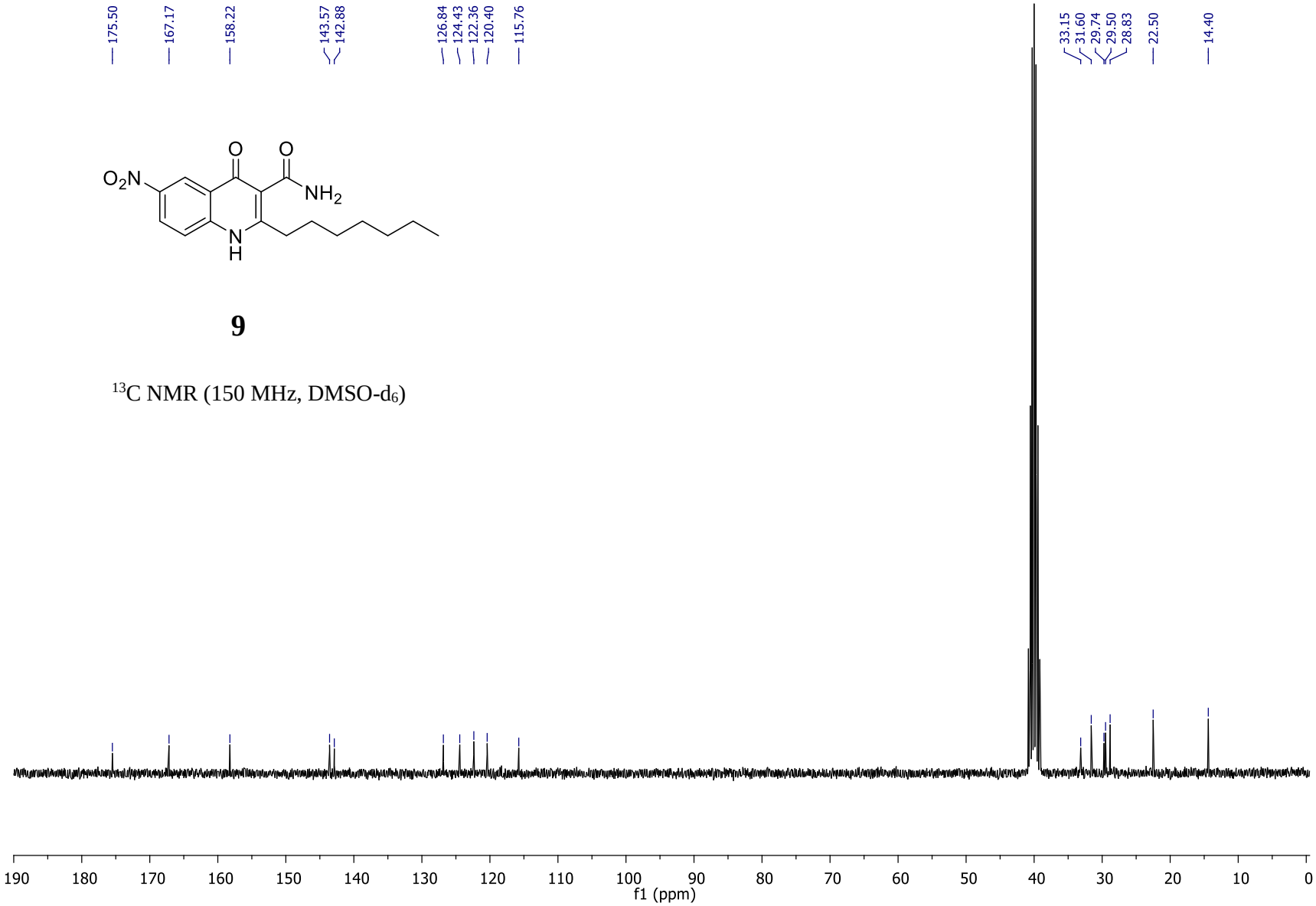
^{1}H NMR (300 MHz, DMSO-d_6)


174.719
157.881
143.377
143.244
126.429
122.285
121.109
120.486
108.304
108.145
108.001
33.413
31.211
28.535
28.436
28.360
22.133
14.020
O
O2N
D
N
H
8
13C NMR (150 MHz, DMSO-d6)
f1 (ppm)



