SUPPORTING INFORMATION

Hydrogen Sulfide Sensing Using an Aurone-based Fluorescent Probe

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Materials and methods

ACS grade chemicals and solvents used as received without further purification. All the reactions were conducted under an air atmosphere. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL AS (500 and 300 MHz) NMR instrument and chemical shifts were recorded in ppm with reference to the tetramethyl silane (TMS) chloroform-*d* or DMSO-d_6.[1] The following conventions are used for multiplicities: s, singlet; *d*, doublet; *t*, triplet; *m*, multiplet; *dd*, doublet of doublet; *dt*, doublet of triplet; *ddd*, doublet of doublet of doublet; *br*, broad. A Cary 630 FT-IR (Agilent Technologies) was used to collect IR spectra. Solid samples were used for collecting the IR-spectra. Fluorescence spectra were collected using a Hitachi F-4500 fluorescence spectrophotometer (Hitachi, Japan) with the slit width for excitation and emission set at 5 nm and photomultiplier voltage set at 700v. Excitation wavelength details are provided in-text as relevant. An HP-8452A Diode Array Spectrophotometer (Agilent Technologies Inc.) was used to record UV-visible absorption spectra. The reactions were monitored by Thin Layer Chromatography (TLC) on aluminum supported silica coated plates (Sorbent Technologies, Inc.). All extracts were concentrated under reduced pressure using a Buchi Rotary Evaporator. Products were purified by flash silica gel (32-63u) column chromatography.

General procedure for the synthesis of 4′-azido benzaldehyde derivatives (4)

In a 3-dram glass vial containing a magnetic stir bar, 1.0 mmol of fluorinated aromatic aldehyde and 1.5 mmol (1.5 equivalent) of sodium azide (NaN_3) were dissolved in 2 mL of dimethylsulfoxide (DMSO) and heated for 2-3 h at 70-90 ^{o}C (70 ^{o}C for multi-fluorosubstituted) in a sand bath. After the completion of the reaction, it was diluted with DI water and extracted with ethylacetate (×4). To remove residual DMSO the organic fraction was further washed with DI water and brine followed by drying over anhydrous $MgSO_4$. The organic fraction was concentrated *in vacuo* and purified by flash column chromatography using 2-10% ethyl acetate: hexane to obtain the desired 4′-azido benzaldehydes.

General procedure for the synthesis of aurones (6a-6g)

In a 3-dram glass vial, benzofuranone (1 equivalent) was dissolved in 5 mL of glacial acetic acid containing a magnetic stir bar. To this solution 4′-azidobenzaldehyde (1.5 equivalent) and 2-3 drops (0.2 mL) of concentrated HCl were added and stirred at room temperature for up to 3 hours. In most

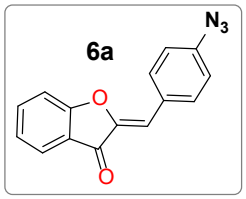
cases the reaction afforded a precipitate, normally after 30 min, indicating completion of the reaction. After the reaction was complete, it was poured into ice cold DI water. The precipitate obtained was filtered and the residue was washed multiple times with water and allowed to air dry. No further purification was required. For some of the aurones which did not precipitate out efficiently, the water diluted reaction mixture was neutralized with saturated $NaHCO_3$ and extracted with ethyl acetate, concentrated under reduced pressure using a Rotary Evaporator and purified by flash column chromatography.

General procedure for the synthesis of amino derivatives (7a-7g)

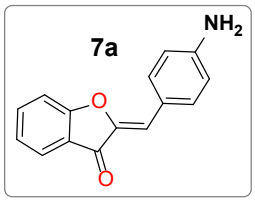
For the synthesis of the corresponding amines (**7a-7g**) of the probe candidates, 1 equivalent of the aurone **(6a-6g)** was mixed with an excess of NaHS (4 equivalents) in a 1-dram glass vial containing a magnetic stir bar. To the mixture, 1.5 mL of MeCN with a few drops of DI H_2O was added and stirred for 30 minutes (acetone can also be used as a solvent). The solution turned bright red. Once the reaction was complete based on the TLC, the mixture was concentrated under vacuum and subjected to flash column chromatography (eluted with 5% MeOH: DCM).

Characterization data

***(Z)-2-(4-azidobenzylidene)benzofuran-3(2H)-one (6a)*:** Reaction scale (1.80 mmol), Yield: 70% (331 mg); yellow solid, mp 128-129 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.92 (d, J = 8.5 Hz, 2H), 7.81 (d, J = 7.5 Hz, 1H), 7.66 (t, J = 7.5 Hz, 1H), 7.33 (d, J = 8.0 Hz, 1H), 7.23 (t, J = 7.4 Hz, 1H), 7.10 (d, J = 8.5 Hz, 2H), 6.85 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 184.7, 166.1, 146.8, 141.6, 137.0, 133.2, 129.3, 124.8, 123.7, 121.8, 119.7, 113.0, 112.2. IR (neat) 2121, 1704, 1655, 1596, 1508, 1302, 1186, 1108, 829, 750 cm^{-1}.

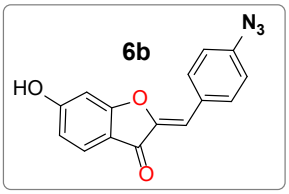


***(Z)-2-(4-aminobenzylidene)benzofuran-3(2H)-one (7a)*:** Reaction scale (0.114 mmol), Yield: 94% (25.4 mg); orange red solid, mp 173-175 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (d, J = 8.5 Hz, 1H), 7.77 (d, J = 8.5 Hz, 2H), 7.61 (ddd, J = 8.5, 7.0, 1.0 Hz, 1H), 7.31 (d, J = 8.0 Hz, 1H), 7.19 (t, J = 7.5 Hz, 1H), 6.87 (s, 1H), 6.72 (d, J = 8.5 Hz, 2H), 4.09 (s, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 184.4, 165.6, 148.7, 145.4, 136.3, 133.9, 124.5, 123.2, 122.5, 122.3, 115.1, 114.8, 112.9. IR (neat)



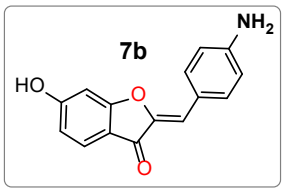
3439, 3312, 3216, 1683, 1577, 1518, 1488, 1302, 1182, 1100, 832, 754 cm^{-1}. HRMS (EI): *m/z* [M + H] calcd for $C_{15}H_{12}NO_2$ 238.0868, found 238.0871.

***(Z)-2-(4-azidobenzylidene)-6-hydroxybenzofuran-3(2H)-one (6b)*:** Reaction scale (0.114 mmol),



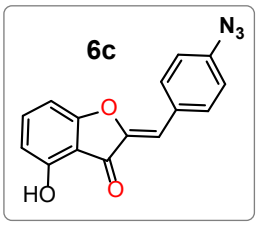
Yield: 94% (25.4 mg); faint orange red solid, mp 194-196 °C.^{1}H NMR (500 MHz, DMSO-D6) δ 11.23 (s, 1H), 7.99 (d, *J* = 8.5 Hz, 2H), 7.63 (d, *J* = 8.5 Hz, 1H), 7.24 (d, *J* = 8.5 Hz, 2H), 6.81 (s, 1H), 6.79 (d, *J* = 2.0 Hz, 1H), 6.72 (dd, *J* = 8.5, 2.0 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-D6) δ 181.2, 167.8, 166.5, 147.1, 140.5, 132.7, 128.9, 125.9, 119.6, 113.0, 112.8, 109.5, 98.6. IR (neat) 3074-2600 (br), 2117, 1681, 1577, 1506, 1458, 1294, 1112, 825 cm^{-1}.

***(Z)-2-(4-aminobenzylidene)-6-hydroxybenzofuran-3(2H)-one (7b)*:** Reaction scale (0.11 mmol),



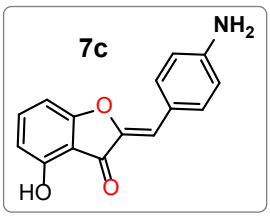
Yield: 92% (25.6 mg); orange red solid, mp > 260 ºC. ^{1}H NMR (500 MHz, DMSO-D6) δ 11.00 (s, 1H), 7.66 (d, *J* = 8.5 Hz, 2H), 7.57 (d, *J* = 8.5 Hz, 1H), 6.77 (d, *J* = 2.0 Hz, 1H), 6.69 (dd, *J* = 8.5, 2.0 Hz, 1H), 6.65 (s, 1H), 6.64 (d, *J* = 9.0 Hz, 3H), 5.94 (s, 2H). ^{13}C NMR (125 MHz, DMSO-D6) δ 180.7, 166.9, 165.6, 151.1, 144.5, 133.3, 125.3, 119.0, 113.8, 113.6, 112.9, 112.5, 98.4. IR (neat) 3461, 3361, 3300-2500 (br), 1666, 1629, 1551, 1454, 1287, 1108, 821 cm^{-1}. HRMS (EI): *m/z* [M + H] calcd for $C_{15}H_{12}NO_3$ 254.0817, found 254.0813.

***(Z)-2-(4-azidobenzylidene)-4-hydroxybenzofuran-3(2H)-one (6c)*:** Reaction scale (0.30 mmol),



Yield: 90% (75 mg); red solid, mp 179-181 ºC. ^{1}H NMR (500 MHz, DMSO-D6) δ 11.16 (s, 1H), 7.98 (d, *J* = 8.5 Hz, 2H), 7.54 (t, *J* = 8.0 Hz, 1H), 7.23 (d, *J* = 8.5 Hz, 2H), 6.85 (d, *J* = 8.0 Hz, 1H), 6.77 (s, 1H), 6.64 (d, *J* = 8.5 Hz, 1H). ^{13}C NMR (76 MHz, DMSO-D6) δ 181.2, 165.9, 157.2, 146.31, 140.5, 138.7, 132.7, 129.1, 119.7, 110.7, 109.2, 109.1, 102.4. IR (neat) 3283, 2125, 2028, 1693, 1652, 1592, 1458, 1316, 1134, 769 cm^{-1}.

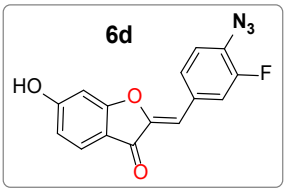
***(Z)-2-(4-aminobenzylidene)-4-hydroxybenzofuran-3(2H)-one (7c)*:** Reaction scale (24 mg, 0.083



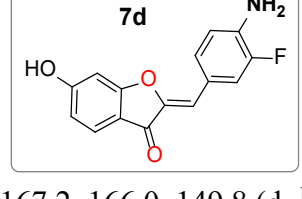
mmol), Yield: 92% (20 mg); red solid, mp >260 ºC. ^{1}H NMR (300 MHz, DMSO-D6) δ 7.54 (d, *J* = 8.5 Hz, 2H), 6.99 (dd, *J* = 8.5, 7.5 Hz, 1H), 6.60 (d, *J* = 8.5 Hz, 2H), 6.25 (s, 1H), 5.86 (d, *J* = 9.0 Hz, 1H), 5.79 (d, *J* = 7.5 Hz, 1H), 5.63 (s, 2H). ^{13}C NMR (75 MHz, DMSO-D6) δ 182.1, 173.9,

165.9, 149.7, 145.9, 136.4, 131.9, 120.4, 117.7, 113.8, 110.7, 107.5, 89.3. IR (neat) 3394-3275 (br), 1674, 1592, 1561, 1477, 1376, 1235, 1127, 1004, 803 cm^{-1}. HRMS (EI): *m/z* [M + H] calcd for $C_{15}H_{12}NO_3$ 254.0817, found 254.0819.

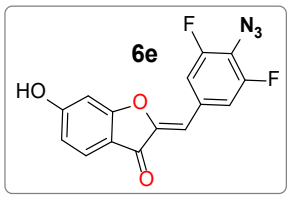
***(Z)-2-(4-azido-3-fluorobenzylidene)-6-hydroxybenzofuran-3(2H)-one (6d)*:** Reaction scale (0.27 mmol), Yield: 76% (61 mg); red solid, mp 156 ºC (decompose). ^{1}H NMR (500 MHz, DMSO-D6) δ 11.24 (s, 1H), 7.80 (d, J = 12.6 Hz, 1H), 7.75 (d, J = 8.6 Hz, 1H), 7.59 (d, J = 8.3 Hz, 1H), 7.34 (t, J = 8.6 Hz, 1H), 6.78 (d, J = 1.8 Hz, 1H), 6.73 (s, 1H), 6.71 (dd, J = 8.3, 1.8 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-D6) δ 181.2, 167.9, 166.7, 153.5 (d, $^1J_{C\text{-}F}$ = 247.3 Hz), 147.6, 130.3 (d, $^2J_{C\text{-}F}$ = 7.5 Hz), 128.3 (d, $^3J_{C\text{-}F}$ = 3.5 Hz), 128.2, 126.0, 121.8, 118.30 (d, $^2J_{C\text{-}F}$ = 20.1 Hz), 113.8, 112.6, 108.43 (d, $^3J_{C\text{-}F}$ = 2.4 Hz), 98.7. IR (neat) 3074-2653 (br), 2136, 2106, 1685, 1573, 1462, 1298, 1119, 851 cm^{-1}.



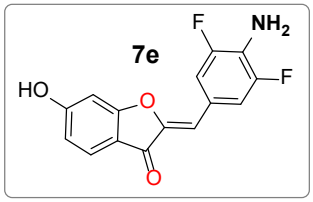
***(Z)-2-(4-amino-3-fluorobenzylidene)-6-hydroxybenzofuran-3(2H)-one (7d)*:** Reaction scale (21.5 mg, 0.069 mmol), Yield: 98% (18.4 mg); orange solid, mp >260 ºC. ^{1}H NMR (500 MHz, DMSO-D6) δ 7.64 (d, J = 12.0 Hz, 1H), 7.58 (dd, J = 8.0, 3.0 Hz, 1H), 7.50 (d, J = 8.0 Hz, 1H), 6.86 – 6.76 (m, 2H), 6.70-6.68 (m, 2H), 5.96 (s, 2H). ^{13}C NMR (125 MHz, DMSO-D6) δ 180.8, 167.2, 166.0, 149.8 (d, $^1J_{C\text{-}F}$ = 237.2 Hz), 145.3, 138.9 (d, $^2J_{C\text{-}F}$ = 12.8 Hz), 129.1, 125.5, 119.5 (d, $^3J_{C\text{-}F}$ = 7.0 Hz, 117.25 (d, $^2J_{C\text{-}F}$ = 18.8 Hz), 115.7, 113.2, 112.8, 111.5, 98.5. IR (neat) 3478, 3372, 3041-2586 (br), 1674, 1566, 1521, 1454, 1294, 1112, 999 cm^{-1}. HRMS (EI): *m/z* [M + H] calcd for $C_{15}H_{11}FNO_3$ 272.07230, found 272.07228.



***(Z)-2-(4-azido-3, 5-difluorobenzylidene)-6-hydroxybenzofuran-3(2H)-one (6e)*:** Reaction scale (0.33 mmol), Yield: 76% (79.3 mg); yellow solid, mp 165 ºC (decompose). ^{1}H NMR (500 MHz, DMSO-D6) δ 11.30 (s, 1H), 7.72 (d, J = 9.5 Hz, 2H), 7.61 (d, J = 8.5 Hz, 1H), 6.83 (s, 1H), 6.74 (s, 1H), 6.72 (dd, J = 8.5, 2.0 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-D6) δ 181.0, 167.9, 166.8, 154.7 (d, $^1J_{C\text{-}F}$ = 247.5 Hz), 148.0, 129.6 (t, $^2J_{C\text{-}F}$ = 10.1 Hz), 125.9, 116.0, 114.3 (d, $^2J_{C\text{-}F}$ = 19.6 Hz), 113.2, 112.3, 107.3, 98.8. IR (neat) 3078, 2117, 1685, 1577, 1506, 1458, 1279, 1138, 1112, 1045, 832 cm^{-1}.

