Supplementary Data for

Structure-activity relationships of novel iodinated quinoline-2-carboxamides for targeting the translocator protein

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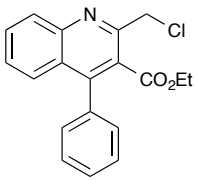
1. General Experimental

All reagents and starting materials were obtained from commercial sources and used as received. All dry solvents were purified using a PureSolv 500 MD solvent purification system. All reactions were performed under an atmosphere of argon unless otherwise stated. Brine is defined as a saturated solution of aqueous sodium chloride. Flash column chromatography was carried out using Fisher Matrix silica 60. Macherey–Nagel aluminium–backed plates pre–coated with silica gel 60 (UV_{254}) were used for thin layer chromatography and were visualized using UV light. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX 400 spectrometer or Bruker 500 spectrometer with chemical shift values in ppm relative to tetramethysilane (δ_H 0.00 and δ_C 0.0) or residual chloroform (δ_H 7.26 and δ_C 77.2) as the standard. Proton and carbon assignments are based on two-dimensional COSY and DEPT experiments, respectively. Infrared spectra were recorded using Golden Gate apparatus on a JASCO FTIR 410 spectrometer and mass spectra were obtained using a JEOL JMS-700 spectrometer. Melting points were determined on a Gallenkamp melting point apparatus.

2. Experimental Procedures and Spectroscopic Data For All Compounds

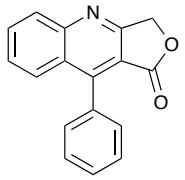
Ethyl 2-chloromethyl-4-phenylquinoline-3-carboxylate (8)[1]



2-Aminobenzophenone (**6**) (4.00 g, 20.3 mmol) and ethyl 4-chloroacetoacetate (**7**) (3.34 g, 20.3 mmol) were placed in a sealed tube with *N*,*N'*-dimethylformamide (40 mL). Chlorotrimethylsilane (10.3 mL, 81.1 mmol) was then added dropwise, the tube was sealed and heated to 100 °C overnight. After cooling, the reaction mixture was diluted with water (50 mL), extracted with dichloromethane (3 × 50 mL), dried ($MgSO_4$) and concentrated to dryness. Purification was carried out using flash column chromatography (petroleum ether/ethyl acetate, 1:1) to give ethyl 2-chloromethyl-4-phenylquinoline-3-carboxylate (**8**) as yellow crystals (5.67 g, 85%). Mp 109–111 °C (lit.,[1] mp 111–112 °C); ν_{max}/cm^{-1} (KBr) 2980 (CH), 1720 (CO), 1566, 1487, 1404, 1301, 1232, 768; δ_H (400 MHz, $CDCl_3$) 0.92 (3H, t, *J* 7.1 Hz, OCH_2CH_3), 4.04 (2H, q, *J* 7.1 Hz, OCH_2CH_3), 5.04 (2H, s, CH_2Cl), 7.35–7.37 (2H, m, 2 × ArH), 7.48–7.52 (4H, m, 4 × ArH), 7.63 (1H, d, *J* 8.3 Hz, ArH), 7.77 (1H, t, *J* 7.2 Hz, ArH), 8.14 (1H, d, *J* 8.3 Hz, ArH); δ_C (101 MHz, $CDCl_3$) 13.5 (CH_3), 45.9 (CH_2), 61.6 (CH_2), 126.1

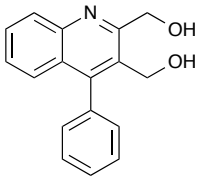
(C), 126.3 (C), 126.7 (CH), 127.8 (2 × CH), 128.3 (CH), 128.6 (2 × CH), 129.3 (CH), 129.6 (CH), 130.8 (CH), 135.7 (C), 147.4 (C), 148.2 (C), 153.1 (C), 167.7 (C); *m/z* (EI) 325.0868 (M^+. $C_{19}H_{16}{}^{35}ClNO_2$ requires 325.0870), 280 (67%), 262 (61), 217 (63), 176 (22), 151 (11), 84 (100).

9-Phenylfuro[3,4-*b*]quinolin-1(3*H*)-one (9)[2]



Ethyl 2-chloromethyl-4-phenylquinoline-3-carboxylate (**8**) (7.00 g, 21.5 mmol) was dissolved in 6 M hydrochloric acid (100 mL) and ethanol (100 mL) and heated under reflux for 9 days. The reaction mixture was concentrated to dryness and diluted with water (100 mL), made alkaline with solid sodium carbonate (pH ~8–9) and extracted with chloroform (100 mL). The organic layer was dried ($MgSO_4$) and concentrated under vacuum. Purification was carried out by flash column chromatography eluting, with petroleum ether/ethyl acetate to give 9-phenylfuro[3,4-*b*]quinolin-1(3*H*)-one (**9**) as a yellow solid (5.15 g, 91%). Mp 197–199 °C (lit.,[2] mp 203–204 °C); ν_{max}/cm^{-1} (neat) 3044 (CH), 1764 (CO), 1605, 1582, 1495, 1443, 1136, 1030, 777; δ_H (400 MHz, $CDCl_3$) 5.46 (2H, s, CH_2), 7.44–7.49 (2H, m, 2 × ArH) 7.57–7.62 (4H, m, 4 × ArH), 7.88–7.94 (2H, m, 2 × ArH), 8.21 (1H, d, *J* 8.3 Hz, ArH); δ_C (101 MHz, $CDCl_3$) 68.9 (CH_2), 112.8 (C), 126.4 (C), 126.7 (C), 127.4 (CH), 127.6 (2 × CH), 128.7 (CH), 128.8 (CH), 129.1 (2 × CH), 130.9 (CH), 131.9 (CH), 150.6 (C), 150.8 (C), 162.9 (C), 167.2 (C); *m/z* (FAB) 262.0867 (MH^+. $C_{17}H_{12}NO_2$ requires 262.0868), 232 (11%), 204 (18), 147 (14), 109 (14), 69 (57), 57 (100).

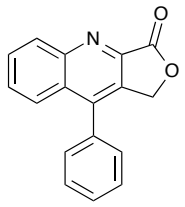
2,3-Bis(hydroxymethyl)-4-phenylquinoline (10)[2]



9-Phenylfuro[3,4-*b*]quinolin-1(3*H*)-one (**9**) (1.95 g, 7.47 mmol) was added to tetrahydrofuran (100 mL) and cooled to 0 °C. Lithium aluminium hydride (30.0 mL, 1.0 M in tetrahydrofuran) was then added slowly and the reaction mixture stirred at room temperature.

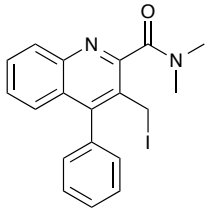
After 1 h, the reaction was quenched with 1 M hydrochloric acid (pH ~2) and the mixture extracted with ethyl acetate (2 × 50 mL), dried ($MgSO_4$) and concentrated under vacuum. The residue was then dissolved in methanol (100 mL) and 10% palladium on carbon (1.00 g) was added. The reaction mixture was stirred at room temperature overnight. The catalyst was removed by filtration, washed with hot methanol and the filtrate was concentrated to dryness under vacuum to give 2,3-bis(hydroxymethyl)-4-phenylquinoline (**10**) as a colourless solid (1.52 g, 77%). Mp 166–169 °C (lit.,[2] mp 175–177 °C); ν_{max}/cm^{-1} (neat) 3420 (OH), 2884 (CH), 1570, 1441, 1022, 1005, 765; δ_H (400 MHz, CD_3OD) 4.58 (2H, s, CH_2), 5.11 (2H, s, CH_2), 7.33–7.48 (4H, m, 4 × ArH), 7.52–7.59 (3H, m, 3 × ArH), 7.72 (1H, ddd, *J* 8.4, 6.8, 1.6 Hz, ArH), 8.10 (1H, d, *J* 8.4 Hz, ArH); δ_C (101 MHz, CD_3OD) 59.1 (CH_2), 64.7 (CH_2), 127.8 (CH), 127.9 (CH), 128.6 (C), 129.4 (CH), 129.5 (2 × CH), 129.6 (CH), 130.1 (CH), 130.8 (2 × CH), 130.9 (C), 137.3 (C), 147.4 (C), 150.4 (C), 160.9 (C); *m/z* (CI) 266.1180 (MH^+. $C_{17}H_{16}NO_2$ requires 266.1181), 248 (28%), 218 (6), 116 (3), 85 (6).

9-Phenylfuro[3,4-*b*]quinolin-3(1*H*)-one (11)[2]



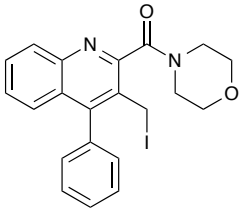
2,3-Bis(hydroxymethyl)-4-phenylquinoline (**10**) (0.10 g, 0.38 mmol) was dissolved in chloroform (20 mL). Activated manganese dioxide (1.02 g, 11.7 mmol) was then added and the reaction mixture stirred at room temperature for 1 h. The reaction mixture was filtered through Celite® and washed with chloroform (100 mL). Concentration of the filtrate gave 9-phenylfuro[3,4-*b*]quinolin-3(1*H*)-one (**11**) as a colourless solid (0.09 g, 92%). Mp 188–190 °C (lit.,[2] mp 189–192 °C); ν_{max}/cm^{-1} (neat) 3061 (CH), 1769 (CO), 1582, 1370, 1344, 1153, 1051, 1009, 768; δ_H (400 MHz, $CDCl_3$) 5.41 (2H, s, CH_2), 7.43–7.48 (2H, m, 2 × ArH), 7.56–7.69 (4H, m, 4 × ArH), 7.85 (1H, ddd, *J* 8.4, 6.8, 1.2 Hz, ArH), 7.91 (1H, d, *J* 9.0 Hz, ArH), 8.43 (1H, d, *J* 8.4 Hz, ArH); δ_C (101 MHz, $CDCl_3$) 67.9 (CH_2), 125.8 (CH), 127.9 (C), 128.9 (CH), 129.4 (2 × CH), 129.5 (2 × CH), 129.6 (CH), 130.7 (CH), 131.4 (CH), 132.3 (C), 133.6 (C), 143.9 (C), 144.3 (C), 150.7 (C), 168.8 (C); *m/z* (EI) 261.0788 (M^+. $C_{17}H_{11}NO_2$ requires 261.0790), 217 (23%), 204 (100), 176 (14), 151 (9), 95 (9), 84 (18).

3-Iodomethyl-4-phenylquinoline-2-*N*-dimethylcarboxamide (12)



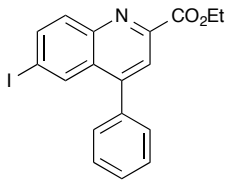
Dimethylamine (0.38 mL, 0.75 mmol) was dissolved in dichloromethane (30 mL) and a 2 M solution of trimethylaluminium in toluene (0.30 mL, 0.75 mmol) was added slowly to the mixture. After 0.25 h, 9-phenylfuro[3,4-*b*]quinolin-3(1*H*)-one (**11**) (0.15 g, 0.57 mmol) was added to the reaction, which was heated under reflux for 48 h. The reaction was quenched with 1 M hydrochloric acid (20 mL), extracted with dichloromethane (3 × 50 mL), dried ($MgSO_4$) and concentrated under vacuum. The resulting residue was washed through a plug of silica gel (dichloromethane/ethyl acetate 5:2) to give 3-hydroxymethyl-4-phenylquinoline-2-*N*-dimethylcarboxamide as a yellow oil (0.10 g, 60%). δ_H (400 MHz, $CDCl_3$) 3.05 (3H, s, NC*H*$_3$), 3.17 (3H, s, NC*H*$_3$), 4.34 (2H, s, C*H*$_2$OH), 7.33–7.48 (7H, m, 7 × ArH), 7.62–7.66 (1H, m, ArH), 8.03 (1H, d, *J* 8.4 Hz, ArH). To a solution of 3-hydroxymethyl-4-phenylquinoline-2-*N*-dimethylcarboxamide (0.10 g, 0.31 mmol) in dichloromethane (10 mL), oxalyl chloride (0.52 mL, 7.18 mmol) was added. The reaction mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated *in vacuo* and excess oxalyl chloride removed by azeotroping with toluene (3 × 20 mL). A solution of sodium iodide (0.20 g, 1.4 mmol) and acetonitrile (30 mL) were de-gased for 0.5 h. The chloride was then added and the reaction mixture heated under reflux for 2.5 h. The mixture was cooled to room temperature and then concentrated to dryness. Purification was carried out by flash column chromatography (dichloromethane/ethyl acetate, 9:1) to give 3-iodomethyl-4-phenylquinoline-2-*N*-dimethylcarboxamide (**12**) as a yellow oil (0.01 g, 52%). ν_{max}/cm^{-1} (neat) 2927 (CH), 1625 (CO), 1485, 1391, 1169, 1059, 757; δ_H (400 MHz, $CDCl_3$) 3.02 (3H, s, NCH_3), 3.18 (3H, s, NCH_3) 4.54 (2H, s, CH_2I), 7.28–7.39 (4H, m, 4 × ArH), 7.42–7.53 (3H, m, 3 × ArH), 7.62–7.66 (1H, m, ArH), 8.03 (1H, d, *J* 8.4, Hz, ArH); δ_C (101 MHz, $CDCl_3$) 0.0 (CH_2), 35.0 (CH_3), 39.1 (CH_3), 126.5 (CH), 127.5 (CH), 128.0 (C), 128.4 (2 × CH, C), 128.5 (2 × CH), 128.6 (CH), 129.2 (CH), 129.9 (CH), 134.9 (C), 145.6 (C), 148.4 (C), 154.9 (C), 168.2 (C); *m/z* (CI) 417.0459 (MH^+. $C_{19}H_{18}IN_2O$ requires 417.0464), 329 (5%), 291 (100), 220 (5), 137 (10), 81 (8).

3-Iodomethyl-4-phenylquinoline-2-*N*-morpholinecarboxamide (13)



The lactone opening reaction was performed as described above using 9-phenylfuro[3,4-*b*]quinolin-3(1*H*)-one (**11**) (0.08 g, 0.27 mmol), morpholine (0.03 mL, 0.75 mmol), trimethylaluminium (0.03 mL, 0.34 mmol) and gave 3-hydroxymethyl-4-phenylquinoline-2-*N*-morpholincarboxamide as a yellow oil (0.09 g, 86%). δ_H (400 MHz, $CDCl_3$) 3.56 (2H, t, *J* 5.2 Hz, NCH_2), 3.68 (2H, t, *J* 5.2 Hz, NCH_2), 3.76–3.83 (2H, m, CH_2O), 3.87–3.90 (2H, m, CH_2O), 4.38 (2H, d, *J* 6.5 Hz, $ArCH_2$), 7.32–7.53 (7H, m, 7 × ArH), 7.61–7.70 (1H, m, ArH), 8.04 (1H, d, *J* 8.4 Hz, ArH). Chlorination and subsequent conversion to the iodide was performed as described above using 3-hydroxymethyl-4-phenylquinoline-2-*N*-morpholinecarboxamide (0.09 g, 0.31 mmol), oxalyl chloride (0.30 mL, 4.17 mmol), then sodium iodide (0.12 g, 0.82 mmol), which gave 3-iodomethyl-4-phenylquinoline-2-*N*-morpholinecarboxamide (**13**) as yellow oil (0.07 g, 59%). ν_{max}/cm^{-1} (neat) 2848 (CH), 1629 (CO), 1617, 1469, 1246, 1012, 766; δ_H (400 MHz, $CDCl_3$) 3.51 (2H, t, *J* 4.8 Hz, NCH_2), 3.79 (2H, t, *J* 4.8 Hz, NCH_2), 3.82–3.90 (4H, m, 2 × CH_2O), 4.58 (2H, s, CH_2I), 7.28–7.33 (3H, m, 3 × ArH), 7.37–7.41 (1H, m, ArH), 7.46–7.55 (3H, m, 3 × ArH), 7.65 (1H, ddd, *J* 8.2, 5.6, 1.2 Hz, ArH), 8.02 (1H, d, *J* 8.2 Hz, ArH); δ_C (101 MHz, $CDCl_3$) 0.0 (CH_2), 41.9 (CH_2), 47.4 (CH_2), 66.1 (CH_2), 66.3 (CH_2), 126.5 (CH), 127.6 (CH), 128.3 (CH), 128.5 (C), 128.5 (2 × CH, C), 128.6 (2 × CH), 129.3 (CH), 129.9 (CH), 134.7 (C), 145.0 (C), 148.2 (C), 153.8 (C), 166.6 (C); *m/z* (FAB) 459.0571 (MH^+. $C_{21}H_{20}IN_2O_2$ requires 459.0570), 410 (70%), 365 (95), 331 (100), 262 (42), 219 (34), 126 (25), 86 (39).

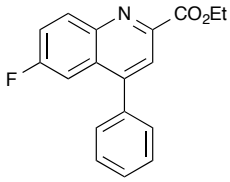
Ethyl 6-iodo-4-phenylquinoline-2-carboxylate (16)



To a solution of 4-iodoaniline (0.657 g, 3.00 mmol) in nitromethane (5 mL) was added phenylacetylene (**14**) (0.494 mL, 4.50 mmol), ethyl glyoxalate solution (50% in toluene) (**15**)

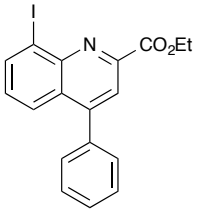
(0.595 mL, 3.00 mmol) and iodine (0.152 g, 0.600 mmol). The resultant solution was stirred vigorously at ambient temperature for 72 h and then diluted with ethyl acetate (20 mL). The organic layer was washed with 0.1 M sodium thiosulfate solution (20 mL) and water (20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by trituration with diethyl ether gave ethyl 6-iodo-4-phenylquinoline-2-carboxylate (**16**) (0.608 g, 50%) as a pale yellow solid. Mp 200–201 °C; ν_{max}/cm^{-1} (neat) 3048 (CH), 1721 (CO), 1481, 1366, 1250, 1111, 1018, 826, 702; δ_H (400 MHz, $CDCl_3$) 1.49 (3H, t, *J* 7.2 Hz, CH_2CH_3), 4.56 (2H, q, *J* 7.2 Hz, CH_2CH_3), 7.49–7.61 (5H, m, 5 × ArH), 8.03 (1H, dd, *J* 8.8, 1.6 Hz, ArH), 8.09 (1H, d, *J* 8.8 Hz, ArH), 8.13 (1H, s, ArH), 8.33 (1H, d, *J* 1.6 Hz, ArH); δ_C (101 MHz, $CDCl_3$) 14.4 (CH_3), 62.4 (CH_2), 95.4 (C), 122.0 (CH), 128.9 (2 × CH), 129.0 (CH), 129.3 (C), 129.5 (2 × CH), 132.7 (CH), 134.6 (CH), 136.9 (C), 138.9 (CH), 147.1 (C), 148.2 (C), 148.8 (C), 165.2 (C); *m/z* (EI) 403.0067 (M^+. $C_{18}H_{14}INO_2$ requires 403.0069), 358 (10%), 330 (100), 203 (45), 176 (17), 150 (4), 83 (38).

