Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Squaramides for Colorimetric and Fluorescent Anion Sensing

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Experimental

General methods and materials

All chemicals and solvents were of reagent grade (≥ 95%) and used as received unless otherwise noted. Reactions were magnetically stirred and monitored by thin-layer chromatography (TLC) carried out on Merck alumina silica gel plates (60F-254) using UV light as visualising agent, and potassium permanganate and heat as developing agents. Melting points were manually observed using a Stanford Research Systems Optimelt melting point apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded using at 300 K using either a 600 Bruker Avance (equipped with a high resolution cryogenic triple nucleus probehead), 500 Bruker Avance DPX or a Bruker Avance 300 spectrometer and are reported as parts per million (ppm), referenced to residual undeuterated solvent. The data are reported as chemical shift (δ), multiplicity (br = broad, s = singlet, d = doublet, t = triplet, m = multiplet), coupling constant (*J* Hz) and relative integral. Low resolution mass spectra were recorded on a Bruker amaZon SL mass spectrometer (ESI) operating in positive or negative mode as indicated. High resolution ESI spectra were recorded on a Bruker BioApex Fourier Transform Ion Cyclotron Resonance mass spectrometer (FTICR) with an Analytica ESI source, operating at 4.7 T or a Bruker Daltonics Apex Ultra FTICR with an Apollo Dual source, operating at 7 T and are reported as *m/z* (relative intensity). Infrared absorption spectra were recorded on a Bruker Alpha-E FT-IR spectrometer using attenuated total reflection (ATR) of either a solid or a thin film. Notable vibrational wavenumbers are given in cm^{-1}.

pK_a values were determined using the absorbance wavelength of maximum difference between the protonated and deprotonated species. UV-Vis pK_a titrations were performed by firstly acidifying a solution of receptor (20 – 25 μM) in DMSO (10% water) containing $TBAPF_6$ (0.1 M) with aqueous perchloric acid (1M) to pH <4. The pH probe was calibrated in a solution of in standard calibrant solution (solutions of pH 4, 7 and 10). The solution was slowly basified with additions of small aliquots aqueous NaOH (0.1 M), and UV-Vis spectra recorded after each addition to obtain the change in absorbance profile with increasing pH. The absorbance values of the wavelength of maximal difference were plotted against the pH values, and the data points fitted to a Boltzmann S curve to obtain the pK_a value at the inflection point of the curve. The pK_a values obtained represent the average of two independent pH titrations.

UV-Vis anion binding titrations were performed by additions of aliquots of the putative anionic guest as the TBA salt (10 – 30 mM) made up in a solution of the receptor (20 – 25 μM) in DMSO (1% water). UV-Vis spectra were recorded on a Cary 400 UV-Vis spectrophotometer at 25 °C in a 1 cm quartz cuvette after background subtraction of the cuvette and solvent. The solution was stirred after each addition. Binding affinities were obtained from a global fit of the absorbance data between 330 – 430 nm, fitting to a 1:1 binding model in Bindfit. The obtained K_a values represent the average of two independent titrations, and experimental error is estimated to be less than 15% for each *K*a value obtained.

Fluorescence anion binding titrations were performed by additions of aliquots of the putative anionic guest as the TBA salt (10 – 30 mM) made up in a solution of the receptor (20 – 25 μM) in DMSO (1% water). Fluorescence spectra were recorded on a Horiba Duetta fluorescence and absorbance spectrophotometer with temperature controlled enabled (25 °C) in a 1 cm quartz cuvette. The solution was stirred after each addition. Binding affinities were obtained from a global fit of the absorbance data between 330 – 430 nm, fitting to a 1:1 binding model in Bindfit. The obtained K_a values represent the average of two independent titrations, and experimental error is estimated to be less than 15% for each K_a value obtained.

3-[(9H-Fluoren-2-yl)amino]-4-(butylamino)cyclobut-3-ene-1,2-dione (1)

Compound **9** (20 mg, 0.07 mmol), butylamine (7 mg, 0.1 mmol) zinc trifluoromethanesulfonate (4 mg, 0.01 mmol) were stirred in methanol (10 mL) and the solution heated to reflux for 24 hours. The solution was allowed to cool to room temperature and the yellow precipitate was collected by vacuum filtration, then washed with cold methanol to afford **1** as a yellow solid (18 mg, 83%). Mp: 319 °C (decomposition); ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.64 (s, 1H), 7.84–7.81 (m, 2H), 7.69 (s 1H), 7.59 (s, 1H), 7.59–7.55 (d, J = 10.1 Hz, 1H), 7.42–7.40 (dd, J = 10.1 Hz, 1H), 7.38–7.35 (t, J = 5.2 Hz, 1H), 7.28–7.25 (td, J = 5.2 Hz, 1H), 3.92 (s, 2H), 3.66–3.62 (q, J = 6.8 Hz, 2H), 1.62–1.56 (m, 2H), 1.43–1.36 (m, 2H), 0.96–0.93 (t, 8.0 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 184.5, 180.9, 169.8, 163.9, 145.0, 143.1, 141.3, 138.6, 136.4, 127.2, 126.5, 125.4, 121.1, 120.0, 117.6, 115.5, 43.9, 37.0, 33.1, 19.5, 13.9; IR (solid) ν_{max}: 3178, 3116, 3040, 2956, 2932, 2871, 1792, 1656, 1601, 1560, 1431, 1353, 1130 1095, 951, 871, 781, 764, 729, 729 cm^{-1}; HRMS (ESI) *m/z*: calc'd for $C_{21}H_{21}N_2O_2$ $[M+H]^+$ 333.1598, found 333.1597.

3,4-Bis[(9H-fluoren-2-yl)amino]cyclobut-3-ene-1,2-dione (2)

A mixture of 3,4-diethoxy-3-cyclobutene-1,2-dione (45 mg, 0.26 mmol) and zinc trifluoromethanesulfonate (19 mg, 0.05 mmol) was stirred in methanol (20 mL). 2-aminofluorene (100 mg, 0.55 mmol) was added and the mixture was heated to reflux. After 48 hours, the precipitate was collected *via* hot filtration and the solid was washed with hot methanol to give 3,4-bis((9H-fluoren-2-yl)amino)cyclobut-3-ene-1,2-dione as a yellow solid (42 mg, 36%). Mp: 325 °C (decomposition); ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.03 (s, 2H), 7.29–7.27 (d, J = 8.0 Hz, 2H), 7.84–7.83 (d, 8.0 Hz, 2H), 7.77 (s, 2H), 7.58–7.56 (d, J = 10 Hz, 2H), 7.51–7.49 (d, J = 10 Hz, 2H), 7.39–7.36 (t, J = 7 Hz), 7.30–7.27 (t, J = 7 Hz, 2H), 3.95 (s, 4H); ^{13}C NMR (125 MHz, DMSO-d_6): 182.3, 166.1, 145.0, 143.2, 141.2, 138.1, 137.1, 127.3, 126.7, 125.5, 121.1, 120.0, 118.1, 116.0, 37.1; IR (solid) ν_{max}: 3150, 3039, 2932, 1788, 1668, 1600, 1550, 1448, 1401, 1309, 820, 764, 729, 561 cm^{-1}; HRMS (ESI) *m/*z: calc'd for $C_{30}H_{21}N_2O_2$ $[M+H]^+$ 441.1598; found 441.1597. The spectral data was in good agreement with those previously reported.[1]

3-(Butylamino)-4-(pyren-1-ylamino)cyclobut-3-ene-1,2-dione (3)

A mixture of **10** (30 mg, 0.09 mmol), butylamine (10 mg, 0.13 mmol) and zinc trifluoromethanesulfonate (3 mg, 0.01 mmol) were stirred in methanol (10 mL) and the solution was heated to reflux for 24 hours. A red precipitate was formed which was collected by vacuum filtration, and washed with cold methanol to yield **3** as a red solid (14 mg, 43%). Mp: 315 °C (decomposition); ^{1}H NMR (400 MHz, DMSO-d_6): 11.26 (s, 1H), 9.62 (s, 1H), 8.45–8.43 (d, J = 8 Hz, 1H), 8.29–8.27 (m, 3H), 8.22–8.20 (m, 1H), 8.17–8.11 (m, 2H), 8.10–8.06 (m, 2H), 3.69–3.65 (t, J = 8 Hz, 2H), 1.63–1.56 (m, 2H), 1.39–1.31 (m, 2H), 0.94–0.90 (t, J = 8 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 182.3, 173.9, 131.6, 131.4, 130.9, 128.5, 127.8, 127.7, 127.1, 127.0, 125.8, 125.6, 125.4, 124.7, 124.3, 122.6, 122.3, 122.2, 44.0, 32.5, 19.5, 13.9; IR (solid) ν_{max}: 3152, 3042, 2925, 1796, 1639, 1559, 1534, 1489, 1343, 1310, 1259, 1242, 1183, 1093, 962, 939, 834, 817, 680, 636; HRMS (ESI) *m/*z: calc'd for $C_{24}H_{21}N_2O_2$ [M+H] 369.1598; found 369.1596.

3,4-Bis(pyren-1-ylamino)cyclobut-3-ene-1,2-dione (4)

A mixture of **10** (20 mg, 0.12 mmol) and zinc trifluoromethanesulfonate (8 mg, 0.02 mmol) was stirred in methanol (25 mL). 1-amimopyrene (50 mg, 0.25 mmol) was added and the mixture was heated to reflux. After 48 hours, the precipitate was collected *via* hot filtration and

the solid was washed with hot methanol to give **4** as an orange solid (11 mg, 20%). Mp: 351 °C (decomposition); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.0 (s, br, 2H), 8.49 – 8.08 (m, 18H); IR (solid) ν_{max}: 3153, 3040, 2931, 1797, 1592, 1557, 1531, 1505, 1403, 1323, 1259, 1188, 941, 706, 677 cm^{-1}; HRMS (ESI) *m/*z: calc'd for $C_{36}H_{21}N_2O_2$ $[M+H]^+$ 513.1598; found 513.1598. The spectral data are in agreement with those previously reported.[2]

3-(Butylamino)-4-[(naphthalen-2-ylmethyl)amino]cyclobut-3-ene-1,2-dione (5)

A mixture of 3,4-diethoxy-3-cyclobutene-1,2-dione (50 mg, 0.29 mmol) and naphthalen-2-ylmethanamine (46 mg, 0.0.29 mmol) was stirred in ethanol (10 mL) at 25 °C. After 1 hour, *n*-butylamine (21 mg, 0.29 mmol) was added and the mixture stirred for a further 2 hours at 25 °C. A colourless precipitate was collected by vacuum filtration and washed with ethanol to give **5** as a colourless solid (63 mg, 70%). Mp: 199–201 °C; ^{1}H NMR (500 MHz, DMSO-d_6): 8.14-8.12 (d, J = 9 Hz, 1H), 8.00-7,.99 (m, 1H), 7.92 (m, 1H), 7.73 (s, 1H), 7.67 (m, 4H), 7.32 (s, 1H), 5.23 (d, J = 10 Hz, 2H), 3.50 (m, 2H), 1.48 (m, 2H), 1.33-1.25 (m, 2H), 0.87 (t, J = 7 Hz, 3 H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 183.0, 182.8, 168.4, 167.7, 134.7, 133.9, 131.1, 129.2, 128.7, 128.7, 127.0, 126.5, 126.1, 123.7, 45.1, 43.4, 33.2, 19.5, 13.9; IR (solid) ν_{max}: 3155, 3051, 2951, 2927, 2869, 1800, 1633, 1557, 1451, 1430, 1213, 1185, 791, 704 cm^{-1}; HRMS (ESI) *m/*z: calc'd for $C_{19}H_{20}N_2O_2Na$ [M+Na] 331.1417, found 331.1417.

3,4-Bis[(naphthalen-2-ylmethyl)amino]cyclobut-3-ene-1,2-dione (6)

A mixture of 3,4-diethoxy-3-cyclobutene-1,2-dione (50 mg, 0.29 mmol) and naphthalen-2-ylmethanamine (115 mg, 0.73 mmol) was stirred in ethanol (10 mL) at room temperature for 12 hours. The precipitate formed was collected by vacuum filtration and washed with cold ethanol to yield **6** as a colourless solid (96 mg, 83%). Mp: 232–234 °C; ^{1}H NMR (500 MHz, DMSO-d_6): 8.10 (d, J = 8 Hz, 2H), 7.98 (d, J = 8 Hz, 2H), 7.90 (m, 2H), 7.6 (s, 2H), 7.64 (m, 8H), 5.22 (d, J = 3 Hz, 4H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 183.1, 168.0, 134.6, 133.9, 131.1, 129.2, 128.7, 128.7, 127.1, 126.5, 126.1, 123.7, 45.2; IR (solid) ν_{max}: 3149, 3048, 2930, 1795, 1645, 1598, 1561, 1456, 1258, 943, 775 cm^{-1}; HRMS (ESI) *m/z:* calc'd for $C_{26}H_{20}N_2O_2Na$ $[M+Na]^+$ 415.1417, found 415.1413.

3-(Butylamino)-4-[(pyren-1-ylmethyl)amino]cyclobut-3-ene-1,2-dione (7)

A mixture of 3,4-diethoxy-3-cyclobutene-1,2-dione (50 mg, 0.29 mmol) and pyren-1-ylmethanamine (67 mg, 0.29 mmol) was stirred in ethanol (10 mL) at 25 °C for 2 hours. Butylamine (21 mg, 0.29 mmol) was subsequently added and the mixture stirred for another 2

hours. A white precipitate formed which was collected by vacuum filtration and watched with cold ethanol to yield white precipitate which was collected by vacuum filtration and washed with water to give **7** as a colourless solid (45 mg, 40%). Mp: 248 °C (decomposition); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.47-8.45 (d, *J* = 10 Hz, 1H), 8.36-8.31 (m, 4H), 8.20-8.20 (d, 1 Hz, 2H), 8.13-8.10 (m, 2H), 7.90 (s, 1H), 7.34 (s, 1H), 5.52 (m, 2H), 3.51-3.50 (m, 2H), 1.49 (m, 2H), 1.28 (m, 2H), 0.86-0.83 (t, 8 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 182.9, 183.0, 168.5, 167.7, 132.7, 131.3, 131.0, 128.6, 127.9, 127.8, 127.3, 126.9, 126.0, 125.9, 125.5, 124.6, 124.4, 123.3, 79.6, 45.3, 43.4, 33.2, 19.4, 13.9; IR (solid) ν_{max}: 3163, 3042, 2955, 2930, 2872, 1797, 1638, 1543, 1343, 1311, 1257, 1244, 1146, 1092, 756, 682 cm^{-1}; HRMS (ESI) *m/z*: calc'd for $C_{25}H_{22}N_2O_2Na$ $[M+Na]^+$ 405.1574, found 405.1571.

3,4-Bis-[(pyren-1-ylmethyl)amino]cyclobut-3-ene-1,2-dione (8)

A mixture of 3,4-diethoxy-3-cyclobutene-1,2-dione (30 mg, 0.18 mmol), pyren-1-ylmethanamine hydrochloride (100 mg, 0.44 mmol) and Et_3N (70 mg, 0.70 mmol) was stirred in ethanol (20 mL) at 25 °C. After 4 hours, water (50 mL) was added to the reaction mixture to produce a white precipitate which was collected by vacuum filtration and washed with water to give **8** as a colourless solid (55 mg, 58%). Mp: >250 °C ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.42-8.40 (d, *J* = 10 Hz, 2H), 8.32-8.26 (m, 8H), 8.19-8.14 (m, 4H), 8.10-8.07 (m, 4H), 7.90 (s, 2H), 5.51-5.49 (d, *J* = 10 Hz, 4H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 183.2, 167.9, 132,.4, 131.2, 131.0, 130.7, 128.5, 127.9, 127.8, 127.3, 126.9, 126.0, 125.8, 125.5, 124.6, 124.3, 123.2, 45.4 IR (solid) ν_{max}: 3160, 3046, 2931, 1797, 1640, 1561, 1459, 1310, 826, 681 cm^{-1}; HRMS (ESI) *m/z*: calc'd for $C_{38}H_{24}N_2O_2Na$ $[M+Na]^+$ 563.1730, found 563.1723.

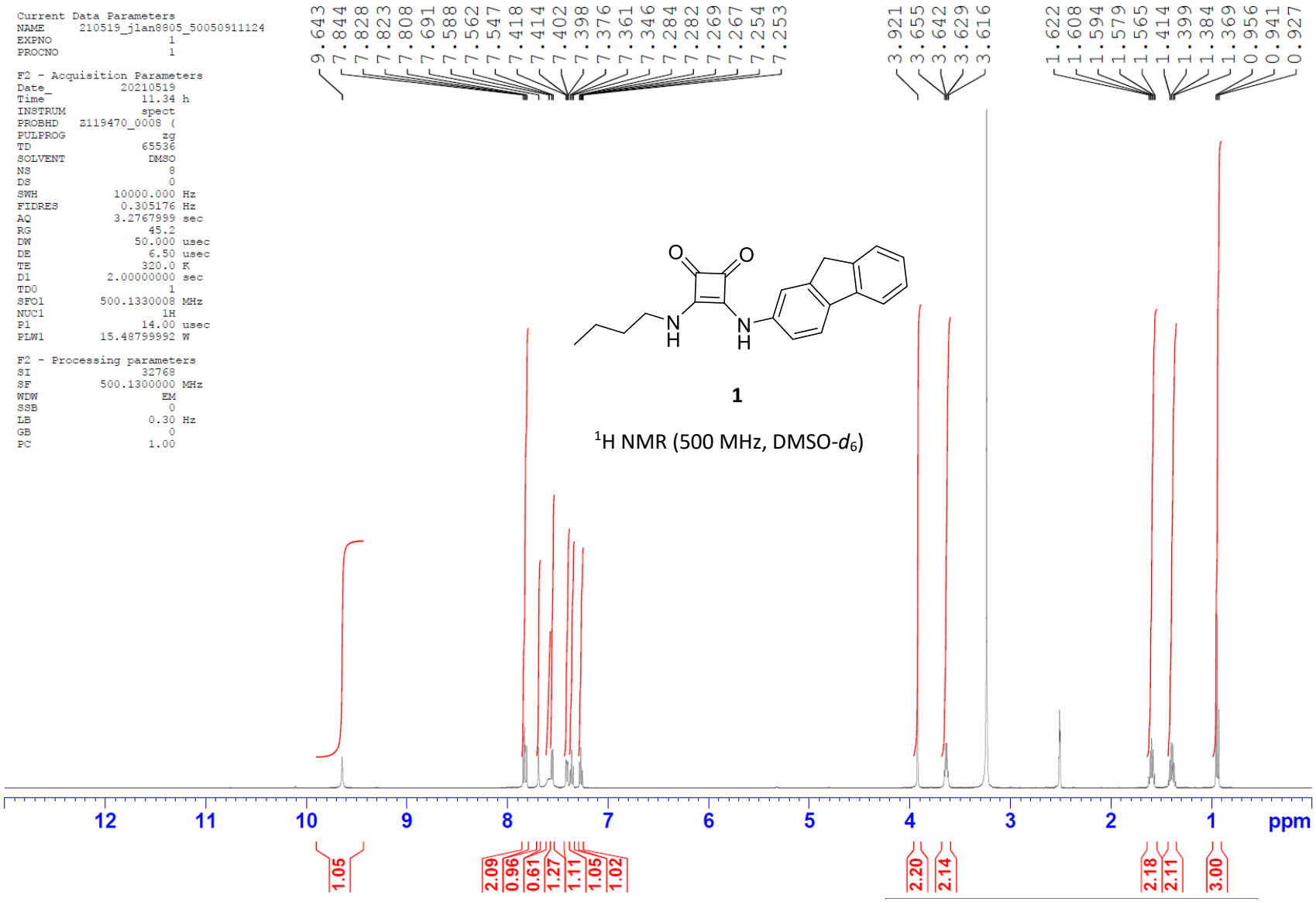
3-[(9H-Fluoren-2-yl)amino]-4-ethoxycyclobut-3-ene-1,2-dione (9)

A mixture of 3,4-diethoxy-3-cyclobutene-1,2-dione (50 mg, 0.30 mmol) and zinc trifluoromethanesulfonate (12 mg, 0.03 mmol) was stirred in methanol (10 mL). 2-Aminofluorene (52 mg, 0.28 mmol) was added and the mixture was stirred at room temperature. After 48 hours, the precipitate was collected by filtration and the solid was washed with cold methanol to give **9** as a yellow solid (55 mg, 65%). Mp: 212–215 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.80 (s, 1H), 7.86-7.83 (t, *J* = 5 Hz, 2H), 7.60 (s, 1H), 7.58–7.56 (d, *J* = 10 Hz, 1H) 7.41–7.36 (m, 2H), 7.31–7.27 (td, *J* = 5, 2, 1H), 4.82–4.78 (q, *J* = 5 Hz, 2 H), 3.92 (s, 2H), 1.46– 1.43 (t, *J* = 5 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 188.5, 184.1, 178.7, 170.0, 144.5, 143.3, 141.1, 137.8, 137.4, 127.3, 126.9, 125.5, 120.8, 120.1, 119.2, 117.1, 70.0, 37.0,

16.1; IR (solid) ν_{max}: 3238, 3196, 3112, 3022, 2979, 2934, 1792, 1704, 1603, 1574, 1514, 1469, 1436, 1377, 1347, 1311, 1212, 1180, 1071. 810, 765, 613 cm^{-1}; HRMS (ESI) *m/z*: calc'd for $C_{19}H_{16}O_3N$ $[M+H]^+$ 306.1122; found 306.1125.