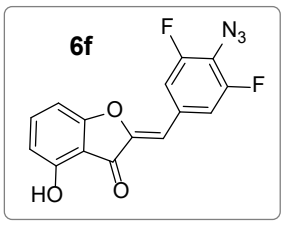


***(Z)-2-(4-amino-3, 5-difluorobenzylidene)-6-hydroxybenzofuran-3 (2H)-one (7e)*:** Reaction scale



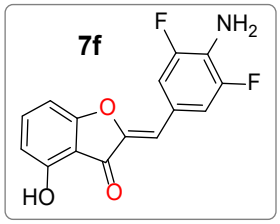
(31 mg, 0.098 mmol), Yield: 80% (22.7 mg); orange solid, mp >260 ºC. ^{1}H NMR (300 MHz, DMSO-D6) δ 7.62 – 7.49 (m, 3H), 6.81 (d, J = 2.0 Hz, 1H), 6.71 (s, 1H), 6.69 (dd, J = 7.5, 2.5 Hz, 1H), 6.00 (s, 1H). ^{13}C NMR (75 MHz, DMSO-D6) δ 180.9, 167.4, 150.4 (dd, J_{C-F} = 238.8, 10.0 Hz), 146.0, 128.0 (t, J_{C-F} = 16.5 Hz), 125.7, 117.85 (t, J_{C-F} = 9.1 Hz), 113.95 (d, J_{C-F} = 13.3 Hz), 113.9, 113.8, 113.0, 110.4, 98.7. IR (neat) 3469, 3368, 1678, 1581, 1532, 1454, 1324, 1287, 1115, 821, 769 cm^{-1}. HRMS (EI): m/z [M + H] calcd for $C_{15}H_{11}FNO_3$ 272.07230, found 272.08226.

***(Z)-2-(4-azido-3,5-difluorobenzylidene)-4-hydroxybenzofuran-3(2H)-one (6f)*:** Reaction scale



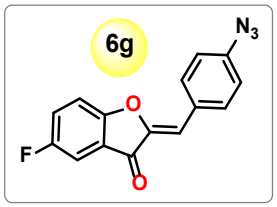
(0.36 mmol), Yield: 70% (80 mg); yellow solid, mp 165-170 ºC. ^{1}H NMR (300 MHz, DMSO-D6) δ 11.27 (s, 1H), 7.75 (d, J = 10.0 Hz, 2H), 7.56 (t, J = 8.0 Hz, 1H), 6.92 (d, J = 8.0 Hz, 1H), 6.76 (s, 1H), 6.65 (d, J = 8.0 Hz, 1H). ^{13}C NMR (125 MHz, DMSO-D6) δ 180.9, 165.8, 157.2, 154.9 (d, $^{1}J_{C-F}$ = 245.9, 4.6 Hz), 147.2, 138.9, 129.68 (t, $^{3}J_{C-F}$ = 9.8 Hz), 117.32 (t, $^{2}J_{C-F}$ = 14.0 Hz), 114.22 (dd, $^{2}J_{C-F}$ = 19.2, 4.7 Hz), 111.0, 108.6, 106.8, 102.5. IR (neat) 3379, 2121, 1696, 1607, 1495, 1339, 1305, 1179, 1041, 799 cm^{-1}.

***(Z)-2-(4-amino-3,5-difluorobenzylidene)-4-hydroxybenzofuran-3(2H)-one (7f)*:** Reaction scale

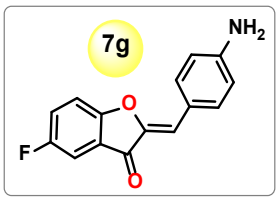


(13 mg, 0.041 mmol), Yield: 84% (10 mg); red solid, mp 222-225 ºC. ^{1}H NMR (500 MHz, DMSO-D6) δ 7.44 (dd, J = 8.0, 2.1 Hz, 2H), 7.08 (t, J = 8.0 Hz, 1H), 6.33 (s, 1H), 6.00 (d, J = 8.0 Hz, 2H), 5.74 (s, 2H). ^{13}C NMR (125 MHz, DMSO-D6) δ 181.2, 165.8, 150.6 (dd, $^{1}J_{C-F}$ = 238.4, 9.8 Hz), 149.6 (d, J_{C-F} = 10.4), 147.4, 136.6, 126.3 (t, $^{2}J_{C-F}$ = 11.2 Hz), 119.5, 112.7 (d, $^{2}J_{C-F}$ = 16.7 Hz), 109.9, 104.9, 104.8, 104.7. IR (neat) 3346, 1689, 1596, 1566, 1566, 1480, 1354, 1235, 1115, 1000, 803 cm^{-1}. HRMS (EI): m/z [M + H] calcd for $C_{15}H_{10}F_2NO_3$ 290.06288, found 290.06290.

***(Z)-2-(4-azidobenzylidene)-5-fluorobenzofuran-3(2H)-one (6g)*:** Reaction scale (0.40 mmol), Yield: 80% (90 mg); yellow solid, mp 137-138 ºC. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89 (d, J = 8.5 Hz, 2H), 7.45 (dd, J = 7.0, 3.0 Hz, 1H), 7.38 (td, J = 8.5, 3.0 Hz, 1H), 7.29 (dd, J = 9.0, 3.5 Hz, 1H), 7.10 (d, J = 9.0 Hz, 2H), 6.85 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 183.9, 162.1, 159.02 (d, $^{1}J_{C\text{-}F}$ = 244.7 Hz), 147.4, 141.9, 133.3, 129.0, 124.4 (d, $^{2}J_{C\text{-}F}$ = 26.1 Hz), 122.5 (d, $^{3}J_{C\text{-}F}$ = 8.0 Hz), 119.7, 114.21 (d, $^{3}J_{C\text{-}F}$ = 7.8 Hz), 113.08, 110.36 (d, $^{2}J_{C\text{-}F}$ = 24.3 Hz). IR (neat) 2128, 1707, 1655, 1598, 1484, 1305, 1268, 1190, 892, 832 cm^{-1}.



***(Z)-2-(4-aminobenzylidene)-5-fluorobenzofuran-3(2H)-one (7g)*:** Reaction scale (18 mg, 0.064 mmol), Yield: 73% (12 mg); red solid, mp 178-180 ºC. ^{1}H NMR (500 MHz, DMSO-D6) δ 7.74 (d, J = 8.5 Hz, 2H), 7.65 – 7.58 (m, 2H), 7.57 (dd, J = 7.0, 2.4 Hz, 1H), 6.88 (s, 1H), 6.66 (d, J = 8.5 Hz, 2H), 6.16 (s, 2H). ^{13}C NMR (76 MHz, DMSO-D6) δ 181.64, 160.5, 158.18 (d, $^{1}J_{C\text{-}F}$ = 240.9 Hz), 152.1, 144.3, 134.2, 123.7 (d, $^{2}J_{C\text{-}F}$ = 26.0 Hz), 122.6 (d, $^{3}J_{C\text{-}F}$ = 8.0 Hz), 118.7, 116.2, 114.72 (d, $^{3}J_{C\text{-}F}$ = 8.1 Hz), 113.9, 109.48 (d, $^{2}J_{C\text{-}F}$ = 24.2 Hz). IR (neat) 3420, 3320, 3216, 1689, 1633, 1562, 1514, 1480, 1272, 1156, 817, 762 cm^{-1}. HRMS (EI): *m/z* [M + H] calcd for $C_{15}H_{11}FNO_2$ 256.0774, observed 256.0071.



References

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UV-visible absorption and emission spectra

The 10 mM stock solution of all the 4'-azidoaurones **6a-g** and corresponding amines **7a-g** were prepared in DMSO. Unless otherwise stated 1X, pH = 7.4 PBS was used for the preparation of samples. All the UV-absorption and emission spectra were collected using a 50 μM solution of the compounds in different solvent systems (90% DMSO/10% PBS, 5% DMSO in PBS, and 0.5% DMSO in PBS) in the presence or absence of CTAB.

Sample preparation for UV-Vis absorption

a) 50 μM solution of **6g** and **7g** in 0.5% DMSO in PBS
   15 μL 10 mM stock solution of **6g** or **7g** in DMSO was diluted with 2985 μL of PBS (1x pH = 7.4) in a 3 mL cuvette.
b) 50 μM solution of **6g** and **7g** in 5% DMSO in PBS
   150 μL 1.0 mM stock solution of **6g** or **7g** in DMSO was diluted with 2850 μL of PBS (1x pH = 7.4) in a 3 mL cuvette.
c) 50 μM solution of **6g** and **7g** in 90% DMSO in PBS
   150 μL 1.0 mM stock solution of **6g** or **7g** in DMSO was diluted with 2550 μL DMSO and 300 PBS (1x pH = 7.4) in a 3 mL cuvette.
d) 50 μM solution of **6g** v **7g** in 0.5% DMSO in PBS and 1mM CTAB (in PBS)
   To a 15 μL 10 mM **6g** and **7g**, 600 μL of CTAB (5 mM stock solution in PBS) and 2385 μL of PBS in a 3 mL cuvette.

Similarly, for collecting the emission spectra the 50 μM solutions of **6g** and **7g** in a 1.5 mL cuvette were excited at their respective absorption maxima (λmax) obtained for the specific solvent condition e.g., **6g** and **7g** were excited at 405 and 460 nm respectively in 0.5% v/v DMSO in PBS with 1mM CTAB.

Effect of pH on spectral properties of 6g and 7g

The effect of pH on the absorption and fluorescence behavior of probe **6g** and the corresponding amine **7g** in the presence or absence of 1 mM CTAB was studied. This study was done in 0.5% v/v DMSO in PBS (1X) solvent. For the study, five different PBS (1X) solutions of pH = 3, 4.8, 7.5, 9.15, and 11 were prepared by adjusting pH using a pH meter. Five different 10 mM stock solution of CTAB (pH = 3, 4.8, 7.5, 9.15, and 11) were prepared using the corresponding PBS (1X, pH = 3,

4.8, 7.5, 9.15, and 11) solutions. The UV-Vis absorption spectra of **6g** and **7g** were collected with and without the inclusion of 1mM CTAB in 0.5% v/v DMSO in PBS (1X) of the specific pH as described above.

Sample preparation (example)

1. 50 µM solution of **6g** and **7g** in 0.5% DMSO in PBS (pH = 3) with 1mM CTAB
   To a 15 µL 10 mM stock solution of **6g** and **7g**, 600 µL of CTAB (5 mM stock solution in PBS, pH =3) and 2385 µL of PBS (pH = 3) were mixed in a 3 mL cuvette and excited at the corresponding λmax to collect the emission spectra.

Similarly, the emission spectra of **6g** and **7g** at specific pH were collected by preparing and exciting their 50 µM solution in a 1.5 mL cuvette at their corresponding absorption maxima (λmax)**.**

Determination of detection limit

Using the stock solutions of **7g** the emission spectra of its various concentrations (0.1 - 50 µM) were collected in either 0.5% v/v DMSO in PBS/ 1 mM CTAB or 90% v/v DMSO/ 10% v/v PBS (IX, pH 7.4) solvent systems. Each solution of **7g** (0.1, 0.5, 1, 2, 5, 10, 15, 20, 25, 30, 40 and 50 µM) prepared in a particular solvent system in a 1.5 mL cuvette was excited at the 465 nm. For each concentration, at least three independent trials were performed. The corresponding emission intensity observed at 560 nm was then plotted as a function of [**7g**] to obtain the linear range of detection. This generated the calibration curve for each solvent condition revealing the useful working range of **7g**.

Sample preparation in 0.5% DMSO in PBS (1X, pH = 7.4) with 1mM CTAB)

a) 0.1 µM **7g** in 0.5% DMSO in PBS with 1mM CTAB
   In a cuvette containing 3.0 µL of **7g** (50 µM stock in DMSO), 4.5 µL DMSO, 300 µL CTAB (5 mM in PBS) were added and diluted to the final volume of 1500 µL with PBS (1192.5 µL, 1X, pH = 7.4)
b) 0.5 µM **7g** in 0.5% DMSO in PBS with 1mM CTAB
   In a cuvette containing 7.5 µL of **7g** (100 µM stock in DMSO), 300 µL CTAB (5 mM in PBS) were added and diluted to the final volume of 1500 µL with PBS (1192.5 µL)
c) 1.0 µM **7g** in 0.5% DMSO in PBS with 1mM CTAB
   In a cuvette containing 7.5 µL of **7g** (200 µM stock in DMSO), 300 µL CTAB (5 mM in PBS) were added and diluted to the final volume of 1500 µL with PBS (1192.5 µL)

d) 2.0 μM **7g** in 0.5% DMSO in PBS with 1mM CTAB

In a cuvette containing 3.0 μL of **7g** (1 mM stock in DMSO), 4.5 μL DMSO, 300 μL CTAB (5 mM in PBS) were added and diluted to the final volume of 1500 μL with PBS (1192.5 μL)

e) 5.0 μM **7g** in 0.5% DMSO in PBS with 1mM CTAB

In a cuvette containing 7.5 μL of **7g** (1 mM stock in DMSO), 300 μL CTAB (5 mM in PBS) were added and diluted to the final volume of 1500 μL with PBS (1192.5 μL)

f) 10 μM **7g** in 0.5% DMSO in PBS with 1mM CTAB

In a cuvette containing 3.0 μL of **7g** (5 mM stock in DMSO), 4.5 μL DMSO, 300 μL CTAB (5 mM in PBS) were added and diluted to the final volume of 1500 μL with PBS (1192.5 μL)

g) 15 μM **7g** in 0.5% DMSO in PBS with 1mM CTAB

In a cuvette containing 4.5 μL of **7g** (5 mM stock in DMSO), 3.0 μL DMSO, 300 μL CTAB (5 mM in PBS) were added and diluted to the final volume of 1500 μL with PBS (1192.5 μL)

h) 20 μM **7g** in 0.5% DMSO in PBS with 1mM CTAB

In a cuvette containing 3.0 μL of **7g** (10 mM stock in DMSO), 4.5 μL DMSO, 300 μL CTAB (5 mM in PBS) were added and diluted to the final volume of 1500 μL with PBS (1192.5 μL)

i) 25 μM **7g** in 0.5% DMSO in PBS with 1mM CTAB

In a cuvette containing 7.5 μL of **7g** (5 mM stock in DMSO), 300 μL CTAB (5 mM in PBS) were added and diluted to the final volume of 1500 μL with PBS (1192.5 μL).

j) 30 μM **7g** in 0.5% DMSO in PBS with 1mM CTAB

In a cuvette containing 4.5 μL of **7g** (10 mM stock in DMSO), 3.0 μL DMSO, 300 μL CTAB (5 mM in PBS) were added and diluted to the final volume of 1500 μL with PBS (1192.5 μL).

k) 40 μM **7g** in 0.5% DMSO in PBS with 1mM CTAB

In a cuvette containing 6.0 μL of **7g** (10 mM stock in DMSO), 1.5 μL DMSO, 300 μL CTAB (5 mM in PBS) were added and diluted to the final volume of 1500 μL with PBS (1192.5 μL)

l) 50 μM **7g** in 0.5% DMSO in PBS with 1mM CTAB

In a cuvette containing 7.5 μL of **7g** (10 mM stock in DMSO), 300 μL CTAB (5 mM in PBS) were added and diluted to the final volume of 1500 μL with PBS (1192.5 μL).

Reaction kinetics study

The time course reaction kinetics for the reduction of the azide (**6g**) to amine (**7g**) was performed by incubating 20 μM **6g** with 100 μM NaHS in either 0.5% v/v DMSO in PBS (1X, pH = 7.4) +/- 1 mM CTAB or 90% v/v DMSO/10% v/v PBS (1X, pH = 7.4) solvent systems. Samples were excited at 465 nm and emission spectra spanning from 480 to 700 nm were collected at 2-minute intervals.

Sample preparation (solvent system 0.5% v/v DMSO in PBS (1X, pH = 7.4)/ 1 mM CTAB):

In a cuvette, 3.0 μL of **6g** (10 mM stock in DMSO) was added followed by 4.5 μL DMSO, 300 μL CTAB (5 mM in PBS), and 1042.5 μL PBS (1X, pH = 7.4). To this solution, 150 μL of NaHS (1 mM stock solution in PBS) was mixed and placed in the fluorescence spectrophotometer to monitor the progress of the reaction by exciting the sample at 465 nm. The emission spectra corresponding to the amine were collected every 2-minutes for 60 minutes. At least 3 trials were performed for each experiment. For the reaction that employed 90% v/v DMSO/10% v/v PBS (1X, pH = 7.4) solvent system, the emission spectra were collected every 2-minutes for 30 minutes

Selectivity study

To evaluate the reaction specificity, a series of fluorescence time courses were collected using the same methodology as described for the kinetics study. In place of NaHS, three potential sulfide-donors were substituted: L-cysteine, reduced glutathione, and 2-mercaptoethanol. All emission spectra reflect the reaction of 100 μM sulfide-donor with 20 μM **6g** in 0.5 % v/v DMSO, 99.5 % v/v PBS, and 1 mM CTAB. At least 3 trials were performed for each experiment.