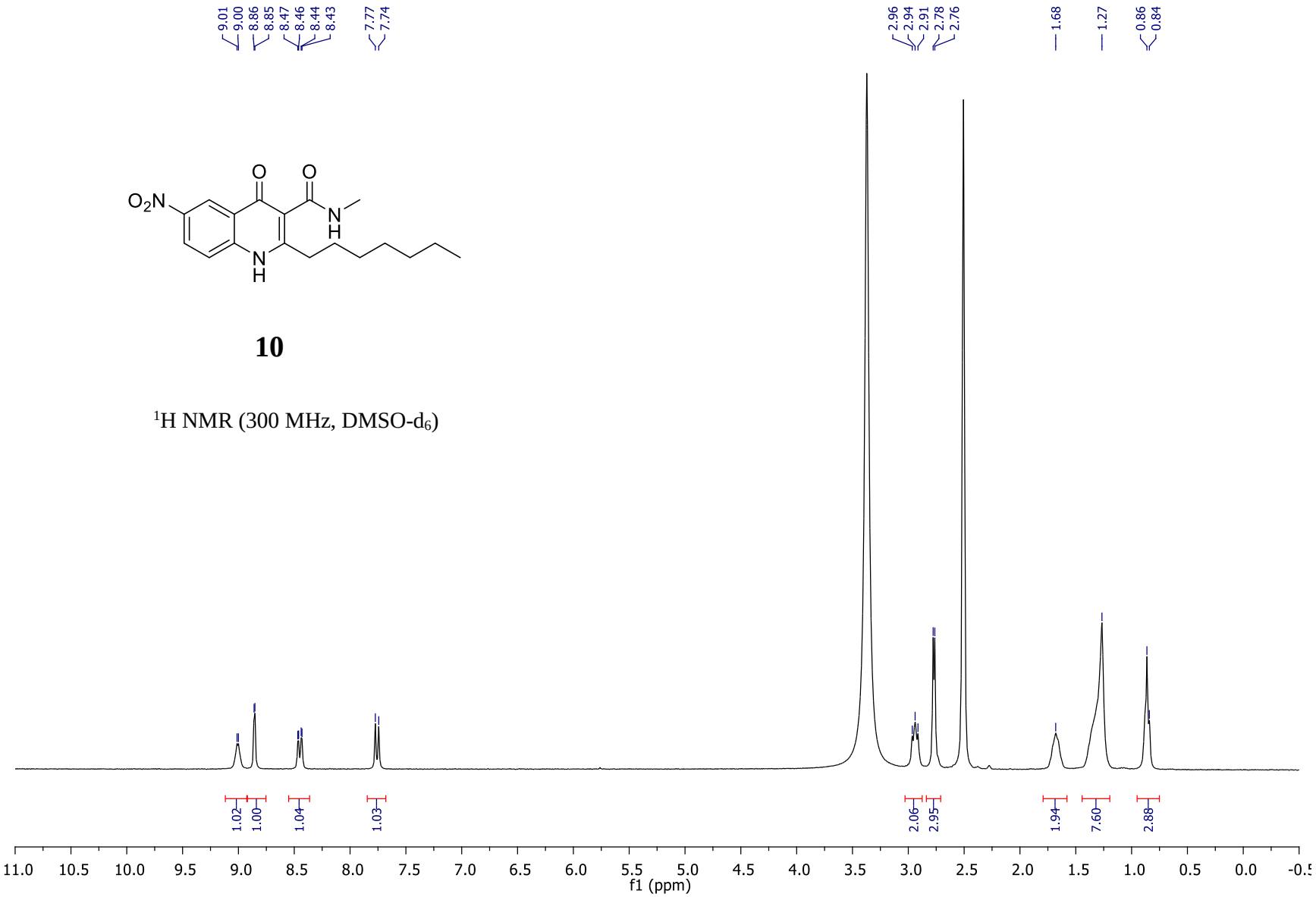
9

^{1}H NMR (300 MHz, DMSO-d_6)