3-Ethoxy-4-(pyren-1-ylamino)cyclobut-3-ene-1,2-dione (10)

A mixture of 3,4-diethoxy-3-cyclobutene-1,2-dione (43 mg, 0.25 mmol) and zinc trifluoromethanesulfonate (8 mg, 0.02 mmol) was stirred in methanol (10 mL). 1-amimopyrene (50 mg, 0.23 mmol) was added and the mixture was stirred at room temperature. After 72 hours, the precipitate was collected by filtration and the solid was washed with cold methanol to give **10** as an orange solid (41 mg, 51%). Mp: 163 °C (decomposition); ^{1}H NMR (600 MHz, DMSO-d_6): 11.32 (s, 1H), 8.33–8.32 (d, J = 5.2 Hz, 1H), 8.30–8.28 (m, 2H), 8.24–8.33 (d, J = 5.2 Hz, 1H), 8.16–8.8.16 (d, J = 2.0 Hz, 2H), 8.09–8.07 (t, J = 5.1 Hz, 1H), 7.91–7.90 (d, J = 5.1 Hz, 1H), 4.69–4.66 (q, J = 3.9 Hz, 2H), 1.32–1.30 (t, J = 3.9 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 192.1, 179.9, 131.8, 131.3, 131.1, 130.9, 130.1, 129.1, 128.0, 127.6, 127.5, 127.1, 125.6, 125.3, 124.7, 124.2, 124.1, 123.1, 122.8, 122.6, 69.7, 16.1; IR (solid) ν_{max} 3182, 3021, 2933, 1805, 1703, 1693, 1599, 1579, 1528, 1459, 1406, 1387, 1155, 1010, 970, 870, 819, 762, 709, 684 cm^{-1}; HRMS (ESI) *m/z*: calc'd for $C_{22}H_{15}NO_3Na$ $[M+H]^+$ 364.0944, found 364.0942.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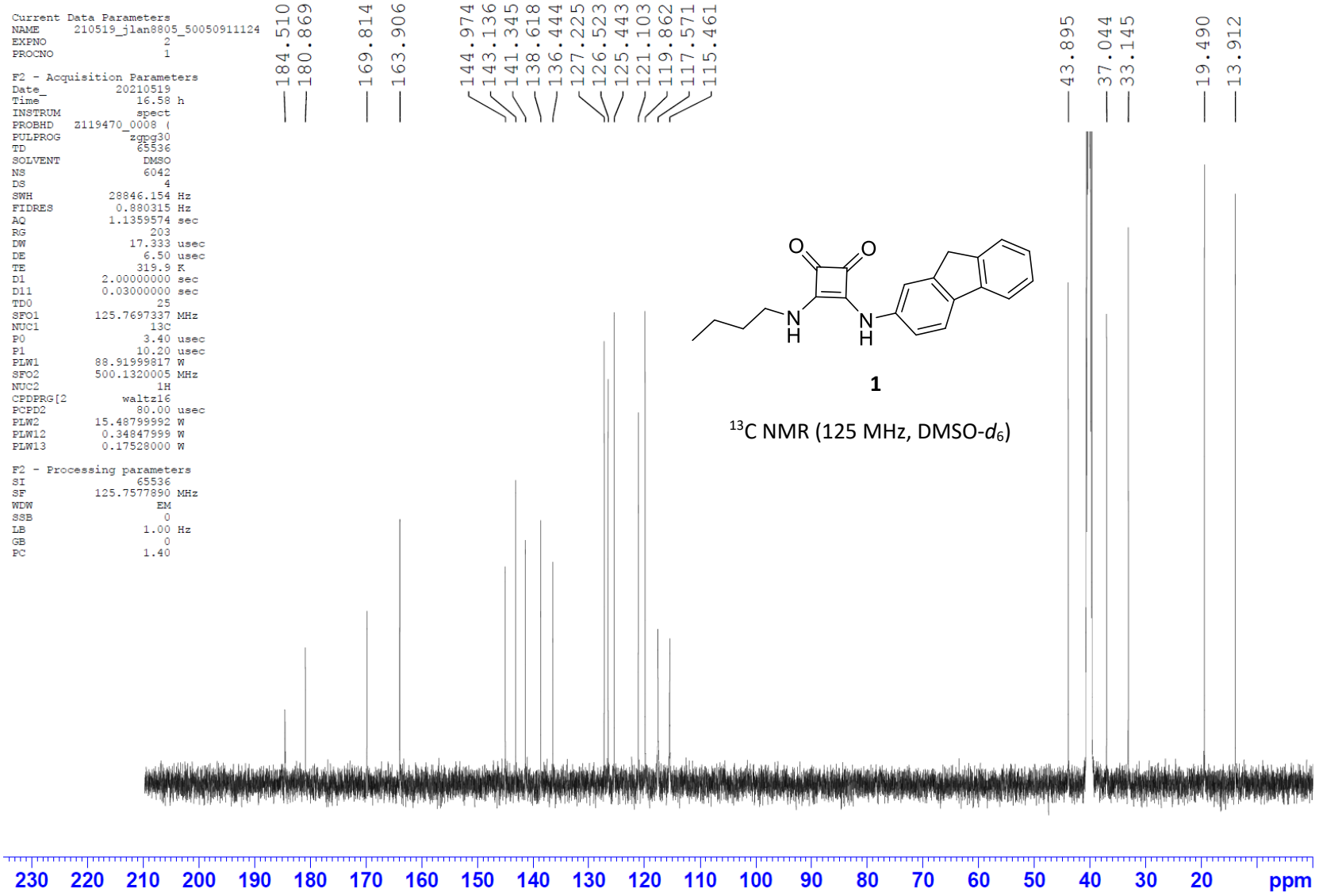

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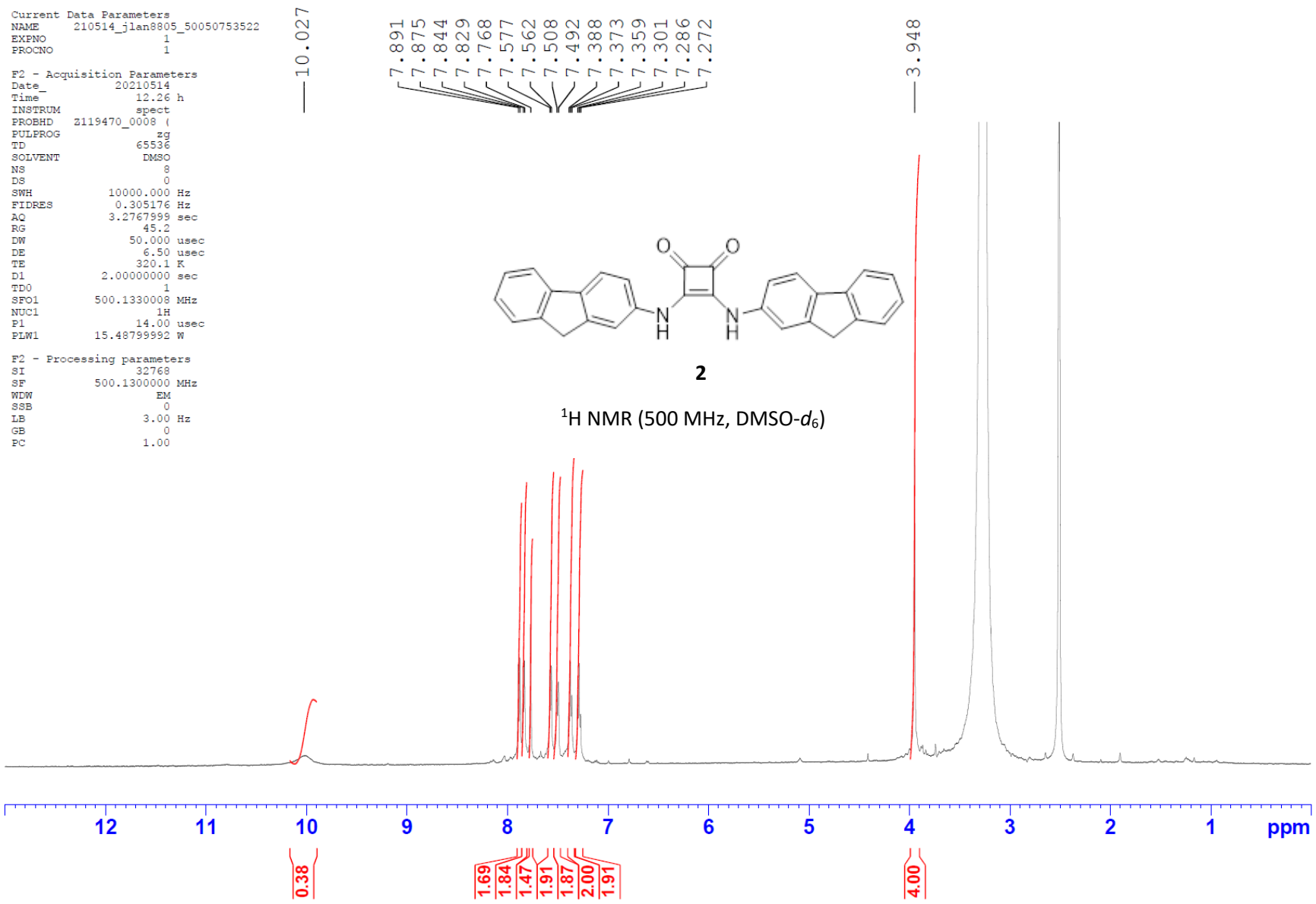

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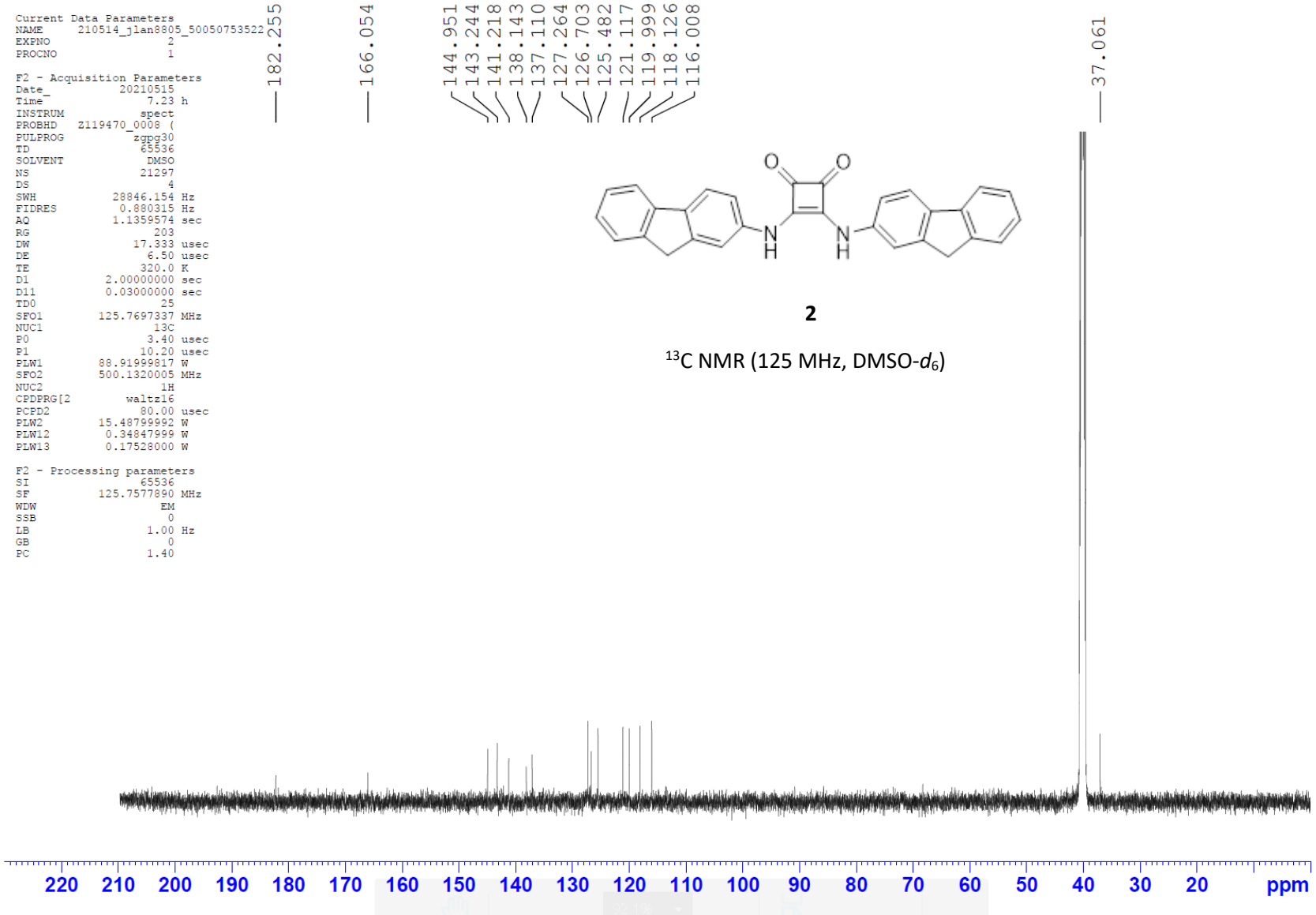

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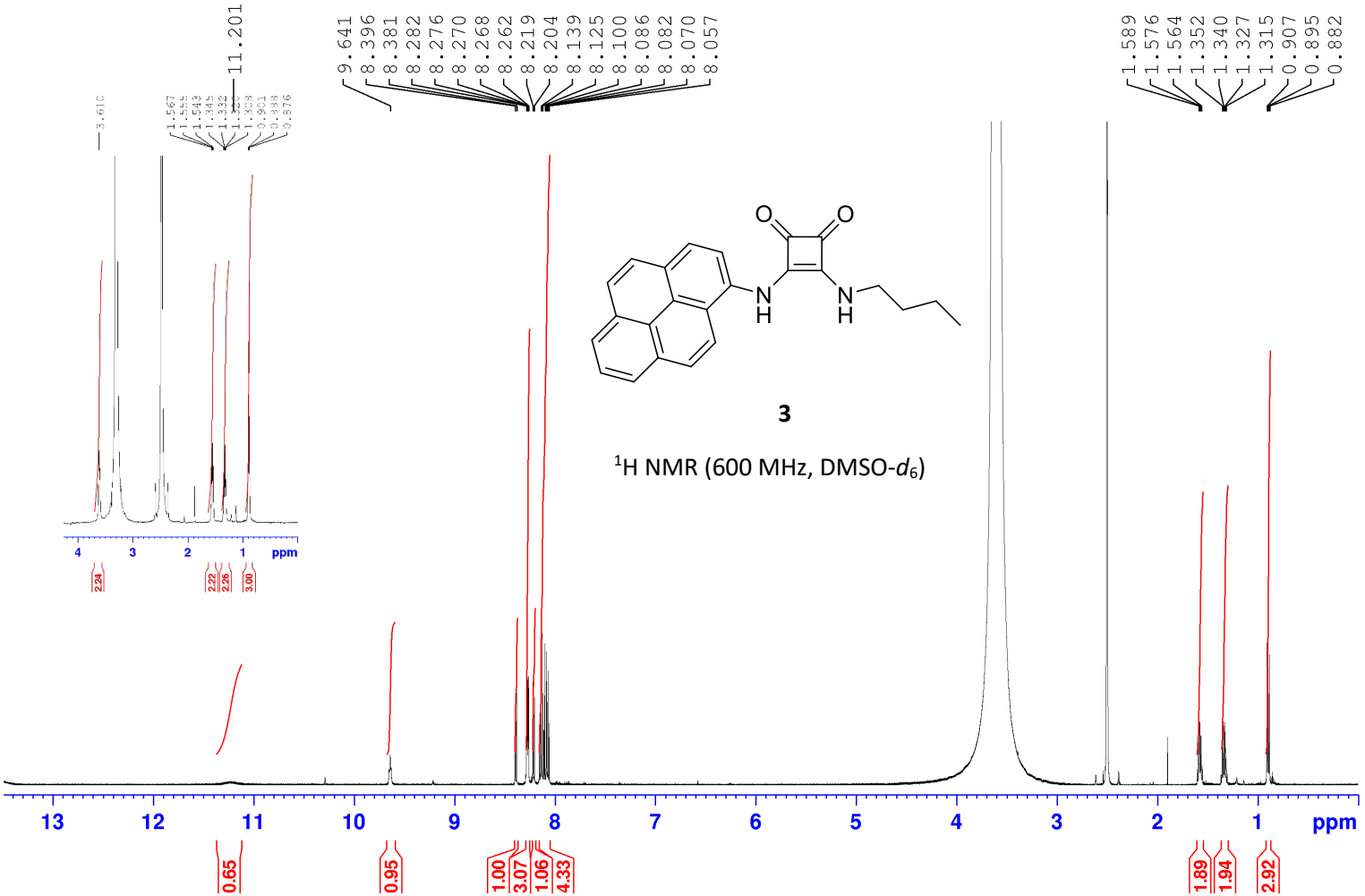
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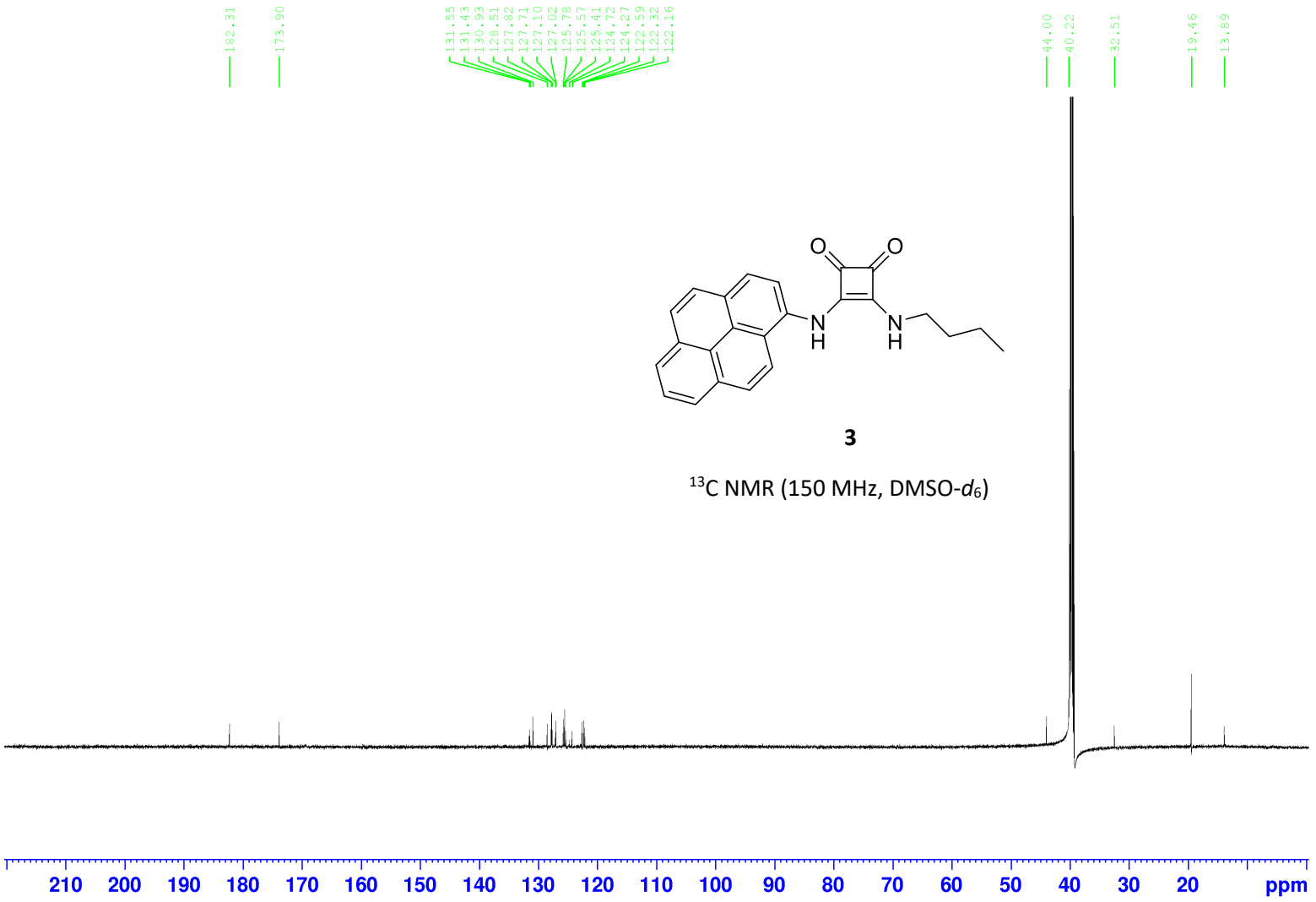