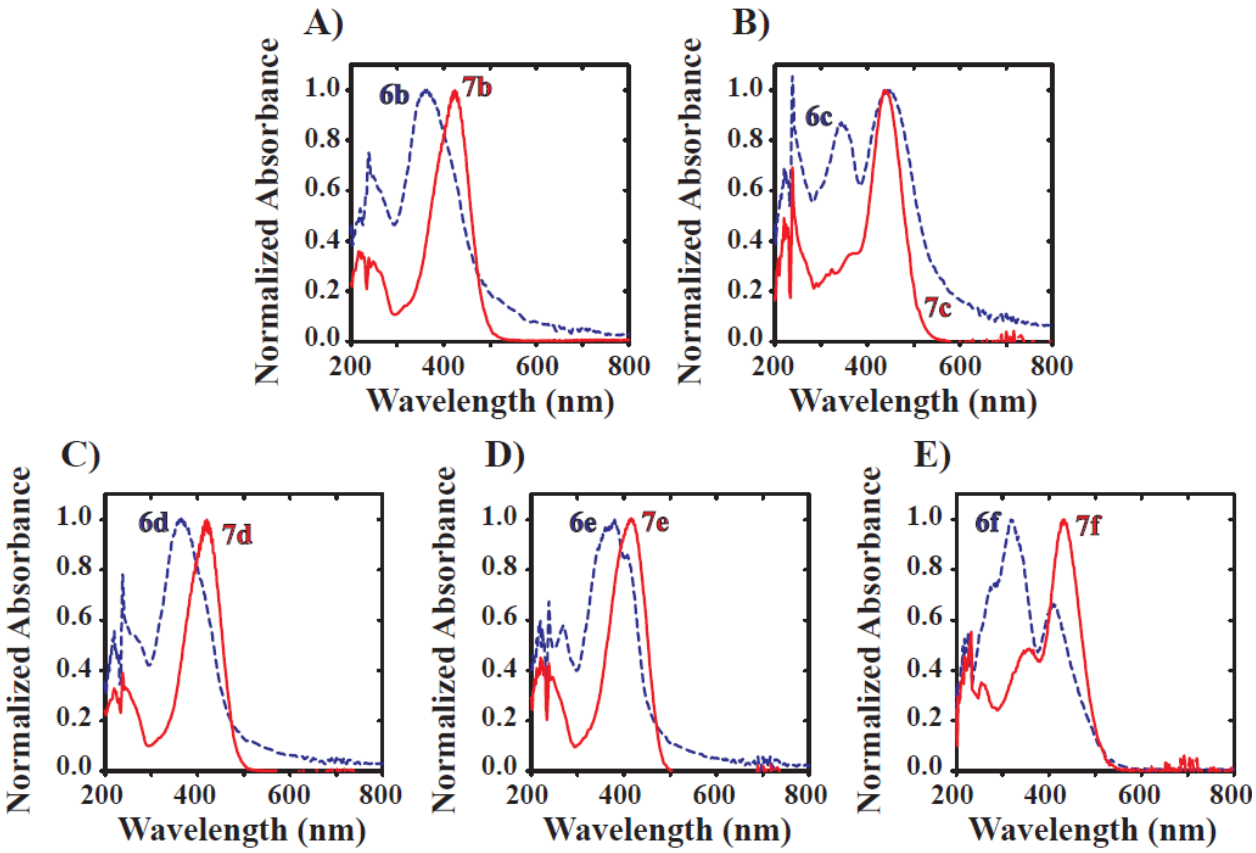


Figure S1. UV-visible Spectra Illustrating Spectral Overlap. Samples (50 μM) were prepared in 5% DMSO in PBS (v/v) for the study.

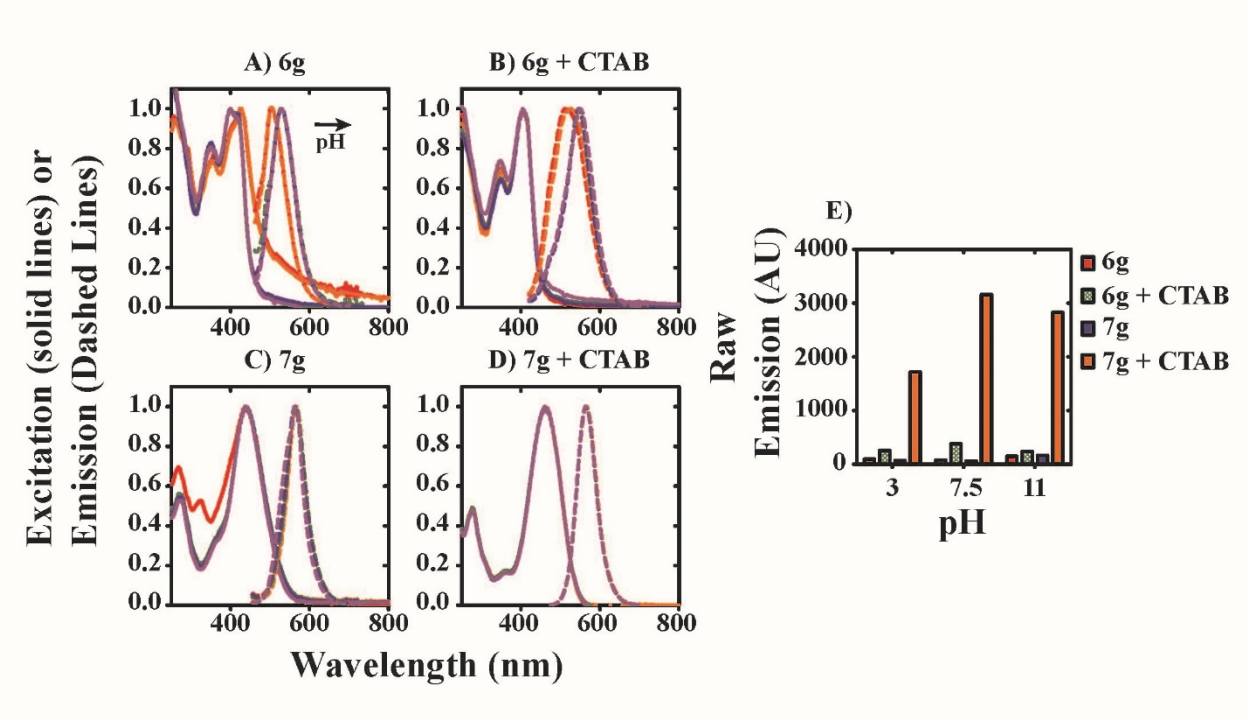


Figure S2. Effects of pH on 6g/7g Spectral Properties. Excitation spectra collected in 0.5 % DMSO/PBS buffer with pH adjusted to 3.0, 4.8, 7.5, 9.2, and 11.0 are represented as solid red, orange, green, blue, and purple lines, respectively. Corresponding emission spectra collected at pH = 3.0, 4.8, 7.5, 9.2, and 11.0 are represented as dashed red, orange, green, blue, and purple lines, respectively. Excitation/emission spectra were collected for (A) **6g** in the absence of CTAB, (B) **6g** in the presence of 1 mM CTAB, (C) **7g** in the absence of CTAB, and (D) **7g** in the presence of 1 mM CTAB. **6g** emission spectra were collected using excitation wavelengths of 425, 425, 405, 405, and 405 nm for conditions with pH = 3.0, 4.8, 7.5, 9.2, and 11.0, respectively, in the absence of CTAB. In the presence of CTAB, **6g** emission spectra were collected using an excitation wavelength equal to 405 nm at all pH values. **7g** emission spectra were generated using excitation wavelengths equal to 440 or 460 nm depending on whether 1 mM CTAB was absent or present, respectively. (E) Raw emission intensities plotted for **6g** without CTAB (red bars), **6g** with 1 mM CTAB (green bars), **7g** without CTAB (purple bars), and **7g** with 1 mM CTAB (orange bars) as a function of pH.

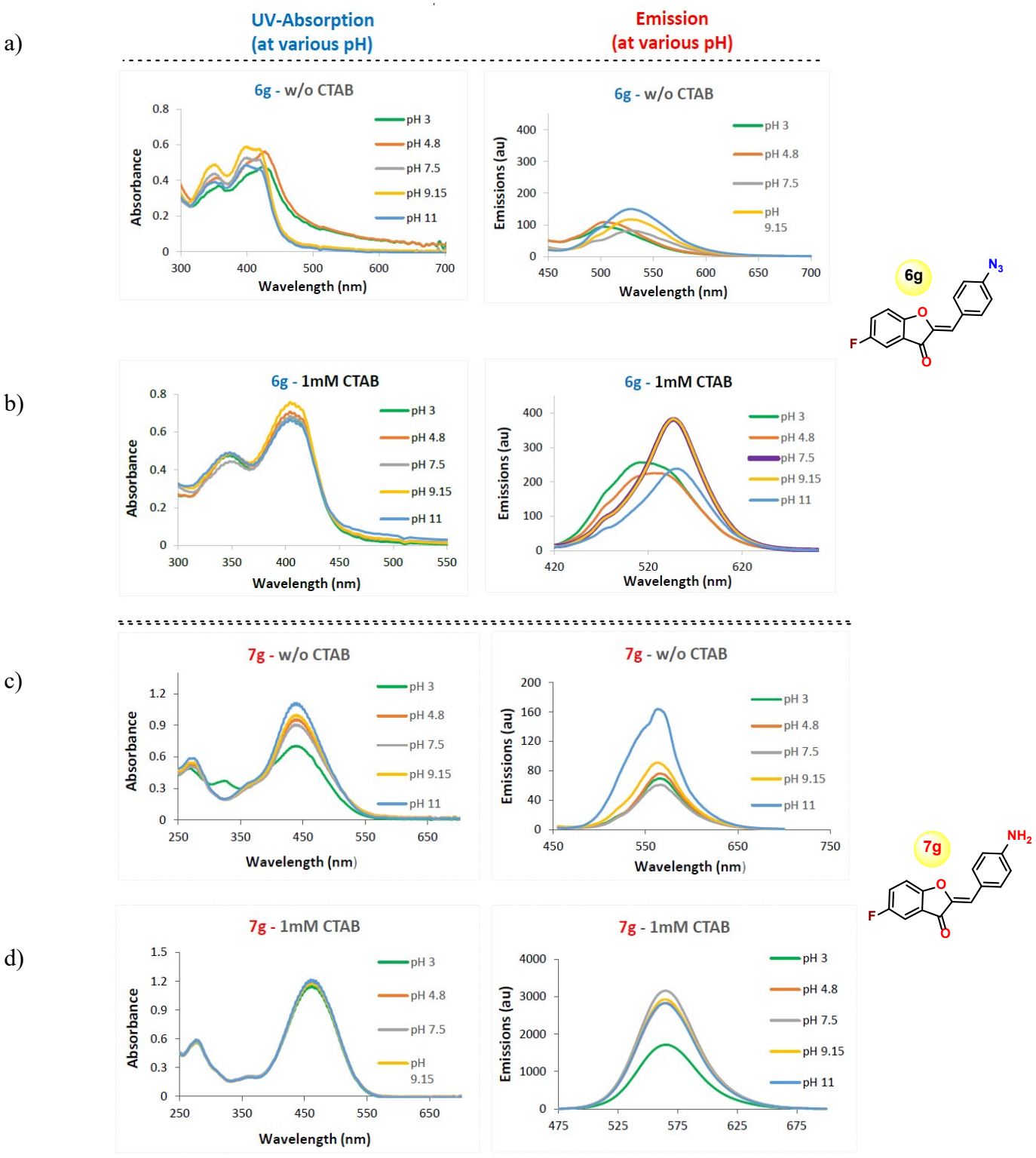
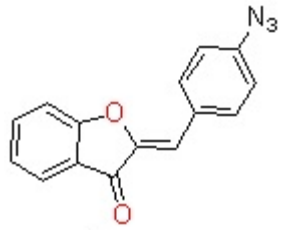


Figure S3. Effects of CTAB on Fluorescence Intensity of 6g/7g at various pH. Solvent system; 0.5%v/v DMSO in PBS.

AK_3_82_2_1H

7.926
7.909
7.815
7.801
7.677
7.660
7.646
7.335
7.319
7.244
7.229
7.214
7.109
7.092
6.851



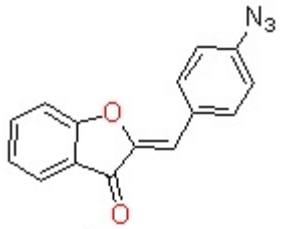
6a

2.15
1.03
1.11
1.06
1.09
2.06
1.00

f1 (ppm)

AK_3_82_2_13C

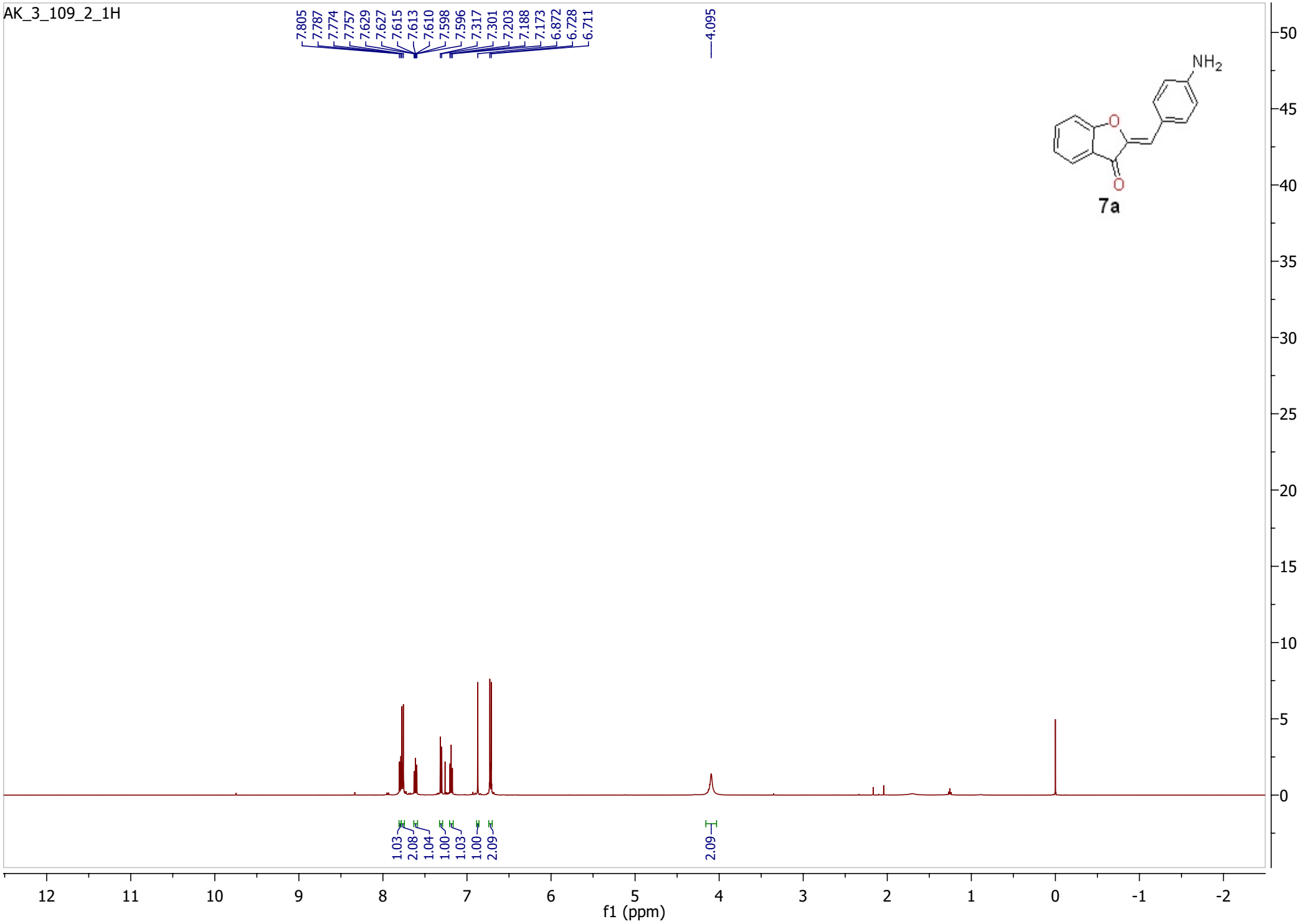
184.709
166.113
146.804
141.630
137.025
133.219
129.283
124.837
123.696
121.796
119.668
113.048
112.216