Ethyl 6-fluoro-4-phenylquinoline-2-carboxylate (17)



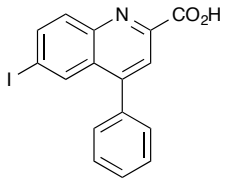
Ethyl 6-fluoro-4-phenylquinoline-2-carboxylate (**17**) was synthesised using the procedure described above using 4-fluoroaniline (94.6 μL, 1.00 mmol), phenylacetylene (**14**) (0.165 mL, 1.50 mmol), ethyl glyoxalate solution (50% in toluene) (**15**) (0.198 mL, 1.00 mmol) and iodine (0.0508 g, 0.200 mmol) in nitromethane (2 mL). Purification by trituration with diethyl ether afforded ethyl 6-fluoro-4-phenylquinoline-2-carboxylate (**17**) (0.169 g, 57%) as a pale yellow solid. Mp 154–155 °C; ν_{max}/cm^{-1} (neat) 2986 (CH), 1713 (CO), 1512, 1466, 1373, 1227, 1196, 1026, 833, 702; δ_H (400 MHz, $CDCl_3$) 1.49 (3H, t, *J* 7.1 Hz, CH_2CH_3), 4.57 (2H, q, *J* 7.1 Hz, CH_2CH_3), 7.49–7.60 (7H, m, 7 × ArH), 8.15 (1H, s, ArH), 8.35–8.41 (1H, m, ArH); δ_C (100 MHz, $CDCl_3$) 14.4 (CH_3), 62.3 (CH_2), 109.2 (CH, d, $J_{C\text{-}C\text{-}F}$ 23.4 Hz), 120.5 (CH, d, $J_{C\text{-}C\text{-}F}$ 26.2 Hz), 121.8 (CH), 128.9 (C, d, $J_{C\text{-}C\text{-}C\text{-}F}$ 10.0 Hz), 128.9 (2 × CH), 129.0 (CH), 129.4 (2 × CH), 133.8 (CH, d, $J_{C\text{-}C\text{-}C\text{-}F}$ 9.4 Hz), 137.1 (C), 145.4 (C), 147.3 (C, d, *J* 2.9 Hz), 149.3 (C, d, $J_{C\text{-}C\text{-}C\text{-}C\text{-}F}$ 5.9 Hz), 161.9 (C, d, $J_{C\text{-}F}$ 251.0 Hz), 165.3 (C); *m/z* (CI) 296.1088 (MH^+. $C_{18}H_{15}FNO_2$ requires 296.1087), 224 (6%), 198 (5), 113 (22), 85 (45), 73 (100).

Ethyl 8-iodo-4-phenylquinoline-2-carboxylate (18)



To a suspension of magnesium sulfate (1.0 g) in dichloromethane (20 mL) was added 2-iodoaniline (0.30 g, 1.37 mmol) followed by ethyl glyoxalate solution (50% in toluene) (**15**) (0.27 mL, 1.37 mmol). The resultant suspension was stirred at ambient temperature for 24 h and then filtered. Phenylacetylene (**14**) (0.15 mL, 1.37 mmol) and copper(II) triflate (0.099 g, 0.274 mmol) was added to the filtrate and the reaction mixture stirred at ambient temperature for a further 48 h. The mixture was washed with water (2 × 20 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification using silica column chromatography (diethyl ether/petroleum ether, 1:9) followed by crystallisation in hexane afforded ethyl 8-iodo-4-phenylquinoline-2-carboxylate (**18**) (0.121 g, 22%) as a colourless solid. Mp 102–104 °C; ν_{max}/cm^{-1} (neat) 2987 (CH), 1722 (CO), 1601, 1488, 1373, 1244, 1120, 1026; δ_H (500 MHz, $CDCl_3$) 1.52 (3H, t, *J* 7.0 Hz, CH_3CH_2), 4.55 (2H, q, *J* 7.0 Hz, CH_3CH_2), 7.28 (1H, dd, *J* 8.5, 7.5 Hz, ArH), 7.49–7.58 (5H, m, 5 × ArH), 7.95 (1H, dd, *J* 8.5, 1.0 Hz, ArH), 8.15 (1H, s, ArH), 8.42 (1H, dd, *J* 7.5, 1.0 Hz, ArH); δ_C (126 MHz, $CDCl_3$) 14.3 (CH_3), 62.2 (CH_2), 105.5 (C), 122.1 (CH), 126.7 (CH), 128.5 (C), 128.7 (2 × CH), 128.9 (CH), 129.4 (CH), 129.6 (2 × CH), 137.1 (C), 140.8 (CH), 147.0 (C), 148.6 (C), 150.8 (C), 165.0 (C); *m/z* (ESI) 425.9947 (MNa^+. $C_{18}H_{14}INNaO_2$ requires 425.9961).

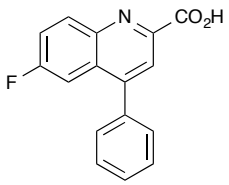
6-Iodo-4-phenylquinoline-2-carboxylic acid (19)



To a solution of ethyl 6-iodo-4-phenylquinoline-2-carboxylate (**16**) (0.078 g, 0.19 mmol) in a 50% aqueous ethanol mixture (10 ml) was added ground sodium hydroxide (0.031 g, 0.77 mmol), and the reaction mixture stirred under reflux for 18 h. On cooling to ambient temperature, the ethanol was removed *in vacuo*, and the aqueous layer acidified (pH ~4) using

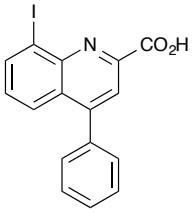
a 1 M hydrochloric acid solution (~10 mL). The crude product was extracted using dichloromethane (3 × 20 mL), washed with water (2 × 20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification by trituration with diethyl ether afforded 6-iodo-4-phenylquinoline-2-carboxylic acid (**19**) (0.051 g, 70%) as a yellow solid. Mp 198–200 °C; ν_{max}/cm^{-1} (neat) 2901 (CH), 1705 (CO), 1582, 1458, 1366, 1250, 1134, 972, 787; δ_H (400 MHz, $CDCl_3$) 7.48–7.63 (5H, m, 5 × ArH), 7.94 (1H, d, *J* 9.0 Hz, ArH), 8.09 (1H, dd, *J* 9.0, 1.8 Hz, ArH), 8.24 (1H, s, ArH), 8.40 (1H, d, *J* 1.8 Hz, ArH); δ_C (101 MHz, $CDCl_3$) 96.0 (C), 120.2 (CH), 129.1 (2 × CH), 129.4 (CH), 129.5 (2 × CH), 129.9 (C), 131.2 (CH), 135.1 (CH), 136.4 (C), 139.8 (CH), 145.5 (C), 145.8 (C), 150.6 (C), 164.0 (C); *m/z* (CI) 375.9832 (MH^+. $C_{16}H_{11}INO_2$ requires 375.9835), 332 (8%), 250 (41), 206 (4), 113 (3), 69 (7).

6-Fluoro-4-phenylquinoline-2-carboxylic acid (20)



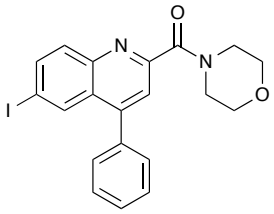
6-Fluoro-4-phenylquinoline-2-carboxylic acid (**20**) was synthesised using the procedure described above using ethyl 6-fluoro-4-phenylquinoline-2-carboxylate (**17**) (0.160 g, 0.542 mmol) and ground sodium hydroxide (0.087 g, 2.17 mmol) in a 50% aqueous ethanol solution (10 mL). Purification by trituration with diethyl ether yielded 6-fluoro-4-phenylquinoline-2-carboxylic acid (**20**) (0.119 g, 82%) as a colourless solid. Mp 129–131 °C; ν_{max}/cm^{-1} (neat) 3228 (OH), 3053 (CH), 1704 (CO), 1588, 1485, 1451, 1373, 1315, 1061, 810; δ_H (500 MHz, $CDCl_3$) 7.49–7.68 (7H, m, 7 × ArH), 8.23–8.27 (2H, m, 2 × ArH); δ_C (126 MHz, $CDCl_3$) 109.8 (CH, d, $J_{C\text{-}C\text{-}F}$ 23.6 Hz), 120.0 (CH), 121.4 (CH, d, $J_{C\text{-}C\text{-}F}$ 26.3 Hz), 129.0 (2 × CH), 129.3 (2 × CH), 129.4 (CH), 129.7 (C, d, $J_{C\text{-}C\text{-}C\text{-}F}$ 9.7 Hz), 132.6 (CH, d, $J_{C\text{-}C\text{-}C\text{-}F}$ 9.4 Hz), 136.6 (C), 143.7 (C), 145.0 (C), 151.1 (C, d, $J_{C\text{-}C\text{-}C\text{-}C\text{-}F}$ 5.7 Hz), 162.2 (C, d, $J_{C\text{-}F}$ 252.5 Hz), 164.2 (C); *m/z* (EI) 267.0694 (M^+. $C_{16}H_{10}FNO_2$ requires 267.0696), 223 (16%), 135 (5), 82 (100), 46 (18).

8-Iodo-4-phenylquinoline-2-carboxylic acid (21)



8-Iodo-4-phenylquinoline-2-carboxylic acid (**21**) was synthesised using the procedure described above using ethyl 8-iodo-4-phenylquinoline-2-carboxylate (**18**) (0.050 g, 0.124 mmol) and ground sodium hydroxide (0.020 g, 0.496 mmol) in a 50% aqueous ethanol solution (5 mL). Purification by trituration with hexane gave 8-iodo-4-phenylquinoline-2-carboxylic acid (**21**) (0.039 g, 84%) as a colourless solid. Mp 128–130 °C; ν_{max}/cm^{-1} (neat) 3310 (OH), 3061 (CH), 1763 (CO), 1599, 1489, 1443, 1408, 1383, 1319; δ_H (500 MHz, $CDCl_3$) 7.37 (1H, dd, *J* 8.0, 7.5 Hz, ArH), 7.47–7.60 (5H, m, 5 × ArH), 8.03 (1H, dd, *J* 8.0, 0.5 Hz, ArH), 8.28 (1H, s, ArH), 8.45 (1H, dd, *J* 7.5, 0.5 Hz, ArH); δ_C (126 MHz, $CDCl_3$) 103.6 (C), 120.3 (CH), 127.1 (CH), 128.9 (2 × CH), 129.3 (C), 129.4 (CH), 129.6 (2 × CH), 130.1 (CH), 136.5 (C), 141.4 (CH), 145.3 (C), 145.8 (C), 152.9 (C), 163.7 (C); *m/z* (EI) 374.9752 (M^+. $C_{16}H_{10}INO_2$ requires 374.9756), 330 (83%), 282 (22), 199 (68), 176 (21), 115 (100), 105 (80), 83 (60).

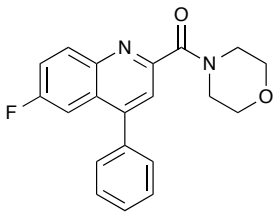
6-Iodo-4-phenylquinoline-2-*N*-morpholinecarboxamide (22)



A solution of 6-iodo-4-phenylquinoline-2-carboxylic acid (**19**) (0.051 g, 0.136 mmol) in dichloromethane (10 mL) was cooled to 0 °C, and to this was added a few drops of *N*,*N*'-dimethylformamide followed by oxalyl chloride (17.3 μL, 0.204 mmol). The resultant solution was allowed to warm to ambient temperature and then stirred under reflux for 18 h. After cooling to ambient temperature, the reaction mixture was concentrated *in vacuo* and excess oxalyl chloride removed by azeotroping with toluene (3 × 20 mL). The crude residue was then reconstituted in dichloromethane (10 mL) and cooled to 0 °C. Morpholine (59.5 μL,

0.680 mmol) was added to the solution dropwise and the reaction mixture stirred under reflux for a further 5 h. On cooling to ambient temperature, the mixture was diluted with water (10 mL) and the aqueous layer extracted using dichloromethane (3 × 10 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification *via* silica column chromatography (methanol/dichloromethane, 1:9) afforded 6-iodo-4-phenylquinoline-2-*N*-morpholinecarboxamide (**22**) (0.055 g, 92%) as a pale yellow solid. Mp 116–118 °C; ν_{max}/cm^{-1} (neat) 2853 (CH), 1628 (CO), 1468, 1439, 1271, 1244, 1111, 1028; δ_H (400 MHz, $CDCl_3$) 3.72–3.91 (8H, m, 4 × CH_2), 7.48–7.59 (5H, m, 5 × ArH), 7.69 (1H, s, ArH), 7.86 (1H, d, *J* 8.8 Hz, ArH), 8.00 (1H, dd, *J* 8.8, 2.0 Hz, ArH), 8.30 (1H, d, *J* 2.0 Hz, ArH); δ_C (101 MHz, $CDCl_3$) 42.9 (CH_2), 47.9 (CH_2), 66.9 (CH_2), 67.1 (CH_2), 94.1 (C), 121.8 (CH), 128.3 (C), 128.9 (2 × CH), 129.0 (CH), 129.4 (2 × CH), 131.7 (CH), 134.7 (CH), 136.8 (C), 138.8 (CH), 146.1 (C), 148.8 (C), 153.3 (C), 167.2 (C); *m/z* (CI) 445.0415 (MH^+. $C_{20}H_{18}IN_2O_2$ requires 445.0413), 319 (100%), 206 (4), 116 (5), 69 (10).

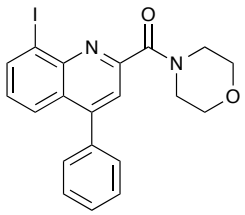
6-Fluoro-4-phenylquinoline-2-*N*-morpholinecarboxamide (23)



6-Fluoro-4-phenylquinoline-2-*N*-morpholinecarboxamide (**23**) was synthesised using the procedure described above using 6-fluoro-4-phenylquinoline-2-carboxylic acid (**20**) (0.048 g, 0.180 mmol), a few drops of *N*,*N*'-dimethylformamide, oxalyl chloride (22.8 μL, 0.269 mmol) and morpholine (78.6 μL, 0.898 mmol) in dichloromethane (10 mL). Purification by silica column chromatography (diethyl ether/petroleum ether, 9:1) afforded 6-fluoro-4-phenylquinoline-2-*N*-morpholinecarboxamide (**23**) (0.037 g, 61%) as a pale yellow oil. ν_{max}/cm^{-1} (neat) 2855 (CH), 1622 (CO), 1474, 1435, 1229, 1196, 1111, 1028; δ_H (400 MHz, $CDCl_3$) 3.71–3.93 (8H, m, 4 × CH_2), 7.48–7.59 (7H, m, 7 × ArH), 7.73 (1H, s, ArH), 8.16 (1H, dd, *J* 9.2, 5.6 Hz, ArH); δ_C (101 MHz, $CDCl_3$) 42.9 (CH_2), 47.9 (CH_2), 66.9 (CH_2), 67.1 (CH_2), 109.3 (CH, d, $J_{C\text{-}C\text{-}F}$ 23.4 Hz), 120.3 (CH, d, $J_{C\text{-}C\text{-}F}$ 25.9 Hz), 121.7 (CH), 127.7 (C, d, $J_{C\text{-}C\text{-}C\text{-}F}$ 9.5 Hz), 128.9 (2 × CH), 129.0 (CH), 129.3 (2 × CH), 132.7 (CH, d, $J_{C\text{-}C\text{-}C\text{-}F}$ 9.3 Hz), 137.1 (C), 144.2 (C), 149.4 (C, d, $J_{C\text{-}C\text{-}C\text{-}C\text{-}F}$ 5.3 Hz), 152.3 (C), 161.4 (C, d, $J_{C\text{-}F}$ 250.6

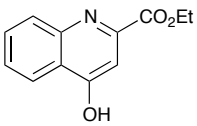
Hz), 167.3 (C); *m/z* (CI) 337.1348 (MH^+. $C_{20}H_{18}FN_2O_2$ requires 337.1352), 220 (5), 188 (4), 69 (7).

8-Iodo-4-phenylquinoline-2-*N*-morpholinecarboxamide (24)



8-Iodo-4-phenylquinoline-2-*N*-morpholinecarboxamide (**24**) was synthesised using the procedure described above using 8-iodo-4-phenylquinoline-2-carboxylic acid (**21**) (0.039 g, 0.104 mmol), a few drops of *N*,*N*'-dimethylformamide, oxalyl chloride (13.2 μL, 0.156 mmol) and morpholine (45.5 μL, 0.520 mmol) in dichloromethane (3 mL). Purification by silica column chromatography (methanol/dichloromethane, 1:99) gave 8-iodo-4-phenylquinoline-2-*N*-morpholinecarboxamide (**24**) (0.026 g, 57%) as a colourless solid. Mp 119–120 °C; ν_{max}/cm^{-1} (neat) 2855 (CH), 1626 (CO), 1460, 1437, 1267, 1246, 1109, 1026; δ_H (400 MHz, $CDCl_3$) 3.88–3.93 (4H, m, 2 × NCH_2), 3.96 (2H, t, *J* 4.6 Hz, OCH_2), 4.20 (2H, t, *J* 4.6 Hz, OCH_2), 7.27 (1H, m, ArH), 7.47–7.56 (5H, m, 5 × ArH), 7.91 (1H, s, ArH), 7.93 (1H, dd, *J* 8.4, 1.2 Hz, ArH), 8.37 (1H, dd, *J* 7.3, 1.2 Hz, ArH); δ_C (101 MHz, $CDCl_3$) 43.5 (CH_2), 48.4 (CH_2), 67.1 (CH_2), 67.9 (CH_2), 104.5 (C), 123.0 (CH), 126.8 (CH), 127.6 (C), 128.7 (2 × CH), 128.9 (2 × CH), 129.6 (2 × CH), 137.1 (C), 140.6 (CH), 145.5 (C), 150.7 (C), 153.0 (C), 166.1 (C); *m/z* (EI) 444.0334 (M^+. $C_{20}H_{17}IN_2O_2$ requires 444.0335), 358 (25%), 331 (100), 203 (91), 176 (30), 86 (31), 83 (42).

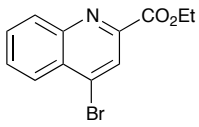
Ethyl 4-hydroxyquinoline-2-carboxylate (27)[3]



A suspension of aniline (**25**) (2.20 mL, 23.8 mmol), diethyl oxalacetate (**26**) (5.00 g, 23.8 mmol) and *p*-toluenesulfonic acid (4.53 g, 23.8 mmol) in cyclohexane (100 mL) was stirred vigorously under reflux with Dean-Stark conditions for 48 h. After cooling to ambient temperature, the suspension was filtered, washed with cyclohexane and the filtrate

concentrated *in vacuo* to yield the desired imine as a yellow oil. Neat polyphosphoric acid (~10 g) was added to the imine and stirred vigorously at 120 °C for 1 h. After cooling to ambient temperature, excess acid was quenched by the slow addition of a saturated solution of aqueous sodium hydrogen carbonate (100 mL). The aqueous mixture was diluted by the addition of chloroform (100 mL) and separated. The aqueous fraction was washed with chloroform (3 × 100 mL), and the combined organic layers dried ($MgSO_4$), filtered and concentrated *in vacuo* to yield a dark yellow solid. Purification using flash column chromatography (methanol/dichloromethane, 1:9) afforded ethyl 4-hydroxyquinoline-2-carboxylate (**27**) (2.41 g, 48%) as a light tan solid. Spectroscopic data in accordance with the literature.[3] Mp 215–216 °C (lit.,[3] mp 213 °C); ν_{max}/cm^{-1} (neat) 2882 (CH), 1736 (CO), 1607, 1560, 1518, 1312, 1267, 1233, 1009; δ_H (400 MHz, $CDCl_3$) 1.44 (3H, t, *J* 7.2 Hz, OCH_2CH_3), 4.49 (2H, q, *J* 7.2 Hz, OCH_2CH_3), 6.99 (1H, d, *J* 1.6 Hz, ArH), 7.39 (1H, t, *J* 8.0 Hz, ArH), 7.43 (1H, d, *J* 8.4 Hz, ArH), 7.67 (1H, ddd, *J* 8.4, 8.0, 1.6 Hz, ArH), 8.35 (1H, dd, *J* 8.0, 0.4 Hz, ArH), 9.01 (1H, br s, OH); δ_C (101 MHz, $CDCl_3$) 14.1 (CH_3), 63.4 (CH_2), 111.7 (CH), 118.0 (CH), 124.5 (CH), 126.4 (CH), 126.4 (C), 133.1 (CH), 136.4 (C), 139.0 (C), 163.0 (C), 179.7 (C); *m/z* (EI) 217.0735 (M^+. $C_{12}H_{11}NO_3$ requires 217.0739), 189 (6%), 171 (22), 143 (98), 115 (30), 89 (27), 83 (27), 49 (29).