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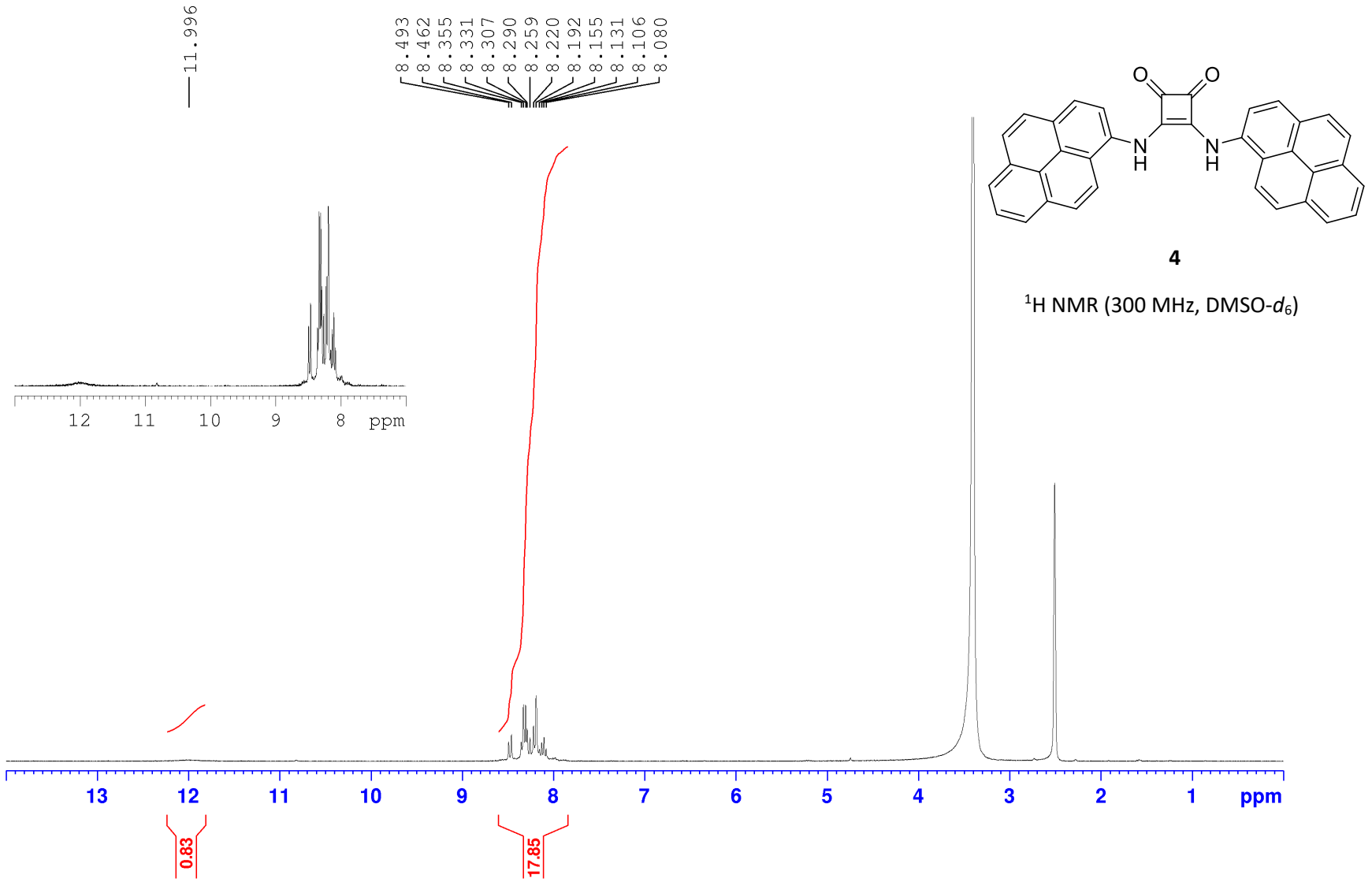

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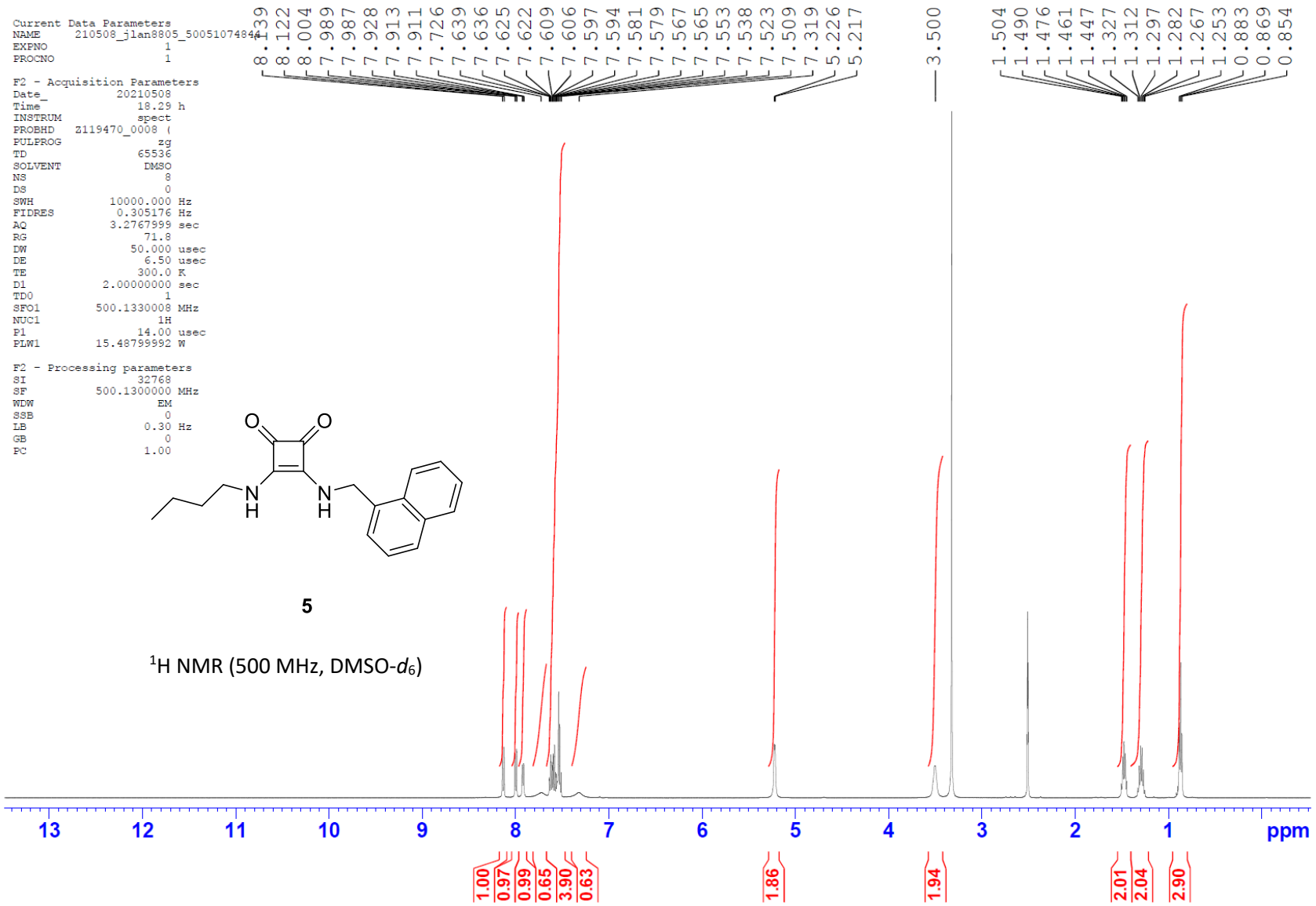

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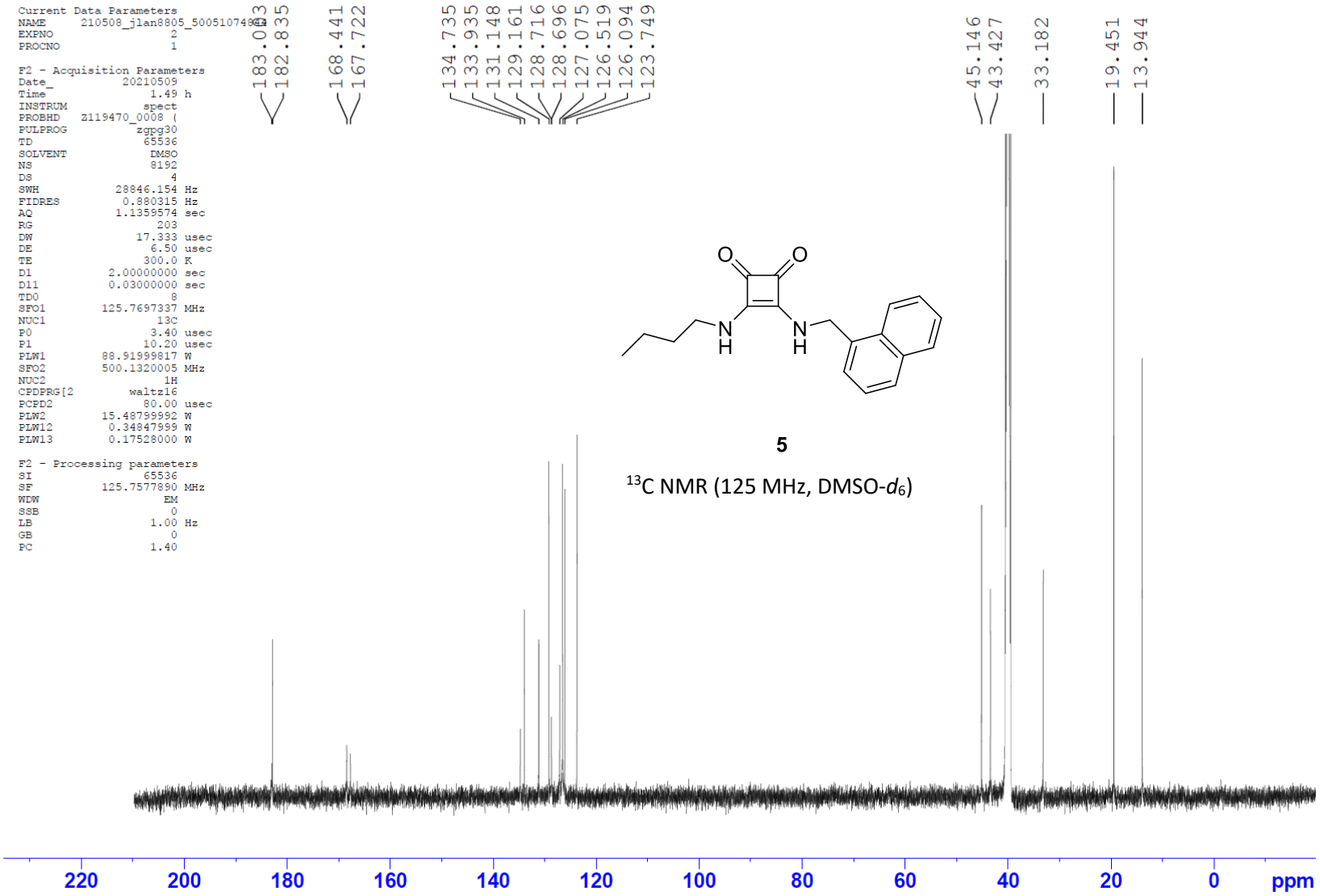
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128.696
127.075
126.519
126.094
123.749
45.146
43.427
33.182
19.451
13.944
O
O
N
H
N
H
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160
140
120
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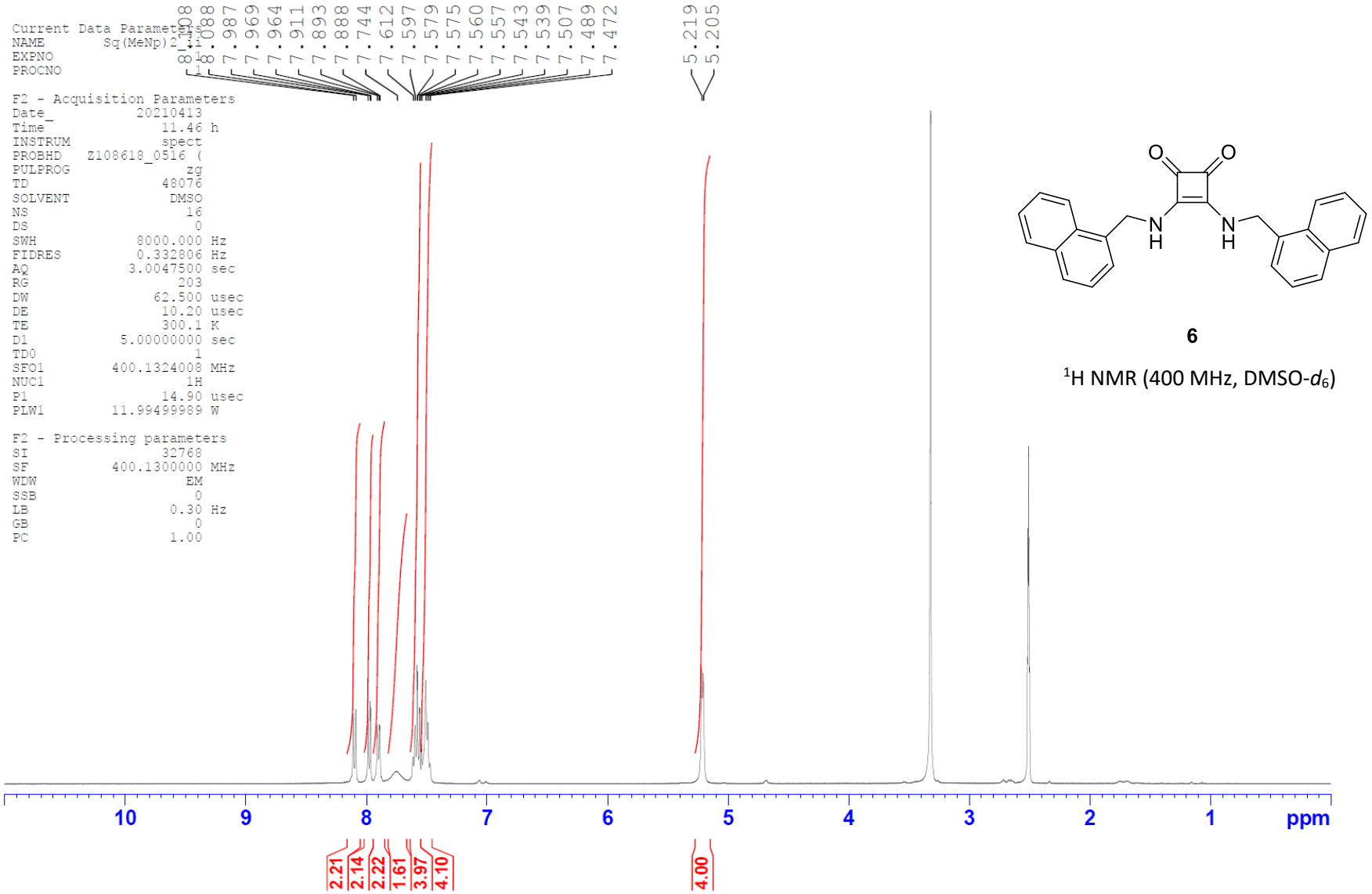
80
60
40
20
0
ppm



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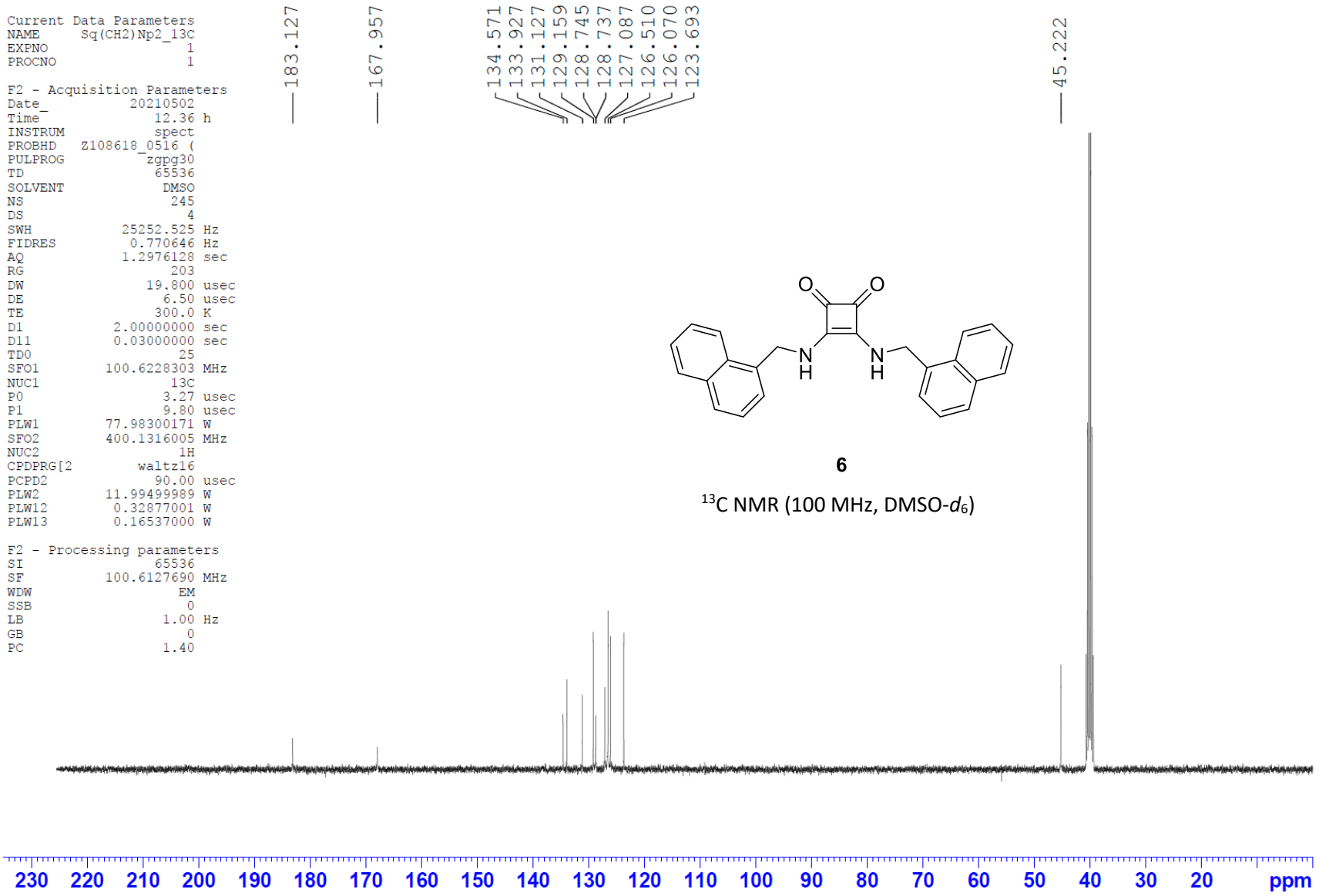