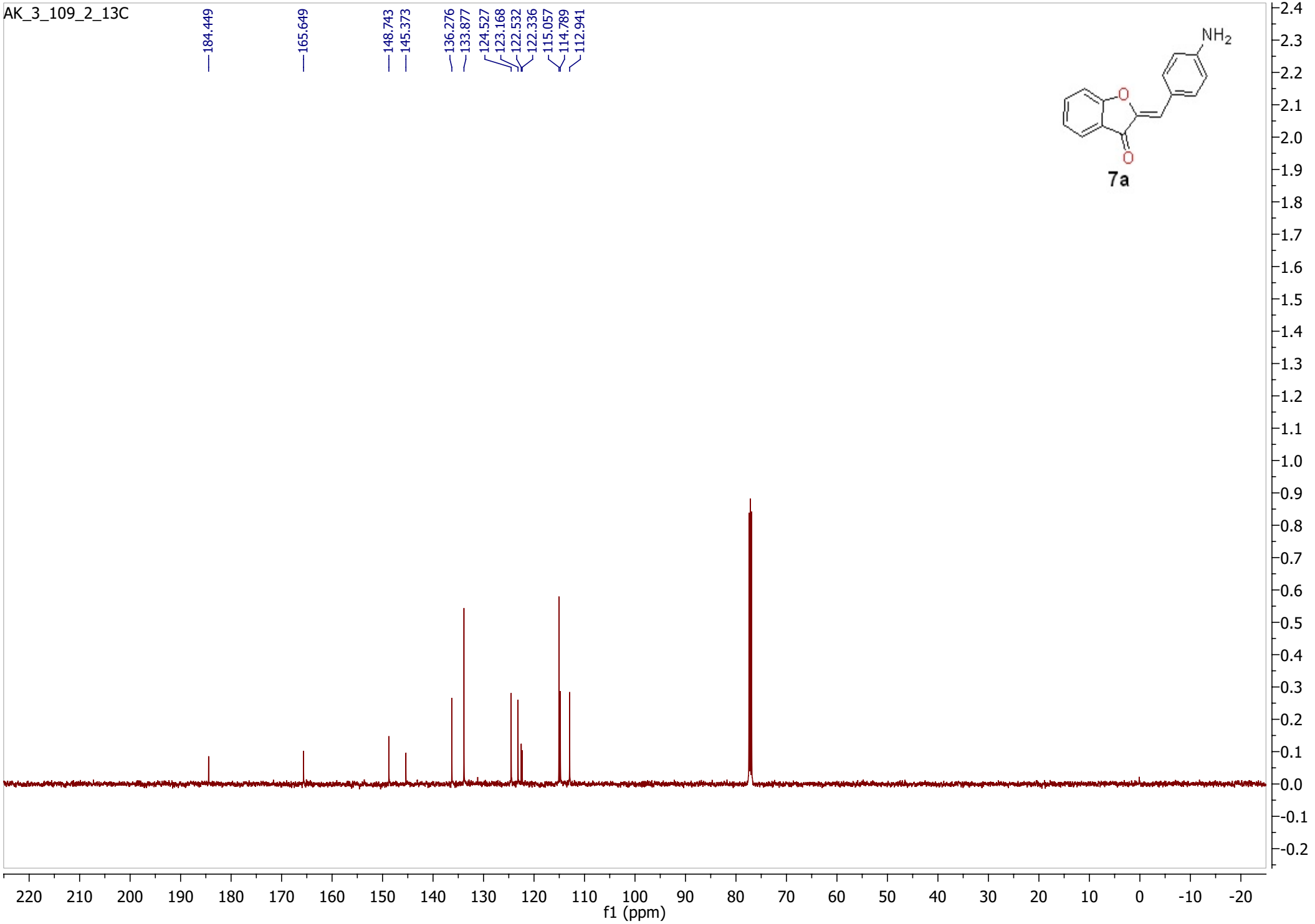


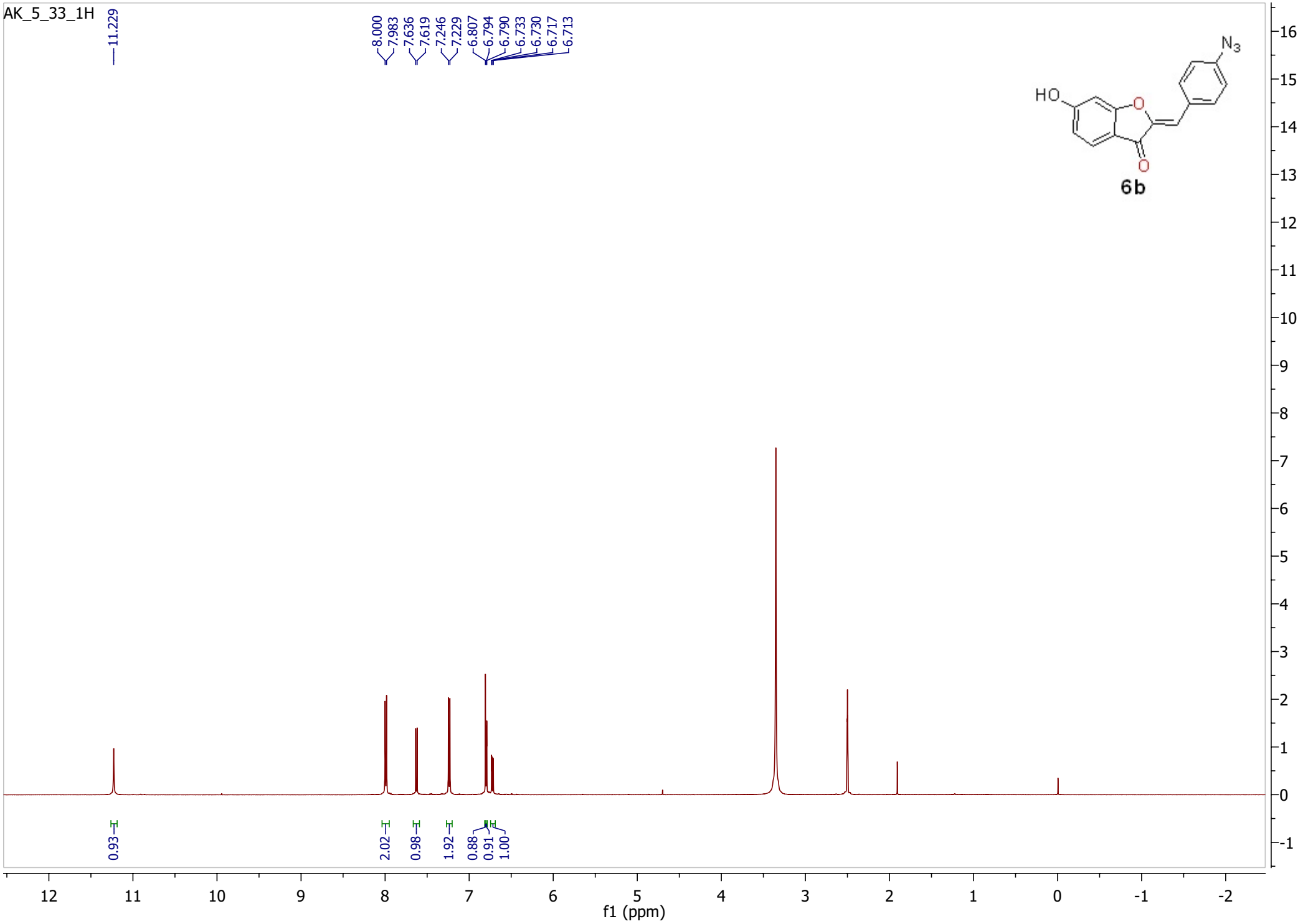
6a

f1 (ppm)

AK_3_109_2_1H

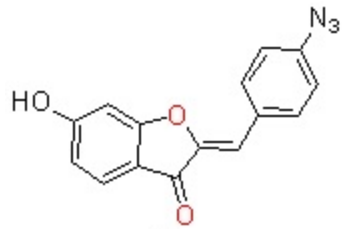

7.805
7.787
7.774
7.757
7.629
7.627
7.615
7.613
7.610
7.598
7.596
7.317
7.301
7.203
7.188
7.173
6.872
6.728
6.711
4.095
NH2
O
O
7a
1.03
2.08
1.04
1.00
1.03
1.00
2.09
2.09
f1 (ppm)


AK_3_109_2_13C
184.449
165.649
148.743
145.373
136.276
133.877
124.527
123.168
122.532
122.336
115.057
114.789
112.941
NH2
O
O
7a
f1 (ppm)


AK_5_33_1H
11.229
8.000
7.983
7.636
7.619
7.246
7.229
6.807
6.794
6.790
6.733
6.730
6.717
6.713
HO
O
O
N3
6b
0.93
2.02
0.98
1.92
0.88
0.91
1.00
f1 (ppm)

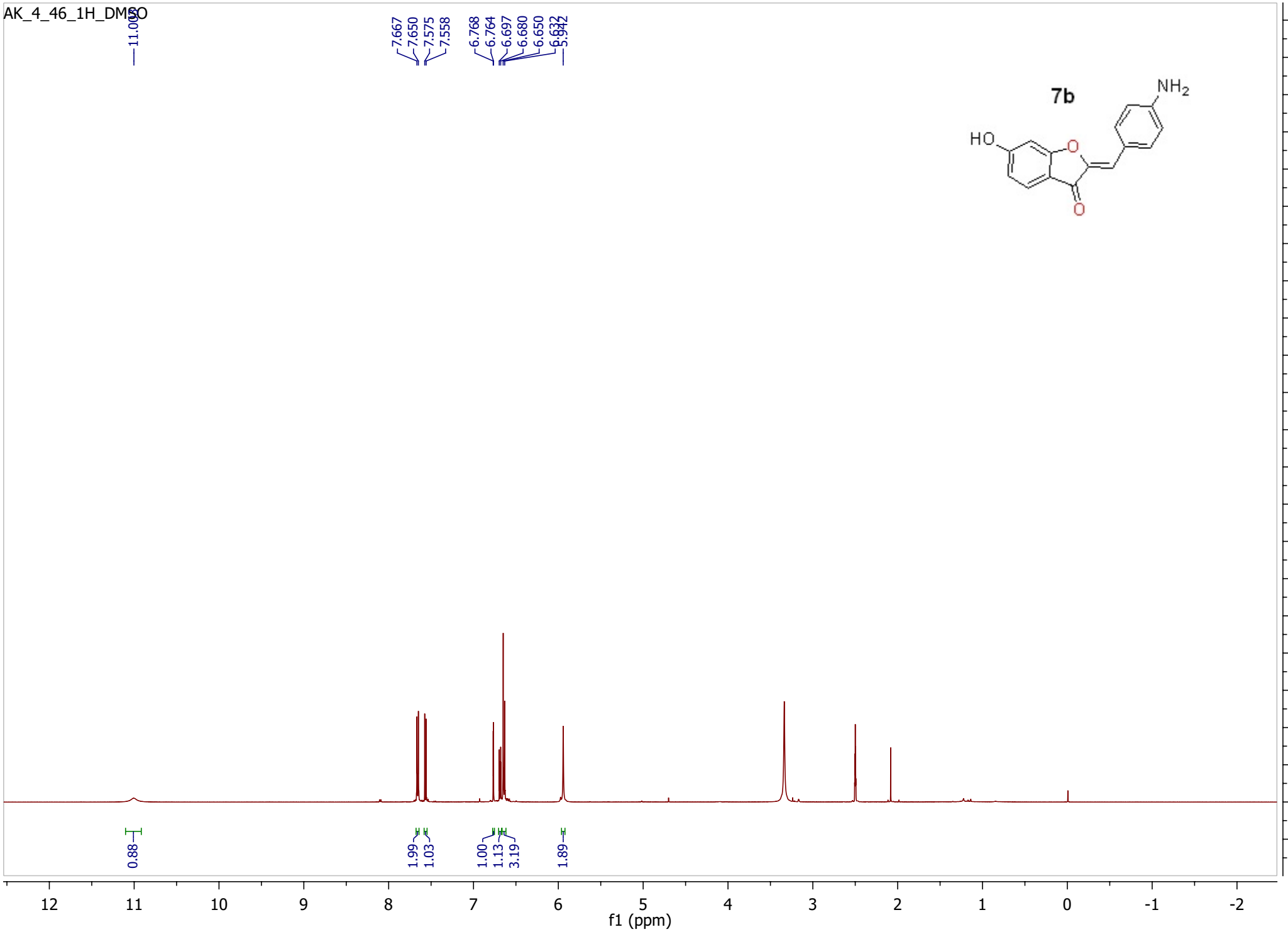
AK_5_33_13C

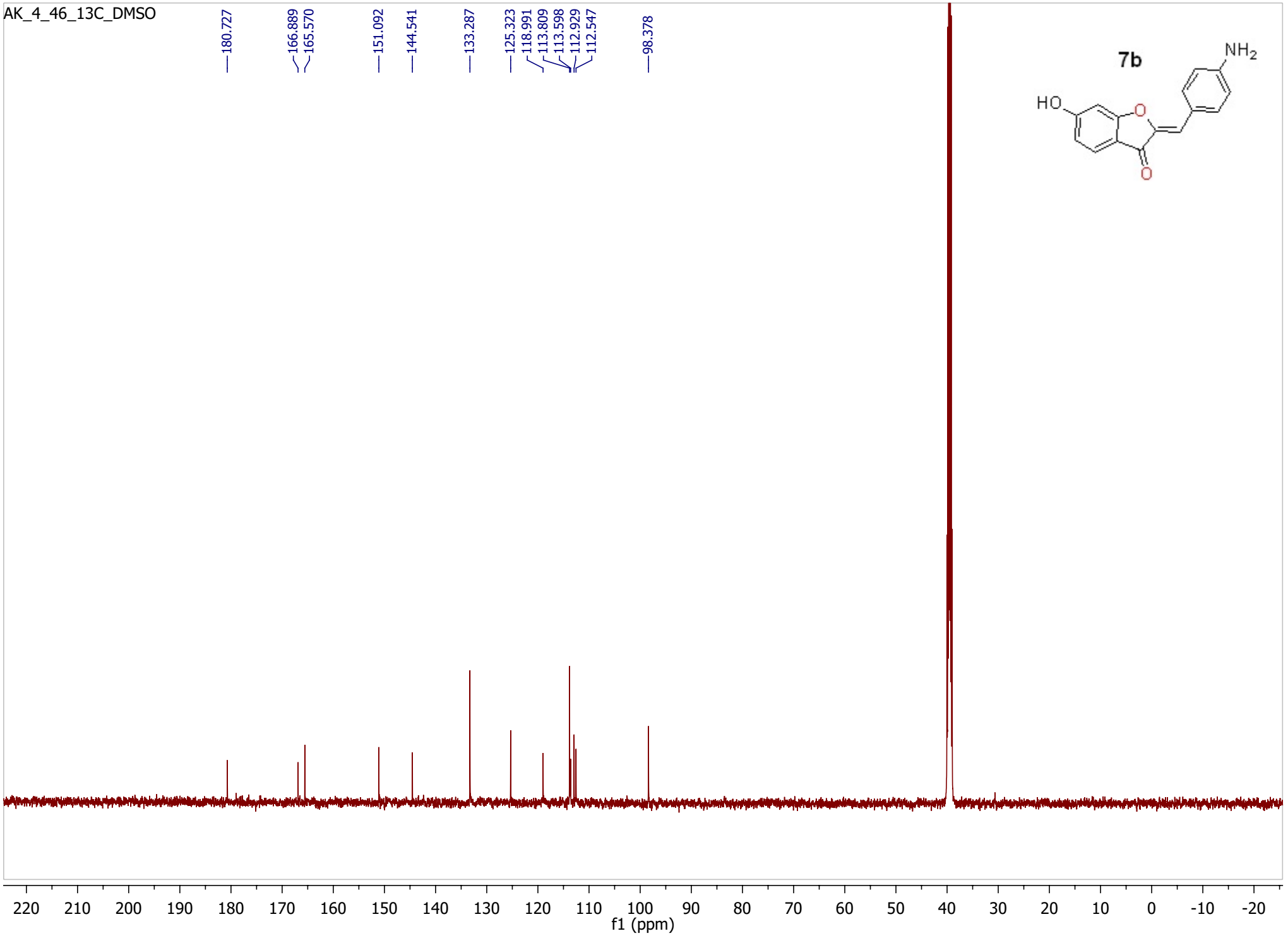
181.255
167.773
166.513
147.097
140.481
132.688
128.971
125.902
119.654
113.053
112.781
109.555
98.580