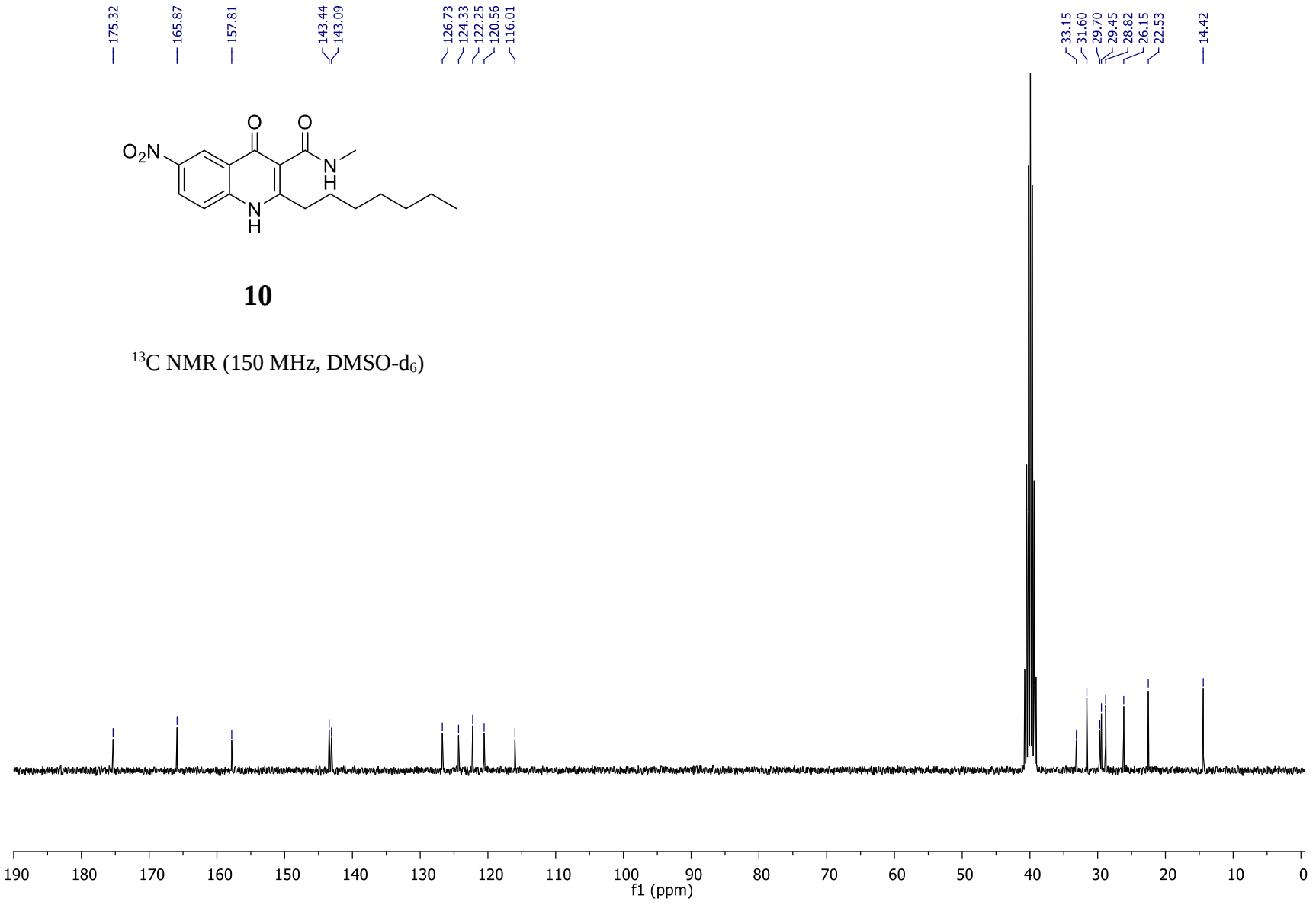
9

^{13}C NMR (150 MHz, DMSO-d_6)