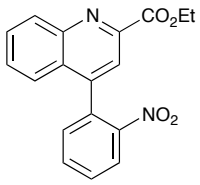
Ethyl 4-bromoquinoline-2-carboxylate[4]



To a solution of ethyl 4-hydroxyquinoline-2-carboxylate (**27**) (3.36 g, 15.5 mmol) in acetonitrile (150 mL) was added phosphorus oxybromide (13.3 g, 46.4 mmol) followed by potassium carbonate (6.41 g, 46.4 mmol). The resultant suspension was heated under reflux and stirred for 2 h. After cooling to ambient temperature, the solution was concentrated *in vacuo* and water (100 mL) slowly added. The crude product was extracted into ethyl acetate (3 × 100 mL), and the combined organic layers were concentrated *in vacuo* to yield a dark brown oil from which ethyl 4-bromoquinoline-2-carboxylate (4.28 g, 99%) crystallised. Spectroscopic data in accordance with the literature.[4] Mp 87–89 °C (lit.,[4] mp 91–92 °C); ν_{max}/cm^{-1} (neat) 2996 (CH), 1709 (CO), 1553, 1458, 1366, 1312, 1196, 1146, 1105; δ_H (400 MHz, $CDCl_3$) 1.49 (3H, t, *J* 7.2 Hz, OCH_2CH_3), 4.56 (2H, q, *J* 7.2 Hz, OCH_2CH_3), 7.75 (1H, ddd, *J* 8.4, 6.8, 1.2 Hz, ArH), 7.84 (1H, ddd, *J* 8.4, 6.8, 1.2 Hz, ArH), 8.24 (1H, dd, *J* 8.4, 1.2

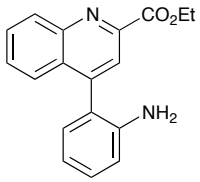
Hz, ArH), 8.34 (1H, d, *J* 8.4 Hz, ArH), 8.47 (1H, s, ArH); δ_C (101 MHz, $CDCl_3$) 14.4 (CH_3), 62.7 (CH_2), 125.1 (CH), 126.7 (CH), 128.9 (C), 130.0 (CH), 131.1 (CH), 131.2 (CH), 135.4 (C), 147.8 (C), 147.9 (C), 164.2 (C); *m/z* (CI) 279.9974 (MH^+. $C_{12}H_{11}{}^{79}BrNO_2$ requires 279.9973), 218 (52%), 202 (47), 157 (3), 85 (9).

Ethyl 4-(2-nitrophenyl)quinoline-2-carboxylate (28)



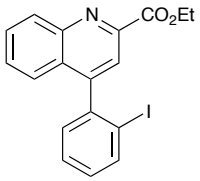
To a solution of ethyl 4-bromoquinoline-2-carboxylate (0.196 g, 0.699 mmol) in *N*,*N*'-dimethylformamide (10 mL) were added 2-nitrophenylboronic acid (0.140 g, 0.839 mmol), potassium phosphate (0.178 g, 0.839 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.081 g, 0.070 mmol). The resultant suspension was stirred at 120 °C for 24 h. An additional aliquot of 2-nitrophenylboronic acid (0.140 g, 0.839 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.081 g, 0.070 mmol) was added and the suspension stirred for a further 24 h. After cooling to ambient temperature, the reaction mixture was concentrated *in vacuo* and reconstituted in dichloromethane (20 mL). The organic layer was washed with water (3 × 20 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification using silica column chromatography (ethyl acetate/petroleum ether, 2:3) afforded ethyl 4-(2-nitrophenyl)quinoline-2-carboxylate (**28**) (0.205 g, 91%) as a yellow solid. Mp 156–157 °C; ν_{max}/cm^{-1} (neat) 2990 (CH), 1713 (CO), 1512, 1350, 1251, 1136, 1107, 1020; δ_H (500 MHz, $CDCl_3$) 1.49 (3H, t, *J* 7.1 Hz, CH_2CH_3), 4.52–4.62 (2H, m, CH_2CH_3), 7.43–7.48 (2H, m, 2 × ArH), 7.55 (1H, ddd, *J* 8.3, 6.9, 1.2 Hz, ArH), 7.70 (1H, ddd, *J* 8.0, 7.5, 1.5 Hz, ArH), 7.75–7.80 (2H, m, 2 × ArH), 8.05 (1H, s, ArH), 8.24 (1H, dd, *J* 8.0, 1.5 Hz, ArH), 8.39 (1H, d, *J* 8.3 Hz, ArH); δ_C (126 MHz, $CDCl_3$) 14.3 (CH_3), 62.2 (CH_2), 120.4 (CH), 124.4 (CH), 124.8 (CH), 127.5 (C), 129.1 (CH), 129.9 (CH), 130.2 (CH), 131.4 (CH), 132.3 (CH), 132.7 (C), 133.3 (CH), 146.2 (C), 147.7 (C), 148.0 (C), 148.7 (C), 165.2 (C); *m/z* (EI) 322.0951 (M^+. $C_{18}H_{14}N_2O_4$ requires 322.0954), 278 (7%), 250 (100), 205 (10), 165 (9), 131 (11), 103 (9), 77 (7), 43 (15).

Ethyl 4-(2-aminophenyl)quinoline-2-carboxylate



Tin(II) chloride dihydrate (0.700 g, 3.10 mmol) was added in one portion to a stirred solution of ethyl 4-(2-nitrophenyl)quinoline-2-carboxylate (**28**) (0.200 g, 0.621 mmol) in ethanol (10 mL) and the reaction mixture stirred under reflux for 15 h. After cooling to ambient temperature, a saturated solution of sodium hydrogen carbonate (20 mL) was added, and the crude product extracted with ethyl acetate (3 × 30 mL). The organic layer was washed with water (30 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo* to afford ethyl 4-(2-aminophenyl)quinoline-2-carboxylate (0.180 g, 99%) as a yellow solid, which was used without further purification. Mp 138–139 °C; ν_{max}/cm^{-1} (neat) 3356 (NH), 2926 (CH), 1716 (CO), 1494, 1452, 1375, 1247, 1230, 1108; δ_H (400 MHz, $CDCl_3$) 1.48 (3H, t, *J* 7.1 Hz, CH_2CH_3), 4.56 (2H, q, *J* 7.1 Hz, CH_2CH_3), 6.86 (1H, d, *J* 8.0 Hz, ArH), 6.92 (1H, td, *J* 7.5, 1.2 Hz, ArH), 7.15 (1H, dd, *J* 7.5, 1.2 Hz, ArH), 7.32 (1H, td, *J* 8.0, 1.5 Hz, ArH), 7.59 (1H, ddd, *J* 8.0, 6.8, 1.2 Hz, ArH), 7.74–7.82 (2H, m, 2 × ArH), 8.17 (1H, s, ArH), 8.38 (1H, d, *J* 8.0, 0.7 Hz, ArH); δ_C (101 MHz, $CDCl_3$) 14.4 (CH_3), 62.3 (CH_2), 115.8 (CH), 118.6 (CH), 122.2 (CH), 122.6 (C), 125.9 (CH), 128.0 (C), 128.8 (CH), 130.0 (CH), 130.3 (CH), 130.6 (CH), 131.3 (CH), 143.7 (C), 147.5 (C), 148.2 (C), 148.4 (C), 165.4 (C); *m/z* (EI) 292.1208 (M^+. $C_{18}H_{16}N_2O_4$ requires 292.1212), 219 (18), 190 (3), 165 (2), 83 (3), 47 (18).

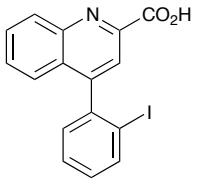
Ethyl 4-(2-iodophenyl)quinoline-2-carboxylate (29)



To a solution of ethyl 4-(2-aminophenyl)quinoline-2-carboxylate (0.030 g, 0.103 mmol) in acetonitrile (1 mL) was added *p*-toluenesulfonic acid monohydrate (0.059 g, 0.308 mmol) at ambient temperature. The resulting solution was then cooled to 0 °C and a solution of potassium iodide (0.043 g, 0.256 mmol) and sodium nitrite (0.014 g, 0.205 mmol) in water

(0.10 mL) was added dropwise over a period of 0.25 h. The reaction mixture was stirred at 0 °C for 1 h, and then allowed to gradually warm to ambient temperature and stirred overnight. The reaction mixture was made alkaline (pH ~9) by the addition of a saturated solution of sodium hydrogen carbonate, and excess iodine quenched by the addition of a 0.5 M sodium thiosulfate solution (~2 mL). The crude mixture was extracted with dichloromethane (2 × 10 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Purification using silica column chromatography (ethyl acetate/petroleum ether, 3:2) gave ethyl 4-(2-iodophenyl)quinoline-2-carboxylate (**29**) (0.034 g, 83%) as a pale yellow solid. Mp 107–109 °C; ν_{max}/cm^{-1} (neat) 2928 (CH), 1717 (CO), 1555, 1458, 1370, 1250, 1231, 1105, 1015; δ_H (500 MHz, $CDCl_3$) 1.50 (3H, t, *J* 7.1 Hz, CH_2CH_3), 4.54–4.61 (2H, m, CH_2CH_3), 7.20 (1H, td, 8.0, 1.5 Hz, ArH), 7.32 (1H, dd, *J* 7.5, 1.5 Hz, ArH), 7.49–7.50 (2H, m, 2 × ArH), 7.57 (1H, ddd, *J* 8.5, 6.5, 1.5 Hz, ArH), 7.79 (1H, ddd, *J* 8.5, 6.5, 1.5 Hz, ArH), 8.04 (1H, s, ArH), 8.05 (1H, d, *J* 1.5 Hz, ArH), 8.40 (1H, d, *J* 8.5 Hz, ArH); δ_C (126 MHz, $CDCl_3$) 14.3 (CH_3), 62.2 (CH_2), 98.2 (C), 121.5 (CH), 125.7 (CH), 127.6 (C), 128.2 (CH), 128.6 (CH), 130.0 (CH), 130.1 (CH), 130.2 (CH), 131.2 (CH), 139.5 (CH), 142.5 (C), 148.0 (C), 148.1 (C), 151.5 (C), 165.3 (C); *m/z* (EI) 403.0065 (M^+. $C_{18}H_{14}INO_2$ requires 403.0069), 388 (12%), 358 (6), 330 (70), 205 (18), 136 (24), 84 (100).

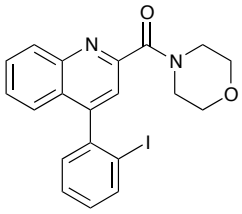
4-(2-Iodophenyl)quinoline-2-carboxylic acid (30)



4-(2-Iodophenyl)quinoline-2-carboxylic acid **(30)** was synthesised as described for 6-iodo-4-phenylquinoline-2-carboxylic acid (**19**) using ethyl 4-(2-iodophenyl)quinoline-2-carboxylate (**29**) (0.133 g, 0.330 mmol) and ground sodium hydroxide (0.053 g, 1.32 mmol) in a 50% aqueous ethanol solution (5 mL) to yield 4-(2-iodophenyl)quinoline-2-carboxylic acid (**30**) (0.124 g, 100%) as a yellow solid, which was used without further purification. Mp 166–168 °C; ν_{max}/cm^{-1} (neat) 2922 (CH), 2340 (OH), 1707 (CO), 1593, 1462, 1377, 1227, 1015; δ_H (500 MHz, $CDCl_3$) 7.22 (1H, td, *J* 8.0, 1.0 Hz, ArH), 7.31 (1H, dd, *J* 7.6, 1.5 Hz, ArH), 7.52 (1H, td, *J* 7.6, 1.0 Hz, ArH), 7.57 (1H, d, *J* 8.3 Hz, ArH), 7.64 (1H, t, *J* 7.6 Hz, ArH), 7.85 (1H, t, *J* 7.6 Hz, ArH), 8.05 (1H, d, *J* 8.0 Hz, ArH), 8.16 (1H, s, ArH), 8.25 (1H, d, *J* 8.3 Hz, ArH); δ_C (126 MHz, $CDCl_3$) 97.8 (C), 119.8 (CH), 126.3 (CH), 128.3 (CH),

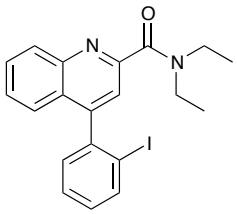
128.4 (C), 129.2 (CH), 129.8 (CH), 130.1 (CH), 130.3 (CH), 130.9 (CH), 139.6 (CH), 142.0 (C), 145.7 (C), 146.3 (C), 153.3 (C), 164.0 (C); *m/z* (EI) 374.9755 (M^+. $C_{16}H_{10}INO_2$ requires 374.9756), 331 (87%), 277 (100), 204 (45), 176 (22), 152 (10), 77 (14).

4-(2-Iodophenyl)quinoline-2-*N*-morpholinecarboxamide (31)



4-(2-Iodophenyl)quinoline-2-*N*-morpholinecarboxamide (**31**) was synthesised as described for 6-iodo-4-phenylquinoline-2-*N*-morpholinecarboxamide (**22**) using 4-(2-iodophenyl)quinoline-2-carboxylic acid (**30**) (0.045 g, 0.119 mmol), a few drops of *N*,*N*'-dimethylformamide, oxalyl chloride (15.1 μL, 0.178 mmol) and morpholine (51.9 μL, 0.593 mmol) in dichloromethane (5 mL). Purification using silica column chromatography (ethyl acetate) afforded 4-(2-iodophenyl)quinoline-2-*N*-morpholinecarboxamide (**31**) (0.032 g, 60%) as a yellow solid. Mp 118–120 °C; ν_{max}/cm^{-1} (neat) 2853 (CH), 1628 (CO), 1551, 1466, 1404, 1273, 1244, 1111; δ_H (400 MHz, $CDCl_3$) 3.68–3.96 (8H, m, 4 × CH_2), 7.20 (1H, td, *J* 7.8, 1.6 Hz, ArH), 7.33 (1H, dd, *J* 7.8, 1.6 Hz, ArH), 7.47–7.57 (3H, m, 3 × ArH), 7.58 (1H, s, ArH), 7.77 (1H, ddd, *J* 8.4, 6.6, 1.7 Hz, ArH), 8.03 (1H, dd, *J* 7.8, 1.2 Hz, ArH), 8.18 (1H, d, *J* 8.4 Hz, ArH); δ_C (101 MHz, $CDCl_3$) 42.9 (CH_2), 47.9 (CH_2), 66.9 (CH_2), 67.1 (CH_2), 98.3 (C), 121.3 (CH), 125.9 (CH), 126.5 (C), 127.9 (CH), 128.2 (CH), 130.1 (CH), 130.1 (CH), 130.2 (CH), 130.3 (CH), 139.5 (CH), 142.3 (C), 147.0 (C), 151.5 (C), 152.9 (C), 167.5 (C); *m/z* (EI) 444.0332 (M^+. $C_{20}H_{17}IN_2O_2$ requires 444.0335), 359 (8%), 331 (100), 203 (75), 176 (15), 83 (66).

4-(2-Iodophenyl)quinoline-2-*N*-diethylcarboxamide (32)



4-(2-Iodophenyl)quinoline-2-*N*-diethylcarboxamide (**32**) was synthesised as described for 6-iodo-4-phenylquinoline-2-*N*-morpholinecarboxamide (**22**) using 4-(2-iodophenyl)quinoline-2-carboxylic acid (**30**) (0.045 g, 0.119 mmol), a few drops of *N*,*N*'-dimethylformamide, oxalyl chloride (15.1 μL, 0.178 mmol) and diethylamine (61.4 μL, 0.593 mmol) in dichloromethane (5 mL). Purification using silica column chromatography (ethyl acetate/petroleum ether, 9:1) afforded the desired product (**32**) (0.021 g, 51%) as a pale yellow oil, which crystallised on standing. Mp 64–66 °C; ν_{max}/cm^{-1} (neat) 2969 (CH), 1626 (CO), 1464, 1404, 1275, 1096, 1015; δ_H (400 MHz, $CDCl_3$) 1.25 (3H, t, *J* 7.1 Hz, CH_2CH_3), 1.33 (3H, t, *J* 7.1 Hz, CH_2CH_3), 3.39–3.60 (2H, m, CH_2CH_3), 3.64 (2H, q, *J* 7.1 Hz, CH_2CH_3), 7.19 (1H, td, *J* 7.8, 1.4, Hz, ArH), 7.33 (1H, dd, *J* 7.8, 1.7 Hz, ArH), 7.45–7.54 (4H, m, 4 × ArH), 7.76 (1H, ddd, *J* 8.3, 6.6, 1.6 Hz, ArH), 8.02 (1H, dd, *J* 7.8, 1.4 Hz, ArH), 8.19 (1H, d, *J* 8.3 Hz, ArH); δ_C (101 MHz, $CDCl_3$) 13.0 (CH_3), 14.6 (CH_3), 40.4 (CH_2), 43.5 (CH_2), 98.4 (C), 120.6 (CH), 125.8 (CH), 126.3 (C), 127.5 (CH), 128.2 (CH), 130.0 (2 × CH), 130.1 (CH), 130.2 (CH), 139.3 (CH), 142.4 (C), 147.1 (C), 151.2 (C), 154.4 (C), 168.6 (C); *m/z* (EI) 430.0544 (M^+. $C_{20}H_{19}IN_2O$ requires 430.0542), 359 (23%), 331 (28), 203 (35), 176 (9), 84 (100).

3. Radioligand Binding Methodology[5]

Preparation of Rat Brain Membranes

Whole brains from male Sprague-Dawley rats (200–300 g) were obtained and immediately added to 25 mL of ice-cold Tris-Base buffer (50 nM, pH 7.4) and thoroughly homogenised using a Polytron. The resultant homogenates were centrifuged at 39100 g for 10 min (4 °C) using a Beckman J2-21M/E centrifuge. After discarding the supernatant, the pellet was resuspended in 25 mL of ice-cold buffer and the centrifugation process repeated. The resultant pellet was then resuspended in 10 mL of ice-cold buffer and the homogenate stored

at −50 °C until further use. Protein content was measured using Bovine Serum Albumin standards.

Competition Binding Assays

Brain homogenate stock was diluted to give a final assay concentration of approximately 0.6 mg of protein. Total binding was determined using a final concentration of ~1 nM [^{3}H]-PK11195, and non-specific binding was measured using a final concentration of ~8 μM unlabelled PK11195 in the presence of [^{3}H]-PK11195. The K_i value of the compound was determined using a range of competitor concentrations (3 pM – 300 μM). Assays were carried out in triplicate, and a typical assay consisted of 100 μL labelled competitor, 100 μL test compound and 200 μL of brain homogenate to give a final assay volume of 400 μL (with < 1% ethanol present). The assay components were thoroughly mixed and then incubated at 5 °C for 90 min. The binding assay was terminated by the rapid filtration through Whatman GF/B glass fiber filters, which were pre-soaked in 0.3% w/v polyethylenimine, using a 24-well Brandel cell harvester. Filters received 3 rapid washes with ice-cold tris-base buffer and were added to prepared scintillation vials containing 10 mL of eco-scint. After a minimum of 48 h at ambient temperature, tritium counts were measured using a liquid scintillation counter. The resultant radioactive counts were used to determine the K_i value of the compound of interest by performing non-linear regression analysis using GraphPad Prism 4.0 (GraphPad Softare Inc). Standard deviation was calculated using Excel 2008 software.

4. HPLC Methodology[6]

All physicochemical analyses were performed using a Dionex Ultimate 3000 series, and data acquisition and processing performed using Chromeleon 6.8 Chromatography software. Standard and test compounds were dissolved in 1:1 organic/aqueous phases, and prepared to a concentration of 0.5 mg/mL. The HPLC system was set to 25 °C, and UV detection achieved using a diode array detector (190 – 800 nm). Analysis was performed using 5 μL sample injections.