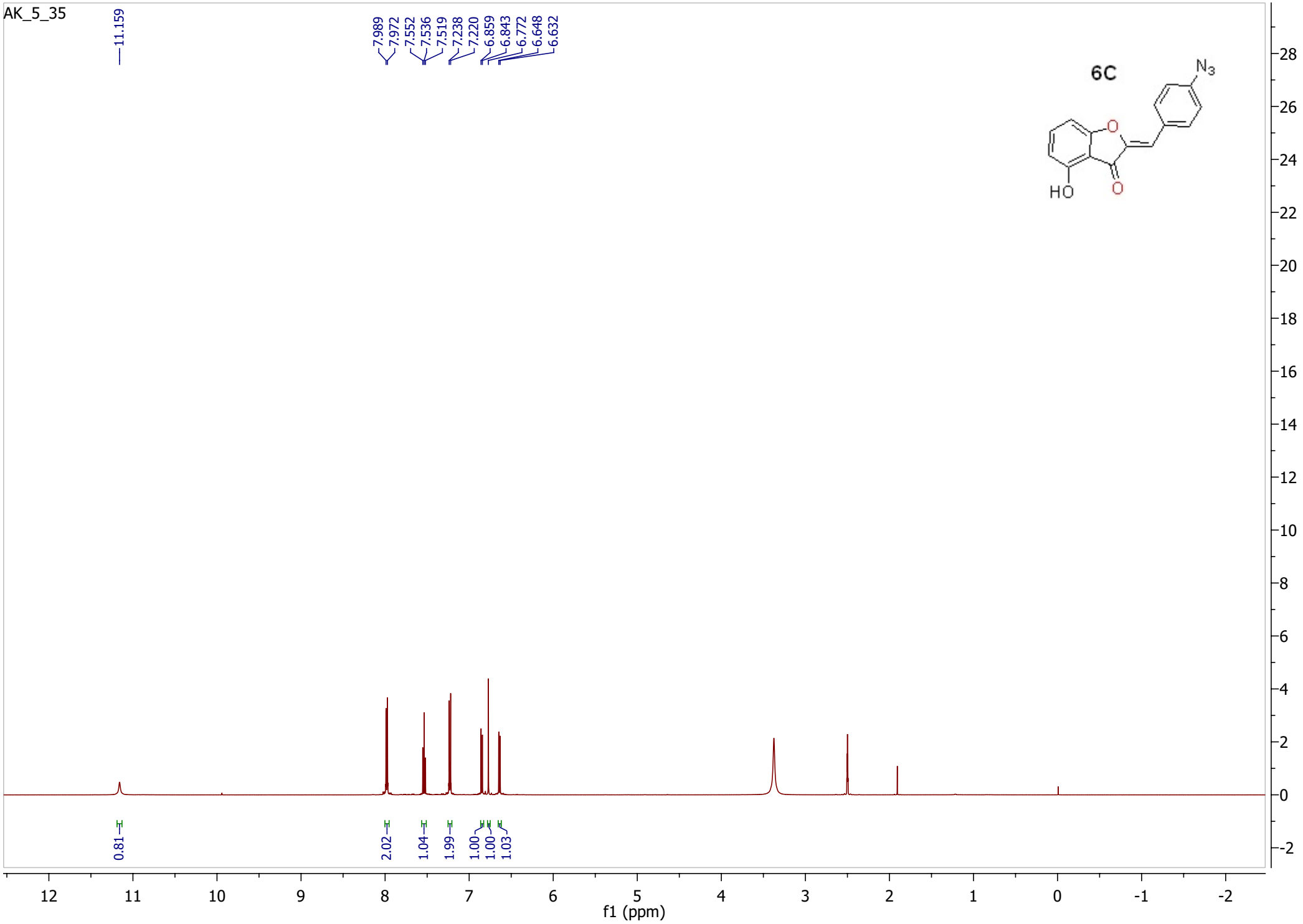


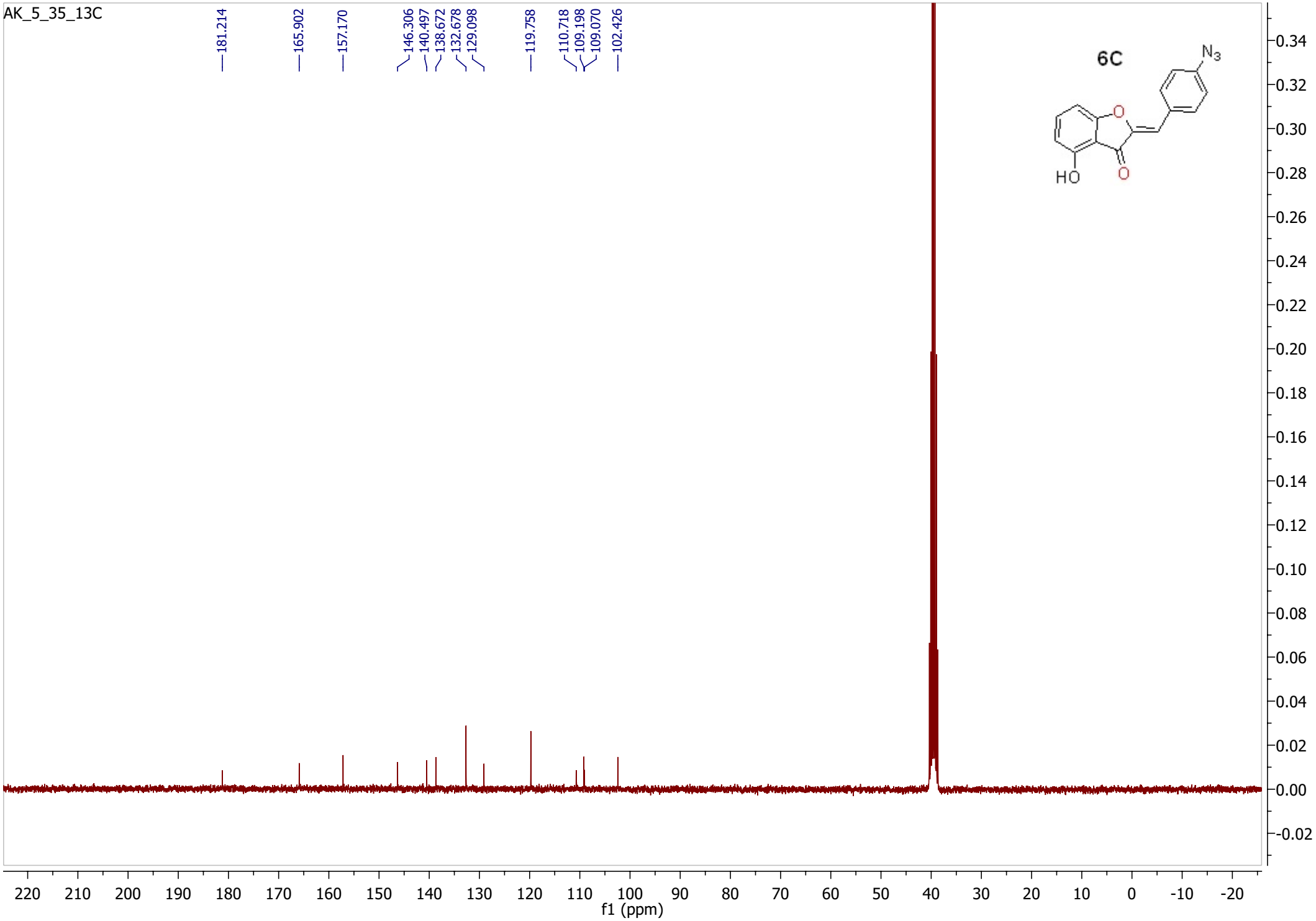
6b

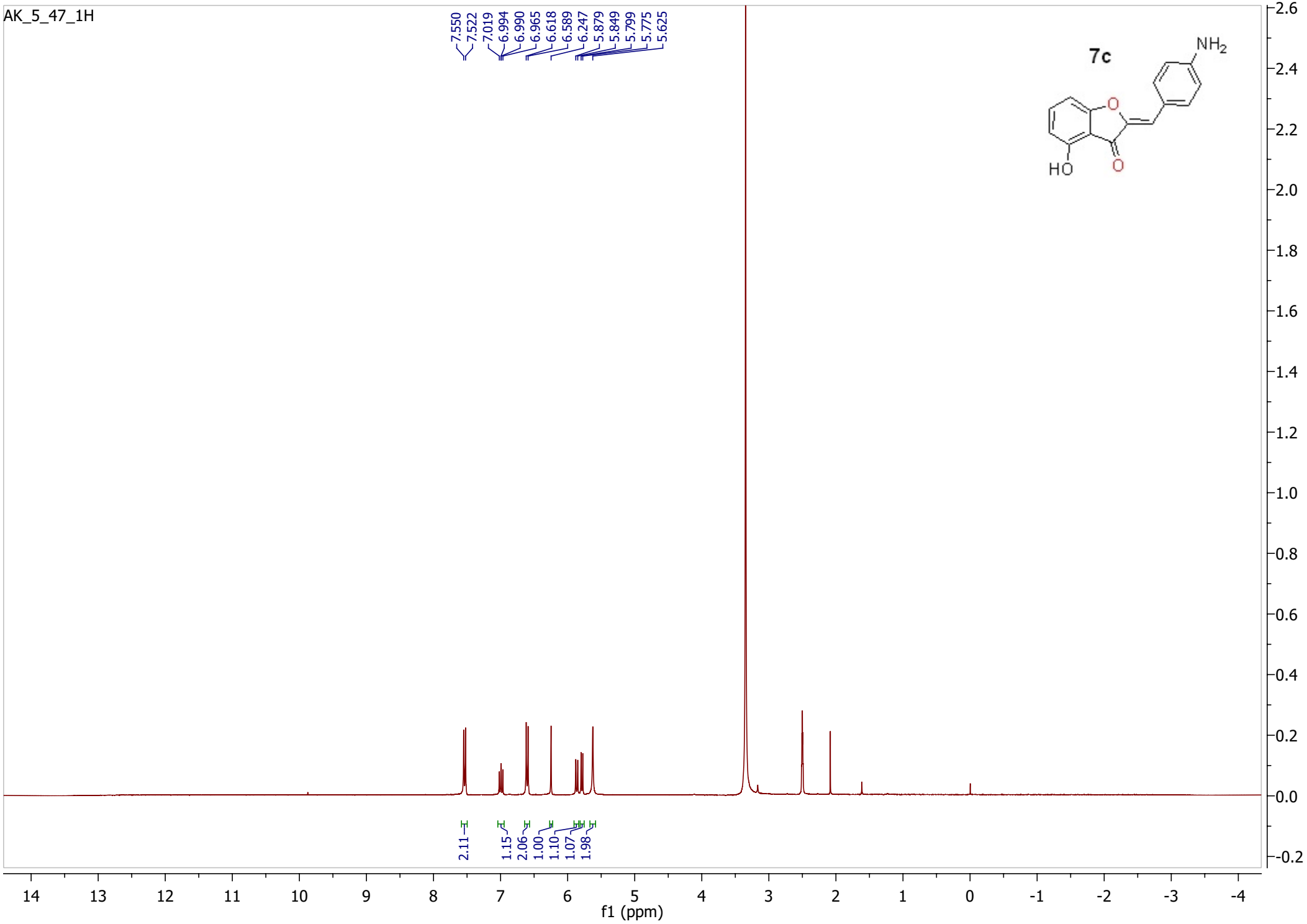
f1 (ppm)

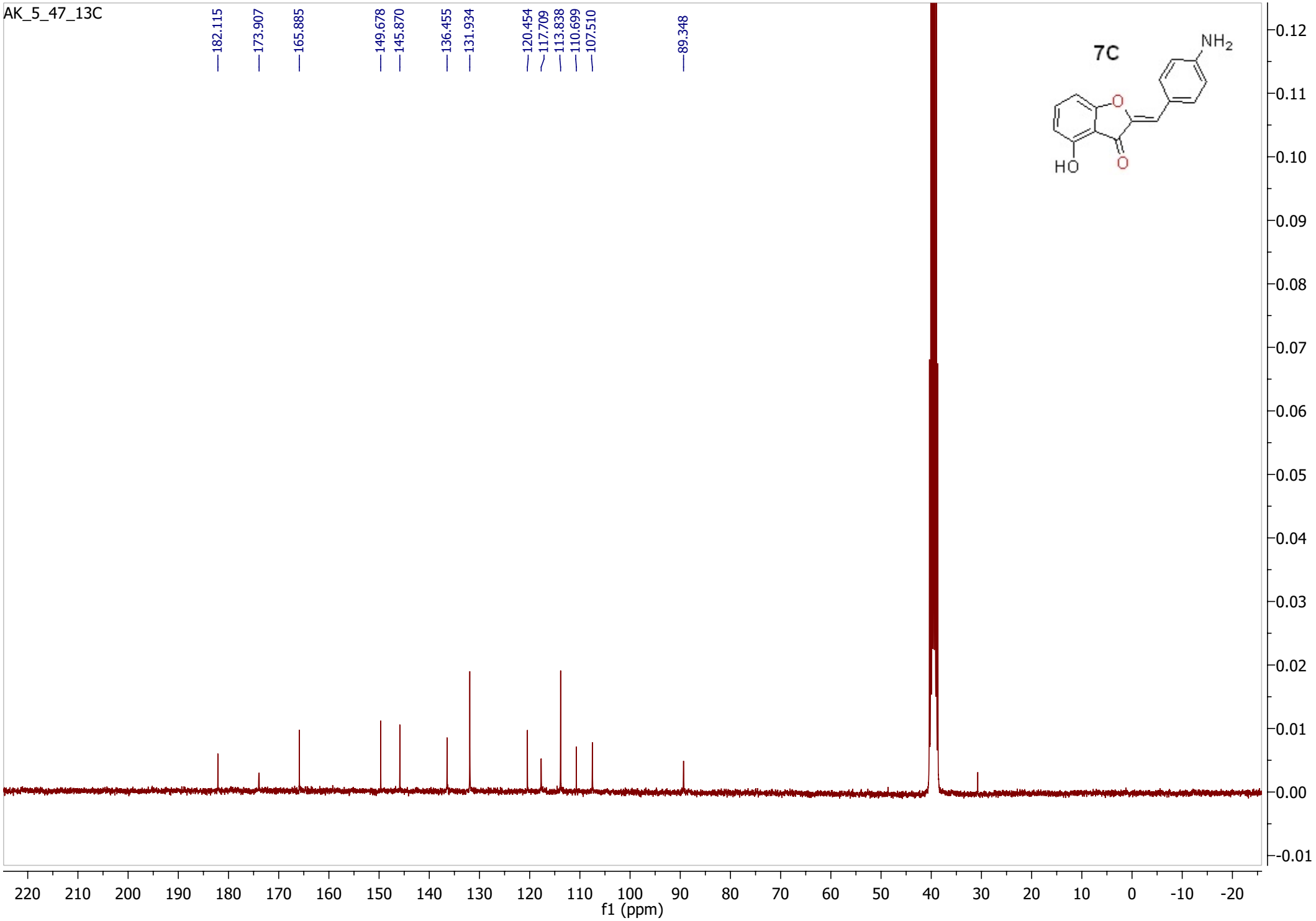

AK_4_46_1H_DMSO
11.008
7.667
7.650
7.575
7.558
6.768
6.764
6.697
6.680
6.650
6.632
5.942
7b
NH2
HO
O
O
0.88
1.99
1.03
1.00
1.13
3.19
1.89
f1 (ppm)