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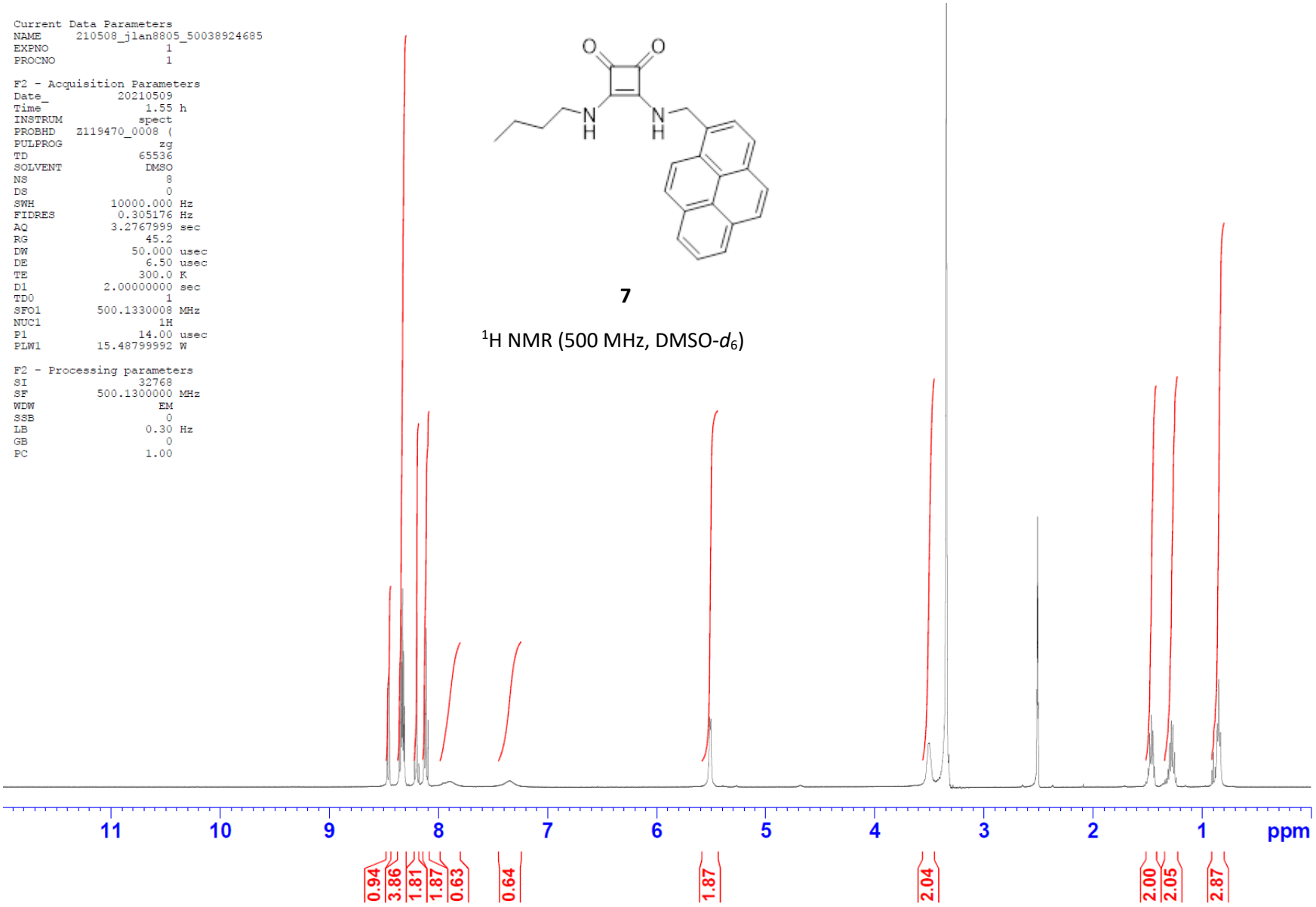

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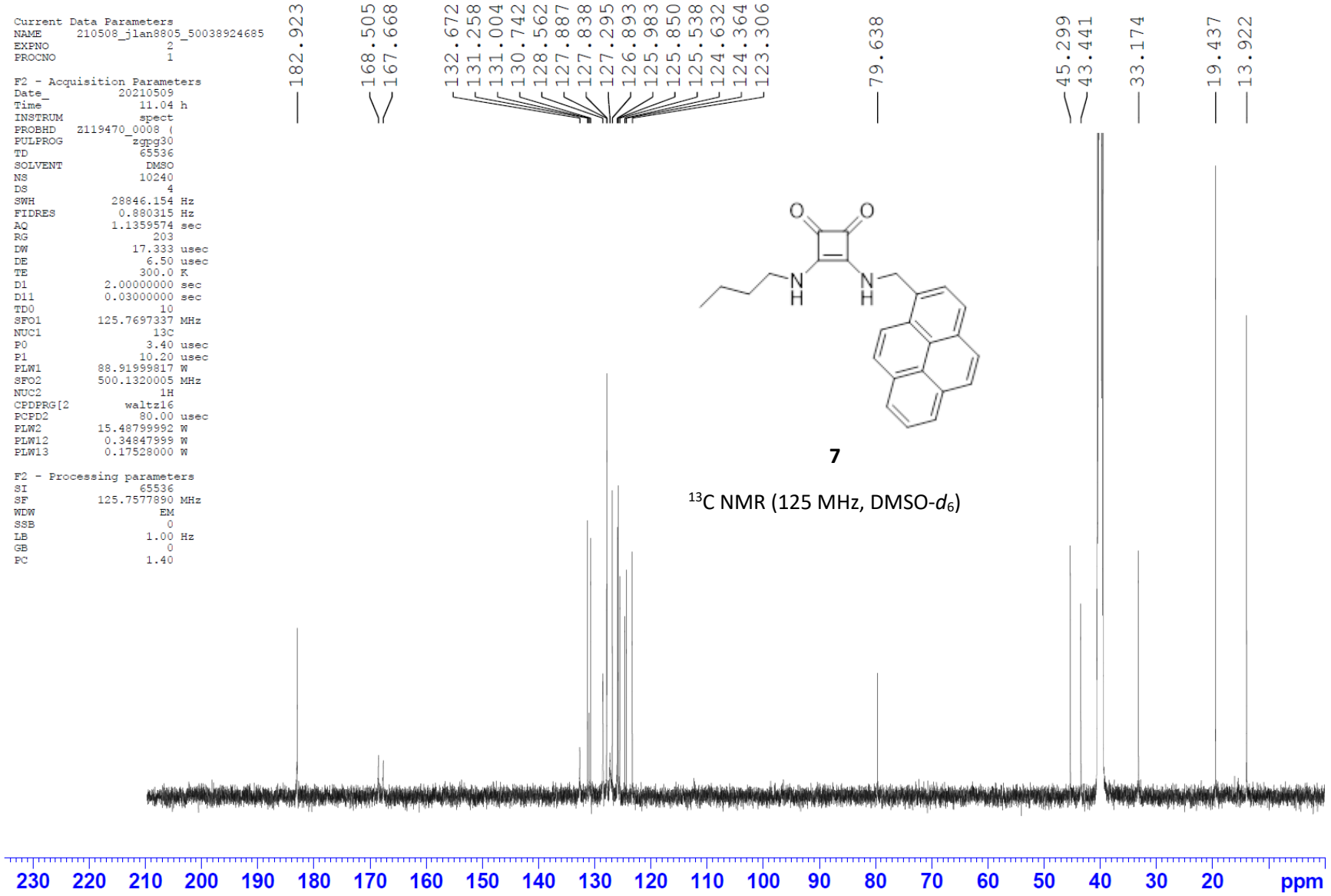

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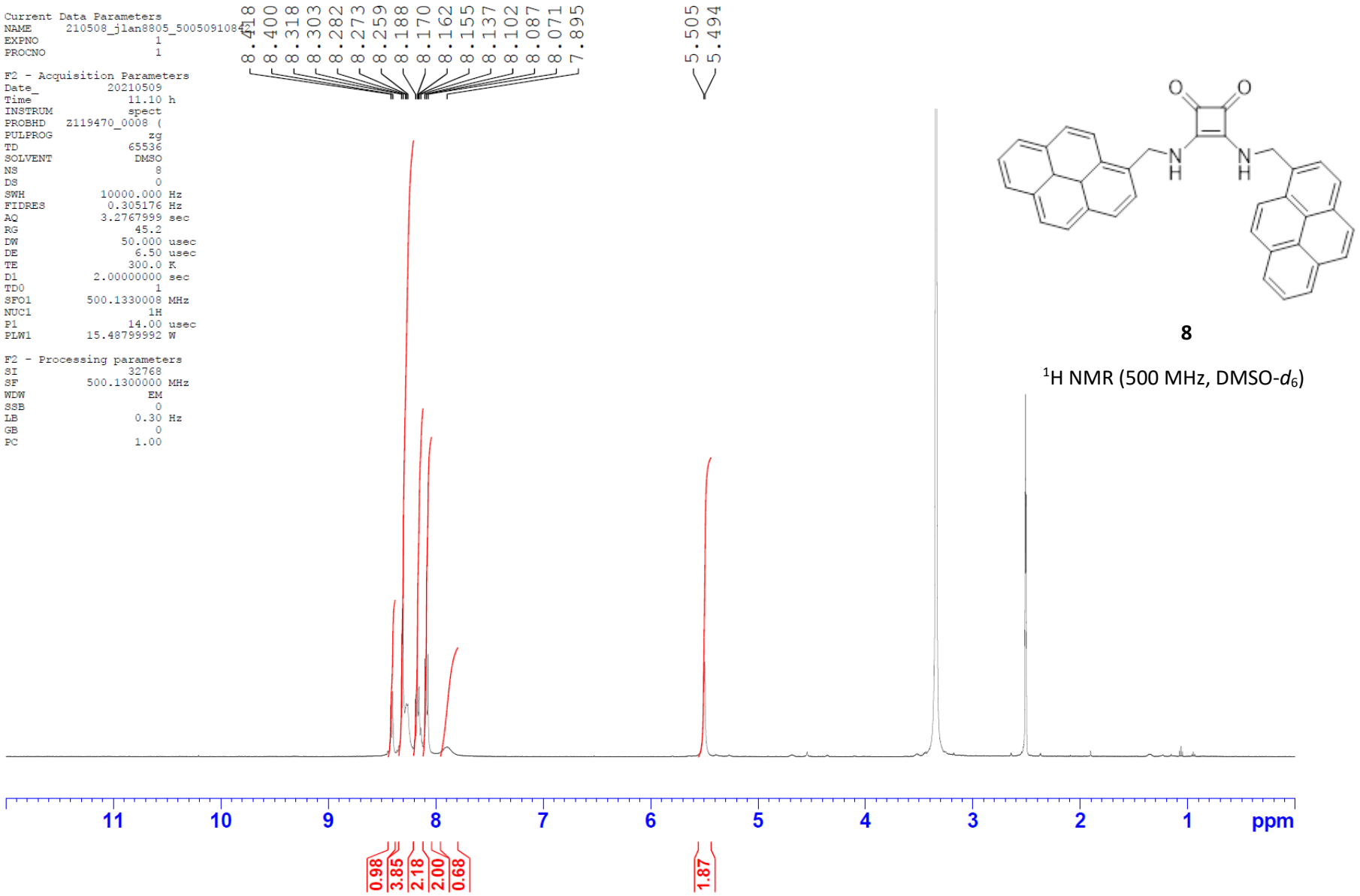

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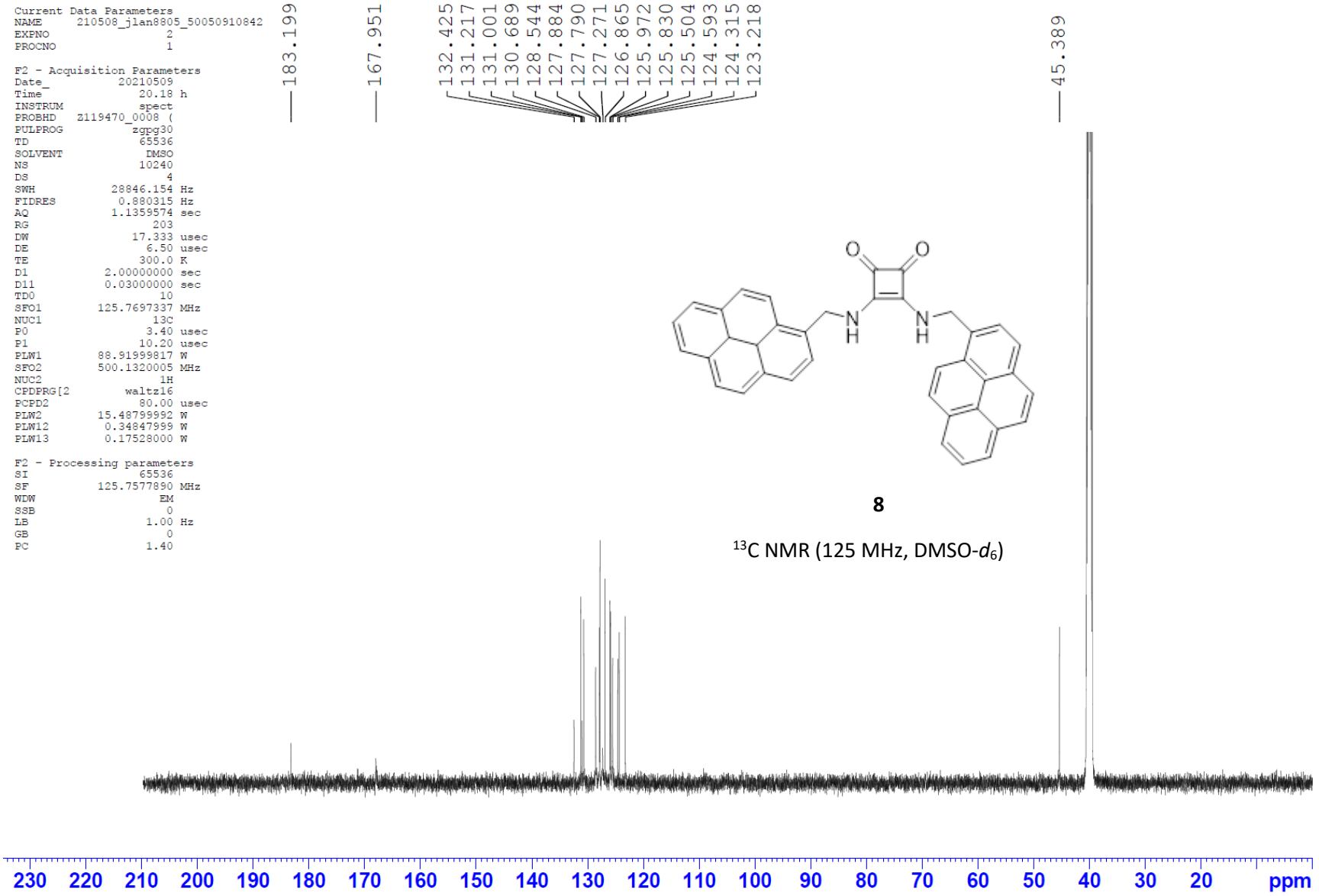

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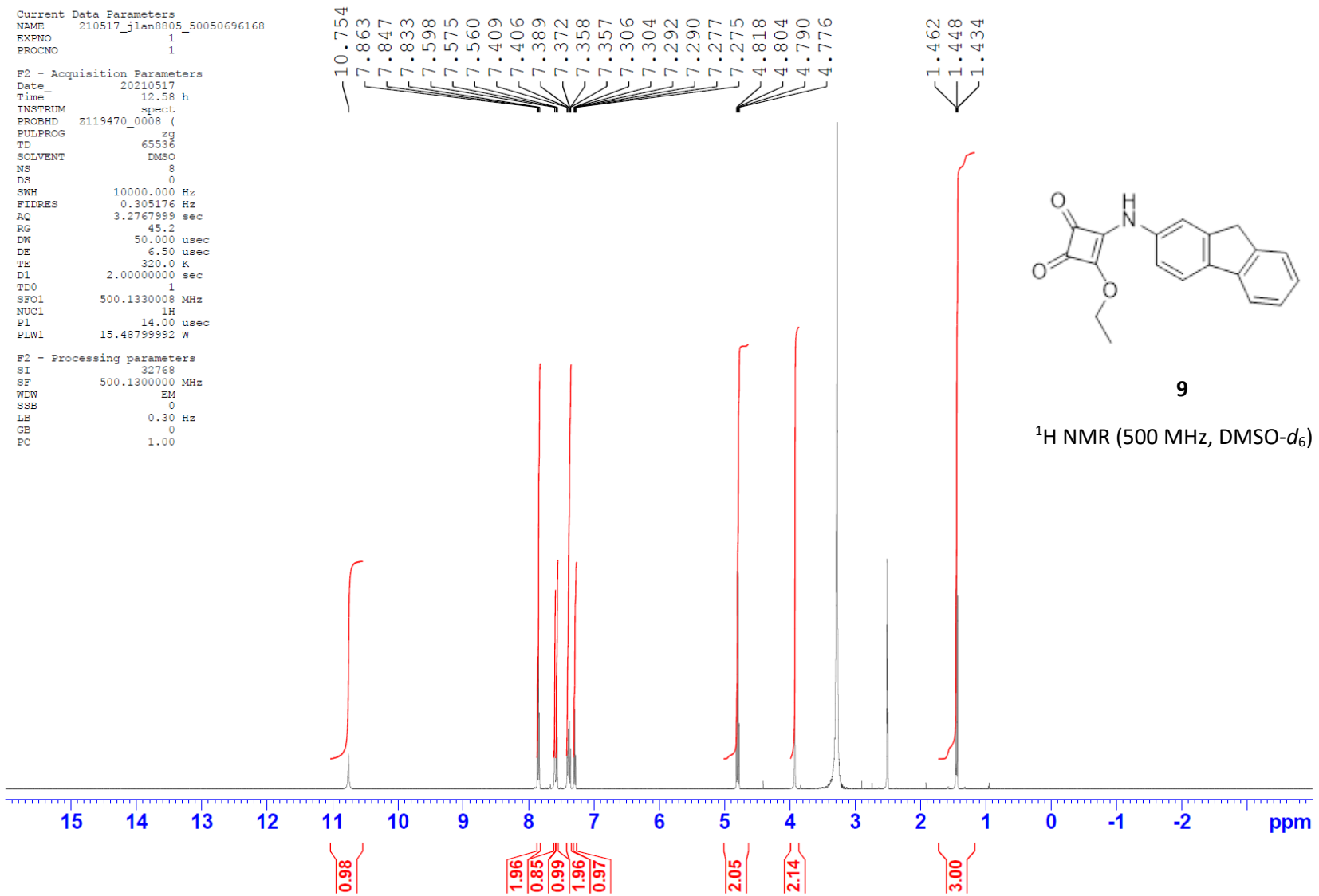