Immobilised Artificial Membrane (IAM) chromatography for determination of membrane permeability (P_m) and membrane partition coefficient (K_m)

P_m and K_m values were determined using previously developed methodology on a Registech IAM.PC.DD2 (15 cm × 4.6 mm) column. Acetonitrile and 0.01 mM phosphate buffered saline at pH 7.4 was used as the mobile phase, with a flow rate of 1.0 mL/min. The retention

time of each compound was measured under an isocratic mobile phase with the percentage acetonitrile ranging from 30–40%. The retention time of citric acid, as an unretained compound, under an isocratic mobile phase of 100% phosphate buffered saline was used for system corrections. The following equations were used to calculate P_m and K_m of the compounds of interest using Excel 2008 Software.

$$k_{IAM} = \frac{(t_r - t_0)}{t_0}$$

where k_{IAM} = solute capacity factor on the column, t_r = compound retention time and t_0 = unretained compound retention time

$$k_{IAM} = \left(\frac{V_s}{V_m}\right) \times K_m$$

where V_s = volume of the IAM interphase created by the immobilized phospholipids, V_m = total volume of the solvent within the IAM column and K_m = membrane partition coefficient

$$V_m = \frac{W_{PhC}}{\delta_{PhC}} + \frac{W_{C10}}{\delta_{C10}} + \frac{W_{C3}}{\delta_{C3}}$$

where the specific weight of PhC (δ_{PhC}) = 1.01779 g/mL and C_{10}/C_3 ($\delta_{C10/C3}$) = 0.86 g/mL; W_{PhC} = 133 mg, W_{C10} = 12.73 mg and W_{C3} = 2.28 mg

$$V_m = f_r' \, t_0$$

where f_r = flow rate

$$P_m = \frac{K_m}{MW}$$

where P_m = permeability and MW = molecular weight

Human Serum Albumin (HSA) chromatography for determination of percentage of plasma protein binding (%PPB)

%PPB values were determined using previously developed methodology on a ChromTech HSA 5 μm (3.0 × 50 mm) column. Isopropanol and 0.01 mM phosphate buffered saline at pH 7.4 was used as the mobile phase, with a flow rate of 1.8 mL/min. The retention time of each compound was measured under the following mobile phase conditions: 0–3 min, 0–30% IPA; 3–10 min, 30% IPA; 10.5–11.0 min, 30–0% IPA; 11.0–15.0 min, 0% IPA. System calibration was achieved using the following compounds and plotting %PPB values against their mean retention times: warfarin (%PPB = 98.0), nizatidine (%PPB = 35.0), bromazepam (%PPB = 60.0), carbamazepine (%PPB = 75.0), budesonide (%PPB = 88.0), nicardipine (%PPB = 95.0), ketoprofen (%PPB = 98.7), indomethacin (%PPB = 99.0) and diclofenac (%PPB = 99.8). For each standard compound, the literature %PPB value was converted to its corresponding Log k value, which when plotted against t_r on the HSA column, afforded a line equation from which the Log k value of the unknown compounds could be extracted. The Log k values of the unknown compounds could then be converted to %PPB. Log k and subsequent %PPB calculation for the compounds of interest were performed using Excel 2008 Software.

$$Logk = Log\left[\frac{\%PPB}{(101-\%PPB)}\right]$$

$$\%PPB = \left[\frac{(101-10^{\log k})}{(1+10^{\log k})}\right]$$

5. References

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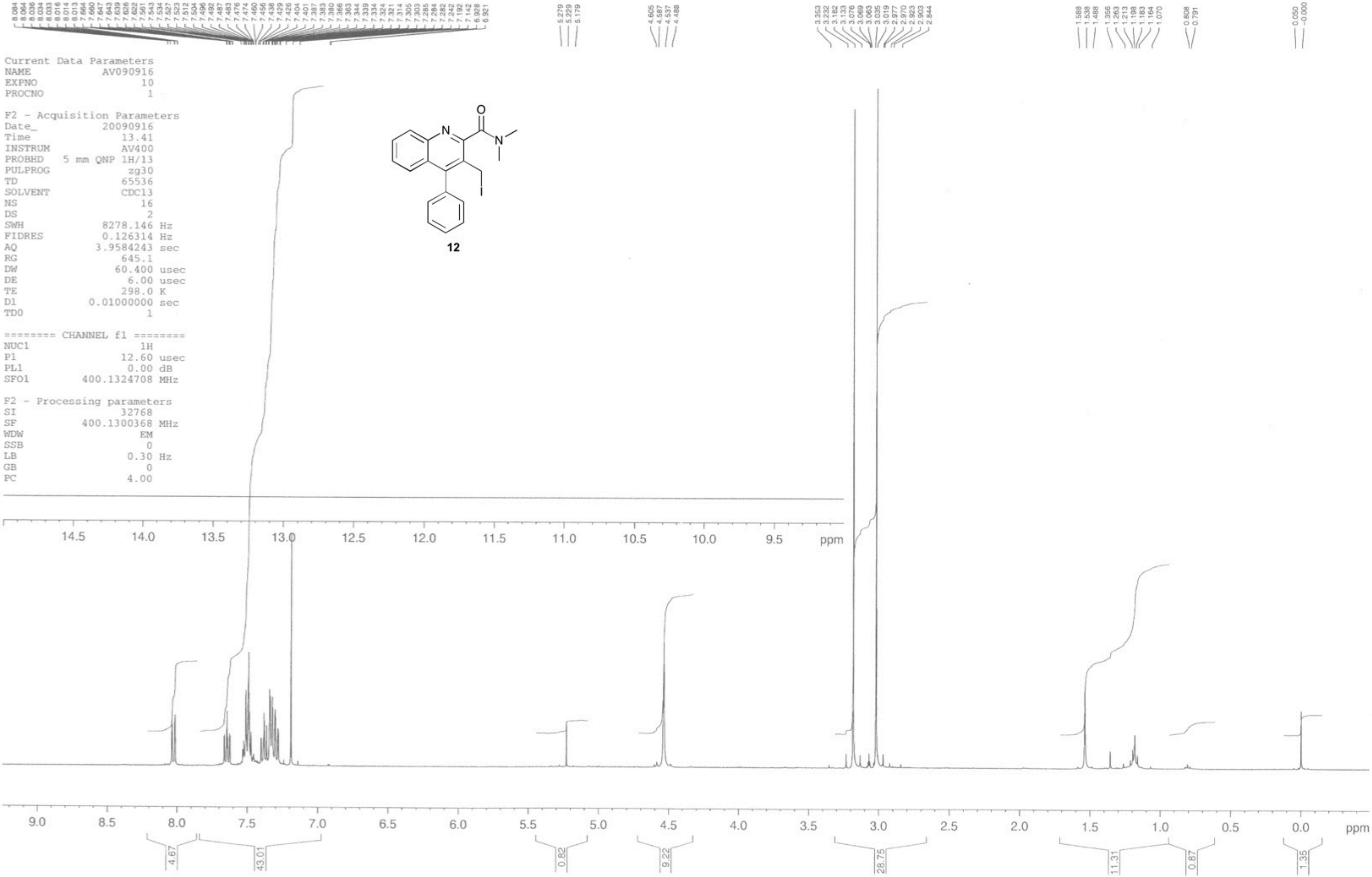
5. Y. Itzhak, L. Baker and M. D. Norenberg, *GLIA*, 1993, **9**, 211.

6. A. A. S. Tavares, J. Lewsey, D. Dewar and S. L. Pimlott, *Nuc. Med. Biol.*, 2012, **39**, 127.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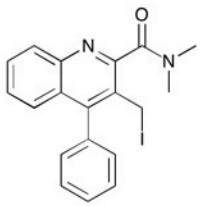

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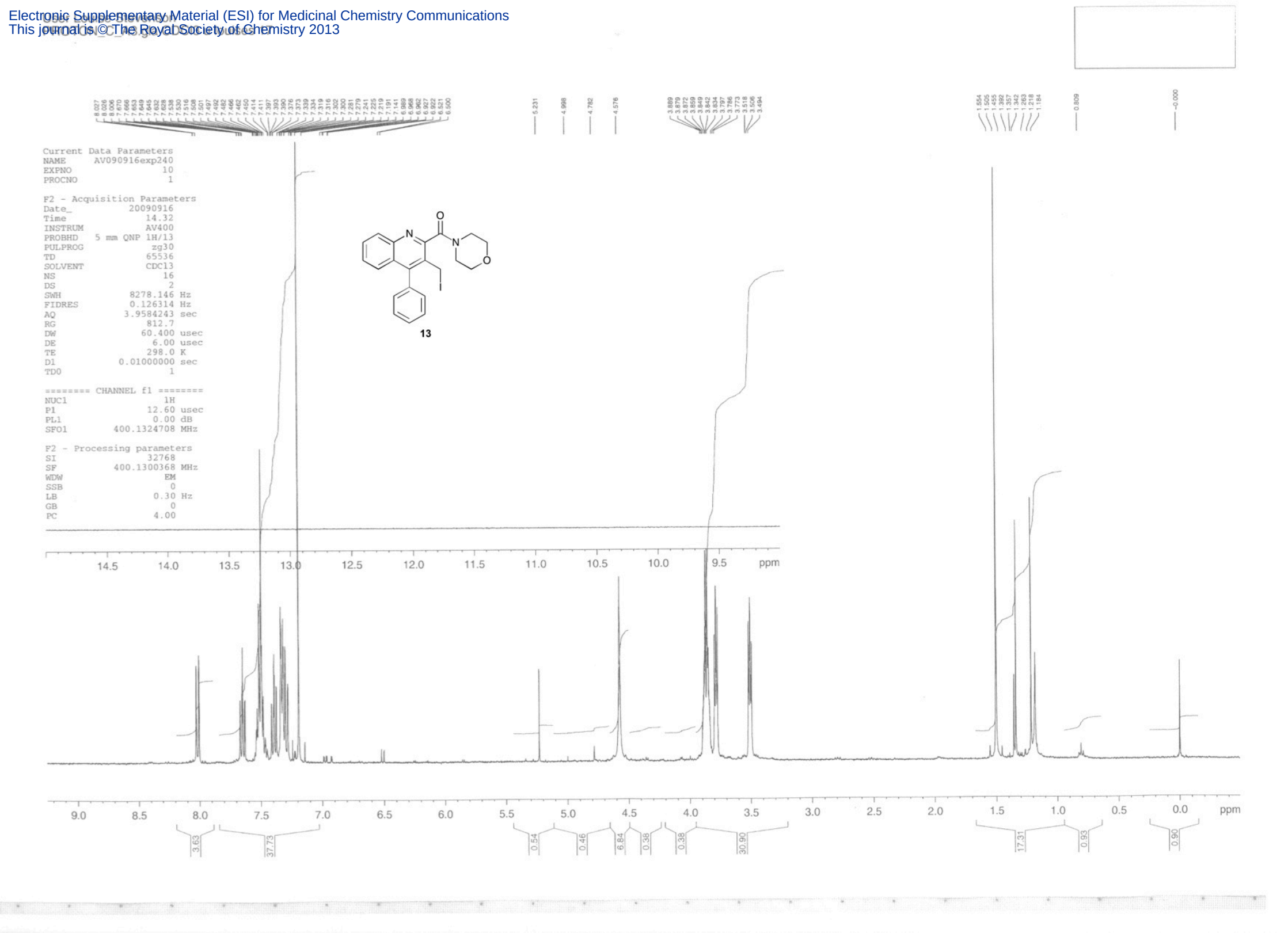

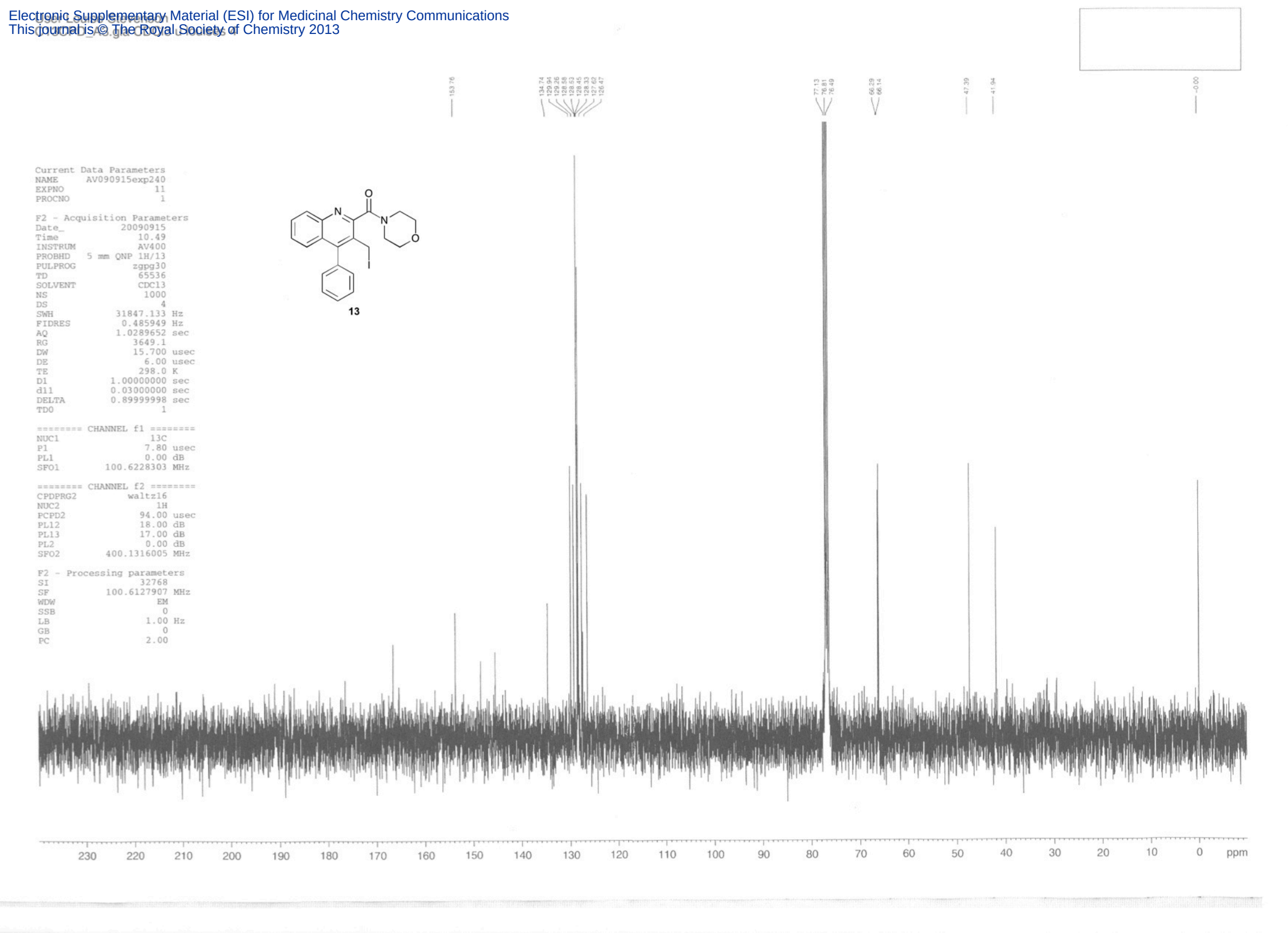

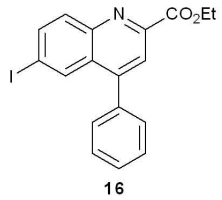

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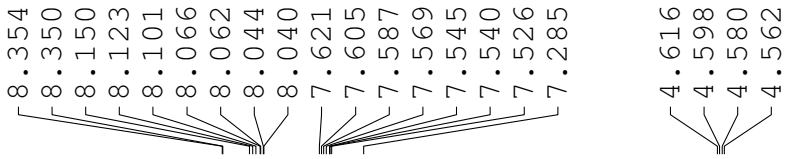