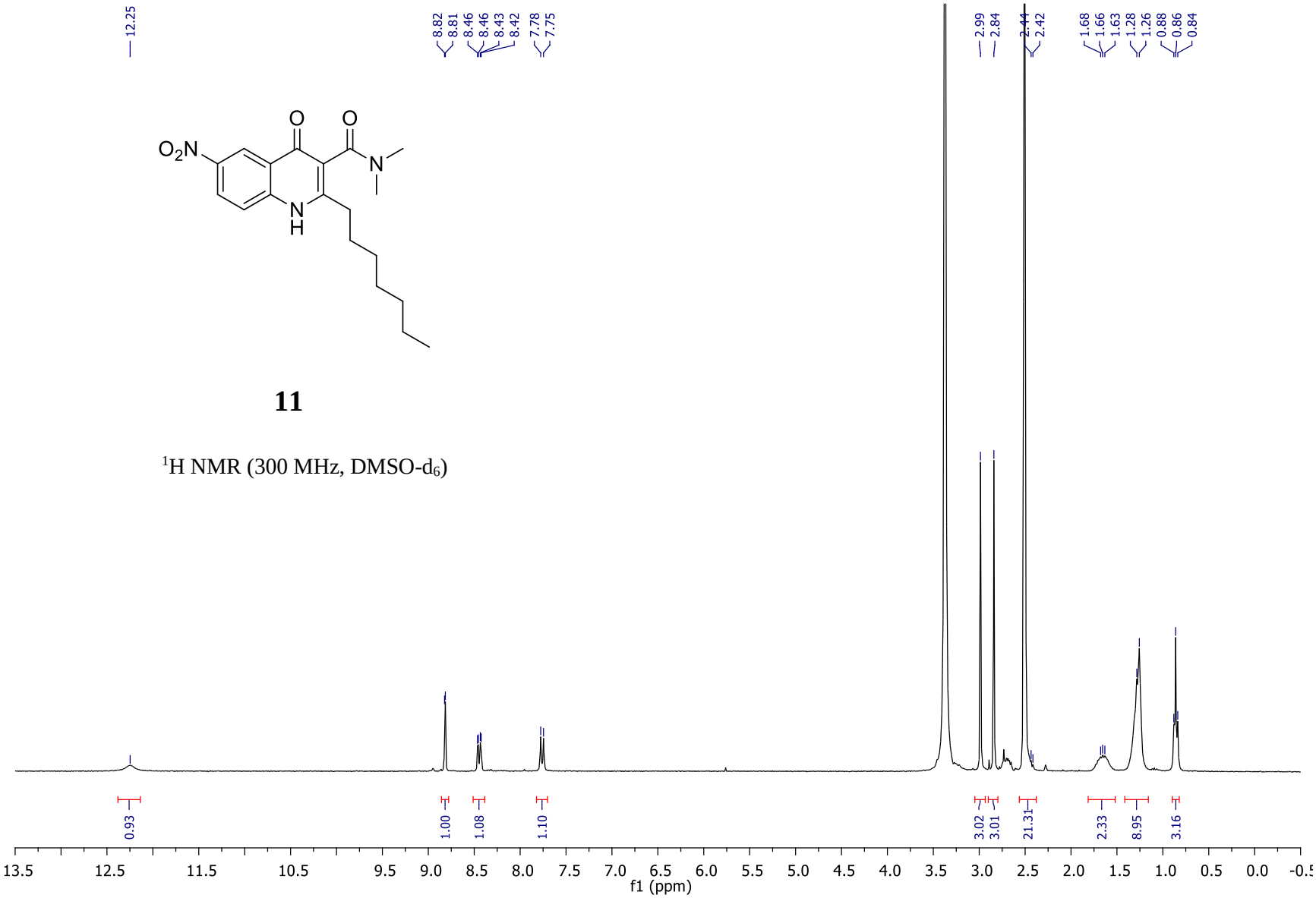
10

^{1}H NMR (300 MHz, DMSO-d_6)