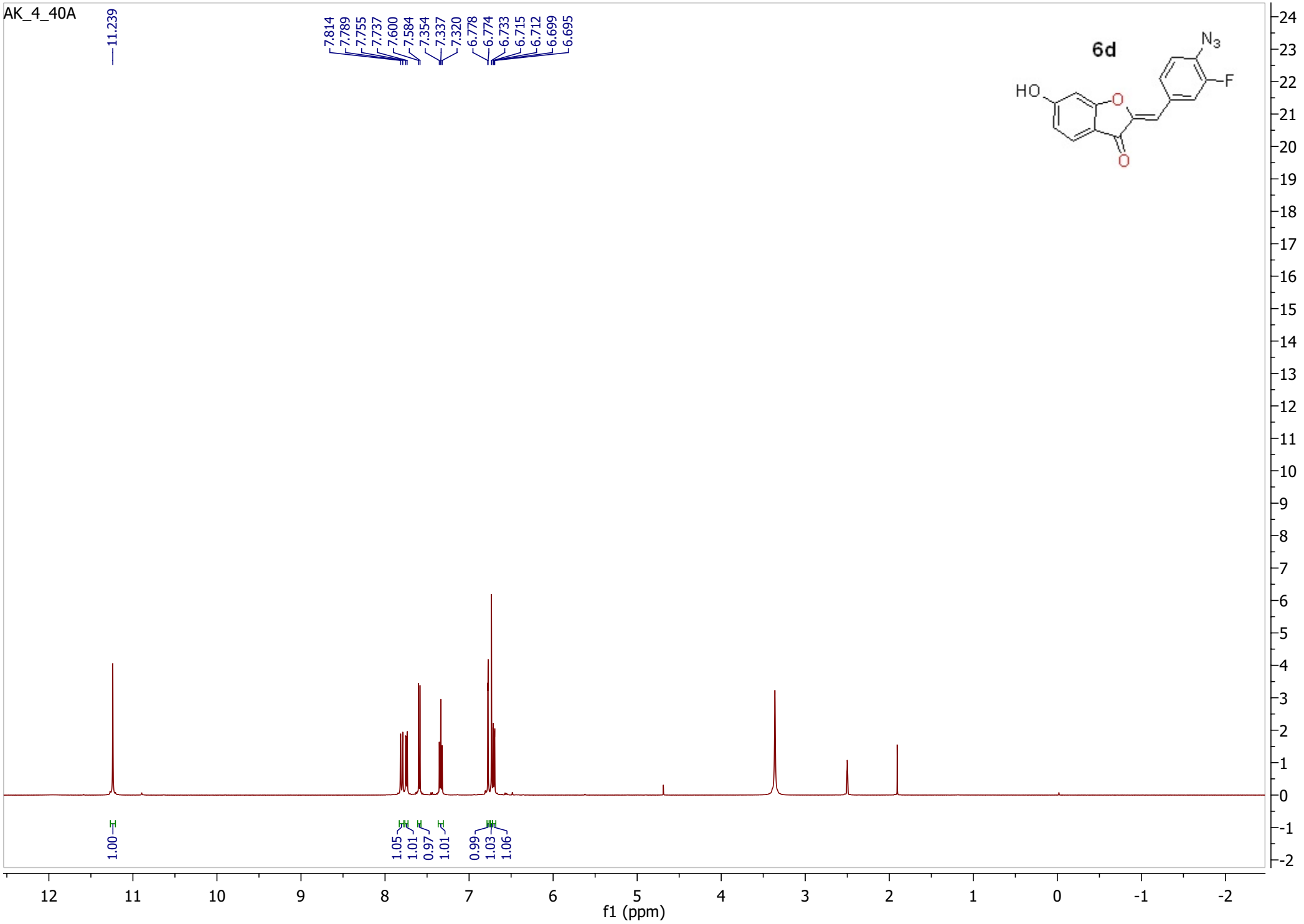

AK_4_46_13C_DMSO
180.727
166.889
165.570
151.092
144.541
133.287
125.323
118.991
113.809
113.598
112.929
112.547
98.378
7b
NH2
HO
O
O
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
f1 (ppm)


AK_5_35
11.159
7.989
7.972
7.552
7.536
7.519
7.238
7.220
6.859
6.843
6.772
6.648
6.632
6C
N3
O
HO
O
0.81
2.02
1.04
1.99
1.00
1.00
1.03
f1 (ppm)


AK_5_35_13C
181.214
165.902
157.170
146.306
140.497
138.672
132.678
129.098
119.758
110.718
109.198
109.070
102.426
6C
N3
O
HO
O
0.34
0.32
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)

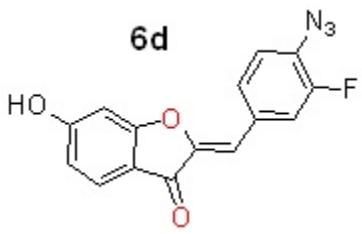

AK_5_47_1H
7.550
7.522
7.019
6.994
6.990
6.965
6.618
6.589
6.247
5.879
5.849
5.799
5.775
5.625
7c
NH2
O
HO
O
2.11
1.15
2.06
1.00
1.10
1.07
1.98
f1 (ppm)


AK_5_47_13C
182.115
173.907
165.885
149.678
145.870
136.455
131.934
120.454
117.709
113.838
110.699
107.510
89.348
7C
NH2
O
HO
O
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
f1 (ppm)


AK_4_40A
11.239
7.814
7.789
7.755
7.737
7.600
7.584
7.354
7.337
7.320
6.778
6.774
6.733
6.715
6.712
6.699
6.695
6d
HO
O
O
N3
F
1.00
1.05
1.01
0.97
1.01
0.99
1.03
1.06
f1 (ppm)

AK_4_40A_13C

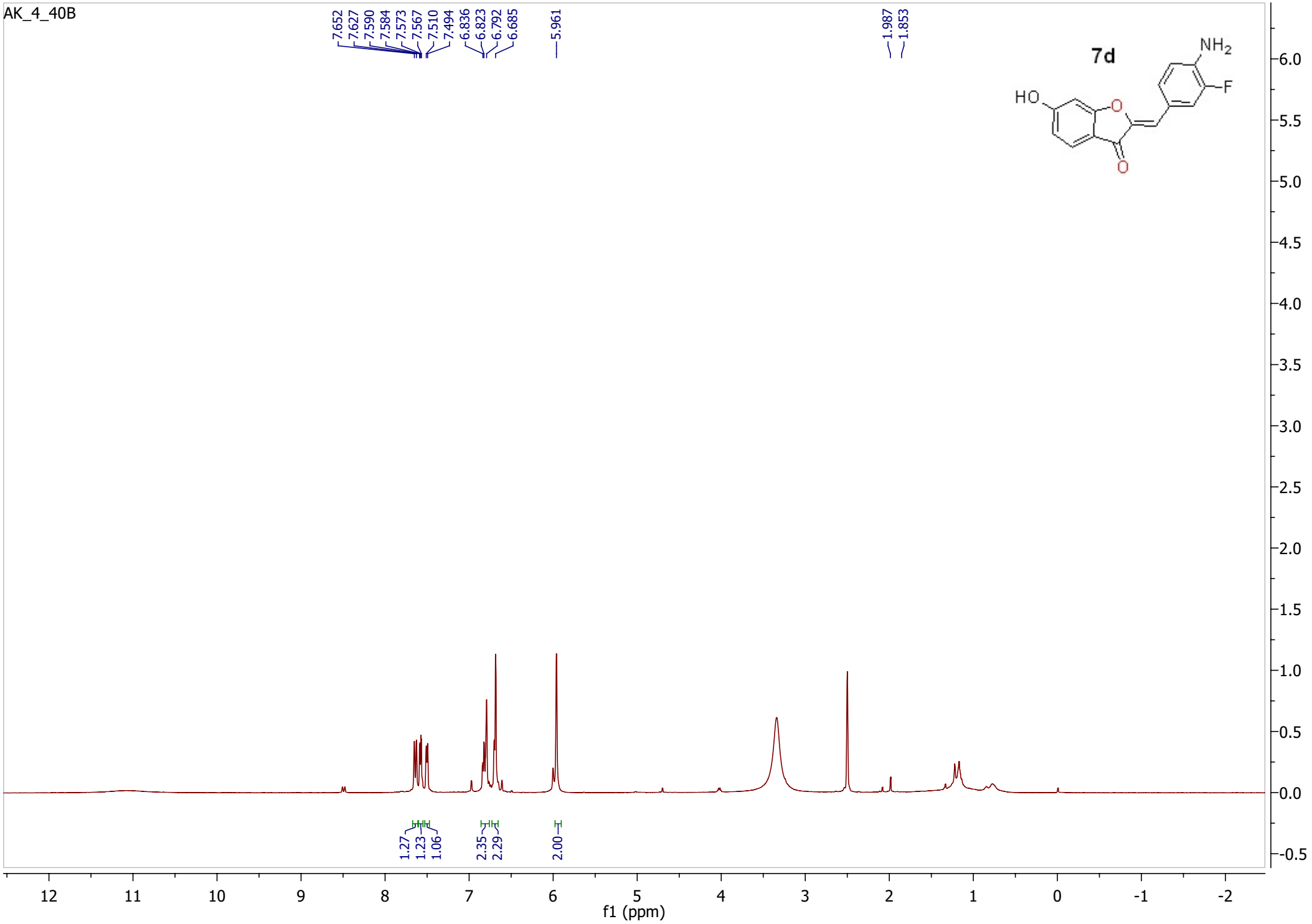
181.204
167.880
166.709
155.157
151.885
147.588
130.326
130.223
128.278
128.232
128.136
126.001
121.754
118.430
118.164
113.179
112.610
108.448
108.416
98.742

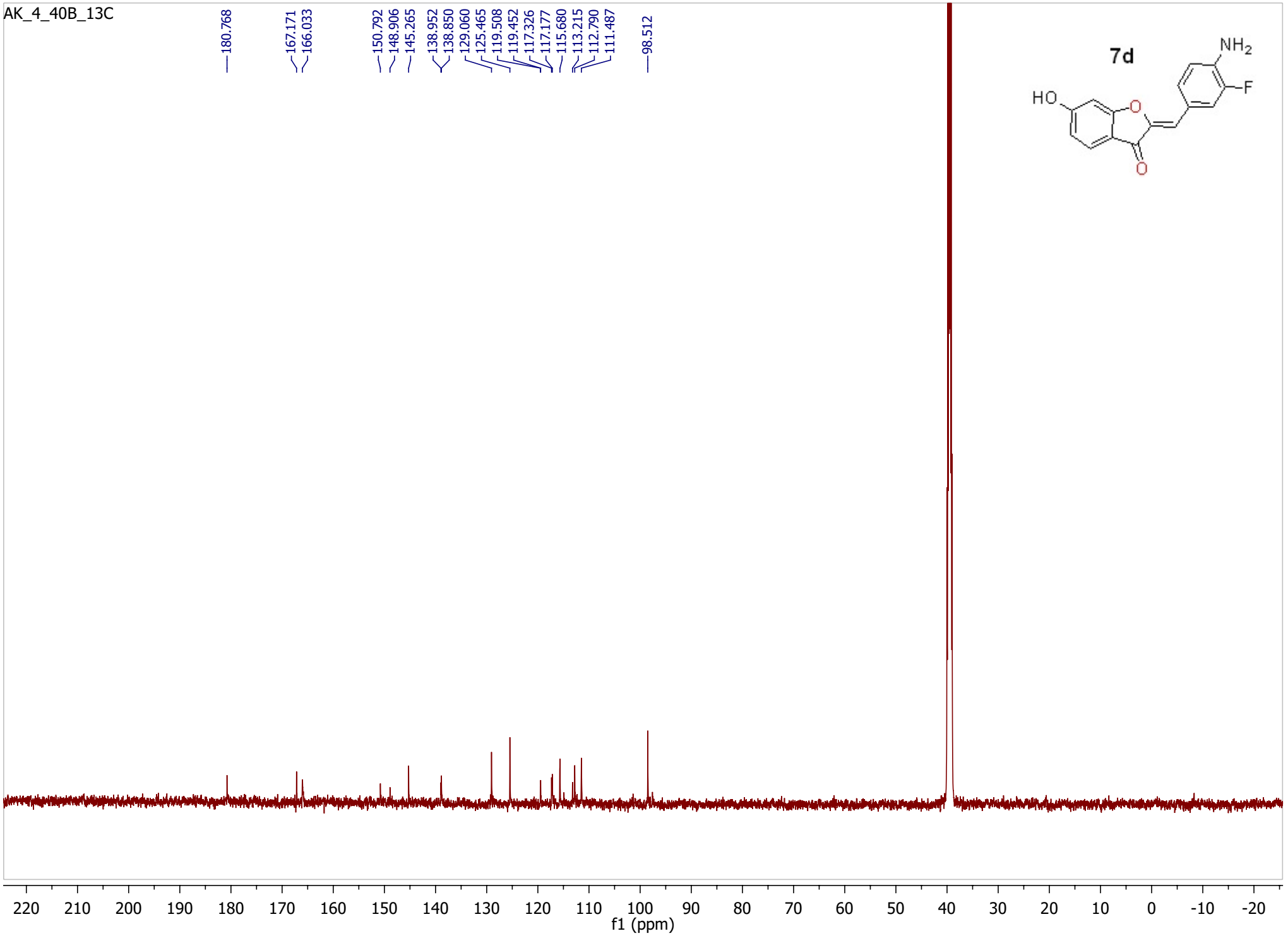


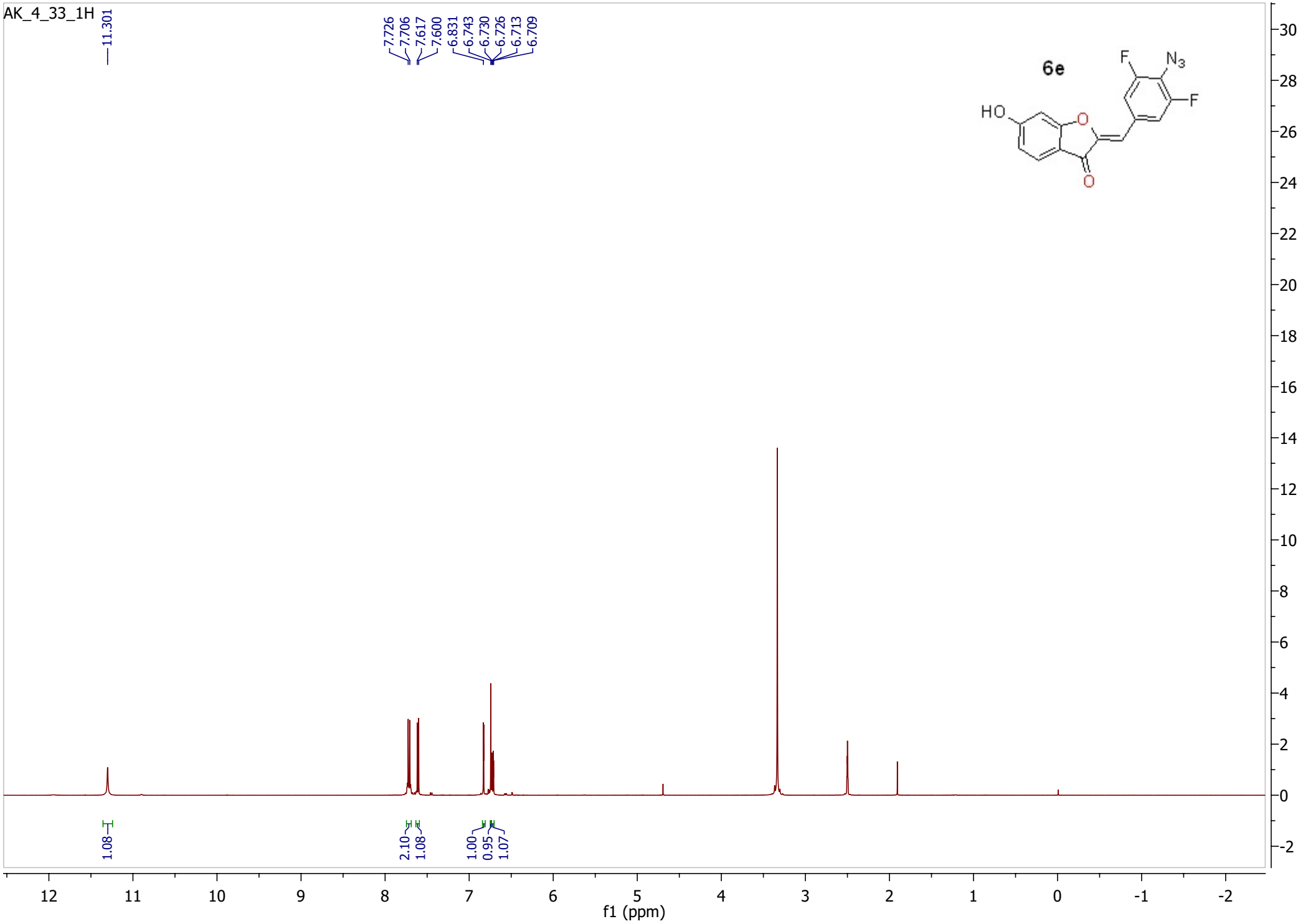
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00