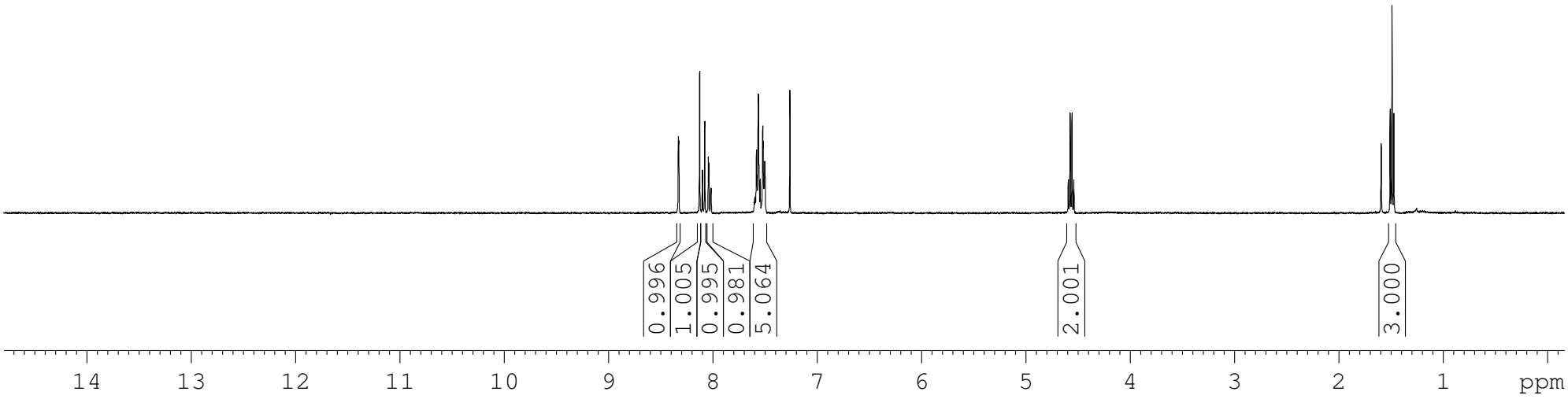

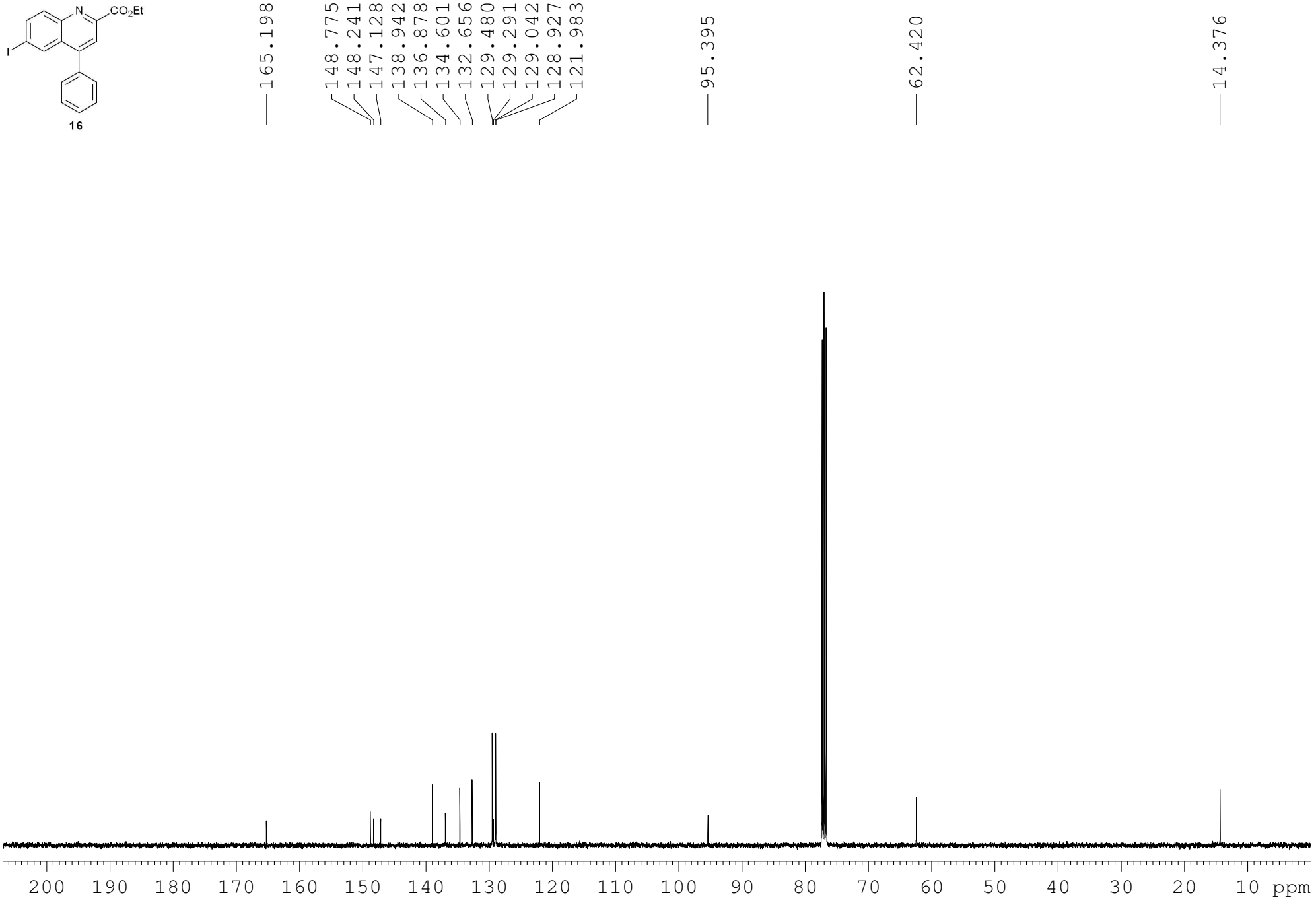

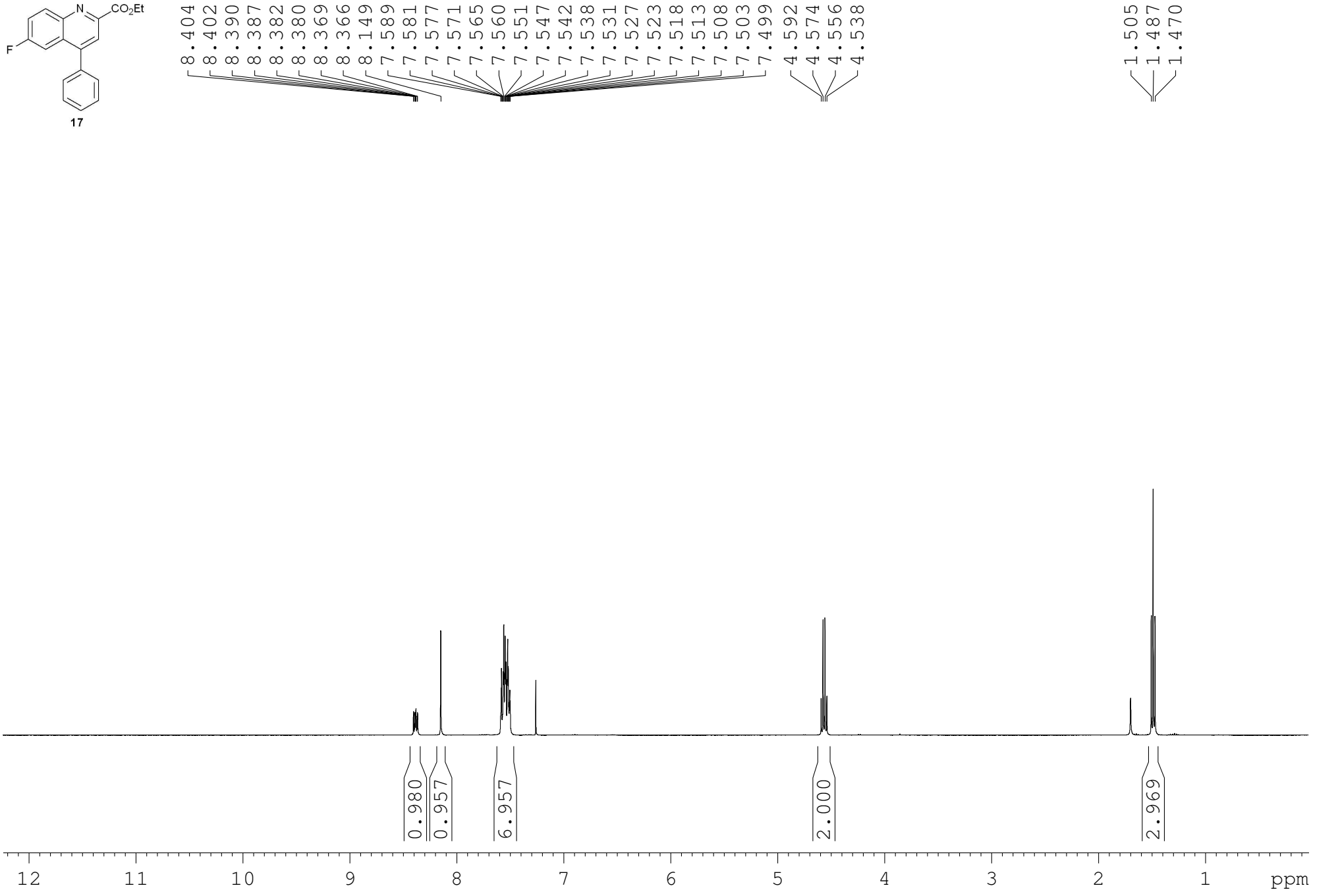

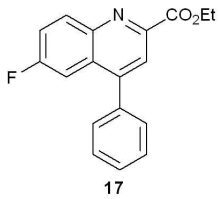

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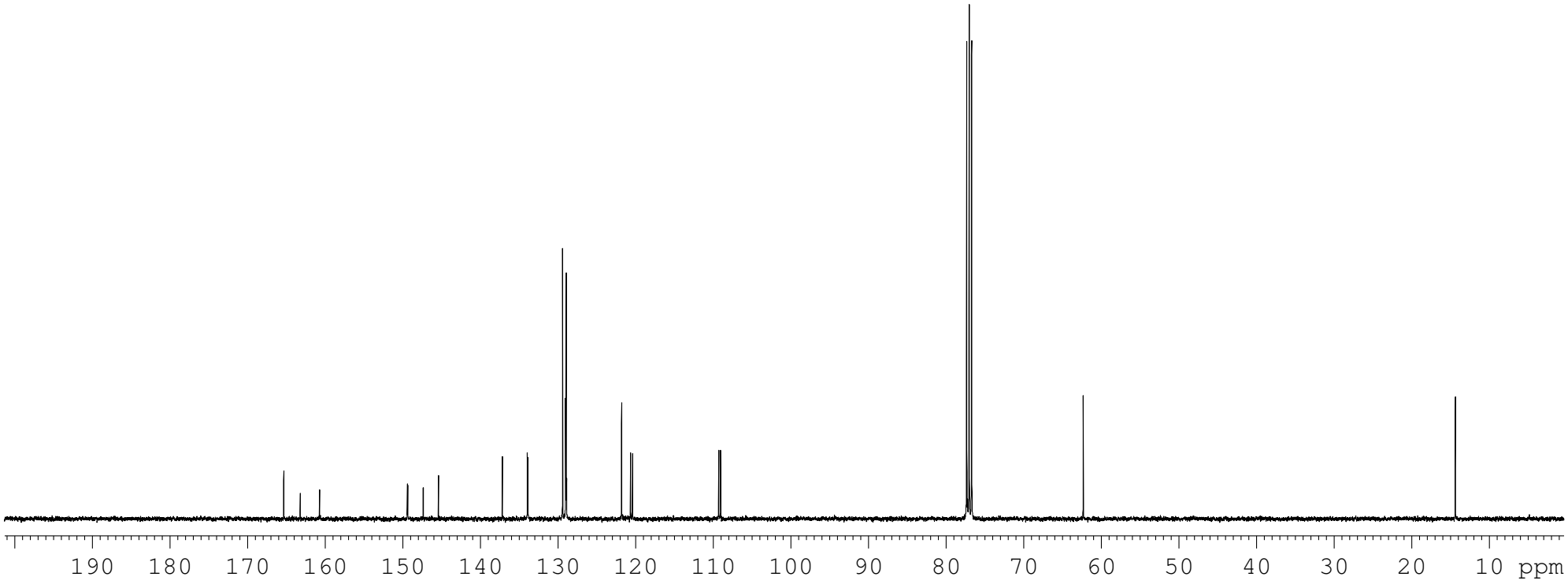

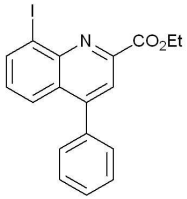

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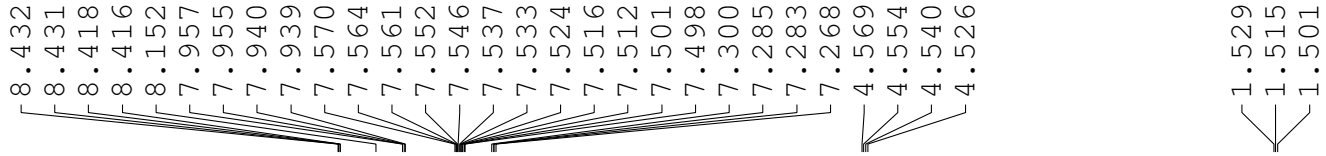

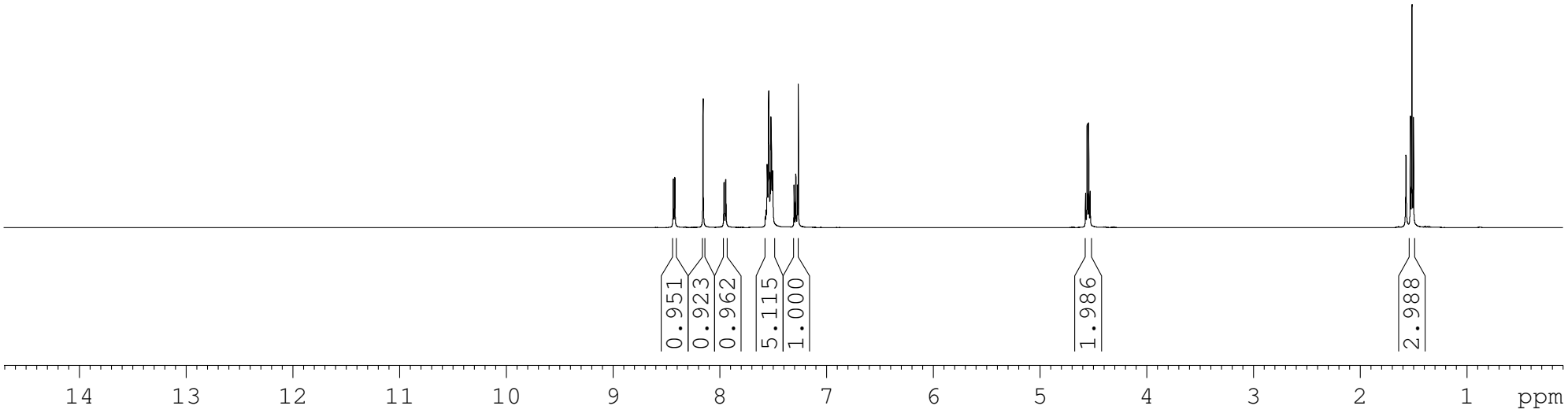

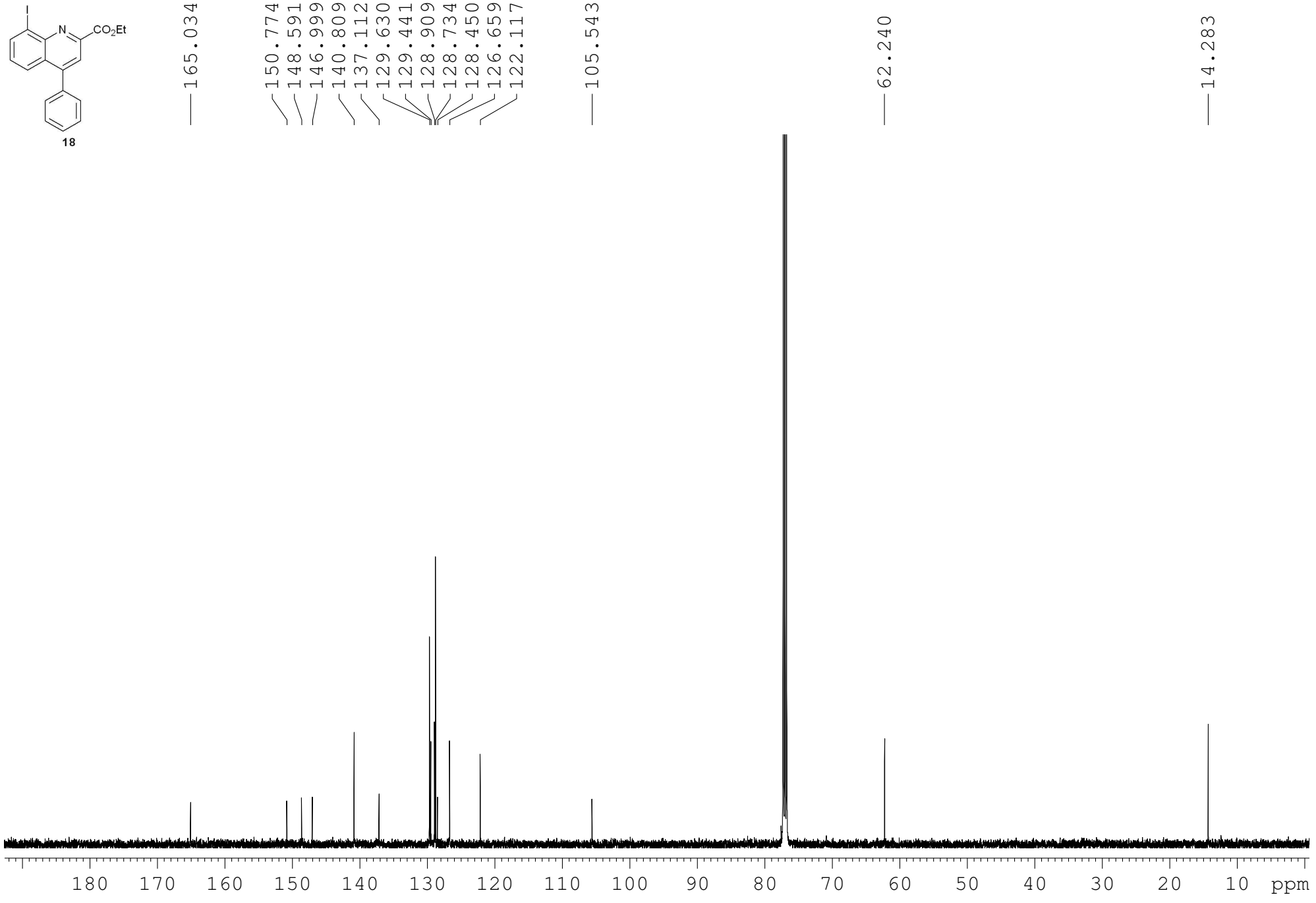

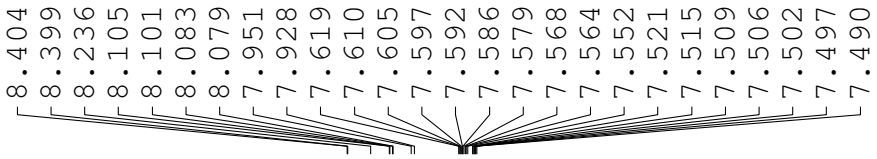

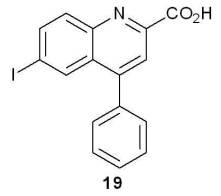

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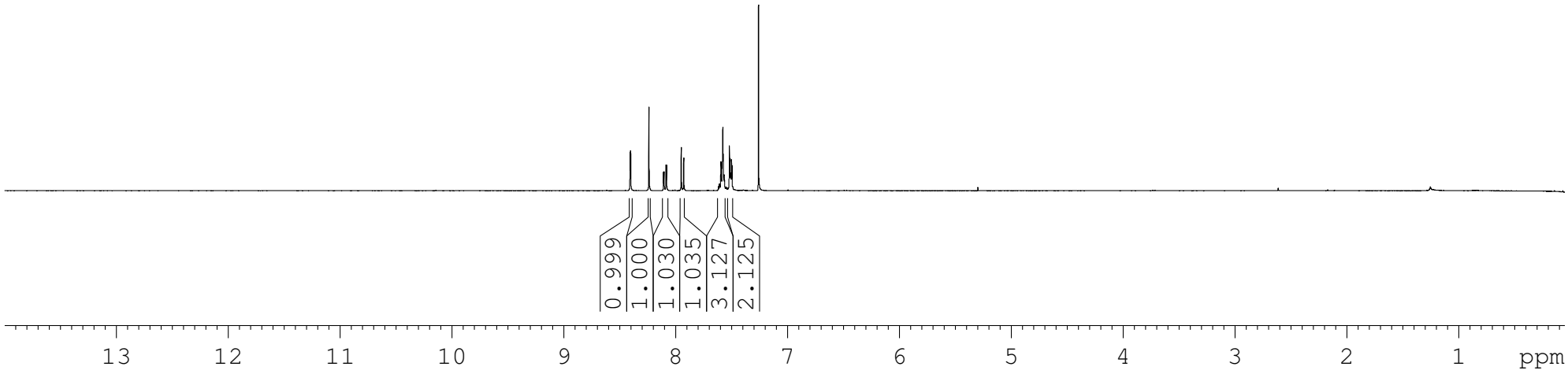