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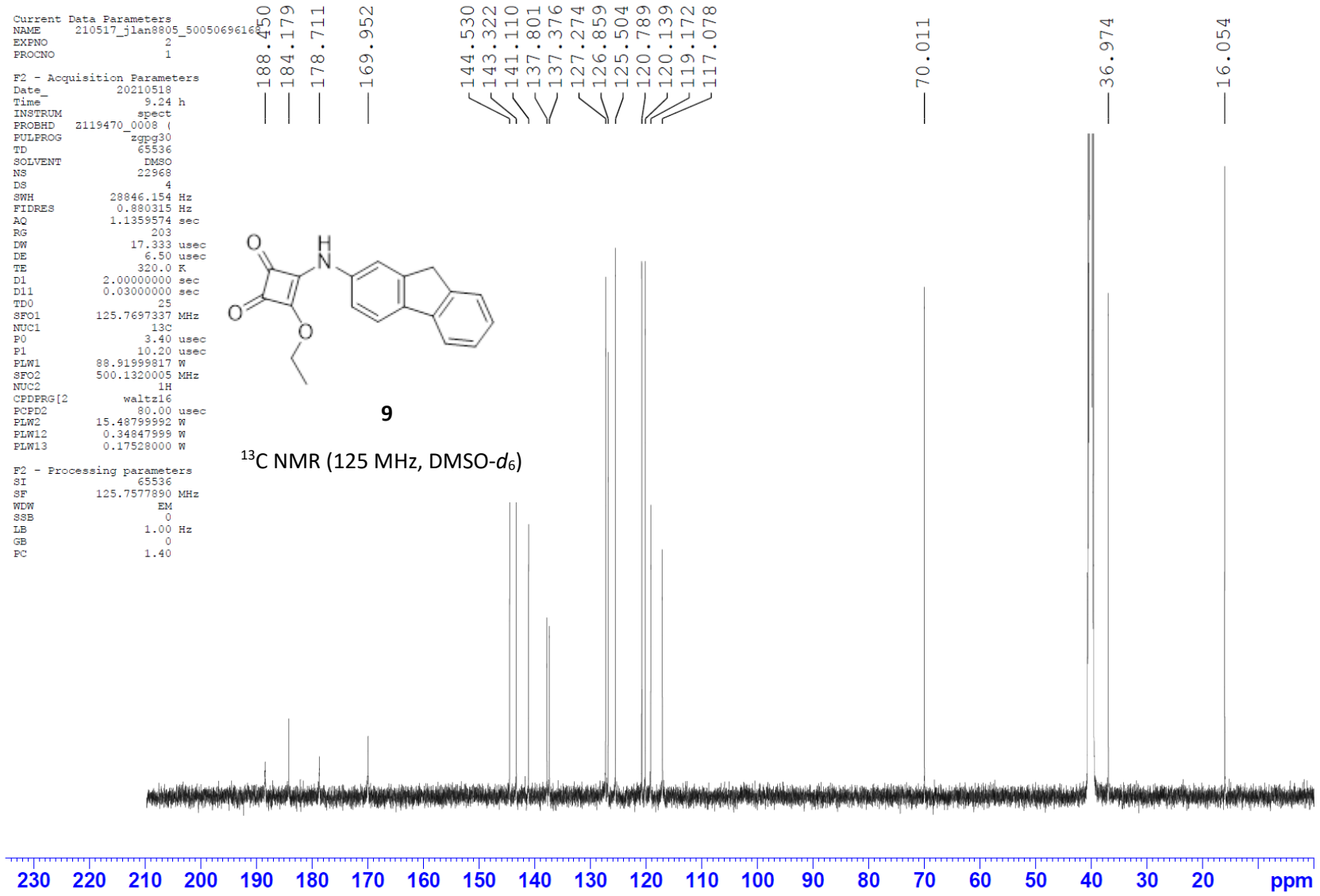
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DE 6.50 usec
TE 320.0 K
D1 2.00000000 sec
D11 0.03000000 sec
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NUC1 13C
P0 3.40 usec
P1 10.20 usec
PLW1 88.91999817 W
SFO2 500.1320005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 15.48799992 W
PLW12 0.34847999 W
PLW13 0.17528000 W
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126.859
125.504
120.789
120.139
119.172
117.078
70.011
36.974
16.054
O
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N
O
O
9
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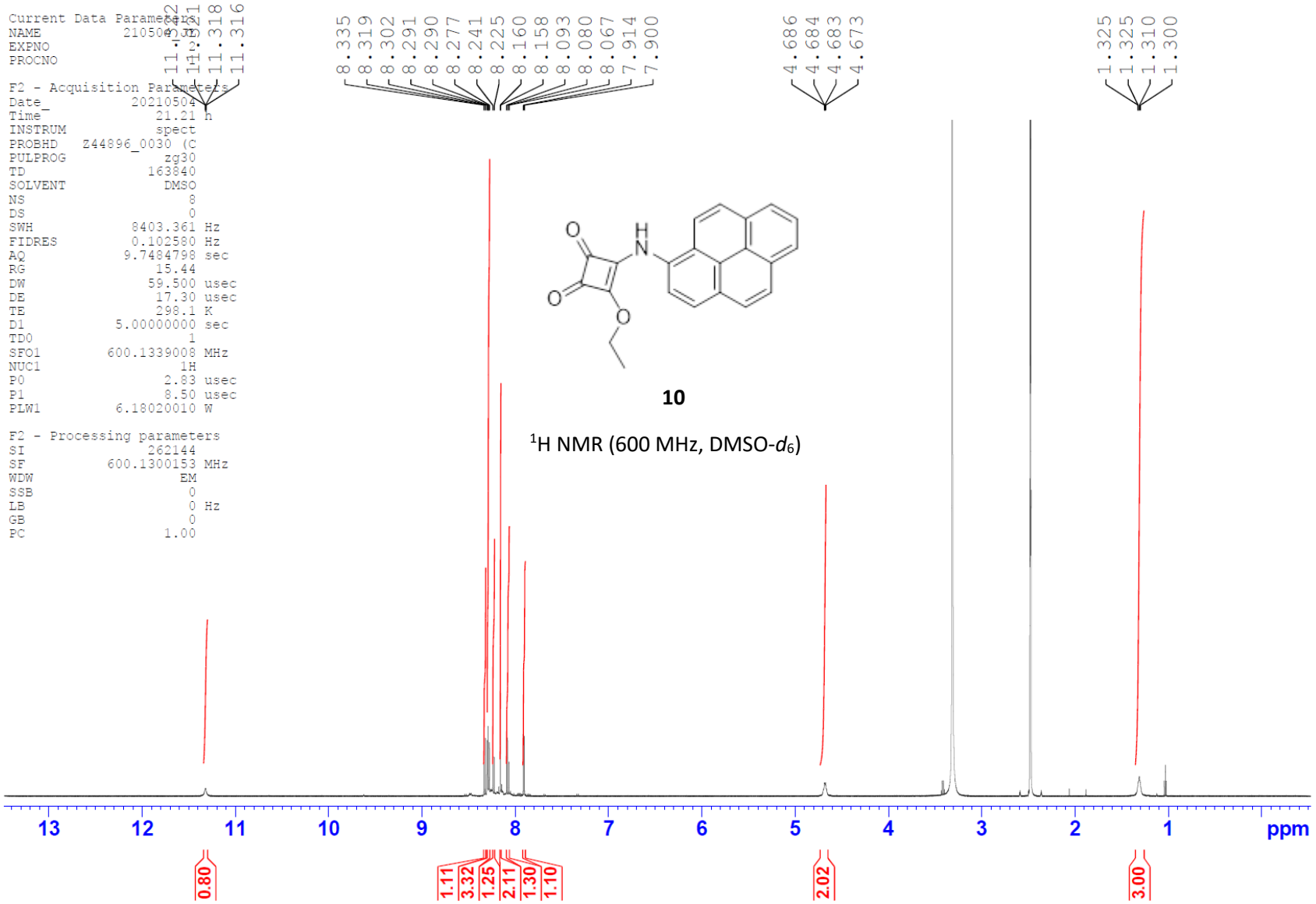
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TE 298.1 K
D1 5.00000000 sec
TD0 1
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NUC1 1H
P0 2.83 usec
P1 8.50 usec
PLW1 6.18020010 W
F2 - Processing parameters
SI 262144
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WDW EM
SSB 0
LB 0 Hz
GB 0
PC 1.00
11.322
11.321
11.318
11.316
8.335
8.319
8.302
8.291
8.290
8.277
8.241
8.225
8.160
8.158
8.093
8.080
8.067
7.914
7.900
4.686
4.684
4.683
4.673
1.325
1.325
1.310
1.300
O
H
N
O
O
10
1H NMR (600 MHz, DMSO-d6)
13
12
11
10
9
8
7
6
5
4
3
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ppm
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2.11
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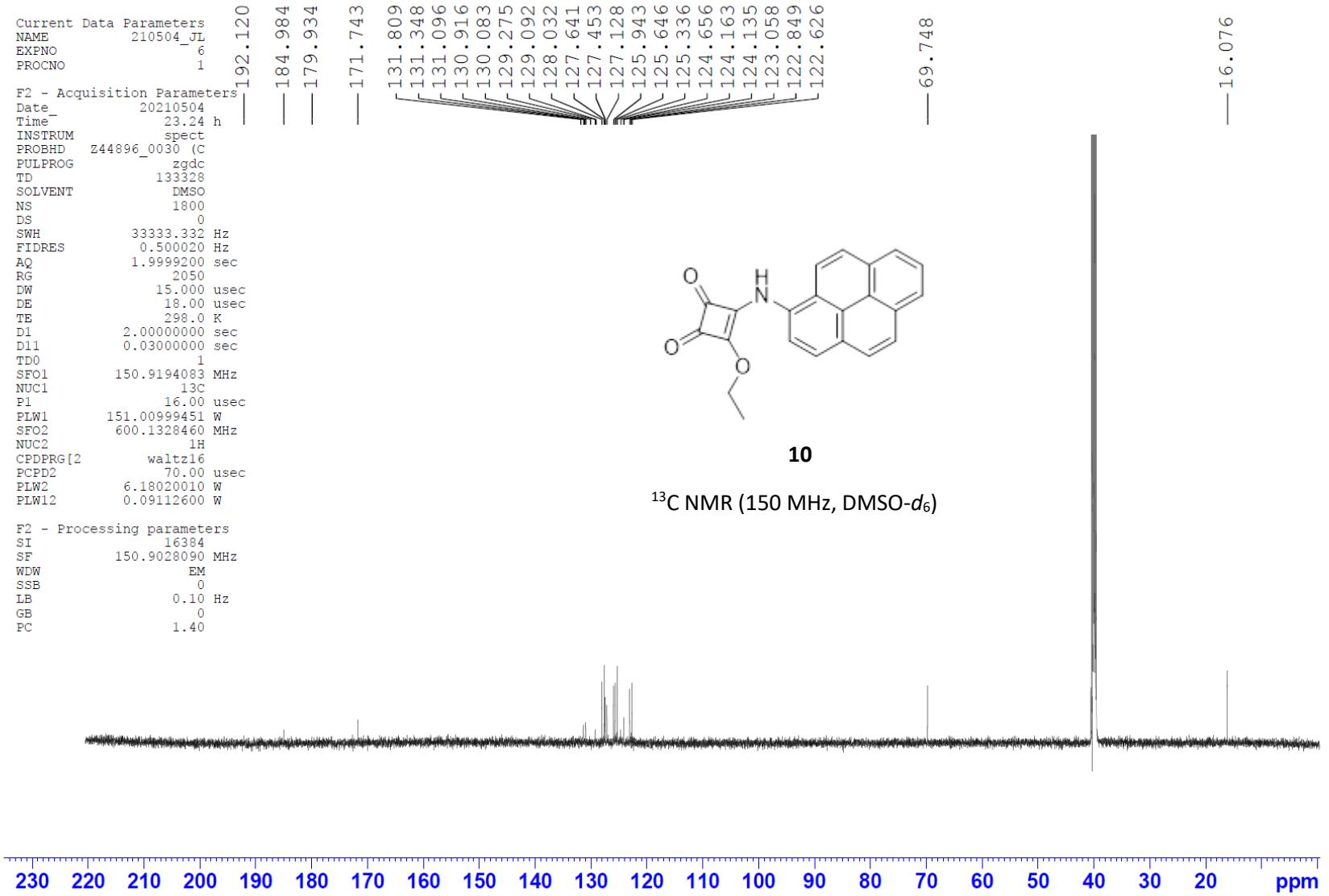
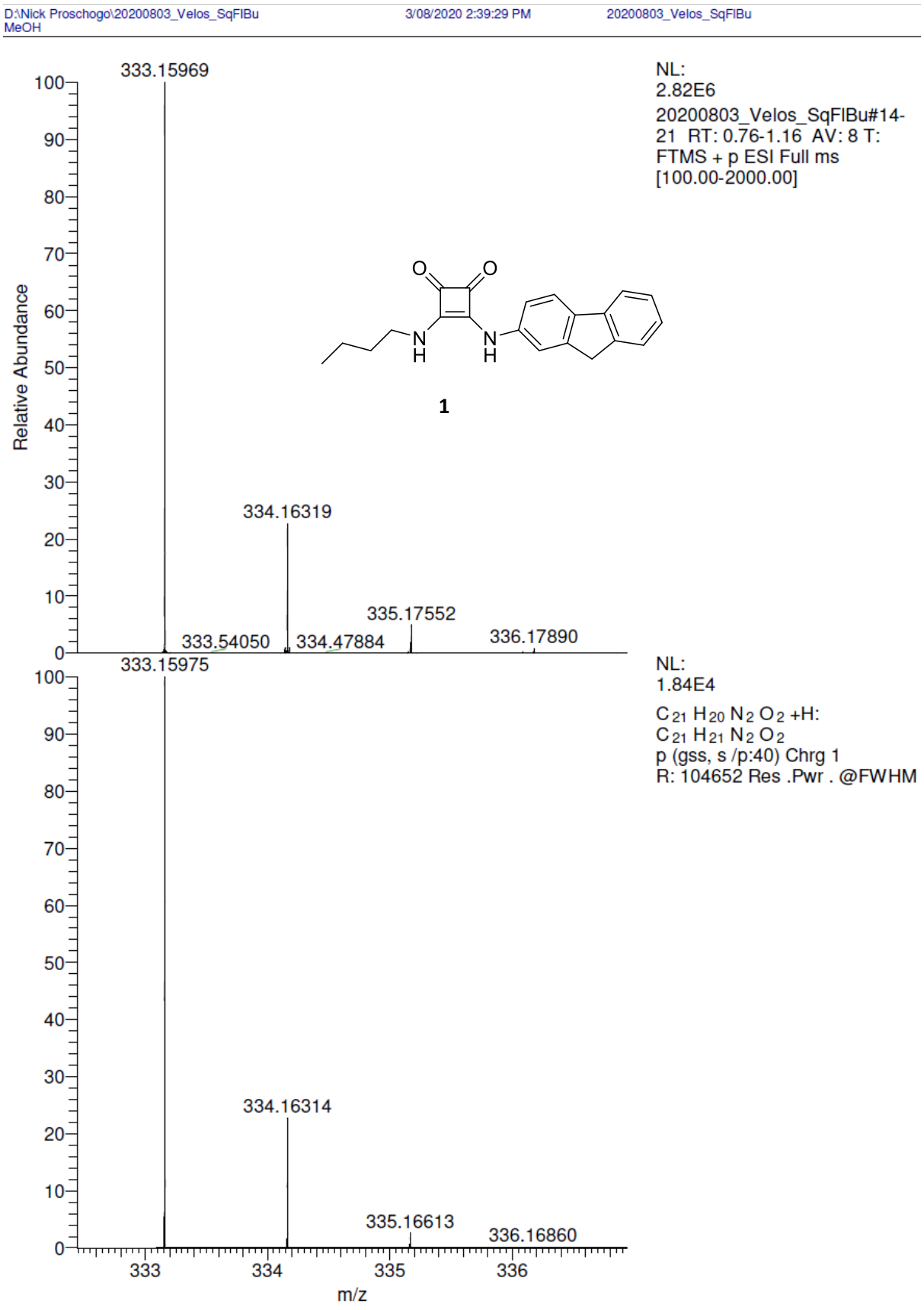
1.10
2.02
3.00