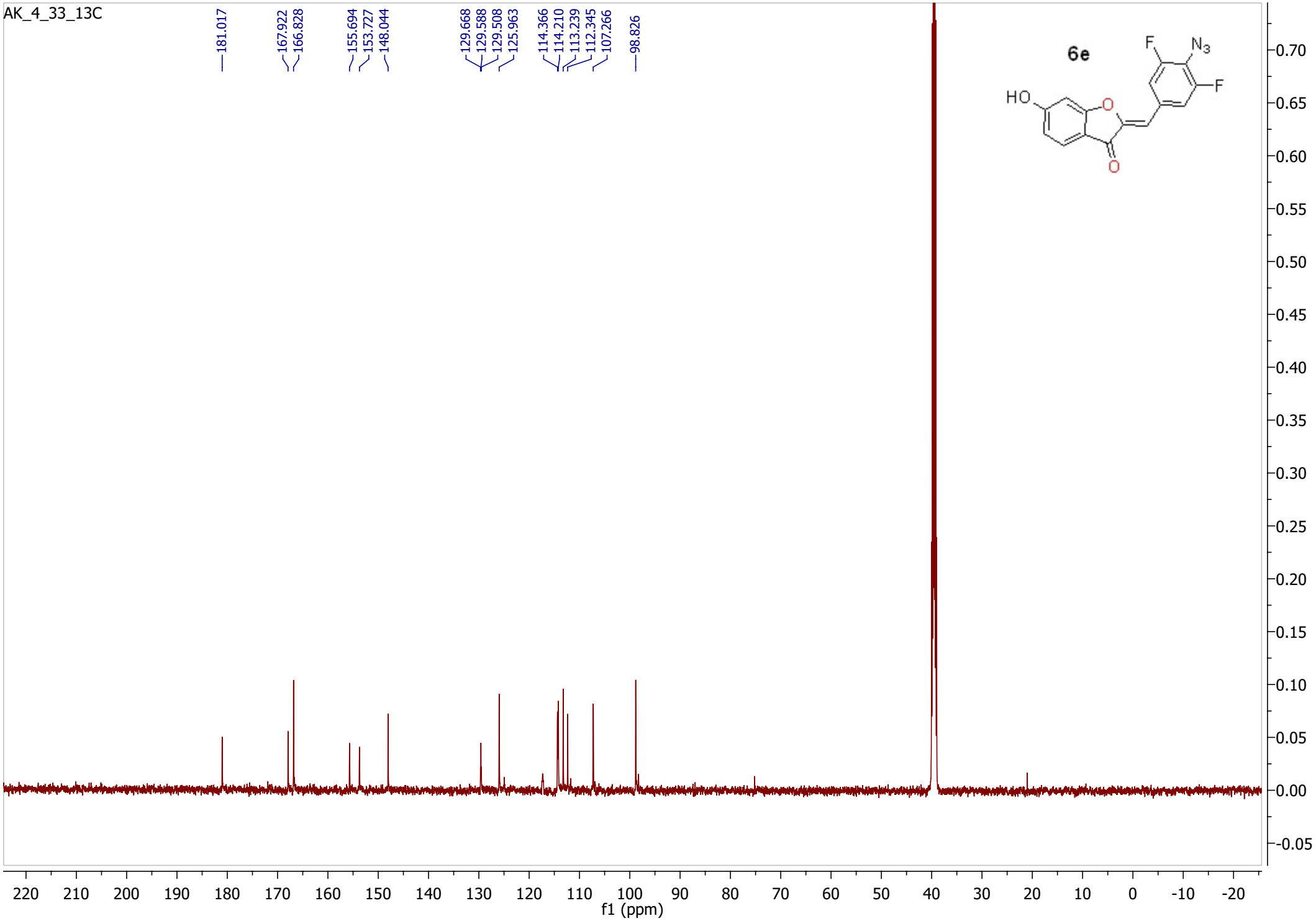
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20

f1 (ppm)


AK_4_40B
7.652
7.627
7.590
7.584
7.573
7.567
7.510
7.494
6.836
6.823
6.792
6.685
5.961
1.987
1.853
7d
NH2
F
HO
O
O
1.27
1.23
1.06
2.35
2.29
2.00
f1 (ppm)


AK_4_40B_13C
180.768
167.171
166.033
150.792
148.906
145.265
138.952
138.850
129.060
125.465
119.508
119.452
117.326
117.177
115.680
113.215
112.790
111.487
98.512
7d
NH2
F
HO
O
O
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20
f1 (ppm)


AK_4_33_1H
11.301
7.726
7.706
7.617
7.600
6.831
6.743
6.730
6.726
6.713
6.709
6e
F
N3
F
HO
O
O
1.08
2.10
1.08
1.00
0.95
1.07
f1 (ppm)

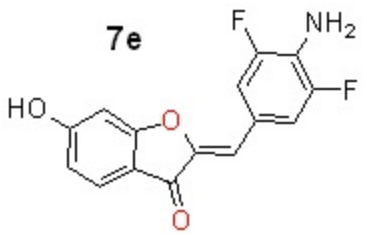

AK_4_33_13C
181.017
167.922
166.828
155.694
153.727
148.044
129.668
129.588
129.508
125.963
114.366
114.210
113.239
112.345
107.266
98.826
6e
F
N3
F
HO
O
O
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
f1 (ppm)

AK_5_39

11.375

7.593
7.564
7.555
7.536
7.528

6.811
6.805
6.710
6.698
6.685

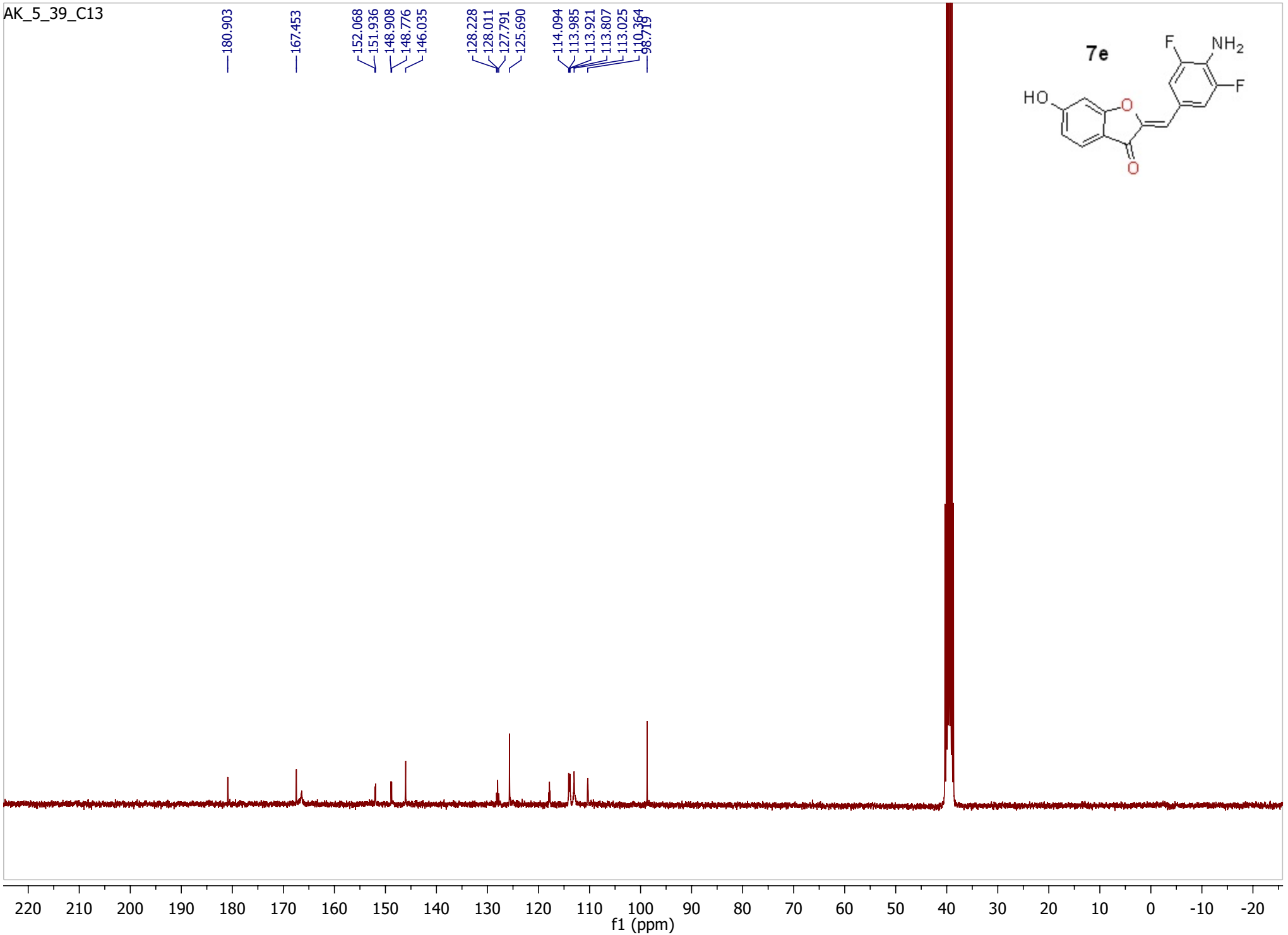


3.03
1.00
2.06
1.97

14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 -3 -4

f1 (ppm)

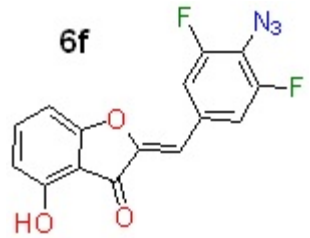
5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5


AK_5_39_C13
180.903
167.453
152.068
151.936
148.908
148.776
146.035
128.228
128.011
127.791
125.690
114.094
113.985
113.921
113.807
113.025
110.364
98.719
7e
F
NH2
F
HO
O
O
0.38
0.36
0.34
0.32
0.30
0.28
0.26
0.24
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
f1 (ppm)

AK-4-32-1H-dmso

11.271

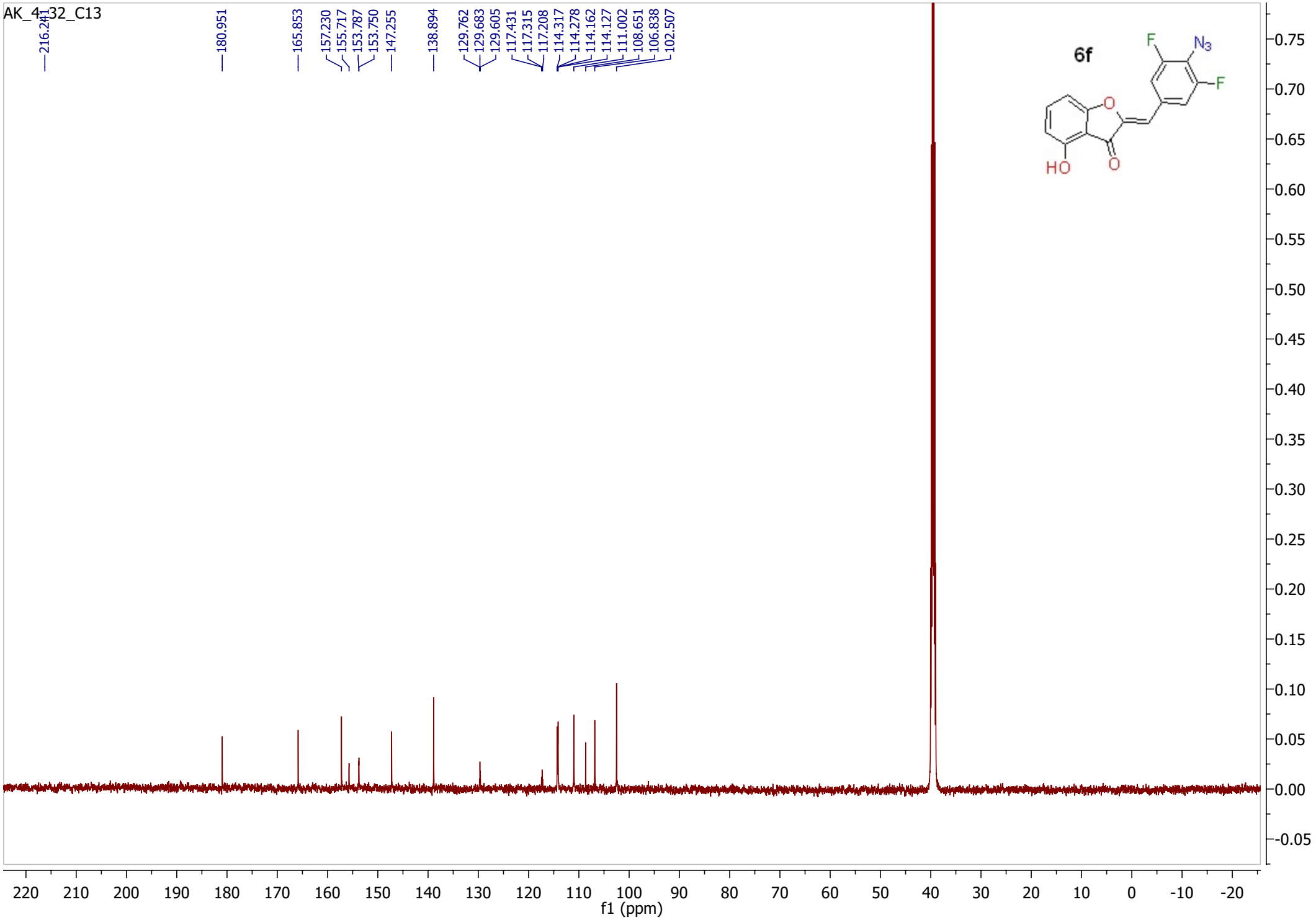
7.770
7.737
7.585
7.558
7.531
6.938
6.911
6.761
6.666
6.639