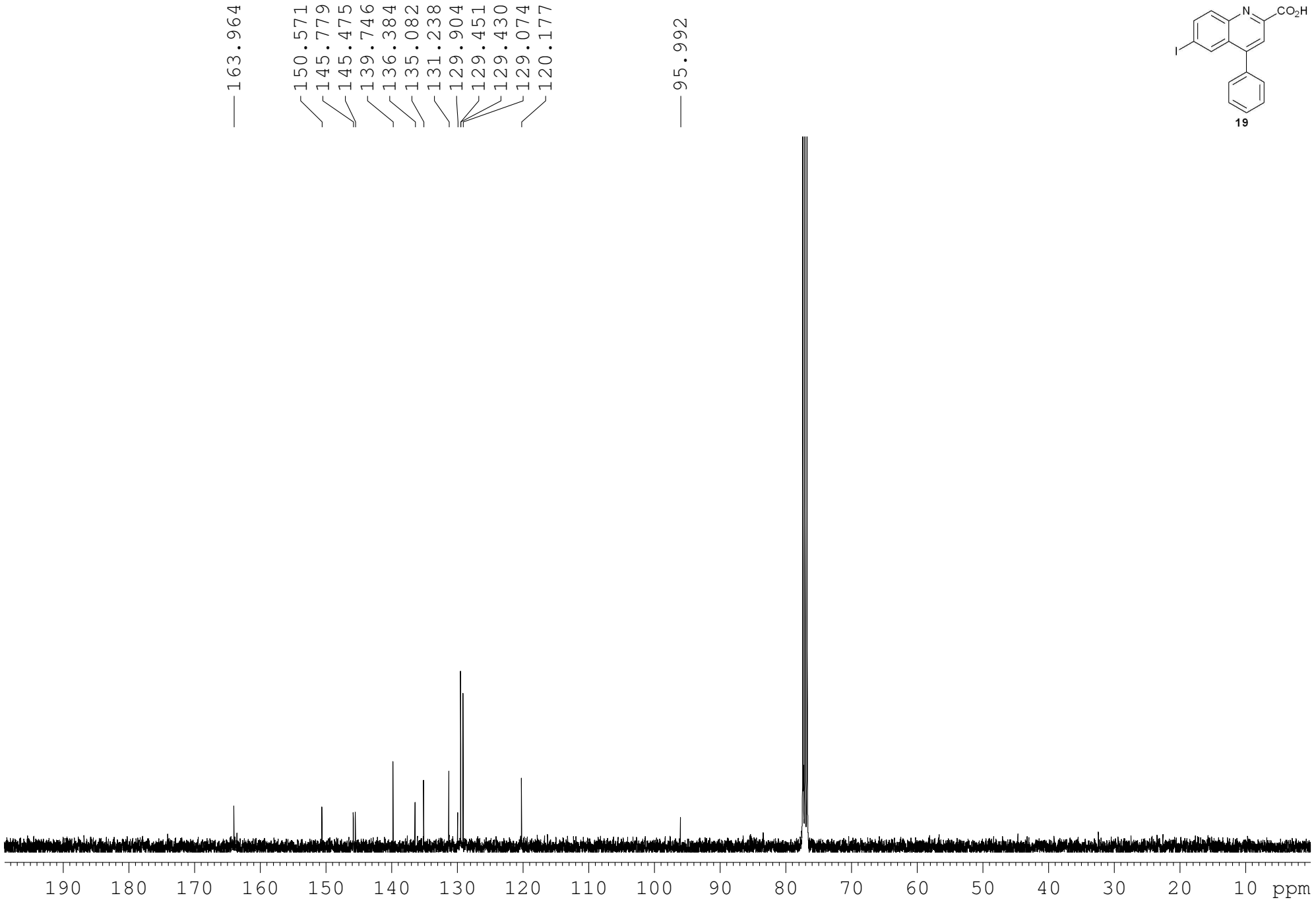

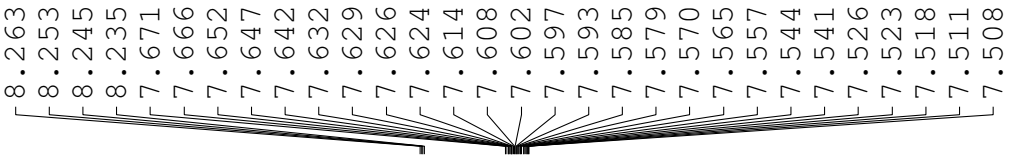

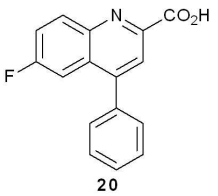

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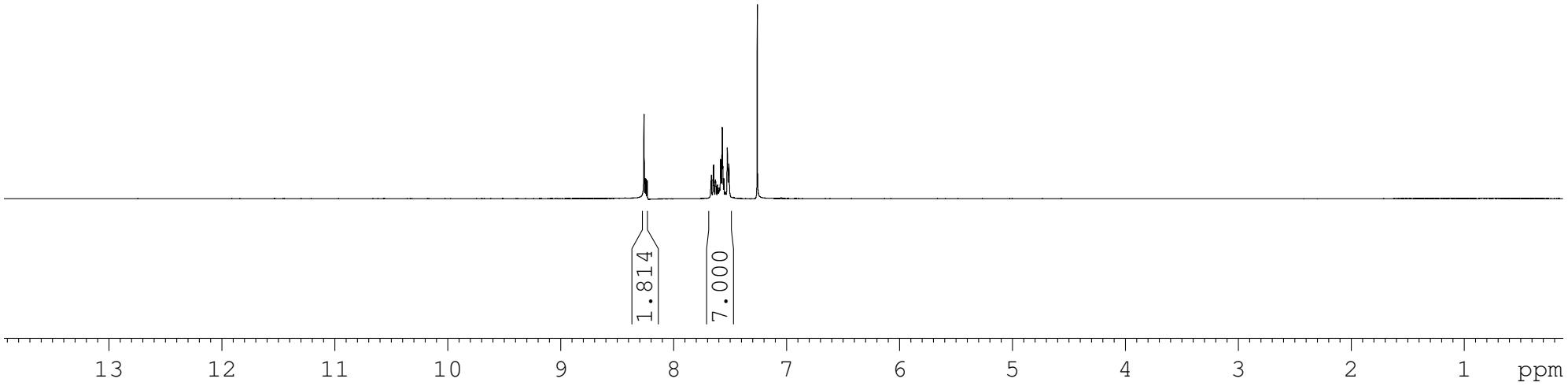

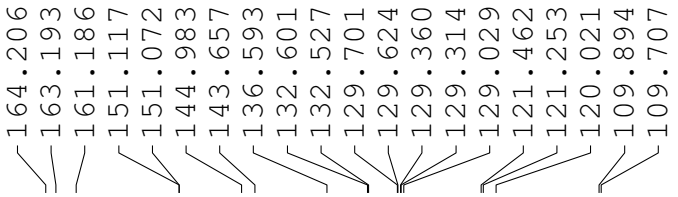

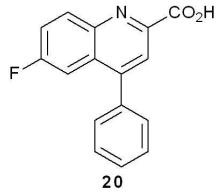

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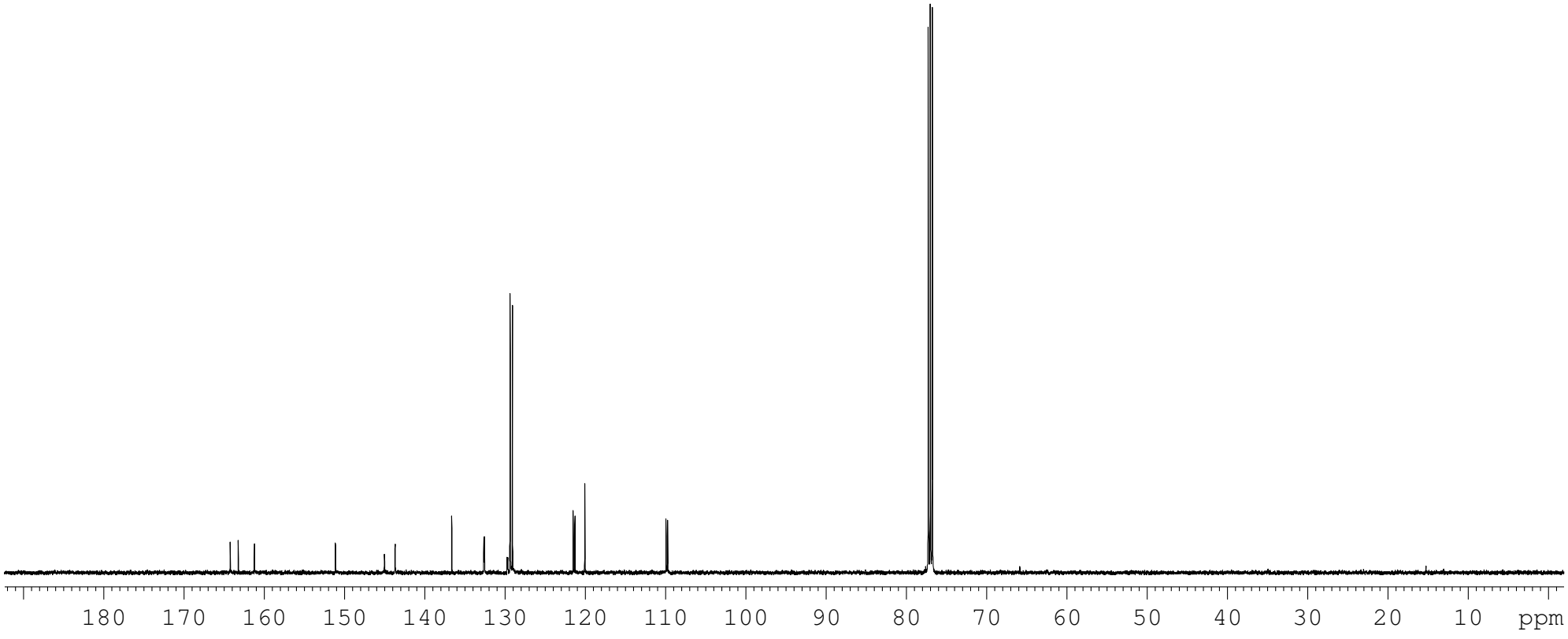

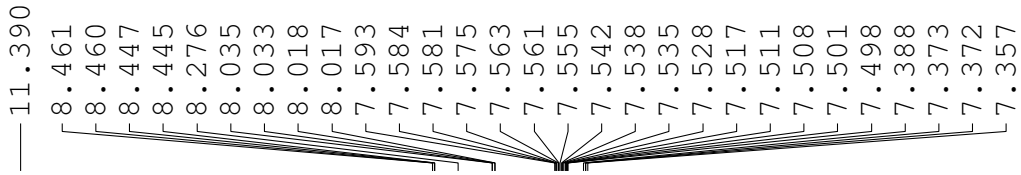

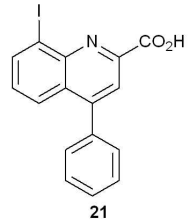

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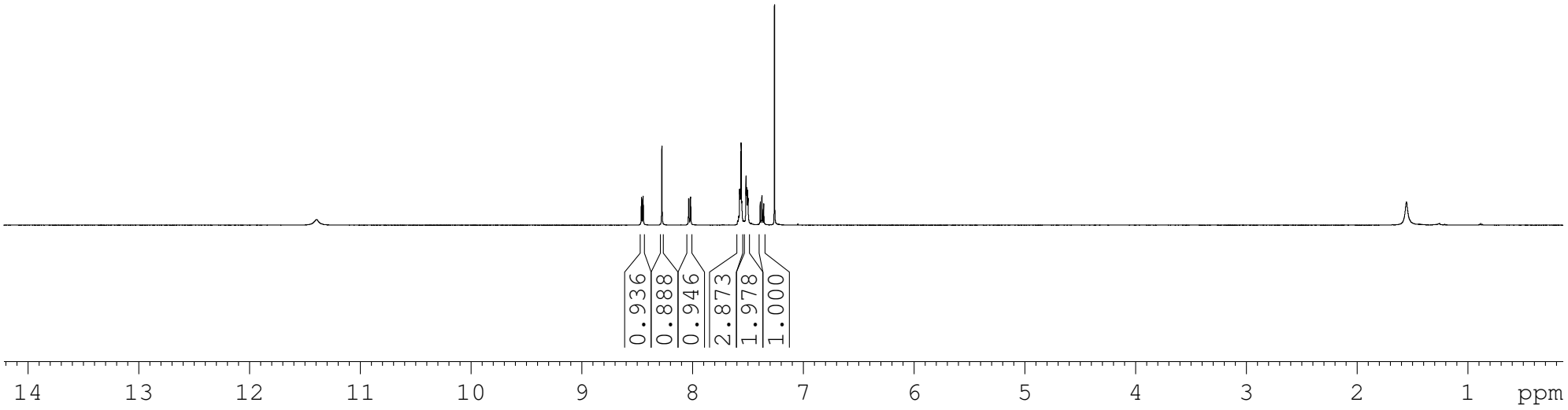

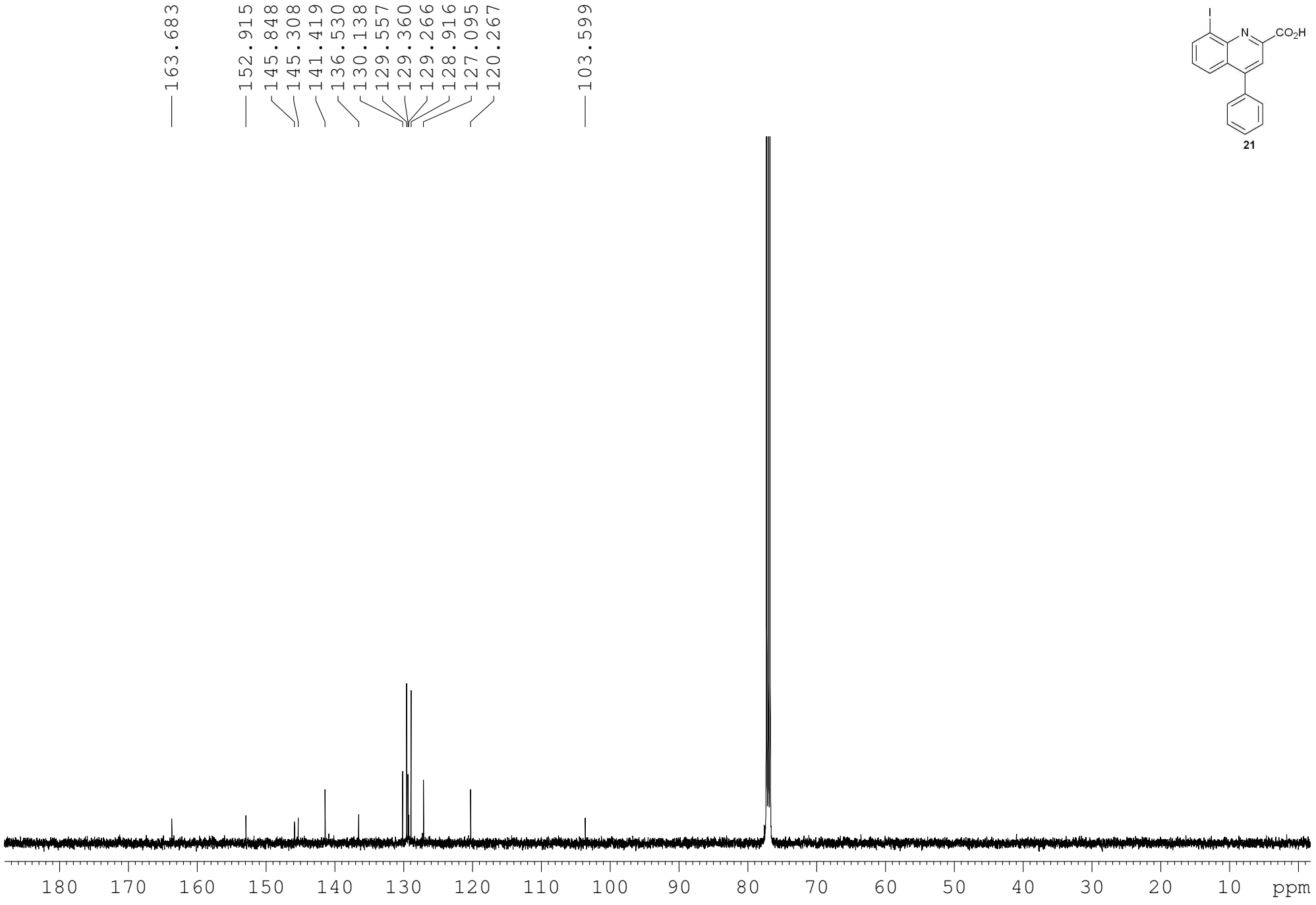

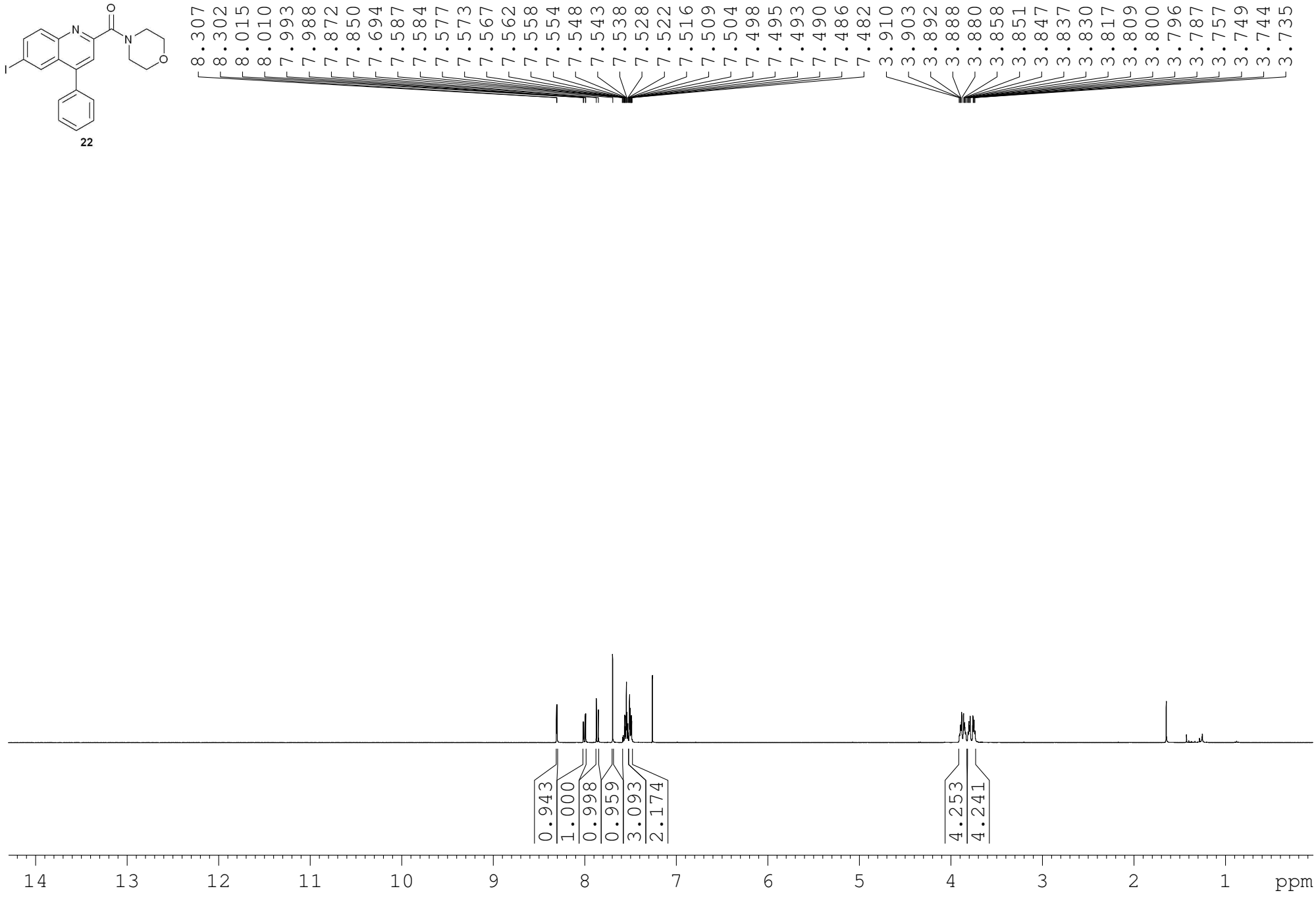

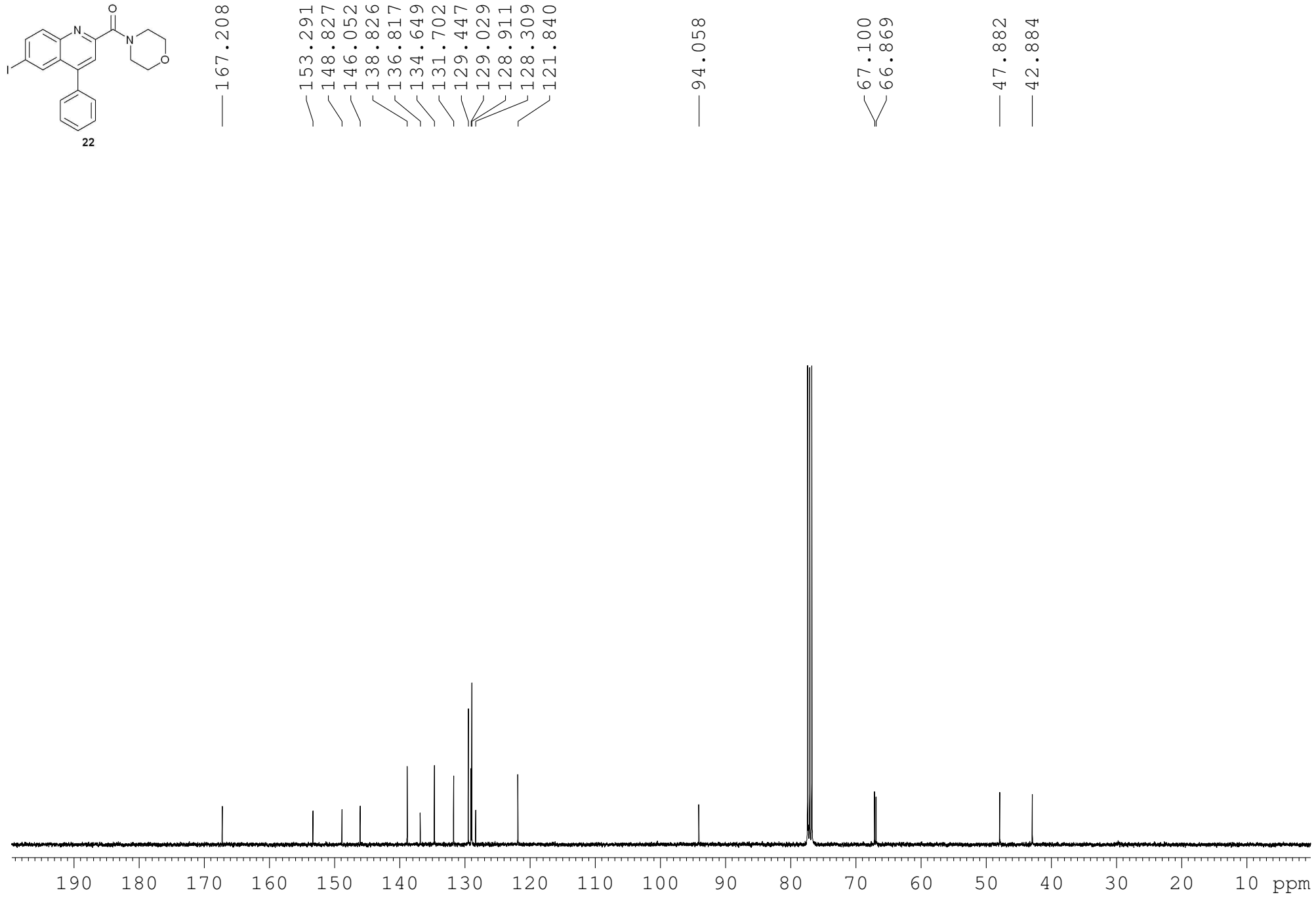

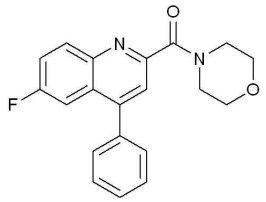

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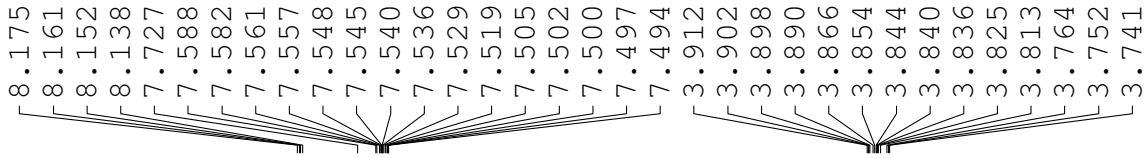