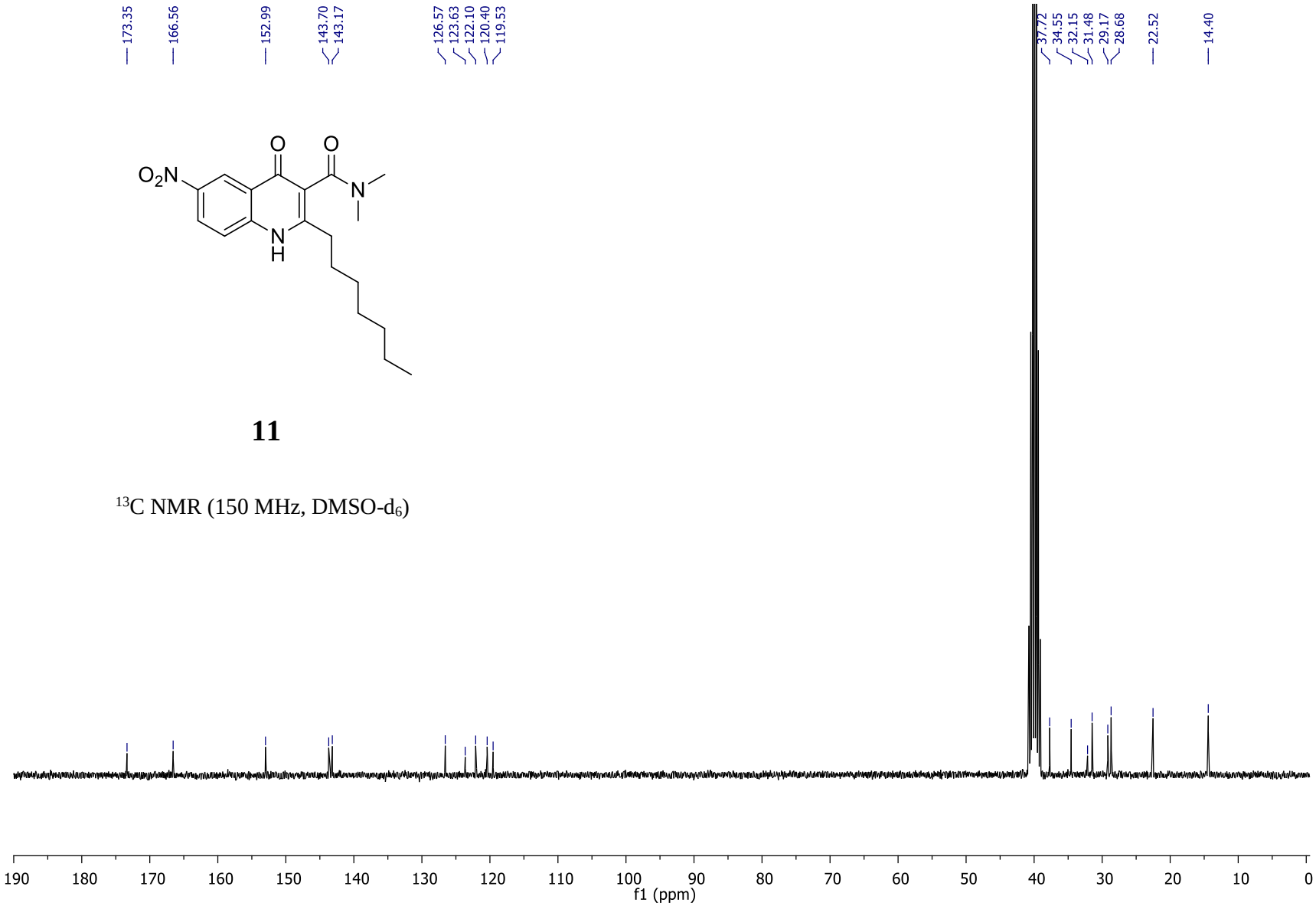
10

^{13}C NMR (150 MHz, DMSO-d_6)