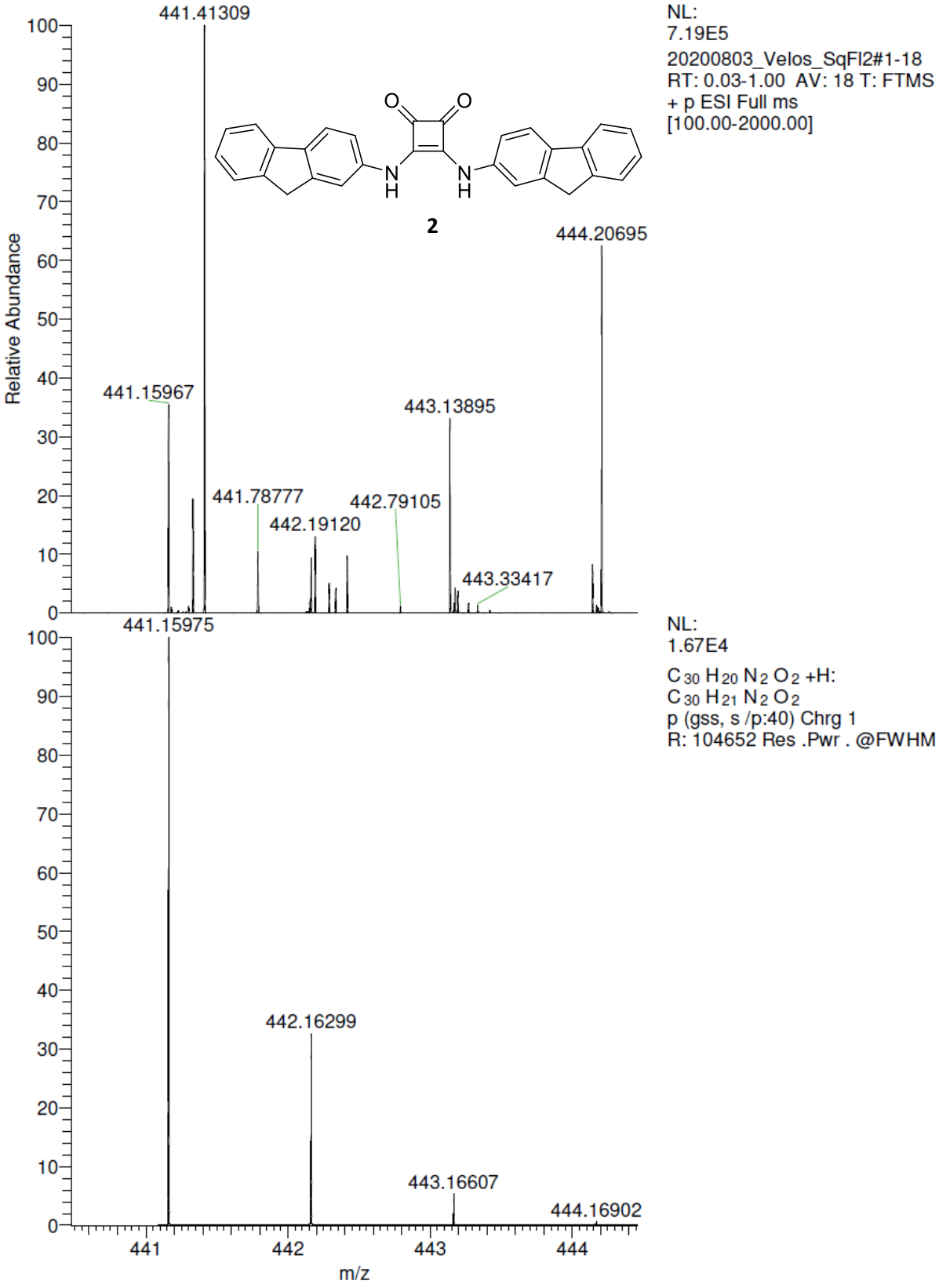


10

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

HRMS characterisation

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+ p ESI Full ms
[100.00-2000.00]
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O
N
H
N
H
2
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441.15967
443.13895
441.78777
442.79105
442.19120
443.33417
Relative Abundance
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NL:
1.67E4
C30 H20 N2 O2 +H:
C30 H21 N2 O2
p (gss, s /p:40) Chrg 1
R: 104652 Res .Pwr . @FWHM
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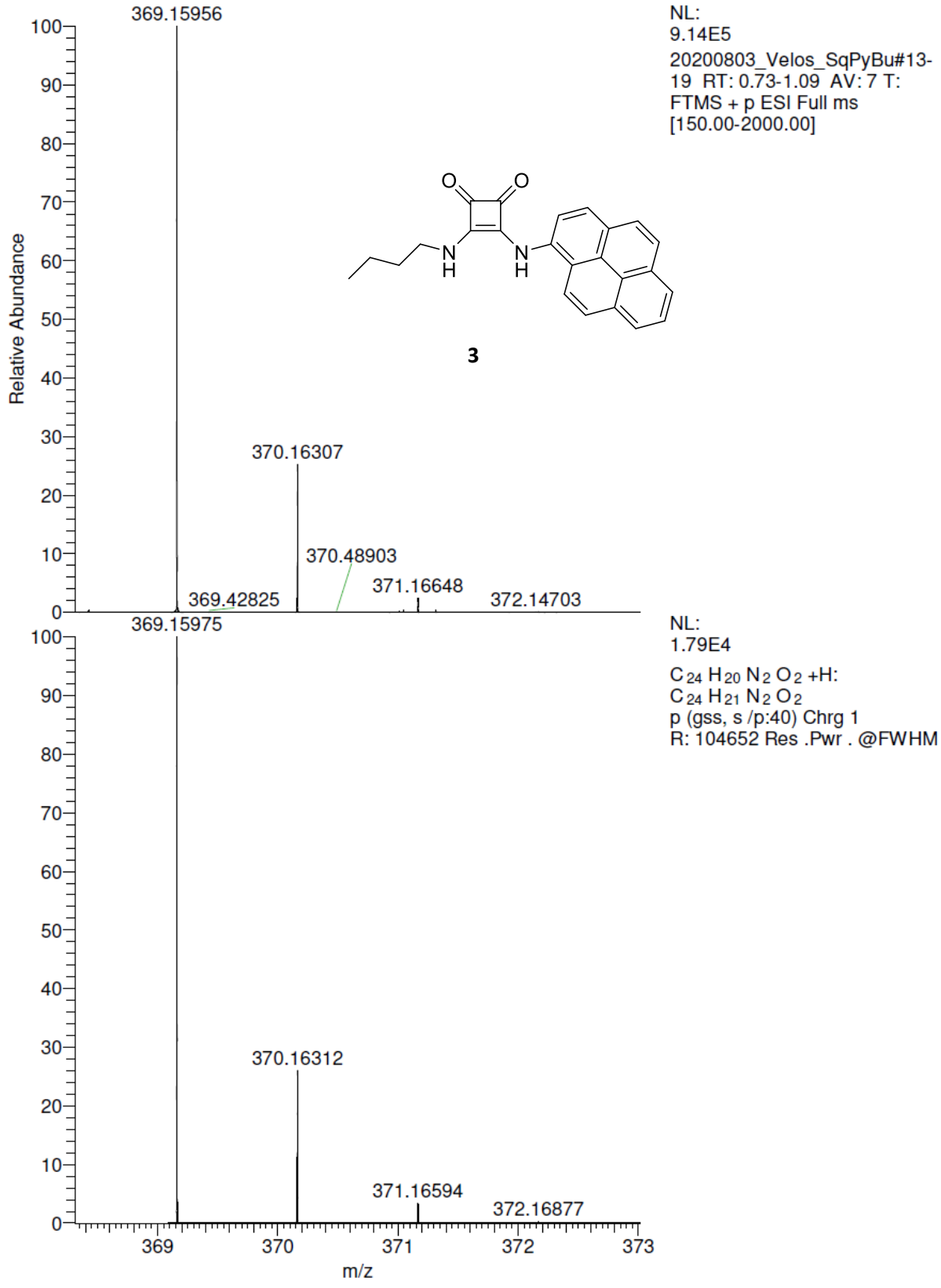
443.16607
444.16902
441
442
443
444
m/z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NL:
9.14E5
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O
N
H
N
H
3
Relative Abundance
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370.48903
371.16648
369.42825
372.14703
369.15975
NL:
1.79E4
C24 H20 N2 O2 +H:
C24 H21 N2 O2
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R: 104652 Res .Pwr . @FWHM
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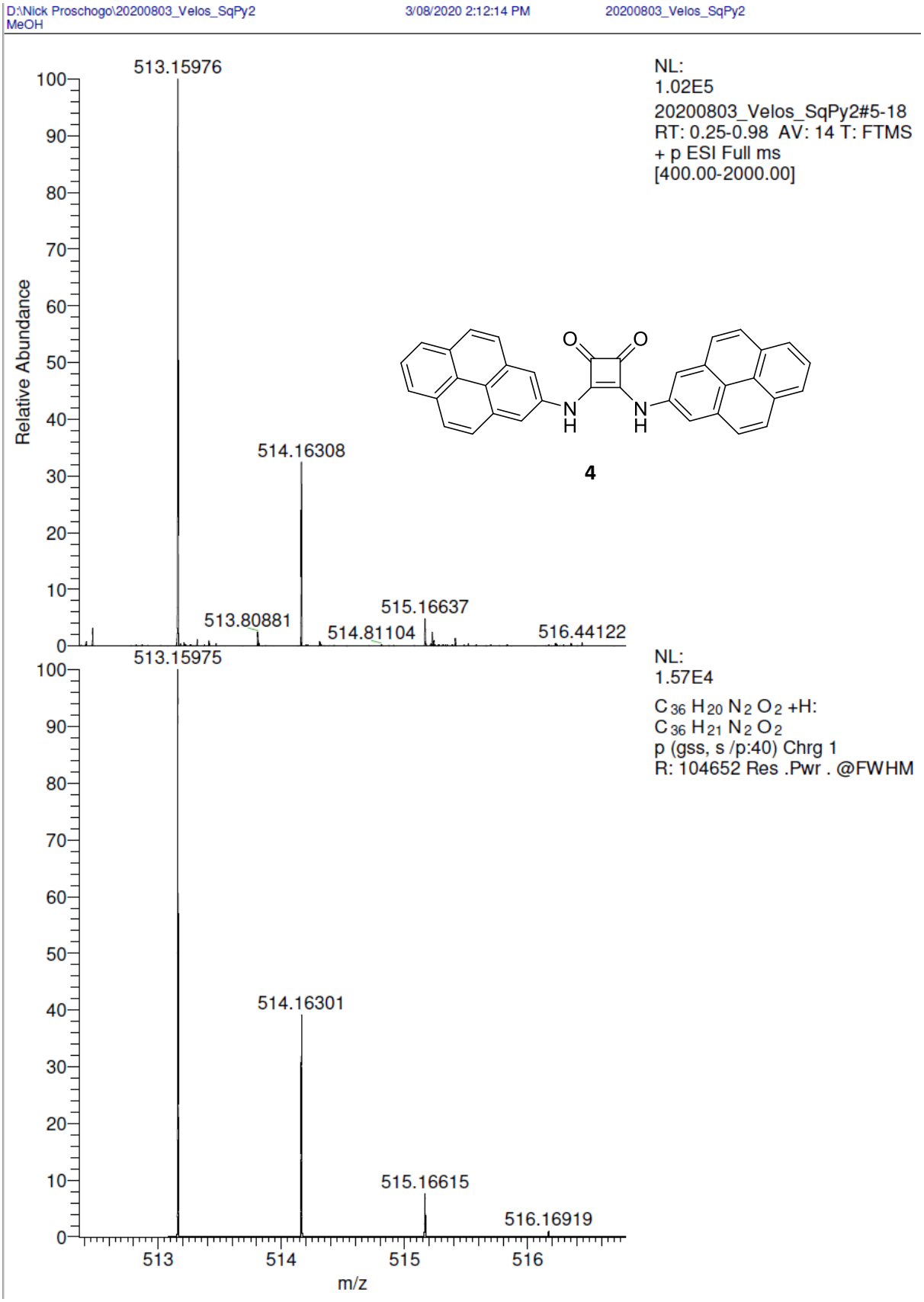
372.16877
m/z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3/08/2020 2:12:14 PM
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20200803_Velos_SqPy2#5-18
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+ p ESI Full ms
[400.00-2000.00]
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513.80881
515.16637
514.81104
516.44122
Relative Abundance
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513.15975
NL:
1.57E4
C36 H20 N2 O2 +H:
C36 H21 N2 O2
p (gss, s /p:40) Chrg 1
R: 104652 Res .Pwr . @FWHM
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515.16615
516.16919
m/z

Generic Display Report

Analysis Info

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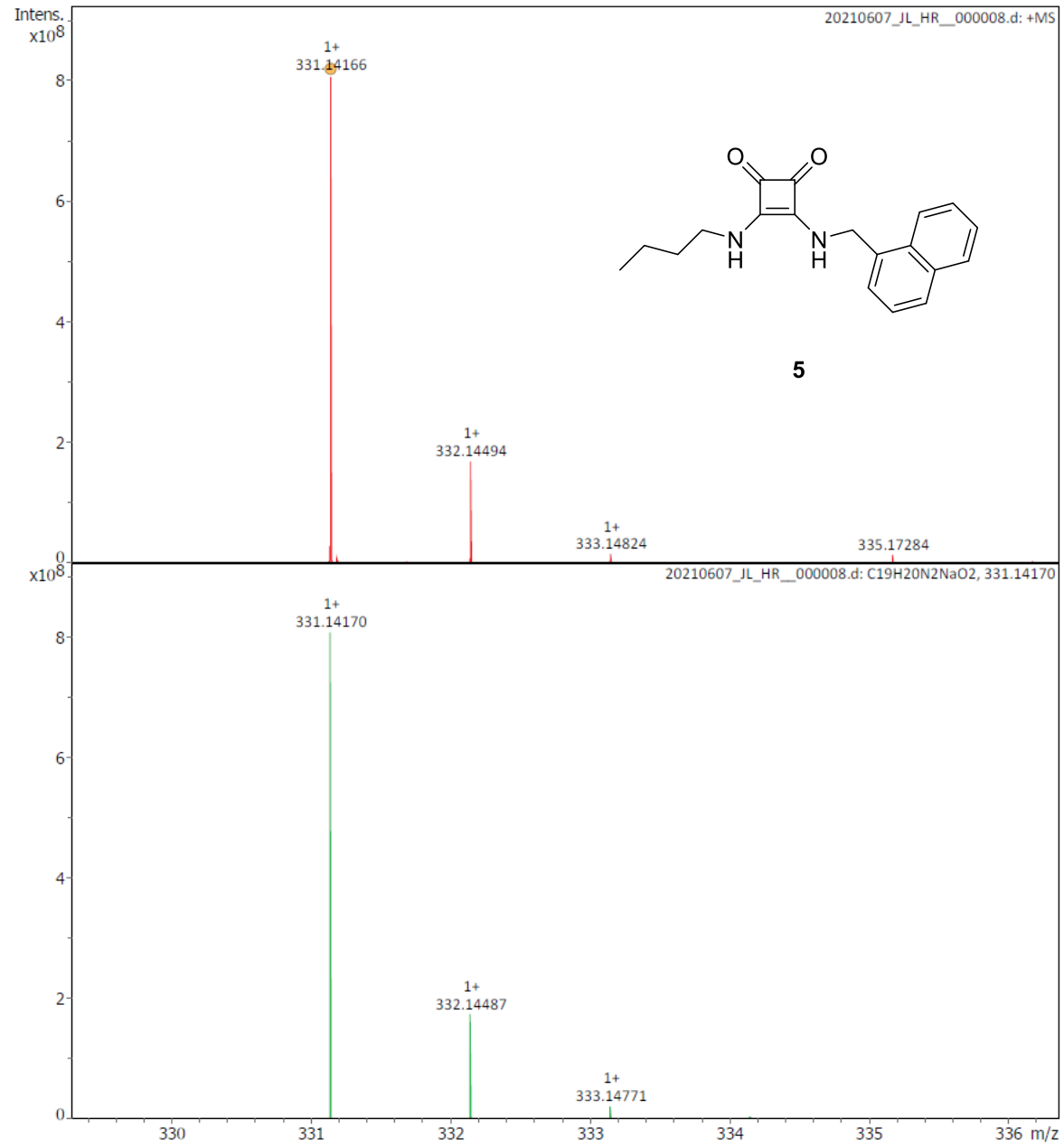
Comment			



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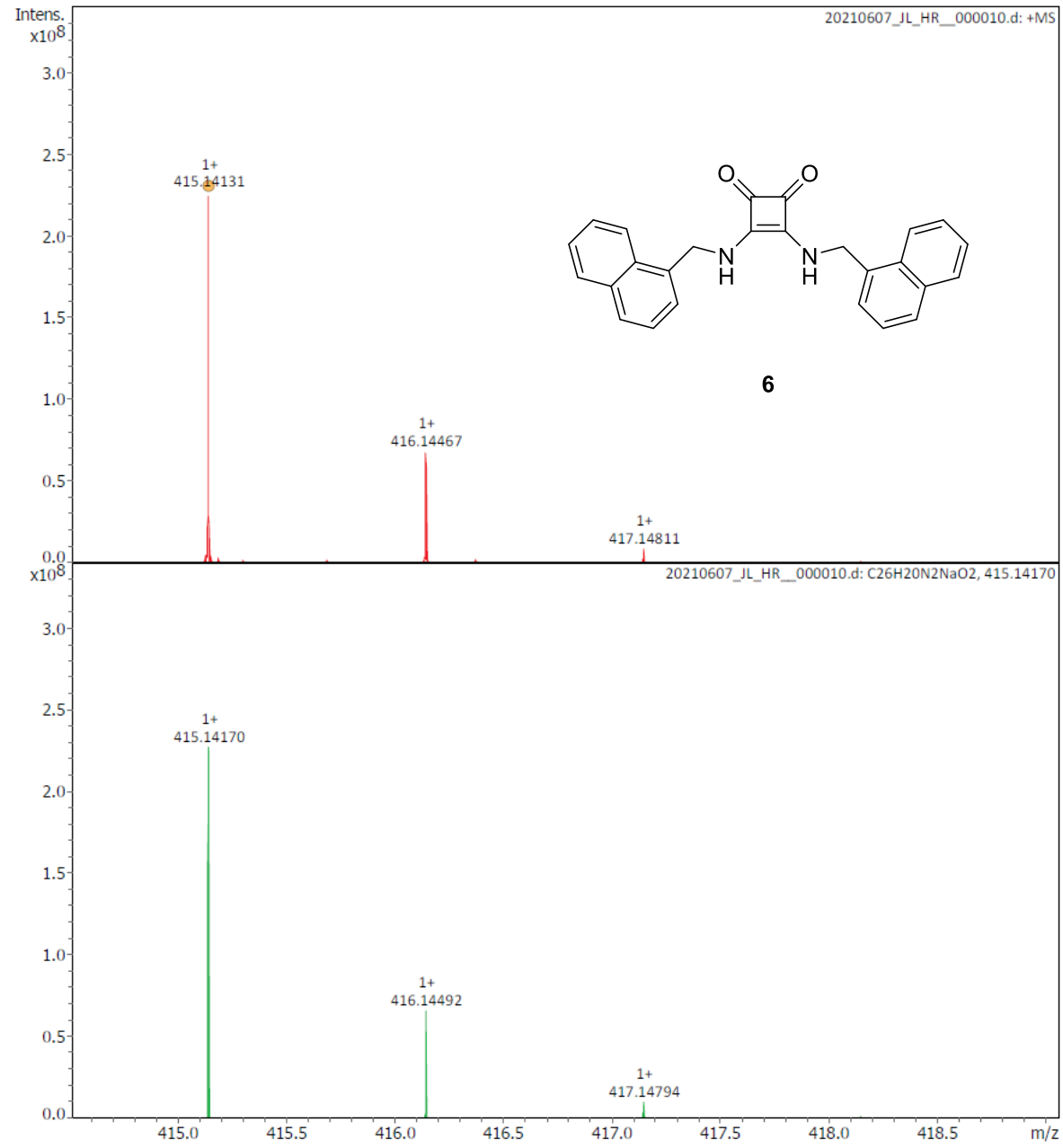

Generic Display Report

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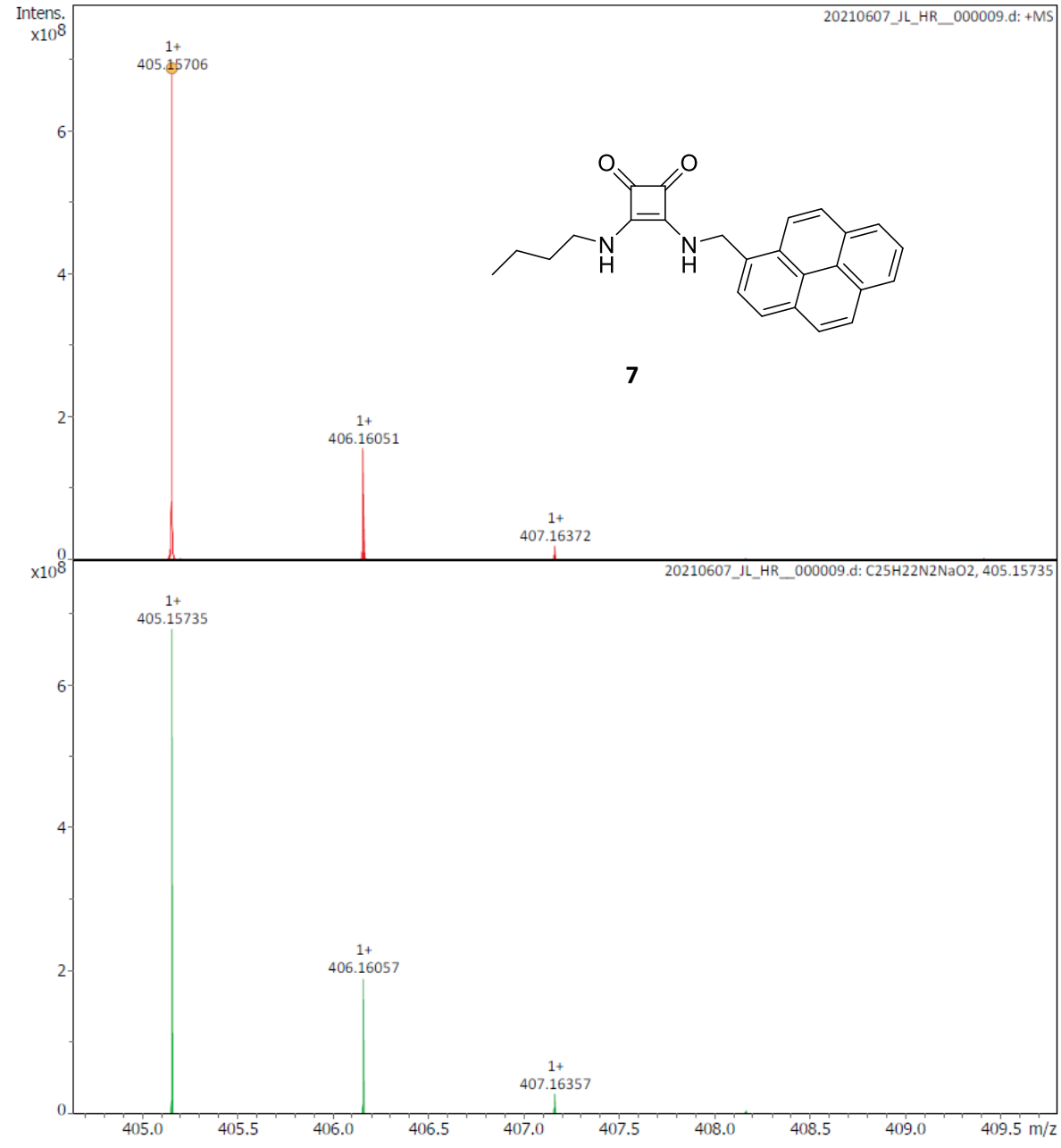
Sample Name	SqCh2PyBu	Instrument	solariX 2xR
Comment			