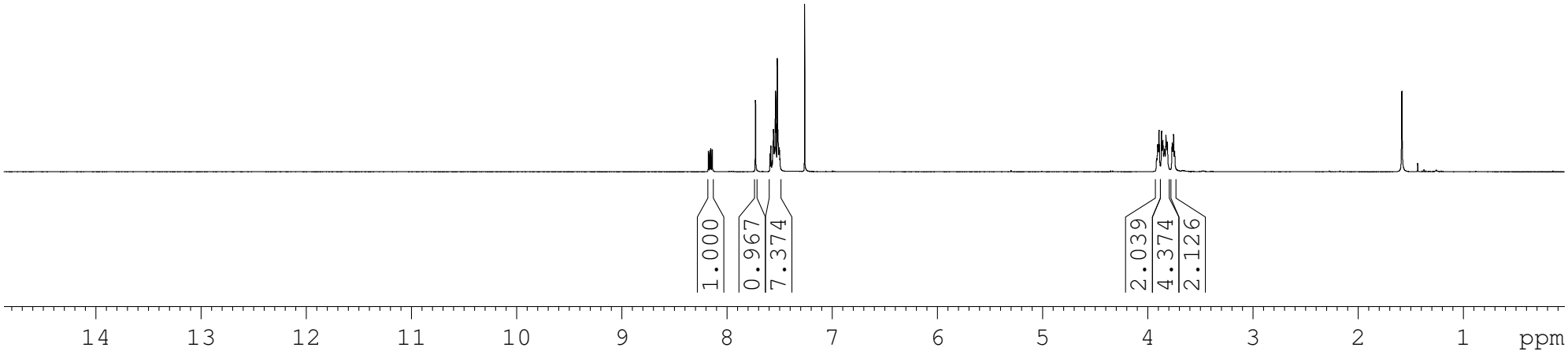

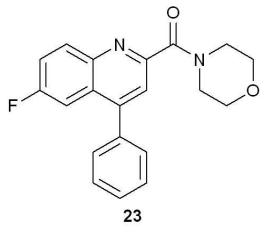

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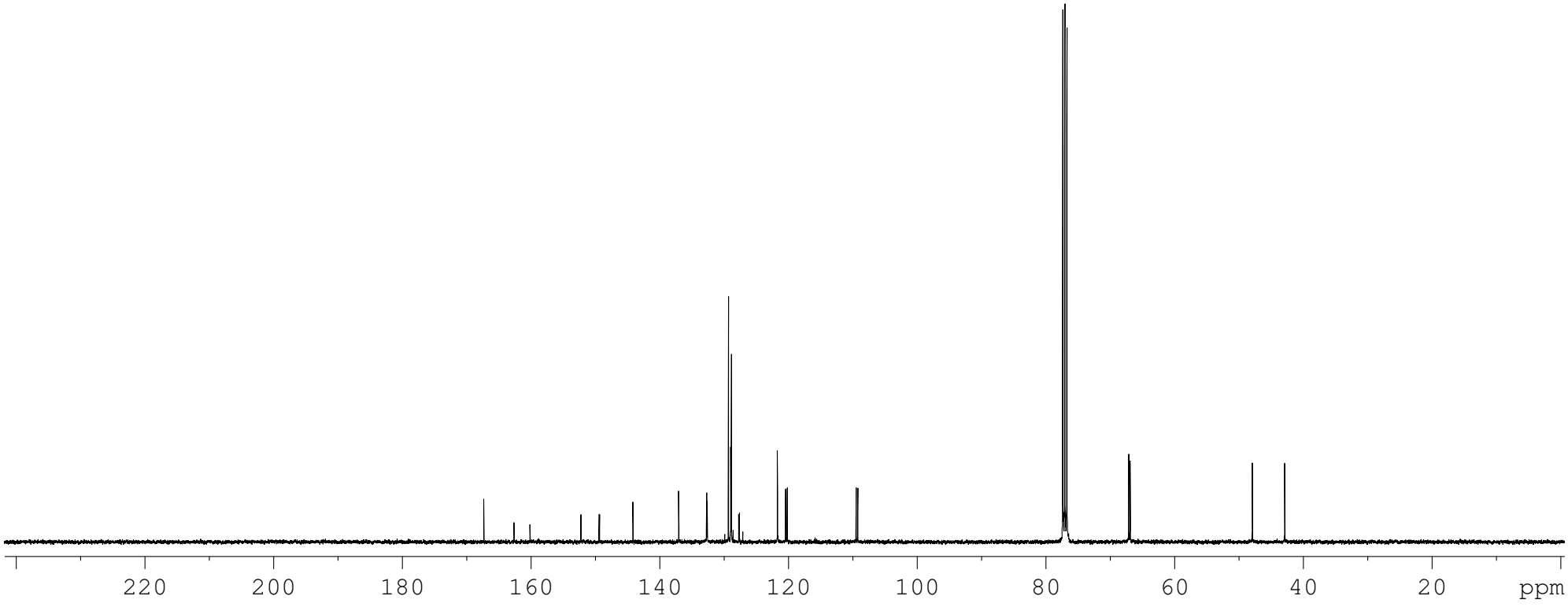

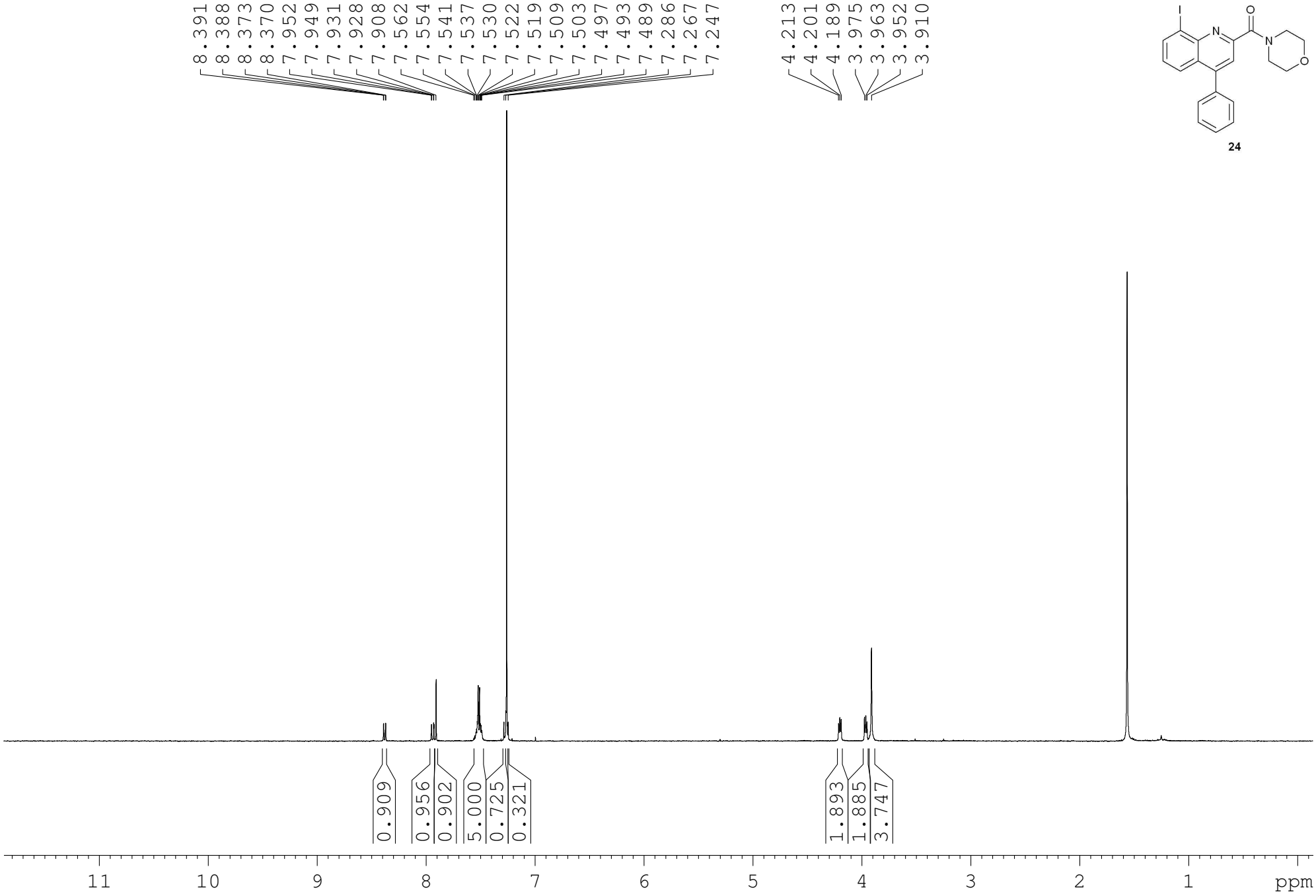

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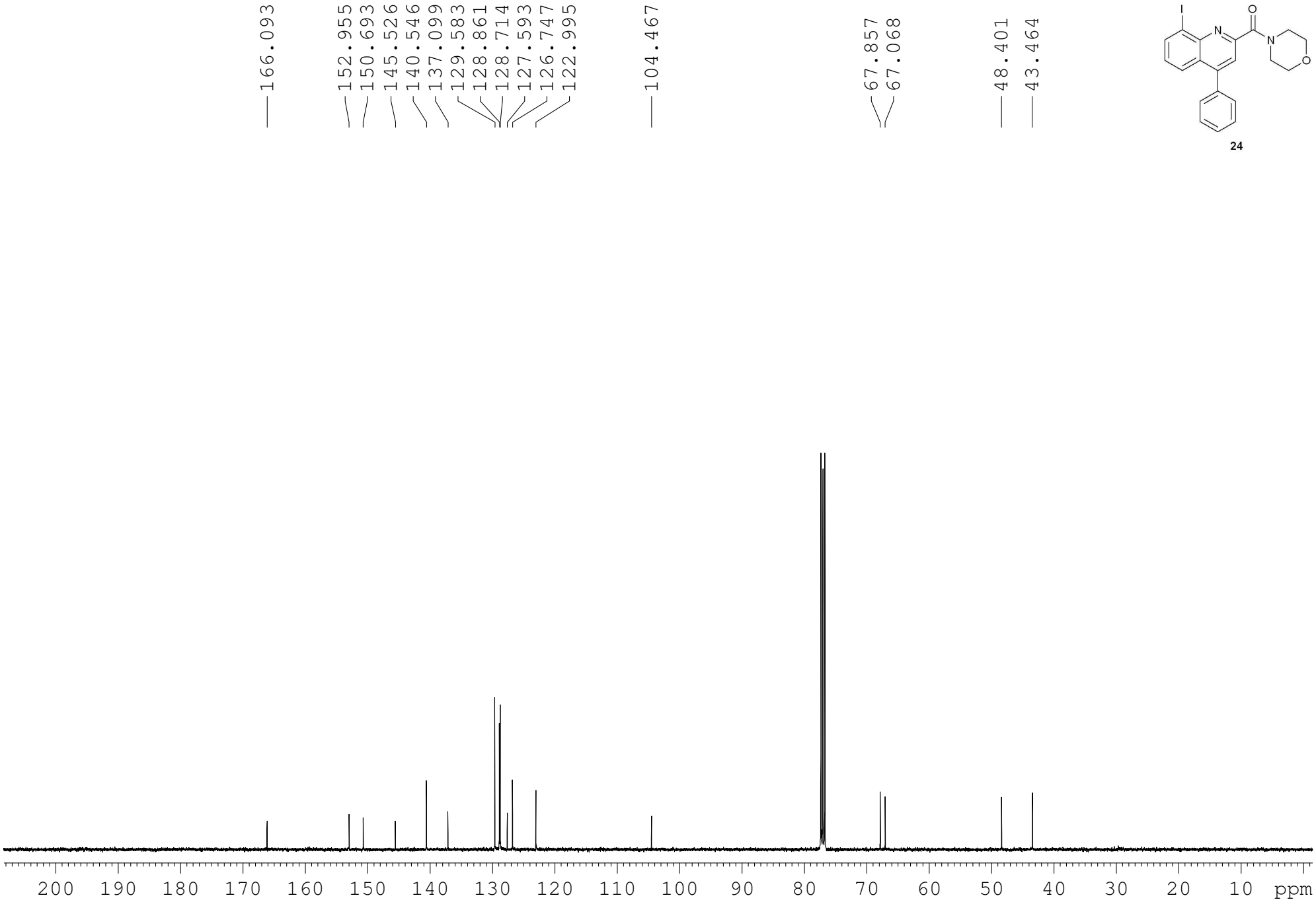

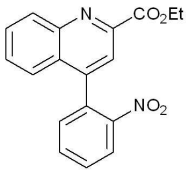

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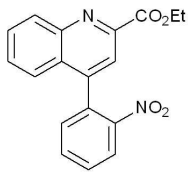

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165.146
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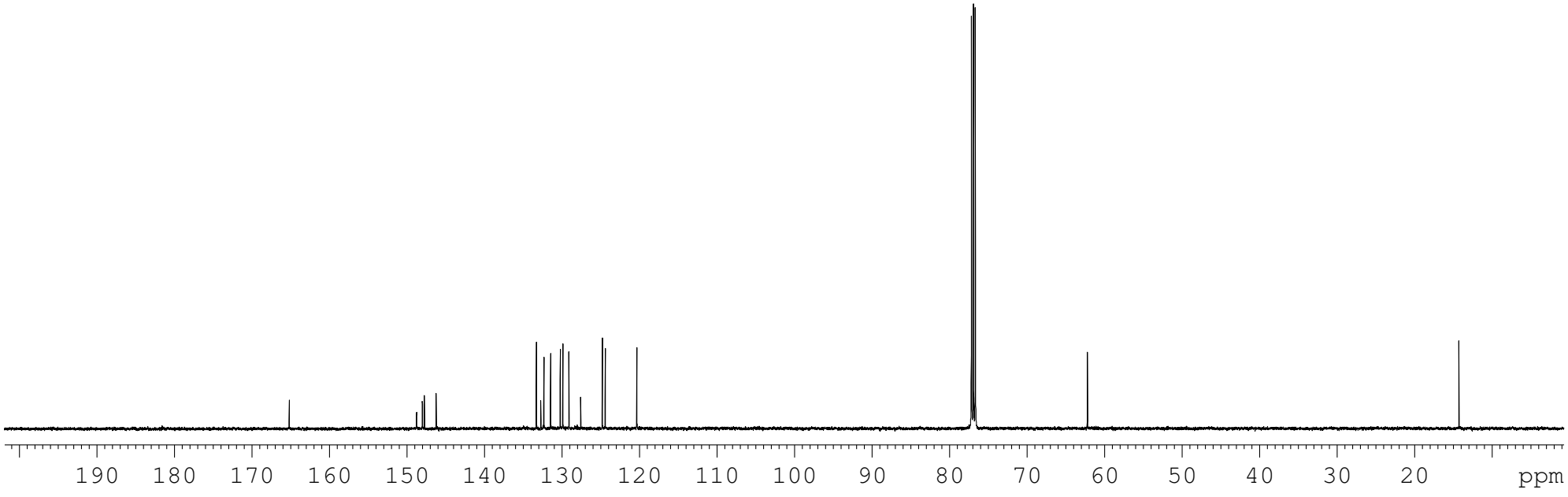