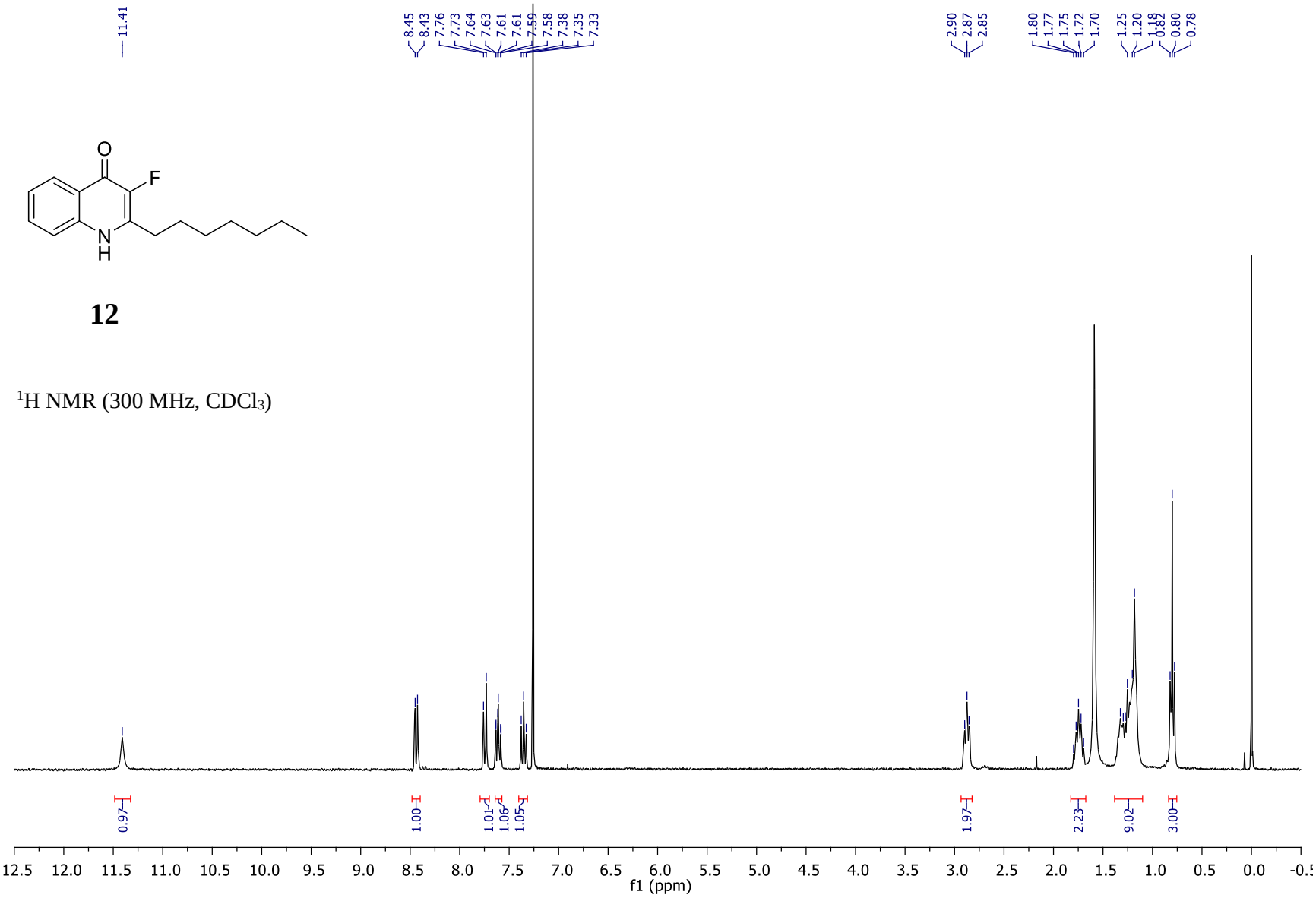
11

^{1}H NMR (300 MHz, DMSO-d_6)