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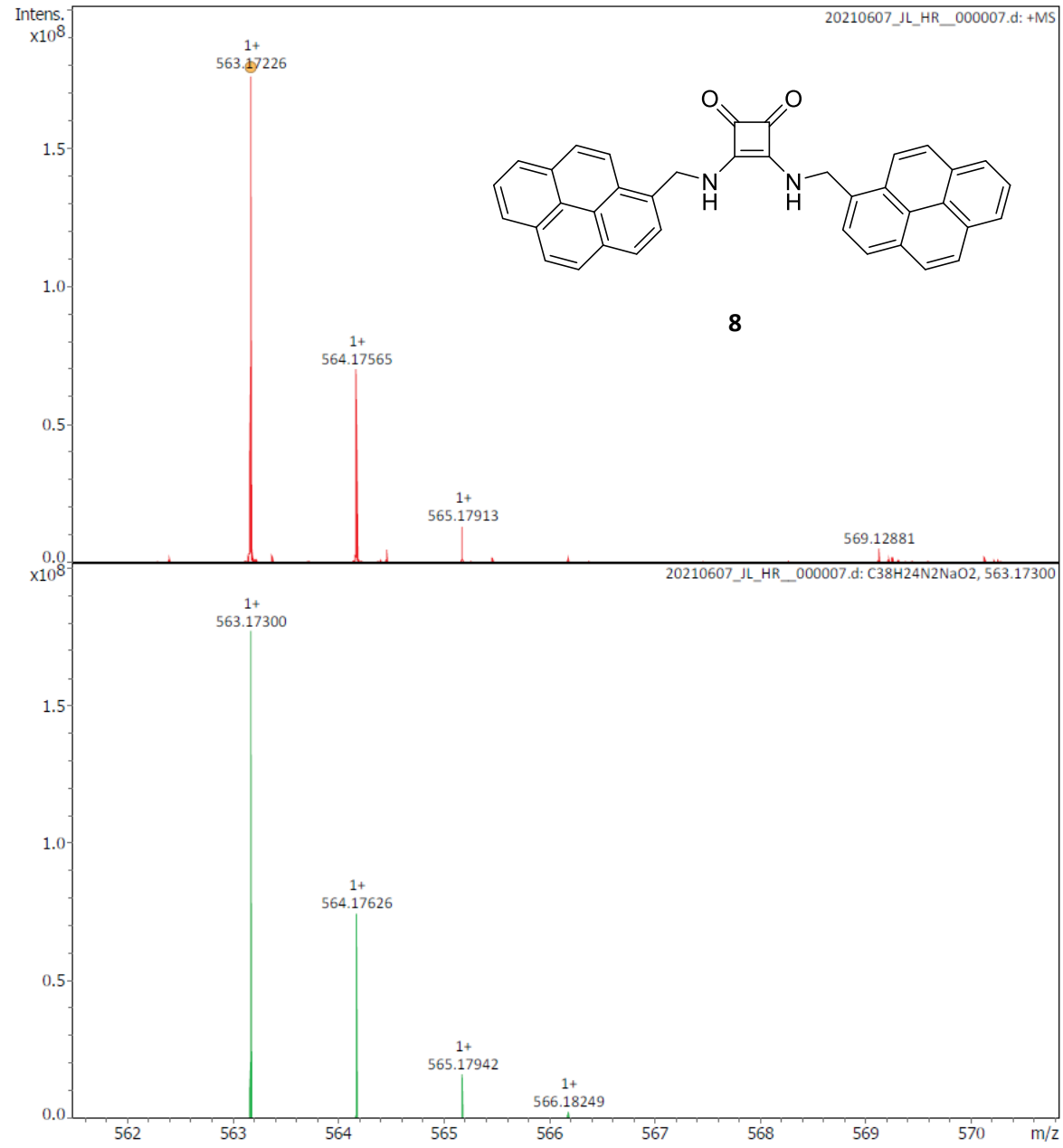

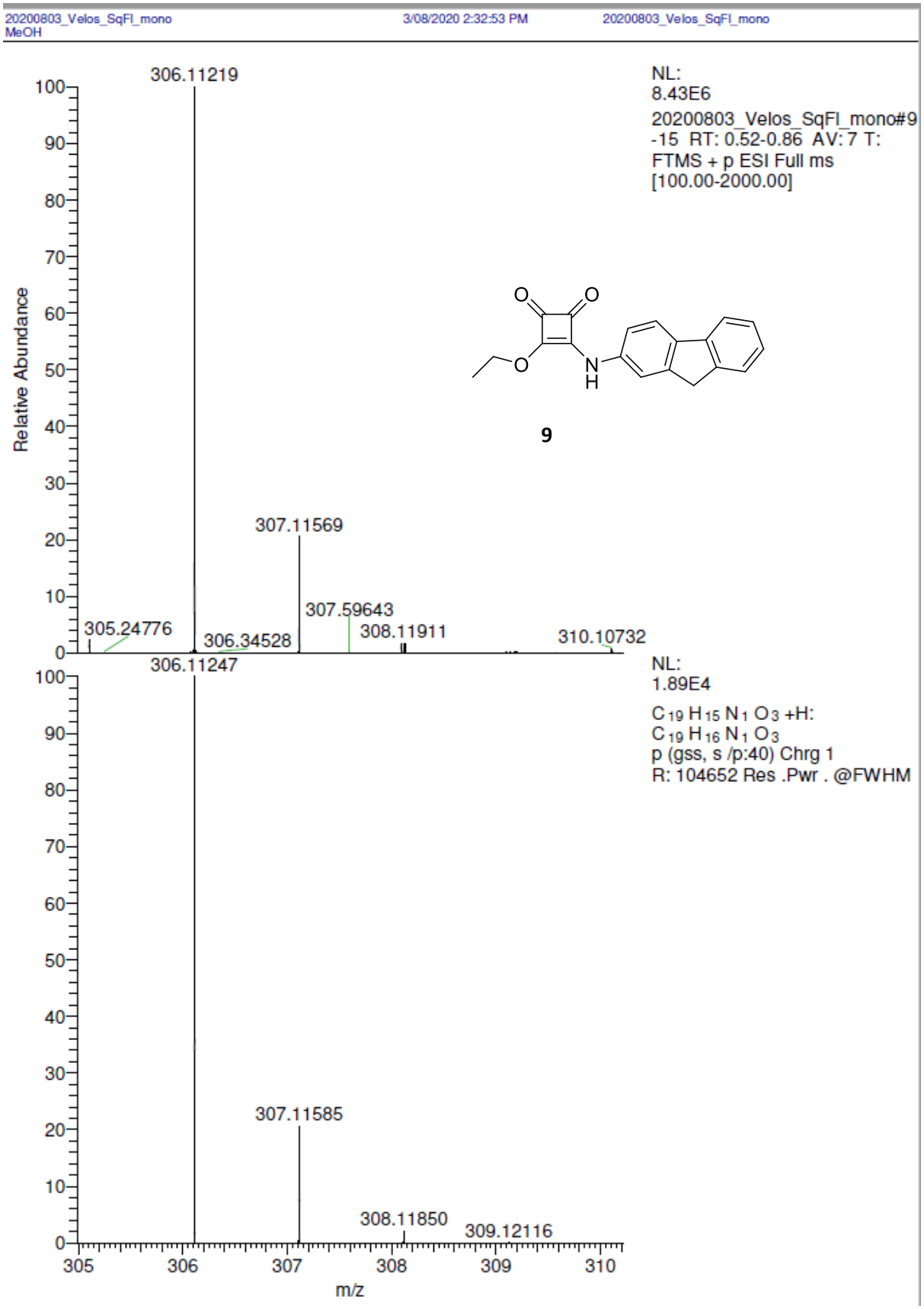
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8.43E6
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FTMS + p ESI Full ms
[100.00-2000.00]
O
O
O
N
H
9
Relative Abundance
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308.11911
305.24776
306.34528
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306.11247
NL:
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C19 H16 N1 O3
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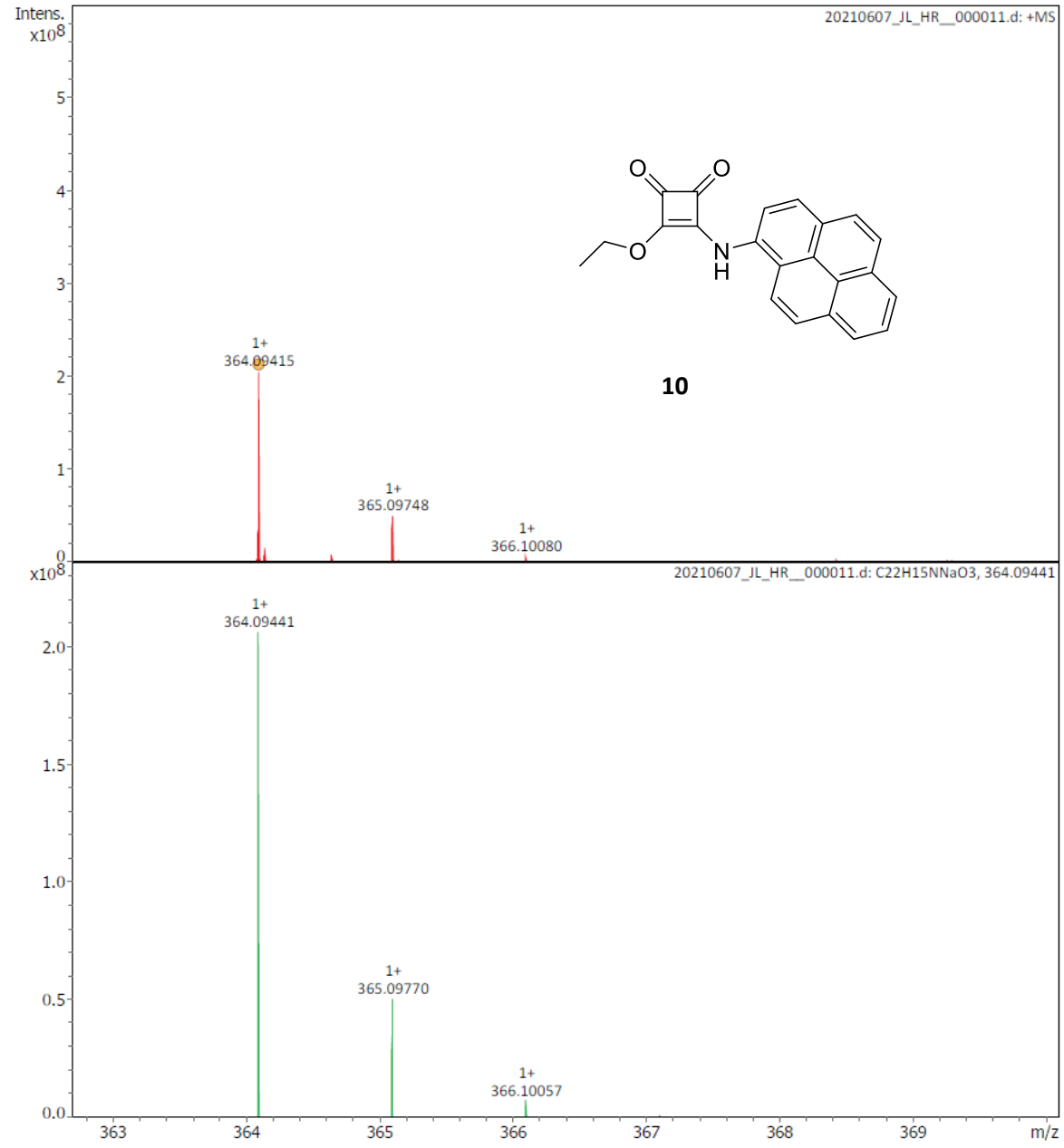


pK_a titration data

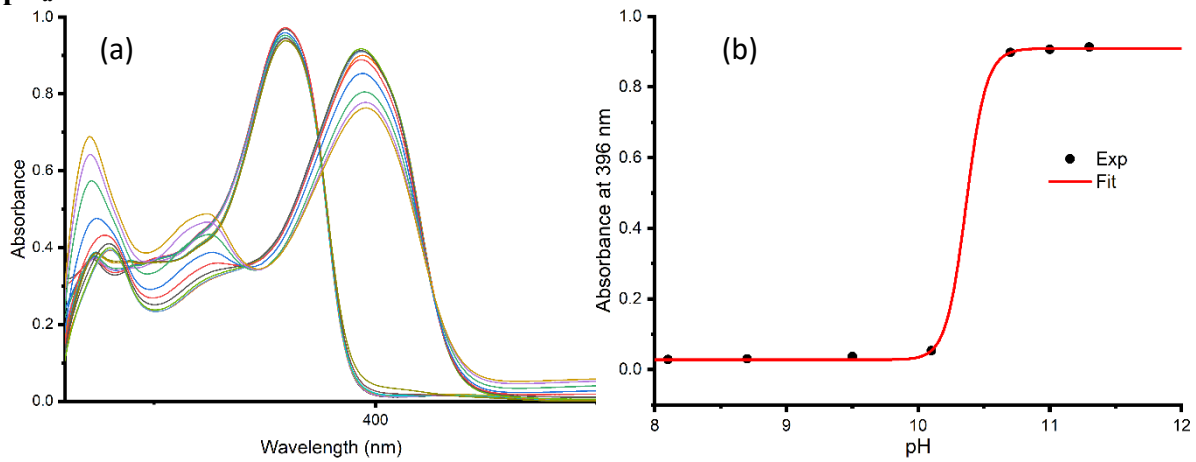


Figure S1: (a) pK_a spectrophotometric titration of **1** in DMSO (10% water) and (b) absorbance at 395 nm for **1** as a function of pH, fitted to a Boltzmann S curve

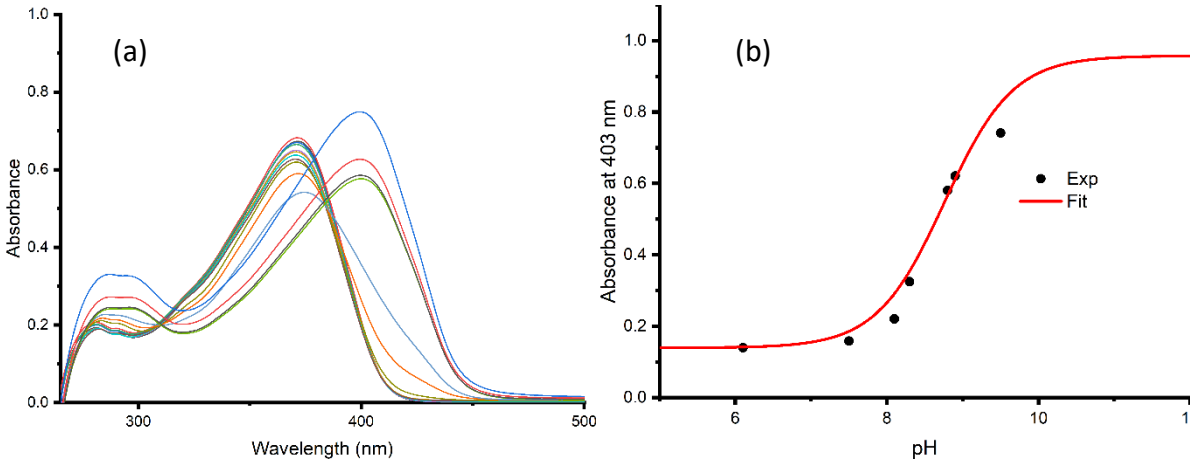


Figure S2: pK_a spectrophotometric titration of **2** in DMSO (10% water) and (b) absorbance at 403 nm for **2** as a function of pH, fitted to a Boltzmann S curve

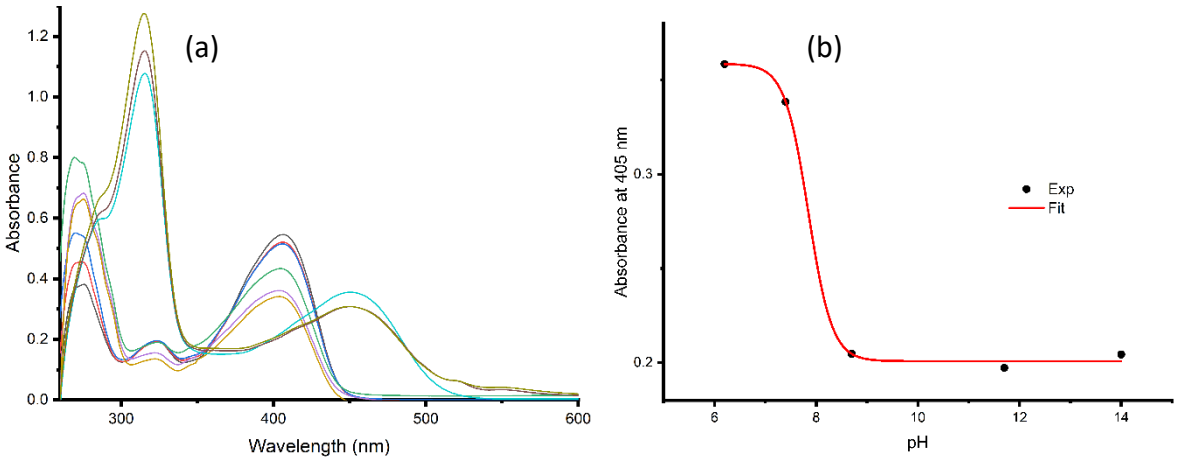
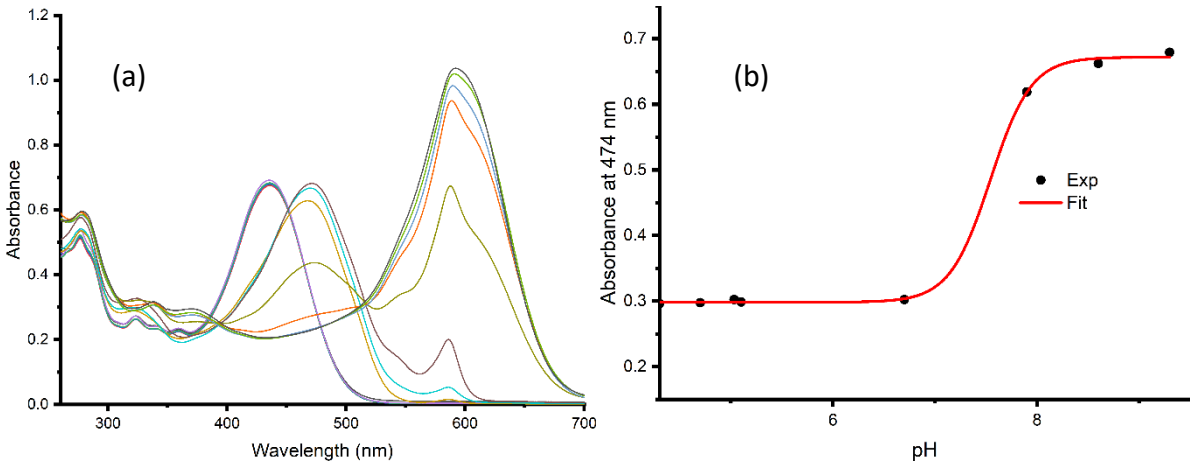


Figure S3: pK_a spectrophotometric titration of **3** in DMSO (10% water) and (b) absorbance at 405 nm for **3** as a function of pH, fitted to a Boltzmann S curve



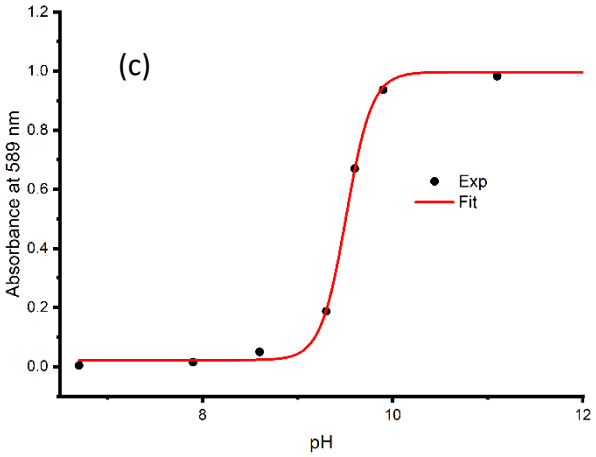


Figure S4: pK_a spectrophotometric titration of **4** in DMSO (10% water), and absorbance at (b) 474 nm and (c) 589 nm for **4** as a function of pH, fitted to a Boltzmann S curve.

Estimation of pK_a by ^{1}H NMR spectroscopy

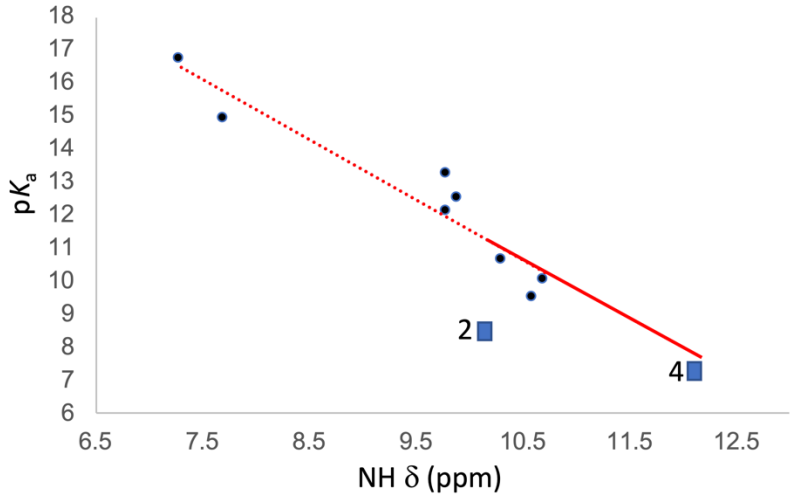
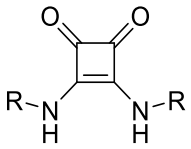


Figure S5: Plot of pK_a against chemical shift in the ^{1}H NMR spectrum in DMSO-d_6 for squaramide NH protons. Dotted line is the trendline for known compounds; solid line is extrapolated.