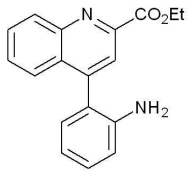

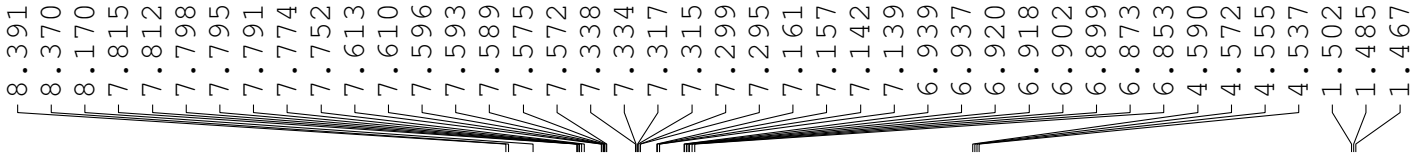

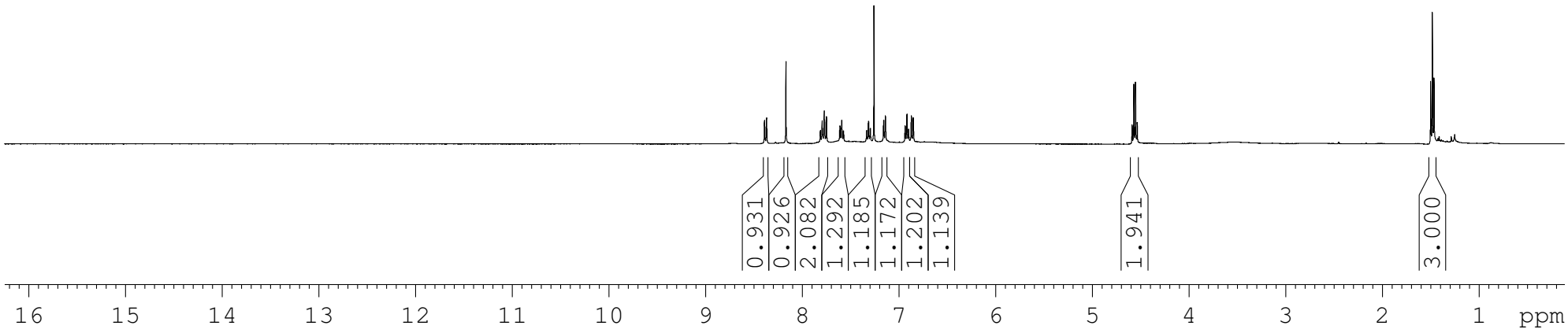

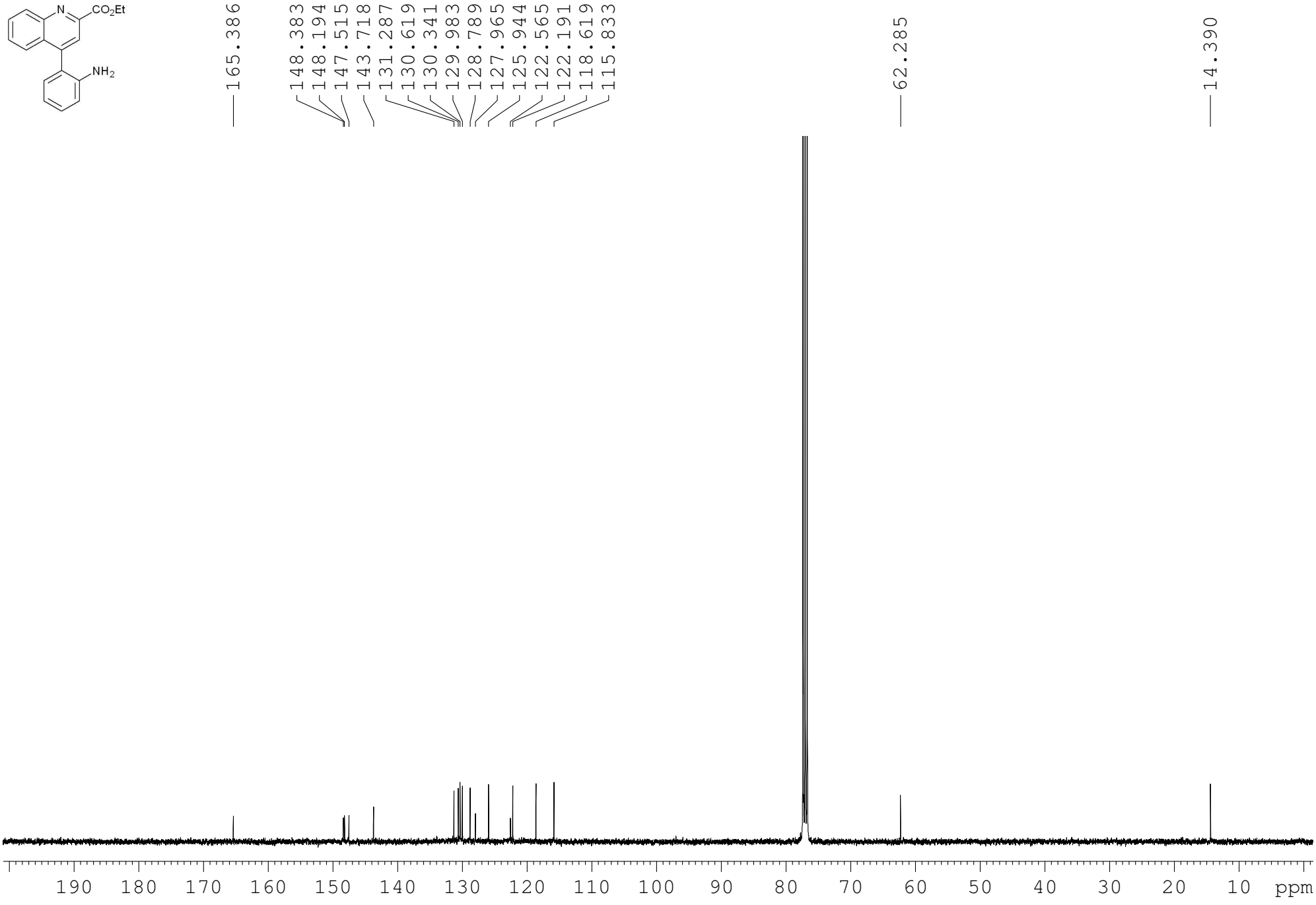

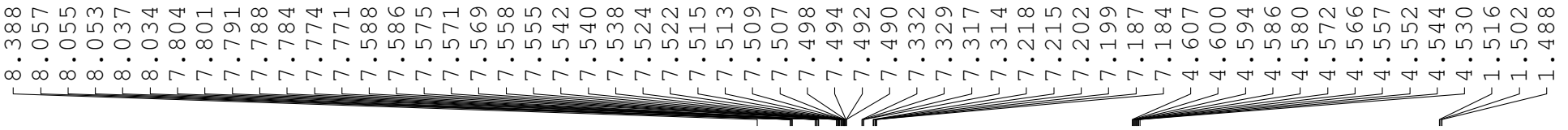

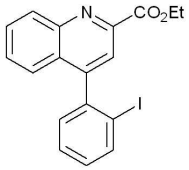

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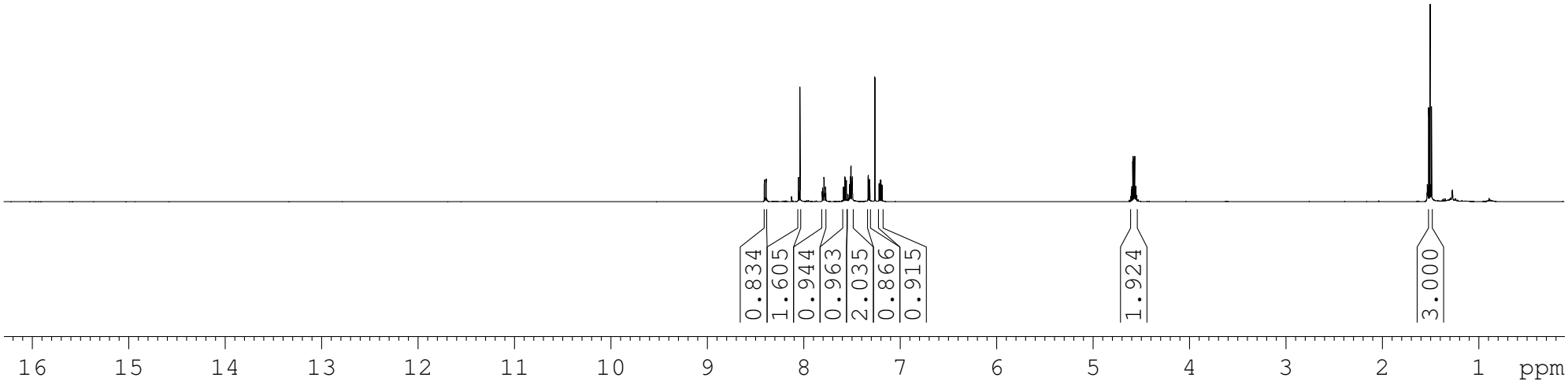

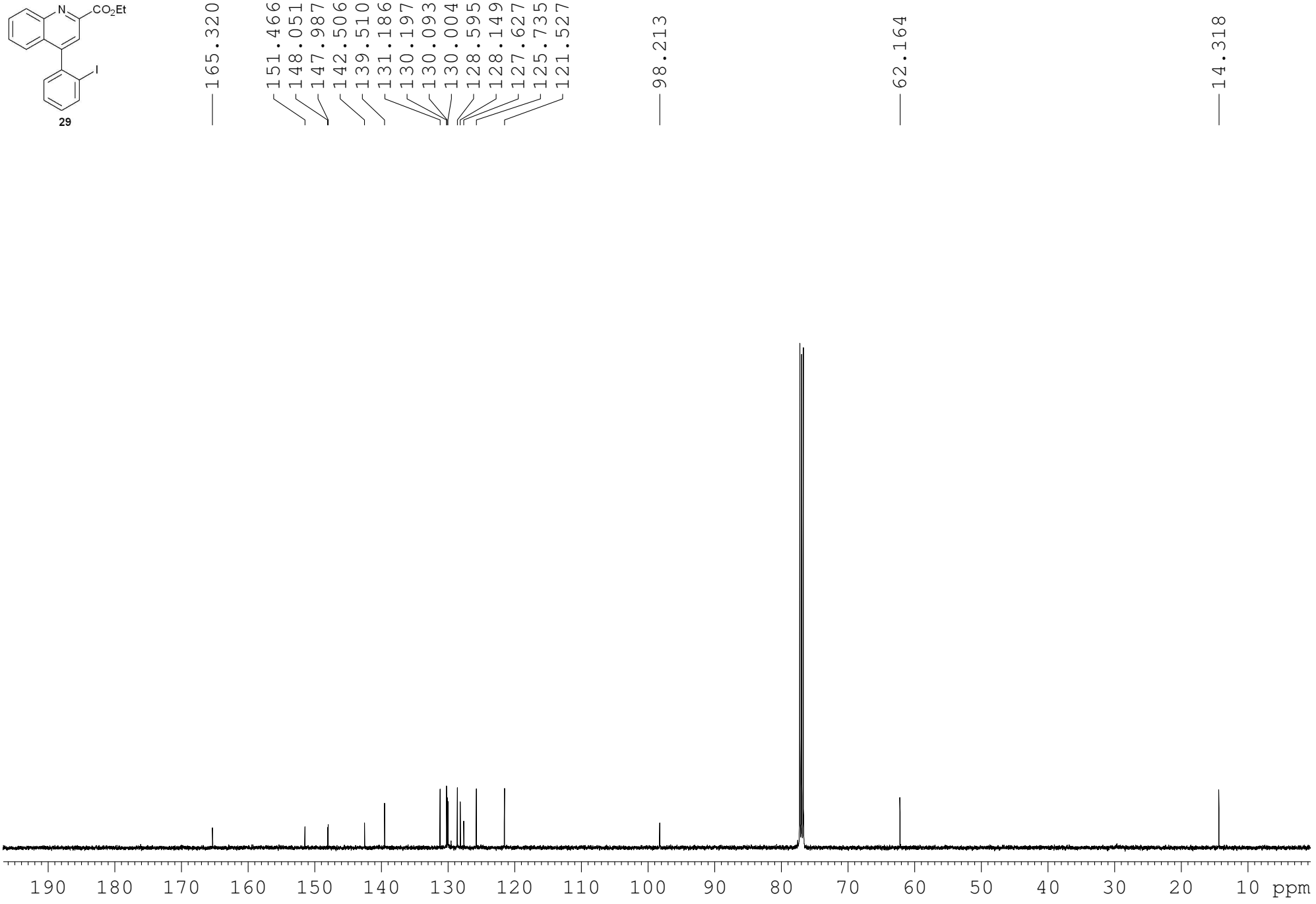

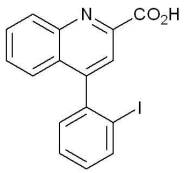

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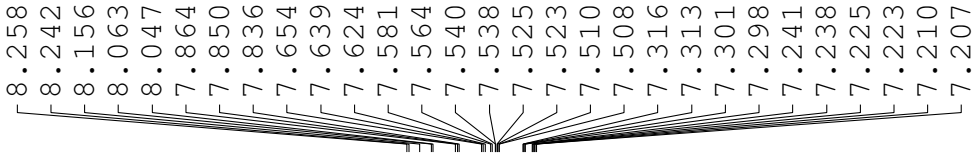

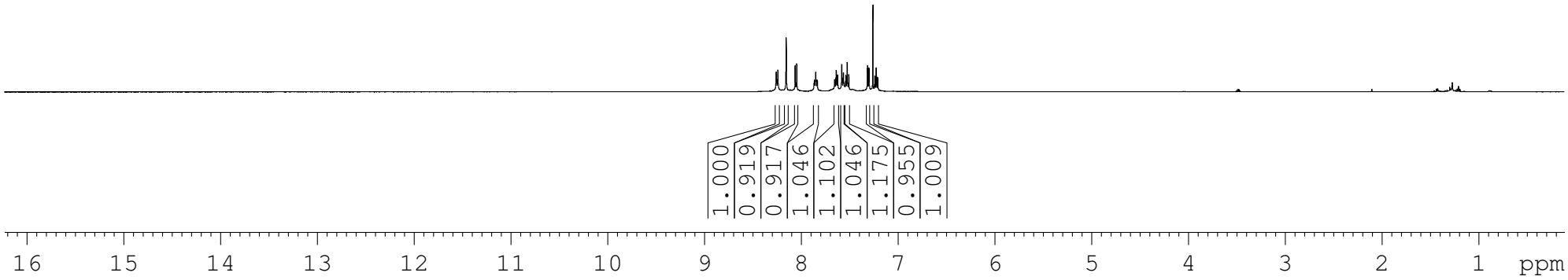

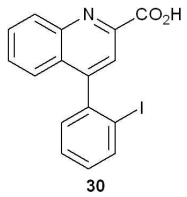

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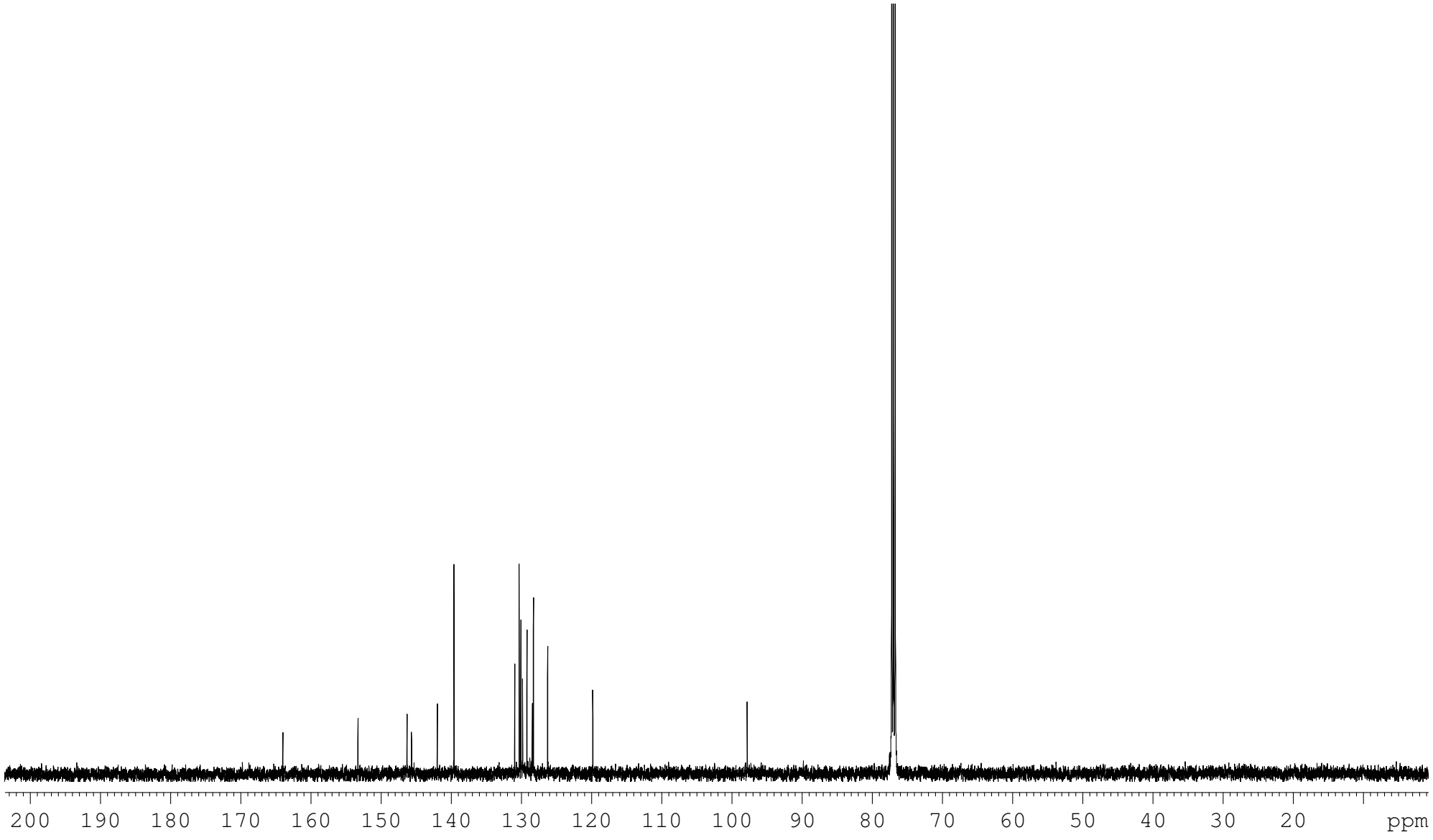

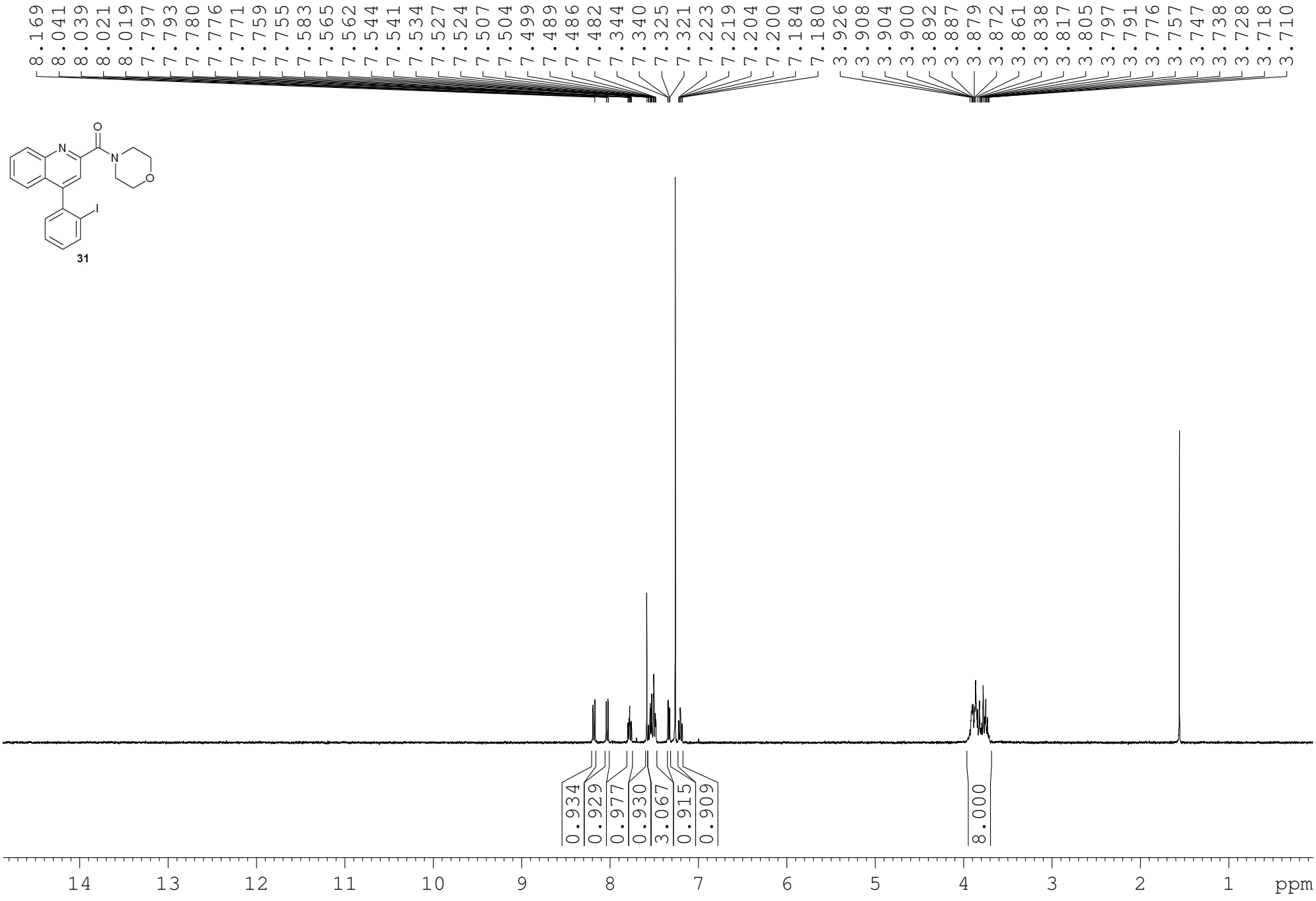

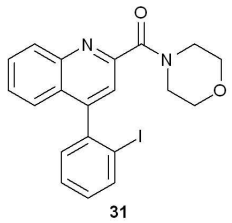

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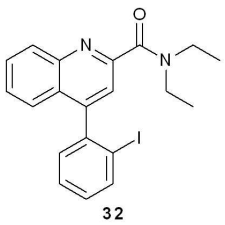

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