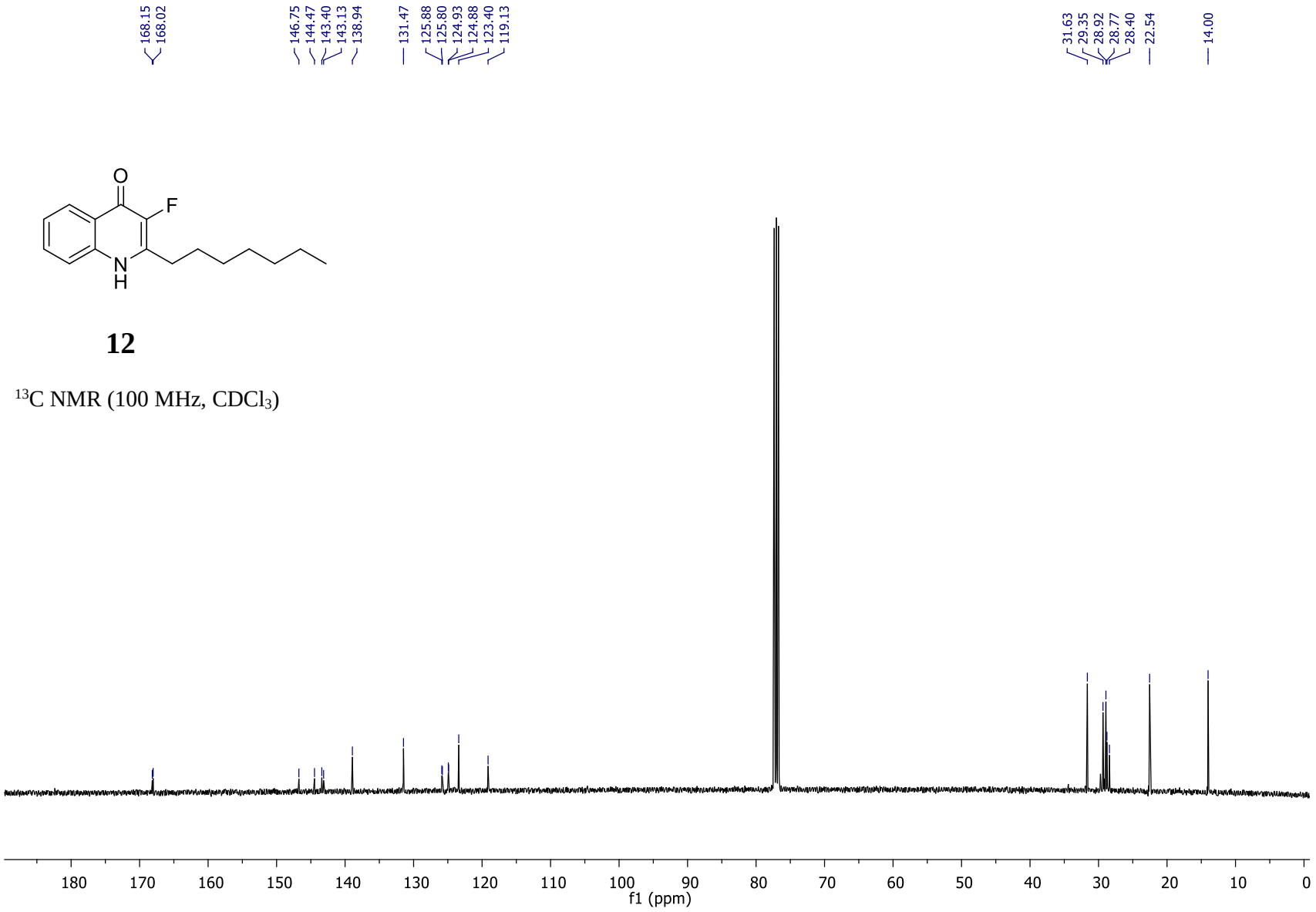
11

^{13}C NMR (150 MHz, DMSO-d_6)



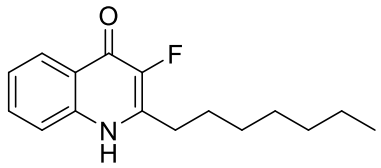
12

^{1}H NMR (300 MHz, $CDCl_3$)



12

^{13}C NMR (100 MHz, $CDCl_3$)



12

^{19}F NMR (282 MHz, $CDCl_3$)

Biological Methods:

Bacterial Culture Conditions

Bacterial cultures of *P. aeruginosa* PAO1 and a PAO1*pqsA* mutant, both containing the pLP0996 *pqsA-lacZ* promoter fusion (McGrath et al., 2004), were maintained on Luria Bertani (LB) plates supplemented with carbenicillin (200 µg/ml), and routinely grown at 37^{o}C. Fresh cultures were prepared prior to inoculation of each experiment.

Promoter fusion analysis

Promoter activity of the *pqsA-E* biosynthetic operon as a proxy for AHQ signalling was monitored using the *pqsA-lacZ* promoter fusion pLP0996 in both the wild-type PAO1 strain and its isogenic *pqsA* mutant.[5] Overnight cultures were transferred to conical flasks containing fresh LB media (carbenicillin 200 µg/ml) at a starting OD_{600nm} 0.02 in the presence of HHQ, PQS and derivative compounds (10 µM) or equivalent volumes of DMSO or methanol. In the absence of endogenous activation, 50 nM PQS was added to each *pqsA* mutant flask to elicit activation of the *pqsA* promoter, with the exception of the PQS 10 µM treated sample and the untreated baseline control. Concentration and time-dependent promoter fusion assays were performed as above, upon addition of 100 nM, 1 µM, and 10 µM concentrations of selected compounds and PQS. In each case, compounds were prepared and added to the test cultures in equal volumes of carrier solvent to ensure standardization throughout the experiments. Samples were taken at mid log phase or early stationary phase growth and β-galactosidase activity was measured as before.[6,7]