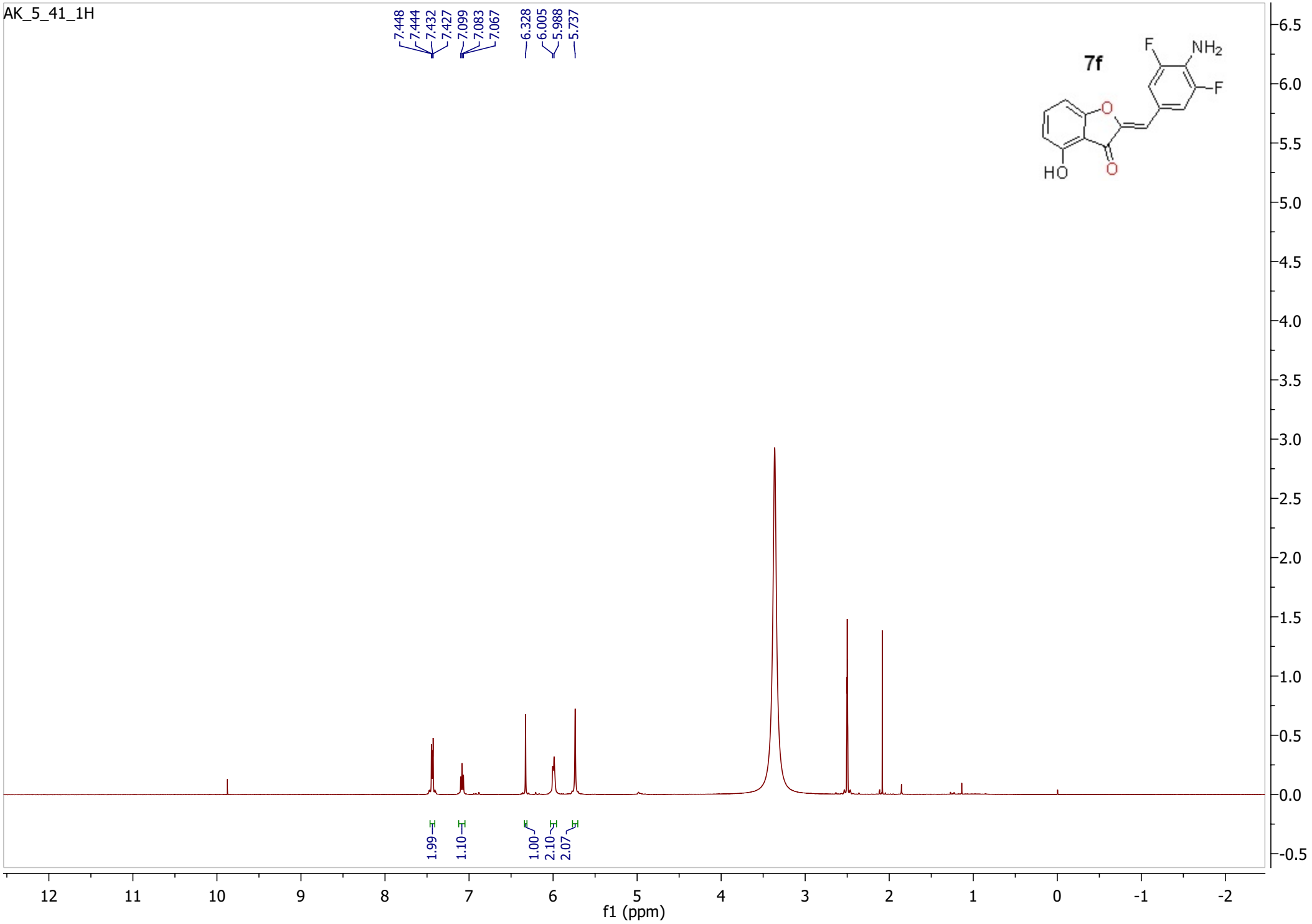


1.03
1.84
1.15
1.08
1.00
1.10

f1 (ppm)

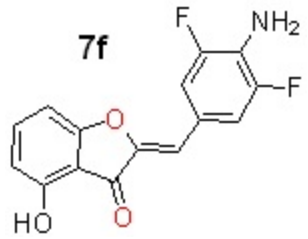
AK_4_32_C13


216.241
180.951
165.853
157.230
155.717
153.787
153.750
147.255
138.894
129.762
129.683
129.605
117.431
117.315
117.208
114.317
114.278
114.162
114.127
111.002
108.651
106.838
102.507
6f
F
N3
F
O
HO
O
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
f1 (ppm)


AK_5_41_1H
7.448
7.444
7.432
7.427
7.099
7.083
7.067
6.328
6.005
5.988
5.737
7f
F
NH2
F
O
O
HO
1.99
1.10
1.00
2.10
2.07
f1 (ppm)

AK_5_41_13C

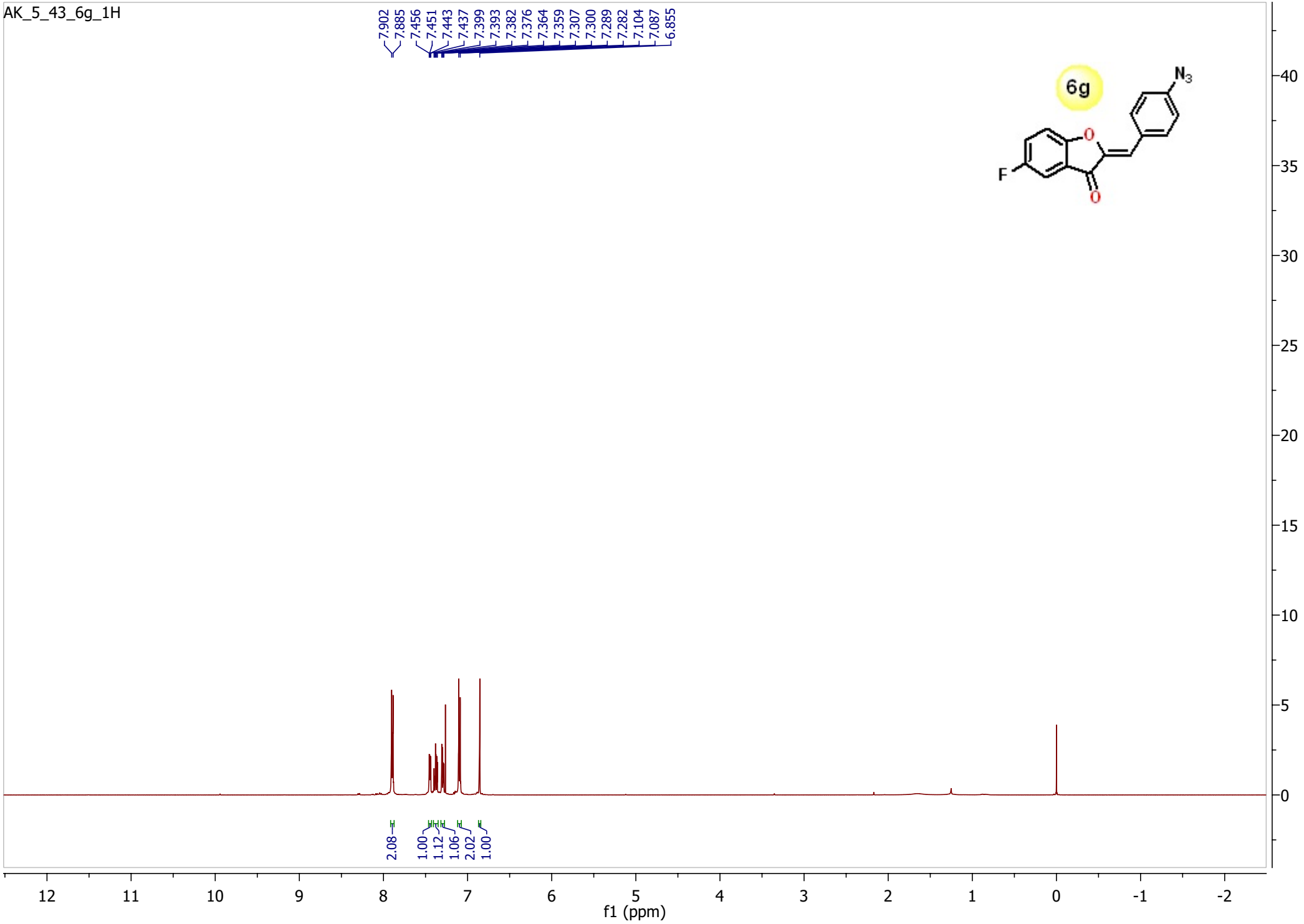
181.163
165.802
151.568
151.489
149.672
149.594
147.579
147.496
147.428
136.641
126.370
126.272
126.191
119.535
112.750
112.617
109.981
104.916
104.844
104.741



0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
-0.01

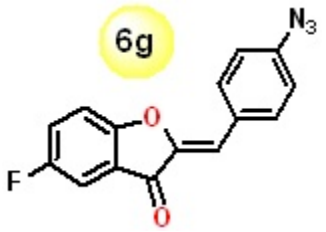
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -20

f1 (ppm)


AK_5_43_6g_1H
7.902
7.885
7.456
7.451
7.443
7.437
7.399
7.393
7.382
7.376
7.364
7.359
7.307
7.300
7.289
7.282
7.104
7.087
6.855
6g
N3
O
F
O
2.08
1.00
1.12
1.06
2.02
1.00
f1 (ppm)

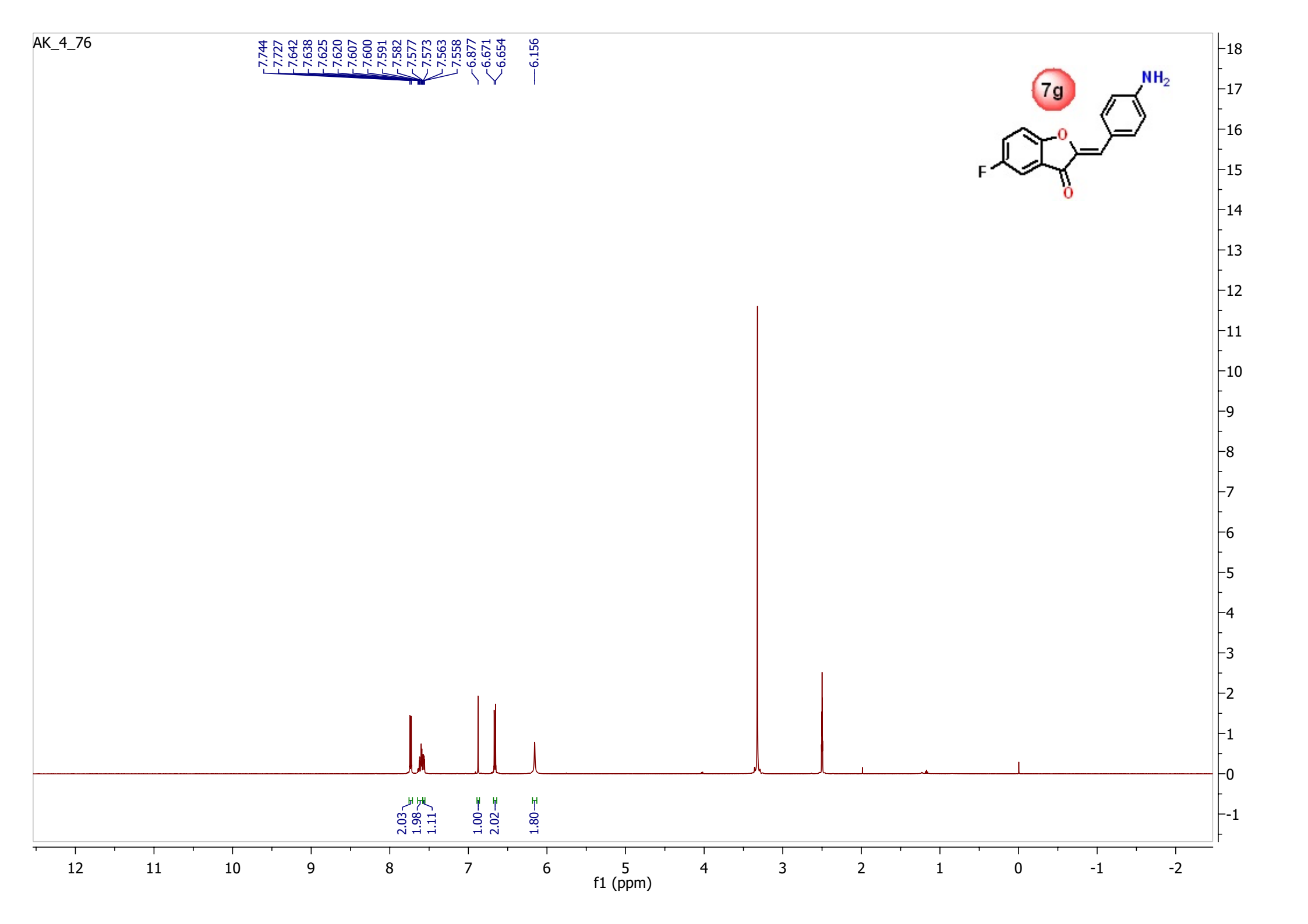
AK_5_43_6g_13C

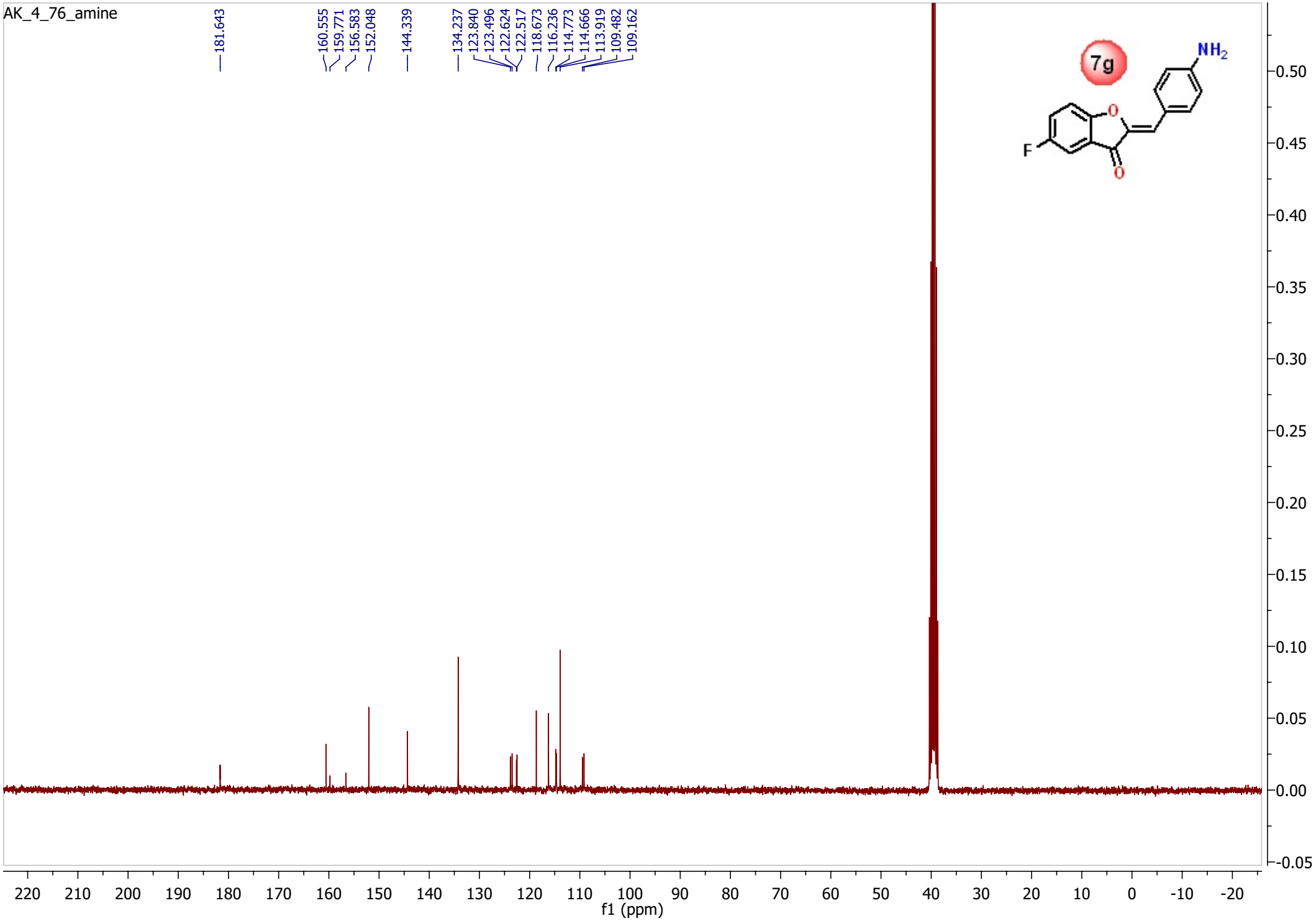
183.973
162.084
159.992
158.046
147.362
141.969
133.343
129.031
124.471
124.264
122.561
122.497
119.722
114.239
114.177
113.085
110.458
110.265



f1 (ppm)

AK_4_76


7.744
7.727
7.642
7.638
7.625
7.620
7.607
7.600
7.591
7.582
7.577
7.573
7.563
7.558
6.877
6.671
6.654
6.156
7g
NH2
F
O
O
2.03
1.98
1.11
1.00
2.02
1.80
f1 (ppm)


AK_4_76_amine
181.643
160.555
159.771
156.583
152.048
144.339
134.237
123.840
123.496
122.624
122.517
118.673
116.236
114.773
114.666
113.919
109.482
109.162
7g
NH2
F
O
O
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
-20
f1 (ppm)