Table S1: Relationships between squaramide NH proton chemical shift in DMSO-d_6 and their pK_a in DMSO



Substituent (R)	^{1}H NMR NH proton chem. shift DMSO (ppm)	Experimental or calculated pK_a (obtained from literature)	Predicted pK_a based on chemical shift	Δ chemical shift (ppm) (Exp. – Pred. pK_a)
Bu	7.3	16.7[a, b]	16.8	-0.1
Bn	7.7	14.9[b]	15.9	-1.0
3,5-bis(tBu)Ph	9.8	12.1[a]	12.1	+0.0
Ph	9.9	12.5[a, b]	12.0	+0.5
4-tBu-Ph	9.8	13.2[b]	12.1	+1.1
PhCF$_3$	10.3	10.6[b]	11.2	-0.6
PhNO$_2$	10.7	10[b]	10.4	-0.4
3,5-bis(CF$_3$)Ph	10.6	9.5[b]	10.5	-1.0

[a]Experimentally derived,[3-5] [b]Computationally calculated, error estimated ±1 pK_a unit[6]

UV-vis titration data

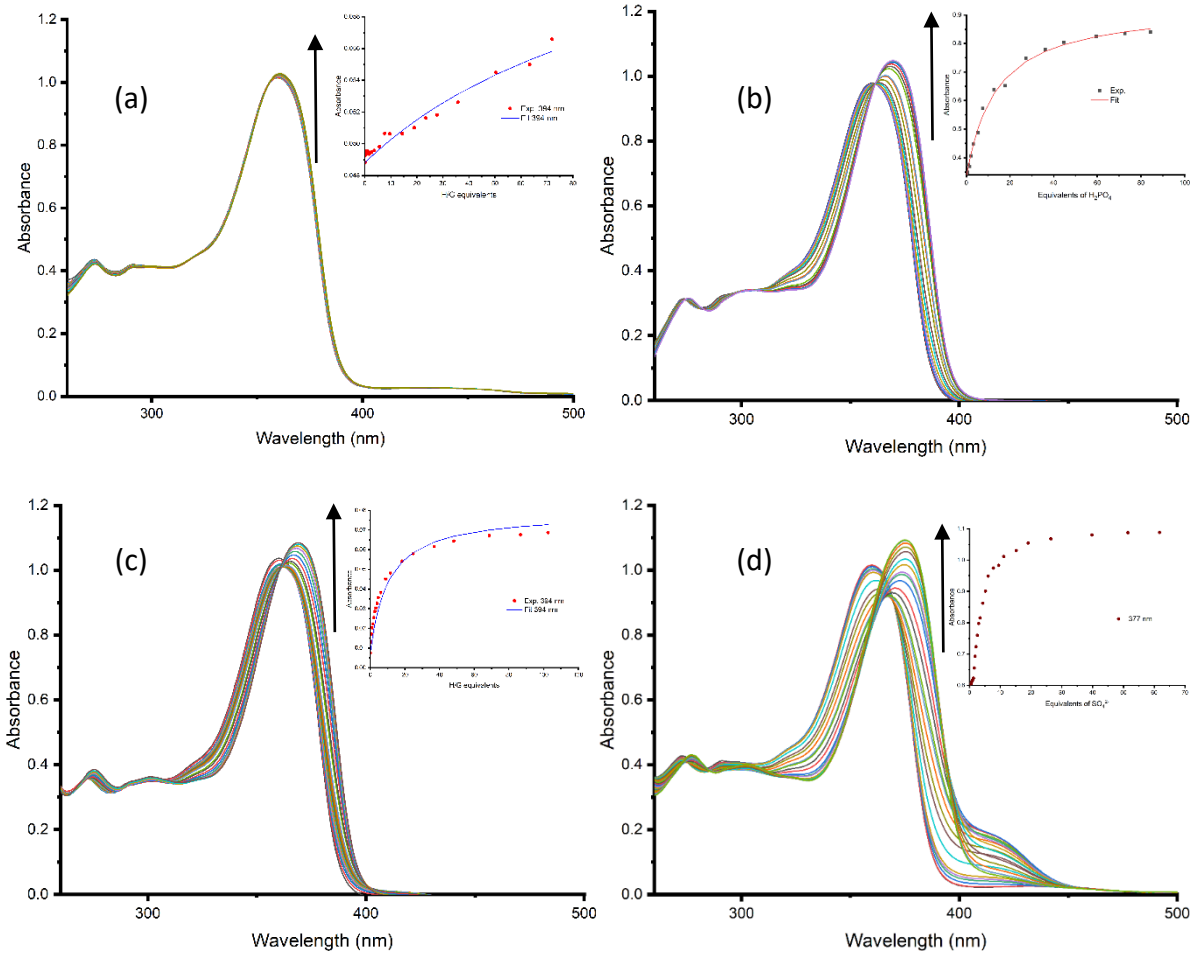


Figure S6: UV-Vis titration spectra of **1** (20 μM) with (a) TBACl, (b) $TBAH_2PO_4$, (c) TBAOAc and (d) TBA_2SO_4, 0 – 80 equivalents of anion in DMSO (1% water) at 298 K. Inset shows fitting of titration data to a 1:1 binding model where possible.

Excitation – emission spectra for 1, 5 – 8 and reference amines 11 and 12

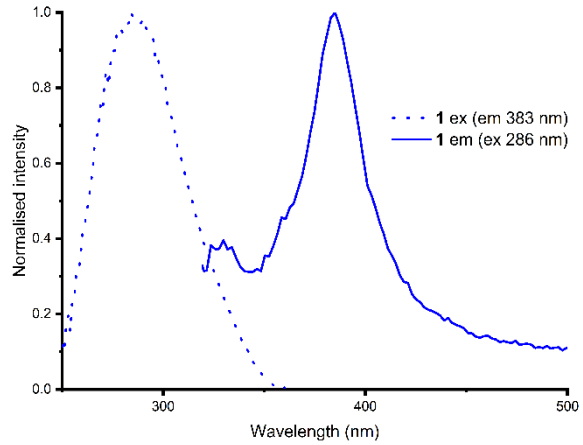


Figure S7: Excitation – emission spectra for compound **1** in DMSO (1% water)

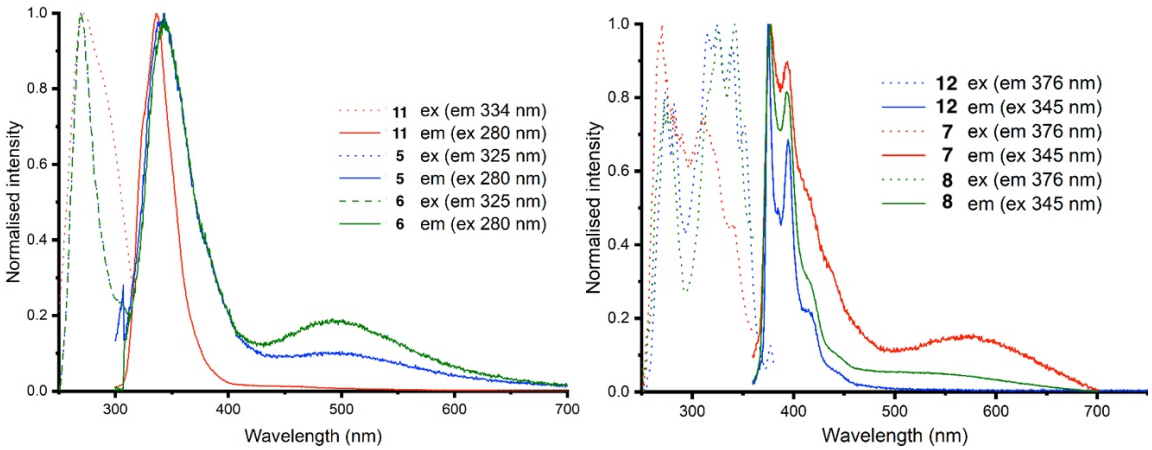


Figure S8: Excitation – emission spectra for (left): 2-naphthalenylmethylamine (**11**), **5** and **6** in DMSO (1% water); (right): 1-pyrenylmethylamine (**12**), **7** and **8** in DMSO (1% water).

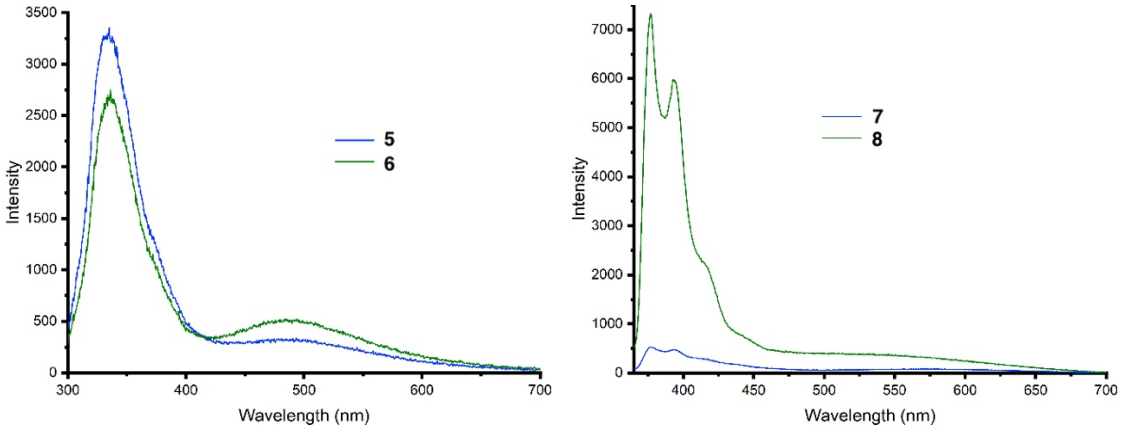


Figure S9: Emission spectra of compounds **5**, **6** (excitation 280 nm) and **7**, **8** (excitation 345 nm) in DMSO (1% water) at 25 μM, illustrating the relatively low emission of **7**.

Fluorescence dilution data

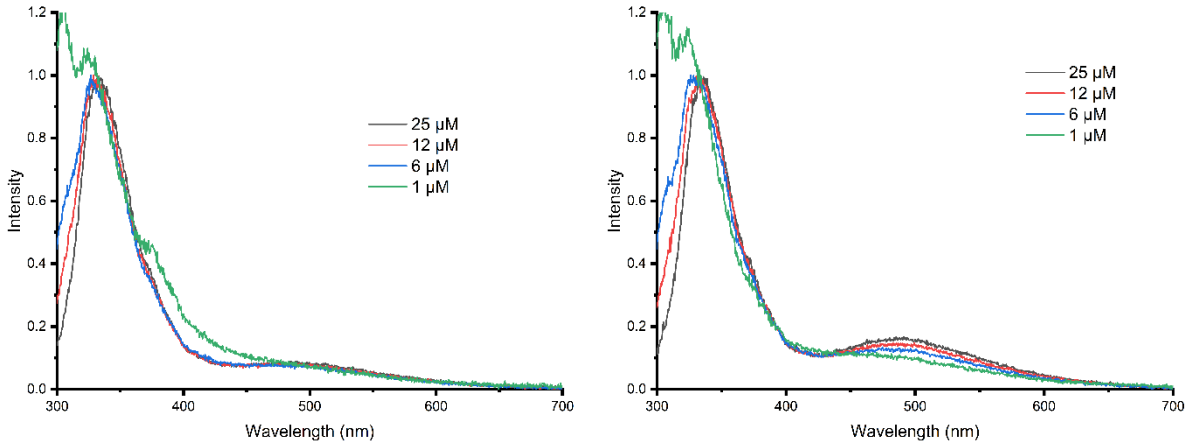


Figure S10: Normalised emission spectra collected of a) **5** and b) **6** at increasing dilution in DMSO (1% water) (excitation at 280 nm).

Fluorescence titration data

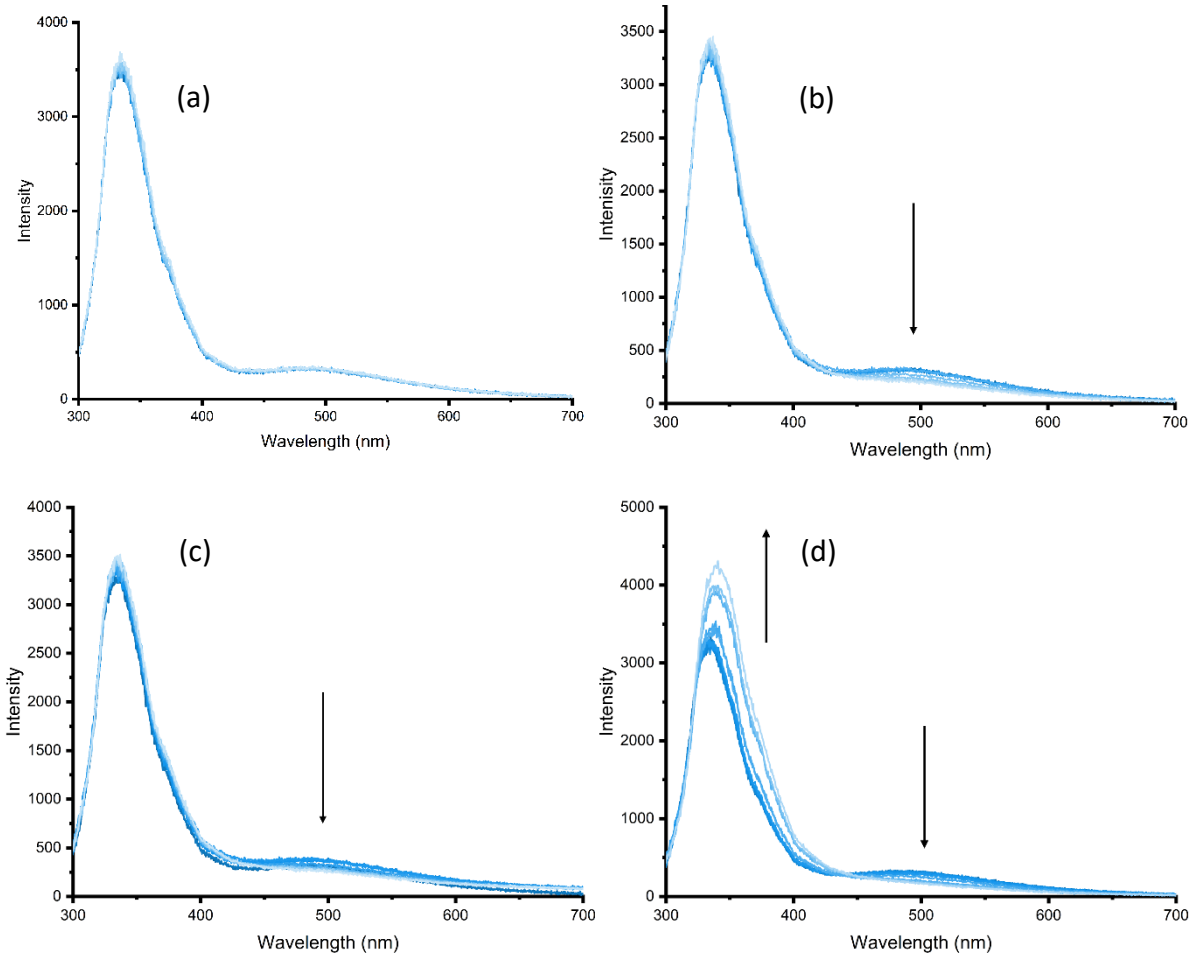


Figure S11: Fluorescence spectra of titration of **5** (20 μM) with (a) TBACl, (b) $TBAH_2PO_4$, (c) TBAOAc and (d) TBA_2SO_4, 0 – 80 equivalents of anion in DMSO (1% water) at 298 K (excitation 280 nm).

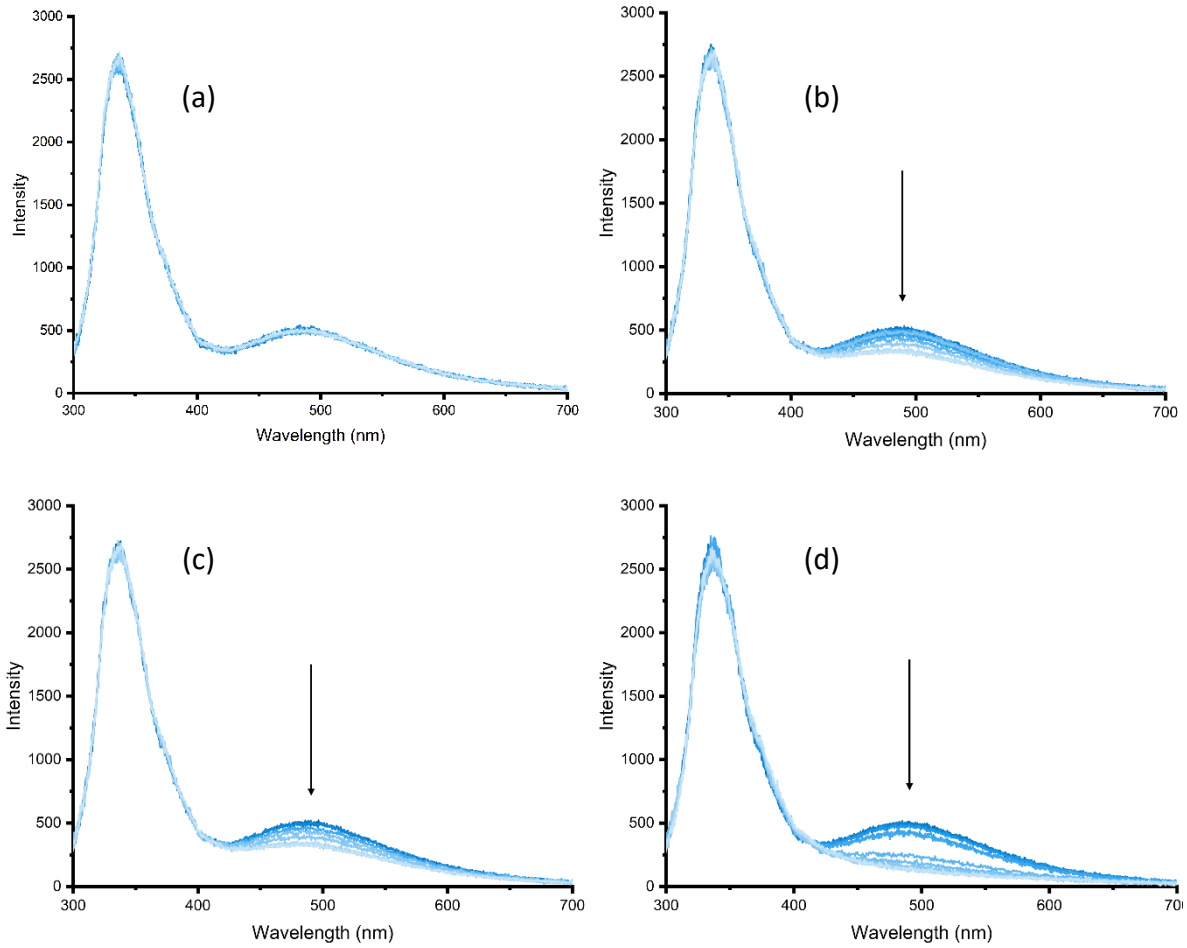


Figure S12: Fluorescence spectra of titration of **6** (20 μM) with (a) TBACl, (b) $TBAH_2PO_4$, (c) TBAOAc and (d) TBA_2SO_4, 0 – 80 equivalents of anion in DMSO (1% water) at 298 K (excitation 280 nm).

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