Briefly, at the same time as measuring the OD_{600nm} of the cultures, 20 µl aliquots of cells were transferred to 80 µl of permeabilisation solution (Na_2HPO_4, 100 mM; KCl, 20 mM; $MgSO_4$, 2 mM; CTAB (hexadecyltrimethylammonium bromide), 0.8 mg/mL; sodium deoxycholate, 0.4 mg/mL). Beta-mercaptoethanol (5.4 µL/mL) was added to the permeabilisation solution

immediately prior to use. After incubation at 30ºC, 600 µl of substrate solution was added and the incubation time monitored. Substrate solution consisted of 60 mM Na_2HPO_4 and 40 mM NaH_2PO_4 to which 1 mg/ml o-nitrophenyl-β-D-Galactoside (ONPG) and 2.7 µL/mL β-mercaptoethanol was added immediately prior to use. The reaction was stopped upon appearance of a yellow colour by addition of 1 M Na_2CO_3. β-Galactosidase activity was calculated as a measure of $(OD_{420nm} * 1000)/(OD_{600nm} * 0.02 * \text{Time (min)})$.

Growth Curve Analysis

PAO1 pLP0996, PAO1 *pqsA*$^-$ pLP0996, and a wild-type PA14 strain were inoculated into Luria Bertani (LB) medium (supplemented with carbenicillin 200 µg/ml in the case of the former two strains) and incubated overnight with shaking at 37ºC. Cells were transferred to fresh media, starting OD600nm 0.02, aliquoted into multi-well plates and incubated at 37ºC static (with shaking for 10 sec prior to measurements at 30 min intervals) on a BioScreen growth curve system. Four technical replicates were included for each biological datapoint.

Statistical Analysis

Statistical analysis was performed on a minimum of three independent biological replicates using the Bootstratio algorithm for normalized fold change data.[8]

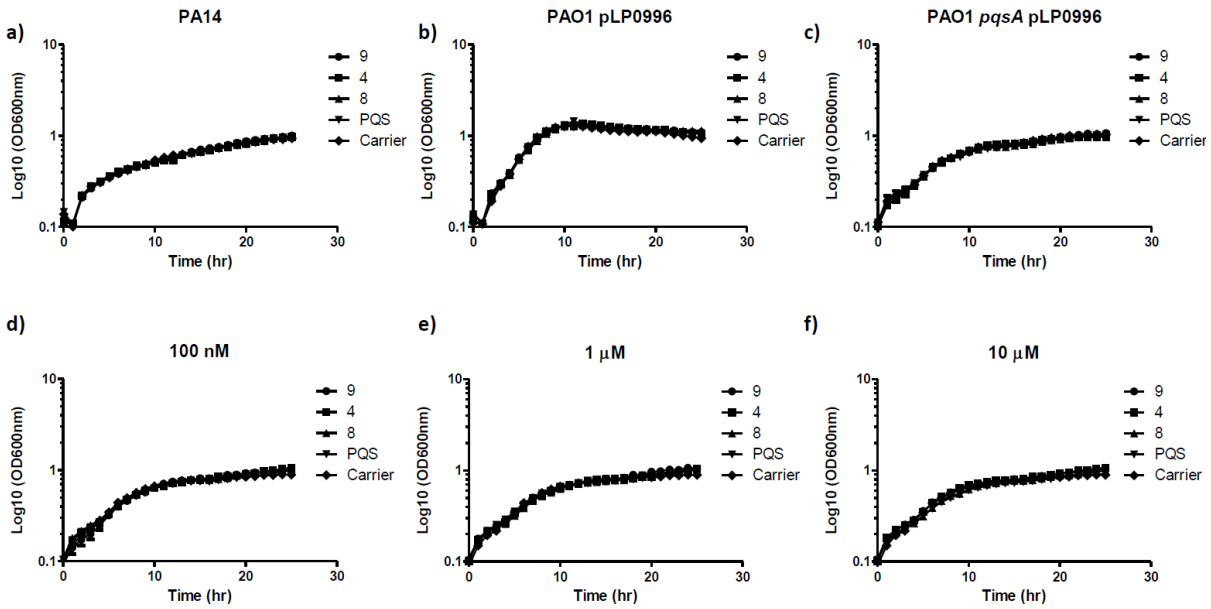


Figure S1. Growth curve analysis of *P. aeruginosa* strains **(a)** PA14, **(b)** PAO1 pLP0996, and **(c)** PAO1 *pqsA*$^{-}$ pLP0996, in the presence of lead compounds 4, 8, 9 and PQS. Additional concentration dependent growth curves were performed on the PAO1 *pqsA*$^{-}$ pLP0996 strain at **(d)** 100 nM, **(e)** 1 µM, and **(f)** 10 µM. All experiments were carried out on at least three independent biological replicates.

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